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Sunflower Pollinators in South Dakota: An Evaluation of Species Composition, Abundance and Influence on Yield

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SUNFLOWER POLLINATORS IN SOUTH DAKOTA: AN EVALUATION OF SPECIES COMPOSITION, ABUNDANCE AND INFLUENCE ON YIELD

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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SUNFLOWER POLLINATORS IN SOUTH DAKOTA: AN EVALUATION OF SPECIES COMPOSITION, ABUNDANCE AND INFLUENCE ON YIELD

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In 2017 and 2018, South Dakota was the largest producer of sunflowers, *Helianthus annuus* L. (Asterales: Asteraceae) in the US, grossing approximately $178 million per year. In South Dakota, both confection and oilseed sunflower cultivars are grown. Despite breeding efforts to increase sunflower's ability to self-pollinate there is evidence that pollinator visitation results in increased sunflower yields. In addition, there is evidence that sunflower pollen is beneficial for pathogen resistance in various bee species, suggesting the importance of a mutualistic relationship between pollinators and sunflowers. In some areas, observed increases in sunflower yield is attributed to the presence of *Apis mellifera* (L.) as well as species of native bees. Annually, honey bee hives are moved to be within close proximity of sunflower fields. The effect that honey bees as well as other pollinators have on yield has been investigated in various crop studies including confection sunflowers. However, in South Dakota, the identity of the insect pollinators visiting sunflowers is unknown. Additionally, there is no evidence to suggest that oilseed sunflower production in South Dakota is positively impacted by the presence of pollinators. The objectives of this research were to identify the species of insect pollinators visiting South Dakota sunflower during flowering and determine if pollinator visitation positively impacted sunflower yield. This experiment was conducted

at across four locations in South Dakota. The first location, Volga, SD was selected due to the absence of sunflower production in the area. The second, Onida (2016), third, Highmore, SD (2017), fourth, Gettysburg, SD (2018) and fifth, Pierre, SD (2018) locations were selected due to the large presence of sunflower production in the surrounding area.

To explore these objectives 59 mL pan traps were modified by being painted with fluorescently dyed paint (i.e., bee bowls). After the onset of flowering, a cluster of three bee bowls were placed in the middle of sunflower plots at approximately 10:00 AM during sunny days with no forecasted precipitation and were removed 24 hours later. This procedure was repeated weekly until sunflower senesced. Each bee bowl contained a mixture of soap and water to capture the specimens. Once collected, specimens were cleaned using warm water, and then dried and stored until they were identified to the lowest taxonomic unit possible.

Our results indicate that *Melissodes trinodis* Robertson was the most abundant pollinator at Volga, (2016), Highmore (2017) and both Gettysburg, and Dakota Lakes Research Station in Pierre, (2017 and 2018), while hover flies were the most abundant pollinators at the Volga (2017 and 2018) location. The pollinator community differed between locations and less than 5% of honey bees were observed across the years and locations of the experiment. We also observed that oilseed varieties on average yielded 26% greater when pollinators were not excluded. These results suggest that native pollinators likely play a more important role in sunflower production in South Dakota. This suggests that future studies should evaluate the impact that routine or contracted

insecticide applications have on pollinator abundance, composition and their impact on yield.

Keywords: *Melissodes trinodis*, *Helianthus annuus*, pollinator, yield.

Chapter 1: Introduction and Literature Review

Thesis Organization

This thesis contains four chapters. The first chapter is an introduction and literature review that covers the main topics of sunflower production history, the implications of sunflower production, and sunflower growth, the relationship between people, pollinators and plants, pollinators, including honey bees, native bees, bumble bees, dipterans, and finally the relationship between sunflower and pollinators including yield. The second chapter evaluates the abundance and species diversity of pollinators in South Dakota, separated into eastern and western South Dakota locations. Chapter three investigates the impact that pollinators have on South Dakota sunflower yield. Chapter four summarizes the conclusions reached in the thesis regarding pollinator species abundance and diversity visiting South Dakota sunflower, in addition to the impact pollinators have on South Dakota sunflower yield.

Literature Review

Sunflower History, Importance and Growth

Sunflower, *Helianthus annuus* L. (Aterales: Asteraceae), is a major crop as well as ornamental plant cultivated on six continents (Gleason 1991). The *Asteraceae* family is a large and widespread group of angiosperms that account for 13 subfamilies, 1,911 genera and 32,913 species (Gleason 1991). The floral head resembles a star-like structure as found with many plants within the *Asteraceae* family (Schneiter and Miller 1981). Sunflowers, specifically, have been documented since the 26th century B.C and are a part of the genus *Helianthus* (Pope et al. 2001, Lentz et al. 2008). Sunflowers are dicotyledonous plants that produce two opposite leaves during the germination period (Schneiter and Miller 1981). During vegetative emergence (VE) the cotyledons expand above the soil to open up the two leaves. Traditionally, VE occurs 10-15 days post planting (Schneiter and Miller 1981). The successive growth stages (V2, V4, V6, …etc.) are characterized by counting the pairs of true leaves that develop at least 4 cm above the ground as the sunflower develops (e.g., V2 occurs when there are two leaves over 4 cm high and if there are four leaves the growth stage would be V4). Growth stage V2 to V8 are particularly important to growers as this is when many post emergence herbicides are applied (Schneiter and Miller 1981, Thomas Gulya 2019) Sometimes leaves will fall off the stem due to mechanical damage, if this has occurred the growth stage can be determined by counting the number of leaf scars (Schneiter and Miller 1981). The reproductive stages are determined as the florescence of the plant develops. The R1 growth stage occurs when the terminal bud develops into a small floral head rather than a cluster of vegetative leaves.

As the reproductive stages progress their classification follows suit. Reproductive stage 2 (R2) is reached when the star-like bud elongates to 0.5 to 2.0 cm above the nearest leaf found on the stem. R3 is reached when the bud elongates over 2.0 cm above the nearest leaf, and R4 is reached when the florescence starts to open. At R4 if you observe the flower from above the immature ray petals are visible (Schneiter and Miller 1981, Federal Crop Insurance 1995). R5 is classified as the beginning of flowering, this stage is characterized by eight sub-stages (R5.1, R5.2, R5.3, R5.4, R5.5, R5.6, R5.7, and R5.8). The decimal placements are defined by the percentage of head area that is flowering or has completed flowering. For instance, R5.2 is classified by 20 percent flowered and R5.3 is classified by 30 percent flowered (Schneiter and Miller 1981). R6 is regarded as the end of anthesis and the seed begins to develop. R6 is identified by the wilting of the ray flowers (Schneiter and Miller 1981, Berglund 2007). R7 occurs as the back of the head begins to turn a pale-yellow, which begins near the stem. R8 occurs when the sunflower head is entirely yellow, except for the bracts which remain green (Schneiter and Miller 1981, Berglund 2007). Plants are physiologically mature when the bracts turn brown.

For sunflower production, the two major types include oilseed and confection (Berglund 2007). Oilseed sunflower is largely used for livestock meal and silage (Konyali 2017), and vegetable oil production due to the seeds containing approximately 38-50 percent oil (at 10 percent moisture). Compared to soybean and other meals, sunflower meal has the lowest percentage of protein at 20 percent (Berglund 2007, Weisz et al. 2009). According to Schneiter et al. (1997), for every 100 pounds of oilseed, approximately 38 pounds of oil and 35 pounds of high-protein meal are produced. Confection sunflower, or non-oil sunflower is largely used for human and bird consumption, and its popularity in use in horticultural gardens and cutting flowers (Berglund 2007, Konyali 2017). Confection sunflower seed is generally striped, and larger than its oilseed counterpart. Confectionary seed is divided into three categories. Food-grade sunflower are composed of seed with highest qualitative attributes, that is, the largest and cleanest seed after processing. Ingredient sunflower is composed of high quantitative seed, this seed is desirable in products that require additional processing compared to food-grade sunflower. The seed that does not pass inspection for human consumption will be used for bird seed and are smaller, low quality seed. Of the confection sunflowers that are produced, approximately 30 percent are utilized for human consumption, and 70 percent as bird feed. During the peak period of U.S. production, approximately 15 percent of the world's sunflower production occurred within the U.S. Total combined acreage of oilseed and non-oilseed sunflower increased rapidly in the 1970s, reaching a peak in 1979 of 5.5 million acres planted, which has remained consistent (NSA 2019).

Sunflower is within the top four most important oilseed crops in the world, 14 percent of crude vegetable oil production is supplied by sunflower and approximately 41 million metric tons are produced annually on 26 million hectares of land (Konyali 2017). In 1966, interest in oilseed sunflower increased as new varieties from Russia with 40-50 percent oil were introduced to the United States. The introduction of these new sunflower varieties drastically increased the interest in the cultivation of sunflower as an economically beneficial crop for farmers across the United States (Geise 1974, Berglund

2007, Lentz et al. 2008). Today, the United States is within the top ten producers of oilseed sunflower in the world, reaching peak production in 1979 with 5.4 million acres planted and 7.3 billion pounds of sunflower produced (Berglund 2007). Annually, the U.S. production accounts for 3 to 5 percent of the total world production (NSA 2019). Among all oilseed crops, sunflower ranks fifth behind soybean, cottonseed, peanut and rapeseed (Seiler et al. 2017). Plant breeders make interspecies crosses within the *Helianthus* genus aiming for higher yields, higher oil content, and cytoplasmic male sterility. Therefore, high yielding hybrid sunflowers have replaced the open-pollinated varieties and have improved the marketability of sunflower products (Carter 1978).

Sunflower have been grown in the U.S. Northern Plains since 1900 (Federal Crop Insurance 1995). But the adoption of sunflower production in South Dakota, North Dakota, and Minnesota did not take place until the early 1970's. In 2018, 971,245 hectares of sunflower were harvested in The United States (NSA 2019). Of the harvested hectares, 930,777 were oilseed varieties and the remaining 40,468 hectares were confection varieties (NSA 2019). The top sunflower producing states in the United States are South Dakota, North Dakota, Colorado, Minnesota, Texas and Kansas and only a small number of acres are grown in states outside the Midwest and Northern Great Plains (Berglund 2007, NSA 2019).

The United States growing season is from mid-May through September. Like most crops, the increase of seeding rate (population) for sunflower may lead to a reduction in overall yield. According to Grady et al. (2008), sunflower seeding rates should be 10-15 percent higher than the desired mature population rate. Generally, oilseed hybrids are planted at a higher seeding rate than confection type hybrids. In central and western South Dakota, oilseed hybrid seeding rates should be approximately 6,400-8,000 plants/hectare. Confection type sunflower should be planted at a lower population rate; 6,000-7,200 plants/hectare. Sunflowers are grown in northern regions were cold winter temperatures can reduce some pest pressure compared to southern double-cropping systems (Geise 1974).

Sunflowers are usually ready for harvest in late September or early October typically within 120 days after initial planting (Geise 1974, Schneiter and Miller 1981). Seed moisture at this stage is usually about 35-40% (Berglund 2007). A killing frost will facilitate crop dry down in most years. Desiccants (Paraquat or Sodium Chloride) may be applied after physiological maturity to aid the drying process (Carter 1978, Berglund 2007). Seed must be dried to 9.5% moisture or less for storage (Schneiter et al. 1997).

