POLLINATOR-MEDIATED INTERACTIONS BETWEEN THE INVASIVE SHRUB
*Lonicera maackii* AND NATIVE HERBS: THE ROLES OF SHADE,
FLOWERING PHENOLOGY, SPATIAL SCALE, AND FLORAL DENSITY

DISSERTATION

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By

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ABSTRACT

Plant invasions affect native plant reproductive mutualisms, such as biotic pollination, in negative and positive directions. Whether increases or decreases in pollination and native plant reproduction occur in response to plant invasions should depend on environmental context, and mechanisms are poorly understood. I therefore examined how shade, phenology, spatial scale, and floral density influence interactions between a non-native invasive shrub in the USA, Lonicera maackii, and two native herbaceous species, Geranium maculatum and Hydrophyllum macrophyllum.

I designed a field experiment to investigate direct (via shading) and indirect pathways (via pollinators) by which invasive plants may interfere in native plant reproduction in a forest invaded by L. maackii. I measured pollinator visitation, pollen deposition, and native plant reproduction in forest plots invaded by L. maackii and two removal treatments: removal of L. maackii shrubs and removal of L. maackii flowers. Potted G. maculatum plants in treatments containing L. maackii shrubs (with and without flowers) received fewer pollinator visits and conspecific pollen grains than plots in which L. maackii was removed. Hydrophyllum macrophyllum did not co-flower with L. maackii and also received fewer visits in the presence of L. maackii. Thus, invasive plants decrease pollinator visitation and pollen deposition to native plants (via shade),
regardless of whether they co-flower or share pollinators. Although potted *G. maculatum* and *H. macrophyllum* also produced fewer seeds in plots containing *L. maackii*, results of a pollen addition treatment suggested that light limited seed set in both native plants, not pollen receipt. Therefore, the mechanism of impact on native plant reproduction was increased understory shade. At a different site, where *H. macrophyllum* and *L. maackii* co-flowered, I measured pollination and reproduction of potted *H. macrophyllum* in plots in which *L. maackii* was naturally present or absent. Here, pollinator visitation was higher and the level of pollen limitation lower in the presence of *L. maackii*. Comparing *H. macrophyllum* results across these two sites in which flowering phenology was synchronous or asynchronous with *L. maackii* suggests that effects may vary depending on flowering phenology.

Because pollinators are mobile, interactions between plants for pollinators may occur beyond the local neighborhood in which interactions for abiotic resources occur. In an experiment in an old field habitat, I examined the distance over which *L. maackii* may affect pollination and subsequent reproductive success of *G. maculatum*. *Geranium maculatum* pollen receipt and seed set of potted plants decreased, and magnitude of pollen limitation of seed set increased from 0-40m from forest edge habitat invaded by *L. maackii*, suggesting pollinator-mediated facilitation of reproduction. Presence of *L. maackii* was confounded with presence of forest edge habitat, but pollinator composition data and pollinator nesting biology support the interpretation that *L. maackii* affected pollination of *G. maculatum* over a 40m spatial scale.
Finally, relative floral density may influence whether plants compete for pollinators or facilitate one another’s pollination. In an array experiment in old field habitat, potted *G. maculatum* received more visits and conspecific pollen grains and produced slightly more fruits in arrays containing a low density of *L. maackii* flowers compared to control arrays. In contrast, the duration of pollinator visits and seeds per fruit were lower, and pollen limitation of seeds per fruit stronger, when *L. maackii* was present at a high density compared to controls. However, the number of *G. maculatum* seeds per flower did not differ among array treatments, suggesting that altered pollinator foraging behavior is unlikely to lead to population-level impacts on *G. maculatum* reproduction.

This research shows that light availability, floral density, and perhaps flowering phenology all influence whether invasive plants have positive or negative effects on native plant pollination. Invasive plants may alter pollination of native plants at distances beyond the local neighborhood, and the spatial scale of pollinator-mediated interactions between plant species deserves further investigation.
DEDICATION

For Seth.
Listener,
thinker, and encourager.
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I would first like to thank my advisor, Dr. Karen Goodell, for accepting me as her first graduate student. She provided sound advice throughout my graduate career. Her ideas substantially improved my dissertation research proposal, and she taught me a great deal about science, especially scientific writing, experimental design, and designing presentations. I appreciate the independence she allowed me to develop my ideas. She provided crucial funding for some of my field work supplies and supported me as an RA several times. I am also grateful for her statistical prowess, which made my life easier on several occasions.

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LIST OF ABBREVIATIONS

alpha

g gram(s)

h hour(s)

k kilo

L liter(s)

m milli-

micro

min minute(s)

s second(s)
CHAPTER 1
SHADING BY INVASIVE SHRUB REDUCES SEED PRODUCTION AND
POLLINATOR SERVICES IN A NATIVE HERB

INTRODUCTION

The mechanisms behind impacts of non-native plant invasion on natural systems are often poorly understood (Levine et al. 2003). Invasive plants can impact native plants through multiple pathways (Mitchell et al. 2006). Invaders directly reduce success of native plants via competition for soil resources and light but also indirectly affect native plant success via altering ecosystem processes, such as fire frequency and soil nutrient cycling (Vitousek 1990; Ehrenfeld 2003), or ecosystem services, such as pollination (reviewed in Bjerknes et al. 2007; Goodell 2008). Mutualisms maintain and generate biodiversity (Herrera and Pellmyr 2002), and understanding the role invasive plants play in mediating pollination to native plants is of interest because of recent evidence of declines in pollinator populations and pollination services (Biesmeijer 2006; Mazer 2007).

Invasions of non-native plants that produce copious floral resources can change pollinator behavior and therefore subsequent pollination services to native plants (Ghazoul 2002; Bjerknes et al. 2007; Goodell 2008), and our understanding of mechanisms for these types of interactions is insufficient (Traveset and Richardson
Invasive plants can alter the frequency of visits to native plants (Ghazoul 2004; Totland et al. 2006; Bartomeus et al. 2008b; Nielsen et al. 2008), which can impact the quantity of pollen delivered to native plant stigmas (Larson et al. 2006). Individual pollinators that forage simultaneously on invasive and native flowers may also alter the sequence of pollinator visits to native plants and cause heterospecific pollen transfer to native stigmas. In some cases this reduces the quality of pollen loads (Brown et al. 2002; Bartomeus et al. 2008a; Cariveau and Norton 2009) and reduces female reproductive success in native plants if pollen quantity or pollen quality limits seed production. Altering the sequence of visits could also increase transfer of geitonogamous pollen and pollen loss (in a pair of native plants, Campbell 1985), which can reduce male plant success (Waser 1983). Overall, empirical support for competition and neutral effects of invasive plants on pollination services to natives outweighs support for facilitative effects (Bjerknes et al. 2007; Goodell 2008; Morales & Traveset 2009). However, only one study has tested for pollen limitation in natives (Munoz and Cavieres 2008), so the overall importance of reduced pollinator services on reproductive success cannot be assessed.

In addition to changing the relative abundance and composition of flowers available to pollinators, invasive plants may alter other environmental conditions that affect pollinator foraging. It is often unclear whether pollinators are responding to floral resources or these other environmental conditions, such as increased shade. Therefore, I designed a study to tease apart the contributions of altered floral resources and foliage cover, caused by the invasion of a flowering shrub, on the pollinator services and reproductive success of a native herb. Shrubs make up the largest percentage of invasive
plant life forms (including trees, vines, herbs, and grasses), comprising 37% (29/78 species) of some of the worst invasive plants in North America (Martin et al. 2009). Globally, shrubs comprise 23% of plants listed as invasive (Martin et al. 2009). Shrubs may increase shade and reduce temperatures below their canopies. Increased understory shade directly reduces growth and reproductive output of forest understory plants (Luken et al. 1997; Gould and Gorchov 2000) but also decreases pollinator foraging (Herrera 1995a, 1997). Increased shade can also decrease temperature (Herrera 1997), which influences foraging of some pollinator species (Herrera 1995a, b; Totland 2001). In sum, invasion of flowering shrubs may impact pollinator services and native plant reproduction through many pathways (Fig. 1.1). If pollinators respond to increased shade associated with plant invasions, then the impact of invasions on pollinator services to native plants would not be limited to those plants that have overlapping flowering phenologies with the invader. In this case I would predict more widespread effects of plant invasions on pollinator services than previously considered under the hypothesis of competition for pollination.

This study tested the effect of invasive non-native Lonicera maackii on pollinator services and female reproductive success of the native co-flowering understory herb Geranium maculatum. I determined the mechanism of these effects by comparing reproductive success, pollinator visitation, and pollen deposition to potted native G. maculatum plants in deciduous forest plots that contained dense L. maackii invasion or one of two removal treatments: removal of aboveground L. maackii plant material or of L. maackii flowers. The pots excluded competition with L. maackii for soil resources and isolated pollinator- and shade-mediated effects on G. maculatum reproductive success at
the post-flowering stage. My specific objectives were 1) to determine local-scale effects of *L. maackii* on pollinator visitation, pollen deposition, and female reproductive output of *G. maculatum* and 2) to determine the underlying mechanism of interaction by distinguishing the effects of foliage from those of flowers on the above response variables (Fig. 1.1). Because *L. maackii* produces large numbers of nectar-rich flowers and creates shade, I expected that *G. maculatum* flowers would receive fewer pollinator visits and conspecific pollen grains on stigmas in the presence of *L. maackii*. These two species overlap in their visitor fauna, so I also expected more heterospecific pollen grains on stigmas in the presence of *L. maackii* flowers. Consequently, fruit and seed set of *G. maculatum* would be lower in the presence of *L. maackii* if reproductive success was pollen limited. In sum, I expected that *L. maackii* foliage alone would reduce visitation, but that *L. maackii* flowers would have an additional negative impact on visitation and cause heterospecific pollen transfer. Reproductive success, then, should be lowest in *G. maculatum* plants near flowering *L. maackii* and highest in the absence of *L. maackii*.

**METHODS**

**Study site**

I conducted this study during spring 2007 and 2008 in a mesic deciduous forest in Three Creeks Metro Park, Groveport, Ohio, USA. The study area was used for agriculture until the late 1960’s, when it was permitted to re-grow naturally and was invaded by *Lonicera maackii* during forest regeneration (J. Snyder, park naturalist, personal communication). I set up experimental plots within a heavily invaded forest where understory plant growth still occurred. Average *L. maackii* density at this site was
43.2 ±2.7 shrubs per plot, as determined from counting all *L. maackii* stems in a random sample of half of the plots (*N*=6 per treatment; no differences in density among treatments *F*_{15,2} = 0.92; *P*=0.42). The dominant canopy species in the study area was *Acer negundo*. There were no naturally occurring *G. maculatum* plants at the study site, so that natural variation in *G. maculatum* plant density was not a confounding factor in this study. The herb community consisted of common spring ephemerals *Claytonia virginica, Dicentra cucullaria, Erythronium americanum, Mertensia virginica* and *Trillium sessile* (A.M. McKinney, personal observation). Herbs co-flowering with *L. maackii* at this site (early May to early June) included *Erigeron annuus, Hydrophyllum appendiculum* and *Hesperis matronalis* (A.M. McKinney, personal observation).

**Study species**

*Lonicera maackii* (Rupr.) Herder was introduced to North America from China in the late 19\textsuperscript{th} century as an ornamental shrub and is now invasive in forests and fields of eastern USA (Luken and Thieret 1996). In central Ohio, *L. maackii* has fully developed leaves by mid-April, prior to full leaf-out of native shrubs and the tree canopy (Trisel and Gorchov 1994); mature plants such as those in the study site produce thousands of medium-sized (2-2.5 cm wide) white to pinkish-white flowers in early May. *Lonicera maackii* cover is negatively correlated with herb cover (Hutchinson and Vankat 1997; Collier et al. 2002), fecundity, survival, growth, and final size (Gould and Gorchov 2000; Miller and Gorchov 2004). These impacts on herbs were generally attributed to competition for light, but pollinator services were not assessed. Non-native honeybees
(Apis mellifera) and native bees in the genera Halictus, Ceratina, Lasioglossum, Nomada, Augochlorella, Andrena and Bombus forage on L. maackii flowers in central Ohio (Goodell and Iler 2007; Goodell et al. 2010).

*Geranium maculatum* L. is a common, widespread understory herb of eastern North American deciduous forests. It was selected for this study because it was likely to interact for pollinators with *L. maackii*; it co-occurs with *L. maackii*, has an open floral morphology, and its geographic range, habitat, flowering time, and pollinators overlap with *L. maackii*. In addition, *G. maculatum* reproduction is limited by pollen receipt in some populations (Agren and Willson 1992) and by light in others (Willson et al. 1979; McCall and Primack 1987), making it well-suited for an investigation of light and pollinator effects on reproduction. *Geranium maculatum* reproduces sexually by seeds and asexually by rhizomes (Martin 1965). It produces 2-30 large (2.5-4 cm wide) purple-pink flowers per plant. Hermaphroditic flowers are protandrous (Bertin and Sholes 1993), and pollinators are required for seed set to occur (Willson et al. 1979); flowers are self-compatible and produce more seeds when fertilized by outcross pollen (Martin 1965). Visitors to *G. maculatum* are mainly solitary bees of the genera Andrena, Ceratina, Dialictus, Halictus, Nomada and Osmia (Robertson 1928; McCall and Primack 1987; Bertin and Sholes 1993) but also include *Apis mellifera*, Lepidopterans (Bertin and Sholes 1993) and syrphid flies (Agren and Willson 1991).

**Experimental design**

I randomly assigned 36 circular plots of 10m radius to one of three experimental treatments: plant removal (P), flower removal (F), and intact (I) (*N* = 12 plots per treatment). In plant removal plots, I cut all *L. maackii* shrubs down to a small stump (no
shade, no flowers). In flower removal plots, I removed all flower buds from *L. maackii* shrubs approximately one week before flowering commenced, and new buds were removed throughout the experiment (shade, no flowers). *Lonicera maackii* retained branches and flowers in intact plots (shade and flowers). Plots were separated by at least 5m to minimize interactions among adjacent treatments. I selected a 10m radius because it was the largest area that could be manipulated within the experimental time frame, which was limited by flower removal. My aim was to create a treatment large enough to detect a response in pollinator foraging. Because bees, the main pollinators in this study, can forage over a range greater than 15m (Eickwort and Ginsberg 1980; Gathmann and Tscharntke 2002), individual bees could choose among treatment plots within a foraging bout. Therefore, this study is relevant to local scale foraging decisions that bees make in response to biotic (e.g. floral) and abiotic (e.g. light) factors in the environment.

In the center of each plot I placed four *G. maculatum* plants, each in a 2.8 L pot, in holes in the ground so that tops of pots were flush with the ground. Holes were dug in a square-shaped pattern and separated by 0.5m. Experimental plants were housed in a screen house in a light environment similar to that of plant removal plots before I moved them to the study site each year. *Geranium maculatum* plants with at least two buds were randomly assigned to a treatment each year, so that years were independent and plants assigned to treatments did not differ systematically. Plants were put into the field approximately one week before *L. maackii* began to flower. I erected wire fences around *G. maculatum* to prevent deer browsing and watered them as needed. Experimental *G. maculatum* plants were removed after all fruits matured and were overwintered in the screen house.
I measured visible light levels at the center of each plot with a digital light meter (model EA30, Extech Instruments, Massachusetts, USA). One light measurement was taken in the center of each plot at the height of *G. maculatum* flowers and upper leaves. Measurements were recorded during the same two hour time interval over the course of two overcast days at the end of the *L. maackii* bloom in 2007.

I recorded the number of pollinator visits to *G. maculatum* in all plots during 10 min intervals between 10:00am and 4:30pm (time of maximum pollinator activity) on sunny or partly cloudy days from 13 May to 1 June, when air temperature was above 15.5°C. I randomly selected plots for observations each day and continued selecting from a list of un-sampled plots, until all plots were observed once; I repeated this protocol throughout the field season. In intact plots, I also recorded visitation to ~100 *L. maackii* flowers in a separate 10 min interval. A visit was defined as a visitor contacting any reproductive organs of the flower. I calculated visitation rate as the number of visits/flower/10 min. Visitors were identified ‘on the wing’ to lowest taxonomic level possible, usually genus. In addition, I recorded air temperature next to flowers at each observation period with a mercury thermometer and collected reference specimens of pollinators in passive water bowl traps set out for 24 periods in each plot. Plastic 20 mL cups painted with blue or yellow fluorescent paint were used for traps, following Droege (http://www.docstoc.com/docs/2541488/Sam-Droeges-Tips-on-How-to-Use-Bee-Bowls-to-Collect-Bees). I used filter paper wicks to sample standing nectar crop of each species and collected nectar from 10 open flowers on 10 different plants per species (N=10). I followed Kearns & Inouye (1993) to determine nectar sugar content of each species.
To assess pollen deposition I collected two post-receptive stigmas in 2007 and five in 2008 from all open-pollinated *G. maculatum* plants. Stigmas are receptive only during the last day of the lifetime of a flower (4-6 days), and pollen tubes reach the ovary in less than 2 h 30 min (Mulcahy et al. 1983). Therefore, all pollen tubes should have reached the ovary when stigmas were collected. In addition, stigma removal did not appear to affect fruit set, as several mature fruits were missing stigmas (A.M. McKinney, personal observation). I mounted stigmas on microscope slides in the field with fuschin-dyed gel and later counted the number of *G. maculatum, L. maackii*, and other heterospecific pollen grains on each stigma.

I recorded total number of buds and open flowers on each *G. maculatum* plant as the season progressed, and *G. maculatum* and *L. maackii* commenced and completed flowering within four days of one another. I collected fruits as they matured through mid-June and counted seeds to quantify female reproductive success. Treatments did not significantly differ in number of *G. maculatum* flowers per plot (P: 17.1 ± 2.5 flowers; F: 11.7 ± 1.1 flowers; I: 16.0 ± 2.6 flowers; \(X^2 = 3.0, P = 0.2179, N = 12\) (proc npar1way, SAS), indicating it was appropriate to quantify seed set as seeds/plant. To ensure this response was legitimate, I also analyzed the number of seeds/flower. Each variable responded to treatments in the same pattern (see results), so I used seeds/plant because this response is more informative of potential population level effects (Knight et al. 2006).

To test for pollen limitation of reproduction I added supplemental outcross pollen to receptive stigmas of all flowers on two *G. maculatum* plants per plot, and the remaining two plants were open pollinated. However, pollen-supplemented plants set
fewer fruits per flower on average than open pollinated plants in 2007 (open: 0.22 ± 0.07 fruits/flower; supplemental: 0.18 ± 0.08 fruits/flower). *Geranium maculatum* requires 30 grains for maximum seed set (Mulcahy et al. 1983). Therefore, in 2008 I deposited ~30 grains per stigma with the aid of a 20x magnifying glass and collected five stigmas from one hand-pollinated plant per plot to quantify pollen deposition in this treatment. In another study, *G. maculatum* plants that produced more seeds as a result of supplemental pollination did not produce fewer flowers the following year (Agren and Willson 1992), so I did not track fruit production the following season. To assess pollen limitation I compared fruit and seed production of open and pollen-supplemented *G. maculatum* plants within each treatment, using only 2008 data because the adjusted methods increased confidence that the supplemental pollination treatment was successful (see results). In contrast, higher fruit or seed production under optimal pollen receipt in plant removal plots compared to those shaded by *L. maackii* (flower removal and intact treatments combined) would indicate direct light limitation of *G. maculatum* reproduction.

