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Direct and Indirect Effects of Invasive Cirsium arvense on Pollination in Southern Appalachian Floral Communities

Jesse Daniels
East Tennessee State University

Direct and Indirect Effects of Invasive *Cirsium arvense* on Pollination in Southern Appalachian
Floral Communities

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Jesse D. Daniels

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Dr. Gerardo Arceo-Gómez, Chair

Dr. Timothy McDowell

Dr. Darrell Moore

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ABSTRACT

Direct and Indirect Effects of Invasive *Cirsium arvense* on Pollination in Southern Appalachian
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Invasive plants can alter pollination dynamics in invaded communities by disrupting patterns of
pollinator visitation, pollen transfer dynamics (conspecific [CP] and heterospecific [HP]), and
reproductive success. The direction of invasive effects (competitive, neutral, and facilitative)
may be partially determined by spatial scale and species' floral traits. Here, we investigated
pollinator visitation, CP and HP receipt, and pollen tube growth for species in a *C. arvense*
present community and non-present community at two scales. At the community-level, the effect
of *C. arvense* on pollinator visitation varied among species. Floral symmetry seemed to explain
this variation. At the floral neighborhood-level, we found competitive effects for pollinator visits
and mixed effects on CP deposition. The overall structure of plant-plant HP deposition networks
was slightly altered. We observed lower average centrality across shared species in the *C.
arvense* present community suggesting *C. arvense* had subverted their roles as pollen donors.

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CHAPTER 1

INTRODUCTION

Invasive Plant Effects on Local Plant Species

Invasive plants are plants that have been introduced outside of their natural range and have spread to a degree capable of harming human health, economic activities, or the environment (Simberloff et al. 2013; van Kluenen et al. 2015). Recent global studies estimate that 13,168 plant species have invaded areas outside their natural range, and projections indicate further spreading of plant invasions across most continents (Bellard et al. 2013; van Kleunen et al. 2015). Most evidence suggests that invasive plants can have strong, competitive effects on native plants (Vila et al. 2011; but see Rodriguez 2006). For instance, when invasive *Centaurea diffusa*, a plant native to Eurasia, was grown with three native species to the Rocky Mountains of Montana, i.e., *Festuca idahoensis, Koelaria cristata,* and *Pseudoroegneria spicata*, native species' biomass decreased up to 86% (Callaway and Ashehoug 2000). Moreover, a meta-analysis on the effects of up to 167 invasive plant species across the globe determined that invasive plants decreased native species abundance by 43.5% and their diversity by 50.7% (Vila et al. 2011). Invasive species can further reduce plant fecundity, productivity, and mineral or nutrient content in plant tissue as well as species richness in native plant communities (Pysek et al. 2012). However, recent studies have shown that negative effects of invasive species on native plants can vary with spatial scale (e.g., landscape or neighboring), history of interactions (e.g., decades to centuries), or with the environmental context (e.g., regional climate) (Powell et al. 2011; Zavorka et al. 2018). Therefore, a more thorough knowledge of invasive species' effects, their underlying mechanisms, and how these effects vary across spatial and temporal scales is

imperative to mitigate their adverse effects and prevent further native plant population decline (Levine et al. 2003).

Invasive species can negatively affect native plant communities through several different mechanisms. First, invasive plants can negatively affect native plants through direct exploitative competition (Levine et al. 2003). When introduced into a community, invasive plants can acquire space, light, and nutrients more efficiently than native plants (e.g. Vila and Weiner 2004) and alter nutrient cycling (Vila et al. 2011). For example, when Woo and Zedler (2002) compared biomass and percent cover of native *Carex* species in wetland plots invaded by *Typha glauca* after adding fertilizer, *T. glauca* increased in cover and biomass while native species did not. *T. glauca* increased in cover because it was able to capitalize on the increase in limiting nutrients (Woo and Zedler 2002). Furthermore, a meta-analysis of 79 studies showed invasive species shift carbon and nitrogen availability (temporal and spatial) in the soil particularly when their phenology differs with native species, which enhances invasive plant uptake of local nutrients (Ehrenfeld 2003). In this sense, carbon and nitrogen availability may be diminished by the invasive species when native species need the nutrients most creating a competitive advantage for the invasive species to acquire nutrients with fewer competitors (Ehrenfield 2003). An efficient uptake of nutrients can also aid invasive species with rapid asexual reproduction or clonal growth to proliferate quickly (Maurer and Zedler 2002). For example, studies of Japanese Knotweed, *Fallopia japonica*, from Britain, the United States, and other parts of Europe indicate individuals are all the same clone (Hollingsworth and Bailey 2000). Overall, by efficiently acquiring nutrients invasive plants can rapidly exploit local resource pools and limit the amount of resources available for native species (Levine 2003).

Second, invasive plants can alter native species success via interference competition, specifically via allelopathy (the release of phytotoxins through plant substrates that inhibit growth of neighboring plant species). Allelopathy is an important mechanism that has been associated with high invasion success (Callaway and Aschehoug 2000; Inderjit et al. 2011). According to Callaway and Ridenour (2004), allelopathy may act as a 'novel weapon' when an invasive plant has developed biochemical interactions with co-existing species in in their natural range that do not exist in invaded native plant communities. Since native plants have never been exposed to the invasive phytotoxins, native plants have not developed any natural defenses against their adverse effects (Callaway and Ridenour 2004). Therefore, allelopathy is considered a strong 'weapon' favoring invasion because introduction of novel chemicals secreted by roots, bark, leaf litter, or other parts of the plant suppress germination and growth of native seeds and saplings (Callaway and Ridenour, 2004). For example, chemicals secreted by the bark and roots of *Ailanthus altissima* can inhibit growth of native seedlings (Heisy 1990). In addition, secretions from leaf litter of *Eucalypus globulus* are known to suppress plant growth and chlorophyll content (Jayakumarand Eyini 1990). Furthermore, foliage and root extracts from invasive *Cirsium arvense* have been shown to reduce growth of native *Hordeum jubatum, Amaranthus retroflexus,* and *Setaria viridis* (Stachon and Zimdahl 1980).

A third mechanism by which invasive species can alter native species success is by indirectly disrupting vital mutualisms (Richardson et al. 2000; Traveset and Richardson 2006). For instance, many plant roots require fungal associations to aid in the uptake of nutrients while providing fungi with energy; however, invasive plants can suppress this symbiotic relationship though the exudate of chemicals to the soil (Richardson et al. 2000). For instance, when invasive garlic mustard, *Alliaria petiolata*, was grown in soils across the United States, mycorrhizal plant

species' biomass decreased 59% (Calloway et al. 2008). On the other hand, some invasive species can take advantage of these mutualisms rather than suppressing them. When invasive *Centaurea maculosa* was present with arbuscular mycorrhizal fungi, competition and consequent negative effects on growth of native *Festuca idahoensis* increased (Marler et al. 1999). Invasive species can also disrupt native interactions by disproportionally attracting animal seed dispersers to their fleshy fruits and seeds, so native plant-seed disperser interactions can be affected when invasive plants colonize an area (e.g. Richardson et al. 2000). For example, introduction of *Ulex europaeus* to New Zealand altered the identity and magnitude of small mammals and birds seed dispersers for native *Kunzea ericoides*, thus negatively affecting subsequent germination of native seeds (Williams and Karl 2002).

Invasive Plant Effects on Pollination

Plant-pollinator interactions have shown great importance for generating, organizing, and maintaining plant biodiversity in nature (Fenster et al. 2004; Ollerton et al. 2009). Furthermore, an estimated 87% of flowering plant species require animal pollination to produce seeds (Ollerton et al. 2011). Pollination is also an important ecosystem service with 75% of leading crop plant species worldwide requiring animal pollination for optimal yields (Klein et al. 2007). Thus, disruption of pollination mutualisms by invasive species can have important effects on plant biodiversity as well as on the well-being of human populations.

Invasive plants have the potential to disrupt native species pollination success because they are typically considered 'super-generalists' (species that interact with many pollinator species) with numerous showy flowers that produce large amounts of floral rewards i.e. nectar, pollen, and scents (Traveset and Richardson 2006; Aizen et al. 2008; Vila et al. 2009, Powell et al. 2011). Super-generalist invasive plants often attract generalist pollinators that help integrate

them into native communities (Memmot and Waser 2002). As a result, invasive flowers can steal pollinator visits from native flowering species (Morales and Traveset 2009). For example, when comparing visitation rates between invasive *Lythrum salicaria* (purple loosestrife) and native *Lythrum alatum* (winged loosestrife), pollinator visitation and seed set on the latter were significantly reduced when the invasive was present (Brown et al. 2002). A meta-analysis of 40 studies also showed an overall decrease in pollinator visitation to native plants when co-flowering near an invasive plant (Morales and Traveset 2009).

However, it has also been shown that invasive plants have the potential to facilitate pollinator visitation to native flowers. For instance, Molina-Montenegro et al. (2008) found increased visitation and seed set in *Carduus pycnocephalus* when flowering with invasive *Lupinus polyphyllus.* This facilitation may result from invasive flowers attracting more pollinators into the community that may also visit native plants near the invasive (Thomson 1978; Moeller 2004). For instance, when comparing invaded sites to invasive flower removal sites, insect species richness and abundance were higher on native plants when the invasive plant was present (Lopezaraiza-Mikel et al. 2007). Likewise, Ghazoul (2006) found that low densities of invasive *Cirsium arvense* facilitated pollinator visitation to *Raphanus raphanistrum*. Here, facilitation was enhanced by resource partitioning when insects visited *C. arvense* for nectar and *R. raphanistrum* for pollen (Ghazoul 2006).

However, effects on pollinator visitation may not reliably indicate effects on overall plant reproductive success. When comparing pollinator visitation to *Lotus corniculatus* alone and in sites invaded by *Lupinus polyphyllus*, pollinator visitation to *L. corniculatus* increased in invaded sites but seed set remained unaffected (Jakobsson and Padron 2014). In addition, when co-flowering with invasive *Solidago canadensis*, pollinator visitation and seed production of native

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Ixeris chinensis decreased while visitation to native *Sonchus arvensis* increased but seed set was not affected (Sun et al. 2013). As a result, quantifying invasive species effects solely via changes in pollinator visitation may lead to unreliable results, so studies should also evaluate changes in visitation quality (quantity and quality of pollen deposition onto stigmas) and reproductive success (e.g., fruit and seed production).

One explanation for the potential unreliable association between pollinator visitation and reproductive success is that insect species have varying rates of pollen removal from anthers and deposition onto stigmas. For example, when evaluating pollen removal and deposition from apple and almond trees by *Apis* and *Bombus* species, Thomson and Goodell (2001) found similar pollen removal by both species, but *Bombus* deposited four times more pollen onto the stigmas of apple trees. Similarly, Wilson and Thomson (1991) found different rates of pollen removal from anthers and deposition on stigmas between genera that primarily collected nectar and passively collected pollen (i.e. *Bombus*) and genera that primarily collected pollen and passively collected nectar (i.e., *Apis* and *Dialictus)*. At a community level, King et al. (2013) also showed that the frequency of pollinator visits to flowers in an entire community does not necessarily indicate successful pollen deposition onto stigmas, so the strength of plant-pollinator interactions might be misrepresented. Therefore, in order to understand the mechanisms responsible for invasive plant effects on pollination, effects on pollinator visit quality should also be assessed. This can be done by observing the quantity and identity of pollen deposited on the stigma in addition to quantifying effects through changes in pollinator visitation rates.

It is known that invasive plants can disrupt deposition of pollen to native stigmas through pollinator sharing (Morales and Traveset 2008) and, as a result, reproductive success (e.g. Flangan et al. 2009). However, changes in the origin (self- versus outcross) of conspecific pollen

(CP) deposited onto stigmas may also affect plant reproductive success. For instance, more than 50% of flowering species have mixed mating systems (Goodwillie et al. 2005), but self-pollen from the same plant may not be as successful as outcross pollen from unrelated plants in siring ovules and producing seeds (Aizen and Harder 2007). This can be due to self-pollen germinating slower (Aizen et al. 1990) or self-pollen tubes failing to reach ovules (Montalvo 1992). Thus, invasive species have the potential to alter the origin and, therefore, quality of pollen delivered to stigmas. These effects can be even stronger in self-incompatible species where deposition of self-pollen would lead to complete germination failure (e.g. Waser and Price 1991).

Finally, pollinators can mediate interactions between invasive and native plant species by transferring pollen from one plant species to the stigmas of another. This heterospecific pollen (hereafter HP) transfer from invasive species can chemically or physically interfere with conspecific ovule fertilization (Arceo-Gomez and Ashman 2016). HP can allelopathically inhibit conspecific pollen germination and pollen tube growth (Kanchan and Chandra 1980), physically block space for conspecific pollen by clogging the stigma (Waser and Fugate 1986), and potentially grow pollen tubes down the style to compete with conspecific pollen tubes (Ashman and Arceo-Gomez 2013). A meta-analysis of HP receipt and subsequent fitness costs across 20 pairwise comparisons showed invasive plants incurred the most negative effects when donating HP (Arceo-Gomez and Ashman 2016). Invasive plants have been shown to alter the species composition of pollen loads carried by pollinators (Lopezaraiza-Mikel et al. 2007), hence potentially influencing patterns of HP transfer in native communities. However, the extent to which invasive species alter patterns of pollen transfer dynamics among co-flowering species and its potential consequences are not well understood, so the magnitude of invasive effects in the entire community may be underestimated.

Ultimately, invasive plant effects on native plants can vary with each stage of the pollination process e.g. pollinator visitation, pollen deposition, and seed set (Ferrero et al. 2013; Albrecht et al. 2016). Thus, in order to develop a more complete mechanistic understanding of how invasive plants perturb pollination, it is imperative to evaluate potential effects on all levels of the pollination process: pollinator visitation, patterns of conspecific and heterospecific pollen deposition, and reproductive success.

Predicting Variation in Invasive Plant Effects Using Floral Traits

Finding generalizations in invasive plant effects is critical for increasing our ability to predict their effects in natural plant communities (Morales and Traveset 2009) and designing efficient strategies to minimize their impact. However, invasive plant effects have been shown to vary widely across plant species (Sun et al. 2013). For example, when comparing pollinator visitation in single native species stands (*Cistus monspeliensis*, *Cistus salviifolius*, *Anthyllis cytisoides*, and *Lotus cytisoides*) versus those invaded by *Carpobrotus sp*., a facilitative effect was observed for *L. cytisoides*, a competitive effect on both *C. salviifolius* and *A. cytisoides*, and no effect on *C. monspeliensis* (Moragues and Traveset 2005). Variation in invasive species effects among native plant species can also be carried throughout the entire pollination process since Ferrero et al. (2013) found mixed effects in pollinator visitation, CP deposition, pollen tube growth, and fruit set before and after removal of invasive *Oxalis pes-caprae* for *Fumaria muralis*, *Raphanus raphanistrum*, *Vicia faba*, *Brassica oleracea*, *Melilotus italicus*, and *Vicia sativa*. Therefore, even though negative effects of invasive species have been commonly reported (Morales and Traveset 2009), it is clear that we are still far from being able to successfully predict the potential effects of invasive species on native plant communities (Charleboi and Sargent 2017).

