POLLINATOR RESPONSE TO ANNUAL FORB PLANTINGS AND SELF-POLLINATING

DRY BEAN CROPS IN AGROECOSYSTEMS

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota

State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Intensive agricultural systems have had several ecological effects on the surrounding ecosystem, including contributing to widespread pollinator declines. In order to help supplement bee communities and potentially improve crop production, we set out to study annual forb plantings adjacent to dry bean crops to determine the pollinator response to both plantings and any potential effects on dry bean yield. We found that annual forb plantings provided continual floral resources throughout the sampling period, which can support bees and their pollination services in agroecosystems. We also found cross-pollination had no effect on dry bean yield in Carrington and a negative effect in Hettinger, which could be due to methodological issues. While we did not observe a yield increase in dry bean production, the addition of annual forb plantings in agroecosystems could help support the local bee community, and potentially encourage pollination services in other crops that do benefit from insect pollination.

ACKNOWLEDGMENTS

DEDICATION

I would like to dedicate this dissertation to those who supported my most during my project:

Zane Ricks, my husband, for love and support always;

Clyde and Denise Adams, my parents, for their unconditional support and enthusiasm; and

Izzy and Charlie.

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CHAPTER 1: EVALUATING ANNUAL FORB PLANTINGS FOR POLLINATORS IN

AGROECOSYSTEMS

Introduction

Conversion of grasslands and expansion of intensive agricultural has negatively affected

ecosystem services and functions (Allan et al., 2015 and Mooney et al., 2009). For example, total

cultivated cropland has transitioned heavily to corn (*Zea mays*) due to the demand for ethanol

production (Searchinger et al., 2008), which has removed or degraded crucial habitat for many

important bee communities (Steffan-Dewenter and Westpahl, 2008; Connelly et al., 2015 and

Otto et al., 2016). These insect pollinators fulfill necessary roles that are essential to functioning

ecosystems, especially in agroecosystems where pollination services are currently suffering the

most (Kevan and Viana, 2003). This issue has become a conservation crisis, and is why there is

such a need for proactive conservation approaches for bees such as conservation plantings within

agroecosystems (Brandt et al., 2017).

Bee communities are among the groups experiencing the most detrimental effects of a

shift towards more intensive agricultural practices (Potts et al., 2010 and Weiner et al., 2014).

The primary drivers of bee declines include habitat loss and fragmentation, increased agricultural

chemicals, and an increase in competition due to fewer floral resources available (Potts et al.

2010). These losses are of major concern because pollination services play a large role in global

food security with roughly 75% of the world's food crops being dependent on pollinators (Klein

et al., 2007). Even some of the remaining 25% of food crops, while not being dependent on

insect pollination, have shown increases in the quality and quantity of yield with additional

insect pollination (Bartomeus et al., 2014; Klatt et al., 2014; and Milfont et al., 2013). The

introduction of non-crop floral resources to cropland and surrounding agroecosystems can

encourage and support crop pollination by increasing the local abundance of insect pollinators (Blaauw and Isaacs 2014; Feltham et al., 2015; Sutter et al., 2017; and Venturini et al., 2017). Thus, pollination services in agroecosystems should be encouraged to benefit both bee communities and crop production (Williams et al., 2015; Orford et al., 2016; and Castle et al., 2019).

Bees need areas of floral resources to support and encourage their services, especially in intensive agroecosystems where non-crop floral resources are becoming scarce (Sutter et al., 2017 and Venturini et al., 2017). Supplemental bee-focused forb plantings can support the local bee community by providing a variety of floral resources (Venturini et al., 2017). Ideally, supplemental forb plantings should include multiple different floral morphologies to attract a diverse bee community with a variety of traits (i.e., flower anatomy related to tongue length), while also including varied phenologies to provide temporally diverse and sustained resources (Williams et al., 2015). For example, honey bees (*Apis mellifera*) and bumblebees (*Bombus spp.*), both social genera in the family Apidae, often have similar responses to the same type of floral resources while solitary, native bees often have different responses and potentially different preferences (Mallinger et al., 2019 and Bendel et al., 2019). The inclusion of complementary floral resources, both morphologically and phenologically, can support the bee community and their services with a variety of options when other non-crop floral resources are limited (Williams et al., 2015; Venturini et al., 2017).

Annual forb plantings may provide consistent and diverse floral resources necessary to help support bees in agriculturally-dominated landscapes (Venturini et al., 2017). There are multiple benefits to using annual forb plantings as opposed to perennial plantings within agroecosystems such as more control year to year and faster floral expression (Rundlöf et al.,

2018 and Mallinger et al., 2019). Annual forb plantings only flower for one season, so relocation from year to year depending on the land allocation or crop rotation is easier for producers (Mallinger et al., 2019). Additionally, annual forb plantings bloom quickly in a season which can elicit a quick bee response (Carreck and Williams, 2002). Moreover, the beginning of the growing season can be a temporal food desert in many agricultural landscapes so early blooming floral resources are critical to support the bee community, especially bumblebees (Westpahl et al., 2009; Williams et al., 2015; and Rundlöf et al., 2018).

There is a need to support bees within agroecosystems by increasing floral resources on the landscape (Williams et al., 2015). An over-arching goal of our study is to add supplemental floral resources in the form of an annual forb planting to promote bee community diversity and abundance in agroecosystems as well as providing important information on best management practices for supporting these conservation actions. We planted annual forb plantings across two agriculturally-dominated landscapes in North Dakota to support bees in a low-resource area while also potentially encouraging their services to the surrounding crops. Our specific objectives for this study are to (1) quantify the floral expression and abundance of the planted seed mixture across sites and (2) quantify bee visitations within the annual forb planting by creating plant-pollinator interaction networks. We expect to see differences in species abundance across fields due to localized environmental differences. Additionally, we expect to observe variable plant-pollinator interactions across fields due to the predicted differences in floral expressions, and therefore, bee response.

Methods

Site Description

We conducted research at the Carrington Research Extension Center (CREC) and Hettinger Research Extension Center (HREC) in North Dakota. Carrington is in the eastern part of the state, in Foster county, where cropland is the most common land use (USA-NASS, 2019). In 2017, roughly 83%, or about 138,800 hectares, of land in Foster county consisted of cropland and mainly was planted in soybeans, corn, and wheat (USDA-NASS, 2019). Cropping management in Carrington is more traditional, with heavy tillage and more intensively managed cropland. The 30-year average temperatures for sampling months (July - August) range from 18°C - 21°C (NDSU, 2015). The 30-year average total rainfall during the sampling months in Carrington ranges from 5.8 - 9.5 cm (NDSU, 2015). The average temperatures and rainfall during the 2019 and 2020 field season for Carrington fall mostly within these 30-year averages, with an exception for rainfall in 2020 (Table 1.1).

Hettinger is in southwestern corner of North Dakota in Adams county. Adams county has a variety of land uses with agricultural land being the most common (USDA-NASS, 2019). In 2017, roughly 61%, or around 157,000 hectares, of land in Adams county was cropland consisting mainly of wheat, hay for forage, and corn (USDA-NASS, 2019). Management of the cropland in Hettinger includes less intensive methods such as no-tillage. The 30-year average temperatures for sampling months (July - August) in Hettinger range from 17.5°C - 21°C, (NDSU, 2015). The 30-year average rainfall during the sampling months in Hettinger ranges from 4.9 - 8.1 cm (NDSU, 2015). The average temperatures and rainfall during the 2019 and 2020 field season for Hettinger fall mostly within these 30-year averages, with an exception for rainfall in 2020 (Table 1.1).

Experimental Design

Each location had four experimental field sites for 2019 and 2020, totaling eight sites for both years. Due to annual cropping rotations, the 2020 field sites in Carrington were in different locations, while the field sites in Hettinger were at the same locations in 2019 and 2020. Each field site consisted of two main components: an annual forb planting and an edible dry bean planting (Figure 1.1). We seeded the annual forb plantings directly adjacent to dry bean crops, but for this chapter we will only focus on the annual forb plantings and bee response. The forb planting measured 60 m wide by 40 m long totaling 2400 m^2. We planted each field site at least 100 m away from any other field site. The planting equipment and timing of the forb planting varied by location but both followed general agronomic practices for the area (Table 1.1). Multiple variables such as precipitation, soil moisture, and air temperature determined when seeding took place in each location.

Seed Mixture

We developed a seed mixture composed of 18 different annual forb and annual cover crop species (Table 1.2). We included some commonly planted cover crop species due to their inexpensiveness, availability to farmers, and fast floral expression (Mallinger et al., 2019). We designed this mixture to maximize varied floral traits such as flower color, phenology, and flower morphology to provide resources for many different bee types. In 2020, we removed millet (*Panicum miliaceum*) from the seed mixture to reduce any potential establishment outside of the intended planting area.
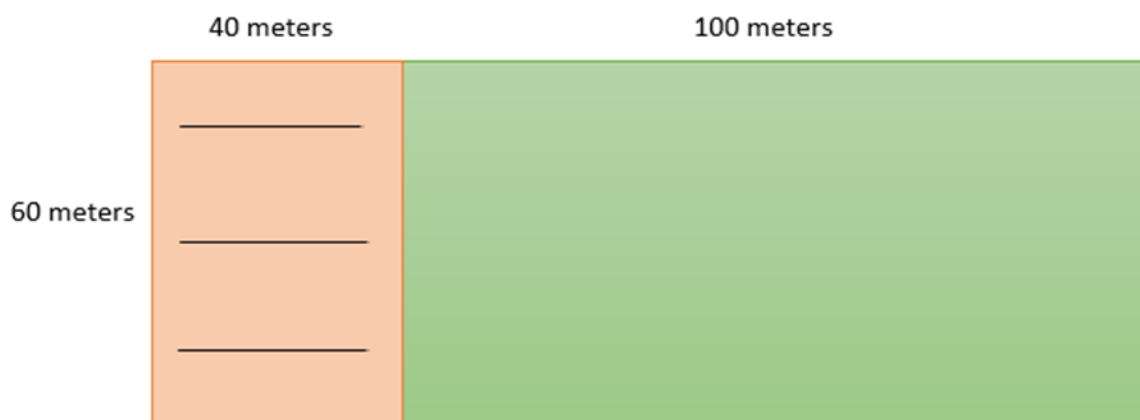
Figure 1.1. Layout of an experimental field site. The forb planting is located on the left side (orange) and the dry bean planting is on the right (green). The three lines within the forb planting are the locations of the surveys within each planting.

Table 1.1. Average weather data (NDAWN, 2020) and planting details for forb plantings by location.

	Hettinger	Hettinger	Carrington	Carrington
	2019	*2020*	*2019*	*2020*
Weather				
Average temperature (June - August)	16.6°C, 20.5°C, and 18°C	18.9°C, 20.6°C, and 20.6°C	18°C, 20.5°C, and 17.7°C	20°C, 21.6°C, and 19.4°C
Average precipitation (June - August)	9.9 cm, 5.4 cm, and 7.7 cm	4.4 cm, 6.2 cm, and 4.7 cm	7.6 cm, 9.2 cm, and 7.8 cm	3.12 cm, 12.7 cm, and 2.7 cm
Annual Forb Planting				
Planter	Truax no-till drill		5' 3-point press drill	
Planting date	May 28 - June 2	May 15 – May 22	May 28 - May 29	May 29
Seeding rate	48.2 kg/ha	46 kg/ha	48.2 kg/ha	46 kg/ha
Fertilizer	11-52-0 fertilizer		none	
Herbicide	none		Select herbicide	
Floral Composition				
Planted species	47.8%	46%	90.8%	77.8%
Volunteer species	52.2%	54%	9.2%	22.2%
Species Richness				
Planted species	15	14	13	13
Volunteer species	16	19	14	12

Table 1.2. Annual forb planting seed mixture.

