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Joshua R. Morris

University of Akron Main Campus, JoshMorris0144@gmail.com

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Behavior of Pollinators That Share Two Co-Flowering
Wetland Plant Species

Joshua Morris

Department of Biology

Honors Research Project

Behavior of Pollinators that share Two Co-Flowering Wetland Plant Species

Abstract:

Intermixed, co-flowering plant species often attract the same pollinators and may therefore compete for pollinator visits. *Mimulus ringens* and *Verbena hastata* are sympatric wetland plants that flower in synchrony and share many pollinators, the most common being bumblebees. The possibility of competition between these two plant species led to this observational study tracking pollinator movements in an area intermixed with both *M. ringens* and *V. hastata*. We identified pollinator species and tracked them as they visited flowers and moved from plant to plant. Smaller bees seemed to prefer the smaller flowers present on the *V. hastata*, and the larger bees frequently visited *M. ringens*. Interspecific moves were highly limited, 4.7% of total moves observed by most abundant pollinators. Therefore, the intermixing of *M. ringens* and *V. hastata* appears to have a neutral effect on each other and should not affect reproductive success. This neutral effect could be due to different foraging behavior due to differing floral morphologies, heights, colors, or even pollinator reward.

Introduction:

Co-existing plants in a community can influence each other in many ways. Although most research focuses on competition for abiotic resources, like light and water, the influences of plants on one another can also include pollination. Sharing pollinators can alter pollinator behavior in ways that may be either beneficial or detrimental to a species. Facilitation of pollination occurs when the presence of one plant increases the pollination rate and reproductive success of another species (Rathcke, 1983, Ghazoul, 2006). Increased floral diversity and flower size in an area can attract more pollinators to plant species not receiving much activity (Essenberg, 2007). Inversely, two or more plant species in a community that flower in synchrony could share and compete for pollinators.

When different plant species compete for pollinators, their reproductive success can be affected by a change in the quality and/or quantity of pollinator visits. Pollinators could be dividing their visits between two species of plants instead of one and even switching between species in a single bout. Moving interspecifically between plants can decrease pollen transfer and reduce an individual plant's reproductive success (Waser, 1983, Flanagan et al. 2009). A lack of floral constancy can cause interspecific pollen transfer, which can result in the pollen from one plant species being wasted or even clogging the stigma of another plant species. Interspecific movements often arise when there is similar floral morphology, height, and reward for the pollinator in a plant community. It becomes more efficient for the pollinator to visit closest flowers if both the energy required to forage and the reward is the same (Waser, 1986). This can render interspecific pollinator movements detrimental in the perspective of plant species reproduction.

Through evolution, some co-existing plants attract different pollinators or flower sequentially to lessen competition (Proctor, 1996). Pollinator preference can affect the quantity of visits for one plant species (Waser, 1978).

There can also be a neutral effect between two sympatric species in which the presence of each species does not significantly affect the other (Waser, 1983). In these cases, the sympatric species could either fill different niches or utilize different pollinators in order to avoid detrimental effects. Avoiding direct competition can increase reproductive success.

In this study, I investigated the behavior of pollinators that are shared by two native, co-flowering wetland plants that have different floral morphology and similar color patterns. The synchronous flowering of these plants could result in interspecific pollinator movements. A greater understanding of how different plant species influence each other can be obtained by studying pollinator behavior. The plant species that I focused on were *Verbena hastata* and *Mimulus ringens*. *M.*

ringens is a native perennial forb that is commonly found throughout wetlands in central and eastern North America (Grant, 1924). It has blue flowers that open around 5am and each flower lasts for about one day before falling off. At the northeastern Ohio site of observation, *M. ringens* began flowering in July and continued flowering through August. Common pollinators included bumblebees and solitary bees (Karron et al 2004). *V. hastata* is also a native perennial forb that is found throughout wetlands in North America (Lovell, 1939). It has violet flowers that are on spikes extending from the stem and the flowers typically last for two days before falling off (Lovell, 1939). *V. hastata* flowered from July till September at the northeastern Ohio site of observation. Common pollinators included bumblebees, *Apis mellifera*, and solitary bees (Lovell, 1939).