In this sense, most of the debate has centered on the potential for overall negative or facilitative effects of invasive species on the pollination of native species (e.g. Morales and Traveset 2009). However, there is no reason to expect that all native species will be affected equally by the invasive. For instance, while humans classify plant species as native/invasive, pollinators prioritize visitation to flowering species based on floral characteristics (e.g. flower size, color, and rewards) available in the community regardless of their invasive status (Charleboi and Sargent 2017). As a result, some native plant species may possess flower/flowering characteristics that make them more susceptible to invasive effects while others may possess characteristics that make them more resistant. One floral trait that could help predict how an invasive may affect a native species' pollination success is floral color (Morales and Traveset 2009). Insect pollinating species typically use floral color as an indicator when deciding which plant species to visit since flower color can be associated with a specific type and/or quantity of floral rewards while foraging (Menzel and Shmida 1993; Fenster 2004). For example, Internicala et al. (2007) showed bumblebees took longer to discriminate visits to deceptive artificial flowers from rewarding artificial flowers when they shared a similar floral color. Therefore, it is possible that native species that share a similar flower color to that of the invasive may be more affected as pollinators may be less likely to discriminate between the two.

Another native plant characteristic that may help predict susceptibility to invasive species effects is the degree of pollinator interaction generalization based on floral symmetry. For instance, insect memory can be programmed to recognize floral symmetry (radial/actinomorphic and bilateral/zygomorphic) as an indicator of floral resources (Neal et al. 1998; Giurfa et al. 1999). In this sense, radial flowers are typically associated with generalist pollinators while bilateral flowers are associated with specialist pollinators. Since invasive plants are typically

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super-generalists, radial flowers may be more vulnerable to effects than bilateral flowers that may possess specialized relationships with specific pollinators. For example, Free (1970) found that honeybees had learned to visit radial floral models over bilateral models even after training for floral rewards on bilateral models. Thus, when native flowers share generalist floral traits with invasive flowers, there could be strong competition for pollinators (Fenster et al. 2004; but see Moeller 2004).

Finally, floral abundance may also be another important factor in determining invasive plant effects on natives (Bartomeus et al. 2008; Ghazoul 2006). Rathcke (1983) proposed that higher floral density would influence pollinator visitation by creating greater chances for interactions between co-flowering species since pollinators efficiently allocate energy for less travel between flowers (Marden and Waddington 1981). Therefore, abundant native species may receive more incidental heterospecific pollen transfer and interact more frequently with the invasive via pollinators compared to less abundant native species (Mitchell et al. 2009).

Spatial Variation in Invasive Plant Effects

Differences in invasive species effects on biodiversity are prominent when comparing small (plot/patch level) study areas (typically negative effects) to community/landscape studies (typically neutral or positive effects) (Powell et al. 2013). Similarly, there can be scale-dependent invasive effects on pollination driven by differential foraging patterns employed by pollinators (Bjerknes et al. 2007; Bartomeus et al. 2010). At the community level, invasive plants may attract more pollinators that concentrate foraging toward large resource pools into the community such as honeybees and bumblebees (Steffan-Dewenter et al. 2002). For example, Jakobsson et al. (2009) found increased pollinator visitation to *Diplotaxis erucoides* when invasive *Oxalis pes-caprae* was present within the community but absent at the smaller-floral neighborhood level. At

the floral neighborhood level, pollinators efficiently allocate energy for less travel between flowers (Marden and Waddington 1981). As a result, invasive flowers with larger numbers of flowers and/or amount of floral rewards (nectar, pollen, floral scents) can steal visits from neighboring native flowers (Totland et al. 2006). For instance, Cariveau and Norton (2009) found decreasing pollinator visitation rates to *Monarda fistulosa* when potted invasive *Carduus nutans* were placed within 1-5 meters. In a different study, Albrecht et al. (2016) found invasive *Oxalis pes-caprae* had a facilitative effect on seed set in *Diplotaxis erucoides* when present at both the floral neighborhood and community levels but a competitive effect when present only at the community level. While scale-dependent invasive effects on pollinator visitation have been well studied (Morales and Traveset 2009), scale-dependent variation in pollen deposition and reproductive success are less understood (Cawoy et al. 2012). Hence, it is prudent to consider spatial scale when determining effects on various pollination stages since focusing on only one scale could misrepresent overall invasive species effects.

Community-level Invasive Plant Effects

Although many studies have evaluated invasive plant effects on the pollination success of a single native species (Morales and Traveset 2009), knowledge of their impacts at the community-level is lacking (but see Traveset and Richardson 2006; Lopezaraiza-Mikel 2007). Knowledge of community-level effects on pollination is essential to predict how entire communities will respond to the increasing spread of invasive species.

Invasive plants typically establish central roles in plant-pollinator interaction networks by donating and receiving pollen from many pollinators (Vila et al. 2009; Albrecht et al. 2014). Even though it seems the inherent generality of plant-pollinator interactions based on visitation makes communities robust to alterations in pollinator and plant community composition

(Bartomeus et al. 2008), super-generalist invasive plants can still affect pollination of native plants by altering pollen transfer dynamics (Aizen et al. 2008; Albrecht et al. 2014). For instance, invasive plants may change the nature of species relationships by functionally replacing key native plant species in the community that typically donate or receive pollen from many species (Vila et al. 2009; Albrecth et al. 2014). Eventually, as native plants adapt to new interactions, changes in patterns of HP receipt can lead to selection for mechanisms that help avoid or tolerate other species' HP and potentially influence their evolutionary trajectory (Ashman and Arceo-Gomez 2013). For example, Dante et al. (2013) found that alien plant species increased flowering synchrony among co-flowering species in a hay field, so flowers receiving facilitative effects from the invasive were clustered with respect to their flowering time. On the other hand, flowers incurring competitive effects may diverge in flowering time and become temporally extirpated from the community (i.e. flowering occurs during a different period) (Aizen and Vazquez 2006; Morales and Traveset 2008). Moreover, potential shifts in the identity and magnitude of HP transfer in communities may cause evolutionary changes favoring reproductive characteristics and mating strategies that avoid or tolerate HP effects resulting from new species interactions via HP transfer (Waser 1978; Ashman and Arceo-Gomez 2013). Consequently, selection may favor pollination mechanisms that avoid pollinators as pollen vectors altogether i.e. selfing (Campbell 1985). In order to cope with increases or altered patterns of HP transfer, native plants may also undergo character displacement, which may alter speed and direction of evolutionary trajectories in floral traits (Armbruster et al. 1994; Ashman and Arceo-Gomez 2013). This can result in reinforcement (when adverse effects cause selection toward traits that reduce reproduction between two species) or isolation of native flowering species (Hopkins and Rausher 2012; Fang and Huang 2016). Such divergence can occur in traits such as flower color

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(Hopkins and Rausher 2012), stigma size and surface area (Yang et al. 2002), stigma secretions, and receptivity (Madjidian et al. 2012). Finally, it is possible that competition for pollinators could alter patterns of species coexistence by decreasing overall native plant reproductive success and ultimately change the assembly of floral species in a community (Ghazoul 2006; Ashman and Arceo-Gómez 2013; Arceo-Gomez et al. 2015). However, in spite of their potential ecological and evolutionary importance, community-level changes in HP transfer among plant species (e.g. Fang and Huang 2013; Tur et al. 2016) as a result of plant invasion have rarely been studied (but see Emer et al. 2015; Johnson and Ashman 2018); therefore, the full effects of plant invasion on native plant communities are only beginning to be understood.

Utilizing Network Analyses in Community-level Studies

Network analyses can be an important tool that helps broaden understanding of how invasive plants affect plant communities in space and time (Olesen et al. 2008; Dupont et al. 2009; Tylianakis and Morris 2017). Network construction is not a new concept in ecology (Elton 1927), and quantitative network analyses have been used in studies on food webs (Memmott et al. 1994), gene and protein interaction studies, and studies on community-level plant-animal interactions (Bascompte and Jordano 2007). These networks are constructed by representing network components (species, proteins, genes) as nodes and interactions connecting them (predation, travel, methylation, and parasitism) as links. In the context of plant-pollinator interactions, plants and pollinators are depicted as nodes and each visit of a specific pollinator to an individual plant species as a link. From these networks, several metrics can be estimated that reflect an ecological aspect of the overall community (Bascompte and Jordano 2007). The average number of links per species shows how many partners with which each species interacts and reflects the magnitude of interconnectivity within the overall network (i.e. community)

(Emer et al. 2015). Linkage density (number of links per species divided by the total number of species) describes the diversity in links per species as an average of vulnerability (e.g. average number of pollen donor species received per stigma species) and generality (average stigma species per pollen donor species) (Bersier et al. 2002). Weighted connectance is the linkage density divided by the total number of species (number of links per species divided by the total number of species divided by the total number of species) (Tylianakis et al. 2007). Weighted nestedness reflects the frequency to which generalist species interact with specialized ones and ranges from 0, perfect chaos, to 1, perfect nestedness (Galeano et al. 2007). H_2' measures the specialization level in interacting species that is derived from the Shannon entropy and ranges from 0, no specialization, to 1, complete specialization (Bluthgen et al. 2006; Emer et al. 2015; may appear as H2' or H'2 in later references). Finally, the number of modules reflects groups of species that interact with one another more closely than with species in other modules (Dormann and Strauss 2014; Newman 2003; Leicth and Newman 2008).

Species' roles in networks can also be described through analyzing network indices at the species level. Some of the species-level metrics commonly used are normalized degree (number of links adjusted by the total number of possible partners), species strength (number of dependents), weighted closeness (centrality or measure of focally connected species based on shortest path length), and d' specialization (interaction specialization) (Bluthgen et al. 2006; Dormann et al. 2008; Popic et al. 2012; Ballantyne et al. 2015; Ballantyne et al. 2017). Even if there are no changes in overall network structure (e.g. mean quantity of HP deposition across species), changes in species-level metrics (i.e. frequency and identity to which individual species interact) may still have ecological and evolutionary consequences in the community (e.g. character displacement, divergence of floral traits, and reinforcement) (Armbruster et al 1994;

Hopkins and Rausher 2012; Ashman and Arceo-Gomez 2013). For example, high values for

weighted closeness or centrality would suggest generalization (links with many other species) as

a focal species by occupying a central position within the network, so this species would have

shorter path distances to all other nodes and interconnect the network (Ballantyne 2017).

Furthermore, a low value would suggest specialization (links with few other species) as a

marginal species occupying a peripheral position within the network, so this species would have

longer path distances to all other nodes (Gonzalez et al. 2010). Should a species' weighted

closeness (centrality) within the network change, it may influence the identity of transfer for

many other species and influence evolutionary trajectory for the entire community.

Network analyses can be also used to uncover novel interaction patterns among plant

species via pollen transfer using data on HP found on stigmas of plants in a community known as

plant-plant HP deposition networks (e.g. Fang and Huang 2013). These networks provide

community-level information on direct, plant-plant interactions in floral communities that may

lead to evolutionary developments in community assembly and floral evolution (Ashman and

Arceo-Gomez 2013). For example, Fang and Huang (2013) described specialization and

generalization of a co-flowering community and found that floral characteristics such as stigma

position correspond to a species' position within the pollen deposition network. Furthermore,

Tur et al. (2016) used HP deposition networks to compare quantitative and qualitative

relationships between plant species across various elevations and found facilitation in more harsh

environments at high elevations whereas lower elevations contained more neutral or competitive

relationships. However, information concerning invasive plant effects on HP deposition

networks between invaded and non-invaded communities is mostly lacking. In the two studies

conducted to date, Emer et al. (2015) found no difference in network properties between sites

invaded by *Impatiens glandulifera* and non-invaded sites while Johnson and Ashman (2018) showed fewer connections between species in networks invaded by *Pennisetum setaceum*.

Study Species: *Cirsium arvense* (Asteraceae) Canadian Thistle

Cirsium arvense, Asteraceae, commonly known as creeping or Canadian thistle, is a perennial native to Eurasia that flowers from June to September (Tiley 2010). Usually occurring in open or disturbed areas, *C. arvense* has spread throughout a large portion of the United States (Tiley 2010). Harmful economic effects of *C. arvense* are due to its piercing leaf spines and its seeds that spread to agricultural fields and contaminate feed (Tiley 2010). However, speedy asexual reproduction through prolific underground root systems allow *C. arvense* to grow in dense patches (part of a site covered mostly or entirely by *C. arvense*) and resist management techniques (Tiley 2010). *C. arvense* stands also threaten to extirpate native species by efficiently competing for space, light, and nutrients (Levine 2003). In addition to highly successful exploitative competition, the roots and leaf litter may also suppress local plant growth through allelopathy (Stachon and Zimdahl 1980). Even though *C. arvense* may adversely affect nearby plants, it may also influence pollination dynamics of more distant plant species in the community.

C. arvense belongs to the composite family (formerly Compositae) Asteraceae. This family is distinguished by its floral arrangement in which many small flowers are clustered into a capitulum or composite flowering "head", which may be commonly mistaken for a single flower. In this study, I have used the term "floret" to refer to a single flower within the capitulum. In *C. arvense* a single capitulum (head) comprises approximately 100 florets. The florets are all tubular, with five short lobes. The florets are subtended (enclosed basally) by a broad ring of prickly, leafy bracts called the involucre. Although a head or capitulum contains many individual

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florets, the entire capitulum functions as the unit of attraction for pollinators (Morhardt and Morhardt 2004). In keeping with other pollination studies of *Cirsium* and other Asteraceae species, the entire head is here regarded as a "flower". Thus, the floral symmetry for *Cirsium arvense* flowers is radial, owing to the circular arrangement of the many florets comprising the head. (Moore 1975).

