Effects of Patch Size and Light Conditions on the Visitation Rates of Flying Pollinators to Common Marigolds (*Calendula officinalis*)

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Abstract
Biotic pollination takes place in 87.5% of all angiosperms and utilizes animals to transfer pollen. Our study focused on how patch size and light conditions affect pollinator visitation rates to common marigolds. We hypothesized that patches receiving more sunlight would receive more pollinators than shaded patches and that larger patches would receive more pollinators than smaller patches. We manipulated patch size and light conditions by placing marigold plots of different sizes (small, medium, and large) onto a field in areas that received differing amounts of sunlight throughout the day. We then counted the number of pollinators visiting each patch for 10 minute intervals four times a day. A linear regression analysis indicated a significant positive relationship between light availability and pollinators, supporting our first hypothesis. The results of an ANOVA test demonstrated a significant difference between the number of pollinators visiting the large patches over the small patches, supporting our second hypothesis. Our findings corroborate studies of a similar nature that examine patch size and light conditions in correlation with pollinator visitation rates. These studies have global implications for the management of agricultural lands.

Introduction

Pollination is the process by which pollen is transferred from the stamen of a plant to the stigma of a conspecific plant. Pollen transfer can occur within the same plant (selfing), or to a different plant of the same species (outcrossing), the latter of which increases genetic diversity. Pollination results in plant fertilization and the production of seeds, both crucial components of plant fitness. There are two methods of pollination. Abiotic pollination relies on non-living mechanisms, such as wind or water, to disperse pollen. This method is employed by fewer plant species than biotic pollination and can be somewhat unreliable (Ackerman, 2000). Meanwhile, biotic pollination is more reliable and takes place in 87.5% of all angiosperms (Ollerton et al., 2011), which make up 90% of all plant species on Earth (Crepet and Niklas, 2009). Biotic pollination relies entirely on animals to transfer the pollen (Dafni et al., 2005). The most common animal pollinators are insects such as bees, wasps, butterflies, moths, flies, and ants (Ingram et al., 1996).

Pollinators and plants form a symbiotic relationship in which pollinators receive nectar in exchange for collecting and dispersing pollen for the plant (Heinen, 2015). Most plants outside of tropical regions are pollinated by multiple generalist pollinators, though some relationships can be species-specific (Heinen, 2015). The relationship between plant and pollinator is mutualistic; therefore natural selection has selected for certain plant traits that attract pollinators (Dafni et al., 2005). Pollinators are predominantly driven by senses such as sight and smell (Chittka and Menzel, 1992), so many flowers are brightly colored and/or emit specific odors (Dafni et al., 2005).

Light intensity is a main factor that affects pollination. Kilkenny and Galloway (2008) tested how light directly and indirectly affects reproductive success in the American bellflower (*Campanulastrum americanum).* Their study examined how light affects resource distribution in natural populations and how this affects floral display size which could also affect pollinator attraction. They also mention that cooler temperatures in shaded areas could influence pollinator visitation rates. The researchers found that plants receiving more sunlight have higher average pollinator visitation rates than those in the shade (Kilkenny and Galloway, 2008).

For this study, we focused on how patch size and light conditions affect the rates of flying pollinator visitation in common marigolds. Marigolds are extremely common in gardens across America and have a bright yellow or orange color to attract pollinators. Common marigolds (*Calendula officinalis*), such as those used in this study, are domestic flowers that have the interesting adaptation of repelling pest species such as aphids and flea beetles through an unknown mechanism while still attracting pollinators (Jankowska et al., 2009). For this reason, they are often intercropped in gardens with vegetables such as cabbages to keep pests away.

Since pollinators are largely attracted to plants through sight, lighting and patch size of flowers may play important roles in the number of visitations (Chittka and Raine, 2006). If light level is low, a plant might not trigger the pollinator's search image. The same could be true of patch size: If there are too few flowers in a patch, they may not be able to efficiently attract a pollinator's attention (Kilkenny and Galloway, 2008). Patch size and lighting may also affect the types of pollinators visiting each plot. Our first hypothesis is that higher pollinator visitation rates will occur in plants that are exposed to more sunlight, as opposed to those in the shade. Our second hypothesis is that larger patch sizes will result in higher pollinator visitation rates.

