Pollinator limitation in the endangered sunflower, *Helianthus schweinitzii*

BRITNEY PHIPPEN

Department of Biology, Queens University, Charlotte, North Carolina 28274

Abstract

Native to the piedmont of North and South Carolina *Helianthus schweinitzii,* commonly named Schweinitz's sunflower, was added to the endangered species list in 1991. The effectiveness and frequency of pollinators may have contributed to the decline of this once plentiful flower in the prairie fauna. In this experiment, the effects of hand-pollination verses open pollination were examined as well as observational trends of pollinators. Six 1x1 meter plots were flagged off at Redlair in Belmont, NC and three plots were open pollinated while the other three were hand pollinated once a week from the time flowers bloomed until seeds had fully formed. Fifteen seed heads were collected from each plot and tested for durability using a metal probe and dissecting microscope. Observation of pollinators occurred at Redlair and Latta Plantation both of which had healthy populations of the flower. The results showed a significant difference between plots that were hand-pollinated and plots that were open-pollinated only. On average, 91.48% of total seeds were viable in hand-pollinated plots compared to 73.37% in open-pollinated plots. Trends in pollinators included limited amount and types of pollinators, sporadic visitation rates and ineffective motility by the pollinators for effective pollination of the flowers. These observations support the results of the pollination experiment,

that inadequate natural pollination may result in lower seed set than that for which the plants are capable.

Introduction

Pollinator limitation can be a large factor in the reproductive success of a plant in which their primary means of pollination is by insects and/or birds. Without a continuous visitation rate of pollinators to a plant species, the species is in danger of becoming endangered or even extinct. The sunflower family (Helianthus), which is native only to North America has six species that are either rare or endangered (Seiler and Gulya 2003). The reason for these species becoming endemic is not known, but pollinator limitation may be a leading cause of higher extinction rates in these flowers.

Helianthus schweinitzii, commonly named the Schweinitz's sunflower of the family Asteraceae, was added to the endangered species list in 1991 by the U.S. Fish and Wildlife Service (Matthews and Howard 1999). Seiler and Gulya (2003) describe that this particular species is frequently found in areas near roadsides or in highly disturbed areas in the Piedmont of North and South Carolina. The exploration and collection of this species was first established by Seiler and Gulya (2003) and before they collected the flowers, they were not readily available for research. These flowers were once part of a vast Piedmont prairie ecosystem that has been displaced through years of habitat fragmentation and development (Davis et al 2002). It is not enough to just observe the type and frequency of pollinators who are visiting the flowers, because the habitat fragmentation that the H. schweinitzii has undergone can affect biological interactions (Mustajarvi et al 2001). Since its

discovery, H. schweinitzii has declined approximately 30% (Matthews and Howard 1999).

Matthews and Howard (1999) explored the genetic variation of the sunflower when compared to common ancestors H. giganteus and H. microcephalus. They concluded that the H. schweinitzii had a moderate genetic diversity (H=0.108) but less population differentiation than other species with restricted ranges. There was no clear correlation between geographic pattern and genetic relationships among the 18 different populations they sampled: some were close in relation and others were further apart. These authors discuss several explanations to explain the genetic similarities. One reason may have been the use of the plants by Native Americans and that they may have actively cultivated the plant for food in areas that they moved to. Another explanation may be on a molecular level, and that there was not adequate time for the species to have a high level of variation. They stated that it would be incorrect to explain that the H. schweinitzii sunflower is endangered due to low levels of genetic variation. This research sparks many questions that need to be examined before understanding this endemic species and requires much more research.

In a study done by Fischer and Matthies (1997), the endangered plant Gentianella germanica was studied for relationships between mating structure and inbreeding and outbreeding depression. They found that increased extinction rates were due to loss of habitat and changing environmental stressors. In small plant populations, the pollinator limitation of reproduction has had a negative effect on plant-pollinator mutualisms, thus further reducing reproductive output. In their

observations, the natural seed set of the G. germanica greatly depended on the frequency of pollinators visiting each plant and on pollen transfer between patches of the flowers. They stressed the importance of the negative correlation between plant population size and the importance of the pollinator. Supplementary hand-pollination was not investigated and some further testing would be needed to determine if seed yield grew if flowers were open-pollinated and additionally hand-pollinated.

