

NATURAL SELECTION BY INSECT POLLINATORS AND SEED PREDATORS ON
FLORAL HEAD TRAITS OF HELIANTHUS GROSSESERRATUS (SAWTOOTH
SUNFLOWER)

by

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ABSTRACT

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The University of Wisconsin-Milwaukee, 2016
Under the Supervision of Professor Gretchen Meyer

Flowering plants must invest energy and resources to produce floral displays that are attractive to pollinators, but these same displays may also attract detrimental insects. How floral traits are shaped by the preferences of both pollinators and herbivores/seed predators is not fully understood. Using *Helianthus grosseserratus* (sawtooth sunflower) as my study species, I investigated these conflicting selective pressures on floral head traits through a 2-year study in a large, unbroken tract of mesic prairie in Wisconsin. In the first season, I followed individual heads over time and recorded insect visitation patterns and phenological changes to floral head traits. I also dissected seed heads at the end of the flowering period and identified all seed predators to order. In the second year, I measured floral head traits (including disc area, ray area, and UV reflectance patterns) on the day when most florets were presenting pollen. I also performed a hand-pollination experiment to determine if the plants were pollen-limited or resource-limited. I recorded the number and percent developed seeds per head as measures of reproductive success and also counted and identified the seed predators in each head. I also measured the number of flowers surrounding the study head as an additional factor that may affect pollinator and herbivore/seed predator preference. Floral heads were visited by a diverse group of insects: 16 species from 7 orders were recorded. Hymenoptera, Coleoptera, and

Diptera were the most common visitors. These 3 orders had highest visitation on the second or third day of pollen presentation. Seed head dissection revealed 6 orders of insect, with Thysanoptera and Diptera being the most common. In year 2, I found that pollinators were required for seed set in this system, as heads that were bagged produced negligible seed. Heads in the hand-pollination treatment had fewer developed seeds and a lower percentage of developed seeds than heads that were open-pollinated, although these differences were not significant. These results suggest that the plants were more likely to be resource-limited than pollen-limited. However, hand-pollinated heads did have significantly more seed predators than open-pollinated heads, which likely reduced seed set. Disc area was the most important trait affecting both the number of developed seeds and the number of seed predators, with larger discs having both greater seed production and more seed predators. Disc area did not influence the percentage of developed seeds, suggesting that the effects on seed number reflect the fact that a larger head has more ovules rather than pollinator attraction. The UV patterning on study heads showed significant polymorphism, where some plants had a strong bulls-eye pattern on rays, while others had no clear demarcation (50% of heads in 2013 had no demarcation; 44% in 2014). My results showed there was no relationship between this patterning and number or percentage of developed seeds, but plants with a stronger bulls-eye pattern (likely because of a reduced amount of UV-absorbing defensive pigments) had more seed predators. These results suggest that UV patterning was important for defense against seed predators. In addition, floral heads with a large ray area had fewer seed insects, while those with a short head height and a large number of flowers in surrounding area had higher number and percentage of developed seeds. Such results highlight the complexities involved in the generalist pollination syndrome and the need to consider a multitude of floral head traits when analyzing plant/insect interactions.

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INTRODUCTION

An estimated 87.5% of all flowering plant species are pollinated by insects and other animals (Ollerton et al., 2011), who visit flowers in search of rewards such as nectar and pollen (Haydak, 1970; Roulston and Kane, 2000). Many flowering plants invest substantial energy on attractive floral displays, which may enhance pollinator visitation and promote cross-fertilization (Ashman, 1994). Floral trait phenotypes often vary among flowers of a given population and are subject to strong natural selection by visiting pollinators (Neff and Simpson, 1990; Stuessy, et al., 1986; Conner and Rush, 1995).

When analyzing the effectiveness of floral displays in promoting cross-fertilization, however, it is essential to also consider the attraction of insects which are detrimental to reproduction. Insect florivores and seed predators, for example, do not transport pollen and have the potential to cause substantial damage to developing seeds and other reproductive parts of a plant (Pilson, 2000; Cardel and Koptur, 2010). Previous studies have shown that florivores and seed predators can influence the prevalence of floral trait phenotypes in a plant population (Pilson, 2000; Irwin, 2003; Strauss and Whittall, 2006; Bertin et al., 2010).

Flowering plants, therefore, are under simultaneous selective pressure from pollinators and insects which damage flower parts (de Jager and Ellis, 2014). Strong evolutionary pressure on flowering plants from florivores and seed-damaging insects can result in floral displays that are not favored by pollinators (de Jager and Ellis, 2014). A small floral display, for instance, may reduce damage from florivores and seed-damaging insects, but may also be less attractive to insect pollinators. Opposing selection between the pollinators and damaging insects may result in the maintenance of floral trait variation within plant populations (de Jager and Ellis, 2014) This evolutionary tradeoff between maximizing pollinator visitation and minimizing damage to

flower parts has not been well-studied in many flowering plant and pollinator systems. In addition, it is not known in many systems which floral trait phenotypes are favored by florivores and seed damaging insects, and which are favored by pollinating insects.

Some studies have attempted to address this question of how flowering plants have adapted to these opposing pressures on floral trait phenotypes by only focusing on isolated traits such as flower size (Danderson and Molano-Flores, 2010). The consideration of just one floral trait, however, is likely inadequate in understanding the entire dynamics of a plant/pollinator/damaging insect system (Connor and Rush, 1996). Instead, it is critical to examine multiple floral traits of a system for both pollinator and damaging insect selection.

These tradeoffs between attracting pollinators and flower-damaging insects may be particularly important in generalist-pollinated flowers. Generalist flowers typically attract a large number of insect orders: Dermaptera, Hemiptera, Orthoptera, Plecoptera, Diptera, Coleoptera, Lepidoptera, and Hymenoptera are all commonly found (Lane, 1996; Dickinson and McKone, 1992). This generalist pollination syndrome is common within Asteraceae, the largest family of flowering plants (Funk et al., 2005; Lane, 1996).

One primary identifying trait of this family is the floral head. Each floral head, or inflorescence, is actually a group of florets massed together. A common head type in the Asteraceae is the radiate head, which has two types of florets: disc florets and ray florets. Disc florets are found within the central disc of the head and are often bisexual, producing both pollen and seeds. Ray florets, in contrast, surround the central disc of the head and are often sterile.

Several floral head traits may influence attraction of insect pollinators, florivores, and seed predators. Ray florets, being often bright and showy, are likely candidates for such insect attractants. However, while the presence of these ray florets has been shown to be important in

attracting pollinating insects (Stuessy et al., 1986; Bertin and Kerwin, 1998), there is limited knowledge of how other aspects of the rays, such as size, may influence insect attraction.

Ultraviolet patterning is an additional ray floret trait likely to affect insect attraction. The ultraviolet range of sunlight, 190-380 nm, though imperceptible to humans, is visible to pollinating insects (Peitsch et al., 1992). Flavonoids and dearomatized isoprenylated phoroglucinols contained within the ray florets are responsible for absorbing this spectrum of light, and may defend against florivore insects (Gronquist et al., 2001). Floral heads commonly have a UV-absorbent zone near the center of the display and a UV-reflectance zone composing the outer portion of the display (Daumer, 1958). In essence, this creates a "bull's-eye" pattern which is thought to orient and direct a pollinating insect to the pollen and nectar rewards (Daumer, 1958). Such patterning, therefore, may increase the chances of successful flower head reproduction. While UV coloration in flowering plants has been documented through photography since the 1920s (Richtmyer, 1923), there is limited knowledge regarding insect pollinator and damaging insect preference for UV phenotypes within a plant population. Other characteristics of the floral display such as the size of the inflorescence (Danderson and Molano-Flores, 2010), the disc size, and floral head height may differ significantly within a population and may affect insect attraction. The number of flower heads surrounding the focal flower head, though a trait not acted on by natural selection, may also be an important factor in insect visitation.

Pollinator attraction to floral heads can be difficult to measure. Direct observation of pollinator visits to particular floral heads suffers from the limitation that long observation periods may be needed to gain an adequate count of insects for each floral head. In self-incompatible species where a pollinator visit is required for successful seed set, the number of developed seeds

can be used as a measure of pollinator visitation because each developed seed represents a pollinator visit that successfully transferred pollen. However, there are two limitations to this approach. The first is that seed production could be limited by the amount of resources available to the plant. If this were the case, an ovule that received pollen may not develop into a seed, and the number of developed seeds could under-estimate pollinator visitation. Hand-pollinations can be used to test whether or not plants are resource-limited in seed production by ensuring that all florets receive out-cross pollen. If hand-pollinated heads produce a similar number of developed seeds compared to open-pollinated heads, plants are likely resource-limited. If hand-pollinated heads produce significantly more developed seeds than open-pollinated heads, then plants are likely pollen-limited. The second limitation in using the number of developed seeds as a measure of pollinator visitation is that the number of developed seeds for a particular floral head will also depend on the size of the head, as a larger floral disc can hold more ovules and has a greater potential to produce more seeds. To separate the effects of disc size from pollinator attraction, percent seed set can be examined. A greater percent seed set would suggest higher visitation rates from pollinators. The attraction of damaging insects to the head can be measured simply by counting the number of seed predators in the floral head.

In order to more closely examine the effects of floral head traits on developed seeds, percent developed seeds, and seed predator visitation, *Helianthus grosseserratus* (sawtooth sunflower, Asteraceae) plants and their insect visitors were examined over a two-year study. For year one, there were two primary goals. The first was to identify common insect visitors to floral heads of *H. grosseserratus*, analyze insect visitation over time, and identify insects feeding in the seed heads. The second goal was to record the phenology of the study plant in terms of duration of pollen presentation and development of ray flowers.

In year two, four specific goals were identified. The first was to verify that *H. grosseserratus* requires insect pollination for seed development. The second goal was to test for resource limitation in seed set through a hand-pollination treatment. The third goal was to collect data on floral traits at the time heads were most attractive to pollinators, and to measure number of developed seeds, percent developed seeds, and the number of seed predators when seeds were fully developed. Natural selection on floral traits was inferred by using multiple regression techniques to measure the influence of floral traits on the number and percentage of developed seeds and the number of seed predators. Finally, I wanted to determine if flower heads within a plant had consistent UV patterning.