People, plants, and pollinators

Flower visiting insects that utilize nectar and pollen as nutrition sources can be considered pollinators due to their potential to transfer pollen from male parts of the flower to the female counterparts. This transfer of pollen results in the transmission of genetic material which is a vital step in angiosperm reproduction (Kevan 1991, Proctor et al. 1996, Klein et al. 2007) and an animal based ecosystem service that is critical for human survival (Kremen et al. 2004). Ecosystem services are defined as excavation of goods and services that benefit the human population (Costanza et al. 1997). These services are diverse and have either a direct or indirect effect on human viability. For example, the excavation of lumber, minerals, and fuel are direct ecosystem services, while soil biodiversity, biological pest control and crop pollination are indirect ecosystem services. The existence of pollinators can enhance the production of agricultural crops and their services create a mutualistic relationship involving animals and plants (Kremen et al. 2004, Greenleaf and Kremen 2006, Klein et al. 2007, Gallai et al. 2009, Dorado and Vázquez 2014). The literature has yet to define the precise number of insect pollinators required to maximize yield, however, animal pollination serves as a vital ecosystem service. An estimated 87.5% of flowering plant species require their service for seed set and fruit. Within that, insects are responsible for 70% of pollination services (Klein et al. 2007). Estimates show that globally, pollinators are valued at $173 billion USD per year (Gallai et al. 2009). Therefore, insect pollinators, both managed and native pollinators, are a crucial component of enhancing crop production worldwide (Klein et al. 2007), which is at the highest production rate in recorded human history (Edgerton 2009).

According to Kremen (2004) and Williams et al. (2004), increases of production within 39 of the leading 57 grain and dry crops occurs due to insect pollinator visitation. These crops are responsible for 35% of global food production but do not account for commodity crops such as fruits and vegetables. Approximately 20% of the global crop production comes from commodity crops. Kein (2007) and Vaissiere et al. (2007) found that the production of 48 of the leading 67 commodity crops also increases due to pollinating insects. Like grain and dry crops, these commodity crops are important culturally, economically and physiologically to humans. They provide essential micro- and macronutrients that contribute to a healthy diet (Kremen et al. 2004, Steffan-Dewenter et al. 2005, Klein et al. 2007). The opinion of Steffan-Dewenter et al. (2005) concludes that human diet and human culture would be substantially, negatively affected by the further decline of insect pollinators. Basic population identification and occurrence data for bees and other insect pollinators is missing in many regions of the United States.

With a particular void of data from the western United States. This knowledge gap of species abundance and diversity and understanding the biology and population dynamics of native bee species is detrimental to study of pollinators and understanding their role in agricultural settings. Prior to the study conducted for this thesis, an inventory of native bees has never been completed for eastern South Dakota.

Honey Bees

Apis mellifera (Hymenoptera: Apidae), or more commonly known as the Western or European Honey Bee provides valuable pollinator services in both agricultural and natural habitats (Allen-Wardell et al. 1998, Edgerton 2009, Gallai et al. 2009). When wild bees are not prevalent in agricultural landscape, managed honeybee hives are frequently used to ensure pollination during bloom. Many apiaries transport their colonies throughout the US, and the value of the ecological services provided by this species is estimated to be worth $14.6 billion annually (Morse and Calderone 2000). Honey bees are eusocial (highly social) and commonly managed in colonies to aid crop-pollination services (Seeley 2009, Kremen 2018).

A colony of honey bees consists of one reproductively mature female, called the queen; up to thousands of underdeveloped females, collectively called workers, and depending on the time of the season, many male drones whose sole purpose is to mate with the queen. *Apis* queens are produced in large cells and develop in a mere 16 days (Jernigan 2017b), in addition, they differ morphologically, physiologically, and behaviorally (Phillips and Charles 1917, Slessor et al. 2005, Seeley 2009, Seeley 2010). Queens are polyamorous, that is, they mate with multiple male drones to produce up to 2,000 eggs per day (Jernigan 2017b, a). The sex of the eggs is determined by their fertilization. Eggs that are fertilized will develop into female workers, and those that are unfertilized will develop into males (Pirk et al. 2004, Seeley 2010). In addition, queens are also responsible for secreting pheromones that regulate the behavior of the hive (Phillips and Charles 1917, Pirk et al. 2004, Slessor et al. 2005, Seeley 2010).

Mature bees require consistent access to proteins, lipids, and carbohydrates to meet their energy demands. These nutrients are found in pollen and nectar and are necessary for the bees to meet the daily energy demands and cope with environmental stressors (Brodschneider and Crailsheim 2010, Bernklau et al. 2019). It was previously believed that honey bees can travel up to 2-3km (Visscher and Seeley 1982), however more recent evidence proves that many agricultural pollinators can travel greater distances. Honey bees travel up to 6 km from their nests to primary forage sources (Beekman and Ratnieks 2000).

Honey bee dance language, in which female workers perform dances to communicate information about the distance and direction to food sources, is highly developed and essential for exploration and discovery of new forage sources and nest-site selection (Seeley and Visscher 1988, Sherman and Visscher 2002). The dance allows bees to quickly utilize newly discovered food sources that may be sparsely distributed or far away, making them difficult to locate (Beekman and Ratnieks 2000, Sherman and Visscher 2002, Donaldson-Matasci and Dornhaus 2012). In addition, a recent modeling study concluded that the dance language shared within a hive likely increases long term longevity of a hive (Schürch and Grüter 2014).

Native Bees

In addition to the European Honey bee, there are more than 4,000 species of native bees in North America (Michener 2000). In highly heterogeneous landscapes, native bees can perform most pollination services (Winfree et al. 2008). However, the amount of pollination services they provide and the variation that occurs due to land management is largely unknown. These native bees are primarily solitary ground nesting species. The belowground nests are used to store pollen and nectar as food for their brood. There are some semi-social species that nest in hollow stems or wood (Michener 2000). There is evidence that natural habitats which provide nesting opportunities adjacent to agricultural fields increases the stability of pollination, which in turn leads to increase of yield and income (Zhang et al. 2007). Further, agriculture is not always expected to reduce pollination services. Some wild bees may benefit from agriculture, because they utilize disturbed areas for their ground nests (Sardiñas and Kremen 2014). In addition, pollinators may benefit from pollen-rich crop systems, such as sunflower or oil-seed rape, and agricultural ecosystems have the potential to provide more abundant resources versus their primitive habitat (Klein et al. 2007, Rader et al. 2016).

The relationship between native pollinators and agronomic systems has become the focus of many recent experiments. Evidence suggests that honey bees may not be the most efficient pollinator in agronomic systems, but instead native bees are more efficient at pollinating commodity and grain crops. For example, crops including field bean (Kremen et al. 2004), oilseed rape (Bartomeus 2014), watermelon (Kremen et al. 2002), raspberries and blackberries (Cane 2005) cherries (Bosch et al. 2006), coffee (Klein et al. 2003a, Klein et al. 2003b) and field tomatoes (Klein et al. 2003b) were all pollinated more effectively by native bees when compared to honey bees. The Klein et al. (2007) survey found that 42% of the world's leading crops are visited and pollinated by at least one wild bee species. Of these crops, 107 are valuable for direct human use. Losey and Vaughan (2006) estimate the value of native pollinators in respect to the US agronomic market is over $3.1 billion annually.

The sex of the pollinators can influence efficacy of pollination services due to differences of morphology and behavior. Polyectic native species have females that nest in more social dwellings and forage on more diverse plant hosts (Cane et al. 2011). In general, female bees tend to be more effective per flower visit, however males do contribute to pollination when nectar is needed to replenish their caloric needs for flight (Cane et al. 2011, Mallinger et al. 2018). Oligolectic (host specific, specialized) male bees are prevalent members of many flora groups, including many of the crops that Klein et al. (2007) list as beneficiaries of insect pollination. Well documented examples of plant species that are visited by both female and male oligolectic bees include but aren't limited to, sunflowers (Greenleaf and Kremen 2006, Mallinger et al. 2018), blueberries (Garibaldi et al. 2013) and alfalfa (Winfree et al. 2008). However, male efficacy has not yet been investigated in the literature.

Bumble Bees

Bumble bees (*Bombus spp.*) are another important pollinator of both agricultural crops and native plants worldwide. The *Bombus* family includes approximately 250 known species that are located in the Northern Hemisphere (Goulson et al. 2008), and 49 located in the United States (Koch 2012). In South Dakota, *Bombus impatiens* and *B. terricola* are the most commonly observed species (Cameron et al. 2011); however the literature has yet to quantify specifically how many species of *Bombus* are in the state.

Bumble bees are considered eusocial because queens develop colonies (Koch 2012). Queens emerge from underground nests in the spring and begin foraging to feed their offspring. Bumble bees generate heat with their flight muscles to incubate their brood and speed up development of worker bees. After the first generation of workers hatches (about four weeks after deposition) (Koch 2012), the empty cocoons may be used for short-term storage of nectar, however no long-term storage is required. In addition, a *Bombus* queen only lives one year, but some species are capable of producing over a thousand eggs during that time (Koch 2012). Bumble bees can be separated into three classes of tongue (proboscis) length: long, medium, and short. The variation in tongue length allows different species to visit and forage on many different floral resources. Bumble bees found in agricultural landscapes tend to consist mainly of short-tongued species, and range in size from 65mg (workers of the smallest species) to 830mg (large species queens) (Heinrich 2004, Goulson et al. 2008). Some short tongued bumble bees including, *B. affinis*, and *B. terricola*, chew holes underneath the sepals of flowers allowing them to extract nectar from otherwise unattainable sources (Winter et al. 2006). This “nectar robbing” behavior often leaves the anthers undisturbed and pollination will not occur (Winter et al. 2006, Goulson et al. 2008). However, it does allow these short-tongued species to directly compete with long-tongued bees.

There is evidence that many bumble bees forage over relatively long distances, up to 20km (Osborne et al. 1999, Heinrich 2004, Graystock et al. 2013) from their nests. Individual behavior during foraging is often driven by immediate energy requirements. Foraging not only depends on pollen vitality but also on nectar availability. Smaller bees have overall smaller energy requirements and tend to stay in the nest during low air temperatures (Heinrich 2004). This is probably due to difficulties they experience when trying to keep their physiological temperature at a desired level (Heinrich 2004). They counter these shortfalls with physiological mechanisms, for example, instead of flying to separate nectar rich flowers, they'll instead walk from flower to flower of dense inflorescence. Dense hairs on the bodies of bumble bees allow a large pollen transfer from flower to flower. Bumble bee individual foraging behavior has no implications to other organisms, and Heinrich (2004) states that their behavior can even be mutualistic in nature due to pollen drop.

