

RELATIVE FLORAL DENSITY OF AN INVASIVE PLANT AFFECTS POLLINATOR FORAGING BEHAVIOUR ON A NATIVE PLANT

Amy M. Iler^{1,3*} & Karen Goodell²

¹Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, 318 W 12th Ave, Columbus, OH 43210 USA

²Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, 1179 University Dr, Newark, OH 43055 USA

³Current address: Department of Biology, University of Maryland, College Park, Maryland 20742 USA

Abstract—Interactions between invasive and native plants for pollinators vary from competition to facilitation of pollination of native plants. Theory predicts that relative floral densities should account for some of this variation in outcomes, with facilitation at low floral densities and competition at high floral densities of the invader. We tested this prediction by quantifying pollination and female reproductive success of a native herb, *Geranium maculatum*, in three experimental arrays that varied in floral density of the invasive shrub *Lonicera maackii*: control (no *L. maackii*), low floral density of *L. maackii*, and high floral density of *L. maackii*. A low density of *L. maackii* flowers was associated with an increase in pollinator visitation rate to *G. maculatum* flowers and an increase in conspecific pollen deposition compared to controls and high density arrays. Increased visitation rates were not associated with an increase in the number of visitors to low density arrays, suggesting instead that a behavioural switch in visitation within the array accounted for increased pollen deposition. In contrast, the only evidence of competition in high density arrays was a shorter duration of visits to *G. maculatum* flowers relative to the other treatments. The number of seeds per flower did not vary among treatments, although trends in seeds per flower were consistent with patterns of pollinator foraging behaviour. Given increased pollinator visits and pollen deposition at a low density of the invader, our study indicates that complete eradication of invasives as a management or restoration technique may have unintended negative consequences for pollination of native plants.

Keywords: *biological invasion; density-dependence; experimental array; indirect interaction; Lonicera; pollination*

INTRODUCTION

Invasive plant species can directly out-compete native plant species for space and other abiotic resources, depending on resource availability and past disturbance regimes (Daehler 2003; Levine et al. 2003). Despite increasing interest in indirect effects of invasive plants on the biotic interactions of native plants, such as those mediated through pollinators, indirect effects are still not as well understood as direct effects (Strauss 1991; White et al. 2006). Establishment of mutualisms in the introduced range can be a crucial component of successful plant invasions (Mitchell et al. 2006). Mutualists are resources for which plants can compete (Waser 1983), and invasive plants are likely to be particularly strong competitors for mutualists because they occur at high densities (Traveset & Richardson 2006). Indeed, invasive plants compete for pollinators with native plants and reduce native plant reproductive success, an effect that is more pronounced as invasive plant density increases (Chittka & Schürkens 2001; Morales & Traveset 2009; Kandori et al. 2009; Takakura et al. 2009; Matsumoto et al. 2010; Dietzsch et al. 2011). On the other hand, facilitation of pollination and native plant seed set by invasive plants has also been observed (Muñoz & Cavieres

2008; McKinney & Goodell 2011). Whether native plants experience facilitation, no effect, or competition for pollination with an invasive plant species seems to depend on relative floral densities and the spatial scale over which interactions are studied (Muñoz & Cavieres 2008; Cariveau & Norton 2009; McKinney 2010). Because invasive plant densities increase as plant invasions progress, studying the role of invasive-native relative floral density in mediating pollination services can serve as a model for understanding ecological processes at different stages of invasion.

Pollinator foraging behaviour depends on processes operating at the level of patches of floral resources within a broader floral landscape and processes within the patch (Fig. 1). Total floral density and floral diversity affect pollinator choice among patches, whereas relative floral density should affect pollinator foraging decisions among floral resources within a patch (Ghazoul 2006; Yang et al. 2011). If attraction to larger patches of floral resources is an accelerating function of floral density, facilitation of pollinator visits to plant species within the patch is possible (sensu Feinsinger et al. 1991; Rathcke 1983; Feldman 2004; Fig. 1A & B). However, facilitation is not expected at any floral density if pollinator attraction increases linearly with patch-level floral density (Feldman 2004). After initial pollinator attraction to an invaded patch, facilitation is more likely at low relative floral density of the invader (Fig. 1B vs. C). As a plant invasion progresses, floral availability is likely

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*Corresponding author; email: amy.marie.iler@gmail.com

