

Effect of Road Dust Deposition on the Floral Lifespan of Scarlet gilia, *Ipomopsis aggregata*

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ABSTRACT

The Rocky Mountains are one of the world's most diverse areas in all of Western United States. Like most wonders of the world, the Rocky Mountains are also subjected to unpaved roads, and these roads often produce fine particulates of dirt that are able to rise into the air with ease. In this study, we investigate how these dust particulates influence the life span of flowers. The plant used in this study was the sub montane perennial Scarlet gilia, *Ipomopsis aggregata*. We unveil how road dust particulates may be the mechanism that could explain of lower pollen loads on Scarlet gilia near unpaved roads. When exposed to the dusty treatment, the stigma on the flowers remain receptive only a fraction of the time as those that are undusted. This evidence supports the idea that road dust reduces the amount of time the stigma is receptive to pollen, which would explain the low pollen loads of Scarlet gilia near unpaved roads.

INTRODUCTION

Road dust rising in the air is a common sight for most people who travel on unpaved roads. Many see the site as an unavoidable product of driving on these roads; and may not pay too much attention to dust, much less to the effects it may have on basic ecosystem interactions, near the unpaved roads (Field, et al. 2010). Also considering that an estimated 15%-20% of the United States is affected by roads (Forman & Alexander, 1998), the ecological impacts may be under estimated. Unpaved roads produce fine particulates that are able to travel in the air with a minimal disturbance (Waser et al, 2017). Dust particles can range in linear diameter from 1 μ m to about 40 μ m which is well within the sizes of pollen grains (Waser et al, 2017). These fine particulates ultimately may land on many roadside wildflowers.

Only a few studies have ever looked at the effects of dust on plants. In 2008, one study investigated whether road dust had an effect on the pollination patterns of four wildflower species (e.g. *Ipomopsis aggregata*, *Delphinium nuttallianum*, etc.) and suggested that dust deposition from unpaved roads lowered the pollen grain count on plants closer to the road relative to plants farther from the road (Waser et al, 2017). The study also found that seed set output was inconsistently related to levels of dusting, which was surprising because seed sets are often limited by pollen (Waser et al, 2017).

The mechanism by which road dust decreases pollen receipt is not known. One possibility that could operate with *Ipomopsis aggregata*, one of the species studied by Waser et al. (2017) is that dust causes stigmas to close. Milet-Hinheiro et al. (2007) found that Brazilian plants in the Bignoniaceae have sensitive stigmas that close within seconds of pollen being introduced on the stigmas. When dirt was applied to the stigma, the stigma responded by closing, though they reopened later on (Milet-Pinheiro et al. 2007). Similar stigma-closing behavior has been observed in other species, including *Ipomopsis aggregata*, one of the species included in the road dust study (Stritongchuay et al. 2010, Waser et al. 2017). Could road dust cause stigmas to close prematurely, thus reducing the amount of time stigmas are open and available to receive pollen?

In the summer of 2016 and 2017 I studied effects of road dust on scarlet gilia, *Ipomopsis aggregata*. The question I addressed was: Does road dust have effects on the floral lifespan of *Ipomopsis aggregata*? If so, could this affect the amount of pollen it receives per visit of a pollinator? If floral lifespan is reduced by dust, it might contribute to the lower pollen counts on dusted flowers.

METHODS AND MATERIALS

Location

The study was conducted near the Rocky Mountain Biological Laboratory in west-central Colorado at an elevation of 9,500 ft (2895m). The location where the study was conducted was at a field called Upper Maxfield Meadow (106° 59' 57.8''W 38°56'57.8''N) (See Figure 1). The plant that was of primary focus was sub montane perennial plant scarlet gilia, *Ipomopsis aggregata*. The flowering stalks of these natives of the western USA vary in height, some reaching as much as approximately 40cm. The telltale sign that it is a Scarlet gilia is the bright red tubular flowers, with some being all red or having some white on the opening of the floral tube.

