Research Project Title: North Dakota Soybeans & Pollinators: Beginning to Investigate Their Potential Interactions and Mutual Benefits
Principal Investigator: Jason Harmon, North Dakota State University
Co-Investigators: Torre Hovick and Kevin Sedivec, North Dakota State University

Research Overview and Objectives: background information and research gaps

Pollinators provide a crucial service by transferring pollen across plants and facilitating plant reproduction. About 75% of the world's flowering plants and 35% of the world's food crops depend on animal pollinators to reproduce. However, soybeans are not one of those plants that require a pollinator to reproduce. While that may suggest that soybeans and pollinators need nothing to do with each other, recent evidence suggests that this is far from true (Fig. 1). A landmark review showed that most crops can benefit from pollinators, regardless of how they reproduce, and another review showed a large potential for pollinators to benefit from visiting these plants.

Fig. 1. A bumblebee visiting a soybean flower. Photo credit: A. Varenhorst.

Additional research suggests that pollinator visits to soybeans have the potential for boosting yield. When looking at decades of research from across the world's soybean producing regions, a review found that soybean yield can increase as much as 126%, pod set by 91%, and seed set by 249% with high pollination compared to no pollination. On average, the increase to soybean yield across 23 comparisons was conservatively set at 21%, which can be higher than the yield benefit from genetic and other management factors. These are tantalizing results; however, it is crucial to recognize that some studies found absolutely no benefit from increased pollination. This variation is incredibly important, especially as we do not yet know why there are different results in different studies. Most importantly for North Dakota soybean growers, are results regionally specific such that we would never see dramatic increases in yield, or is it possible that we have the pollinator species and soybean varieties that could benefit from pollinator visits? Answering that question will require a great deal of effort, particularly in terms of knowing how general any benefits might be and what management options could be used in North Dakota to encourage the right pollinators. However, important first steps towards this goal include identifying what different pollinators currently visit North Dakota soybeans.

While the potential for soybeans to benefit from pollinator visits is an exciting possibility, it is also worth exploring how soybeans may benefit North Dakota's pollinators. Pollinators require nectar to give them energy for their activities, and many types of pollinators, including bees, use pollen to help them reproduce. Soybeans can provide valuable nectar and pollen rewards in row-crop dominated landscapes that often have few other flowers. Soybean flowers are known to provide nectar high in sugar, which is particularly nutritious for pollinators. Even though soybeans only flower during a part of the growing season, that pulse is incredibly large and potentially very beneficial for pollinators that can use it. Honey bees, in particular, could benefit from this pulse, which would be of great interest to North Dakota soybean farmers as honey bees are a crucial industry to our state. Not only is North Dakota one of the top honey-producing states, but the bees that pollinate crops across the country come to North Dakota during the summer to rest and reproduce. This could lead to mutually beneficial collaborations between bee keepers and soybean producers. To build towards this long-term goal, we again need concrete first steps, including a better understanding of the species of pollinators that visit soybean flowers and the floral rewards that North Dakota soybeans may provide pollinators.

Objectives:

1. **Reveal which pollinator species are visiting and collecting pollen from ND soybean.**
2. **Measure the floral rewards (nectar and pollen) provided by North Dakota soybean.**

Materials and Methods

Objective 1. Reveal what pollinators are visiting and collecting pollen from ND soybeans.

While honey bees are the most iconic pollinator, there are many different insects that visit and pollinate different flowers in North Dakota. This includes different types of insects like bees, butterflies, and ants, as well as different species within those groups. For example, we recently found over 300 different bee species in ND grasslands. But not all pollinators visit all types of flowers, so it is important to identify what pollinators could be important for ND soybeans.

To accomplish this objective, we performed multiple pollinator surveys across soybean fields in central North Dakota. Our surveys were located at the Central Grasslands Research Extension Center and the Carrington Research Extension Centers near Streeter, ND, and Carrington, ND, respectively. The field sites we used were in collaboration with Drs. Miranda Meehan and Kevin Sedivec in their ongoing research project: *Enhancing Profitability of Soybean Production and Soil Health through Livestock Integration* (funded by North Dakota Soybean Council).