Almost one million commercially produced bumblebee colonies are imported annually on a global scale for the pollination of greenhouse crops, and 95% of reared bumble bees are used in production of tomatoes (Winter et al. 2006, Graystock et al. 2013). Bumble bees are helpful in greenhouse operations because they work well in enclosed spaces (Graystock et al. 2013), and exhibit "buzz" pollination which is 400 times more effective than *Apis mellifera* (Winter et al. 2006). Buzz pollination is defined by the behavior of a bee when it flies up to a specific flower, sits on the corolla of the flower, folds its wings backward and vibrates its wing muscles at 100-200hz to effectively drop pollen from the flower and allow for seed set. Five species of bumble bees are currently commercially reared for crop pollination: *Bombus terrestris*, *B. lucorum*, *B. occidentalis*, *B. ignitus* , and *B. impatiens* (Winter et al. 2006). These species belong to two closely related subgenera, *Bombus* and *Pyrobombus* who are considered "pollen storers" making them particularly useful as managed colonies. The value of bumblebees comes from their pollination services in agriculture, which are worth billions of dollars annually (Heinrich 2004, Winter et al. 2006, Goulson et al. 2008, Graystock et al. 2013).

Dipterans

Diptera (flies) is one of the largest insect orders, and the one of the most frequent visitors to flowers (Larson et al. 2001, Ssymank et al. 2008, Mallinger et al. 2018). Dipterans are often overlooked; however, they can be important pollinators. Overall, flies are less effective pollinators when compared to bees, but the high frequency of their visits often mitigates their inefficiency resulting in pollination services that are proportionate to bee species. Compared to bees, flies have low energy requirements and because they don't support their brood, they are capable of depending on less rewarding flowers. Rader et al. (2016) concluded that fruit set increased with fly visitation separately of bee visits, confirming their hypothesis that flies offer a unique benefit that bees cannot. Dipterans are a diverse group composing 120,000 species, and 70 families observed in North America (Larson et al. 2001). Of these families, *Syrphidae* (hover flies herein), *Bombyliidae*, and *Tachinidae* visit flowers for nutritional resources (Larson et al. 2001, Ssymank et al. 2008). Of these three families, hover flies are likely the most important flower visitors and are found in most agricultural landscapes (Larson et al. 2001, Mallinger et al. 2018). The shape of their mouth parts suggests that virtually all adults of the 6,000 hover fly species utilize nectar and some also consume pollen (Larson et al. 2001, Klein et al. 2003b). Nectar provides a good source of energy, and pollen protein can be essential for reproduction and fecundity (Kevan 2002). Flowers can also provide meeting sites during the mating period. Syrphid flies are not only useful as pollinators in agroecosystems, but also as biological control agents. The larvae of many hover flies are predators of small, soft-bodied insect pests (Schneider 1969). Some hover flies have evolved large tarsi on their front legs which enables them to extract pollen out of anthers (Ssymank et al. 2008), and others have sub-erect, thick hairs which are useful for pollen accumulation (Thompson and Rotheray 1998). The role that syrphid flies have in agricultural landscapes like South Dakota has yet to be investigated but their presence is has been documented (Mallinger et al. 2018).

Sunflower and Pollinators

The loss of insect pollinators has a profound impact on pollination services, and a crucial step in maintaining pollination services is understanding the community of pollinators native to agronomic ecosystems. Factors that limit or benefit yield are the abundance and diversity of the pollinator community, their competence as pollinators, visitation frequency, and the requirements of the crop itself (Kremen et al. 2002, Rader et al. 2016, Mallinger et al. 2018). Sunflower breeding has focused on developing a self-compatible plant that does not require insect pollination for seed production. However, sunflowers are still a viable nectar and/or pollen resource for native and managed bees. Additionally, there is evidence that even self-compatible sunflowers benefit from insect pollination (Mallinger et al. 2018). Mallinger et al. (2018) suggest that conservation of native wild bees may improve yield in sunflower. In addition, Chambó et al. (2011) observed hybrid sunflower yield increases by up to 43% when pollinators were present. Greenleaf et al. 2006 found that interactions between honeybees and wild bees increased pollination of sunflower by 5-fold. Additionally, these findings suggest that protecting wild bee communities can help mitigate the shortcomings that honeybees experience (Greenleaf and Kremen 2006). These findings also demonstrate the economic importance of interspecific interactions for ecosystem services and suggest that protecting wild bee populations can help buffer the human food supply from honey bee shortages (Greenleaf and Kremen 2006). Mallinger et al. (2018) recently described an abundant and diverse community of pollinators that were observed visiting sunflower for pollen in the Northern Great Plains. For example, *Melissodes spp.* and *Andrena helianthi* have been documented as the most common, and most effective female large-bodied bees visiting sunflowers in North Dakota (Mallinger et al. 2018).

Variation in visitation frequency and self-pollination rates are due to a combination of factors including genotype and pollinator interaction and variation in floral traits. For example, the amount of nectar and pollen rewards, or volatile emissions, may contribute to variation in pollinator visitation rates across hybrids (Greenleaf and Kremen 2006, Garibaldi et al. 2013, Mallinger et al. 2018). In most environments, both wild pollinators and honeybees will exploit flowers of crop species. Strawberry flowers visited by both wild bees and honeybees are more likely to be completely developed in contrast to flowers that are visited by only honeybees or only wild bees (Chagnon et al. 1993). Males of wild bees that were searching for mates disturbed honeybees that were foraging, which caused the honeybees to switch between lines of hybrid sunflower more often. In addition, the honeybees were observed carrying more pollen, thereby increasing the overall pollination service (Greenleaf and Kremen 2006).

A common practice among bee keepers is to place their colonies adjacent to sunflower fields to increase their pollen collection (Valido et al. 2019). Placing colonies near sunflower not only increases their pollen collection, but may also reduce pathogens. Recent research suggests that bees that eat sunflower pollen dramatically and consistently reduced a protozoan gut pathogen (*Crithidia bombi)* infection in the common eastern bumble bee (*Bombus impatiens)* and reduced a microsporidian pathogen (*Nosema ceranae)* of the European honey bee (Bernklau et al. 2019). This raises the possibility that sunflowers may provide a simple solution to improve the health of economically and ecologically important pollinators. The reduction of pathogens is consistent with other limited research suggesting that sunflower and related taxa pollen may have medicinal qualities useful for bees. Additionally, Spear et al. (2016) observed lower survival rates of parasitoid larvae in *Osmia spp*. bees that fed on *Asteraceae* pollen. These discoveries suggest that sunflower pollen may have positive effects that could aid bees to resist parasites and pathogens, however, the literature has not investigated this phenomenon across other plant families.

Sampling Methods

To determine species richness and abundance in a given area, unbiased and reliable sampling must be obtained. Historically, many different sampling methods have been used to determine pollinator diversity and abundance. Each sampling method differs on how it performs to obtain these objectives. For example, to obtain species richness across time and across multiple locations, a single sampling method may be utilized.

However, to collect an exhaustive data set of the species that may be present within an area or across time multiple sampling methods may need to be utilized. The most popular sampling methods in pollination studies are often observational, where field workers will travel to a site and record the insects visiting the flowers in a given plot or transect (Westphal et al. 2008). Another sampling method is capture and release. For this method, workers use insect nets to collect and record the taxa in a given area, and then release the insects unharmed. However, both of these methods are susceptible to sampler bias. As bee faunas and floral resources are highly variable in space and time (Michener 2000, Klein et al. 2007), a fixed observational transect may not represent the full spatial foraging patterns of a site and would result in only sampling a small portion of the community. In addition, active sampling often only samples the largest, slower moving insects because they are easier to be captured. An alternative passive sampling method is the use of pan traps, and trap nests. Pan traps for pollinator sampling are typically constructed using 59 mL cups that are painted with UV fluorescent paint, then filled with water and a small amount of detergent (Nielsen et al. 2011). The pan traps are mounted to a pole and then placed at the vegetative height in order to drown specimens and store them effectively for later analysis. Trap nests, are often human generated nests that include plant material, or hollow sticks that are attractive to ground dwelling, or solitary bee species (Nielsen et al. 2011). These nests tend to stay in the field for a prolonged period of time in comparison to pan traps. These nests will remain in the field until the season is over, and then are collected. After collection these nests are stored around 4 degrees Celsius (Nielsen et al. 2011) in order for larvae to hatch into adults and be pinned for later analysis.

Census methods or active sampling (i.e. transects walks, catch and release) are comparatively more time consuming and often require experienced surveyors in order to obtain accurate identification. Passive methods (i.e. pan traps and nest traps) generally take less time; however, they may be biased towards a limited taxonomic collection. Westphal et al. (2008), states that pan traps catch small-bodied bees and may be subject to under sampling large bodied bees, and trap nests are limited to solitary, cavity nesting bees. Often, it is valuable to have more than one collection method within a study to counteract these biases and accurately collect and identify the taxa in a given area.

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CHAPTER 2

DETERMINING THE SPECIES DIVERSITY AND ABUNDANCE OF POLLINATORS VISITING SOUTH DAKOTA SUNFLOWER

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Abstract

In South Dakota, sunflowers are an economically important crop. As a result, sunflowers are closely monitored and treated with insecticides to prevent yield loss from insect pests. However, these treatments do not take into consideration the presence of native pollinators that are likely foraging on the flowering crop. Despite breeding efforts to increase self pollination within commercial sunflower varieties, there is evidence that pollinator visitation results in increased sunflower yields. In addition, there is evidence that sunflower pollen can benefit pollinator health. However, in South Dakota, the pollinator community visiting sunflowers has not been evaluated. The objective of this study was to identify the species of insect pollinators visiting South Dakota sunflower and evaluate their abundance. To do this, a study using modified pan traps (i.e., bee bowls) was conducted during 2016, 2017 and 2018. Each year, at least two sunflower fields were utilized, which were either in

Eastern or Central SD. Bee bowls were placed in the field at the onset of flowering and were used to sample pollinators once per week. Specimens were identified after preparation. The results indicate that the species assemblage between Eastern and Central SD differ slightly. Over three field seasons a total of 3,509 individual specimens were collected representing 41 species. A species accumulation curve for each site-year determined that our sampling methods were robust. We observed an increased abundance of sunflower specialists at experimental sites in Central SD where sunflower are routinely grown.

