THE EFFECT OF AN INVASIVE PLANT SPECIES ON

THE REPRODUCTIVE SUCCESS OF A NATIVE

CONGENER

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Abstract: Invasive plants may affect the reproductive success of native species in shared habitats via competition for abiotic resources and by altering pollinator behavior. This 2-year study investigates the effect of the presence and density of the invasive plant species *Lespedeza cuneata* on the pollination and reproductive output of its native congener *L. virginica* using three groups of metrics: pollinator visitation and assemblage, pollen deposition and pollen tube formation, and pollen limitation of fruit set. No effect was found on pollen deposition, pollen tube formation, fruit set, or pollen limitation as a function of *L. cuneata* density. Pollinator assemblages had considerable overlap, but the relative visitation of some taxa, most notably *Apis mellifera*, varied between each species. Notably, *A. mellifera* was not observed visiting *L. virginica* in plots where *L. cuneata* had been removed. These results suggest that while *L. cuneata* may cause changes in pollinator composition, it neither facilitates nor inhibits the reproduction of *L. virginica* via pollinator interactions.

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CHAPTER I

INTRODUCTION

Background

Biological invasions are ecological changes defined by the introduction of a non-native species

into a new environment that results in one or more of the following: (1) harm to native species,

(2) change to overall ecological function, (3) harm to the economy, or (4) harm to human health

(Clinton 1999). Commonly, however, invasive species are described in the literature in terms of

their demographic features, such as regional abundance and both potential and realized range,

rather than their detrimental effects to humans and the environment, as demographic features

often provide insight into invasions (see Catford et al. 2016). Across the globe, over 13,000

vascular plants have become naturalized outside of their native range due to human activity (van

Kleunen et al. 2015). Of these naturalized plants, a small subset become invasive. There are

around 4,300 identified incidents of biological invasion in the United States, and that number is

expected to continue to grow as new invasions are identified and human activity introduces more

species to new habitats (Sakai et al. 2001, Turbelin et al. 2017). In the United States alone,

invasive species cause over 120 billion dollars in damages and in control costs each year, largely

due to agricultural pests (Pimentel et al. 2005, Paini et al. 2016), and are a contributing factor to

the imperilment of roughly 49% of all threatened and endangered species (Wilcove et al. 1998).

However, the understanding of how invasive species affect ecosystem function and services is

still developing (Gordon 1998, Dukes and Mooney 2004, Crowl et al. 2008, Pejchar and Mooney,

2009, Walsh et al. 2016). Regardless, invasives are considered a major driver in global change. (Simberloff et al., 2013; Kumschick et al., 2015).

Understanding the ecology of biological invasions requires an understanding of the invasiveness of the non-native species. The biological characteristics that influence invasiveness vary by the taxon of the invader (Sakai et al. 2001); in many taxa, species with r-selected traits are more likely to become invasive, and invading populations are more likely to be have r-selected traits when compared to populations of the same species in its native range (McMahon 2002, Phillips 2009). For example, Davis (2005) found that *Spartina alterniflora* sourced from an invasive population on the Pacific US coast invested substantially more in reproduction than *S. alterniflora* from its native populations by flowering earlier, producing more inflorescence per unit of biomass, producing more seeds per inflorescence, and self-pollinating more frequently. Phenotypic and reproductive plasticity are also relatively common in invasive plants, allowing them to invade novel habitats or fit into a previously unoccupied niche (Goodwin et al. 1999, Grotkopp et al. 2002, Richards et al. 2006, van Kleunen et al. 2010, Raje et al. 2016, Razanajatovo et al. 2016).

Invasibility of a habitat is largely dependent on the frequency and intensity of disturbance events, with habitats suffering frequent disturbances of moderate to severe intensity being the most likely to suffer a biological invasion (Lonsdale 1999, Davis et al. 2000, Thuiller et al. 2005). Lonsdale (1999) also explores other qualities of a habitat that influence its invasibility in terms of survivorship of a non-native species; open niches can reduce competitive pressure from native species on non-natives that are able to fill those niches, ecosystems that support few or specialized predators can release non-native species from predation pressures, and native species

that are poor competitors will have little effect on limiting the spread of non-natives. One final factor explored by Lonsdale (1999) is the propagule pressure of non-native species on the habitat, as more introductions allow for more opportunities for non-native species to become established.

Invasive plant species most often cause ecological or economic damage by direct competition with native species, particularly abiotic resource competition (Dyer and Rice, 1999, Seabloom 2003, Vila and Weiner 2004, Funk and Vitousek 2007, Tabassum and Leishman 2016). There is, however, a growing interest in interactions between invasive and native species at the ecosystem level, especially regarding mutualisms (Grabas and Laverty 1999, Richardson et al. 2000, Brown and Mitchell 2001, Burns et al. 2011, Herron-Sweet et al. 2016). Richardson et al. (2000) discuss the role of several mutualisms in the establishment of invasive species including those with animal pollinators, animal seed dispersers, mycorrhizae, and nitrogen-fixing bacteria. While Richardson et al. (2000) note a lack of previous studies examining the effect of invasive plants on the pollination and reproductive success of native species, a number of empirical studies on this topic were published soon after. Among the first, Chittka and Schürkens (2001) found that the presence of the invasive plant, *Impatiens glandulifera*, reduced pollinator visitation to the native *Stachys palustris* by about 50% and seed set by about 25%, even when controlling for abiotic resource competition. Similarly, invasive *Lythrum salicaria* reduced the pollinator visitation rate, enhanced heterospecific pollen transfer, and reduced the reproductive success of native *L. alatum* (Brown and Mitchell 2001, Brown et al. 2002). More recently, a meta-analysis by Morales and Traveset (2009) of 40 studies that examined either the effect of non-native plants for the pollinator visitation to or reproductive success of natives found that, overall, non-native plants had a negative effect on both pollinator visitation and reproductive success of native species. However, Morales and Traveset (2009) also found that the negative effect that non-natives had on

the reproductive success of natives was not unique; native plants affected each other's reproductive success just as negatively.

While the Morales and Traveset's (2009) review found an overall trend towards competitive interactions between native and nonnatives for pollination, there were exceptions, including neutral or facilitative effects. A review by Bjerknes et al. (2007) examined 15 different non-native/native pairs for which at least pollinator visitation had been studied, most of which were included in Morales and Traveset's (2009) meta-analysis. Like Morales and Traveset (2009), they found that the presence of a non-native resulted in competitive, neutral, and facilitative effects on pollinator visitation to the native, depending on the species pair and even the year of study (4 instances of increased visitation, 4 instances of decreased visitation, 5 instances of no effect, and 2 instances in which the effect varied by year) (Bjerknes et al. 2007). However, the non-native species always had either a negative or neutral effect on fruit set and seed set of the native species (4 instances of reduced seed or fruit set, 5 instances of no effect, and 6 studies that did not examine fruit or seed set) (Bjerknes et al. 2007).

