

Plant–pollinator interactions between an invasive and native plant vary between sites with different flowering phenology

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Abstract Floral displays of invasive plants have positive and negative impacts on native plant pollination. Invasive plants may also decrease irradiance, which can lead to reduced pollination of native plants. The effects of shade and flowers of invasive plant species on native plant pollination will depend on overlap in flowering phenologies. We examined the effect of the invasive shrub *Lonicera maackii* on female reproductive success of the native herb *Hydrophyllum macrophyllum* at two sites: one with asynchronous flowering phenologies (slight overlap) and one with synchronous (complete overlap). At each site, we measured light availability, pollinator visitation, pollen deposition, and seed set of potted *H. macrophyllum* in the presence and absence of

L. maackii. At both sites, understory light levels were lower in plots containing *L. maackii*. At the asynchronous site, *H. macrophyllum* received fewer pollinator visits in the presence of *L. maackii*, suggesting shade from *L. maackii* reduced visitation to *H. macrophyllum*. Despite reduced visitation, *H. macrophyllum* seed set did not differ between treatments. At the synchronous site, *H. macrophyllum* received more pollinator visits and produced more seeds per flower in the presence of co-flowering *L. maackii* compared to plots in which *L. maackii* was absent, and conspecific pollen deposition was positively associated with seed set. Our results support the hypothesis that co-flowering *L. maackii* shrubs facilitated pollination of *H. macrophyllum*, thereby mitigating the negative impacts of shade, leading to increased seed production. Phenological overlap appears to influence pollinator-mediated interactions between invasive and native plants and may alter the direction of impact of *L. maackii* on native plant pollination.

Keywords Biological invasion · Competition · Facilitation · *Lonicera maackii* · Phenological overlap

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Introduction

Co-flowering plant species often interact via shared pollinators because most plant–pollinator interactions

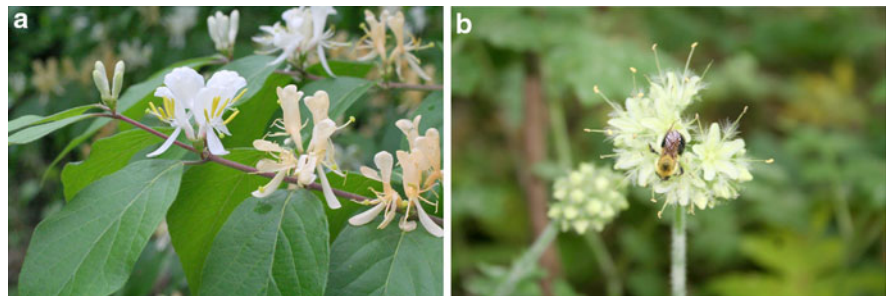
are generalized; that is, several pollinator species visit many different plant species within a community (Vazquez et al. 2009; Waser et al. 1996). Perhaps because of this broad trend toward generalization, non-native plants appear to be well-integrated into native plant-pollinator communities (Memmott and Waser 2002). Novel interactions between non-native plants and resident pollinators sometimes result in reduced pollinator visitation to native plants and losses in seed production, although in some cases they lead to facilitation of pollinator visitation to native plants (Bartomeus et al. 2008; Bjerknes et al. 2007; Chittka and Schürkens 2001). Changes in pollinator visitation to native plants can affect both the amount and quality of pollen delivered to native plant stigmas (Brown et al. 2002; Larson et al. 2006; Lopezaraiza-Mikel et al. 2007; Moragues and Traveset 2005; Totland et al. 2006). In addition to directly affecting pollinator foraging behavior, plant invasions may also affect pollinator population dynamics and community composition, which in turn could indirectly impact pollinator foraging to native plants (Carvalho et al. 2008; Moron et al. 2009). Over an evolutionary time scale, these new interactions between invasive and native plants for pollinators may select for divergence (competition) or convergence (facilitation) of floral traits such as flowering phenology, floral morphology, and timing of reward production (among native plants: Campbell et al. 1996; Pleasants 1980; Raine et al. 2007; Stone et al. 1998; Thomson 1980; Waser 1978). The direction of evolutionary change will depend on the consistency of these interactions across space and time, yet few studies of invasive-native plant interactions for pollinators include multiple sites (see Grabas and Lavery 1999).