Keywords: *Melissodes trinodis, Helianthus annuus*, pollinator, yield, native pollinators

Introduction

In the United States, sunflowers, *Helianthus annuus L.*, have been a part of the top grossing oilseed commodities for five years. Additionally, sunflower is well suited for growth in central and western South Dakota due to its tolerance of drought, extreme heat and cold. For this reason, sunflower is often implented in crop rotations in South Dakota (Jean 2014). Although, wild sunflower, is native to the United States and can commonly be observed growing in South Dakota, there are many differences between it and its commercial relative. For instance, commercial sunflower varieties that are capable of self pollinating are selected and used for breeding to reduce the dependence on insect pollinators and ensure uniform seed set (Mallinger et al. 2018). However, there is evidence that regardless of these breeding efforts, pollinators flower visitations still provide benefits to sunflower (Greenleaf and Kremen 2006, Mallinger et al. 2018)

In many other agricultural crops, pollinators are known to play an important role in providing pollination services. For example, wild bees have been observed pollinating crops including field bean (Kremen et al. 2004), oilseed rape (Bartomeus 2014), watermelon (Kremen et al. 2002), raspberries and blackberries (Cane 2005) cherries (Bosch et al. 2006), coffee (Klein et al. 2003a, Klein et al. 2003b) and field tomatoes (Klein et al. 2003b). Pollinators have even been observed in other major agricultural crops including corn and soybean (Morse and Calderone 2000, Kremen et al. 2002, Gill and O’Neal 2015, Wheelock et al. 2016). Pollination services are defined as activities on flowers that result in the transport of pollen (Jean 2014). An estimated 87.5% of flowering plant species require their service for seed set and fruit. Within that, insects are responsible for 70% of pollination services (Klein et al. 2007). Estimates show that globally, pollinators are valued at $173 billion USD per year (Gallai et al. 2009). Therefore, insect pollinators, both managed and native pollinators, are a crucial component of enhancing crop production worldwide (Klein et al. 2007), which is at the highest production rate in recorded human history (Edgerton 2009). Mallinger et al. (2018) recently determined that sunflower in North Dakota are visited by an abundant and diverse community of pollinators. Both *Melissodes spp.* and *Andrena helianthi* were documented as being the most common, and most effective female large-bodied bees visiting sunflowers in North Dakota (Mallinger et al. 2018). However, the presence of pollinators in agricultural crops, specifically sunflower, may present additional concerns regarding pollinator health and habitat (Wheelock et al. 2016).

Of the insect pests that affect sunflower, those that affect the developing seed present a great threat to the yield and quality of the sunflower. For this reason, sunflower growers are contracted to spray their sunflower fields with at least one and in some cases multiple passes of insecticides. However, the insecticides that are used to manage several of these insect pests in sunflowers have active ingredients with broad-spectrum insecticidal activity, which have negative impacts on both pest and beneficial (i.e., pollinators) populations that are present at the time of application. Although there have been efforts in South Dakota to reduce the impact of insecticides applied to sunflowers on honey bee hives (Beck 2014), there has been minimal to no effort to identify and protect populations of native pollinators or determine the impact that insecticide applications have on their populations.

It is assumed that native and managed pollinators are visiting sunflowers in South Dakota, but there is no evidence of what species are present or their abundance.

Furthermore, this information is necessary to determine long-term impacts of prophylactic or targeted insecticide use in sunflower on the native pollinator community. To gather these data, a survey of the pollinators visiting sunflower fields in South Dakota is necessary. Therefore, the objective of this study was to conduct a survey of pollinators visiting sunflower in both Eastern and Central South Dakota. The survey will determine the species of pollinators that visit sunflower, overall species diversity as well as pollinator abundance. The culmination of this data may be useful for providing sunflower growers with information to reduce the impact of insecticide applications on pollinators.

Materials and Methods

Data for this survey was collected during 2016, 2017 and 2018. For this experiment, two to three locations in South Dakota were used each year. For 2016, 2017 and 2018 sunflower were grown in Eastern South Dakota at the South Dakota State University (SDSU) Volga Research Farm (Volga, SD 44°18'13.9"N 96°55'38.1"W). This site was selected based on the absence of sunflower production in the surrounding landscape to provide a comparison to the Central South Dakota locations, where sunflowers are routinely grown. The specific locations in Central South Dakota varied by year due to field availability and weather events (e.g., the 2018 Onida location was destroyed by a severe hail storm). In 2016, the site was near Onida, SD (44°43'25.3"N, 100°15'05.9"W). During the 2017 season, the site was located near Highmore, SD (44°31'23.5"N, 99°40'46.6"W) and in 2018 there were two sites. One was located near Gettysburg, SD (45.0875° N, 99.9904° W) and the other site was at the Dakota Lakes Research Station (i.e., Pierre, SD; 44°17'35.0"N, 100°00'23.8"W).

In the 2016 growing season, sunflowers were planted at the SDSU Volga Research Farm on 22 June, and at the Onida, SD location on 15 June. In 2017, sunflowers were planted at SDSU Volga Research Farm on 19 June. At the Highmore, SD location sunflowers were planted 21 June. In 2018, sunflowers were planted at the SDSU Volga Research Farm on 24 June, 21 June at the Gettysburg, SD location, and 28 June at the Dakota Lakes Research Farm location. At each location a randomized complete block design with 10 blocks was implemented to account for varietal differences and potentional impacts of sampling locations within the field (i.e., edge vs. center). Each plot consisted of four rows, each 3m wide and 8.5m long. At each location three treatments were utilized, each treatment was composed of a different sunflower hybrid. The varieties consisted of one confection variety (i.e., LD5009; Nuseed) and two oilseed hybrids (i.e., P63HE90; Pioneer, and Hornet; Nuseed).

Pollinator Collection and Identification

Pollinators were collected using bee bowls (i.e., modified pan traps) that have been demonstrated as an effective pollinator sampling method in prevous studies (Gill and O'Neal 2015, Wheelock et al. 2016). A 3m steel post with three 3.2cm PVC sleeves was placed at the center of each plot. Each sleeve had either a white, flourescent yellow or flourescent blue bee bowl placed on the upper ring during selected sampling days. The individual bee bowls were constructed from 59 mL clear plastic portion cups (Uline, Hudson, WI). The cups were initially sanded to provide a suitable substrate for paint, and then painted using either white (East Coast Guerrra Paint and Pigment, New York, NY), fluorescently dyed yellow (East Coast Guerrra Paint and Pigment, New York, NY) or flourescently dyed blue (East Coast Guerrra Paint and Pigment, New York, NY) paint.

The colors were selected based on evidence of success from previous studies (Gill and O'Neal 2015, Wheelock et al. 2016). During sampling, each bee bowl was filled with a 50% dish detergent and water solution and then placed in the PVC sleeves at 10:00 AM on days with no rain forecasted for at least 24 hrs. Bee bowls remained in the field for 24 hrs and then were retrieved. Upon removing the bee bowl from the PVC sleeve it was capped using a lid and placed into cooler at SDSU until the pollinators could be processed and identified. Pollinator sampling began when sunflower reached the R5.1 growth stage and continued until the end of flourescence.

All pollinators were processed using methods described by Gill and O'Neal (2015). Briefly, pollinators were removed from the soapy water solution using a strainer and washed using a water spray bottle. The polliantors were then placed into a 354mL glass jar (Uline, Hudson, WI) with a modified mesh lid. A hair dryer that was set on the no-heat, low speed was then used to dry the specimens for an individual sample. This process was repeated for each collected sample. Once dry, the pollinators were identified to the lowest possible taxonomic unit using the DiscoverLife dichotomus key (www.discoverlife.org). Identification was then confirmed by comparing samples to individuals in the Severin-McDaniel South Dakota State University Insect Museum. A reference collection for this study has been submitted to the Severin-McDaniel South Dakota State University Insect Museum.

Statistical Analysis

Interpolation (rarefaction) and extrapolation were utilized to create an estimation of the unmeasured pollinator community (i.e., determine if sampling methods were robust). These estimations were calculated using sampling models within Estimate S version 9.1.0 (Colwell et al. 2012) and demonstrate how species richness increases per sample to the total number of individuals that were collected. This is also referred to as the abundance reference sample (Colwell et al. 2012). Because sampling an representative for every possible species present is nearly impossible for most environments the reference sample is defined as one that is random within our capabilities within the sampling method in relation to the taxa collected. Interpolation uses the rarefaction technique to estimate species abundance within a dataset, using random subsampling, and extrapolation is utilized to estimate asymptotic species abundance beyond the abundance obtained from the reference sample (Colwell et al. 2012, Gotelli and Chao 2013).

Species accumulation curve graphs were created for 2016, 2017, and 2018 for both eastern and western locations in South Dakota. Two types of graphs were generated, one that compares the number of collected individuals to the number of species and a second that compares the number of collected samples to the number of species. For this project, a sample is defined as the number of bee bowls that were collected from each site-year. S_{obs} is the number of species that were observed during that year, while S_{est} is the estimated sampling effort that would be required to maximize the collection of individuals as well as species. It is based on the logarithmic curve's point of stabilization.

The data for this study were analyzed using Program R version 3.5.3 (R Core Team 2018). In addition, species diversity for all locations were calculated using Simpson's Diversity Index for 2016, 2017 and 2018 using the vegan: Community Ecology package version 2.5-5. Two sided t-tests were utilized to compare the significance between locations in a given year, indicating if one location was more diverse than another. All statistical comparison tests used to interpret results of data utilized $\alpha = 0.05$.

Results

Collection Summary

A total of 1,309 pollinator specimens were collected in 2016, 462 specimens in 2017 and 1,393 specimens in 2018. A total of 14 species were observed in 2016, 28 species in 2017 and 32 speceis in 2018. For 2016, a total of 795 specimes were collected from the SDSU Volga Research farm with 10 observed species (Table 1). In comparison, a total of 795 specimens were collect from the 2016 Onida, SD location with 13 observed species (Table 1). During 2017, a total of 89 specimens were collected from the SDSU Volga Research Farm with 10 observed species (Table 2). Meanwhile, a total of 373 specimens were collected from 2017 Highmore, SD location with 24 observed species (Table 2). In 2018, a total of 886 specimens were collected form the SDSU Volga Research Farm with 19 observed species (Table 3). At the 2018 Gettysburg, SD location a total of 197 specimens were collected with 24 observed species, and at the Pierre, SD location a total of 312 speciemens were collected with 22 observed species (Table 3). These resultes indicate that for all years, a greater number of pollinator species were collected from sunflower fields in areas where sunflowers are routinely grown.

Species Accumulation Curve

All sampling was conducted from mid-August through mid-September (i.e., R5.1 to R7). For 2016, pollinator samples were collected from the Onida location during 2 days from 40 plots. For the species accumulation curves, this represented 80 total samples. The species accumulation curves for the number of samples reveal that approximately six additional species could have been collected if 200 additional samples had been collected. Similarily, five additional species could have been observed if 2,450 additional individuals had been collected from the 2016 Onida location (Fig. 1). For the 2016 Volga location sampling was conducted on 3 days from 40 plots for total of 120 samples. For Volga, the species accumulation curve indicated that two additional species could have been collected if the number of samples collected was increased by 218 and the number of individuals were increased by 925 (Fig. 1).

For 2017, pollinator samples were collected from the Highmore location during 4 days from 26 plots. For the species accumulation curves, this represented 104 total samples. The species accumulation curves for the number of samples reveal that approximately 12 additional species could have been collected if 420 additional samples had been collected (Fig. 2). Similarily,12 additional species could have been observed if 1,522 additional individuals had been collected from the 2017 Highmore location (Fig. 2). For the 2017 Volga location sampling was conducted on 3 days from 40 plots for total of 120 samples. For Volga, the species accumulation curve indicated that two additional species could have been collected if the number of samples collected was increased by 203 and the number of individuals were increased by 151 (Fig. 2).