The purpose of this study was to: 1) determine if *M. ringens* and *V. hastata* share common pollinators, and 2) determine if certain pollinators are more likely to make interspecific moves than others and how common interspecific moves are in general.

Methods:

In July of 2014, we scored pollinator visits at field sites that contained both *Mimulus ringens* and *Verbena hastata*. The field sites were wetlands at Bath Nature Preserve in Bath, Ohio. The regions in the Nature Preserve that were used in the study were the "Garden Bowl" and the "Soup Bowl". Both regions are restored wetlands that contain native *M. ringens* and *V. hastata* intermixed with other native plant species. Observations began at 9:00 a.m. and continued until 12:00 p.m. on five separate days. The observation days had similar weather with clear skies and about 75 degrees Fahrenheit.

Pollinators were identified and followed as they moved to and from plant species. A team of researchers tracked and recorded time of day and the number of flowers (*ringens*) and the number of spikes (*hastata*) pollinators visited on each individual plant as they moved through the area. We also noted when interplant moves were between the same species of plant and when movements were

between different plant species. As soon as a bee was seen, the observer began following and scoring its behavior. Pollinators were followed for their entire bout or until the observer lost sight of the individual. The aim of the methods was to get a mix of bees starting on either of the two plant species. A total of 295 foraging bouts were scored in those days of observation, with each bout ranging from 1 to 79 plant visits (mean of 10) and 1 to 771 flower or spike visits (mean of 46). Plant species that were co-flowering was also recorded.

To evaluate some factors likely to affect pollinator sharing, I recorded the abundance and flower heights of *M. ringens* and *V. hastata* in the "Soup Bowl". To do this, on July 22nd I started in an average portion of the "Soup Bowl" where both *M. ringens* and *V. hastata* were located. Next, I took three steps in one direction and where they stopped was the center of a 1m² quadrat. The species and abundance (number of individuals) of each plant species flowering in the quadrat was recorded. The heights of the highest and lowest flowers from both *M. ringens* and *V. hastata* in the quadrat were scored as well (see Plate 1). The highest and lowest flowers were not necessarily from the same plant, but were contained within the quadrat. I then went to the edge of the quadrat and took three steps in a random direction to form the next quadrat. This process was repeated for a total of 18 distinct quadrats with species, abundance, and flower heights recorded. In addition to the quadrats, on August 1, 27 points were chosen haphazardly along a transect in an area adjacent to the quadrats described above. For each point (3 steps from the last), I found the nearest *Mimulus* and used that plant as focal. The distance from that focal plant to its nearest conspecific neighbor, and the high and low flower heights for that plant were scored. Then the distance of nearest *Verbena* plant from the focal point was scored, and the height of its highest and lowest flowers was recorded.

