Assessing the pollination contribution of bees within the southeastern United States

By

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Abstract

Over 80% of the world's flowering plants rely on animal pollinators, and approximately 75% of the world's food crops depend on insect pollinators. Insects are crucial in the pollination of many cultivated and wild flowering plants, yet specific details (including the insects they attract) are not often fully understood. For this reason, my dissertation focused on studying the pollination requirements of both a specialty crop and native wildflowers in the southeastern United States.

Kiwifruit (*Actinidia chinensis* var. *chinensis* Planch) has been grown commercially within the United States since the 1960s, yet the pollination biology of specific cultivars grown within the United States is largely unknown. For this reason, orchard managers implement multiple pollination methods on a single crop to achieve high fruit yields, but this is an inefficient use of resources. My two-year study on the pollination of kiwifruit revealed that artificial pollination results in higher quantity and quality of fruit compared to flowers pollinated by wind or by insects. Additionally, my study suggests that managed bee species (*Apis mellifera* L. or *B. impatiens* Cresson,) which are commonly used in commercial kiwifruit operations, contribute minimally to the pollination of kiwifruit and may be negatively influenced by the presence of non-kiwifruit flowers. I conclude that orchard managers should implement artificial pollination of their crops to ensure high fruit yields.

Anthropogenic pressures, including habitat loss, agricultural intensification, diseases, pesticides, and climate change, are thought to be main drivers of insect declines worldwide. About 20% of all plant species are at risk of extinction, and estimates suggest 40% of the world's insect species could become extinct in a few decades. Recent government incentives within the United States have recognized the importance of providing sufficient forage in the form of wildflowers to support pollinators, yet the pollination biology including the attractiveness and dependence on pollinators of common and widespread wildflower species is lacking. I planted eighteen species of wildflowers native to the southeastern United States to assess their attractiveness towards native

bees. Additionally, I conducted an insect exclusion experiment to assess the dependence of thirteen wildflower species on pollinators. My experiments suggest that not all wildflower species are equal when it comes to attracting pollinators, and that many wildflower species depend on insect pollinators to complete their life cycles.

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Chapter 1
Dissertation introduction

Over 80% of the world's flowering plants rely on animal pollinators (National Research Council 2007), and approximately 75% of the world's food crops are dependent upon insect pollination (Klein et al. 2007, Vanbergen and Initiative 2013, Kremen 2018). Pollination is the transfer of pollen from male to female parts of a flower, and accomplished via wind, or by animals (including insects) while they are seeking out nectar and pollen rewards (Moissett et al. 2010). For many flowering plant species, insects are crucial pollen vectors for production of seeds and fruits. The global value of insect pollination, including managed and wild bees, was estimated to be about $180 billion in 2009 (Gallai et al. 2009). Within the United States, the total economic value of insect pollination is estimated to be about $20 billion with native bees contributing about 15% of this value (Losey and Vaughan 2006). Within the United States, many insect groups are considered important pollinators to both crops and to wildflowers, including bees, wasps, beetles, flies, moths, and butterflies (Campbell et al. 2019). Bees are arguably the most important pollinators (Kremen et al. 2002) and there are approximately 4,000 bee species native to the United States, yet only a small number are managed for crop pollination (Kremen et al. 2018).

Within the United States, several native and non-native bee species, including *Apis mellifera, Bombus impatiens, Megachile rotundata, Osmia lignaria*, and *Osmia cornifrons* (Klein et al. 2018) are managed to aid in the pollination of multiple crops, including alfalfa, almond, apple, blueberry, cherry, cucumber, pepper, pumpkin, tomato, and kiwifruit (Shipp et al. 1994, Morse and Calderone 2000, Pomeroy and Fisher 2002, Artz and Nault 2011, Pitts-Singer and Cane 2011, Vaudo et al. 2020). Kiwifruit is a fruit-bearing deciduous vine native to the Yangtze River Valley, People's Republic of China. The two most economically important kiwifruit varieties grown in commercial operations worldwide are *Actinidia chinensis* var. *deliciosa* and *A. chinensis* var. *chinensis*

(Ferguson 2015, Zhang et al. 2020) and have been grown in the United States since the 1960s (Beutel 1990), yet little information on the pollination biology, or the pollination contribution of wild or managed bee species, exists within the United States. *Actinidia chinensis* var. *deliciosa* and *A. chinensis* var. *chinensis* both produce perfect flowers, yet plants are functionally dioecious and require a pollen vector to produce fruits (Beutel 1990). Fruit size is positively correlated with the number of seeds produced, and thus with the number of pollen grains deposited on the stigma of a flower (Ferguson 1991). In kiwifruit's native habitat, wind and insect pollination are sufficient in setting fruit (Craig et al. 1988), yet they result in small fruits which are not commercially acceptable. For this reason, producers have developed several methods of artificial pollination.

Studies outside of the United States have come to conflicting conclusions on whether wind, insect (wild and managed bees), or artificial pollination is the most effective method to results in high fruit yields (Palmer-Jones and Clinch 1974, Bellini et al. 1989, Donovan and Read 1991, Matheson 1991a, 1991b, Costa et al. 1993, Vaissiere et al. 1996, Gonzalez et al. 1998, Morley-Bunker and Lyford 1999, Howpage et al. 2001, Pomeroy and Fisher 2002). For this reason and due to a lack of knowledge on pollination requirements of kiwifruit cultivars within the United States, commercial kiwifruit producers implemented a mosaic of pollination methods including wind pollination, insect pollination (stocking *Apis mellifera* and *B. impatiens* colonies within an orchard), and artificial pollination (hand pollination or pollen dusting via boom sprayers), to ensure high fruit yields. The implementation of multiple pollination methods is costly, time consuming, and possibly an inefficient use of resources.

Similar to *A. chinensis* var. *chinensis* being dependent upon a pollen vector, many native plants depend on the specialized pollination services of native bees to complete produce viable seeds (Moissett et al. 2010). Plant-pollinator relationships are one of the most important relationships among plant-animal interactions, and without pollinators, many pollinator dependent plants cannot successfully set seeds. Unfortunately, declines of numerous insect lineages have been reported worldwide (Hallmann et al. 2017, Leather 2018, Lister and Garcia 2018, Janzen and Hallwachs 2019, Sánchez-Bayo and Wyckhuys 2019). Estimates suggest 40% of the world's insect species could become extinct in the next few decades, with members of the Coleoptera, Lepidoptera, and Hymenoptera being many of the most vulnerable (Sánchez-Bayo and Wyckhuys 2019). For this reason, planting of native wildflowers can aid in supporting these vulnerable (Williams et al. 2015). Although native bees are ecologically and economically valuable, there remains a high level of uncertainty concerning the status of native bee populations throughout the United States, and concerns about declines in both managed and native bee populations have sparked scientific evaluations at the national level (Koh et al. 2016).

Anthropogenic pressures including habitat loss, agricultural intensification, diseases, pesticides, and climate change are thought to be the main drivers in the decline of biodiversity worldwide (Barnosky et al. 2011). About 20% of all plant species are at risk of extinction, which directly impacts the pollinators that rely upon them for floral and nesting resources (Willis 2017). Within the United States, the pollination biology of rare plant species and agricultural crops has been evaluated to design effective management plans (Boyd 1994, Dute et al. 2004, Isaacs and Kirk 2010, Garratt et al. 2014, Paris and Boyd 2018), yet the pollination biology of common and widespread wildflowers remains largely unknown (Corbet et al. 1991, Cane 2008). Recent government incentives within the United States have recognized the importance of providing sufficient forage to support bee populations within human impacted landscapes (Williams et al. 2015), yet the pollination biology of these forge plants, such as the species of pollinators they attract and the degree to which they depend on pollinators, is lacking.

For my dissertation, I investigated the contribution of wild and managed bees to some cultivated plants and native wildflower species in the southeastern United States. I also investigated the pollination of two *A. chinensis* var. *chinensis* cultivars grown within the southeastern United States. I chose to research the pollination biology of kiwifruit because it is a new and emerging specialty crop in the southeastern United States, and little is known about its pollination biology. I did this by assessing the effectiveness of existing pollination methods on kiwifruit, and the foraging behavior of two commonly managed bee species (*A. mellifera* and *B. impatiens*) within a kiwifruit orchard. Determining the pollination biology of kiwifruit grown within the United States could help develop a science-based pollination management plan for the kiwifruit industry. Additionally, I investigated the attractiveness of native wildflower species to bees in the southeastern United States, and their dependence upon insect pollinators. The Natural Resources Conservation Service (NRCS) has recognized the importance of planting native wildflowers to support bee populations, yet little is known about the attractiveness of commonly recommended wildflower species to bees. Understanding the pollination biology of wildflowers commonly used in plantings could assist in developing conservation management plans that not only conserve wildflower diversity, but also conserve the insect pollinators that visit them.

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Chapter 2

The pollination and fruit quality of two kiwifruit cultivars (Actinidia chinensis var. chinensis-‘AU Golden Sunshine’ and ‘AU Gulf Coast Gold’) (Ericales: Actinidiaceae) grown in the southeastern United States

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Abstract

Kiwifruit is a new emerging crop for the southeastern United States that requires cross-pollination to set fruit. However, the pollination requirements for varieties grown in the southeastern United States are unknown. Through insect surveys and a bagging experiment, we assessed the pollination requirements of two female kiwifruit cultivars (*Actinidia chinensis* var. *chinensis* ‘AU Golden Sunshine’ and *A. chinensis* var. *chinensis* ‘AU Gulf Coast Gold’). For each, fruit quantity (fruit set) and fruit quality (weight, size, seed count, firmness, soluble solid content, and dry matter content) were compared among three pollination treatments (wind, insect, and artificial pollination). Low abundance of insects were observed visiting female flowers of both kiwifruit cultivars, and therefore insects likely minimally influenced kiwifruit pollination. Artificial pollination resulted in fruits that were greater in weight, size, and contained more seeds, than insect and wind pollinated fruits. Firmness and soluble solid content did not vary greatly between pollination treatments, yet were greater in ‘AU Golden Sunshine’. Dry matter content was statistically different between pollination treatments for ‘AU Golden Sunshine’, and was not statistically different between pollination treatments for ‘AU Gulf Coast Gold’. Dry matter content was only statistically different between the two cultivars for the artificial pollination treatment. To maximize yields and optimize fruit quality, these results suggest that kiwifruit producers should place more effort into artificial

pollination compared to wind and insect pollination. Future research should explore the use of managed bees (e.g., honey bees and bumble bees) within kiwifruit orchards to determine ways to utilize them as a secondary source for pollination needs.

Introduction

Kiwifruit is a fruit-bearing deciduous vine native to the Yangtze River Valley, People's Republic of China (Takeoka et al. 1986, Beutel 1990). The flowers of male plants produce viable pollen but lack developed ovaries, ovules or styles, whereas the flowers of female plants, although perfect, lack viable pollen (Ferguson 1999). Great variability in fruit characteristics such as size, shape, firmness, hairiness, internal and external colors, flavor, and length of storage life, are observed among *Actinidia* species and between cultivars (Given 1993, Ferguson 1999). For this reason, numerous plant-breeding programs have strived to improve the crop for commercial operations worldwide (Ferguson and Seal 2008).

The two most economically important kiwifruit species grown in commercial operations worldwide are *Actinidia chinensis* var. *deliciosa* and *Actinidia chinensis* var. *chinensis* (Ferguson 2014, Zhang et al. 2020). The former is known for its green-fleshed fruit, brown skin with pubescence, and oval shape, while the latter is known for its golden-yellow fruit, hairless golden-brown skin, and rounded fruit. Globally, *A. chinensis* var. *deliciosa*- 'Hayward' is the preferred variety of consumers because of its large fruit. Despite the lower commercial production of *A. chinensis* var. *chinensis* compared to *A. chinensis* var. *deliciosa*, the fruit is thought to be generally superior (Ferguson 1999). For this reason, efforts to develop specific *A. chinensis* var. *chinensis* cultivars with desirable characteristics have been pursued. In the southeastern United States, several male and female *Actinidia chinensis* var. *chinensis* cultivars have been developed and patented for commercial operations, including two female cultivars in Alabama – 'AU Golden Sunshine' and 'AU Gulf Coast Gold' (Dozier et al. 2011, Dozier et al. 2018). As of 2018, the global production of kiwifruit was approximately 4.0 million metric tons (MMt), with the majority of kiwifruits being produced in China (2.0 MMt) Italy (0.56 MMt), New Zealand (0.41 MMt), Iran (0.27 MMt), Greece (0.27 MMt), Chile (0.23 MMt), Turkey (0.06 MMt), France (0.05 MMt), and the United States

(0.03 MMt) (FAOSTAT 2020). Despite the United States producing far fewer metric tons of kiwifruit per year compared to other countries (FAOSTAT 2020), it is a growing industry. Within the country, California produces ninety-eight percent of all kiwifruits, with Alabama, North Carolina, Oregon, and South Carolina making up the difference (NASS 2018).

Because nearly all kiwifruit plants are dioecious (Wang et al. 2018), a pollen vector is required for successful pollination. In kiwifruit's native habitat, wind and insect pollination are sufficient in setting fruit (Craig et al. 1988), yet result in small fruits. Due to the demands for high production and fruit quality within commercial orchards, producers rely on multiple pollen vectoring techniques such as wind, insects, and human technologies (artificial pollination) to yield fruits of marketable size and quality. Although studies have evaluated the pollination requirements of kiwifruit in multiple geographic regions including Argentina (Sáez et al. 2019), Australia (Howpage et al. 2001), France (Vaissière et al. 1996), India (Sharma et al. 2013), Italy (Testolin et al. 1991), New Zealand (Clinch 1984, Costa et al. 1993, Pomeroy and Fisher 2002, Goodwin et al. 2013), and Spain (Miñarro and Twizell 2015), no information concerning the pollination requirements of kiwifruit cultivars grown within the United States exists. As a result, growers currently employ a "shotgun" approach by implementing multiple pollination methods on a single crop in hope of ensuring satisfactory yields of marketable fruits, and thus profits (Costa 1999). Implementing multiple pollination strategies on a single crop over the course of the bloom period is costly and potentially an inefficient use of resources.

The primary purpose of this study is to determine the pollination requirements of two female *Actinidia chinensis* var. *chinensis* cultivars ('AU Golden Sunshine' and 'AU Gulf Coast Gold') grown in the southeastern United States. Our specific objectives are to 1) identify insect flower visitors of each female kiwifruit cultivar and to 2) comparatively evaluate the effectiveness of common pollination methods (wind, insect, artificial pollination) on kiwifruit quantity (fruit set) and quality (fruit weight, size, seed count, soluble solids content (SSC), firmness, and dry matter content (DMC)).

Material and Methods

Study Site

I performed this work during the 2019 and 2020 kiwifruit growing seasons (April-October) within a 72.8 ha (180 acre) kiwifruit orchard located in central Alabama (Reeltown, Alabama; lat. 32° 35’ N, long. 85°47’ W). Blocks A (5.5 ha) and B (6.5 ha) of the orchard contained female *Actinidia chinensis* var. *chinensis* ‘AU Gulf Coast Gold’ and ‘AU Golden Sunshine’ cultivars, respectively (Figure 1) and hereafter called Gulf Coast Gold and Golden Sunshine. Each block contained rows that alternated between one female cultivar and one of three male cultivars (*A. chinensis* var. *chinensis* ‘AU Golden Tiger’, ‘CK3’ and *A. chinensis* var. *deliciosa* ‘Chieftain’) and each row was separated by 2.74 m. To ensure an abundance of blooming female kiwifruit flowers during the spring, I winter pruned all plants within each block between December 2018 – February 2019 according to best management practices for commercial kiwifruit operations in the southeastern United States (Clint Wall, pers. comm.). Additionally, the Southeastern Kiwi Farming Cooperative obtained both honey bee (*Apis mellifera* L.) and bumble bee colonies (*Bombus impatiens* Cresson) to aid in kiwifruit pollination during the bloom period. Honey bees were stocked at 12 colonies per hectare, and bumble bees were stocked at 10 colonies per hectare (Koppert Biological Systems, Howell, MI).

Figure 1: Arrangement of kiwifruit rows within Block A and Block B of the kiwifruit orchard. **A)** Female *Actinidia chinensis* var. *chinensis* - 'AU Gulf Coast Gold', **B)** Female *A. chinensis* var. *chinensis* -'AU Golden Sunshine', **1)** Male *A. chinensis* var. *chinensis* -'AU Golden Tiger', **2)** *A. chinensis* var. *chinensis* -'CK3', and **3)** *A. chinensis* var. *deliciosa* - 'Chieftain'. Image from Google Earth 2020.

Pollinating insect visitors

To determine the common insect visitors for both female kiwifruit cultivars (Golden Sunshine and Gulf Coast Gold), I conducted insect surveys (N = 37) on the female flowers over two seasons (April 10-20 of 2019 and 2020) (Miñarro and Twizell 2015). In each insect survey, I walked down a female kiwifruit row for 10 minutes capturing any insects observed visiting the female kiwifruit flowers. To maximize insect capture and standardize sampling procedures, netting surveys were conducted over multiple days and time periods (morning n = 12, midday n = 15, afternoon n = 10) (Prado et al. 2017), and when weather conditions were appropriate for sampling foraging insects (Hopwood 2008). Appropriate sampling conditions consisted of warm (>20°C) sunny days (<60% cloud cover) with ≤40% chance of precipitation (Hopwood 2008). To limit variability, experimental plants selected for sweep-netting surveys occurred near the center of each block, and were of similar size, age, and flowering state (Sharma et al. 2013). Insects were netted and stored in 70% ethanol to be identified in the laboratory unless they were captured, positively identified in the field and released.

Comparative assessment of common pollination methods

I conducted a bagging experiment on each female kiwifruit cultivar to comparatively assess how each pollination treatment (wind, insect, and artificial pollination) influenced fruit quantity (fruit set) and fruit quality (fruit weight, fruit size, firmness, soluble solid content (SSC), seed count, and dry matter content (DMC)). The bagging experiment was initiated in mid-April 2019, approximately two days prior to each female cultivar's bloom period. I applied each pollination treatment (wind, insect, and artificial pollination) to each plant, and replicated the treatments across 100 kiwifruit plants per cultivar. Treatments are described in the following sections.

Wind pollination

I bagged one unopened cluster of female flower buds with a 4.5 L fine mesh (150 micron) polyester pail strainer (Cary Company part #21WII1) (Figure 2) to exclude all insects (McIver and Erickson 2012), but allow the passive passage of pollen through the mesh (Neal and Anderson 2004). I tied flagging tape around the kiwifruit vine and counted the number of flower buds within each bag. Prior to the experiment, I confirmed using a hand puffer (Antles Pollen Supplies, INC., Post Falls, ID) that kiwifruit pollen can pass through the 150 micron mesh (Neal and Anderson 2004). Each bag remained on the cluster of flower buds until every flower within the bag bloomed and senesced, at which point the bags were removed and fruits were left to develop until the fall harvest.

Insect pollination

I tagged but did not bag one unopened cluster of female flower buds per plant and recorded the number of unopened flower buds present before the bloom (Figure 2). Tagging, but not bagging a cluster of flowers enabled insects and the passive passage of pollen via wind to freely access the blooming female kiwifruit flowers. All fruits were left to develop naturally throughout the growing season until the fall harvest.

Artificial pollination

On each plant, I tagged but did not bag an additional flower cluster like the insect pollination treatment. All flowers were counted, but then artificially pollinated every other day using a hand puffer (Antles Pollen Supplies, INC., Post Falls, ID) that applied two large puffs of ‘Chieftain’ pollen to the open female flowers (Dozier et al. 2018). This occurred from time of flower anthesis to flower senescence/petal drop, approximately a 6 days window. All fruits were left to develop naturally throughout the growing season until the fall harvest.

Figure 2: Two clusters of *Actinidia chinensis* var. *chinensis* flower buds, one representing the wind pollination treatment (left) with a meshed bag fitted around the flower cluster to exclude all insects, and the other, the insect pollination treatment (right) to allow the passage of pollen via wind and insect vectors.

Fruit quantity assessments

I conducted fruit quantity assessments for both varieties at four weeks post peak bloom on 18 May 2019, and at harvest on 29 August 2019 for Golden Sunshine and 3 September for Gulf Coast Gold. Fruit quantified at four weeks post peak bloom were referred to as "fruit set," whereas fruits quantified at harvest were termed "mature fruit." The harvest date was chosen once the kiwifruits of each cultivar reached two threshold parameters including a SSC over 6.5 °Brix (Burdon 2015), and a fruit hue angle below 111° (Minchin et al. 2003). Fruit set was documented by recording the number of experimental flowers that bore fruit and was defined as the period when petals abscise from the flower and fruit growth begins (Richardson et al. 2011). Fruits were divided into two sub-categories at harvest - Marketable or Unmarketable - depending upon size, shape, and if imperfections were observed. Marketable fruits were symmetrical, showed no visual imperfections, possessed a weight > 70 grams and a Fruit Size Index (FSI) >46 mm (Snelgar and Hopkirk 1988, Testolin et al. 1991, Tacconi et al. 2016). All fruits were individually labeled, placed into paper bags, and stored within a walk-in cooler at 0.5°C and 90% RH (Brantley et al. 2019) for two weeks, and then stored at room temperature (20°C) for 48 h prior to conducting fruit quality assessments (Xie 2017).

Fruit quality assessments

To assess fruit quality for both kiwifruit cultivars, I randomly selected twenty fruits per pollination treatment with the use of a random number generator and measured fruit weights, size, firmness, SSC, seed counts, and DMC. Fruit weights were measured using a digital scale (VWR International, Radnor, PA, USA) to the nearest gram. To quantify fruit size, I calculated the fruit size index (FSI) for each fruit using three measurements: length (L), major/widest width (W1), and minor/shortest width (W2) [$FSI = (L + W1 + W2) * 3^{-1}$] (Brantley et al. 2019). I measured fruit firmness measurements with a hand-held penetrometer equipped with an 8 mm diameter plunger

(Agricultural Solutions, Strong, ME, USA). Prior to measuring the fruit firmness, I removed a 1 cm^2-1 mm thick equatorial section of the skin, and the force required to penetrate the fruit to a maximum depth of 8 mm was recorded in kilograms of force (kgf) (Hopkirk et al. 1996, McGlone and Kawano 1998, Li et al. 2016). I estimated soluble solid content by expressing the juice from two 10 mm-thick slices (one from the stem end and one from the calyx end of the fruit) (McGlone and Kawano 1998, Schaare and Fraser 2000). The soluble solid content of each slice was recorded and averaged in °Brix using a digital refractometer (Leica Mark II Abbe, Leica Inc., Buffalo, NY, USA). To quantify the number of seeds contained within each fruit, I placed each fruit into a plastic 0.95 L Ziploc® freezer bag and manually pulverized it. I then forced the pulverized fruit through a wire test sieve (1.00 mm mesh) (Newark Wire Cloth Company, Newark, NJ) with pressurized tap water leaving all seeds behind. A 0.15 mm polyester mesh cloth was placed below the wire sieve to ensure the collection of all seeds. Due to the destructive process of seed quantification, an additional subset of 20 kiwifruits per pollination treatment per cultivar were randomly sampled with a random number generator to obtain DMC. I measured dry matter content by drying a 2 mm transverse slice from the middle of each kiwifruit in a food dehydrator (Excalibur products, Sacramento, California, USA) at 65 °C for approximately 24h (Burdon et al. 2014). Each slice was weighed before and after to determine DMC.

Statistical analyses

A Kruskal-Wallis one-way nonparametric analysis of variance was used to test for differences in fruit set, marketable fruit, fruit weight, FSI, firmness, SSC, seed count, and DMC among the three pollination treatments for each kiwifruit cultivar. Kruskal-Wallis tests were used because square-root and logarithmic transformations applied to the data failed to eliminate heteroscedasticity. In cases with significant differences among pollination treatments, a post hoc multiple comparison test was used to determine which treatment groups were significantly different from one another. To comparatively test for differences between cultivars for the variables measured, a Wilcoxon rank sum test was used. All statistical tests were performed using Statistix 9.0 Analytical Software, Tallahassee, Florida, U.S.A.

Results

Insect visitors

Our 37 ten-minute surveys conducted over the course of two bloom periods documented a total of 35 insects belonging to 5 families visiting the female flowers of Golden Sunshine and Gulf Coast Gold (Table 1). The most abundant flower visitor was *Bombus impatiens* (Apidae), followed by *Toxomerus politus* (Syrphidae), *Apis mellifera* (Apidae), and *Lasioglossum* spp. (Halictidae).

Table 1: Insect flower visitors collected from *Actinidia chinensis* var. *chinensis* 'AU Golden Sunshine' and *A. chinensis* var. *chinensis* 'AU Gulf Coast Gold' during 37 ten-minute sweep netting surveys. An * represents an additional insect taxon collected during the 2020 kiwifruit season.

