Abiotic and biotic determinants of pollinator activity on smooth blackberry (*Rubus canadensis*) in Waldron Fen, Alanson, MI

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Abstract

The purpose of our experiment was to determine the abiotic and biotic variables that affect pollinator visitations rates of *Rubus canadensis* at Waldron Fen in northern Lower Michigan. Five 1m^2 plots were set up, each testing a separate factor and/or acting as a control. These factors included: sunlight/large patch, shade, small patch, petals off, and petals on. Notes were also taken on temperature throughout the day. Number of pollinator visits—determined by pollinator contact with each blossom—was recorded for each plot in ten-minute intervals during two half-day and two all-day sessions. Our resulting data concluded that pollinator activity increased as the day progressed and the temperature rose in all but the sun and large patch plot. Additionally, the plots with direct sun and petals had significantly higher mean numbers of visits than the plot with petals removed as well as the plot in the shade. Finally, we observed that the smaller patch had a significantly higher mean number of visits than the large patch. All in all, we found pollination rates to be highest during the afternoon in warmer, drier conditions where petals were present on *R. canadensis*. The results from this experiment could prove useful in future experiments and studies of ideal conditions for high pollination rates.

Introduction

Pollination is the process by which grains of pollen are moved from one flower and transferred to another flower's stigma, thus resulting in fertilization and the creation of viable seeds. The focus of our study, *Rubus canadensis,* is a cross-pollinated plant that relies on bees as well as wasps and flies to carry its pollen to other *R. canadensis* blossoms. These insects are dependent on several factors when it comes to their effectiveness as pollinators, including environmental variables such as temperature, as well as their ability to identify correct flowers. Temperature influences many pollinators' travel abilities since most insects are cold-blooded and need to be warmed by the sun in order to get moving. Petal presence and relative patch size are also significant in visually aiding pollinators to their desired flower—in this case, *Rubus canadensis*.

A member of the Rosaceae family, *R. canadensis* is a semi-woody perennial that grows in open fields and meadows. The erect or arching canes are reddish with thorns and can grow up to 3 meters tall. Its leaves are alternate, compound, palmate, serrate and acuminate with 3 to 5 leaflets and the flower typically consists of 5 white petals in a corymb with numerous stamens that bloom from early to mid-summer (Naegele 1996). In Michigan, *R. canadensis* ranges from the Upper Peninsula down to the northern section of the Lower Peninsula, where this study was conducted.

The purpose of this study is to determine the influence of sunlight received, petal presence, patch size, time of day, and temperature on the pollination rates of *R. canadensis* flowers in N. Lower Michigan. We predict that pollination rates will increase when the flowers are exposed to more direct sunlight. As bees are primarily visual organisms, the increased reflectivity and subsequent visibility of the blossoms in direct sunlight will most likely result in higher pollination rates. In addition, a study by Dolen et al. regarding the foraging behavior of honeybees (*Apis mellifera*) and other pollinators showed that there was a greater preference for patches of honeysuckle (*Lonicera spp.*) with higher sunlight exposure (Dolen et al. 2000).

We also predict that warmer temperatures will yield higher pollination rates primarily because the majority of pollinators in Waldron Fen are small, cold-blooded insects that require higher temperatures in order to heat their own bodies and become active. Following the logic of these hypotheses, we predict that pollination rates will be highest later in the afternoon when temperature is at its peak and sunlight is most direct. In a study that examined pollinator visitation rates with respect to time of day, the number of visits reached its peak in late afternoon (Jacques et al. 1999) when the temperature was relatively high.

Our final hypotheses predict that *R. canadensis* blossoms with petals and those in larger patches will experience a greater number of pollination events due to their attractiveness and ability to provide landing for the target pollinators. Since petals also serve as visual cues allowing insects to identify flowers, we expect pollination rates to be relatively low for the flowers that do not have petals (Kudoh and Whigham 1998). Furthermore, in a study observing the effects of patch size of catnip (*Nepeta cataria*) on pollinator visitation rates, rates were shown to be higher in larger patches for honeybees and bumblebees. Additionally, the study also indicated that isolated patches received fewer visits (Sih and Baltus 1987). Because of this we expect a lower number of pollinator visits for smaller, isolated patches of *R. canadensis*. From this study we hope to observe how pollinators behave in differing environmental conditions and discover which of these conditions generate the greatest rates of pollination.