There have also been many experimental studies done on the outcomes of natural and experimental high pollen loads and their effect on seed production and fitness. Young and Young (1992) described that there are two outcomes when hand-pollination studies are used, either no change or a decrease in reproductive success of the female plant. These studies are generally only testing how sufficient natural pollination is in an area. Young and Young (1992) predicted that excess pollination would result in a lower level of seed production and that the seeds would be less viable. In their study of 99 cases, they found significant reproductive success in flowers that were hand-pollinated. They showed that there was significantly reduced success in 17.2% compared to 42.4% success in hand-pollinated flowers.

The more information that can be gathered on H. schweinitzii, the higher chance it has for survival and the possible conservation of the species. This particular flower is not spreading when moved to undisturbed locations. The need for pollinator studies would be very beneficial in predicting the best place for the flowers to be moved and its best chance for reproduction. To find out why t H. schweinitzii is not growing in new habitats and if it is related to the pollination of

the plants will be a huge advantage when it comes to conservation of the species. The goal is to find out why a species that clearly used to thrive and prefer disturbed soil cannot seem to reproduce and therefore move to new locations, furthering diversity and increasing population size.

This study observed types and frequencies of pollinators that visited the sunflower as well as a direct comparison between groups of flowers only pollinated by pollinators with groups that have been artificially pollinated. This information will be geared toward direct causal relationships between pollination and the effect it has on species dispersal and what might be holding the species back from flourishing. The comparison of artificially pollinated flowers to naturally pollinated flowers will begin to determine what is going on in terms of reproduction in the plants. Is it not spreading due to limited pollinator interaction or is it something else that makes these plants unable to thrive in their environments?

Methodology

Plant Population

This study on *H. schweinitzii* was performed at Red Lair in Belmont, North Carolina where there has been a continuous population of the flower. The study began in August when the flowers began to bloom and continued until all data was collected (Matthews and Howard 1999). Ideally, there would have been four test groups as described in the study done by Giblin and Hamilton (1999); however, due to the species being endangered, open-pollination and artificial hand-pollination within patches will be studied. After assessing plant population density three test

groups were chosen. The test areas were chosen in areas that would reduce trampling on the flowers that were not being tested.

Artificial Hand-Pollination/Open-Pollination Field Study

After location and density of flowers were determined the test area was divided and flagged off creating 1x1 meter plots (as shown in Figure 1) for distinction between open-pollinated side and the hand-pollinated side. The open-pollinated side was marked with blue flags and pink flags marked the hand-pollinated side. The open-pollinated area remained untouched and allowed only pollination by pollinators. The hand-pollination study followed protocol developed by Giblin and Hamilton (1999) and Mustajarvi et al (2001) and was performed once flowers had emerged and pollen was visible on the stamens. The ripe pollen was rubbed across the stigmas of plants (as shown in Figure 2) by a paintbrush in the area of hand-pollination, making sure to get pollen from multiple stamens rubbed on multiple stigmas. After all the flowers were hand-pollinated, a 10x hand lens was used to make sure pollen has been properly

Figure 1 1x1 meter plots of testing areas

Figure 2 Hand pollination process on each flower in plots

dispersed. Hand-pollination was repeated once a week to ensure optimal pollination.

Seed Collection/Durability Testing

Once seeds formed, specimens from each tested area were collected to perform basic fitness tests. In each plot 15 fully developed seed heads were collected. It was important to be careful about the amount of collected specimens due to the fact that *H. schweinitzii* is endangered. Approximately 10 percent of the studied area was collected. Seeds will be collected in separate paper bags; one bag for each test area and transported to the lab. In the lab, a dissecting microscope (10x power) was used to compare the number of filled seeds to empty seeds. Pressing the individual seeds with a metal probe made the distinction: empty seeds were easily flattened and filled seeds were durable. The same amount of pressure was used for each seed. If there was any uncertainty, the seed was opened by scalpel. The number of filled and empty seeds were recorded for each test area and statistically analyzed. After testing was complete, the filled seeds were returned to Red Lair so that they can grow next season.

Types and Frequency of Pollinators

To determine specific pollinators and frequency of visitation, a simple observational field study was performed. The flowers were observed at Red Lair as well as at Latta Plantation, which both populations of the flower. The population of flowers was monitored from August until the first frost once a week for 1 hour

intervals. The observations took place once a week at alternating time intervals, morning, afternoon and dusk (before dark). The order at each site was random so that it did not bias the study in terms of conditions and weather patterns. This meant that every week the flowers were observed for one of these time intervals and repeated until the study was complete. It was important to visit the population at different times of the day and different weather conditions to get an accurate view of pollinators. All flower-visiting insects were recorded and classified as they interact with the flowers; they also will be photographed with a Canon Powershot© camera that has 10x zoom capabilities in order to specifically identify each insect.

