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Using Insects to Improve Seed Quality and Farming Profitability in *Phaseolus vulgaris*

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Introduction

Phaseolus vulgaris, or dry beans, are generally cultivated for their seeds, which add flavor to the diets of millions of people throughout the world. This crop was originally domesticated in South America more than 7,000 years ago, eventually spreading north through Mexico and across most of the United States (NDBGA, n.d.). *Phaseolus vulgaris* belongs to the legume family, and like other legumes it can fix nitrogen in the soil from the air through a symbiotic relationship with a bacterium called *Rhizobia.* In addition to this, *P. vulgaris* is high in protein, and in many parts of the world, it is considered the cheapest way to acquire protein (Plants of the World Online, n.d.). In Nebraska, dry bean producers plant around 140,000 to 200,000 acres of beans annually, producing approximately 1 billion servings for human consumption. The production is concentrated in Western Nebraska where the climate is arid, and the warm days and cool nights provide excellent growing conditions for dry, edible beans. The state of Nebraska produces a greater amount of northern beans than any other state in the nation, is second in pinto and light red kidney production, and fourth in black bean production (Ostdiek, 2018).

Insect pollinators provide critical ecosystem services to many fruit, vegetable, and field crops that depend on pollination for fruit and seed production (Gill & O'Neal, 2015). *Phaseolus vulgaris* is a self-pollinating crop, and therefore does not require any pollinator agents for its reproduction. However, it is thought that flowers attract pollinators. Due to past research in crops from *Phaseolus spp.* and observations of pollinators in dry bean fields, it is believed that they increase yield and seed quality. Previous research papers such as Du Preez et al. (1975), and recent papers such as Kingha et al. (2012) and Doukal et al. (2013) confirmed that Hymenopterans are related to the increased of yield, size, and seed quality of *Phaseolus spp.* crops. They also recommended the planting of dry bean fields close to bee hives or nests to improve pod and seed production. Therefore, the conservation of pollinators such as Hymenopterans and a better understanding of pesticide application on fields are important. Lastly, the improvement of yield in the crop is essential to meet the demand in world nutrition.

The objectives of this study are to describe the pollinator community within dry bean fields, gain insight of pollinator taxa that may contact dry bean pollen, and verify if they affect yield and seed quality of the plant.

Field Site and Experimental Design (First season 2016)

The field site is located in Scottsbluff, NE at the experimental fields of the Panhandle Research & Extension Center from University of Nebraska-Lincoln (PHREC-UNL). Confection type sunflower seeds (*Helianthus annuus)* were first planted at the field site, and then intercalated with different market classes of dry edible beans *Phaseolus vulgaris* on June 20, 2016 (Figure 1). The field was divided by 4 replicates with 24 plots in total, and 50 feet in length each. Each plot had 4 rows of dry beans, and sunflowers were planted between each plot (Figure 2). We assigned different bean cultivars randomly to each plot. The different types of dry beans were pinto, great northern, black, cranberry, red kidney, and dark red kidney.

(**Figure 1**)

(**Figure 2**)

After the field was planted, irrigation pipes were installed, and the field was treated with insecticide to control populations of Mexican bean beetle weeks before starting with the data and pollinator collection. During pollinator and data collection, pesticide was not used. The field was managed by technicians from PHREC-UNL Entomology Program.

Bee Bowl Stands

Pollinators were collected at nine sampling points around the field using bee bowl stands. The collection started as soon as the beans bloomed. Bee bowl stands were built from polyvinyl chloride pipe (PVC) and cut to be two feet long. These stands were painted in satin claret wine (249083 Rust-Oleum paint can) and installed in a vertical position supported by an iron stake. The red color was selected to not interfere with the color of the traps and be clearly visible in the field. The bee bowls were 96-ml cups (3.25 oz. SOLO brand plastic soufflé cups, Bluff Sanitary Supply, Scottsbluff, NE) painted fluorescent yellow, fluorescent blue, and silica flat base (East

Coast Guerra Paint and Pigment, New York, NY) shown in Figure 3. After painting them, the bee bowls were installed onto the stands using two inch twisted brackets, one shelf brackets, and steel clamps (Figure 4). In the field, the bee bowls were filled with 50 milliliters of a soapy water solution made from Ultra Gain brand 2% aqueous solution. The bee bowls were set up three times for 24 hours over a time span of 3 weeks and during favorable environmental conditions (<30% cloud cover, no precipitation, and limited wind gusts). These are the conditions in which the majority of pollinator species are considered to be most active. The bee bowl stands were adjusted to canopy height according to the plant growth.

(**Figure 3**)

(**Figure 4**)

Sticky Traps and Sweep Nets

Yellow sticky traps and sweep nets were also used for sampling pollinators. Sticky traps were sheets of glue-coated cardboard dyed yellow. The yellow sticky traps were folded to create a double-sided trapping surface with each side measuring 9 inches by 5.5 inches. These traps were installed in the center of the field and adjusted at canopy height. Two yellow sticky traps were used each week (3 weeks in total) for 5 days in a row. Sweep nets were used for collecting pollinators flying around bean plots during the day (11:00am – 2:00pm). The bees collected with the sweep nets had visited the bean flowers.

Specimen Processing and Identification

Prior to identification, insects captured by bee bowls were processed according to methods described by Droege's (2015) *The Very Handy Manual: How to Catch and Identify Bees and Manage a Collection*. Books and identification guides from Evans (2007), BugGuide.net, and DiscoverLife.org were used to identify bees, flies, and other insects captured in the bee bowls. Individuals were identified from Order to Family. After identification, specimens were placed and identified in Whirl-Pak bags filled with 70% ethanol and stored inside a freezer. Insects with pollen grains attached and visible through a stereoscope were stored and identified for further investigations (Figures 5 & 6).

(**Figure 5**)

(**Figure 6**)