Scientific name	Common name	Seeding rate per species (lb/ac)	Flower color	Surveyed?	Observed bee interaction?
Fagopyrum esculentum	Buckwheat	4.8	white	Yes**	Yes
Lens culinaris	Lentil	5	white-purple	Yes**	No
Helianthus annuus	Sunflower	0.5	yellow	Yes	Yes
Raphanus sativus	Radish	0.4	white	Yes**	Yes
Carthamus tinctorius	Safflower	0.5	red-orange	Yes	Yes
Linum usitatissimum	Flax	4	blue	Yes**	Yes
Phacelia tanacetifolia	Phacelia	3	purple	Yes**	Yes
Brassica napus	Rape oil	0.5	yellow	Yes	No
Trifolium alexandrinum	Berseem clover	3	red-white	Yes	Yes
Vigna unguiculata	Cowpea	3	pink-purple	No	n/a
Coreopsis tinctoria	Plains coreopsis	0.5	red-yellow	Yes**	Yes
Camelina sativa	Winter camelina	0.5	yellow	Yes	No
Cichorium intybus	Chicory	0.5	light blue-purple	No	n/a
Cleome serrulata	Rocky Mountain bee plant	2	pink-purple	Yes	No
Lathyrus sativus	Chickling vetch	10	white-blue-pink-purple	Yes**	Yes
Gaillardia pulchella	Indian blanket	1	yellow-red	Yes	Yes
Trifolium incarnatum	Crimson clover	2	red	Yes**	Yes
*Panicum miliaceum**	Millet	1.3	n/a	n/a	n/a

*only included in the 2019 seed mixture; **within the top 17 most commonly transected species by proportion, which made up more than 5% by proportion of total observed floral observations

Data Collection

Along each 20 meter by 2 meter transect, we conducted two surveys: a floral visitor survey and a floral vegetation survey. We conducted the floral visitor surveys first followed immediately by the floral vegetation surveys. Each floral visitor survey consisted of walking along the transect line and observing any insect-flower interaction within a 15-minute timeframe. After the floral visitor survey, we started the floral vegetation surveys where we focused on accurate counts of all flowering forbs within two meters of the transect line with no set time limit. All sampling occurred between 0800h – 1800h when temperatures were between 20°C – 35°C, sustained winds were <24km/h, and cloud cover was <75% (modified from Harmon-Threatt and Hendrix, 2015). We followed these parameters to improve insect detection due to bee activity and flower visitations (Harmon-Threatt and Hendrix, 2015). We returned to each field site a total of four times within a single growing season, and performed all surveys between July 11 to August 24 in 2019 and between July 3 and August 19 in 2020. These visits took place throughout the growing season to document the phenology of the planted forbs, and to account for phenological differences in bee response.

Both surveys combined provided us with comprehensive data of the bees and forbs present and the interactions between them. During the floral visitor surveys, any insect observed on the floral parts of a flower were aerial netted and the time was paused for the duration of the insect handling period. We euthanized all the collected bees in the field with a vial of ammonium carbonate (Bendel et al. 2019). We made sure to collect all individuals observed within the 15-minute limit with an exception for honey bees (*Apis mellifera*). Due to the close proximity to multiple apiaries and our confidence in site identifying honey bees, we decided to only collect the first honey bee within a single survey. However, we recorded any subsequent honey bee

interactions without collection making sure to only count each individual once. We took each collected specimen back to the lab for later identification. We identified the specimens to at least family level to determine the bee community composition and richness. The floral vegetation surveys consisted of walking along the same transect line while counting all reproductively open flowering stems. We counted only the basal stem for each flowering forb due to some species having a high number of flowering inflorescences, but we assigned a floral cover percentage to each forb species observed within a single survey (Otto et al., 2020).

Data Analysis

We analyzed the flowering stem counts and percent cover using an analysis of variance (ANOVA) using JMP (version 14.0.0). We used this to evaluate the relative floral expression and abundance across the forb plantings to satisfy our first objective. We first evaluated the cumulative effects over the growing year and used site, year, and their interaction as the independent variables and either average number of flowering stems per transect or average total cover as the dependent variable. We then analyzed the different dynamics of flowering within year. We did this as a repeated measures in JMP (version 14.0.0), but this time separately analyzing each of the two years since the exact dates of sampling did not match between year. Therefore, our repeated measure analyses for each year focused on site, time (across samples), and the time*site interaction, and again used either stems or cover as the dependent variable.

We made plant-pollinator interaction networks using the 'bipartite' package in R (bipartite version 2.14, Dormann, Gruber, Fruend 2008; R version 3.6.2, R Core Team, 2020). We included the collected female native bees, excluding honey bees, and all planted and volunteer plant species where an interaction was observed. We only included female bees in our interaction networks due to the different usage of floral resources between male and female bees

(e.g. males only use nectar resources, while females use nectar and collect pollen; Roswell et al., 2018). These interaction networks provide us information on which bee groups interacted with the different forbs in the forb plantings. The 'bipartite' package also allows us to calculate the H_2' index of each interaction network (bipartite version 2.14, Dormann, Gruber, Fruend 2008). The H_2' index is on a scale of 0-1 that shows the degree ofspecialization of the interaction network (Blüthgen et al., 2006). In other words, the H2' index shows how the interactions are partitioned between different plant and bee groups and if they more random (closer to 0) or if they have easily identifiable trends of specific interactions (closer to 1).

Results

Floral Vegetation Surveys

We observed 15 forbs out of the 17 forbs in the planted seed mix across both years and locations (Table 1.2.). We found a total of 48 unique flowering plants over both years with 33 species being volunteer, ruderal plant species.

We found no significant difference in overall average flowering stem counts between years and locations ($F_{3,12}$=2.92, P=0.07), but Hettinger in 2020 showed slightly fewer average flowering stems. However, we did find a significant difference between average floral cover with Carrington in 2019 and 2020 being significantly higher than Hettinger 2020 only ($F_{3,12}$=6.31, P=0.008), while Hettinger 2019 was not significantly different than either.

In 2019, we found a significant difference of average flowering stem counts over time across both locations ($F_{3,4}$=8.0, P=0.02; Figure 1.2.) with the peak bloom being between July 26 and August 11. Likewise, average floral cover differed over the course of the season ($F_{3,4}$=54.29, P=0.0006) with a peak bloom between July 26 and August 11. In 2020, we observed a significant difference in average flowering stems over time for both locations ($F_{3,4}$=18.82, P=0.005; Figure

1.3.) with a peak bloom around July 27. We also saw a significant increase in the average floral cover around July 27 ($F_{3,4}=79.07$, $P=0.0003$).

Despite the similarities in overall vegetation trends across time, there was a great deal of variation among our eight field sites in vegetation composition. Examining the expression of the seed mixture compared to other vegetation, we observed differences from field to field. Specifically, the proportion of planted species to volunteer species varied greatly between years and locations (Table 1.2) and individual field sites within each location each year (Figure 1.4.). Furthermore, the composition of both planted and volunteer species substantially varied between each field site (Figure 1.5.). Hettinger had a higher proportion of volunteer plants both years when compared to Carrington ($F_{1,2}=32.48$, $p=0.029$; Table 1.2. and Figure 1.5.). However, there was much variation within a location as seen with one site in Hettinger (Lagoon) having over 90% volunteer species while the rest of the Hettinger sites had at least 40% expression of planted forbs.
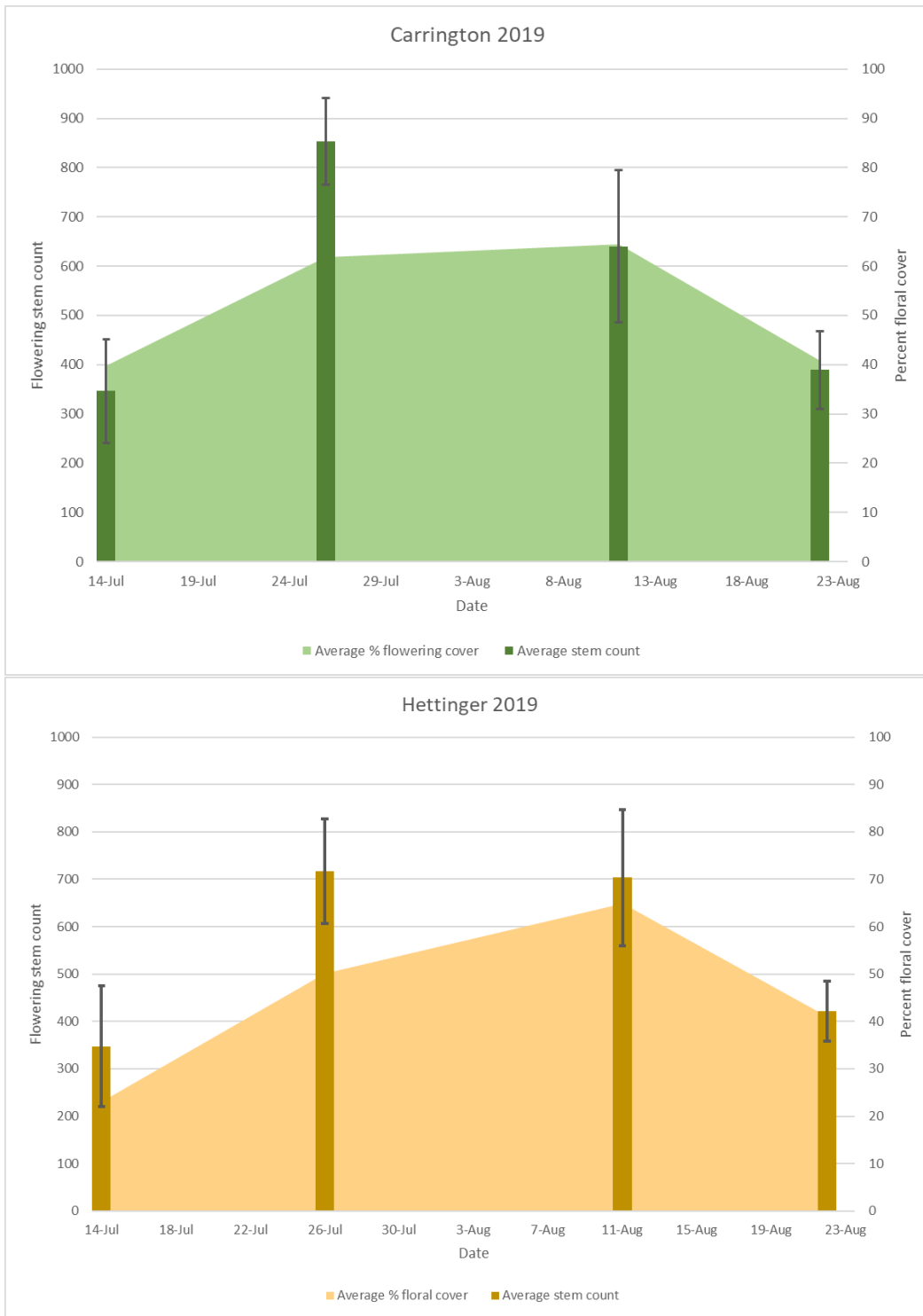
Figure 1.2. Flowering stem count and percent floral cover. Data collected in July and August in 2019 of the average flowering stems and percent floral cover by location. The secondary y-axis shows the area of average percent floral cover for each survey. Standard error bars included on the flowering stem count data points.