Results

Hand-pollinated plots had more seeds overall as well as a significant difference between viable and non-viable seeds based on the durability tests that were performed on each seed. Plot 1 had 129 viable seeds compared to 52 non-

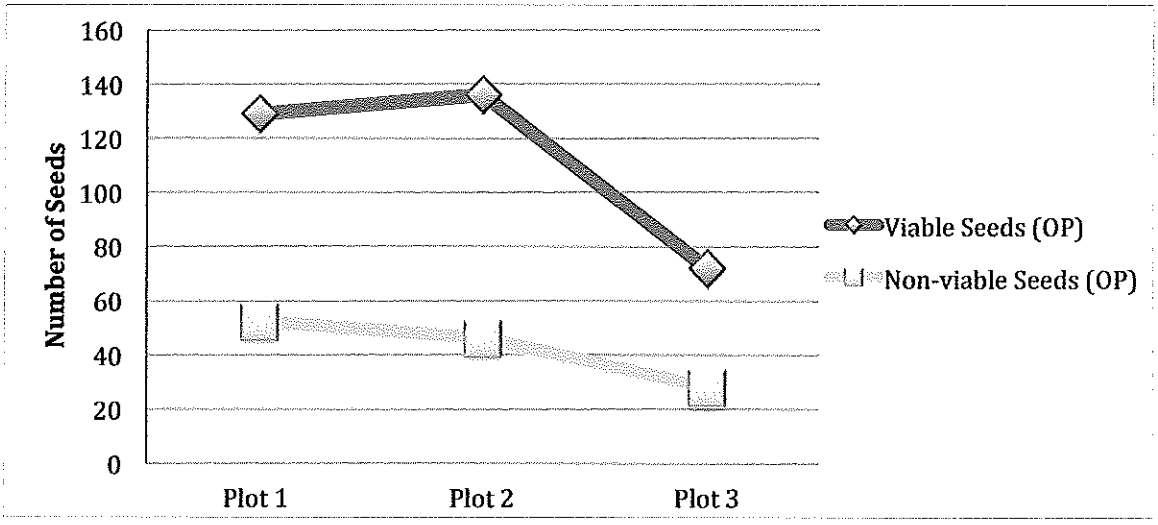

Figure 3 Number of viable and non-viable seeds in all three open-pollinated test plots

viable seeds, plot 2 had 136 compared to 46 and plot 3 had 72 compared to 28 respectively in open-pollinated areas (Figure 3). In hand-pollinated areas plot 1 had 228 viable seeds compared to 25 non-viable, plot 2 had 224 compared to 20 and plot 3 contained 97 compared to 9 respectively (Figure 4). In general, plots that were hand-pollinated every week had a higher yield of seeds (viable and non-viable).

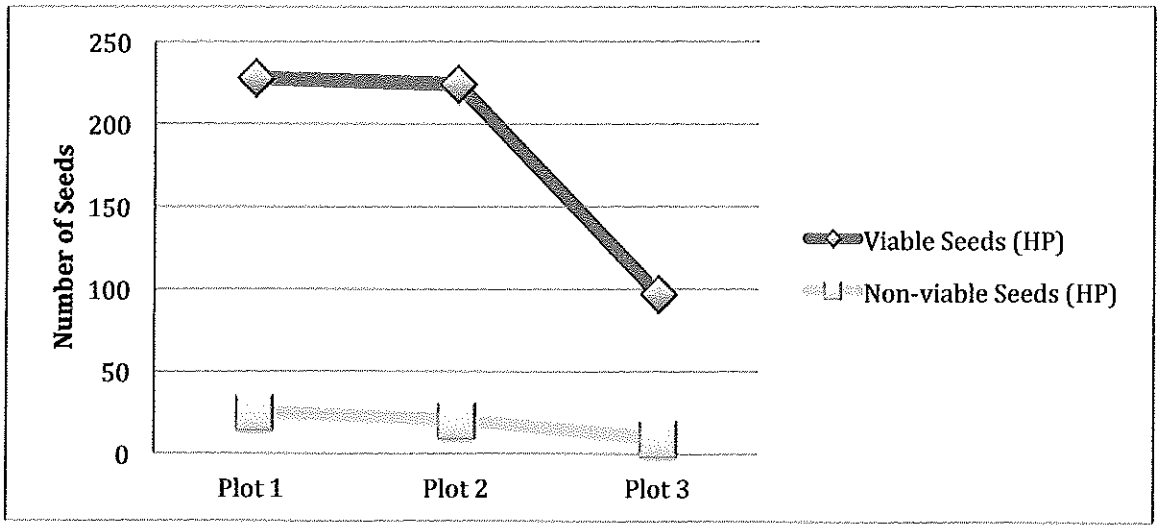

Figure 4 Number of viable and non-viable seeds in all three hand-pollinated test plots