Study System

To answer the question, I used the following methods. In the study 31 pairs of plants were used, for a total of 62 plants. All Scarlet gilia selected in Maxfield were flagged and put in

pairs, and each pair was paired because it would eliminate any variables relating to a single plant (e.g. only taller plants are dusted). The plants were paired by four criteria: (1) similar base stem diameter and (2) similar height (3) similar initial flower counts (4) and being in close proximity to one another. In order to measure similar base diameter, each potential pair's stem was measured using a digital caliper at surface of the soil. To measure the height of the plants, a wooden meter ruler was used. Once a pair had been identified, the number of existing buds and flowers were counted for each pair. This was just one more step in ensuring similar plants and to keep things randomized. All pairs were flagged in order to keep track of them more easily and a flip of a coin was used to randomly select one of the members of each pair to be dusted. If the coin landed on heads, the individual of the pair closer to the road was dusted.

The dusting treatment was applied using a garden duster (Spray Dog Gilmore Gardening Innovation). Each dusted plant received 10 "puffs" of dust. The dust used during the study was from a dirt road near the RMBL that had not been treated with magnesium chloride. The dirt was sieved down to .250 micrometers to mimic dust particulates caused by traffic on unpaved roads. In order to ensure that 10 "puffs" of road dust particulates was not over an amount that would mimic natural dusting condition, nor an amount that would be so low that no effects would be seen, a small quantification experiment was set up. An experimental dusting treatment was set up to determine if the amount of dust applied was within reasonable range. Four separate Scarlet gilia were used for this experiment and were dusted with 5, 10, 15, 20 puffs respectively. After 4 days of dusting the plants, 5 open flowers were taken from each plant. The flower was placed between wax paper and a glass slide and pressed and tapped firmly for 10 seconds to displace dust onto the wax paper. Once on the wax paper, the relative middle of the flower was marked. In order to count the dust particulates two transect lines were made using a compound microscope, (1) toward the top area of the flower, and (2) toward the middle of the flower. The area of each transect was $.22\text{mm}^2$ at a magnification of 50x. Within those ocular units, dust particulates were counted for the two transect lines. The counts were then compared and statistically analyzed with open flowers taken from a naturally dusted site we call dusty corner that were counted the same way.

To observe the floral lifespan, two phases were explored. Each census began on the Monday of every week, and one flower per plant was looked at each week. In Scarlet gilia

typically the bud expands to “elongated bud” stage, it then tends to opens in the afternoon. This is when male phase opens, the style grows through the male phase and eventually the female phase begins when the stigma opens and is receptive to pollen, and then the corolla falls off. The total floral lifespan was considered when both male and female phases are completed. The male phase was considered when the corolla first opened up, exposing the pollen on the dehiscent anthers. However the stigma must remain closed to still be considered in male phase. The male phase was considered over when the stigma began to open up to start the female phase, regardless of any pollen remaining on the anthers. The female phase was considered finished when the stigma recloses again, and at that point it also completed the floral lifespan of the flower. To keep track of the floral phases, the corolla, anthers, and the stigma were scored. The corolla was scored as (1) bud (2) partly opened (3) reflexed or (4) off. The anthers were scored as (1) immature (2) dehiscent with pollen (3) stripped of all pollen. The female phase was scored as (1) closed (2) partly opened (3) reflexed. If the corolla also falls off, it would be another indicator that the floral lifespan has been completed, however it is to be noted it is not an absolute indicator of a completed lifespan because the stigma may remain open, which was considered still receptive to pollen. One flower for each plant in the study was checked using a hand-lens. Each flower, studied was tagged in elongated bud stage with a string using one color for its respective census. The tag was tied around the pedicel of the elongated bud.

In order to check for the presence of a *Hylemya* fly egg, the sepals on the calyx were peeled back using forceps (Brody and Morita 2000). Every flower throughout the census was checked for eggs.

Each tagged flower was checked twice a day throughout the flowering season of study plants at Maxfield Meadow. The times were recorded when the stigma is visually open or opening and the rate of closure was monitored until the stigma closed. During every check, the stigmas were also checked to see if they were exerted or inserted. Based on the data, several ANOVAs were used to analyze differences among means between the treatments, and JMP was used to conduct the analysis. The analysis was to compare dusting/control treatments this year, but also compare them to the analysis of last year’s study.

RESULTS

Was 10 “Puffs” Enough?

A Tukey’s HSD pairwise comparison of dust densities of flowers of the 10-puff, natural “near road”, and natural “far from road” treatments indicated that our experimental 10-puffs treatment had significantly more dust than natural far, collected from dusty corner ($\text{Prob} > |t| = .0008$, $r^2 = .74$, $DF = 60$). From this we conclude that 10-puffs is enough dust on the plants without being an unrealistic measure.