Study Organism

Helianthus grosseserratus is an insect-pollinated species very common in mesic (moist) prairies throughout the eastern half of the United States. Sawtooth sunflower is known to form rhizomes and clonal colonies. While plants are often found in high densities, each colony can typically be identified by especially tight clumping and close proximity of stems.

H. grosseserratus has both sterile ray florets and bisexual central disc florets. Through the course of development, the disc florets undergo both a male and female phase. During the initial male phase, pollen is produced by the stamens. This pollen is then pushed out of a tube made from fused anthers by the style as it elongates. Once the pollen is presented, the flower becomes attractive to pollinators. The floral disc typically develops with the outer florets first and subsequently progressing toward inner rings of florets (Harris, 1995).

After the disc florets have presented pollen, they then undergo the female phase of development. The style continues to grow through the tube of anthers and separates. The stigma

then is ready to accept pollen from neighboring plants. It has been documented that *H. grosseserratus* is self-incompatible during this process (Heiser, 1969).

The most common order of pollinators visiting *Helianthus* species is Hymenoptera (Dickinson and McKone, 1992). However, additional insect species are known to contribute to pollination (Dickinson and McKone, 1992). Florivores, particularly those from the order Coleoptera, are also known to commonly visit *Helianthus* plants (Dickinson and McKone, 1992).

Study Site

I selected the Scuppernong River Habitat Area in Waukesha County, Wisconsin as the study site. Owned and leased by the Wisconsin Department of Natural Resources, this expanse covers 815 hectares of land, making it the largest mesic prairie east of the Mississippi River (Wisconsin DNR, 2014). The study site was selected for this large, unbroken tract of mesic prairie surrounded by forest. The large area and low level of human development and disturbance make the site as representative as possible of original prairies of this area.

MATERIALS AND METHODS

Year 1 (August-September 2013)

Plant Selection

Within the Scuppernong River Habitat Area, a 7040 m² area was designated as the study area for the first year. The site was monitored until it appeared that the majority of plants had most of their floral heads in bloom. At this time, on August 26th, 30 plants were randomly selected. In order to ensure plants were from different clones, each plant was separated by at least 3.0 meters. One floral head was selected on each plant under the criteria that all ray florets were developed and that no more than half of the outermost ring of disc florets on the chosen head were developed. Data collection began on the following day, the first full day that heads

were actively presenting pollen. Individual plants were given a number from one to thirty and were marked with a 1/2 inch x 48 inch hardwood dowel. Each individual floral head was identified by tagging it with a 1/8 inch-wide and 6 inch-long tan rubber band wrapped around the stem of the head.

In order to keep track of each plant and ensure that all plants could be visited on each day, plants were placed into one of four groups based on location. Two groups contained 6 plants each and the other two groups contained 9 plants each. All photography and plant visitation was randomized every day based on an online number generator for both the order of groups visited and again for the plants visited within each group.

Insect Observation

Each of the 30 selected floral heads were observed daily to record insects that were visiting the floral heads. Observations were conducted on each head for a six minute period every day from August 27th through September 5th. In order to coincide with the most active period for visiting insects, all observations were conducted between the hours of 0930-1230 and 1330-1630. Plants were visited in random order, as described previously. Every insect which landed on the floral head was identified to order and recorded as "visiting". Insect visitors were identified further to family, genus, or species when possible, based on initial insect collection prior to the study. Select insect specimens from this research will be permanently deposited in the University of Wisconsin-Madison Insect Research Collection (WIRC).

Color Photography

Visible light photographs were taken daily to collect data on floral head traits. These photographs were taken of each floral head in a randomized order between the hours of 0930-1230 and 1330-1630 from August 27th through September 5th. A Nikon D3100 digital camera

with a Nikon Micro-Nikkor 55mm 1:3.5 lens was used to take the photographs. This camera was modified for UV-photography (see next section), so for visible light photographs a Baader UV/IR-Cut/L filter was used to block ultraviolet light below 400nm and infrared light above 680nm. The camera was set to manual and adjustments were made to the f-stop and shutter speed in order to create a clear picture. In order to enhance contrast between floral heads and surrounding vegetation in the photos, an 8.5x11" background was created using a piece of solid black construction paper glued onto a piece of cardboard. A notch in the background with a covering flap was created to allow the stem of the plant to be placed in it without damaging the head. A 9mm scale bar was included in each picture.

ImageJ (Abramoff et al., 2004) was used to obtain the majority of the measurements for floral head characteristics (Figure 1A). The total head area was measured by altering the color threshold and separating the flower head from the background in the picture. Using the wand (tracing) tool, the entire highlighted head was then selected, and the measurement tool was clicked to retrieve the area (Figure 1B). I obtained a measurement of the disc area by selecting the "oval" shape tool, stretching the shape to fit the border of the floral head disc, and clicking the measurement tool (Figure 1C).

Ray area was also measured from the photographs. Some rays had insect damage when photographed; so the "expected ray area", or area that the rays would have had in the absence of damage, was calculated. In order to obtain the expected ray area, I first had to obtain the actual ray area; by subtracting the disc area from the total head area (Figure 1D). I then determined the length of each ray by measuring from the base of each ray to the tip. To measure percent damage, I used the length of the rays to calculate the proportion of the rays on each head that was missing due to damage. In order to account for damage which was anywhere other than the

apical end of the ray, (i.e. a hole chewed out of the center of the ray), I measured the area of the damaged portion and subtracted this area measurement from the top of the ray. Thus, the length of the ray was adjusted based upon area damaged. I calculated the actual ray length by adding all of the damaged and undamaged ray lengths together for each head and came up with the total length of all rays measured. I then calculated a total "expected ray length" by adding all of the undamaged ray lengths together, determining their average, and multiplying that average by the total number of rays present. There was always at least one undamaged ray on every floral head to be used as the expected ray length. By dividing the expected ray length by the actual total ray length, a percentage damage could be obtained. With this percent damage known, I was able to calculate the expected ray area (the area the rays would have had if undamaged) by the formula; (expected ray area = measured ray area/estimated proportion of ray area missing).

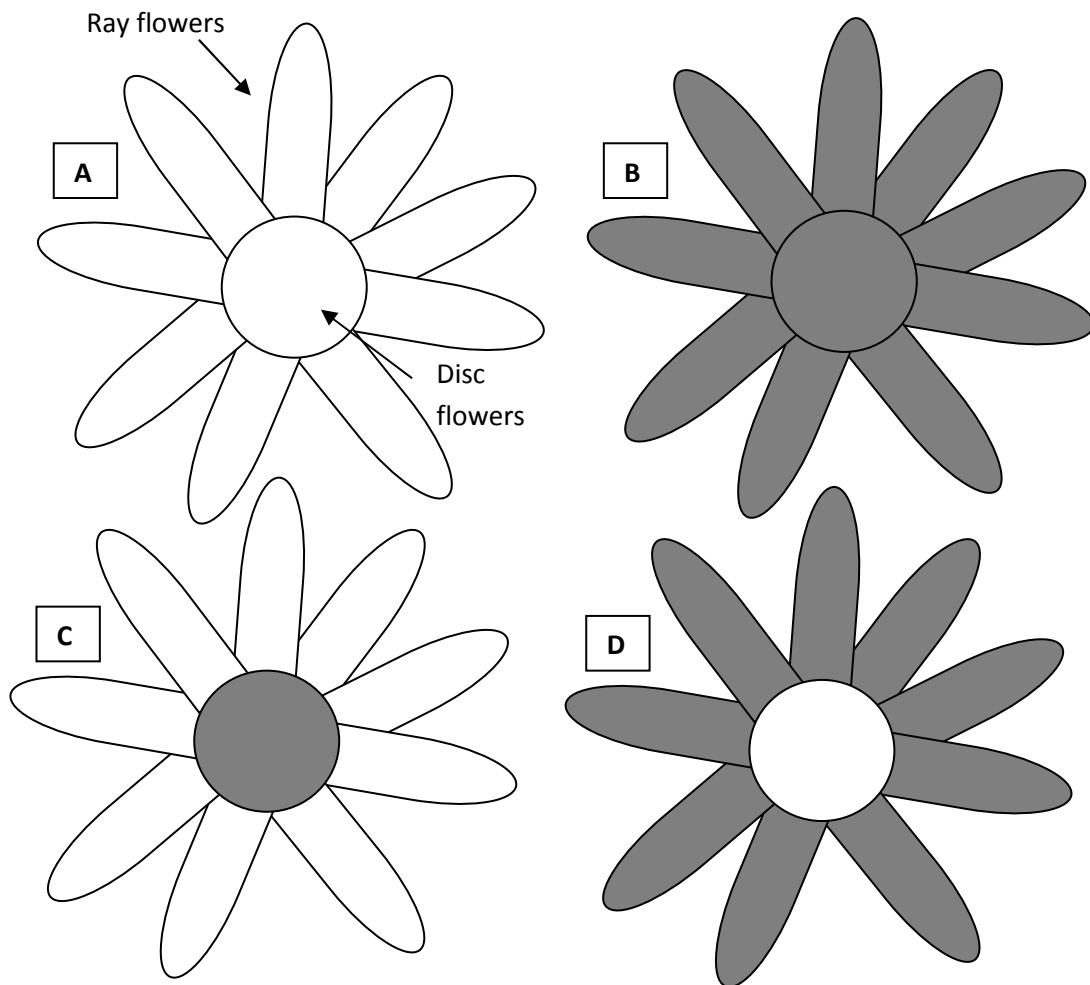


Fig. 1. Measurements of floral head traits derived from color photographs. (A) head showing disc and rays. (B) total head area. (C) disc area. (D) ray area.

UV Photography

In order to collect data on the ultraviolet attributes of the floral head display, ultraviolet photographs were taken daily. These photographs were taken of each floral head in a randomized order between the hours of 1230 and 1330 from August 27th through September 5th. The Nikon D3100 camera was modified to allow UV photography by removing the manufacturer's filter and installing a full spectrum clear filter (Life Pixel, Mukilteo WA). The camera was used with a Nikon micro-nikkor 55mm f/3.5 lens. This was an older lens that lacked modern lens coatings that can block UV light. A reverse-mounted Baader U-filter was used in order to fully

block visible and infrared light from the photograph for UV photography. Care was taken to avoid shadows as much as possible and to avoid taking photographs when clouds blocked full sun conditions.