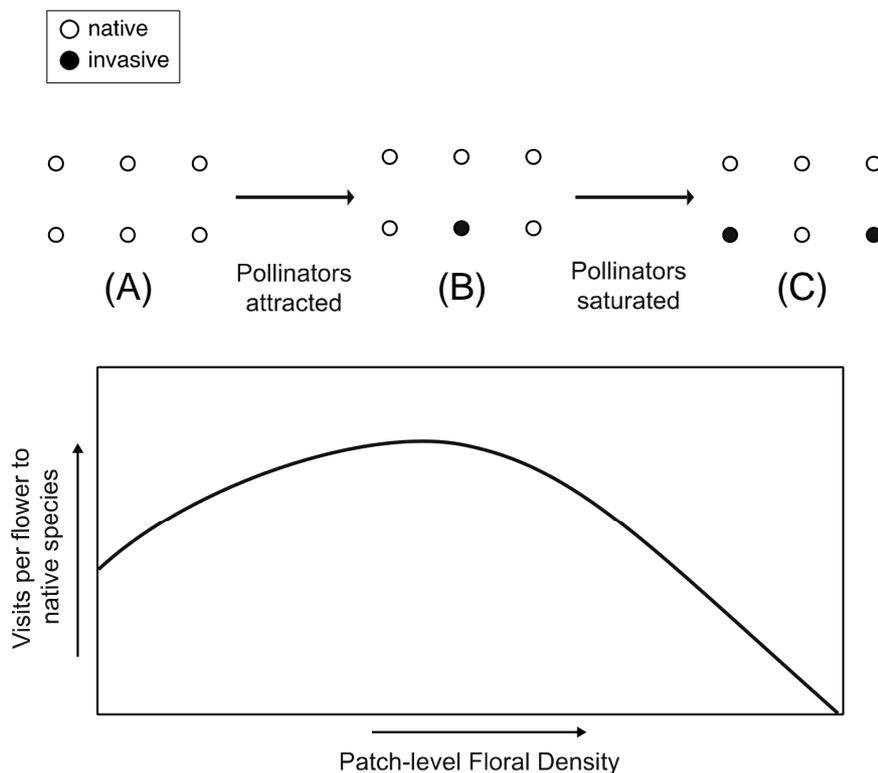


FIGURE 1. Conceptual diagram of pollinator foraging responses to increasing floral density in patches of flowers. Total patch-level floral density increases from (A) to (C), as patches become increasingly invaded by an invasive plant species. Circles represent individual plants. As pollinators are attracted to patches with a higher density of floral resources, facilitation of visits to the native species is possible (B), until pollinators become saturated and competition for visits is likely (C). In this study, we use plant arrays in which total floral density increases in invaded arrays, similar to those portrayed here (see Table 1 for experimental design).

to saturate the resident pollinator community, and competition for pollinators within the patch is more likely (Feinsinger 1987; Rathcke 1983; Fig. 1C).

The reproductive consequences of altered pollinator foraging behaviour to native plants depend on subsequent patterns of pollen removal and deposition, in addition to whether conspecific pollen receipt limits native plant reproduction (McKinney & Goodell 2010; Tscheulin & Petanidou 2013). For example, heterospecific pollen deposition can offset increased visitation rates to native plants and reduce native plant reproductive success (Grabas & Laverty 1999; Brown & Mitchell 2001; Lopezaraiza-Mikel et al. 2007; Jakobsson et al. 2008; but see Bartomeus et al. 2008). Transfer of heterospecific pollen can counteract any positive effects of increased pollinator visitation if pollen clogs plant stigmas or styles or if pollen allelopathy interferes in plant reproduction (among native plants: Waser 1978; Galen & Gregory 1989; Murphy & Aarssen 1995). Sequential visits to flowers of the invader may also result in loss of native plant pollen and lower seed production for the native plant, even when more pollinators are attracted to invaded patches (Flanagan et al. 2010). Finally, changes in pollinator foraging behaviour may have no effect on native plant reproduction if seed production is not pollen-limited (Ghazoul 2004; Totland et al. 2006; Kaiser-Bunbury & Muller 2009; Bartomeus et al. 2010).

Here we focus on pollination services to a native plant species within invaded and uninvaded artificial patches of floral resources. We use experimental arrays that vary in relative floral densities of the invasive shrub *Lonicera maackii* and the native herb *Geranium maculatum*, two

species that have been shown to interact for pollinators in our previous work (McKinney & Goodell 2010). The goal of the treatments is to emulate different stages of invasion. In a previous experiment at this site, we detected evidence of facilitation of *G. maculatum* pollination at an approximate 20:1 floral ratio (and at a distance up to 20 m away from *L. maackii*; McKinney 2010). Here we investigate whether increasing the floral density of the invader will reduce the facilitative effect or even result in competition with the native plant (McKinney 2010). We specifically ask how a low (20:1) and high (60:1) relative floral density of the invader affects pollinator foraging (number of visitors, visitation rate, and duration of visits), heterospecific and conspecific pollen deposition, and seed set of *G. maculatum* relative to control plots containing only *G. maculatum* flowers.

MATERIALS AND 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 the eastern USA (Luken & Thieret 1996). Mature plants, such as those in our study site, produce thousands of medium-sized (2-2.5 cm wide), zygomorphic, white to light pink flowers in early May. Non-native honeybees (*Apis mellifera*) and native solitary bees forage on *L. maackii* flowers in central Ohio (Goodell & Iler 2007). *Lonicera maackii* invasions seem to negatively affect native flora and fauna through direct pathways, such as competition for abiotic resources and by increasing nest predation of native

birds (Hutchinson & Vankat 1997; Gould & Gorchov 2000; Collier et al. 2002; Borgmann & Rodewald 2004; Miller & Gorchov 2004) and through indirect pathways, such as increasing human disease risk via altering white-tailed deer host dynamics (Allan et al. 2010; Mattos & Orrock 2010).