Although most studies of pollinator-mediated interactions between plant species focus on species that co-flower and share pollinators, indirect interactions between plant species for pollinators are not limited to co-flowering plants. 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 factors, such as temperature and light (Heinrich 1979; Herrera 1995, 1997; Thomson and Plowright 1980; Thomson 1981; Totland 1994), which can change following invasions. For example, the invasive shrub *Lonicera maackii* is associated with increased forest

understory shade and can reduce reproductive success of understory herbs (Gould and Gorchov 2000; McKinney and Goodell 2010). The shadier understory environment in patches invaded by *L. maackii* appears to alter pollinator foraging habitat, with negative consequences for pollinator visitation and pollen deposition independent of the presence of invasive flowers (McKinney and Goodell 2010). Therefore, impacts of invasive plants on pollination of native plants conceivably extend beyond the period of flower overlap to plant species that do not co-flower or even share pollinators. Negative effects of shading by invaders could be widespread because shrubs compose 23% of invasive plant species globally (Martin et al. 2009). Furthermore, variation in flowering phenology across populations could mean that interactions affecting pollination between specific pairs of invasive and native plants vary geographically. If invasive shrubs co-flower with understory native plants, the shrub's flowers may compete for pollinators and exacerbate the negative effects of shade on pollinator foraging. Conversely, the shrub's flowers may facilitate pollination of native plants, potentially mitigating the negative impacts of shade on pollinator foraging to native herbs.

This study investigated the role of phenological overlap between an invasive shrub and a native herb in mediating plant-pollinator interactions. We compared the pollination and reproduction of the native understory herb *Hydrophyllum macrophyllum* in the presence and absence of the invasive non-native shrub *L. maackii* at two sites: one with synchronous flowering phenologies (*H. macrophyllum* flowering during *L. maackii* flowering) and one with asynchronous flowering phenologies (*H. macrophyllum* flowering beginning at the end of *L. maackii* flowering). At each site, we determined light availability, pollinator visitation, pollen deposition, and seed set of potted *H. macrophyllum* plants in deciduous forest plots in which *L. maackii* was either present or absent (see Fig. 1 for pictures of study species). Because the pots excluded competition with *L. maackii* for soil resources, we effectively isolated pollinator- and light-mediated effects on *H. macrophyllum* reproductive success at the post-flowering stage. Our objectives were (1) to determine the underlying mechanism of the impact of *L. maackii* on *H. macrophyllum* pollination and female reproductive success at sites

Fig. 1 Study species: flowers of the invasive shrub *Lonicera maackii* (Amur honeysuckle) (a) and flowers of the native herb *Hydrophyllum macrophyllum* (large-leaf waterleaf), with *Bombus impatiens* (eastern bumble bee) foraging for nectar (b)



with different phenological overlaps and (2) to determine variation across sites in local-scale effects of *L. maackii* on *H. macrophyllum* pollination and reproduction.

Methods

Study species

Lonicera maackii (Rupr.) Herder (Amur honeysuckle), a native of China, was introduced to North America in the late nineteenth 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* fully develops leaves by mid-April, prior to full leaf-out of native shrubs and trees (Trisel and Gorchov 1994). Individual shrubs produce thousands of white to pale pink flowers from May through early June in central Ohio, and flowers offer pollen and nectar rewards (see McKinney and Goodell 2010 for nectar sugar content). *L. maackii* negatively affects the growth, fecundity, and survival of native understory herbs (Gould and Gorchov 2000; Miller and Gorchov 2004). Pollinators influence reproductive output of *L. maackii*, and non-native honeybees (*Apis mellifera*) and native bees in the genera *Andrena*, *Augochlorella*, *Bombus*, *Ceratina*, *Halictus*, *Lasioglossum*, and *Nomada* forage on *L. maackii* flowers in the central Ohio area (Goodell and Iler 2007). *H. macrophyllum*, a native biennial woodland herb in eastern North America, produces one or more inflorescences of 5–40 white hermaphroditic flowers that bloom sequentially from mid-May to mid-June and produce nectar for 72–84 h (Baskin and Baskin 1983; Beckmann 1979). *H. macrophyllum* is self-compatible but requires pollinators to set seed (Beckmann 1979). Flowers are visited by a variety of

