

ABSTRACT

O'BRIEN, JENNIFER ERIN. The Contribution of Roadside Wildflower Plantings for Enhancing Pollinator Habitat in the Piedmont of North Carolina. (Under the direction of Dr. Danesha Seth Carley.)

Pollinators play a significant role in our ecosystem. Insect pollinators are responsible for pollinating up to 75% of the world's food crops (Lutz et al. 2014; Klein et al. 2007). Over the past few decades, insect pollinator populations have been declining. One of the major driving forces of decline is the changing landscape, primarily due to urbanization. With increasing urbanization across the world, the availability of natural pollinator habitat is decreasing. This decline of habitat reduces foraging and nesting resources in human modified landscapes (Arbol 2012). One way to improve urbanized areas for pollinating insects is to increase food resource availability in these areas (Fortel et al. 2014). The objectives of this study were to determine if 1) planting pollinator habitat along interstates would increase pollinator abundance and richness compared to lightly-managed turf and if 2) land use surrounding pollinator sites had an effect on bee abundance. Sites were established in the Piedmont of North Carolina in 6 counties. For pollinator sites, an acre of wildflower seeds were seeded by NCDOT in medians, shoulders, and interchanges in 2014. Control sites were managed monthly by mowing. All locations were sampled every three weeks from May 2015 through August 2015. Insect pollinators including bees, butterflies, and flies were identified. Pollinator abundance was determined for all insect orders and species richness was determined for bees and butterflies. Floral cover was recorded for sites at each visit. Land use was determined using ArcGIS and categorized as urban/suburban, natural/forested, and agriculture at radii of 500 m, 1000 m, and 2000 m surrounding each site. Pollinator habitat along roadsides supported significantly greater bee abundance as well as higher species richness compared to weedy grassy roadsides, but had no effect on fly or butterfly abundance. Floral cover had a significant impact on bee abundance and diversity, especially for *Apis mellifera*, *Bombus* spp., and *Xylocopa* spp. Land use impacted bee diversity (but not abundance) in the early part of the sampling season. Most impacted by land use were small native bees and *Bombus* spp. Roadside pollinator plantings supported more bees than the sites along roadsides dominated by lightly managed grasses and "weedy" flowers. Our research provides some insight to the importance of establishing and protecting habitat that can be used by insect pollinators, especially bees.

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The Contribution of Roadside Wildflower Plantings for Enhancing Pollinator Habitat in the Piedmont of North Carolina

by
Jennifer Erin O'Brien

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APPROVED BY:

Dr. Danesha Seth Carley
Committee Chair

Dr. Thomas Rufty

Dr. David Tarpy

Dr. Richard McLaughlin

DEDICATION

To my parents, Susan and Stephen O'Brien, thank you for always being there for me and showing your support.

BIOGRAPHY

Jennifer O'Brien was born August 31st, 1990 in Mattoon, IL. An only child, she spent much of her time playing sports and being outdoors. Science sparked her interest in high school, thanks to her superior biology teachers. She received her B.S. in Biological Sciences from Eastern Illinois University in 2013. In 2014, she accepted a position in Danesha Seth Carley's lab in the Crop Science Department of North Carolina State University.

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CHAPTER 1 – Literature review

Importance of Pollinators. Pollinators are responsible for the yields of 75% of the world's food crops (Lutz et al. 2014; Klein et al. 2007). Several fruit, nut, and vegetable crops including apples, blueberries, citrus, cucumbers, mangos, olives, and many others require pollination in order to provide vitamins and minerals to the human diet (Lutz et al. 2014; Arbol 2012; Brittain and Potts 2011). Agriculture has become more pollinator-dependent over time, and this trend is more pronounced in the developing than developed world. Due to our greater reliance on pollinators, it is likely that pollinator shortages will intensify demand for agricultural intensity, a trend that will be more pronounced in the developing world, but still of concern globally. Unfortunately, changes away from natural- and semi- natural lands to agricultural and urbanized lands has significantly contributed to global environmental changes that have negatively impacted pollinator populations.

Insect pollinators can include: bees, wasps, moths, butterflies, and flies. Of all insects, bees are considered to be the most beneficial pollinator for human needs (Arbol 2012). Certain bees such as honey bees and bumblebees are reared for pollination services, although it is not possible for managed hives alone to keep up with agricultural demand (Button and Elle 2014; Breeze et al 2011; Aizen and Harder 2009). Wild bees are important pollinating communities that will help in the agricultural pollination deficit (Lowenstein et al. 2012; Aizen and Harder 2009). Without these pollinators, there will likely be severe negative impacts to biodiversity and agricultural yields (vanEngelsdorp and Meixner 2010; Kevan and Phillips 2001).

Honey bees. Honeybees, mainly *Apis mellifera*, remain the most economically valuable pollinators of crop monocultures worldwide. This is primarily because honey bees are one of the easiest insect pollinators to manage for honey production and agricultural crop pollination. Compared with the management of other bees, honeybees are versatile, cheap and convenient, but for some crops they are not the most effective pollinators on a per flower basis reviewed in Parker et al. (1987). Honey bees are generalists when foraging, have the ability to travel relatively far distances (4.5 km) while foraging, and have a surplus of workers that forage (vanEngelsdorp and Meixner 2010). While crops such as almond, apple, melon, alfalfa, blueberry, avocado, sunflower, cranberry, kiwi, and many more (Robinson et al. 1989) requiring pollination have increased over time (>300% over the last 50 years), the number of managed bees have not (Aizen and Harder 2009). There are several factors that impact honey bee colony growth. Stressors of the honey bee have created more strain on the success of managed hives and have limited growth in

populations for honey bees. Colony collapse disorder (CCD) was first documented in 2007 for the substantial loss of colonies in the United States. While the proximate cause is unknown, it is commonly thought that CCD is a complex of factors resulting in stress to honey bees. These include pesticides such as neonicotinoids and parasites, most notably the parasitic mite *Varroa destructor* (Hanley et al. 2014; Lutz et al. 2014; Arbol 2012).

Wild Bees. When the general public thinks about bees, they think of honey bees. While honey bees are important, wild bees comprise a majority of the bees in the world. In fact, there are over 4,000 species of native bees in North America. Members of the five most common families are Apidae, Halictidae, Andrenidae, Megachilidae, and Colletidae. These native pollinators have been pollinating plants since long before the arrival of honey bees and come in all sizes, shapes, and colors. They also have a wide variety of niches including the types of flowers they visit, seasons of activity, and where they build nests. Ground nesting bees dig themselves a nest underground at varying depths dependent upon the species (Cane 1991). Cavity nesting bees live in sticks, trees, and even the lumber used to build homes (Cane et al. 2007). Social bees, like honey bees, live in colonies and have varying degrees of sociality, but most bees are solitary. Solitary bees live by themselves and raise their young on their own. Overall, native bees are more plentiful and more efficient than honey bees, and as such, play a very important role in the natural world; it has been proposed that diverse native bee communities may be key for continued pollination of economically important crops.

Challenges facing insect pollinators. Over the past few decades, insect pollinator populations have been declining. One of the major driving forces of decline is the changing landscape, primarily due to urbanization. The resulting habitat loss, fragmentation, and alteration leads to the reduction in foraging and nesting sites (Blackmore et al. 2014; Fortel et al. 2014; Hanley et al. 2014; Arbol 2012). Urbanization also increases the number of small patches suitable to support pollinators, which can be problematic, especially for small solitary bees. The distance a bee will travel to forage is dependent upon the species and can range from less than 300 m up to 1.75 km (Button and Elle 2014). Smaller bees, for example, have a much smaller range for foraging due to body size limitations.

Fragmentation and urbanization also increase the likelihood of inbreeding among pollinator populations by both decreasing the amount of pollinator habitat and increasing the distances between habitats. Since bees can only travel limited distances, wildflower patches that are spatially separated create isolated habitats, and isolated mating populations. A positive trend was found between proximity of nesting sites and genetic similarity of wild bee

populations in an urban setting (López-Urbe et al. 2015). The results from this study suggest the need for the amount of pollinator habitat to increase while the distance between habitat decreases.

The decline in forage by way of reduction in floral biodiversity is another challenge pollinators face. The reduction in wildflower acreage limits the availability of forage, resulting in a reduction of nutritional requirements necessary for bee survival (Arbol 2012). Bees are attracted to wildflowers that produce sufficient amounts of pollen and nectar (Potts et al. 2010). In several studies, it has been noted that plant diversity increases pollinator abundance (Tonietto et al. 2011; Noordijk et al. 2009; Hopwood 2008), but flower richness did not have an effect on bee species richness (Fortel et al. 2014). More studies support the idea that flower abundance and richness are important for bee communities. Being able to provide abundant floral supplies across seasons is important for pollinators in order to maintain their food supply. “Bridging plants” help maintain a food sources for foraging pollinators year round (Menz et al. 2011). Small fragments of pollinator-friendly habitat attract fewer pollinators compared to larger pollinator patches (Blaauw and Isaacs 2014; Aizen and Feinsinger 1994), but Hopwood (2008) determined 18 m of wildflowers was sufficient in buffering the negative effects of urbanization along roadways. Another study along roadways demonstrated that reduced mowing regimes allowed for more flowering plants which increased butterfly abundance (Halbritter et al. 2015).

The reduction of native habitat caused by agricultural practices has put further constraints on pollinator communities. Crop monocultures and agriculture contributes to a reduction in nesting areas and provides less nutritional pollen for pollinators (Arbol 2012). Yearly tilling disrupts and displaces ground nesting bees. Several studies have suggested farming practices reduce the quality of the soil in which wild bees prefer to build their nest (Kim et al. 2006). Williams et al. (2010) determined tilling reduces floral coverage and has other effects which negatively impact social bees and specialists. Weedy species found in agricultural land helps support wild bee communities. Maintaining acceptable levels of weeds within a crop can be beneficial for pollinators (Nicholls and Altieri 2013). For farms that eradicate nearly all weeds, it is important to establish hedgerows, wildflower rich field borders, and other patches of perennial flora to provide nesting and foraging sites (Goulson et al. 2015; Lowenstein et al. 2012).