*Analysis*

Light, temperature (log transformed), and number of seeds/fruit were analyzed with a one way ANOVA (proc glm, SAS). *Lonicera maackii* treatment was an independent variable. Other response variables (visitation rate, number of pollen grains, fruits/flower, and seeds/plant) had skewed, long-tailed distributions and violated normality assumptions. I used a non-parametric ANOVA with Wilcoxon Mann-Whitney tests for planned comparisons among treatments and Wilcoxon Mann-Whitney tests to test for pollen and light limitation (proc npar1way, Wilcoxon, SAS). I combined data
from flower removal and intact treatments to create a shade treatment in the analysis of light limitation because these two treatments did not differ in amount of light (see results). Nonparametric tests compare the mean ranks of variables as opposed to means, but for readability, the term ‘mean rank’ is not included in results. I also used Wilcoxon Mann-Whitney tests to assess yearly differences for each response variable; none were significantly different except pollen deposition (see results), so results are reported with data combined between years.

For a more powerful test of limitation of *G. maculatum* fruit set, I modeled the probability of a flower setting a fruit with maximum-likelihood estimation (proc catmod, SAS). Fruit set was a binomial dependent variable weighted by the number of flowers on each plant that did or did not produce a fruit. I tested two models, one for pollen limitation and one for light limitation. To test for pollen limitation of fruit set, pollination treatment (open or supplemental), *L. maackii* treatment (intact, flower removal, or plant removal) and their interaction were categorical independent variables. I removed data for two highly productive supplemental plants in shaded treatments that were discovered to be in light gaps in the forest understory, for a conservative test of pollen limitation. (Data from these plants were flagged prior to analysis as potentially erroneous.) To test for light limitation, I compared the probability of pollen-supplemented flowers setting a fruit between plant removal and shade treatments. (Flagged data remained in the analysis for a conservative test of light limitation.)
RESULTS

Light intensity was significantly different among treatments (plant removal: 1241.67 ± 164.66 lux, flower removal: 431.83 ± 80.64 lux, intact: 334.25 ± 29.8 lux; \( F_{2, 33} = 25.1, P < 0.0001 \)). Mean ± one standard error is shown in text, and \( N = 12 \) unless otherwise specified. Planned comparisons showed that plant removal plots (P) had a significantly higher light intensity than both flower removal (F) and intact plots (I) (\( t > 2.03, P < 0.0001 \)), while flower removal and intact plots did not differ (\( t < 2.03, P = 0.4377 \)). Temperature did not differ significantly among treatments (\( F_{2, 33} = 0.7, P = 0.4934 \)).

Pollinator services

I conducted over 16.5 h of pollinator observations over two years, with 5 h 40 min of observations in plant removal and intact plots and 5 h 20 min in flower removal plots, approximately 30 minutes per plot on average. *Lonicera maackii* and *G. maculatum* shared bee pollinators belonging to the genera *Ceratina*, *Andrena*, *Augochlorella*, *Halictus*, and *Apis*. *Ceratina* accounted for by far the highest percentage of visits in all treatments and to both species (*G. maculatum* 65%, *L. maackii* 36%); the main difference in visitor composition was the presence of honey bees on *L. maackii*, accounting for 19% of total visits, compared to 1% of visits to *G. maculatum*. *Geranium maculatum* plants in flower removal and intact treatments received significantly fewer pollinator visits per flower than in the plant removal treatment, and there was no difference in visitation rate between the former two treatments (Fig. 1.2a; Table 1.1). Per flower visitation rate to *L. maackii* was similar to that of *G. maculatum* in flower removal and intact treatments.
Geranium maculatum nectar contains 40% more sugar on average than L. maackii (171.8 ± 17.3 µg/flower and 103.8 ± 19.2 µg/flower, respectively; \( t = 2.6; P = 0.0168; N=10 \)). L. maackii has many more flowers per plant, however, and the volume of nectar reward may also differ between species.

There were significantly more G. maculatum (Gm) pollen grains deposited on stigmas in each treatment in 2007 compared to 2008 (2007: P: 26.3 ±4.4 Gm grains, F: 17.6 ±4.1 Gm grains, I: 10.5 ±2.4 Gm grains; 2008: P: 11.1 ± 2.2 Gm grains, F: 4.7 ±1.6 Gm grains, I: 2.4 ±0.6 Gm grains; between year comparison \( z =4.4, P <0.0001 \)), but because I collected fewer stigmas in 2007, and the patterns of deposition between treatments were the same between years, I combined data from each year. Conspecific pollen deposition followed the same pattern as visitation to G. maculatum. Pollinators delivered significantly fewer conspecific pollen grains to G. maculatum in flower removal and intact treatments compared to the plant removal treatment, and the former two treatments did not differ significantly (Fig. 1.2b; Table 1.1). In contrast, pollinators delivered very few L. maackii (Lm) pollen grains to G. maculatum stigmas across all treatments (P: 1.6 ±0.4 Lm grains; F: 1.6 ±0.4 Lm grains; I: 1.2 ±0.2 Lm grains; \( X^2 =0.6, P =0.7347 \)). There was also very little deposition of other heterospecific (hs) pollen grains, with no difference among treatments (P: 2.0 ±0.7 hs grains, F: 0.3 ±0.1hs grains, I: 0.3 ±0.1hs grains; \( X^2 =1.9, P =0.3904 \)).

**Female reproductive success**

Geranium maculatum plants in flower removal and intact treatments set significantly fewer fruits/flower, seeds/plant, and seeds/flower than plants in the plant removal treatment, and there were no differences in fruit or seed set between the former
two treatments (Fig. 1.2c and d; Table 1.1). In the plant removal treatment, 58.9% of the plants produced at least one fruit, compared to 17.5% and 7.0% of plants in flower removal and intact treatments, respectively. *Geranium maculatum* plants produced 6.3 times more seeds/plant on average in the plant removal treatment than in treatments containing *L. maackii*. There were no differences in average number of seeds/fruit produced among treatments (P: 1.6 ± 0.3 seeds/fruit, F: 1.2 ± 0.5 seeds/fruit, I: 2.0 ± 0.9 seeds/fruit; $F_{2, 18} = 0.51, P = 0.6077$); sample sizes were reduced for this comparison because several plots contained plants that failed to produce fruit (P: $N = 11$, F: $N = 6$, I: $N = 4$).

**Pollen vs. light limitation**

I deposited on average 32.4 ± 4.2 pollen grains ($N = 72$ stigmas) in the supplemental pollination treatment in 2008 (the only year stigmas were collected for this pollination treatment), compared to an average of 10.3 ± 0.8 conspecific pollen grains ($N = 394$ stigmas) on open-pollinated stigmas (see Fig. 1.2 for breakdown by treatment). There was no evidence of pollen limitation of *G. maculatum* reproductive success within any treatment when data were analyzed with nonparametric tests; pollen-supplemented plants did not set significantly more fruits/flower or seeds/plant than open pollinated plants (fruits/flower: P: $z = 1.1, P = 0.2834$; F: $z = 0.1, P = 0.9498$; I: $z = 0.3, P = 0.8013$, Fig. 1.3a; seeds/plant: P: $z = 0.7, P = 0.4802$; F: $z = 0.3, P = 0.7464$; I: $z = 0.9, P = 0.3740$, Fig. 1.3c). However, pollination treatment and *L. maackii* treatment were both significant factors in the categorical analysis; pollen-supplemented flowers were significantly more likely to set a fruit than open pollinated flowers (probability =0.070 vs. 0.038, respectively, Table 1.2).
Both nonparametric and categorical analyses supported the hypothesis of light limitation of \textit{G. maculatum} reproductive success. Fruit and seed set of pollen-supplemented plants were significantly higher in the plant removal treatment than the shade treatment (fruits/flower: \(z = 2.0, P = 0.0408, \) Fig. 3b; seeds/plant: \(z = 2.4, P = 0.0170, \) Fig. 1.3d). Flowers of pollen-supplemented plants in the plant removal treatment were more than twice as likely to produce a fruit as flowers in the shade treatment (probability =0.15 vs. 0.069, respectively, Table 1.2).

**DISCUSSION**

*Impacts on pollinator services*

The presence of flowering invasive plants can decrease pollinator services and reproductive success of native plants (e.g. Munoz and Cavieres 2008), but this is the first study to my knowledge to experimentally determine that shade overrides effects of competition for pollination between flowers and has a larger impact on pollinator foraging behavior. As predicted, \textit{L. maackii} invasion was associated with locally reduced pollinator services to a native herb, quantified as visitation rate and pollen deposition. This effect appears to reflect lower light under the \textit{L. maackii} canopy compared to uninvaded areas, as \textit{L. maackii} significantly reduced the amount of visible light reaching the forest understory. \textit{Geranium maculatum} and \textit{L. maackii} shared pollinators, but the results did not support the hypothesis of competition for pollinators because \textit{G. maculatum} flowers received similar visitation and pollen deposition in flower removal and intact treatments (Fig. 1.2a and b). Therefore, shade appears to have caused the reduction in pollinator visitation, which resulted in lower pollen deposition. These results
are consistent with those of Herrera (1995b), who found that Hymenopterans preferred to forage in areas with high irradiance. Bertin and Sholes (1993) found that *G. maculatum* received less pollen as either the density of the tree canopy or cloud cover increased in a forest with no shrub layer, results consistent with shade as a mechanism causing reduced pollinator services to *G. maculatum*. Hymenopteran visitation is also influenced by air temperature, which was positively related to irradiance in a meadow habitat (Herrera 1995a). Irradiance and air temperature were unrelated in this study, which may be a result of habitat differences or differences in measuring instruments (see Herrera 1995a). Low light may be associated with lower visitation in the absence of a temperature effect if *Geranium* flower color is less vibrant and therefore less visible in low light areas, or if the *L. maackii* shrub layer obscures the flowers, making them more difficult for pollinators to find.

Contrary to predictions, heterospecific pollen deposition did not differ between treatments, providing further support for the result of a neutral effect of *L. maackii* flowers on pollinator services. Several factors may account for this result: bees may carry pollen from each species in separate foraging bouts, bees may not have switched from *L. maackii* to *G. maculatum* flowers frequently, or bees may have carried *L. maackii* pollen on body parts that did not generally contact *G. maculatum* stigmas. While pollinator observations did not directly address these mechanisms, bees were seldom observed switching between *L. maackii* and *G. maculatum* flowers but were often observed making intraspecific flower transitions.
Mechanism of effects on reproduction

As predicted, *G. maculatum* female reproductive success was significantly lower in the presence of *L. maackii*, in terms of both fruit and seed set (Fig. 1.2c and d). Contrary to my predictions, however, pollinator services had little effect on reproductive patterns (Fig. 1.4). I found only weak evidence that *G. maculatum* fruit production was pollen limited. The categorical analysis, but not the nonparametric analysis, showed significant pollen limitation of fruit production. Because nonparametric tests are prone to type II error, treatment effects must be relatively large to be detected. Pollen limitation does not appear to depend on *L. maackii* treatment because the interaction between *L. maackii* and pollination treatment was not significant. These results are consistent with long term data from a deciduous forest herb community that show that late spring/early summer herbs, such as *G. maculatum*, generally have low percent fruit set (<15%), are light limited, and pollen addition has no effect or results in only a small increase in fruit set (Kudo et al. 2008).

Light reduction caused by *L. maackii* foliage appears to limit reproduction in *G. maculatum*. Among pollen-supplemented plants, the number of fruits/flower and seeds/plant were significantly higher and flowers significantly more likely to produce a fruit in plant removal compared to shaded treatments (Fig. 1.3a and b). Therefore, shading by *L. maackii* appears to be the primary mechanism of reduced fruit and seed set in *G. maculatum* in both flower removal and intact treatments (Fig. 1.4). Consistent with this interpretation, two plants that received supplemental outcross pollen occurred in light gaps in the *L. maackii* canopy and produced several fruits and seeds. Occasionally, *G. maculatum* may grow in ideal light conditions and experience pollen limitation, but
generally, its reproduction seems to be light limited within dense *L. maackii* invasions. In an experiment in a field habitat where light is plentiful, *G. maculatum* reproduction was pollen limited, and *L. maackii* facilitated pollination and reproduction of *G. maculatum* (McKinney, ch. 3). Other studies in forests have found *G. maculatum* populations to be either light or pollen limited (McCall and Primack 1987; Agren and Willson 1992). In forests with a sparser *L. maackii* shrub canopy, I therefore expect *G. maculatum* reproduction to be limited by both light and pollen receipt; if pollen limited, they may even benefit from being near the *L. maackii* bloom, although this would likely also depend on relative floral abundance of each species (McKinney, ch. 4; Munoz and Cavieres 2008).

Finally, heterospecific pollen transfer (HPT) was not an important factor influencing reproduction in this experiment because little *L. maackii* pollen was transferred to *G. maculatum* stigmas across all treatments. Support for HPT reducing native plant reproductive success under natural pollination conditions is lacking. Although I did find significantly lower reproduction with addition of mixed pollen loads when hand pollinations delivered a large number of *L. maackii* pollen grains (17.2 ± 3.6), these levels of HPT were never realized in our study (McKinney, ch. 4). Other studies report similar results from hand pollinations, although not all found HPT to reduce reproduction in native species (Brown and Mitchell 2001; Moragues and Traveset 2005).

Competition for other resources may underlie patterns consistent with competition for pollination; the results of this study emphasize the importance of looking broadly for mechanisms of impact of invaders and fully exploring their consequences for reproduction in native plants. Simply comparing plant removal and intact plots revealed
results consistent with the competition for pollination hypothesis, but the flower removal and supplemental pollination treatments elucidated a different mechanism. The removal of *L. maackii* shrubs was associated with significantly increased light in this experiment. While I measured irradiance and temperature in each treatment, I did not directly manipulate them, and removal of shrubs undoubtedly affected other environmental parameters besides irradiance. Direct light manipulations would make for an insightful investigation of the aspects of the light environment to which pollinators respond. Within the context of this study, however, it would be difficult to alter the same spectrum of light as shrubs without altering other environmental factors. Other potential limiting resources besides light and pollen limitation that were controlled for in experimental design but not directly investigated include water and nutrient availability, and allelopathy. However, empirical evidence supports light as an important resource for plants competing with *L. maackii*. Shading by *L. maackii* has been shown to exert a stronger negative influence on native tree seedling growth and survival than belowground resource competition with *L. maackii* (Gorchov and Trisel 2003). *Lonicera maackii* has negative allelopathic affects on germination of some herbs (Dorning and Cipollini 2006), but field experiments suggest that light is a more important mechanism than allelopathy in reducing understory herb reproductive output and survival (Cipollini et al. 2008).

**Conclusions**

Empirical studies showing that flowers of invasive plants can alter visitation patterns and reproduction in native plants have made us aware that competition between invaders and natives can include pollinators as resources (Grabas and Laverty 1999; Chittka and Schürkens 2001). While competition for light is often investigated in studies
of invasive-native plant interactions (e.g. Braithwaite et al. 1989; Woods 1993), most do 
not consider shade effects on pollinators or other invertebrates that could affect the 
outcome of such interactions, such as herbivores. An important conclusion that I can 
draw from this study is that co-occurring invasive and native plant species need not co-
flower or share pollinators for negative impacts on pollination of the native plant to 
occur. This study suggests that native plants can experience reduced fruit set under a 
wide set of environmental conditions, either through indirect effects of shade on 
pollinators if pollen limited or through direct light limitation if not pollen limited (Fig. 
1.4). Indeed, wildflowers blooming after the *L. maackii* bloom also experienced reduced 
pollinator services and reproductive output in the shade of the *L. maackii* canopy 
(McKinney, ch. 2). *Lonicera maackii* leaves fully develop before those of native shrubs 
and before *G. maculatum* begins to flower (Trisel and Gorchov 1994; Schmidt and 
Whelan 1999). Many spring wildflowers of deciduous forests bloom prior to full leaf-out 
of the forest canopy, are adapted to higher light environments, exhibit pollen limited 
reproduction, (e.g. Kudo et al. 2008), and bloom for short periods, making them 
particularly susceptible to negative effects of shade on pollinator services and 
reproduction.

Based on my results I recommend that future studies investigate mechanisms of 
plant invasions on plant and pollinator populations and communities, how changes in 
native plant communities feedback on pollinator communities and vice versa, and the role 
of floral reward and relative density in mediating competition for pollination. The 
experimental design allowed me to investigate how *L. maackii* affected bees within 
foraging bouts and local-scale effects on native plant reproduction but not how *L. maackii*
may be affecting bee foraging and plant reproduction over larger spatial scales. Specifically, comparisons of historically invaded vs. un-invaded sites will increase understanding of the threats that plant invasions pose to native plant reproduction and the stability of pollination as an ecosystem service.

