

Does road dust have an effect on nectar production in *Ipomopsis aggregata*?

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**Abstract:**

Road dust has potential impacts on plant metabolism and reproduction even in small amounts. Dust is everywhere and is increasing due to human influence, this has the potential to reduce overall plant pollination worldwide. Since dust is roughly the size of a grain of pollen, it might possibly clog the stigma of plants. It appears that there is already a correlation between road dust and flower life span as well as pollination success (Mary Price personal communication 2019; Waser et al. 2017). For these reasons it is an interesting question to determine whether road dust affects nectar production in *Ipomopsis aggregata*.

In order to explore this question, we located 30 pairs of flowering *I. aggregata* plants. We simulated roadside dust levels with 5 puffs of sieved dust from a garden duster to one half of the plants, while the other half received a controlled 5 puffs of normal air from another garden duster. Treatment was assigned at random within each pair. Elongated flower buds on these plants were capped with a piece of drinking straw for 21-29 hours to prevent pollinators from removing nectar. Then the nectar was collected utilizing a 10-microliter microcapillary tube, and the concentration of the nectar was measured using a refractometer. The refractometer refracts light to give a mass percentage of sucrose within nectar. To determine the mass of sugar in each sample the Handbook of Chemistry and Physics was used to convert from percentage mass to mass to percentage mass to volume. The corolla length of each flower was measured as a covariant to determine if dust affects flower size. It was found that road dust had no effect on nectar production however, there seems to be a yearly affect that influences the response to the dusting treatment. **There was also found to be a correlation where corolla length and nectar volume increase, sugar mass decreases.**

**Introduction:**

Road dust can have serious ecological impacts on scales that range from a single plant to global scale (Field et al. 2010). Dust production is at an all-time high and is expected to increase as climate change increases in severity and human land use intensifies (Field et al. 2010). It was found that road dust may directly have positive and negative consequences whether it be providing nutrients for plant growth, changing the pH of soil, or plant communities (Santelman et al. 1988). The range of road dust is usually <10-20m but may extend to 200 m downwind. In arid land, soil erosion and drainage are common road problems (Iverson et al. 1981) that can cause an influx of road dust. It was found that dust deposition is at 1/2 of roadside dust levels at a 10m distance from the road, ~1/4 at 20m, and increasingly smaller after 20m (Waser et al. 2017). Dust particles vary in size between 1  $\mu\text{m}$  to about 40  $\mu\text{m}$  which is roughly within the size of a pollen grain (Waser et al. 2017). It is possible that this road dust can occupy space on the stigma that is normally meant for pollen, therefore lowering pollen loads.

Indeed, plants exposed to dusty conditions had lower pollen loads than those not exposed to dust (Waser et al. 2017). There are several possible mechanisms that may contribute to the effects of dust on pollen loads; dust reduces flower longevity or stigma surface area, stigma adhesion, resource limitation, and pollinator aversion. Pollinators may avoid dusted plants because they may have learned that dusted plants yield less nectar, which is what was investigated by my study. These mechanisms open doors to entirely new studies on dust, pollination, and plant reproduction.

In 2018, the same study was done in order to gauge whether road dust had an effect on nectar production. In both years the same study site was used to perform the experiment as well as the same methods. It was suggested that nectar production rates dropped when roadside dust levels were replicated with a possible positive correlation between flower length and nectar production. However, this data was collected from a drought year and the sample sizes were too low (Centeno. RMBL 2018 student paper).

The formula for photosynthesis ( $6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2$ ) is crucial since a catalyst for the reaction to happen are photons however, road dust can play a role in limiting the number of photons a plant receives. This limitation of photons causes a decrease in photosynthetic abilities causing an overall decrease in vital processes in plants (Thompson et al. 1984). This limitation can cause an overall partitioning of resources within the plant forcing it to choose between nectar or growth. Therefore, we measured nectar production, nectar concentration, and mass of sucrose from nectar. Corolla length of each flower was also measured as a covariant to test further physiological differences between dusted and undusted plants. The purpose of this study is to determine if nectar production is affected by road dust. We hypothesized that an increased amount of road dust will inhibit the nectar production of *I. aggregata*.

## Methods:

This experiment was conducted at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado. The field site used was Maxfield Meadow, (106°59' 26.5666"W, 38°56' 57.8" N) approximately one kilometer from the gothic townsite, since the site yielded many *I. aggregata* that bloomed at the height of summer. Maxfield has a seasonal cycle with the blooming of *I. aggregata* and other plants in the summer. One nickname for *I. aggregata* is Scarlet Gilia or Scarlet Trumpet, due to its elongated buds, which flower in the afternoon. It is possible to tell which buds will flower by observing the elongated buds on the plants. In order to have an accurate representation of how road dust affects nectar production, utilizing the randomized block design, the experiment utilized 30 pairs of *I. aggregata*. The first of the pair was thoroughly dusted, while the second of the pair remained undusted; treatment was decided at random by flipping a coin to ensure equivalent treatments. The data were analyzed by comparing plant mean

values of dependent variables through the use of analysis of variance to compare treatment and year effects. There was also a correlation analysis to compare the relationships between flower length and nectar variables between the years. The plants paired together were decided by similar microsites, stem diameter, and stalk length to verify the plants were similar. The initial size data was input and analyzed, and it was found that the design had achieved its goal since there were no initial size differences between treatment groups.