C. arvense is near-dioecious (Lloyd and Myall 1976). The floret structure includes both male (stamen) and female (pistil) reproductive structures, but each individual plant functions physiologically as either male or female (Tiley 2010) since the heads (hereafter "flowers") of each individual plant produce all physiologically male or female florets. Female plants bear vestigial stamens that do not produce pollen, while male individuals bear sterile pistils (Tiley 2010). In some cases, male individuals have been shown to produce seeds, albeit smaller with reduced viability (Tiley 2010). As a result, pollen transfer between individuals is imperative for fertilization. *C. arvense* plants grow large, showy capitula (10-22 mm in diameter) with about 100 florets per capitulum on average (Moore 1975). Individual *C. arvense* shoots have been observed producing between 32-69 capitulate heads (Bakker 1960). These capitula produce a wide range of floral rewards (Tiley, 2010). For instance, a single *C. arvense* capitulum contains approximately 4,000 pollen grains and produces 2609µg (+/- 239) of nectar sugar, which makes it highly attractive to pollinators (Tiley 2010; Hicks et al., 2016).The introduction of common chemical scents may also alter the dynamics of native, mutualistic interactions by further integrating *C. arvense* into native pollination webs. For example, the main compounds in invasive *Cirsium arvense* floral scent (phenylacetaldehyde, methyl salicylate, dimethyl salicylate, pyranoid linalool oxide, and benzaldehyde) attract many pollinators (El-Sayed et al. 2007). However, these also attract florivores that may also visit native plants nearby (Theis

2006). Even though these emissions decrease when florivores are present (Theis 2007), this trait potentially attracts florivores to native plants if they grow near *C. arvense.*

Study Questions

In this study, we will evaluate the effect of invasive *C. arvense* on the pollination and reproductive success of local plant species in a community. We will also evaluate if the effects depend on specific characteristics of local species or on spatial scale and assess its effect on community-wide interactions via pollen transfer. Specifically, we ask the following questions:

Q1. Do invasions by *C. arvense* affect pollinator visitation, average conspecific and heterospecific pollen deposition, and reproductive success of local plant species?

Q2. Are *C. arvense* effects on the pollination success of local species stronger on species that share similar flower color, have generalized pollinator interactions, and/or are more abundant in the community?

Q3. Do *C. arvense* effects on pollination of local plant species vary with spatial scale (floral-neighborhood vs community-wide scale)?

Q4. Does invasion by *C. arvense* alter the overall structure of plant-plant heterospecific pollen deposition networks or cause changes in individual species roles within invaded networks?

CHAPTER 2

MANUSCRIPT FORMATTED FOR SUBMISSION TO PLOS ONE

Direct and Indirect Effect of Invasive *Cirsium arvense* on Pollination in Southern Appalachian Floral Communities

Jesse D. Daniels and Gerardo Arceo-Gomez

East Tennessee State University

Abstract

Invasive plants can alter pollination dynamics in invaded communities by disrupting patterns of pollinator visitation, pollen transfer dynamics (conspecific [CP] and heterospecific [HP]), and reproductive success. The direction of invasive effects (competitive, neutral, and facilitative) may be partially determined by spatial scale and species' floral traits. Here, we investigated pollinator visitation, CP and HP receipt, and pollen tube growth for species in a *C. arvense* present community and non-present community at two scales. At the community-level, the effect of *C. arvense* on pollinator visitation varied among species. Floral symmetry seemed to explain this variation. At the floral neighborhood-level, we found competitive effects for pollinator visits and mixed effects on CP deposition. The overall structure of plant-plant HP deposition networks was slightly altered. We observed lower average centrality across shared species in the *C. arvense* present community suggesting *C. arvense* had subverted their roles as pollen donors.

Introduction

Globalization and international trade has granted plant species unprecedented distribution to new areas across the globe. As a result, 13,168 plant species have become naturalized outside their native range (van Kleunen et al., 2015). These invasive plant species can directly exploit local resources and directly or indirectly interfere with local plant growth and

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development (Callaway and Aschehoug, 2000; Levine et al., 2003) and contribute to a decline in native plant biodiversity (Vila et al., 2011).

One of the most studied impacts of invasive species is their effect on mutualistic associations between native plants and their pollinators (Traveset and Richardson, 2006; Richardson et al., 2007). Pollination by animal vectors is paramount in generating and maintaining angiosperm biodiversity (Fenster et al., 2004; Ollerton et al., 2009) since approximately 87% of flowering plant species require animal pollinators to produce seeds (Ollerton et al., 2011). Invasive flowers can affect pollination of local species by modifying pollinator behavior because they typically produce large, showy floral displays containing copious amounts of rewards (i.e. pollen, scent, and nectar) that attract a plethora of generalist pollinators (Traveset and Richardson, 2006; Vila et al., 2009; Powell et al., 2011). These generalist pollinators can then help integrate invasive plants into local pollination systems (Memmot and Waser, 2002). Once integrated, invasive plants can exert positive (Thomson, 1978; Moeller, 2004; Ghazoul, 2006; Molina-Montenegro et al., 2008), neutral (Jakobsson and Padron, 2014; Sun et al., 2013; Goodell and Parker, 2017), or negative (Brown, Mitchell, and Graham, 2002) effects onto local plants.

While some invasive species have been shown to facilitate pollinator visitation to local flowers (Thompson, 1981; Ghazoul, 2006; Lopezaraiza-Mikel et al., 2007), most studies have found a decrease in flower visitation to native, local species (reviewed in Morales and Traveset, 2009). This suggests that negative invasive species effects might be pervasive. However, facilitative or competitive effects on pollinator visitation may not always translate to differences in plant reproductive success (e.g., Jakobsson and Padron, 2014; Sun et al., 2013; Goodell and Parker, 2017). For instance, pollinators vary in pollen removal and deposition rates (Thomson

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and Goodell, 2001; Wilson and Thomson, 1991); therefore, quantifying pollinator visits alone does not necessarily reflect visit quality i.e. quantity and quality of pollen deposited (King et al., 2013). For example, even if pollinator visitation remains unaltered, reductions in the amount of conspecific pollen (hereafter CP) deposited on stigmas can lead to a decrease in reproductive success (Flanagan et al., 2009). Furthermore, invasive species may also influence CP quality by decreasing pollinator visitation in self-compatible species, which may increase selfing rates. While many species have mixed mating systems (Goodwillie et al., 2005), self-pollen is typically less successful at siring seeds (Aizen and Harder, 2007) due to slower germination (Aizen et al., 1990) and self-pollen tubes failing to reach the ovules (Montalvo, 1992). In self-incompatible species, self-pollination can lead to seed abortion and reduce the number of ovules that would otherwise be available for legitimate outcross pollination (Waser and Price, 1991).

Invasive plants have also been shown to alter pollen species composition carried by pollinators and influence patterns of HP transfer in native communities (Lopezaraiza-Mikel et al., 2007). Heterospecific pollen from other plant species (hereafter HP) can physically block space for conspecific pollen by clogging the stigma (Waser and Fugate, 1986), chemically (i.e. allelopathically) inhibit conspecific pollen germination and pollen tube growth (Kanchan and Chandra, 1980, Morales and Traveset, 2008), and/or potentially grow pollen tubes down the style to compete with conspecific pollen tubes (Arceo-Gomez and Ashman, 2013). Ultimately, these mechanisms can lead to decreased ovule fertilization and reproductive success of local plant species (Arceo-Gomez and Ashman, 2016). A meta-analysis of HP receipt and subsequent fitness costs across 20 pairwise comparisons showed invasive plants incurred the most negative effects when donating HP (Ashman and Arceo-Gomez, 2013). However, even though the effects

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of invasive species on native species pollination success have been well studied, the underlying

mechanism have not (Charlebois and Sargent, 2017).

While invasive plants typically impose negative effects on native plant species (Morales

and Traveset, 2009), wide among-species variation in invasive species effects have been

observed (Sun et al., 2013; Moragues and Traveset, 2005). Consequently, a consensus regarding

the direction of their effects has been hard to achieve (Charlebois and Sargent, 2017). Although

most studies to date have centered in testing for overall negative or facilitative effects (Morales

and Traveset, 2009), there is no reason to expect that all native species will respond similarly to

the presence of the invasive (Charlebois and Sargent, 2017). Thus, when considering multiple

native species, a better approach would be to examine when positive or negative effects can be

expected based on a species' floral characteristics.

Insect pollinating species typically use floral color as an indicator of specific types and/or

quantities of floral rewards (nectar, pollen, oils) while foraging through learning or innate

behavior (Menzel and Shmida, 1993; Fenster, 2004). For example, Internicola et al., (2007)

showed bumblebees took longer to discriminate visits to deceptive artificial flowers from

rewarding artificial flowers when they shared a similar floral color. Therefore, an invasive plant

may facilitate pollinator visitation to local flowers with similar coloration since they will receive

visits from pollinators that have learned to visit the invasive flower color (Johnson et al., 2003;

Moeller, 2004; Bjerknes et al, 2007; but see Morales and Traveset 2009)

Flower-pollinator interaction generalization based on floral symmetry

(radial/actinomorphic versus bilateral/zygomorphic) may also help determine invasive effect

(Free, 1970; Neal et al., 1998; Giurfa et al., 1999). Radial flowers typically attract many

pollinators that can easily collect their resources while bilateral flowers attract few pollinators

that specialize in collecting their resources. In this sense, we could expect that local generalized species (e.g. flowers with radial symmetry) may be more likely to experience competition (Morales and Traveset, 2009) for pollinators (but see Charlebois and Sargent, 2017). Thus, we expect that super-generalist invasive plants may be more likely to impose competitive effects on local species sharing generalist characteristics (Fenster et al., 2004; Morales and Traveset, 2009; Gibson et al., 2012; Goodell and Parker, 2017) because they will attract and share the same community of pollinators.

Floral abundance may be another factor mediating invasive species effects (Ghazoul, 2006; Bartomeus et al., 2008; Olesen et al., 2008). Rathke B. (1983) proposed higher floral densities influence pollinator visitation by creating greater chances for interactions since pollinators efficiently allocate energy for less travel between flowers (Marden and Waddington 1981). For instance, abundant local species may have increased potential of pollinator-mediated interactions with invasive species and, as a result, receive more incidental heterospecific pollen (Mitchell et al., 2009).

Invasive species effects on the pollination of local plant species may also be scale-dependent due to differential foraging behavior employed by pollinators (Bjerknes et al., 2007). Some pollinators have different foraging ranges. For example, honeybees and bumblebees can travel kilometers to find resources while flies, beetles, and moths travel shorter distances (meters). Hence, the scale at which invasive species impose effects on local plant species may depend on the dominant pollinator groups in the community. At a large-scale community-scale, invasive flowers may attract more pollinators that concentrate foraging on large resource pools or dense patches, such as honeybees and bumblebees, potentially increasing pollinator availability in the entire community (Thomson, 1981; Steffan-Dewenter et al., 2002; Jakobsson

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et al., 2009). However, at the smaller floral neighborhood scale, pollinators may optimize energy use (Marden and Waddington 1981) and concentrate foraging on patches of invasive flowers with superior floral rewards (nectar, pollen, floral scents). Thus, dense invasive patches can decrease visits to neighboring local species (Westphal et al., 2003; Totland et al., 2006; Cariveau and Norton, 2009). While scale-dependent invasive effects on pollinator visitation have been well studied (Morales and Traveset, 2009), scale-dependent variation in pollen deposition and reproductive success is less understood (Cawoy et al., 2012; Albrecht et al., 2016; Bruckman and Campbell, 2016).

Furthermore, while much is known on the effects of invasive species on individual native species, little is known about effects on entire plant communities. Knowledge of community-level effects on pollination is essential in order to predict how entire communities will respond to the increasing spread of invasive species. For instance, even if average HP deposition to local stigmas is not affected, there may be shifts in patterns of plant-plant interactions via pollen transfer (Fang and Huang, 2013) that alter community structure such as connectivity between local species (Aizen et al., 2008).

Since invasive plants have varying impacts on local plants, it is necessary to focus on population-level impacts at the species-level to help explain community-level effects (Stout and Tiedeken, 2016). Invasive plants can also fill key roles in pollen transfer networks in invaded communities when they are focal species in their native range (Emer et al., 2016) or fill unoccupied niches (Stouffer et al., 2014), so new connections between local species and the invader may shift pollen donor identity throughout the community. Adverse effects on reproduction resulting from new HP donors could drive reinforcement process or exert selection pressures on local flowering species toward traits that reduce or eliminate new plant-plant

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interactions via HP transfer (e.g., Campbell, 1985; Yang et al., 2002; Aizen and Vazquez, 2006; Hopkins and Rausher, 2012; Madjidian et al., 2012; Fang and Huang, 2016). However, in spite of their importance, community-level changes in HP transfer among plant species (Tur et al., 2016) as a result of plant invasion (Emer et al., 2015) have rarely been studied; therefore, the full effects of plant invasion on local plant communities may be underestimated.

In order to develop a more complete mechanistic understanding of how invasive plants affect local species pollination success, it is imperative to evaluate potential effects on all levels of the pollination process (pollinator visitation, patterns of conspecific and heterospecific pollen deposition, and reproductive success) at various scales. It is also important to consider the role of floral traits and other plant characteristics in mediating invasive species effects. Combined, such knowledge will help take our understanding of invasive species effects from a descriptive to a more predictive stage. In this study, we ask the following specific questions: 1) Do invasions by *C. arvense* affect pollinator visitation, average conspecific and heterospecific pollen deposition, and reproductive success of local plant species? 2) Are *C. arvense* effects on the pollination success of local species stronger on species that share similar flower color, have generalized pollinator interactions, and/or are more abundant in the community? 3) Do *C. arvense* effects on pollination of local plant species vary with spatial scale (floral-neighborhood vs community-wide scale)? 4) Does invasion by *C. arvense* alter the overall structure of plant-plant heterospecific pollen deposition networks or cause changes in individual species roles within invaded networks?

Materials and Methods

Invasive species: *Cirsium arvense* (Asteraceae)

Cirsium arvense, Asteraceae, commonly known as creeping or Canadian thistle, is a perennial herb native to Europe and northern Asia that occurs in open or disturbed areas and has spread throughout a large portion of the United States (Tiley, 2010). *C. arvense* plants typically flower from June to September (Tiley, 2010). *C. arvense* is a near-dioecious species (separate male and female plants) (Lloyd and Myall, 1976; Tiley, 2010). Because of their near-dioecious syndrome, pollen transfer between different individuals is imperative for fertilization. The floret structure is hermaphroditic containing both male and female reproductive structures but the entire plant functions as either male or female (Tiley, 2010). In some cases, male individuals have been shown to produce smaller seeds with reduced viability (Tiley, 2010). *C. arvense* plants grow large, showy capitula (10-22 mm diameter) with each containing about 100 florets on average (Moore, 1975). Individual *C. arvense* shoots have been observed producing 32-69 capitulate heads (Bakker, 1960). Although a capitulum contains individual florets, the entire capitulum functions as the unit of attraction for pollinators (Morhardt and Morhardt, 2004). A single capitulum produces floral scents that commonly attract pollinators (mainly benzaldehyde and phenylacetaldehyde) (Theis, 2006; El-Sayed *et al.*, 2008), approximately 4000 pollen grains (Tiley, 2010), and 2609μg (+/- 239) of nectar sugar per male capitula (Hicks et al., 2016). This combination of floral signals and rewards attracts a diverse community of floral visitors including bees, wasps, moths, beetles, butterflies, and other varieties of insects (Tiley, 2010) thus; the spread of *C. arvense* to local plant communities has the potential to alter pollinator visitation rates and pollen deposition to local floral species.