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Table 1.1  Nonparametric analysis of ranked variables table showing responses of pollinator services and *Geranium maculatum* reproduction to *Lonicera maackii* removal treatments (plant removal (P), flower removal (F) and intact (I)). Nonparametric ANOVAs were conducted for each response variable with treatment as the independent variable ($X^2$), and Wilcoxon Mann-Whitney tests were used for planned comparisons between each treatment ($z$)

<table>
<thead>
<tr>
<th>Source</th>
<th>Visitation rate</th>
<th>Pollen deposition</th>
<th>Fruits per flower</th>
<th>Seeds per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X^2$</td>
<td>$P$</td>
<td>$X^2$</td>
<td>$P$</td>
</tr>
<tr>
<td>Treatment</td>
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<td>0.0023</td>
<td>12.7</td>
<td>0.0018</td>
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<td>$z$</td>
<td>$z$</td>
<td>$z$</td>
</tr>
<tr>
<td>P vs. F</td>
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<td>2.3</td>
<td>0.0194</td>
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</tr>
<tr>
<td>F vs. I</td>
<td>1.0</td>
<td>0.9206</td>
<td>0.9</td>
<td>0.3864</td>
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</table>
Table 1.2 Pollen and light limitation of *Geranium maculatum* reproduction. Maximum likelihood analysis of variance table showing effects of pollination treatment (open vs. supplemental), *Lonicera maackii* removal treatment (plant removal (P), flower removal (F) and intact (I)), their interaction, and light treatment (P vs. shade= F and I combined) on the proportion of *G. maculatum* flowers to set fruit.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>$X^2$</th>
<th>P</th>
</tr>
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<tr>
<td>Pollination</td>
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<td>8.78</td>
<td>0.0031</td>
</tr>
<tr>
<td><em>L. maackii</em></td>
<td>2</td>
<td>65.55</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pollination x <em>L. maackii</em></td>
<td>2</td>
<td>2.56</td>
<td>0.2781</td>
</tr>
<tr>
<td>Light</td>
<td>1</td>
<td>21.12</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Contrasts

<table>
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<tr>
<th>Contrasts</th>
<th>df</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>P vs. F</td>
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<td>8.18</td>
<td>0.0042</td>
</tr>
<tr>
<td>P vs. I</td>
<td>1</td>
<td>2.3</td>
<td>0.1296</td>
</tr>
<tr>
<td>F vs. I</td>
<td>1</td>
<td>4.23</td>
<td>0.0398</td>
</tr>
</tbody>
</table>
Figure 1.1  Concept map showing potential mechanisms of effects of an invasive shrub on pollinator services (visitation rate and conspecific pollen deposition) and reproductive output of a native herb. Response variables have incoming arrows and independent variables outgoing arrows. Signs above arrows show hypothesized direction of interactions and should be interpreted as multiplicative effects. E.g. An invasive shrub will be associated with decreased irradiance; irradiance will be positively associated with fruit set, but the overall impact of an invasive shrub acting through irradiance will be negative to fruit and seed set $[(-) \times (+) = (-)]$
Figure 1.2 Response of native Geranium maculatum to Lonicera maackii removal treatments: a) pollinator visitation rate shown as number of visits/flower/10 minutes, b) conspecific pollen deposition, c) fruit set and d) seed set. Data from 2007 and 2008 were combined in all graphs, and responses were averaged by plot so that N = 12 for all treatments. Boxes are 25th and 75th percentiles, and error bars are 5th and 95th percentiles. Solid squares are means and horizontal lines across boxes are median values; if no horizontal line is visible, median value is zero. Significant differences from Wilcoxon Mann-Whitney tests are indicated by letters: $P < 0.05$ in b and d and $P < 0.01$ in a and c.
Figure 1.3 Pollen limitation (a and c) and light limitation (b and d) of *Geranium maculatum* female reproductive success in *Lonicera maackii* removal treatments. Data are from 2008. N = 12 plots for all samples in a and c, except for open pollinated plants in the plant removal treatment (N = 11). In graphs b and d N = 12 for plant removal and 24 for shade treatments (flower removal and intact combined because light availability for *G. maculatum* did not differ significantly between these two treatments). Boxes are 25-75th percentiles and solid squares are mean values. Median values are represented by horizontal lines and are zero if otherwise not visible. Significant differences from Wilcoxin Mann-Whitney tests are indicated by asterisks: P < 0.05
Figure 1.4  Concept map showing mechanisms of effects of the invasive shrub *Lonicera maackii* on reproductive output of the native herb *Geranium maculatum*. Response variables have incoming arrows and independent variables outgoing arrows. Signs above arrows show direction of interactions in this study and should be interpreted as multiplicative effects. Arrows correspond to evidence for each mechanism; solid arrows were significant in both nonparametric and categorical analyses, while dotted arrows were significant only in a categorical analysis. Our results suggest that on average, decreased irradiance directly reduces herb reproduction, and when light is not limiting, decreased irradiance will indirectly reduce herb reproduction via reduced pollination services.
CHAPTER 2

PLANT-POLLINATOR INTERACTIONS BETWEEN A NATIVE AND INVASIVE PLANT VARY BETWEEN SITES WITH DIFFERENT FLOWERING PHENOLOGY

INTRODUCTION

Pollination is an important stage in the angiosperm life cycle, and animal pollinators deliver pollen that is either required or beneficial for reproduction of the majority of flowering plants (Brown 1990). However, widespread pollen limitation of plant reproduction means that many plants do not receive enough pollen to reach their reproductive potential (Knight et al. 2005). In plant-pollinator communities, most plant species are visited by several pollinator species (i.e. generalization), and co-occurring plant species commonly interact via shared pollinators (Waser 1996, Fontaine et al. 2006, Mitchell et al. 2009, Vazquez et al. 2009). Invasive plants integrate into plant-pollinator communities in the invaded range and can affect seed production of native plant species through effects on pollinator visitation to native plants (Chittka and Schürkens 2001, Memmott and Waser 2002, Bartomeus et al. 2008). Co-flowering plant species may increase, decrease, or have no effect on one another’s pollination (for reviews see Waser 1983, Bjerknes et al. 2007, Goodell 2008). Most studies of pollinator-mediated interactions between plant species focus on co-flowering plant species that share pollinators, but plant invasions can also alter abiotic aspects of the pollinator foraging
environment, which affects pollinator visitation and pollen deposition to native plants (McKinney and Goodell 2010). Therefore, we must look more broadly for impacts of invasive plants on pollination of native plants, including plant species that do not co-flower or share pollinators. Furthermore, variation in flowering phenology of invasive or native plants across populations or regions could mean that interactions that affect pollination between specific pairs of invasive and native plants vary widely with phenological overlap.

Competition and facilitation of pollination may have demographic consequences for native plants, depending on the change in magnitude of pollen limitation of reproduction (Ashman et al. 2004). Pollinator-mediated interactions among plant species influence plant community structure (reviewed in Palmer et al. 2003), and they may affect various facets of flowering through selection for divergence (via competition) or convergence (via facilitation) of floral traits such as flowering phenology, floral morphology, and timing of reward production throughout the day (Waser 1978, Pleasants 1980, Thomson 1980, Campbell et al. 1996, Stone et al. 1998, Raine et al. 2007). It is therefore important to measure how plant invasions impact pollen limitation of native plant reproduction. However, very few studies of pollinator-mediated interactions between invasive and native plants quantify pollen limitation (Munoz and Cavieres 2008, McKinney and Goodell 2010).

Interestingly, pollinator-mediated interactions between invasive and native plant species are distinct from those among native plant species. A meta-analysis of studies of plant-pollinator interactions between invasive and native plant species revealed that, of documented cases, the presence of invasive plants is associated with lower pollinator
visitation and reproductive success of native plants; in contrast, neighboring native plant species have no effect on one another’s pollinator visitation or reproductive success (Morales and Traveset 2009). Therefore, the magnitude of impacts on pollination may be stronger between invasive and native plants than between native plants. Invasive plants may have larger effects on pollination of neighboring native species because invasive plants have relatively large floral displays compared to native species (Bjerknes et al. 2007), and they are often more common than native species. Invasive plants both compete for pollinators with native plants and facilitate pollinator visitation to native plants, thereby affecting the quantity of pollen delivered to native plant stigmas (Moragues and Traveset 2005, Larson et al. 2006, Totland et al. 2006). When pollinators frequently move between invasive and native plant species during foraging bouts, pollinators may deliver heterospecific pollen to native plant stigmas (Brown et al. 2002, Lopezaraiza-Mikel et al. 2007). Finally, plant invasions can alter pollinator communities, with potential subsequent effects on pollen deposition and native plant reproduction (Carvalheiro et al. 2008, Moron et al. 2009). Changes in pollen quantity and quality will impact reproductive success when pollen receipt limits plant reproduction.

Interspecific interactions for pollinators between invasive and native plants are not limited to co-flowering individuals, however. In addition to the myriad floral cues to which pollinators respond, such as floral density, nectar and pollen rewards, and floral scent, pollinator activity is also influenced by abiotic environmental factors, such as temperature and light (Heinrich 1979, Thomson and Plowright 1980, Thomson 1981, Totland 1994, Herrera 1995, 1997). Invasive shrubs are associated with increased forest understory shade, reduced pollinator visitation to understory herbs, and negative impacts.
on reproductive success of understory herbs (Gould and Gorchov 2000, McKinney and Goodell 2010). The negative effects of shade on pollinator foraging to understory plants may be widespread, given that shrubs comprise 23% of plant species listed as invasive throughout the world (Martin et al. 2009). In North America, shrubs make up the largest percentage of invasive plant life forms (including trees, vines, herbs, and grasses), comprising 37% (29/78 species) of some of the worst invasive plant species (Martin et al. 2009).

The phenology of understory plant flowering and fruit production in relation to canopy closure may influence how altered light conditions affect reproduction of understory plants. Reproduction in understory herbs that flower during canopy closure and fruit after canopy closure is often resource limited, as opposed to pollen limited (Kudo et al. 2008, McKinney and Goodell 2010). Fruit set of herbs that flower and fruit either before or after the canopy closes, however, seems to be influenced much less by light, perhaps because fruit and flower production is in proportion to the amount of photosynthate available (Taylor and Pearcy 1976). It is therefore more likely that pollinator services influence reproductive success of herbs flowering and fruiting pre- and post-canopy closure (Barrett and Helenurm 1987, Kudo et al. 2008). In such cases, I expect decreased understory irradiance from an invasive shrub to reduce reproductive success of native herbs via pollinator-mediated pathways. If shrubs co-flower with understory native plants, the shrub’s flowers may compete for pollinators and exacerbate the negative effects of shade. Conversely, the shrub’s flowers may facilitate pollination of native plants, thereby balancing or overcoming the negative impacts of shade on pollinator foraging to native herbs, depending on the strength of facilitation.
This study examined effects of flowering phenology and understory light environment on pollinator-mediated interactions between the native understory herb *Hydrophyllum macrophyllum* and the invasive non-native shrub, *Lonicera maackii*, at two sites: one with synchronous flowering phenologies (*H. macrophyllum* flowering during *L. maackii* bloom) and one with asynchronous flowering phenologies. At each site, I examined the mechanism behind reproductive effects in *H. macrophyllum* by comparing pollinator visitation, magnitude of pollen limitation of reproductive success, and correlation between pollen deposition and reproductive success of potted native *H. macrophyllum* plants in deciduous forest plots in which *L. maackii* was present and absent. The pots excluded competition with *L. maackii* for soil resources and isolated pollinator- and light-mediated effects on *H. macrophyllum* reproductive success at the post-flowering stage. The specific objectives were 1) to determine variation across sites in local-scale effects of *L. maackii* on female reproductive output of *H. macrophyllum* and 2) to determine the underlying mechanism of the impact on reproductive output at sites with different phenological overlaps.

**METHODS**

*Study species*

*Lonicera maackii* (Rupr.) Herder (Amur honeysuckle), a native of China, was introduced to North America in the late 19th century as an ornamental shrub and is now invasive in forests and fields of the Eastern US (Luken and Thieret 1996). In central Ohio, *L. maackii* has fully developed leaves by mid-April, prior to full leaf-out of native shrubs and the tree canopy (Trisel and Gorchov 1994, Shustack et al. 2009). Individual
shrubs produce thousands of white to pinkish-white flowers. Flowers are present from May through early June in central Ohio and offer pollen and nectar rewards (see McKinney and Goodell 2010 for nectar sugar content). *Lonicera maackii* has negative impacts on forest flora and fauna, and its presence is negatively correlated with the growth, fecundity, and survival of herbs (Gould and Gorchov 2000, Miller and Gorchov 2004, Leston and Rodewald 2006). Pollinators influence reproductive output of *L. maackii*; it exhibits pollen-limited fruit and seed production in urban woodlands and is partially self-incompatible (Goodell and Iler 2007). Non-native honeybees (*Apis mellifera*) and native bees in the genera *Apis, Halictus, Ceratina, Lasioglossum, Nomada, Augochlorella, Andrena*, and *Bombus* forage on *L. maackii* flowers in the Columbus, Ohio, area (Goodell and Iler 2007).

*Hydrophyllum macrophyllum* (largeleaf waterleaf) is a biennial woodland herb native to eastern North America. Individual flowers are hermaphroditic and last approximately 72-84 hours, and nectar is produced throughout this time (Beckmann 1979). *Hydrophyllum macrophyllum* produces one or more inflorescences of 5-40 white actinomorphic flowers that bloom sequentially from mid-May to mid-June, and seeds mature in mid- to late July (Baskin and Baskin 1983). *Hydrophyllum macrophyllum* is self-compatible but requires pollinators to set seed (Beckmann 1979). The most common visitors observed on *Hydrophyllum* flowers in observations performed throughout its eastern range include *Apis mellifera, Bombus* spp. (bumble bees), and *Osmia* spp. (leafcutter bees) (Beckmann 1979). *Hydrophyllum macrophyllum* is also visited by several species of small solitary bees in the genera *Lasioglossum, Augochlorella, Halictus, and Ceratina*, but they rarely contact the sexual parts of the flower (K. Goodell,
personal observation). This species did not occur within the invaded areas of either of these sites, which allowed me to control the effects of conspecific floral density on pollinator foraging behavior. Within the central Ohio region, *H. macrophyllum* grows within invaded areas, and large populations have been found < 1 m from *L. maackii* shrubs (K. Goodell, unpublished).

**Study site**

I conducted this study during spring 2007 in a mesic deciduous forest at two locations: Denison University Bioreserve, Granville, Ohio, USA, and Three Creeks Metro Park, Groveport, Ohio, USA. The Dennison University Bioreserve site was located in a patch of ca 50 year old secondary hardwood forest containing a patchy *Lonicera maackii* invasion. Canopy species included *Acer saccharum* (sugar maple), *Cornus florida* (flowering dogwood), *Fraxinus spp.* (ash), *Juglans nigra* (black walnut), *Malus* spp. (apple), and *Prunus serotina* (black cherry). A shrub layer of mixed native and exotic species included *Rosa multiflora*, *Rubus* spp., *Viburnum* spp., *Lonicera maackii*, *L. tartarica* (tartarian honeysuckle), and *L. morrowii* (Morrow’s honeysuckle). The herb layer consisted of *Alliaria petiolata* (garlic mustard), *Aster* spp., *Fragaria virginiana* (wild strawberry), *Galium* spp., *Hesperis matronalis* (dame’s rocket), *Hydrophyllum macrophyllum*, *H. appendiculatum* (great waterleaf), *Parthenocissus quinquefolia* (Virginia creeper), *Rhus toxicodendron* (poison ivy), and *Viola* spp. I set up experimental plots within the invaded forest where understory plants were present. Natural populations of *H. macrophyllum* did not occur within at least 100 m of the plots. At this site, *L. maackii* flowered throughout the entire *H. macrophyllum* flowering period
(Fig. 2.1), with peak *H. macrophyllum* flowering overlapping broadly with peak *L. maackii* flowering.

The study site at Three Creeks was also located in a patch of ca 50 year old secondary hardwood forest containing a relatively continuous *L. maackii* invasion (0.14 *L. maackii* shrubs per m\(^2\)). The dominant canopy species in the study area was *Acer negundo* (box elder). The shrub layer was comprised mainly of *L. maackii* but also included *L. morrowii*. The herb community included *A. petiolata, Aster* spp., *Claytonia virginica, Dicentra cucullaria, Erigeron annuus* (daisy fleabane), *Erythronium americanum* (trout lily), *H. matronalis, H. appendiculatum, Mertensia virginica* (Virginia bluebells), *R. toxicodendron*, and *Trillium sessile* (toadshade). There were no populations of *H. macrophyllum* at this site. I set up experimental plots within the invaded forest where understory plants were present. The study species co-flowered for only 4 days, at the end of the *L. maackii* flowering period and beginning of the *H. macrophyllum* flowering period (Fig. 2.1).

**Experimental design**

At Denison, I randomly selected 24 circular plots of 10m radius, 12 without *L. maackii* (absent) and 12 with *L. maackii* (present). At Three Creeks, I followed the same procedure, but because the forest was more continuously invaded, I randomly assigned plots to either *L. maackii* removal (absent) or *L. maackii* present (*N*=12 plots per treatment). In removal plots, I cut all *L. maackii* shrubs down to a small stump (ca. 15cm). At both sites, plots were separated by at least 5m to minimize interactions among adjacent treatments. Experimental *H. macrophyllum* plants were removed from the Denison nature preserve at the rosette stage, transferred to 2.8 L pots containing a
mixture of local soil and ProMix potting soil, and housed in a screen house at The Ohio State University-Newark. Here they matured until I moved them to the field, approximately one week before *L. maackii* began to flower at each site. Potted *H. macrophyllum* flowering phenology was similar to natural populations at Denison and other central Ohio populations, with first and last dates of flowering varying by only a couple of days across populations (K. Goodell, personal observation). In the center of each plot at Denison I placed four potted *H. macrophyllum* plants, and three at Three Creeks, separated by 0.5m. Several of the rosettes I collected did not produce flower buds, so I reduced the number of plants per plot at Three Creeks compared to Denison. The potted plants were watered as needed at both sites and 4m tall wire fences were erected around *H. macrophyllum* to prevent deer browsing only at Three Creeks. There was no evidence of deer browsing on *H. macrophyllum* plants at Denison (K. Goodell, personal observation). I removed potted *H. macrophyllum* plants from each site after all flowers had senesced (midway through fruit maturation) to a common, protected environment at The Ohio State University-Newark. The plants were watered regularly until the fruits had completely matured.

At both sites, I measured light availability, recorded pollinator visits, counted conspecific, *L. maackii*, and other heterospecific pollen grains deposited on *H. macrophyllum* stigmas, and counted fruits and seeds as they matured. Light measurements were taken at the center of each plot within both sites at the height of *H. macrophyllum* flowers, using a digital light meter (model EA30, Extech Instruments, Massachusetts, USA). Light measurements were taken on different overcast days at each site.
I recorded the number of pollinator visits to *H. macrophyllum* flowers in all plots during 10 min intervals between 10:00am and 4:30pm (time of maximum pollinator activity) on sunny or partly cloudy days, when air temperature was above 15.5°C. A visit is defined as contact with either the anthers or stigma of the flower. Visitation rate was calculated as the number of visits/plot/10 min. Visitors were identified ‘on the wing’ to lowest taxonomic level possible, usually genus. To assess pollen deposition, two post-receptive stigmas per plant were collected at Three Creeks and 10 stigmas per plant at Denison. Stigmas were placed into vials containing 70% ethanol, stained with fuschin dyed gel, and pollen grains counted with the aid of a microscope.

I recorded total number of open flowers on each *H. macrophyllum* plant as the season progressed. To test for pollen limitation of female reproductive success, supplemental outcross pollen was added to receptive stigmas of all flowers on one *H. macrophyllum* plant per plot at Three Creeks and to most flowers on two plants per plot at Denison, and the remaining plants were open pollinated. Some of the plants were eaten or dug out of pots at Three Creeks, presumably by chipmunks, reducing the sample size of pollen-supplementation at this site (see Fig. 2.3 for sample sizes).

**Analysis**

Response variables were averaged by plot, the sample unit (*N*=12 per treatment per site), and analyses were conducted with JMP v.8 software (SAS institute, Cary, NC). Sites were analyzed separately because phenology classification was replicated once (synchronous and asynchronous), and experimental design differed slightly: natural absence of *L. maackii* at Denison compared to removal of *L. maackii* at Three Creeks. I used t-tests, and non-parametric Mann-Whitney *z*-tests when response variables were
non-normally distributed, to test for differences in light, pollinator visitation rate, pollen deposition, fruits per flower, seeds per fruit, and seeds per flower between *L. maackii* treatments. Light was natural log transformed for Three Creeks to meet normality assumptions. To assess pollen limitation, I used *t*-tests to test for differences in the magnitude of pollen limitation of reproduction between *L. maackii* treatments. Pollen limitation was calculated as the difference in reproductive success between pollen-supplemented and open-pollinated plants.