To survey pollinators in soybean fields, we used a combination of active and passive sampling techniques. We have demonstrated the power of this approach in previous research that showed how different methods capture different parts of the pollinator community. Surveys took place throughout soybean floral bloom, with 4 surveys in each field to account for variation in floral rewards and pollinator activity. Surveys occurred between the hours of 1000 and 1800 on days with less than 50% cloud cover and winds less than 25 km/h to maximize activity of all pollinators and avoid detection biases.

Active pollinator surveys. We actively sampled pollinators by freely searching for bees for 30 minutes within each soybean field. Surveyors walked at a consistent pace that was slow enough not to scare pollinators away. Whenever a surveyor observed a pollinator, they stopped the timer and caught the pollinator using a sweep net and placed it in a catch jar (Fig. 2). Surveyors gave special attention to pollinators actively visiting flowers, with notes about the flower. To identify bees, surveyors took ≥5 clear digital pictures within the jar. We then processed the pollinator using a cotton swab to remove and later identify visible pollen on the pollinator. Our goal was to minimize disruption, but, when warranted, we collected and preserved voucher individuals.

Fig. 2. Captured bees from active pollinator survey. Photo credit: B. Roberton


Fig. 3. Example of a blue vane transect on the edge of a soybean field. Photo credit: B. Roberton.

Passive pollinator sampling. We used transects of blue vane traps to complement active sampling (Fig. 3). Each transect was 6 blue vanes, with each trap placed on hooks to securely hold the trap at canopy height. We used lethal (soapy water or similar collection liquid) and non-lethal (crumpled newspaper or similar) materials inside different traps. In both cases, bees were retrieved, processed, cleaned, any pollen removed, and stored for identification using appropriate keys.

Floral surveys. We complemented every round of pollinator survey with a subsample of soybeans to estimate the percent of soybeans actively flowering.

Objective 2. Measure the floral rewards (nectar and pollen) provided by ND soybeans.


Fig. 4. Soybean plants growing in NDSU greenhouse. Photo credit: B. Roberton.

We grew soybeans within a greenhouse, a laboratory, and growth chambers at NDSU to attempt to quantify their nectar and pollen production. In all locations, artificial lights were used to create a 17h light:7h dark day, humidity was ~30% and temperature was 68-78° F. We used general purpose potting soil in 3-gallon pots and placed each pot on a tray at about 15-20 inches away from each other. We planted 3 soybean seeds per pot that were inoculated with Exceed Superior Seed Inoculant for Soybeans at the recommended dosage. The seeds were watered every other day and misted once a week. Growth stage was recorded three times a week when plants had reached the VE stage. When plants had reached a growth stage of V2-V4, we thinned down each pot to two plants to give them more growing room. We then added Osmocote smart-release plant food (14-14-14) at approximately 1/2 teaspoon per pot which was mixed into the top 1-3 inches of soil. When buds appeared, we added Jack's Blossom Booster 10-30-20 to half the pots. From then on, we gave each pot 3 cups of water three days a week (regular water for control pots and fertilizer water for treatment pots).

Research Results/Outcomes

Objective 1. Reveal what pollinators are visiting and collecting pollen from ND soybeans.

Active pollinator sampling found pollinators at both locations. However, we saw almost nine times more pollinators in soybeans at Streeter than at Carrington (Fig. 5A). Both active samples were made up entirely of honey bees and various species of bumblebees with the ratio of honey bees to bumblebees much higher at Carrington compared to Streeter. Across all active pollinator samples, 73% of bees were actively engaged with a soybean flower in a way that looked like nectaring behavior (Fig. 5B). This is an excellent sign that at least these groups are actively visiting soybean flowers and are likely benefiting from them as well.