Figure 1.3. Flowering stem count and percent floral cover. Data collected in July and August in 2020 of the average flowering stems and percent floral cover by location. The secondary y-axis shows the area of average percent floral cover for each survey. Standard error bars included on the flowering stem count data points.
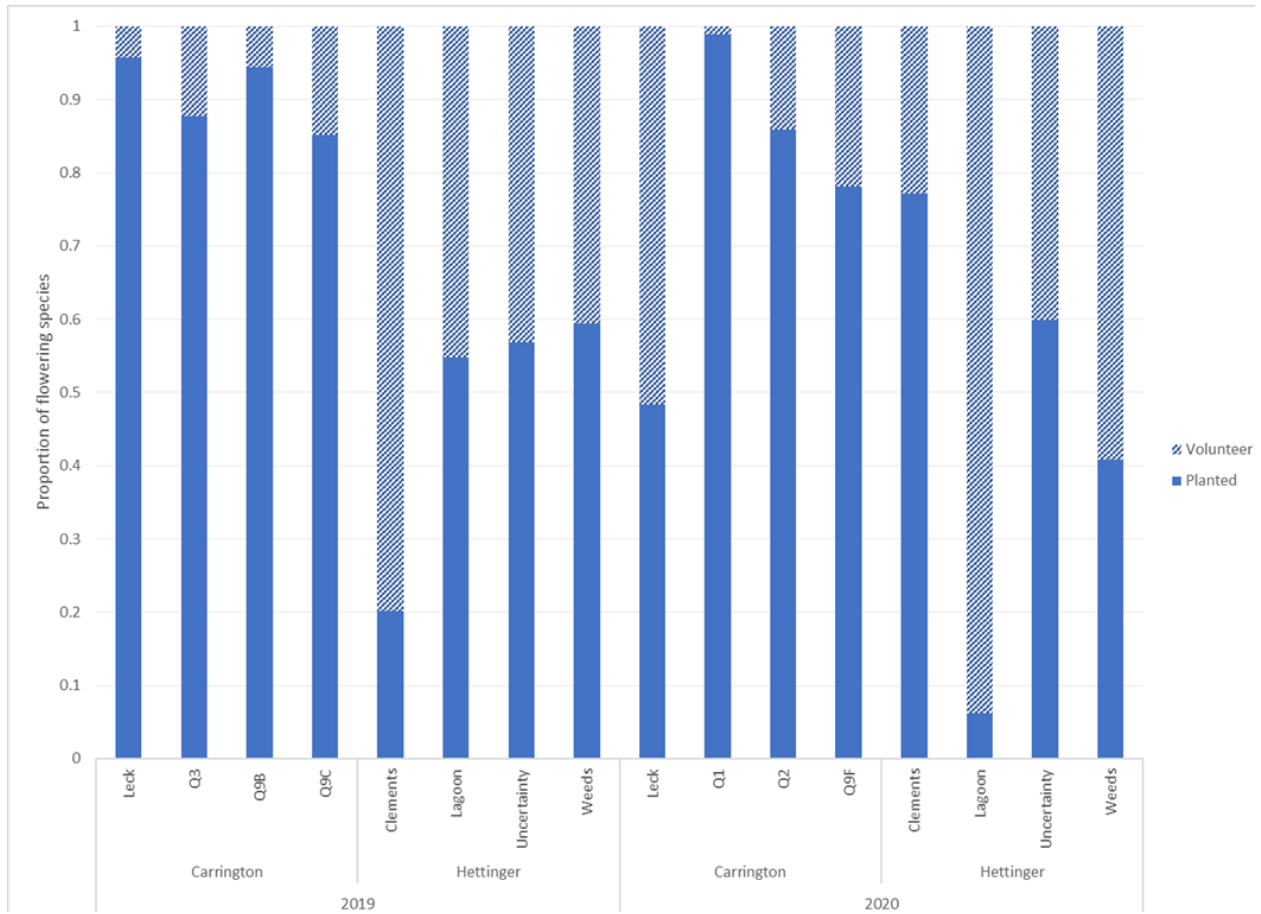
Figure 1.4. Proportion of planted and volunteer species. Field sites broken down by year and location. The y-axis shows the proportion of the flowering species surveyed across both locations in July and August in 2019 and 2020. Planted species are the 17 species from the seed mixture designated with a solid color and volunteer plants are plant which were not planted but were present in the forb plantings designated with a pattern.

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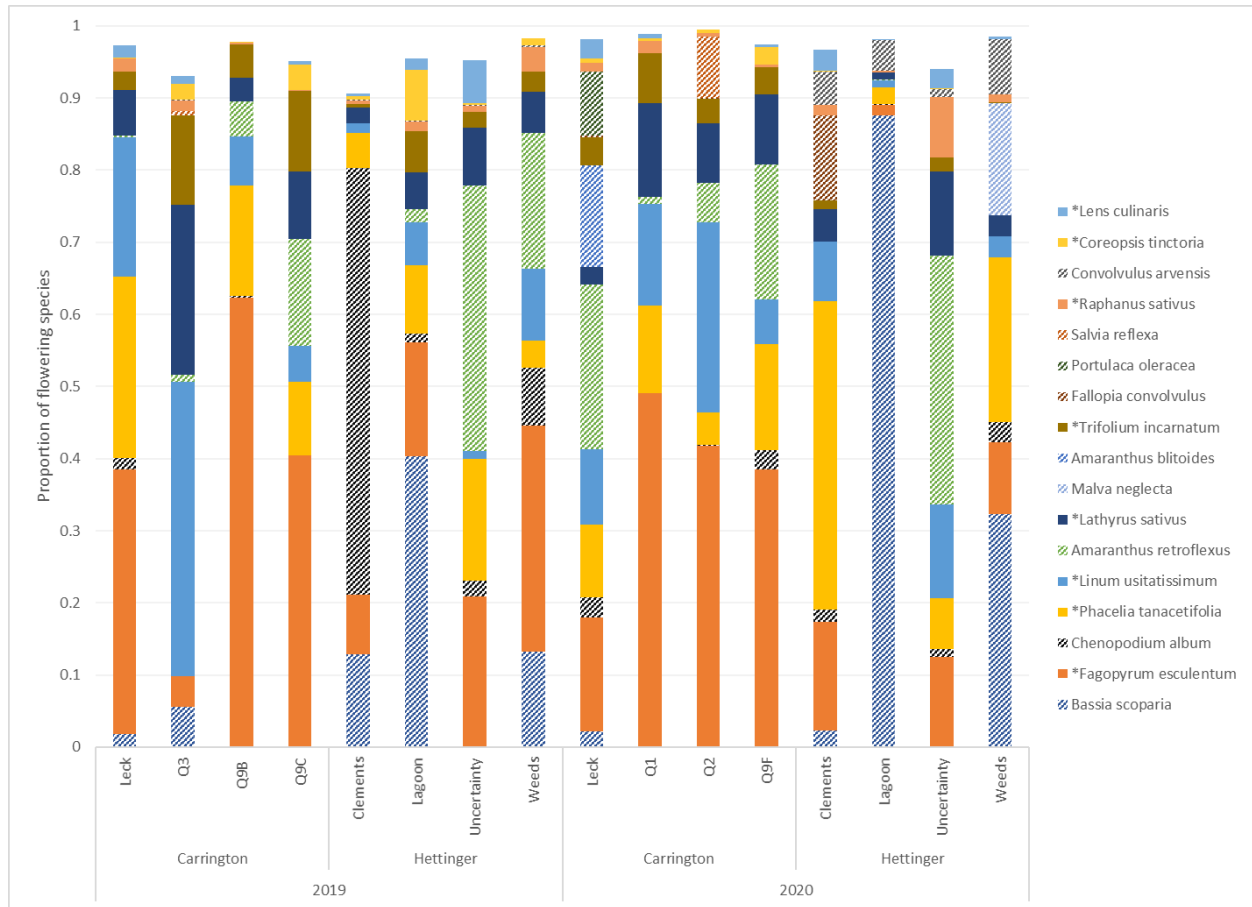
Figure 1.5. Proportion of the planted and volunteer species for each field site. The proportions of the previous figure (Figure 1.4.) broken down by species composition. Each site shows variation within a location and between locations. All the solid colors with an * in the legend are the planted species and all pattern colors without an * represent the volunteer species. **We only included the top 17 most commonly transected species by proportion in this graph. Any flowering species that, proportionally, made up less than 5% of total observed species were not included in this graph.

Network Analyses

In Carrington, we collected a total of 89 bees on 9 flower species in 2019 and 93 bees on 9 flower species in 2020 (Figure 1.6.). Carrington floral interactions consisted mainly of phacelia and buckwheat. In 2019, phacelia (*Phacelia tanacetifolia)* was the most visited forb with 54 total interactions, followed by buckwheat (*Fagopyrum esculentum*) with 15 interactions. However, in 2020, buckwheat was the most visited forb with 54 interactions, followed by phacelia with 36 interactions. The most common bees observed across both years belonged to the family Apidae.

Bumblebees were the most common genus in Apidae with 69 total observations. The H_2' index for Carrington were similar with 0.529 in 2019 and 0.566 in 2020. These H_2' indices show that there was little difference in bee specialization, or how selective bees were, for the overall interactions.

We collected a total of 225 bees on 15 flower species in 2019 and 151 bees on 10 flower species in 2020 in Hettinger (Figure 1.7.). Phacelia was the most visited forb in Hettinger for both years with 130 and 102 interactions, respectively. However, the next most common species in 2019 was Plains coreopsis with 25 interactions and in 2020 was radish (*Raphanus sativus*) with 14 interactions. Apidae was the most common bee family observed in 2019. We collected 107 bumblebees in 2019. Most plant-pollinator interactions in 2020 still consisted of Apidae with 48 bumblebees. However, the bee family Halictidae comprised 71 total interactions in 2020. The H_2'indices for each plant-pollinator network in Hettinger varied significantly between years with 0.499 in 2019 and 0.209 in 2020 (Figure 1.6. and Figure 1.7.). Contrary to Carrington, these H_2' indices show that there was a difference in bee specialization in Hettinger between years for the overall interactions.

We observed 1098 honey bee interactions during transects without collection (Figure 1.8.). Honey bee observations followed similar trends of the plant-pollinator networks with phacelia and buckwheat being the most commonly used species in Carrington. Honey bees in Hettinger most often interacted with phacelia, with relatively fewer observations on buckwheat.

Carrington 2019

Convolvulus arvensis — Agapostemon sp.

*Lathyrus sativus — Megachilidae

*Helianthus annuus — Melissodes bimaculatus

*Trifolium incarnatum

*Phacelia tanacetifolia — Bombus sp.

— Melissodes sp.

*Coreopsis tinctoria

Conyza canadensis — Halictidae

*Linum usitatissimum

— Andrenidae

*Fagopyrum esculentum — Apidae

H₂'=0.529

Carrington 2020

Salvia reflexa — Melissodes bimaculatus

*Helianthus annuus

Bassia scoparia

*Lathyrus sativus

— Bombus sp.

*Phacelia tanacetifolia

*Linum usitatissimum — Andrenidae

*Fagopyrum esculentum — Melissodes sp.

— Apidae

*Coreopsis tinctoria — Halictidae

H₂'=0.566

Figure 1.6. Plant-pollinator interaction networks in 2019. Interaction networks from bees observed and collected in 2019 and 2020 in Carrington, ND. The bars on the left side of the figure are the plant species and the bars on the right side are the different bees collected. The varying bars connecting both sides represent the amount of interactions that occurred between any given bee and plant.