Effects of Road Dust on Total Floral Lifespan

Male phase contributed only about 13% and female phase contributed about 52% of total floral lifespan. Because female phase contributed more to the overall floral lifespan, all analysis focused on the effects on female phase rather than male phase.

Analysis of the main result (Table 1. ANOVA, length of female phase vs. Treatment, Census Week, and Stigma Exsertion) revealed all three factors affected female floral longevity.

Total floral lifespan was affected by road dust treatment. Dusted plants did not last as long as those in undusted conditions. (Table 1, figure 2).

Weather Patterns Affect Longevity

Average Maximum Temperature (AMT) for the month of June was 21.2°C and the Average Rain Fall (ARF) was 0.00 inches. For the month of July the MAT was 22.9°C and the ARF was .08 inches (billy barr, unpublished data).

Over the course of the study, precipitation levels kept rising. Each week there was more recorded precipitation levels for each new census (Figure 7). Each new census for the week began every Monday. The ANOVA performed showed that floral longevity increased in parallel with precipitation (Table 1, Figure 3). Week (1) 1.41 days, (2) 1.95 days, (3) 1.99 days and (4) 2.55 days.

Stigma Exsertion

Stigma exsertion affected floral lifespan. With inserted flowers lasting 1.25 days and exserted flowers lasting an average of 1.58 days. (Table 1, Figure 5).

Hylemya Eggs

A total of 13 flowers were found to have a *Hylemya* egg present on the calyx throughout the study. On average when an egg was found on an elongated bud the floral lifespan lasted about 1.52 days compared to when an egg was not found on the calyx it lasted an average of 1.30 days. This difference was not statistically significant ($P=.0016$), perhaps because the sample of flowers with eggs was very small.

Effect of Dust on Flower Production

When comparing both dusted and undusted treatments we see that flower production was affected by the dusting (figure 4). On the dusted plants, 48.06 flowers were produced on average during the course of 6 censuses, whereas undusted plants on average produced 55.8 flowers. Dusting did not delay the flowering of the plants a difference of about 7.74 flowers between treatments. (Correlation = .48)

DISCUSSION

The results of our study support the hypothesis that dust affects the floral lifespan of *Ipomopsis aggregata*. The study also supports last year's study with consistent patterns in the results (Lopez, Student Paper, 2016).

To our surprise female phase once again showed up as being the phase of the floral lifespan that contributed most of the lifespan (52%), we continued to use it as a way of identifying floral lifespan. The female phase was affected by many factors including; dust particulates, precipitation levels, presence of a *Hylemya* egg, and exertion of the stigma.

Floral lifespan was decreased in flowers that were in the dusted treatment (See figure 2), which could be the mechanism for lower pollen loads as hypothesized in the study by (Waser et al. 2017). If the stigmas on dusted plants are closing sooner, it may mean that stigmas do not have as much time as undusted plants to be receptive to pollen.

One of the surprises from last year's study was that stigma exertion has a significant contribution to female phase longevity. Once again this factor had a significant contribution and suggested that flowers with exerted stigmas were receptive to pollen longer than those flowers

with inserted stigmas. Even more surprisingly this was true for both dusted and undusted treatments (See figure 5). However, these observations support what (Campbell et al. 1994) observed in stigmas that were exerted which were receptive for longer periods of time and also had increased pollen loads. An interesting aspect to investigate is whether exerted stigmas on dusted plants experience higher receipts of pollen compared inserted stigmas.

Another possible mechanism that is hypothesized is of the primary pollinators of *Ipomopsis aggregata* are humming birds, Broadtail Hummingbirds and Rufous Hummingbirds (Campbell. 1989, Waser et al. 2017). It's been a matter of study that suggests that hummingbirds by experience associate the red colored flowers on Scarlet gilia with a high nectar reward compared to other colored flowers on other wildflowers (Meledez-Ackerman et al. 1997, Campbell. 1989). It been observed in this study that the road dust does have an influence on how vibrant and colorful Scarlet gilia can look (See figure 6). The red on the flowers look duller than those on non-dusted plants. It is possible those hummingbird pollinators may avoid the dusted plants because of the color difference and if the hummingbirds do avoid dusted plants it would lead to lower pollen loads as well. The lack of pollinators would have major impacts on different aspects of plants such as setting seeds or reproduction which ultimately leads to diversity in communities (Ollerton et al. 2011). If the dusted plants/flowers are avoided it is plausible that it would contribute to higher pollen loads and higher fitness levels of those undusted Scarlet gilia (Mitchell and Waser 1992).