Ultraviolet photographs were then analyzed using the ImageJ software program. The camera produced a three-color image (red, green and blue). Since humans cannot see in the ultraviolet range, the human-defined colors recorded by the camera have no meaning, and a single grayscale value that represents intensity is suitable for analysis. ImageJ automatically generates a single value of grayscale intensity using the default formula ($\text{Intensity} = (\text{Red} + \text{Green} + \text{Blue})/3$).

The rays generally showed a UV-absorbent zone at the base and a UV-reflective zone at the tip. I determined the mean intensity of both the UV-absorbent zone and the UV-reflective zone, and subsequently calculated the ratio between them. The separation between the absorbent and reflectance zones was often visually identifiable by a sharp contrast in coloration. However, I used ImageJ to precisely demarcate the zones. ImageJ will generate an intensity measurement for a particular point when hovering the cursor over the photograph. The border between the UV-reflectance and UV-absorbance zones was determined by hovering the cursor over each ray and determining the area with the most dramatic shift in intensity measurements. The UV-reflective and absorbent zones were then traced on each ray separately and an overall mean value was obtained for both zones across the entire floral head display, using ImageJ to determine the mean intensity measurement for each area. The intensity of the UV-absorbance zone was then divided by the intensity of the UV-reflectance zone in order to obtain a ratio between the two zones. Any discernible shadows were avoided when tracing the two UV zones. Any ray which had either zone entirely covered in shadows was not included in intensity measurements.

For several floral heads, the intensity measurements of UV-reflectance and UV-absorbance zones were similar and the border between the two zones could not be determined. In order to estimate the intensity of each zone and to calculate the ratio between them, I measured the intensity of the outer 10 percent of the ray length for the UV-reflectance zone and the inner 10 percent of the ray length for the UV-absorbance zone. I chose 10% because the smallest percentage of a head covered by either UV-absorbance or UV-reflectance in the heads with clear zone demarcation was a 10.5 percent UV-absorbance zone.

Seed Head Collection/Dissection

In order to record the numbers and kinds of seed predators, the heads from each of the 30 plants were collected after seeds had set. Once the disc florets stopped presenting and receiving pollen, the floral head display began to erode and dry out, and seed development occurred. The heads were collected once seeds were ready to drop from the plant. I determined this by running my finger across the floral head disc. If seeds began to lift up, I determined that the seeds were on the verge of dropping and the head was cut, placed into a labeled Ziploc bag, and frozen. The heads were all collected between the 6th and 11th of September as they became ready to drop their seeds.

Seeds heads were then thawed and dissected, and insects discovered in the flower head were identified to order, classified as mature or immature, counted, and preserved in alcohol. A majority of the insects found were clearly seed predators. Both adult and immature insects from the order Hymenoptera were also present in the heads. Two Hymenoptera species were identified with the assistance of Steven J. Krauth, curator of the Wisconsin Insect Research Collection, as both being parasitic wasps (one identified as likely being a member of the family Platygasteridae and the other a likely member of the genus *Tetrastichus*). Since both species are parasitic, their

presence directly implied there was an immature herbivorous insect present at one time in the seed head. Each parasitic wasp, therefore, counted as one seed predator.

Year 2 (August-September 2014)

Plant Selection and Treatments

The research in year two was centered on the same area within the Scuppernong River Habitat Area that was used in year one. The area, however, was expanded to account for a significant increase in plants monitored. There were 3 categories of plants in year 2: bagged, to test whether pollinator visitation is required for seed set (10 plants), open-pollinated (100 plants), and hand-pollinated, to test for resource limitation of seed set (100 plants). On August 17th, 210 plants were chosen at random while walking through the mesic prairie. These plants were randomly assigned to one of the three categories. Care was taken to avoid clones and each selected plant was separated from others by no less than 3.0 meters. Each plant was assigned a number for identification purposes and was marked with a wooden dowel. A map was also created to detail where each plant was in relation to each other and assist in revisiting each area.

Bagged Heads

On August 18th, for each of the 10 plants in the bagged treatment, one head was selected to be bagged and isolated from insect visitation. All of the heads chosen were in the beginning stages of ray flower formation and had no florets that were presenting pollen. A square piece of insect netting was then cut and wrapped around the head. When this was done, the rigidity of the netting allowed for the creation of a dome-like enclosure for the head. Care was taken to ensure enough space was provided for floral head growth. Netting was secured with a rubber band around the stem of the head. A strip of green flagging tape was placed on the wooden dowel to indicate the plant was a member of the bagged group.

Open-Pollinated Heads

The selection of one head on the 100 plants in the open-pollinated group occurred over the course of three days. The criterion for selection was that the heads needed to have at least half of their florets presenting pollen. With this criterion in mind, 63 heads were selected on August 19th. On August 20th, 13 heads were selected. The final 24 heads were selected on August 21st. Upon selection, each head was marked by placing a rubber band around the stem leading to the head. In addition, a strip of pink flagging tape was placed directly above a strip of blue flagging tape on the nearby marker dowel to indicate that the plant belonged to the open-pollinated group.

Hand-Pollinated Heads

On August 20th, one head was chosen from each of the 100 plants in the hand-pollinated group. The criteria for selection were that the heads needed to be in the early stages of ray flower development and have no florets presenting pollen. Selection in early stages allowed for pollen supplementation throughout the duration of floral development. Upon selection, each head was marked with a rubber band wrapped around the stem leading to the head. In addition, a strip of blue flagging tape was placed directly above a piece of pink flagging tape on the nearby marker dowel to indicate that the plant belonged to the hand-pollinated group.

Every day, 100 floral heads were collected from random sawtooth sunflower plants located at least 100 meters outside of the research plot in the Scuppernong River Habitat Area in order to ensure the two groups were not closely related. The only criterion for head selection was that they had to have the majority of their florets presenting pollen. The 100 heads were placed in a canvas bag and taken to the research area. For each of the hand-pollinated heads, one head was arbitrarily chosen from the canvas bag and the two heads were brushed together. In the

process, pollen was transferred from the collected head onto the hand-pollinated head. This process was repeated daily until the heads were collected.

Color Photography

Using the methods described for year 1, color photographs were taken of the open-pollinated and hand-pollinated flower heads when at least half of their florets were presenting pollen. Color photographs of the open-pollinated group occurred immediately after the head was selected, following the randomized order that occurred during initial head selection. Therefore, 63 heads were photographed on August 19th, 13 heads were photographed on August 20th, and the final 24 heads were photographed on August 21st. For the hand-pollinated group, heads were initially selected at the early stages of floral development. Heads were photographed once half of the florets in the central disc were presenting pollen. Fifty eight heads were photographed on August 22nd. On August 23rd, 41 heads were photographed. The final 1 head was photographed on August 24th. For both groups, a black cardboard background was created with an attached scale bar for measurement. With the use of the image-analysis software program, ImageJ, I used these photos to obtain values for expected ray area, disc area, and percent ray damage, as described for year 1.

UV Photography

UV photographs were only taken of the open-pollinated group. UV intensity ratios were calculated using methods described previously. In addition to the UV intensity ratio, the mean percentage of undamaged rays covered by the UV-reflectance zone was also calculated. In order to do so, the length of UV-reflectance zone on undamaged rays was first calculated using the straight line tool in ImageJ to measure the distance from the distal end of the absorbance zone to the UV-reflective tip. From these measurements, the mean UV-reflectance zone length could be

calculated for every floral head. For the heads where it was impossible to determine the demarcation between ultraviolet zones, length measurements were not calculated. The mean ray length for each head was then divided by the mean ray length covered by the UV-reflective zone and multiplied by 100 in order to obtain a percent UV-reflectance.

The area of UV-absorbance on the floral head was also calculated. This was accomplished by taking the percentage of each undamaged ray covered by the UV-absorbent zone, dividing by 100, and multiplying it by the ray area calculated from the color photographs.

UV Photography of Rays Within Each Plant

On August 26th, UV photography was used in order to determine if all heads on a plant shared similar ultraviolet patterning. For each of 81 open-pollinated plants, one ray was removed from every additional floral head for analysis (19 of the 100 open-pollinated plants did not have additional open heads besides the study head, and could not be included). The collected rays from each plant were then placed on a black cardboard background with a scale bar and were photographed under sunny conditions. ImageJ was then used to determine similarities between the study head and all other heads on the plant in both UV ratio and percent UV-reflectance.

Seed Head Collection and Dissection

Similar to the first year, seed heads from the three plant groups were collected and dissected in order to count the number of seed predators. The heads were all collected according to the criteria described in year one. The number of heads collected on each day was recorded for every group (as seen in Table 1).

Table 1. Number of heads collected daily in each of three plant groupings from August 26th-September 3rd.

Group	Aug.26	Aug.27	Aug.28	Aug.29	Aug.30	Aug.31	Sept.1	Sept.2	Sept.3
Bagged	0	10	0	0	0	0	0	0	0
Open	30	22	33	4	1	6	3	0	1
Hand	0	4	64	7	0	15	5	4	1

In addition to seed predator collection, the number of developed seeds was counted in order to measure reproductive success. Seeds with a hardened, black seed coat were counted as developed (Fig. 2A). Developed seeds were further grouped into the categories of developed/filled and developed/unfilled. Developed/filled seeds (Fig. 2B) had a fully developed seed embryo within the black seed coat. Developed/unfilled seeds (Fig. 2C) had either a minimal seed embryo or none entirely within the black seed coat. Those with a tan/transparent seed coat were considered undeveloped (Fig. 2D). All of these undeveloped seeds lacked a developed or developing embryo. For purposes of analysis, developed seeds included both filled and unfilled seeds. The percentage of developed seeds was also calculated by dividing the number of developed seeds by the total number of ovules (developed + undeveloped seeds) in the seed head and multiplying by 100.