Results

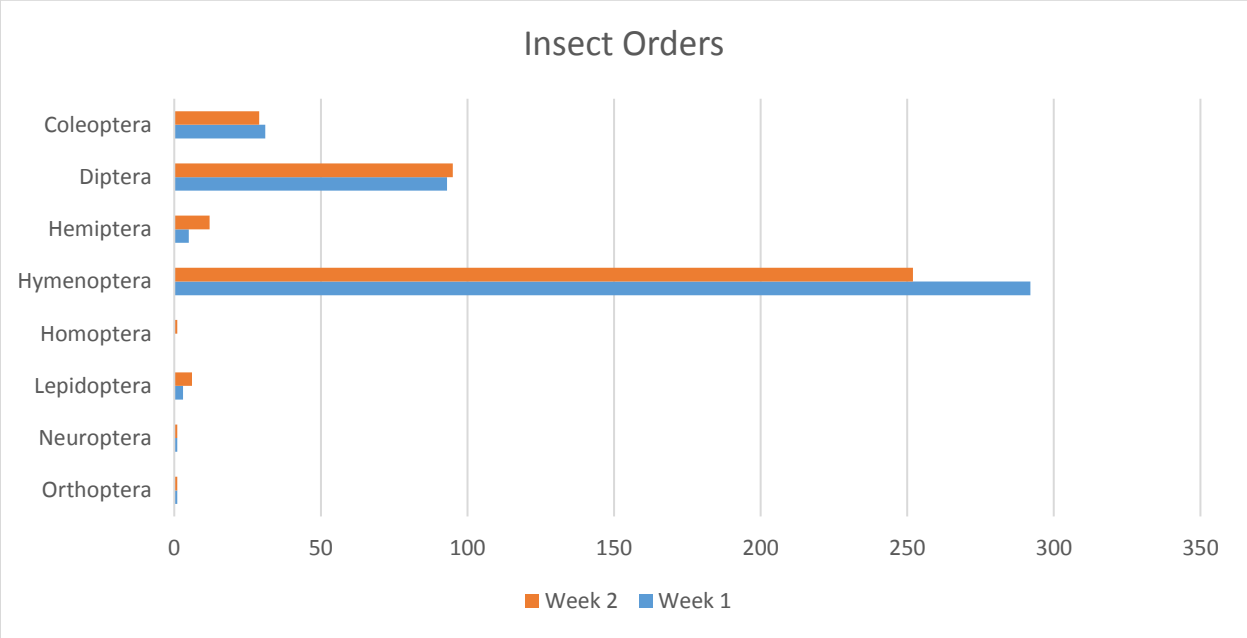
Bee Bowl Samples

Dates	Yellow Bowl	Blue Bowl	White Bowl
8/1/16	**141**	**46**	**8**
8/3/16	**158**	**55**	**19**
8/5/16	/	/	/
8/8/16	/	/	/
8/10/16	**117**	**45**	**14**
8/12/16	**173**	**48**	**2**
8/15/16	/	/	/

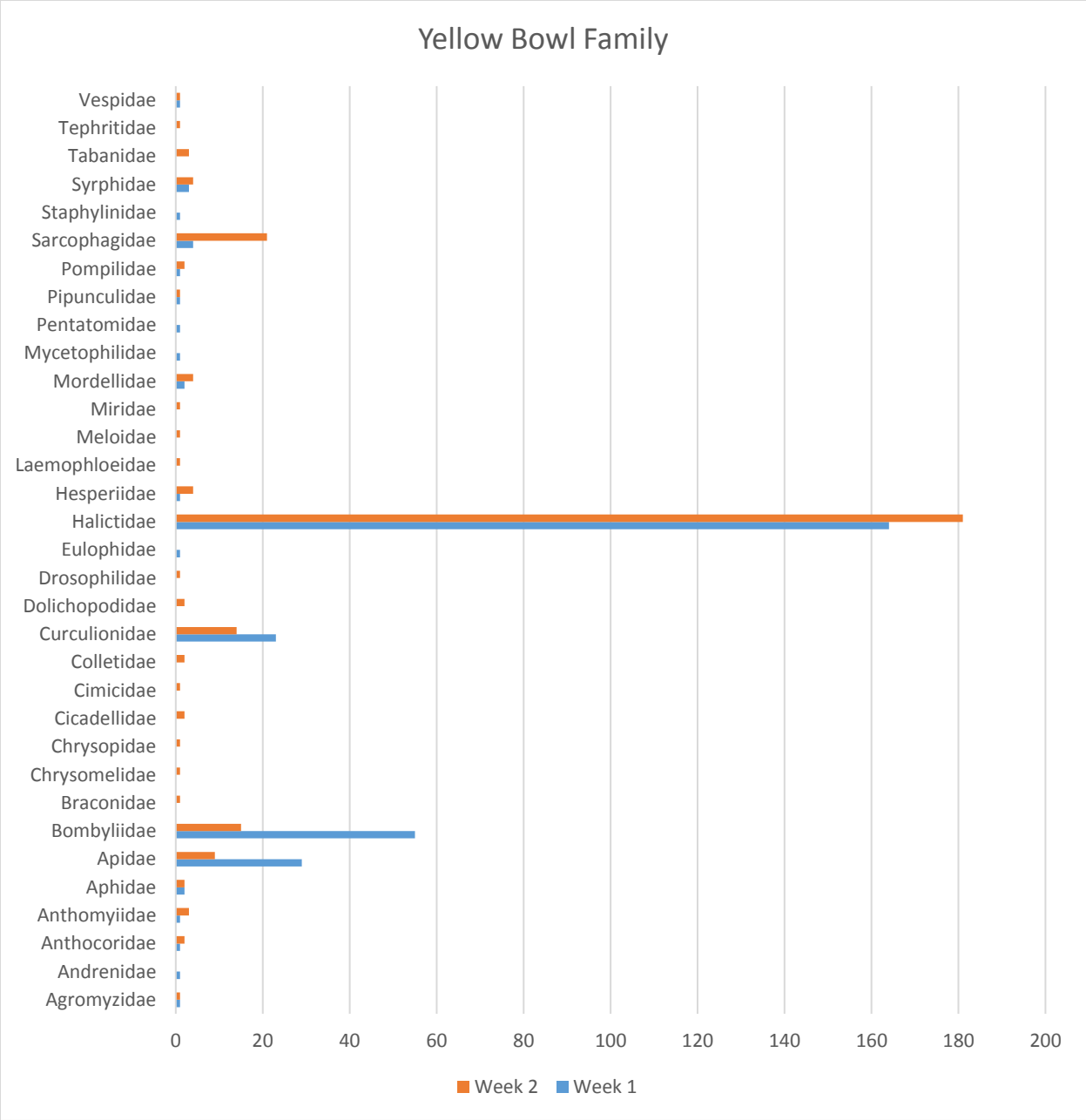
(**Table 1.** Number of insects collected on each bee bowl during the flowering)

Week	Yellow Bowl	Blue Bowl	White Bowl	Total of Insects
Week 1	**299**	**101**	**27**	**427**
Week 2	**290**	**93**	**16**	**399**
Week 3	/	/	/	/