Farming practices commonly employed in agriculture also negatively impact pollinators (Kim et al. 2006). These practices include tilling, monoculture plantings, and chemical applications. Population decline due to

agricultural intensification is dependent on ecological and life-history traits of the species being studied. Ground-nesting bees, for example, are especially impacted by tillage regimes while distance from natural habitat negatively affected above ground nesting bees (Williams et al. 2010; Kim et al. 2006). A few ways to help reduce the impact these agricultural practices imposed on bee communities is to minimize or completely reduce the amount of tillage in a field.

Insecticides, commonly used in landscapes and conventional agricultural practices, can impose strain on beneficial insect populations. Accidental bee deaths as a result of improper insecticide use occurred as early as the 1870s (Johansen 1977). A number of these insecticides, especially neonicotinoids, are controversial in their implicated role in the reduction of wild and managed bee populations. A majority of the studies on insecticide effects on bees have focused on managed bee species, specifically, *Apis mellifera* and *Bombus* species, with less focus has been on wild bees. In one study focusing on bee pollinators, it was determined that wild bees were negatively impacted by insecticide applications that occurred two or more times over the season, while bumble bees and butterflies were not affected (Brittain et al. 2010). Longley and Sotherton (1997) suggest sublethal effects can be detrimental to populations within Lepidoptera due to the long life-cycle and number of times these butterflies are exposed to applications over the course of a growing season.

Neonicotinoids have been found harmful to bees in laboratory studies, but in a field study on bumblebees, the effects of neonicotinoids were not as harmful when comparing conventional corn fields and organic corn fields (Cutler and Scott-Dupree 2014). According to Cutler and Scott-Dupree (2014), bumble bees rarely collected pollen from conventional corn fields with seed treated with neonicotinoid. Despite have little effect on the health of the colonies, except worker numbers, there were significantly fewer workers at the conventional fields. This could be due to the lack of nutritional value corn pollen provides to bumble bees. Bumble bees are choosy and prefer nectar that is of high quality (Somme et al. 2015). In a meta-analysis executed by Cresswell (2011), it was determined that typical doses of imidacloprid, a neonicotinoid, did not cause lethal effects in honey bees; however, seeds coated with imidacloprid may still pose a threat to honey bee health. Although lethal effects may not be a contributing factor to colony loss, sublethal effects included reduced honey bee pollinating performance (Cresswell 2011). Ultimately, the best way to protect bees and other insect pollinators from exposure to petrochemicals, is to reduce pesticide use, reduce pesticide drift and implement correct timing of pesticide applications so that chemicals are not being applied

while bees are present in the field (Nuyttens et al. 2013; Reimer and Prokopy 2012; Vaughan and Hoffman Black 2008; Pimentel 2005).

Since there are differences between genera and even within species, the effects of chemicals on pollinators are difficult to generalize across all pollinator groups. For bees, it has been determined social bees are more negatively affected by pesticide use than solitary species (Benjamin et al. 2014; Gill et al. 2012; Henry et al. 2012; Laycock et al. 2012; Williams et al. 2010). Several insecticides including fenitrothion, imidacloprid, deltamethrin, and spinosad have been found to cause both lethal and sub-lethal effects on *Apis* and *Bombus* species (Brittain and Potts 2011). While the research is still unclear as to the exact impacts of specific chemicals on bees, scientists generally agree that pesticide exposure (i.e. insecticides, fungicides, herbicides, and miticides) does contribute in some way to a decline bee health (Finley et al. 1996; Hanley et al. 2014; Henry et al. 2012).

Habitat. Just as fragmentation and degradation of near- and semi-natural habitats can be detrimental to bee communities, quality habitat provides pollinators with nesting sites, nutrition, and protection from pesticides. As urbanization increases, it is important to understand how this change in landscape affects pollinators. In a study which examined the abundance of bees and hoverflies in urban, suburban, and rural habitats, it was determined that both bee and hoverfly abundance was greater in rural areas versus urban/suburban areas (Bates et al. 2011). The difference in sites was attributed to greater floral abundance and quality of habitat in the rural areas (Bates et al. 2011). Another study looking at richness and abundance of hoverflies and bees determined that natural areas benefited hoverfly populations, but only if floral cover was high (Kleijn and van Langevelde 2005). Additionally, Kleijn and van Langevelde (2005) found that bee richness was only affected by floral cover when surrounding areas were lacking flowering plants. Another study which observed natural, urban, and agricultural landscapes determined that bee visitation rates were highest in urban and agricultural settings (Leong et al. 2014). However, they only recorded visitation rates to *Centaurea solstitialis*, yellow starthistle, which may affect the results because other floral resources were not taken into account. One European study reported semi-natural, urban, and agricultural habitats affected bee populations differently (Carré et al. 2009). Based on these studies, we can speculate that the effects of landscape use will positively affect some species and has the potential to negatively affect other species. Since landscape is a driver in bee composition, it stands to reason that only the most resilient bee species will survive (Carré et. al 2009).

Several studies report that floral abundance and quality of nesting sites have significant effects on pollinator abundance and diversity (Bates et al. 2011; Roulston and Goodell 2011; Tonietto et al. 2011; Hopwood 2008) while Küshel and Blüthgen (2015) suggest temperature has a greater effect. Not only does floral abundance affect pollinator presence, thermal niches of pollinators can determine the success of certain species in different landscape contexts. In grasslands that were intensely managed, Küshel and Blüthgen (2015) found that pollinators present had a higher tolerance to heat than in areas that were semi-natural. They suggest pollinators that have wider thermal niche ranges should succeed as climate change advances (Küshel and Blüthgen 2015).

Increasing habitat. Landscape context and habitat quality likely has a pronounced effect on pollinator species richness and abundance. Conservation of natural- and semi-natural habitats adjacent to agricultural landscapes help to increase and protect resources needed by pollinators and may be useful to improve pollination services, through the protection of pollinator populations. There are many important reasons to increase the amount of pollinator habitat in both the semi-natural, agricultural, and urban environment. This pollinator habitat can beautify landscape, provide habitat and food sources for other wildlife, and improve soil quality and water quality by providing organic matter and filtering water and increase biodiversity (Wratten et al. 2012). In a study focusing on ground-nesting bees and sunflower fields, it was determined that it would be beneficial to plant nesting habitat patches along the field because of the cultural practices that go hand-in-hand with agriculture (Kim et al. 2006). Goulson et al. (2015) recommends planting flower hedgerows or field margins that provide benefits to both farmers and pollinators. They also suggest providing nesting sites such as bare ground and hollow plant stems along agricultural fields (Goulson et al. 2015). Morandin and Kremen (2013) stress the importance of hedgerows in maintaining wild bee and syrphid populations. Hedgerows are important as they provide not only foraging habitat, it also provides nesting habitat for pollinators. Another benefit of hedgerows is that they provide a greater variety of flowers during the early summer when agricultural fields are not in full bloom (Hannon and Sisk 2009). In urban settings, studies suggest that small urban gardens can be an important part of supporting pollinator health (Matteson and Langellotto 2010; Matteson et al. 2008; Tommasi et al. 2004), but it has not yet been established what plants should be make the best habitat in urban landscapes.

The debate between planting native and non-native vegetation is an ongoing one. To date, research does not agree on the best vegetation for pollinators. Chrobock et al. (2013) found that pollinators visited native flowers more

than the invasive and non-invasive flower species in Switzerland. They attributed the higher visitation rates on native flowers to smaller, but more numerous “flower units” on the native flowers (Chrobock et al. 2013). Another study found that native plants had greater bee abundance (Paredee and Philpott 2014). Gardens that had natives planted instead of only non-natives had higher bee abundance across all bee types (Paredee and Philpott 2014). A study in the UK by Lopezaraiza-Mikel et al. (2007) reported that invasive species plots had more pollinator visitor richness and abundance than the control plots. While another study suggests pollinators were dependent on alien plant species more so than native plant species (Vilà et al. 2009). Vilà et al. (2009) determined pollinators visited alien plants more frequently than native plants due to the attractiveness of the flowers since they were introduced as ornamentals. Some ornamentals and other non-native plants are beneficial for pollinators. Salisbury et al. (2015) suggests that planting flowers other than natives would benefit pollinators more than solely planting natives because exotic plants can extend the flowering season.

Few studies have covered natives versus adapted or near-native species; most have focused on native versus invasive species. Although the origin of flowers planted for pollinator habitat may not be crucial for the success of pollinators, quality of pollinator habitat should be the main concern when establishing habitat (Bruckman and Campbell 2014; Kleijn and van Langevelde 2006). Bees are active from February through November (Vaughan and Hoffman Black 2008), so regardless of flower origin, it is important to extend flowering into fall in order to maintain a food source for the bees.