In the cases where reproductive success of a native plant is affected by the presence of a non-native species (e.g., Brown et al. 2002), it is challenging to disentangle the effects of altered pollinator visitation from other forms of competition; losses in reproductive success of the native may be as a result of the presence of the invader imposing greater resource limits than pollen limits. Pollen supplementation experiments, however, disentangle potential resource limits caused by the abiotic competition with an invasive species from pollen limitation caused by the invasive competing with the native for pollinator services. By comparing the seed or fruit set of plants that received pollen supplementation to those that were open-pollinated, researchers are able to assess

the level of pollen limitation those plants are experiencing; greater reproductive output of supplemented compared with unsupplemented plants indicates that plant reproduction is limited by pollen receipt. In their review, Knight et al. (2005) hypothesized that invasives could alter the magnitude of pollen limitation of co-occurring native species. Since the Knight et al. (2005) review, a handful of empirical studies have examined the effect of non-native species on the pollen limitation of co-occurring natives (see Table 1, Chapter 2). In many cases, the presence of a non-native species had no effect on the visitation or pollen limitation of the native species, although only in two cases did it not affect either (see Table 1, Chapter 2). While Knight et al. (2005) hypothesized based on the empirical evidence available at the time that decreased pollinator visitation to natives growing in the presence of an invasive will cause an increase in the pollen limitation of the native species, none of the empirical studies that subsequently tested this hypothesis have found such a relationship (see Table 1, Chapter 2).

As there are so few studies that examine the pollen limitation of a native plant in the presence of a non-native plant, it is difficult to determine why there is so much variation in findings across studies. Investigation of pollen limitation of native plants in invaded habitats did not begin until after the Knight et al. (2005) meta-analysis, and few studies include measurements of conspecific pollen loads or pollen tube formation (but see Spellman et al. 2015), both of which might explain the presence or absence of pollen limitation in certain systems. Likewise, the spatial and temporal scales at which these experiments are conducted varies greatly (but tend towards large spatial scales and short temporal scales), both of which may greatly influence the effects that are observed (Bjerknes et al. 2007, Bartomeus et al. 2010). Additionally, a topic that has seen almost no attention is the effect of the severity of the invasion, especially considering that it is possible that the effects on pollinator visitation and reproductive success do not relate linearly with the density or proximity of the invader (Dietzsch et al. 2011). Shelby and Peterson's (2015) study

uses distance from an invasive plant to represent the relative effect that the invasive will have on native species, finding no effect on pollen limitation. Bruckman and Campbell (2016) also investigated the effect that distance from the invasive *Brassica nigra*, as well as the floral density of the invasive, has on the pollinator visitation, pollen deposition, and seed set of native *Phacelia parryi*. They found that *P. parryi* plants that were immediately surrounded by low density patches of *B. nigra* were visited more frequently, had greater conspecific pollen deposition, and had a greater mean seed set compared to *P. parryi* that were 3m away from high densities of *B. nigra*, and both of these treatments had a greater facilitative effect on *P. parryi* compared with individual *P. parryi* that were immediately surrounded by high density patches of *B. nigra* or 5-7m away from high density patches. Because this experiment was conducted with a simulated invasion, lacked a treatment group in which *B. nigra* was not present, and did not include treatments in which *P. parryi* was located at a distance from low densities of *B. nigra*, it has methodological limitations.

An important factor that likely contributes to the lack of consistency in measured effects of invasives on pollen limitation of natives is the stochastic nature of biological invasions in particular and ecosystems in general (Pysek and Hulme, 2005). Many plant invaders rely partly on vegetative reproduction or are capable of self-pollination (Razanajatovo et al. 2016), and may invest less in attracting pollinators as a result, explaining the presence of neutral pollinator visitation interactions in the literature. It is also likely that biological invasions result in changes to the ecosystem beyond co-flowering natives and their pollinators (such as predators and parasites of native species and their pollinators), potentially confounding non-manipulative studies.

Overview of thesis research

During 2004 and 2005, Woods and colleagues (2012) conducted an experimental study that investigated how the invasive legume *Lespedeza cuneata* altered pollinator visitation to three native members of the *Lespedeza* genus: *L. virginica*, *L. capitata*, and *L. violacea*. They discovered that *L. cuneata* had variable effects on pollinator visitation to the native species during 2005, promoting visitation to *L. virginica*, reducing visitation to *L. capitata*, and having no effect on visitation to *L. violacea*. My thesis is an extension of this study; Woods and colleagues (2012) collected robust data on pollinator visitation, but did not examine how *L. cuneata* affects pollen deposition or pollen limitation in the native species. In order to address these questions, in addition to collecting data on pollinator visitation, I limited my study to the interaction between *L. cuneata* and *L. virginica*. My methods varied from those used in the Woods et al. (2012) study to address these research questions. While Woods et al. (2012) examined invaded and uninvaded sites in Kansas, I experimentally manipulated the density of *L. cuneata* at a single study site (Lake Carl Blackwell, Payne County, Oklahoma. 36° 6' N, 97° 13' W). This additional component of *L. cuneata* density manipulation added a layer of complexity to my study and was only reasonable to investigate on a small spatial scale.

CHAPTER II

CHANGES IN DENSITY OF A NON-NATIVE SPECIES DOES NOT ALTER THE

REPRODUCTIVE SUCCESS OF A NATIVE CONGENER

Introduction

Biological invasions, the deleterious introductions of species to areas outside their native range,

have received increased attention over the last 30 years as their environmental and economic

costs have become better understood (Wilcove et al. 1998, Pimentel et al. 2005, Paini et al. 2016).

Many invasive plants are able to invade new habitats due to high propagule pressure (Warren et

al. 2012, Woods et al. 2009), allowing the establishment of large, dense populations of the

invasive and the reduction in size of native plant populations (Dyer and Rice 1999, Woods et al.

2009). As such, there exists a need to understand the ecological interactions that both influence

the likelihood of a biological invasion as well as the effects of these invasions. Additionally,

invasions offer a unique opportunity to investigate the interaction between ecological and

evolutionary mechanisms, as invasive species often must evolve rapidly to the challenges of their

new environment (Lambrinos 2004). Many invasive plants rely on mutualisms for pollination,

seeds dispersal, and nutrient uptake, and most often form mutualistic relationships with generalist

pollinators, seed dispersers, and mycorrhizae (Richardson et al. 2000). Understanding these

relationships is not only important in understanding the ecological and evolutionary dynamics

that allows a non-native species to become invasive, but also its effect on the invaded ecosystem.