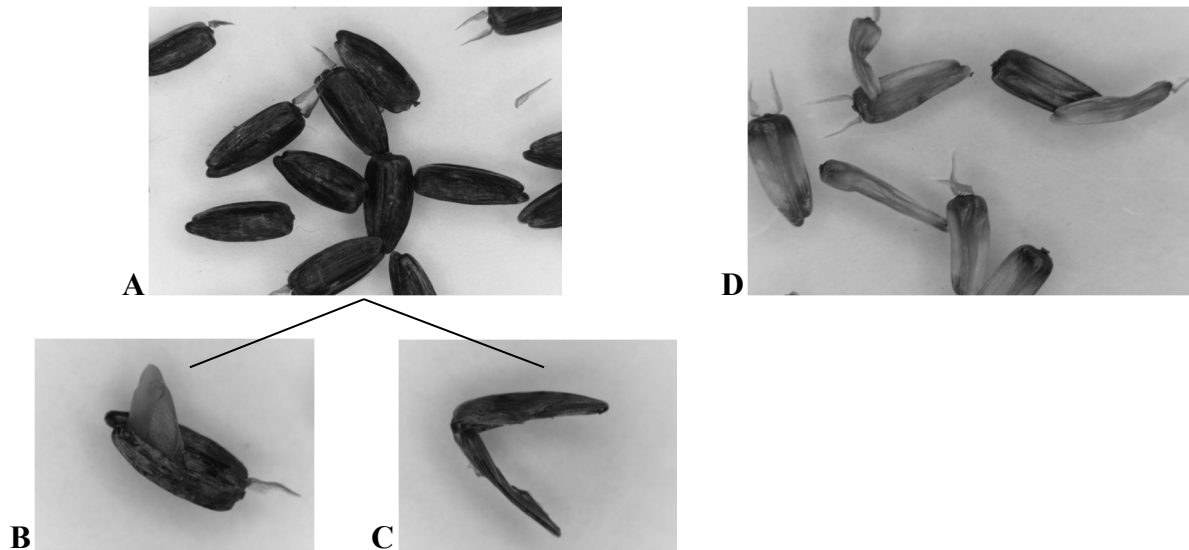


Fig. 2. Photographs of seeds removed from floral heads. (A) developed seeds. (B) developed/filled seeds. (C) developed/unfilled seeds. (D) undeveloped seeds.

Any damage the insects caused to seeds was also recorded. Damage to developed or developing seeds would imply that seed predators may have reduced the initial reproductive potential achieved by visiting pollinators.

2015 Test of Hand Pollination Methods

Contrary to expectations, the hand-pollination treatment did not result in increased seed number (see Results), so the effectiveness of the hand pollination methods was examined in August of 2015 at the University of Wisconsin-Milwaukee Field Station in Saukville, Wisconsin. The tests were conducted on a garden population of *Helianthus grosseserratus* once the majority of floral heads were open and presenting pollen. There were 4 separate plots of planted *Helianthus grosseserratus* plants in the garden. Within each of these 4 plots, 4 heads with at least half of their florets presenting pollen were selected from 4 separate plants to be pollen-recipients, for a total of 16 heads. Sixteen additional heads chosen with the same criteria and using the same selection pattern were used as pollen-sources.

In order to determine if damage occurred during pollen transfer and if pollen was successfully transferred during the process, 10 pollen-recipient and 10 pollen-source heads were taken back to the lab. All reproductive parts in each of the 10 pollen-recipient heads were examined under the microscope and any initial damage was documented. Each of the 10 pollen-recipient heads were then randomly matched up with a pollen-source head, and pollen was transferred using the same methods as was previously described for the 2014 field season. Pollen-recipient heads were analyzed and photographed under the microscope for any new damage to floral reproductive parts caused by hand-pollination. During this time, heads were also examined to determine if excess pollen was successfully transferred to the recipient head.

These initial tests suggested that florets potentially could be bent during pollen transfer. A bent floret that failed to rebound could result in reduced pollination success. To test the ability of florets to rebound following hand pollination, the remaining 6 pollen-recipient heads were not removed from the plants and were marked using a rubber band. Hand-pollination was then performed on the pollen-recipient heads using the remaining 6 pollen-source heads, as previously described. Heads were examined with a hand lens, photographed, and bent florets were documented. Following a 3 hour break to allow florets to rebound, pollen-recipient heads were re-visited, photographed, and examined with a hand lens for bent florets.

Additionally, it was necessary to determine if any insects were transferred from the pollen-source head to the pollen-recipient head during the procedure. To test for this, recipient heads were examined before and after pollen transfer for the presence of additional insects. In order to obtain a clear picture of pollen and insect transfer, an additional group of heads were collected from the field and rubbed against a blank white paper. The paper was then examined for any insects that had dropped out of the head.

Measurements of the Focal Plant and Surrounding Area

Additional measurements were taken on the focal plant and surrounding area. On August 27th, the number of additional open sawtooth sunflower heads within a 1.5 meter radius (both on the study plant and surrounding plants) were recorded for each of the 100 open-pollinated heads. The floral head height above the ground of each open-pollinated head was also measured.

Data Analysis

Floral Head Characteristics and Insect Visitors

All calculations were completed using Systat 9. Pearson correlation tests were used to test for associations between pairs of variables: UV intensity ratio and percent UV reflective area for open-pollinated heads in 2014 (using only heads with a distinguishable bulls-eye pattern), expected ray area and area of UV-absorbance for open-pollinated heads in 2014 (using only heads where there was a distinguishable bulls-eye pattern), UV intensity ratio for the study head and the average UV intensity ratio for all other heads on the same plant in 2014, and percent UV-reflectance for the study head and average percent UV-reflectance for all other heads on the study plant in 2014. Trends over time for percent ray damage were analyzed with linear regression (using data from 2013). Day was used for the independent variable and the average percent ray damage for each day was used as the dependent variable.

Natural Selection on Floral Traits

T-tests were used to determine whether or not differences in the number of developed seeds, percent of developed seeds, and number of seed predators between the open-pollinated and hand-pollinated treatments in 2014 were significant. Pearson correlation tests were used to compare the number of seed predators in the seed head with the grayscale intensity of the UV reflective area and the grayscale intensity of the UV absorbent area.

A Pearson correlation test was used to determine whether or not the number of ovules in a floral head disc was significantly correlated with disc area (the open-pollinated and hand-pollinated treatments were combined for this test).

Multiple regression was used to show the effects of floral traits (disc area, ray area, UV ratio, head height) and the number of *H. grosseserratus* heads within 1.5m of the study head on the number of developed seeds, percent developed seeds, and the number of seed predators. The techniques outlined by Lande and Arnold (1983) to estimate the strength and direction of natural selection were followed. The dependent variables (number of developed seeds, percent developed seeds, and seed predators) were transformed into relative measurements by dividing each absolute measure by the mean value. The x-variables (floral traits and number of heads) were standardized by calculating the mean for each variable, subtracting this mean from the value of each individual measurement, and dividing this quantity by the standard deviation of the data set. This resulted in new, standardized values for each individual measurement. Thus, relative dependent variables were used in the multiple regression with standardized variables rather than absolute measurements. The residuals from the regression were found to be normally distributed. The variance inflation factor (VIF) was used to measure the impact of collinearity on regression model variables (Frey 2010). The VIF was calculated as $1/\text{Tolerance}$. No values greater than 10 were found, indicating that multicollinearity was not a problem.

RESULTS

Floral Head Characteristics and Insect Visitors

The *H. grosseserratus* study heads in 2013 had florets that were actively presenting pollen for 5 full days (Fig. 3). The number of florets presenting pollen was highest on the second

day, peaking at a mean of 37 florets/head, and by day 5 had dropped to just above 1, with 23 of the 30 heads not having any florets presenting pollen.

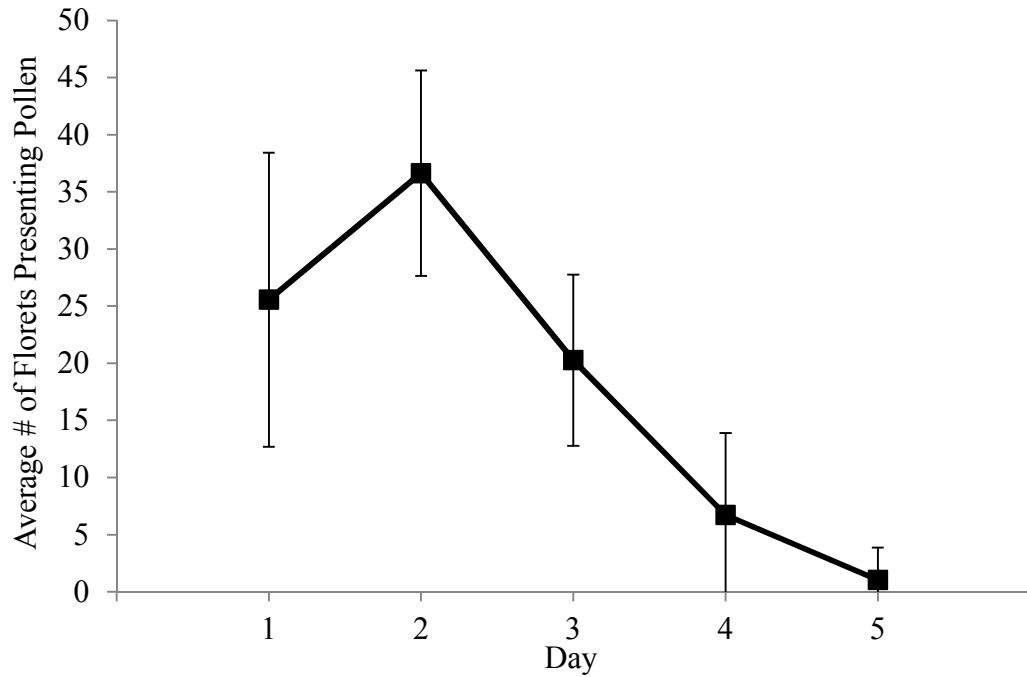


Fig. 3. Trends over time in the number of florets presenting pollen on floral heads in 2013 (mean with standard deviation error bars, N=30).

Rays continued expanding from day 1 to day 2, reaching their full size on day 2 (Fig. 4). After day 2, expected ray area (or the area expected if the rays were undamaged) remained constant for the remaining 3 days.