Pollinator Studies in NC. Relatively few studies have been published on pollinator populations in North Carolina. One study by Rogers et al. (2013) evaluated the efficiency of bees as pollinators of *Vaccinium corymbosum* ‘O’Neal’ “highbush blueberry”. They determined small native bees were more efficient and less affected by environmental factors than the non-native honey bee and that different bee groups contribute to pollination differently (Rogers et al. 2013). Another study conducted in North Carolina examined the effect of urbanization on forest bee communities (Carper et al. 2014). Carper et al. (2014) determined that flower abundance in suburban areas, along with open space, can support native bees in an urban setting. A study in Virginia and North Carolina determined pollen preference of a member of Megachilidae between orchards and other competing flowering trees. They determined that the *Osmia lignaria* lignaria “blue orchard mason bee” preferred *Cercis Canadensis* “Eastern redbud” pollen compared to orchard and other tree pollen (Kraemer et al. 2014). In her

unpublished thesis, Hamblin (2015) examined bee communities across Raleigh, NC to determine the effects of impervious surface and floral coverage on bee abundance and richness. Increasing impervious surface decreased bee abundance, but increasing floral cover and diversity increased bee diversity (Hamblin 2015).

Based on the literature, it is likely that increasing pollinator habitat and floral diversity should also increase abundance and diversity of pollinators (Tonietto et al. 2011; Noordijk et al. 2009; Hopwood 2008). The increase in bee diversity can be attributed to the fact that bees are attracted to wildflowers that produce sufficient amounts of pollen and nectar (Potts et al. 2010). With a variety of flowers to choose from, bees should be better able to reach their nutritional requirements. When land has low floral diversity, the area is not as enticing to bees. In roadside restorations, weedy areas with fewer flowering plants resulted in lower bee abundance and richness due to the lack of nesting sites and foraging capacity for ground nesting bees (Hopwood 2008). Although it ideal to have habitat rich in flowers, sporadic flowering may be enough to support pollinator communities when the land is typically sterile of flowers.

Roadside areas cover more than 10 million acres of land in the United States (Forman et al. 2003). This land can offer valuable pollinator habitat because it is lightly managed, set aside from further development, and because it stretches across the landscape, connecting multiple habitat patches. Lands along roadsides and other marginal lands have the potential to support a variety of pollen and nectar sources and unlike agricultural fields, are unplowed, and therefore can also provide potential nesting sites for ground-nesting bees. The question remains: To what degree does roadside habitat impact insect pollinators in North Carolina? And specifically, does adding pollinator habitat to marginal lands, such as roadsides, increase pollinator abundance and richness?

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CHAPTER 2 – Impacts of pollinator habitat and land use on insect pollinator abundance and richness

For the past 60 years the world has undergone major landscape transformation through urbanization (United Nations 2014). A majority of people across the world live in urbanized areas compared to rural areas, with the United States being one of the most urbanized locations (United Nations 2014). By 2050, urbanization is expected to increase as much as 66% (United Nations 2014). As urbanization increases, natural and semi-natural lands are replaced by buildings, and with them, impervious surface also increases. Increasing land dedicated to urbanization and farming reduces naturalized habitat, and ultimately limits the amount of nesting and foraging sites for wildlife of all types, and especially for important insect pollinators.

Pollination of flowering plants is an essential ecosystem service provided by animals such as bees, flies, wasps, and butterflies, among others. These ecosystem services include pollination of wildflowers which increases aesthetic appeal of the landscape and also increases habitat for other wildlife (Wratten et al. 2012) as well as provide essential macronutrients, vitamins, and minerals in human diets (Nicole 2015). Insect pollinators are responsible for pollinating 75% of the world's food crops (Lutz et al. 2014; Klein et al. 2007). Of all insects, bees are considered to be the most beneficial pollinator for human needs (Arbol 2012), although flies, butterflies, moths, and wasps are also important pollinators. Over the past few decades, insect pollinator populations have been declining across the world. While no single factor is responsible, increasing threats from anthropogenic sources including habitat loss and fragmentation (Blackmore et al. 2014; Fortel et al. 2014; Hanley et al. 2014; Arbol 2012), agricultural intensification (Kremen et al. 2002; Klein et al., 2007), and pesticide exposure (Brittain et al. 2010; Kearns et al., 1998) all greatly contribute to the general decline.

Concerns about the global decline of pollinators (Memmott et al. 2007; Kearns et al., 1998) have increased interest in investigating, promoting, and conserving habitat that can support pollinators. Pollinator habitat must provide pollinators with nesting sites, protection from pesticides, and foraging sites. Incorporating suitable habitat for insect pollinators throughout urbanized landscapes is potentially one way to reduce the negative effects of urbanization on pollinators (Miller 2005).

Based on the literature, it is likely that increasing pollinator habitat and floral diversity should also increase abundance and diversity of pollinators (Tonietto et al. 2011; Noordijk et al. 2009; Hopwood 2008). With a variety of flowers to choose from, bees should be better able to reach their nutritional requirements. Although it is ideal to

have habitat rich in flowers, sporadic flowering may be enough to support pollinator communities where land is typically devoid of flowers. Pollinator habitat acts as a buffer from surrounding impervious surfaces in urbanized areas by increasing foraging and nesting sites, and should be sufficient to support greater species numbers in areas with few resources.

It has been well established that floral diversity increases pollinator abundance and diversity (Tonietto et al. 2011; Noordijk et al. 2009; Hopwood 2008). The increase in bee diversity can be attributed to the fact that bees are attracted to wildflowers that produce sufficient amounts of pollen and nectar (Potts et al. 2010). In highly human-impacted environments, marginal lands such as field buffers, utility rights-of-way, and roadsides become especially important for the conservation of biodiversity. Roadside habitats, for example, have been shown (in a limited number of studies) to increase insect species abundance under reduced management (Halbritter et al. 2015, Hopwood 2008). This leads us to posit that roadsides in the Piedmont of North Carolina have the potential to support suitable pollinator habitat. And unlike agricultural fields, these areas are generally undisturbed, and therefore provide potential nesting sites for ground-nesting bees, plant debris for cavity-nesting bees, in addition to a variety of pollen and nectar sources to attract other insect pollinators.

Roadside vegetation management in the southern United States has frequently included wildflower species plantings to help reduce long-term maintenance costs, enhance roadside wildlife habitat, provide an attractive roadside environment, and promote natural establishment of wildflowers (AASHTO 2013; FLDOT 2016; NCDOT 2016; TXDOT 2016). North Carolina's Department of Transportation (NCDOT) established their roadside flowering habitat initiative in 1985, and today they plant 1,500 acres of wildflowers including annuals, perennials, and natives across North Carolina interstates in efforts to increase wildlife habitat and beautify roadsides. With the understanding that habitat plays an important role in the success of pollinators, we addressed the following questions 1) Does planting wildflowers along North Carolina interstates increase insect pollinator abundance and richness? 2) Does percent flower cover impact pollinator species abundance? And 3) Does urbanization and land use around pollinator sites affect pollinator abundance and richness?

Methods

Study sites. Sites for this study were located along interstates in the Piedmont of North Carolina. A total of 18 pollinator habitat sites (which are defined, for the purposes of this body of work, seeded wildflower patches

intentionally planted and maintained) and 12 control sites (defined as primarily lightly managed turfgrass areas) were sampled in Alamance, Guilford, Johnston, Nash, Orange, Wake, and Wilson counties. The pollinator habitat sites were established and maintained by North Carolina Department of Transportation (NCDOT). In fall of 2014, North Carolina DOT planted various wildflowers at each site by hydroseeding after preparing the soil and applying pre-emergent herbicide in 0.4 hectare patches in medians and along exit and entrance ramps along North Carolina highways. Pollinator habitat sites consisted of flowering vegetation ranging from monocultures to mixed species (Appendix C). Pollinator habitat sites chosen were generally a minimum of 8 km apart, however, two pollinator sites were separated by 3.2 km and 6.8 km. Control sites were established on lightly managed turf with intermittent weedy flowering species (Appendix C cont). Control sites were paired with a pollinator habitat site and located within 1.75 km of with the exception of one control site that was 3.2 km from the paired pollinator site. Not all wildflower sites had a paired control site due to locality constraints.

As with any field study, there were challenges that arose due to unscheduled maintenance of a number of our sites. Many pollinator plots were mowed without warning at various times during our study, making for an asynchronous sampling schedule.

Sampling. Sampling occurred every third week from May 2015 through August 2015 on sunny days above 15.5°C. Each site was walked at a moderate pace for 20 minutes using a modified sweep net, or zip net, (Sepsenwol 2014) to collect bees, flies, and butterflies. An aerial net was used to collect butterflies to protect specimens and kill jars were also used. Three transects 15 m long were established 1.5 m apart. Blue, white, and yellow pan traps were placed along each transect for a total of 15 pan traps (5 blue, 5 white, and 5 yellow) per sampling. Pan traps were filled with unscented, soapy water and were collected after 5 hours between 8:15 and 16:45 (Polatto et al. 2014; Albrecht et al. 2012; Stone et al. 1999). Ambient temperature and general weather conditions were recorded at the beginning and end of sampling periods.

Floral Cover. On the same date sampling occurred, digital images were taken of the sites. Flowering species were documented and relative floral abundance was determined during each visit by visually estimating floral cover across the site. For species with less than 5% floral coverage, those plants were not identified to species but were included in overall percent flower cover analysis.

Specimens. Insects collected from sweep netting were stored in a freezer until the specimens were cleaned and/or pinned. Insects collected from pan traps were maintained in Whirl-Paks® with ethyl alcohol (95% denatured with methyl and IPA; Fisher Science Education) until washed (Droege 2015) and pinned. All insects collected were pinned. Bees were identified to species with the exception of *Lasioglossum*, which was identified to genus. Flies were identified and categorized as syrphid and non-syrphid groups. Butterflies were identified to species. Insects were identified using “IDnature guides” from an online identification key, Discover Life (www.discoverlife.org).

Land use. In order to determine land use surrounding wildflower sites at 500 m, 1,000 m, and 2,000 m radii, all sites were imported into ArcMap (ESRI: ArcGIS Desktop v. 10.3.1) for analysis. At these radii, using Gap Analysis Program Land Cover data (US Geological Survey 2011), land use was divided into 3 categories: urban/suburban, natural/forested, and agriculture. Urban/suburban included developed land from low through high intensities. Natural/forested included forests, fields of herbaceous plants, and wetlands. Agriculture included pasture and cultivated land. Land use cover was determined for each site using Excel.