Finally, to assess whether pollinator services affected reproduction, I used linear regression analysis (standard least squares) to investigate the correlation between average plant reproductive success and average pollen deposition per plot. I combined data across *L. maackii* treatments at each site. Pollen deposition was a continuous explanatory variable and reproductive success was a continuous response variable. I performed separate regressions for fruits per flower, seeds per fruit, and seeds per flower. If reproductive responses were pollinator-mediated, I expected conspecific pollen deposition to be positively correlated with *H. macrophyllum* female reproductive success. In contrast, if *H. macrophyllum* reproductive success responded more to the abiotic environment, I expected conspecific pollen deposition to be uncorrelated with female reproductive success (but not negatively correlated). All p-values are two-tailed.
RESULTS

Pollination

Understory light levels were significantly lower in the presence of *L. maackii* at both sites (Denison: \( t = 5.42, P < 0.0001; \) Three Creeks (natural log transformed): \( t = 4.84, P < 0.0001; \) Fig. 2.2). A total of 121 pollinators were observed in 16 hours of observations at Denison and 42 pollinators in 8 hours of pollinator observations at Three Creeks. The pollinator communities were similar between the two sites, as shown by identification of bees to genera, and other pollinators to the family level (Table 2.1). At Denison where *H. macrophyllum* and *L. maackii* flowering phenologies were synchronous, a total of 6 pollinator taxa were recorded visiting *H. macrophyllum* in the presence of flowering *L. maackii* but only 3 pollinator taxa were recorded visiting *H. macrophyllum* in the absence of *L. maackii* (Table 2.1). There were significantly more pollinator taxa on average visiting *H. macrophyllum* in the presence of flowering *L. maackii* compared to plots in which *L. maackii* was absent at Denison (absent: 1.83±0.21 pollinator taxa; present: 2.83±0.21 pollinator taxa; \( z = 2.79, P = 0.0053, N = 12 \)). At Three Creeks, where *H. macrophyllum* and *L. maackii* flowering phenology only slightly overlapped, I recorded a total of 6 pollinator taxa visiting *H. macrophyllum* in the absence of *L. maackii* compared to 3 total pollinator taxa in the presence of *L. maackii* (Table 2.1). There were significantly more pollinator taxa on average visiting *H. macrophyllum* in plots in which *L. maackii* was absent compared to plots in which *L. maackii* was present (absent: 1.46±0.18 pollinator taxa; present: 0.55±0.25 pollinator taxa; \( z = 2.64, P = 0.0082, N = 11 \)).
At Denison, pollinator visitation rate to *H. macrophyllum* was significantly higher in the presence of flowering *L. maackii* compared to when *L. maackii* was absent (absent: 0.92±0.13 visits per plot, *N*=12, present: 1.94 ±0.18 visits per plot, *N*=12; *t*=4.50; *P*=0.0002), but there was no significant difference between treatments in the number of conspecific pollen grains delivered to *H. macrophyllum* stigmas (absent: 6.90±1.96 grains, *N*=10, present: 7.37±1.57 grains, *N*=11; *z*=0.297; *P*=0.77). At Three Creeks, pollinator visitation rate exhibited the opposite trend and was significantly lower in the presence of *L. maackii* compared to when *L. maackii* was absent (absent: 4.70±1.22 visits per plot, present: 0.77±0.42 visits per plot, *N*=11, *z*=3.12; *P*=0.0018). Again, there was no difference between treatments in average conspecific pollen deposition to *H. macrophyllum* stigmas (absent: 8.47±1.88 grains, *N*=12; present: 5.48±1.95 grains, *N*=11; *t*=1.56, *P*=0.13).

Heterospecific pollen transfer to *H. macrophyllum* stigmas was uncommon at both sites. At Denison, only 4.3% of *H. macrophyllum* stigmas contained *L. maackii* pollen, and there was no difference in the amount of *L. maackii* pollen between treatments (absent: 0.12±0.11 *L. maackii* grains, present: 0.088±0.044 *L. maackii* grains; *z*=0.88, *P*=0.38). I found only one unknown heterospecific pollen grain on *H. macrophyllum* stigmas. Because the end of the *L. maackii* bloom overlapped for a few days with the beginning of the *H. macrophyllum* bloom at Three Creeks, I found a total of two *L. maackii* pollen grains on *H. macrophyllum* stigmas, both in plots in which *L. maackii* was present. Only 5.3% of *H. macrophyllum* stigmas contained other heterospecific pollen grains at Three Creeks, and there was no difference between
treatments (absent: 0.17±0.17 heterospecific grains, present: 0.27±0.14 heterospecific grains; z=0.98, P=0.33). I conclude that there was insufficient heterospecific pollen transfer to affect *H. macrophyllum* reproduction at either site.

*Reproductive output*

At Denison, *H. macrophyllum* flowers produced marginally significantly more fruits and seeds in the presence of flowering *L. maackii* compared to when *L. maackii* was absent (t=1.87, P=0.08; t=2.03, P=0.06; Fig. 2.3a,c respectively). There was no difference in the number of seeds per fruit between treatments, although the trend was consistent with the other measures of female reproductive success at this site (t=1.61, P=0.13; Fig. 2.3b). In addition, *H. macrophyllum* plants produced almost double the seeds in the presence of co-flowering *L. maackii* compared to when *L. maackii* was absent, but the number of seeds each plant produced was quite variable, and the difference between treatments was marginally significant (41.40±7.9 seeds, 22.24±5.52 seeds, respectively; N=12, z=2.09, P=0.07). At Three Creeks where flowering phenologies were asynchronous, *H. macrophyllum* reproduction showed the opposite trend. The average number of seeds per fruit was significantly lower in the presence of *L. maackii* compared to the absence of *L. maackii* (t=3.09, P=0.006; Fig. 2.3b). The average number of fruits per flower and seeds per flower did not significantly differ between treatments (t=0.70, P=0.49 and t=0.24, P=0.82; Fig. 2.3a, c, respectively).

*Pollen limitation*

At Denison, the magnitude of pollen limitation of *H. macrophyllum* fruits per flower and seeds per flower was significantly lower in the presence of flowering *L. maackii* compared to the absence of *L. maackii* (t=2.29, P=0.03; t=2.32, P=0.03,
respectively; Fig. 2.4a, c). The level of pollen limitation of seeds per fruit was marginally significantly lower in the presence of flowering *L. maackii* compared to when *L. maackii* was absent (*t*=1.97, *P*=0.06; Fig. 2.4b). At Three Creeks, however, there was no evidence that the magnitude of pollen limitation of fruits per flower, seeds per fruit, or seeds per flower differed between treatments (*t*=0.089, *P*=0.93; *t*=1.47, *P*=0.17; *t*=1.56, *P*=0.14, respectively; Fig. 2.4).

Because pollen deposition data showed trends consistent with reproductive responses at both sites but did not significantly differ between treatments at either site, I investigated the correlation between *H. macrophyllum* pollen receipt and reproductive success. At Denison, the average number of conspecific pollen grains was positively correlated with all measures of reproductive success (fruits/flower: *F*=3.9, *P*=0.063; seeds/fruit: *F*=8.2, *P*=0.01; seeds/flower: *F*=8.9, *P*=0.008; Fig. 2.5a, b, c). However, at Three Creeks the average number of conspecific pollen grains was not correlated with reproductive success (fruits/flower: *F*=2.7, *P*=0.11; seeds/fruit: *F*=2.2, *P*=0.17; seeds/flower: *F*=1.0, *P*=0.33; Fig. 2.5e, d, f).

**DISCUSSION**

Denison: synchronous flowering phenology

Results suggest that flowering *L. maackii* shrubs alleviated pollen limitation of *H. macrophyllum* reproduction through increased pollinator visitation, leading to a slight increase in overall *H. macrophyllum* fruit and seed production compared to when *L. maackii* shrubs were absent. Conspecific pollen deposition was positively correlated with *H. macrophyllum* reproductive success, supporting the link between pollination and plant
reproduction. In addition, *H. macrophyllum* received more pollinator visits from a larger number of pollinator taxa when near flowering *L. maackii* compared to when *L. maackii* was absent. Facilitation of visitation occurred despite shade created by the *L. maackii* canopy, which is associated with decreased pollinator visitation to the native herb *Geranium maculatum* (McKinney and Goodell 2010). Instead, the relatively high density of *L. maackii* flowers may have attracted pollinators to the plots from a distance (sensu Thomson and Plowright 1980, Rathcke 1983, Feldman et al. 2004, Hegland et al. 2009), and spill over of visitors from the *L. maackii* flowers may have increased visitation to *H. macrophyllum* despite decreased irradiance under the *L. maackii* canopy. Plant density and diversity affect both the composition of pollinators on plants and pollinator visitation (Conner and Neumeier 1995, Hegland and Totland 2005, Moeller 2005, Lazaro et al. 2009), and *L. maackii* seemed to attract a variety of pollinators to plots that also visited *H. macrophyllum*, compared to when *L. maackii* was absent. Similarly, Lopezaraiza-Mikel et al. (2007) found a higher diversity and abundance of pollinators and higher visitation to native plants in plots invaded by *Impatiens glandulifera* compared to uninvaded plots. Insect diversity was higher in pan traps near invasive *Heracleum mantegazzianum*, and native plants received higher visitation near the invader (Nielsen et al. 2008). Specifically, *Bombus spp.* and *Ceratina spp.* were observed on *H. macrophyllum* only when *L. maackii* flowers were present, and *Bombus spp.* are listed as pollinators of *Hydrophyllum* in the literature (Beckmann 1979). Bee species diversity is correlated with plant reproductive success (Slagle and Hendrix 2009), a pattern that may arise simply because as the number of pollinator species increases so does the chance of including species that are effective pollinators, which may have occurred at this site.
The mechanism of alleviation of pollen limitation of *H. macrophyllum* fruits and seeds per flower may have been an increase in pollen quantity or in pollen quality. However, pollen quality is unlikely to have large impacts on a self-compatible plant species. Our results suggest pollen quantity as a mechanism because as pollen quantity increased, so did *H. macrophyllum* reproduction, although pollen quality may also increase as quantity increases, a distinction I am unable to make with my data. It should be noted that I did not detect a significant difference in pollen deposition between treatments, perhaps because the pollen deposition results were quite variable. Such highly variable pollen deposition is common (Engel and Irwin 2003, Price et al. 2005, Burd et al. 2009). Other studies of invasive-native plant interactions for pollinators also report inconsistencies between visitation and pollen deposition (Larson et al. 2006, Cariveau and Norton 2009), and the sample sizes for pollen deposition were comparatively smaller in this study. Higher variation in pollen deposition compared to reproductive success is statistically expected because pollen deposition represents a sample of stigmas from individual plants, whereas reproductive success includes the entire plant. It is therefore telling that I detected a significant correlation between average pollen deposition and reproductive success, despite the variation in pollen deposition.

Flowering *L. maackii* seems to alleviate pollen limitation of *H. macrophyllum*, based on results showing decreased magnitude of pollen limitation in the presence of the invader. *Lonicera maackii* also seems to alleviate pollen limitation of reproduction in native co-flowering *Geranium maculatum* in an old field habitat; seed set increased and pollen limitation of seed set decreased with proximity to an invaded forest edge.
containing *L. maackii* (McKinney, ch. 3). Theory and empirical work suggest that pollen limitation is stronger in environments rich in abiotic resources in which plant reproduction is unlikely to be resource-limited (Totland 2001, Burd 2008, Goodell et al. 2010) (but see Niesenbaum 1993). Consistent with these studies, understory light was more abundant in the absence of *L. maackii* where pollen limitation of *H. macrophyllum* was stronger. Pollen limitation may also have been stronger in the absence of *L. maackii* because of lower pollinator visitation and fewer pollinator taxa visiting *H. macrophyllum* compared to plants near flowering *L. maackii*.

*Three Creeks: asynchronous flowering phenology*

I detected the opposite pattern of pollinator visitation and *H. macrophyllum* reproduction at Three Creeks. Decreased visitation in the presence of *L. maackii* foliage supports my previous work showing that invasive and native plants need not co-flower for invasive plants to negatively affect pollinator foraging to native plants (McKinney and Goodell 2010). Fruit set was similar to other understory deciduous forest herbs sharing the same flowering and fruiting phenology (~50%) (Kudo et al. 2008). In general, measures of *H. macrophyllum* reproductive output did not differ between treatments, but the number of seeds per fruit was significantly lower in the presence of *L. maackii* foliage compared to when *L. maackii* was absent. A factor other than pollination likely caused the decrease in seed production because no measures of *H. macrophyllum* reproduction were significantly correlated with pollen deposition at this site, and the magnitude of pollen limitation was unaffected by *L. maackii*. Decreased light under the *L. maackii* canopy is a possible mechanism of reduced seeds per fruit because irradiance was significantly lower in invaded compared to *L. maackii* removal plots, and I
controlled for below-ground competition by placing experimental *H. macrophyllum* plants in pots. Fruits of pollen-supplemented plants (i.e. pollination held constant) produced significantly fewer seeds in the presence of *L. maackii* foliage compared to when *L. maackii* was absent (*t*=2.81, *P*=0.017, *N*=12, data not shown), providing further evidence of light limitation. (Reproductive success of pollen supplemented *H. macrophyllum* plants did not differ between *L. maackii* treatments at Denison, data not shown.) Because I correlated average pollen deposition with average reproduction per plot, it is possible that the regression analysis missed a relationship between decreased pollen deposition to shaded *H. macrophyllum* and decreased seeds per fruit for some individual plants, but my results suggest that direct negative effects of shade reduced the average number of seeds per fruit in *H. macrophyllum*.

Comparing results across sites reveals insights into the effect of flowering phenology on interactions between *L. maackii* and *H. macrophyllum*. *Lonicera maackii* exerts both positive (through flowers) and negative (presumably through shade) impacts on pollinator foraging to *H. macrophyllum*. When *L. maackii* and *H. macrophyllum* flowering phenologies were synchronous, facilitation of pollinator visitation seemed to overcome the negative impacts of foliage on visitation when flowering phenologies were asynchronous. In contrast, in a flower removal experiment at Three Creeks, I found that flowers of *L. maackii* had no effect on pollinator services and reproductive success of co-flowering *G. maculatum* beyond that of the negative impacts of shade (McKinney and Goodell 2010). These differences in the effect of *L. maackii* flowers on pollination of two native plant species may reflect different reward levels or different relative floral densities. *Hydrophyllum macrophyllum* plants produce more flowers than *G. maculatum*.
plants (41.5± 3.1 flowers vs. 15.5 ±1.4 flowers, respectively). Experimental studies indicate that facilitation of visitation tends to occur when the invader occurs at a lower relative floral density (Munoz and Cavieres 2008, McKinney, ch. 4). Flowering *L. maackii* shrubs seemed to moderately facilitate the number of fruits and seeds produced by *H. macrophyllum* flowers compared to no effect of *L. maackii* when they flower asynchronously. A rigorous test of effects of flowering phenology will require comparisons across multiple sites in which flowering phenology of the two species is synchronous and asynchronous, which would require a substantial amount of work and challenging site selection. My results from two sites with different *L. maackii* flowering phenology suggest that the flowering phenology of invasive plants may ultimately affect pollen limitation of native plant reproduction and whether invasive plants compete for pollinators with native plants or facilitate pollination of native plants.

Regardless of whether flowering phenology was the factor that influenced *H. macrophyllum* reproductive patterns, my results clearly show that interactions between invasive and native plants are quite variable across space and time, even between the same pair of species with similar pollinator communities. In addition, the amount of understory light was similar between the two sites, but I did not statistically compare light availability between sites because light measurements were taken on different days. At Three Creeks, shade from *L. maackii* had a negative impact on pollinator foraging to *H. macrophyllum*, showing that invasive plants may have more widespread negative impacts on pollinator foraging to native plants than previously realized, through non-floral pathways (McKinney and Goodell 2010). Indirect interactions, i.e. those mediated by a third species, between invasive and native species are likely more ubiquitous than
realized and merit further study (White et al. 2006). Indirect mutualisms such as pollination and seed dispersal may be important factors that shape plant community structure (Bascompte et al. 2003). Although flowering *L. maackii* shrubs alleviated pollen limitation of *H. macrophyllum*, it is difficult to predict impacts at the population level because pollination comprises one aspect of the potential direct and indirect interactions between co-occurring plant species (Hunter and Aarssen 1988, Callaway and Walker 1997). For example, small mammal consumers that take refuge under *L. maackii* prefer fruits and seeds of native plants, exerting a negative pressure on native plant reproduction (refuge-mediated apparent competition) (sensu Orrock et al. 2008, Dutra & Orrock, unpublished data). Therefore any pollinator-mediated benefit derived from growing near *L. maackii* may be negated by increased seed consumer pressure near *L. maackii*, depending on the spatial scale of indirect interactions. *Lonicera maackii* may alleviate pollen limitation of *G. maculatum* up to 40m from invaded forest edge habitat (McKinney, ch. 3). Below ground competition may also cancel out any benefits of decreased pollen limitation in a natural *H. macrophyllum* population, but the main effects of *L. maackii* on native plants seem to take place above ground (Gould and Gorchov 2000, Gorchov and Trisel 2003, Miller and Gorchov 2004). Studies that incorporate multiple direct and indirect effects between invasive and native plant species will be especially informative to the mechanisms and overall outcomes of interactions between invasive and native plant species.
ACKNOWLEDGEMENTS

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Table 2.1  Bee genera and family of other visitors accounting for percentage of total visits to *Hydrophyllum macrophyllum* in the presence and absence of the invasive shrub *Lonicera maackii* (Lm). Data are shown for two sites separately, in which flowering phenology of *H. macrophyllum* and *L. maackii* was synchronous (Denison, *N*=12) and asynchronous (Three Creeks, *N*=11).