Materials and Methods

This study was conducted on the ball field within the University of Michigan Biological Station (UMBS). The tract of land that is now the UMBS was donated to the university in 1909 by the Bogardus family (Gates, 1985). The area had been clearcut and burned prior to university ownership, limiting native species (Heinen and Vande Kopple, 2003). The ball field at UMBS is now surrounded by forest and is home to many common native plant species, including but not limited to oak, white pine, red pine, red maple, bracken fern, milkweed, and mosses.

We created small, medium, and large patches of marigolds. The large patches were composed of twenty plants (one flat), medium patches composed of ten plants, and small patches composed of 5 plants. The marigold patches were placed intact on the southern half of the ball field, as opposed to being planted and introducing an exotic species. One patch of each size (small, medium, and large) was placed in the middle of the south side of the field, where they would receive full sun all day. The second set of patches was placed one meter from the edge of the forest on the west side of the field, where plants would receive sun in the morning and shade in the afternoon. The third set of patches was placed one meter from the edge of the forest on the east side of the field, where plants would receive sun in the afternoon and shade in the morning. The final set of patches was placed five to twenty meters within the forest just southeast of the field, in areas where plants would be shaded all day. The treatments were placed at least 30 feet apart and were clearly marked with white flags (Figure 1). Cylindrical chicken wire barriers were placed around each plot to prevent herbivory. The patches were placed in their positions on the ball field three days before the first observation period to allow pollinators to become acquainted with the sites before data collection.

Data collection took place during four days throughout two weeks in late May and early June. Each patch was observed four times per day at 9 a.m., 12 p.m., 3 p.m., and 6 p.m. for a period of ten minutes. At the beginning of each data collection session we recorded temperature, weather conditions, light levels, and wind speed at each patch. Light levels were measured using lux meters while wind speed was determined using a wind meter and temperature was recorded using a smartphone weather application. We recorded the general weather conditions and coded them as sunny, overcast, or rainy.

During observations, the number of visitations of flying pollinators to the patches was recorded. Each time a pollinator landed on a flower, one visit was counted. If a pollinator flew from one flower to another within the same patch without exiting the patch, it was counted as only one visit. If a pollinator landed on a flower, flew outside the bounds of the patch, then re-entered the patch and landed again on a flower, two visitations were counted. The general type of pollinator (fly, bee, butterfly, or moth) at each visit was also recorded.

After the data were collected, a linear regression analysis was performed to determine the relationship between light level and the number of pollinators. Two more linear regression analyses were performed to examine the relationships between pollinator visitation, wind speed, and temperature. To determine the relationship between patch size and pollinator visitation, an ANOVA multiple comparisons test was performed. Another ANOVA multiple comparisons test was used to determine the relationship between time of day and number of pollinators. Two chi-square tests of independence were also used to investigate whether light conditions and patch size had an effect on the type of pollinators visiting each patch. SPSS and an alpha of 0.05 were used for all statistical analyses.

Results

We conducted a linear regression analysis and found a significant positive relationship between the amount of sunlight the flowers received and the number of pollinator visitations (p-value <0.001). This supported our first hypothesis that higher visitation rates will occur in patches that receive more sunlight. We also ran a linear regression to test the relationship between temperature and number of pollinators and found a significant positive relationship with a p-value $<.05$ (Figure 2).

The first ANOVA multiple comparisons test determining whether patch size had an effect on the number of pollinator visitations demonstrated a significant difference between the number of pollinators visiting the small and the large patches (p-value=.02), but failed to find a significant difference between the number of pollinators visiting the small and medium plots (p-value=.742) or the medium and large plots (p-value=.063). This data supports our second hypothesis that more pollinator visitation will occur in larger patches.

The second ANOVA multiple comparisons test determining whether pollination visitation changed with the time of day found a significant difference between the number of pollination visits between 9:00 a.m. and 12:00 p.m. (p-value=.04), but failed to find a significant difference between any other times (9:00 a.m.-3:00 p.m., 9:00 a.m.-6:00 p.m., 12:00 p.m.-3:00 p.m., 12:00 p.m.-6:00 p.m.). We performed a linear regression test between wind and number of pollinators that determined there was no significant relationship between wind speed and number of pollinators at the 95% confidence level. Using two chi-square tests of independence, we also found that there was no significant difference in distribution between the flies, bees, butterflies, and moths that visited the marigold patches in different light conditions (p-value=.15) or patch sizes (p-value=.40).