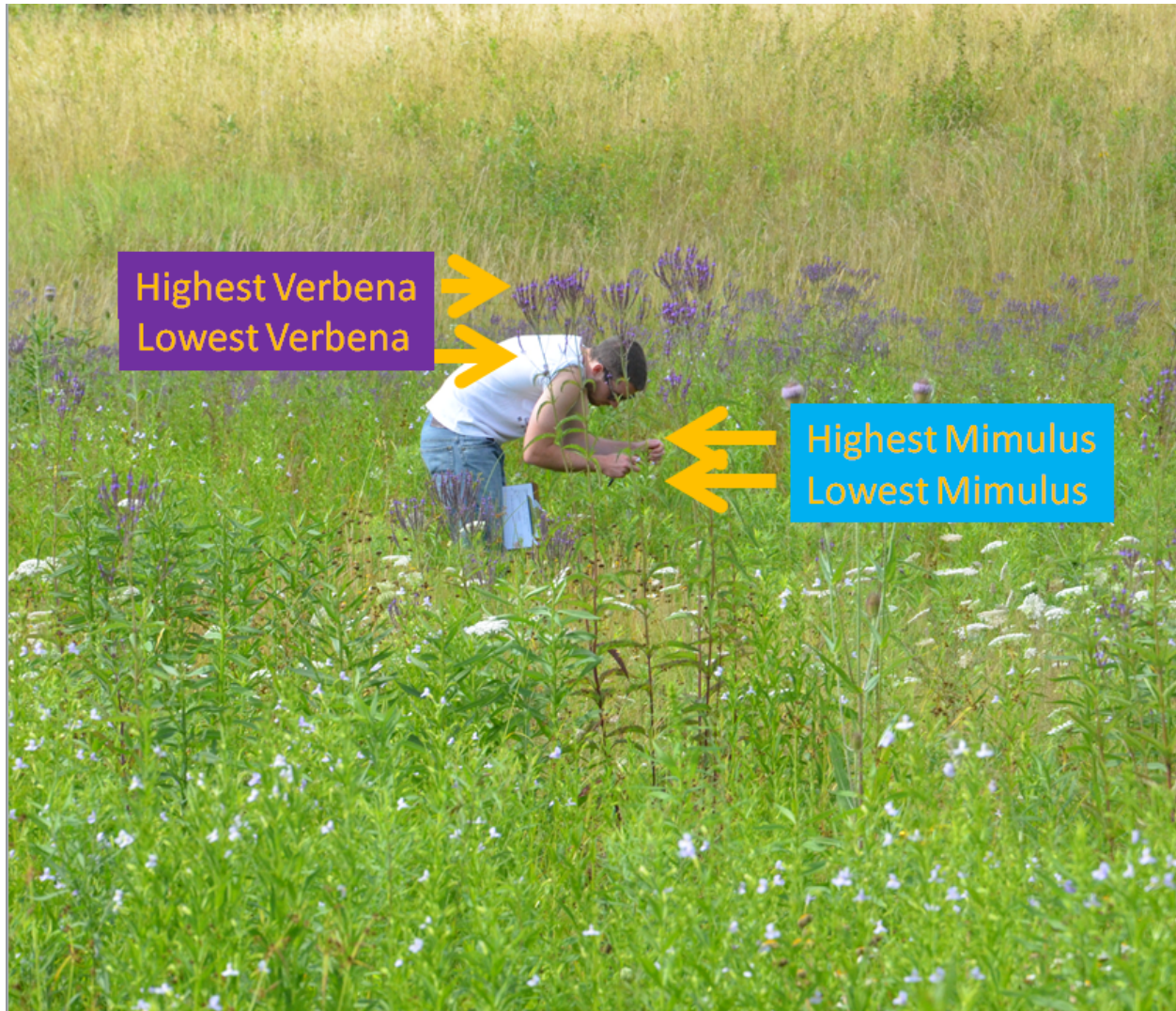


Plate 1. Field Site in Garden bowl with the author measuring flower height for *Mimulus ringens*. Arrows indicate highest and lowest flowers on two plants as described in the text.

Results:

Visitors to *M. ringens* during my observations included *Bombus fervidus*, *Bombus Impatiens*, *Apis mellifera*, *Xylocopa virginica*, *Bombus vagans*, *Hemaris thysbe*, *Megachile mendica*, and *Bombus griseocollis* (in order of most to least frequent; Table 1). Visitors to *V. hastata* during my observations included *Apis mellifera*, *Bombus impatiens*, *Bombus griseocollis*, *Bombus fervidus*, *Xylocopa virginica*, *Bombus vagans*, *Bombus bimaculatus*, and two solitary bees from the halictidae family (in order of most to least frequent; Table 1). The two plant species share many common pollinators; a chi squared test

determined the different visitor species showed different proportions of visits to *M. ringens*, and *V. hastata* ($\chi^2 = 8239.2$, $df = 6$).