<table>
<thead>
<tr>
<th>Bee Genera</th>
<th>Denison Lm present</th>
<th>Denison Lm absent</th>
<th>Three Creeks Lm present</th>
<th>Three Creeks Lm absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Augochlorella/Augochlorea</td>
<td>7.5</td>
<td>13.0</td>
<td>-</td>
<td>21.7</td>
</tr>
<tr>
<td>Bombus spp.</td>
<td>10.8</td>
<td>-</td>
<td>-</td>
<td>9.8</td>
</tr>
<tr>
<td>Ceratina spp.</td>
<td>23.7</td>
<td>-</td>
<td>80.0</td>
<td>55.4</td>
</tr>
<tr>
<td>Halictus ligatus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.5</td>
</tr>
<tr>
<td>Lasioglossum spp.</td>
<td>9.7</td>
<td>43.5</td>
<td>15.0</td>
<td>4.3</td>
</tr>
<tr>
<td>Diptera</td>
<td>48.4</td>
<td>43.5</td>
<td>5.0</td>
<td>-</td>
</tr>
<tr>
<td>Lepidoptera</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.2</td>
</tr>
<tr>
<td>Site</td>
<td>First Date</td>
<td>Last Date</td>
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<tr>
<td>Denison</td>
<td>May 22</td>
<td>June 7</td>
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<td></td>
<td>June 12</td>
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<tr>
<td>Three Creeks</td>
<td>May 7</td>
<td>May 25</td>
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<tr>
<td></td>
<td>May 29</td>
<td>June 8</td>
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</table>

**Figure 2.1** First and last date of flowering for the invasive shrub *Lonicera maackii* and the native herb *Hydrophyllum macrophyllum*, at two sites in central Ohio. Solid line represents *H. macrophyllum* flowering phenology and dotted line represents *L. maackii* flowering phenology.
**Figure 2.2** Forest understory light availability compared between plots that contained the invasive shrub, *Lonicera maackii*, and plots that did not at two sites differing in flowering phenology of the invasive shrub *L. maackii*. Sites were analyzed separately and responses compared using t-tests: **significantly different at** $P<0.05$. Sample sizes shown at bottom of bars represent number of plots. Untransformed light levels are shown for Three Creeks.
Figure 2.3 *Hydrophyllum macrophyllum* reproductive success compared between forest plots that contained the invasive shrub, *Lonicera maackii*, and plots that did not at two sites differing in flowering phenology of the invasive shrub *L. maackii*. Flowering phenologies were synchronous at Denison and asynchronous at Three Creeks. Sites were analyzed separately and responses compared using t-tests: *significantly different at P<0.10, **significantly different at P<0.05. Sample sizes shown at bottom of bars represent number of plots.
Figure 2.4  Magnitude of pollen limitation of Hydrophyllum macrophyllum reproductive success (pollen-supplemented minus open-pollinated plants) compared between forest plots that contained the invasive shrub, Lonicera maackii, and plots that did not at two sites differing in flowering phenology of the invasive shrub L. maackii. Flowering phenologies were synchronous at Denison and asynchronous at Three Creeks. A positive value indicates pollen limitation. Sites were analyzed separately and responses compared using t-tests: Double asterisks (**) denote significant differences at $P<0.05$. Sample sizes shown at bottom of bars represent number of plots.
Figure 2.5  Linear regression of average conspecific pollen deposition (per plot) with average *Hydrophyllum macrophyllum* reproductive success at two sites differing in flowering phenology of the invasive shrub *L. maackii*. Flowering phenologies were synchronous at Denison and asynchronous at Three Creeks. $P<0.05^{**}$ and $P<0.10^*$. $N=21$ plots at Denison and $N=20$ plots at Three Creeks.
CHAPTER 3
FACILITATION OF POLLEN RECEIPT AND SEED SET IN A NATIVE PLANT
ATTENUATES WITH DISTANCE FROM INVASED FOREST EDGE HABITAT

INTRODUCTION

Biological invasions are one of the primary agents of global environmental change and threaten biodiversity through their impacts on the abundance of native species, community composition, and biotic relationships within invaded communities (Wilson, 1992; Simberloff 2004; Mitchell et al. 2006). Exotic plant invasions affect biotic interactions between native plants and their mutualist partners, such as pollinators and seed dispersers, with potential subsequent effects on native plant reproduction (reviewed in Traveset and Richardson 2006; Bjerknes et al. 2007; Goodell 2008;). Species invasions that disrupt native plant reproductive mutualisms can result in community-level changes in plant composition, a result that highlights the important role of mutualisms in maintaining biodiversity (Christian 2001; Herrera and Pellmyr 2002). Evidence shows that invasive plants successfully integrate into plant-pollinator communities, creating new pollinator-mediated interactions with native plants in the invaded range (Memmott and Waser 2002).
A meta-analysis of studies of pollinator-mediated interactions between pairs of invasive and native plants found that invasive plants often have a negative impact on pollinator visitation and reproductive output in native plants (Morales and Traveset 2010). Competition for pollination may occur when 1) native plants receive fewer pollinator visits and/or conspecific pollen grains in the presence of invasive plants (i.e. decreased pollen quantity) (Grabas and Laverty 1999; Chittka and Schürkens 2001; Totland et al. 2006; Larson et al. 2006) and when 2) pollinators make interspecific plant movements, causing a decrease in the quality of pollen delivered to native plants (Brown et al. 2002; Jakobsson et al. 2008). The direction (competition or facilitation) of pollinator-mediated interactions is also highly variable across studies because some invasive plants facilitate pollinator visits to native plants (Moragues and Traveset 2005; Lopezaraiza–Mikel 2007; Bartomeus et al. 2008). In addition, neighboring plant species can affect the composition of pollinators visiting focal plant species, which is associated with increased visitation to the focal species (Moeller 2005, Lazaro et al. 2009). It is therefore possible that invasive plants may facilitate native plant reproduction by altering the composition of pollinators on native plants. However, increases in visitation to native plants may result in increased in heterospecific pollen deposition and loss of conspecific pollen and therefore to negative effects on native plant reproduction (e.g. in native plants Waser, 1978; for review see Morales and Traveset, 2008).

To move forward in our understanding of interactions for pollination between invasive and native plant species, information on mechanism and spatial scale should be particularly insightful (Traveset and Richardson, 2006). Alterations in pollen quantity and quality will cause changes in plant female reproductive success only if reproduction
is pollen limited (Waser 1978; Aizen and Harder 2007). If pollen limitation in native plants increases or decreases with plant invasions, the viability of native plant populations may be diminished or enhanced, respectively (sensu Ashman et al. 2004). In contrast, if plants compete for space and other resources, reproduction may not be pollen-limited, in which case changes in pollination would not affect reproductive success (sensu Feldman et al. 2004). Therefore, to gain a mechanistic view of interactions for pollinators between invasive and native plants, it is important to measure pollen receipt, reproductive success, and pollen limitation of reproduction in native plants, and few studies of invasive-native plant interactions have incorporated all of these aspects (see Munoz and Cavieres 2008; McKinney and Goodell 2010).

Plant invasions may interfere in pollinator-mediated native plant reproduction at distances far from the invasion when pollinators are highly mobile. While competition for light and soil resources occurs in the immediate vicinity of plants and their neighbors, interactions with pollinators may extend over much larger distances. For example, some common bee pollinators (i.e. *Apis mellifera*, *Bombus* spp., and wild solitary bees) forage from 150m to <1km away from nest sites (Eickwort and Ginsberg 1980; Waddington et al. 1994; Gathmann and Tscharntke 2002). Cariveau and Norton (2009) showed that competition for pollinator visits can occur up to 5m away from an invasive plant. Transport of invasive plant pollen to native plant stigmas, on the other hand, has been documented at least 100m from stands of an invasive plant (Larson et al. 2006). However, to my knowledge, no information exists regarding the consequences for native plant reproduction beyond the interactions that play out between adjacent invasive and native plants.
The objectives of this study were 1) determine the distance over which the invasive exotic shrub *Lonicera maackii* influence pollinator foraging and subsequent reproductive success of the native herb *Geranium maculatum* and 2) describe the pattern of changes in pollination and reproduction with distance from *L. maackii* (e.g. linear or curvilinear). I compared per flower visitation rate, pollen receipt, and fruit and seed set of potted *G. maculatum* plants in an old field at increasing distances from a forest edge invaded by *L. maackii*. To functionally link any changes in visitation rate and pollen receipt to reproduction, I determined the level of pollen limitation of fruit and seed set in *G. maculatum*. Based on empirical foraging ranges of bee taxa that I observed at the study site, I expected pollinator-mediated effects of invasive plants on native plants to extend beyond adjacent interactions.

**METHODS**

*Study species*

*Lonicera maackii* (Rupr.) Herder (Caprifoliaceae) was introduced to North America from China in the late 19th century as an ornamental shrub and is now invasive in forests and fields of the Eastern USA (Luken and Thieret 1996). Mature plants such as those in the study site produce thousands of medium-sized (2-2.5cm wide) white to pinkish-white tubular flowers in early May in central Ohio. *Lonicera maackii* cover is negatively correlated with herb cover, fecundity, survival, growth, final size, and pollinator visitation to herbs (Hutchinson and Vankat 1997, Gould and Gorchov 2000, Collier et al. 2002, Miller and Gorchov 2004, McKinney and Goodell 2010).
*Geranium maculatum* L. (Geraniaceae) is a common, widespread understory herb of eastern North American deciduous forests and open fields (Martin 1965). *Geranium maculatum* plants produce 3-8 protandrous, hermaphroditic flowers that require pollinators to set seed (Willson et al. 1979; Bertin and Sholes 1993). Flowers have an open morphology (2.5-4 cm wide), are purple-pink in color, are self-compatible, and produce more seeds when fertilized by outcross pollen compared to self pollen (Martin 1965). *Geranium maculatum* plants produce more flowers when in full sun in open field habitat compared to shadier forest habitat (Martin 1965). I selected *G. maculatum* for this study because it co-flowers over a broad geographical range (eastern USA), shares habitat type (forests and fields), and overlaps in flower visitors with *L. maackii*, most commonly solitary bees (in the genera *Ceratina*, *Halictus*, *Andrena*, and *Augochlorella* (Goodell et al. 2010, McKinney unpublished data). Because both study species have open floral morphologies, rewarding flowers, and generalized pollinator interactions, they constitute an appropriate study system for invasive-native plant interactions.

**Study site**

I performed this experiment at Three Creeks Metro Park in Groveport, OH, USA, in an approximately 1ha strip of abandoned agricultural land that is classified as old field habitat. The study site was used for agriculture until the late 1960’s, when it was permitted to re-grow naturally, and *L. maackii* invaded forest areas during regeneration (J. Snyder, park naturalist, personal communication). *Lonicera maackii* density in the adjacent forest is 0.14 shrubs per m$^2$. At this site in 2008, *L. maackii* and potted *G. maculatum* plants co-flowered from May 5 to June 3. There were no other species flowering during the experiment within the old field. I selected a site that did not have a
natural population of *G. maculatum* to avoid potentially confounding effects of variation in *G. maculatum* density. A white pine forest heavily invaded by *L. maackii* bordered the site to the west, and small shrubs (not yet flowering) were beginning to colonize the adjacent field. A paved bicycle path bordered the site to the east. A small (~3ha) restored prairie was located 40m east of the study area. The prairie was mainly composed of *Andropogon gerardii* and *Sorghastrum nutans*; *Chrysanthemum leucanthemum* was also present, and its flowering period partly overlapped with *L. maackii* and *G. maculatum* (from May 15 to June 3).

**Experimental design**

In spring 2008, I arranged potted *G. maculatum* plants in rows parallel to the edge of the invaded forest, starting adjacent to the edge of the *L. maackii* canopy so that the shrubs did not shade *G. maculatum* plants and continuing at 20m increments up to 120m away from *L. maackii*. Distances between rows (20m) were uniform to avoid confounding density with distance. Higher floral density is correlated with higher pollinator visitation rates and higher plant reproductive success (Kunin 1993; Kunin 1997; but see Spigler and Chang 2008). The composition of pollinator visitors to *G. maculatum* is quite similar between the old field habitat and adjacent deciduous forest habitat, as indicated by visitation data collected in 2008 in both habitat types (Table 3.1). This suggests that variation in pollinator composition with distance from invaded forest edge should not be strongly impacted by the transition between old field and forest edge habitat. All *G. maculatum* plants were located in full sun for most of the day and were watered as necessary throughout the season. I erected six wooden fence posts along each row and draped plastic deer netting over the posts to protect *G. maculatum* plants from
mammal herbivory. The largest pollinators at this site moved through the 2cm holes in the netting with ease (*Bombus impatiens* and other *Bombus* spp. queens and Lepidopterans, A. M. McKinney, personal observation).

Within each row I placed 15 potted *G. maculatum* plants 1m apart (*N*=15). The pots were placed into recesses that were dug in the ground so that the top of the pots were flush with the ground, to prevent tipping and drying of soil. Potting the plants eliminated competition for soil nutrients and water and isolated pollinator-mediated effects on *G. maculatum* reproduction. *Geranium maculatum* fruit set does not appear to be affected by pots (McKinney and Goodell 2010) and is naturally low (< 20%), similar to other temperate late spring/early summer flowering plants (Kudo et al. 2008). By the end of the experiment, the number of *G. maculatum* plants varied at each distance from *L. maackii* because of plant removal from pots and damage to plants (see Table 3.2 for sample sizes).

I recorded the number of pollinator visits to *G. maculatum* during 10 min intervals between 10:00am and 4:30pm on sunny or partly cloudy days when air temperature was above 15.5°C (conditions for maximum pollinator activity). My *a priori* definition of a visit required that the visitor touch reproductive parts of the flower. During each observation period, I observed flowers on two randomly selected neighboring *G. maculatum* plants at each distance. I also conducted 10 min pollinator observations on ~100 flowers of *L. maackii* plants along the forest edge within the study area. All distances were observed on the same day for most observation sessions; when weather or time occasionally prevented this, I observed at least four distances per session. I recorded number of open flowers per plant, number and identity of each visitor to lowest
taxonomic level possible (genus of bees and family of all other visitors), and number of visits made per visitor, so that visitation rate was calculated as the number of visits per flower per 10 min. Many pollination studies lump solitary bees into one taxonomic group, making identification to genus a relative improvement. Identification of bees to species is difficult and typically requires the use of a microscope, making species identifications on the wing almost impossible.

To assess pollen receipt in open pollinated stigmas, I collected a total of five mature stigmas from a subsample of five randomly selected *G. maculatum* plants at each distance (*N*=5). Collecting stigmas does not appear to affect fruit or seed production (McKinney and Goodell 2010). I mounted stigmas on microscope slides prepared with fuschin-dyed gel in the field and later counted the number of *G. maculatum* and *L. maackii* pollen grains on each stigma. The total number of open flowers on each *G. maculatum* plant were counted as the season progressed. I collected fruits as they matured through mid-June and counted seeds to quantify female reproductive success.

I measured pollen limitation of seed set in *G. maculatum* by adding supplemental outcross pollen to all flowers on ten potted plants that were not part of the distance experiment but were located within the old field. Open flowers were pollinated every other day until all flowers senesced, and donor pollen was taken from ten randomly selected plants each time (one donor per plant). I collected fruits and seeds as described above.

Because proximity to forest and *L. maackii* may both affect pollinator visitation to *G. maculatum* and were confounded at my site, I conducted pollinator observations on potted *G. maculatum* plants adjacent to invaded and uninvaded sections of forest edge
within the same oldfield site the following year, in spring 2009. Although pollinator communities often experience turnover through time (Petanidou et al. 2008), pollinator responses to *L. maackii* should remain constant through time. I observed 10-20 flowers of *G. maculatum* in 10 minute sessions and recorded variables previously described for pollinator observations. Plants were set out at six different locations adjacent to forest (three invaded, three uninvaded) and allowed to acclimate for 20 minutes before observations were conducted.

Statistical Analysis

For pollinator visitation rate, each observation session was a sample. Because I randomly selected plants for observation to avoid bias in plant selection, some plants may have been sampled more than once (across different days). Therefore, visitation rate was averaged by observation session. Pollinator visitation rate data exhibited a zero-inflated distribution and violated normality assumptions required for regression analysis; transformations did not improve normality. Therefore, I converted visitation rate to a binomial response variable (received visit or not) weighted by the number of flowers on each plant that did or did not receive a visit. Distance class was a continuous independent variable. I used nominal logistic regression to analyze the correlation between the probability that a *G. maculatum* flower received a visit and distance from the *L. maackii* invasion. All analyses were performed in JMP v. 8 (SAS Institute, Cary, NC).

All other response variables were averaged per plant. I fit linear and curvilinear (2nd, 3rd, and 4th order polynomial) regressions of *G. maculatum* and *L. maackii* pollen deposition, *G. maculatum* seed production, and magnitude of pollen limitation of seed set as a function of distance from invaded forest edge. I examined the percentage of
variation explained ($R^2$ values) and F-statistics from lack of fit tests to determine the best fit for each response. A linear fit explained a significant amount of variation in seed set and magnitude of pollen limitation of seed set and a marginally significant amount of variation in conspecific pollen receipt with distance from invaded forest edge (results not shown). However, the 3rd order polynomial fit was best for pollen receipt and 4th order for seed set and pollen limitation of seed set, and I report only results for these functions.

I analyzed _G. maculatum_ pollen deposition with and without 3 plants that were borderline outliers at 100m. Statistics are reported for both analyses and results shown only for the analysis without the outliers. _Lonicera maackii_ pollen deposition on _G. maculatum_ stigmas was natural log transformed to meet normality assumptions.

Seed set was analyzed as the number of seeds produced per plant because this measure is relevant to population level effects in plants (Ashman et al. 2004). The number of flowers per plant did not vary among distances (Nonparametric ANOVA $X^2=1.66, P=0.95$), suggesting seeds per plant is not biased by differences in total flowers per plant. The number of seeds per plant was natural log transformed to meet normality assumptions required for regression analysis.

I used t-tests to test whether seed set was pollen limited at each distance from invaded forest edge, using overall per plant averages of open and supplemental pollinated plants. Next, I quantified the magnitude of pollen limitation as the difference between average seeds per plant of supplemental and open-pollinated plants. Transformations did not improve normality of these data, so I removed 10 outliers from the dataset. The outliers were plants that exhibited no pollen limitation or low levels of pollen limitation: 4 of the outliers were at 0m, 2 at 20m, 1 at 40m, 1 at 60m, and 2 at 80m. Because the
trend was for weaker pollen limitation of *G. maculatum* seed set closer to *L. maackii*, removing these outliers makes the analysis a conservative test of effects of distance from invaded forest edge on pollen limitation.

I compared visitation rate to potted *G. maculatum* flowers adjacent to invaded and uninvaded forest using ANOVA and iterative fitting (general linear model). The final model included visitation rate as a continuous response variable and treatment, percent cloud cover, date, and their interaction with treatment as explanatory variables. Explanatory variables excluded from the final model, because they did not account for a significant amount of variation, were time of day and temperature.

**RESULTS**

Hymenopterans accounted for 92.20% of the total visits to *G. maculatum*, Dipterans 6.80%, and Coleopterans and Lepidopterans each 0.50% of total visits (*N*=87 total observation sessions). All of the visitors recorded to *L. maackii* during observations were Hymenopterans. Specifically, *Ceratina spp.* accounted for 75.0% of total pollinator visits to *L. maackii*, and *Halictus ligatus* and *Apis mellifera* for 16.67% and 8.3%, respectively. The solitary bees *Halictus ligatus* and *Ceratina spp.* accounted for most of the visits to both plant species (Table 3.2). There was some variation in bee genera visiting *G. maculatum* with distance from *L. maackii* (Table 3.2).

There was a significant relationship between the probability of a *G. maculatum* flower receiving a visit and distance from invaded forest edge ($R^2=0.01$, $X^2=9.78$, $P=0.0018$); however, the $R^2$ value was too low to explain a meaningful amount of
variation (Fig. 3.1). The average visitation rate to L. maackii flowers was 0.05 ±0.02 (mean ± 1SE, N=14).

A cubic polynomial fit best described average G. maculatum pollen receipt per flower when the potential outliers from the 100m distance were excluded ($F=3.24$, $P=0.037$, Fig. 3.2a). Pollen receipt decreased in general from 0-40m, remaining somewhat constant at 40-80m and showing a slight increase at 80-120m from invaded forest edge (Fig. 3.2a). When the potential outliers at 100m were included, a 4th order polynomial fit was best. There was still a significant relationship between G. maculatum pollen receipt and distance from invaded forest edge, with a more pronounced increase in pollen receipt from 60-120m ($R^2=0.37$, $F = 4.47$, $P=0.0059$). There was no significant relationship between average L. maackii pollen deposition per G. maculatum flower (natural log transformed) and distance from the invaded forest edge ($R^2=0.12$; $F=0.98$; $P=0.43$; Table 3.3).

A 4th order polynomial best described the number of G. maculatum seeds per plant (natural log transformed) with distance from invaded forest edge and showed a decrease in seed set from 0m to 40m, no change from 40-100m, and a further decrease between 100-120m from invaded forest edge ($F=4.59$, $P=0.0024$, Fig. 3.2c). Geranium maculatum seed set was significantly pollen limited at every distance (Table 3.4). Consistent with the pattern of seed set, a 4th order polynomial also best fit the magnitude of pollen limitation of seed set and approximately mirrored seed set ($F=2.66$, $P=0.042$, Fig. 3.2c). The magnitude of pollen limitation increased from 0m to 40m, leveled off between 40m and 100m, and increased again from 100m to 120m from invaded forest edge (Fig. 3.2c).
Geranium maculatum flowers that were adjacent to invaded forest edge received significantly more visits per flower than flowers adjacent to uninvaded forest edge, suggesting pollinators respond independently to L. maackii despite the presence of forest edge habitat, at least when directly adjacent to the forest (invaded: 1.30 ± 0.14 visits per flower; uninvaded: 0.76 ± 0.16 visits per flower; Table 3.5).