Discussion

Our results support the hypothesis that light level has a significantly positive relationship with pollinator visitation rates. In general, when the light level was higher, higher rates of pollinator visitation occurred. The relationship between light level and pollinators is likely due to a combination of sight enhancement and temperature. Flowers have many traits to attract pollinators, including color. Pollinators, which are greatly reliant on vision to locate flowers, are highly receptive to the colors of different flowers. The colors of flowers trigger the stored memory of pollinators and help them to differentiate between plant species, which influences whether or not they visit a certain flower (Chittka and Raine, 2006). Colors are more easily observed when light levels are higher, which could be a significant contributing factor to pollinator visitation. This relationship could also be very important when flowers are in areas with dense vegetation that hides the flowers from the sight line of pollinators.

Light level might also correlate with pollination because of temperature. Although temperature was not a variable in our original hypotheses, we performed a linear regression test to investigate the effect of temperature on pollinator visitation rates. It is important to note that the data are not completely reliable because only the general temperature of Pellston, MI was recorded from a weather application. However the results did reveal a statistically significant positive relationship between temperature and visitation rates. On colder days, there were significantly less pollinators than on warmer days. Since light level has an effect on temperature (Kilkenny, 2008), if we had recorded temperature at individual plots during each observation time it is likely that we would have found a correlation between light level and temperature and a correlation between temperature and pollinator visitation rates. This may be due to the fact that many pollinators have an optimal temperature window in which foraging flight can be sustained and prefer warmer temperatures to colder temperatures (Corbet, 1993). The effect of the time of day on pollinator visitation rates was investigated here, and it was found that the rates of pollinator visitation were significantly higher at 12:00 p.m. than at 9:00 a.m. The differences in pollinator visitation could be due to variations in light level and temperature, as we recorded higher averages of each at 12:00 p.m. than at 9:00 a.m.

Our results also support the hypothesis that there is a significantly positive relationship between patch size and number of pollinator visits. The significant difference between the small and large patches may be attributed to sight. The ball field is predominantly covered by bracken ferns, plants that may have inhibited the view of the marigolds. Larger patches have more flowers and are easier to see from a distance and through other vegetation than smaller patches. These results may also be attributed to optimal foraging. A pollinator has to expend less energy to acquire nectar if there are many flowers within a close proximity, which is the case in the larger patches. It may not be worth it for a pollinator to visit smaller patches based on the tradeoff of energy cost and amount of nectar received (Heinen, 2015). The reason this relationship was not significant between the small and medium patches or the medium and large patches could be because there was not enough variation in size between these patches to make a considerable difference to the pollinators. This trend may have been significant on each level if the patch sizes had been larger overall and if there had been a greater size difference between the patches.

Furthermore, there are several potential reasons for why no significant relationship was found between wind speed and pollinator visitation rates. The wind speeds may not have been high enough to discourage some flying pollinators. Pollinators such as monarch butterflies are able to migrate vast distances in turbulent wind conditions (Gibo, 1986). Additionally, the wind speeds recorded with a wind meter at the beginning of each data collection session may not have been representative of the entire ten-minute period; we observed that the wind speed could vary substantially even within short periods of time.

When the relationship between type of pollinator and light conditions were examined, no statistically significant differences were found. Also, no statistically significant differences were found between patch size and type of pollinator. This means that the light and patch size preferences of the pollinators did not appear to vary between the different types of pollinators. This may be due to the small sample sizes of pollinators other than flies. While a total of 185 pollinators were observed, 174 were flies. We recorded only 7 bees, 3 butterflies, and 1 moth visiting the marigolds. The lack of flying pollinator diversity could be attributed to the types of plant species present around the marigolds. The ball field was populated largely by bracken fern and cattails, which rely upon abiotic pollination to reproduce. There seemed to be only a few other plants relying on biotic pollination in the area, including milkweed, which was not flowering at the time. This may have skewed the results, since most pollinators likely spend more time in areas with a higher density of plants requiring biotic pollination, where they could forage more efficiently.