Statistics. Insect abundance and richness were analyzed with an ANOVA using R 3.2.3 with the following packages: lattice, car, scatterplot3d, MASS, permute, plyr, Matrix, vegan, nlme, lme4, date, and lsmeans (Lenth 2016; Oksanen et al. 2016; Pinheiro et al. 2016; Simpson 2016; Xie 2016; Therneau et al. 2014; Fox and Weisberg 2011; Wickman 2011; Sarkar 2008; Ligges and Mächler 2003; and Venables and Ripley 2002). The model used included pairtype (paired sites, pollinator habitat site or control site, and the interaction), month, and site (as a random effect). To determine insect biodiversity and evenness at each site, the Shannon-Wiener index (H') was calculated using the following formula:

$$H' = -\sum p_i \ln p_i$$

Following Aitchinson (1982), the three land use variables were transformed to two centered log ratios, $\log(\text{natural-forested/urban})$ and $\log(\text{agricultural/urban})$ prior to analysis (Figure 1, Figure 2, and Figure 3). Bee abundance data were first analyzed using multiple linear regression with the two log ratios and floral cover as independent variables. Flowering percentage was the only variable with a significant effect in the model. A second multiple regression model, this time with a better fitting nonlinear relationship for flowering percentage, was tested. Again, the effect of land use variables was not statistically significant, and its magnitude was negligible relative to the magnitude of the effect of flowering percentage. We thus present the results of the simple nonlinear model of bee count as a two parameter hyperbolic function of flowering percentage. The model used for analyzing bee

abundance included pairtype (paired sites, pollinator habitat site or control site, and the interaction), month, and site (as a random effect). All analyses were performed using SAS software, Version 9.4 of the SAS System for Windows (SAS Institute, Cary, NC, USA).

Results

Species Composition. A total of 1223 bees, 251 syrphids, 744 non-syrphids, and 166 butterflies were collected at pollinator and control sites. A total of 29 individual bee species were collected throughout the sampling period (Appendix A and Appendix B). The most abundant bee species was *Halictus ligatus/poeyi* Say/Lepeletier (235) while other abundant species included *Bombus impatiens* Cresson (164) and *Apis mellifera* Linnaeus (150) (Figure 4). Since *Lasioglossum* was not identified to species it was not included in the most abundant species list although there were 226 individuals within this genus. *Andrena rudbeckiae* Robertson, *Andrena sp.*, *Bombus perplexus* Cresson, *Halictus parallelus* Say, and *Melissodes communis* Cresson were represented by a single individual. Halictidae and Apidae were the most represented families comprising 50.4% and 44.9% of the individuals, respectively (Figure 5). Ground nesting (50.2%) and cavity nesting (49.8%) bees comprised roughly the same percentage of individuals collected over all. A total of 17 butterfly species were collected throughout the sampling period. The most abundant species was *Pieris rapae* L. comprising nearly 50% of the total species collected. There were 5 species that were represented by a single individual.

Abundance and Species Richness. Bee abundance was significantly greater at the pollinator sites than at the control sites ($p=0.002$) and bee abundance was significantly lower in May ($p<0.001$, Figure 6). Over the entire sampling period, the mean bee abundance was 18.5 ± 2.3 individuals per sampling (Table 1). Syrphid, non-syrphid, and butterfly abundance were not significantly different ($p>0.05$). Overall abundance of all pollinators collected was significantly greater at the pollinator sites than the control sites ($p=0.034$). Neither fly abundance nor butterfly abundance was significant ($p=0.191$ and $p=0.443$, respectively) (Figure 7).

Overall bee and butterfly diversity was significantly greater for pollinator sites than control sites ($p=0.007$). Bee diversity was significantly greater at the pollinator sites than at the control sites ($p=0.005$) and bee diversity was significantly lower in May ($p=0.001$), with an average, 4.2 ± 0.3 different species collected at each site (Table 1). Butterfly diversity was not significant at 0.05 significance level.

Insect biodiversity by Site. Site 19 had 9 species represented and an H' value of 1.95. Site 24 had the second highest H' value with 12 species represented and an H' value of 1.93. Four sites (20, 22, 27, and 30) had only a single species sampled, and an H' of 0.00 (Table 2).

Floral Cover. Mean floral abundance was $48.55 \pm 5.80\%$ at pollinator habitat sites compared to $5.13 \pm 4.47\%$ at control sites (Table 1), but these values were not significant. Maximum floral cover was 100% at pollinator habitat sites and 15% at control sites (Table 1). A variety of weedy species and wildflowers were flowering throughout the sampling period (Table 3). At pollinator habitat sites, floral diversity was 2.61 ± 0.30 compared to 1.07 ± 0.16 at control sites (Table 1). Floral abundance was greatest during May 2015 compared to any other month during sampling (Table 4).

Land Use.

Floral Cover. Total bee abundance was significantly greater when more flowers were in bloom (Table 5). Bee diversity was significantly greater during early spring at all radii and during summer at the 2000m radius ($p=0.043$) (Table 5). *Apis mellifera* had an early spring effect at all distances ($p=0.041$, 0.044 , and 0.047), but not during later samplings. *Bombus* spp. presence was significantly greater during late spring at small ($p=0.000$) and large radii ($p=0.000$ & $p=0.000$) (Table 5). *Xylocopa* spp. had a late spring effect at all distances ($p= 0.022$, 0.038 , and 0.043), but not during later samplings (Table). Small native bees, flies, and butterflies were not significantly affected by floral cover.

Forested/Urban. *Bombus* spp. had an early spring effect at larger radii ($p= 0.002$ & $p= 0.019$) and a summer effect at the largest radii ($p=0.024$) (Table 5 and Figure 8). Total bee abundance, bee diversity, *Apis mellifera*, *Xylocopa* spp., flies, and butterflies were not affected by forested/urban land use.

Agriculture/Urban. Bee diversity was significantly greater at the largest radius during early spring ($p= 0.036$) and at the largest radius during summer ($p= 0.011$) (Table 5). *Bombus* spp. had a late spring/early summer effect at the larger radii (Table 5). Small native bees were significantly greater at urban sites during early and late spring at all radii, but not during any other sampling. Total bee abundance, *Apis mellifera*, *Xylocopa* spp., and butterflies were not significantly affected by agriculture/urban land use.

Discussion

Though roadsides are not a substitute for all types of native vegetation, they do have value as habitat for birds (Adams 1984), small mammals (Camp and Best 1994), and a variety of insect life (Hopwood 2008; Vermeulen 1993; Keals and Majer 1991). In this study, we were able to demonstrate that roadside habitats also provide refuge for pollinators. Specifically, we demonstrated that pollinator-friendly roadside plantings increased both bee abundance and diversity.

Bee abundance was significantly increased when areas along the interstate in North Carolina had increased floral coverage. This suggests that North Carolina Department of Transportation's practice of sowing wildflowers along roadsides have been beneficial for bees, since pollinators rely on flowers for pollen and nectar for food. By increasing floral cover or diversity, pollinator abundance and diversity have been shown to increase (Blaauw and Isaacs 2014; Hopwood 2008; Kleijn and van Langevelde 2005). In our study, sites with some floral cover had increased bee abundances compared to sites with no or minimal floral cover. We determined 100% flower cover is not necessary. From 0-45% flower cover, there was a steady increase of bee abundance. However, bee abundance did not increase as rapidly when percent flower was greater than 45%. This suggests that as long as there is some floral cover, the flowers will attract and support bee communities. Dicks et al. (2015) reported hedgerows with a minimum of 2% coverage was enough flowering habitat to support pollinators in an agricultural setting. According to Blaauw and Isaacs (2014), in smaller scale landscapes (100m²), flowering patches with greater diversity were needed to support wild bee communities, but even small strips of flowering patches 18 m wide along roadways has been shown to be sufficient for pollinators (Hopwood 2008). Our study suggests that larger areas of pollinator habitat (0.4 hectare), can benefit bee communities with moderate levels of floral cover. This is extremely valuable considering the amount of marginal land surrounding interstates across the U.S.

Although not all pollinator types were affected equally by the presence of pollinator habitat along roadsides, our study found that bee abundance and diversity did increase with the presence of pollinator-friendly wildflower plots. This is an important point as bees are regarded as the most efficient pollinator group (Arbol 2012). In a recent study examining butterfly abundance along roadways, it was determined that not mowing grassy roadsides significantly increased butterfly abundance, and mowing less frequently increased butterfly abundance, although not as dramatically as the no-mow treatments (Halbritter et al. 2015). Flies and butterflies, less efficient

pollinators than bees, do not rely entirely on pollen and nectar from flowers for their nutritional needs making floral resources not as vital as compared to bees. It is likely that the grassy areas along roadsides provide enough suitable habitat to sustain their populations. While we did find more flies as compared to bees at the control sites, this did not prove to be significantly different. Our findings are similar to that of Hopwood (2008) who also reported that habitat along roadways increased bee abundance and diversity.

Several habitat studies suggest floral abundance and quality of nesting sites have positive effects on pollinator abundance and diversity (Bates et al. 2011; Roulston and Goodell 2011; Tonietto et al. 2011; Noordijk et al. 2009; Hopwood 2008). In our study, we determined that floral cover was most important in supporting bee communities; at sites with little to no floral cover, bee abundance was greatly reduced as compared to sites that had greater floral cover. This is not surprising since pollinators are attracted to wildflowers that produce sufficient amounts of pollen and nectar (Potts et al. 2010). We did not look at how individual sites may or may not have been attractive to pollinators, nor did we consider the quality of pollen and nectar produced by the various flowering species at our sites. Both of these topics would be something worth researching in the future.