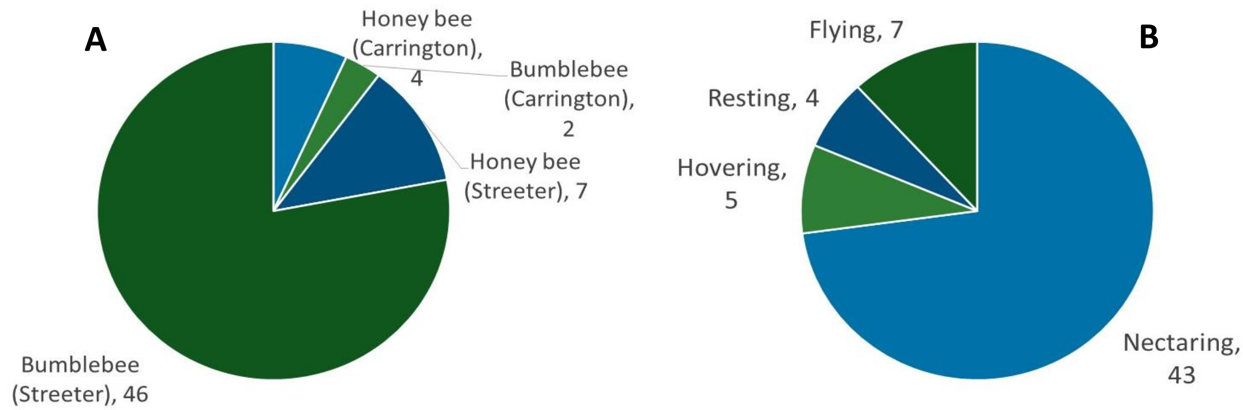

Fig. 5. Active sampling results from Carrington and Streeter broken down by (A) site and taxonomy or (B) the behavior of the pollinator when observed during the sample.

Passive sampling results complemented active sampling in that pollinators were caught in and adjacent to soybean fields and that there were substantial differences between the two locations (Fig. 6 A vs. B). Carrington soybean flowered earlier than Streeter, but pollinator counts were low during Carrington's peak flowering. Numbers increased to some extent during the later portion of flowering, but total trap catch across all sampling dates at Carrington was less than half what it was in Streeter (428 vs. 930). In Streeter, the number of pollinators caught appeared to increase as more soybean plants were flowering, however, the number of pollinators was even higher in the last sampling date when flowering was lower. Overall, it is difficult to use this data to understand how much the abundance of pollinators in our traps was related to the timing of flowering and how much was associated with the timing of other things in the landscape (e.g., timing of other plants, timing of pollinator life cycles, environmental conditions, etc.).

We identified bees caught in the two dates at each location with the highest trap catch (Table 1). Besides the previously mentioned difference in total abundance by location, there was also a difference in the composition of bees caught in each location. Longhorn bees were the most common bees found and made up a similar percentage of the bees caught in each location. Honey bees were relatively rare but were about 4% of bees caught in each location. The biggest compositional differences were that sweat bees were more commonly found in Carrington whereas bumblebees were more commonly seen in Streeter. Given that we actively observed bumblebees visiting flowers but did not see the same for sweat bees might mean that such locational differences could be important for how much species-specific soybean-pollinator interactions are seen in each soybean field.