Geranium maculatum L. is a common, widespread, perennial 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 phenology overlap with *L. maackii*. *Geranium maculatum* relies on sexual reproduction as well as vegetative reproduction, but seeds are required for dispersal and population expansion (Martin 1965). Flowering plants bear 2–30 large (2.5–4 cm wide), actinomorphic, purple-pink flowers and produce more flowers in full sun than plants in shaded habitat (Martin 1965). Some populations are gynodioecious, with a small proportion of female plants relative to hermaphrodites (Agren & Willson 1991). The hermaphroditic flowers of *G. maculatum* are protandrous (Bertin & Sholes 1993). Plants are self-compatible, but pollinators are required for movement of pollen between flowers (Martin 1965; Willson et al. 1979). At our study site, the most common visitors to *G. maculatum* are native solitary bees that also visit *L. maackii*; Lepidopterans and syrphid flies also occasionally visit *G. maculatum* (McKinney 2010). Reproduction of potted hermaphroditic *G. maculatum* plants at our study site was pollen limited in spring 2008, suggesting that pollinators generally play an important role in seed production of potted *G. maculatum* plants at this location (McKinney 2010).

Study site

We 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 our 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* (J. Snyder, park naturalist, personal communication). The old field was approximately 160 m (E to W) × 350 m (N to S) and was bordered to the west and north by a mixed white pine and deciduous forest that was invaded by *L. maackii* and to the east by a restored prairie that was separated from the old field site by a paved bicycle path. A park entrance road bordered the site to the south, approximately 200 m from the experiment. *Lonicera maackii* density in the forest adjacent to the old field was 0.14 ± 0.0086 shrubs per m² (mean \pm 1 SE here and throughout). The prairie was 40 m 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 15–25 May (the latter 2/3 of the experiment). 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* shrubs started to colonize the field. There were no naturally occurring *G. maculatum* plants at our study site, probably because of clearing of natural vegetation for agriculture, so that natural variation in *G. maculatum* plant density was not a confounding factor in this study. There were no other flowering plants in the old field during the study.

Experimental design

We established 24 1 m × 0.5 m plots within the old field that were at least 40 m away from invaded forest edge habitat and ca. 20 m apart from one another. Plots were randomly assigned to one of three array treatments: control, low floral density invasion, or high floral density invasion ($N = 8$ plots per treatment; Table 1). We arranged potted *G. maculatum* plants in rectangular arrays in which each plant was separated by 0.5 m (3×2 plants in controls; Table 1). In low and high density invasion arrays, *L. maackii* branches replaced one and two *G. maculatum* plants, respectively (Table 1). *Lonicera* branches were cut each morning and placed in brown 1L pitchers containing water, which were attached to a fence post at ca. 1.25 m from the ground (vertical separation of ca. 0.75 m between flowers of each species). *Geranium maculatum* rhizomes were purchased from Prairie Moon Nursery (Minnesota, USA), planted in 2.8 L plastic pots containing ProMix potting soil, fertilized with Osmocote smart-release® plant food to encourage flowering, and watered as necessary. Plants were maintained in a screen house at The Ohio State University Newark until transportation to the field. All plants used in the study were hermaphrodites. The low density ratio of 20:1 invasive:native flowers was based on a previous experiment at this site in which we detected evidence of facilitation (McKinney 2010). We aimed to keep relative floral densities relevant to densities observed in the field by setting the high floral density treatment at 60:1, based on floral densities of *L. maackii* and other native herbs within the forest at this site (A. Iler, personal observation). While the total number of *G. maculatum* plants varied across treatments, the average number of *G. maculatum* flowers open per day per plot did not significantly differ across treatments (ANOVA, $F_{2,21} = 0.86$, $P = 0.44$; control: 11.5 ± 2.3 flowers, low: 8.7 ± 1.3 flowers, high: 7.4 ± 0.96 flowers).

TABLE 1. Experimental arrays of native *Geranium maculatum* and invasive *Lonicera maackii* used to simulate effects of plant invasion on pollination services to native plants. *Lonicera maackii* branches were placed in containers of water daily to create target floral ratios, and *G. maculatum* plants were permanently contained in pots. Plants were arranged in two rows of three plants in each treatment. $N = 8$ arrays per treatment.

	Control	Low density invasion	High density invasion
# Native plants	6	5	4
# Invasive branches	0	1	2
Invasive:native floral ratio	–	20:1	60:1

The spatial scale of this experiment is within the foraging ranges of most native bee species (Gathmann & Tscharntke 2002; Greenleaf et al. 2007), yet relevant to foraging decisions of bees within and among patches of floral resources (Ghazoul, 2006). It is a reasonable representation of plant densities in natural systems because flowers are often patchily distributed (e.g. Sih & Baltus, 1987). Our experimental design intentionally confounds pollinator responses to presence of *L. maackii* with higher overall flower density, because flowering *L. maackii* shrubs increase community-level floral density relative to uninvaded areas (A. Iler, personal observation). Furthermore, from an invasion perspective, *L. maackii* is likely to replace herbs as the invasion progresses (Hutchinson & Vankat 1997), a pattern with copious support from studies of other invasive species (e.g., Woods 1993; Tilman 1997; D'Antonio et al. 1998; Christian & Wilson 1999).