Overall, we found that increasing flowering plants along interstates in North Carolina was beneficial for bee pollinators. Other research has also shown that incorporating a variety of flowering species and ensure greater floral coverage has resulted in greater pollinator abundances and diversity (Blaauw and Isaacs 2014; Hopwood 2008; Kleijn and van Langevelde 2006). In our study, the pollinator habitat sites were in peak bloom in May although bee abundances were highest later in the season. This stresses the idea that there should always be flowering habitat during months when bees are active. Pollinator habitat should include blooming flowers, which supply pollinators with high quality pollen and nectar, ideally through multiple seasons. These sites, if left undisturbed, can also provide nesting sites for many native bees. Many bees dig in-ground nests in suitable areas, while other bee species nest above ground in plant stems or cavities in dead wood. Ideally, flowering habitat along roadsides should be no farther than a mile apart to allow for connectivity between flower patches, and should remain un-tilled and ideally, unsprayed. We also suggest selecting a variety of flowers that will provide pollen and nectar throughout the entire season when pollinators are active.

Despite an increase in urbanization in many parts of the Southeast and across the US, it is possible to protect pollinators by increasing wildflower habitat along roadsides. These marginal habitats are often the only

semi-natural areas remaining in heavily altered urban landscapes, and can provide pollinators with places to forage for food and to nest and rear their young. In areas where flowers cannot be planted, we recommend reducing scheduled mowing to allow weedy species to flower, as even this seems to provide suitable habitat. If managed properly the thousands of hectares of land associated with roadsides could offer substantial refuge for pollinators.

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FIGURES

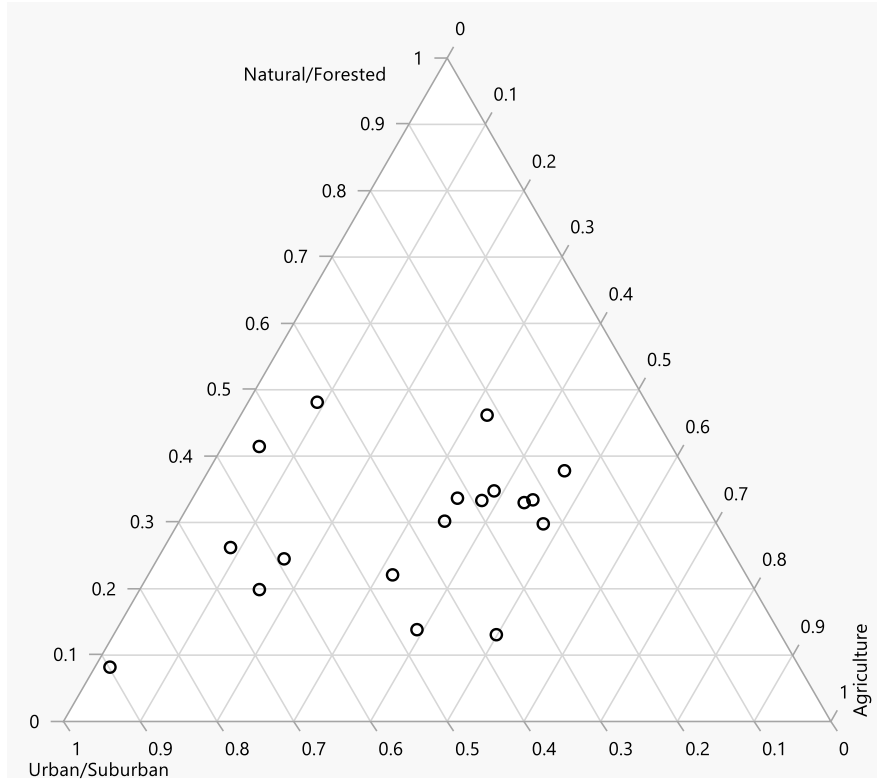


Figure 1. The distribution of sites comprised of urban/suburban, natural/forested, and agriculture at a 500m radius surrounding each site.

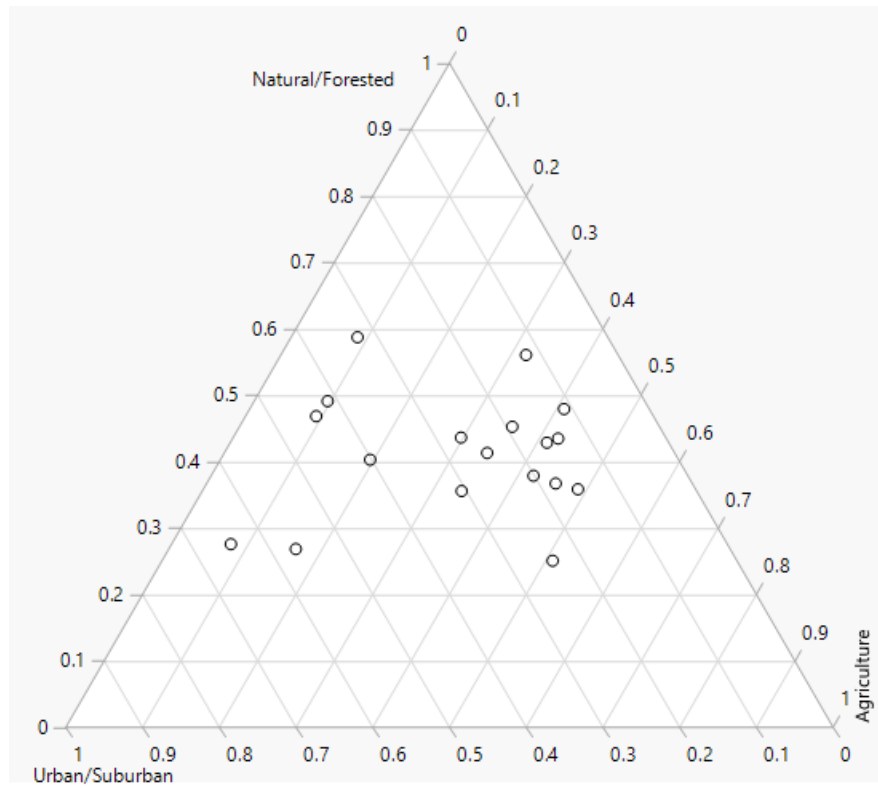


Figure 2. The distribution of sites comprised of urban/suburban, natural/forested, and agriculture at a 1000m radius surrounding each site.

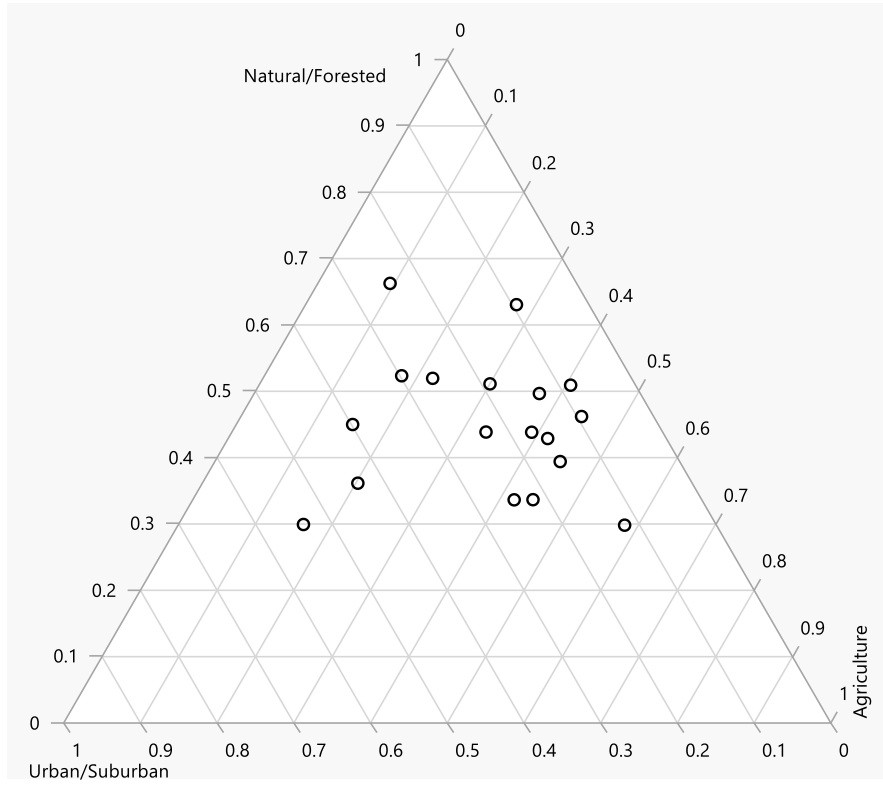


Figure 3. The distribution of sites comprised of urban/suburban, natural/forested, and agriculture at a 2000m radius surrounding each site.