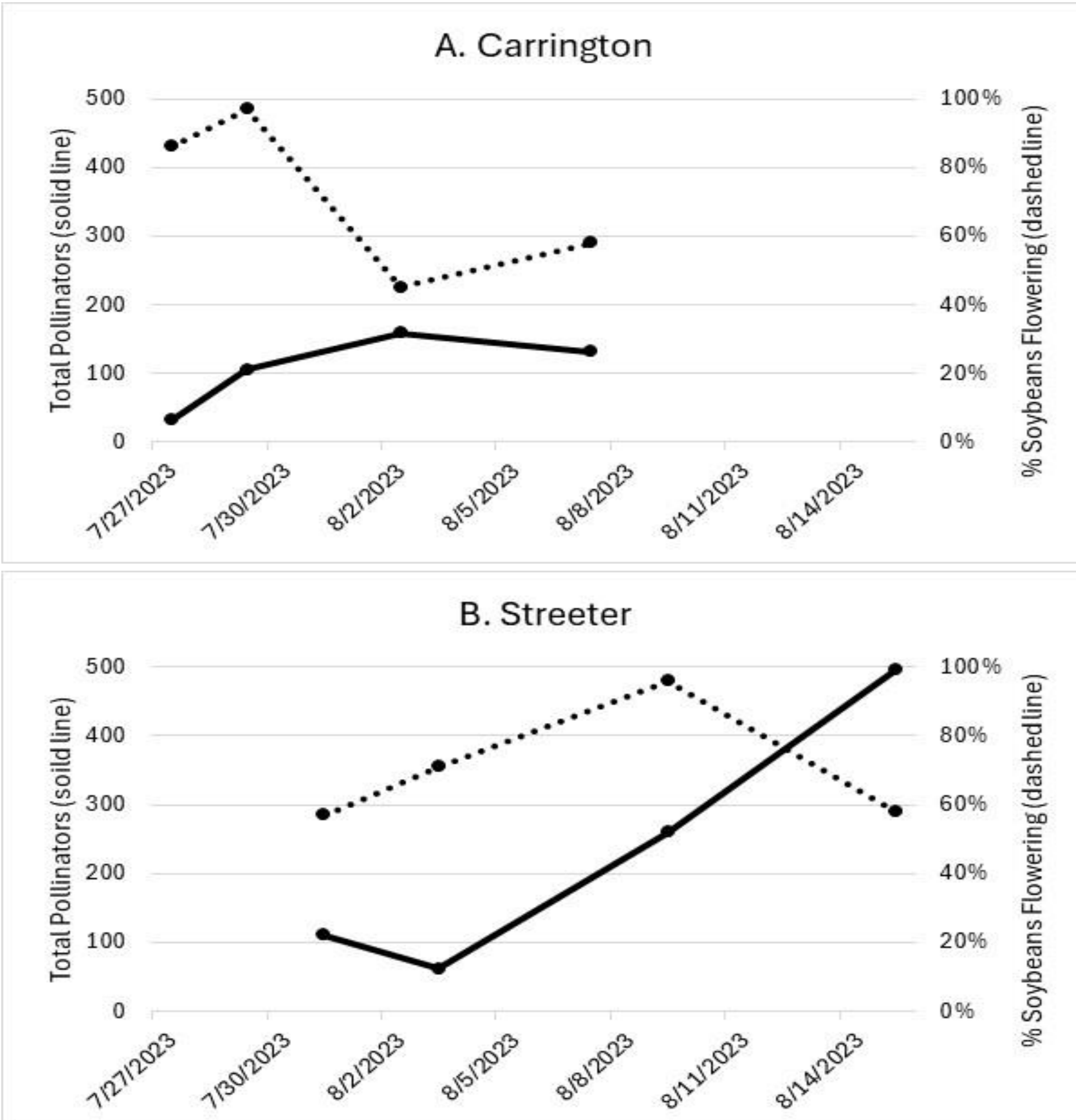

Fig. 6. Passive sampling results from (A) Carrington and (B) Streeter. Each graph shows the total number of pollinators caught at each site on a given trap at a given date (solid line) and the percent of soybean plants that were flowering at the time of the sampling. broken down by (A) site and taxonomy or (B) the behavior of the pollinator when observed during the sample (dashed line).

Bee	Carrington	Streeter
Longhorn bee	181 (74.2%)	547 (72.4%)
Sweat bee	50 (20.5%)	65 (8.6%)
Bumblebee	1 (0.4%)	106 (14.0%)
Honey bee	10 (4.1%)	28 (3.7%)
Cleptoparasitic bee		5 (0.7%)
Leafcutter bee	1 (0.4%)	1 (0.1%)
Masked bee		2 (0.3%)
Miner bee		2 (0.3%)
Mason bee	1 (0.4%)	

Table 1. Identity of bees caught in passive traps in and adjacent to soybean in Carrington and Streeter. The number is the total number of bees from that group and the % is the percentage of bees from a location that belonged to that group.

Objective 2. Measure the floral rewards (nectar and pollen) provided by ND soybeans.
Unfortunately, three separate attempts with plants growing in a combination of a greenhouse, open lab space, and controlled growth chamber, all failed to produce flowering soybeans. Plants produced buds, but skipped opening flowers and went straight to pod development (Fig. 7A). This is apparently common with stressed soybean plants (*C. Miranda pers. com.*), so we will try to find better conditions and potentially use multiple varieties of soybean in the future.