Cultivar	Order	Family	Species	Count
'AU Gulf Coast Gold'	Diptera	Syrphidae	*Toxomerus politus* Say	2
	Hymenoptera	Apidae	*Apis mellifera* L.	1
			Bombus impatiens Cresson	12
			Xylocopa micans Lepeletier	2
			**Xylocopa virginica* L.	2
	Lepidoptera	Nymphalidae	*Junonia coenia* Hübner	1
'AU Golden Sunshine'	Diptera	Calliphoridae	Unknown	1
		Syrphidae	*Toxomerus politus* Say	5
	Hymenoptera	Apidae	*Apis mellifera* L.	3
			Bombus impatiens Cresson	3
		Halictidae	*Lasioglossum* spp.	3

Comparative assessment of common pollination methods

Fruit quantity assessments

Overall, 2,160 kiwifruit flowers were included in the fruit quantity assessments (1,169 Golden Sunshine flowers and 991 Gulf Coast Gold flowers). Several kiwifruit vines were damaged during the season reducing replications from 100; a total of 94, 93, and 92 plants were analyzed for Golden

Sunshine, and 96, 89, and 96 plants for Gulf Coast Gold for wind, insect, and artificial pollination, respectively (Figures 3 and 4). Four weeks post peak bloom, the average percentage of kiwifruit flowers that set fruit was significantly different among the three pollination treatments for both Golden Sunshine and Gulf Coast Gold ($z = 2.4$, $P < 0.0001$; $z = 2.4$, $P < 0.0001$, respectively). For both cultivars, artificial pollination resulted in the greatest percent fruit set followed by insect pollination and wind pollination (Figure 3a). When comparing the mean percentage of fruit set between each cultivar at four weeks peak bloom, Gulf Coast Gold set significantly more fruit when compared to Golden Sunshine for wind and insect pollination, but not for artificial pollination ($z = 5.2$, $P < 0.0001$; $z = 6.3$, $P < 0.0001$; $z = 1.7$, $P = 0.09$) (wind, insect, artificial pollination, respectively) (Figure 3a). At harvest, the mean percentage of mature kiwifruits present was significantly different between the three pollination treatments for both Golden Sunshine and Gulf Coast Gold ($z = 2.4$, $P < 0.0001$; $z = 2.4$, $P < 0.0001$, respectively) (Figure 3b). Artificial pollination resulted in the greatest percentage of mature kiwifruits, followed by insect and wind pollination (Figure 3b).

A similar trend was observed in the percentage of marketable kiwifruits present at harvest between the three pollination treatments for both Golden Sunshine and Gulf Coast Gold ($z = 2.4$, $P = <0.0001$; $z = 2.4$, $P = <0.0001$, respectively) (Figure 4). Artificial pollination produced significantly greater percentages of marketable kiwifruits followed by insect pollination and wind pollination for both kiwifruit cultivars (Figure 4). When comparing the percentage of marketable kiwifruits between the two cultivars and pollination treatments, insect and artificial pollination were statistically different from one another ($z = 3.5$, $P = 0.0006$; $z = 8.3$, $P < 0.0001$), yet no significant differences were observed within the wind pollination treatment ($z = 1.0$, $P = 0.3$) (Figure 4). Additionally, Golden Sunshine dropped 53% of its fruit between four weeks peak bloom and harvest compared to 10% fruit drop in Gulf Coast Gold.

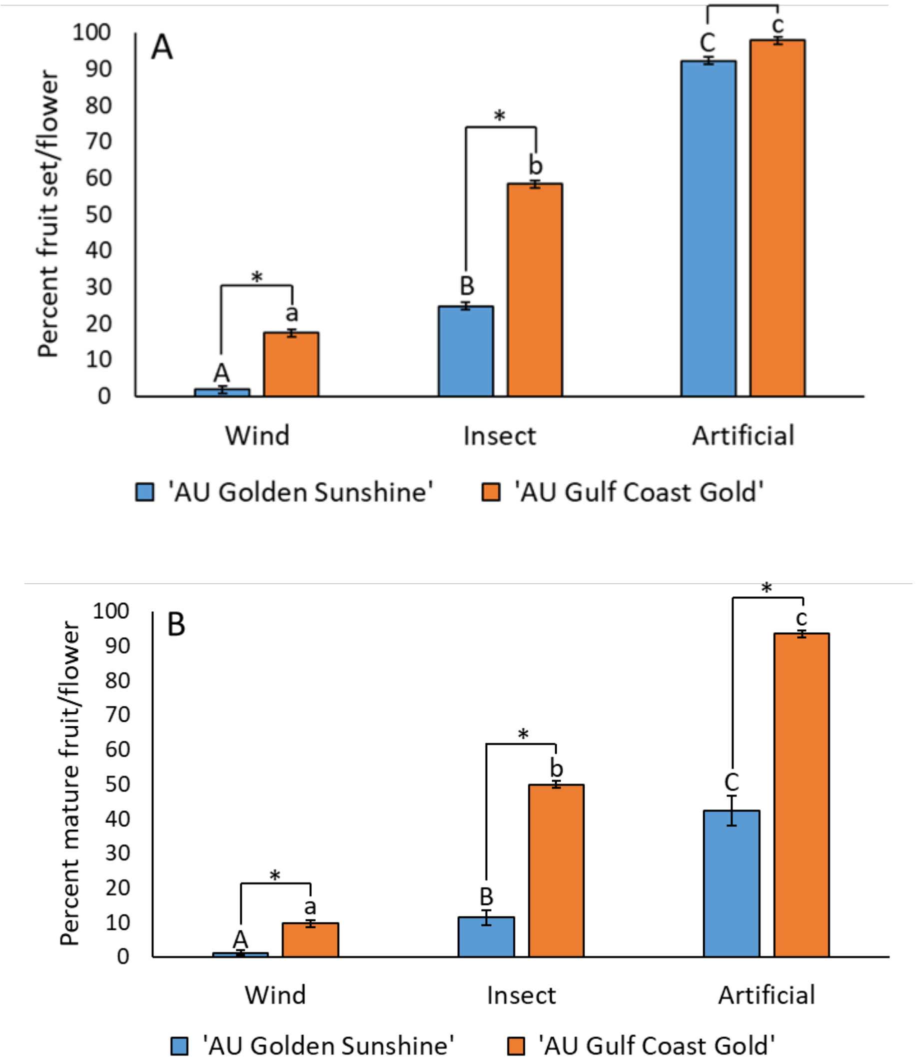

Figure 3: Mean percent fruit set per flower (±SE) for *Actinidia chinensis* var. *chinensis* 'AU Golden Sunshine' and *A. chinensis* var. *chinensis* 'AU Gulf Coast Gold' across the three pollination treatments at **A)** four weeks post peak bloom and **B)** at harvest. Different letters (uppercase for 'AU Golden Sunshine' and lowercase for 'AU Gulf Coast Gold') represent significant differences ($P < 0.05$) within each cultivar. An * represents a significant difference at ($P<0.05$) between 'AU Golden Sunshine' and 'AU Gulf Coast Gold' for a specific pollination treatment.

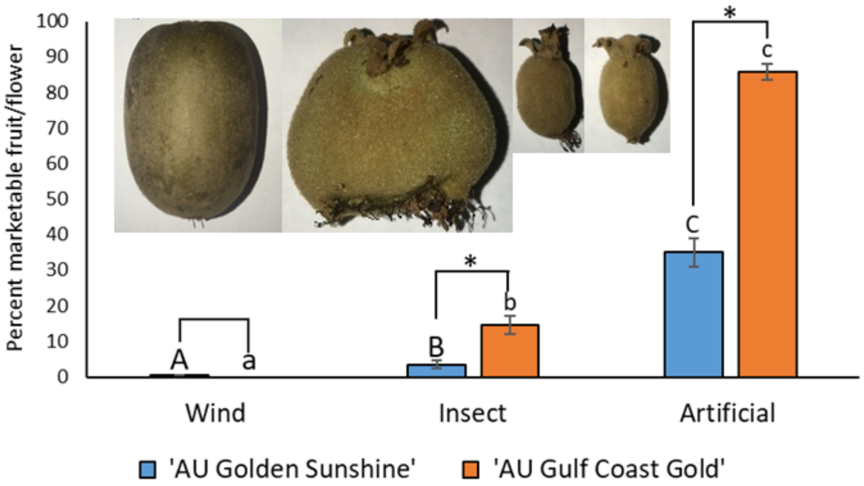

Figure 4: Mean percent marketable fruit per flower (± SE) for *Actinidia chinensis* var. *chinensis* ‘AU Golden Sunshine’ and *A. chinensis* var. *chinensis* ‘AU Gulf Coast Gold’ across three pollination treatments at harvest. Different letters (uppercase for ‘AU Golden Sunshine’ and lowercase for ‘AU Gulf Coast Gold’) represent significant differences (P<0.05) within each cultivar. An * represents a significant difference at (P<0.05) between ‘AU Golden Sunshine’ and ‘AU Gulf Coast Gold’ for a specific pollination treatment. Fruit image furthest to the left represents a marketable kiwifruit while all others represent unmarketable kiwifruit. All kiwifruit images are to scale.

Fruit quality assessments

One hundred and twenty fruits per cultivar were used to conduct the fruit quality assessments. This included twenty fruits per treatment to assess fruit weight, fruit size index (FSI), firmness, soluble solid content (SSC), and seed counts. Due to the destructive process of acquiring seed counts, an additional twenty fruits per treatment and per cultivar were needed to assess dry matter content (DMC). Due to low fruit set percentages within the wind pollination treatment for Golden Sunshine, only four fruits were available to assess fruit weight, FSI, firmness, SSC, and seed counts, and no fruits were available for the DMC analysis. Furthermore, only 13 fruits were available for the DMC analysis within the insect pollination treatment for Golden Sunshine. Similarly, only 11 fruits were available to conduct the DMC analysis for the wind pollination treatment for Gulf Coast Gold (Table 2).

In Golden Sunshine, significant differences in average fruit weight ($z = 2.4$, $P < 0.0001$), FSI ($z = 2.4$, P = 0.004), seed count ($z = 2.4$, P < 0.0001), and DMC ($z = 2.0$, P < 0.0001) were observed among the three pollination treatments (wind, insect, and artificial pollination). No statistical differences in average SSC ($z = 2.4$, P = 0.4) or firmness ($z = 2.394$, P = 0.3) were observed among the three pollination treatments. Artificially pollinated Golden Sunshine fruits were heavier and larger in size (FSI) than wind pollinated fruits. There were no statistical differences in the average weights, FSI, and seed counts between artificially pollinated fruits and insect pollinated fruits, although the average percent DMC was statistically greater in artificially pollinated fruits compared to insect pollinated fruits in AU Golden Sunshine (Table 2).

For Gulf Coast Gold, significant differences in average kiwifruit weight ($z = 2.4$, P < 0.0001), FSI ($z = 2.4$, P < 0.0001), firmness ($z = 2.4$, P < 0.0001), and seed counts ($z = 2.4$, P < 0.0001) were observed among the three pollination treatments (wind, insect, and artificial pollination). No statistical differences in average SSC ($z = 2.4$, P = 0.05) and DMC ($z = 2.4$, P = 0.6) were observed (Table 2). On average, artificially pollinated fruits weighed more, were larger in size (FSI), and contained a more seeds when compared with both wind and insect pollinated fruits. Fruit firmness did not differ between pollination methods in AU Golden Sunshine, yet artificially pollinated fruits were firmer than wind and insect pollinated fruits in AU Gulf Coast Gold.

Within the wind pollination treatment, the comparison of fruit quality variables between both cultivars revealed that Golden Sunshine and Gulf Coast Gold differed statistically in their average weight, ($z = 3.1$, P = 0.002), FSI ($z = 3.1$, P = 0.002), firmness ($z = 3.2$, P = 0.001), SSC ($z = 2.9$, P = 0.004), and seed count ($z = 3.1$, P = 0.002) (Table 2). Due to the lack of fruits within the wind pollination treatment, DMC comparisons were not conducted. Within the wind pollination treatment, Golden Sunshine produced fruits that were heavier, larger (FSI), more firm, and contained more seeds, yet were lower in SSC than Gulf Coast Gold fruits (Table 2).

When comparing fruit quality variables within the insect pollination treatment, we observed statistical differences in the average fruit firmness ($z = 3.5$, P = 0.0005), and SSC ($z = 4.8$, P < 0.0001) between Golden Sunshine and Gulf Coast Gold (Table 2). No significant differences were observed for average weight ($z = 0.92$, P = 0.4), FSI ($z = 0.69$, P = 0.5), seed count ($z = 1.1$, P = 0.3), or DMC ($z = 0.055$, P = 0.9). Within the insect pollination treatment, Golden Sunshine produced fruits that were more firm, yet were lower in SSC, than insect pollinated Gulf Coast Gold fruits (Table 2).

Lastly, when comparing fruit quality variables within the artificial pollination treatment, statistical differences were observed in average fruit size (FSI) ($z = 2.2$, P = 0.03), firmness ($z = 3.1$, P = 0.001), SSC ($z = 4.7$, P<0.0001), seed counts ($z = 3.9$, P = 0.0001), and DMC ($z = 3.1$, P = 0.002) between the two kiwifruit cultivars. No statistical differences were observed in weight between Golden Sunshine and Gulf Coast Gold ($z = 0.12$, P = 0.9) fruits. Overall, Golden Sunshine produced fruits that were smaller in size (FSI), more firm, lower in SSC, contained fewer seeds, and contained smaller percentages of dry matter content than artificially pollinated Gulf Coast Gold fruits (Table 2).

Table 2: Summary of kiwifruit quality variables for *Actinidia chinensis* var. *chinensis* 'AU Golden Sunshine' and *A. chinensis* var. *chinensis* 'AU Gulf Coast Gold'**.** Mean numbers (±SE) followed by different letters (a, b, or c) are significantly different at ($P < 0.05$) among pollination treatments (wind, insect, artificial) for each fruit quality variable. Numerical values followed by an * represent a significant difference at ($P < 0.05$) between 'AU Golden Sunshine' and 'AU Gulf Coast Gold' for a specific pollination treatment and fruit quality variable.

Cultivar	Variable	Wind	*N*	Insect	*N*	Artificial	*N*
'AU Golden Sunshine'	**Weight (g)**	97.50(20.10)**a***	4	67.46(6.85)**ab**	20	132.05(10.19)**b**	20
	Fruit size index (mm)	50.85(3.88)**a***	4	44.50(1.66)**ab**	20	52.47(3.01)**b***	20
	Firmness (kgf)	1.98(0.33)**a***	4	1.46(0.22)**a***	20	1.94(0.26)**a***	20
	SSC (°Brix)	12.68(1.87)**a***	4	14.64(0.47)**a***	20	13.85(0.47)**a***	20
	Seed count	238.0(113.59)**a***	4	178.8(41.26)**ab**	20	456.7(36.52)**b***	20
	Dry matter content	-	NA	19.90(0.28)**a**	13	18.80(0.11)**b***	20
'AU Gulf Coast Gold'	**Weight (g)**	19.75(2.03)**a***	20	58.70(7.55)**b**	20	124.4(4.00)**c**	20
	Fruit size index (mm)	29.50(1.10)**a***	20	42.37(2.11)**b**	20	58.55(0.64)**c***	20
	Firmness (kgf)	0.25(0.07)**a***	20	0.40(0.08)**a***	20	0.83(0.09)**b***	20
	SSC (°Brix)	18.93(0.46)**a***	20	18.41(0.28)**a***	20	17.64(0.23)**a***	20
	Seed count	6.85(1.55)**a***	20	153.4(47.01)**b**	20	671.3(27.69)**c***	20
	Dry matter content	19.22(1.13)**a**	11	19.90(0.22)**a**	20	19.50(0.24)**a***	20

Discussion

Understanding the pollination requirements of kiwifruit grown within the United States is an important step in increasing kiwifruit yields while simultaneously decreasing pollination costs for farmers. Few insects visited the female flowers of kiwifruit during the bloom period, and greater fruit quantity and quality was achieved through artificial pollination. Producers in the southeastern United States should explore Gulf Coast Gold further with less emphasis on Golden Sunshine because artificially pollinated Gulf Coast Gold flowers produce significantly greater numbers of higher quality marketable fruits compared to Golden Sunshine. Furthermore, Gulf Coast Gold does not prematurely drop fruit, which results in greater overall yields when compared to Golden Sunshine.

Several studies have demonstrated that honey bees (Vaissière et al. 1996, Howpage et al. 2001), bumble bees (Pomeroy and Fisher 2002), and other wild insects including coleopterans, dipterans, hymenopterans, and lepidopterans readily visit female kiwifruit flowers, and are considered effective pollinators (Sharma et al. 2013, Miñarro and Twizell 2015). Few insects were observed visiting the female flowers of the two *A. chinensis* var. *chinensis* cultivars assessed in this study, and their pollination contribution is likely minimal. Several possibilities could help explain the reason for few pollinator visits to flowers of kiwifruit in the southeastern United States. *Actinidia chinensis* var. *chinensis* flowers do not produce nectar, and thus may be unattractive to insects (Clinch 1989, Hopping 1990). It is also possible that the visitation by pollinators to female kiwifruit flowers was reduced by competition from other pollen and nectar sources. We observed both honey bees and bumble bees visiting white clover (*Trifolium repens* L.), wild radish (*Raphanus raphanistrum* L.), and other flowering plants directly below kiwifruit blooms (Tew et al. 2014). Goodwin (1986) also observed honey bees visiting competing non-kiwifruit flowers, and determined that supplementing honey bee colonies with sugar syrup during the kiwifruit bloom promoted kiwifruit flower visitation. Additionally, Clinch (1989) and Pomeroy and Fisher (2002) documented that non-kiwifruit flowers lure pollinators away from kiwifruit flowers and can result in an insufficient transfer of pollen required for sufficient fruit set. Honey bee colonies in our study were not supplemented with sugar syrup during the kiwifruit bloom period, which could explain why they were visiting non-kiwifruit flowers.

Through our fruit quality assessments, we determined that the largest percentage of fruit set was for the artificial pollination treatment, followed by insect and wind pollination treatments. Pollination of female kiwifruit flowers by hand is a common practice used by kiwifruit growers to ensure successful pollination of their crop, and the effective pollination period for *A. chinensis* var. *chinensis* is reported as within 6 days of anthesis (Brantley et al. 2019). Therefore, our results align well with the results of multiple studies (Testolin et al. 1991, Gonzalez et al. 1998, Howpage et al. 2001, Pomeroy and Fisher 2002, and Goodwin et al. 2013). Others have documented that wind pollination results in appreciable fruit set but results in low seed counts and fruit weights (Palmer-Jones and Clinch 1974, Clinch 1984, Burge et al. 1988, Razeto et al. 2005). We documented very low percentages of fruit set and mature fruits produced by the wind pollination treatment at both four weeks post-bloom and at harvest. However, our fruit quantity assessments also did not align with the findings of Sáez et al. (2019) or Miñarro and Twizell (2015). Sáez et al. (2019) concluded that bee-pollinated flowers produced 40% more fruits than artificially pollinated ones, but our method of artificial pollen application differed from Sáez et al. (2019). We applied 100% pure dry pollen stored at freezer conditions until just before application to each female flower with the use of a hand puffer, whereas Sáez et al. (2019) suspended their kiwifruit pollen in pure water just before application. Borghezan et al. (2011) determined that kiwifruit pollen could be adequately stored in refrigerator or freezer conditions for approximately forty days, and Hopping and Jerram (1980) showed kiwifruit pollen suspended in water had significantly reduced viability, swelled, and ultimately burst. Therefore, the study by Sáez et al. (2019) might have underestimated the potential role of artificial pollination and overestimated the role insects can play in the pollination of kiwifruit flowers. Similarly, Miñarro and Twizell (2015) concluded that fruit set did not differ between insect pollinated and hand pollinated flowers. Adequately pollinated *A. chinensis* var. *chinensis* fruits can contain up to 700 seeds (Goodwin et al. 2013) and the number of seeds is directly proportional to the number of transferred pollen grains. Miñarro and Twizell (2015) touched the stigmas with anthers of three different male flowers within their hand pollination treatment, yet did not document how many pollen grains were transferred in the process. Although Miñarro and Twizell (2015) documented a high abundance and diversity of pollinators visiting kiwifruit flowers, they too might have underestimated the role artificial pollination played in fruit set.

Through our fruit quantity assessments, we determined that 'AU Golden Sunshine' dropped 53% of its fruit between four weeks post bloom and harvest compared to 10% fruit drop in Gulf Coast Gold. It is well documented that Golden Sunshine blooms and ripens earlier than Gulf Coast Gold (Dozier et al. 2011), but the prematurely dropped Golden Sunshine fruits contained low SSC values (<6.5 °Brix), and were not considered ready for harvest. This is a major problem for farmers who cultivate Golden Sunshine, as no fruits were harvested and sold during the 2019 season due to the loss of the fruit. Despite the fruit drop problem in Golden Sunshine, the highest percentage of fruit set was only achieved by the artificial pollination treatment. Thus, artificial pollination is the only pollination treatment that resulted in high percentages of fruit set and marketable fruits for both kiwifruit cultivars when compared to wind and insect pollination. The pollination method chosen for kiwifruit not only influences fruit set, but also greatly influences fruit quality.

Fruit weight, size, and SSC are three of the most important parameters when grading kiwifruit for export (Jordan et al. 2000, Sáez et al. 2019, Qiu et al. 2020). Fruit weight has been shown to be positively correlated with the number of seeds and fruit size (Costa et al. 1993, Gonzalez et al. 1998, Pomeroy and Fisher 2002). Soluble solid content and DMC are also important variables used in determining the quality of kiwifruit at harvest, during cold storage, and post cold storage, with positive relationships being documented between consumer acceptability and SSC and DMC levels (Scott et al. 1986, Mitchell et al. 1991, Jordan et al. 2000). The retention of fruit firmness is important in maintaining high fruit quality during cold storage and results in fewer damaged fruits (Mcdonald and Harman 1982). The wind pollination treatment did not provide female kiwifruit flowers of both Golden Sunshine and Gulf Coast Gold with adequate numbers of pollen grains to produce high quality marketable kiwifruits. For this reason, wind pollination should not be relied upon as a sole pollination method. No significant differences were observed in weight, FSI, firmness, SSC, or seed count, between insect pollinated and hand pollinated flowers for Golden Sunshine, but differences were observed for DMC. Additionally, no significant differences were observed for SSC and DMC between insect and artificially pollinated flowers for Gulf Coast Gold, but differences were observed for weight, FSI, firmness, and seed count. With fruit weight, size and SSC being the most important parameters used to grade kiwifruit, artificial pollination resulted in fruits with greater weights and sizes in Gulf Coast Gold. Of the three pollination treatments, our study suggests that farmers should invest more time and resources in hand pollination to maximize yields and profits.

In conclusion, very few insects visited kiwifruit flowers of Golden Sunshine and Gulf Coast Gold. Due to high fruit drop of Golden Sunshine, Gulf Coast Gold appears to be the best option for commercial kiwifruit growers in the southeastern United States. Artificial pollination resulted in the greatest percentage of flowers that set fruits and higher quality fruits when compared with wind and insect pollination. Thus, kiwifruit growers of the southeastern United States should not rely solely on insects for pollination needs. The use of managed bees (e.g., honey bees and bumble bees) within kiwifruit should be further explored to determine ways to potentially utilize them as a secondary source for pollination.

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Chapter 3

Managed honey bees (*Apis mellifera*) and bumble bees (*Bombus impatiens*) are inadequate pollinators of kiwifruit (*Actinidia chinensis* var. *chinensis*) in Alabama

Abstract

Kiwifruit is a relatively new specialty crop grown within the United States, where 98% of all fruits are produced in California. Due to a lack of knowledge on the pollination requirements of kiwifruit cultivars grown within the United States, farmers implement multiple pollination methods simultaneously on a single crop including wind pollination, utilizing managed bee species such as honey bees (*Apis mellifera*) and bumble bees (*Bombus impatiens*), and artificially pollinating plants to achieve high fruit yields. Implementing multiple pollination methods is costly and possibly not an efficient use of resources. We assessed the contribution of two managed bee species (*A. mellifera* and *B. impatiens*) to the pollination of kiwifruit by 1) determining the relative abundance of kiwifruit pollen collected by foragers of each species, and 2) comparing fruit set among insect pollinated and artificially pollinated flowers of through an insect exclusion experiment. Overall, *A. mellifera* collected the greatest relative abundances of pollen from *Trifolium/Melilotus* (21.7%), followed by *A. chinensis* (21.3%), *Rhus* (15.6%), Rosaceae (12.6%), and *Brassica* (12.1%). For, *B. impatiens*, the greatest relative abundances of pollen collected were from *A. chinensis* (66.6%), *Rhus* (8.5%), *Nyssa* (7.2%), Rosaceae (7.1%), and *Trifolium/Melilotus* (3.5%). A significant difference was observed between mean relative abundance of kiwifruit pollen carried in the corbicula of *A. mellifera* and *B. impatiens* over the eight day period, with *B. impatiens* carrying on average 46% more kiwifruit pollen than *A. mellifera*. We conclude that *A. mellifera* and *B. impatiens* contribute minimally to the pollination of kiwifruit in the southeastern United States, possibly because they were drawn to competing non-kiwifruit blooms. Artificially pollinated

kiwifruit flowers set significantly greater numbers of fruit per flower at four weeks post bloom and at harvest. Kiwifruit producers should put more effort into artificially pollinating their crops rather than relying on managed or wild insects for kiwifruit pollination. Future research should comparatively evaluate other methods of artificial pollination to determine their effectiveness and efficiency in the pollination of kiwifruit grown within the United States.