Study Sites and Communities

The study was conducted in eastern Tennessee, which is a highly biodiverse area projected to fall within one of the largest hotspots for future biological invasions resulting from global trade and global climate change (Bellard et al., 2013). Therefore, it is critical to understand the overall effects of invasive species on local floral communities in this region. This study was carried out at the Hampton Creek Cove (hereafter HCC) State Natural Area in Carter County, Tennessee. Within HCC, one early successional field with *C. arvense* present (hereafter SCAP; Site with *Cirsium arvense* present) and another without *C. arvense* (hereafter SCAA; Site with *Cirsium arvense* absent) were selected. The SCAP (N 36°08.843', W 82°02.794', elevation – 3171ft.) was an abandoned field adjacent to a creek and highway containing a *C. arvense* infestation with an estimated ground cover of approximately 30% and 22 capitula per square meter (Daniels J. unpublished data). The SCAA (N 36°08.840', W 82°02.791, elevation – 3453ft.) was located within grazed pastureland along a hiking trail and contained no *C. arvense*. Sites were approximately 1 km apart and embedded in a forest and grazed field matrix.

Because our goal was to evaluate the effects of *C. arvense* on pollination success and pollen transfer dynamics of local co-flowering plants, only plant species that overlap in flowering with *C. arvense* (between June and July) were considered in this study. Hereafter, all floral displays are referred to as flowers (see Table 9 for classification of floral structures). We excluded rare plant species (less than 0.1% of the total number of flowers at each community across the entire season; $N = 8$) because these contributed very little to the overall pollination dynamics in these communities (less than 1% accumulated total pollinator visits throughout the season). In total, we included 22 species at the SCAA and 24 at the SCAP of which 20 species (91%) were shared between the two sites (Table 9). Voucher specimens for all flowering plants

within each site are deposited at the John C. Warden Herbarium (ETSU). The similarity in the species composition of the pollinator community between sites was 72.4% (Morisita-Horn index; Daniels J. unpublished data).

To evaluate *C. arvense* effects on the pollination success of local plants, we established 1x2m plots at each site (31 plots in the SCAA and 26 plots in the SCAP site, 57 total). Plots were at least 5m apart and distributed across the site ensuring all plant species were present in at least one plot and that plots captured site-level floral abundance for each species at each site. The study was conducted during the *C. arvense* flowering season (June-July) of 2017.

Pollinator Visitation Rate

To determine if *C. arvense* lowers pollinator visitation to local flowers, we recorded pollinator visitation to flowers of local plant species within each plot (see above). Each plot was observed for 5 minutes during peak time of pollinator activity from 8 a.m. to 5 p.m in the morning, midday, and evening when possible. During each observation period, the number of pollinator visits to each plant species and floral abundance were recorded. A visit was only recorded if the pollinator made contact with the anther and/or stigma. Observations took place once a week for 7 weeks at the SCAA (27.3 hrs. total observation hours) and 6 weeks at the SCAP (25.8 hrs. total). Visitation was recorded during sunny and partially cloudy but warm weather conditions. Before recording pollinator visitation, each plant species within each plot was identified and the number of open flowers or capitula in the case of composite flowering heads (e.g., Asteraceae) was recorded for every species (Table 9). Visitation rates were calculated as number of visits/number of open flowers/minutes observed for every species in each plot.

Pollen Deposition and Reproductive Success

To determine if *C. arvense* lowers average CP, increases average HP deposition, and decreases reproductive success (pollen tube growth), approximately 10 styles per plant species/day were sampled once a week for 7 weeks. 10-37 total styles were collected per species depending of the length of the flowering period and their availability at a site (except for GE where we could only collect 5 styles at the SCAP). Styles were collected from wilting flowers from randomly selected plants (one style per plant) inside the plots at each site. In the case of composite flowering structures (Asteraceae), individual styles were collected by alternating selection from the middle, side, or edge per plant. If not enough plants were found inside the plots, styles were collected from plants near the plots until 10 styles were collected. Styles taken near plots were categorized as styles taken from those plots. Styles were stored in 1.5mL microcentrifuge tubes containing 70% isopropyl alcohol in the field. To quantify CP and HP deposition, styles were softened with KOH and stained following standard procedures (e.g. Arceo-Gomez and Ashman 2014, Arceo-Gomez et al. 2015), and mounted onto slides. Pollen grains on stigmas were identified and counted for every style using a compound light microscope. In order to identify each pollen grain accurately, a pollen grain reference library was created by harvesting pollen from the anthers of field and voucher specimens for every species to reference with pollen found on stigmas. Some pollen morphs of related species which were similar and could not be differentiated and were grouped together (12 species in 5 pollen donor groups, see appendix). To determine if *C. arvense* decreases average pollen tube growth, the number of pollen tubes at the base of collected styles was counted using a fluorescence microscope (Dafni A. 1992; Arceo-Gomez and Ashman, 2014). The number of pollen tubes at the base of the style is considered a reliable measure for capturing quantitative and qualitative

pollination effects on the likelihood of reproductive success (Weller and Ornduff, 1989; Snow

and Spira, 1991; Waser and Price 1991; Aizen and Feinsinger, 1994; Ghazoul et al,. 1998;

Cascante et al., 2002; Aguilar and Galetto, 2004; Ashman and Arceo-Gomez, 2011; Alonso et

al., 2012; Alonso et al., 2013; Bruckman and Campbell, 2016; Mazer et al., 2016).

Floral Traits and Abundance

To evaluate if local species that are similar in flower color to *C. arvense* experience

positive effects compared to those species that are not, we considered plant species with a purple

hue color similar to *C. arvense* capitula (in the human vision spectrum) as 'similar' in color (6

species). All other species with a different hue were considered dissimilar (18 species). To

determine if plant species with generalized pollinator relationships incur negative effects from *C.*

arvense, we categorized all local species based on their floral symmetry with actinomorphic

(radial symmetry) (17 species) as generalist and zygomorphic (bilateral symmetry) (7 species) as

specialist (Table 9). Although *Trifolium* species contain a radial cluster of flowers, their lipped,

bilaterally symmetrical floral shape produces specialized pollinator relationships with bees (N=3)

(Fenster et al., 2004; Van Kluenen et al., 2008; Lazaro and Totland, 2014). To evaluate if

abundant local species are more negatively affected by the presence of the invasive species,

relative floral abundance for each species at each site was calculated by taking the number of

flowers recorded throughout the season divided by the total number of flowers in the site (Table

9). This metric represents seasonal proportional abundance for each species relative to the total

abundance of flowers at a site.

Data analysis

Generalized mixed models were used to compare pollinator visitation rates

(visits/flower/min), pollen deposition (total number of CP and HP grains), and pollen tube

number at the base of the style between sites. To evaluate overall differences between the two plant communities, we considered site, plant species, and their interaction as fixed effects. Week and plot nested within site were considered as random effects. Visitation rate and was analyzed using a gamma error distribution while CP, HP, and pollen tube success were analyzed using a Poisson error distribution. Because we wanted to test for an interaction between site and plant species, only the species that were shared between the two sites were considered. However, the significance of the main effects (i.e. site and plant species) did not change when the entire data set was analyzed (Daniels J. unpublished). Thus, 19 species were used for analyses of visitation rate and 17 species for analyses of pollen deposition (CP and HP) and pollen tube success. When significant site x species interactions were found, mixed models were used to determine if floral symmetry, floral abundance, or flower color mediated the differential responses of plant species at each site. For this, site, symmetry, color, abundance, and the interactions of site with each predictor were considered as fixed effects. Species identity was considered a random effect.

To evaluate *C. arvense* effects at the level of the immediate floral neighborhood at the SCAP, plots were categorized by when *C. arvense* flowers were present, hereafter PCAP (*N*=11), and when *C. arvense* was absent, hereafter PCAA (*N=23*). As a result, plots could switch from week to week between PCAP and PCAA depending on *C. arvense* flowering phenology. Although there were a low number of PCAP, the entire *C. arvense* flowering patch was represented. There were 17 shared flowering species within both plot types. For floral neighborhood level analyses (PCAP vs. PCAA), plot status, plant species, and their interactions were considered fixed effects. Week was considered a random effect. Visitation rate was analyzed using gamma error distribution while CP, HP, and pollen tube growth were analyzed using a Poisson error distribution. In order to test for interaction between plot status and plant

species, only shared species in both PCAA and PCAP were considered. 10 species were used for pollinator visitation analysis and 17 species were used for pollen deposition (CP and HP), and pollen success analyses. When significant plot status x species interactions was found, mixed models were used to determine if floral symmetry, floral abundance, or flower color mediated the differential responses of plant species in each plot type. Here, plot status, symmetry, color, abundance, and the interactions of plot status with each predictor were considered as fixed effects. Species identity was considered a random effect. All analyses were conducted in SAS 9.3.

HP Deposition Network Construction and Analysis

To determine if *C. arvense* alters the structure of pollen deposition networks, we constructed matrices of plant-plant interactions via HP transfer using the average number of HP grains from each pollen donor species found on each recipient stigma (e.g. Fang and Huang 2013). For each matrix, rows represent plant species that receive pollen while columns represent pollen donor species. We then used this matrix to construct a bipartite interaction network in R 3.4.2 (e.g. Emer et al., 2015). The following metrics were used to describe the overall structure of each network: links per species (total number of links divided by the total number of species), linkage density (number of links per species divided by the total number of species) (Bersier et al. 2002), weighted connectance (realized HP transfer links/all possible HP transfer links)(Tylianakis et al. 2007), weighted nestedness (frequency of generalist species interacting with specialized species) (Galeano et al., 2007), H_2' is an indicator of overall network specialization, and modularity (where a module contains species that interact with one another more closely than other species) Dormann and Strauss, 2014; Newman 2003; Leicth and Newman 2008; Fang and Huang, 2013, Emer et al., 2015). All these metrics have commonly been used to describe

pollen transfer networks and other interaction networks (Memmott, 1999; Bascompte et al., 2003; Olesen et al., 2008; Popic et al., 2012; Ballantyne et al., 2015; Ballantyne et al., 2017). To determine if links per species, linkage density, weighted connectance, weighted nestedness, and H_2' specialization were significantly different from random in both communities, the observed values were compared against average values from 1000 randomly generated networks (Dormann et al., 2009). To determine if the presence of *C. arvense* alters individual species' roles as key pollen donors or pollen recipients in the network, we calculated species-level metrics from the same networks. These include: normalised degree (number of links calibrated with number of possible partners), species strength (number of dependents), weighted closeness (centrality), and d' specialization (based on difference from random partners) (Dormann et al., 2008; Popic et al., 2012; Ballantyne et al., 2015; Ballantyne et al., 2017). All network metrics were calculated using the bipartite package in R 3.4.2. Species-level metrics were compared between shared pollen donors and shared pollen recipients in both communities using mixed models. Site was considered a fixed effect. Plant ID was considered a random effect. Mixed models were conducted in SAS 9.3.

Results

Community-Level

   Pollinator Visitation. We recorded 10,707 visitation events from pollinator species belonging to the orders Hymenoptera, Hemiptera, Diptera, Coleoptera, and Lepidoptera. Bees were represented by the following families: Apidae, Halicitidae, Megachilidae, Colletidae, Andrenidae, and Melittidae. Wasps were represented by the following families: Pompilidae, Leucospidae, Cynipidae, Vespidae, and Sphecidae. Percentages of total visits for each group varied between communities (Table 1).

Table 1. Percentages of total pollinator visits from morph groups at both communities.

Community	Hymenoptera				Coleoptera and Hemiptera	Diptera	Lepidoptera
	Apis	*Bombus*	**Bees**	**Wasps**			
SCAA	4.19%	16.1%	29.8%	6.66%	12.9%	24.1%	6.38%
SCAP	9.76%	14.9%	36.3%	2.1%	13.7%	21.7%	1.52%

1730 visits were recorded at the SCAA and 8977 visits were recorded at the SCAP. We did not find significant differences in pollinator visitation rate to flowers of local plant species at the SCAP (0.006 ± 0.0007 visits per flower per minute, mean $\pm$ SEM; Table 2A) versus the SCAA (0.00653 ± 0.0012 visits per flower per minute, mean $\pm$ SE; Table 2A). We found significant differences in visitation rate (Table 2A) among plant species. However, we also found a significant plant species x site interaction (Table 2A) suggesting that pollinator visitation rate to each plant species depended upon which site it was found. When we tested if this interaction was explained by any of our predictors (floral symmetry, color, and abundance), we found a marginally significant interaction between site and floral symmetry (Table 3) where radial flowers at the SCAA received higher visitation rates than radial flowers at the SCAP ($P=0.0526$, Pre-planned Comparison). None of the other predictors (flower color and abundance) were significant (Table 3).

Table 2. *Community-Level Invasive Effects on Pollination.* Results from generalized mixed models on community-level pollination between SCAA and SCAP. (A) Visitation Rate. (B) CP Deposition. (C) Pollen Tube Growth. (D) HP Deposition.

Effect	A. Visitation Rate				B. CP Deposition				C. Pollen Tube Growth				D. HP Deposition			
	Num DF	Den DF	*F* value	*P* value	Num DF	Den DF	*F* value	*P* value	Num DF	Den DF	*F* value	*P* value	Num DF	Den DF	*F* value	*P* value
Site	1	50	1.5	0.23	1	1	0.39	0.64	1	1	1.43	0.44	0	0	0.18	0.74
Plant Species	18	330	6.33	**<0.01**	16	1	17.5	0.19	16	1	6.67	0.3	16	1	4.83	0.34
Site*Plant Species	13	330	2.11	**0.01**	16	1	1.96	0.51	16	1	1.51	0.57	16	1	1.2	0.63

Table 3. *Site Floral Trait Mixed Model Results*. Results from mixed model for which floral traits explain the site and plant species interaction.