An interesting component that the road dust had was the physiological effect it appeared to have on the plant in general. Last year it was observed the plants that had the dusted treatment didn't look as healthy as the plants in the control group (See Figure 6). A phenomenon that was observed was that the dusted plants did not produce as many flowers, and indeed the plants as a whole looked like they were giving up. In fact there was a trend as the study continued the number of flowers on the dusted plants decreased more than those in the control group (See figure 4). These observations prompted thoughts on why the dusted plants are reacting to the treatment in this matter, and we hypothesized that photosynthetic rates were being affected by the dusting treatment. Using a Li Cor machine, photosynthetic rates and stomatal conductance were measured on 10 dusted and 10 undusted plants (Aug 8th, 2017). Preliminary results revealed that dusted and undusted plants did not differ in photosynthetic rates ($r^2=.14$, $\text{Prob}>|t|=.0001$)

(Least Sq Mean: Dusted=.23, Undusted .32) but undusted plants higher stomatal conductance values rates than did dusted plants ($\text{Prob} > |t| = <.0001$). Our data supports (Sharifi et al. 1997) in which we observed the same effect of conductance being reduced in plants that were dusted. The consequence of dust deposition is that it disrupts the natural life cycle of a plant (Sharifi et al. 1997). In the same study by (Sharifi et al. 1997), observed that plants exposed had increased leaf temperatures, and also had reduced leaf area as compared to those not exposed which affected photosynthetic rates and stomatal conductance (Sharifi et al. 1997). Considering the impacts of dust deposition on the Mojave plants, there may be possibility of similar results with Scarlet gilia. However, it is still not known if road dust particulates have effects on rosettes of Scarlet gilia. If dust did have an effect, the vegetative damage studies suggest that damage occurring early in life; cost of repair may affect reproductive outputs (see Brody, Price et al. 2007). Again, it is not known if dust has this kind of effect on Scarlet gilia, but it is an interesting topic for further investigation.