Pollinator Species	<i>Mimulus</i>	<i>Verbena</i>	Grand Total
<i>A. mellifera</i>	371	4367	4738
<i>B. bimaculatus</i>	8	5	13
<i>B. fervidus</i>	2607	187	2794
<i>B. griseocollis</i>	1	328	329
<i>H. thysbe</i>	4	0	4
<i>B. impatiens</i>	472	4622	5094
<i>M. mendica</i>	3	0	3
<i>B. vagans</i>	57	6	63
<i>X. virginica</i>	120	44	164
Grand Total	3643	9559	13202

Table 1. Scored observed pollinator species and number of times each species visited *Mimulus* flowers, or *Verbena* spikes.

Very few pollinator species were observed moving between *M. ringens* and *V. hastata* in the same bout (98% of the two most frequent pollinator's total moves were intraspecific). Species observed moving between these two plant species included *Bombus fervidus*, *Bombus impatiens*, and *Apis mellifera* (Table 2). For the three most abundant pollinator species, only 39 out of a total of 2364 inter-plant movements (1.6%) were interspecific. Chi squared test for homogeneity was conducted to determine if the pattern of pollinator movement differed between the 3 most frequent pollinators (those with expected values over 5). Test for homogeneity indicated a p -value < 0.00001 ($\chi^2 = 972$, $df = 9$), rejecting the null hypothesis that all species show the same pattern. Interspecific moves from *M. ringens* to *V. hastata*, and *V. hastata* to *M. ringens* were approximately equally likely overall, and for each bee species. But some species were more likely to visit *Mimulus* (e.g., *B. fervidus*), while others were more likely to visit *Verbena* (e.g., *A. mellifera*).

Pollinator Movement	<i>B. fervidus</i>	<i>B. impatiens</i>	<i>A. mellifera</i>	Grand Total
<i>Mimulus</i> to <i>Mimulus</i>	1204	253	184	1641
<i>Mimulus</i> to <i>Verbena</i>	6	11	4	21
<i>Verbena</i> to <i>Mimulus</i>	7	7	4	18
<i>Verbena</i> to <i>Verbena</i>	22	330	332	684
Grand Total	1239	601	524	2364

Table 2. Inter-plant moves for the three most abundant visitor species. 91% of all observed interplant moves between *Mimulus* and *Verbena* are accounted for here (2364 out of 2610).

Co-flowering species
<i>Verbena hastata</i>
<i>Mimulus ringens</i>
<i>Daucus carota</i>
<i>Dipsacus sylvestris</i>
<i>Hyperica perforatum</i>
<i>Erigeron annuus</i>
<i>Asclepias incarnata</i>

Table 3. Co-flowering species present during observation in the “Soup Bowl” and “Garden Bowl”

The height of the highest *M. ringens* flower and the height of the lowest *V. hastata* flower were compared to determine overlapping flower heights in each quadrat (Figure 1.1 and Figure 1.2). *M. ringens* and *V. hastata* measured in the study rarely had overlapping floral heights. The extent of overlap in floral heights could be important for constancy in pollinators and possible interspecific moves (Waser, 1986). Only 3 of the 18 quadrats had both species of plants with overlapping floral heights (17%). The *V. hastata* plants typically had higher flowers than *M. ringens* (Figure 1.1 and Figure 1.2). On average, *V. hastata* flower height ranged from 113 cm-125 cm and *M. ringens* flower height ranged from 74 cm-85 cm.

These two plant species were abundant, and frequently occurred in close sympatry. The average number of *M. ringens* was 2.78 plants/m² (standard deviation = 2.13), while *V. hastata* averaged

only 1.56 plants/m² (standard deviation = 1.82; n = 18, t = -1.85, p = 0.073). 50% of the quadrats included both species of plants. The average distance from *M. ringens* to the nearest neighboring *M. ringens* was 41.8 cm (standard deviation = 26.96), and the average distance from *M. ringens* to the nearest neighboring *V. hastata* was 79.7 cm (standard deviation = 62.11; n = 27, t = -2.91, p = 0.0062). Table 3 contains all co-flowering plants that were present at the sites of observation (“Soup Bowl” and “Garden Bowl”).