Effect	Mean Visitation Rate			
	Num DF	Den DF	*F* value	*P* value
Site	1	14	0.64	0.44
Symmetry	1	14	0	0.99
Color	1	14	0.32	0.58
Abundance	1	14	0.01	0.92
Site*Symmetry	1	14	5.01	**0.04**
Site*Color	1	14	2.65	0.13
Site*Abundance	1	14	0.73	0.41

Pollen Deposition and Pollen Tube Growth. At the SCAA, we analyzed 363 styles from which we counted a total of 17407 CP grains, (range: 8.8-200.5/species), 872 HP grains (range: 0.06-16.2/species), and 669 pollen tubes (range: 0.35-6.8/species). At the SCAP we analyzed 343 styles from which we counted 16665 CP grains (range: 8.41-214.62/species), 786 HP grains (range: 0.15-12.23/stigma), and 557 pollen tubes (range: 0.29-5.9/species). We did not find any significant differences between sites nor interactions between site and individual plant

species for conspecific pollen deposition, pollen tube success, or heterospecific pollen deposition (Table 2B-D).

Floral-neighborhood

Pollinator Visitation. 5410 visits were recorded in PCAA and 792 visits were recorded in PCAP. We found significantly higher pollinator visitation rate in PCAA (0.00717 ± 0.001, mean ± SEM; Table 4A) compared to PCAP (0.00252 ± 0.00063, mean ± SEM; Table 4A). Visitation rates were significantly different among plant species, but there was no significant interaction between plant species and site (Table 4A).

Table 4. *Floral Neighborhood Level Invasive Effects on Pollination*. Results from generalized mixed models on floral neighborhood-level pollination between plot statuses. (A) Visitation Rate (B) CP Deposition (C) Pollen Tube Growth (D) HP Deposition.

Effect	A. Visitation Rate				B. CP Deposition				C. Pollen Tube Growth				D. HP Deposition			
	Num DF	Den DF	*F* value	*P* value	Num DF	Den DF	*F* value	*P* value	Num DF	Den DF	*F* value	*P* value	Num DF	Den DF	*F* value	*P* value
Plot Status	1	191	4.15	**0.04**	1	332	0	0.95	1	332	0	0.97	1	332	0	0.98
Plant Species	7	191	3.45	**<0.01**	16	332	524	**<0.01**	16	332	20.2	**<0.01**	16	332	3.22E29	**<0.01**
Site*Plant Species	3	191	0.09	0.97	16	332	33.6	**<0.01**	15	332	1.15	0.31	14	332	0.65	0.82

Pollen Deposition and Pollen Tube Growth. We analyzed 298 styles from PCAA from which we scored a total of 16131 CP grains (range: 5.6-359.72/species), 1022 HP grains (range: 0.1-20.72/species), and 636 pollen tubes (range: 0.31-9.89/species). In PCAP, we analyzed 178 styles and scored a total of 4155 CP grains (range: 0-358/species), 131 HP grains (range: 0-9.83/species), and 205 pollen tubes (range: 0-8/species). We found significant differences among plant species in CP deposition, pollen tube success, and HP deposition (Table 4B-D). For CP

deposition, we also found a significant interaction between plant species and site (Table 4B).

When we tested if this difference was explained by any of our floral predictors, there were no

significant interactions between plot status and floral symmetry, color, or abundance (Table 5).

We did not find any significant site effects or interactions between site and plant species for

pollen tube growth or HP deposition (Table 4C and D).

Table 5. *Plot Status Floral Trait Mixed Model Results*. Results from mixed model for which
floral traits explain plot status and plant species interaction for CP deposition.

Effect	Mean CP Deposition			
	Num DF	Den DF	*F* value	*P* value
Plot Status	1	13	0.67	0.43
Symmetry	1	13	0.14	0.72
Color	1	13	0.13	0.73
Abundance	1	13	0.93	0.35
Plot Status*Symmetry	1	13	3.15	0.1
Plot Status*Color	1	13	3.1	0.1
Plot Status*Abundance	1	13	0.02	0.88

Plant-Plant Heterospecific Pollen Deposition Networks

The SCAA pollen deposition network contained 21 recipient species and 21 pollen donor

morphs with 104 links (Figure 6). The SCAP pollen deposition network contained 24 recipient

species and 28 pollen donor morphs with 143 links (Figure 6). We found that links per species,

weighted connectance, linkage density, and weighted nestedness all tended to be larger in the

invaded pollen deposition network while H_2' specialization and modularity were lower (Table 6).

We found that links per species, weighted connectance, linkage density, weighted nestedness,

and H_2' specialization were significantly different from 1000 null models for both communities

(Table 7). The SCAA network had 7 modules (Figure 7) while the SCAP network had 6 modules

(Figure 8).

Table 6. *Community Plant-Plant Heterospecific Pollen Deposition Network Metrics.* Summary of network metrics used to describe the bipartite pollen deposition networks in the SCAA and the SCAP.

Network Metric	SCAA	SCAP
Links per Species	2.47	2.75
Weighted Connectance	0.076	0.095
Linkage Density	3.25	4.93
H2' Specialization	0.58	0.46
Weighted Nestedness	0.42	0.48
Modularity	0.51	0.44

Table 7. *Null Model Test Results.* Results from t tests of 1000 null network models.

SCAP Network

Network Metric	obs	null mean	lower CI	upper CI	t	*P*
Links per Species	2.75	1.651	1.647	1.656	-507.91	<.001
Weighted Connectance	0.09	0.1769	0.1762	0.177	237.16	<.001
Linkage Density	4.93	5.31	5.29	5.33	36.37	<.001
H2' Specialization	0.46	0.194	0.191	0.196	-243.07	<.001
Weighted Nestedness	0.48	0.578	0.572	0.585	29.1	<.001

SCAA Network

Network Metric	obs	null mean	lower CI	upper CI	t	*P*
Links per Species	2.47	1.389	1.385	1.393	-504.18	<.001
Weighted Connectance	0.076	0.172	0.171	0.173	222.71	<.001
Linkage Density	3.25	4.31	4.28	4.33	97.35	<.001
H2' Specialization	0.578	0.171	0.167	0.174	-264.64	<.001
Weighted Nestedness	0.412	0.59	0.582	0.598	42.57	<.001

The SCAA and SCAP networks contained 18 shared pollen donors and 17 shared pollen recipients. We found a significant site effect for weighted closeness (Table 8) meaning that

pollen donors at the SCAA (0.027±0.005, mean ± SEM) had a higher weighted closeness than

pollen donors at the SCAP (0.018±0.003, mean ± SEM).

Table 8. *Species-level Mixed Model Results.* Results from mixed models comparing species-level network metrics between shared pollen donors and pollen recipients between HP deposition networks.

Site Effect	Pollen Donors				Pollen Recipients			
	Num DF	Den DF	*F* value	*P* value	Num DF	Den DF	*F* value	*P* value
Normalized Degree	1	17	0.09	0.77	1	16	2.12	0.16
Species Strength	1	17	1.56	0.23	1	16	0.29	0.6
Weighted Closeness	1	17	9.13	**0.01**	1	16	0.6	0.45
d' Specialization	1	17	3.87	0.07	1	16	1.76	0.2

Discussion

At the community-level, generalist species (radial flowers) in the SCAP received less

visits than generalist species in the SCAA, but this difference was not reflected in any other

aspect of pollination. Between PCAP and PCAA at the SCAP (floral-neighborhood scale), all

flowers received less pollinator visits in PCAP. This decrease in pollinator visits seemed to

translate into a decrease in CP deposition, but this effect varied by plant species. However, none

of the species floral characteristics that I predicted would explain this interaction were

significant. Nonetheless, there was no change to overall reproductive success at either scale. *C.*

arvense did not alter the amount of HP deposited on local stigmas at either scale. The SCAP

pollen deposition network contained higher number of links per species, linkage density,

weighted connectance, and weighted nestedness and lower values for H_2' specialization and

modularity. Network structure at both sites were significantly different from random. When

analyzing species-level metrics, weighted closeness (centrality) was lower in shared species

within the SCAP pollen deposition network. These and other results are discussed in detail below.

Our results demonstrate the need to evaluate the effect of invasive flowering species at all stages of the pollination process. Although the presence of *C. arvense* had mixed effects (positive and negative) on pollinator visitation to local flowers at the community-level, this difference was not reflected on patterns of pollen deposition (CP or HP deposition) or reproductive success at the community level. Therefore, invasive effects on one or several aspects of the pollination process cannot be expected to extend to all aspects, especially if effects neutralize one another (Cawoy et al., 2012; Ferrero et al., 2013; Albrecht et al., 2016). This further illustrates the need to investigate the entire pollination process in order to uncover the mechanism by which invasive plants may ultimately affect reproductive success or lack thereof.

At the floral-neighborhood level, *C. arvense* decreased pollinator visitation by 65% to all local flowers nearby and this resulted in decreased CP deposition for some species. However, *C. arvense* is also known to outcompete local species for space, light, and nutrients (Tiley, 2010) and have allelopathic effects (Stachon and Zimdahl, 1980). As a result, there were fewer local flowers in plots invaded by *C. arvense* (Daniels J. unpublished data). Therefore, this may have contributed to the decrease in pollinator visitation in invaded plots since pollinators could be attracted to larger patches of local flowers.

Scale-dependent effects may be explained by shifts in identity and differential foraging patterns of local pollinators (Bjerknes et al., 2007; Bartomeus et al., 2010; Albrecth et al., 2016). Effects on pollinator visitation were mixed at the community level depending on species generalization but competitive at the floral-neighborhood-level, so pollinators might be

responding more strongly to the presence of *C. arvense* at smaller spatial scales. Similar results have been found in other study systems (Cariveau and Norton, 2009; Powell et al., 2013). At the community-level, pollinators, such as bees, travel long distances and perceive patches of flowers as resources, so they quickly move between flowers throughout the resource-rich patch and may be the principle mediators of pollination dynamics compared to other pollinator groups such as flies (Rader et al., 2011). It may be possible that the reduction in visitation rate to local radial flowers at the community-level was largely due to a decrease in visitation of generalist (and perhaps inefficient) floral visitors attracted to the patch of generalized *C. arvense* flowers (Thompson, 1981; Ghazoul, 2006; Ferrero et al., 2013) while visitation from the more specialized pollinators remained constant to local species (Lavert and Plowright, 1988; but see Bruckman and Campbell, 2014).

At the floral neighborhood-level, different pollinators that travel shorter distances and distinguish between individual flowers may promote pollination dynamics; therefore, the *C. arvense* flowers may have attracted all of these pollinators away from local flowers (Steffan-Dewenter et al., 2002; Bjerknes et al., 2007; Bartomeus et al., 2010). Pollinators efficiently forage for less travel between flowers to optimize energy use (Marden and Waddington 1981, Kevan and Baker, 1983), so invasive plants with more flowers and/or amount of floral rewards (nectar, pollen, floral scents) stole visits from all neighboring local flowers (Totland et al., 2006). When *C. arvense* decreased pollinator visitation to local flowers, this may have also increased the number of pollinator movements between individuals of the same species and influenced the quality of CP deposited on stigmas (Kevan and Baker, 1983; Tur et al., 2016). In plots with *C. arvense*, all bee species (*Apis, Bombus*, and other solitary bees) and lepidopterans decreased in their contribution of overall visits to local flowers while wasps, flies, and beetles increased

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(Daniels J. unpublished data). For instance, Larsson (2005) found that a solitary bee, *Andrena hattorfiana*, had far greater pollinator effectiveness than generalist pollinators but constituted a small percent of the total visits and pollination to *Knautia arvensis*. Therefore, changes in the identity and behavior of principle pollinators depending on scale may influence invasive effect on visitation rates and pollen delivery. Other studies, however, have found conflicting results regarding scale (Jakobsson et al., 2009; Albrecht et al., 2016), so more studies are needed to uncover the importance of scale and the underlying mechanisms by which it may mediate invasive species effects

In our study, however, effects on pollinator visitation and the quantity of pollen delivered to stigmas of local species did not translate into differences in reproductive success (i.e. number of pollen tubes at the base of the style). Even though the presence of *C. arvense* decreased pollinator visitation and CP deposition in some species while increasing CP deposition and in others, these plants still grew the same number of pollen tubes that reached the base of the style. It is possible that self-pollination mechanisms safeguarded plants from adverse effects on pollen deposition caused by *C. arvense* (e.g. Delmas et al., 2016). Future studies should take into account potential plant mechanisms for ensuring reproductive success when considering invasive species effects on plant reproductive success (Bjerknes et al., 2007).

Several attempts have been made to find generalizations regarding the effects of invasive species on the pollination of native plant species (e.g. Morales and Travset 2009; Arceo-Gomez and Ashman 2016). However, although valuable, these have mainly focused on finding overarching invasive species effects as either positive or negative (e.g. Morales and Traveset, 2009; Arceo-Gomez and Ashman 2016; Charlebois and Sargent, 2017). Nevertheless, it is unrealistic to expect that all plant species will respond similarly to the presence of the invasive

(Charlebois and Sargent, 2017). Increasing evidence suggests that invasive plant effects on plant pollination is highly dependent on the identity (or specific characteristics) of local plant species (Ferrero et al., 2013; Sun et al., 2013; Charlebois and Sargent, 2017). In this sense, our results also show that local species did not respond equally to the presence of *C. arvense*. We found significant interactions showing mixed effects (positive and negative) on pollinator visitation rates for various local plant species at the community-level and CP deposition at the floral neighborhood level. Even though we were able to show that species generalization based on floral symmetry explained effects on community-level pollinator visitation, it is important to note that our sample sizes for floral traits were limited (e.g. 6 species with bilateral symmetry and 4 with color similar to *C. arvense*) and that other traits were not evaluated here such as floral scent and phylogenetic distance. Therefore, future studies should focus on determining which traits or combination of traits mediate invasive plant effects across multiple species. Only through these types of studies we will be able to predict invasive species' effects that help design more efficient strategies to mitigate their impacts on native plant communities.

It is important to note that we did not observe any change in the magnitude of HP received by local species at either scale. Preliminary results also suggest that deposition of *C. arvense* pollen on stigmas has little effect on pollen tube success of local species (Daniels J., unpublished data). This suggests that HP transfer is not a main mechanism by which *C. arvense* interferes with reproductive success of local plants in the community. However, there were important variations in HP transfer between the SCAP and SCAA.