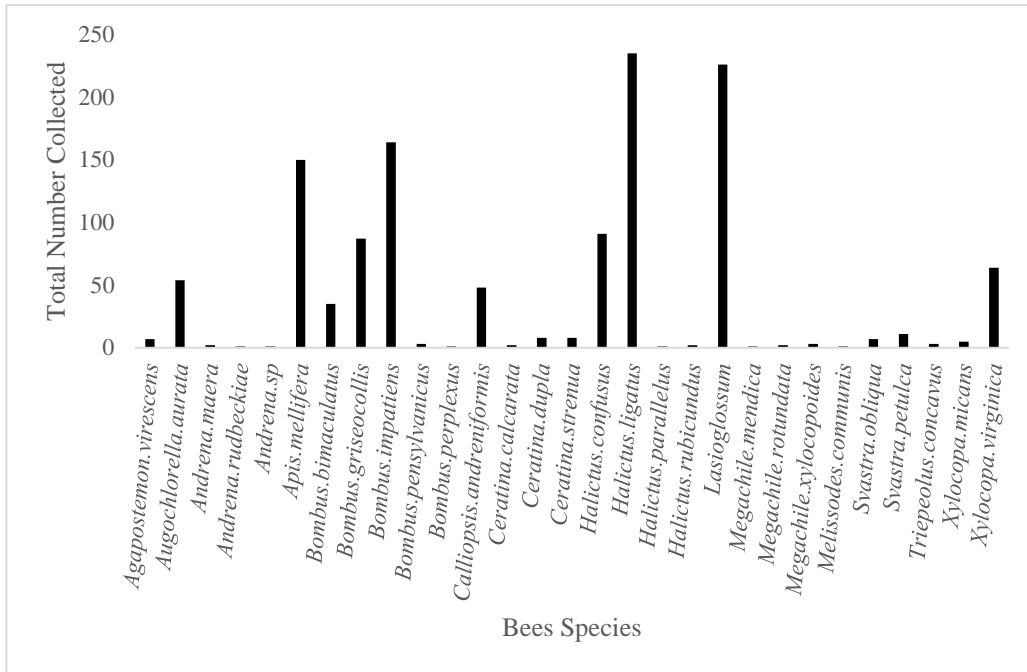


Figure 4. Total number of bees collected from May through August, 2015 at all sites.

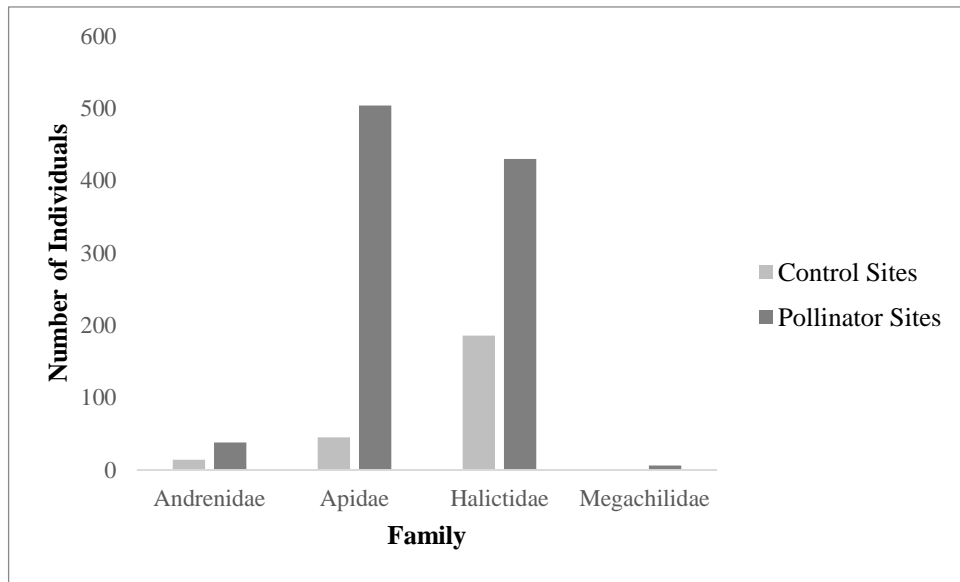


Figure 5. Number of bees collected at pollinator and control sites May through August, 2015 belonging to various families.

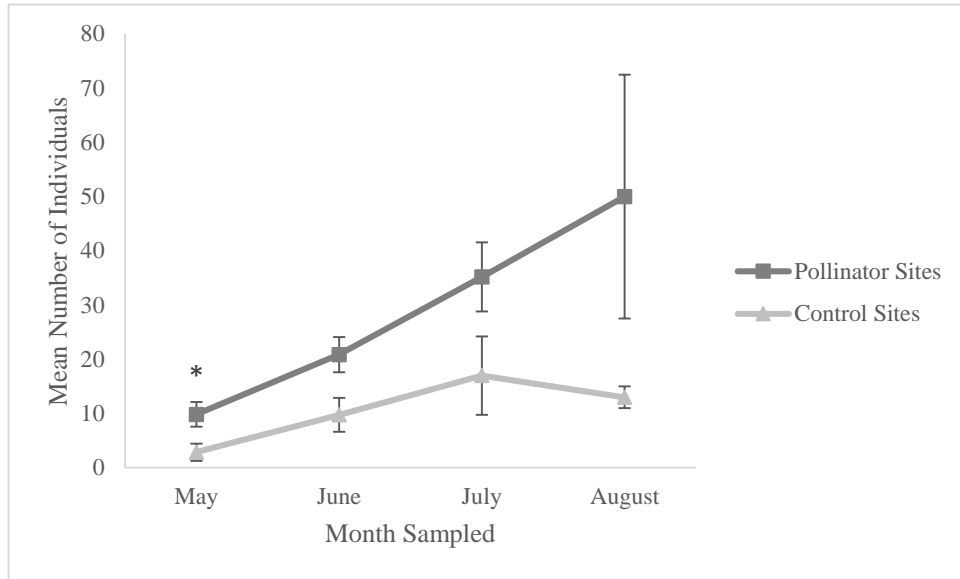


Figure 6. Mean number of bees collected May through August, 2015.

*Mean number of bees was significantly lower for the month of May compared to other months sampled ($p < 0.001$).

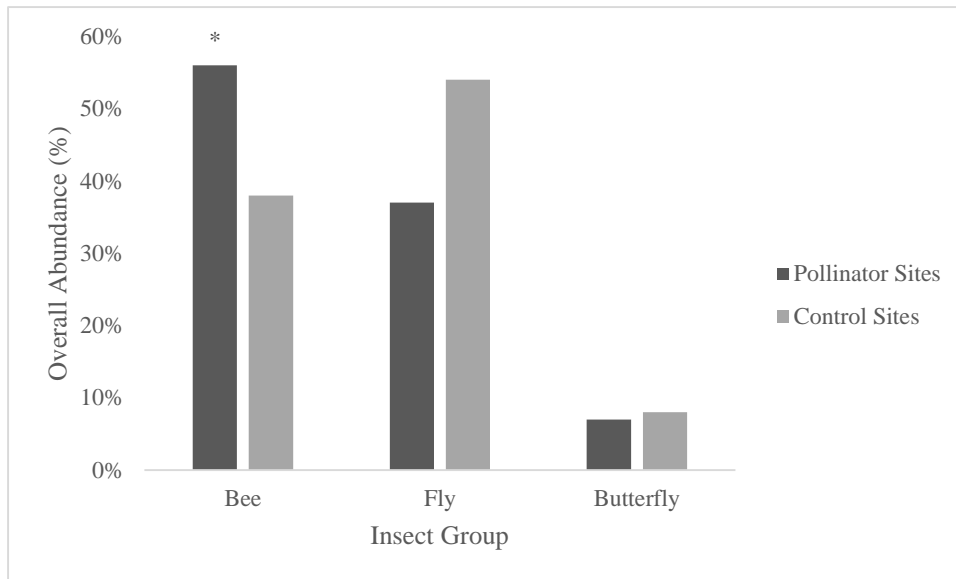


Figure 7. Percentage of bees, flies, and butterflies collected at pollinator habitat sites and control sites from May through August, 2015.

*Overall bee abundance at pollinator habitat sites was significantly greater than at the control sites ($p= 0.002$).