There were several confounding variables that may have affected the results of this experiment. General weather conditions represent one possible confounding variable. Pollinator activity is highly dependent on weather variables such as temperature, rain, and wind speed (Vicens and Bosch, 2015). Pollinators avoid emerging in overcast and windy conditions because rain and wind can negatively affect a flying pollinator's ability to navigate successfully (Vicens and Bosch, 2015). It rained on the first day of data collection, which may have decreased the number of pollinators actively pollinating plants that day. We also recorded increased wind speeds on the first and last days of data collection, which also may have negatively affected the pollinator visitation rates at the marigolds (Vicens and Bosch, 2015). The wind and rain may have posed a greater danger to the pollinators of the ball field, particularly at the patches in full sun, which were in a relatively unsheltered area of the field.

Another confounding variable is that the marigolds may not have been included in the pollinators' search images. Marigolds are not a native species in the UMBS ball field, which could mean that marigolds were excluded from the search image of local pollinators, causing pollinators to ignore them. Search images may not have been triggered because the patch sizes were too small to draw attention and attract the local pollinators. In addition, pollinators generally follow optimal foraging patterns and might exclude marigolds from their search images if the benefits of pollinating marigolds do not outweigh the energy required to do so.

The proximity of insect nests and hives could have been another confounding variable. If a colony of pollinators was close to one of the marigold patches, that patch could have seen an increase in pollinator visitation rates unrelated to the variables being tested. The opposite might occur in reverse conditions. However, we did not notice any nearby colonies of flying insects, and the number of bees observed in the area was low enough that it is reasonable to assume that there were no nearby beehives. Again, the reason there may not have been any nearby beehives or significant pollinator diversity is because many of the native plants species on the ball field do not utilize biotic pollination.

There are several limitations of our experiment. Twice, individual plots fell victim to herbivory, resulting in the purchase of additional flats of marigolds as well as chicken wire. The chicken wire was cut and formed into enclosures, which were then placed around each of the twelve plots. No additional incidents of herbivory were observed after the chicken wire was placed, but we found that the chicken wire itself introduced another source of error. It was noted that the chicken wire prevented approaching bees from landing on the marigolds and pollinating. Bees are very sensitive to external forces and avoid narrow spaces that could harm their wings and prevent flight. Upon noticing this, we decided to remove each barrier from its plot during the observation period. Furthermore, observing each plot for only four ten-minute intervals per day may have not been enough time to collect data that accurately reflects true pollination patterns. While we did spread out observation times to get a wide range of light and weather conditions, it is certain that we missed many pollination events. Observing the plots for longer periods of time may have added validity to our results. Other pollination studies have used stationary cameras to observe plots for a much larger duration of time, which would have made their results more significant (Vicens and Bosch, 2000). This method of observation would also alleviate the effects of a researcher hovering nearby the plots, as human presence may have deterred pollinators from visiting the flower patches.

Conclusion

Our findings demonstrate that light conditions and patch size have significant effects on pollinator visitation rates. Studies that test how multiple variables affect pollinators are of utmost importance because the crops grown and eaten by humans around the world are predominantly angiosperms that utilize biotic pollination are. Animal pollination leads to increased fruit or seed sets in around

75% of the world's leading food crops, increasing the amount of food available (Ollerton et al., 2011). The economic value of pollination as an ecosystem service is an estimated $153 billion annually, or 9.5% of the value of world agricultural production (Ollerton et al., 2011). Honey bees alone pollinate approximately $10 billion worth of crops in the United States each year (Watanabe, 1994). Animal pollinators also support biodiversity. There is a correlation between plant diversity and pollinator diversity and declines in pollinators may make plants more vulnerable to extinction (Heithaus, 1974). It is important that these studies continue in order to optimize crop pollination throughout the world and promote biodiversity.

For future studies, we recommend using a digital or mercury thermometer to measure the temperature at the site of each plot. In our study we observed a general relationship between temperature and number of pollination visits, but a more accurate reading of temperature is necessary to further support this relationship. Additionally, our study has shown that there is a significant difference in pollinator visitation rates between small and large patch sizes, yet not between small and medium patches or medium and large patches. It may be beneficial to repeat our experiment with larger patch sizes and increased size differences among them, as it could increase the significance of results and make conclusions with greater confidence. We also recommend that future studies use a fenced exclosure to prevent herbivory rather than chicken wire to decrease potential error associated with pollinator exclusion. To further expand upon this study, we also suggest investigating whether the types of surrounding plants have an impact on the pollinator visitation rates of marigolds, such as plant size or its method of pollination (biotic or abiotic). It may also be interesting to actually plant individuals of a native species of flower in the soil to observe and compare the pollinator visitation rates of potted and planted flowers.