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TABLES & FIGURES

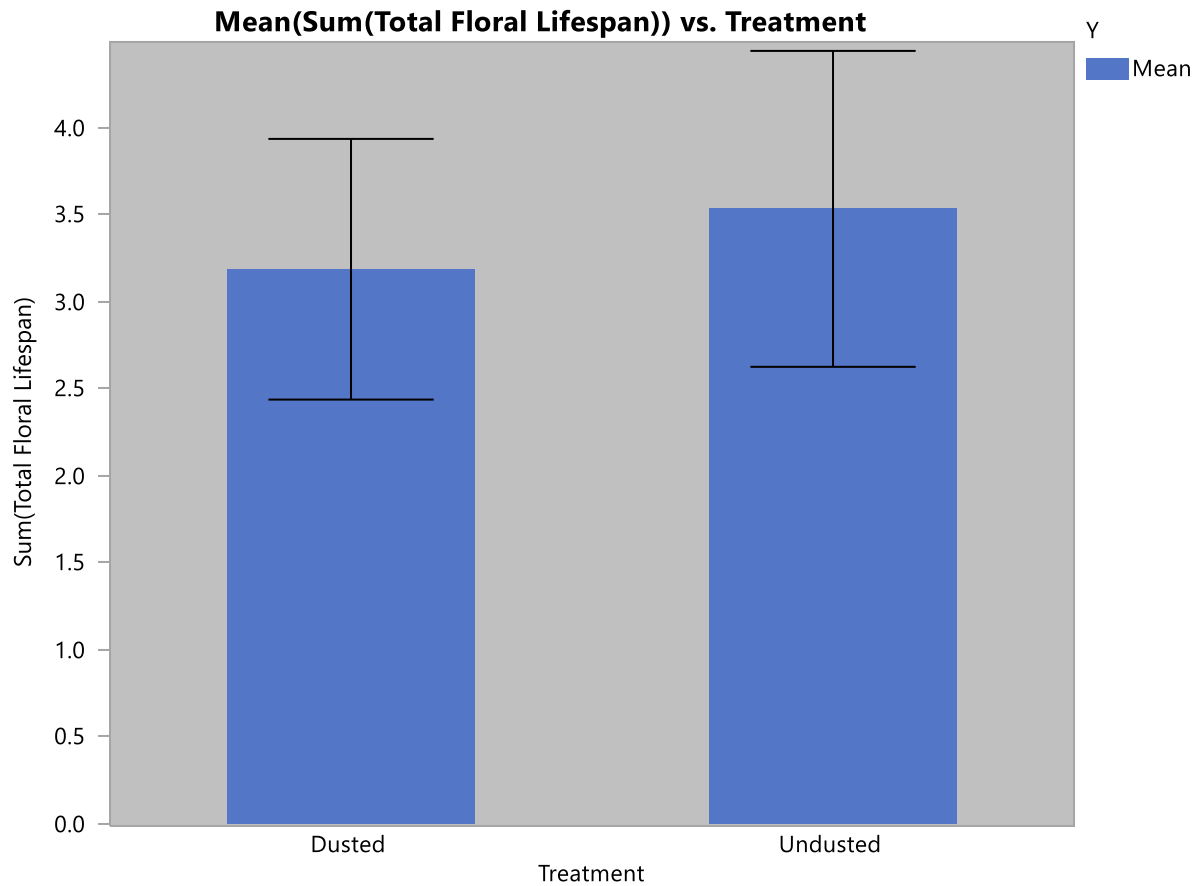
Summary of fit

R^2	0.137923
R^2 Adjusted	0.111601
Root Mean Square Error	0.822201
Mean of Response	1.648693
Observations	136

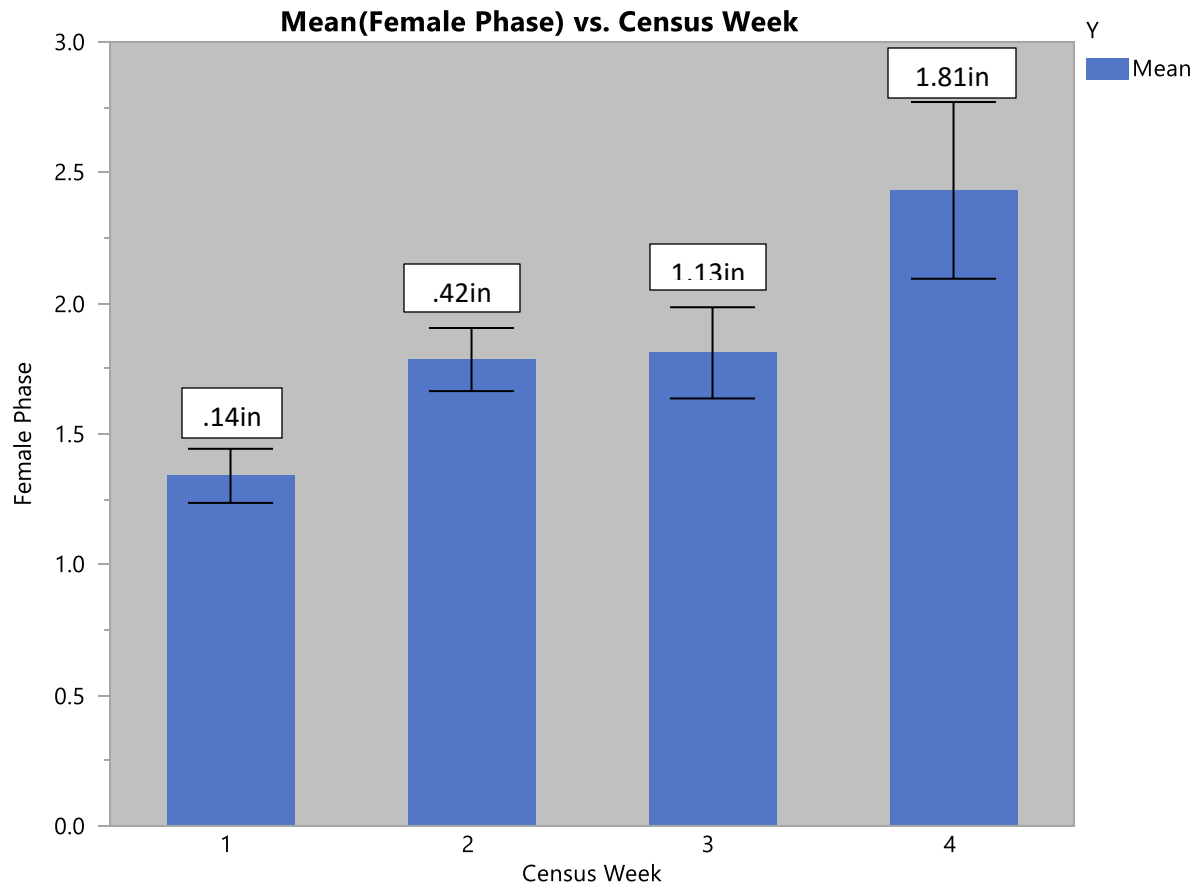
(Table 1) Table shows a summary of ANOVA performed for female phase longevity with treatment, week of census, whether a hylemia egg was present, and stigma exsertion.