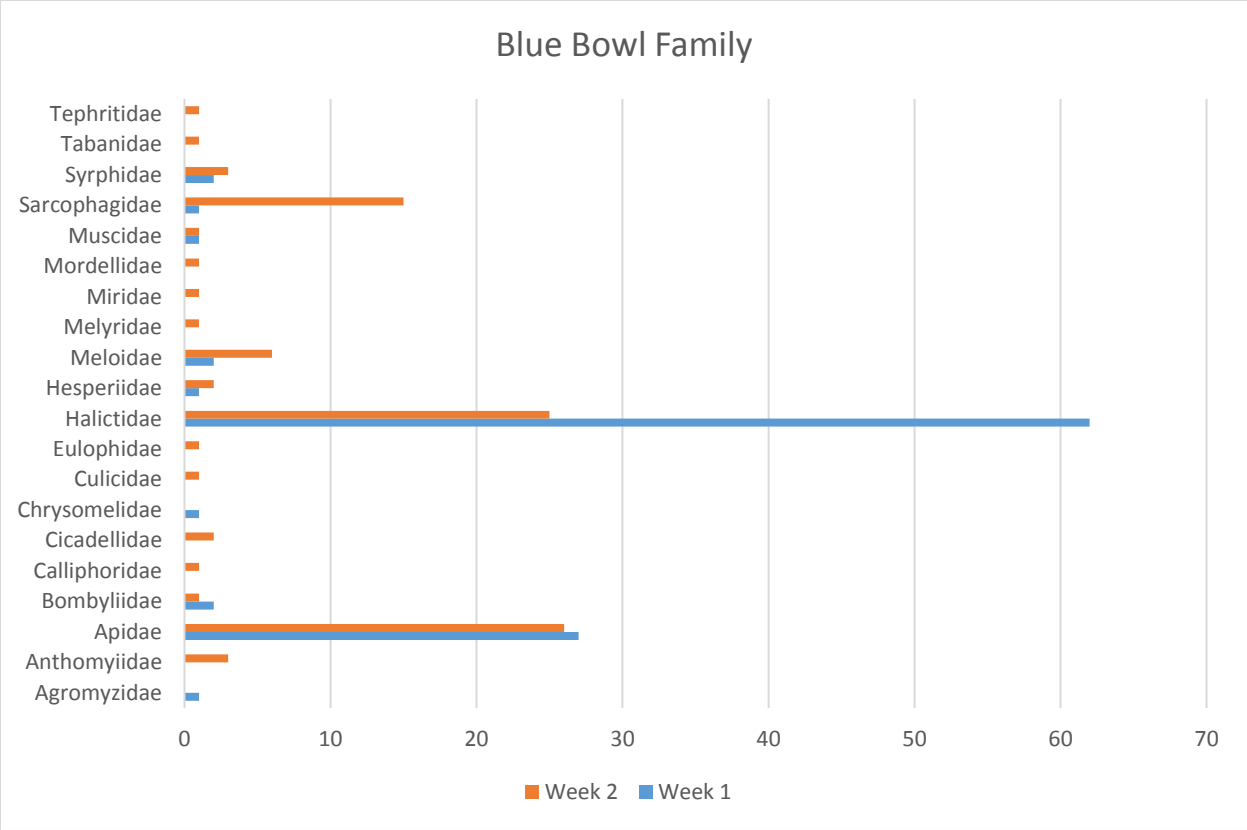
(**Table 2.** Total number of insects collected during each week)



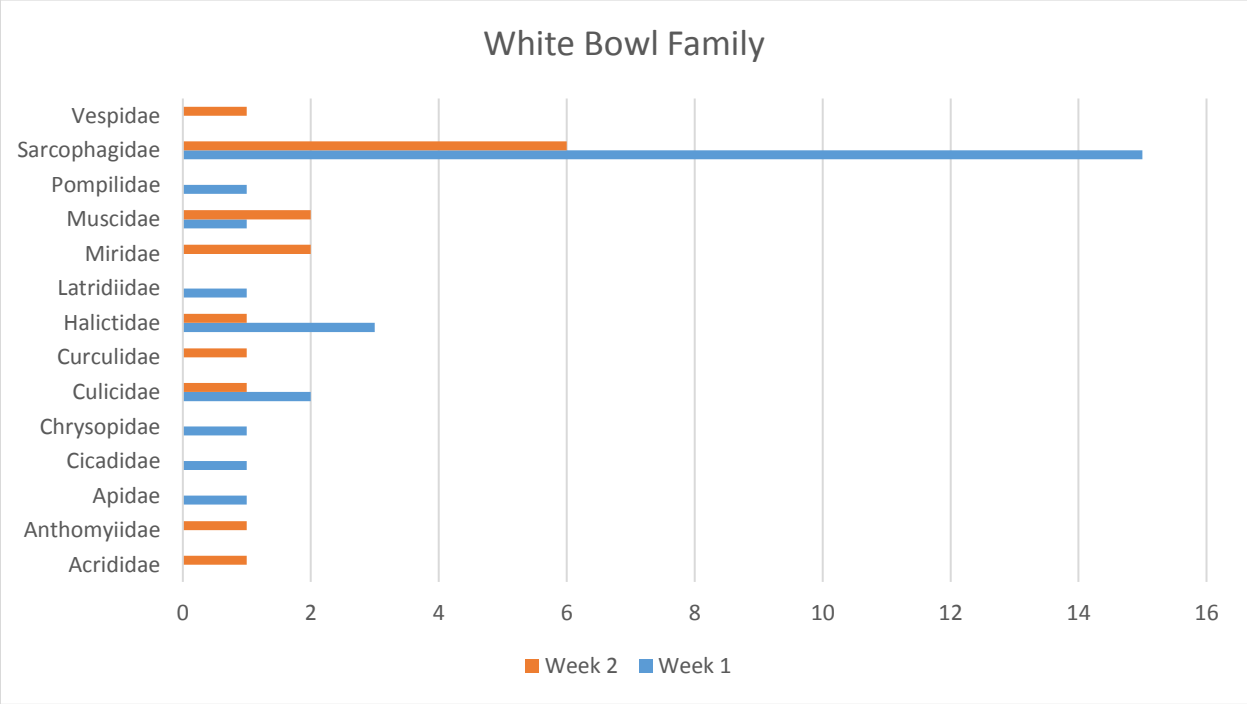
(**Graph 1.** Total insect orders identified on bee bowls)



(**Graph 2.** Number of families identified on week 1and 2 in yellow bowls)



(**Graph 3.** Number of families identified on week 1 and 2 in blue bowls)

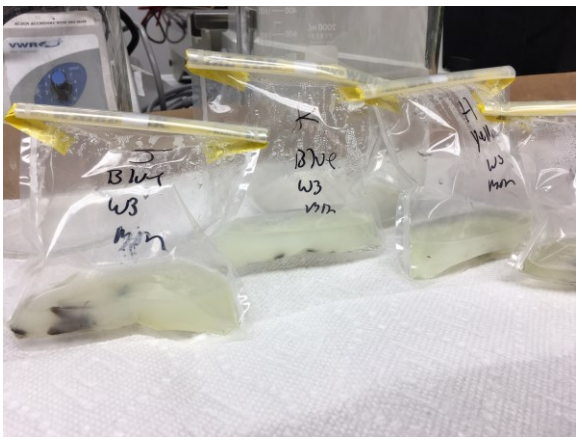


(**Graph 4.** Number of families identified on week 1 and 2 in white bowls)

Dates	T-High (F)	T-Low (F)	Rel Hum %	Solar Lang	Precip (in.)
8/1/16	**92.9**	**61.5**	**48.7**	**588.4**	**0**
8/3/16	**93.5**	**62.2**	**48.9**	**501.4**	**0**
8/5/16	**72.1**	**59.8**	**72.2**	**195.4**	**0**
8/8/16	**89.4**	**62.7**	**63.8**	**489.1**	**0.2**
8/10/16	**95.9**	**60.6**	**48.9**	**464.7**	**0**
8/12/16	**84.0**	**52.1**	**60.4**	**575.0**	**0**