Introduction

Kiwifruit is a fruit-bearing deciduous vine native to the Yangtze River Valley, P.R. China (Takeoka et al. 1986, Beutel 1990). The two most economically important kiwifruit species grown worldwide are *Actinidia chinensis* var. *deliciosa* and *Actinidia chinensis* var. *chinensis* (Ferguson 2015, Zhang et al. 2020). Great variability in fruit characteristics such as size, shape, firmness, pubescence, internal and external colors, flavor, and length of storage life, are observed among the *Actinidia* species and between cultivars (Given 1993, Ferguson 1999). The flowers of male plants produce viable pollen but lack developed ovaries, ovules or styles, whereas the flowers of female plants, although perfect, lack viable pollen (Ferguson 1999). Thus, despite *A. chinensis* var. *deliciosa* and *A. chinensis* var. *chinensis* both producing perfect flowers, they are functionally dioeceous and require a pollen vector to accomplish sufficient pollination (Beutel 1990).

Pollination of kiwifruit flowers is the most influential factor affecting fruit set, size, and yield (Ferguson 1991). Furthermore, fruit size is positively correlated with the number of seeds produced, and kiwifruit growers therefore go to great lengths to ensure their crops are adequately pollinated (Ferguson 1991). Managed bees such as honey bees (*Apis mellifera* L.) are considered the main pollinators of kiwifruit, and several studies quality (Palmer-Jones and Clinch 1974, Matheson 1991a, 1991b, Vaissiere et al. 1996, Howpage et al. 2001) have demonstrated the effectiveness of deploying 8-10 colonies/ha within kiwifruit orchards to increase kiwifruit quantity and quality (Palmer-Jones and Clinch 1974, Matheson 1991a, 1991b, Vaissiere et al. 1996, Howpage et al. 2001). However, there are conflicting results concerning the contribution of the honey bees to kiwifruit pollination. Some researchers (Donovan and Read 1991, Costa et al. 1993, Pomeroy and Fisher 2002) have stated that honey bees are not efficient pollinators of kiwifruit because they are not attracted to the blooms due to the lack of nectar produced by pistillate flowers. Furthermore, honey bees stocked within kiwifruit orchards have been shown to be drawn towards competing non-kiwifruit blooms, and may collect as little as 2 to 4% kiwifruit pollen (Ford 1971, Blanchet et al. 1990). Honey bees acquire all of the nutrients they need for growth and development from the pollen and nectar sources they collect from flowers (Day et al. 1990), and pistillate kiwifruit flowers contain pollen nutrients below the minimum level required by honey bees (De Groot 1953, Day et al. 1990). For this reason, honey bee colonies weaken when stocked within kiwifruit orchards, and workers collect decreasing amounts of kiwifruit pollen towards the end of the flowering season (Goodwin et al. 1994). Therefore, it is suggested that beekeepers supplement their colonies with sugar water during the kiwifruit bloom as an energy source in case bees ignore competing blooms that provide nectar (Goodwin et al. 1986).

In addition to managed pollinators, wild species have been documented visiting kiwifruit blooms, including hoverflies, butterflies, beetles and several *Bombus* species*,* with the latter being evaluated as potential pollinators (Miñarro and Twizell 2015). The contribution of *Bombus* species to kiwifruit pollination is considered minimal due the low numbers of workers (Clinch 1984). Additionally, some studies have documented that wind pollinated kiwifruit flowers produce similar sized fruits when compared to insect-pollinated flowers (Bellini et al. 1989); however, most evidence suggests wind is ineffective for setting and producing kiwifruits of marketable size (Donovan and Read 1991, Costa et al. 1993, VaissiÉre et al. 1996, Gonzalez et al. 1998, Morley-Bunker and Lyford 1999, Howpage et al. 2001, Pomeroy and Fisher 2002). Therefore, other pollination methods in addition to wind pollination are suggested to achieve the high fruit set and yields required by commercial operations (Brantley et al. 2019).

Due to varying results by wind and insect pollination on kiwifruit pollination, several methods of artificial pollination have been developed. Artificial pollination methods include the spraying of aqueous pollen solutions, pollen dusting, and hand pollination when the female flowers are receptive (Razeto et al. 2005, Brantley et al. 2019). Like wind and insect pollination, artificial pollination methods have varying degrees of pollination success (Razeto et al. 2005). Spraying of pollen in aqueous suspensions onto open pistillate flowers can damage pollen (while formulating suspensions and during spraying), and cause an inadequate number of pollen grains to be deposited on stigmas of sprayed flowers (Hopping and Hacking 1983). A pollen suspension sprayed via a boom sprayer is one of the most commonly used artificial pollination methods (Hopping and Jerram 1980, Sáez et al. 2019), yet evidence has shown 50% fewer pollen tubes form compared to hand pollination with dry pollen (Hopping and Hacking 1983). Several studies have shown wind and insect pollinated (mainly by honey bees) kiwifruit flowers result in greater or similar fruit set and fruit weights when compared to artificially pollinated flowers (Miñarro and Twizell 2015, Sáez et al. 2019), while others have found the opposite (Costa et al. 1993, Gonzalez et al. 1994, 1998).

Within the United States, California is the leading producer of all kiwifruits (98%), with Alabama, North Carolina, Oregon, and South Carolina making up the rest (NASS 2018). Due to the varying results across kiwifruit pollination studies, and the lack of knowledge of the pollination requirements of kiwifruit cultivars within the United States, orchard managers have turned to implementing a mosaic of pollination methods to ensure fruit yields. Orchard managers currently plant male and female kiwifruit plants in close proximity to one another to facilitate cross-pollination via wind, have introduced managed honey bees and bumble bees to increase fruit set and yields, and artificially pollinate their crops via hand pollination during a single bloom (Brantley et al. 2019). Implementing multiple pollination methods is costly, time consuming, and possibly an inefficient use of resources. In the southeastern United States, a successful female kiwifruit cultivar called *A. chinensis* var. *chineisis* ‘AU Gulf Coast Gold’ was developed and patented for commercial operations, yet its pollination requirements remain largely unknown (Dozier et al. 2018).

Although commonly used for kiwifruit pollination, no studies have comparatively evaluated the foraging behavior of honey bees and bumble bees within kiwifruit orchards in the United States to determine their contribution to kiwifruit pollination. Furthermore, no studies within the country have comparatively evaluated existing pollination methods including wind, insect, and artificial pollination on kiwifruit fruit set. The primary objectives of this study are to 1) compare the foraging behavior of managed honey bees and bumble bees in a managed kiwifruit agricultural setting typical of the southeastern United States by quantifying the corbicular pollen of foraging bees stocked within a kiwifruit orchard, and 2) compare the effectiveness of common pollination methods (wind, insect, artificial pollination) on fruit set of kiwfruit through an exclusion study while stocking honey bees and bumble bees within the kiwifruit orchard. I hypothesized that managed honey bees and bumble bees would differ in their foraging behaviors within the kiwifruit orchard, because of the unattractiveness of kiwifruit blooms, and the attractiveness of competing non-kiwifruit blooms (Pomeroy and Fisher 2002), and that artificial pollination would result in the greatest fruit set compared to wind and insect pollination because more pollen grains would be deposited on the female flowers than the other pollination methods (Costa et al. 1993, Gonzalez et al. 1994, 1998). The results of this research could help orchard managers within the United States develop a science-based pollination management plan for the kiwifruit industry.

Methods

This work was performed during the 2020 kiwifruit growing season (April-October) within a 72.8 ha (180 acre) kiwifruit orchard located in central Alabama (Reeltown, Alabama; lat. 32° 35’ N, long. 85°47’ W). All of the experiments were concentrated within a single 5.5 ha block (Figure 5a) and within five consecutive kiwifruit rows. The block contained rows that alternated between one female cultivar (*Actinidia chinensis* var. *chinensis* ‘AU Gulf Coast Gold’) and one of three male cultivars (*A. chinensis* var. *chinensis* ‘AU Golden Tiger’, ‘CK3’, and *A. chinensis* var. *deliciosa* ‘Chieftain’), with 2.74 m spaced between each row. According to best management practices for commercial kiwifruit operations in the southeastern United States (Clint Wall, pers. comm.), I winter pruned all female and male kiwifruit plants within the five rows (2 female and 3 male rows; Figure 5b) between December 2019 and February 2020 (Figure 6a and 6b). In mid-April 2020, four bumble bee (*Bombus impatiens*) quads (16 colonies total; four colonies per quad) with weights between 832 and 845 grams were purchased from BioBest, and 12 honey bee (*Apis mellifera*) colonies, each consisting of at least 10 frames of brood and 15 frames of adults, were transferred to the kiwifruit orchard 1 day prior to the start of the *A. chinensis* var. *chinensis* 'AU Gulf Coast Gold' bloom. To ensure numbers of colonies for both managed bee species reflected common stocking rates of commercial kiwifruit operations (Blanchet et al. 1990, Pomeroy and Fisher 2002), I created four circular 0.40 ha plots spread evenly (distance of 61 m between each plots' center point) across the entire length of the kiwifruit block (244 m) (Figure 5a). Within each 0.40 hectare plot, I placed one *B. impatiens* quad (10 colonies/ ha) on a wooden pallet and three *A. mellifera* colonies (7 colonies/ha) on another wooden pallet, with each wooden pallet spaced by a distance of 9.1 m (Figure 7).

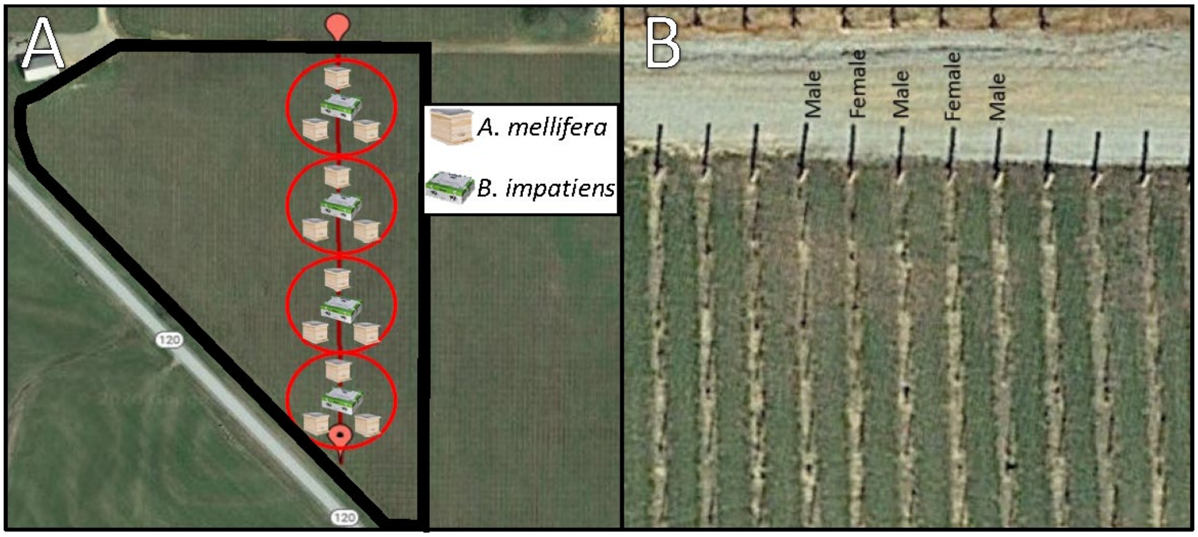

Figure 5: Experimental design within the kiwifruit orchard. **A)** Orientation of the four 0.40 ha experimental plots each separated by 61 m from each center, spread out along a 244 m transect. Each 0.40 ha plot contained one bumble bee (*Bombus impatiens*) quad (10 colonies/ha) and three honey bee (*Apis mellifera*) colonies (7 colonies/ha), and **B)** arrangement of alternating female *Actinidia chinensis* var. *chinensis* 'AU Gulf Coast Gold' and male *A. chinensis* var. *chinensis* 'AU Golden Tiger', 'CK3,' and *A. chinensis* var. *deliciosa* 'Chieftain' rows within the 5.5 ha block.

Figure 6: **A)** Female *Actinidia chinensis* var. *chinensis* 'AU Gulf Coast Gold' before winter pruning and **B)** after winter pruning.

Figure 7: Configuration of the bumble bee (*Bombus impatiens*) quad with plastic weather covering (lower left), and three honey bee (*Apis mellifera*) colonies (upper right) each placed on wooden pallets separated by 9.1 m.

Palynological Study

Pollen Collection

During the kiwifruit bloom period, which occurred 9-17 April 2020, *A. mellifera* and *B. impatiens* foragers were netted when returning to their colonies during three sampling periods (morning, midday, and afternoon) for a total of 8 sampling days (sweep netting did not occur on 12 April due to poor weather conditions) (Leonhardt and Blüthgen 2012). For this, I netted the first four *A. mellifera* and *B. impatiens* foragers as they returned to their colonies per each 0.4 ha plot, totaling 16 bees per species per sampling period. I temporarily placed netted bees in individual plastic 15 mL vials stored in a cooler with an icepack to anesthetize them. Once anesthetized, I removed the corbicular pollen with forceps and the bees were left to regain consciousness to fly back to their colonies unharmed (Figure 8a and 8b). To prevent cross contamination of pollen between bee species, sampling days, and sampling periods (morning, midday, afternoon), I used designated nets for each bee species and washed them daily in soapy water. I also used new plastic 15 mL vials, and cleaned the forceps after each sampling event and day with soapy water. I pooled collected corbicular pollen from each bee species at each sampling period before they were processed.

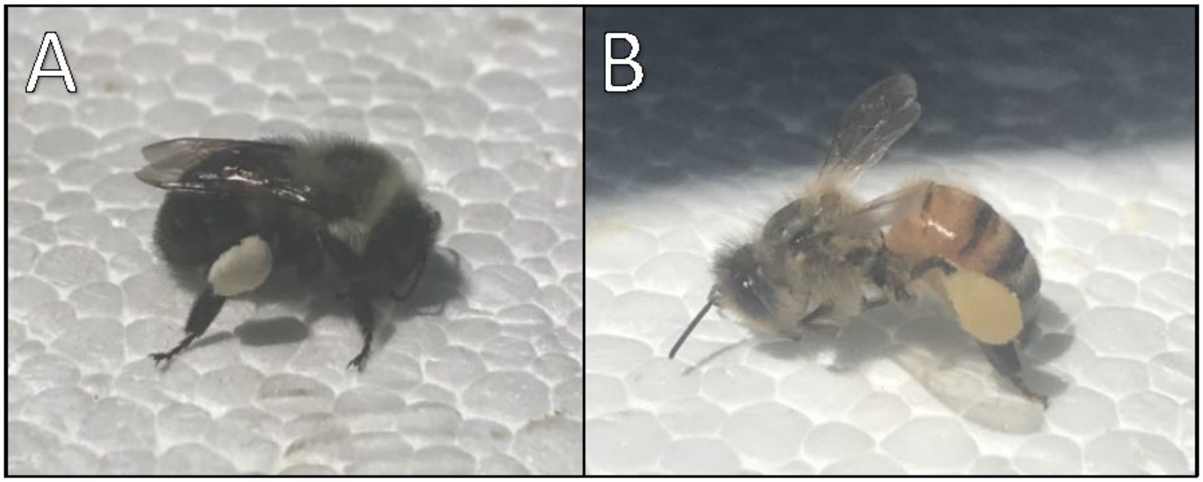

Figure 8: A) Bumble bee (*Bombus impatiens*) with white corbicular pollen, and **B)** Honey bee (*Apis mellifera*) with yellow corbicular pollen.

Palynological Analyses

Pollen samples were chemically processed by Global GeoLab Limited following standard palynological laboratory methods (Brown 2008), and then quantified at the CENEX Laboratory at Louisiana State University. Pollen samples received by Global Geolab Limited were dehydrated and dissolved in glacial acetic acid, and the lipids, waxes, and cytoplasm were removed via an acetolysis chemical treatment to allow for easier identification of the pollen grains. After the acetolysis chemical treatment, pollen grains were rinsed with ethanol and water, and then suspended in glycerine. Pollen samples received by the CENEX Lab were in 2 mL vials containing processed pollen suspended in glycerine. The vial content was stirred thoroughly for one minute, then a small drop of the suspension was mounted on a 75 x 25 mm microscope slide and covered with an 18 x 18 mm #1-thickness glass coverslip. Coverslips were sealed with clear nail polish to prevent leakage and were examined at 600x and 1000x magnification with an Olympus BX41 to identify the pollen types. To establish statistically valid relative abundances of each pollen taxon, a minimum of 300 identified pollen grains were counted for each of the samples using traverses that prevented duplicate counts of pollen, and all slides were scanned for unique and rare types of pollen after the initial count was completed (Sophie Warny, pers. comm.). Pollen types were identified to family, genus, or species when possible.

Exclusion Study

Study Design

I conducted an exclusion study to assess the contribution of managed *A. mellifera* and *B. impatiens* to *A. chinensis* var. *chinensis* ‘AU Gulf Coast Gold’ (hereby referred to as AU Gulf Coast Gold) pollination by quantifying fruit set among four pollination treatments including Insect Pollination (i.e. managed bee and potential wild pollinators), Wind Pollination, Artificial Pollination, and a Pollen Exclusion treatment for comparisons. The experiment was initiated in mid-April 2020, approximately two days prior to the bloom period of AU Gulf Coast Gold. Each pollination treatment (Insect Pollination, Wind Pollination, Artificial Pollination, and Pollen Exclusion) was applied to each plant, and were replicated across 114 kiwifruit plants.

To document the contribution of managed bees and wild pollinators to the pollination of AU Gulf Coast Gold, I tagged, with the use of flagging tape, a cluster of unopened female flower buds on each plant and recorded the number of flower buds present (Figure 9a). During the bloom, managed *A. mellifera* and *B. impatiens* and any wild pollinators cold visit the tagged floral cluster for pollen rewards. In the Wind Pollination treatment, I bagged one unopened cluster of female flower buds per plant with a 1,000 micron nylon monofilament mesh liquid filter bag (Cary

Company part #NMO1000P1DS) to exclude all insects, but to allow the passive passage of pollen through the mesh bag (Neal and Anderson 2004, McIver and Erickson 2012). The vine containing the cluster of flower buds was tagged with flagging tape and the number of flower buds present was recorded (Figure 9b). In the Artificial Pollination treatment, I bagged one unopened cluster of female flower buds per plant with a 25 micron polyester felt liquid filter bag (Cary Company part #PES25P1DS) that prevented the passage of pollen via insects and wind from reaching the female kiwifruit flower buds during the bloom (Figure 9c). To ensure kiwifruit pollen was unable to pass through the 25 micron bags, I followed methods used by Neal and Anderson (2004) and confirmed kiwifruit pollen was not capable of passing through the material. Wooden clothespins were used to ensure a tight fit around the bag opening, and around the kiwifruit bine (Figure 9c). Clothespins were necessary only for the 25 micron bags due to the difference in material compared to the 1,000 micron bags used in the Wind Pollination treatment. Brantley et al. (2019) determined the effective pollination period of *A. chinensis* var. *chinensis* is approximately 4 days after anthesis (flower opening). For this reason, I opened each bag within the Artificial Pollination treatment every other day and checked for recently bloomed flowers. If open flowers were present, I applied two puffs of 'Chieftain' pollen with the use of a hand puffer (Antles Pollen Supplies, INC., Post Falls, ID) to ensure adequate pollination (Dozier et al. 2018). Pollen application occurred from time of flower anthesis to flower senescence/petal drop (approximately a 6-day window). Lastly, for the Pollen Exclusion treatment, I used the same 25 micron polyester felt liquid filter bags used for the artificial pollination treatment to exclude all pollen via insects and wind, but did not apply any pollen to the recently opened flowers to serve as a control. For each treatment requiring an exclusion bag (Wind, Artificial Pollination, and Pollen Exclusion), bags remained on the cluster of flower buds until every flower within the bag opened and senesced, at which point the bags were removed and fruits were left to develop until the fall harvest.

Figure 9: Three clusters of *Actinidia chinensis* var. *chinensis* 'AU Gulf Coast Gold' representing **A)** Insect Pollinated flowers, with flagging tape tied to the kiwifruit vine, **B)** a kiwifruit vine with a 1,000 micron insect exclusion bag representing the Wind Pollinated treatment, and **C)** a kiwifruit vine with a fine meshed 25 micron filter bag representing both the Pollen Exclusion and the Artificial Pollination treatments.

Fruit Quantity Assessments

I conducted fruit quantity assessments at four weeks post bloom (11 May 2020) and at harvest (15 September 2020). Fruits quantified at four weeks post bloom were referred to as "Fruit Set", whereas fruits quantified at harvest were termed "Mature Fruit". I chose to quantify fruit set at four weeks post bloom in case kiwifruit plants or fruits became damaged, for example by storms or insect pests. This ensured that, during the growing season, fruit set could be accurately estimated prior to harvest. I also chose to quantify the number of mature fruits at harvest to give an accurate estimation of total fruit production of each pollination treatment at harvest. The harvest date was chosen once the kiwifruits reached two threshold parameters, including a SSC (soluble solid content) over 6.5 °Brix (Burdon 2015) and a fruit hue angle below 110° (Minchin et al. 2003). Fruit set was documented by recording the number of experimental flowers that produced a fruit, and was defined as when petals abscise from the flower and fruit growth begins (Richardson et al. 2011).

Statistical Analyses

Palynological Study

A Wilcoxon Rank Sum test was used to test for differences in the relative abundance of kiwifruit pollen carried in the corbicula of *A. mellifera* and *B. impatiens* over the eight-day period. A Kruskal-Wallis one-way nonparametric analysis of variance was used to test for differences in the relative abundance of kiwifruit pollen collected by *A. mellifera* and *B. impatiens* among each sampling date. Additionally, a Kruskal-Wallis one-way nonparametric analysis of variance was used to test for differences in the relative abundance of kiwifruit pollen carried by *A. mellifera* and *B. impatiens* among the three sampling periods (morning, midday, afternoon). Kruskal-Wallis tests were used after square-root and logarithmic transformations applied to the data failed to eliminate heteroscedasticity. In cases with significant differences among treatments, a post hoc multiple comparison test was used to determine which treatment groups were significantly different from one another.

Exclusion Study

A Kruskal-Wallis one-way non parametric analysis of variance was used to test for differences in fruit set and mature fruit among the four pollination treatments (Insect Pollination, Wind Pollination, Artificial Pollination, and Pollen Exclusion) for *A. chinensis* var. *chinensis* 'AU Gulf Coast Gold' at four weeks post bloom and at harvest. Kruskal-Wallis tests were used after square-root and logarithmic transformations failed to eliminate heteroscedasticity in the data. In cases with significant differences among pollination treatments, a post hoc multiple comparison test was used to determine which treatment groups were statistically different from one another. All statistical tests were performed using Statistix 9.0 Analytical Software, Tallahassee, Florida, U.S.A.

Results

Palynological Study

Overall, 15,616 pollen grains were counted (7,666 from *A. mellifera* and 7,950 from *B. impatiens*), representing a total of 26 distinct pollen taxa over the eight sampling days (Appendix Table 1). Nineteen pollen taxa were identified from the corbicular pollen of *A. mellifera* and 24 from the corbicular pollen of *B. impatiens*. Overall, *A. mellifera* collected the greatest relative abundances of pollen from *Trifolium/Melilotus* (21.7%), followed by *A. chinensis* (21.3%), *Rhus* (15.6%), Rosaceae (12.6%), and *Brassica* (12.1%) (Figure 10). For, *B. impatiens*, the greatest relative abundances of pollen collected were from *A. chinensis* (66.6%), *Rhus* (8.5%), *Nyssa* (7.2%), Rosaceae (7.1%), and *Trifolium*/*Melilotus* (3.5%) (Figure 11). A significant difference was observed between mean relative abundance of kiwifruit pollen carried in the corbicula of *A. mellifera* and *B. impatiens* over the eight day period (z=5.04, P<0.0001), with *B. impatiens* carrying on average 46% more kiwifruit pollen than *A. mellifera*. The relative abundances of kiwifruit pollen collected by *A. mellifera* foragers among each sampling day (9 April 2020 - 13 April 2020) were statistically different (z=14.98, P=0.04), yet the results from the post hoc test failed to illustrate which sampling dates were statistically different from one another (Figure 10). For *B. impatiens,* the relative abundance of kiwifruit pollen collected between day 1 (9 April 2020) and day 8 (13 April 2020) was statistically different (z=17.99, P=0.0003), but no other sampling dates were statistically different from one another (Figure 10). Lastly, no significant differences were observed in the relative abundance of kiwifruit pollen collected between the three sampling periods (morning, midday, afternoon) for both *A. mellifera* (z=1.71, P=0.42) and *B. impatiens* (z=1.30, P=0.52).