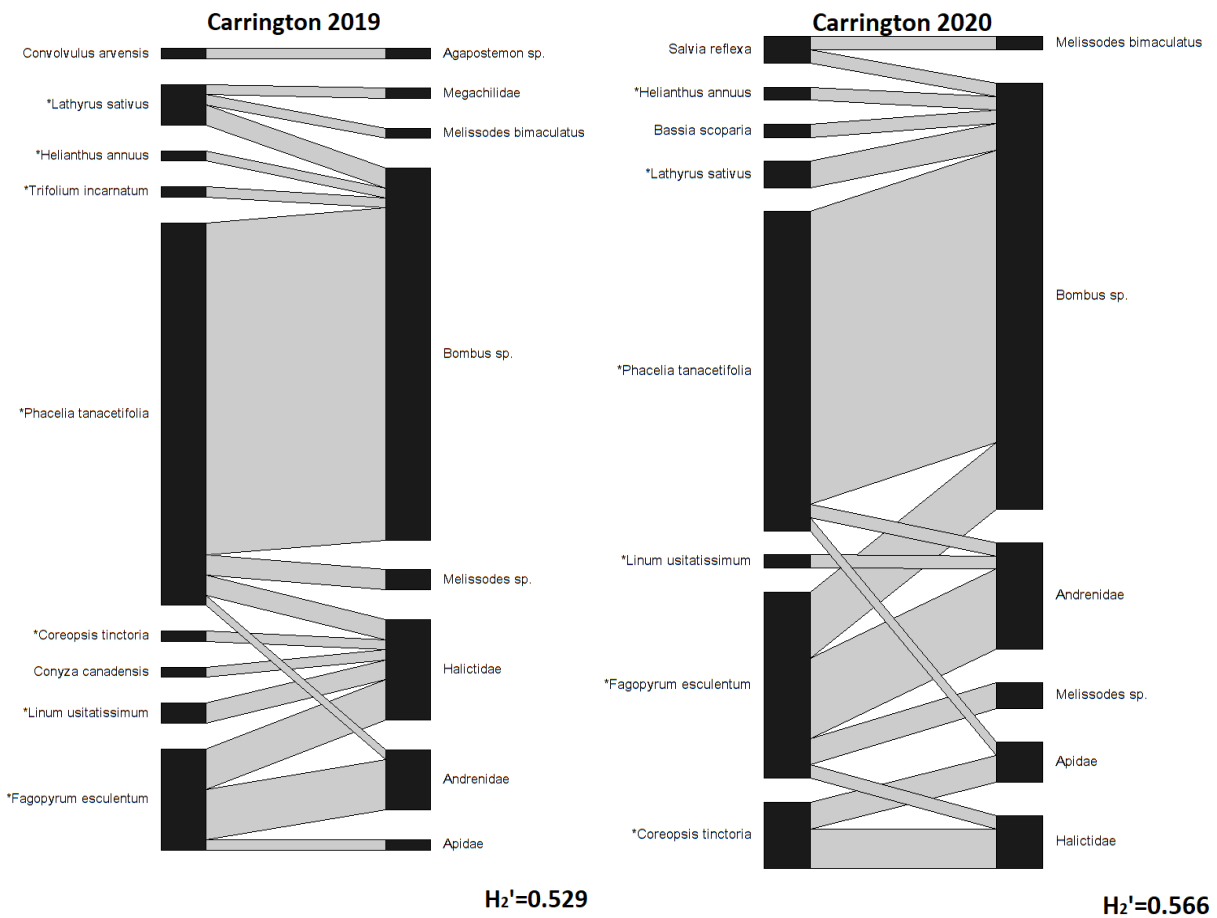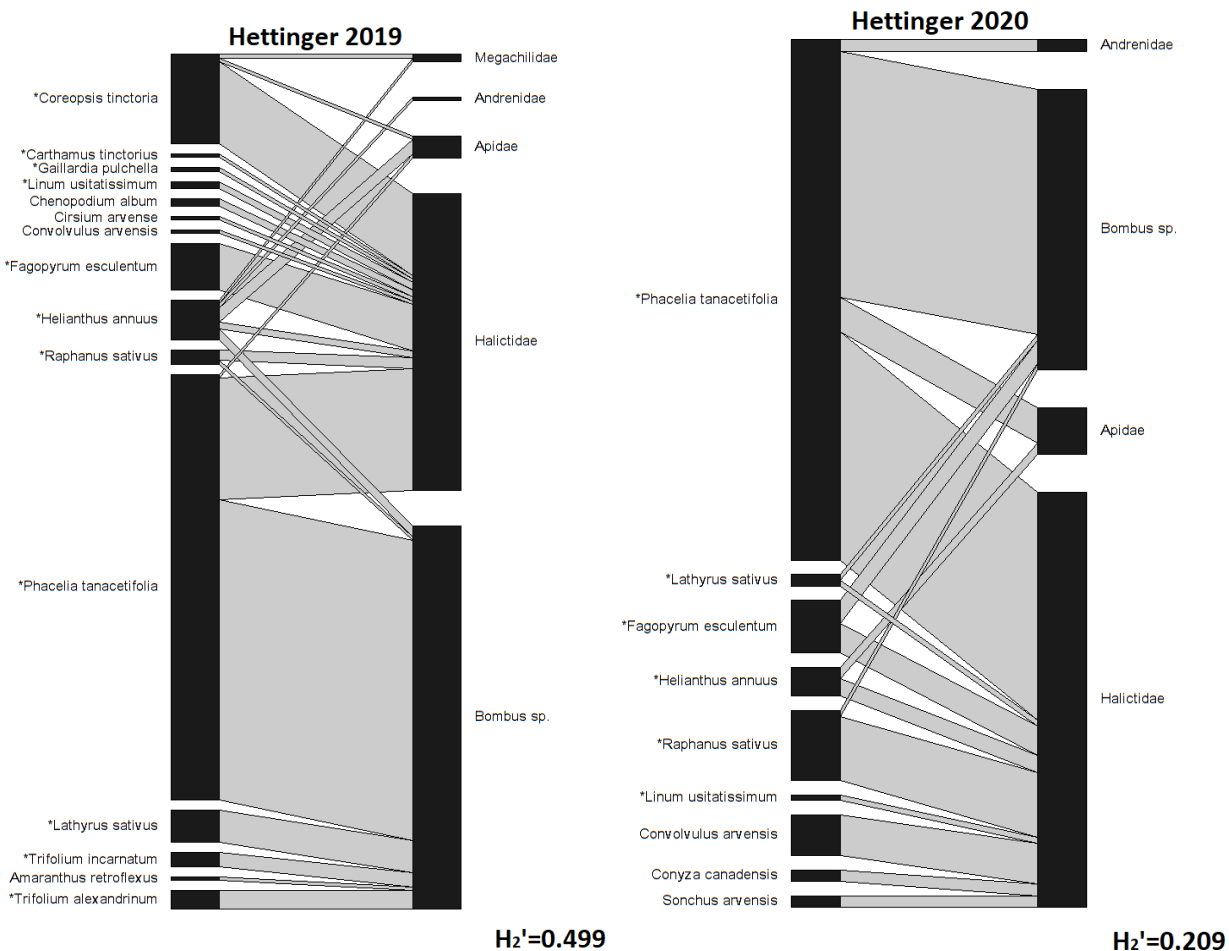Figure 1.7. Plant-pollinator interaction networks in 2020. Interaction networks from bees observed and collected in 2019 and 2020 in Carrington, ND. The bars on the left side of the figure are the plant species and the bars on the right side are the different bees collected. The varying bars connecting both sides represent the amount of interactions that occurred between any given bee and plant.
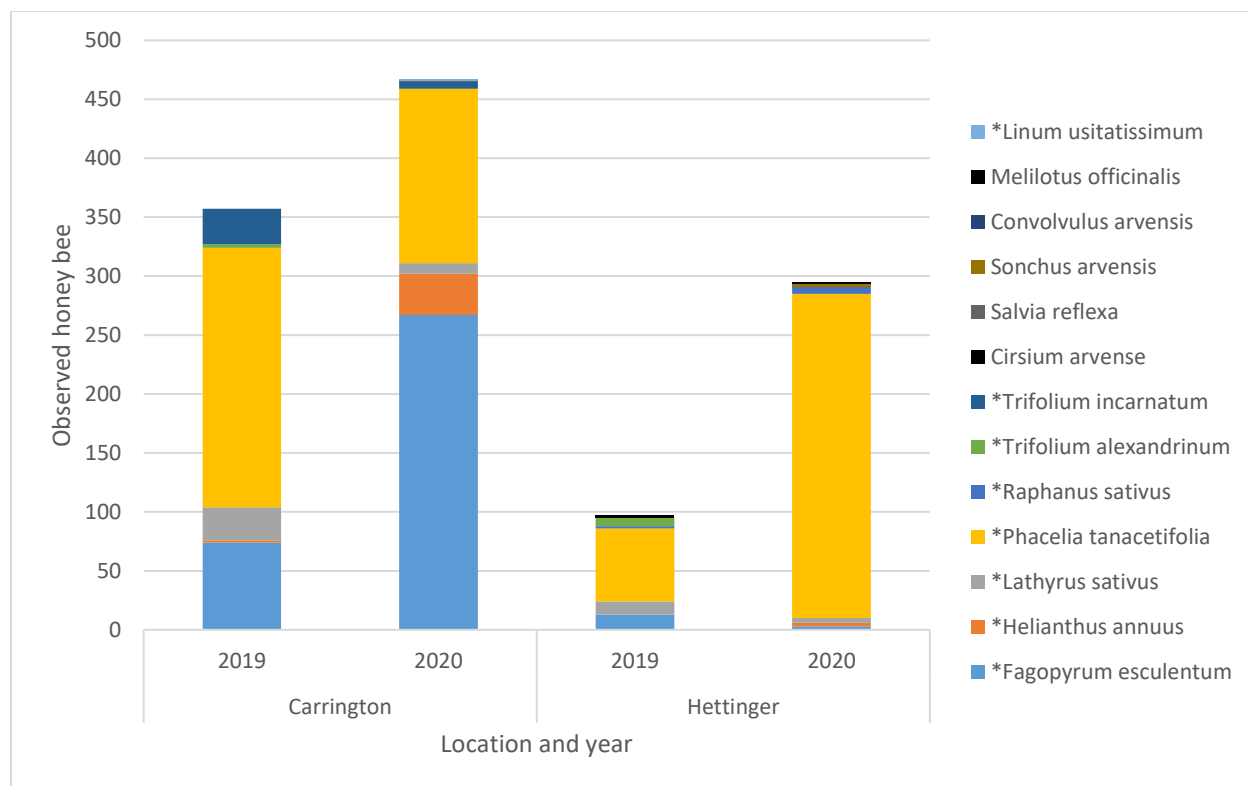
Figure 1.8. Total honeybee observations during the floral visitor surveys. These observations occurred following the initial capture of a single honey bee during any given floral visitor survey. Each bar represents all honey bees collected and/or observed on different forb species during all floral visitor surveys for each location and year, respectively. *indicates planted forb species.

Discussion

Globally, bees are facing declines that have led to the need for proactive conservation practices, especially in agroecosystems where pollination services are in high demand (Steffan-Dewenter and Westpahl, 2008; Potts et al., 2010; and Brandt et al., 2017). We planted an annual mixture of wildflowers and cover crops adjacent to crop fields in North Dakota to determine the floral expression and abundance of the planted seed mixture while also quantifying bee usage of the annual forb plantings. We observed very different expression of the seed mixture across locations and field sites within a location. We observed 15 of the 17 planted forbs across both years, and 8 of those planted species made up the top 17 most common species by proportion

across both years and locations. Our plant-pollinator networks showed 5 different bee families interacting with 21 plants which included 11 planted forb species. These results show that annual forb plantings can add floral resources into areas with low natural floral cover, which is consistent with other studies (Rundlöf et al., 2018 and Mallinger et al., 2019), despite varied expression of our planted seed mixture.

Our seeded forb plantings provided diverse floral resources throughout the surveyed months (July – August). Planted forbs like buckwheat, phacelia, and flax were the most consistently successful forbs in expression by total stems and floral cover. The addition of prevalent cover crop species, such as buckwheat, flax, and radish, in annual plantings can help reduce soil erosion, reduce soil compaction, and increase soil fertility (Ellis and Barbercheck, 2015 and Mallinger et al., 2019). However, despite planting the same seed mixture at both locations, there was significant variation between locations and even field sites within a location. These differences could be due to localized conditions, local climate differences, and the existing seed bank of weedy plants among other factors. Some of these weedy volunteer plants, while not desirable for some farmers, did serve as extra floral resources, which previous studies have also found (Requier et al., 2015 and Bretagnolle and Gaba, 2015). Different management practices, such as herbicide application and tillage, could affect the establishment and persistence of weedy volunteer species in the forb plantings (Conn, 2006 and Bretagnolle and Gaba, 2015). Despite a drought in 2020, which hit Hettinger especially hard, we still saw an average of nearly 40% floral cover within a transect in Hettinger and just about 70% in Carrington during the peak bloom in late July. This demonstrates the resiliency of annual forb plantings and suggests that they could help provide a source of floral resources on the landscape even during drought which

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is particularly important for some species (i.e., *Bombus spp*.; Thomson et al., 2016 and Neilson et al., 2017).