(**Table 3.**Weather data through the 2 weeks of collection)

In total, 826 insects, including bees and flies, were captured in the bee bowls. The yellow bee bowls caught the greatest number of insects, with 299 insects during week 1, and 290 insects during week 2. They were followed by blue bee bowls, which captured 101 insects during week 1 and 93 insects during week 2. The white bee bowls caught the fewest insects, with 27 insects during week 1, and 16 insects during week 2 (Tables 1 &2). On August 5th and August 8th, bee bowls were not deployed because of unfavorable weather conditions (>30% cloud cover, high relative humidity, and low temperatures). Also, bee bowls were not deployed during week 3 due to pollen cross-contamination from sunflowers (Figure 7). By containing sunflower pollen, the captured insects will be contaminated, and it will not be possible to identify which insects contain only bean pollen. The most common insects identified during the two weeks were the Hymenopterans followed by the Dipterans and Coleopterans (Graph 1). The three families identified with the highest individuals were the Halictidae, followed by the Apidae, and lastly the Bombiilydae.



(**Figure 7**)

Sample	Pollen grain	Order	Name
#1	Yes	Hymenoptera	Carpenter Bee
#2	No	Hymenoptera	Bumblebee (brown belted)
#3	No	Hymenoptera	Mason bee
#4	Few pollen grains	Hymenoptera	Mason bee
#5	No	Hymenoptera	Carpenter Bee
#6	No	Hymenoptera	Mason bee
#7	Yes	Hymenoptera	Mason bee
#8	Few pollen grains	Hymenoptera	Bumblebee (brown belted)

(**Table 4.** Sweep netting samples on bees close to beans flower)

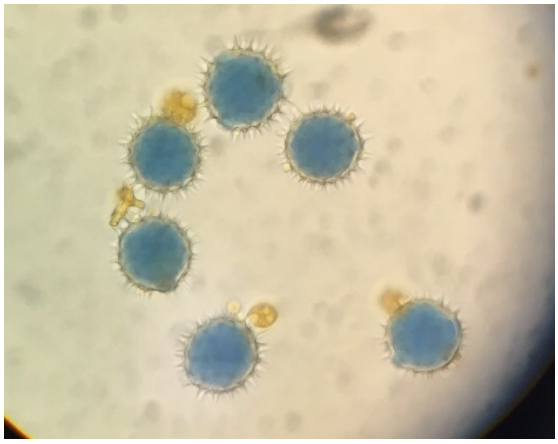
Sweep net samples were from dry bean fields and individuals were captured close or inside the bean flower during good weather conditions (Figures 8 & 9).

(**Figure 8**)

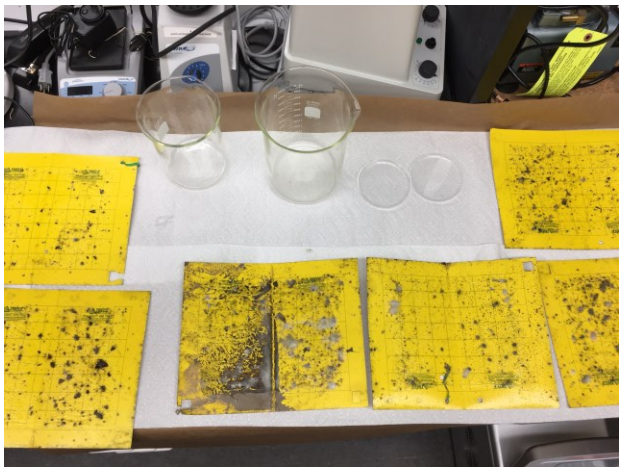
(**Figure 9**)

During the pollen analysis, different types of pollen grains were found on four different bees, as shown in Table 4, with pollen attached to the abdomen, thorax, and head. With help of the Phytopathology Department at the Panhandle Research & Extension Center (UNL), the bee samples that contained pollen grains were analyzed. First, a sample of *Helianthus annuus* was used to see and learn its shape and identify it from the other types of pollen found in the body of

the insects (Figure 10). Then pollen samples were taken from the pollinators' mouthparts. Microscope plates were made in the laboratory with pollen samples from insects, and a drop of lactophenol blue was added to give color for easier identification.



(**Figure 10**)



(**Figure 11**)

The yellow sticky traps were collected from the field, and damage from abiotic factors due to long exposure through the week was observed (Figure 11). During the three weeks of collection, the sticky traps did not show significant individuals. Insects found on yellow sticky traps were Thysanopterans (thrips), Dipterans (fruit flies, flesh flies, and mosquitoes), and Lepidopterans (moths).

Field Site and Experimental Design (Second season 2017)

The field is located N 41º 53'40.9" W -103º 40'14.8" on private land in Scottsbluff, NE near a city water tower, and was planted on June 6, 2017 with only one commercial line named Draco. In addition, it was spaced with 30 inches rows and a population around 45,000 plants per acre (Figure 12). The owner performed all field maintenance, irrigation, and agronomic practices necessary. Pesticides were not used in the field.

(**Figure 12**)

Bee Bowl Stands

Pollinators were collected at ten sampling points randomly in the field and divided by two replicates using bee bowl stands. The collection started as soon as beans bloomed for two weeks. Bee bowl stands were the same from the first season, and the bowls were repainted with the same paint codes. Additionally, the bowls were filled with the same specifications of soapy water from the previous season.

(**Figure 13)**

Bee bowls were deployed three times for 24 hours each over a course of 2 weeks, and during favorable environmental conditions (<30% cloud cover, no precipitation, and limited wind gusts). The bee bowl stands were adjusted to canopy height two times during the floral time (Figure 13).

Dry Beans Screened Cages

Previous research papers such as Du Prees et. al (1975) and recent papers such as Kingha et. al (2012) and Doukal et. al (2013) confirmed that Hymenopterans are related to increased yield, size and seed quality of *Phaseolus spp.* crops. Based on the previous research, it was decided to build cages inside the row to exclude them from pollinators. The cages measure 2 inches by2 inches by 20 inches, and they were built using galvanized metal wire and screens (ADFORS 48" x 84" Clear Advantage Fiberglass) from the local hardware store (Figure 14).

(**Figure 14**)

The cages were assigned randomly in the field, and 20 cages divided by two replicates. In addition, the cages were deployed only for two weeks during flowering. Soon after the season ended, they were harvested and compared with the control treatment right next to the row.

Specimen Processing and Identification

Prior to identification, insects captured by bee bowls were processed again according to methods described by Droege (2015) in *The Very Handy Manual: How to Catch and Identify Bees and Manage a Collection*. Books and identification guides from Evans (2007), BugGuide.net, and DiscoverLife.org were used to identify bees, flies, and other insects captured in the bee bowls. Individuals were identified from Order to Family. After identification, the specimens were placed and identified in Whirl-Pak bags filled with 70% ethanol and stored inside a freezer. Insects with pollen grains attached and visible through a stereoscope were stored and identified separated from the others.