generalist bee species, including introduced *A. mellifera* and native bees in the genera *Bombus* and *Osmia* (Beckmann 1979). *H. macrophyllum* is also visited by several species of native, relatively small solitary bees in the genera *Augochlorella*, *Ceratina*, *Halictus*, and *Lasioglossum*, but they rarely contact the sexual parts of the flower (K. Goodell, personal observation). Within the central Ohio region, *H. macrophyllum* grows within areas invaded by *L. maackii*, and large populations occur <1 m from *L. maackii* shrubs (K. Goodell, unpublished data).

Study site

We conducted this study during spring 2007 in a mesic deciduous forest at two locations: Denison University Bioreserve, Granville, OH, USA, and Three Creeks Metro Park, Groveport, OH, USA. The Denison University Bioreserve site was located in a patch of ca. 50-year-old secondary hardwood forest containing a patchy *L. maackii* invasion (see Goodell and Iler 2007 for vegetation description). The elevation of this site is ca. 323 m. We 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, which allowed us to control the effects of conspecific floral density on pollinator foraging behavior. At this site, *L. maackii* flowered throughout the entire *H. macrophyllum* flowering period (Fig. 2), 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 (see McKinney and Goodell 2010 for vegetation description). The elevation of this site is ca. 226 m,

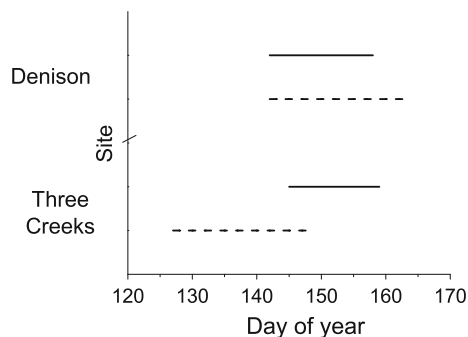


Fig. 2 Flowering phenophase (duration of flowering) for the invasive shrub *Lonicera maackii* and the native herb *Hydrophyllum macrophyllum*, at two sites in central Ohio: *top pair of lines* corresponds to Denison and *bottom set of lines* to Three Creeks. Day of year is shown on x-axis for the year 2007, with day 121 = May 1 and day 152 = June 1. *Solid lines* represent *H. macrophyllum* flowering phenophase, and *dotted lines* represent *L. maackii* flowering phenophase

and it is approximately 29.5 km west and 21.5 km south of the Denison site. We set up experimental plots within the invaded forest where understory plants were present. Because *L. maackii* flowered earlier at this site, 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).

Experimental design

At Denison, we randomly selected 24 circular plots of 10 m radius, 12 without *L. maackii* (absent) and 12 with *L. maackii* (present). We followed the same procedure at Three Creeks, but because the forest was more continuously invaded, we randomly assigned plots to either *L. maackii* removal (absent) or *L. maackii* present ($N = 12$ plots per treatment). In removal plots, we cut all *L. maackii* shrubs down to a small stump (ca. 15 cm high). At both sites, plots were separated by at least 5 m. Our aim with this design was to create plots of sufficient size and separation to detect a response in pollinator foraging behavior on *H. macrophyllum*. Because the bees we observed at this site can forage over distances greater than 15 m (Eickwort and Ginsberg 1980; Gathmann and Tscharrntke 2002), individual bees could choose among treatment plots within a foraging bout. Experimental *H. macrophyllum* plants were dug from two natural populations at the Denison Bioreserve prior to

flowering, transferred to 2.8 l pots containing a mixture of soil from Denison and ProMix potting soil, and housed in a screen house at The Ohio State University-Newark. When *H. macrophyllum* plants started to produce flower buds, we moved them to the field (approximately May 11 at Denison, May 14 at Three Creeks). The potted *H. macrophyllum* plants were placed at the center of each plot; in invaded plots, placement was always under or within 10 cm of the *L. maackii* canopy. Potted *H. macrophyllum* flowering phenology tracked that of natural populations at Denison and other central Ohio populations, with first and last dates of flowering varying by ca. 2 days across populations (K. Goodell, personal observation).