Animal pollinators are a particularly conspicuous mutualist, and evidence suggests that their relationships with invasive plants can affect the reproductive success of native plants (Bjerknes et al. 2007, Morales and Traveset 2009). Pollen limitation of plant reproduction occurs when a plant has lower reproductive output than it would with adequate quantity or quality of pollen receipt (Knight et al. 2005). Evolutionary theory predicts that plants should optimize their resource allocation to reproduction so that pollen limitation and resource limitation are balanced (Haig and Westoby 1988). Despite this theoretical prediction, 63% of plants studied to date show significant pollen limitation of reproductive output (Knight et al. 2005). Both ecological factors and intrinsic traits of a plant may contribute to pollen limitation of plant reproduction, and these factors may interact (Vamosi, Steets, and Ashman 2013). For example, plant population size and density (Knight 2003, Moeller 2004), pollinator species richness (Vamosi, Steets, and Ashman 2013) and habitat size (Cunningham 2000) are often negatively correlated with pollen limitation, while plant species richness is often positively correlated with pollen limitation (Alonso et al. 2010, Lázaro et al. 2014), especially for self-incompatible species (Vamosi, Steets, and Ashman 2013). These findings suggest that pollen limitation is partly driven by pollinator-mediated plant-plant interactions, and that these interactions are often competitive (Gross 1996, Campbell and Motten 1985). Alternatively, coflowering plant species may facilitate each other's pollination by attracting more pollinators to the habitat (Moeller 2004). The relative strength of these interactions appears to be highly context-dependent (Lázaro et al. 2014).

Pollen limitation in native species may be more prominent in cases of invasion. There are several modes by which a non-native species can have an indirect competitive effect on the pollination of native species. A non-native plant may reduce the pollinator visitation rates to native plants by stealing pollinators by means of more attractive displays or larger quantities of rewards (Woods et al. 2012, Sun et al. 2013). If the non-native plant is visited by a different assemblage of

pollinators than the native, it may cause the pollinator community to become more heavily

weighted towards species that are territorial or otherwise drive off pollinators of native plants

(Abe et al. 2011, Bruckman and Campbell 2014). The presence of large quantities of non-native

plants may cause an increase in the amount of heterospecific pollen deposited on flowers of the

native (Jakobsson et al. 2008). Ultimately, these competitive interactions may result in greater

pollen limitation, and reduced reproductive output of the native species when growing in the

presence of a non-native.

However, it is possible that the non-native species, especially if it is present in large quantities,

has attractive flowers, and yields large amounts of nectar, may attract more pollinators, a greater

richness of pollinators, or pollinators from a greater distance away from the location (Chung et al.

2014, Stiers et al. 2014, Sun et al. 2013). This change to the community may facilitate the

pollination of the native species, decreasing its pollen limitation and increasing its reproductive

output. A decrease in pollen limitation is not necessarily an indication of a facilitative interaction

between two species. If the non-native species competes strongly and directly with the native

species for resources, as is often the case with biological invasions (Dyer and Rice 1999, Gioria

and Osborne 2014, Mattingly and Reynolds 2014, Palladini and Maron 2013), it is possible that

the native plant's reproduction may be more resource limited than pollen limited, even if the non-

native also competes with the native for pollinators. As such, strong direct resource competition

may cause a reduction in pollen limitation and a reduction in reproductive output of the native.

The research of Knight (2003) suggests that community floral density is inversely related to

pollen limitation. Thus, removing flowering invasive plants, and thus reducing the community

floral density, reduces pollinator visitation rates within the community and more specifically to

natives in almost all cases (Waters et al. 2014). Aggressive invasive species removal may result in fragmented and sparse populations of flowering species, which would typically result in reduced pollinator services to the community (Dauber et al. 2010), although this is not universally the case (Elliott and Irwin 2009). This has implications in the management of invasive species, but also suggests that invasive plants may partly, or even completely, fill the role of the native flowering species that were displaced in the invasion. This is supported by a meta-analysis by Morales and Traveset (2009), who found that invasive plants had a negative effect on the reproductive success of paired native plants, but not to a greater degree than native plants have on other native plants.

Many studies have examined the effect of non-native plants on the pollination and reproductive success of native species, finding mixed but overall negative effects on pollinator visitation rate and seed and fruit set (Bjerknes et al. 2007, Morales and Traveset 2009). This suggests that invasive plants generally compete with native plants for pollinator services, although variation between different study systems and even between years within a study system suggests that this interaction is highly context-dependent. Furthermore, seed and fruit set measurements alone may be confounded by competition for abiotic resources, which is often strong in invasions (Dyer and Rice 1999). While pollen supplementation studies address the problem of abiotic competition by assessing pollen limitation in addition to overall reproductive success (Knight et al. 2005), there are relatively few pollen supplementation studies to date that examine the interaction between invasive and native species (Table 1). Like the studies on reproductive success reviewed by Bjerknes et al. (2007), the effect of non-native species on natives varies by study system, but in most cases, no effect was observed for either pollinator visitation rate or pollen limitation. This suggests that pollen limitation as a function of the presence of a non-native is highly context-dependent, and often there is no pollinator-mediated interaction between non-native and native

plants. Likewise, it appears that differences in pollinator visitation between treatments is not a good predictor for pollen limitation between treatments; in none of these pairs does significantly increased visitation result in significantly decreased pollen limitation, or vise versa (Table 1). However, Tscheulin and Petanidou (2012) found that *Apis mellifera* visitation to the native *Glaucium flavum* decreased in the presence of the non-native *Solanum elaeagnifolium*, which they suggest is the cause of the increased pollen limitation, even though total pollinator visitation increased with the presence of *Solanum elaeagnifolium*. Additionally, in the studies that examined heterospecific pollen transfer to the native plant, the presence of a non-native species was not observed to have an effect, although these data were collected for few of the studied non-native/native pairs (Table 1). There are no strong trends between the type of study and the interaction between the two species, although studies that introduced a non-native plant more frequently found that this introduction treatment increased pollinator visitation to the native compared to the control treatment (Table 1).

Table 1. Summary of the findings of pollen supplementation experiments conducted on native plants growing in the presence and absence of a non-native co-flowering species.