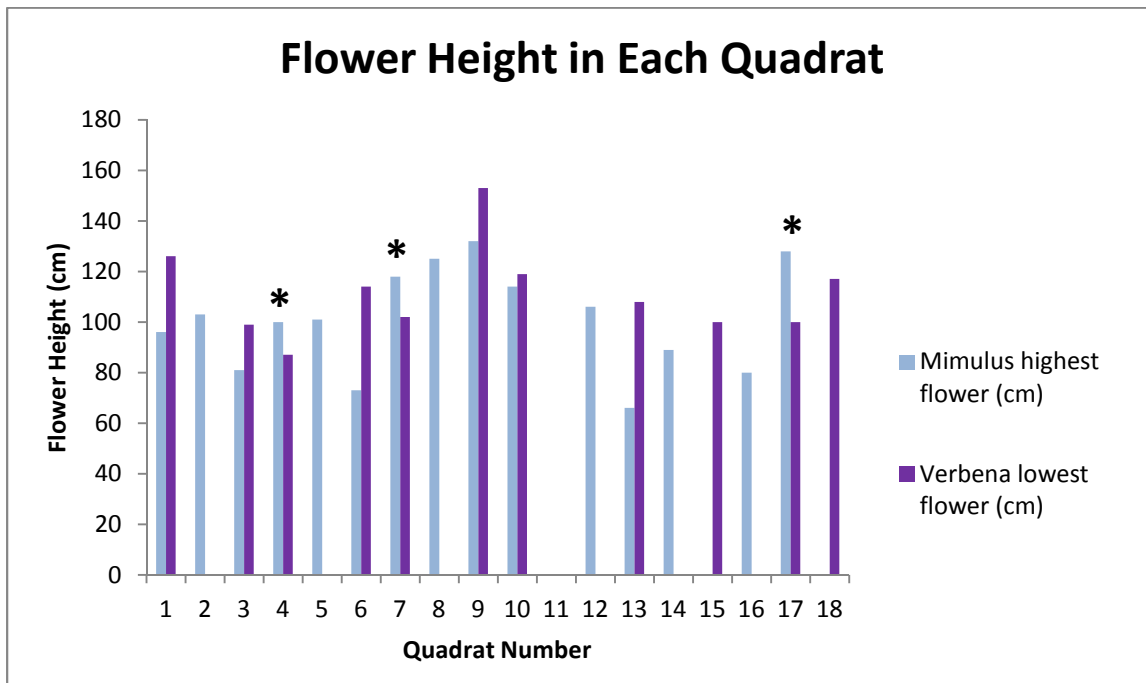


Figure 1.1. Highest *Mimulus* flower (light blue) compared to the lowest *Verbena* flower (purple) found in quadrats 1-18. When highest *Mimulus* flower is greater than lowest *Verbena* flower, (light blue > purple) overlapping floral heights is occurring.