**DISCUSSION**

The results of this study lend credibility to the notion that plant invasions can have impacts through mobile mutualist partners on native plant reproductive success at spatial scales beyond the local neighborhood (e.g. defined as < 4m in Spigler and Chang 2008). My results show that conspecific pollen receipt and seed set of the native herb G. maculatum decreased with distance from forest edge invaded by L. maackii. Pollen receipt limited G. maculatum seed set at every distance, supporting the hypothesis that patterns of seed set are influenced by pollen receipt. Further supporting the link between pollen receipt and seed set is the result that pollen limitation of G. maculatum seed set was weaker nearer to L. maackii.

The presence of L. maackii was confounded with forest edge habitat in this study, but pollinator foraging behavior data, pollinator composition data, and nesting biology of the most common visitors suggest that changes in pollen deposition and G. maculatum seed set with distance from invaded forest edge were likely a response to L. maackii. Higher pollinator visitation rate to G. maculatum in the presence of L. maackii, when proximity to forest edge was held constant, suggests that L. maackii rather than forest
edge habitat accounted for changes in pollinator services to *G. maculatum*, at least near to *L. maackii*. This increase in visitation next to *L. maackii* compared to uninvaded forest may be a pollinator response to higher floral abundance. Consistent with this interpretation, community floral abundance principally affects solitary bee abundance, and solitary bee abundance is unaffected by surrounding landscape context (Bartomeus et al. 2010). These visitation data were collected one year following the distance experiment. While pollinator communities often experience turnover through time (Petanidou et al. 2008), I observed the same pollinator fauna on *G. maculatum* in 2009 as in 2008 (McKinney, unpublished data). Pollinator composition changes over space in response to plant density, and the number of flowers on neighboring plant species affects the composition of pollinator visitors to focal plant species (Conner and Neumeier 1995, Lazaro et al. 2009). *Halictus ligatus* seemed to be more prevalent near invaded forest edge. It is more likely that *H. ligatus* individuals were responding to the presence of *L. maackii* as opposed to their abundance near *L. maackii* being an artifact of nest location or proximity to forest edge because *Halictus ligatus* is a ground nesting species (Richards and Packer, 1996). Packer and Knerer (1986) found no evidence of non-random nest dispersion in *H. ligatus* across two study sites in open field habitat, one of which was adjacent to a forested area. Finally, the other most common visitor in this study, *Ceratina* spp., nest in the pith of dead twigs (Rehan and Richards 2010). If the visitation pattern of *Ceratina* spp. is a response to forest edge habitat, I expect to see more visits by *Ceratina* spp. nearer to the forest edge, a pattern I did not observe.
While invasive plants can decrease pollinator visitation to native plants over a spatial scale of 1-5m from the invader (Cariveau and Norton 2009), to my knowledge, this study is the first to detect variation in reproductive success of a native plant species at a distance beyond adjacent or local invasive-native plant interactions. Bonferroni comparisons of all distances revealed significant differences in seed set and pollen limitation of seed set only between 0m and 120m from *L. maackii* and in conspecific pollen receipt 0m and 80m from invaded forest edge (results not shown). These tests are so conservative that other differences are likely missed (significant at $P<0.002$), and the regression analyses show that *G. maculatum* pollen receipt and seed set increased from 40m to 0m from invaded forest edge, suggesting facilitation occurred over a 40m spatial scale. This spatial scale is far beyond the local neighborhood within which interactions for soil resources and light are expected to occur (sensu Mack and Harper 1977).

Similarly to this study, Nielsen et al. (2008) found more insect visits to native plants adjacent to a stand of invasive plants compared to native plants at distances up through 200m away. However, only one other study of which I am aware has documented facilitation of native plant reproduction by an invasive plant, which occurred at a low density of the invasive species (Munoz and Cavieres 2008).

Interestingly, there was a distinct nonlinear shape to the relationship between distance from invaded forest edge and pollination of *G. maculatum*, suggesting that effects of invasive species do not trail off consistently with distance but instead may vary in level of impact on native plants depending on the spatial scale examined. There was some discordance between pollen receipt and seed set at distances farther from invaded forest edge; pollen receipt gradually increased from 80-120m while seed set decreased
over this spatial scale. My methods introduce some variation in pollen receipt data, which are variable by nature (Burd 1994; Engel and Irwin 2003; Price et al. 2005; Burd et al. 2009). Pollen samples were taken over a span of 10 days, while seed set reflects pollinator services throughout a four week period. In addition, pollen receipt data represented a subset of the total plants in the experiment (44%), and average pollen receipt for these plants was a subsample of all the stigmas, while seed set reflects the entire plant. The number of flowers and ovules in a plant ultimately restricts plant reproductive success and is therefore expected to be less variable than pollen receipt, especially in a plant with low maximum seed set such as *G. maculatum* (Martin 1965; Cane and Schiiffhauer 2003). Therefore, the level of *G. maculatum* pollen limitation is probably more reliable evidence of a pollinator-mediated effect than conspecific pollen deposition.

The probability of *G. maculatum* flowers receiving a visit with distance from invaded forest edge did not match the pattern of *G. maculatum* pollen receipt. There are many other factors related to foraging behavior of different pollinator taxa that may have accounted for the observed pattern of pollen deposition, such as the duration of pollinator visits (Manetas and Petropoulou 2000, Munoz and Cavieres 2008, McKinney ch. 4) or changes in the composition of the pollinator community with proximity to *L. maackii*. Pollinators differ in their efficiency of transferring pollen for several reasons, such as body size, pollen placement on bee bodies, the likelihood of contacting the stigma, the amount of pollen removed, or other behaviors adopted while foraging (Wilson and Thomson 1991; Harder and Barrett 1993; Rademaker et al. 1997; Goodell and Thomson 2007). The percentage of visits by *H. ligatus* decreased with distance from invaded forest
edge, while *Ceratina* and other solitary bees were more prevalent on *G. maculatum* at distances greater than 40m from invaded forest edge. *Halictus ligatus* spend more time per visit to *G. maculatum* flowers compared to *Ceratina spp.*, the most common visitor at this site, which could have accounted for the trend in increased pollen receipt from 40m to 0m from invaded forest edge (McKinney, unpublished data). My results are consistent with the interpretation that a behavioral difference in pollinator fauna associated with a change in pollinator composition resulted in higher pollen receipt closer to *L. maackii*, but further investigation is required to verify this mechanism.

An alternative explanation for higher seed set closer to the invasion is variation in resource availability, but this is unlikely because *G. maculatum* plants were grown in pots to eliminate competition for soil nutrients, plants were watered when necessary, and all plants received full sun for most of the day. The forest shaded *G. maculatum* plants nearer to *L. maackii* sooner than plants farther away. The first plants became shaded around 3:45 p.m. at 0m from invaded forest edge. Pollinators make fewer visits and deliver fewer conspecific pollen grains to *G. maculatum* that occurs in the shade of the *L. maackii* canopy compared to plants in areas where *L. maackii* is removed, and shade from *L. maackii* reduces seed set of *G. maculatum* (McKinney and Goodell 2010). Therefore if late afternoon shade from the forest were affecting pollen delivery and seed set in *G. maculatum* in this study, I expect to see lower pollen receipt and seed set nearer to *L. maackii*, or the opposite of what was observed. It should also be noted that distance from invaded forest edge accounts for 21-26% of the variation in seed set and pollen receipt. Nest location may explain some of the remaining variation in pollen receipt and seed set.
because floral resources and nesting sites are essential resources for solitary bees such as those at this study site (Gathmann and Tscharntke 2002).

*Lonicera maackii* pollen receipt was substantially below levels found to decrease *G. maculatum* seed set (McKinney, ch. 4). Pollinators may not make frequent transitions from *L. maackii* to *G. maculatum* flowers, they may exhibit floral constancy after switching flower species, or they may carry pollen on different parts of their bodies because of dissimilar floral morphologies (Harder and Barrett 1993; Chittka 1999). However, *L. maackii* pollen grains were detected on *G. maculatum* stigmas 120m from invaded forest edge. Taken with results of Larson et al. (2006), who reported pollen from an invasive plant on native plant stigmas 100m away from stands of the invader, these results suggest that competition via transfer of heterospecific pollen has the potential to extend over substantial distances (e.g. Brown and Mitchell, 2001).

Although invasions could extend their influence on pollinator interactions beyond 120m, this distance is consistent with the foraging ranges of the bees that primarily visit *L. maackii* and *G. maculatum* (Greenleaf et al., 2007). Invasive-native plant pairs that share pollinators with larger foraging ranges may show interactions for pollination over even larger spatial scales. Solitary wild bee abundance and diversity responds to landscape context over a spatial scale ≤ 750m, much smaller than the spatial scale to which social bees (*Bombus* spp. and *Apis mellifera*) respond to landscape structure (Steffan-Dewenter et al. 2002). *Bombus* spp. are attracted to stands of the invasive plant *Impatiens glandulifera*, independent of the surrounding landscape context (Bartomeus et al. 2010). Impacts of plant invasions may therefore vary across a larger regional scale compared to the scale of this study. For example, if patches of attractive invasive plants
draw pollinators from areas beyond the footprint of the invasion, pollinator visitation to native plants may be increased in comparison to local areas in which invasive plants are removed, but competition or neutral effects for pollinator visits may still occur at larger spatial scales between invaded and uninvaded patches (Lopezaraiza-Mikel et al. 2007; Jakobsson et al. 2009a).

In light of conservation concerns surrounding pollination and the accelerating rate of plant invasions, it is important to understand the impacts of plant invasions on pollinator services and subsequent native plant reproductive success (Kearns et al. 1998; Kremen and Ricketts 2000; Reid et al. 2005; Mazer 2007). Specifically, identifying how mutualisms change over space in response to plant invasions will help to define the scope of impacts of invasive plants. This study represents an important first step in understanding the spatial scale of pollinator-mediated interactions between invasive and native plant species. My results suggest that impacts of an invasive plant on pollination of a native plant can extend over farther distances than previously shown (up to 40m) and that reproductive effects in the native plant extend beyond the local neighborhood.

Although pollinator data and the nesting biology of the most common visitors are consistent with the interpretation that effects on *G. maculatum* pollen deposition and seed production were a response to *L. maackii*, the presence of *L. maackii* was associated with a habitat transition to forest edge that also may have affected these responses. The next step to further understand the spatial scale of pollinator-mediated interactions is to measure pollination and native plant reproduction of plants at various distances up to 40m from individual *L. maackii* shrubs compared to plants at the same distances from a native shrub or focal native plant species, all within the same habitat. Future work that
compares pollen limitation of native plants at various distances across paired invaded and uninvaded sites (sensu Jakobsson et al. 2009b) will also enhance our understanding of the spatial scale of pollinator-mediated interactions between invasive and native plants.

ACKNOWLEDGEMENTS

Funding for this project was provided by The Ohio State University’s Department of Evolution, Ecology and Organismal Biology Janice Carson Beatley Award to A.M. for graduate students studying plant ecology. I thank Three Creeks Metro Park, especially J. O’Meara and C. Morrow, for allowing off-trail access for this experiment. R. Snyder assisted with experimental set-up and pollinator observations, and K. Phetlasymongkhon and M. Clement provided additional assistance with pollinator observations. I also thank K. Goodell, C. Lin, and K.R. Greenwald for providing valuable feedback on earlier drafts.

REFERENCES


Goodell, K. and J.D. Thomson. (2007) Pollen movement in a mustard, Brassica rapa (Brassicaceae), and the muskmelon Cucumis melo (Cucurbitaceae), is influenced by bee species (Hymenoptera: Apiformes) with contrasting behaviors. Entomologia Generalis 29: 237-252


Table 3.1 Percentage of total visits to *G. maculatum* by pollinator taxa in open field and deciduous forest habitat in 2008. Visitation was recorded during 10 min observation sessions in each habitat. The ‘other’ group includes Lepidoptera, Coleoptera, and unknown Hymenoptera. *N* = 87 observation sessions in old field and 74 observation sessions in forest.

<table>
<thead>
<tr>
<th>Pollinator Taxa</th>
<th>Old field</th>
<th>Forest</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ceratina</em> spp.</td>
<td>44.0%</td>
<td>69.0%</td>
</tr>
<tr>
<td><em>Halictus ligatus</em></td>
<td>26.6%</td>
<td>4.4%</td>
</tr>
<tr>
<td><em>Augochlorella</em> spp.</td>
<td>1.9%</td>
<td>13%</td>
</tr>
<tr>
<td><em>Andrena</em> spp.</td>
<td>9.7%</td>
<td>2.7%</td>
</tr>
<tr>
<td><em>Lasioglossum</em> spp.</td>
<td>6.8%</td>
<td>-</td>
</tr>
<tr>
<td><em>Apis mellifera</em></td>
<td>-</td>
<td>1.1%</td>
</tr>
<tr>
<td>Diptera</td>
<td>6.7%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Other</td>
<td>4.3%</td>
<td>7.1%</td>
</tr>
</tbody>
</table>
Table 3.2 Percentage of total visits to *G. maculatum* in open field habitat by pollinator taxa that were recorded during 10 min observation sessions at each distance from forest edge invaded by *L. maackii*. The ‘other’ group includes Lepidoptera, Coleoptera, and unknown Hymenoptera. Sample sizes are number of 10 min observation sessions.

<table>
<thead>
<tr>
<th>N</th>
<th>0m</th>
<th>Ceratina spp.</th>
<th>Halictus ligatus</th>
<th>Andrena spp.</th>
<th>Augochlorella spp.</th>
<th>Diptera</th>
<th>Lasioglossum spp.</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>5.3%</td>
<td>89.5%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.2%</td>
</tr>
<tr>
<td>13</td>
<td>45.5%</td>
<td>36.4%</td>
<td>-</td>
<td>-</td>
<td>18.1%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>62.1%</td>
<td>19.7%</td>
<td>3.0%</td>
<td>1.5%</td>
<td>7.7%</td>
<td>4.5%</td>
<td>1.5%</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>9.4%</td>
<td>34.4%</td>
<td>25.0%</td>
<td>6.3%</td>
<td>3.1%</td>
<td>18.8%</td>
<td>3.0%</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>54.8%</td>
<td>-</td>
<td>19.4%</td>
<td>-</td>
<td>12.9%</td>
<td>9.7%</td>
<td>3.2%</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>53.1%</td>
<td>37.5%</td>
<td>-</td>
<td>3.1%</td>
<td>-</td>
<td>6.3%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>43.8%</td>
<td>18.8%</td>
<td>25.0%</td>
<td>-</td>
<td>12.5%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3.3 *Lonicera maackii* pollen deposition on *G. maculatum* stigmas. Untransformed mean number (± 1SE) of *L. maackii* pollen grains per *G. maculatum* flower shown for every distance. *N*=5 plants per distance

<table>
<thead>
<tr>
<th>Distance (m)</th>
<th><em>L. maackii</em> pollen grains</th>
</tr>
</thead>
<tbody>
<tr>
<td>0m</td>
<td>3.49 ± 1.38</td>
</tr>
<tr>
<td>20m</td>
<td>1.41 ± 0.71</td>
</tr>
<tr>
<td>40m</td>
<td>2.01 ± 0.96</td>
</tr>
<tr>
<td>60m</td>
<td>0.46 ± 0.16</td>
</tr>
<tr>
<td>80m</td>
<td>1.04 ± 0.89</td>
</tr>
<tr>
<td>100m</td>
<td>1.26 ± 0.17</td>
</tr>
<tr>
<td>120m</td>
<td>0.96 ± 0.47</td>
</tr>
</tbody>
</table>
Table 3.4 Pollen limitation of *G. maculatum* seed set (natural log transformed seeds per plant) at increasing distance from the invasive shrub *L. maackii*. Student’s t-tests were used to compare average seed set of pollen-supplemented plants to open pollinated plants within each distance $df=1$, critical $t>1.99$. $N=9$ pollen supplemented plants and sample sizes are shown for number of open pollinated plants at each distance.

<table>
<thead>
<tr>
<th>Distance (m)</th>
<th>$P$</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0m</td>
<td>0.0089</td>
<td>13</td>
</tr>
<tr>
<td>20m</td>
<td>0.0002</td>
<td>11</td>
</tr>
<tr>
<td>40m</td>
<td>&lt;0.0001</td>
<td>13</td>
</tr>
<tr>
<td>60m</td>
<td>&lt;0.0001</td>
<td>9</td>
</tr>
<tr>
<td>80m</td>
<td>&lt;0.0001</td>
<td>10</td>
</tr>
<tr>
<td>100m</td>
<td>&lt;0.0001</td>
<td>11</td>
</tr>
<tr>
<td>120m</td>
<td>&lt;0.0001</td>
<td>13</td>
</tr>
</tbody>
</table>
Table 3.5 Pollinator visitation to *G. maculatum* next to un-invaded forest edge and forest edge invaded by *L. maackii*. Maximum likelihood analysis of variance table showing effects of forest edge invasion treatment, date, percent cloud cover, and their interactions with invasion treatment on pollinator visitation rate to *G. maculatum* flowers. df=1 for each source. N=15 10min observation sessions per treatment

<table>
<thead>
<tr>
<th>Source</th>
<th>$X^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>invasion</td>
<td>7.53</td>
<td>0.006</td>
</tr>
<tr>
<td>date</td>
<td>5.29</td>
<td>0.02</td>
</tr>
<tr>
<td>invasion * date</td>
<td>0.04</td>
<td>0.84</td>
</tr>
<tr>
<td>cloud cover</td>
<td>8.72</td>
<td>0.003</td>
</tr>
<tr>
<td>invasion * cloud cover</td>
<td>0.003</td>
<td>0.95</td>
</tr>
</tbody>
</table>
Figure 3.1 The probability of a *G. maculatum* flower receiving a visit as a function of distance from a forest edge invaded by *Lonicera maackii*. Each point represents the average probability that flowers will receive a visit during one 10 minute observation session. Some points overlap, and sample sizes range from 11-14 for each distance.
Figure 3.2 Geranium maculatum (a) average conspecific pollen receipt per flower, (b) natural log transformed number of seeds set per plant, and (c) magnitude of pollen limitation of seeds per plant (seed set in pollen supplemented – open pollinated plants) as a function of distance from a forest edge invaded by Lonicera maackii. R² values are from fit of a (a) 3rd order polynomial equation and (b, c) 4th order polynomial equation. Each point represents one G. maculatum plant. (b) Distance class at 100m includes only 2 plants because 3 outliers were removed from the analysis (see text)
CHAPTER 4
RELATIVE FLORAL DENSITY OF AN INVASIVE PLANT AFFECTS POLLINATOR FORAGING BEHAVIOR ON A NATIVE PLANT

INTRODUCTION

Invasive exotic plants interfere in plant-animal interactions in invaded habitat through a number of ecological pathways. Indirect biotic effects of invasive plants, defined as the effect an invasive plant has on one native species through effects on an additional species, may occur more frequently than presently acknowledged (Strauss 1991, White et al. 2006). For example, woodland birds experience higher predation when nesting in invasive shrubs compared to native shrub species (Borgmann and Rodewald 2004). Seed predators of native plants take refuge under invasive plants and increase consumer pressure on seeds of neighboring native plant species (Orrock et al. 2008). Invasive plants successfully integrate into plant-pollinator and plant-seed disperser communities (Memmott and Waser 2002, Kawakami et al. 2009), sometimes competing for pollinators and seed dispersers with native plants (Chittka and Schürkens 2001, Lafleur et al. 2007). Under some circumstances, invasive plants may benefit native plants if they support larger pollinator populations (Tepedino et al. 2008). Examples from two invasive plant species that comprise an important part of a Mediterranean plant-
pollinator community show contrasting effects on pollinator visitation to native plants; one species facilitates pollinator visits to native species, while the other species competes for pollinator visits with native species (Bartomeus et al. 2008).