Native species	Non-native species	Type of study	Fitness-related effect in native			Reference
			Pollinator Visitation Rate	Heterospecific Pollen Deposition	Pollen Limitation	
Mimulus guttatus	*Heracleum mantegazzianum*	Introduction of non-native	Increased[d]	-	No effect	Nielsen et al. (2008)
Sisyrinchium campestre	*Euphorbia esula*	Observation in pure vs. mixed stands	Reduced	No effect	No effect	Montgomery (2009)
Decodon verticillatus	*Lythrum salicaria*	Observation in pure vs. mixed stands[a]	No effect	-	Reduced	Da Silva *et al.* (2011)
Clarkia pulchella	*Euphorbia esula*	Introduction of native[b]	Reduced	-	No effect	Palladini and Maron (2012)
Glaucium flavum	*Solanum elaeagnifolium*	Introduction of non-native	Increased[e]	-	Increased[e]	Tscheulin and Petanidou (2012)
Camassia leichtlinii	*Cytisus scoparius*	Observation in pure vs. mixed stands	Reduced	-	No effect	Muir and Vamosi (2015)
Collinsia parviflora	*Cytisus scoparius*	Observation in pure vs. mixed stands	No effect	No effect	No effect	Muir and Vamosi (2015)
Geranium molle	*Cytisus scoparius*	Observation in pure vs. mixed stands	No effect	No effect	Increased	Muir and Vamosi (2015)
Sidalcea hendersonii	*Rubus armeniacus*	Introduction of native	No effect	No effect	No effect	Shelby and Peterson (2015)
Heterotheca villosa	*Centaurea stoebe*	Introduction of non-native[c]	No effect	-	No effect	Herron-Sweet *et al.* (2016)
Dasiphora fruticosa	*Lythrum salicaria*	Observation in pure vs. mixed stands and Removal of non-native	Reduced	-	No effect	Goodell and Parker (2017)

a "Observation in pure vs. mixed stands" studies compare the fitness-related variables between native plants in wild stands without the non-native and native plants in wild stands with the non-native.

b "Introduction of native" studies compare the fitness-related variables between native plants introduced into stands without a wild population of the non-native to native plants introduced into stands with a wild population of the non-native.

c "Introduction of non-native" studies compare the fitness-related variables between native plants in wild stands without the non-native and native plants in wild stands in which the non-native was experimentally introduced.

d *Mimulus guttatus* received the greatest pollinator visitation at the closest distance from *Heracleum mantegazzianum*.

e Tscheulin and Petanidou (2012) attribute the increase in pollen limitation to a decreased in *Apis mellifera* visitation, despite increased overall pollinator visitation.

Lespedeza cuneata (sericea lespedeza) is an invasive species found throughout the central plains of North America (Woods 2009). It readily invades fields, especially in ditches and along the edges of wooded areas, forming thick, tall, monotypic stands, reproducing both sexually and asexually; it is capable of selfing, but has greater reproductive success if outcrossed (Woods 2009). It shares its range with a number of native congeners, including *L. virginica* (slender lespedeza)*, L. capitata* (roundhead lespedeza)*,* and *L. violacea* (violet lespedeza), often co-existing with these species in close proximity (Woods 2009). A field study by Woods et al. (2012) examined pollinator-mediated interactions between *L. cuneata* and *L. virginica, L. capitata*, and *L. violacea* in Kansas. Woods and colleagues (2012) found that *L. capitata* experienced competition for pollinator visits in the presence of *L. cuneata*, *L. virginica* experienced increased pollinator visitation in the presence of *L. cuneata*, and *L. violacea* experienced no change in pollinator visitation in the presence of *L. cuneata.* However, the study was limited to pollinator observation, and thus does not fully address all components of reproductive success, which the Bjerknes et al. (2007) review found to vary considerably in response to the presence of an invasive species.

While Woods and colleagues (2012) collected robust data on pollinator visitation for the study species, they did not examine the effect of *L. cuneata* on the pollen limitation and reproductive success of native lespedezas. This research fills in this knowledge gap. Further, this research was conducted in Oklahoma, whereas the Woods et al. (2012) study was conducted in Kansas, allowing for an understanding of the variation in pollinator-mediated interactions between *L. cuneata* and native lespedezas that is not specific to one location.

In a manipulative field experiment, I address the following questions:

1. Does pollinator visitation rate to *L. virginica* differ depending on the density of *L. cuneata*, and if so, what is the nature of this effect?

2. What similarities and differences exist in the pollinator communities of *L. cuneata* and *L. virginica*?

3. Does the composition of the pollinator community visiting *L. virginica* change with the density of *L. cuneata*?

4. How does stigmatic pollen load and pollen tube growth of *L. virginica* vary with *L. cuneata* density?

5. How does pollen limitation of *L. virginica* vary with *L. cuneata* density?

Given the past findings by Woods et al. (2012), I hypothesize that pollinator visitation rate to *L. virginica* will increase with increasing density of *L. cuneata*. I hypothesize that *L. cuneata* and *L. virginica* are pollinated by many of the same functional groups (Woods et al., 2012), and that pollinators may visit flowers of both species in succession, depositing pollen from one onto the other. I hypothesize that the amount of conspecific pollen grains, as well as pollen tubes, on the stigmas of *L. virginica* will increase with increasing concentrations of *L. cuneata*. Finally, I hypothesize that *L. virginica* will be less pollen-limited in increasing density of *L. cuneata* due to an increased pollinator visitation rate.

Methods

Study sites

In June 2015, thirty 5 x 5 m plots were established at Lake Carl Blackwell (36° 6' N, 97° 13' W) located 15 km west of Stillwater, OK. Plots were established in locations where *L. cuneata* and *L. virginica* co-occur. All plots were separated from one another by at least 5 m and contained between 23-56 *L. cuneata* ramets (mean number of *L. cuneata* per plot ± SE = 37.13 ± 5.21). Plots were randomly assigned to one of three *L. cuneata* floral density treatment levels. The floral density of *L. cuneata* was manipulated by removing the aboveground portion of the plant so that three different *L. cuneata* floral density treatment-levels were established: (1) No Removal – ambient *L. cuneata* floral density (mean number of flowering *L. cuneata* per plot ± SE = 36.72 ± 5.72); (2) Partial Removal – half of all *L. cuneata* floral stems were clipped to remove them (mean number of flowering *L. cuneata* per plot ± SE =17.33 ± 5.46); (3) Full Removal – flowering stems of all *L. cuneata* were clipped. Clipping was repeated weekly to maintain the experimental floral density of *L. cuneata* from summer 2015 to fall 2016. The floral densities of *L. virginica* and other flowering plants were not manipulated. The floral densities (flowers/plot) for *L. cuneata* and *L. virginica* were recorded before the experiment and after each weekly clipping. Floral density was estimated for both *L. cuneata* and *L. virginica* by quantifying the mean number of flowers per plant of at least 10 representative plants of that species multiplied by the total number of individuals of that species in the plot.