Arrays were set up on all sunny or partly cloudy days (days when pollinators were active), commencing when *G. maculatum* began to flower on 10 May and ending when *L. maackii* stopped flowering on 25 May. We removed the few remaining *G. maculatum* flower buds after this date; the few plants with remaining flowers were equally distributed across treatments and should therefore not affect our ability to detect differences in seed production across treatments. Out of a total of 16 days of co-flowering between our study species, experimental arrays were set up on 13 days, 10 of which included pollinator observations; the remaining three days were rainy and overcast. New *L. maackii* branches were cut each morning before pollinators became active (prior to 10:00am) and flowers removed as needed to create target floral ratios of 20:1 and 60:1. The average floral ratios were $20.4 \pm 0.7:1$ and $57.6 \pm 2.3:1$, respectively.

Data collection & analysis

To measure pollinator services to *G. maculatum*, we quantified pollinator visitation and pollen deposition. We conducted multiple 10 min pollinator observation sessions per plot, on all open *G. maculatum* flowers within arrays on a total of ten sunny to partly cloudy days when air temperature was above 15°C. Conditions of maximum pollinator activity are based on previous *L. maackii* observations in central Ohio (Goodell et al. 2010). Observations were conducted within the hours of 10:30am to 4:00pm, and all plots were observed in a random order on each observation date. Although we were unable to observe all 24 plots each time pollinator observations were conducted, we observed an equal number of randomly selected arrays from each treatment on each day. The number of open *G. maculatum* flowers, number of visitors and visits to all open *G. maculatum* flowers in the array (measures of visitation quantity), visitor identification, and visit duration per flower (a measure of visit quality, following Muñoz & Cavieres 2008) were recorded during each observation session. A visit was defined as contact with the stigma or anthers. We did not track visits to individual plants during observations. We followed individual bees foraging on *G. maculatum* until they left the plot; bees were not collected and therefore could have returned to the same plot in a later foraging bout. Bees were identified on the wing to genus

with the use of a reference collection, and all other visitors were identified to family. If too many visitors were present during a 10-min session to track both the number and duration of visits, we conducted a separate 10-min observation to record duration of visits. Because of time constraints, we have fewer observations of the duration of individual visits (duration data from a total of 106 10-min observation sessions vs. a total of 161 observation sessions for quantity of visits). Pollinator observations were not conducted on *L. maackii* branches because of time constraints, but pollinators were observed foraging on these flowers.

Ten post-receptive *G. maculatum* stigmas were collected haphazardly from each plot on six separate days throughout the flowering period and placed in microcentrifuge tubes containing 75% ethanol. Post-receptive stigmas are easily identifiable because they begin to close and dry out. 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, pollen tubes should have reached the ovary when stigmas were collected. Stigma removal did not appear to affect fruit set because several mature fruits were missing stigmas (A. Iler, personal observation). Pollen grains were stained with fuchsin dye and mounted on slides with glycerin gel (Kearns & Inouye 1993). We counted pollen grains adhered to stigmas and classified them as *G. maculatum*, *L. maackii*, or unknown pollen grains using a compound microscope. Our protocol may underestimate the amount of *L. maackii* pollen that arrives on *G. maculatum* stigmas because we placed stigmas in ethanol after collection, and any pollen that had not adhered to the stigma may have washed away. Nevertheless, we observed similar numbers of *L. maackii* pollen grains on *G. maculatum* stigmas in another study within the old field in which we mounted stigmas immediately after collection (McKinney 2010). We collected *G. maculatum* fruits from experimental arrays as they matured (1-3 weeks after all flowering ended) and counted the number of seeds.

We used one-way ANOVAs to compare mean responses across treatments (except for the number of unknown pollen grains, which was analyzed with a nonparametric ANOVA). When ANOVAs revealed significant differences among treatments, we used Tukey-Kramer HSD (honest significant difference) tests to compare means between each pair of treatments. The number of visitors to *G. maculatum* per plot, visitation rate (number of visits per *G. maculatum* flower), and duration of visits were each averaged across all 10-min observation sessions for each plot, the unit of replication ($N = 8$ plots). We obtained visit durations in all but one plot in the high-density treatment, so $N = 7$ for this treatment. The number of conspecific, *L. maackii*, and unknown pollen grains per flower were first averaged by plant then by plot. We calculated reproductive output as the number of seeds per flower instead of seeds per plant because the number of flowers per plant differed across treatments ($F_{2,21} = 6.13$, $P = 0.008$). Plants in the low density treatment had significantly fewer total flowers per plant than plants in the control ($P = 0.0058$), and neither the control nor the low density treatment differed from the high density

treatment ($P = 0.20$, $P = 0.22$, respectively; all P -values for comparisons between treatments are from Tukey's HSD tests). Therefore, seed production per plant could reflect this pattern of flower production rather than treatment effects. The mean number of seeds per flower for each plant was averaged by plot and was log transformed to meet normality assumptions. All analyses were performed in R v 2.15.3 (R Development Core Team 2013, <http://www.r-project.org/>).

Pollinator foraging choices across plots could reflect a response to relative density of flowers or absolute abundance of flowers, both of which varied across plots. We conducted a regression analysis to investigate evidence for effects of total plot-level *G. maculatum* floral abundance on pollinator foraging responses to *G. maculatum*, focusing on variation in total flower number across control arrays. We used simple linear regression to analyze the relationship between the number of *G. maculatum* flowers open in control arrays and pollinator responses: number of visitors (patch-level attraction), number of visits, and duration of visits. Daily means were calculated for each pollinator response from 10-min observation sessions ($N = 10$ days). Response variables were log transformed as necessary to account for unequal variances. One extreme outlier was removed from the analysis of the number of visits (Bonferroni $P = 0.0002$); removing the outlier made the trend weaker but does not affect any of our conclusions. If *G. maculatum* floral abundance determines pollinator choice among arrays, as opposed to relative floral density, we expect the number of visitors per plot to increase with increasing *G. maculatum* floral abundance.