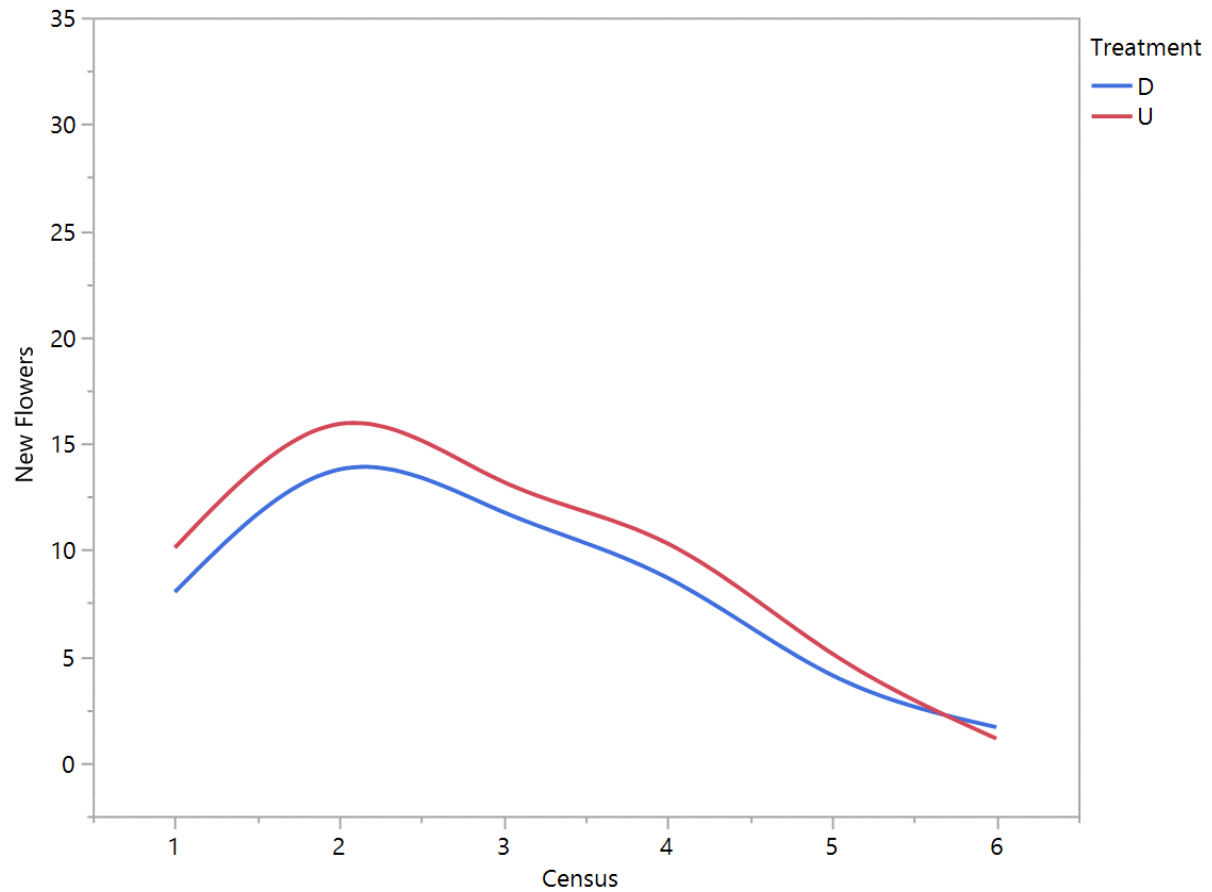
(Figure 1) Aerial view of our field site which is south of RMBL and west of the Gothic road.