Pollinator visitation

Pollinator observations were taken for each of the thirty plots to determine whether *L. cuneata* influences pollinator visitation to *L. virginica*. Pollinator observations were conducted during late August through late September in both 2015 and 2016, when both species of *Lespedeza* were in bloom. Observations were made between 10:00 and 19:00 hours in 2015 and between 7:00 and 19:00 hours in 2016. During a given observation period, a representative focal plant within a

randomly selected 1 x 1 m section within each 5 x 5 m plot was chosen for observation. Two to three 1-hour pollinator observation periods were conducted within each plot during 2015. In 2016, seven to eight 15-minute observation periods were conducted for each plot. Insects were considered pollinators only if they came into contact with the flowers. Pollinator visitation was recorded by the taxonomic group of the visitor (Coleoptera, Diptera, Lepidoptera, Hemiptera, and Hymenoptera; members of Hymenoptera and Lepidoptera were differentiated by family, genus, or species when possible) for both *L. cuneata* (in plots in which it was not removed) and *L. virginica*. Visitation rates were calculated at the flower (number of visits per flower per plant per hour) and the plant (number of visits per plant per hour) level. Total pollinator visitation rates for *L. virginica* were analyzed using ANOVA with the *L. cuneata* density treatment as the predictor variable. The structure of pollinator visitors to *L. virginica* and *L. cuneata* were compared using a *G*-test of heterogeneity (Sokal and Rholf 1969). The structure of pollinator visitors to *L. virginica* in each *L. cuneata* removal treatment were compared using a canonical correspondence analysis (1000 repetitions). All analyses were conducted in IBM SPSS 23, except for the *G*-test of heterogeneity, which was conducted in R, and the canonical correspondence analysis, which was conducted in CANOCO 5.

Stigmatic pollen load and pollen tube formation

To explore whether *L. virginica* stigmatic pollen load and pollen tube formation changes with *L. cuneata* density, I collected 3 senesced *L. virginica* flowers from a *L. virginica* plant in each plot (not one used in the pollen supplementation experiment, described below) and preserved them in 70% ethanol. The number of pollen tubes and pollen grains were counted using epifluorescence (as per Alonso et al. 2013); the senesced flowers were softened in 1M KOH for 30 minutes at 70°C, then stained with decolorized aniline blue for 15 minutes at 70°C. After staining, I removed

and crushed the style. I visualized the pollen tubes and pollen grains using a Leica DM IL light microscope with Leica EL6000 fluorescent illumination. *L. cuneata* is not known to hybridize with *L. virginica* (Clewell 1967, Hanson and Cope, 1955, see Appendix A). Pollen grain deposition and pollen tube formation were analyzed with ANOVA with the *L. cuneata* density treatment as the predictor variable.

Pollen limitation

To determine whether the reproductive success of *L. viriginia* is pollen limited and whether this depends on the density of *L. cuneata*, I performed a pollen supplementation experiment. Within each of the thirty experimental plots, I randomly selected two pairs of *L. virginica* plants in June 2015. Pairs were selected for similarity in size. All of the flowers on one of plants within each pair was hand-pollinated every two days using pollen collected from *L. virginica* plants at least 5 m from the plot (i.e., hand-pollination treatment level). All of the flowers of the other paired plant were unmanipulated and thus were subject to ambient pollination (i.e., open-pollination treatment level). At the end of the reproductive season, I harvested all plants in the pollen supplementation experiment and enumerated flower and fruit number on each plant in the laboratory. The sepals remain on the plant after the flowers senesce, and these were counted to determine the number of flowers per plant. Fruits also remain attached to *L. viriginica* allowing for enumeration in the lab. Fruit set for each plant was calculated as the number of fruits produced divided by the total number of flowers on the plant. For each pair of plants, I estimated the pollen limitation effect size as the log response ratio (following Knight et al. 2005):

Pollen limitation effect size= ln (Fruit set $_{hand-pollinated}$/ Fruit set $_{open-pollinated}$)

L. virginica only produces one seed per fruit, so this analysis was not repeated for seeds. Pollen limitation effect size was analyzed using ANOVA with the *L. cuneata* density treatment as the

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predictor variable. Fruit set was also analyzed using ANOVA with the *L. cuneata* density

treatment the pollination method as predictor variables.

Results

Density

L. virginica floral density did not vary significantly across *L. cuneata* removal treatments in either

2015 ($F_{2, 117}$=0.978, *P*=0.379) or in 2016 ($F_{2, 116}$=0.818, *P*=0.444). On average, the floral density

across all plots was 601.15 per 5m^2 in 2015 and 564.85 per m^2 in 2016. Prior to implementing the

L. cuneata removal treatments, *L. cuneata* ramet density did not vary across plot types ($F_{2, 27}$=0.252, *P*=0.779). The *L. cuneata* removal treatment levels were effective at manipulating *L.*

cuneata floral density across both years (see methods for mean L. cuneata density/treatment level;

$F_{2, 57}$=239.961, *P*<0.001).

Pollinator visitation

Across treatment-levels, pollinator visitation was recorded for a total of 42 hours in 2015 and 56

hours in 2016. Overall *L. virginica* per-flower visitation rate did not differ significantly with *L.*

cuneata removal treatment (2015: $F_{2,47}$=0.131, *P*=0.877; 2016: $F_{2,118}$=1.494, *P*=0.229; Fig. 1a &

b). Overall *L. virginica* per-plant visitation rate also did not differ significantly with *L. cuneata*

removal treatment (2015: $F_{2,47}$=0.274, *P*=0.631; 2016: $F_{2,118}$=1.867, *P*=0.159 Fig. 1c & d).

L. virginica plants received a significantly different composition of pollinators compared to *L.*

cuneata plants in both 2015 (not shown) and 2016 (G_2=36.31 in 2015, G_2=43.14 in 2016,

P<0.001 in both years, Fig.2a). *L. cuneata* received a relatively greater number of visits from