RESULTS

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 each day. On average, the low density arrays contained 163.8 ± 19.0 *L. maackii* flowers, and the high density arrays contained 411.8 ± 48.1 *L. maackii* flowers.

Eight bee genera were recorded visiting *G. maculatum* flowers in over 28 hours of observation (approximately 9 hours per treatment). The most common visitors were native bees in the genera *Ceratina*, *Halictus*, and *Osmia*, and we observed only one honey bee visit (*Apis mellifera*) and no bumble bees (*Bombus* spp.) during our observations (see McKinney 2010 for a list of visitors). This result is consistent with previously published records of bee communities in old field habitat (Ginsberg 1983). The average number of individual insect visitors per 10 min observation session observed on *G. maculatum* flowers differed among treatments ($F_{2,21} = 4.48$, $P = 0.024$, Fig. 2a). The number of visitors to *G. maculatum* did not differ between the control and either invaded array (low density: $P = 0.52$, high density: $P = 0.18$), but *G. maculatum* in low density arrays had more visitors than in high density arrays ($P = 0.02$; Fig. 2A). Mean pollinator visitation rate to *G. maculatum* differed among treatments ($F_{2,21} = 6.58$, $P =$

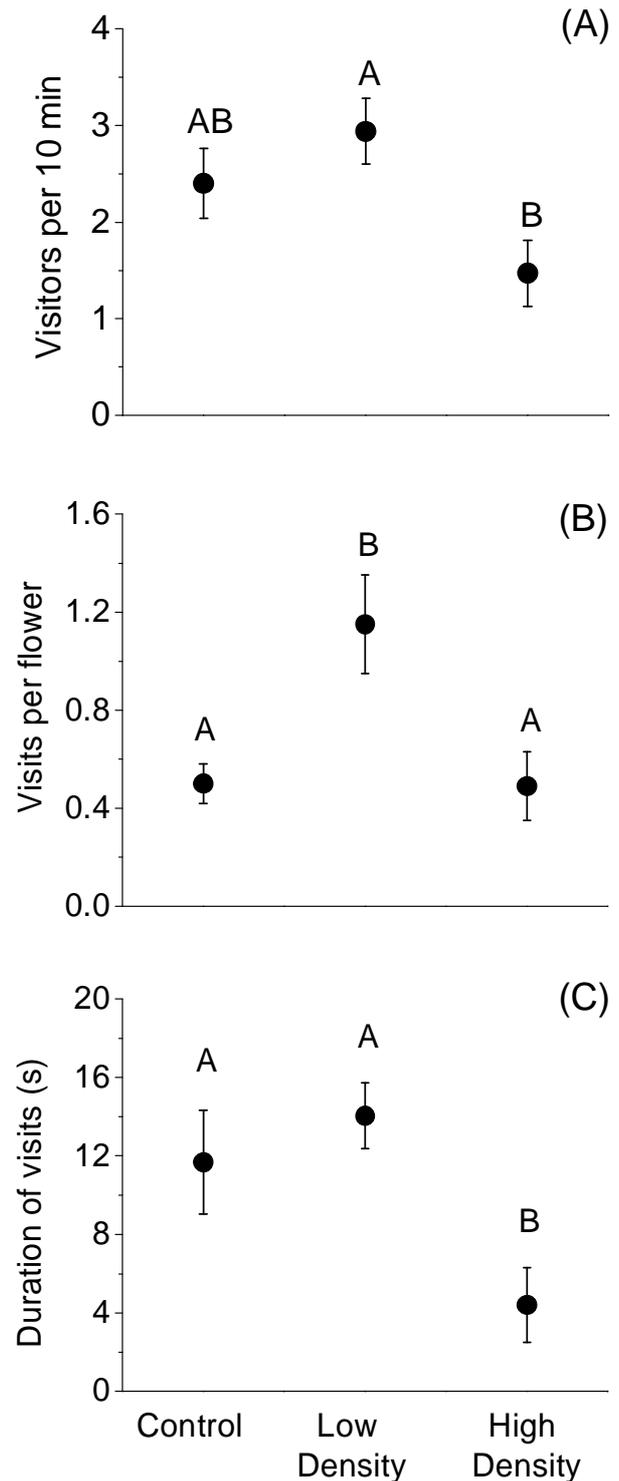


FIGURE 2. Pollinator foraging on *G. maculatum* flowers in three array treatments varying in relative floral density of the invasive shrub *L. maackii* (control: no *L. maackii*). (A) Number of visitors. (B & C) Pollinator foraging behaviour. Bars are mean values and error bars ± 1 SE. Capital letters represent significant differences at $P < 0.05$ from Tukey's multiple comparisons.