Although we only had one SCAP and one SCAA HP deposition network (Popic et al., 2012), we were still able to observe important qualitative differences in overall network structure. For instance, the number of links per species shows a larger number of species

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interacting via pollen transfer (i.e. higher connectivity) with higher intensity at the SCAP. The larger linkage density at the SCAP shows that these species have a higher diversity of interactions (Bersier et al. 2002). The SCAP network also had a larger weighted connectance, which is a more sensitive metric than the number of links per species, and showed that the SCAP network tends to be more interconnected. Furthermore, the higher value of weighted nestedness in the SCAP network compared to the SCAA suggests that the SCAP network may contain a larger number of generalist plant species interacting with specialist plant species via HP transfer (Galeano et al., 2007). All these results combined suggest that the introduction of *C. arvense* may increase the overall connectivity of the network, which is supported by a lower degree of specialization (H_2') in the SCAP. This suggests *C. arvense* has become a central species for HP transfer donating and receiving pollen from many local species. Interestingly, similar results have been reported for other invaded plant-pollinator interaction networks (Bartomeus et al., 2008). Lastly, when comparing the shared pollen donors (N=18) between the SCAP and SCAA pollen deposition networks, shared species had a lower weighted closeness (centrality) in the SCAP. Therefore, these local species had more central pollen donor roles focal to pollen transfer within the pollen deposition network at the SCAA but were replaced by *C. arvense* at the SCAP.

While local pollen donors were subverted in network roles, the modules in which species were present also shifted between sites and altered the identity of HP receipt (Figure 7 and Figure 8). Potential shifts in identity and magnitude of HP transfer in communities may cause evolutionary changes in order to avoid or tolerate HP effects because of new species interactions (Waser, 1978, Ashman and Arceo-Gomez 2013). This can result in diverging evolutionary trajectories of local flowering species toward traits (i.e. character displacement, Armbruster et al, 1994; Ashman and Arceo-Gomez 2013) and mechanisms (i.e. selfing , Campbell, 1985) that

reduce adverse effects from HP transfer (Hopkins and Rausher, 2012, Fang and Huang, 2016).

These may include divergence in flower color (Hopkins and Rausher, 2012), flowering

synchrony (Aizen and Vazquez, 2006), stigma size, surface area (Yang et al., 2002), secretions,

and receptivity (Madjidian et al., 2012).

Our study reiterates the need to evaluate the effects of invasive species across multiple

stages of the pollination process while simultaneously evaluating potential impacts depending

upon spatial scale (Cariveau and Norton, 2009; Albrecth et al., 2016). Thus, we join others in

emphasizing the need for single studies that integrate a multitude of predictors to assess invasive

plant effects on pollinator-mediated interactions across multiple species (Charlebois and Sargent,

2017; Johnson and Ashman, 2018). By understanding which species are likely to experience

facilitation or competition in the presence of *C. arvense*, conservation goals will be better

informed when deciding how to allocate resources toward control.

Acknowledgments

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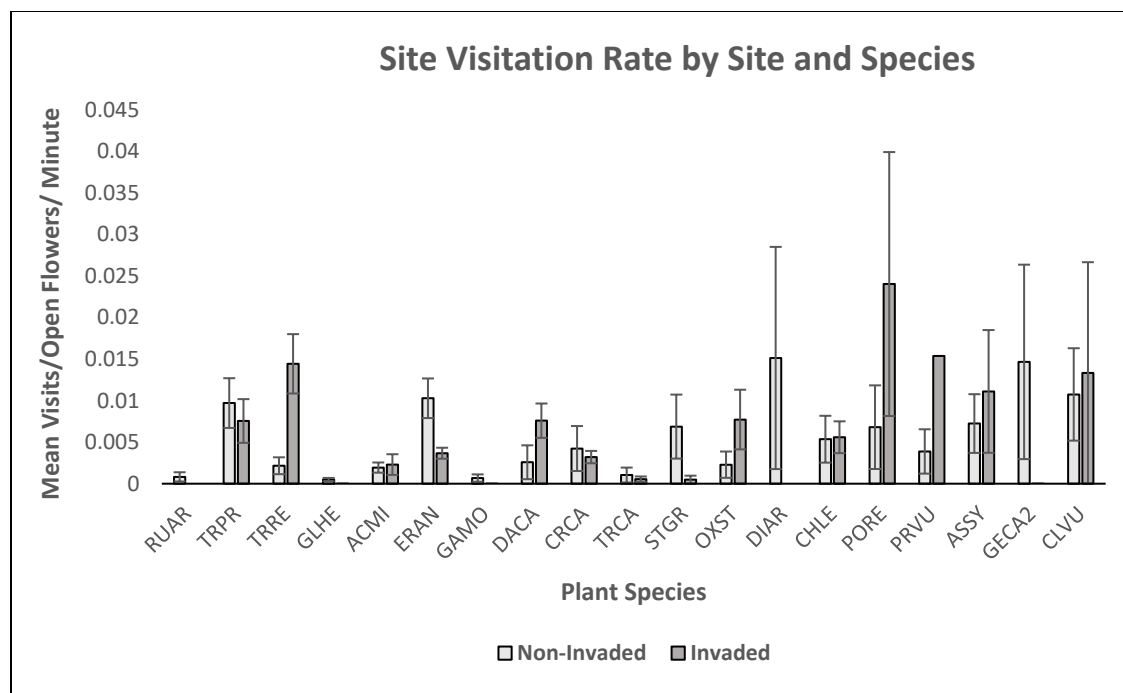
Figure 1. Means ± SEM for visitation rate to local flowers in SCAP and SCAA. Species codes are labelled as first two letter of genus then species name. Species names and codes are listed in appendix Table 9.
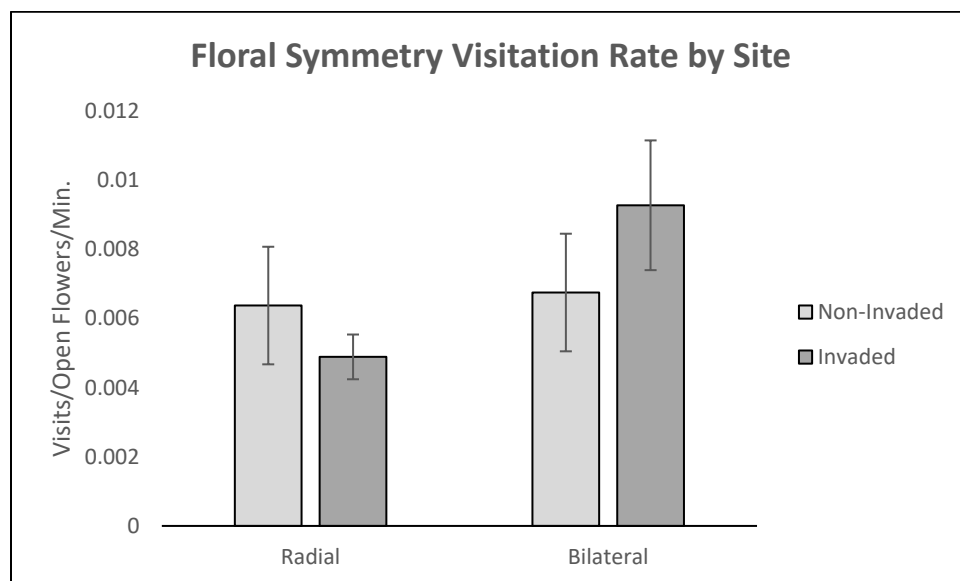
Figure 2. Means ± SEM for visitation rate to radial flowers in the SCAP and SCAA

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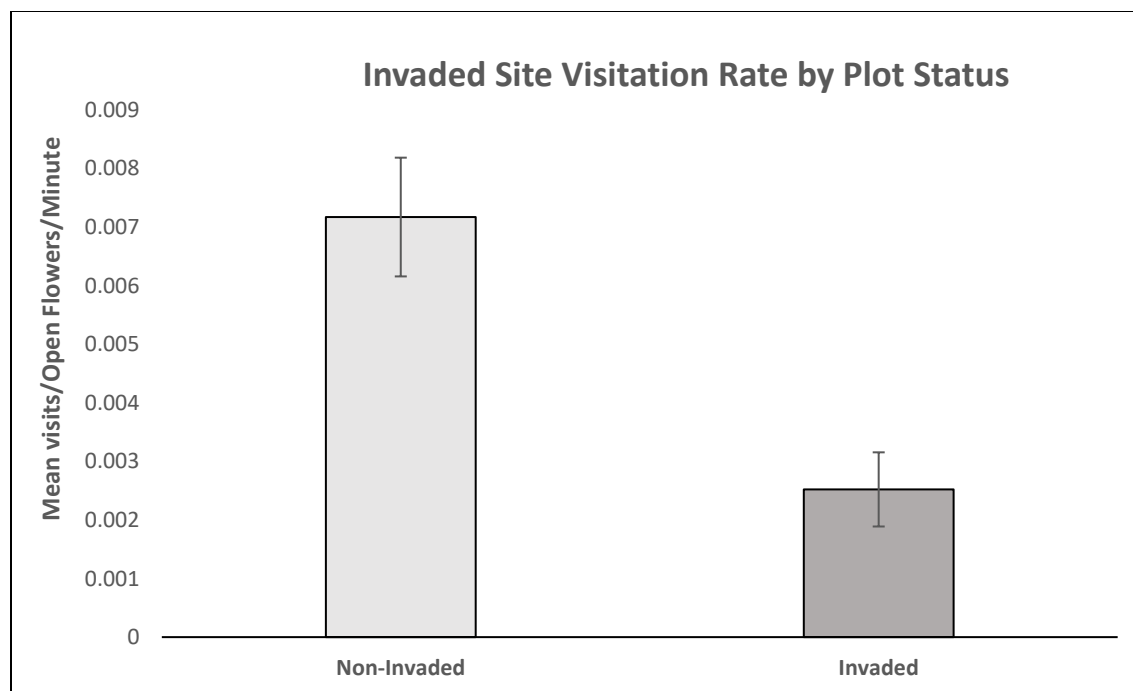
Figure 3. Means ± SEM for visitation rate to flowers inside PCAP and PCAA.
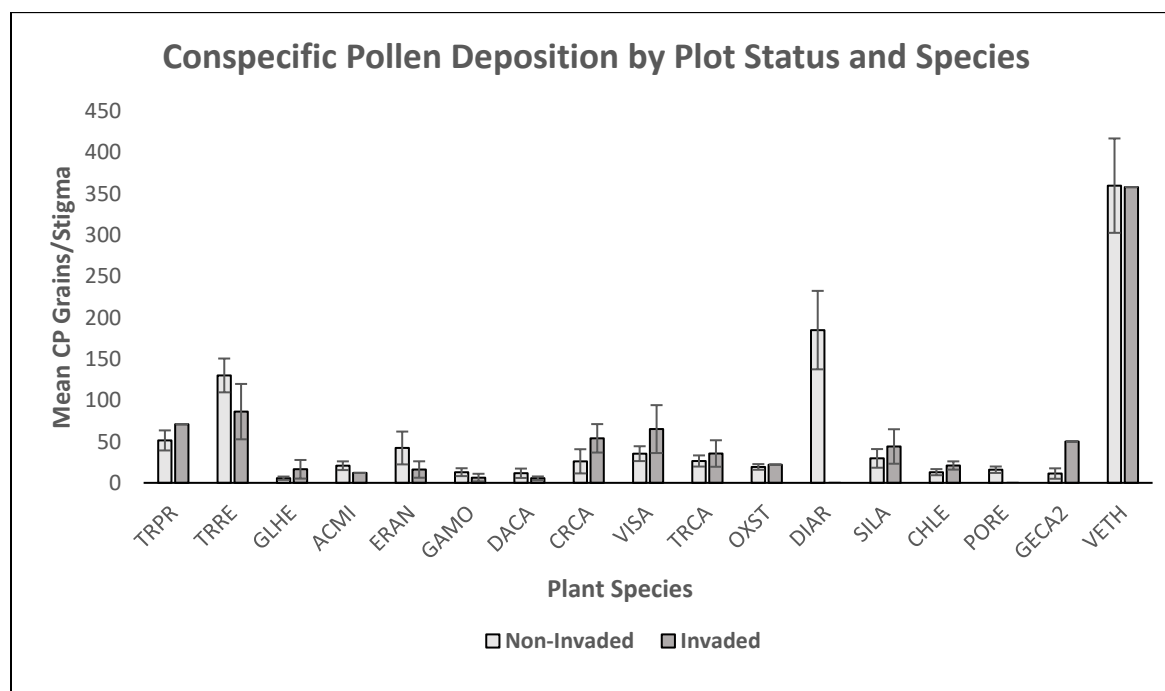
Figure 4. Means ± SEM for conspecific pollen deposition onto local stigmas inside PCAA and PCAP. Numbers correspond to plant species. Species names and codes are listed in appendix Table 9.