The center of each plot contained either one (Denison) or two (Three Creeks) potted and naturally pollinated *H. macrophyllum* plants, separated by 0.25–0.5 m. Several of the plants we collected did not produce flowers, which resulted in fewer flowering *H. macrophyllum* plants per plot at Denison compared to Three Creeks. However, by the end of the experiment, 15 of 24 plots at Three Creeks contained only one open-pollinated *H. macrophyllum* plant because of digging in pots, presumably by the eastern chipmunk, *Tamias striatus*. At both sites, each plot also contained one potted hand-pollinated plant to which we added supplemental outcross pollen; comparisons between open and hand-pollinated plants are addressed elsewhere (McKinney 2010). The potted plants were watered as needed at both sites and 4 m tall wire fences were erected around *H. macrophyllum* to prevent deer browsing at Three Creeks; prior observations revealed little deer browsing on naturally occurring *H. macrophyllum* at Denison, so we did not erect fences at Denison. We removed potted *H. macrophyllum* plants from each site after all flowers had senesced (midway through fruit maturation) to a common shaded courtyard at The Ohio State University-Newark. The plants were watered regularly until the fruits had completely matured. We recorded total number of open flowers on each *H. macrophyllum* plant as the season progressed, and we collected mature fruits to count seeds.

At both sites, we used a digital light meter to measure light availability (model EA30, Extech Instruments, Massachusetts, USA). We placed the sensor at the center of each plot at the height of *H. macrophyllum* flowers. Light measurements were taken on a single overcast day in each site but different

days across sites. To reduce variation between treatments and sites caused by different sunlight intensity, we chose to measure light on overcast days, which means this measure is a conservative test of light differences between treatments. We also recorded the number of pollinator visits to *H. macrophyllum* flowers in all plots during 10 min intervals between 10:00 a.m. and 4:30 p.m. on sunny or partly cloudy days, when air temperature was above 15.5°C. This timeframe of pollinator activity is based on previous observations on *L. maackii* in central Ohio forest (Goodell et al. 2010, McKinney, personal observation). A visit is defined as an insect landing on a flower and seeking pollen or nectar reward, which often brought it in contact with the anthers, stigma, or both. Visitation rate was calculated as the average number of visits per plant per 10 min observation session, summarized by plot, the sample unit. Visitors were identified 'on the wing' to lowest taxonomic level possible, usually genus, with the use of a reference collection. 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 light microscope.

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 (synchronous and asynchronous) was un-replicated. In addition, experimental design differed slightly: natural absence of *L. maackii* at Denison compared to removal of *L. maackii* at Three Creeks. We used t tests or non-parametric Mann–Whitney z tests, when response variables were non-normally distributed, to test for differences between *L. maackii* treatments in light, pollinator visitation rate, pollen deposition, and seeds per flower. Light measurements (lux) from Three Creeks were natural log transformed to satisfy normality assumptions.

To assess the degree to which pollen receipt affected seed production, we used linear regression analysis (standard least squares) to investigate the relationship between average number of seeds per

flower per plot (continuous response variable) and average pollen deposition per plant per plot (continuous explanatory variable), because plot was our sampling unit. We combined data across *L. maackii* treatments at each site to obtain sufficient sample sizes. If responses in seed set were influenced by pollinators, we expected conspecific pollen deposition to be positively related to *H. macrophyllum* seed set. In contrast, if *H. macrophyllum* seed set responded more to the abiotic environment, we expected conspecific pollen deposition to be unrelated to seed set. All P values are two-tailed, and in text we report mean \pm 1 SE.