Interactions for pollinators between plants may occur along a continuum from competition to facilitation that is mediated by relative floral density (Rathcke 1983). As floral density of one species increases, facilitation is hypothesized to change to competition. Consistent with this idea, hummingbird, bee, and fly visitation exhibit density-dependent responses to varied floral density (Feinsinger et al. 1991, Ghazoul 2006). Interspecific variation in floral traits such as color and morphology may also play a role in determining whether facilitation or competition for pollination occurs, or these traits may influence the relative floral densities at which facilitation changes to competition. In some cases, similar floral morphology and color seem to be conducive to facilitation (Le Corff et al. 1998, Moeller 2004, Hegland et al. 2009), while Ghazoul (2006) found that increased floral diversity attracts more pollinators to a focal species, with morphologically distinct flowers facilitating pollinator visitation and seed set of the focal species. Communities under different stages of plant invasion should therefore experience varying interactions for pollinators, with facilitation at incipient stages of invasion and competition at later stages, assuming flower density increases as the invasion matures. Because invasive plants can provide a pulse of relatively dense floral resources in communities (Goodell 2008), I expect to see competition for pollinators within the most mature or extensive plant invasions.
There are several ways in which pollinator behavioral responses to invasive floral resources may translate into consequences for native plant reproductive success. If pollinators visit native plants less frequently when an invasive plant is present because of competition for pollinators, native plant stigmas may receive fewer conspecific pollen grains (Larson et al. 2006), which in turn can decrease native plant seed production (Munoz and Cavieres 2008). Alternatively, facilitation of pollinator visitation (e.g. Moragues and Traveset 2005) may increase both conspecific pollen deposition and heterospecific pollen deposition (Lopezaraiza-Mikel et al. 2007), leading to either increases or decreases in native plant reproductive success, respectively. However, for alterations in pollinator behavior to cause reproductive consequences for native plants, reproductive success must be pollen limited, a link that has rarely been investigated in studies of invasive-native plant interactions (but see Munoz and Cavieres 2008, McKinney and Goodell 2010). Therefore, neutral effects of invasive plants on native plant pollination and reproduction may occur in the absence of pollen limited reproduction and/or when relative floral densities do not influence pollination (sensu Grabas and Laverty 1999, Ghazoul 2004, Totland et al. 2006, Bartomeus et al. 2010). In addition, pollinator visitation rate and pollen deposition are often uncorrelated (e.g. Larson et al. 2006), which could be an effect of sample size, variation in pollen placement on pollinator bodies (Harder and Barrett 1993), or other aspects of pollinator behavior that can affect plant reproductive success, such as time spent during a flower visit (Manetas and Petropoulou 2000).
Studies of how pollinator-mediated interactions between invasive and native plants change through time are necessary to fully understand the impact of invasive plants (Bjerknes et al. 2007). Plant-pollinator interactions fluctuate across years and between months within a year (Basilio et al. 2006, Petanidou et al. 2008), and much of this variation may relate to changes in absolute or relative floral density of plant species within a plant-pollinator community. Invasion of exotic plant species may influence fluctuations in floral density and subsequent responses in the pollinator community. For example, invasive goldenrod species decrease pollinator abundance and diversity in wet meadows (Moron et al. 2009). In this study I experimentally alter relative floral densities of an invasive and native plant to model different stages of a plant invasion. I use experimental arrays to investigate how relative floral density of the invasive shrub, *Lonicera maackii*, impacts pollinator behavior (visitation rate, visit duration, and pollen deposition) and subsequent female reproductive success of the native herb, *Geranium maculatum*. I also conduct two hand pollination experiments, one to test for pollen limitation of *G. maculatum* reproductive success and one to determine the impact of *L. maackii* pollen deposition on *G. maculatum* reproductive success. Based on Rathcke’s (1983) hypotheses, I expect to see increased pollinator services and reproductive success at a low density of *L. maackii* flowers (a model for an incipient stage of invasion), and reduced *G. maculatum* pollinator services and reproductive success at a high density of *L. maackii* flowers (a model for a mature invasion), compared to when *L. maackii* flowers are absent.
METHODS

Study species

*Lonicera maackii* (Rupr.) Herder was introduced to North America from China in the late 19th century as an ornamental shrub and is now invasive in forests and fields of eastern USA (Luken and Thieret 1996). Mature plants such as those in the study site produce thousands of medium-sized (2-2.5 cm wide) white to pinkish-white flowers in early May. *Lonicera maackii* cover is negatively correlated with herb cover, fecundity, survival, growth, final size, and pollinator visitation to herbs (Hutchinson and Vankat 1997, Gould and Gorchov 2000, Collier et al. 2002, Miller and Gorchov 2004, McKinney and Goodell 2010). Non-native honeybees (*Apis mellifera*) and native bees in the genera *Halictus, Ceratina, Lasioglossum, Nomada, Augochlorella, Andrena* and *Bombus* forage on *L. maackii* flowers in central Ohio (Goodell et al. 2010).

*Geranium maculatum* L. is a common, widespread understory herb of eastern North American deciduous forests and open fields (Martin, 1965). It was selected for this study because it shares pollinators and co-occurs with *L. maackii*, and its geographic range, habitat, and flowering time overlap with *L. maackii*. *Geranium maculatum* reproduces sexually by seeds and asexually by rhizomes (Martin 1965). It produces 2-30 large (2.5-4 cm wide) purple-pink flowers per plant. Plants in full sun produce more flowers than plants in shady habitat (Martin 1965). Hermaphroditic flowers are protandrous (Bertin and Sholes 1993), and pollinators are required for seed set to occur (Wilson et al. 1979); flowers are self-compatible and produce more seeds when fertilized
by outcross pollen (Martin 1965). Visitors to G. maculatum at Three Creeks are mainly solitary bees that also visit L. maackii but also include Lepidopterans and syrphid flies (McKinney, unpublished data).

**Study site**

I conducted this study during May 2009 in an old field habitat in Three Creeks Metro Park, Groveport, Ohio, USA. The area of the park in which this experiment was conducted was used for agriculture until the late 1960’s, when it was permitted to re-grow naturally and was invaded by Lonicera maackii during forest regeneration (J. Snyder, park naturalist, personal communication). The old field is approximately 160m (E to W) x 350m (N to S) and is bordered by a mixed white pine and deciduous forest that is invaded by L. maackii to the west and south and a restored prairie to the east. A park entrance road borders the site to the north, approximately 200m from the experiment. Average L. maackii density in the forest adjacent to the old field is 0.14 shrubs per m². A bicycle path separates the old field from the prairie. The prairie was 40m away from the nearest experimental plants and was mainly composed of Andropogon gerardii and Sorghastrum nutans; Chrysanthemum leucanthemum was also present and co-flowered with L. maackii and G. maculatum from May 15-25. To deter the spread of weedy species, park management mowed the old field in the spring. It was not mowed during spring 2008 or 2009 because of other experiments, and small (mostly non-flowering) L. maackii and Eleagnus umbellata (flowering prior to study species) shrubs have started to colonize the field. There were no naturally occurring G. maculatum plants at the study site, so that natural variation in G. maculatum plant density
was not a confounding factor in this study, and there were no other flowering plants in the old field during the study. To determine whether *G. maculatum* was an appropriate study species for pollinator-mediated interactions, I tested for pollen limitation (PL) of *G. maculatum* in the old field habitat in spring 2008. I added outcross pollen to all of the flowers on ten potted *G. maculatum* plants in the old field, and ten other potted plants received open pollination. Plants were hand pollinated every other day. Plants were watered as necessary and received full sunlight. Comparison of pollen supplemented to open pollinated plants revealed significant pollen limitation of fruit set, supporting *G. maculatum* as an appropriate study species for my research question (open: 0.36±0.099 fruits/flower; supplemental: 0.66±0.059 fruits/flower; \( t=2.59, P=0.010 \)).

**Experimental design**

I established 24 plots within the old field habitat that were at least 40m away from the invaded forest edge and approximately 20m apart from one another. Plots were randomly assigned to one of three array treatments: control, low floral density invasion or high floral density invasion (N=8). I arranged *G. maculatum* plants in 2.8L plastic pots in 3x2 rectangular arrays; each plant was separated by 0.5m. In low and high density invasion arrays, *L. maackii* branches replaced one and two *G. maculatum* plants, respectively. Control arrays contained 6 *G. maculatum* plants, low density arrays contained 5 *G. maculatum* plants with an approximate 20:1 ratio of *L. maackii* to *G. maculatum* flowers, and high density arrays contained 4 *G. maculatum* plants with an approximate 60:1 ratio of *L. maackii* to *G. maculatum* flowers. The low density floral ratio was based on a previous experiment in which I detected increasing facilitation of pollen deposition and seed set, and decreasing pollen limitation of seed set in *G.
maculatum, with proximity to the forest edge invaded by L. maackii at this site (McKinney and Goodell, ch. 3). The ratio of L. maackii flowers to G. maculatum flowers was estimated at 20:1 in this case (McKinney, unpublished data). This suggests that a large number of L. maackii flowers may be necessary for competition for pollination with native plants to occur, so I estimated a high floral density at 60:1, based on approximations of local floral densities of L. maackii and other native herbs within the forest at this site. While the total number of G. maculatum plants varied across treatments, the average number of G. maculatum flowers open at any given time did not significantly differ across treatments (see Results section, pg. 112).

The replacement series design is an appropriate choice for my research question because the goal of the array treatments is to emulate the invasion process (see Jolliffe 2000 for a discussion of replacement series). Thus, my design purposefully confounds pollinator responses to presence of the invasive plant with presence of higher overall flower density because overall floral density increases in areas in which L. maackii invades (McKinney, personal observation). As L. maackii cover increases, herb cover decreases, suggesting that L. maackii replaces herbs as the invasion matures (Hutchinson and Vankat 1997). There is general support for the pattern of invasive plants displacing native plant species (Woods 1993, Tilman 1997, D'Antonio et al. 1998, Christian and Wilson 1999). In addition, study of the age structure of an L. maackii population in SW Ohio showed that the population remained small for approximately 10 years and then substantially increased (Deering and Vankat 1999), suggesting that L. maackii flowers may be present at low densities for at least a few years when invading new areas before increasing to higher densities. Arrays were set up on all days that were sunny or partly
cloudy (days when pollinators were active), commencing when *G. maculatum* began to flower on May 10 and ending when *L. maackii* stopped flowering on May 25. Any remaining *G. maculatum* flower buds were removed after this date.

*Lonicera maackii* branches were cut each morning before pollinators became active (prior to 10:00am) and placed in 1L brown pitchers containing water. Pitchers were attached to dark green metal fence posts approximately 1.25m from the ground, to mimic the natural height of *L. maackii* branches. Low density plots contained one pitcher and high density plots contained two. Flowers were quickly counted and removed to create target floral ratios of 20:1 and 60:1 every morning. The actual floral ratios were on average 20.5:1 and 57:1, respectively.

**Pollinator services**

To measure pollinator services to *G. maculatum*, I quantified pollinator visitation and pollen deposition. Ten minute pollinator observation sessions were conducted on all open *G. maculatum* flowers within each array on sunny to partly cloudy days when air temperature was above 15°C (conditions for pollinator activity). The number of open *G. maculatum* and *L. maackii* flowers, number of visits to all open *G. maculatum* flowers in the array, visitor identification, and visit duration were recorded during each observation session. Bees were identified to the genus level and all other visitors to family. If too many visitors were present during a 10 min session to keep track of both the number and duration of visits, a separate 10 min observation was conducted to record duration of visits. A visit was defined as contact with the stigma or anthers. Pollinator observations were not conducted on *L. maackii* branches because of time constraints, but pollinators were observed foraging on the branches (McKinney, personal observation). Ten *G.*
maculatum stigmas were collected from each plot throughout the flowering period and placed in microcentrifuge tubes containing 75% ethanol. Pollen grains were stained with fuschin dye, mounted on slides with glycerin gel, and pollen grains counted and classified as *G. maculatum*, *L. maackii*, or unknown pollen grains. I only counted pollen grains that contacted the stigmatic surface because these are presumably capable of growing pollen tubes and most closely linked with female reproductive success.

The number of visitors to *G. maculatum*, visitation rate (the number of pollinator visits per flower during 10 minute observations), duration of visits (seconds), and the number of conspecific and *L. maackii* pollen grains were each analyzed with a one-way ANOVA with array treatment as a categorical independent variable. I used planned contrasts to compare the control treatment to both the low and high density treatment (significant at *P*<0.05). The number of unknown pollen grains was analyzed with a nonparametric ANOVA, and Mann-Whitney z-tests were used to compare the control to the low and high density treatment. Responses were averaged by plot for each analysis (*N*=8). All analyses were performed in JMP v. 8 (SAS institute, Cary, NC).

**Native plant reproductive success**

I collected *G. maculatum* fruits from experimental arrays as they matured (1-3 weeks after all flowering ended) and counted the number of seeds within each fruit. Fruit set was calculated as the number of fruits produced per flower and seed set was quantified as the number of seeds produced per fruit and per flower. The number of seeds per flower represents combined effects of fruit set and seeds per fruit and is more closely related to population level effects (Ashman et al. 2004). I used seeds per flower in place of seeds per plant because the total number of flowers per plant differed across
treatments. Throughout the entire experiment, a total of three high density treatments accidentally received a low *L. maackii* density for only one day each. I marked individual *G. maculatum* flowers on the day they opened, so I removed fruit and seed set data from these flowers from the analysis. Fruit set had a left-skewed, zero-inflated distribution because 35% of plants failed to set fruit. Therefore, I used the relative neighbor effect index (RNE) to analyze fruit set (Weigelt and Jolliffe 2003, Munoz and Cavieres 2008). The RNE calculates performance relative to the best performer in a sample pair, making it appropriate for detecting either competition or facilitation (Markham and Chanway 1996).

\[
RNE = \frac{(X_c - X_t)}{X_c} \text{ if } X_c > X_t
\]

\[
RNE = \frac{(X_c - X_t)}{X_t} \text{ if } X_t > X_c
\]

where \(X\) is the average response per plot, \(c=\)control and \(t=\)treatment. I calculated the average number of fruits per flower (on a per plant basis) for each plot and then randomly paired control with treatment plots to calculate RNE separately for low and high density treatments \((N=8)\). Each RNE value was multiplied by -1 so that negative values represented competition and positive values facilitation (Munoz and Cavieres 2008). I then used z-tests to calculate whether the RNE was significantly different from zero for low and high density treatments and for a random sample of control plots paired up with other control plots to gauge any potential bias from randomly pairing plots. Seed set was log transformed to meet normality assumptions and was analyzed with an ANOVA and planned contrasts.
To test for pollen limitation (PL) of *G. maculatum* female reproductive success in the experimental arrays, average fruit and seed set were compared between pollen-supplemented plants and open pollinated plants within each array treatment, using t-tests. The magnitude of PL was quantified as the difference between fruit and seed set of supplemental and open pollinated plants. Because fruit set violated normality assumptions, I randomly paired each plant in the array experiment with one of the ten pollen-supplemented plants to calculate the magnitude of PL of fruit set. I averaged the magnitude of PL per plant for each plot. For seed set, I subtracted average seed set (on a per plant basis) in each plot from the average seed set of pollen-supplemented plants to calculate average magnitude of PL of seed set. The number of seeds per flower was natural log transformed to improve normality. I used an ANOVA and planned contrasts as described above to analyze differences among array treatments in the magnitude of PL of fruit and seed set.

*Heterospecific pollen transfer*

To determine the effect of *L. maackii* pollen on *G. maculatum* female reproductive success, I conducted an additional hand-pollination experiment in the spring of 2008, one year prior to the array experiment. I used 12 potted *G. maculatum* plants that were separate from the array study and hand-pollinated all of the flowers, as stigmas matured, on 12 *G. maculatum* plants with either a mixture of conspecific and *L. maackii* pollen or only conspecific pollen (*N*=6 plants). I collected 5 stigmas from each plant, one day following pollinations, and counted pollen grains. Pollen tubes in *G. maculatum* reach ovaries in less than 2h 30min, so stigma collection should not impact fruit maturation (Mulcahy et al. 1983).
To analyze the effect of *L. maackii* pollen on *G. maculatum* fruit set, the number of fruits per flower was compared between mixed and pure hand pollination treatments with a Mann-Whitney z-test. I used two sample t-tests to compare *G. maculatum* seeds per fruit and seeds per flower between treatments. I expected female reproductive success to be lower in the mixed treatment and used one-sided p-values to determine significance. Natural *L. maackii* pollen transfer was compared to *L. maackii* transfer from hand pollinations using a Mann-Whitney z-test.

**RESULTS**

The number of *G. maculatum* flowers open at any given time did not differ significantly among treatments but tended to decrease with the number of plants per array treatment as expected, with the control arrays having the most *G. maculatum* flowers open at any given time (ANOVA, $F_{2,21} = 0.86$, $P=0.44$; control: 11.52±2.25 flowers, low: 8.69±1.30 flowers, high: 7.37±0.96 flowers). The ratio of *G. maculatum* to *L. maackii* flowers remained constant within treatments throughout the experiment, but the absolute number of flowers changed through time, depending on how many *G. maculatum* flowers were open. The low density arrays on average contained 163.8±19.02 *L. maackii* flowers, and the high density arrays contained 411.80±48.07 *L. maackii* flowers on average (±1 SE). P-values given throughout the results are two-tailed for ANOVA tests and one-tailed based on my hypotheses for planned comparisons of control plots to treatment plots (expect increases in low compared to control and decreases in high compared to control).
Pollinator services

A total of 7 different bee genera, syrphid flies, and Lepidopterans (butterflies and hawk moths) were recorded visiting *G. maculatum* flowers in over 28 hours of observation (approximately 9 hours per treatment) (Table 4.1). Hawk moths typically did not contact *G. maculatum* stigmas or anthers when foraging for nectar but did on occasion contact anthers or stigmas. Pollinator visitation rate to *G. maculatum* was significantly different among treatments ($F_{2,21} = 6.89, P=0.005$). Planned comparisons revealed significantly higher visitation rates in the low density arrays compared to control arrays ($t>2.08, P=0.0024$) and no difference in visitation rates between control and high density arrays ($t<2.08, P=0.98$, Fig. 4.1a). The duration of pollinator visits was also significantly different among treatments ($F_{2,21}=5.79, P=0.01$). In contrast to visitation rate, the duration of visits was significantly shorter in high density arrays compared to controls ($t>2.08, P=0.010$), and there was no difference in the duration of visits between control and low density arrays ($t<2.08, P=0.23$, Fig. 4.1b).