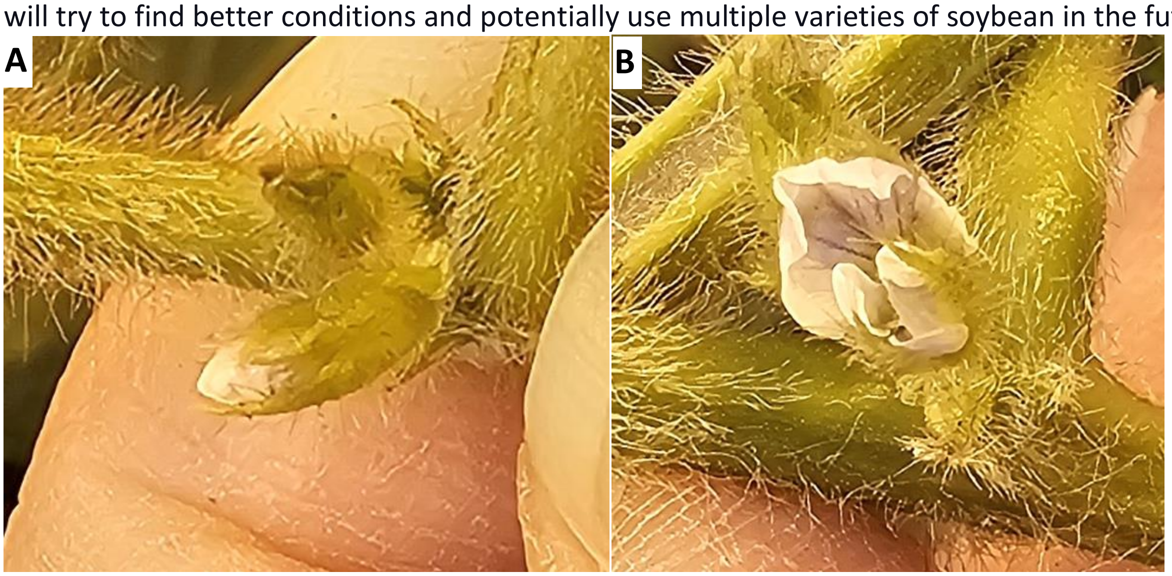

Fig. 7. (A) Typical results of growing soybeans inside where soybean flowers began to peek out, but did not open further. (B) Physical manipulation of flowers to open them further than they would on their own. Photo credit: B. Roberton.

Listings of any disclosures of inventions or plant varieties
Nothing to disclose.

Discussion
Our active sampling showed honey bees and bumblebees visiting soybean flowers, especially at Central Grasslands REC at Streeter, ND. This was likely an attempt to receive nectar and/or pollen from open flowers, which is a positive sign that bees are receiving floral rewards from flowering soybeans. The bees seen in our active surveying were a fraction of the bees seen in passive traps and in neighboring rangelands. We are hoping to identify pollen on captured bees to better understand whether more species are actively visiting soybean flowers or if they are only traveling nearby or through flowering soybean fields. Another season of surveys will help us better understand the temporal pattern of visitations as it relates to the period of flowering as well as whether there are differences in the pollinator community across years.

Our efforts to grow soybean flowers in the off-season have not been successful so far. We have plans in place to modify methodologies and identify new approaches to accomplish this task. If it is not successful going forward, we will pivot to focus on quantifying floral rewards of traditionally grown soybeans during the summer.

Conclusion/Benefits to the North Dakota Soybean Farmers and the Industry
Our pollinator sampling successfully identified a pollinator community that is found around flowering soybeans and a smaller, but important group of species that are actively interacting with flowers from North Dakota soybeans. These results have also been very helpful in developing more efficient plans for next summer. This includes trying to better understand other indications of interacting with soybean flowers, specifically by identifying what pollinators are carrying pollen from soybean flowers. Knowing that there are pollinators that are interacting with soybean flowers is a crucial prerequisite for any potential benefits that soybeans may receive from pollinator visitations. So, we have taken a very good first step to understanding the potential mutual benefits of North Dakota soybeans and pollinators.