Results

Pollination

Understory light levels were lower in the presence of *L. maackii* at both sites (Denison: $t = 5.42$, $P < 0.0001$; Three Creeks: $t = 4.84$, $P < 0.0001$, Fig. 3). A total of 121 pollinators were observed in 16 h of observations at Denison and 42 pollinators in 8 h of pollinator observations at Three Creeks. The pollinator communities were similar between the two sites (Table 1). At Denison, where *H. macrophyllum*

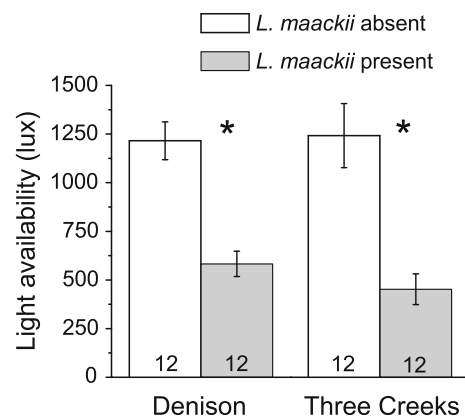


Fig. 3 Forest understory light availability compared between plots that contained the invasive shrub, *Lonicera maackii*, and plots that did not at two sites differing in flowering phenology of the invasive shrub *L. maackii*. Sites were analyzed separately and responses compared using t tests: *Significantly different at $P < 0.05$. Sample sizes shown at bottom of bars represent number of plots. Untransformed light levels are shown for Three Creeks

Table 1 Percentage of total visits to the native herb *Hydrophyllum macrophyllum* in the presence and absence of the invasive shrub *Lonicera maackii* (Lm) shown for bee and other taxa identified on the wing

	Denison % of total visits		Three Creeks % total visits	
	Lm present	Lm absent	Lm present	Lm absent
<i>Augochlorella/Augochlora</i> spp.	7.5	13.0	0	21.7
<i>Bombus</i> spp.	10.8	0	0	9.8
<i>Ceratina dupla dupla/calcarata</i> .	23.7	0	80.0	55.4
<i>Halictus ligatus</i>	0	0	0	6.5
<i>Lasioglossum</i> spp.	9.7	43.5	15.0	4.3
Diptera	48.4	43.5	5.0	0
Lepidoptera	0	0	0	2.2

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, $N = 11$). At Denison, there were 93 and 46 visits in the presence and absence of *L. maackii*, respectively, compared to 20 and 92 visits at Three Creeks, respectively

and *L. maackii* flowering phenologies were synchronous, a total of six pollinator taxa were recorded visiting *H. macrophyllum* in the presence of flowering *L. maackii*, compared to only three total pollinator taxa in the absence of *L. maackii* (Table 1). Here, there were more pollinator taxa on average visiting *H. macrophyllum* in the presence of co-flowering *L. maackii* (absent: 1.83 ± 0.21 pollinator taxa; present: 2.83 ± 0.21 pollinator taxa; $z = 2.79$, $P = 0.0053$, $N = 12$). In contrast, at Three Creeks, where *H. macrophyllum* and *L. maackii* flowering phenology only slightly overlapped, we recorded a total of six pollinator taxa visiting *H. macrophyllum* in the absence of *L. maackii* compared to three total pollinator taxa in the presence of *L. maackii* (Table 1). There were fewer pollinator taxa on average visiting *H. macrophyllum* in 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, average pollinator visitation rate to *H. macrophyllum* plants was significantly higher in the presence of co-flowering *L. maackii* ($t = 4.50$; $P = 0.0002$; Fig 4a), 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; *H. macrophyllum* plants received fewer visits in the presence of *L. maackii* ($z = 3.05$, $P = 0.0023$; Fig 4a). Again,

these differences in visitation rate did not result in a significant 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 we found only one unknown heterospecific pollen grain on *H. macrophyllum* stigmas. At Three Creeks, we 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. Therefore, there seems to be insufficient heterospecific pollen transfer to affect *H. macrophyllum* reproduction at either site.