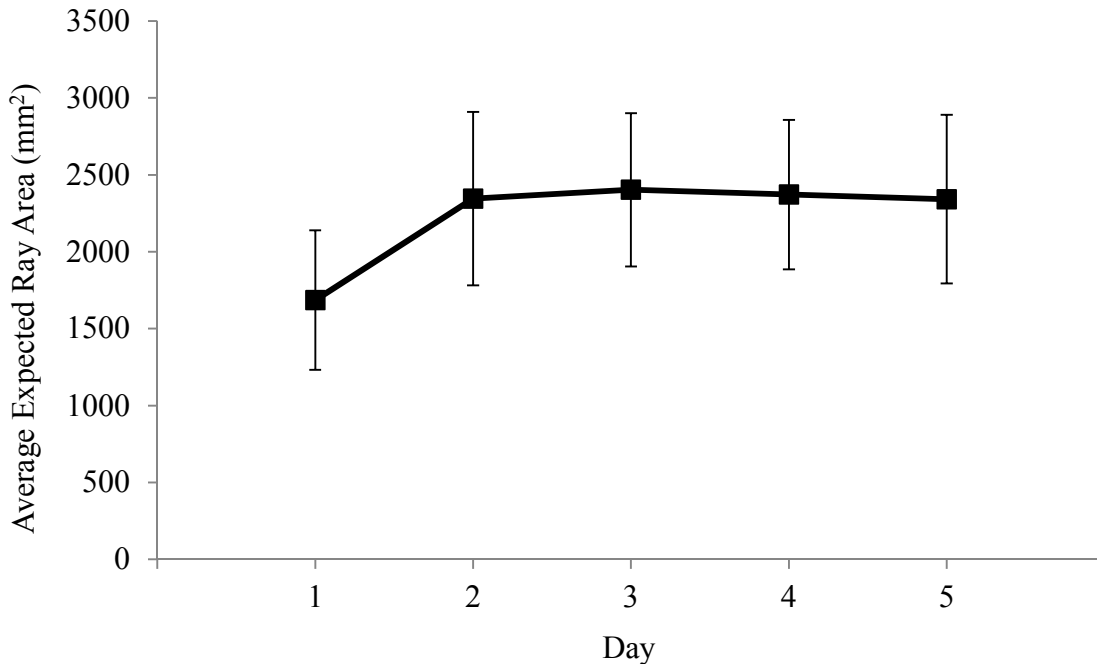


Fig. 4. The trends over time of expected ray area on floral heads in 2013 (mean with standard deviation error bars, N=30).

The UV ratio, which is the grayscale intensity of the UV-reflective tip of the rays divided by the grayscale intensity of the UV-absorbent base of the rays, trended towards increasing over time but this was not statistically significant (linear regression, N=5, R=0.731, p=0.161). A large grayscale value indicates a highly-reflective UV pattern, while a small grayscale intensity value is indicative of a UV pattern which is not highly-reflective. A large UV intensity ratio, therefore, indicates that there is a strong bulls-eye pattern and a distinct demarcation between the UV-reflective tip and UV-absorbent base.

The UV ratio had a large range in values, from 1.432 to 6.919 (N=30, Mean=3.343, SD=1.601) on the second day of sexual maturity among heads in 2013 and 1.030 to 6.891 (N=100, Mean=2.442, SD=1.262) for open-pollinated heads at a similar phenological stage in 2014. The floral heads exhibited a distinct pattern of polymorphism (Fig. 5), where rays on heads either had a well-defined UV bulls-eye pattern with a large UV intensity ratio (Fig. 5A), or

had no distinguishable bulls-eye UV pattern and a small UV ratio (Fig. 5B). There were 50% of heads on the second day of sexual maturity in 2013 and 44% of open-pollinated heads at a similar phenological stage in 2014 that had no identifiable demarcation between absorbent and reflective zones.



Fig. 5. The pattern of polymorphism evident from floral heads with (A) a strong bulls-eye pattern (B) no identifiable bulls-eye pattern.

For heads showing a bulls-eye pattern (i.e., there was a strong demarcation between the reflective and absorbent zones), rays with brighter tips also had a larger UV reflective zone (Pearson correlation between average UV ratio and average percent UV reflectance: $N=56$, $r=0.380$, $p=0.004$, using 2014 open-pollinated data). Heads having the identifiable bulls-eye pattern also showed a significant correlation between ray area and area of UV-absorbance; where heads with a larger ray area had a larger area of UV-absorbance. (Pearson correlation between average expected ray area and average area of UV-absorbance, $N=56$, $r=0.908$, $p<0.001$, using 2014 open-pollinated data).

The patterns of both UV intensity ratio and percent UV-reflectance were shared on open floral heads within a plant (Fig. 6). There was a significant relationship between UV intensity ratio of the study head and the average UV intensity ratio for all other heads on the same plant (Pearson correlation, $N=81$, $r=0.862$, $p<0.001$) (Fig. 6A). There was also a significant relationship between the percent UV-reflectance of the study head and the average percent UV-reflectance for all other heads on the study plant ($N=45$, $r=0.816$, $p<0.001$) (Fig. 6B). There was a smaller range of values for the UV intensity ratio measured on the study head (1.03 to 6.40) compared to all other heads on the same plant (1.48 to 11.00) (Fig. 6A). This could have been caused by differences in lighting or the background used for the photographs.

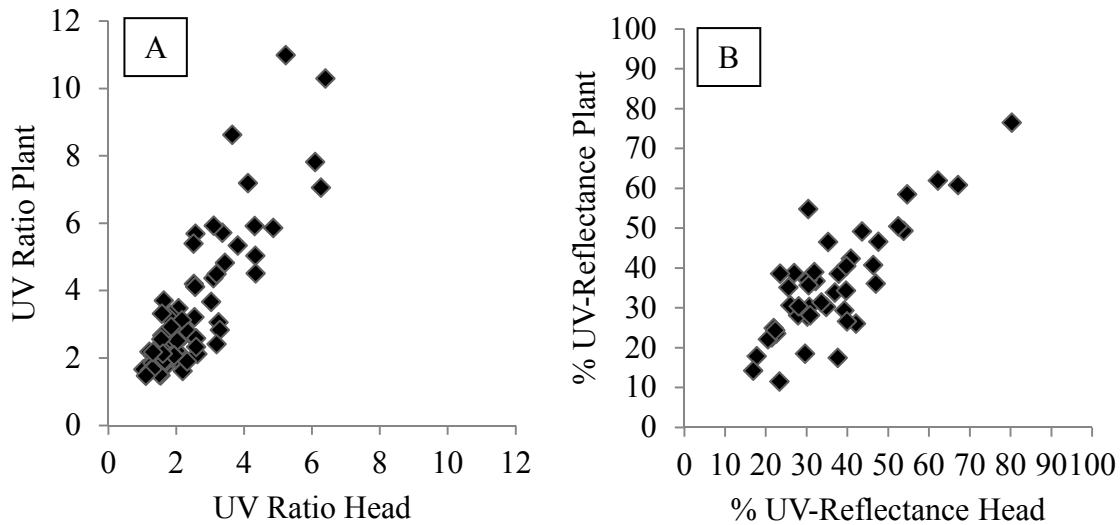


Fig. 6. The consistency of both (A) UV intensity ratio and (B) % UV-reflectance between the study head and the average of all other heads on the same plant. Data from open-pollinated heads in 2014.

Sixteen species from 7 insect orders visited the floral heads (Table 2). Of these, 23% were Hymenoptera. Most of the Hymenoptera visitors were bumble bees (*Bombus*, 52%), although other bees were also observed. The most common insect visitors to the floral heads were soldier beetles (*Chauliognathus pensylvanicus*, 29%), which feed on pollen and nectar. Multiple insects from the order Diptera also visited the heads, as gall midges (*Pilodiplosis helianthibulla*, 13%) and hover flies (*Toxomerus marginatus*, 13%) were the most common. An insect from the order Hemiptera, the minute pirate bug (*Orius insidiosus*), also accounted for 10% of all insect visitors to the heads.

Table 2. Total number of insect visitors for all 30 heads over the first 5 days of the study.

Order	Species	Common Name	# of Insects	Insects/Head
Coleoptera	<i>Chauliognathus pensylvanicus</i>	Soldier Beetle	27	0.90
	<i>Olibrus</i> sp.	Shining Flower Beetle	2	0.07
Diptera	<i>Bombylius major</i>	Black-Tailed Bee Fly	1	0.03
	<i>Pilodiplosis helianthibulla</i>	Gall Midge	12	0.40
	<i>Musca domestica</i>	Housefly	2	0.07
	<i>Eristalis transversa</i>	Transverse Flower Fly	4	0.13
	<i>Toxomerus marginatus</i>	Hover Fly	12	0.40
Hemiptera	<i>Orius insidiosus</i>	Minute Pirate Bug	9	0.30
Hymenoptera	<i>Apis mellifera</i>	Honey Bee	2	0.07
	<i>Bombus</i> spp. ¹	Bumble Bee	11	0.37
	<i>Megachile latimanus</i>	Broad-handed Leaf-cutter Bee	7	0.23
	<i>Onycholyda rufofasciata</i>	Sawfly	1	0.03
Lepidoptera	<i>Alsophila pometaria</i>	Fall Cankerworm	1	0.03
Orthoptera	<i>Melanoplus femurrubrum</i>	Red-Legged Grasshopper	1	0.03
Total			92	3.06

¹ *Bombus* spp. includes *Bombus griseocollis*, *Bombus impatiens*, and *Bombus rufocinctus*, which could not confidently be identified while in flight.

Insect visitation numbers (Fig. 7) for the two most common insect pollinator orders (Diptera and Hymenoptera) both peaked on day 3 (Fig. 7A). For the two most common non-pollinators (Coleoptera and Hemiptera), Coleoptera peaked on day 2, while Hemiptera did not reach a peak until the fourth day of the study and only slightly decreased thereafter (Fig. 7B). Other insect orders that were identified as visiting the floral heads included one Orthoptera on day 3, and one Lepidoptera on day 5.