Materials and Methods

This study was conducted on the southwestern edge of Waldron Fen in Alanson, Michigan. This northern region of the Lower Peninsula has a temperate climate influenced by lake effect weather that can cause summer temperatures as well as blossoming periods to vary greatly over a short period of time. *Rubus canadensis*, commonly referred to as “smooth blackberry,” was chosen in this pollination study due to its abundance at Waldron Fen as well as the timing of its blossoms, which proved concurrent with our observational time frame. It is also pollinated by a wide variety of bees, wasps, flies, and other pollinators, which was critical in the data collection and subsequent completion of our project studying the various factors affecting pollinator visit frequency. All selected plots of *R. canadensis* were identified at a stage of partial blooming so as to assure blooms were present for the duration of the two-week study.

Initially, five $1m^2$ plots were marked with flagging tape, so the same plot could be located and measured each day of the experiment. Each quadrant selected had 3-5 individual *R. canadensis* plants, all about 1 meter high, with several blossoming branches each. In plot 1, petals were removed from the currently blossoming flowers on every plant within the quadrant leaving the sepals, pistils and stamens untouched. In plot 2, petals were left on the flowers, but each flower was touched by a human hand in order to control for human oils or aromas that could have been left by petal removal in plot 1. To test the effect of light availability on pollinator activity, plot 3 was marked in a patch of *R. canadensis* that received constant natural shade from larger surrounding trees. Plot 4 was marked in a patch that received constant sunlight and was used as a control for both the shade and large patch variables since both controls required the same conditions. This also allowed us more observation periods at each plot. Lastly, for plot 5, our small patch variable, we selected a cluster of three *R. canadensis* branches within a 1 m^2 plot that were isolated from the larger patch in which our other four $1m^2$ plots were set up.

As a group of 4, each member observed a plot for ten minutes, counting each pollinator visit to a blossom within the given plot. A pollinator visit was defined as contact with a blossom, including when the same pollinator made multiple visits. After each ten-minute segment, the temperature and total visits were recorded for each variable and the observers rotated plots, so as to randomize counting and avoid any biases in data due to variance in human observation. Observation times were divided into three categories: morning from 9:00am - 12:00pm, afternoon from 12:00pm - 2:00pm, and evening from 2:00pm - 4:00pm. Throughout the experiment the petal plots (plots 1 & 2) were observed for 82 ten-minute intervals and the remaining three plots (sunlight/large patch, shade, and small patch) were observed during 52 ten-minute intervals.

Data were gathered on May 30th, 2012 in the morning hours from 9:00am – 12:00pm, in the afternoon on June 3rd from 1:00pm – 4:00pm, and on June 6th and 9th from 9:00am – 4:00pm. The four days of data collection also represented varying weather conditions, from full sun, to overcast clouds, to rain, with varying wind speeds. The first two dates were colder with an average temperature of 55 degrees Fahrenheit. The final two had an average temperature of 75 degrees Fahrenheit, with a three-hour rainy period on the morning of June 9th (*Table 1)*.

Linear regression tests were conducted for the number of visits in the ten-minute observation period in each of the treatment groups (petals on, petals off, sun, shade, large patch, and small patch) as a function of temperature and again as a function of time of day. We also performed one-sample t-tests to examine if there was a difference in the mean number of visits between each treatment and its control (i.e. sun versus shade, petals on versus petals off, and large patch versus small patch).

Results

Number of visits for all treatments had a significant positive linear relationship corresponding to time of day, with p-values <.001. Number of visits was significant and linearly positive in relation to temperature for the petals off treatment, the petals on treatment, the shade treatment, and the small patch treatment, with p-values for the slopes of all the lines <.001. The p-value for the slope of the line for the sun and large patch treatment was .278, and therefore statistically insignificant (*Table 2*).

The petals off treatment had a mean number of visits of 19.72 per ten-minute interval and the petals on treatment had a mean number of visits of 48.52. The one-sample t-test showed a significant difference in these sample means with a p-value <.001, a sample size of N=67, and t-statistics of 7.830 and 7.170 for petals off and petals on, respectively. The shade treatment had a mean number of visits per ten minutes of 38.93, while the sun treatment had a mean number of visits of 90.88. These sample means were also significantly different, with a p-value of <.001, a sample size of N=42, and t-statistics of 8.992 and 6.751 for shade and sun, respectively. Finally, the large patch treatment had a mean number of 90.88 visits per ten minutes, while the small patch treatment had a mean number of visits of 114.3. These sample means were significantly different with a p-value of <.001, a sample size of N=42, and t-statistics of 6.751 and 8.038 for the large patch and small patch, respectively.