Reproductive output

At Denison, *H. macrophyllum* plants produced more seeds per flower in the presence of co-flowering *L. maackii* ($t = 2.38$, $P = 0.029$; Fig 4b). At Three Creeks, *H. macrophyllum* seeds per flower did not differ between *L. maackii* treatments ($t = 0.24$, $P = 0.82$; Fig 4b). At Denison, the average number of conspecific pollen grains accounted for a significant amount of variation in seeds per flower ($F_{1,19} = 6.56$, $P < 0.05$; Fig. 5a), while at Three Creeks, conspecific pollen deposition was unrelated to seed set ($F_{1,18} = 1.01$, $P < 0.05$; Fig. 5b).

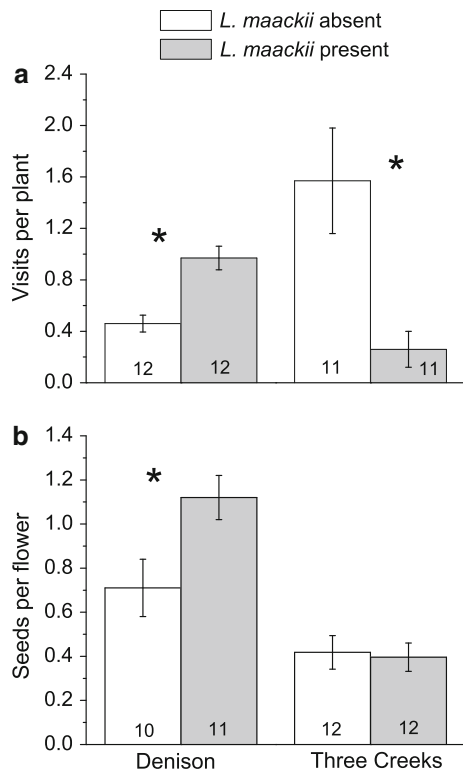


Fig. 4 Pollinator visitation and seed set of native *Hydrophyllum macrophyllum* compared between forest plots that contained the invasive shrub *Lonicera maackii* and plots that did not at two sites differing in flowering phenology of *L. maackii*. Flowering phenologies were synchronous at Denison and asynchronous at Three Creeks. In **a**, pollinator visitation to *H. macrophyllum* was averaged per plant for 10 min observation sessions and then averaged by plot, our sample unit. In **b**, seeds per flower were analyzed by plant and then by plot. Sites were analyzed separately and responses compared using *t* tests or *z* tests (see text): *Significantly different at $P < 0.05$. Sample sizes shown at bottom of bars represent number of plots

Discussion

Three Creeks: asynchronous flowering phenology

Our investigation of the indirect interaction between invasive *L. maackii* and native *H. macrophyllum* at Three Creeks, where their flowering phenologies were asynchronous, detected negative impacts of the invader on pollinator visitation to the native. Shade from the *L. maackii* canopy seems to decrease pollinator foraging to *H. macrophyllum*, consistent with other work showing that Hymenopteran pollinators prefer to forage in areas with high light

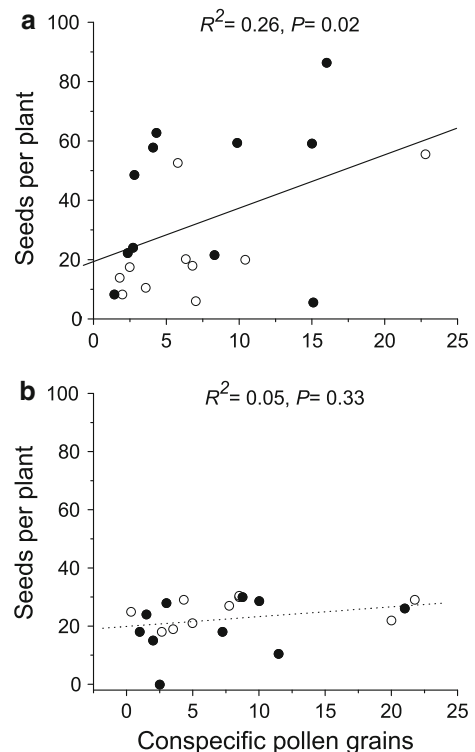


Fig. 5 Linear regression of average conspecific pollen deposition (per flower per plant per plot) with average *Hydrophyllum macrophyllum* seeds per flower (per plant per plot) at two sites differing in flowering phenology of the invasive shrub *Lonicera maackii*. Flowering phenologies were synchronous at Denison (**a**) and asynchronous at Three Creeks (**b**). Open circles represent plots in which *L. maackii* was absent, and filled circles represent plots in which *L. maackii* was present. A solid regression line corresponds to a significant regression and dotted line to a non-significant regression