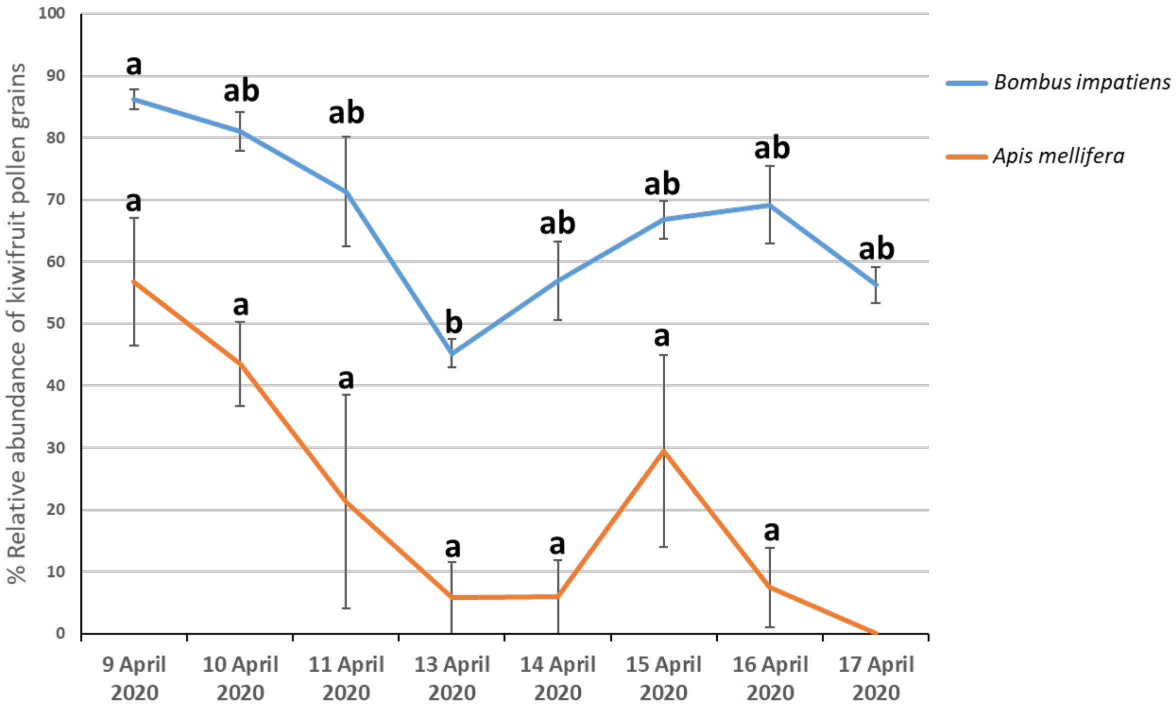

Figure 10: Percent relative abundance of *Actinidia chinensis* var. *chinensis* 'AU Gulf Coast Gold' kiwifruit pollen per day averaged across each sampling period (morning, midday, and afternoon) for honey bee (*Apis mellifera*) and bumble bee (*Bombus impatiens*) during an 8 day sampling period. For each species, days with different letters represent a significant difference in relative abundance of kiwifruit pollen collected each day at P≤0.05.

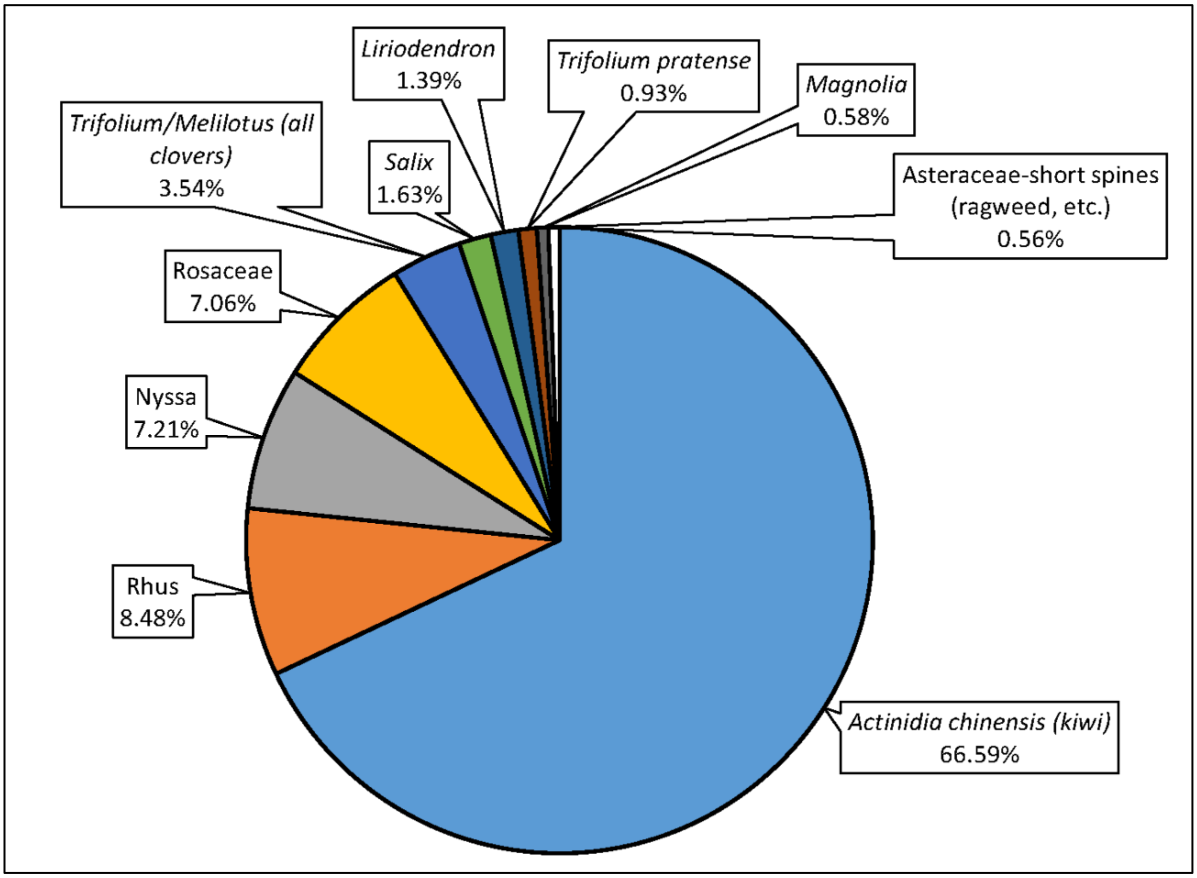

Figure 11: Corbicular pollen of bumble bee (*Bombus impatiens*) representing the top ten plant species visited per day. Relative abundances of corbicular pollen were averaged over the eight day sampling period.

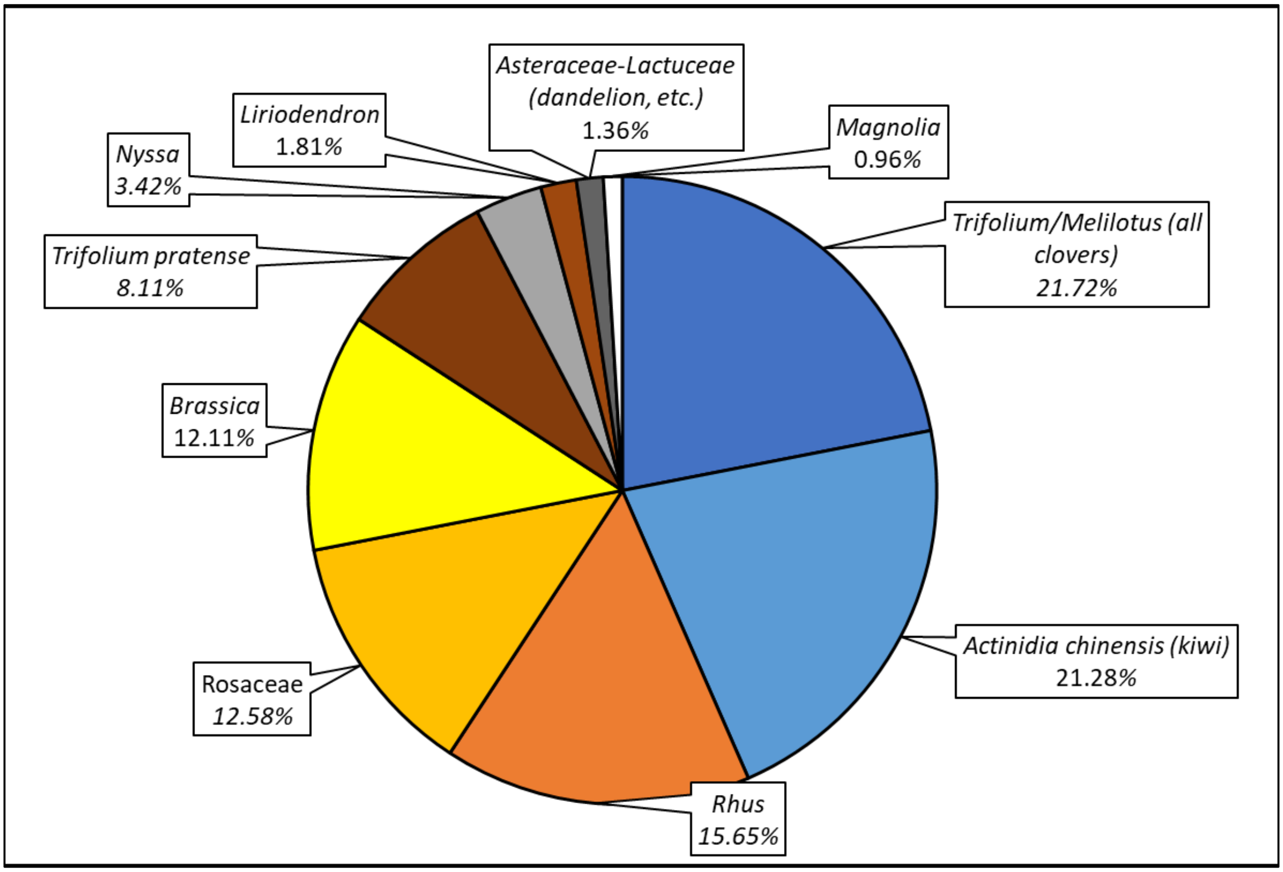

Figure 12: Corbicular pollen of honey bees (*Apis mellifera*) representing the top ten plant species visited per day. Relative abundances of corbicular pollen were averaged over the eight day sampling period.

Exclusion Study

Overall, 1,674 kiwifruit flowers were included in the fruit quantity assessments, with 436, 442, 364, and 432 flowers utilized for the Insect Pollination, Wind Pollination, Artificial Pollination, and Pollen Exclusion treatments, respectively. Several kiwifruit bines were damaged during the growing season, thus a total of 114, 113, 106, and 109 kiwifruit plants were utilized for each pollination treatment (Insect Pollination, Wind Pollination, Artificial Pollination, and Pollen Exclusion, respectively). At four weeks post full bloom, the mean percentage of kiwifruit flowers that set fruit was statistically different among the four pollination treatments (z=338.2, P<0.0001). Post hoc multiple comparison tests revealed that Artificial Pollination resulted in the greatest percent fruit set per flower with Insect Pollination, Wind Pollination, and Pollen Exclusion treatments not differing statistically from each other (Figure 13). At harvest, the percentage of kiwifruit flowers that produced mature kiwifruits was significantly different among the four pollination treatments (*z*=297.5, P<0.0001). Post hoc multiple comparison tests revealed that Artificial Pollination resulted in the greatest percent of mature fruits per flower, with Insect Pollination, Wind Pollination and Pollen Exclusion treatments not statistically different from one another (Figure 14).

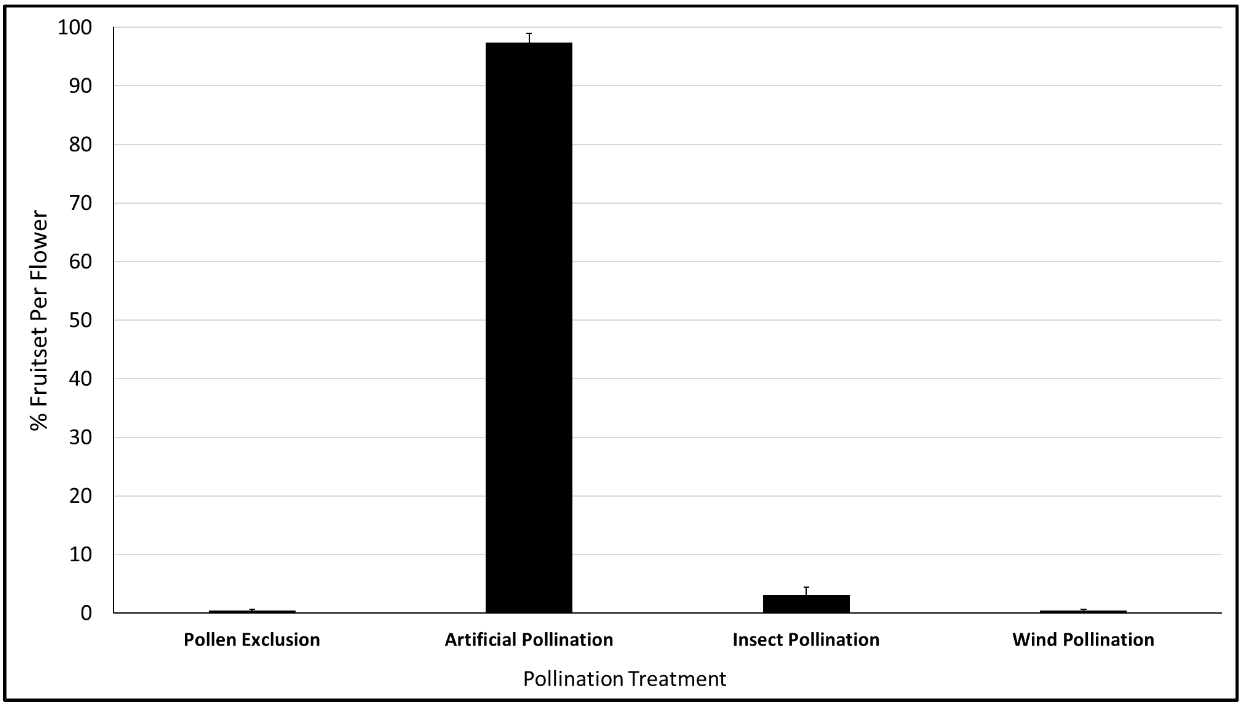

Figure 13: Mean percentage of kiwifruit flowers that set fruit at four weeks post peak bloom (11 May 2020) for the four pollination treatments (Insect Pollination, Wind Pollination, Artificial Pollination, and Pollen Exclusion). Means with different letters are significantly different at P=0.05.

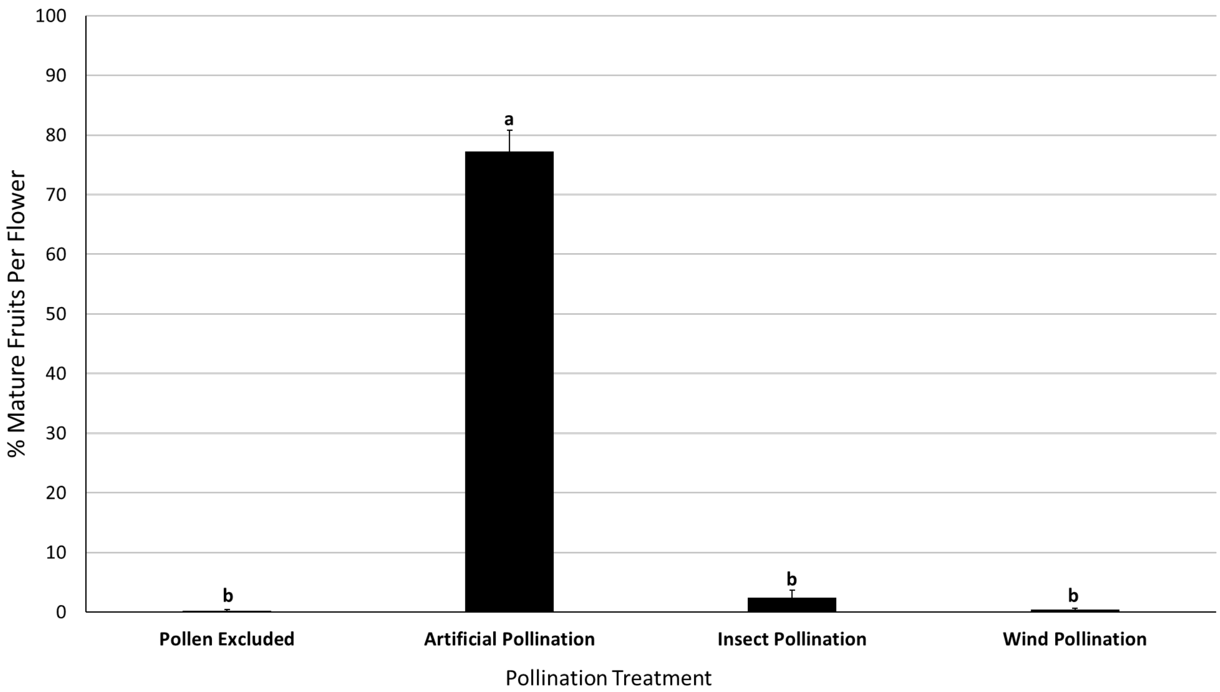

Figure 14: Mean percentage of kiwifruit flowers that produced mature kiwifruits per flower at harvest (September 2020) for the four pollination treatments (Insect Pollination, Wind Pollination, Artificial Pollination, and Pollen Exclusion). Means with different letters are significantly different at P=0.05.

Discussion

Determining the contribution of managed bees to the pollination of kiwifruit within the southeastern United States is an important step in developing a pollination management plan that will increase kiwifruit yields while simultaneously decreasing pollination costs. *Apis mellifera* and *B. impatiens* to played a small role in the pollination of kiwifruit in this study. Stocking *A. mellifera* and *B. impatiens* within the kiwifruit orchard did not result in high fruit set or fruit yield at harvest, due to low percentages of kiwifruit pollen collected by both bee species. Furthermore, both managed bee species likely were influenced by competition for visits by of non-kiwifruit blooms, based on quantification of their corbicular pollen loads. For this reason, artificial pollination is the best pollination method. Kiwifruit producers should put a greater emphasis on artificial pollination if they are to efficiently pollinate their crop to increase profits.

Apis mellifera and *B. impatiens* are both floral generalists (Ascher and Pickering 2009), and in this study they collected pollen from a diversity of flowering plant species growing within the kiwifruit orchard. These results aligned well with previous studies (Clinch 1984, Pomeroy and Fisher 2002). Clinch (1984) investigated the foraging behavior of *A. mellifera* within a New Zealand kiwifruit orchard and determined that foragers were the most abundant visitor to kiwifruit blooms, yet they were influenced by the presence of competing non-kiwifruit blooms. He also noted that although bumble bees were observed visiting the kiwifruit blooms, their abundance was too low to contribute to kiwifruit pollination. Similarly, Pomeroy and Fisher (2002) compared the pollination contribution of two managed bee species (*A. mellifera* and *B. terrestris*) stocked within a New Zealand kiwifruit orchard, and concluded that although *B. terrestris* displayed a greater fidelity towards collection of kiwifruit pollen than *A. mellifera*, both bees were influenced by the presence of competing blooms. Many flowering plant species begin to bloom during the spring in central Alabama, and I observed both *A. mellifera* and *B. impatiens* visiting the flowers of white clover

(Trifolium repens), wild radish *(Raphanus raphanistrum*), and other flowering plant species within the kiwifruit orchard, and these observations are consistent with the results from our palynological study. Elimination of all competing flowers in an orchard is a near impossible task and could be ecologically damaging. Additionally, being larger bodied bees, *A. mellifera* and *B. impatiens* are can fly large distances (up to 1.4 km for *A. mellifera* and 1.8 km for some *Bombus* spp.) (Walther-Hellwig and Frankl 2000, Greenleaf et al. 2007, Couvillon et al. 2015), and competing blooms could occur well outside of orchard's boundaries.

Although *A. mellifera* foragers collected pollen from fewer plant taxa than *B. impatiens*, *A. mellifera* collected smaller relative abundances of kiwifruit pollen. Studies on the foraging behavior of *A. mellifera* have noted that its foragers display high floral constancy, often foraging on blue or yellow flowers with high nutritional rewards (Grant 1950, Wells and Wells 1983, Grüter et al. 2011). Comparatively, *B. impatiens* foragers are generally less constant in foraging behavior (Grant 1950), often change their foraging behavior based on the quality of pollen and nectar rewards available (Gegear and Thomson 2004). Because *A. mellifera* and *B. impatiens* must acquire all essential amino acids, vitamins, fats, sterols, and minerals required for growth and development solely from the pollen in their diets (Dietz 1975, Moerman et al. 2016), they may have targeted other species of flowering plants to meet these needs. Kiwifruit pollen derived from staminate flowers contains quantities of essential amino acids above the minimum level required for *A. mellifera*, yet pistillate kiwifruit flowers do not (De Groot 1953, Day et al. 1990). Both *A. mellifera* and *B. impatiens* could have altered their foraging behavior due to the inadequate nutrition supplied by pistillate kiwifruit flowers. Alternatively, kiwifruit pollen collected and stored in the corbicula by both bee species could be representative of staminate pollen rather than the infertile lower nutritional quality pistillate kiwifruit pollen. It is possible that both *A. mellifera* and *B. impatiens* concentrated foraging efforts on staminate kiwifruit flowers while bypassing nutritionally lower quality pistillate pollen (Pomeroy and Fisher 2002). This would result in decreased outcrossing and thus decrease in fruit formation.

Within other agricultural crops, flowers visited by *Bombus* spp. consistently displayed lower abortion rates and higher seed sets than *A. mellifera* visited flowers, so that *Bombus* spp. might be more consistent at outcrossing than *A. mellifera* (Skinner and Lovett 1992, Stanghellini et al. 1998, Drummond 2012, Campbell et al. 2019a). Despite our stocking *A. mellifera* and *B. impatiens* at commonly recommended rates for kiwifruit pollination, their contribution (along with the contribution of wild pollinators) to the pollination of kiwifruit was minimal. *Apis mellifera* and *B. impatiens* appeared to be influenced by competing non-kiwifruit flowers and, as a result, no significant differences in fruit set per flower were observed between Insect Pollinated, Wind Pollinated, and Pollen Excluded flowers. The artificial Pollination treatment yielded the greatest percentage of fruits per flower compared to all other pollination treatments (Insect Pollination, Wind Pollination, and Pollen Exclusion). Therefore, due to the low percentages of fruits set per flower in Insect Pollinated and Wind Pollinated treatments, orchard managers should plant only female plants and artificially pollinate them instead of intermixing male cultivars and introducing managed bees. Since the planting of male kiwifruit plants may occupy 25% of a typical orchard's area, this act alone would increase yields (and profits) for an orchard. Due to the ime and costs associated with hand pollination, future studies should compare the pollination efficiency of other methods of artificial pollination (e.g. spraying dry pollen via boom sprayers) to hand pollination, as boom spraying requires less time and is less expensive for applying pollen. Lastly, future studies should investigate how different stocking rates of *A. mellifera* and *B. impatiens* influence fruit set and yields, and how stocking them at different times during the kiwifruit bloom influences fruit set and yields.

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Chapter 4

Assessing the attractiveness of native wildflower species to bees (Hymenoptera) in the southeastern United States

Abstract

Habitat loss and agricultural intensification have led to declines in numerous insect groups including native bees. Recent government incentives have recognized the importance of supplementary wildflower plantings to help support native bee populations, yet little information exists on the attractiveness of recommended plant species to native bees. In this study, I evaluated the attractiveness to bees of 18 wildflower species belonging to six families (Apiaceae, Apocynaceae, Asteraceae, Fabaceae, Lamiaceae, and Verbenaceae) and 17 genera. Through timed sweep netting surveys, I highlighted numerous plant-pollinator interactions to support the idea that some species of native wildflowers vary in the abundance, diversity, and richness of native bees they attract. My results suggest that *Asclepias tuberosa, Gaillardia pulchella,* and *Verbena hastata* are highly attractive to native bees. While other wildflower species (*Echinacea purpurea, Monarda punctata, Rudbeckia hirta, Ratibida pinnata, Symphyotrichum pilosus,* and *Solidago rugosa*) attracted a lower abundance, diversity, and richness of native bees, they still are ecologically important to bees.