Discussion

From our statistical analysis we observed two general trends in terms of time of day and temperature. As the day progressed from morning to evening, pollinator activity increased. This increase was most likely due to multiple factors including blossoms opening, dew evaporation, cloud dissipation, and temperature increase. As temperature rose, we also saw a significant increase in pollinator activity in all but the sun and large patch plot. Higher temperatures enable small, cold-blooded insects to pollinate more actively as they rely on external heat to remain active. The temperature increase did not seem to significantly affect the visitation rates in the large patch and sun plot. This could be a result of that plot receiving constant direct sunlight and having smaller fluctuations in temperature throughout the day.

When comparing mean number of visits per ten-minute interval between each opposing treatment it was found that the plot with petals had a significantly higher mean number of visits than the plot with petals removed. This reinforces our hypothesis that pollinators rely on visual cues while foraging. When pollinators are unable to detect petals they are less likely to visit that plot. Similarly, we found that mean number of visits to the shade plot were significantly lower than in the sun treatment. Again, with increased sunlight, flowers are more visible and temperatures are typically higher, two conditions which are ideal for increased pollination rates. Finally, we observed that the smaller patch had a significantly higher mean number of visits than the large patch. This finding did not support our initial hypothesis that the larger patch would receive higher rates of pollination. We expected the larger patch to be more accessible and more visible to pollinators, when in fact, pollinators, especially small wasps, tended to remain in the small patch and pollinate at very high rates. The large patch provided an opportunity for the pollinators to spread out and access a great number of flowers whereas the smaller patch offered a more condensed *R. canadensis* population and allowed the small pollinators to expend less energy while still visiting a reasonably large number of flowers. In this case, both patch sizes were relatively large in comparison to the body size of the smaller pollinators. Those pollinating in the smaller patch were observed to spend more time on one flower cluster, thereby focusing their pollinating activity and energy on a smaller region.

The results of this study lead us to conclude that pollination rates were highest during the afternoon in warmer, drier conditions where petals were present on *R. canadensis.* With the exception of our patch size hypothesis, the results supported our initial hypotheses. We determined from our observations that pollinator size was the decisive factor in choosing either large or small patch size. Future studies could test whether pollinator size is significantly correlated to patch size and determine whether small pollinators prefer small patches or if the presence of larger pollinators deters them from foraging in larger patches.

Conducting this study as a field experiment presented significant barriers in data collection, mainly due to the inconsistent weather and temperatures experienced in Northern Michigan. We originally set out to study pollination rates on *Prunus serotina* (Wild Cherry); however, its blossoming period was considerably reduced by temperature fluctuations during the week of May 28th, 2012, which ultimately caused the blossoms to senesce earlier than expected. It was also impossible to control for various factors such as high wind speeds and/or precipitation that could significantly affect the presence of small pollinators. During our two colder days the larger *Bombus* (bumblebee) was virtually the only pollinator present, likely due to their functional endothermic abilities to warm their own bodies—a trait small bees and wasps do not exhibit. Additionally, during the rainy period we observed an increase in the presence of herbivorous beetles on the leaves and petals of the *R. canadensis* blossoms and a near total absence of any pollinators. In future studies, it could be useful to conduct corresponding laboratory experiments for each variable in order to control for factors that deter pollinator visitation such as inclement weather and other insects that prey on *R. canadensis* plants. The results from both field and controlled laboratory experiments could prove useful in examining the relationships between plant, pollinator, and the abiotic environment.

Works Cited

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Time of Day	May 30, 2012	June 3, 2012	June 6, 2012	June 9, 2012
Morning	Overcast, ~55° F	Overcast, ~55° F	Sun, ~75° F	Rain, ~65° F
Afternoon	Overcast, ~55° F	Overcast, ~55° F	Sun, ~75° F	Sun, ~75° F
Evening	Overcast, ~55° F	Overcast, ~55° F	Sun, ~75° F	Sun, ~75° F

Table 1: Weather conditions for observation days, by time of day.

Relationship	Treatment	R^2	Sig
# Visits/Time of Day	Petals off	.321	<.001
	Petals on	.517	<.001
	Shade	.425	<.001
	Sun	.271	<.001
	Large Patch	.271	<.001
	Small Patch	.417	<.001
# Visits/Temperature	Petals off	.592	<.001
	Petals on	.659	<.001
	Shade	.442	<.001
	Sun	.029	.278
	Large Patch	.029	.278
	Small Patch	.638	<.001

Table 2: P-values and square of the correlation for number of visits versus time of day and versus temperature for each treatment plot.