0.0061); flowers in low density arrays received more than twice as many pollinator visits as those in control and high density arrays ($P = 0.013$, $P = 0.013$; Fig 2B). In contrast, visitation rates in high density arrays did not differ from

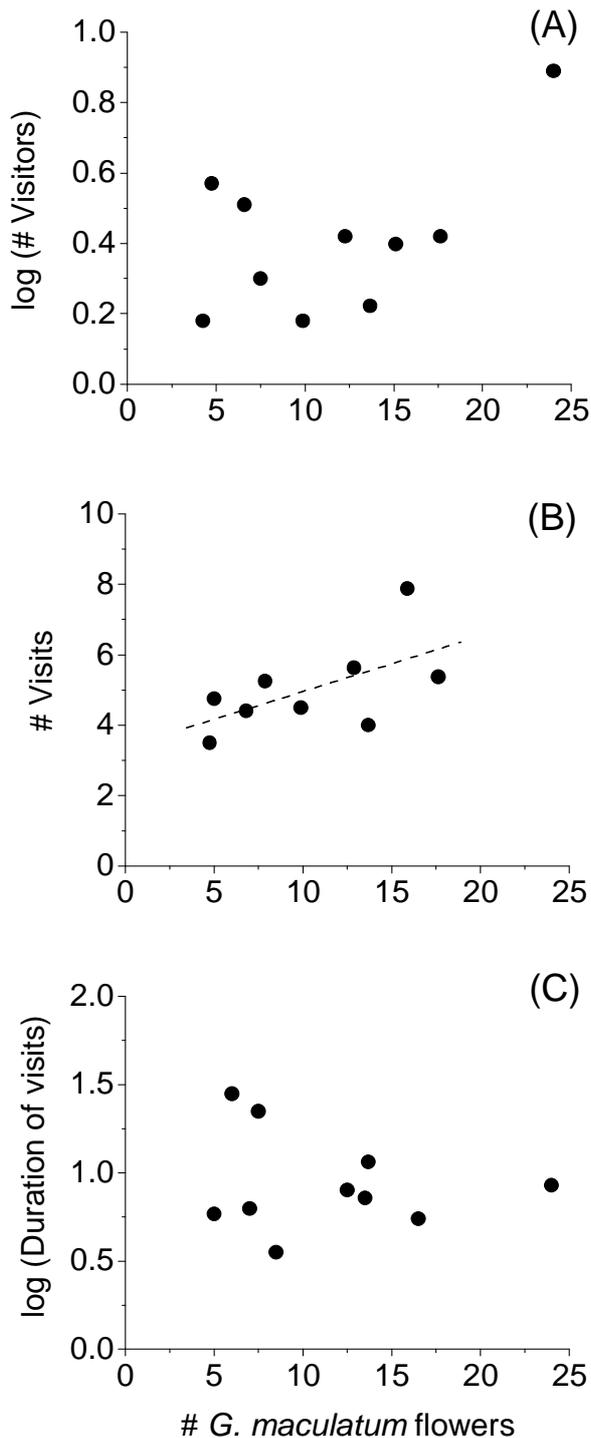


FIGURE 3. Relationship between pollinator foraging behaviour and total number of conspecific flowers in control arrays (only *G. maculatum* flowers). Pollinator foraging was quantified as (A) visitor attraction at the patch level (log transformed number of visitors), (B) pollinator visits, and (C) log transformed duration of visits (seconds). Each point represents mean daily values calculated for each 10-min observation session in control arrays (each plot was observed on each day, $N=10$ days). Dashed line is from simple linear regression and represents a marginally significant relationship ($0.05 < P < 0.10$).

control arrays ($P = 0.99$, Fig. 2B). The average duration of pollinator visits (log transformed) also differed among

treatments ($F_{2,20} = 10.86$, $P = 0.0006$). Visitors spent less than half as much time on *G. maculatum* flowers in high density compared to control and low density arrays ($P = 0.0047$; $P = 0.0007$, respectively; Fig. 2C), and there was no difference in the duration of visits in low density compared to control arrays ($P = 0.69$, Fig. 2C).

There was no effect of *G. maculatum* floral abundance in control arrays on the number of visitors attracted to these patches of flowers (log transformed), the number of visits observed to these flowers, or in the duration of visits (log transformed) ($R^2 = 0.29$, $F_{1,8} = 3.31$, $P = 0.11$, Figure 3A; $R^2 = 0.35$, $F_{1,7} = 3.76$, $P = 0.094$, Figure 3B; $R^2 = 0.03$, $F_{1,8} = 0.25$, $P = 0.63$, Figure 3C). These results do not support the hypothesis that *G. maculatum* flower abundance, at least across the range used in this experiment, determined attraction of visitors and visitor foraging behaviour and instead support the hypothesis that relative floral densities had an effect on pollinator foraging behaviour. Although there is a marginally significant trend of increased visits at higher *G. maculatum* floral abundances, this trend is in the opposite direction expected if *G. maculatum* floral abundance was accounting for increased visitation rates to low density arrays.