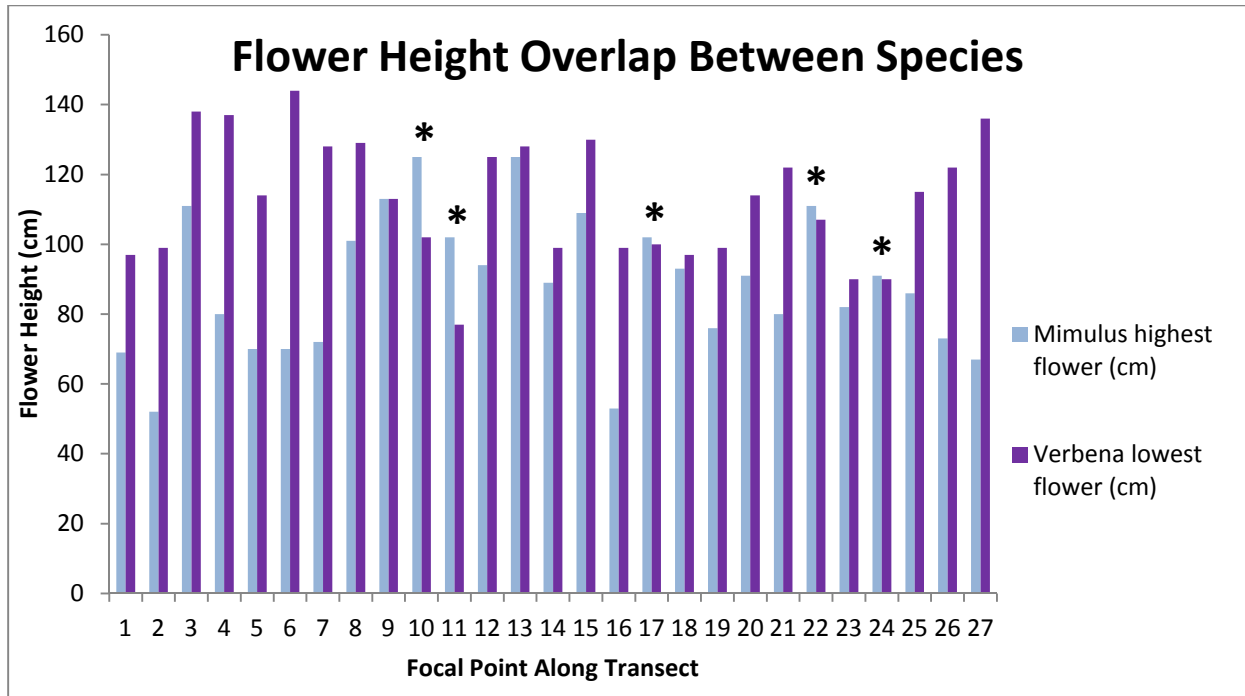


Figure 1.2. Highest Mimulus flower (light blue) compared to the lowest Verbena flower (purple) along a transect (27 total focal points). When highest Mimulus flower is greater than lowest Verbena flower, (light blue > purple) overlapping floral heights is occurring.

Discussion:

Pollinators almost exclusively visited one or the other species of plant when *V. hastata* was flowering with *M. ringens* (Table 2). Movements between plant species were rare - for the three most abundant pollinator species only 1.6% of inter-plant movements were interspecific, and none of the less common visitors were observed moving between plant species. *B. impatiens* moved interspecifically between species of plants more than any other pollinator species, 4.7% of the time. This shows how rare interspecific movements were in the study and that pollinator species differ in their probability of making interspecific movements.

Plant species abundance in a community can affect a pollinator's preference. A pollinator may visit the most abundant plant species to increase efficiency. *M. ringens* ranged from 50%-100% more abundant than *V. hastata* in the field quadrats. Only 17% of the quadrats contained overlapping flower

heights for the two plant species (Figure 1.1 and Figure 1.2). By occupying different floral heights, the two plant species can have a set of pollinators that forage at constant levels (Waser, 1986). The fact that the two species flowers are typically at different heights may suggest resource partitioning, which can decrease competition for limited resources. This could contribute to the fact that the bee species differ in their pattern of movement (Table 2; $p < 0.00001$). Therefore, these two plant species likely do not compete for pollinators even though they have overlapping geographic ranges, flowering periods, and pollinators. This means that the presence of both plant species together has a neutral effect and does not alter pollinator visitation.