Appendix

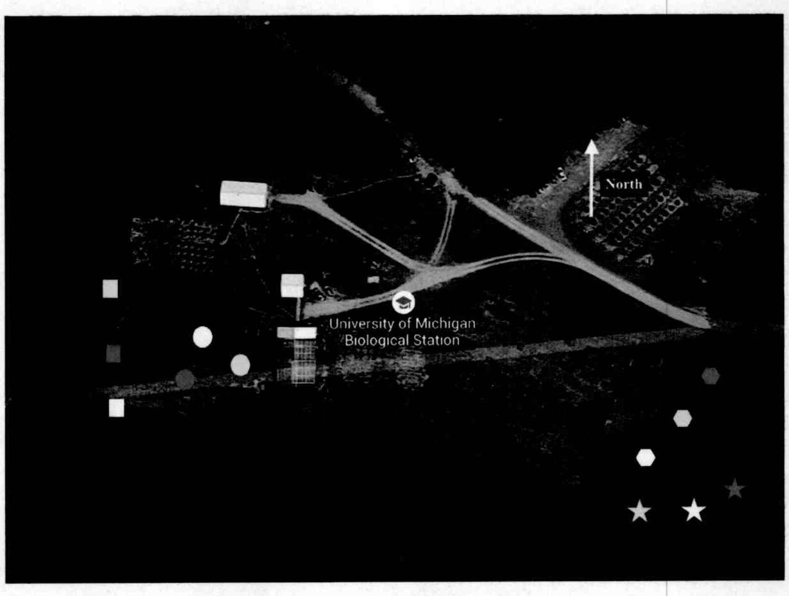

Figure 1. illustrates the approximate locations of the marigold patches. Yellow - large; blue - medium; white - small; square - morning sun; circle - full sun; hexagon - afternoon sun; star - full shade.

Linear Regression of Light Level (lux) and Number of Pollinators

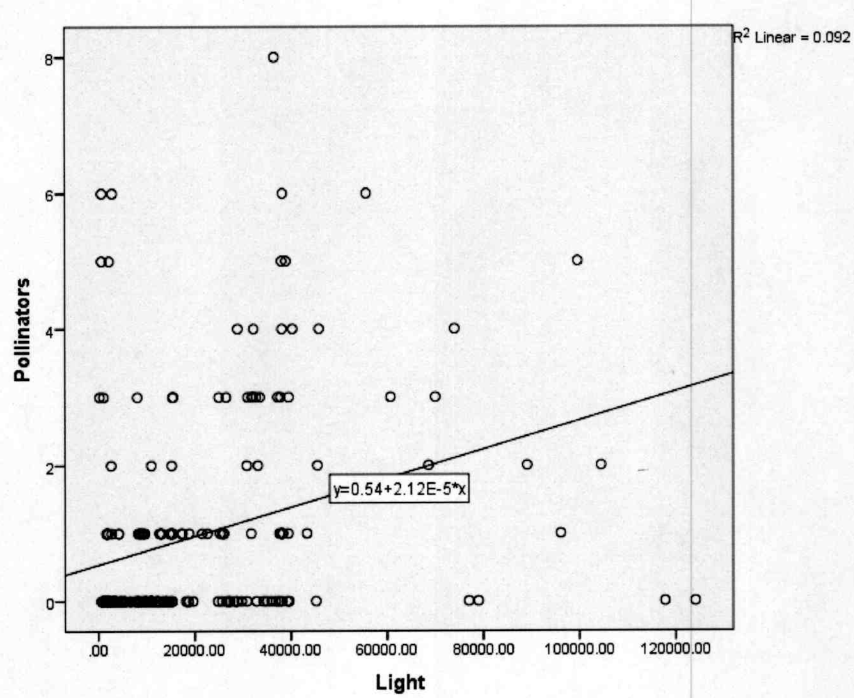

Figure 2. Linear regression analyses of light level and number of pollinators

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