Apis mellifera. The composition of pollinators visiting *L. virginica* did not vary by *L. cuneata* removal treatment in 2016 (*P*=0.412, Fig.2b).
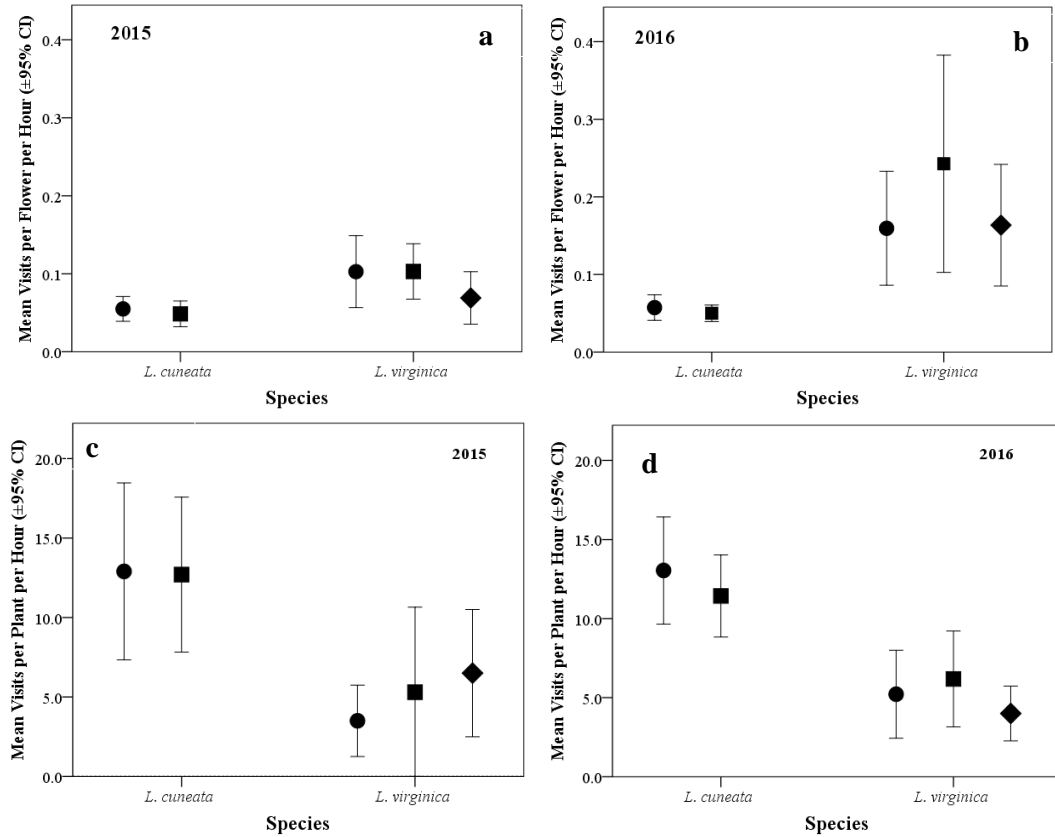

Figure 1. The mean number of pollinator visits per flower (a, b) or per plant (c, d) per hour during the early fall of 2015 (a, c) and 2016 (b, d). Observations were made in one-hour periods in 2015 (a, c) and in 15-minute periods in 2016 (b, d), and were conducted on unmanipulated plants in the treatment plots. Circles represent plants in the no *L. cuneata* removal treatment, square plants in the partial *L. cuneata* removal treatment, and diamonds in the full *L. cuneata* removal treatment. *L. cuneata* was not present in the full removal treatment. Error bars represent 95% confidence intervals.
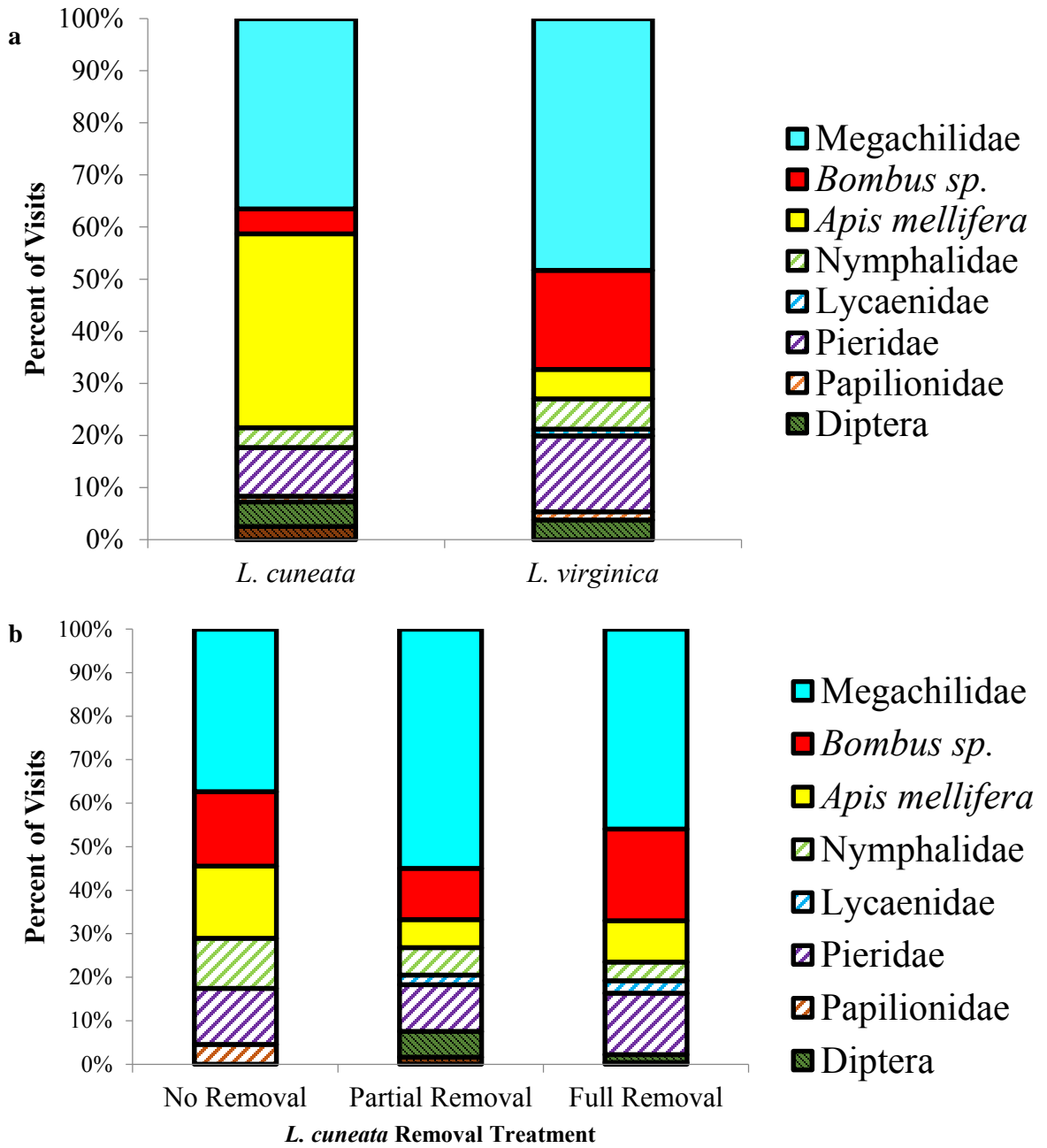
Figure 2. Relative abundance of insect visitors by taxa to both *L. cuneata* and *L. virginica* (a) and to *L. virginica* between removal treatments (b).