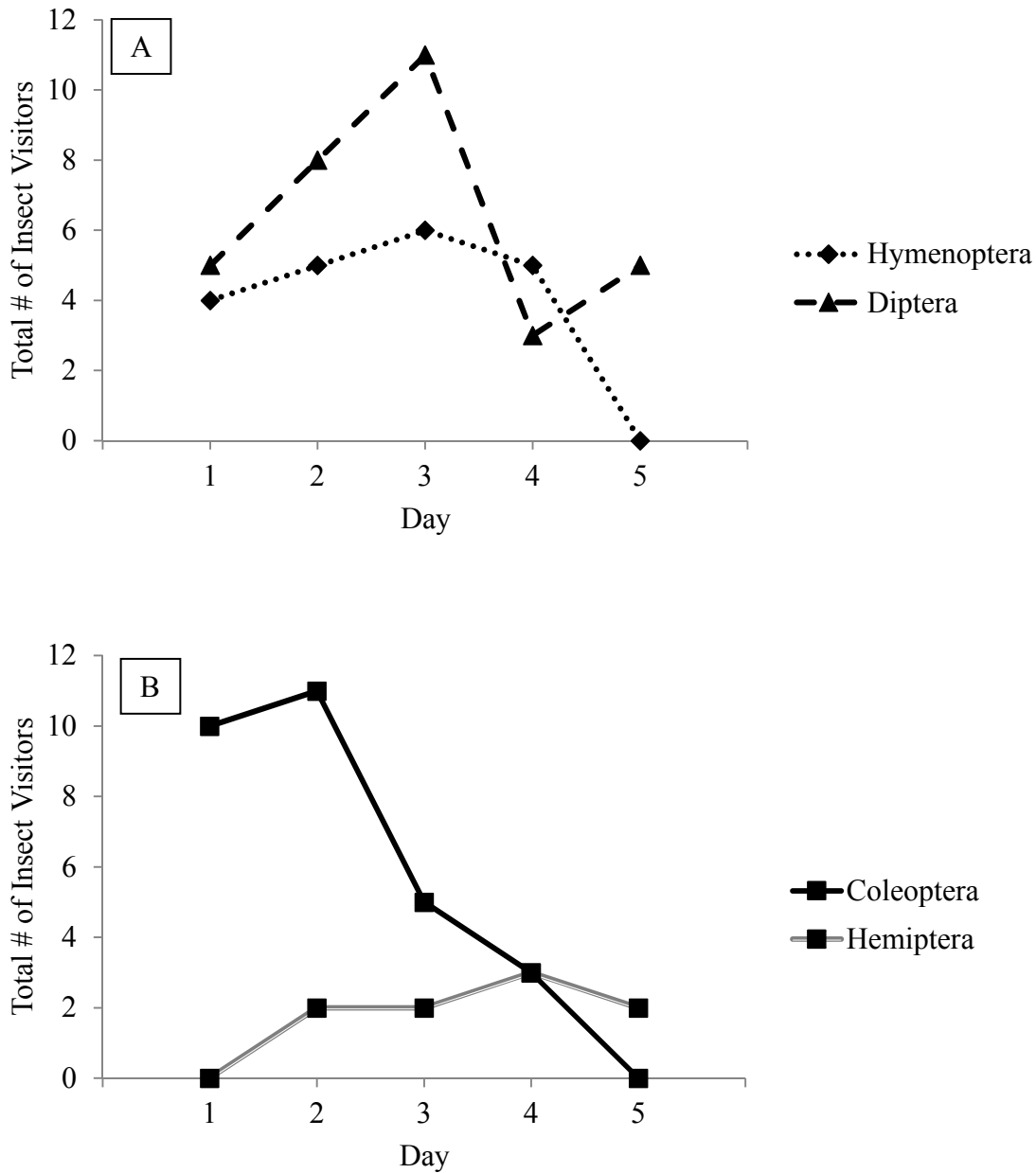


Fig. 7. Total number of insect (A) pollinators and (B) non-pollinators from 4 orders during the 5 days that study heads were presenting pollen. Insect numbers are summed over all 30 plants in 2013.

Dissection of heads after seeds had developed revealed that the most common seed predators were immature Diptera (1.37 insects/head and 3.07 insects/head, for 2013 and 2014, respectively, Table 3). There also were a significant number of immature Thysanoptera (1.13

insects/head and 1.22 insects/head). Immature Lepidoptera in 2014 (1.07 insects/head), and immature Hemiptera (0.43 insects/head and 0.46 insects/head, for 2013 and 2014, respectively) were also common insects found in the head.

Table 3. Total number of insects per seed head found at the end of 2013 and 2014. Only the open-pollinated treatment is shown for 2014.

Order	Life Stage	2013	2014
Coleoptera	Immature	0.13	0.15
	Mature	0.00	0.00
Diptera	Immature	1.37	3.07
	Mature	0.03	0.00
Hemiptera	Immature	0.43	0.46
	Mature	0.10	0.09
Hymenoptera (parasitic) ¹	Immature	0.07	0.53
	Mature	0.10	0.20
Lepidoptera	Immature	0.03	1.07
	Mature	0.00	0.00
Thysanoptera	Immature	1.13	1.22
	Mature	0.17	0.33
Total/Head		3.56	7.12

¹ Two species of parasitic hymenoptera were identified; one belonging to the family Platygastridae and the second belonging to the genus *Tetrastichus*.

Rays incurred damage that significantly increased over the 5 days that the heads were presenting pollen in 2013 (linear regression, N=5, R= 0.999, p<0.001). On day 1, rays had just under 5 % damage; this rose to just over 32 % by day 5 (Fig. 8). Percent damage to rays was very low in 2014 (mean= 2.34), and was not followed over time.

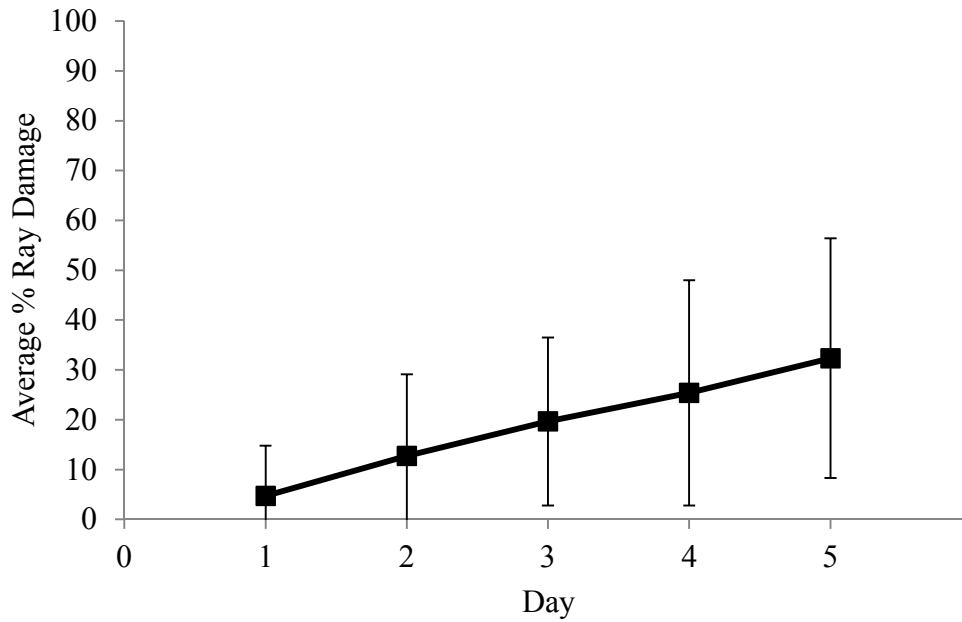


Fig. 8. Increase in percent ray damage over time in 2013 (mean of 30 heads with standard deviation error bars).

Natural Selection on Floral Traits

The bagged treatment established that pollination is required for successful seed production. With a mean of only 3.5 developed seeds, reproduction was negligible.

The average number of developed seeds was lower in the hand-pollinated treatment (mean= 40) compared to the open-pollinated treatment (mean= 46), although this difference was not significant (Fig. 9, t-test. N= 200, $t= 1.831$, $p= 0.070$).

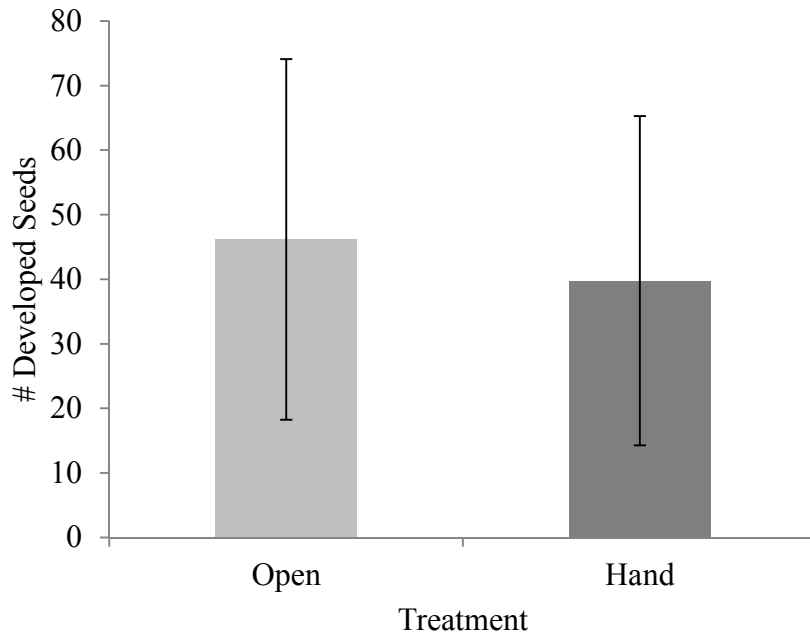


Fig. 9. The average number of developed seeds in the floral heads at the end of the study in 2014 (open and hand treatments). Mean and standard deviation are shown.

The average percent developed seeds was lower in the hand-pollinated treatment (mean= 42) compared to the open-pollinated treatment (mean= 48), although this difference was not significant (Fig. 10, t-test. N= 200, $t= 1.849$, $p= 0.067$).

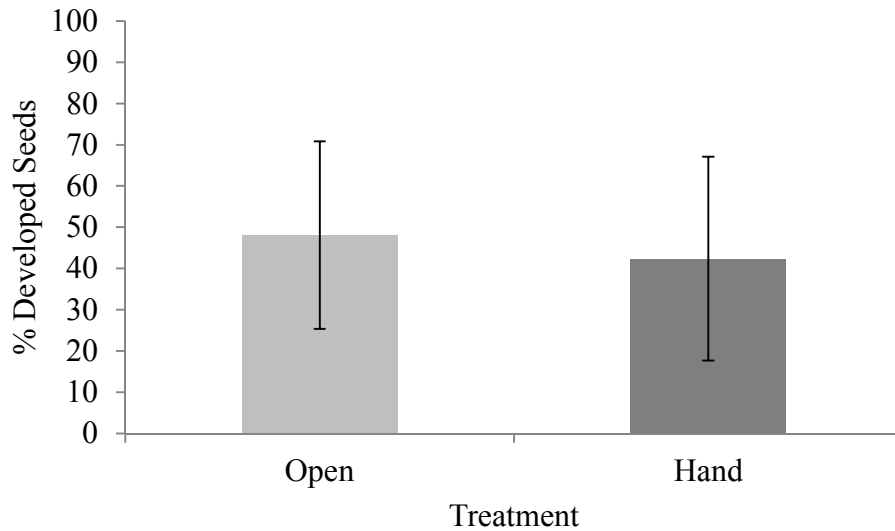


Fig. 10. The percent developed seeds in the floral heads at the end of the study in 2014 (open and hand treatments). Mean and standard deviation are shown.