SCAA
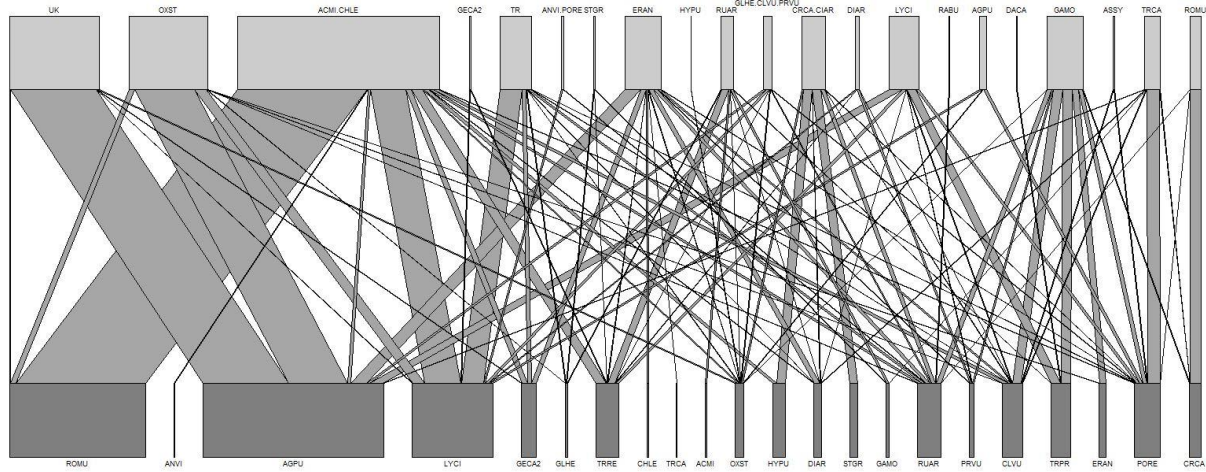
SCAP
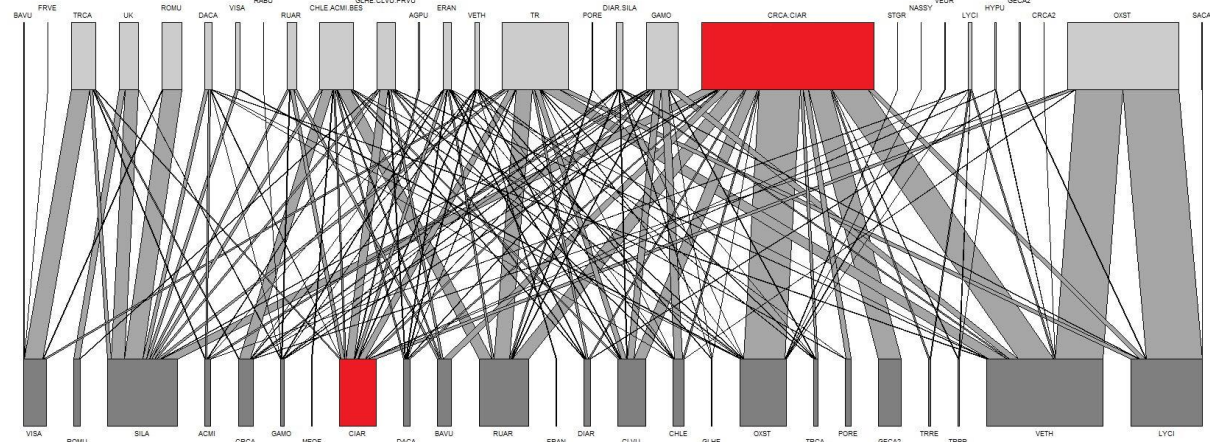
Figure 5. *Plant-Plant Heterospecific Pollen Deposition Networks.* The SCAA pollen deposition network (top) shows links between pollen donor species (top row), N=21, with receptive stigma species (bottom row), N=21. The thickness of the links represent average heterospecific pollen grains per stigma. The SCAP pollen deposition network (bottom) shows links between pollen donor species (top row), N=28, with receptive stigma species (bottom row), N=24. The thickness of the links represent average heterospecific pollen grains per stigma. *Cirsium arvense* pollen is labelled as CRCA.CIAR and stigmas as CIAR while both are colored red.
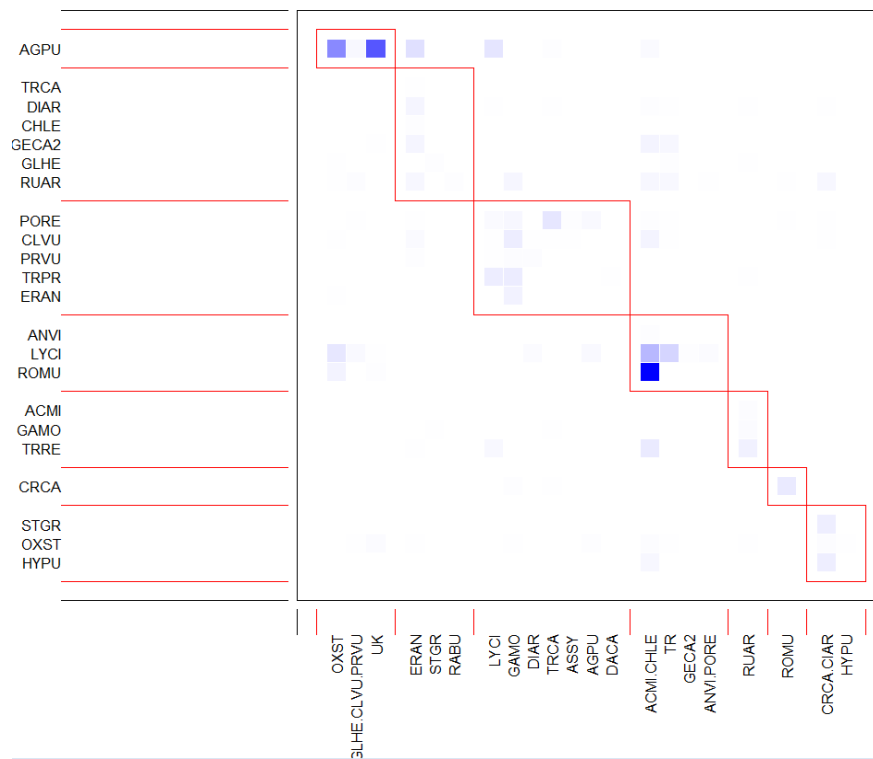
Figure 6. *Cirsium arvense Absent Network Module Web.* Web diagram for number of modules in the SCAA pollen deposition network. Species in the left column are stigma receptor species. Species on the bottom row are pollen donor species. Red boxes indicate modules. The darker squares indicate stronger interactions.
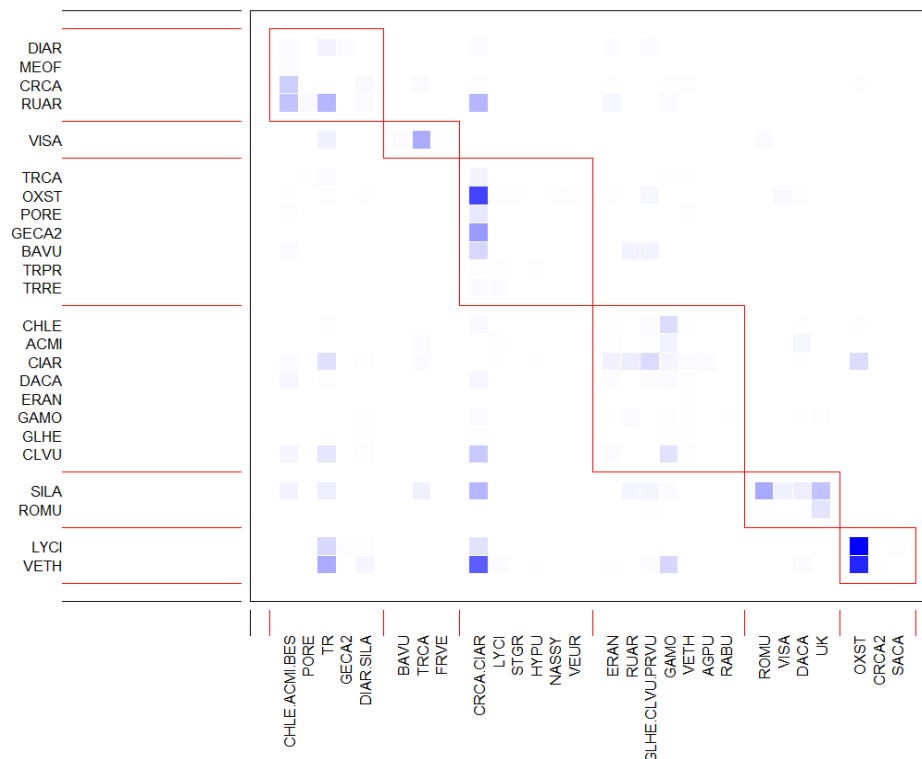
Figure 7. *Cirsium arvense Present Network Module Web.* Web diagram for number of modules in the SCAP pollen deposition network. Species in the left column are stigma receptor species. Species on the bottom row are pollen donor species. Red boxes indicate modules. The darker squares indicate stronger interactions.

Table 9. *Species List and Characteristics.* Numbers and codes correspond to species used in statistical analyses and network construction. Pollen that could not be identified from the anther reference library are coded as UK. Characteristics for species not used in analyses are denoted by a period.

Number	Code	Genus	Species	Family	Floral Unit	Symmetry	Color	Community	Abundance
1	RUAR	*Rubus*	*argutus*	Rosaceae	Flower	Radial	Dissimilar	Both	Abundant
2	TRPR	*Trifolium*	*pratense*	Fabaceae	Flower	Bilateral	Similar	Both	Abundant
3	TRRE	*Trifolium*	*repens*	Fabaceae	Flower	Bilateral	Dissimilar	Both	Abundant
4	RABU	*Ranunculus*	*bulbosa*	Ranunculaceae	Flower	.	.	Both	Rare
5	GLHE	*Glechoma*	*hederacea*	Lamiaceae	Flower	Bilateral	Similar	Both	Abundant
6	ACMI	*Achillea*	*millefolium*	Asteraceae	Flower	Radial	Dissimilar	Both	Abundant

7	ER	*Erigeron*	*annuus*	Asteraceae	Capitula	Radial	Dissimilar	Both	Abundant
8	ROMU	*Rosa*	*multiflora*	Rosaceae	Flower	Radial	Dissimilar	Both	Rare
9	GAMO	*Gallium*	*mollugo*	Rubiaceae	Flower	Radial	Dissimilar	Both	Abundant
10	POSI	*Potentilla*	*simplex*	Rosaceae	Flower	.	.	SCAP	Rare
12	TAOF	*Taraxacum*	*officinale*	Asteraceae	Capitula	.	.	Both	Rare
13	CIAR	*Cirsium*	*arvense*	Asteraceae	Capitula	Radial	Similar	SCAP	Abundant
17	DACA	*Daucus*	*carota*	Umbellifers	Flower	Radial	Dissimilar	Both	Abundant
19	DUIN	*Duchesnia*	*indica*	Rosaceae	Flower	.	.	SCAP	Rare
20	FRVE	*Fragaria*	*vesca*	Rosaceae	Flower	.	.	Both	Rare
21	BAVU	*Barbarea*	*vulgaris*	Brassicaceae	Flower	.	.	SCAP	Rare
22	CRCA	*Crepis*	*capillarus*	Asteraceae	Capitula	Radial	Dissimilar	Both	Abundant
23	CEBR	*Cerastium*	*brachypetalum*	Caryophyllaceae	Flower	.	.	Both	Rare
24	GECA	*Geranium*	*carolinianum*	Geraniaceae	Flower	.	.	SCAP	Rare
25	VISA	*Vicia*	*sativa*	Fabaceae	Flower	Bilateral	Similar	SCAP	Abundant
26	TRCA	*Trifolium*	*campestre*	Fabaceae	Flower	Bilateral	Dissimilar	Both	Abundant
28	STGR	*Stellaria*	*graminea*	Caryophyllaceae	Flower	Radial	Dissimilar	Both	Abundant
29	OXST	*Oxalis*	*stricta*	Oxalidaceae	Flower	Radial	Dissimilar	Both	Abundant
31	DIAR	*Dianthus*	*armeria*	Caryophyllaceae	Flower	Radial	Similar	Both	Abundant
32	SIAT	*Sisyrinchium*	*atlanticum*	Iridaceae	Flower	.	.	Both	Rare
33	SILA	*Silene*	*latifolia*	Caryophyllaceae	Flower	Radial	Dissimilar	SCAP	Abundant
34	CHLE	*Chrysanthemum*	*leucanthemum*	Asteraceae	Capitula	Radial	Dissimilar	Both	Abundant
36	MEOF	*Meliotus*	*officinalus*	Fabaceae	Flower	.	.	SCAP	Abundant
39	PORE	*Potentilla*	*recta*	Rosaceae	Flower	Radial	Dissimilar	Both	Abundant
41	TRPE	*Triodanis*	*perfoliata*	Campanulaceae	Flower	.	.	SCAP	Rare
42	SACA	*Sambuca*	*canadensis*	Adoxaceae	Flower	.	.	Both	Rare
43	PRVU	*Prunella*	*vulgaris*	Lamiaceae	Flower	Bilateral	Similar	Both	Abundant
44	ASSY	*Asclepias*	*syriaca*	Apocynaceae	Flower	Radial	Dissimilar	Both	Abundant
45	ANVI	*Anemone*	*virginiana*	Ranunculaceae	Flower	.	.	SCAA	Abundant
47	GECA 2	*Geum*	*canadense*	Rosaceae	Flower	Radial	Dissimilar	Both	Abundant
49	CRCA 2	*Cryptotaenia*	*canadensis*	unknown	Flower	.	.	SCAP	Rare

50	PELO	*Persicaria*	*longiseta*	Polygonaceae	Flower	.	.	SCAP	Rare
51	VETH	*Verbascum*	*thapsus*	Scrophulariaceae	Flower	Radial	Dissimilar	SCAP	Abundant
52	HYPU	*Hypericum*	*punctatum*	Hypericaceae	Flower	.	.	Both	Rare
53	SOCA	*Solanum*	*carolinense*	Solanaceae	Flower	.	.	SCAP	Rare
54	AGPU	*Agrimonia*	*pubescens*	Rosaceae	Flower	.	.	SCAA	Abundant
55	CLVU	*Clinopodium*	*vulgare*	Lamiaceae	Flower	Bilateral	Similar	Both	Abundant
56	LYCI	*Lysimachia*	*ciliata*	Primulaceae	Flower	Radial	Dissimilar	Both	Abundant
61	CASE	*Calystagia*	*sepium*	Convolvulaceae	Flower	.	.	SCAA	Rare
62	RUHI	*Rudbeckia*	*hirta*	Asteraceae	Capitula	.	.	SCAP	Rare
63	VEUR	*Verbena*	*urticifolia*	unknown	Flower	.	.	SCAP	Rare
64	LEVI	*Lepidium*	*virginicum*	Brassicaceae	Flower	.	.	SCAA	Rare
65	GAPI	*Galium*	*pilosum*	Rubiaceae	Flower	.	.	SCAA	Rare
66	CLVI	*Clematis*	*virginiana*	Ranunculaceae	Flower	.	.	SCAA	Rare
UK	UK	*unknown*	*unknown*	unknown	.				

CHAPTER 3

SUMMARY AND FUTURE STUDIES

Summary

In this study, we investigated whether the presence of invasive *Cirsium arvense* alters pollinator visitation, CP and HP deposition, and overall reproductive success for local plant species. We further evaluated if the observed effects differ depending upon local species' pollinator generalization level, flower color, and relative abundance and if these varied with scale (community vs floral-neighborhood). At the community-level (comparisons between the site with *Cirsium arvense* present (SCAP) and absent (SCAA), generalist species (radial flowers) received less visits than specialized ones (bilateral flowers) at the SCAP, but this difference was not reflected in any other aspect of pollination. Between PCAP and PCAA at the floral-neighborhood scale (close proximity of 1-2 meters) within the SCAP, all flowers received less pollinator visits in PCAP. This decrease in pollinator visits seemed to translate into a decrease in CP deposition, but this effect varied by plant species. However, none of the species floral characteristics that we predicted would explain this interaction showed a statistically significant effect. However, none of these effects caused the number of pollen tubes reaching the bases of styles for any local plant species to change. Even though the presence of *C. arvense* did not alter the amount of HP deposited on local stigmas at either scale, patterns of HP transfer among species differ between communities. Specifically, the SCAP pollen deposition network contained higher number of links per species, linkage density, weighted connectance, and weighted nestedness and lower values for H_2' specialization and modularity. Network structure at both sites were significantly different from random. When analyzing species-level metrics, weighted

closeness (centrality) was lower in shared species within the SCAP pollen deposition network. Therefore, *C. arvense* replaced shared species central roles in HP transfer as donors.