Native bees used 21 different forbs in our forb plantings (11 species planted in the seed mixture and 10 species were weedy volunteers). Planted forb species accounted for over 95% of the interactions in plant-pollinator networks, with < 5% of interactions with nonplanted, volunteer species. Phacelia and buckwheat, the two most visited species and planted in the seed mixture, have distinctly different morphologies and they both flowered throughout the surveyed months (July and August). We also found that bumblebees were the most common and abundant phacelia visitor, but buckwheat had a more diverse group of bees in both locations. Furthermore, all of plant species visited within the networks show a wide variety of flower morphology and phenology which should help promote a diverse bee community to use the floral resources. Other studies have similarly shown that a morphologically and phenologically diverse seed mix can attract and provide resources for many different bee types (Carreck and Williams, 2002; Williams et al., 2015; and Mallinger et al., 2019). Buckwheat was the most prevalently surveyed planted species, however, phacelia was the most visited in Hettinger in 2019 and 2020 and in Carrington in 2019. Similarly, both species, especially phacelia, have been shown to be highly attractive to honey bees and bumblebees, which both make up the largest proportion of the bees we observed and collected (Mallinger et al., 2019). Additionally, we also saw in Hettinger in 2020 a lower specialization H_2' index (0.209) compared to 2019 in Hettinger and both years in Carrington by over half. This H_2' index shows that Hettinger bees in 2020 were "less specialized," which means the bees were less specific about what forb they visited for resources. However, this could be directly related to the drought in the area in 2020 which reduced overall floral species and abundance, leaving less room for bees to have a more specialized diet.

We found a large amount of variation both across locations and between sites within a location in seed mixture expression and bee response that could be due to a variety of factors. There were differences in seeding method, agrochemical application, and surrounding landscape which all could contribute to the differences between locations. These differences in methodologies have led to some difficulty in interpreting exactly why we saw some of our differences between locations. Due to this, we are unable to confidently conclude how much differences in environment versus methodology may have affected the overall outcome of the forb planting and bee community response. However, there was still a large amount of variation within locations as well. This could mean that the observed variation could be due to localized conditions for each field site within a location in addition to overall differences between locations. Additionally, we encountered issues in 2020 due to a statewide drought across North Dakota (NDAWN, 2020). The 2020 precipitation was lower than average in Hettinger for all surveyed months and was lower for one month in Carrington. In July 2020, Carrington saw above average rainfall for the month and one field site (Q9F) flooded following a large rainstorm which negatively affected some floral cover. The variability observed across locations, due to multiple environmental/geographic/management differences, preclude some interpretation of the results and associated conclusions. Future research efforts with annual forb planting expression and the bee visitors could help isolate some of these factors to better understand the role of each in our results.

The large-scale conservation effort focused on insect pollinators can coincide with the need to maximize production and ecosystem services in agroecosystems (Blaauw and Isaacs, 2014; Sutter et al., 2017; Venturini et al., 2017). Annual forb plantings can serve as a connecting thread between future bee conservation efforts and the need to optimize crop yield quality and

quantity to reduce future land use conversion (Klein et al., 2007). Management practices within agroecosystems, especially ones with bee-friendly mass flowering crops, should be assessed and reevaluated to better support bees for their services. We found that our annual forb plantings can be extremely variable depending on localized conditions and factors, but they all provided some form of floral resources to support local bee communities. Additionally, management of weedy growth may be necessary in such plantings to reduce unfavorable volunteer species while better expressing higher quality floral resources included in a seed mixture. This allows for a farmer or land manager to directly add floral resources to the landscape, while also supporting the bee community and soil health within an area to allow for better overall growing conditions for future plantings, whether they be forb plantings or crop fields. These annual forb plantings can serve as a supportive method to boost local bee abundances, and hopefully their pollination services, particularly in the agroecosystems where bees and their services are steadily declining.

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CHAPTER 2: INSECT POLLINATOR VISITATIONS AND THE POTENTIAL EFFECT ON YIELD IN SELF-POLLINATING DRY BEAN CROPS

Introduction

Insect pollinators play a critical role in agroecosystems (Kevan and Viana, 2003; Winfree et al., 2011; and Potts et al., 2010). However, pollination services are becoming a threatened ecosystem service, especially in intensively managed agricultural systems (Lautenbach et al., 2012). Many agroecosystems have replaced native ecosystems which has contributed to pollinator declines due to habitat loss and degradation (Otto et al., 2016 and Steffan-Dewenter and Westpahl, 2008). This can be detrimental to both pollinators and the crops within agroecosystems, as up to 75% of the world's food crops are dependent on or benefit from pollination (Klein et al., 2007). However, globally, the leading 28 crops do not require insect pollination (Klein et al., 2007). Despite not requiring pollination to produce a crop yield, some of these crops can provide resources to pollinators (e.g., soybean (*Glycine max*); Gill and O'Neal, 2015) while others may benefit from pollinator visitation (e.g. field bean (*Vicia faba*); Garratt et al., 2013). These crops may provide a source of floral resources for pollinators in low floral resources agroecosystems, and the pollinators could potentially benefit production by increasing pollination of the crops and increasing yields which is a main goal for producers (Garibaldi et al., 2014). There is potential for self-pollinating, flowering crop and pollinator interactions to be mutually beneficial in many cropping systems, and there is a need to find the insect communities that use these crops to determine the effect on yield in self-pollinating cropping systems.

The influence of insect pollination on many self-pollinating crops has not been explicitly studied despite the prevalence of self-pollinated cropping systems across the United States (Klein et al., 2007 and Bartomeus et al., 2014). Because production is satisfactory without additional

insect pollinators, pollinator communities that may be present in self-pollinating crops get overlooked (McGregor, 1976). Similarly, there is limited research about the use of self-pollinating crop floral resources by pollinators and even less about the quality of floral resources, e.g. nectar and pollen, that these species provide (McGregor, 1976). Native pollinators provide an estimated $3.07 billion in pollination services to fruits and vegetables in the United States (Losey and Vaughan, 2006). Additionally, several studies show insect pollinators can increase both yield quality and quantity in a variety of self-pollinated and wind-pollinated crops (Mireille et al., 2012; Milfont et al., 2013; and Bartomeus et al., 2014). Yet, the community composition and effect of pollinator interactions still need to be assessed in many, more globally important crops, to better understand these interactions.

Bean crop varieties are prevalent across the world and vary in terms of pollination requirements with most being self-pollinating species (McGregor, 1976). However, bean crops that do not require outside pollination have some known insect pollinator interactions (Garratt et al., 2014; Bartomeus et al., 2014; and Gill and O'Neal, 2015). For example, field beans are a mostly self-pollinating crop but are known to benefit from bumblebee visitors (Hymenoptera: Apidae: *Bombus;* Garratt et al., 2013). Bumblebees use some bean crops for pollination visits as well as to raid floral resources, where they bypass the reproductive floral parts to access nectar only, and they can be effective bean pollinators due to the flower morphology and location of floral resources within the flower (Garratt et al., 2013). Soybeans are a prevalent self-pollinating crop that offer floral resources that are attractive to insect pollinators and have relatively higher quality pollen and nectar than other prevalent wind- and self-pollinated crops (Knopper et al., 2016). Therefore, we need to evaluate other prevalent self-pollinating bean crops where there are

gaps for the use of their floral resources by insect pollinators, what pollinator community is present, and how that affects the yield and overall production.

The United States plants 600,000 to 800,000 hectares of dry beans annually with North Dakota leading production at around 285,300 hectares planted in 2017 (US Dry Bean Council, 2020; UDSA-NASS, 2019). The prevalence of self-pollinating dry bean crops across the United States, specifically North Dakota, calls for more research to identify insect pollinator communities within the crops and any potential effects on yield. In this study, we will plant pinto bean crops (a variety of the common bean, *Phaseolus vulgaris*) in agroecosystems within North Dakota to assess the pollinator community and the effects of outside pollination in a self-pollinating crop. Our objectives for this study are to (1) assess dry bean crops for pollinator presence, abundance, and richness and (2) evaluate the effect of insect pollination on dry bean yield. For the first objective, we hypothesize that we will observe floral visitors to our dry bean flowers. Specifically, we expect to see bumblebees using the bean flowers within our plots due to other self-pollinating bean crops having similar pollinator interactions. For the second objective, we hypothesize that insect pollination will result in an increase in dry bean yield due to similar responses in other self-pollinating cropping systems.

Methods

Site Description

We conducted research at the Carrington Research Extension Center (CREC) and Hettinger Research Extension Center (HREC) in North Dakota. Further information regarding county land uses, major crops, and 30-year weather averages can be found in the "Site Description" section in the methods of Chapter 1. The average temperatures and rainfall for this chapter fell within the 30-years averages aside from Hettinger rainfall in 2020 (Table 2.1.).

Dry Beans

Our study crop is pinto bean, which are a dry bean variety of the common bean plant (*Phaseolus vulgaris*). Pinto bean plants are herbaceous annual plants grown as dry bean crops worldwide (Graham and Ranalli, 1997). We used a slow-darkening cultivar of the pinto bean called ND-palomino. North Dakota State University and the United States Department of Agriculture – Agricultural Research Service jointly developed this cultivar (Osorno et al., 2017). We chose to use pinto beans as the study crop due to its prevalence as a major dry bean crop within North Dakota (US Dry Bean Council, 2020 and USDA-NASS, 2019). North Dakota produces the most dry bean crops of any other states with over 283,000 hectares planted in 2017 (US Dry Bean Council, 2020; USDA_NASS, 2019); and pinto beans are the most important market class of those dry bean crops (NDSU Extension, 2019).

The morphology of the pinto bean plant is characteristic of Fabaceae crops (McGregor, 1976). Our variety, ND-palomino, has an indeterminate growth, meaning that the plant will continue to produce flowers and vines throughout the season (NDSU Extension, 2019). Each plant has multiple flowers that are often white, but can also be pink or purple (Graham and Ranalli, 1997). The flowers are papilionaceous, meaning that they are irregular and "butterfly-like," which is common for plants in the Faboideae subfamily (McGregor, 1976). There is no information readily available about the quality or quantity of floral resources (e.g. nectar and pollen) provided by pinto bean crops. However, other related bean crops are known for having relatively high-quality resources as compared to other self- or wind-pollinated crops (Knopper et al., 2016). Additionally, the pinto bean morphology could indicate what floral visitors use the flowers since they are harder to reach for small, short-tongued native bees, while larger native

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bees with long-tongues, such as some bumblebee species, allows for easier access to the nectar and/or pollen (Garratt et al., 2013).

Experimental Design

Each location had four different experimental field sites for 2019 and 2020, totaling eight sites for each year. Each field site had two main components: an annual forb planting and an edible dry bean planting (Figure 2.1). We seeded the dry bean plantings directly adjacent to the forb plantings. The dry bean planting measured 60 m wide by 100 m long with a total area of 6000 m^2. We planted each field site at least 100 m away from any other field site. The planting equipment and timing of the planting varied slightly by location but followed general, standard protocols (Table 2.1). Multiple variables such as precipitation, soil moisture, and air temperature determined when the seeding took place.