The number of *G. maculatum* pollen grains adhering to the stigmatic surface was significantly different among treatments ($F_{2,21}=6.58, P=0.006$). Conspecific pollen deposition was significantly higher in low density arrays compared to controls ($t>2.08, P=0.012$), and there was no difference in conspecific pollen deposition between high density and control arrays ($t<2.08, P=0.15$, Fig. 4.1c). Conspecific pollen grains accounted for 78.55%, *L. maackii* grains for 14.20%, and unknown pollen grains for 7.43% of total pollen deposition to *G. maculatum* stigmas. The numbers of *L. maackii* (Lm) and unknown (un) pollen grains on *G. maculatum* stigmas were relatively consistent across treatments, with no significant differences detected among arrays in
either response \((F_{2,21}=0.32, P=0.73, \text{control}: 4.80\pm0.61 \text{ Lm grains, low: } 4.16\pm0.60 \text{ Lm grains, high: } 4.18\pm0.72 \text{ Lm grains}; X^2=5.42, P=0.067, \text{control: } 3.16\pm0.85 \text{ un grains, low: } 2.26\pm0.77 \text{ un grains, high: } 1.72\pm0.84 \text{ un grains})\).

**Native plant reproductive success**

The RNE index for fruit set was marginally significantly higher than zero in the low density treatment compared to the control, indicating slight facilitation of fruit set by a low density of *L. maackii* flowers \((z=1.63, P=0.051)\), and was not significantly different from zero in the high density treatment compared to the control, indicating no effect of a high density of *L. maackii* flowers on fruit set \((z=0.24, P=0.59, \text{Fig. 4.2a})\). In addition, control plots were randomly paired with one another and a control RNE was calculated for fruit set; it was not significantly different from zero \((z=-0.19, \text{two-sided } P=0.85, \text{Fig. 4.2a})\). This suggests that by chance alone, the RNE should overlap with zero (i.e. no effect). The number of *G. maculatum* seeds per fruit (natural log transformed) was marginally significantly different among treatments \((F_{2,21}=3.35, P=0.055)\). In contrast to fruit set, seeds per fruit (natural log transformed) was significantly lower in high density arrays compared to controls \((t>2.08, P=0.011)\) and did not differ between low density and control arrays \((t<2.08, P=0.16, \text{Fig. 4.2b})\). Finally, there was no significant difference among treatments in *G. maculatum* seeds per flower (natural log transformed), and planned comparisons revealed no significant differences between control and treatment plots \((F_{2,21}=0.056, P=0.95; \text{low: } t<2.08, P=0.47; \text{high } t>2.08, P=0.37, \text{Fig. 4.2c})\).

Both fruit and seed set were significantly limited by pollen receipt in all treatments (Table 4.2). The magnitude of pollen limitation (PL) of fruit set did not differ among treatments \((F_{2,21}= 0.55, P=0.58, \text{Fig. 4.3a})\). Planned contrasts did not reveal any
significant differences in the magnitude of PL of fruit set between control and low density or control and high density arrays (low: $t<2.08$, $P=0.16$; high: $t<2.08$, $P=0.68$, Fig. 4.3a). The magnitude of PL of seeds per fruit did not differ among treatments ($F_{2,21}=2.86$, $P=0.076$, Fig. 4.3b). However, planned comparisons revealed significantly stronger PL of seeds per fruit in high density compared to control arrays ($t>2.08$, $P=0.015$) and no difference between low density and control arrays ($t<2.08$, $P=0.22$, Fig. 4.3b). The magnitude PL of seeds per flower (natural log transformed) did not differ among treatments ($F_{2,21}=0.67$, $P=0.52$, Fig. 4.3c). Planned contrasts did not reveal any significant differences in the magnitude of PL of seeds per flower between control and low density or control and high density arrays (low: $t<2.08$, $P=0.44$; high: $t<2.08$, $P=0.19$, Fig. 4.3c).

**Heterospecific pollen transfer**

I delivered similar numbers of conspecific pollen grains to *G. maculatum* (Gm) stigmas in mixed and pure pollination treatments (mixed: $26.5 \pm 2.7$Gm grains, pure: $30.8 \pm 7.1$Gm grains, $t=0.56$, $P=0.59$) and significantly greater numbers of *L. macackii* (Lm) pollen grains in the mixed compared to the pure treatment (mixed: $18.3 \pm 5.0$ Lm grains; pure: $2.2 \pm 0.7$Lm grains, $z=2.80$, one-sided $P=0.0025$). The number of fruits and seeds per flower were not significantly different between hand pollination treatments, and mixed pollen loads were associated with significantly fewer seeds per fruit compared to pure pollen loads (Table 4.3). The hand pollinations delivered over four times as many *L.
maackii pollen grains to G. maculatum stigmas compared to natural L. maackii transfer by pollinators in the experimental arrays with L. maackii (hand: 18.26 ±5.0 Lm grains, open: 4.17±0.45 Lm grains; z=3.43, two-sided $P=0.0006$, $N=6$ (hand), 16 (open)).

**DISCUSSION**

Results of this study indicate the importance of floral density in shaping outcomes of interactions for pollinators between co-flowering plant species and how these outcomes may or may not translate to subsequent consequences for plant reproduction. Because pollen receipt limits both fruit and seed set of G. maculatum in all treatments, changes in pollinator services affect female reproductive success. Consistent with Rathcke’s (1983) hypothesized continuum, pollinator services and G. maculatum fruit set in arrays with a low L. maackii floral density are higher compared to control arrays in which L. maackii is absent. Facilitation of pollinator visitation to G. maculatum changes to competition for pollination and reduced G. maculatum seeds per fruit at higher densities of L. maackii flowers. However, these changes in fruits per flower and seeds per fruit in the presence of L. maackii do not combine to affect the number of G. maculatum seeds per flower, which is more relevant to population-level impacts (Ashman et al. 2004). For invasive plant species in which floral density increases as the invasion progresses, facilitation of native plant pollination may occur during early stages of invasion and switch to competition as the invasion matures, but changes in pollinator foraging behavior may not have reproductive consequences for native plants (e.g. Grabas and Laverty 1999).
When *L. maackii* flowers are present at a low density, pollinators visit over twice as many *G. maculatum* flowers, delivering more conspecific pollen relative to controls. Facilitation of pollination corresponds to a slight increase in the number of *G. maculatum* flowers setting fruit. Several studies suggest that a higher density or diversity of flowers in mixed arrays compared to control arrays may initially attract more bees to mixed arrays, which then visit both plant species (sensu Schemske 1981, Kunin 1993, Feldman et al. 2004, Ghazoul 2006). Because visitation rate to *G. maculatum* does not differ between control and high density arrays that contain the same number of species as low density arrays, it seems likely that floral density is the major influence on increasing pollinator visitation rate to *G. maculatum*. My results are consistent with Ghazoul (2006) in showing that facilitation of pollinator visitation to a focal species occurs between species with distinct floral morphologies. There are several cases of invasive plant facilitation of pollinator visitation to native plants in the literature (for reviews see Bjerknes et al. 2007, Morales and Traveset 2009); only one other case of which I am aware shows evidence of a positive reproductive effect in the native species (Munoz and Cavieres 2008). However, increased *G. maculatum* fruit set did not translate into increased overall seed production, perhaps because pollinators delivered enough pollen to reach the threshold pollen: ovule ratio required for fruit production (Mitchell 1997) in more flowers compared to controls but not enough to increase overall seed production compared to controls. Many studies of interactions between invasive and native plant species measure only pollinator visitation and/or pollen deposition; based on my results,
caution should be used when extrapolating from pollinator services to reproduction of native species. I detected only a slight increase in fruit set, despite a two-fold increase in pollinator visitation rate and increased conspecific pollen deposition.

Heterospecific pollen transfer (HPT) may counteract any positive effects of increased pollinator visitation to native plants if pollen from invasive plants clogs native plant stigmas or styles or if pollen allelopathy interferes in plant reproduction (among native plants Waser 1978, Galen and Gregory 1989, Murphy and Aarssen 1995). In this study, natural levels of HPT were consistently low (~4 L. maackii grains) across arrays and did not affect G. maculatum reproductive success. Results from the hand pollination experiment suggest that substantially more L. maackii grains are required to disrupt G. maculatum female reproductive success than observed in the array study. Similar hand pollination experiments show either no effect or negative effects on native plant reproduction (Brown and Mitchell 2001, Moragues and Traveset 2005, Nielsen et al. 2008). Because stigmas were placed in ethanol after collection, some pollen may have washed off of stigmas. However, in another study in which I immediately mounted G. maculatum stigmas on slides after collection, I observed similar levels of L. maackii pollen deposition (McKinney and Goodell, ch. 3). I may detect little HPT here because pollinators do not frequently switch between G. maculatum and L. maackii, because pollinators exhibit floral constancy after switching between floral species (Chittka et al. 1999), or because dissimilar floral morphologies may lead to differential pollen placement on pollinator bodies (Harder and Barrett 1993).
In high density arrays, pollinators spend less time per visit to *G. maculatum* flowers compared to controls. Pollinators make a similar number of visits to *G. maculatum* flowers in high density and control arrays, delivering a similar amount of conspecific pollen on a per flower basis, which ensures a similar number of fruits produced per flower; however, flowers in high density arrays make fruits that contain fewer seeds, corresponding to shorter pollinator visits. In addition, the magnitude of pollen limitation of seed set is stronger in high density compared to control arrays. The duration of pollinator visits is associated with seed abortion rate and seed yield, especially when plants are pollinator-limited (Manetas and Petropoulou 2000). It should be noted, however, that conspecific pollen deposition is significantly lower in high density compared to low density arrays but not compared to controls. In addition, the magnitude of the reduction in seeds per fruit was not strong enough to translate into lower seeds per flower in high density compared to control arrays. However, a study in an alpine meadow found that native plants compete for pollinators and experience decreased seed production at high densities of an invasive dandelion species (Munoz and Cavieres 2008).

It is unclear whether a behavioral shift in pollinators (e.g. Ghazoul 2006) or a shift in the pollinator community visiting *G. maculatum* resulted in shorter visits in high density arrays. The results point to some evidence of a shift in the bee community. *Halictus ligatus* accounts for a smaller percentage of visits to *G. maculatum* when *L. maackii* is present at high densities, and *H. ligatus* spends significantly more time per visit to *G. maculatum* flowers across treatments compared to the most common pollinator.
in this study, Ceratina spp. (McKinney, unpublished data: H. ligatus: 42.56±12.49 secs, Ceratina: 16.16±2.80 secs; z=2.18, P=0.03). Regardless of the reason behind shorter visit duration, my results emphasize the importance of considering various pollinator visitation responses; if I had not measured duration of visits, I would have missed important patterns in pollinator foraging.

This study illustrates that the relative floral density of invasive plants can affect pollinator foraging behavior on native plants, with competition only occurring at a high floral density of the invader. The particular floral density at which competition occurs probably varies considerably among plant species. For example, Munoz and Cavieres (2008) found density-dependent effects at relative floral densities of 1:1 (low invader) and 5:1 (high invader), compared to 20:1 and 60:1 in this study. Floral reward and plant breeding system are likely to influence the relative floral densities at which facilitation and competition for pollination occur. Native species with less rewarding nectar than co-flowering invasive species may experience competition at lower relative floral densities of the invasive, compared to native plants with more rewarding nectar or a higher rate of nectar production (Chittka and Schürkens 2001). Indeed, G. maculatum nectar contains on average 40% more total sugar in comparison to L. maackii nectar (McKinney and Goodell 2010), which may explain why facilitation of pollination was observed at a relatively high density of the invader compared to Munoz and Cavieres (2008). Similarly, self-incompatible native species may experience competition at lower relative floral densities of an invasive plant compared to self-compatible native species. Self-
incompatible plant species also become mate-limited at lower densities (Elam et al. 2007). In addition, the prevalence of HPT and its effect on native plant reproduction will also influence transitions from facilitation to competition for pollination.

My results are relevant to local-scale foraging decisions of bees among and within patches of floral resources. This design is a reasonable representation of plant densities in natural systems because flowers are often patchily distributed (e.g. Sih and Baltus 1987). The old field habitat at this site supports populations of relatively small bees, consistent with Gathmann et al. (1994). The distance between arrays is well within the foraging distance of the smallest bees observed at this site (~150m) (Gathmann and Tscharntke 2002, Greenleaf et al. 2007), so that any given bee is likely to choose between control, low, and high density arrays of varying total floral density and relative floral density (Ghazoul 2006). It is important to keep in mind that these results specifically apply to the relative floral densities in this experiment. I designed this study to represent \textit{L. maackii} invasions at incipient and mature stages (low and high density, respectively). It seems reasonable to assume that \textit{L. maackii} floral densities encompassing those modeled in this study will occur through time in a more natural setting, as an area transitions from incipient to mature stages of invasion.

My experimental design isolates pollinator-mediated effects. Under field conditions, neighboring plant species can impact several aspects of a focal plant’s ecology, such as microclimate, soil resources, herbivory, and fruit and seed dispersal, that apply various pressures on individual fitness and ultimately may impact community structure (Hunter and Aarssen 1988). Above-ground interactions may comprise a large portion of the net interactions between \textit{L. maackii} and native plants (Gould and Gorchov
2000, Miller and Gorchov 2004, McKinney and Goodell 2010). For example, Gorchov and Trisel (2003) found that above-ground competition between *L. maackii* and native tree seedlings was stronger than below-ground competition.

Indirect biotic interactions are complicated, difficult to understand mechanistically, and often overlooked in studies of interactions between invasive and native species (White et al. 2006). Indirect mutualisms between invasive and native plants exhibit a range of outcomes (i.e. competition to facilitation) (Traveset and Richardson 2006, Bjerknes et al. 2007, Morales and Traveset 2009). This study suggests that relative floral densities of invasive to native plants explain at least part of the variation in interactions for pollinators between invasive and native plants. It is important to consider density-dependent effects when drawing conclusions about pollinator-mediated interactions between invasive and native plants because interactions for pollinators may change as the invasion becomes more or less severe. In this study, changes in pollinator foraging behavior did not ultimately affect *G. maculatum* seed production per flower. Long term data will be particularly insightful in understanding how plant-pollinator interactions change through time as an invasion progresses.

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Table 4.1 Composition of visitors to *Geranium maculatum* in three array treatments: control, low floral density of the invasive shrub *Lonicera maackii* and high floral density of *L. maackii*. Shown for each pollinator category are the percentage of total visits for which each accounted. The other category includes Lepidoptera, Coleoptera, and unknown visitors.

<table>
<thead>
<tr>
<th>Category</th>
<th>Control</th>
<th>Low density</th>
<th>High density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrena spp.</td>
<td>3.8%</td>
<td>3.5%</td>
<td>7.7%</td>
</tr>
<tr>
<td>Apis mellifera</td>
<td>-</td>
<td>-</td>
<td>0.48%</td>
</tr>
<tr>
<td>Augochlorella spp.</td>
<td>1.6%</td>
<td>8.2%</td>
<td>1.9%</td>
</tr>
<tr>
<td>Ceratina spp.</td>
<td>48.7%</td>
<td>42.0%</td>
<td>39.6%</td>
</tr>
<tr>
<td>Halictus ligatus</td>
<td>28.7%</td>
<td>29.0%</td>
<td>14.5%</td>
</tr>
<tr>
<td>Lasioglossum spp.</td>
<td>5.1%</td>
<td>3.1%</td>
<td>7.2%</td>
</tr>
<tr>
<td>Osmia spp.</td>
<td>6.1%</td>
<td>9.3%</td>
<td>23.7%</td>
</tr>
<tr>
<td>Syrphid flies</td>
<td>0.66%</td>
<td>0.66%</td>
<td>0.97%</td>
</tr>
<tr>
<td>Other</td>
<td>5.4%</td>
<td>4.2%</td>
<td>3.4%</td>
</tr>
</tbody>
</table>
One way ANOVAs ($df=3$) of effects of array treatment on pollen limitation of *Geranium maculatum* reproductive success and planned contrasts of pollen limitation of *Geranium maculatum* reproductive success. Nonparametric tests were used for seeds per flowers. Supp = Plants hand pollinated with supplemental conspecific pollen ($N=10$ plants); open pollinated plants within each treatment ($N=8$ plots, average of all plants in the plot)

<table>
<thead>
<tr>
<th>Source</th>
<th>Fruits per flower</th>
<th>Seeds per fruit</th>
<th>Seeds per flower</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F$</td>
<td>$P$</td>
<td>$F$</td>
</tr>
<tr>
<td>Treatment</td>
<td>22.45</td>
<td>0.0002</td>
<td>11.81</td>
</tr>
<tr>
<td>Contrasts</td>
<td>7.08</td>
<td>&lt;0.0001</td>
<td>3.10</td>
</tr>
<tr>
<td>Control v supp</td>
<td>5.13</td>
<td>&lt;0.0001</td>
<td>3.98</td>
</tr>
<tr>
<td>Low v. supp</td>
<td>6.38</td>
<td>&lt;0.0001</td>
<td>5.1</td>
</tr>
<tr>
<td>High v. supp</td>
<td>6.38</td>
<td>&lt;0.0001</td>
<td>5.1</td>
</tr>
</tbody>
</table>
Table 4.3 Mann-Whitney z tests and t tests of responses of *Geranium maculatum* female reproductive success to plants hand pollinated with *G. maculatum* pollen (pure) and plants hand pollinated with a mixture of *L. maackii* and *G. maculatum* pollen (mixed). Means ±1 SE and one-sided p-values are shown, $df=1$, $N=6$ plants.

<table>
<thead>
<tr>
<th></th>
<th>Fruits per flower</th>
<th>Seeds per fruit</th>
<th>Seeds per flower</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$z$</td>
<td>$P$</td>
<td>$t$</td>
</tr>
<tr>
<td>pure</td>
<td>0.38±0.08</td>
<td>1.15</td>
<td>0.065</td>
</tr>
<tr>
<td>mixed</td>
<td>0.25±0.06</td>
<td></td>
<td>1.70±0.50</td>
</tr>
</tbody>
</table>
Figure 4.1 Differences in a) pollinator visitation rate, b) duration of visits, and c) conspecific pollen deposition to *Geranium maculatum* flowers in three experimental arrays consisting of varied relative floral densities of the invasive shrub *Lonicera maackii* (control- no *L. maackii*). Solid circles are mean values and error bars ± 1SE. Capital letters represent significant differences at $P<0.05$, as determined by one-sided t-tests.
Figure 4.2 *Geranium maculatum* reproductive success in three experimental arrays consisting of varied relative floral densities of the invasive shrub *Lonicera maackii* (control- no *L. maackii*). a) Solid circles are mean relative neighbor effect index RNE values for fruit set and error bars ± 1SE. Asterisks represent significant differences from zero at $P<0.05$, as determined from one-tailed z-tests. b-c) Solid circles are mean values of natural log transformed seed set and error bars ± 1SE. Capital letters indicate significant differences, as determined by one-tailed t-tests from planned contrasts $P<0.05$. 
Figure 4.3 Magnitude of pollen limitation (PL) of *Geranium maculatum* female reproductive success a) fruit set and b–c) seed set in three experimental array treatments. c) PL of seeds per flower was natural log transformed. Capital letters indicate significant differences, as determined by one-tailed t-tests for planned contrasts $P<0.05$. 

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COMPREHENSIVE REFERENCE LIST


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