Second Season Results

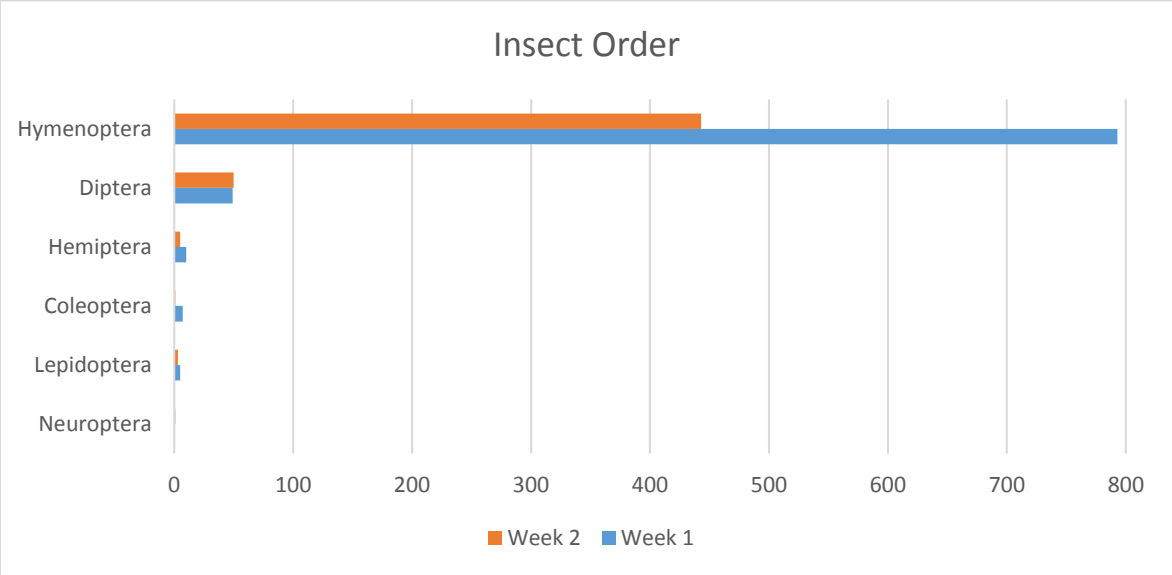
Bee Bowl Samples

Dates	Yellow bowl	Blue bowl	White bowl
25-Jul	143	196	13
27-Jul	/	/	/
29-Jul	196	294	23
31-Jul	107	131	10
2-Aug	40*	58*	4*
4-Aug	61*	78*	14*

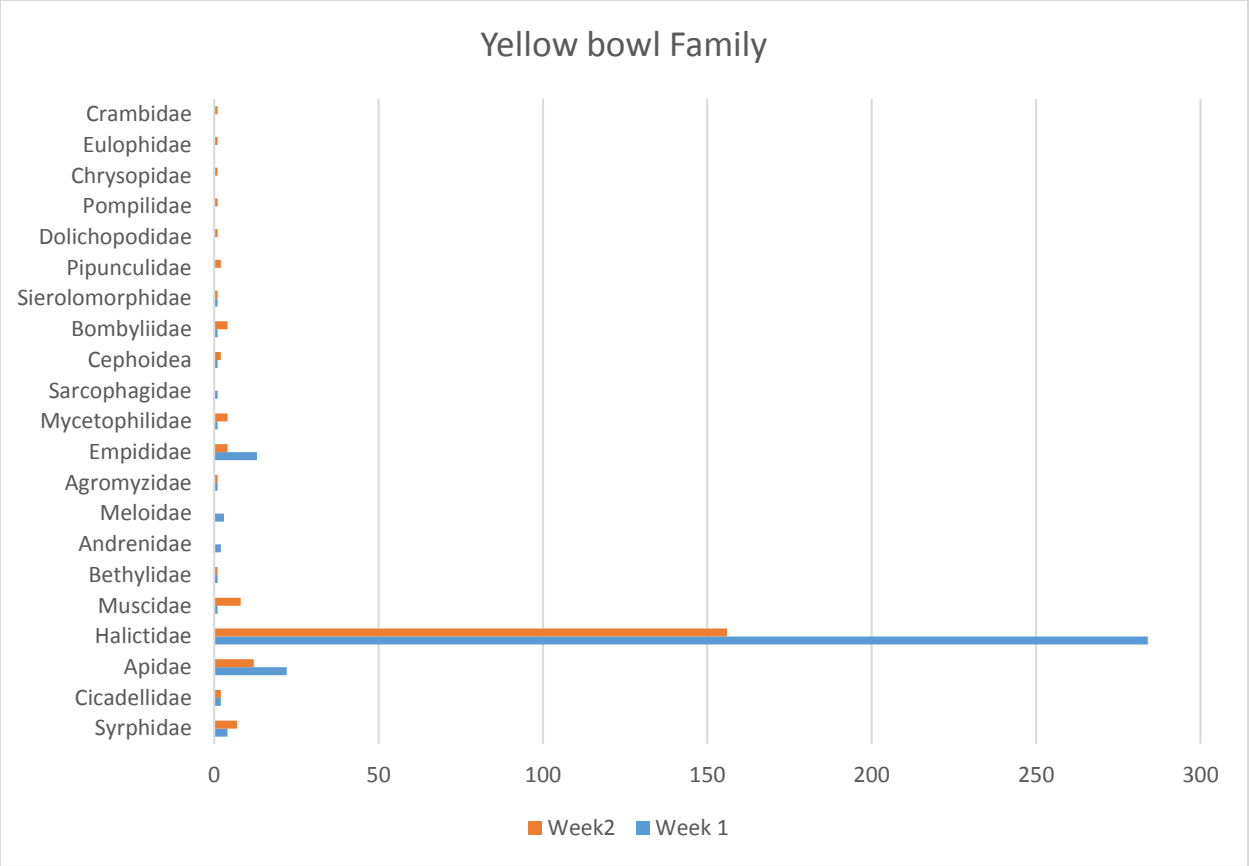
(**Table 5.** Number of insects collected on each bee bowl during flowering.
* only five bee bowls where deployed.)