availability (Herrera 1995). Lower visitation in the presence of post-flowering *L. maackii* corroborates our previous work showing that foliage of invasive shrubs negatively affects pollinator foraging to native plants (McKinney and Goodell 2010). Because the impacts of invasive plants on pollinator foraging to neighboring plants can occur through non-floral pathways, invasive plants have more potentially negative impacts than previously considered. Despite lower pollinator visitation in the presence of *L. maackii*, there was no effect on *H. macrophyllum* seed set, probably because seed set was not pollinator-limited. In contrast, early spring herbs often exhibit pollen-limited reproduction and produce their fruits and seeds prior to canopy closure (Kudo et al. 2008). Spring herbs may therefore be especially prone to

negative impacts of early season shade on pollinator visitation because *L. maackii* leaves fully develop weeks before leaf-out of native woody species (Shustack et al. 2009).

Denison: synchronous flowering phenology

Flowering *L. maackii* shrubs appear to have facilitated pollinator visitation to *H. macrophyllum*, leading to a 63% increase in seed set compared to plots in which *L. maackii* was absent. The positive relationship between *H. macrophyllum* pollen deposition and seed set corroborates this link between pollination and plant reproduction. Facilitation of pollinator visitation to *H. macrophyllum* occurred despite shade created by co-flowering *L. maackii* shrubs, which is associated with decreased pollinator visitation to native *Geranium maculatum* (McKinney and Goodell 2010). Instead, the relatively high density of *L. maackii* flowers may have attracted pollinators to the plots, and spillover of visitors from *L. maackii* flowers may have increased visitation to *H. macrophyllum* (sensu Feinsinger 1987; Ghazoul 2006; Rathcke 1983). Plant density and diversity affect both the composition and abundance of pollinators on plants (Conner and Neumeier 1995; Hegland and Totland 2005; Lazaro et al. 2009; Moeller 2005), consistent with the idea that *L. maackii* affected the composition and abundance of pollinators on *H. macrophyllum*. Data from our pollinator observations suggest that *L. maackii* attracted to the plots a variety of pollinator species that also visited *H. macrophyllum*; we found more pollinator taxa on *H. macrophyllum* in plots in which *L. maackii* was present. Specifically, *Bombus* spp. and *Ceratina* spp. were observed on *H. macrophyllum* only where *L. maackii* flowers were present, which is significant because *Bombus* spp. are important pollinators of *Hydrophyllum* (Beckmann 1979). Consistent with this study, 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. Under some circumstances, native plants receive higher visitation near invasive plants compared to distances farther away from the invader (McKinney 2010; Nielsen et al. 2008).

Either increased pollen quantity or pollen quality may have lead to facilitation of *H. macrophyllum*

seed set in the presence of flowering *L. maackii*. Ultimately, pollen quality may increase in tandem with increases in pollen quantity, a distinction we are unable to make with our data (Aizen and Harder 2007). However, pollen quality seems unlikely to have a large impact on seed set of a self-compatible plant species such as *H. macrophyllum*. Instead, the significant correlation between average *H. macrophyllum* pollen deposition and seed set suggests pollen quantity as the mechanism behind increased seed production. This significant correlation is a relatively conservative measure of the importance of pollen receipt for reproduction because of our sampling procedure; pollen deposition data come from a subsample of all the stigmas on individual plants, whereas seed set represents the entire plant. The magnitude of pollen limitation of *H. macrophyllum* seed set is significantly smaller in the presence of flowering *L. maackii*, providing further support for the link between pollination and seed set (McKinney 2010). It should be noted, however, that despite increased pollinator visitation in the presence of *L. maackii*, we did not detect a significant difference in pollen deposition to *H. macrophyllum* between treatments, perhaps because of the variation surrounding pollen deposition. Other studies of invasive-native plant interactions for pollinators also report inconsistencies between visitation and pollen deposition, but it is unclear why these inconsistencies arise (Cariveau and Norton 2009; Larson et al. 2006). Finally, we measured pollinator visitation on a per plant basis, but the number of flowers per plant could influence pollinator visitation. We only have data for pollinator visits on a per flower basis at Three Creeks, where we find the same results as when we use average visits per plant (McKinney, unpublished data).