For 2018, pollinator samples were collected from the Gettysburg location during 4 days from 10 plots. For the species accumulation curves, this represented 40 total samples. The species accumulation curves for the number of samples reveal that approximately seven additional species could have been collected if 160 additional samples had been collected. Similarily, nine additional species could have been observed if 783 additional individuals had been collected from the 2018 Gettysburg location (Fig.

3). For the 2018 Pierre location sampling was conducted on 4 days from 10 plots for total of 40 samples. For Pierre, the species accumulation curve indicated that ten additional species could have been collected if the number of samples collected was increased by 217 and the number of individuals were increased by 1,652 (Fig. 3). For the Volga location, the species accumulation curve indication that 29 additional species could have been collected if the number of samples collected was increased by 1,300 and the number of individuals were increased by 8,557 (Fig. 3).

Simpson's Diversity

The Simpson's Diversity Index values in Volga, Onida, Highmore, Gettysburg, and Pierre, SD in 2016, 2017 and 2018 range from zero to one. An index value of zero indicates low or no diversity and an index value of one indicates high diversity. In 2016, the species diversity index for Volga was D = 0.59, and for Onida was D = 0.64, which was significantly higher than Volga (t = 24.675; df = 1; $P \leq 0.05$) (Table 4). The average diversity index for both locations in 2016 was D = 0.62. In 2017, the species diversity index for Volga was D = 0.67, and for Highmore was D = 0.84 (Table 4). The average diversity index for both locations in 2017 was D = 0.76 for the two locations. In 2017, species richness was not significantly impacted by location. In 2018, the species diversity index for Volga was D = 0.44, Gettysburg was D = 0.84 and Pierre was D = 0.84. The average diversity index for all locations in 2018 was D = 0.71. In 2018, location significantly affected species richness (t = 5.2786; df =2; $P \leq 0.05$) (Table 4).

Discussion

In total, 3,169 pollinators were captured and identified for this survey. Our results indicated that the pollinator community, with the exclusion of Syrphidae, was more abundant and diverse at locations in Central SD when compared to the location in Eastern SD. However, our results also suggest that native pollinators are more likely to be observed visiting sunflower than the European honey bee. Interestingly, the number of pollinator species that were observed at the Volga location increased over time, with the greatest number of species being observed in 2018. This may be due in part to the increased planting frequency of sunflowers at the location from 2016-2018 for research projects including this one. We hypothesize that soybean aphid (Hemiptera: Aphididae) populations that were present in adjacent soybean fields likely influenced the increased observation of syrphids at the Volga location when compared to the locations in Central SD across the site-years. For instance, syrphids were the most abundant pollinator found in the Volga location, composing 69% of all specimens collected.

Of the bees that were collected, the most abundant one across all years was *Melissodes trinodis,* accouting for 38.5% of the pollinators collected in 2016, 48% of the pollinators collected in 2017 and 34.5% of the pollinators collected in 2018. This finding is consistent with results observed by Mallinger et. al. (2018) that *Melissodes* spp. have a noticable preference for sunflower. In contrast, studies show that honey bees have a limited preference for cultivated sunflower and will instead visit other flowers in the surrounding landscape (Andrada et al. 2004, Mallinger et al. 2018)

In South Dakota, apiaries are a $28 million dollar industry, with ~225,000 hives located in the state (DOA 2012). Despite honey bee stocking in the surrounding area, only 35 *A. mellifera* were collected in 2016, 2017 and 2018, composing 2% of the pollinator community. These findings confirm our hypothesis that native pollinators compose the majority of the pollinator community in South Dakota. Thus, the results of this survey indicate that honey bees may not have a significant impact on sunflower yields in the Northern Great Plains. However, this may vary across the region and be influenced by the field's proximety to hives. Additionaly, other related projects have also noted that bee bowls may not be an effective sampling technique for monitoring *A. mellifera*. Furthermore, the community of hymenopterans we observed within sunflower fields have similarities to those defined in a survey of 90 studies that examined pollinator communities present in field crops across North America (Klein et al. 2006, Gill and O'Neal 2015)

To determine if our sampling technique was robust we analyzed our data using species accumulation curves. With the exception of the Volga 2018 site-year, all of the locations were sampled adequately. We defined this based on the extra resources that would be necessary to attain additional species. Our results indicate that in most site-years, additional sampling would only result in collecting a few additional species. The benefit of the additional species would be outweighed by the required increase in sampling and also the diversification of the implemented sampling method. Due to budget constraints and the short blooming period of sunflower, additional samples would be difficult if not impossible to attain. In South Dakota, sunflower blooms between mid-August and September (Berglund 2007). This period is considerably shorter than other field crops, which in turn, limits the number of species active during this time (Greenleaf and Kremen 2006, Mallinger et al. 2018).

The Simposon's Diversity Index in 2016 and 2018 indicated that the diversity between locations was statistically significant from one another. This indicates that overall, site-years in Central SD (e.g. Gettysburg, Highmore, Pierre, and Onida) had a significantly higher diversity (i.e., more species). We hypothesize this difference may be due to *Asteraceae* specialized bees found in those locations. In addition, the increased adoption of no till practices may contribute to undisturbed ground, which would result in more available nesting areas for for solitary native bees in the area.

These results suggest that pollinator surveys can provide valuable data on the abundance and diversity of pollinators that are present in South Dakota sunflower. Furthermore, understanding the pollinator community allows for future projects to evaluate pollinator population trends and determine if agricultural practices are reducing the native pollinator community that has been documented. This also provides the framework for additional research projects that can evaluate the impact of routine pest management on ground nesting bees within sunflower fields and evaluation of the impact of pollinator activity on sunflower yields.

Awknowledments

We would like to thank Phil Rozeboom, and Brady Hauswedell with assistance with experimental set up and sample collection. In addition, we would like to thank the numerous hourly employees, especially Madeline St. Clair, and Aaron Hargens for their hardwork, dedication, and comradery during the years of pollinator collection. Without them this project wouldn't have been possible. This project was funded by a South Dakota Department of Agriculture Specialty Crop Block Grant and the South Dakota Oilseeds Council.

Table 1. 2016 Pollinator abundance in South Dakota sunflower.

Order	Pollinator Taxa Family	Species	2016 Abundance Onida	Volga
Hymenoptera				
	Andrenidae			
		Andrena spp.	117	26
		Calliopsis andreniformis	0	1
	Apidae			
		Apis mellifera	4	9
		Bombus citrinus	0	3
		Epeolus ainsliei	0	6
		Melissodes agilis	3	0
		Melissodes trinodis	277	231
		Nomada spp.	0	6
	Halictidae			
		Agopostemon texanus	1	2
		Halictus confusus	0	5
		Halictus ligatus	1	2
		Lasioglossum spp.	75	31
	Megachilidae			
		Megachile brevis	1	0
Diptera				
	Syrphidae		45	449
Totals				
Hymentoptera			**479**	**305**
Diptera			**45**	**449**
Grand total			**524**	**754**

Table 2. 2017 Pollinator abundance in South Dakota sunflower.

Pollinator Taxa Order	Family	Species	2017 Abundance Highmore	Volga
Hymenoptera				
	Andrenidae			
		Andrena spp.	51	5
	Apidae			
		Apis mellifera	8	12
		Bombus citrinus	2	0
		Bombus hunterii	1	0
		Bombus spp.	2	0
		Epeolus ainsliei	1	1
		Florilegus spp.	1	0
		Melissodes bidentis	1	0
		Melissodes bimaculatus	0	1
		Melissodes desponsus	1	0
		Melissodes trinodis	86	30
		Mellisodes spp.	0	1
		Nomada spp.	1	0
		Perdita ignota	1	0
	Halictidae			
		Agopostemon texanus	3	6
		Agopostemon virescens	26	3
		Collectidae spp.	4	0
		Dieunomia triangulifera	1	0
		Duforea marginata	4	0
		Halictus confuses	1	0
		Halictus ligatus	7	0
		Lasioglossum paraforbesii	1	0
		Lasioglossum spp.	78	3
		Lasioglossum vierecki	1	0
		Lasioglossum zonulium	25	31
	Megachilidae			
		Megachile fortis	3	0
Diptera				
	Syrphidae		61	27
Totals				
Hymentoptera			**311**	**93**
Diptera			**61**	**27**
Grand total			**372**	**120**

Table 3. 2018 Pollinator abundance in South Dakota sunflower.

Order	Pollinator Taxa Family	Species	2018 Abundance Gettysburg	Pierre	Volga
Hymenoptera					
	Andrenidae				
		Andrena geranii	4	1	0
		Andrena helianthin	3	1	0
		Andrena illinoiensis	6	1	0
		Andrena spp.	2	0	0
	Apidae				
		Apis mellifera	7	3	10
		Bombus affinis	0	0	2
		Bombus citrinus	1	0	0
		Bombus griseocollis	0	1	1
		Bombus pennsylvanicus	1	1	0
		Bombus rufocinctus	2	1	1
		Bombus spp.	1	1	1
		Epeolus ainsliei	0	1	2
		Melissodes agilis	24	39	60
		Melissodes bidentis	3	1	4
		Melissodes bimaculatus	6	0	8
		Melissodes communis	0	2	3
		Melissodes desponsus	0	0	1
		Melissodes fumosus	1	1	0
		Melissodes trinodis	24	64	83
		Mellisodes spp.	30	69	50
		Svastra obliqua.	1	0	0
	Halictidae				
		Agopostemon sericeus	0	3	4
		Agopostemon splendons	4	2	3
		Agopostemon texanus	0	23	0
		Agopostemon virescens	2	57	0
		Agopostemon femoratus	0	0	1
		Halictus ligatus	0	0	1
		Lasioglossum spp.	16	32	5
	Megachilidae				
		Megachile brevis	1	0	0
		Megachile parallela	1	1	0
Diptera					
	Syrphidae		58	7	649
Totals					
Hymentoptera			**140**	**305**	**240**
Diptera			**58**	**7**	**649**

Grand total	**198**	**312**	**889**

Table 4. 2016, 2017 and 2018 Simpson's Diversity Index.

Location	Simpson's Diversity Index (D)		
	2016	**2017**	**2018**
Volga	0.59	0.67	0.44
Highmore	N/A[1]	0.84	N/A
Gettysburg	N/A	N/A	0.84*
Pierre	N/A	N/A	0.84*
Onida	0.64*	N/A	N/A
Average	**0.62**	**0.76**	**0.71**

[1]N/A refers to locations that weren't sampled during a given year.

*Refers to signficance less than $P \leq 0.05$.

Figure 1.