Figure 2.1. Layout of a field site in 2019. The annual forb planting is located on the left side (orange) and the dry bean planting in on the right (green). The three lines within the forb and dry bean planting are the locations of the surveys within each planting. Each transect was 20 m x 2 m long, along which each survey is conducted.
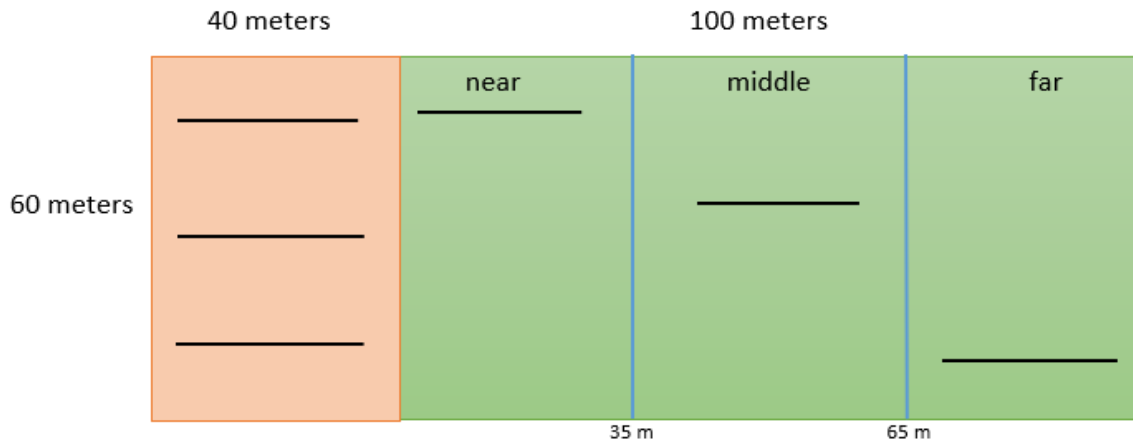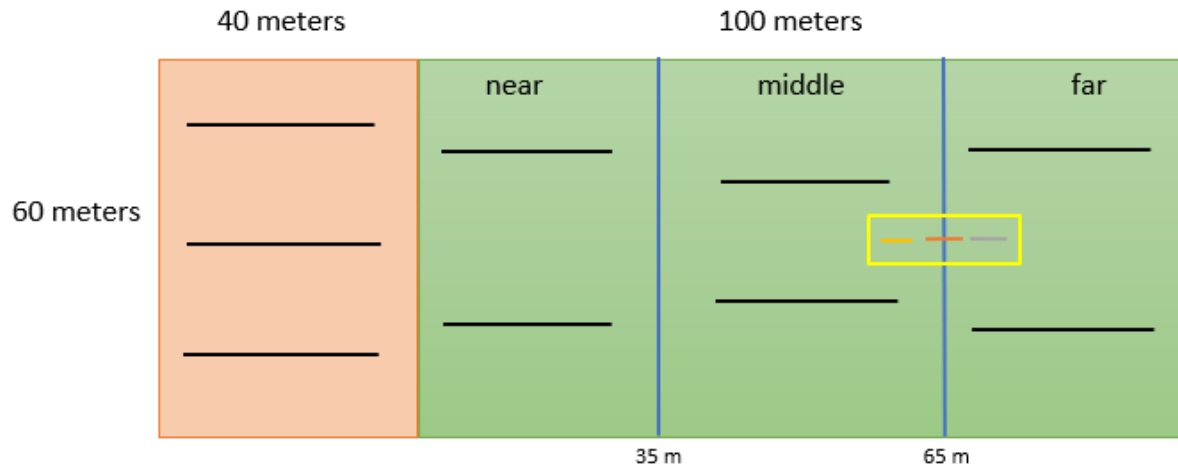
Figure 2.2. Layout of a field site in 2020. The annual forb planting is located on the left side (orange) and the dry bean planting in on the right (green). The three lines within the forb plantings and the six lines within the dry bean planting represent the survey locations at each field site. Each transect was 20 m x 2 m long along which both surveys were conducted. The yellow box represents the new experimental plot with the bean plantings. Within each experimental plot there are three lines that represent each pollination treatment: hand pollination with outside pollen, hand self-pollination with no outside pollen, and a control with no pollination treatment.

Table 2.1. Average weather data (NDAWN, 2020) and planting details for dry bean plantings by location.

	Hettinger	Hettinger	Carrington	Carrington
	2019	*2020*	*2019*	*2020*
Weather				
Average temperature (June - August)	20.5°C and 18°C	20.6°C and 20.6°C	20.5°C and 17.7°C	21.6°C and 19.4°C
Average precipitation (June - August)	5.4 cm and 7.7 cm	6.2 cm and 4.7 cm	9.2 cm and 7.8 cm	12.7 cm and 2.7 cm
Dry Bean Planting				
Planter	John Deere 1700 planter, side banded 71.7kg/ha urea (=33.6 kg/ha nitrogen)		Plate planter	Bulk planter
Planting date	June 7 - June 11	June 3 - June 5	May 29 – June 3	June 2
Harvest date	Oct 9	Sept 12	Sept 17	Sept 11
Seeding rate	98,800 seeds/ha*	197,700 seeds/ha	185,000 seeds/ha	
Row spacing	76.2 cm rows		55.9 cm rows	76.2 cm rows

Table 2.1. Average weather data (NDAWN, 2020) and planting details for dry bean plantings by location (continued).

	Hettinger	Hettinger	Carrington	Carrington
	2019	*2020*	*2019*	*2020*
Herbicide	June 12 – BroadAxe XC (sulfentrazone + S-metolachlor) 2.2 kg/ha, Cornerstone 5+ (glyphosate) 1.7 kg/ha, ClassAct (AMS + surfactant) 2.2 kg/ha July 17 – Rezult (Sethoxydim + bentazon) 3740 ml/ha, ClassAct (AMS+surfactant) 0.6 kg/ha, Destiny MSO 1.1 kg/ha	June 3 – Spartan Charge (sulfentrazone + carfentrazone-ethyl) 0.35 kg/ha, Cornerstone 5+ (glyphosate)1.1 kg/ha, ClassAct (AMS + surfactant) 2.2 kg/ha, Destiny MSO (methylated seed oil) 1.1 kg/ha	none	
Floral Composition				
Pinto bean plants	78.8%	46.3%	99.2%	95.9%
Volunteer plants	21.2%	53.6%	0.8%	4.1%

*The low seeding rate in Hettinger 2019 was due to planter issues.
** Floral Composition section are the results from floral vegetation surveys

Data Collection

We performed four main data collection methods to identify the pollinator community within the dry bean crops and their potential effect on dry bean yield: floral visitor surveys, floral vegetation surveys, yield by distance (2019 only), and hand-pollination (2020 only) (Table 2.2; Figures 2.1 and 2.2). To address our first objective of what pollinator community was present within the dry beans, we conducted the floral visitor surveys as many times as possible during the bean flowering period (July – August). In 2019, we were able to perform two rounds of sampling, and in 2020, we conducted three rounds of sampling. All sampling occurred between 0800h – 1800h when temperatures were between 20°C – 35°C, sustained winds were <24km/h, and cloud cover was <75% (modified from Harmon-Threatt and Hendrix, 2015). We followed these parameters to improve insect detection due to bee activity and flower visitations occurring

mainly within these parameters in prairie ecosystems (Harmon-Threatt and Hendrix, 2015). To address our second objective assessing the influence of pollinators on dry bean yield, we had two different approaches in 2019 and 2020. We first wanted to assess if dry bean yield was affected by distance from the annual forb planting. For this, we harvested our dry beans at the end of the growing season by distance from the pollinator planting in 2019 to assess potential impacts on dry bean yield. In the second year, we implemented hand-pollination methodology to see if different pollination treatments had any effect on dry bean yield. The hand-pollination started around July 22, and took place muiltiple times througout the bean flowering period to pollinate as many flowers as possible.

Table 2.2. Methods for data collection.

Data Collection Method	Purpose	Year	Data Collected
Floral visitor surveys	Assess visitors by distance	2019 and 2020	Plant-pollinator interaction recorded and specimen collected for identification
Floral vegetation surveys	N/A – included in Table 2.1.	2019 and 2020	Vegetation species richness and abundance
Yield by distance	Measure yield by distance	2019 only	Harvested dry beans at different distance intervals to assess if there is a distance effect from the annual forb planting on bean yield
Hand-pollination	Evaluate pollination effects on yield	2020 only	Used three different treatments (hand cross-pollinated, hand self-pollinated, and a control) to assess the effects of different pollination on bean yield

Floral Surveys (2019 and 2020)

We chose the location of each transect at each field site by distance from the adjacent forb plantings. These transects within each bean planting were located 0-35 m (near), 35-65 m (middle), and 65-100 m (far) from the forb planting (Figures 2.1 and 2.2; modified from Ganser et al., 2018). We established these intervals to observe if there is any difference in pollinator

abundance or visitations depending on distance from the forb planting. Each transect was 20 meters long by 2 meters wide. Each designated transect had both the floral visitor survey and floral vegetation survey conducted consecutively. We conducted three floral visitor and floral vegetation surveys in 2019, and doubled our sampling efforts in 2020 to increase our sample size (Figure 2.1; Table 2.2). We started with the floral visitor surveys and each lasted for 15 minutes, excluding any pollinator handling time. We netted any insect that interacted with a bean flower during the floral visitor survey. We collected any floral visitor instead of focusing solely on the bee community to get a more accurate representation of the bean visitors present without any bias (Garratt et al., 2013). We collected and euthanized all specimens in a tube filled with ammonium carbonate, and we later identified each in the lab (Bendel et al., 2019). The floral vegetation survey immediately followed the floral visitor survey. The floral vegetation survey consisted of counting any flowering stems within the transect, including the dry bean crops and any agricultural weeds present. We performed these at the same three distances (near, middle, and far) at the same locations as the floral visitor survey. The results of the floral vegetation surveys are presented as a floral composition proportion by location and year to show overall differences (Table 2.1.).

Yield by Distance (2019 only)

To further assess if distance from the forb planting had any effect on dry beans, we collected yield data for three distance intervals in 2019. We left the dry bean plants completely open and unaltered during the sampling period. We allowed the dry bean plants to dry down in the field, and then we harvested two 5-meter sections of dry beans from each distance interval. This was done at the end of the growing season when the plants had started to dry in the fields (see Table 2.1. for harvest dates). For each dry bean planting, we averaged the yield of the two

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harvested strips to produce one number at each of the three locations for each plot (with the exception of one plot at Hettinger which only had a near and middle yield).

Hand-Pollination (2020 only)

In 2020, we tried to determine the effect of pollination on dry bean yield using different pollination treatments. We only treated four of the eight field sites (two in Carrington and two in Hettinger) due to the high amount of time and effort of the hand pollination. Each of the selected field sites for hand pollination had three different treatments within a single section of the dry beans (Figure 2.2). Each section of dry beans was 17 meters long – 5 meters of each treatment with a 1-meter buffer between each treatment. We randomly assigned the three treatments at each location and they consisted of: hand cross-pollination, hand self-pollination, and a control group with no imposed pollination or pollinator deterrent (i.e., open bean plants).

We performed the hand cross-pollination treatment using flowers from nearby plants. We plucked a nearby reproductively-open flower and harvested as much pollen as possible using a small wand marketed for pollination purposes (Carolina Biological Supply Company, 2021). We immediately used this pollen to cross pollinate one specific flower within that 5 m section of treated plants. We then tied a flag marker on the flower stem to track the treated flowers and subsequent bean pods. We performed the hand self-pollination on another 5 m section of plants within the treatment plot. For this method, we continued the use of the pollination wands but without any outside pollen. We used a clean wand for each flower, and we replicated the movement and manipulation used for the cross-pollinated treatment. This is to reduce any potential increase in yield that could be due to the movement alone instead of the result of any additional outside pollen. We repeated this pollination on as many open flowers during each visit to ensure high sample numbers. We tried to hand pollinate as many flowers as possible due to the

potential abortion by the plant or accidental removal from the plant, as the dry bean flowers are very delicate and the high amount of manipulation needed to make the floral reproductive parts available for pollination. We did not manipulate or treat the final 5 m section of plants alone to act as an open control against the hand pollinated plants. All pollination treatments were left in the field until they were appropriately dry for harvest. All dry bean plants were harvested at the end of the growing season (see Table 2.1. for harvest dates). This consisted of ripping the plant from the ground to reduce any bean pods falling from an individual plant. These hand-harvested plants were then taken to the lab, left out for a week to dry fully, and then we counted and weighed all treated bean pods on each plant. We ended up with a total of 471 pods: 137 hand cross-pollination pods, 145 hand self-pollination pods, and 189 pods from the control group with approximately 100 pods from each of the Hettinger plots and 130 from each of the Carrington plots.