Pollen Grain Deposition and Pollen Tube Formation

In 2015, 90 (3 from each of the 30 plots) senesced *L. virginica* flowers were collected, stained, and visualized. There were no significant differences by *L. cuneata* removal treatment in the number of pollen grains deposited per stigma ($F_{2,89}=1.156$, $P=0.320$) nor in the number of pollen tubes that formed per style ($F_{2,89}=1.312$, $P=0.275$).
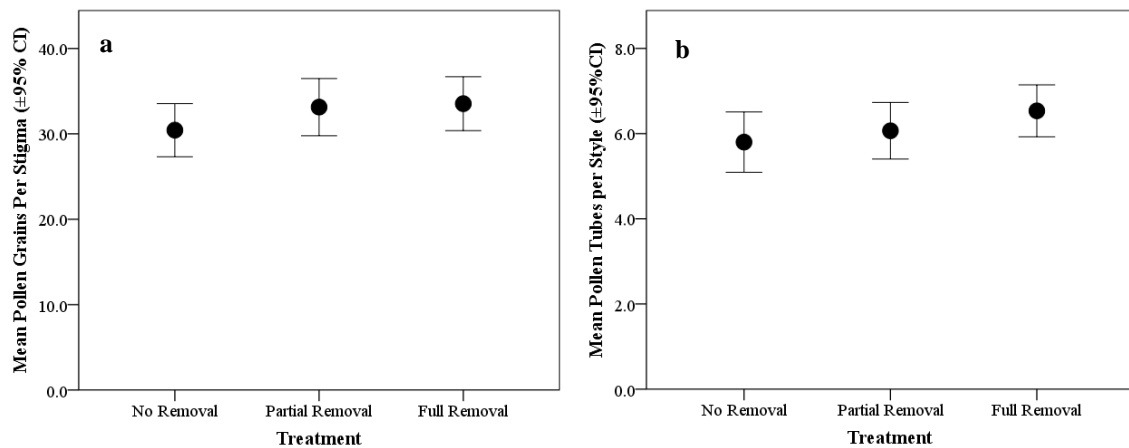

Figure 3. Mean pollen grains per stigma (a) and mean pollen tubes per style (b) from senesced *L. virginica* flowers collected in the fall of 2015, by *L. cuneata* removal treatment. Flowers were collected from unmanipulated plants within the treatment plots. Error bars represent 95% confidence intervals.

Pollen limitation

Across *L. cuneata* removal treatments, *L. virginica* set 4.3% higher fruit set when hand-pollinated compared with open-pollinated control plants ($F_{1,119}=4.240$, $P=0.042$), which indicates that *L. virginica* is significantly pollen limited. However, there were no significant differences in *L. virginica* fruit set ($F_{1,119}=1.387$, $P=0.254$, Fig. 4a) or pollen limitation ($F_{2,29}=1.236$, $P=0.307$, Fig. 4b) by *L. cuneata* removal treatment.
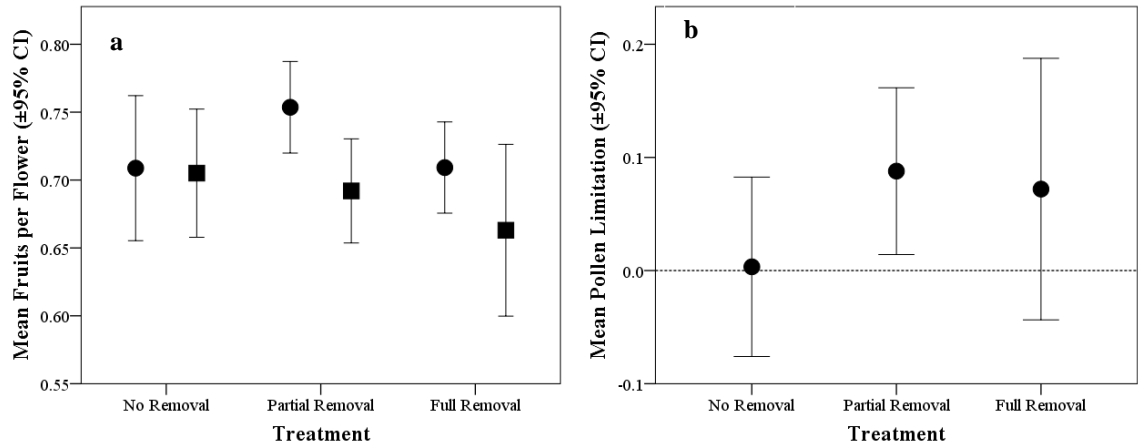
Figure 4. Mean fruit set for hand-pollinated (circles) and open-pollinated (squares) *L. virginica* plants (a) and mean pollen limitation of *L. virginica* plants (b) by *L. cuneata* removal treatment. Plants were pollinated and collected in early fall 2015. Error bars represent 95% confidence intervals.

Discussion

This study examined the relationship between the density of the invasive plant species *Lespedeza cuneata* and several metrics for reproductive success of its native congener *L. virginica*: pollinator visitation rates, pollen deposition and tube formation, and pollen limitation. *L. cuneata* density had no significant effect on the pollinator visitation, pollen deposition, pollen tube formation, nor pollen limitation for *L. virginica*. Pollinator composition between *L. cuneata* and *L. virginica* was similar, but *L. cuneata* received relatively more visits from *Apis mellifera* than *L. virginica*. This result is consistent with the findings of Woods et al. (2012), in which *A. mellifera* visited *L. cuneata* to the exclusion of three native *Lespedeza* species, including *L. virginica*. The hypotheses that *L. cuneata* density would affect the reproductive success of *L. virginica* were not supported.

The lack of effect of *L. cuneata* on the reproductive success of *L. virginica* is consistent with other studies that have shown no significant effect of non-native species on the pollination, pollen

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limitation, or reproductive success of native species (Bjerknes et al. 2007, Muir and Vamosi 2015, Herron-Sweet et al. 2016, see Table 1). There is a weak trend, however, towards invaders reducing visitation to native plants, presumably by competing with natives for pollination services (Morales and Traveset 2009). Even within a single system, annual differences may alter this relationship, transitioning between the invasive having an effect (facilitative or competitive) on visitation to the native and having no effect, or occasionally transitioning between a facilitative and a competitive interaction (Bjerknes et al. 2007, Woods et al. 2012). Differences between systems may be explained by the extent to which the invasive and native share pollinators; if the composition of the pollinating fauna is similar between the two plant populations, it is reasonable to expect that both facilitative and competitive interactions will be stronger. However, an invader may still exert a competitive effect on the pollination of a native if the native requires specialized pollinators and the invasive does not, as an abundant invader may alter the local pollinator community in favor of generalist pollinators (Lopezaraiza–Mikel et al. 2007). This relationship has been borne out in other studies with the *Lespedeza* genus, in which *L. cuneata* exerts a facilitative effect on visitation on congeners with which it shares most of its pollinators, and a competitive effect on congeners with which is shares few pollinators (Woods et al. 2012).