Introduction

Declines across numerous insect lineages have been reported worldwide (Hallmann et al. 2017, Leather 2018, Lister and Garcia 2018, Janzen and Hallwachs 2019, Sánchez-Bayo and Wyckhuys 2019). Estimates suggest 40% of the world's insect species could become extinct during the next few decades, with Coleoptera, Hymenoptera, and Lepidoptera being the most vulnerable orders (Sánchez-Bayo and Wyckhuys 2019). Declines of insect populations, including insect pollinators, are expected to cause cascading effects on food webs and ultimately ecosystem services (Kehoe et al., 2020). Declines in populations of native and non-native bee species within the United States and abroad may be due to pesticides, disease, climate change, agricultural expansion and intensification, and habitat loss (Cane and Tepedino 2001, Vanbergen and Initiative 2013, Garbuzov and Ratnieks 2014, Koh et al. 2016).

The provision of sufficient forage across a diversity of habitats is important in supporting and promoting bee populations within human impacted landscapes (Williams et al. 2015). Native bees rely on many wildflower species that have overlapping bloom periods and provide floral resources in the form of nectar and pollen. Wild and managed bees acquire all of their nutritional needs from the flowers they visit and these recources are vital for bee growth, development, and reproduction (Dietz 1975, Leach and Drummond 2018). The recources bees gain from flowers occur in the form of nectar and pollen. Nectar is a vital energy source for bees and is produced by flowers to attract pollinators (Seeley 2009, Nicolson 2011). Nectar with high sugar concentrations has a positive effect on the fitness of solitary and honey bee offspring (Burkle and Irwin 2009, Kaftanoglu et al. 2011). Differences in sugar concentrations of nectar vary between plant species (Nicolson et al. 2007), and thus the overall energetic reward varies. For this reason, bees could prioritize highly rewarding wildflowers while bypassing wildflowers with lower quality nectar rewards. Pollen is another floral reward but, unlike most nectar, is a rich source of protein (Ahrenfeldt et al. 2019). It is vital to all bees as it is essential to larval growth and development, egg-laying (in females), and positively affects bee immune systems (Génissel et al. 2002, Roulston and Cane 2002, Tasei and Aupinel 2008, Alaux et al. 2010, Nicolson 2011, Brunner et al. 2014, Frias et al. 2016, Lawson et al. 2016). Pollen protein content also differs between plant species (Roulston et al. 2000), and bees can discriminate between flowers of varying pollen qualities (Robertson et al. 1999). Thus, bees often target plant species that produce nutritionally higher quality nectar and pollen (Gegear and Thomson 2004).

Due to variation in nectar and pollen quality between wildflower species, bees must decide upon which flower types to target when the bloom periods of wildflower species coincide (Wells and Wells 1983, Gegear and Thomson 2004, Grüter et al. 2011). Additionally, flowering plants have evolved varying plant and floral characteristics to attract pollinators. These traits include flower size, color, scent, and the time and duration of bloom periods (Pellmyr 2002, Garbuzov and Ratnieks 2014, Williams et al. 2015, Rijn and Wäckers 2016, Rowe et al. 2020). Bee species can have innate floral preferences regarding flower size, color, and scent (Pellmyr 2002, Rowe et al. 2020), but other preferences are learned (Gegear and Thomson 2004). There often are positive correlations between number of bee visits and flower size, along with quantities and qualities of available floral resources such as nectar and pollen (Galen and Newport 1987, Cartar 2004, Gegear and Thomson 2004, Mallinger and Prasifka 2017, Rowe et al. 2020).

Recent government programs in the United States have recognized the importance of supplementary wildflower plantings to native bees and have promoted planting native wildflowers to benefit them, yet the study of individual wildflower species (and seed mixes) on their ability to attract and support native bee populations is in its infancy (Williams et al. 2015). For example, several studies have investigated the attractiveness of wildflower mixtures for insect pollinators in North America (Carvell et al. 2006, Blaauw and Isaacs 2014, Williams et al. 2015, Campbell et al. 2019), yet few studies have been conducted within the southeastern United States. This is important because understanding the attractiveness of each wildflower species to bees native to the southeastern United States is necessary for land managers to implement a science-based approach to wildflower selection for their region.

Here, I investigated bee attractiveness of 18 native wildflower species commonly recommended by the USDA-NRCS to land managers in the southeastern United States. To accomplish this, I planted the 18 wildflower species in a randomized block design and conducted timed sweep netting events to assess each species' attractiveness to bees. I hypothesized that each species would vary in floral characteristics and thus would attract different abundances and diversities of bees. The information gained could aid restoration ecologists in their decision making process to determine which wildflower species to include in a seed mixture when seeking to attract and conserve certain pollinators.

Methods

Site Preparation

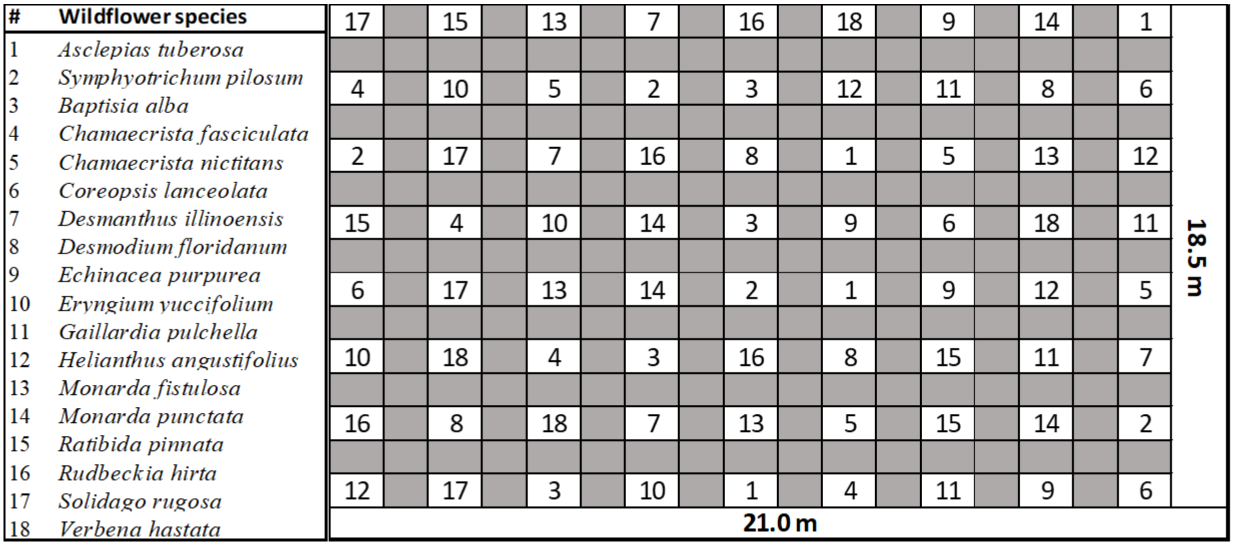
In the spring of 2019, a 388 m^2 field was prepared for the planting of the 18 wildflower species at the Auburn University Poultry and Animal Nutrition Center in Auburn, Alabama (32°41.135'N, 85°30.440'W). Field preparation included taking soil samples to make fertilizer adjustments, reducing weed competition with the use of herbicides, and tilling the plot prior to planting. Fifteen soil samples (depth of 8 cm) were obtained on 29 March 2019 using a soil sample probe, and were combined and tested at the Auburn University Soil Testing Laboratory to quantify pH, phosphorus, potassium, magnesium, and calcium levels. On 7 April 2019, the site was mowed and sprayed with a solution of 6.5 L of 41% glyphosate (Cornerstone® Plus), 0.95 L of surfactant mixed, and 378.5 L of water at a rate of 23 L per hectare (Cornerstone® Plus, Winfield Solutions, LLC. St. Paul, MN) to kill undesirable plants two weeks prior to planting. On 16 April 2019, the site was fertilized with 7.3 kg of triple superphosphate (0-46-0), 90 kg of limestone and 7.3 kg of nitrogen (34-0-0), and then tilled. A second application of 7.3 kg of nitrogen (34-0-0) was broadcasted over the entire study plot six weeks after planting as recommended by the Auburn University Soil Testing Laboratory.

Wildflower Planting

I studied 18 species of wildflowers native to the southeastern United States (Table 3). Each wildflower species met all of the following criteria: (1) is recommended by the USDA/NRCS to enhance native bee habitat, (2) flowers during spring, summer, or fall, and (3) is easily available for purchase. On 30 April 2019, a grid pattern was staked on the surface of the soil using nylon string, creating seventy-two 1 m^2 wildflower subplots each surrounded by a 1.5 m^2 buffer. I transplanted mature annual and second year perennial plants (Superior Trees Inc. Lee, FL) (Table 1) each within a 1 m^2 plot in a randomized block design, with four replicates per species (Figure 15). The number of mature plants transplanted per 1m^2 ranged from four to nine plants to achieve close to one-hundred percent vegetation cover (Superior Trees Inc. pers. comm.) and was dependent upon each species' growth habit at maturity (Garbuzov and Ratnieks 2014). After planting, a 1 m^2 sheet of Kraft paper, followed by a thick layer (5-8 cm) of wheat straw, was placed on top of the soil and around each plant to retain soil moisture and to prevent the growth of unwanted plants. All plants were well watered at transplant (manually with a hose) and watered at four days intervals for three weeks post transplanting to ensure their survival. During the growing season, a 5 hectare area surrounding the experimental wildflower plot, and the 1.5 m^2 buffers within the wildflower plot were mowed on a weekly basis to limit all other flowering plants.

Table 3: Scientific and common names of the 18 native wildflowers planted in the spring of 2019 to assess their attractiveness to bees. The planting rate is based on the target number mature plants per 1 m^2 and is included for each species. [1] USDA Plant Atlas (https://plants.sc.egov.usda.gov/java/)

Family	Species[1]	Common name	Life Span[1]	Plants per m^2
Apiaceae	*Eryngium yuccifolium* Michx.	Rattlesnake Master	Perennial	4
Apocynaceae	*Asclepias tuberosa* L.	Butterfly Milkweed	Perennial	6
Asteraceae	*Coreopsis lanceolata* L.	Lance-leaved Coreopsis	Perennial	9
	Echinacea purpurea (L.) Moench.	Purple Coneflower	Perennial	9
	Gaillardia pulchella Foug.	Blanket flower	Annual	5
	Helianthus angustifolius L.	Narrow-Leaved Sunflower	Perennial	4
	Ratibida pinnata (Vent.) Barnhart.	Greyheaded Coneflower	Perennial	4
	Rudbeckia hirta L.	Black-eyed Susan	Biennial	9
	Solidago rugosa Mill.	Wrinkled-Leaved Goldenrod	Perennial	4
	Symphyotrichum pilosum (Willd.) G.L. Nesom	Frost Aster	Perennial	4
Fabaceae	*Baptisia alba* (L.) Vent.	White Wild Indigo	Perennial	4
	Chamaecrista fasciculata (Michx.) Greene	Partridge Pea	Annual	4
	Chamaecrista nictitans (L.) Moench.	Sensitive Partridge Pea	Annual	9
	Desmanthus illinoensis (Michx.) MacMill. ex. B.L. Rob. and Fern.	Illinois Bundleflower	Perennial	9
	Desmodium floridanum Chapm.	Florida Ticktrefoil	Perennial	9
Lamiaceae	*Monarda fistulosa* L.	Bergamot	Perennial	4
	Monarda punctata L.	Spotted Beebalm	Perennial	4
Verbenaceae	*Verbena hastata* L.	Blue Vervain	Perennial	5



#	Wildflower species
1	*Asclepias tuberosa*
2	*Symphyotrichum pilosum*
3	*Baptisia alba*
4	*Chamaecrista fasciculata*
5	*Chamaecrista nictitans*
6	*Coreopsis lanceolata*
7	*Desmanthus illinoensis*
8	*Desmodium floridanum*
9	*Echinacea purpurea*
10	*Eryngium yuccifolium*
11	*Gaillardia pulchella*
12	*Helianthus angustifolius*
13	*Monarda fistulosa*
14	*Monarda punctata*
15	*Ratibida pinnata*
16	*Rudbeckia hirta*
17	*Solidago rugosa*
18	*Verbena hastata*

17	15	13	7	16	18	9	14	1
4	10	5	2	3	12	11	8	6
2	17	7	16	8	1	5	13	12
15	4	10	14	3	9	6	18	11
6	17	13	14	2	1	9	12	5
10	18	4	3	16	8	15	11	7
16	8	18	7	13	5	15	14	2
12	17	3	10	1	4	11	9	6

Figure 15: Arrangement of the 18 wildflower species planted as mature potted plants in a randomized block design at the Poultry Science Building at Auburn University during the 2019 field season. Each number represents a single wildflower species planted within a 1 m^2 plot surrounded by a 1.5 m^2 buffer (grey).

Wildflower and insect monitoring

I monitored and recorded the number of floral blooms occurring within each 1 m^2 plot for each wildflower species on a weekly basis from May-November 2019 (Garbuzov and Ratnieks 2014). Additionally, any plot containing at least one inflorescence was sweep netted for 10 minutes. Any bees visiting the flowers of each wildflower species were captured and stored in separate plastic 5 mL vials for the entire duration of the 10-minute survey to avoid counting the same individual multiple times. After each ten-minute survey, captured bees were either identified in the field and released (when possible), or placed in a cooler and brought back to the lab to be pinned, identified, and databased. I identified bees to genus or species using keys in Ascher and Pickering (2009) and Mitchell (1962). Identifying information such as plot number, date, and time of day were recorded for each floral visitor bee. To maximize insect capture and standardize sampling procedures, each weekly sweep netting survey was conducted over a two day period, rotating between morning, midday, and afternoon, and when weather conditions were appropriate for foraging insects (Hopwood 2008). Appropriate sampling conditions consisted of warm (>20°C), sunny days (<60% cloud cover), with ≤40% chance of precipitation (Hopwood 2008).

Statistical analyses

Network diagrams

We constructed bee-wildflower interaction networks with R statistical software using the plotweb function within the bipartite package (Dormann et al. 2008). Bee and wildflower phenologies were also constructed using R statistical software using ggplot 2 (Wickham 2016).

Wildflower-bee interactions

I plotted number of inflorescences of all wildflower species and count of captured bee species; I visually binned numbers and counts into one of the following three abundance levels: 1) superabundant, 2) abundant; and 3) rare (see Grodsky et al. 2018). I set the cutoff for inclusion of individual bee and flowering plant species in the analyses at the break between abundant and rare bee and flowering plant species, respectively, thereby excluding all rare bee species with relatively low numbers and flowering plant species with relatively low sweep netting events from analyses. As a result, we set cutoffs for individual bee species with abundances at $n \geq 28$ and individual flowering plant species at $n \geq 25$ sweep netting events. Rare bee species were pooled according to their genus in the analysis. For bee species- and genera-specific models, I excluded from analyses flowering plant species that were not visited by individual bee species or genera.

I conducted Poisson generalized linear models (GLMs) to elucidate patterns in the selection of experimental flowering plant species by bees. I structured the models with count of total bees, bee species/genus richness, and count of individuals for each bee species/genus pooled across all sampling periods on each experimental flowering plant species (i.e., treatment) in each block as response variables, treatment and block as explanatory variables, and the log of sampling effort (i.e., number of sampling events per flowering plant species) as an offset. I followed the same modeling procedure for bee diversity, using the Shannon-Weiner diversity index as a response variable in a generalized linear model with a Gamma rather than Poisson distribution because of the nature of the data (positive, continuous, and not normally distributed) (Dunder et al. 2018). I performed likelihood ratio tests on GLMs to determine significant treatment effects. Then, I conducted post hoc Tukey's pairwise comparisons of treatment means with a Bonferroni adjustment, using general linear hypothesis testing (glht function, single-step method) in the R package 'multcomp' (Hothorn et al. 2017). I set $\alpha = 0.05$ to determine statistical significance.

Results

Wildflower and bee monitoring

A total of 155,185 flowers were counted in plant surveys from all planted wildflower species from May-November 2019. Among the 18 planted wildflower species, only one species (*B. alba*) failed to flower during the 2019 field season and therefore was not included in any analysis. Eleven out of the 17 wildflower species began flowering during the month of May, and each species' peak bloom event varied by season (spring, summer, fall) and in bloom duration. At any given time during the study, 3-16 wildflower species were simultaneously in bloom, therefore giving foraging bee species a choice of which wildflowers to visit. The majority of the wildflower species bloomed from June-November (Figure 16), whereas, *D. illinoensis* and *H. angustifolius* only bloomed for relatively short periods (from mid-July through early September, and from mid-September to early November, respectively).

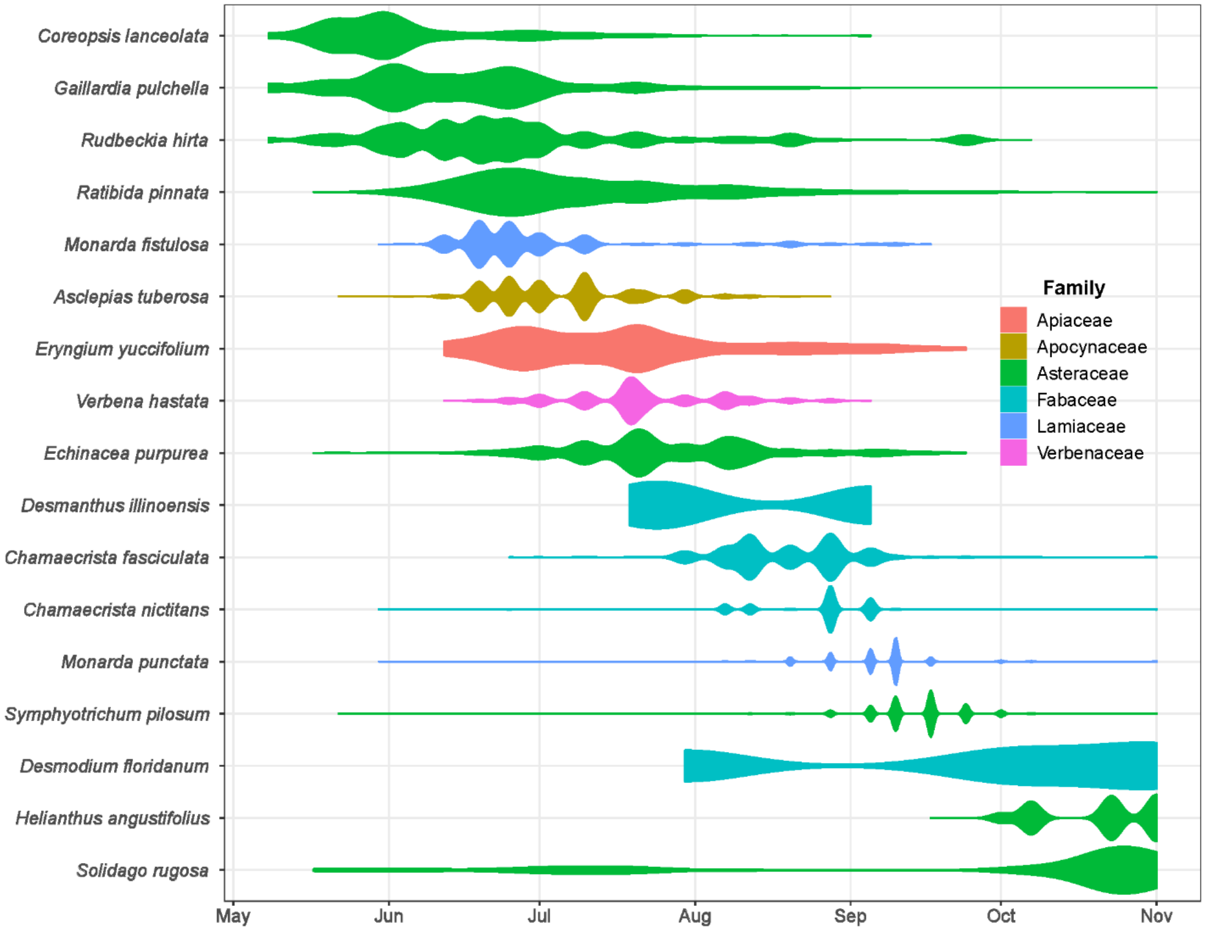


Figure 16: Flowering plant phenology indicating when each plant species was blooming in the wildflower plot. Violin plots are colored by plant family, and the height of each violin plot indicates the relative abundance of flowers within each species.

I spent a total of 7,740 minutes sweep netting from May-November 2019, collecting a total of 1,024 bees visiting the 17 blooming wildflower species. The 1,024 netted bees belonged to 26 genera/species from three families (Apidae, Halictidae, Megachilidae) (Table 4). Halictidae was the most common family, accounting for 648 individuals (63% of total captures), followed by Apidae (346; 34%), and Megachilidae (30; 3%). Four species/genera of bees (*H. poeyi*, *Lasioglossum* spp., *B. bimaculatus*, and *B. impatiens*) were active throughout the entire season, whereas 13 bee species (*A. splendens*, *B. fraternus, M. communis, X. micans, M. brevis*, *M. inimica*, *M. parallela*, *M. albitarsis*, *M. xylocopoides*, *M. comptoides*, *M. policaris*, *A. metallica,* and *M. agilis*) were caught in very low abundances (five or less individuals) (Table 4 and Figure 17). Three species of bees (*M. sculpturalis*, *M. communis*, and *H. parallelus*) were active for approximately two weeks during the end of June and into early July, with the remaining species of bees being active for at least one month.

Table 4: Numbers of bee genera and species captured while visiting the 18 wildflower species from May-November 2019. Number of wildflower flower species is the total number of wildflower species a particular bee species/genera visited.

Family	**Genus/Species**	**Total Number**	**# of wildflower species visited**
Apidae	*Apis mellifera*	60	5
	Bombus bimaculatus	28	7
	Bombus fraternus	5	3
	Bombus griseocollis	84	6
	Bombus impatiens	93	9
	Bombus spp.	12	6
	Melissodes agilis	1	1
	Melissodes communis	4	3
	Melissodes comptoides	1	1
	Svastra obliqua	18	2
	Xylocopa micans	5	2
	Xylocopa virginica	35	10
Halictidae	*Agapostemon splendens*	4	3
	Augochloropsis metallica	1	1
	Halictus parallelus	13	3
	Halictus poeyii	563	14
	Halictus rubicundus	9	3
	Lasioglossum spp.	58	12
Megachilidae	*Megachile albitarsis*	1	1
	Megachile brevis	1	1
	Megachile inimica	1	1
	Megachile mendica	12	4
	Megachile parallela	1	1
	Megachile policaris	1	1
	Megachile sculpturalis	11	1
	Megachile xylocopoides	2	2
Total		**1024**	

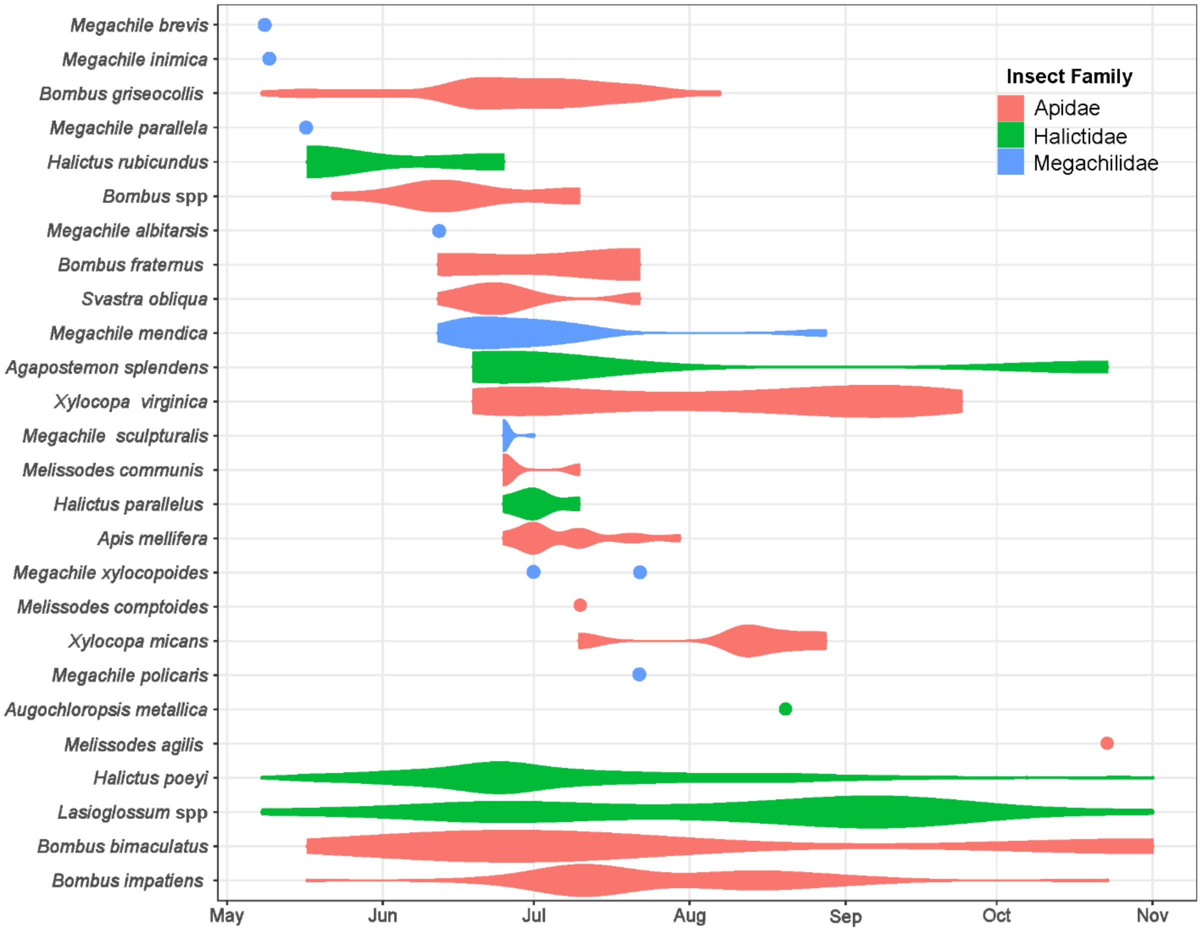


Figure 17: Bee phenology indicating when bee species were captured visiting the wildflower species within the experimental wildflower plot during May-November of 2019. Violin plots are colored by bee family, and the height of each violin plot indicates relative abundance within each bee species. Dots represent single bee captures, and plot and dot color corresponds to bee family.