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 number of *G. maculatum* pollen grains differed significantly among treatments ($F_{2,21} = 5.38$, $P = 0.013$). Consistent with pollinator visitation data, conspecific pollen deposition was higher to flowers in low density arrays compared to control and high density arrays ($P = 0.048$, $P = 0.015$, respectively), and there was no difference in conspecific pollen deposition between high density arrays compared to controls ($P = 0.86$, Fig. 4A). The numbers of *L. maackii* (Lm) and log-transformed unknown (un) pollen grains on *G. maculatum* stigmas were relatively consistent across treatments ($F_{2,21} = 1.22$, $P = 0.31$, $F_{2,20} = 2.18$, $P = 0.14$, respectively; control: 4.84 ± 0.64 Lm grains, 2.96 ± 0.69 un grains; low: 3.52 ± 0.42 Lm grains, 2.75 ± 1.04 un grains; high: 3.93 ± 0.73 Lm grains, 1.27 ± 0.53 un grains). *Geranium maculatum* seeds per flower (log transformed) did not differ among treatments ($F_{2,21} = 0.056$, $P = 0.95$, Fig. 4B).

DISCUSSION

Our results highlight the importance of relative floral density within patches of floral resources in shaping outcomes of interactions for pollinators between co-flowering plant species. Although the total number of flowers in each array varied across days, relative floral density remained constant, and pollinator foraging responses were consistent with our hypotheses about the effects of relative floral densities. We detected increased visitation rates to *G. maculatum* flowers at a low relative density of *L. maackii* flowers compared to our other treatments. Higher densities of *L. maackii* flowers were associated with a lower quality of visits, evidenced by shorter visits to *G. maculatum* flowers in high density arrays relative to other treatments. Bjerknes et al. (2007) emphasize that variation in spatial and temporal

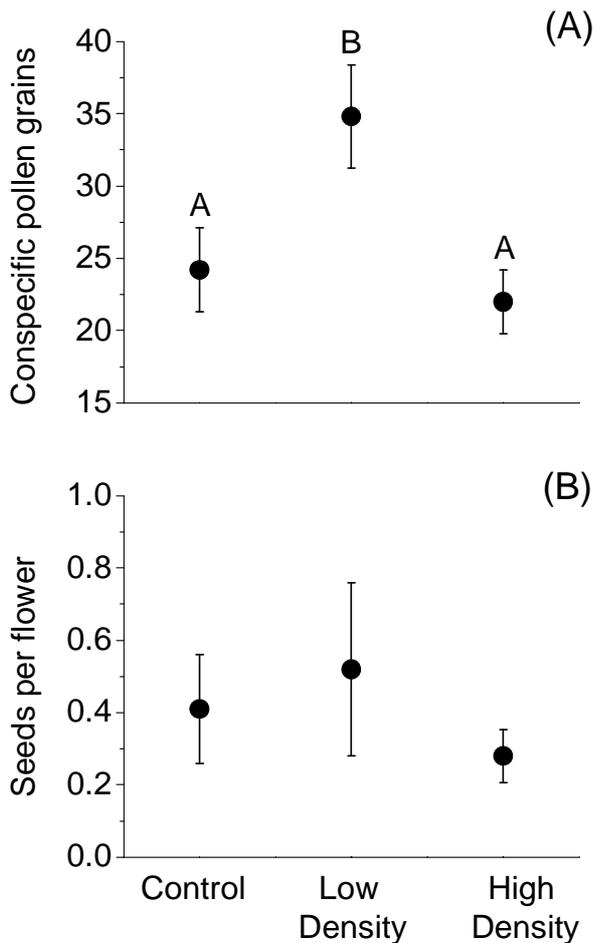


FIGURE 4. *Geranium maculatum* female reproductive success, averaged per plant then by plot, in three arrays varying in relative floral density of *L. maackii* (control: no *L. maackii*). (A) Conspecific pollen deposition to *G. maculatum* flowers (B) Mean values of seeds per flower are shown, although they were log-transformed for analysis. Error bars \pm 1 SE. Capital letters represent significant differences at $P < 0.05$ from Tukey's multiple comparisons.

scales may explain variation among outcomes of studies of pollinator-mediated interactions between invasive and native plant species. Theoretical work and field studies have also confirmed the role of spatial scale in mediating interactions for pollinators between co-flowering plant species (Cariveau & Norton 2009; Hanoteaux et al. 2013). Our study supports the idea that relative floral density within patches of floral resources is also likely to play an important role in mediating these outcomes, perhaps as an explanation for why interactions vary through time, both within seasons as an invasive plant species comes into bloom and across years as an invader increases in density. Our results also suggest that facilitation of pollination is possible in incipient invasions, but that this effect may be neutralized as relative floral density of the invader increases.

The particular floral density at which facilitation or competition for pollination occurs is likely to vary considerably among plant species. We detected density-dependent pollinator foraging behaviour at relative floral densities of 20:1 (low invader) and 60:1 (high invader),

compared to 1:1 and 5:1 in another study (Muñoz & Cavieres 2008). While strong interaction effects for pollination are expected for species of similar growth form and floral morphology (Morales & Traveset 2009), we find that interaction effects, in this case facilitation, can also be quite strong for pairs of species with non-similar morphologies (Ghazoul 2006). In addition to floral morphology, floral reward should also influence the relative floral densities at which facilitation and competition for pollinators occur. Native species with less rewarding nectar than co-flowering invasive species may experience competition at lower floral densities of the invader compared to native plants with more rewarding nectar or a higher rate of nectar production (Chittka & Schürkens 2001). Indeed, *G. maculatum* nectar contains on average 40% more total sugar than *L. maackii* nectar, but differences in nectar volume are unknown (McKinney & Goodell 2010). The higher nectar sugar content in the native species in our study may explain why we detected facilitation of pollination at a relatively high floral density of the invader compared to the findings of Muñoz & Cavieres (2008). Our results suggest that even higher *L. maackii* densities are necessary to observe negative effects on *G. maculatum* pollination.