Durability tests between hand-pollinated and open-pollinated test areas showed a significant (p-value=0.006) difference. Plots were compared using the number of viable seeds in each test area. The mean value of viable seeds in open-pollinated plots is ≈73% and the mean value in hand-pollinated plots is ≈91%. All three plots had very similar percentages with a standard error of 0.017 in open-pollinated plots and 0.005 in hand-pollinated plots (Figure 5). The low errors show

that there was a similarity between the individual plots and location of the testing areas did not play a large role in seed numbers and viability.

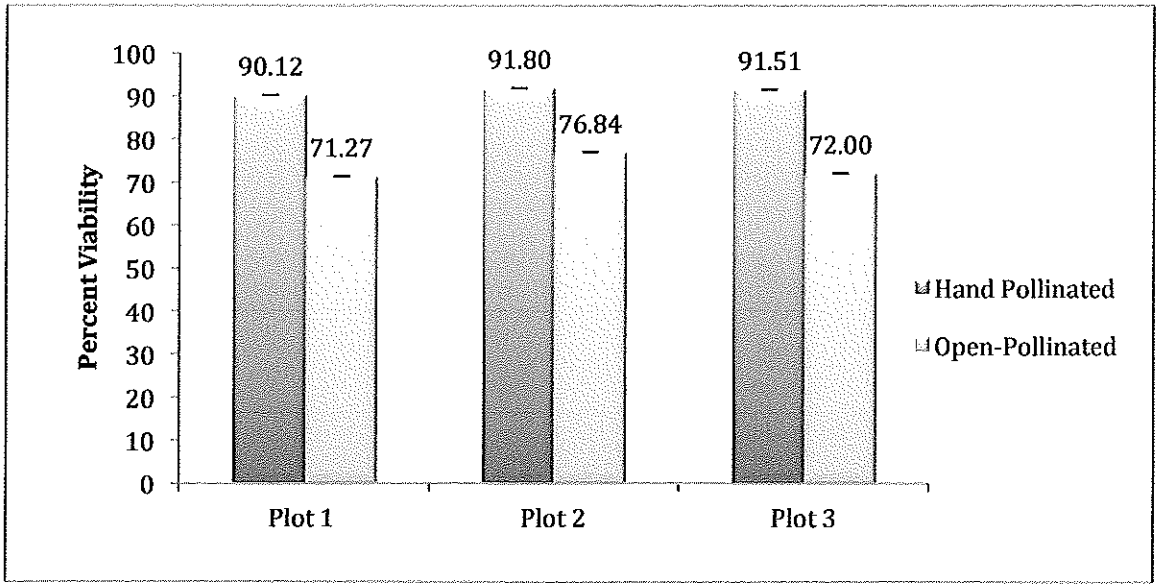

Figure 5 Percent of viable seeds in open-pollinated and hand-pollinated areas as well as standard error for respective plots

Frequencies of visiting pollinators could not be exactly quantified but trends and types were recorded. The most common pollinators were caterpillars, bees, butterflies, crickets and ants (Figure 6). No birds visited the flowers during the observational intervals that were performed during the experiment. Many of the pollinators were more stationary and therefore could not pollinate the flowers as well as other insects or birds. In the morning interval there were more butterflies and caterpillars while in the afternoon there were more bees and ants and in the evening there were more crickets and beetles. Overall the beetles were the most common pollinator and they tended to stay mainly on one flower and occasionally moved from flower to flower but did not go from one area to another area further away. The butterflies seemed to be the most effective pollinators moving from

flower to flower and patch to patch but there were only one or two at a time so the limited number could effect pollination. Ants and caterpillars stayed exclusively on one plant and did not move to other flowers in the 1-hour intervals of observation. Latta Plantation had a larger population of the flower and seemed to have a higher number of pollinators in the range of plant patches. Also, at Latta Plantation the flowers were more concentrated in one big area rather than at Redlair where they were more patchy.