Wildflower-bee interactions

The wildflower-bee network showed a total of 1,024 bee-wildflower interactions, with each bee species/genus visiting an average of four wildflower species, and each plant species visited by an average of six bee species (Figure 18). *Halictus poeyi* was the most commonly collected bee species, (563 individuals), making up over half of all plant-bee interactions, followed in order by *B. impatiens*, *B. griseocollis, A. mellifera, Lasioglossum* spp., *X. virginiana*, and *B. bimaculatus* (Table 2). *Halictus poeyi* visited the greatest number of plant species (14), followed by *Lasioglossum* spp. (12), *X. virginica* (10), *B. impatiens* (9), *B. bimaculatus* (7), *B. griseocollis* (6), and *A. mellifera* (5). Seventeen of the 26 bee genera/species visited three or less different plant species during the flowering season, and nine bee species visited a single plant species (Table 4). *Gaillardia pulchella*, *V. hastata*, *A. tuberosa*, and *E. purpurea* attracted the greatest richness of bee species with 17, 14, 13, and 11 bee species visiting respectively (Figure 18).

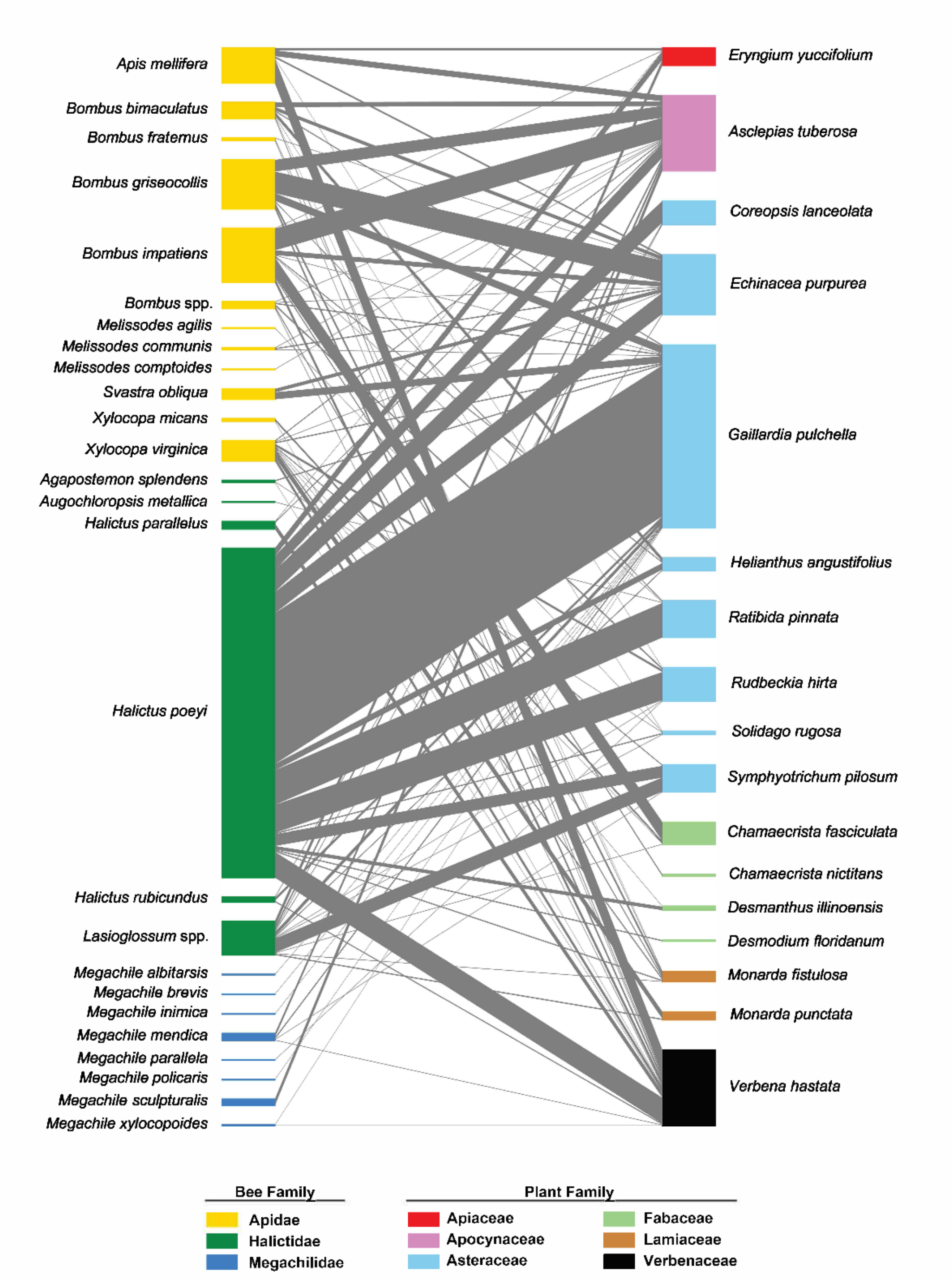


Figure 18: Graphical representation of the plant-pollinator network observed in the wildflower plot during the 2019 field season (May-November). Polygons depicted on the left represent floral visitors by the lowest taxonomic level and are colored by bee family. Polygons depicted on the right symbolize the different plant species the floral visitors were observed visiting and are also colored by plant family. The width of each polygon is proportional to the number of interactions observed per taxonomic unit. Lines represent links between insect and plant species. The line width is proportional to the frequency of interactions.

Significant differences were observed among the wildflower species when comparing mean bee abundance per sampling event (LRT=810, χ^2 <0.0001) (Table 5 and Figure 19a). *Gaillardia pulchella* was visited by significantly greater abundances of bees compared to all other plant species, and the majority of those bee visits were by *H. poeyi* (82%) (Figure 18). *Asclepias tuberosa* and *V. hastata* attracted the second greatest mean abundance of bees per sampling event, yet no significant differences were observed between the two plant species (P=0.47) (Table 5 and Figure 19a). The majority of bee visits (79%) to *Asclepias tuberosa* were primarily by five bee species (*H. poeyi, A. mellifera, B. griseocollis, B. impatiens,* and *M. sculpturalis*), and the majority of bee visits (66%) to *V. hastata* were represented by *H. poeyi* and *A. mellifera.* Lastly, *E. purpurea* attracted the third greatest mean bee abundances per sampling event, but was not significantly different from mean bee abundances per sampling event for *C. fasciculata* (P=1.0)*, C. lanceolata* (P=1.0)*, E. yuccifolium* (P=0.93)*, So. rugosa* (P=0.93)*, M. fistulosa* (P=1.0)*, and M. punctata* (P=0.45) (Table 5 and Figure 19a). The majority of bee visits to *E. purpurea* were by two bee species (*B. griseocollis* and *H. poeyi*), which made up 71% of all bee visits to the plant species (Figure 18). Overall, *M. punctata, D. floridanum,* and *C. nictitans* were visited by the fewest number of bee taxa (represented as links between wildflower and bee species/genera in Figure 18).

When comparing mean bee species richness per sampling event, significant differences were observed among the wildflower species (LRT=241, χ^2 <0.0001) (Table 5 and Figure 19b). *Gaillardia pulchella, A. tuberosa, V. hastata, So. rugosa, and M. fistulosa* attracted the greatest mean bee richness per sampling event (P<0.03) when compared to all other wildflower species*,* with no significant differences observed among them (P>0.13 in all cases). The wildflower species with the lowest mean bee richness per sampling event were *R. hirta, R. pinnata, Sy. pilosus, M. punctata, C. fasciculata,* and *C. nictitans*, with no significant differences observed among them (P>0.32 in all cases).

Significant differences were observed when comparing mean bee species diversity per sampling event among the wildflower species (LRT=158, χ^2<0.0001) (Table 3 and Figure 19c). *Asclepias tuberosa*, *G. pulchella*, and *V. hastata* attracted the greatest bee species diversity per sampling event, yet were not statistically different from *So. rugosa* (P>0.70), *E. yuccifolium* (P>0.16)*, G. pulchella* (P>0.99)*,* and *M. fistulosa* (P>0.25). Lastly, *M. fistulosa* showed no significant differences from any of the plant species (P>0.22) (Table 5 and Figure 19c). The wildflower species with the lowest bee species diversity per sampling event were *Sy. pilosus, C. lanceolata, C. fasciculata, R. hirta, M. punctata, R. pinnata,* and *C. nictitans*, which were not statistically different from one another (P>0.76 in all cases) (Table 5 and Figure 19c).

Table 5: Mean bee abundance, species richness, and Shannon Wiener diversity index, followed by (95% CI) for each wildflower species per replicate per sampling event during the 2019 field season.

			Wildflower species													
	LRT	**Pr(Chi)**	***Asclepias tuberosa***	***Chamaecrista fasciculata***	***Coreopsis lanceolata***	***Chamaecrista nicitians***	***Echinacea purpurea***	***Ernyngium yuccifolium***	***Gaillardia pulchella***	***Monarda fistulosa***	***Monarda punctata***	***Rudbeckia hirta***	***Ratibida pinnata***	***Symphyotrichum pilosus***	***Solidago rugosa***	***Verbena hastata***
Bee Abundance (±95%CI)	810	<0.001	2.94 (0.87)	0.92 (0.78)	0.84 (0.38)	0.06 (0.12)	1.67 (0.82)	0.68 (0.29)	5.53 (0.97)	0.59 (0.24)	0.38 (0.21)	0.76 (0.91)	0.91 (0.56)	0.71 (0.11)	0.64 (1.22)	2.50 (0.64)
Bee Richness (±95%CI)	241	<0.001	1.43 (0.28)	0.29 (0.21)	0.54 (0.21)	0.04 (0.08)	0.94 (0.51)	0.57 (0.21)	1.63 (0.23)	0.51 (0.17)	0.30 (0.12)	0.43 (0.10)	0.40 (0.13)	0.40 (0.16)	0.51 (0.97)	1.35 (0.29)
Bee Diversity (±95%CI)	158	<0.001	0.35 (0.10)	0.04 (0.05)	0.05 (0.03)	0.00 (0.00)	0.21 (0.14)	0.12 (0.11)	0.30 (0.12)	0.10 (0.10)	0.01 (0.03	0.02 (0.03)	0.01 (0.02)	0.06 (0.06)	0.13 (0.25)	0.37 (0.07)

Table 6: Mean bee abundance (95% CI) per replicate per sampling event for the eight most common genera/species of bees netted visiting the planted wildflower species during the 2019 field season.

			Wildflower species																
	LRT	Pr(Chi)	*Asclepias tuberosa*	*Chamaecrista fasciculata*	*Coreopsis lanceolata*	*Chamaecrista nictitans*	*Desmodium floridanum*	*Desmanthus illinoensis*	*Echinacea purpurea*	*Eryngium yuccifolium*	*Gaillardia pulchella*	*Helianthus angustifolius*	*Monarda fistulosa*	*Monarda punctata*	*Rudbeckia hirta*	*Ratibida pinnata*	*Symphyotrichum pilosus*	*Solidago rugosa*	*Verbena hastata*
Apis mellifera	113	<0.001	0.23 (0.17)						0.06 (0.11)	0.11 (0.09)					0.01 (0.02)				0.81 (0.44)
Bombus bimaculatus	36	<0.001	0.18 (0.20)						0.09 (0.09)		0.03 (0.04)	0.50 (0.42)	0.03 (0.06)					0.13 (0.24)	0.06 (0.04)
Bombus impatiens	174	<0.001	0.86 (0.65)	0.77 (0.67)		0.06 (0.12)			0.12 (0.07)	0.03 (0.05)	0.02 (0.03)	0.10 (0.20)				0.01 (0.03)			0.15 (0.18)
Bombus griseocollis	57	<0.001	0.46 (0.20)						0.59 (0.23)		0.23 (0.09)		0.17 (0.15)			0.03 (0.03)			0.12 (0.08)
Halictus poeyi	606	<0.001	0.54 (0.41)		0.76 (0.39)		0.17 (0.33)	1.50 (2.94)	0.59 (0.40)	0.31 (0.22)	4.55 (1.10)	0.77 (0.47)	0.10 (0.15)		0.63 (0.28)	0.85 (0.55)	0.29 (0.16)	0.25 (0.49)	0.85 (0.19)
***Lasioglossum* spp.**	59	<0.001	1.25 (1.47)	0.25 (0.49)	0.50 (0.57)				0.50 (0.57)	2.50 (1.88)	1.75 (0.49)	0.50 (0.57)	0.25 (0.49)	0.50 (0.98)	0.25 (0.49)		6.00 (4.23)	0.25 (0.49)	
***Megachile* spp.**	56	<0.001	0.40 (0.16)								0.15 (0.07)				0.01 (0.03)		0.01 (0.03)		0.04 (0.04)
Xylocopa virginica	50	<0.001	0.50 (0.57)	0.25 (0.49)				0.33 (0.57)	0.25 (0.49)		0.50 (0.57)		0.75 (0.94)	3.00 (1.39)	1.00 (1.80)		0.50 (0.57)		1.75 (0.94)

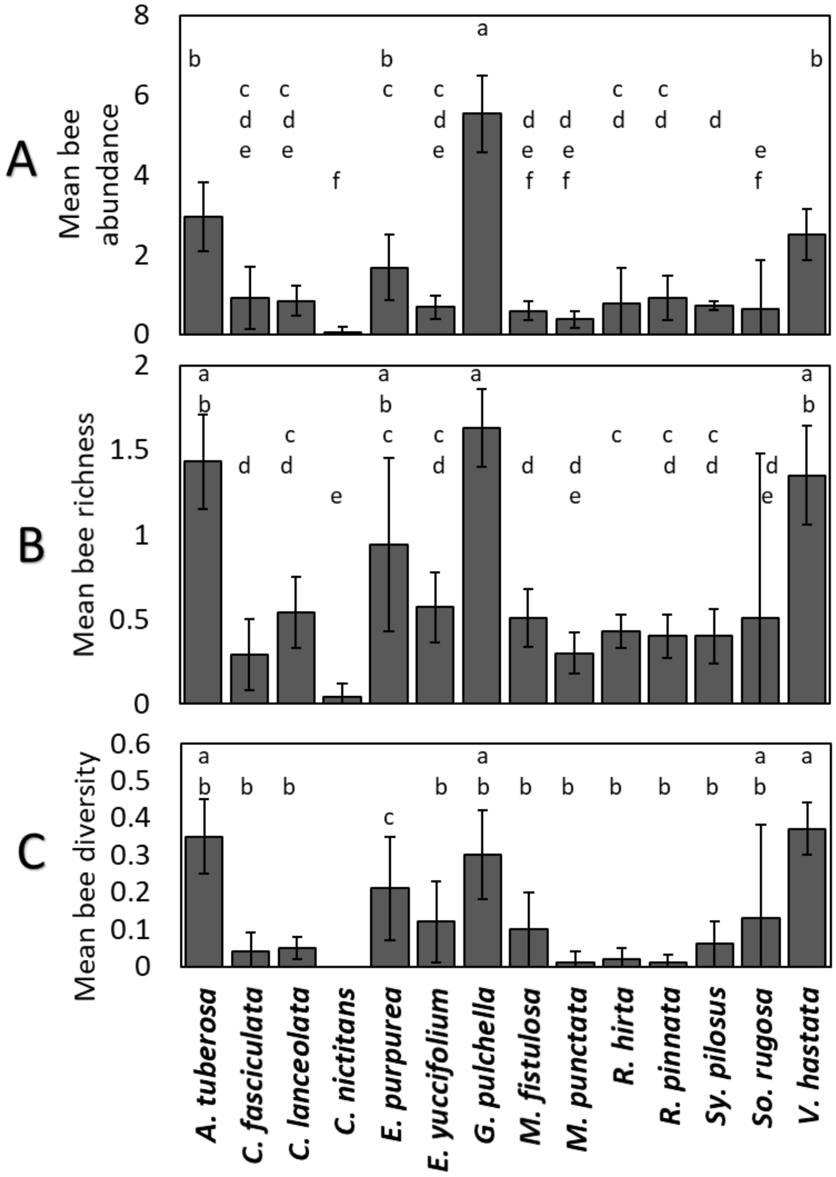


Figure 19: A) Mean bee abundance **B)** mean bee richness, and **C)** mean Shannon Wiener diversity index per replicate per sampling event, for each wildflower species during the 2019 field season. Error bars represent ±95% CI, and groups with different letters represent significant differences at p≤0.05.

Bee species within the genus *Megachile* were captured in abundances too low to be assessed at the species level, so all bees within the genus were grouped for statistical analysis. When investigating the most commonly captured bee species/genus and if they visited certain wildflowers more often than others, we observed statistical differences in the floral selections of *A. mellifera,* (LRT=113, χ^2<0.0001), *B. bimaculatus* (LRT=36, χ^2<0.0001), *B. impatiens* (LRT=174, χ^2<0.0001), *B. griseocollis* (LRT=57, χ^2<0.0001), *H poeyi* (LRT=606, χ^2<0.0001), *Lasioglossum* spp. (LRT=59, χ^2<0.0001), *Megachile* spp. (LRT=56, χ^2<0.0001), and *X. virginica* (LRT=50, χ^2<0.0001) (Table 6 and Figure 20). *Apis mellifera* visited five plant species (*A. tuberosa, E. purpurea, E. yuccifolium, R. hirta,* and *V. hastata*), and was significantly more attracted to *V. hastata* than any of the other wildflower species (P<0.008), followed by *A. tuberosa* and *E. yuccifolium*, which were not statistically different from one another (P=0.54). *Bombus bimaculatus* visited seven out of the 17 plant species (*A. tuberosa, E. purpurea, G. pulchella, H. angustifolius, M. fistulosa, So. rugosa,* and *V. hastata*), and was significantly more attracted to *H. angustifolius* than any other wildflower species (P<0.05), with no significant differences being observed among the remaining wildflower species (P>0.25 in all cases). *Bombus impatiens* visited nine of the 17 plant species and was most attracted to *A. tuberosa* and *C. fasciculata,* with *A. tuberosa* and *C. fasciculata* not being statistically different from one another (P=1.0). *Bombus griseocollis* visited six of the 17 plant species and was significantly more attracted to *A. tuberosa, E. purpurea*, *G. pulchella,* and *M. fistulosa*, than *R. pinnata* and *V. hastata* (P>0.05) which were not statistically different from one another (P=0.23) (Table 6 and Figure 20). *Halictus poeyi* visited the greatest diversity of plant species (14 of 17) and was significantly more attracted to *G. pulchella* than all other plant species (P<0.01) except for *D. illinoensis* (P=0.79). *Lasioglossum* spp. was attracted to 12 different plant species, yet few significant differences were observed between wildflower species they visited by (Table 6 and Table 20). Bees in the genus *Megachile* were attracted to 5 plant species (*A. tuberosa, G. pulchella, V. hastata, S. pilosus,* and *R. hirta*), with *A. tuberosa* being the most attractive plant species (P<0.03) and *G. pulchella, R. hirta, S. pilosum* and *V. hastata* not being statistically different from one another (P>0.07 in all cases) (Table 6 and Figure 20). Lastly, *X. virginica* was attracted to 10 of the 17 plant species, and visited the flowers of *M. punctata* statistically more often per sampling event than *E. purpurea, G. pulchella, R. hirta,* and *Sy. pilosus* (P<0.01), yet was not statistically different from *V. hastata*, *D. illinoensis*, *M. fistulosa*, *A. tuberosa*, or *C. fasciculata* (P>0.08 in all cases).

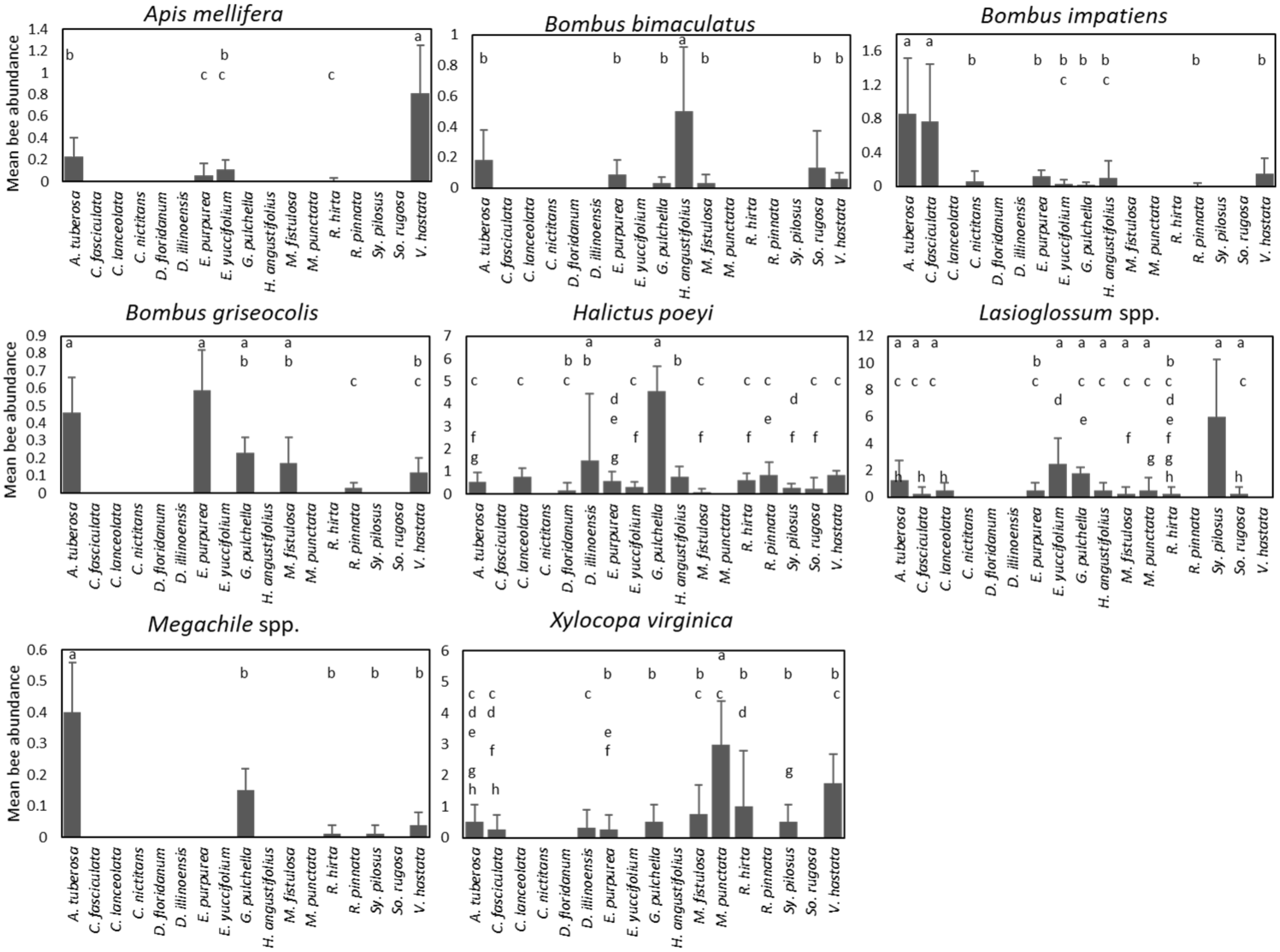


Figure 20: Mean bee abundance per replicate per sampling event for the eight most common genera/species of bees netted while visiting the planted wildflower species during the 2019 field season. Plant species with different letters represent significant differences at P≤0.05.

Discussion

Floral resources are considered a major driver for bee species richness and abundance (Roulston and Goodell 2010). Planting flowering plants, such as native wildflowers, can augment bee abundance and diversity within agricultural and urbanized landscapes (Williams et al. 2015, Abbate et al. 2019, Campbell et al. 2019, Wilson and Jamieson 2019). Our study demonstrated that not all wildflower species are equally attractive to native bees so they should be carefully selected based upon their floral traits, phenology, and the bees they attract.