Indirect interactions between invasive and native species are likely common but are generally poorly understood, and pollination constitutes only one facet of the potential direct and indirect (i.e., those mediated by a third species) interactions between co-occurring plant species (Callaway and Walker 1997; Hunter and Aarssen 1988; White et al. 2006). For example, *L. maackii* provides refuge for small rodent seed consumers and is associated with increased seed predation of some native plant species (Mattos and Orrock 2010; Meiners 2007). Below-ground competition or increased seed predation have the potential to cancel out the benefits of increased

seed production in a natural *H. macrophyllum* population. However, the main impacts of *L. maackii* on native plants seem to take place above ground (Gorchov and Trisel 2003, Gould and Gorchov 2000, Miller and Gorchov 2004). The importance of any ecological interaction that impacts seed production will depend on the relative importance of seed set to population growth. Studies that investigate indirect interactions between invasive and native plants eventually need to consider the consequences of multiple interactions within natural contexts to understand fully the indirect impacts of invaders on native plant populations (sensu Agrawal et al. 2007).

Variation in phenology and impacts of *L. maackii*

Although our study lacks the replication necessary for a rigorous test of the effects of phenological variation on the impacts of *L. maackii* on native plant pollination, comparing results across sites reveals some important insights into this particular system. In two comparable plant and pollinator communities with similar understory light conditions, *L. maackii* exerted both positive (via flowers) and negative (via shade) impacts on pollinator foraging to *H. macrophyllum*, although other environmental variables likely differed between sites. 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. *H. macrophyllum* seed set increased compared to no effect of *L. maackii* on seed set when flowering phenologies were asynchronous. In contrast to this study, we found that experimental removal of *L. maackii* flowers at Three Creeks had no effect on pollinator services or reproductive success of *G. maculatum* beyond the negative impacts of shade, suggesting no effect of *L. maackii* flowers (McKinney and Goodell 2010). These differences in the effect of *L. maackii* flowers on pollination of two native understory herbs may reflect different reward levels of the native plants or different relative floral densities. For example, *H. macrophyllum* plants produce more flowers than *G. maculatum* plants on average (41.5 ± 3.1 flowers and 15.5 ± 1.4 flowers, respectively), and experimental studies indicate that facilitation of pollinator visitation tends to occur

when the invader's flowers occur at a low relative density (McKinney 2010, Munoz and Cavieres 2008).

A rigorous test of the effects of flowering phenology will require comparisons across multiple sites, in which flowering phenology of the plant species is synchronous and asynchronous. Aizen and Rovere (2010) show that increased overlap in flowering phenology is most likely to be associated with decreased seed output, but they also observed increased seed output in some plant species and years. Indeed, plant–pollinator interactions are quite variable across both space and time (Eckhart 1992; Horvitz and Schemske 1990), and we are unsure of the variation surrounding *L. maackii* flowering phenology across the native range of *H. macrophyllum*. Generalizations about the effects of plant invasions on native plant pollination will need to consider how these interactions play out across the range of flowering overlap where phenological differences, as well as variability in relative density, may influence the intensity and even direction of interaction. We can conclude that the interactions between a single pair of invasive and native plants for pollinators can vary over relatively small distances. Flowering phenology of invasive plants may ultimately influence native plant reproduction and whether invasive plants compete for pollinators with native plants or facilitate pollinator visitation to native plants.

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