Data Analysis

We used an analysis of variance (ANOVA) to evaluate the collected insects by plot and location and year with an interaction between year*location (JMP version 14.0.0). This was used to answer our first objective to quantify pollinator presence and abundance within the dry bean plantings. We next determined if distance from the forb planting affected the bean visitors. We divided the total insect captures into whether the insect was captured in the near (to the planting), middle, or far (from the planting) transect to see if the distance had any effect on visitor presence or abundance. We used all Carrington field sites in 2019 and 2020 for this analysis, but we only used 3 sites in Hettinger for 2019 and none for 2020 due to residual forb planting forbs flowering on both sides of the bean planting, meaning there was not an appropriate near and far category. We performed this as a repeated measures (JMP version 14.0.0), but this time separately

analyzing each of the two years since the exact dates of sampling did not match between year. Therefore, our repeated measure analyses for each year focused on site and distance, and we used the visitors as the dependent variable.

We next evaluated bean yield to determine the effect of pollination type on dry bean yield. First, we looked at the 2019 harvest data by distance. We did this with a repeated measures again (JMP version 14.0.0) to focus on location, distance, and a location*distance interaction using the yield weight as the dependent variable. To determine the effects of our pollination treatment, we ran a Tukey's HSD (JMP version 14.0.0) to determine differences among treatments at each location.

<div align="center">

Results

</div>

Floral Visitor Surveys

We collected a total of 77 insects across four orders visiting dry bean flowers in 2019 and 2020 (Figure 2.3). Despite the increased sampling effort in 2020, we collected 52 insects in 2019 and only 25 insects in 2020. Across both years, we found a significant difference in visitors by location ($F_{4,10} = 5.78$, $p = 0.011$) with Carrington sites comprised mainly of flies (Order: Diptera) and Hettinger sites comprised mainly of bees (Order: Hymenoptera). However, we did not find a significant difference between years ($F_{4,10} = 1.19$, $p = 0.37$) or an interaction between year and location ($F_{4,10} = 0.50$, $p = 0.74$). In 2019, there was a trend towards more insects captured in beans in Carrington compared to Hettinger, but it was non-significant ($F_{1,6} = 4.05$, $p = 0.091$). In 2020, there was no difference in captures by location ($F_{1,6} = 0.23$, $p = 0.65$). Furthermore, there was no difference in the number of insects collected on bean flowers based on the distance from the forb planting ($F_{2,7} = 0.77$, $p = 0.50$; Figure 2.4.). Similarly, there were no significant interactions between the location and distance from planting ($F_{4,14} = 0.75$, $p = 0.70$).
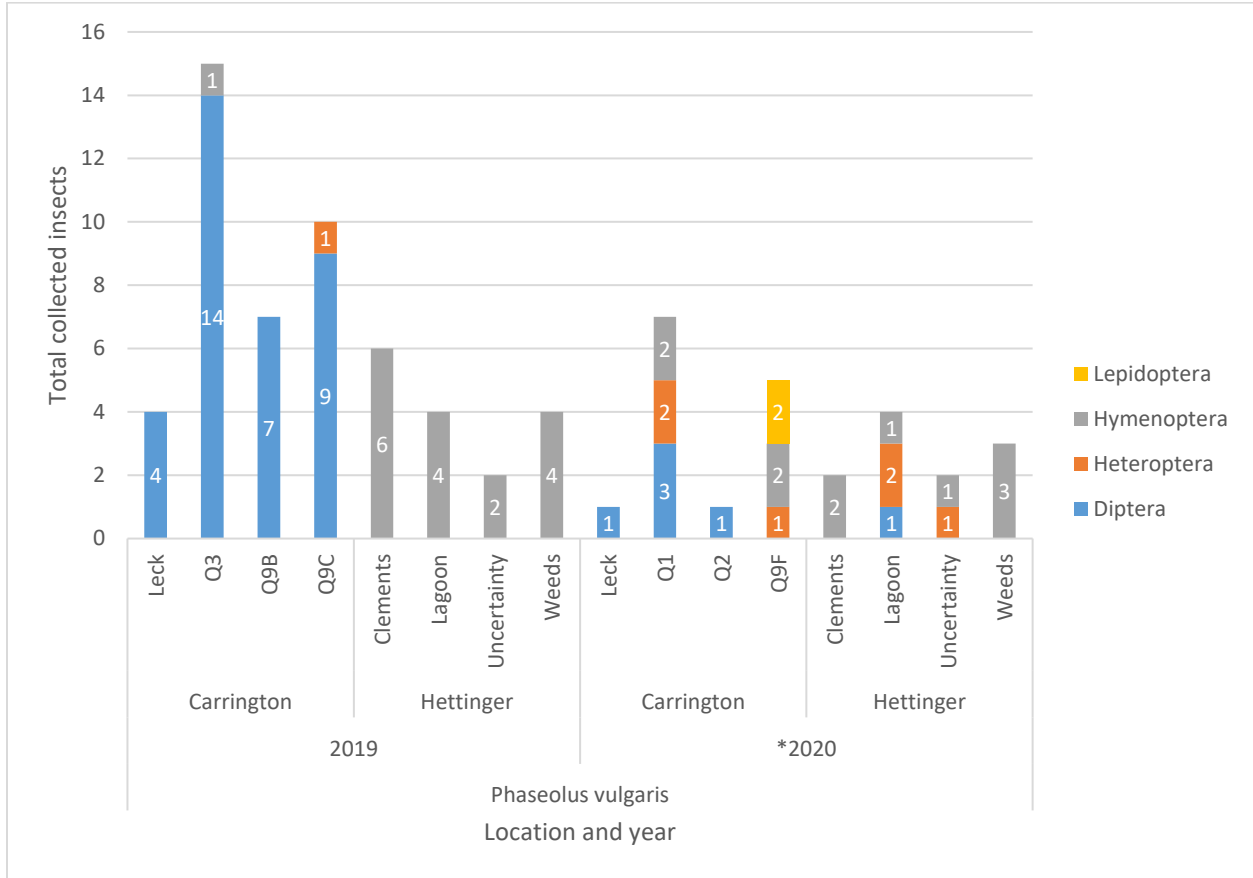
Figure 2.3. Total insects, grouped by order, observed visiting dry bean flowers in 2019 and 2020 by plot. Each bar represents the total collected insects for each field site with the number of each order in white on the bar. The bars represent the average across two sampling rounds (with three surveys per round) in 2019 and three sampling rounds (with six surveys per round) *Sampling effort increased in 2020 to double that of 2019
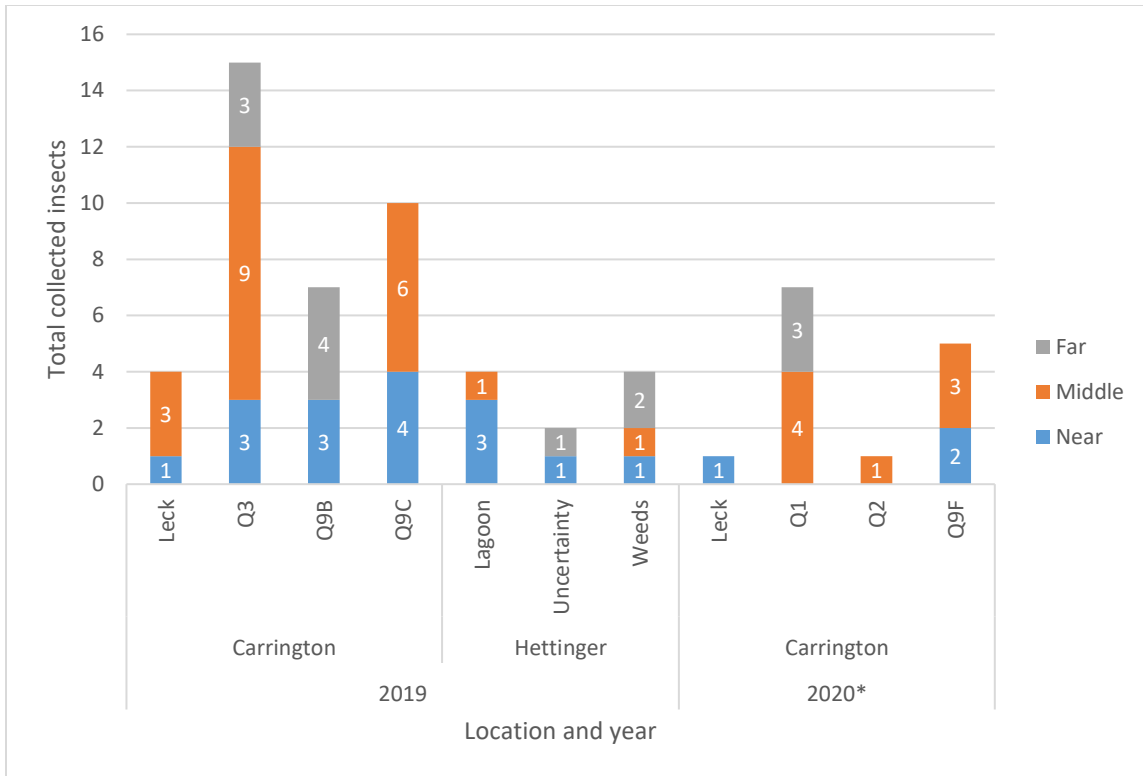
Figure 2.4. Total collected insects by distance from the annual forb planting. Each bar represents all collected insects at that distance. The distance of each collected insect by plot, location, and year. The bars represent the average across two sampling rounds (with three surveys per round) in 2019 and three sampling rounds (with six surveys per round). *2020 sampling effort increased from 2019 due to low sample size