Tests for the effectiveness of the hand pollination procedures revealed that pollen was successfully transferred from the donor head to the recipient head in each of the 10 heads examined. In addition, hand pollination did not result in structural damage to florets of any of the 10 pollen-recipient heads. Florets which were bent during the pollen-transfer process successfully rebounded to a vertical position within a period of 3 hours (Fig. 11).





Fig. 11. Photographs showing (A) the initial vertical position of florets in floral head. (B) florets immediately following hand pollination procedure. Note that some florets are bent. (C) the rebounded vertical position of florets in floral head after 3 hours.

Hand-pollinated heads had more seed predators than open-pollinated heads, and this difference was significant (t-test, $N= 200$, $t= 2.554$, $P= 0.012$)(Fig. 12).

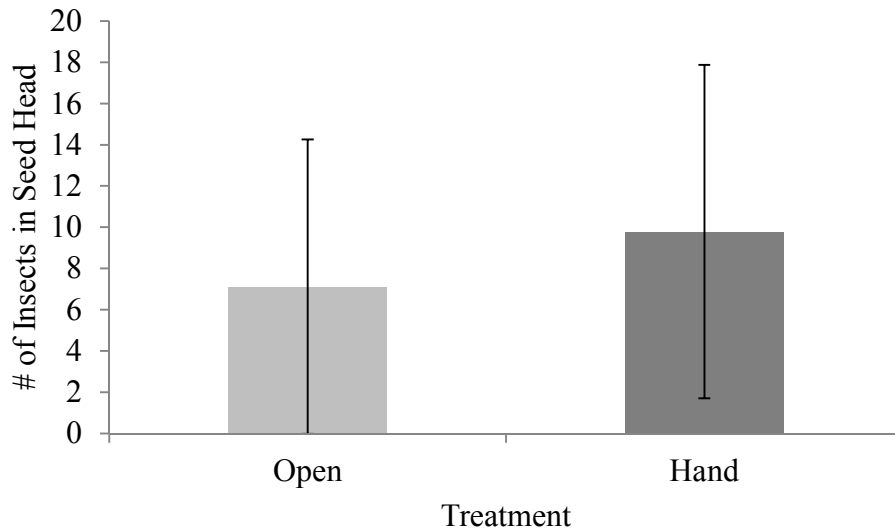


Fig. 12. The average number of insects in the seed heads in 2014 (hand-pollinated and open-pollinated treatments). Mean and standard deviation are shown

Of the insect orders found in the seed heads, only Diptera and Thysanoptera had higher numbers in the hand-pollinated heads (Fig. 13). All other insect orders were slightly lower in hand-pollinated heads. The 2015 test of hand pollination methods showed that only a minimal number of insects were likely to have been transferred from pollen-source to pollen-recipient heads. Fewer than 25% of tests brushing heads against a white piece of paper had insects drop out of the pollen-source floral head. Thysanoptera was the only order of insects shown to drop out during the process.

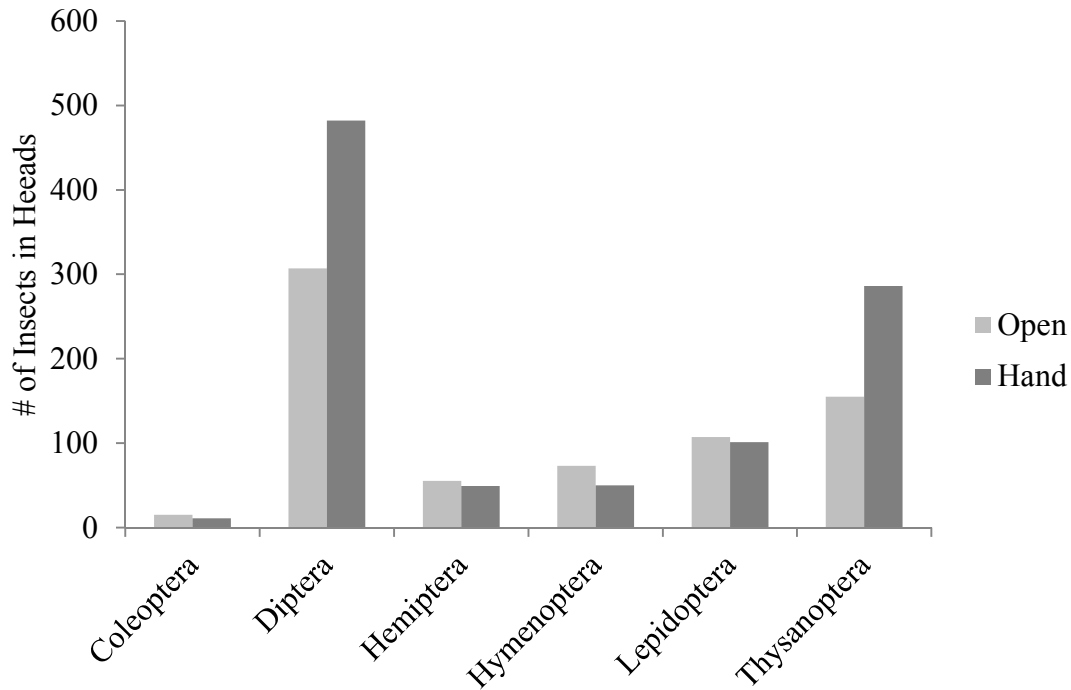


Fig. 13. The number of insects in the seed heads in 2014 (hand-pollinated and open-pollinated treatments). Numbers shown are summed over the 100 heads in each treatment.

There was a strong positive correlation between the number of ovules and disc area for both open-pollinated and hand-pollinated groups combined in 2014 ($N=200$, $r=0.772$, $p<0.001$)(Fig. 14).

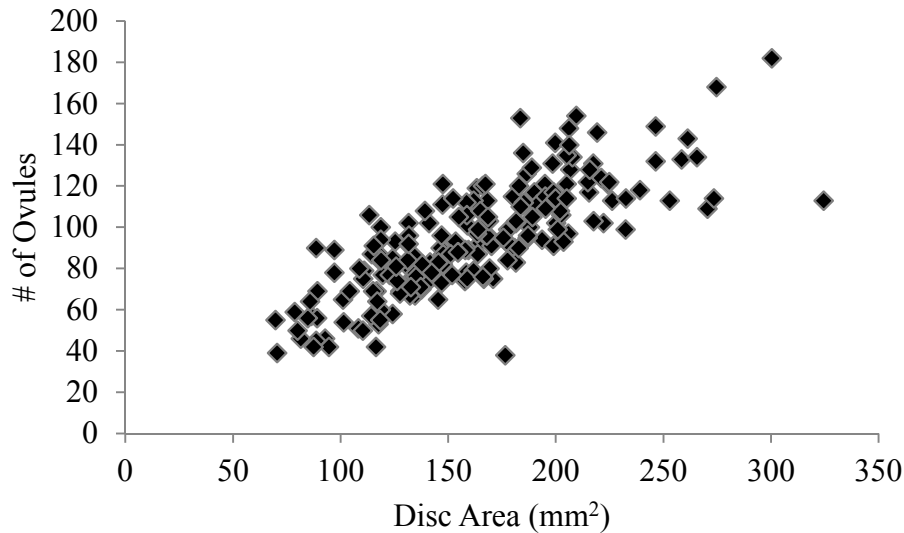


Fig. 14. Strong positive correlation between number of ovules in the seed heads and disc area in 2014 (open-pollinated and hand-pollinated treatments combined)

There was minimal herbivore damage to seeds in both open pollinated (N= 100, Mean percent damage to developed and undeveloped combined= 5.133) and hand pollinated seed heads (N= 100, Mean percent damage to developed and undeveloped combined= 5.298). The vast majority of damage was to undeveloped seeds in the head. Of the total damaged seeds, only 8.4% were developed.

For open-pollinated heads, regression analysis of four floral head traits and the number of surrounding floral heads (Table 4) revealed that disc area had the strongest influence on both number of developed seeds and number of seed predators in the floral head. Both the number of developed seeds and the number of seed predators in the head increased with a larger disc. There was no significant relationship between disc area and percent developed seeds. The expected ray area was significantly and negatively correlated with the number of seed predators in the seed head. Heads with a larger ray area had a lower number of seed predators in the seed head. There was no significant relationship between expected ray area and number of developed seeds or percent developed seeds. There was also a significant relationship between number of seed

predators and the UV intensity ratio. Heads with a large UV intensity ratio had a larger number of seed predators in the seed head. There was no significant relationship between number of developed seeds or percent developed seeds and the UV intensity ratio. There was a stronger relationship between numbers of insects in the head and the grayscale intensity of the UV reflective area (Pearson correlation, N=100, $r= 0.200$, $p= 0.092$) than the grayscale intensity of the UV absorbent area (Pearson correlation, N=100 $r= 0.061$, $p= 0.549$). Head height and heads in a 1.5m radius were both significantly correlated with the number of developed seeds and percent developed seeds. Floral heads closer to the ground and heads surrounded by a large number of open floral heads had a larger number of developed seeds and a larger percent developed seeds. There was no significant relationship between the number of seed predators and head height or number of heads in a 1.5m radius.

Table 4. Multiple regression results from 2014 open-pollinated treatments, showing effects of four floral head traits and number of surrounding *H. grosseserratus* floral heads on number of developed seeds, percent developed seeds, and number of seed predators found in the seed head. Standardized regression coefficients are shown.

Floral Traits	# of Developed Seeds	% Developed Seeds	# of Seed Predators
Disc Area	0.268**	0.072	0.483**
Exp Ray Area	0.029	0.118	-0.317*
UV Ratio	-0.003	0.020	0.243**
Head Height	-0.110*	-0.221*	0.133
Heads 1.5m Radius	0.183**	0.334**	0.027

* $P < 0.05$, ** $P < 0.001$.