Week	Yellow bowl	Blue bowl	White Bowl	Total of insects
Week 1	339	490	36	865
Week 2	208	267	28	503
				1368

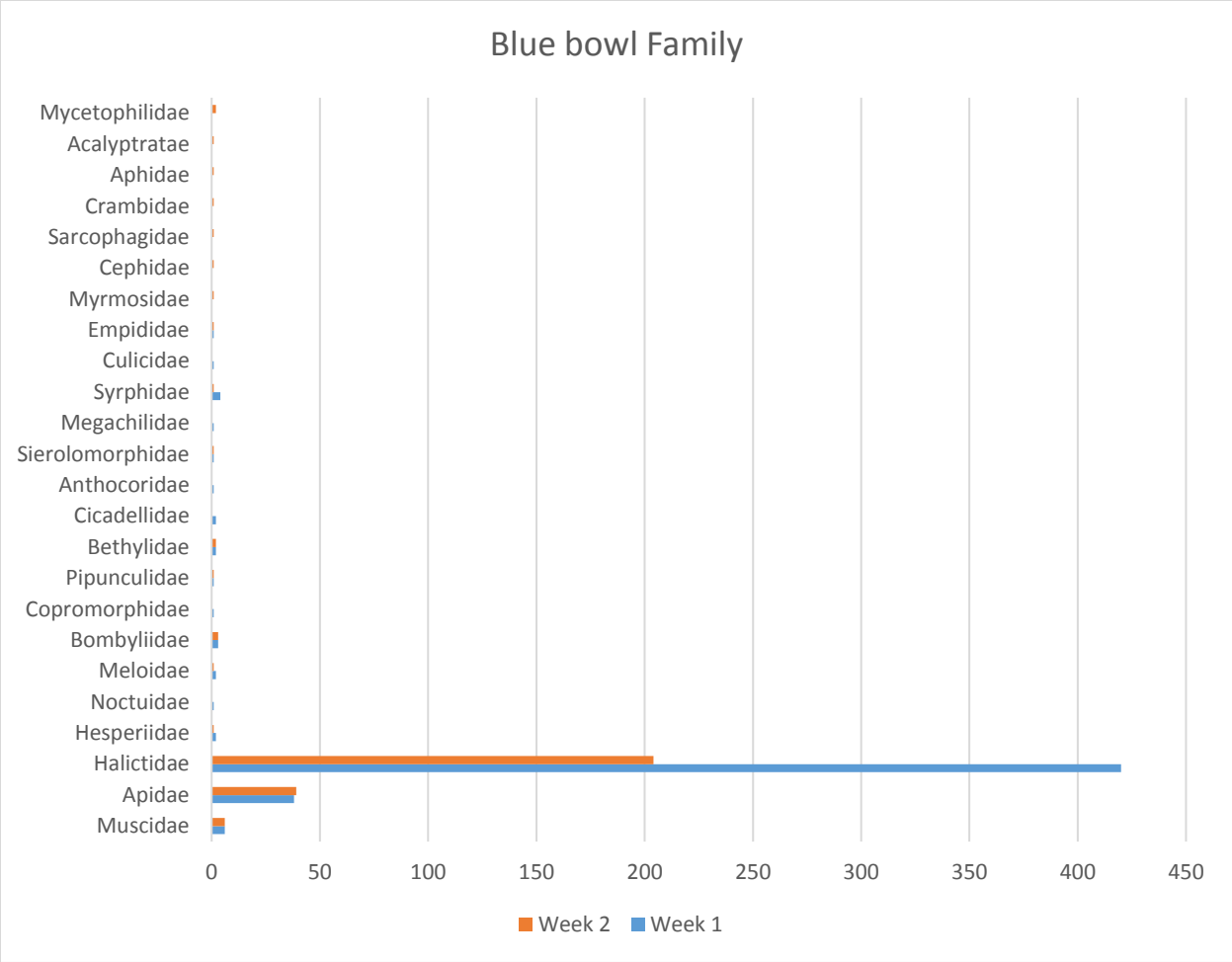
(**Table 6.** Total number of insects collected during each week.)



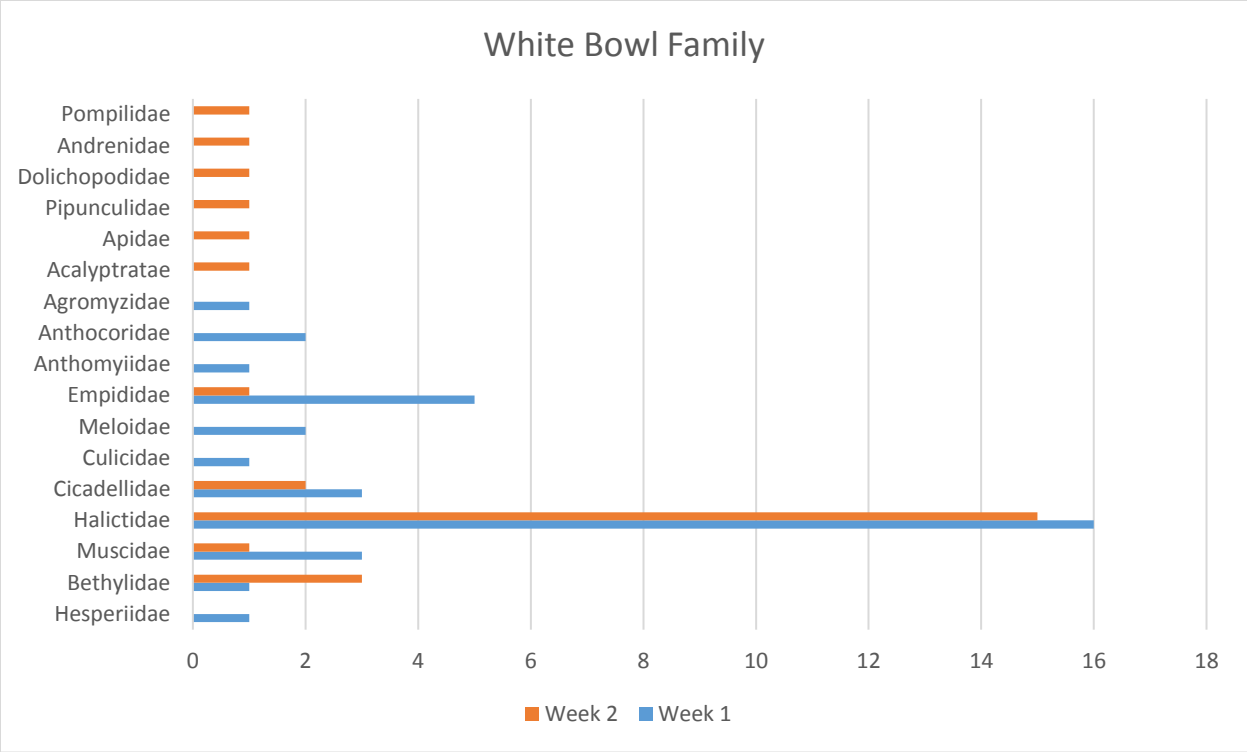
(**Graph 5**. Total insect orders identified on bee bowls.)



(**Graph 6.** Number of families identified in the yellow bowl for 2 weeks.)



(**Graph 7.** Number of families identified in the blue bowl for 2 weeks.)



(**Graph 8.** Number of families identified in the white bowl for 2 weeks.)