The lack of interspecific pollinator movements between *V. hastata* and *M. ringens* could be due to the difference in floral morphology, height, color, or pollinator reward. *M. ringens* have large flowers that most likely are preferred by large insect pollinators like bumblebees. The long tongues of bumblebees allow for easy retrieval of nectar stored at the base of the corolla tube (Mitchell et al. 2004). *B. fervidus* is long-tongued and large in size and was the most common species observed visiting *M. ringens* (Table 1). These characteristics strongly favor foraging specifically on *M. ringens* as opposed to *V. hastata*. Small solitary bees would have to climb deep into the flower in order to retrieve nectar. Few solitary bees were seen visiting *M. ringens*, most likely because it is time consuming and thus inefficient. The size of smaller bees also decreases the chance of contacting anthers and gathering pollen from a *M. ringens* visit. Therefore, solitary bee visits are not beneficial for *M. ringens* and is inefficient and energy consuming for the bee itself.

V. hastata has small flowers in a dense bundle, commonly called a spike. The small shallow flowers select for smaller pollinators or pollinators with short tongues. This is consistent with *A. mellifera* (small body size) and *B. impatiens* (small-tongued species) being the most common observed visitors for *V. hastata* (Table 1). These smaller bees will often forage nectar from *V. hastata* and be able

to collect pollen on their face or body. Larger bumblebees have more difficulty accessing the nectar because their size hinders them from entering into the flower (Levin & Kerster, 1973). Therefore, pollinator size can be a significant factor in foraging behavior and the likelihood of interspecific movements.

A proper foraging behavior is necessary to efficiently forage on nectar in a particular plant species. *M. ringens* and *V. hastata* require different foraging behaviors, and therefore reduce the likelihood of interspecific movements by pollinators (Rathcke, 1983). Different foraging behaviors are necessary because *M. ringens* and *V. hastata* have differing floral morphologies. Floral morphologies are recognized by pollinators and are used to discriminate among flowers. Once a foraging behavior is learned, a pollinator tends to visit the plant species that the behavior is for to be as efficient as possible (Waser, 1986). This promotes visiting only one species of plants and not moving interspecifically. The pollinator is most efficient when it minimizes the time spent learning a new foraging behavior or foraging haphazardly.

Future studies should focus on interspecific movements of pollinators in communities where *M. ringens* and *V. hastata* coexist. First, pollen receipt could be quantified by counting total pollen harvested from the stigmas of both plant species. The pollen could then be analyzed and it could be determined if there is heterospecific pollen deposition or not. Large accounts of heterospecific pollen deposition could indicate competition between *M. ringens* and *V. hastata*. Next, an array of specified density of *M. ringens* and *V. hastata* could be created in order to test density dependent competition between the two species. This would test if pollinators avoid the closest flower in order to visit a particular species of choice. Expending more energy traveling and less time foraging could be a beneficial trade-off. The results of these tests could provide a clearer answer to whether or not there is pollinator competition between these two co-flowering plants.

Conclusion:

Currently, the relationship between *M. ringens* and *V. hastata* is not fully understood. It is known that they share common pollinators, similar floral coloration, overlapping flowering periods as well as geographic ranges. These similarities suggest the possibility of competition for pollinators. An intermixed area of these two similar plant species could have a serious negative effect on each species reproductive success. This observational study of *M. ringens* and *V. hastata* in their natural habitat in a northeast Ohio wetland showed that the presence of both species in conjunction did not cause pollinators to move interspecifically between plant species frequently. A small number of interspecific movements occurred but they only accounted for less than 5% of the total plant to plant movements of the most common pollinators. This indicates that pollinators rarely move interspecifically in the same bout. Since these movements are so uncommon, it is unlikely that *M. ringens* and *V. hastata* compete for pollinators. The size of the pollinator is a key factor in determining which of the two plant species are preferred because of the variation in floral morphology. Height, color, and pollinator reward for visiting the flower are all possible factors that influence pollinator visitation. Further research on the interactions between *M. ringens* and *V. hastata* is needed to determine whether interspecific pollen deposition is occurring and if there is a direct effect on the number of seeds produced by these individual plant species. This study illustrates how co-flowering plants may have a significant or non-significant effect on each other's pollination, and therefore directly affect each other's reproductive success.

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