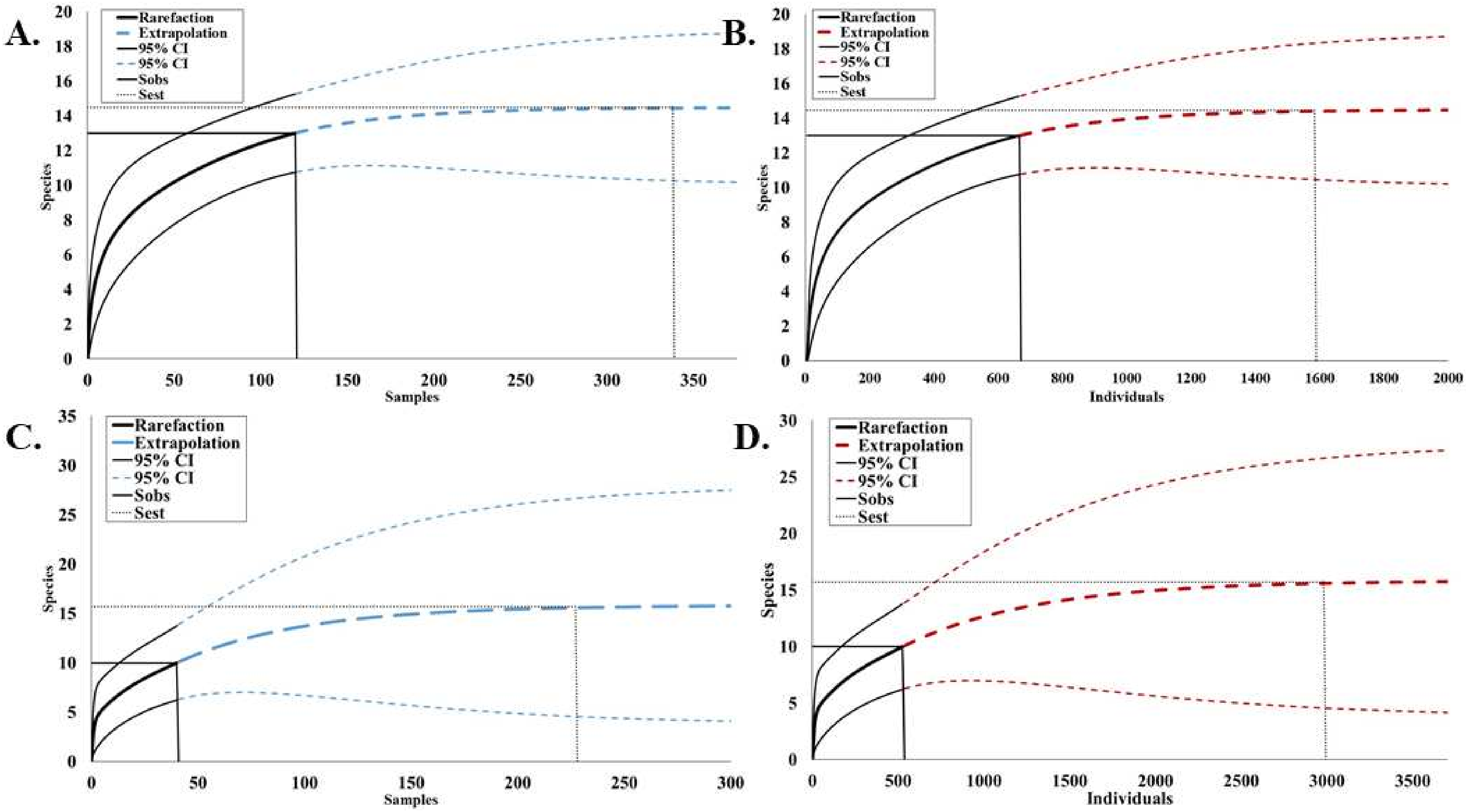


Figure 1. 2016 Species accumulation curves for A) sample number at Volga, B) individuals at Volga, C) sample number at Onida, D) individuals at Onida. Interpolation (solid curve) and extrapolation (dashed curve) with 95% confidence intervals. Intersection of the light gray line represents species observed (S_{obs}), while the intersection of the dotted grey line represents species estimated (S_{est}) at the point where the logistic curve stabilizes. Samples represents collected specimens from each plot at a given collection date.

Figure 2.

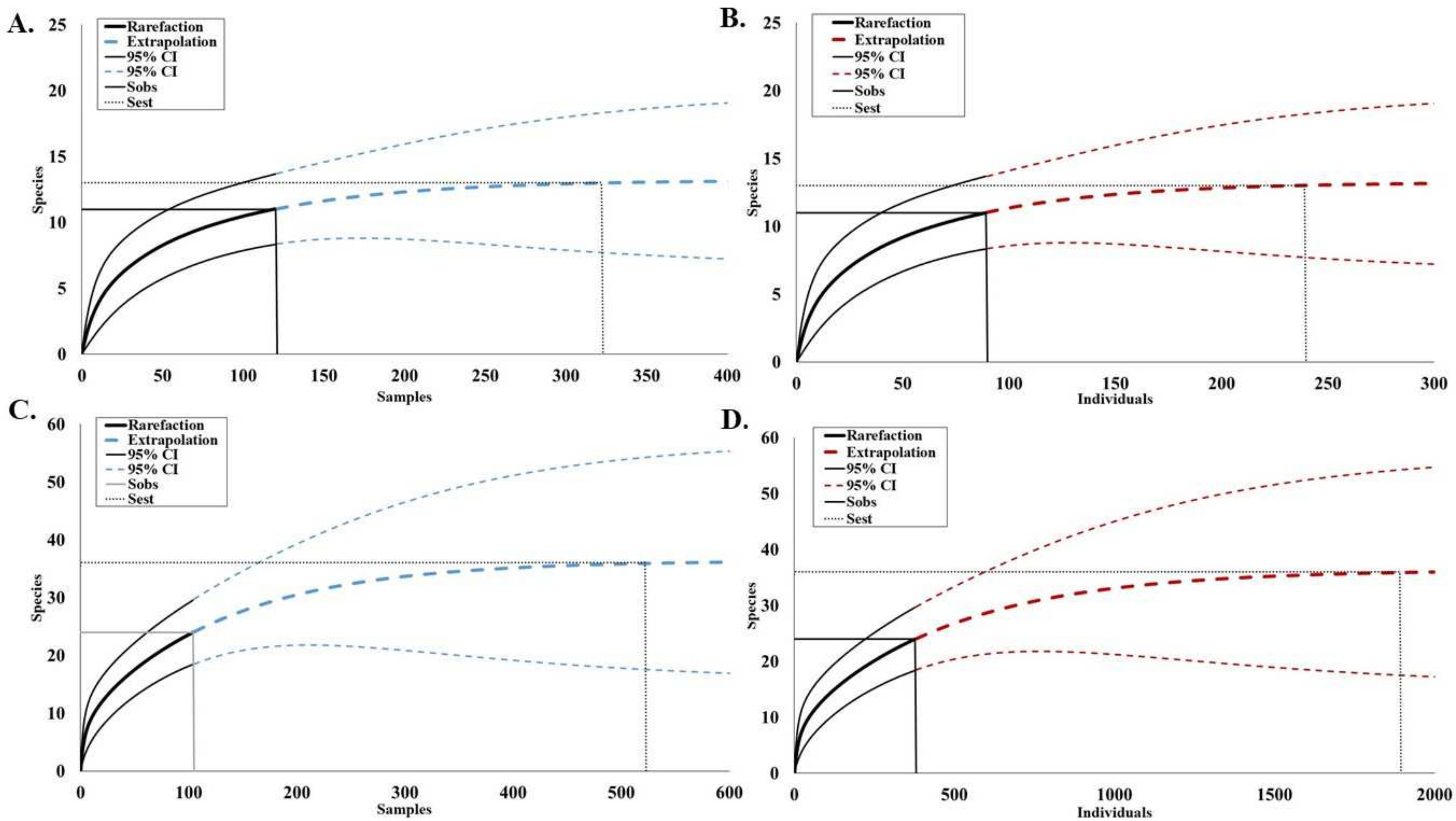
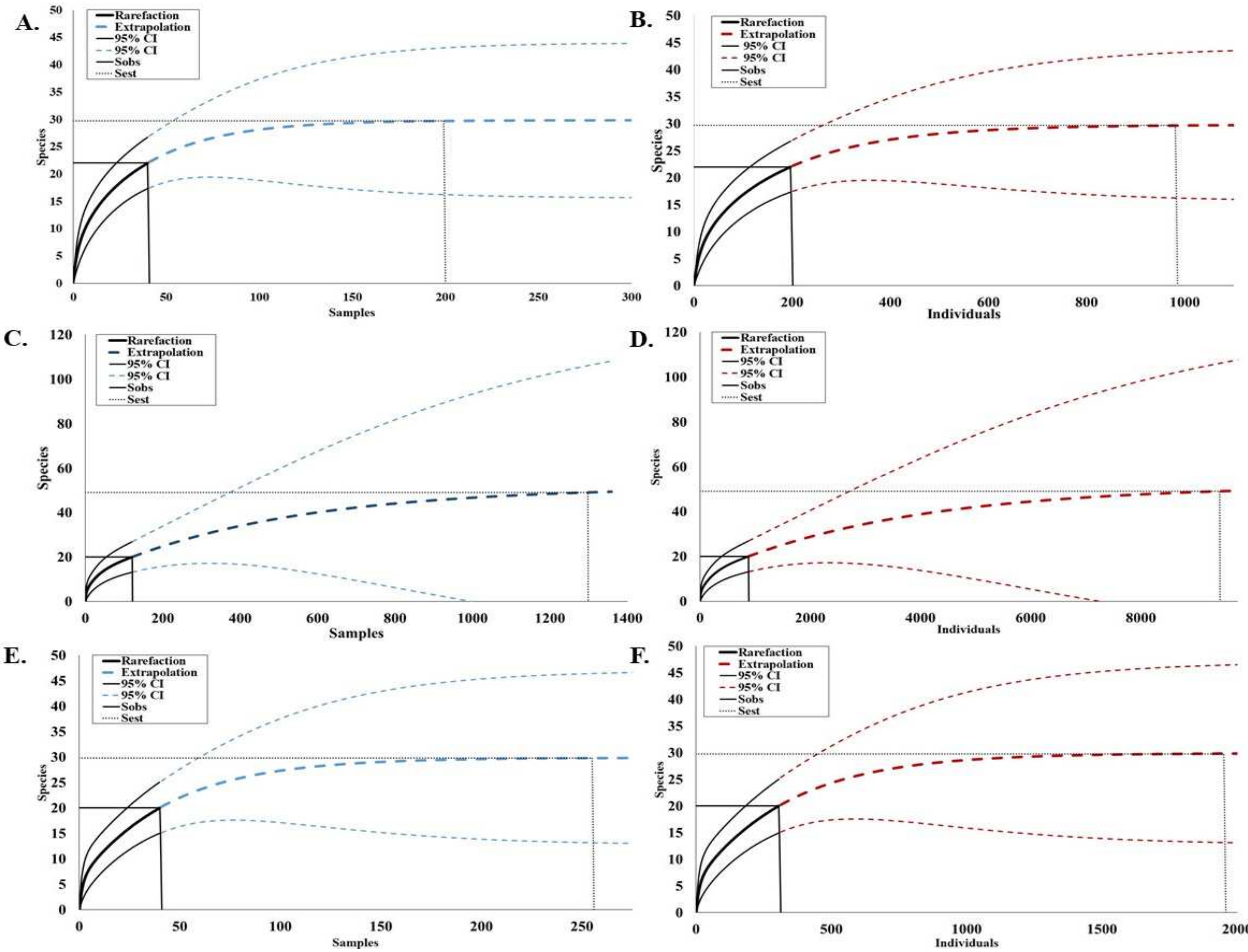


Figure 2. 2017 Species accumulation curves for A) sample number at Volga, B) individuals at Volga, C) sample number at Highmore, D) individuals at Highmore. Interpolation (solid curve) and extrapolation (dashed curve) with 95% confidence intervals. Intersection of the light gray line represents species observed (S_{obs}), while the intersection of the dotted grey line represents species estimated (S_{est}) at the point where the logistic curve stabilizes. Samples represents collected specimens from each plot at a given collection date.