Dry Beans Cages

Replicate 1

TREATMENT

CAGE	#PLANTS	#PODS	YIELD (g)	100seeds (g)
1	6	52	71.6	42.2
2	5	39	58.1	38.5
3	5	44	53.5	33.8
4	5	40	57.8	38.8
5	6	43	44.3	34.8
6	9	61	101.7	42.9
*7	7	52	39.2	30
8	7	47	55.4	35.6
9	9	69	73.1	30.8
10	7	47	60.6	35.5
	66	494	615.3	36.29

CONTROL

CAGE	#PLANTS	#PODS	YIELD (g)	100seeds (g)
1	6	70	95.7	36.8
2	5	56	82.7	35.4
3	5	60	91.2	33.3
4	5	68	99.9	35.2
5	6	55	68.1	31
6	9	146	208.5	37.8
7	7	91	146.6	40.8
8	7	101	169.9	39.1
9	9	139	244.8	40.1
10	7	64	86.8	34.9
	66	850	1294.2	36.44

Replicate 2

TREATMENT

CAGE	#PLANTS	#PODS	YIELD (g)	100seeds (g)
1	8	67	51.8	30.8
2	7	50	46.2	33.7
3	7	64	63.5	28.8
4	6	43	58.7	38.7
5	6	54	62.1	34.9
6	6	53	73.4	39.5
*7	8	50	53.2	33.2
8	5	44	55	34.5
9	7	48	52.2	31
10	6	42	49.7	32.8
	66	515	565.8	33.79

CONTROL

CAGE	#PLANTS	#PODS	YIELD (g)	100seeds (g)
1	8	87	105.4	32.9
2	7	88	103.6	34.1
3	7	131	156.3	32.1
4	6	95	148.8	36
5	6	140	230	38.1
6	6	53	75.5	36
7	8	83	123.7	37.3
8	5	66	107.8	39
9	7	145	238.6	38.2
10	6	99	173.6	36.7
	66	987	1463.3	36.04

Exclusion Cage Results

BLOCK 1		CAGE	NO CAGE	DIFF
	Pods/plant	**7.57**	**12.59**	**5.01**
	CWT (g)	**0.55**	**0.55**	**0.00**
BLOCK 2				
	Pods/plant	**7.86**	**15.10**	**7.24**
	CWT (g)	**0.51**	**0.55**	**0.03**

(**Table 7.** Cage differences within treatment.)

Weather Data 2017

Dates	T-High (F)	T-Low (F)	Rel Hum %	Solar Lang	Precip (in.)
25-Jul	**93.3**	**66.3**	**49.**	**393**	**0**
27-Jul	**88.1**	**62.6**	**78.**	**427**	**.26**
29-Jul	**85.1**	**63.5**	**84.**	**310**	**0**
31-Jul	**88.9**	**58.2**	**67.**	**523**	**0**
2-Aug	**84.3**	**58.8**	**64.**	**499**	**.06**
4-Aug	**87.2**	**49**	**64.**	**537**	**.01**

(**Table 8.** Weather data through the 2 weeks of collection.)

In total, 1368 insects were captured in the bee bowl stands during the second season. The blue bee bowls captured the greatest number of insects with 490 insects during week 1 and 267 insects during week 2. This was followed by the yellow bee bowls, which captured 339 insects during week 1 and 208 insects during week 2, and finally, the white bee bowls with 36 insects

during week 1 and 28 insects during week 2 (Tables 5 & 6). On July 27th, bee bowls were not deployed because of unfavorable weather conditions (>30% cloud cover, high relative humidity, and low temperatures). In addition, on August 2nd and August 4th, only 5 bee bowls per color were deployed due to pollen cross-contamination from a nearby corn field. By containing corn pollen, the captured insects will be contaminated, and it will not be possible to identify which insects contain only bean pollen. The most common insects identified during the two weeks were the Hymenopterans, followed by the Dipterans and Hemipterans (Graph 5). The four families identified with the highest individuals was the Halictidae, followed by Apidae, Muscidae, and Empididae.

After harvest season, all data from the dry bean cages were collected and analyzed. The first replicate cage treatment had 66 plants, 494 pods, and a yield of 615.3 grams of seeds. In the control treatment, 850 pods were harvested with the same amount of plants and had a yield of 1294.2 grams of seeds. Also, in both treatments 100 seeds were counted and weighed randomly per cage and ended with a similar weight, which averaged 36.36 grams. There was only a difference of 0.15 grams between the two treatments. In the second replicate, both treatments had 66 plants. The cage treatment had 515 pods, a yield total of 565.8 grams of seeds, and an average of 33.79 grams of 100 seeds. The control treatment had 987 pods, a yield total of 1463.3 grams of seeds, and an average of 36.04 grams per 100 seeds. There was only a difference of 2.25g/100seeds between the two treatments.

During the last 4 days of the second week, plants inside the cage started to grow bigger, taking the same shape of the cage. This effect caused reduction or abortion of flowers and created a microclimate inside the cage. This affected the plant performance, resulting in differences from the control.

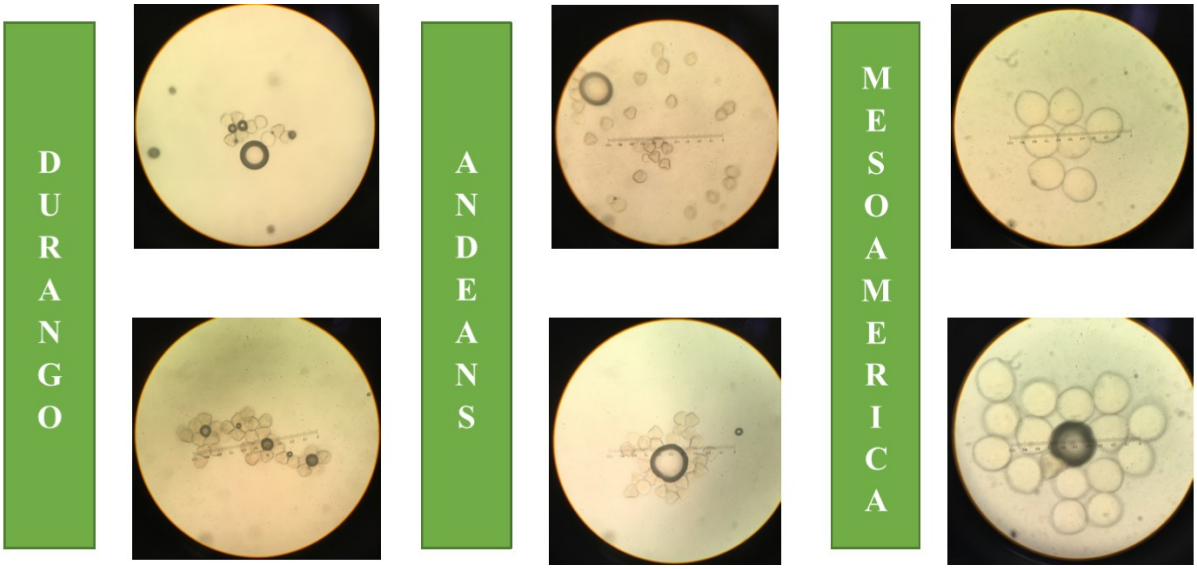
Time-lapse Cameras

Time-lapse cameras were used in the field as another option to verify which pollinators visited the flowers. These cameras were installed in four different areas within the two replicates for 24 hours. To aid in identification, cameras were placed approximately six inches from the flowers to allow for close views of insects (Figures 15 & 16) and were programmed to capture an image every ten minutes. As a result, non-pollinators, like spiders and beetles, were captured with the time-lapse cameras.