Yield by Distance

We found that dry bean yield had an interaction of distance from the forb planting by location in 2019 ($F_{2,4}$=6.91, p=0.016). This is shown with the differences between Carrington (higher overall yield at each distance; Figure 2.5.A) vs. Hettinger (lower overall yield at each distance; Figure 2.5.B). Furthermore, there was no clear effect of distance in Hettinger in 2019, with different patterns of yield occurring with distance across the different plots. However, there was a more consistent effect of distance in Carrington with the closest beans to the forb planting having lower yield compared to the middle or far end of the same bean planting.
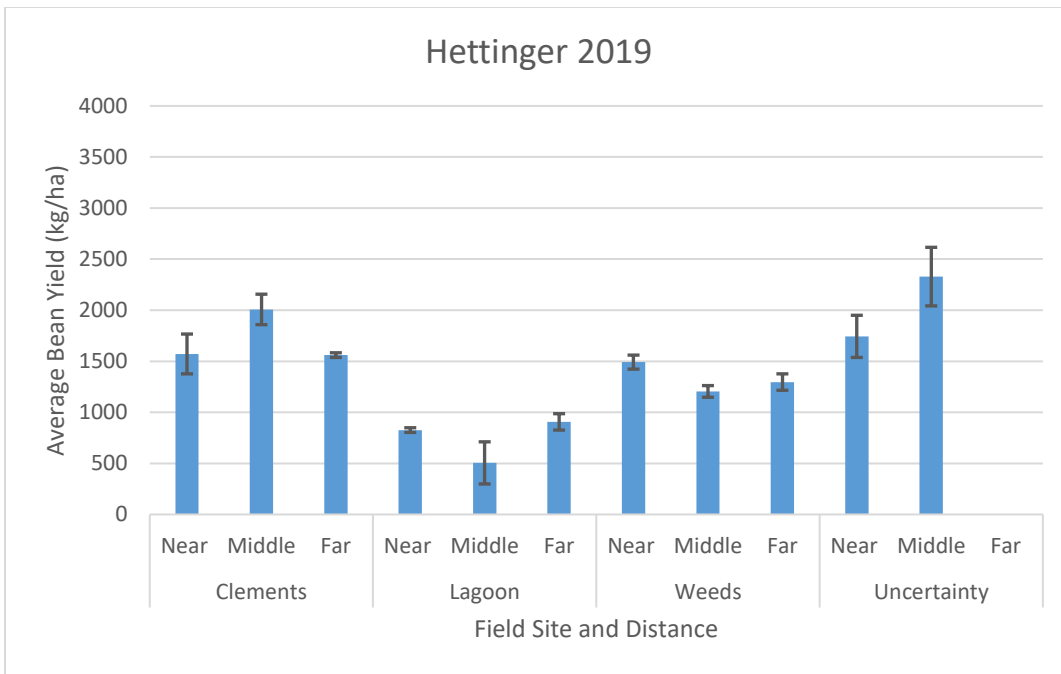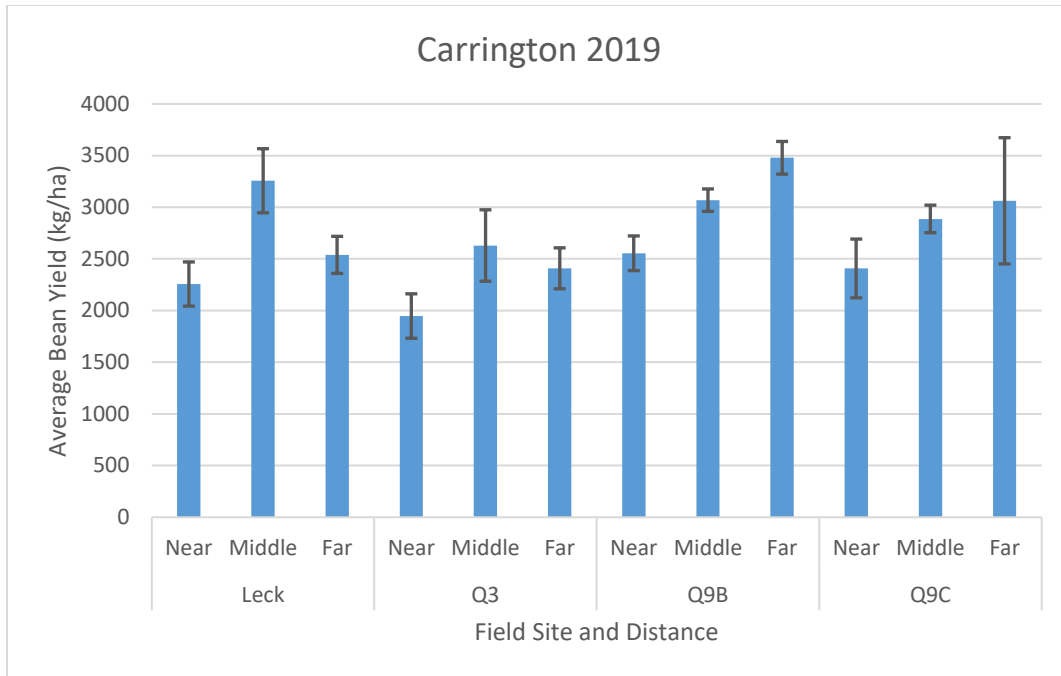
Figure 2.5. Dry bean harvest by distance. We harvested dry beans by distance from the forb planting in 2019 in both Carrington (A) and Hettinger (B) to see if distance affected dry bean yield. Each bar shown here represents the average bean yield (or weight of all harvested dry beans only) for each distance interval at each field site with standard error bars included. We harvested two strips at each distance interval, and averaged those two data points for weight of all dry beans for a 5 m section of beans to get the average shown above. Average bean yield was measured by average kg/ha weight for each distance in all field sites. The far distance in Hettinger Uncertainty was unable to be harvested due to loss of bean plants.

Hand-pollination

In 2020, we found that average dry weight of beans on a plant was significantly affected by the interaction between location and treatment ($F_{2,463} = 4.33$, $p = 0.014$). Across the two plots with pollination treatments, we found in Carrington there were no differences among the three treatments, meaning yields were similar. However, we did find a treatment effect in Hettinger, with the control weights being significantly higher than both pollinated treatments. This shows that the pollinated treatments had significantly lower weight when compared to the control yield.
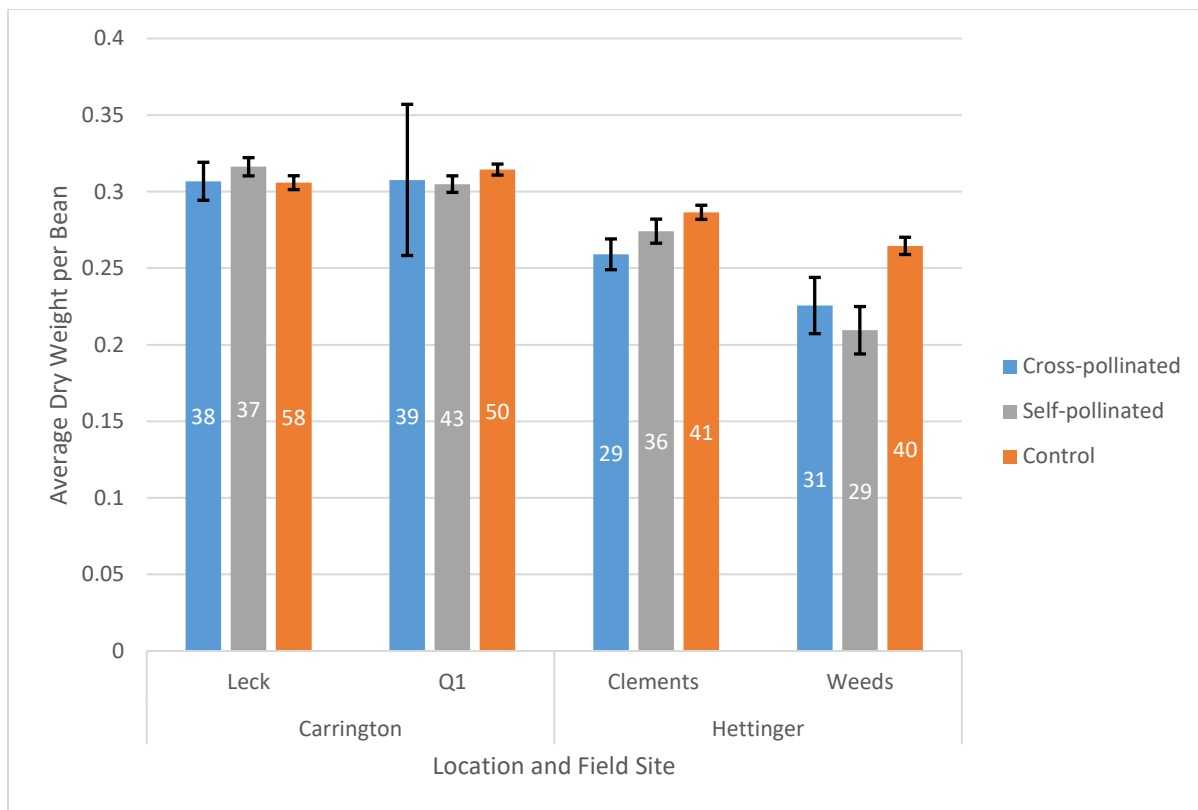

Figure 2.6. Average dry bean weight between pollination treatments. Each bar represents the average weight per bean for that treatment. Each plant's yield was averaged together to give us this overall treatment average, with the total n for each treatment being shown in white on the bar itself. Each treatment was applied to a 5 m section of beans in each bean planting. The cross-pollinated treatment used pollen from donor plants to pollinate a treated flower. The self-pollinated treatment consisted of the same manipulation as the cross-pollinated treatment without any donor pollen. The control was left completely open and left alone during flowering.

Discussion

Self-pollinating crops comprise a large proportion of agricultural cropland across the United States and may benefit from insect pollination (Milfont et al., 2013 and Bartomeus et al., 2014), yet the majority of studies have continually overlooked the presence, composition, and effects of pollinators within self-pollinating crops (McGregor, 1976 and Klein et al., 2007). Through our research, we observed four different insect orders visiting dry bean flowers in 2019 and 2020. Overall, flies (Diptera) were the most abundant dry bean visitor followed by bees (Hymenoptera), true bugs (Heteroptera), and butterflies (Lepidoptera). Our observed crop-pollinator interactions can help influence future dry bean management now that we have evidence of insects visiting their flowers. We were able to quantify average dry bean yield between cross-pollination, self-pollination, and a control in the 2020 treatments. Our sites had no significant differences between pollination treatments, and only Hettinger showed the control having significantly higher yields than both pollination treatments. However, it is unclear if our results are reliable given challenges with manipulating dry bean flowers for manual pollination or exclusion, Other studies have similarly encountered issues with field studies regarding pollination of beans (Bliss, 1980), so future greenhouse studies may be needed to successfully determine the effect on dry bean yield.

We observed and collected a variety of insects visiting the dry bean flowers. We found a significant difference in the composition of the bean flower visitors. Our most common visitor in Carrington were various small fly species such as flower flies (Syrphidae). Non-bee pollinators like flies are globally important crop pollinators, but they are less efficient at transferring pollen (Ssymank et al., 2008 and Rader et al., 2016). In Hettinger, bumblebees were the most commonly observed bean visitor. This follows our prediction as other studies have shown

bumblebees as a common and effective pollinator of bean crops (Garratt et al., 2014). Different factors, such as surrounding landcover and land use, could influence the observed differences in pollinator composition by location. Non-bee pollinators, such as flies, are known to be less affected by proximity to intensive agricultural land uses (Rader et al., 2016). This may explain our findings at Carrington which is the more agriculturally-dominated location which also had a higher proportion of fly visitors.

We did encounter challenges with hand pollination treatments in 2020. Due to the flower morphology, we needed to use significant manipulation to access the reproductive floral parts of the pinto bean. This led to many of the manipulated flowers either immediately falling off the plant or being aborted shortly thereafter. Previous field studies have also had little success due to similar issues with fragile flowers and manipulation (Bliss, 1980). Overall, there was a trend for the control plant yield to be higher than the manipulated treatments at either location. However, Carrington had no significant difference in yield. These results could indicate that the manipulation and pollination methodologies negatively affected yield. Conversely, several other studies have found insect pollinators to either be equal or increase dry bean yield compared to solely self-pollinating (Ibarra-Perez et al., 1999 and Mireille et al., 2012), which leads us to believe our methodologies caused the discrepancies between past studies and our own.

We acknowledge there were several other aspects of the project that affected our final results. We had some planting inconsistencies that led to a much lower seeding rate in Hettinger in 2019. Additionally, it was difficult to observe and capture insects with the pinto bean plants due to the bushy, indeterminate growth of the plant (Osorno et al., 2017; NDSU Extension, 2019). Most of the flowers were located under foliage, so netting insects proved difficult at times. Moreover, the dry bean plantings were located directly adjacent to annual forb plantings in

both locations with Carrington also surrounded by other flowering crops that attract pollinators, which could have caused a competition between the dry beans and the variety of different flowering plants available in the area. Additionally, there was a drought in 2020 which could have led to reduced plant and flower abundances, and subsequently, reduced pollinator visits.

Our objectives were to observe and identify self-pollinating dry bean crop visitors and assess the effect of those visitations on overall dry bean yield. We were able to successfully observe and document a pollinator community that visits dry bean crops in North Dakota. Farmers and other land managers can use this information to direct future management strategies in these self-pollinating systems where there were previously no assumed pollinator interactions. Some pollinator-friendly management such as reduced agrochemical use and the increase of local plant biodiversity (crop or otherwise) could be used to encourage these interactions instead of suppressing them (Garibaldi et al., 2014 and Kovács-Hostyánszki et al., 2017). Further investigation is still needed to fully determine the effect of pollinators on dry bean crops. Due to the issues in the field, greenhouse crosses may be the most effective method to further evaluate the effect of cross-pollination on dry bean yield (Bliss, 1980). However, knowing the pollinator community that visits the dry bean crops can provide useful information when trying to determine the effect of yield in future studies. Additionally, these interactions have the potential to boost local pollinator populations through floral resources independent of the effect on yield, which could still help support the surrounding ecosystem. To better support both crop production and pollinator communities, management practices should be considerate of pollinators and attempts should be made to support crop-pollinator interactions in the future.

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