Figure 3.


A.
B.
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F.
Rarefaction
Extrapolation
95% CI
95% CI
Sobs
Sest
Species
Samples
Individuals

Figure 3. 2018 Species accumulation curves for A) sample number at Gettysburg, B) individuals at Gettysburg, C) sample number at Volga, D) individuals at Volga, E) sample number at Pierre, F) individuals at Pierre. Interpolation (solid curve) and extrapolation (dashed curve) with 95% confidence intervals. Intersection of the light gray line represents species observed (S_{obs}), while the intersection of the dotted grey line represents species estimated (S_{est}) at the point where the logistic curve stabilizes. Samples represents collected specimens from each plot at a given collection date.

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CHAPTER 3

DETERMINING THE IMPACT OF POLLINATORS ON ON SOUTH DAKOTA SUNFLOWER YIELD

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Abstract

South Dakota is routinely the first or second largest sunflower producing state in the United States. In South Dakota, both confection and oilseed sunflower cultivars are economically important, and due to their value are intensively managed for insect pests and diseases. Despite breeding efforts to increase the ability of sunflower to self-pollinate there is evidence that pollinator visitation results in increased confection sunflower yields. In other states, this increase in yield is attributed to the presence of *Apis mellifera* L. as well as species of native bees. In South Dakota, there is no evidence to suggest that sunflower production is positively impacted by the presence of pollinators. To determine the impact that pollinators have on both confection and oilseed sunflower varieties we conducted an experiment evaluating the yield of open and closed sunflower heads. This experiment was conducted in two locations in 2017 and 2018. In 2017, the first location was the South Dakota State University Volga Research Farm in Eastern South Dakota and the second location was in a collaborator field near Highmore, SD in Central South Dakota. In 2018, the first location was at Volga Research Farm and the second location was near Onida, SD. To determine the impact that pollinators have on yield, six random sunflower heads in each plot were covered using no-see-um mesh netting. An additional six random sunflower heads were marked with tape and left uncovered. All covered and marked heads were harvested and seeds were weighed to determine the impact that pollinators had on sunflower yield. Our results indicated that the open heads yielded significantly greater. These results suggest that the impact of pollinators on sunflower production should be further explored.

Keywords: *Melissodes trinodis*, *Helianthus annuus,* pollinator, yield.

Introduction

The existence of pollinators can enhance the production of agricultural crops and the pollination services they offer is a mutualistic process involving animals and plants (Kremen et al. 2004, Greenleaf and Kremen 2006, Klein et al. 2007, Gallai et al. 2009, Dorado and Vázquez 2014). Animal pollinators are valued at an estimated $173 billion USD annually, within that, insects are directly responsible for approximately 70% of pollination services (Klein et al. 2007). Therefore, insect pollinators contribute $121.1 billion dollars to the global economy every year. Previous studies have examined the impact of pollinator visitation on crop yields (Allen-Wardell et al. 1998, Klein et al. 2003, Garibaldi et al. 2013, Bartomeus 2014, Mallinger et al. 2018, Gulya et al. 2019). Studies have shown that bees are the dominant pollinators of flowering plants, and are critical for the production of various crops (Giacomini et al. 2018). Additionally, Kremen et al. (2004) concluded, 48 of the leading 67 commodity crops saw increased yield when pollinators are present. Furthermore, the importance of both managed and native pollinators world-wide is extensive. The presence of pollinators enhances not only the fruit and seed set of plants, but also the genetic diversity of their offspring leading to pathogen resistance (Morse and Calderone 2000, Kremen et al. 2002, Klein et al. 2003, Bommarco et al. 2012, Garibaldi et al. 2013, Bartomeus 2014, Rader et al. 2016, Mallinger et al. 2018).

Sunflowers, *Helianthus annuus* L. (Asterales: Asteraceae), are an important oilseed and confection crop within the states of North and South Dakota. Combined, approximately 384,451 hectares of sunflower are harvested annually (NASS 2018a, b, Gulya et al. 2019). Sunflower is capable of surviving in in dry regions such as Central

South Dakota and is frequently included in crop rotations in that area (Schneiter et al. 1997, Berglund 2007). In South Dakota, sunflowers have been a part of the top grossing commodities for five years, and as a result are monitored for yield. Land allocated for sunflower production peaked in 2016, however yield continues to increase by 0.3 tons/hectare annually (NSA 2019). Additionally, few researchers have investigated the role of bees in the production of sunflower seed set. In fact, so little is known that growers often use self-fertile varieties to ensure sufficient yield (Fick and Rehder 1977, Abrol 2011). Breeding efforts have been able to successfully create cultivars that are self-pollinating, that is, they do not require animal visitation to result in seed set (Horner Jr 1977, Abrol 2011). However, despite breeding efforts to increase sunflower's ability to self-pollinate there is evidence that both oilseed and confection sunflower yields are increased when insect pollination occurs (Greenleaf and Kremen 2006). In other states (e.g. Texas, North Dakota, and Kansas), this increase in yield is attributed to the presence of *Apis mellifera* as well as species of native bees (Mallinger et al. 2018).

There are many factors that may lead to reduced sunflower yield including insect pests and diseases (Berglund 2007). However, the use of broad-spectrum insecticides that are used to manage pest insects and prevent yield loss are known to have negative effects on beneficial insects, which include pollinators. This negative impact on the pollinator populations may negatively affect yield. In South Dakota, there is a need to quantify the impact that pollinators have on sunflower yields so that management recommendations for pollinators can be generated that are specific to the region.

Sunflower is native to North American, which suggests that native pollinators likely co-evolved with this plant (Hurd et al. 1980). In addition, there is a growing body of evidence that sunflowers are a valuable source of these nutritious substances (Abrol 2011, Giacomini et al. 2018, Mallinger et al. 2018). Parker (1981) observed many species of bees visiting commercial sunflower; however, the role that pollinators have in seed production has not yet been investigated. The objective of this research was to determine if pollinator visitation positively impacts sunflower yield in South Dakota.

Materials and Methods

Data for this survey were collected during 2017 and 2018. For this experiment, two locations in South Dakota were used each year. In Eastern South Dakota, the location was at the South Dakota State University (SDSU) Volga Research Farm (Volga, SD 44°18'13.9"N 96°55'38.1"W). This site was chosen based on the absence of sunflower production in the surrounding landscape to provide a comparison to the Central South Dakota location, where sunflower are routinely grown. The specific locations for Central South Dakota varied by year due to field availability and weather events. The 2017 experimental site was located near Highmore, SD (44°31'23.5"N, 99°40'46.6"W) and the 2018 experimental site was located near Onida, SD (45.0875° N, 99.9904° W). Both of the Central SD sites were located on farmer collaborator fields. For this manuscript, the 2018 Onida site will be excluded as it was destroyed during a hail storm.

In 2017, sunflowers were planted at SDSU Volga Research Farm on 19 June. At the Highmore, SD location sunflowers were planted 21 June. In 2018, sunflowers were planted at the SDSU Volga Research Farm on 24 June, and 21 June at the Onida, SD location. However a significant hail storm damaged all plant material at the Onidalocation on 27 June, 2018. For each location, treatments (i.e., sunflower varieties) were planted in a randomized complete block design with 10 blocks. Each plot consisted of four rows, each 3m wide and 8m long. At each location three treatments or sunflower varieties were utilized. The varieties consisted of one confection variety, LD5009 (Nuseed), and two oilseed hybrids, which were P63HE90 (Pioneer) and Hornet (Nuseed). Each plot was planted at 8,093 seeds per hectare.

In order for yield comparisons to be conducted, six random plants in the middle two rows of each plot were chosen using an online random number generator and then bagged with 0.9oz no-see-um mesh (MosquitoCurtains.com, Alpharetta, GA) before flower emergence. The bags were secured tightly to the stems using zip ties (Uline, Hudson, WI), and remained on the flower head until harvest. Six additional unbagged plants were chosen using the same random method. At harvest, these heads were harvested and placed into a separate seed bag. Flowers that had been chosen for comparison were harvested by hand by cutting off the florescence with a pruning tool and placing gently into a seed bag for later analysis. All six of the covered or uncovered heads were measured to determine head diamater (mm), threshed as a pool by plot, weighed on a scale (g) and then tested for moisture content using the MT-16 grain moisture tester (AgraTronix, Streetsboro, OH). Moisutre was then corrected to 10% moisture to account for industry standards for sunflower and weights were adjusted accordingly.

Statistical Analysis

To determine if pollinator visitation increased sunflower yield the data were analyzed using an analysis of variance (ANOVA) and a Welch's unequal variances t-test in Program R version 3.5.3 (R Core Team 2018). To assess the effects of insect pollination on confection sunflower yields, we ran an ANOVA with head weight (kg), and treatment (open vs closed), location (Volga, and Highmore) and variety (n=2), and year (2017, 2018) (function 'aov', R v. 3.5.3). Additionally, we included a three-way interaction between treatment, year, and location, and a multiple two-way interactions between treatment and year, treatment and location, and treatment and variety, in order to assess variation in pollinator benefits across locations, plant genotypes, and years. (function 'aov', R v. 3.5.3). Next, we analyzed the means of each significant factor using a Welch's two sided t-test (function 't.test' R v. 5.3.3). In addition, we conducted post hoc treatment comparisons with a Fischer's Least Significant Difference (LSD) test to solidify statistical significance between groups (function 'LSD.test', R v. 5.3.3). Analysis of the yield count was conducted separately for each year, location, and sunflower type (oilseed versus confection). All statistical comparison tests used to interpret results of data utilized $\alpha < 0.05$.

Results

No significance was found between the two oilseed varieties yield utilized in this study, therefore they were combined for analysis. However, confection type sunflower remained separate from oilseed due to substantial physiological differences. We determined that yield varied significantly by year ($F = 23.967$; df = 1, 330; $P < 0.001$). In addition, location within South Dakota significantly affected sunflower yield ($F = 26.9$; df = 1, 882; $P < 0.001$) (Table 1). No significance was found between the two oilseed varieties; therefore, we combined the varieties into an overall oilseed type. Confection sunflower remained separate than the oilseed due to their significant physiological differences.