(**Figure 15**)

(**Figure 16**)

Pollen Analysis



(**Figure 17**. Six different pollen samples with their domestication regions. From left to right: Durango composed of Pinto Bean and Great Northern; Andeans composed of Cranberry and Red Kidney; and Mesoamerica composed of small red and black beans.)

Six different market classes were collected and observed through a microscope for identification. These pollen samples helped to identify pollen by size and shape, and the pollen found on the pollinators captured in the bee bowls. After potential pollinators with pollen grains were inspected, it was concluded that medium size bees from the family Apidae carry pollen that

is shaped most like *Phaseolus vulgaris* pollen. In addition to this, during the first season it was found that medium size bees from the family Apidae carry Mesoamericans pollen grains, and Durango pollen grains during the second season. This observation matches the field design when during the first season the field was planted with different market classes of beans and the second season only Great Northern beans.

These results indicate that pollinators are carrying dry bean pollen grains when visiting flowers on the field. However, knowing that *P. vulgaris* is a self-pollinated crop, further investigations are required to know what effects bees have on the crop.

Data Discussion

The majority of insects and the diversity of pollinators captured was mainly from the bee bowl samples. Using only 48 bee bowls in the first season (2016) on the first week of collection, 427 individuals were collected. In the second week, a decrease of individuals was observed with 399 insects collected from 45 bee bowls. The three main orders captured in total on bee bowls were the Hymenoptera (544), Diptera (188), and Coleoptera (60). It was also observed that Hymenoptera decreased from week 1 to week 2, while an increase of Dipterans was observed. In the yellow and blue bee bowls, large quantities of the Halictidae (sweat bees) were found, followed by Apidae, Bombyliidae, and Sarcophagidae (flesh flies). During week 2 there was an increase of Halictidae and Sarcophagidae individuals.

During the second season (2017), 60 bee bowls were used in the first week, collecting a total of 865 individuals. In the second week, only 503 individuals were collected using 60 bee bowls. The three main orders captured in total in the bee bowls were the Hymenoptera (1236), Diptera (99), and Hemiptera (13). The decrease observed during the second week was probably due to a nearby blooming corn field that attracted pollinators and predators. Again, during the second season, the yellow and blue bee bowls captured the largest amount of families from Halictidae, followed by Apidae, Empididae, Muscidae, and Syrphidae. These results collected during both seasons is consistent with other studies (Douka et al. 2013, Gill et al. 2015 & Kingha et al. 2012) where the order Hymenoptera made up the majority of insects, and from the families Apidae and Halictidae.

It may be suspected that the decrease of insects captured in bee bowls was due to an increase of sunflower (2016 season), and corn blooming (2017 season). The majority of the

pollinators found during the first season were from the order Hymenoptera, which move to the flowers of *Helianthus annuuss* looking for pollen nectar. Also, it was observed on week 1 that a high population of Bombyliidae individuals were hovering around bean flowers because of the high infestation of grasshoppers. This increase of population from Bombyliidae individuals is because the larvae are parasitoids of the Orthopterans. Dipterans from the family Sarcophagidae were found more during week 2 due to their behavior of parasitoids. Sarcophagidae flies differ from others because they are ovoviviparous, and some flesh fly larvae are internal parasites of other insects. During week 2, we found more orders and families of insects due to the flowering of sunflowers.

Sweep net samples (2016) were only captured in fields with dry beans and with favorable weather conditions for pollinators. Only 8 pollinator insects from the order Hymenoptera were captured with only 4 showing pollen grains. It was hypothesized that bumblebees were going to be the major pollinator with pollen grains because of observations at the field sites, but during the pollen analysis they did not shown any pollen. Mason bees and carpenter bees were the most abundant with pollen grains present on their body. This observation is similar to the Kingha et al. 2012 study. They observed that species of carpenter bees was the main floral visitor of *P. vulgaris.* Also, they observed a high activity of carpenter bees on *P. vulgaris* flowers between 10.00 and 13.00 h. The carpenter bee foragers had a high affinity with respect to *P. vulgaris* when compared to the neighboring plant species, indicating their faithfulness to this Fabaceae, a phenomenon known as "floral constancy" (Kingha et al. 2012). Due to this observation, carpenter bees can provide benefits to pollination management on dry beans.

Although a good number of pollinators were collected in bee bowl stands, in sweep net samples and field observations, *Apis spp* or honey bees were only found one time (2017). This is consistent with other studies that have used similar collection methods for pollinators. There may be several explanations for this including the capacity of *Apis spp.* to either avoid or escape from bee bowls because of their size or a general disinterest in dry beans as a source of nectar or pollen. The size of the bee bowls likely does not explain the lack of *Apis spp.* captured, as we found many similar sized bees in the bee bowls. Another explanation for the low abundance and possibly other species, is that nearby floral displays may have influenced foraging behavior, detracting from the attractiveness of either the dry bean flowers or the traps (Gill et al. 2015). However, in the field plot that was planted, surrounded, and intercalated with *Heliaunthus*

annuuss (season 2016), *Apis spp* were not found either in the samples collected. The effect of Colony Collapse Disorder and the global decline in pollinators reported (Potts et al. 2010) appears to have an effect on the Panhandle area of Nebraska.

Recommendations

Regarding the field design from the first season, an agronomic sized field of dry beans with only common cultivars and with growth habit type 1 is suggested. Dry beans with growth habit type 1 are determinate and have flowers at the end of the branches, making it easily for pollinators to see the bean flowers. Also, field sites should have only the crop for the experiment, in this case dry beans, and not any other crops, such as sunflowers, that attract many insects after blooming. Sunflowers produce allelochemicals and volatiles at early growth stages, and that causes high population of insects from the family Vespidae and Phymatinae. In order to help to increase the number of beneficial insects, it is better to have a more organic crop without the use of pesticides.

The bee bowl stands that were used worked as expected, but for future experiments it is suggested to change the fluorescent yellow and the fluorescent blue to colors that match the colors of the bean flower. Finally, instead of using the dry beans for the production of honey related products with beekeepers, it is better to first try using a greenhouse. Inserting species of *Apis spp.* and inoculating beans plants inside a greenhouse can be another option to observe if honey bees produce honey from the nectar of bean flowers. With expected results and increases of bean yield, the experiment can proceed to a bigger scale.

During the pollen analysis, DNA pollen tests should be conducted to confirm that the pollen grains observed on the head, thorax, and abdomen of pollinators are from *P. vulgaris* and not from another legume crop. Further studies are required and recommended.

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