In our study, *A. tuberosa, G. pulchella,* and *V. hastata* attracted the greatest abundance, richness, and diversity of bees per sampling event, which aligns well with other studies (Baker and Potter, 2018; Campbell et al., 2019; Rollings and Goulson, 2019; Stubbs et al., 1992). Flowers in the genus *Asclepias* exhibit unusual floral morphology and pollination mechanisms compared to the other wildflowers in our study (Willson et al. 1979). Within each flower, large quantities of nectar are secreted within five stigmatic chambers and the nectar is highly attractive to bees (Borders and Lee-Mäder, 2015; MacIvor et al., 2017; Struven et al., 1994). Nectar is the only floral reward associated with *Asclepias* flowers as pollen is massed into structures called pollinia which are not consumed by bees (Struven et al., 1994). Some insects visiting flowers seeking nectar rewards, but almost never extract pollinia, and have been referred to as "nectar thieves" (Willson and Bertin, 1979; Willson et al., 1979). Because of the structure and placement of pollinia within an *Asclepias* flower, larger-bodied bees such as *A. mellifera,* and bees in the genus *Bombus*, make contact with pollinia more often than smaller bodied bees, thus making them more efficient pollinators (Struven et al., 1994). This aligns well with our study as the most common insect visitors of *A. tuberosa* flowers were *A. mellifera, B. griseocollis, B. impatiens, H. poeyi,* and *M. sculpturalis.* Although *H. poeyi* was a common visitor of *A. tuberosa* it could have been a "nectar thief" due to its small size. It is generally thought that bees in the family Megachilidae are less important pollinators of *A. tuberosa* flowers, but non-native Megachile species have not been taken into account (Struven et al., 1994). *Megachile sculpturalis,* a large-bodied bee native to East Asia, could also have been a potential pollinator, but future work should investigate its ability to transfer pollinia between flowers.

Gaillardia pulchella was highly attractive to bees during our study. *G. pulchella* exhibits long bloom periods (Lazri and Barrows 1984, Campbell et al. 2019), provides season-long pollen and nectar resources (Wheeler 2017), and can attract great abundances and diversities of bees (Campbell et al. 2019). Eighty-two percent of all bee visits to *G. pulchella* in our study were by *H. poeyi. Halictus poeyi* is a primitively eusocial native bee (Zayed and Packer 2002), with colony sizes reaching to approximately 25 individuals or more (Packer and Knerer 1986). Female workers of *H. poeyi* provision their brood with pollen, and since *G. pulchella* blooms throughout the year, it could be an important pollen source for them.

Another wildflower species in our study that attracted great abundances, richness, and diversity of bees was *V. hastata*. *Verbena hastata* flowered from June-September with peak blooming in mid-July. Flowers of *V. hastata* grow in such close proximity to one another that honey bees have been observed moving from flower to flower (for their nectar sources) without flying (Lovell and Lovell 1939). The corolla-tube of an individual flower is 3-4 mm long and lined with hairs to exclude small insects from reaching the nectar (Lovell and Lovell 1939), and only large bees such as *A. mellifera* or *Bombus* spp. can reach the nectar rewards while inadvertently picking up pollen (Lovell and Lovell 1939).

Despite some wildflower species in this study attracting a great abundance, richness, and diversity of bees, several wildflower species attracted bee species captured in low abundances, or were pollen specialists, or were non-native. For this reason, the ecological importance of each plant species to the bee community should not be overlooked (Sutter et al. 2017). *Bombus fraternus* is a bumble bee species (Hatfield et al. 2014) that was captured in low abundances in our study while visiting flowers of *E. purpurea*, *M. punctata*, and *V. hastata.* It is thought that their abundances observed today reflect only 15% of their historical numbers (Hatfield et al. 2014). The planting of these wildflower species they visited could provide the floral resources needed to support their populations and should be considered in seed mixtures to promote them.

Melissodes agilis, M. comptoides, Agapostemon splendens, Augochloropsis metallica, and several *Megachile* species were also caught in extremely low abundances during the field season and were observed visiting a combination of *A. tuberosa, G. pulchella, R. pinnata, Sy. pilosus,* and *So. rugosa*, exemplifying the importance of incorporating these wildflower species that might provide floral resources for rare or pollen specialist bees. Two pollen specialists (*M. parallela* and *M. xylocopoides*) (Ascher and Pickering, 2009) were caught while visiting *R. hirta* and *V. hastata* within our wildflower plot. Both *M. parellela* and *M. xylocopoides* are pollen specialists on Asteraceae (Ascher and Pickering, 2009) and explains their visits to *R. hirta.* But pollen specialists do visit flowering plant species outside of their pollen foraging specialization to obtain nectar rewards (Ascher and Pickering, 2009), which could explain why they visited *V. hastata*, and exemplifying the importance of these two wildflower species to promote a diversity of bees. Furthermore, the floral selections by bees in the family Megachilidae could be driven by morphological differences in the bees such as tongue length (Rollings and Goulson 2019). Differences in bee-tongue length at the family, genus, or species level could help explain why some bee species (who are specialists on Asteraceae) ignored some wildflower species while targeting others within Asteraceae.

In addition to attracting rare and pollen specialist bees, planting wildflowers can also be beneficial to managed and introduced bees. *Apis mellifera* is the most economically important managed bee species because of the value of their pollination services and products it provides. *Apis mellifera* and other managed bees such as bees in the genus *Bombus* can alter their foraging preference based upon availability of pollen and upon nectar quality and quantity (Seeley 2009), and it is possible that *V. hastata* and *A. tuberosa* provided the greatest pollen and nectar rewards and were thus utilized more frequently by *A. mellifera* than other wildflower species.

Because *A. tuberosa, G. pulchella,* and *V. hastata* have staggered bloom periods, provide a diversity of floral rewards, and are highly attractive to an abundance, richness, and diversity of bees, they should be favored when developing conservation plots that provides season-long floral resources to attract the greatest abundance and diversity of bees. However, *E. purpurea, M. punctata, R. hirta, R. pinnata, Sy. pilosus,* and *So. rugosa* should not be overlooked because of the rare bee species, and pollen specialists, that they attract. The information gained from this study can aid conservation biologists, farmers, and landowners when choosing which native wildflower species to plant in order to attract the greatest abundance and diversity of bees. Although our study evaluated the attractiveness of 18 wildflower species to native bees, there are thousands of wildflowers (rare, common, and widespread species) that we know little about. Furthermore, there is a lack of knowledge of wildflowers and the non-bee pollinators they attract. Additional research should investigate the attractiveness of other wildflower species to bees and other pollinators so that we can better conserve and support these economically and ecologically important pollinators.

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Chapter 5

Assessing the pollination requirements of native wildflowers in the southeastern United States

Abstract

The diversity of plants and their animal pollinators are in decline due to human activities. It is estimated that approximately 86% of all angiosperms rely on an animal vector for pollination, yet knowledge of the pollination biology of many native wildflowers is lacking. Thirteen flowering plants native to the southeastern United States were used for this study including: *Baptisia alba, Bidens laevis, Coreopsis lanceolata, Coreopsis tinctoria, Coreopsis tripteris, Desmanthus illinoensis, Desmodium floridanum, Echinacea purpurea, Eryngium yuccifolium, Gaillardia pulchella, Monarda punctata, Verbesina alternifolia,* and *Verbesina virginiana*. We evaluated each species' degree of dependence on pollinators by performing an insect exclusion study, and surveyed floral visitors through sweep netting surveys. The mean number of seeds, and mean seed weight per flower head was significantly greater for open pollinated flowers compared to insect excluded flowers for most wildflower species. Many species of wildflowers that had significant reductions in seed counts and weights when excluded from insects also attracted a high diversity of insect visitors. These results suggest that the majority of the wildflower species we examined are dependent upon insect pollinators to optimize the number and size of seeds produced to successfully complete their life cycles.

Introduction

Human activities have drastically altered Earth's landscapes and threaten its biodiversity (Barnosky et al. 2011). It has been estimated that approximately 20% of all plant species are at risk of extinction (Willis 2017), which directly impacts the pollinators that rely on them for floral resources and nesting habitats. Approximately 86% of all angiosperm species rely on pollinators to complete their life cycles (Ollerton et al. 2011), and most declines and extinctions of flowering plant species and their pollinators are caused by anthropogenic pressures including habitat loss, agricultural intensification, diseases, pesticides, and climate change (Vanbergen and Initiative 2013, Koh et al. 2016).

Pollination is an ecosystem service that has evolved over millions of years and benefits both flowering plants and their pollinators. Plant-pollinator relationships are arguably one of the most important relationships among plant-animal interactions, and without pollinators, many pollinator-dependent plants cannot successfully set seed to complete their life cycles (Blaauw and Isaacs 2014). Pollinators visit flowers for nectar and/or pollen rewards, and when doing so, transfer pollen from one flower to another or a different part of the same flower. The transfer of pollen from one flower to another promotes genetic diversity through outcrossing and increased pollen dispersal, which can influence seed counts and weights (Blaauw and Isaacs 2014, Moreira-Hernández and Muchhala 2019).

Seed set and seed weights often are directly related to fecundity and persistence of numerous plant species and generally are enhanced by pollinator visits (Blaauw and Isaacs 2014). Often there is a direct link between the degree of pollinator dependence of a flowering plant and the number of seeds produced (Boyd 1994, Oz et al. 2009, Campbell et al. 2018b). Furthermore, seed weights are an accurate indicator of flowering plant's reliance on pollinators. Seed weights of pollinator dependent plants are reduced when pollinators are excluded from flowers (Atmowidi et al. 2007, Oz et al. 2009, Bommarco et al. 2012) and seeds with reduced weights (due to insufficient pollination) have been shown to be less fit compared to larger seeds produced by the same plant species (Banovetz and Scheiner 1994).

Within the United States, the pollination biology of rare plant species and agricultural crops has been evaluated to design effective management plans (Boyd 1994, Dute et al. 2004, Isaacs and Kirk 2010, Garratt et al. 2014, Paris and Boyd 2018). However, the pollination biology of the majority of common and widespread flowering plant species, including wildflowers, remains largely unknown (Corbet et al. 1991, Cane 2008). The widespread ecosystem services that these species provide, such as filtering of pollution, soil stabilization, nutrient cycling, and provisioning of food and water, warrant investigation into their natural history so that they can be effectively managed and protected (Havens et al. 2014).

Provision of sufficient forage in the form of pollen and nectar is a key element to support and promote pollinator habitats within human impacted landscapes (Williams et al. 2015). Recent government incentives in the United States and abroad have promoted the planting of native wildflowers to benefit pollinators, yet ecological information, including data on pollination biology such as the species of pollinators they attract and the degree to which they depend on pollinators for reproduction is lacking. This is important because plant characteristics, such as floral morphology, bloom period, and floral rewards, attract different insect pollinators that can aid in the pollination and production of viable seeds (Payne et al. 1989, Khan and Chaudory 1995, Stein et al. 2017) and because flowering plants often benefit from being pollinated by multiple species of insects (Waser et al. 1996). Understanding the pollination biology of wildflowers commonly used in plantings could assist development of conservation management plans to not only to conserve wildflower diversity, but also conserve the insect pollinators that visit wildflowers.

The primary purpose of this study was to 1) assess the pollination requirements of commonly planted wildflower species through an insect exclusion study, and to 2) document the common insect floral visitors of each wildflower species. I hypothesized that wildflower species that are dependent upon pollinators to complete their life cycles would show reduced seed set and seed weight in insect excluded flowers compared to open pollinated flowers (Atmowidi et al. 2007, Lowenstein et al. 2015, Campbell et al. 2018a). I also hypothesized that, due to the large variation in flower characteristics of native wildflowers, they would attract varying abundances and diversities of insect visitors (Campbell et al. 2019).

Methods

Study Design

Wildflower selection

I assessed the pollination requirements of 13 species of wildflowers native to the southeastern United States – *Baptisia alba, Bidens laevis, Coreopsis lanceolata, Coreopsis tinctoria, Coreopsis tripteris, Desmanthus illinoensis, Desmodium floridanum, Echinacea purpurea, Eryngium yuccifolium, Gaillardia pulchella, Monarda punctata, Verbesina alternifolia,* and *Verbesina virginica*. These 13 wildflower species are currently recommended by the USDA/NRCS to enhance native pollinator habitat, and their pollination biology is largely unknown. I performed an insect exclusion study and insect floral visitor surveys to document pollination requirements and identify floral visitors. The 13 wildflower species were assessed across three sites in 2018 and 2019, with each wildflower species occurring only within a single site and year. Eleven of the wildflower species were planted, whereas, two occurred as naturally growing populations.

Seed preparation

Of the 11 wildflower species planted, six (*C. tripteris*, *Desmanthus illinoensis*, *Desmodium floridanum, M. punctata*, *V. alternifolia*, and *V. virginiana*) were germinated from seed obtained from Roundstone Seed LLC (Upton KY). Due to differing germination requirements of each species, seeds of *C. tripteris, V. alternifolia* and *V. virginiana* were cold moist stratified by placing them at 1.6 °C for 60 days in separate 0.95 L Ziploc® bags with moist sand (Kirt 1996). Seeds of *Desmanthus illinoensis* and *Desmodium floridanum* did not require cold stratification, but needed to be mechanically scarified. This was performed by placing the seeds of each species in separate 0.95 L Ziploc® bags containing dry sand; each bag was then vigorously shaken for five minutes (Kimura and Islam 2012). *Monarda punctata* seeds require no pre-treatment prior to planting, and were planted directly into seed starter trays. Once seeds of all species were pretreated as needed, two seeds per species were planted in a single cell within a 72-cell seed starter tray in February 2019. Seedlings were exposed to 16 hours of light per day using fluorescent full spectrum (6000K) grow lights (Bubel and Nick 2018) and bottom watered when the soil surface dried to prevent fungal and bacterial growth (Bubel and Nick 2018). Once each seedling produced its first set of true leaves, the weakest of the two seedlings (if both seeds germinated) was thinned by clipping the stem at soil level (Bubel and Nick 2018).

Starting on 16 April 2019, all seedlings were placed under the shade of a tree and were hardened off over a two-week period (Stephens 2014). For this, each seedling was brought outside, and the time spent outdoors increased each consecutive day. The wildflowers were slowly moved from an increasing spectrum of full shade to full sun until they could be left outdoors for the entire day: at this point they were ready for transplanting (Stephens 2014). In addition to the six wildflower species started from seed, five wildflower species (*B. alba*, *C. lanceolata*, *Ec. purpurea*, *Er. yuccifolium*, and *G. pulchella*) were sourced from Superior Trees Inc. (Lee, Florida) as one year old mature potted plants.

Experimental plot preparation and wildflower planting

The 11 wildflower species (*B. alba, C. lanceolata, C. tripteris, Desmanthus illinoensis, Desmodium floridanum, Ec. purpurea, Er. yuccifolium, G. pulchella, M. punctata, V. alternifolia,* and *V. virginiana*) were planted at the Auburn University Bee Lab (32° 35.929'N, 85° 30.101'W) (Figure 1A) in April 2019. Two weeks prior to planting, a 10% concentration of glyphosate (Roundup®) was sprayed over a 2 × 41 m area using a backpack sprayer to reduce weed competition with the wildflowers, and then tilled two weeks later (Norcini and Aldrich 2004). On 30 April 2019, all 11 wildflower species (both started from seed and mature potted plants) were transplanted into one 1 ×3 m plot, each separated by a 1 m^2 buffer. The number of plants per species per 1 × 3 m plot varied from 4 to 9 to achieve close to one-hundred percent vegetation cover, depending upon the size at maturity (Garbuzov and Ratnieks 2014). and based upon the recommendations of Superior Trees Inc. and Roundstone Seed LLC.

Lastly, two wildflower species were observed growing naturally on Auburn University's campus and were utilized for this study. A population of *B. laevis* was growing along the edge of a pond at Auburn University's Ag Heritage Park (32° 35.710'N, 85° 29.456'W) from 24 September – 7 November 2019 (Figure 21c), and a population of *C. tinctoria* was growing within a drainage ditch outside of Auburn University Facilities Management area (32° 35.872'N, 85° 30.132'W) from 14 June – 5 July 2018 (Figure 21c). I utilized a subsection of each naturally occurring wildflower patch for our bagging and sweep netting surveys. The patch sizes for both species were of similar size to the wildflower plots planted at the Auburn University Bee Lab.

Insect Exclusion Study

To determine the self-pollination ability of each wildflower species (Kearns and Inouye 1993, McIver and Erickson 2012), I conducted a bagging experiment, whereby I completely enclosed unopened inflorescences (Insect Excluded) using a 4.5 L fine mesh (150 µm) polyester pail strainer (The Cary Company, Addison, IL) for each wildflower species. Bagging the inflorescences prevents all insects from accessing and depositing pollen on the flowers' reproductive parts, yet does not prevent the passive passage of pollen via wind through the bag (Neal and Anderson 2004). I also tagged, but did not bag, unopened inflorescences (Open Pollinated) using orange flagging tape during the bloom periods of each wildflower species (Figure 21d). Flagging, but not bagging the flowers of each wildflower species enabled insects to freely visit the flower buds once they bloomed, and allowed for transfer of pollen via insects to the floral reproductive parts. All bags and flagging tape remained on the inflorescences for the duration of the experiment.

For each wildflower species, and for each treatment (Insect Excluded and Open Pollinated flowers), once a seeds formed, it was collected, placed in an individual brown paper bag, and allowed to dry naturally (Blaauw and Isaacs 2014). When each seed head was completely dried, it was opened so that the number of mature fruits could be counted and weighed (Blaauw and Isaacs 2014). I counted all mature seeds and weighed them to the nearest hundredth of a milligram with a digital scale (VWR-124B). By allocating flowers to Insect Excluded and Open Pollinated treatments, I was able to directly compare number of seeds produced per inflorescence per treatment for each wildflower species. Furthermore, I was able to directly compare mean weights per seed per flower head for each treatment and wildflower species. Only flower heads that produced one or more seeds were included in the seed weight analysis.

Figure 21: A) Eleven wildflower species at Auburn University's Bee Lab planted in a 2 m X 41 m strip. **B)** Naturally occurring patch of *Bidens laevis* growing along the edge of a pond at Auburn University's Ag Heritage Park with exclusion bags and flagging tape deployed, **C)** Naturally occurring patch of *Coreopsis tinctoria* growing within drainage ditch outside of Auburn University Facilities Management area, and **D)** *Echinacea purpurea* planted in a 1 m X 3 m plot with insect exclusion bags and flagging tape deployed.

<u>Floral Insect Visitor Survey</u>

To determine the floral insect visitors for all 13 wildflower species, I conducted insect surveys (N=419) concurrently with the insect exclusion study over the 2018 and 2019 field seasons. During the 2018 field season, I preliminarily surveyed *C. tinctoria* (N=27)*,* whereas, in 2019 I surveyed *B. alba* (N=29), *B. laevis* (N=60), *C. lanceolata* (N=28), *C. tripteris* (N=30), *Desmanthus illinoensis* (N=14), *Desmodium floridanum* (N=12), *Ec. purpurea* (N=58), *Er. yuccifolium* (N=27), *G. pulchella* (N=35), *M. punctata* (N=29), *V. alternifolia* (N=51), and *V. virginiana* (N=19). The number of insect surveys completed for each wildflower species reflected the duration of their bloom periods. For each insect survey, the surveyor stood at each wildflower plot for 10 minutes to capture all insects observed visiting the flowers. Sweep netting surveys conducted on the two naturally occurring wildflower species were concentrated on randomly selected subplots (by dividing the subplot into three areas and using a random number generator to decide which area to sweep net) that were similar in size as the planted wildflower plots at the Auburn University Bee Lab. To maximize insect capture and to standardize sampling procedures, surveys were conducted over multiple days and sampling times (morning, midday and afternoon) (Prado et al. 2017), and when weather conditions were appropriate for sampling foraging insects – warm (>20°C) sunny days (<60% cloud cover) with ≤40% chance of precipitation (Hopwood 2008). All insects captured that could not be positively identified in the field to species and released (e.g., *Halictus poeyi* (Halictidae) and *Danaus plexippus* (Nymphalidae)) were stored in a freezer in individual 15 mL collection vials until I could identify them in the lab using stereomicroscopy. All insects were identified to the lowest possible taxonomic level (Bohart et al. 1976, McAlpine et al. 1981, Arnett Jr and Thomas 2000, Thomas et al. 2002, Buck et al. 2008, Ascher and Pickering 2009). When I observed but could not collect visiting insects, I recorded their genus.

Statistical Analyses

A Wilcoxon Rank Sum test was used to test for differences in mean seed count and mean weight per seed per flower head between Insect Excluded and Open Pollinated inflorescences for each wildflower species. Wilcoxon Rank Sum tests were used after square-root and logarithmic transformations applied to the data failed to eliminate heteroscedasticity. Species richness and Shannon-Weiner diversity indices were calculated for each wildflower species, and were averaged across all insect surveys resulting in a mean species richness per 10-minute sweep netting survey. All statistical tests were performed using Statistix 9.0 Analytical Software, Tallahassee, Florida, U.S.A.

Results

Insect Exclusion Study

A total of 1,009 Insect Excluded and 1,036 Open Pollinated flowers were evaluated during this study across all wildflower species. The total number of seed heads utilized per wildflower species varied, and was dependent upon the growth habit, numbers of flowers produced, and the duration of the bloom period. The number of flower heads utilized for the seed count analysis per wildflower species were (Insect Excluded: Open Pollinated): *B. alba* (308:359), *B. laevis* (18:26), *C. lanceolata* (114:91), *C. tinctoria* (113:102), *C. tripteris* (19:31), *Desmanthus illinoensis* (25:25), *Ec. purpurea* (24:19), *Er. yuccifolium* (56:28), *G. pulchella* (42:29), *M. punctata* (26:25), *V. alternifolia* (239:264), and *V. virginiana* (25:37). Additionally, the number of seed heads utilized for the seed weight analysis per wildflower species differed from the number of flower heads utilized for the seed count analysis because not all flower heads produced seeds. The number of flower heads utilized for the seed weight analysis per wildflower species were (Insect Excluded: Open Pollinated): *B. alba* (8:19)*, B. laevis* (15:26)*, C. lanceolata* (23:85)*, C. tinctoria* (113:102)*, C. tripteris* (19:30)*, D. illinoensis* (9:25)*, E. purpurea* (24:19)*, E. yuccifolium* (53:28)*, G. pulchella* (38:28)*, M. punctata* (25:25)*, V. alternifolia* (213:263)*,* and *V. virginiana* (23:37). *Desmodium floridanum* was excluded from the study due to inadequate production of seeds by flower heads within both treatments.

Treatments had a significant effect on the mean number seeds produced per flower head in *B. laevis* (z=4.5, P<0.0001), *C. lanceolata (*z=11.9, P<0.0001)*, C. tinctoria* (z=4.8, P<0.0001)*,* *Desmanthus illinoensis* (z=6.2, P<0.0001), *Ec. purpurea* (z=3.2, P=0.001), *M. punctata* (z=4.8, P<0.0001), *V. alternifolia* (z=7.1, P<0.0001), and *V. virginiana* (z=3.5, P<0.0001), but did not have a significant effect on *B. alba* (z=1.8, P=0.07), *C. tripteris* (z=0.35, P=0.73), *Er. yuccifolium* (z=1.7, P=0.09), and *G. pulchella* (z=1.9, P=0.053) (Figure 22). Additionally, mean weights per seed per flower head in Insect Excluded treatments were significantly reduced compared Open Pollinated treatments for in *B. laevis* (z=4.9, P<0.0001), *C. tinctoria* (z=10.8, P<0.0001), *C. tripteris* (z=4.8, P<0.0001), *Desmanthus illinoensis* (z=2.5, P=0.01), *Ec. purpurea* (z=4.1, P<0.0001), *Er. yuccifolium* (z=5.8, P<0.0001), *G. pulchella* (z=4.5, P<0.0001), *V. alternifolia* (z=11.7, P<0.0001), and *V. virginiana* (z=4.9, P<0.0001), but not in *B. alba* (z=0.66, P=0.51), *C. lanceolata* (z=0.95, P=0.34), and *M. puntcata* (z=0.33, P=0.74) (Figure 23).