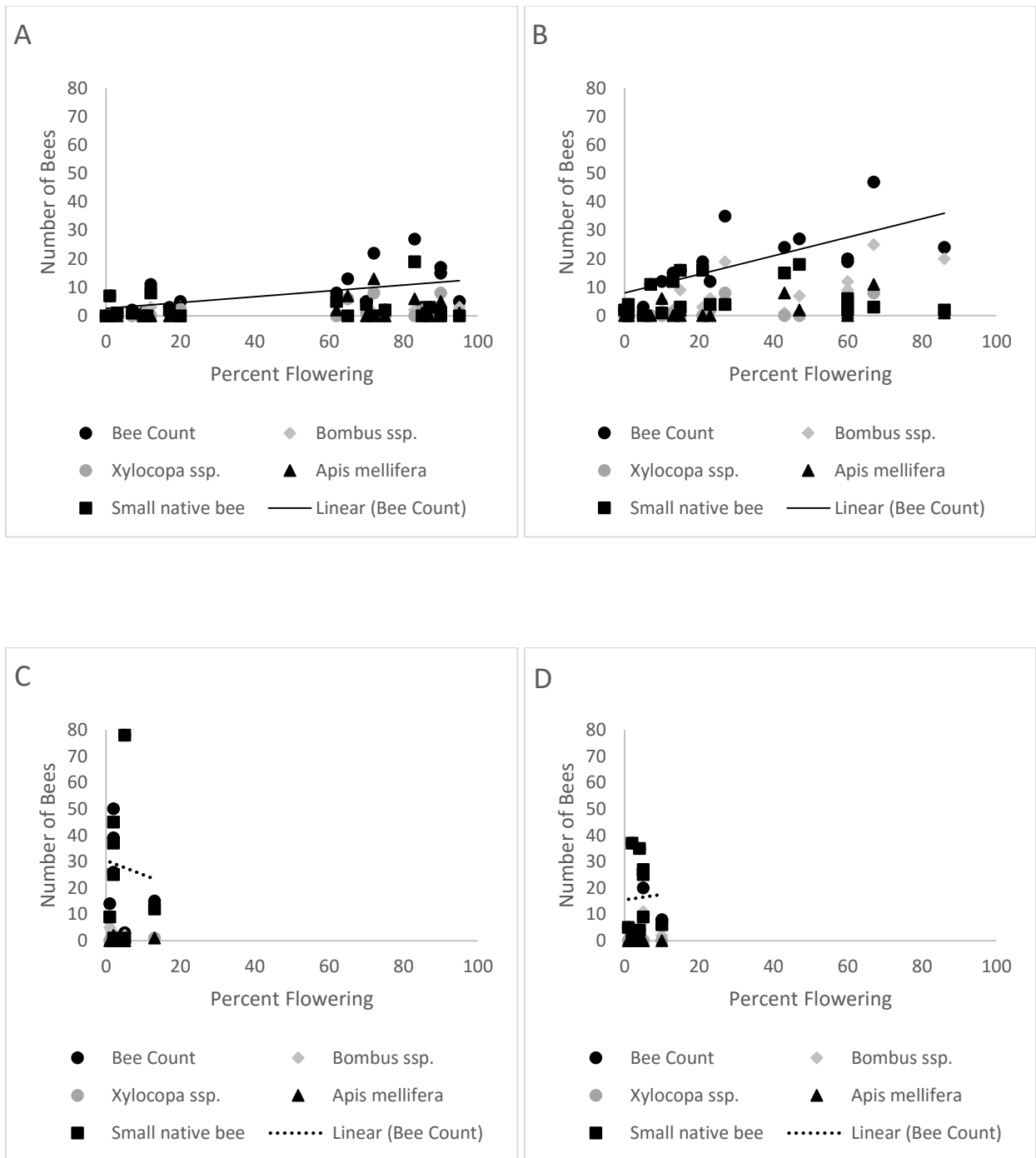


Figure 8. Overall bee count, *Xylocopa* spp., small native bee, *Bombus* spp., and *Apis mellifera* at the 2000m radius for percent flowering for 4 different samplings (A – 1, B – 2, C – 3, and D – 4). Solid lines are significant ($p < 0.05$) and dashed lines are not significant for overall bee count.

TABLES

Table 1. Pollinator habitat and control sites were sampled May through August, 2015. Mean pollinator abundance, pollinator richness, floral abundance, and floral diversity \pm standard error for pollinator as well as the minimum and maximum values for each visit were determined.

Variate		Pollinator Sites			Control Sites		
		Mean \pm se	Min	Max	Mean \pm se	Min	Max
Abundance	Bees	23.3 ^a \pm 3.1	1	92	10.2 \pm 2.5	0	35
	Syrphids	3.0 \pm 1.0	0	32	5.3 \pm 2.0	0	35
	Non-syrphids	12.4 \pm 2.6	0	73	9.1 \pm 2.7	0	46
	Butterflies	2.7 \pm 0.4	0	11	2.1 \pm 0.5	0	7
Species Richness	Bees	4.9 ^a \pm 0.3	1	8	3.0 \pm 0.50	0	8
	Butterflies	1.4 \pm 0.2	0	4	1.0 \pm 0.3	0	5
Habitat	Floral Abundance (%)	48.6 \pm 5.8	1	100	5.1 \pm 4.5	0	15
	Floral Diversity (number of species)	2.6 \pm 0.3	1	10	1.1 \pm 0.2	0	3

^a Significantly greater than control at 0.01 level

Table 2. Shannon-Wiener index and evenness values for all sites.

Site	# of Species	Total Sample	Shannon Index	Evenness
1	9	22	1.89	0.86
2	6	64	1.41	0.79
3	8	35	1.90	0.92
4	7	29	1.65	0.85
5	7	29	1.56	0.80
6	6	15	1.58	0.88
7	7	17	1.68	0.86
8	8	57	1.66	0.80
9	3	5	1.05	0.96
10	12	219	1.56	0.63
11	10	36	1.92	0.84
12	10	63	1.95	0.85
13	11	127	1.74	0.72
14	9	71	1.52	0.69
15	3	41	0.23	0.21
16	8	95	1.52	0.73
17	7	35	1.21	0.62
18	2	17	0.61	0.87
19	9	20	1.95	0.89
20	1	1	0.00	0.00
21	3	4	1.04	0.95
22	1	1	0.00	0.00
23	2	3	0.73	0.67
24	12	92	1.93	0.78
25	3	7	0.96	0.87
26	9	92	1.29	0.59
27	1	1	0.00	0.00
28	5	15	1.44	0.89
29	3	8	1.08	0.99
30	1	1	0.00	0.00

Table 3. Presence (X) of flowering species recorded in June 2015. Species not flowering or not present at sites are represented by (-).

Scientific Name	Pollinator Habitat Sites																		Control Sites											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
<i>Barbarea vulgaris</i>	.	.	.	X	.	.	.	X
<i>Centaurea cynus</i>	.	X	X
<i>Chrysanthemum leucanthemum</i>	X	.	X	X
<i>Chrysanthemum maximum</i>	X	.	X	.	X
<i>Coreopsis lanceolata</i>	X	.	X	.	X	X
<i>Coreopsis tinctoria</i>	.	.	X	.	.	X	X	.	.	X	X	.	X
<i>Cosmos bipinnatus/sulphureus</i>	.	.	.	X	.	.	.	X
<i>Daucus carota</i>	X	X
<i>Delphinium ajacis</i>	.	X	X	X	.	.	.	X	X
<i>Erigeron annuus</i>	X
<i>Eschscholzia californica</i>	X	X	.	.	.	X	X
<i>Helianthus sp.</i>	X	X	X	X	X
<i>Hesperis matronalis</i>	X	X	.	.	.	X
<i>Hypochaeris radicata</i>	X
<i>Lathyrus odoratus</i>	X
<i>Linaria maroccana</i>	.	.	.	X	.	.	.	X
<i>Papaver rhoeas</i>	.	X	X	X	.	X	X	X	X
<i>Phlox drummondii</i>	X
<i>Rudbeckia amplexicaulis</i>	.	.	X
<i>Rudbeckia hirta</i>	X	.	.	X
<i>Trifolium pratense</i>	X	.	X
<i>Trifolium repens</i>	X	X	.	X

Table 4. Percent mean floral cover \pm standard error and mean floral diversity \pm standard error for pollinator habitat sites and control sites from May through August, 2015.

Site	Mean Floral Cover (%)				Species Flowering (Mean)
	May	June	July	August	
Pollinator	70.1 \pm 7.0	32.1 \pm 6.9	47.2 \pm 15.3	42.7 \pm 28.8	2.6 \pm 0.3
Control	6.3 \pm 2.4	5.5 \pm 2.0	4.3 \pm 1.0	2.50 \pm 1.5	1.1 \pm 0.2

Table 5. Significant interactions at 3 radii (500m, 1000m, and 2000m) for bee abundance, bee diversity, *Apis mellifera*, *Bombus* spp., *Xylocopa* spp., small native bees (which include all bees collected not within previously mentioned functional groups), and syrphid for all sites when analyzing land use variables (floral cover, logarithm of forested to urban, and logarithm of agricultural to urban).

Visit	Distance (m)	Functional Group	Variable	p-value
1	500	Bee Abundance	Floral Cover	0.021
1	1000	Bee Abundance	Floral Cover	0.012
1	2000	Bee Abundance	Floral Cover	0.025
1	500	Bee Diversity	Floral Cover	0.007
1	1000	Bee Diversity	Floral Cover	0.005
1	2000	Bee Diversity	Floral Cover	0.050
1	500	<i>Apis mellifera</i>	Floral Cover	0.041
1	1000	<i>Apis mellifera</i>	Floral Cover	0.044
1	2000	<i>Apis mellifera</i>	Floral Cover	0.047
2	500	Bee Abundance	Floral Cover	0.002
2	1000	Bee Abundance	Floral Cover	0.001
2	2000	Bee Abundance	Floral Cover	0.002
2	500	Bee Diversity	Floral Cover	0.004
2	1000	Bee Diversity	Floral Cover	0.002
2	2000	Bee Diversity	Floral Cover	0.003
2	500	<i>Bombus</i> spp.	Floral Cover	0.000
2	1000	<i>Bombus</i> spp.	Floral Cover	0.000
2	2000	<i>Bombus</i> spp.	Floral Cover	0.000
2	500	<i>Xylocopa</i> spp.	Floral Cover	0.022
2	1000	<i>Xylocopa</i> spp.	Floral Cover	0.038
2	2000	<i>Xylocopa</i> spp.	Floral Cover	0.038
4	2000	Bee Diversity	Floral Cover	0.043
1	1000	<i>Bombus</i> spp.	For_Urb	0.002
1	2000	<i>Bombus</i> spp.	For_Urb	0.019
1	500	Small Native Bees	For_Urb	0.025
3	2000	<i>Bombus</i> spp.	For_Urb	0.024
1	2000	Bee Diversity	Ag_Urb	0.036
1	500	Small Native Bees	Ag_Urb	0.001
1	1000	Small Native Bees	Ag_Urb	0.001
1	2000	Small Native Bees	Ag_Urb	0.003
1	500	Syrphid	Ag_Urb	0.000
1	1000	Syrphid	Ag_Urb	0.001
1	2000	Syrphid	Ag_Urb	0.009
2	1000	<i>Bombus</i> spp.	Ag_Urb	0.020
2	2000	<i>Bombus</i> spp.	Ag_Urb	0.025
2	500	Small Native Bees	Ag_Urb	0.000
2	1000	Small Native Bees	Ag_Urb	0.000
2	2000	Small Native Bees	Ag_Urb	0.000
3	2000	<i>Bombus</i> spp.	Ag_Urb	0.041
4	2000	Bee Diversity	Ag_Urb	0.011

APPENDIX

APPENDIX

Appendix A. Presence (X) of bee species collected May through August, 2015 at pollinator habitat sites. Species not collected at sites are represented by (·).