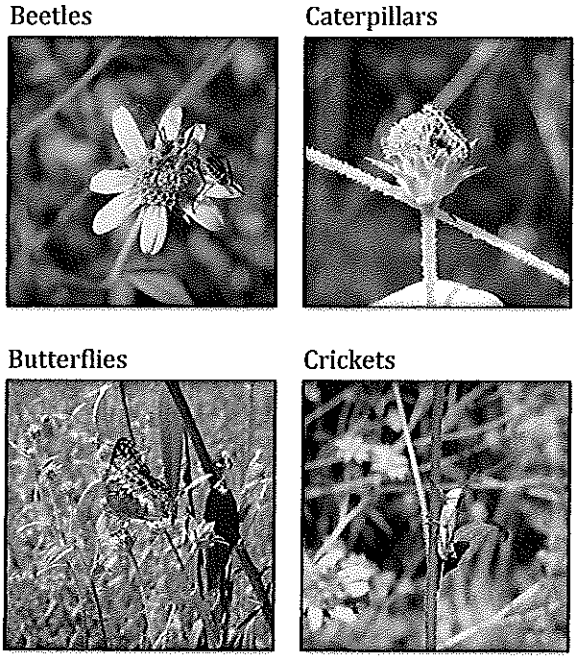

Figure 6 Types of pollinators that were commonly found on flowers at both locations, Redlair and Latta Plantation

Discussion

Hand-pollination had a significant affect on the viability of the seeds produced by the Schweinitz's sunflower. There were approximately 20% more viable seeds in hand-pollinated plots compared to open-pollinated plots. Viable seeds were distinguished by a durability test developed by Young and Young

(1992). Flowers in open-pollinated plots may have had lower viability due to the fact that trends in pollinators suggest ineffective motility and low frequency of visitation. The significant difference of the viability combined with the trends of pollinators could in fact have a correlation on why the Schweinitz's sunflower is not spreading to new areas and only found in specific locations. The results of the experiment paired with the observational study suggest that there is a lack of pollination of the flower in this location and could be one of the reasons for its decline.

There may be several reasons why the experiment showed significance in the pollination study. One reason is that since visitation rates in pollinators was low the hand-pollinated flowers were able to all be pollinated so they had a better probability to produce viable seeds. Another reason for this result may be that although choosing flower heads to count was random, it may have biased the experiment rather than if all of the seeds could have been collected and tested. This does however show that there is some correlation between the pollination and seed viability.

Further research is required to validate the results from this experiment. There are many changes to the experimental design that would help eliminate question and bias. One thing is repetition; the study needs to be repeated over as many flowering seasons as possible in order to control different seasons and to see if there are similar patterns between the seasons. Another thing is to change the location to make sure that the pollinator correlation is not site specific. In testing the seeds a simple durability test was used to determine viability and in further

experiments seeds should undergo the same durability tests as well as germination of the seeds to ensure that the seeds would in fact grow. Ideally there would also be a control group in which all of the area would be netted off to not allow any pollination and create a baseline for seed production numbers. With a few changes there may be enough information to begin to help expand the Schweinitz's population.

References

Davis J, McRae C, Estep BL, Barden LS. 2002. Vascular flora of piedmont prairies: Evidence from several prairie remnants. Castanea 67(1):1-12.

Fischer M, Matthies D. 1997. Mating structure and inbreeding and outbreeding depression in the rare plant *Gentianella germanica* (Gentianaceae). American Journal of Botany 84(12) 1685-92.

Giblin DE, Hamilton CW. 1999. The relationship of reproductive biology to the rarity of endemic *Aster curtus* (Asteraceae). Canadian Journal of Botany 77(1):140-9.

Kirchner F, Luijten SH, Imbert E, Riba M. et al. 2005. Effects of local density on insect visitation and fertilization success in the narrow-endemic Centaurea corymbosa. Oikos 1(1): 130 -142.

Matthews CR, Howard JH. 1999. Genetic variation in the federally endangered Schweinitz's sunflower, *Helianthus schweinitzii* T. & G. (Asteraceae). Castanea [Internet]. [cited 2010 Feb 18]; 64(3):231-42. Available from : http://www.jstor.org/stable/4033907.

Mustajarvi K, Siikamaki P, Rytkonen S, Lammi A. 2001. Consequences of plant population size and density for plant-pollinator interactions and plant performance. Journal of Ecology (1):80-7.

Seiler GJ, Gulya TJ. 2003. Recent explorations to recover seed of rare and interesting *Helianthus* species and future explorations. U.S. Department of Agriculture, Agriculture Research Service. Fargo,ND.

Townsend CR, Begon M, Harper J. 2008. Essentials of Ecology: 3rd Edition. Malden, MA: Blackwell Publishing. 510 p.

Young HJ, Young TP. 1992. Alternative outcomes of natural and experimental high pollen loads. Ecology 73(2):639-47.