Differences in weather between years may account for annual changes in pollinator populations and thus the relationship between invader and natives; as some plants are capable of greater phenotypic plasticity in response to climate than insects, the flowering of the plants and the emergence of specific pollinators may be mismatched some years and matched in others (Forrest and Thomson 2011). As this study lacks pollen deposition, pollen tube formation, and pollen limitation data for 2016, while the pollinator visitation data suggests that no differences in reproductive success would be found, other studies have found pollen limitation without finding

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any effect on pollinator visitation (Da Silva et al. 2012, Muir and Vamosi 2015). Future studies into the pollinator-mediated interaction between invasive and native plants should aim to extend the duration of the experiment as long as possible to control for annual variation, especially because annual variation occurs in other two-year studies (see review by Bjerknes et al. 2007).

The lack of effect *L. cuneata* had on the pollen limitation of *L. virginica* may be due to *L. virginica*'s relatively high autofertility (67%, see Appendix A). Pollen limitation is expected to be lower in autofertile species (Knight et al. 2005; Rodger and Ellis 2016), which may contribute in large part *L. cuneata*'s lack of effect on the pollen deposition or pollen limitation of *L. virginica*. However, this degree of autofertility is substantially greater than the northeastern Kansas, USA population studied by Woods and colleagues (2009). As such, other populations of *L. virginica* may experience pollen limitation to a greater extent than was observed in this study, both overall and as a result of *L. cuneata* invasion.

Another factor that may have contributed to the lack of effect of *L. cuneata* on the pollen limitation of *L. virginica* is the method by which *L. cuneata* was removed from Partial Removal and Full Removal treatment plots. While only aboveground *L. cuneata* biomass was removed in these treatment plots in order to limit the effect of abiotic resource competition, a study by Brandon et al. (2004) suggests that *L. cuneata* competes with native plants primarily through shading, so the removal technique used in this study may have decreased resource limitation on *L. virginica*, making pollen limitation more prominent (potentially obfuscating a facilitative pollinator-mediated interaction between *L. cuneata* and *L. virginica*). Alternatively, the removal of aboveground biomass may have increased belowground competition as *L. cuneata* invested resourses in nutrient acquisition, thus increasing resource limitation on *L. virginica*, decreasing

pollen limitation, and obfuscating competitive pollinator-mediated effects of *L. cuneata* on *L. virginica*.

It is very possible that any effect of the presence of *L. cuneata* on the pollinator-mediated reproductive success of *L. virginica* was too weak to be detected with the methodology used in this study. The spatial scale at which this experiment was conducted was substantially smaller than the scale used by Woods et al. (2012), in which pollinator observations were conducted along transects in several different field sites, some of which were invaded with *L. cuneata* and some were not. This differs from the methodology used in this experiment, in which the field site was invaded, and the presence of *L. cuneata* was manipulated within $5m^2$ plots. While experiments using plots as small as $2m^2$ have examined the pollinator-mediated interaction between native and non-native plants (Bruckman and Campbell 2014), the difference in sampling scale may explain why this study found results that did not corroborate the findings of Woods et al (2012). The effect of spatial scale of the invasion is thought to influence pollinator-mediated plant-plant interactions (Bartomeus et al. 2010), and as such should be considered in when conducting research on this interaction.

This study adds to a growing body of work that suggests the effect of invasive plants on the pollinator-mediated reproductive success of natives is highly context-dependent and frequently minimal (see Table 1). While there is substantial evidence that biological invasions often alter pollinator visitation rates to coflowering native species (Bjerknes et al. 2007, Morales and Traveset 2009), only three studies to date have found evidence that a non-native species affects the degree to which a native species is pollen limited (Da Silva et al. 2011, Tscheulin and Petanidou 2012, Muir and Vamosi 2015). Notably, in all three instances in which the native was

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either more or less pollen limited in the presence of a non-native, the non-native species (*Lythrum salicaria*, *Solanum elaeagnifolium*, and *Cytisus scoparius*, respectively) are considered obligate outcrossers. However, obligate outcrossing does not necessarily indicate that a non-native will affect the pollen limitation of a native; for example, the presence of *C. scoparius* did not alter pollen limitation in the outcrossing native species *Camassia leichtlinii* or *Collinsia parviflora* (Muir and Vamosi 2015). As both *L. cuneata* and *L. virginica* are capable of selfing (Woods et al. 2009), it is unsurprising that the density of *L. cuneata* had no observable effect on the pollen limitation of *L. virginica*. Further research, however, is needed to better understand the conditions under which an invasive plant species affects the reproductive success of co-flowering natives.

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APPENDIX A: AUTOFERTILITY AND HETEROSPECIFIC INCOMPATABILITY OF *LESPEDEZA VIRGINICA*

To determine whether *Lespedeza virginica* is incompatible with *L. cuneata* pollen, and whether L. cuneata pollen will produce pollen tubes within *L. virginica* styles, I conducted a manipulative experiment. In the summer of 2016, 18 *L. virginica* plants were selected from locations at least 5 meters away from any experimental plot detailed in Chapter 2. On each plant, a branch containing 5 flowers was selected and each flower on that segment was emasculated prior to flower opening using forceps; in cases where there were more than 5 flowers per branch, the additional flowers were removed. These plants were randomly assigned to one of three treatment levels (n=6 plants/treatment level): (1) Outcross pollination: flowers were hand-pollinated with *L. virginica* pollen, (2) Pollination with *L. cuneata* pollen: flowers were hand-pollinated with *L. cuneata* pollen, and (3) Emasculation/Bagging Control: flowers were not hand-pollinated. On an additional six plants a branch containing five flowers was marked and the flowers on those branches were left unmanipulated (i.e., not emasculated nor hand-pollinated; (4) Autofertlity Control). All treatment branches on these 24 plants were covered with pollinator exclusion bags to prevent pollinator-mediated pollen transfer.

In half the plants in each group, the flowers were collected (15 per group) upon senescence and preserved in 70% ethanol. The number of style containing at least one pollen tube were counted using epifluorescence (following the methods described in Chapter 2).

In the remaining plants, the fruit was allowed to mature (up to 15 per group) and enumerated.

Table A1. Percentage of flowers that developed fruits and styles that contained pollen tubes per treatment level.

	Percentage of flowers developing fruits	Percentage of styles that contained pollen tubes
Outcross pollination	93	87
Pollination with *L. cuneata* pollen.	0	7
Emasculation/Bagging Control	0	7
Autofertility Treatment	67	73

These trends suggest that *L.virginica* is not cross-compatible with *L. cuneata* (Table A1). Chasmagomous *L. virginica* flowers are capable of auto-fertility in the absence of pollinators (Table A1).

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