Multiple regression was also used to compare open-pollinated and hand-pollinated heads, using only disc area and expected ray area, as the other three variables were not measured on hand-pollinated heads (Table 5). Disc area was significantly correlated with number of developed seeds and number of seed predators in both open-pollinated and hand-pollinated treatments. A larger disc area resulted in a higher number of developed seeds and seed predators

for both treatment types. There was no significant relationship between disc area and percent developed seeds in either treatment. Ray area was significantly correlated with the number of developed seeds, the percent developed seeds, and the number of seed predators in the hand-pollinated group. A larger ray area resulted in a higher number of developed seeds, a higher percent developed seeds, and a lower number of seed predators. There was no significant relationship between ray area and number of developed seeds, percent developed seeds, or number of seed predators in the open-pollinated group. .

Table 5. Multiple regression results from 2014 open- and hand-pollinated treatments, showing effects of two floral head traits on number of developed seeds, percent developed seeds, and number of seed predators found in the seed head. Standardized regression coefficients are shown.

Floral Traits	# of Developed Seeds		% Developed Seeds		# of Seed Predators	
	Open	Hand	Open	Hand	Open	Hand
Disc Area	0.245**	0.175*	0.018	-0.163	0.438**	0.509**
Ray Area	0.046	0.280*	0.156	0.622**	-0.202	-0.433*

* $P < 0.05$, ** $P < 0.001$.

DISCUSSION

Floral Head Characteristics and Insect Visitors

H. grosseserratus heads had a relatively short window when they were attractive to pollinators. My study showed that florets on each *H. grosseserratus* head were only presenting pollen for 5 days; a slightly shorter sexual lifespan than the 6-8 days that was identified by Neff and Simpson (1990) regarding a closely-related species, *Helianthus annuus*, and shorter than the one to two week flowering period that is typical for composites in general (Proctor et al., 1996). Ray damage increased steadily throughout the 5 days.

The second day of floral head sexual maturity is likely the most important to insect attraction. On day 2 of the study, both the number of florets presenting pollen and the expected ray area reached or were near their peak values. The insect orders of Hymenoptera, Diptera, and

Coleoptera all had peak visitation on either day 2 or 3. The different pattern seen with the fourth-most common insect visitor, Hemiptera, of relatively steady visitation and a peak on day four, likely reflects their lifestyle extracting and feeding on plant sap rather than being tied to the increases and decreases of pollen availability. Although plants have multiple heads which all develop at different times over the course of a flowering season, this short window of sexual maturity and peak attraction for each head emphasizes the importance of receiving the attention of pollinators soon after the head begins presenting pollen.

Prior studies have indicated that UV patterning may be a trait used to gain the attention of pollinators and direct them to the floral head (Daumer, 1958; Kevan et al., 2001). Other studies have suggested alternative functions of UV patterning; such as a defense mechanism against abiotic stresses (Rivero et al. 2001; Jansen et al. 1998) or florivores (Gronquist et al. 2001). The knowledge that many florivore/seed predator insects such as Diptera, Coleoptera, and Lepidoptera are also able to see into the UV range (Briscoe and Chittka, 2001) and that such patterning is heritable (Yoshioka et al., 2005; Syafaruddin et al., 2006; Koski and Ashman, 2013) indicates that the preferences of pollinator and non-pollinator insects are both likely to shape natural variation in UV patterning. The polymorphism of floral patterning found in my study (where heads either have a well-defined bulls-eye or no identifiable pattern) has been documented previously (Cruden, 1972; Naruhashi and Ikeda, 1999), but not as part of a complex system involving multiple floral head traits, pollinators, and florivores/seed predators in a natural environment. For both phenotypes to exist within a population to a nearly equal extent, it is likely that both forms have tradeoffs regarding overall seed production.

One likely explanation for the maintenance of polymorphism is the existence of defensive chemicals present in the UV-absorbing portion of the floral display (Gronquist et al., 2001).

Floral heads with a small UV intensity ratio (minimal or no visible demarcation between zones) are darker throughout, and thus more "absorbent" as a whole. It would follow, therefore, that heads with a small UV intensity ratio are likely to be better defended. Heads with a large UV intensity ratio are less "absorbent" as a whole and are less defended, but may also be able to allocate more resources toward seed production and growth.

The importance of florivores and seed predators to the system is highlighted by the high diversity of insect visitors. Simply focusing on Hymenoptera and other pollinating insects would leave out critical information regarding how the system functions as a whole. The heads are likely under strong evolutionary pressure not only to attract pollinators, but also to discourage herbivores from visiting and causing damage.

On a similar note, there was a surprising number and variety of seed predators collected from seed heads in each year. Insects from 6 different orders were found; more than the 3 orders documented by Pilson (2000) in the closely-related species of *Helianthus annuus*. The most common orders of insects, Thysanoptera and Diptera, have the potential to cause significant damage to the floral heads and seeds. There is evidence that feeding from Thysanoptera can result in flower and immature fruit abortion (Childers, 1997). In addition, studies have shown that Diptera larvae are capable of causing significant damage to ovules and stamens, and premature floral abscission (Pena et al., 1989; Edwards and Brown, 1997).

Natural Selection on Floral Traits

The hand pollination treatment in 2014 revealed no evidence for pollen-limitation in this system, and instead suggested that the plants were resource-limited. There were no significant differences in either the number or the percent of developed seeds between open-treatment and hand-treatment heads. This result must be interpreted cautiously, because contrary to

expectations, the trend was for a decreased number and percent of developed seeds in the hand-pollination group, and the difference between the two groups approached statistical significance.

This trend is likely due to an increase in insect-induced damage to floral reproductive parts on hand-pollinated heads. The 2015 garden study showed that the hand pollination techniques used in the 2014 study were, in fact, successful at transferring pollen from pollen-source heads to pollen-recipient heads, and that there was no human-induced damage to styles or other reproductive parts during pollen-transfer.

The number of damaging insects in the seed heads was significantly higher in the hand-pollinated group compared to the open-pollinated group. In addition, the two insect orders which contributed to this difference, Thysanoptera and Diptera, have the potential of causing significant damage to the floral head (Childers, 1997; Pena et al., 1989; Edwards and Brown, 1997). While the garden study in 2015 revealed that the transfer of a small number of Thysanoptera was possible from hand pollination techniques, the significant difference between the two treatments in both Diptera larvae and immature Thysanoptera is unlikely to be solely due to direct insect transfer. It is far more likely that the addition of pollen resulted in the attraction of additional insects to the floral heads. It is possible that seed predators caused damage to the reproductive parts of the plant and prevented full seed development. Hand pollinations are widely used in pollination studies to increase fruit set (Willmer, 2011), but their potential to increase the numbers of seed predators on recipient flowers has not been well-explored.

Regression analysis revealed that disc area was the most important factor affecting both number of developed seeds and number of seed predators, but there was no significant influence of disc area on percentage of developed seeds. If pollinator attraction was the cause of an increased number of developed seeds in heads with a larger disc, I would have also expected to

see a greater percent developed seeds in floral heads with a larger disc. Since there was no relationship between percent developed seeds and disc size, attraction to larger discs was probably not the primary reason that the number of developed seeds increased with disc area. Instead, larger discs had more developed seeds most likely because a larger disc could hold more ovules. Nevertheless, the results suggest that there still is opposing selective pressure on disc size, although not mediated by pollinators. A larger disc has more developed seeds, but also has more damaging seed predators.

A surprising trend related to ray area was revealed following the regression analysis. Contrary to expectations, ray area had no effect on the number or percent of developed seeds. Heads with a larger ray area did have significantly fewer seed-predator insects, however. Such results indicate that ray area may not be as important to pollinator attraction as previously thought (Stuessy et al., 1986; Bertin and Kerwin, 1998). It is possible that, in this system, the larger rays may be providing some defense against seed predators. As was discussed in the results, the area of UV absorbance increased with an increasing ray area among heads with a distinct bulls-eye pattern. Since the UV absorbance zone has been shown to be defensive (Gronquist et al., 2001), it is possible that the larger zone of defense deterred seed insects from visiting heads with large rays.

On a similar note, it was also shown that a large UV intensity ratio was associated with more insects in the seed head. A small UV ratio means the ray area is darker throughout, and thus more "absorbent" as a whole. It is possible that seed insects, identifying this pattern as a warning, avoided the heads more often. There was no significant relationship between UV intensity ratio and the number or percent of developed seeds, indicating that UV patterning may not be as important to pollinator attraction as once thought (Daumer, 1958; Kevan et al., 2001).

Instead, in this system, plants appear to be using UV patterning of floral heads primarily as a means of defense against harmful insects.

My results indicate that other factors such as head height and number of open flowers in the surrounding area may be more important to seed development. A lower head height resulted in a higher number and percentage of developed seeds. A larger number of open flowers in a 1.5m radius resulted in a higher number and percentage of developed seeds. Since both factors had an effect on percent developed seeds, it is likely that pollinators contributed to this trend.

The open-pollinated and hand-pollinated heads were compared using the same multiple regression approach, but only using disc area and ray area. The results for disc area were similar to those presented above: disc area was positively associated with the number of developed seeds and the number of seed predators for both open-pollinated and hand-pollinated heads, and there were no significant effects for the percent developed seeds for either treatment.

Differences between the open-pollinated and the hand-pollinated treatments emerged for ray area. For the hand-pollination treatment, a larger ray area was associated with a greater number and percent of developed seeds, but fewer seed predators in the seed head. There were no significant effects of ray area for the open-pollinated treatment. This difference between the hand-pollinated and open-pollinated treatments may be related to the additional seed predators present in the hand-pollinated group. It is possible that the indirect addition of more seed predators to the hand pollination treatment by increasing the amount of pollen resulted in more damaged seeds and increased the ability to detect trends related to defenses against seed insects. In addition, it is likely that this association between ray area and number of insects is related to UV patterning on the ray itself. A larger ray area corresponded with a larger area of UV

absorbance (and, therefore, more defense against seed predators). Therefore, though not directly measured, the area of UV absorbance may actually be contributing significantly to this trend.

My research showed that multiple insect orders and species are involved in the relatively short sexually-mature phase of *H. grosseserratus* floral heads. Such insects all contribute to the complex network of interactions which contribute to floral trait prevalence and natural selection. Here, my research has shown that the disc area is positively correlated with seed development, head height is negatively correlated with pollinator visitation, and floral heads in a 1.5m radius is positively correlated with pollinator visitation. The disc size, expected ray area, and the UV intensity ratio are the most important variables influencing seed predator visitation. More research is necessary to solidify such findings and discover their applicability to other ecological systems.

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