Increased *G. maculatum* pollen receipt at a low density of the invader seems to be a result of a shift in the behaviour of individual pollinators relative to uninvaded patches. *Geranium maculatum* flowers received more than twice as many visits in low density arrays compared to controls, but not significantly more visitors, indicating that visitation rate increased because individual pollinators made more visits to *G. maculatum*. This is in contrast to the more common trend of invaders acting as a 'magnet species' by attracting more pollinators to mixed patches of floral resources, whose visitors then spill over onto nearby plants (Fig. 1b; Moragues & Traveset 2005; Muñoz & Cavieres 2008; Molina-Montenegro et al. 2010; Flanagan et al. 2010; McKinney & Goodell 2011). Additionally, the non-significant trend towards more visitors in low density arrays relative to controls cannot account for a doubling of visits in low density arrays. These results suggest that intraspecific competition for pollinators between native plants may decrease in the presence of a low floral density of the invader when the number of visitors remains constant. This behavioural switch in the presence of a low density of invasive flowers could reflect perceived reward per unit of foraging effort within the patch of floral resources. Bees that encounter *G. maculatum* flowers after visiting less rewarding *L. maackii* flowers may have a higher 'departure' threshold for leaving the patch, compared to bees that only have the option of intraspecific floral transitions in control arrays. Bees that opt to continue foraging on *G. maculatum* at low densities of *L. maackii* may not do so at high densities merely because of the surplus of *L. maackii* flowers relative to *G. maculatum* in high density arrays. Our results are consistent with this hypothesis because visitors spent less time on *G. maculatum* flowers in high density arrays compared to controls and low density arrays.

Behavioural shifts in pollinator foraging to *G. maculatum* did not translate into differences in *G. maculatum* reproductive success (seeds per flower) among

treatments. This result may reflect numerous issues: pollen quality, variation in maternal resources across treatments, and/or low statistical power. The aim of our experimental design was to represent different *L. maackii* invasion scenarios. However, placing fewer *G. maculatum* plants (i.e. fewer nearby pollen donors) in low and high density arrays compared to controls could have influenced reproduction via pollen quality because *G. maculatum* reproduction shows evidence of inbreeding depression (Ågren & Willson 1991); receipt of self pollen results in a lower probability of seed set in this species (Chang 2007). For our design to affect *G. maculatum* reproduction in this way, bees have to make more intra-plant movements and transfer more self pollen (and insufficient outcross pollen) in mixed arrays compared to controls, a pattern that has empirical support (Yang et al. 2011). We did not distinguish between inter- and intra-plant pollinator movements in our observations and therefore cannot conclusively evaluate of the role of pollen quality vs. pollen quantity. Because all of our plants were hermaphrodites, however, they may be more prone to inbreeding depression than female plants that require outcross pollen to set seed. At the same time, we expect female plants to respond more strongly to changes in pollination because they require outcross pollen; in this sense our experiment is a conservative test for effects of plant invasion on pollination and reproductive success of *G. maculatum*. Heterospecific pollen transfer (HPT) also may have countered positive effects of increased pollinator visitation in low density arrays (sensu Brown & Mitchell 2001), but in our study, HPT was consistently low across arrays (consistent with Bartomeus et al. 2008). We may detect little HPT because pollinators do not frequently switch between species (Chittka et al. 1999; Feldman 2008) or because dissimilar floral morphologies lead to differential pollen placement on pollinator bodies (Harder & Barrett 1993). Finally, if flower production is an indicator of maternal resources, it is possible that *G. maculatum* plants in low density arrays were unable to take full advantage of increased conspecific pollen deposition, because plants in low density arrays had significantly fewer flowers than plants in control arrays. Indeed, conspecific pollen deposition was above that required for maximum seed production in *G. maculatum* (Mulcahy et al. 1983), suggesting resource rather than pollen limitation.

The net effect of invasive plants on pollination of neighboring plants will ultimately depend on how pollinator populations and communities respond to invasion, in addition to pollinator attraction to native plants at local spatial scales (Tepedino et al. 2008; Goodell 2008; Mitchell et al. 2009; Moron et al. 2009; Bartomeus et al. 2010; Yang et al. 2011). Here we show that relative floral density within patches of floral resources may account for some of the variation in the direction of pollinator-mediated interactions between invasive and native plants at local spatial scales (i.e. competition, neutral effects, and facilitation) (Traveset & Richardson 2006; 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. In sites severely degraded from initial or target conditions, like our study site, invasive plants may be important for maintaining or developing plant-pollinator communities (Ewel & Putz 2004). Invaders may serve as facilitators of pollination in early stages of invasion and in some restoration scenarios, at least when HPT does not negatively affect native plant reproduction and when invaders act as magnet species. In this study, we provide novel evidence for facilitation as a result of a behavioural shift in pollinator foraging, independent of a magnet effect. Although this may be another mechanism by which invasive plants can facilitate pollination of native plants, facilitation is still the exception to the more common trend of competition for pollination (Larson 2006; Morales & Traveset 2009).

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