In 2017 at the Highmore location, the closed confection sunflower yielded significantly greater than the open heads ($t = -2.0852$; $df = 51$; $P \leq 0.05$). However, at the Volga location the open oilseed heads yielded signficantly greater than the closed heads ($t = 5.9007$, $df = 377$, $P \leq 0.001$) (Table 1). In 2018, at the Volga location the oilseed opened heads yielded signficantly better than the closed heads ($t = 3.0387$, $df = 207$; $P \leq 0.01$) (Table 2).

Discussion

For this study, we observed that pollinator visitation improved oilseed sunflower yield at two of the three tested site-years. However, we did not observe an improvement in confection sunflower yield. Conversely, Mallinger et al. (2018) noted yield improvements with pollinator presence in confection hybrids in South Dakota. This difference may be attributed to the differences in variety selection and grouping. When analyzed for pollinator influence on confection sunflower in South Dakota, Mallinger et al. (2018) combined 10 varietes of sunflower, some of which are crop wild relatives. The combination of varieties may lead to overall differences in pollinator influence on yield versus our single selected variety. Additionally, we found that yields in the Highmore location were significantly higher than yields in Vogla ($t = 4.3387$; $df = 198$; $P \leq 0.01$). This may be due to seasonal growing conditions (e.g. adequate precipitation and temperatures), as well as soil conditions. Weather during bloom can affect self-pollination rates and contributes to variation in pollinator benefits across locations and growing seasons. Ground nesting semi-social bees prefer softer soils that are easier to dig and nest in, with smaller amounts of precipitation (Potts et al. 2003). Given that about 70% of native bees are solitary ground nesting species, the higher yield may be attributed to more favorable conditions for nesting habitat. Additionally, more abundance and diverse pollinator populations were found in the Western locations across 2016, 2017, and 2018 (Mattern, unpublished). The increase of abundance attributes to higher yield in many field crop species (Klein et al. 2007, Hoehn et al. 2008, Bommarco et al. 2012, Mallinger et al. 2018). The most common pollinator found in all locations were *Melissodes* spp., and Parker (1981) note that *Melissodes agilis* specialize on sunflower pollen. The *Melissodes* genus are pollen specialists of Asteraceae, however, Kim et al. (2006) reported that tillage practices may disturb common nesting areas. Tillage in the Western locations is less common than in the Eastern, Volga location, this may contribute to both the higher pollinator abundance, and higher overall yield. This study highlights the importance of pollinators for achieving maximum sunflower yields, in particular native bee species including sunflower specialists such as *Melissodes* spp., *Svastra obliqua*, and *Adrena* spp.

The benefits of pollination services are less obvious in sunflower seed production systems due to the high self-pollination in these plants. In fact, we found that confection sunflowers do set a significant amount of seed without pollinator presence. Additionally, confection sunflower only had a significantly higher yield in the Highmore location. Across both growing seasons, oilseed sunflower in Volga observed significantly higher yields in the presence of pollinators when compared to the excluded treatment yield. The average yield improvements in oilseed may be attributed to the desirability of the oilseed floret. Among the various traits that influence bee preference, Portlas et al. (2018) notes, that floret size may be critical during pollinator foraging as the depth of the corolla influences the accessibility of nectar. For bees and the flowers they are associated with, tongue length and corolla depth compatibility is pertinent to successful foraging behavior. Some bee species and plants are very closely matched, with bee tongue size directly matched to the corolla depth, others are considered generalists, fitting well with a variety of plant species. Observations on 30 inbred lines showed that floret size attributed to 52% of variation in native bee species preference, with floret lengths of 2mm less more than doubled pollinator visitation (Portlas et al. 2018). In addition, Mallinger and Prasifka (2017), recorded preference for sunflower with shorter corollas and greater quantities of nectar in both native and managed bees. Other factors, such as bee body size, sex and morphology are expected to produce similar results across sunflower cultivars. However, time spent per sunflower head, visitation frequency and per-visit efficacy may affect the influence pollinators have on sunflower seed set. We acknowledge that foraging behaviors may differ between male-sterile, and male-fertile sunflowers, therefore in the future observational data in addition to pollinator diversity and abundance must be collected in order to accurately estimate the pollinator influence on sunflower seed set.

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Table 1. 2017 sunflower yield data.

Location	**Sunflower Variety**	**Yield (g per six heads)**	
		Open*	**Closed**
Highmore*	Oilseed	130.25 ± 83.67	139.80 ± 86.63
	Confection	108.86 ± 93.79*b*	152.12 ± 64.13*a*
Volga	Oilseed	145.80 ± 79.46*a*	115.98 ± 162.71*b*
	Confection	130.25 ± 83.67	139.81 ± 86.64

*Refers to signficance less than $P < 0.05$.

Table 2. 2018 sunflower yield data.

Location	Sunflower Variety	Yield (g per six heads)	
		Open*	**Closed**
Volga	Oilseed	95.50 ± 54.47*a*	74.71 ± 45.7*b*
	Confection	85.04 ± 54.24	75.37 ± 38.44

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CHAPTER 4

GENERAL CONCLUSIONS

The results from chapter two indicate that there is a diverse fauna of pollinators in the state of South Dakota. With noticeable differences between eastern, and western locations. The Simposon's Diversity Index in 2016 and 2018 support this idea. We found that the diversity in the western locations (e.g. Pierre, Gettysburg, Onida, and Highmore) was significantly higher than the eastern Volga location. More specialized *Asteraceae* bees were identified in western locations suggesting that the native populations have adapted to the region and utilize sunflower more actively in these regions. Variation in abundance and diversity is in turn likely due to a combination of factors including plant genotype, environment, and pollinator interaction. In addition, the increased adoption of no till practices may contribute to undisturbed ground, which would result in more available nesting areas for solitary native bees in the area.

In South Dakota, bee stocking is a common practice near sunflower fields, and there are approximately 225 thousand hives in the state (DOA 2012). It has been noted that honey bees can travel up to 3 miles from their colony, and are attractive to farmers due to their generalist foraging behaviors and potential to increase yield (Beekman and Ratnieks 2000, Chambó et al. 2011). Despite multiple honey bee hives stocked near our test-sites only 35 *A. mellifera* were collected in 2016, 2017 and 2018, composing 2% of the pollinator community. These findings confirm our hypothesis that native pollinators compose the majority of the pollinator community in South Dakota. Thus, the results of this survey indicate that honey bees may not have a significant impact on sunflower yields in the Northern Great Plains. Additionally, other related projects have also noted that bee bowls may not be an effective sampling technique for monitoring *A. mellifera* (Kremen et al. 2004). Our results seem to support the suggestion that bee bowls may not be the most efficient way to sample *A. mellifera,* specifically. Moreover, Greenleaf and Kremen (2006) indicated that honey bee and native bee interactions may increase native bee efficacy in sunflower fields, which in turn may contribute to the differences in yield we saw in oilseed, but not confection varieties. Overall, female large-bodied bees, in particular *Melissodes* spp., were the most abundant pollinators identified in South Dakota. This highlights the importance of pollinators for achieving ideal yields, specifically when sunflower specialists such as *Melissodes agilis* and *Melissodes trinodis* are present.

The species accumulation curves suggest that our sampling methods were sufficient in most site-locations across each year, except Volga 2018. In 2018, high numbers of aphids found in the surrounding soybean and corn fields may attribute to the high levels of syrphid (*Syrphidae)* flies collected. Additionally, syrphid flies were grouped as one species. Given the high amount of syrphid flies collected in 2018, this contributes to the high extrapolated value of potential species in this location. Overall, Volga 2018 the species accumulation curve suggests that more sampling, or more diverse sampling could have been conducted to adequately identify the diversity in the region. However, due to both time constraints and plant morphology of sunflower, many alternative sampling methods are nearly impossible. The tall stature, and close proximity of sunflower plants within agricultural fields makes sweep collection disastrous to the plant material itself. Additionally, ground pan traps collect more small body, ground dwelling bees versus higher pan traps. Some studies suggest that honey bees are not as attracted to modified pan traps “bee bowls”, which may explain the small numbers of *Apis mellifera* collected throughout all three site-years.

In all site-years, in order to achieve the potential species abundance, the graph suggests, sampling efforts would need to increase 2-5 times what was implemented in this study. However, sunflower blooms between 2-3 weeks every growing season (Berglund 2007). This short blooming period makes it difficult to accurately represent the diversity of the pollinator community throughout a given growing season. Additionally, in order to collect more bee species more diverse collection methods must be conducted. Census methods (e.g. transect walks, catch and release) are comparatively more time consuming and often require very experienced surveyors in order to obtain quick and accurate identification. Moreover, sunflower is a tall plant, and is traditionally grown in 3ft rows (Berglund 2007). This practice makes it difficult to conduct transect walks and sweeps within a field without damaging plant material.

Chapter three confirms our hypothesis that pollinator presence positively impacts sunflower yields in South Dakota. Across all site-years and locations, we found a significant difference between open sunflowers and closed. In particular, within oilseed varieties. These results suggest there is a preference in oilseed varieties for pollinators. Additionally, we found that yields in the Highmore location were significantly higher than yields in Volga. This may be attributed to abiotic factors (e.g. temperature and precipitation), as well as biotic factors such as soil conditions. Weather during bloom can affect self-pollination rates and contributes to variation in pollinator benefits across locations and growing seasons. (Potts et al. 2003), found that ground nesting bee species prefer dryer, softer soils to build their nests in and given that about 70% of native bees are solitary ground nesting species, the higher yield in Highmore may be attributed to more favorable conditions for nesting habitat. Specifically, the average precipitation in Volga is 2.88 inches per July-September (the flowering period for sunflower) and in Highmore is 1.99 inches per July-September (Woolhiser and Roldán 1986, Kahara et al. 2009).

Because of the high self-pollination rate in sunflower, the benefits of pollination services may seem less obvious. In fact, we found that confection sunflowers do set a significant amount of seed without pollinator presence. Conversely, Mallinger et al. (2018) noted yield improvements with pollinator presence in confection hybrids in South Dakota. This difference may be explained by the grouping of ten different confection sunflower varieties during analysis, some of which are crop wild relatives. The combination of varieties may lead to overall differences in pollinator influence on yield versus our single selected 5009 variety. Additionally, across both growing seasons, oilseed sunflower in Volga observed significantly higher yields in the presence of pollinators when compared to the excluded treatment yield.

These results suggest that understanding the diversity and abundance of pollinator communities in South Dakota provides valuable data that may be used to determine best conservation, and management practices. They also suggest that pollinators do, in fact, have a positive effect on South Dakota oilseed sunflower. Continuation of pollinator surveys supplies the information necessary for researchers to determine if current agricultural practices are having a negative impact on pollinator populations. This also provides the framework for additional research projects that can evaluate the impact of routine pest management on ground nesting bees within sunflower fields and evaluation of the impact of pollinator activity on sunflower yields. As few managed bees were collected during the three-year study, this study also solidifies the importance of native pollinators. Future research should continue to identify the syrphid species visiting South Dakota sunflower, and record the specific visitation patterns to determine which species the most efficient pollinators are found in sunflower fields.

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