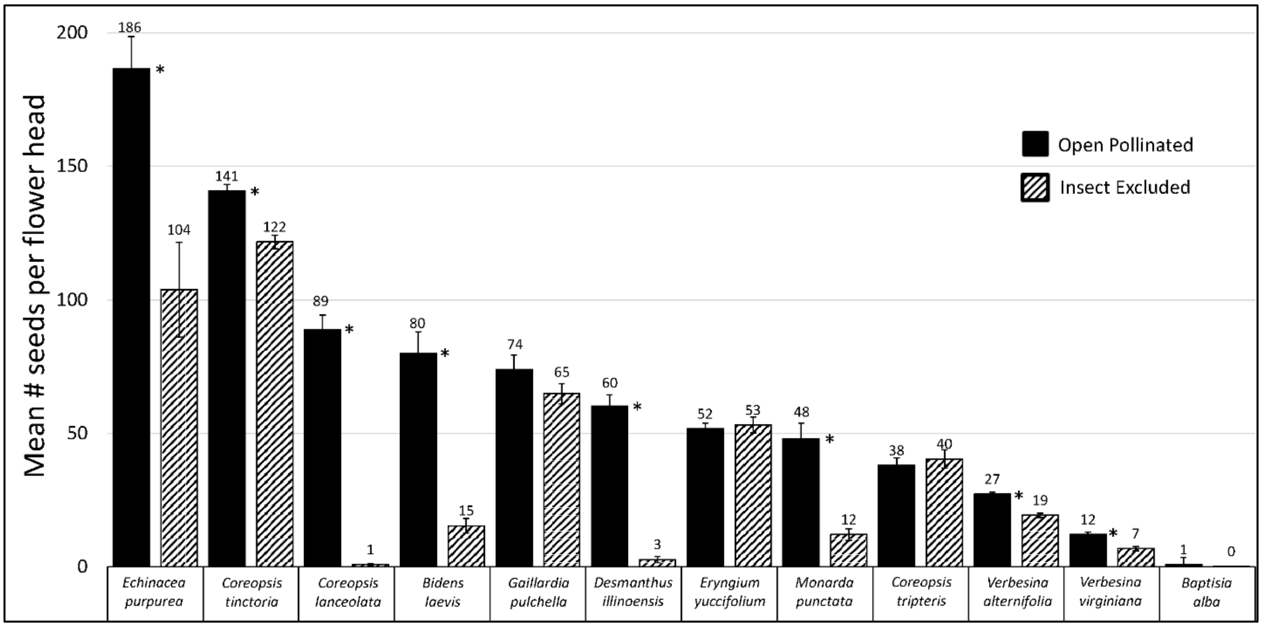

Figure 22: Mean number of seeds (above each plant species and for each treatment) ± SE per flower head for Insect Excluded and Open Pollinated treatments for each wildflower species. Wildflower species with an asterisk represent significant differences in mean seed counts per flower between treatments (P<0.05).

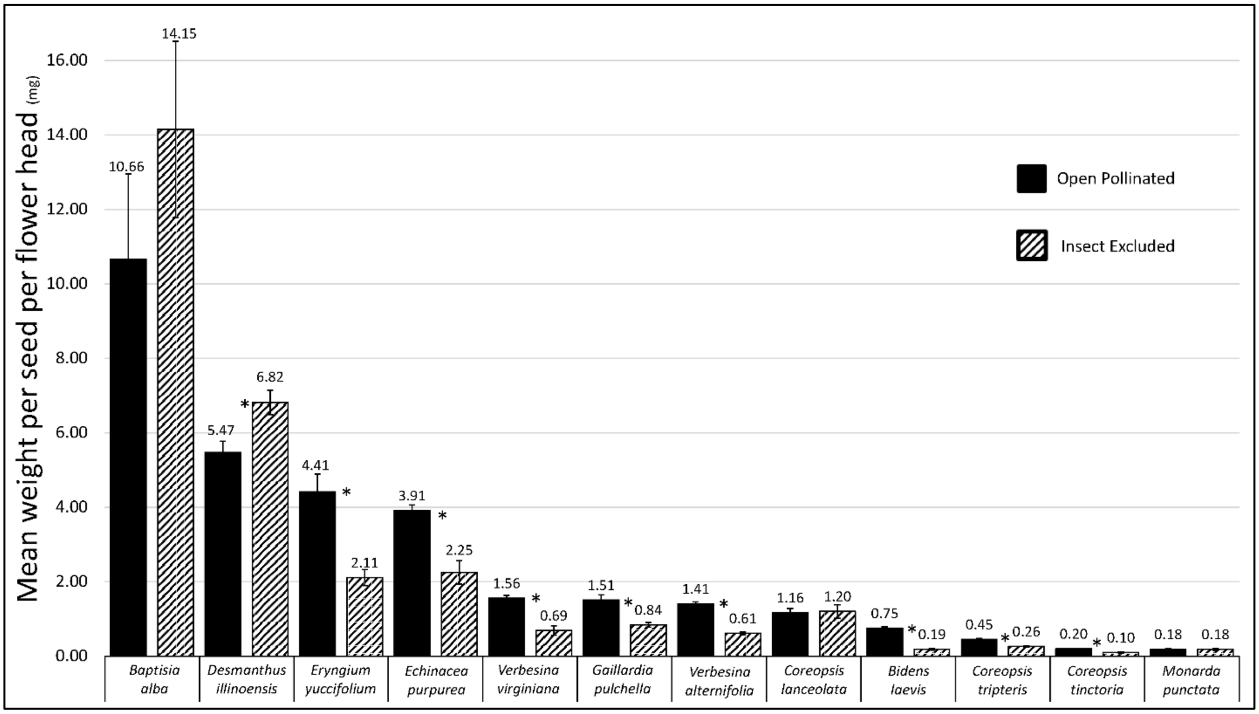

Figure 23: Mean seed weight (above each species) in mg ± SE per flower head for Insect Excluded and Open Pollinated flowers for each wildflower species. Wildflower species followed by asterisk represent significant differences in mean seed weight per flower head between treatments (P<0.05).

Floral Visitor Identification Survey

A total of 1,922 insects, representing 81 species/genera in 23 families within 4 orders, were observed visiting the 13 wildflower species (Appendix Table 1). The majority of floral visits (1,336 or 70%) were by bees, followed by wasps (221 or 11.5%), flies (154 or 8%), butterflies and moths (108 or 5.6%), and beetles (103 or 5.4%). The three wildflower species that attracted the greatest richness of insect species per 10-minure sweep netting survey were *C. tinctoria, G. pulchella,* and *B. laevis*, with *V. virginiana, D. illinoensis,* and *B. alba* attracting the lowest richness of insects per 10-minute sweep netting survey (Table 7). Additionally, the three wildflower species that contained the greatest mean Shannon-Weiner diversity index per 10-minute sweep netting survey were *B. laevis, G. pulchella,* and *C. tinctoria,* with *B. alba, D. floridanum,* and *D. illinoensis* attracting the lowest mean Shannon-Weiner diversity index per 10-minute sweep netting survey (Table 7).

Table 7: Mean insect species richness (S) and mean Shannon Weiner Diversity Index (H')per 10-minute sweep netting event (N) attracted to the 13 wildflower species during the 2018 and 2019 field seasons.

Family	**Wildflower Species**	**S(±SE)**	**N**	**H'(±SE)**	**N**
Apiaceae	*Eryngium yuccifolium*	1.6(0.17)	28	0.28(0.07)	28
Asteraceae	*Bidens laevis*	1.95(0.20)	42	0.42(0.07)	42
	Coreopsis lanceolata	1.55(0.18)	27	0.32(0.08)	27
	Coreopsis tinctoria	2.26(0.37)	19	0.34(0.08)	19
	Coreopsis tripteris	1.16(0.12)	30	0.15(0.05)	30
	Echinacea purpurea	1.54(0.16)	59	0.23(0.05)	59
	Gaillardia pulchella	1.96(0.22)	28	0.42(0.08)	28
	Verbesina alternifolia	1.23(0.11)	51	0.18(0.05)	51
	Verbesina virginiana	0.8(0.14)	20	0.10(0.06)	20
Fabaceae	*Baptisia alba*	0.03(0.04)	29	0.00(0.00)	29
	Desmanthus illinoensis	0.50(0.14)	14	0.00(0.00)	14
	Desmodium floridanum	0.91(0.23)	12	0.09(0.09)	12
Lamiaceae	*Monarda punctata*	1.34(0.23)	29	0.21(0.09)	29

Discussion

Anthropogenic disturbances such agricultural intensification, diseases, pesticides, and habitat loss have led to decreases in the populations of angiosperms and their pollinators (Ollerton et al. 2011, Koh et al. 2016). Evaluating the pollination biology of common and widespread flowering plant species is an important step in conserving their populations, and those of the pollinators that depend upon them. Recent government incentives have promoted planting native wildflowers to benefit pollinators, yet data on their pollination biology, including their reliance upon pollinators and the species of pollinators they attract, is lacking. Through an insect exclusion experiment and sweep netting surveys, several wildflower species examined showed decreased number of seeds produced per flower head, and reduced weights per seed per flower head, when insects were excluded. The differences in seed characteristics between those inflorescences excluded from insects or not appeared to be correlated to richness and diversity of insect pollinators they attracted, and potentially their dependence upon them.

Most wildflower species in this study (*B. laevis*, *C. lanceolata, C. tinctoria,* *Desmanthus illinoensis, Ec. purpurea, M. punctata, V. alternifolia,* and *V. virginica*) had fewer numbers of seeds produced when insect pollinators were excluded. The reduction in the number of seeds produced has been viewed as being directly related to the fecundity of the species (Blaauw and Isaacs 2014). Furthermore, studies have shown a strong positive correlation between the number of floral visits by insects (pollination intensity) and the number of seeds developed per flower head (Snow 1982, Steffan-Dewenter et al. 2001, Bommarco et al. 2012). For this reason, the differences in seed set between pollinator excluded and open pollinated flowers for most of the wildflower species evaluated in this study, show high dependence upon pollinators to set seed. Conversely, of the 13 wildflower species evaluated in this study, four wildflower species (*B. alba, C. tripteris, Er. yuccifolium,* and *G. pulchella*) showed no significant differences in the mean number of seeds produced per flower head between insect excluded and open pollinated flowers. Interestingly, the mean weights per seed per flower head for *C. tripteris, Er. yuccifolium,* and *G. pulchella* were statistically greater in open pollinated flowers compared to insect excluded flowers, , but there were no statistical differences in mean weights per seed per flower head observed *B. alba*. It is possible that smaller seeds produced by insect excluded flowers are non-viable compared to seeds produced by open pollinated flowers, yet little information exists on the relationship between seed size/weight and seed viability (Ne'eman et al. 2010). There is a clear trade-off between the number of seeds produced by an individual flower head and the size of the seed. Lower seed production per unit measured has been associated with greater extinction rates across multiple studies (Meagher et al. 1978, Banks 1980, Mehrhoff 1983, Walck et al. 2002, Miller et al. 2004, Dorsey and Wilson 2011, Combs et al. 2013), and it has been hypothesized that larger seeds have a better chance of survival when compared to smaller seeds (Mojonnier 1998). Banovetz and Scheiner (1994) found that larger seeds tend to have increased viability, germination and emergence rates, and for some plant species that produce smaller seeds (smaller weights) tended to have fewer individuals survive the seedling stage (Dorsey and Wilson 2011). Further work should investigate the viability of seeds produced by insect excluded flowers and open pollinated flowers.

Sweep netting surveys showed that many of the wildflowers with significant reductions in seed set (*B. laevis*, *C. lanceolata, C. tinctoria, Desmanthus illinoensis, Ec. purpurea, M. punctata, V. alternifolia,* and *V. virginica*) or seed weights (*B. laevis, C. tinctoria, C. tripteris, Desmanthus illinoensis, Ec. purpurea, Er. yuccifolium*, *G. pulchella*, *V. alternifolia,* and *V. virginiana*) between pollinator excluded and open pollinated flowers attracted a great richness and diversity of insect pollinators. Due to floral morphology, bloom period, and floral rewards, different wildflower species attract varying degrees of insect taxa that aid in the pollination and production of viable seeds (Payne et al. 1989, Khan and Chaudory 1995, Stein et al. 2017). Generally, when flowering plants are exposed to a greater abundance and a greater diversity of insect pollinators, they produce greater numbers of seeds, and produce seeds of larger size and weights (Jennersten 1988, Atmowidi et al. 2007). For this reason, our study demonstrates the importance of insect pollinators to many wildflower species in the southeastern United States, and how they may increase their reproductive success.

Hymenopterans were the most common insects observed visiting the wildflower species in this study. The most common insect was *H. poeyi*, which was observed visiting 10 of the 13 wildflower species, but most often *G. pulchella* and *C. tinctoria. Halictus poeyi* is well known as a generalist pollinator and has been observed visiting many species of flowering plants to collect pollen across multiple studies (Dunn et al. 1998, Cusser et al. 2018, Campbell et al. 2019). *Apis mellifera* was observed visiting 12 of the 13 wildflower species and was highly attracted to *E. purpurea* and *B. laevis. Echinacea purpurea* produces large quantities of nectar, and bloomed from May-October, thus making it a highly attractive and a largely accessible plant to *A. mellifera* (Wist and Davis 2006). *Bidens laevis* was also highly attractive to *A. mellifera*, which also provides nectar and pollen

(Torres and Galetto 2002). *Bidens laevis* is a late season bloomer (September-November), and could have been targeted by *A. mellifera* when other nectar sources were scarce, and when fewer insect pollinators were active. This is equally true for *B. impatiens*, which was observed visiting only five of the 13 wildflower species, the most common of which was *B. laevis*.

Of the non-hymenopteran insect visitors, beetles in the genus *Mordella* (Mordellidae) were the most commonly observed coleopteran visiting the wildflowers in this study. Beetles in the genus *Mordella* were observed primarily visiting the flowers of *G. pulchella* and *C. lanceolata*. Adult beetles in the family Mordellidae are considered pollinators because pollen grains have been shown to adhere to their body hairs, and they will readily visit flowers to feed on pollen (Liljeblad 1945, Ford and Jackman 1996, Bao et al. 2019). *Gaillardia pulchella* and *C. lanceolata* are both pollen and nectar sources for insects that bloom for long periods of the year (May-August), and mordellid beetles could have been seeking out these plants for their rewards. Of the dipterans, the most common insect captured visiting the wildflower species was *Toxomerus* sp., and it was most commonly collected on *C. tinctoria*. Much like *C. lanceolata*, *C. tinctoria* also provides a pollen and nectar source and blooms during the summer (June-July) when other pollen and nectar sources might be scarce. Syrphids in the genus *Toxomerus* have been documented to be generalist pollinators, feeding on the pollen of multiple flowering plant species (Fleischmann 2016). Lastly, the most common lepidopteran caught while visiting the wildflower species was *Hylephila phyleus*, and was primarily caught visiting the flowers of *E. purpurea.* Adults of *Hylephila phyleus* are known to feed on the nectar of various plants, and due to the large production of nectar in *E. purpurea*, it was most likely targeting this plant for nectar (Clements 2012).

Although I documented the pollination requirements of 13 native wildflower species and their 81 potential pollinators native to the southeastern United States, the pollination requirements for thousands of native wildflower species still remains largely unknown. The provision of floral resources to native pollinators is important to supporting their communities, and for wildflowers, being visited by an abundance and diversity of pollinators is equally important to its persistence and spread throughout landscapes (Blaauw and Isaacs 2014). This study serves as a foundation for future studies, which should explore the pollination requirements of other wildflower species to gain a better understanding how to conserve these ecologically important plants along with their pollinators. Furthermore, the knowledge gained from this study should be utilized in future conservation management plans aimed at conserving a diversity of ecologically important wildflowers and their insect pollinators.

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Chapter 6

Dissertation Conclusions

My dissertation investigated the contribution of wild and managed insects to the pollination of two kiwifruit (*Actinidia chinensis* var. *chineisis*) cultivars and several common and widespread native wildflowers grown within the southeastern United States.

Evaluation of the pollination biology of kiwifruit cultivars in the southeastern United States has been lacking. Due to the lack in knowledge on the pollination requirements of kiwifruit cultivars grown within the southeastern United States, producers implement a mosaic of pollination methods including wind pollination, insect pollination with the use of wild and managed bees, and artificial pollination to achieve high fruit yields (Brantley et al. 2019). My results showed artificially pollinated kiwifruit flowers set significantly greater numbers of fruit per flower when compared with wind and insect pollination. Furthermore, my results suggest that wild and managed bees including *A. mellifera* and *B. impatiens* contribute minimally to the pollination of kiwifruit. For this reason, producers should focus less on the contribution of wild insects, or the utilization of managed bees for kiwifruit pollination, while focusing more on artificially pollinating their crops.

Due to the recent declines in insect populations worldwide (Hallmann et al. 2017, Leather 2018, Lister and Garcia 2018, Janzen and Hallwachs 2019, Sánchez-Bayo and Wyckhuys 2019), recent government incentives within the United States have prompted the planting of native wildflowers to benefit pollinators, yet specific information on the pollination biology of these species, such as the attractiveness to pollinators and the degree to which they depend on pollinators to produce seeds, has been lacking. My results suggest that not all wildflower species are equally attractive to native bees, and even wildflower species that attract low abundances of bees could still be ecologically important and should not be overlooked. Furthermore, my results show that many wildflower species that grow within the southeastern United States depend on pollinators produce seeds.

In summary, my dissertation highlights the importance of artificial pollination over other methods of pollination in commercial kiwifruit operations within the southeastern United States. It also highlights the importance of planting specific wildflowers to attract a diversity of pollinators, and their reliance on pollinators to successfully complete their life cycles. The information gained from my dissertation will help kiwifruit producers implement science-based pollination management plans, and will aid restoration ecologists in their decision making process when determining which wildflower species to include in their restoration plans. Finally, my dissertation also highlights that we have much more to learn, and much more to do to support and conserve the insect pollinators we so greatly depend on.

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Appendix 1 – Chapter 2 – Supplementary material

Table 1. Total number of pollen taxa quantified from the corbicula of honey bee (*Apis mellifera*) and bumble bee (*Bombus impatiens*) over an 8-day sampling period. Numerical values are expressed as mean percentages of pollen grains carried within the corbicula of each species per day.

Pollen Taxa	**Common Name**	**% Pollen *Apis mellifera***	**% Pollen *Bombus impatiens***
Actinidia chinensis	Kiwifruit	21.28	66.59
Asteraceae-Lactuceae	Dandelion	1.36	0.42
Asteraceae-long spines	Sunflower	0	0.01
Asteraceae-short spines	Ragweed	0.49	0.56
Brassica	Cabbage/mustard	12.11	0.04
Campanula	Bellflower	0.34	0.20
Carya	Hickory	0.05	0.03
Diospyros	Persimmon	0.07	0.47
Erodium	Heron's Bill	0	0.03
Ligustrum	Privet	0.00	0.08
Liriodendron	Tulip tree	1.81	1.39
Lonicera	Honeysuckle	0.01	0.45
Magnolia	Magnolia	0.96	0.58
Nyssa	Tupelo	3.42	7.21
Pinus	Pine	0	0.05
Plantago	Plantain	0.01	0
Poaceae	Grasses	0.02	0.04
Prunus	Plum, Cherry, Peach	0	0.06
Rhus	Sumac	15.65	8.48
Rosaceae	Rose	12.58	7.06
Rumex	Sorrel	0	0.01
Salix	Willow	0	1.63
Salvia	Sage	0.01	0
Trifolium pratense	Red clover	8.11	0.93
Trifolium/Melilotus	Clovers	21.72	3.54
Unknown #2		0.01	0.16
Total		**100.00**	**100.00**

Appendix 2 - Chapter 5 - Supplementary material

Table 1: Insect taxa collected through 10-minute sweep netting surveys during the 2018 and 2019 field seasons on thirteen species of wildflowers.

Order	**Family**	**Genus/Species**	***Baptisia alba***	***Bidens laevis***	***Coreopsis lanceolata***	***Coreopsis tinctoria***	***Coreopsis tripsipteris***	***Desmanthus illinoensis***	***Desmodium floridanum***	***Echinacea purpurea***	***Eryngium yuccifolium***	***Gaillardia pulchella***	***Monarda punctata***	***Verbesina alternifolia***	***Verbesina virginica***	**Total**
Coleoptera	Cantharidae	*Chauliognathus* sp.													1	1
	Chrysomelidae	*Diabrotica* sp.											1	1		2
	Mordellidae	*Mordella* sp.			17						12	56			5	90
	Scarabaeidae	*Euphoria sepulcralis*													1	1
		Trigonopeltastes delta								4	5					9
Diptera	Bibionidae	*Plecia nearctica*													7	7
	Calliphoridae	*Phaenicia* sp.		1						1						2
	Conopidae	*Physocephala* sp.										1		1		2
	Syrphidae	*Allograpta obliqua*		1												1
		Dioprosopa clavata						2								2
		Eristalis dimidata		25												25
		Eristalis sp.		5		9										14
		Eristalis tenax														0
		Eristalis transversa		15												15
		Eupeodes sp.		5												5
		Helophilus fasciatus		9												9
		Ocyptamus fuscipennis											1			1

Order	**Family**	**Genus/Species**	***Baptisia alba***	***Bidens laevis***	***Coreopsis lanceolata***	***Coreopsis tinctoria***	***Coreopsis tripsipteris***	***Desmanthus illinoensis***	***Desmodium floridanum***	***Echinacea purpurea***	***Eryngium yuccifolium***	***Gaillardia pulchella***	***Monarda punctata***	***Verbesina alternifolia***	***Verbesina virginica***	**Total**
		Palpada furcata		1							1					2
		Syritta flaviventris									1	1				2
		Toxomerus sp.		2	1	39				3						45
		Unknown sp.			10					1		1			1	13
	Tachinidae	*Archytas* sp.												1		1
		Prosenoides sp.				1	1									2
	Tephritidae	*Tephritis* sp.			5											5
	Tipulidae						1									1
Hymenoptera	Andrenidae	*Andrena accepta*		1												1
	Apidae	*Apis mellifera*	3	94	4	1	17		4	193	10	17	17	41	8	409
		Bombus bimaculatus	1													1
		Bombus impatiens		124	1	7				1				1		134
		Bombus sp.								1						1
		Ceratina floridana					1									1
		Melissodes bimaculatus								1						1
		Melissodes communis												1		1
		Melissodes comptoides										1				1
		Xylocopa virginica		8	1		1	1	1	3		1	16	4		36
	Halictidae	*Augochlora metallica*		3												3
		Augochlora pura					1									1
		Halictus parallelus		1												1

Order	Family	Genus/Species	*Baptisia alba*	*Bidens laevis*	*Coreopsis lanceolata*	*Coreopsis tinctoria*	*Coreopsis tripsipteris*	*Desmanthus illinoensis*	*Desmodium floridanum*	*Echinacea purpurea*	*Eryngium yuccifolium*	*Gaillardia pulchella*	*Monarda punctata*	*Verbesina alternifolia*	*Verbesina virginica*	Total
		Halictus poeyi		5	40	270	41	1	1	22		245		34	19	678
		Lasioglossum spp.		1	1	5	3	18	3	7	7	10	5			60
	Megachilidae	*Megachile integra*		1												1
		Megachile sp.								1		4				5
		Osmia georgica			1											1
	Chalcididae	*Chalcididae*							1							1
	Crabronidae	*Bicyrtes* sp.									1					1
		Cerceris bicornuta									3		6			9
		Philanthus sp.				1					1					2
	Scoliidae	*Scolia bicincta*											3			3
		Scolia dubia												19		19
		Scolia nobilitata				7	5			6	75			26	3	122
	Sphecidae	*Ammophila fernaldi*									1					1
		Ammophila procera					1				1		2	4		8
		Ammophila sp.							1							1
		Eremnophila aureonotata		1									7	2		10
		Sphex dorsalis											5			5
		Sphex flavovestitus							1				2			3
		Sphex ichneumoneus									3		6	4		13
		Sphex pensylvanicus											3			3
	Vespidae	*Eumenes fraternus*		1											1	2
		Euodynerus crypticus											1			1
		Euodynerus hidalgo				1										1

Order	Family	Genus/Species	***Baptisia alba***	***Bidens laevis***	***Coreopsis lanceolata***	***Coreopsis tinctoria***	***Coreopsis tripsipteris***	***Desmanthus illinoensis***	***Desmodium floridanum***	***Echinacea purpurea***	***Eryngium yuccifolium***	***Gaillardia pulchella***	***Monarda punctata***	***Verbesina alternifolia***	***Verbesina virginica***	**Total**
		Monobia quadridens											4			4
		Pachodynerus erynnis			1											1
		Polistes carolina							1							1
		Pseudodynerus quadrisectus											3			3
		Vespula germanica		1												1
		Vespula squamosa		1												1
		Zethus spinipes											1	2	2	5
Lepidoptera	Attevidae	*Atteva aurea*								1						1
	Hesperiidae	*Atalopedes campestris*								2						2
		Copaeodes minima					1			2	1			1		5
		Hylephila phyleus		1	1	1				44		7		1		55
		Lerodea eufala								1						1
		Panoquina ocola								1						1
		Pyrgus albescens								3						3
		Pyrgus sp.								1	2					3
		Unknown sp.		1												1
	Nymphalidae	*Danaus plexippus*		18												18
		Junonia coenia					1			5		1		3		10
		Phycoides tharos				2										2
		Vanessa cardui								3		1				4

Order	**Family**	**Genus/Species**	***Baptisia alba***	***Bidens laevis***	***Coreopsis lanceolata***	***Coreopsis tinctoria***	***Coreopsis tripsipteris***	***Desmanthus illinoensis***	***Desmodium floridanum***	***Echinacea purpurea***	***Eryngium yuccifolium***	***Gaillardia pulchella***	***Monarda punctata***	***Verbesina alternifolia***	***Verbesina virginica***	**Total**
		Vanessa virginiensis				1				1						2
																1922