Overall, our results suggest that *C. arvense* affects the pollination of local species, but these effects depend on particular species, spatial scale studied, and the specific stage of pollination being evaluated. *C. arvense* also altered the overall structure of plant-plant HP deposition networks and individual species dynamics within the networks. However, there are certain considerations, not addressed in this study, that would be important to take into account in future studies. Such considerations could help improve our understanding of invasive species effects on the pollination dynamics of native plant communities and are discussed below.

Pollinator and Pollen Transport Dynamics

While we evaluated the effects of an invasive species on different stages of the pollination process (visitation, CP and HP deposition, reproductive success), potential changes in the behavior or composition of the pollinator community resulting from invasion were not studied. Changes in pollinator species composition, the amount and diversity of pollen carried by pollinators, and pollinator efficiency due to plant invasion, may also be a factor indirectly influencing successful pollination of native species, but it was not addressed in the present study. Even though we found mixed effects of *C. arvense* on patterns of pollen deposition (CP and HP) in local flowers, the underlying causes are still unknown. Investigating pollinator dynamics would then provide a more complete understanding of *C. arvense* effects on local flowers as well as of the underlying mechanisms. For instance, the composition of the pollinator community visiting native flowers is subject to change between sites as *Cirsium arvense* may attract more generalist pollinators into the community (Memmot and Waser 2002). These generalist

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pollinators may then have significant differences in visitation and pollinator efficiency, which is the amount of seeds sired from a single visit onto a virgin flower. Preliminary data collected at the study sites showed a 72.4% similarity in pollinator community composition between sites (Morisita-Horn index; Daniels J. unpublished data). However, there were 58 pollinator species unique to the SCAA and 54 unique to the SCAP, and the extent to which differences in pollinator community composition between sites is due to the presence of *C. arvense* in this study is unknown. To date, most studies have centered on understanding how invasive plant may affect native plant species, but the extent to which invasive plants may alternative pollinator community composition is not well-understood and deserves further attention (but see Bezemer et al. 2014; Stout and Tiedeken 2016; Davis et al. 2018).

However, increasing evidence suggests that invasive plants typically establish central roles in plant-pollinator and pollen-transfer networks (e.g. by donating and receiving pollen from many pollinators), particularly those that are generalists (Vila et al. 2009; Albrecht et al. 2014). Thus, a promising avenue of research would be identifying and quantifying the pollen carried by pollinators in order to show which pollinators are playing a key role in connecting the invasive plant to the local pollen deposition network, as well as which local plant species may be most likely to receive HP (e.g. Lopezaraiza-Mikel et al. 2007).

When determining mechanisms for invasive plant effects, knowing pollinator and pollen transport dynamics would better help associate patterns for visitation, pollen transport, pollen deposition, and ultimately plant reproductive success (Cawoy et al. 2012). It has been shown that effects on pollinator visitation rates do not necessarily equate to effects on pollen deposition (King et al. 2013). In order to explain the apparent discord between pollinator visitation and pollen deposition onto stigmas, it would be necessary to evaluate the pollination efficiency of the

different floral visitors. This would help elucidate which pollinators contribute to changes in visit quality (i.e. quantity and quality of CP grains deposited per visit) so focus could be shifted to the most functional pollinators when discerning mechanisms of disruption. In this sense, Neeman et al. (2010) reviewed various methods and proposed improvements for evaluating pollinator efficiency by evaluating pollen loads deposited from single-visits, which is how much CP is deposited by a pollinator onto a virgin stigma (e.g. King et al. 2013; Ballantyne et al. 2015). For instance, it is possible that a frequent visitor will have low rates of pollen deposition whereas a rare visitor may have high rates of pollen deposition (Thomson et al. 2000). If this is the case, observations of pollinator visitation events and even recording pollen grains on pollinator bodies may be insufficient in describing the efficiency of the plant-pollinator interaction. Knowledge of the overall importance of floral visitors as pollinators (visitation and pollen deposition) would lead to more accurate description of plant-pollinator interactions based on their functional performance (King et al. 2013; Ballantyne et al. 2015). For instance, even though the apparent inherent generality of plant-pollinator interactions makes them robust to changes in pollinator and plant community composition (Bartomeus et al. 2008), such slight changes could ultimately affect patterns of CP and HP deposition.

Predictors of Invasive Plant Effects on Pollination

There were various challenges in testing our predictions for explaining variation in invasive species effects among local flowering species. The most prominent is that due to intrinsic properties of our studied communities (i.e. species composition), there was limited replication of the proposed predictors (flower color and symmetry). For instance, when evaluating flower symmetry we had 17 species with radial flowers but only 7 species with bilateral flowers and this may have been insufficient to detect significant differences.

Consequently, the non-significant results in this study should be interpreted with caution, and future studies should attempt to find communities with enough shared species and replication of floral traits.

Since conducting large field studies with adequate replication of species and traits is logistically daunting, most well replicated studies of predictors of invasive species effects have come from meta-analyses of pollination studies within different environments. However, these studies have led to conflicting conclusions regarding predictors of invasive plant effects on reproductive success and pollinator visitation (Morales and Traveset, 2009; Charleboi and Sargent 2017). One problem may be that each individual study in these meta-analyses was conducted in a unique context of biotic and abiotic conditions. Sometimes data was collected using different methodologies. This may obscure any potential effects and lead to unreliable results. Therefore, even though they may be logistically challenging, more natural or experimental studies should be conducted in order to achieve a better understanding of invasive plant effects and of the potential drivers of these effects.

Furthermore, in this study, floral color was assessed based on human vision and not insect visual spectrums. According to Kevan and Baker (1983), insects are more sensitive to certain wavelengths of light than humans are (e.g. ultraviolet light). This results in trichromatic vision in bees and flies that mainly see ultraviolet, blue-green, and yellow. Other insects may only see black and white but are still extremely responsive to ultraviolet. As a result, flowers that appear similar in color to humans may be appear very different to insects. For instance, a diversely colored community to an insect may only contain three or four colors to a human. Therefore, since color was classified on a similar/dissimilar basis to the human eye in this study, the insects may have observed distinct colors whereas we observed the same color. As a result,

we found floral color to be a non-significant predictor while it may have been significant if

classified considering insect vision. In addition, nectar guides, which are a common visual signal

to some insects, may also be present in the ultraviolet spectrum and invisible to the human eye.

The presence/absence of nectar guides was not considered in this study and their potential role in

mediating invasive species effects has been little studied (Kevan and Baker 1983). Overall,

future studies that evaluate the role that flower color plays in mediating invasive species effects

should consider color in the context of the visual system of the dominant floral visitors in the

community (Kevan 1972).

Although local floral abundance did not significantly explain variation in invasive species

effects in our study, future studies should consider patch size, the spatial distribution of plants

within a site, and their abundance relative to the invasive plant. Invasive plants have been shown

to facilitate pollination at low densities and become competitive at high densities (Ghazoul 2006;

Munoz and Cavieres 2008; Iler and Goodell 2014; Seifan et al. 2014; Bruckman and Campbell

2016). The distribution of plant species within a community (uniform or patches) has also been

shown to influence pollination of co-flowering species (Hanoteaux et al. 2012). However, these

effects may depend upon the size of the community, resource availability, and abundance of

local flowers (Bartomeus et al. 2010; Williams et al. 2010). Thus, it would be important to

evaluate at what density *C. arvense* causes an effect on local species. Furthermore, distance from

the invasive plant has also been shown to mediate invasive species effects on pollination within a

community (Spellman et al. 2016). For example, Cariveau and Norton (2009) found that

pollinator visitation rates to *Monarda fistulosa* decreased when growing in the 1-5 meter range

from *Carduus nutans* but unaffected when growing at 15 meters from or directly beside *C.

nutans.* In this study, all plots were placed at least 5m apart. However, some PCAA in the SCAP

were near patches of *C. arvense* (approximately 2 meters) while others were far away (approximately 30 meters). Thus, it would be prudent to consider the distance from the invasive and the spatial distribution of plants within the community in future studies.

Increasing evidence shows a unique combination of predictors is required for specific communities and for specific invasive species (Muthukrishnan et al. 2018) in order to determine invasive mechanisms of disruption. Floral shape or outline has been shown to influence insect visitation (Leppik 1956; Kevan 1983). For example, *Bombus* visitation increased to *Digitalis purpurea* with larger floral displays and larger densities (Grindeland et al. 2005). On the other hand, the type and size of reward is another important factor in determining foraging patterns. Makino and Sakai (2007) found that bees learned to avoid larger floral displays with no rewards and visit smaller floral displays with nectar. Therefore, local species with a different type of reward or combination of rewards than an invasive species may be more resistant to effects on pollinator visitation. For instance, pollinators are known to visit flowers for nectar, pollen, and oils, but the chemical make-up of each is variable between species (Simpson and Neff 1981; Dobson 1994). However, pollinators do not solely use visual cues to assess rewards since floral scent is another strong learning mechanism, especially when used simultaneously with displays (Kunze and Gumbert 2001; Raguso and Willis 2002; Wright and Shiestl 2009). Overall, multiple combinations of floral attractants influence pollinator visitation and may act as unique predictors when considering which species may experience facilitative, neutral, or competitive effects on pollinator visitation in relation to the invasive species being studied.

Co-Invaders

It has been shown that invasive plants and invasive pollinators can facilitate one another (D'Antonio and Dudley 1993; Olesen et al. 2002), which further enhances effects on the native community (Simberloff and von Holle 1999; Aizen et al. 2014). In this study, *Apis mellifera*, an alien pollinator species, was observed visiting *C. arvense* the most, and their visits in the SCAP as a whole increased 133% compared to the SCAA. *Apis mellifera* has also been observed as an important mutualist facilitating other invasive species such as *Lythrum salicaria* (Grabas and Laverty, 1999), *Centaurea solstitalis* (Barthell et al. 2001) *Cytisus scoparius*, *Genista monspessulana* (Parker and Haubensak 2002), and *Rosa multiflora* (Jesse et al., 2006). Invasive plants have also been shown to provide substantial resources for expanding *Apis mellifera* colonies (Barthell et al. 2001; Wenner et al. 2009). Therefore, it is possible that these two alien species may be assisting one another in establishing a strong community presence and may not need to interact with local species for resources. The inherent generality of both *Apis* foraging patterns and *C. arvense*'s pollinator rewards may be an advantage over specialized plant-pollinator interactions since they may not actually need to interact with other local species. Knowing to what extent invasive plants and pollinators facilitate each other and how this interaction aids in invasion would help predict invasive species effects and to design effective management strategies to minimize effects on local flowering species.

Temporal Variation in Networks

While this study has shown that *C. arvense* alters the overall structure of plant-plant pollen deposition networks and individual species roles therein, it is not known whether these alterations persist across consecutive flowering seasons. As with most pollination studies, long

term assessment of invasive species effects is lacking. It is known that impacts of invasive plants will be reduced over time since the invasive plant evolves to its new environment and local species evolve to tolerate its effects (Dostal et al. 2013). Moreover, pollen transport networks between pollinators and plant species have been shown to vary year-to-year (Alarcon et al. 2008; Petanidou et al. 2008; Devoto et al. 2011). Similarly, plant-plant pollen deposition networks may contain similar variability because they are a result of pollinator-mediated interactions. However, whether this variability exists or how prevalent it is in plant-plant pollen deposition networks across plant communities is not known. In the future, inter-annual variation in invaded plant-plant pollen deposition networks should be studied to determine if invasive plant effects remain constant over time.

There may also be fine-scale temporal variation in plant-plant-pollinator interactions within a single flowering season. For instance, plant and pollinator phenologies vary within a season; hence, pollinator-mediated interactions may change over a relatively short time-span (days, weeks) and effects of the invasive species on plant-plant-pollinator interactions in the community may depend on what stage of the invasive flowering season is being studied. At the floral-neighborhood level, *C. arvense* decreased pollinator visitation to all local flowers nearby and this resulted in decreased CP deposition for some species. This process may have also been gradual through preferential attachments as *C. arvense* approached peak flowering (Barabasi and Albert 1999). Furthermore, it has been shown that floral abundance and flowering phenology influence links between species (Basilio et al. 2006; Olesen et al. 2008; Vazquez et al. 2009) and these typically change throughout a single season. Mutualistic networks may not show links between some species and assume these do not interact. However, some of these species may not interact simply because their phenologies do not overlap within the season (i.e. forbidden links)

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(Olesen et al. 2008), and not because of avoidance (Jordano et al. 2003) via spatial segregation (floral distribution) (Stang et al. 2006; Vazquez et al. 2009), size restrictions (floral morphology) (Stang et al. 2006), mismatching rewards (pollen vs nectar) (Genini et al. 2010), or pollinator foraging behavior (pollinator effort and preference) (Jordano 1987; Olesen et al. 2010). When sampling throughout the *C. arvense* flowering season, approximately 8 species flowered the entire *C. arvense* flowering season. On the other hand, some species flowering in June were not flowering in July and vice versa. However, these were all included in the pollen deposition network (as it was done in this study). As a result, connectivity of the network may seem low as links between some species are simply not possible. Thus, in order to more finely dissect the potential effects of invasive species on the structure of plant-plant HP deposition networks, studies should partition networks into biologically relevant sampling periods (hours, days, or weeks) that accurately captures variation in flowering phenology (Arceo-Gomez et al. 2018) and lead to better depictions of HP transfer dynamics.

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JESSE DURHAM DANIELS

Education:	M. S. Biology, East Tennessee State University, Johnson City, Tennessee 2018
	B.S. in Biology, Radford University, Radford, Virginia July 2015
Professional Experience:	Laboratory Manager, East Tennessee State University, Arceo-Gomez Labs, 2018-Present
	Substitute Teacher, Lee County Public Schools, Jonesville, Virginia, 2015-Present
	Graduate Assistant, East Tennessee State University, College of Arts and Sciences, 2016-2018
	Field Researcher, East Tennessee State University, College of Arts and Sciences, 2016-2018
	Teacher, Harlan Independent Schools, Harlan, Kentucky, 2015
Honors and Awards:	1st Place Oral Presentation
	American Society of Plant Biologists, Southern Section Annual Meeting
	2nd Place Oral Presentation
	Appalachian Student Research Forum
	School of Graduate Studies Research Grant.
	East Tennessee State University
	The Marcia Davis, Biological Sciences Research Award.
	East Tennessee State University
	Annual Biology Research Grant for Honors Capstone.
	Radford University
	Radford University Presidential Scholarship.
	Radford University

The Slemp Foundation Scholarship.

    Radford University

Tobacco Commission Scholarship

    Radford University

The David Buntzman Scholarship

    Radford University

Sigma Phi Epsilon Balanced Man Scholarship

    Radford University