Bee Species	Pollinator Habitat Sites																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<i>Agapostemon virescens</i>	·	·	·	·	·	·	·	·	·	X	·	·	·	·	·	·	·	·
<i>Augochlorella aurata</i>	·	·	·	·	·	·	·	·	·	X	X	X	·	X	·	·	X	·
<i>Andrena maera</i>	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
<i>Andrena rudbeckiae</i>	·	·	·	·	·	·	·	·	·	·	·	·	X	·	·	·	·	·
<i>Andrena sp.</i>	X	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
<i>Apis mellifera</i>	X	X	X	X	X	X	X	X	·	·	·	X	X	X	·	X	·	·
<i>Bombus bimaculatus</i>	·	X	X	X	X	X	·	X	X	·	X	·	·	X	·	X	·	·
<i>Bombus griseocollis</i>	X	X	X	X	X	·	X	X	X	X	X	X	X	X	X	X	·	X
<i>Bombus impatiens</i>	X	X	X	X	X	·	X	X	·	·	X	X	·	X	X	X	X	X
<i>Bombus pensylvanicus</i>	·	·	·	·	·	·	·	·	·	·	·	·	X	·	·	·	·	·
<i>Bombus perplexus</i>	·	·	·	·	·	X	·	·	·	·	·	·	·	·	·	·	·	·
<i>Calliopsis andreniformis</i>	X	·	X	·	·	·	X	·	·	X	X	X	X	·	·	·	·	·
<i>Ceratina calcarata</i>	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
<i>Ceratina dupla</i>	·	·	·	·	·	·	·	·	·	X	X	·	·	·	·	·	·	·
<i>Ceratina strenua</i>	·	·	·	·	·	·	·	·	·	X	·	·	·	·	·	X	·	·
<i>Halictus confusus</i>	·	·	·	·	·	·	·	·	·	X	X	X	X	·	·	X	·	·
<i>Halictus ligatus</i>	X	·	·	X	·	·	·	·	·	·	·	·	·	·	·	·	·	·
<i>Halictus parallelus</i>	·	·	·	·	·	·	X	·	·	·	·	·	·	·	·	·	·	·
<i>Halictus rubicundus</i>	·	·	·	·	·	·	·	·	·	·	·	X	X	·	·	·	·	·
<i>Lasioglossum</i>	X	X	X	X	·	X	X	X	·	X	X	X	X	X	·	X	X	·
<i>Megachile mendica</i>	·	·	·	·	X	·	·	·	·	·	·	·	·	·	·	·	·	·
<i>Megachile rotundata</i>	·	·	·	·	·	·	·	·	·	·	·	X	·	·	·	·	·	·
<i>Megachile xylocopoides</i>	·	·	·	·	X	X	·	X	·	·	·	·	·	·	·	·	·	·
<i>Melissodes communis</i>	·	·	·	·	·	·	·	X	·	·	·	·	·	·	·	·	·	·
<i>Svastra obliqua</i>	·	·	·	·	·	·	·	·	·	X	·	·	X	X	·	·	X	·
<i>Svastra petulca</i>	·	·	·	·	·	·	·	·	·	X	·	·	·	·	·	·	X	·
<i>Triepeolus concavus</i>	·	·	·	·	·	·	·	·	·	X	·	·	·	·	·	·	X	·
<i>Xylocopa micans</i>	·	·	X	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
<i>Xylocopa virginica</i>	X	X	X	X	X	X	X	X	X	·	X	·	X	X	X	·	·	·

Appendix B. Presence (X) of bee species collected May through August, 2015 at control sites. Species not collected at sites are represented by (·).

Bee Species	Control Sites											
	1	2	3	4	5	6	7	8	9	10	11	12
<i>Agapostemon virescens</i>	·	·	·	·	·	X	·	X	·	·	·	·
<i>Augochlorella aurata</i>	·	·	·	·	·	X	·	X	·	·	·	·
<i>Andrena maera</i>	·	·	·	·	·	X	X	·	·	·	·	·
<i>Andrena rudbeckiae</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Andrena sp.</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Apis mellifera</i>	X	·	·	·	·	X	·	·	·	·	·	·
<i>Bombus bimaculatus</i>	·	·	·	·	·	X	·	·	·	·	·	·
<i>Bombus griseocollis</i>	X	·	·	X	·	X	·	·	·	·	·	·
<i>Bombus impatiens</i>	X	·	·	·	·	X	·	·	·	·	X	·
<i>Bombus pensylvanicus</i>	·	·	·	·	·	X	·	·	·	·	·	·
<i>Bombus perplexus</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Calliopsis andreniformis</i>	X	·	·	·	·	X	·	X	·	·	·	·
<i>Ceratina calcarata</i>	·	·	·	·	·	·	·	·	·	X	·	·
<i>Ceratina dupla</i>	·	·	·	·	·	·	·	X	·	·	·	·
<i>Ceratina strenua</i>	·	·	·	·	X	·	·	X	·	X	·	·
<i>Halictus confusus</i>	·	·	X	·	·	X	·	X	·	X	X	·
<i>Halictus ligatus</i>	X	·	X	·	·	X	X	X	·	X	·	X
<i>Halictus parallelus</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Halictus rubicundus</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Lasioglossum</i>	X	X	X	·	X	X	X	X	X	X	X	X
<i>Megachile mendica</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Megachile rotundata</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Megachile xylocopoides</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Melissodes communis</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Svastra obliqua</i>	X	·	·	·	·	·	·	·	·	·	·	·
<i>Svastra petulca</i>	X	·	·	·	·	·	·	·	·	·	·	·
<i>Triepeolus concavus</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Xylocopa micans</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Xylocopa virginica</i>	X	·	·	·	·	·	·	X	·	·	·	·

Appendix C. Presence (X) of flowering species recorded May through August, 2015 at pollinator sites. Species not flowering or not present at sites are represented by (·).

Scientific Name	Site																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<i>Barbarea vulgaris</i>	·	·	·	X	·	·	·	X	·	·	·	·	·	·	·	·	·	·
<i>Centaurea cynus</i>	·	X	X	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
<i>Chrysanthemum leucanthemum</i>	·	·	·	·	·	·	·	·	·	X	·	X	X	·	·	·	·	·
<i>Chrysanthemum maximum</i>	X	·	X	·	X	·	·	·	·	·	·	·	·	·	·	·	·	·
<i>Coreopsis lanceolata</i>	X	·	X	·	X	X	·	·	·	·	·	·	·	·	·	·	·	·
<i>Coreopsis tinctoria</i>	·	·	X	·	·	X	X	·	·	X	X	·	X	·	·	·	·	·
<i>Cosmos bipinnatus/sulphureus</i>	·	·	·	X	·	·	·	X	·	·	·	·	·	·	·	·	·	·
<i>Daucus carota</i>	·	·	·	·	·	·	·	·	·	X	·	·	·	·	·	·	·	·
<i>Delphinium ajacis</i>	·	X	X	X	·	·	·	X	·	·	·	·	X	·	·	·	·	·
<i>Erigeron annuus</i>	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
<i>Eschscholzia californica</i>	X	X	·	·	·	X	X	·	·	·	·	·	·	·	·	·	·	·
<i>Helianthus sp.</i>	·	·	·	·	·	·	·	·	·	·	·	·	·	X	X	X	X	X
<i>Hesperis matronalis</i>	X	·	·	·	·	·	X	·	·	·	X	·	·	·	·	·	·	·
<i>Hypochaeris radicata</i>	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
<i>Lathyrus odoratus</i>	·	·	·	·	·	·	·	·	X	·	·	·	·	·	·	·	·	·
<i>Linaria maroccana</i>	·	·	·	X	·	·	·	X	·	·	·	·	·	·	·	·	·	·
<i>Papaver rhoeas</i>	·	X	X	X	·	X	X	·	·	·	·	X	X	·	·	·	·	·
<i>Phlox drummondii</i>	·	·	·	·	·	·	·	·	·	·	·	·	X	·	·	·	·	·
<i>Rudbeckia amplexicaulis</i>	·	·	X	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
<i>Rudbeckia hirta</i>	·	·	·	·	·	·	·	·	·	X	·	·	X	·	·	·	·	·
<i>Trifolium pratense</i>	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
<i>Trifolium repens</i>	·	·	·	·	·	·	·	·	X	·	·	·	·	·	·	·	·	·

Appendix C cont. Presence (X) of flowering species recorded May through August, 2015 at control sites. Species not flowering or not present at sites are represented by (·).

Scientific Name	Site											
	19	20	21	22	23	24	25	26	27	28	29	30
<i>Barbarea vulgaris</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Centaurea cynus</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Chrysanthemum leucanthemum</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Chrysanthemum maximum</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Coreopsis lanceolata</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Coreopsis tinctoria</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Cosmos bipinnatus/sulphureus</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Daucus carota</i>	X	·	·	·	·	·	·	·	·	·	·	·
<i>Delphinium ajacis</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Erigeron annuus</i>	·	X	·	·	·	·	·	·	·	·	·	·
<i>Eschscholzia californica</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Helianthus sp.</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Hesperis matronalis</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Hypochaeris radicata</i>	·	X	·	·	·	·	·	·	·	·	·	·
<i>Lathyrus odoratus</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Linaria maroccana</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Papaver rhoeas</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Phlox drummondii</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Rudbeckia amplexicaulis</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Rudbeckia hirta</i>	·	·	·	·	·	·	·	·	·	·	·	·
<i>Trifolium pratense</i>	·	·	·	X	·	X	·	·	·	·	·	·
<i>Trifolium repens</i>	·	·	·	·	·	X	·	X	·	·	·	·