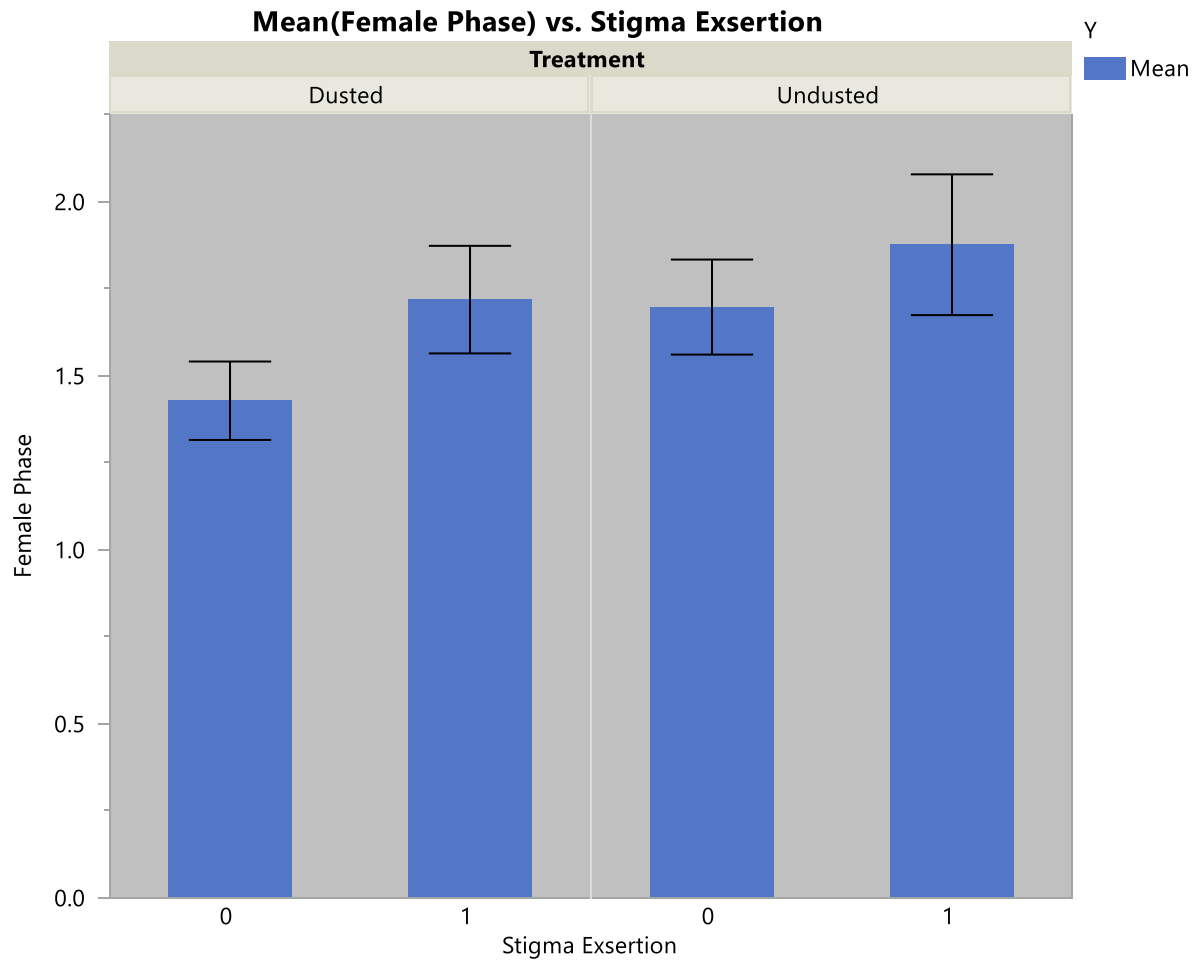
(Figure 2) Bar graph shows how long the total floral lifespan lasted when comparing both dusted and undusted treatments. Undusted flowers lasted on average 3.31 days and dusted flowers lasted on average 2.98 days. Each error bar is constructed using 1 standard deviation from the mean.



(Figure 3) Graph shows mean length of female phase (in decimal days) in each census week. Each week can be interpreted as difference in the amount of precipitation that was available. The numbers above the bars is the amount of accumulated precipitation from June 1st until the start of each weekly. Each error bar is constructed using 1 standard error from the mean.



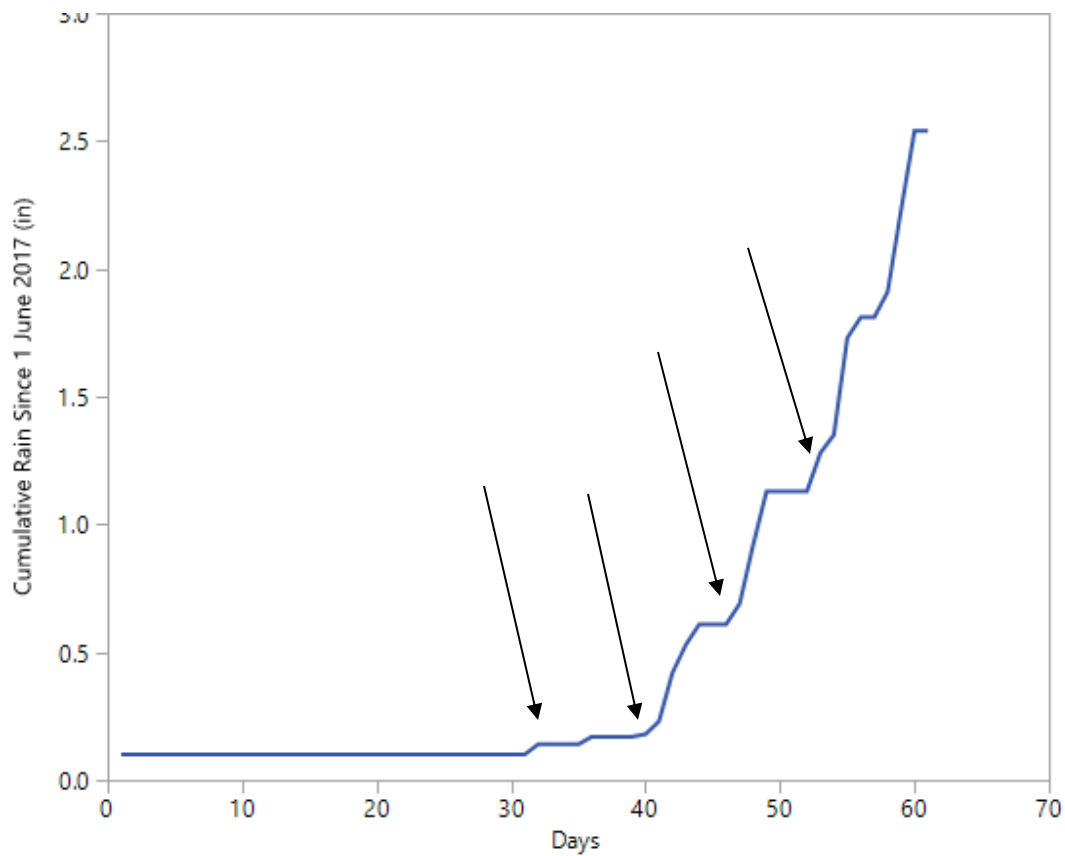
(Figure 4) Graph shows the general pattern of the number of average flowers found for each treatment. D=Dusted. U=Undusted. Notice this is the progress of flower production has the season was progressing.



(Figure 5) Graph shows the differences in female phase longevity by treatment, but as effected by whether or not the flower had an exserted stigma or if it had an inserted stigma. 0=Inserted. 1=Exserted. Each error bar is constructed using 1 standard error from the mean.



(Figure 6) The picture shows the differences between the experimental group (left) and the control group (right). The effects of dusting are clearly shown.



(Figure 7) The graph showcases the cumulative rain over a period of 4 weeks. It shows how much precipitation had fallen since the start of the study. The arrows mark the dates of when a new census began.