Since magnesium chloride had already been applied to the roads, the plants were hand dusted using a garden duster (Spray doc, Gilmour Gardening Innovations) using sieved road dust from the road north of Gothic otherwise known as "Dusty Corner" since it had not been treated with magnesium chloride. The dusted plants received 5 puffs every other day from the duster to simulate the amount of dust plants receive near a dusty road (D. Lopez & C. Rosas, RMBL 2017 student paper). The undusted control plants were covered while dust was applied in order to shelter them from the puffs of dust. Then the undusted controls were blown with 5 puffs of air from a clean duster to simulate the disturbance from the puffs being applied that the dusted plants received while being dusted. In order to track the amount of nectar produced a maximum of two elongated buds per plant were covered with straws, twist tied off to exclude pollinators, then collected within 21-29 hours (Campbell et al. 1991). The process of strawing is quite literal: the elongated buds of the plant are covered with small pieces of drinking straw and then twist tied to the stem to prevent them from falling off the bud. This method excludes pollinators from recovering the nectar within the flower due to them being unable to reach through to collect the nectar. The exclusion of pollinators in the nectar collection process is important in order to accurately gauge the amount of nectar each flower is producing.

To collect and measure nectar samples we utilized 10 microliter microcapillary tubes to collect the samples then measured the amount of nectar collected in millimeters using a caliper. The microcap is a cylinder so the volume per millimeter of nectar column is constant; therefore, since the total length of the microcapillary tube is 41mm, the volume in the nectar column in microliters is its length in mm x 10ul/41mm.. The nectar is then blown onto a refractometer which determines the percent mass of sugar per mass of nectar by measuring the bending of light. The mass of sucrose in the nectar is found by multiplying the sample volume by the percent mass per volume, obtained from a conversion table in The Handbook of Chemistry and Physics (Hodgman, 1946) The independent variable in the study is the plants' dust treatment, while the dependent variables are the volume, concentration, and sugar content of nectar the test plants produce. I also measured corolla length of each flower.

## **Results:**

The study site used was Maxfield Meadow (Figure 1) which yielded many bolting *I. aggregata* this summer. Dusted plants produced a mean nectar volume of 3.54 microliters, whereas control plants produced a mean nectar volume of 3.21 microliters (Figure 4). This is the opposite of 2018's results in which the undusted control produced more nectar than the dusted experimental (Figure 4). After analyzing 60 plants it appears that there is no effect on nectar production due to the dusting treatment.

It was also found that the dusting treatment most likely had no effect on the mass of sucrose produced with a p score of .1362. However, utilization and comparison of data from the summer of 2018, it appears that there is a correlation between flower length, nectar volume, and nectar concentration.

Utilizing figure 3, it is found that there is a direct correlation with nectar volumetric production and sucrose weight in nectar when both are directly compared to soil moisture (Waser, Price 2016). This is an interesting finding since this seems to fall in line with the results achieved in 2018 and 2019 with them being dry and wet years respectively. Snowpack data, in the unit of inches, from billy barr helps reinforce this statement since most of the water comes from the winter snowpack it is evidenced that 2018 was a dry year due to its limited snowfall (barr 2019) (Figure 2). The correlation being a positive correlation between nectar volume and flower length then a negative correlation in comparison to nectar concentration. Meaning, that as flower length and nectar volume increase, nectar concentration decreases. Utilizing figures 4, 5, and 6 respectively, which show the 2018 and 2019 mean data comparisons, that correlations were found to be consistent after two consecutive years of sampling.

### Discussion:

It appears that there was a correlation with nectar production and flower length, which is one of the reasons the study was replicated. (Armenta. RMBL 2018 student paper). This can be explained using figure 1, in which there is a linear correlation between soil moisture and sucrose weight with soil moisture (Waser, Price 2016). There does appear to be a yearly effect however, regarding soil moisture and the amount of sucrose produced with regards to the dusting treatment. Since the experiment was conducted for 10 weeks, an improvement that can be made is to expose the selected pairs to the dusting treatment for a longer period. A further improvement to build upon this experiment would be to conduct it while taking temperature readings and soil moisture readings to further understand correlations in data. One problem that occurred throughout the course of the experiment were sample plants being consumed by deer. This was a problem since replacements had to be found, the new pairs were not the best matches and furthermore, the plants were not exposed to the experimental and control treatments for as long as their original counterparts. While it does not appear that there was a difference in the replaced plants and originals, it would be important if the experiment were run longer to keep differences in plants to a minimum.

An impact of this study was the loss of 194 *I. aggregata* flowers and their seed input to the population due to destructive sampling as well as any seeds lost during dusting. Another impact of this study is the loss of plant life caused by researchers walking through the meadow, which may also cause a decrease in wildlife activity due to researchers working in the meadow. Furthermore, a potential problem could be found within the dust since it came from miles up the Gothic road, it possibly could have a different composition than dirt found next to Maxfield Meadow.

#### **Acknowledgements:**

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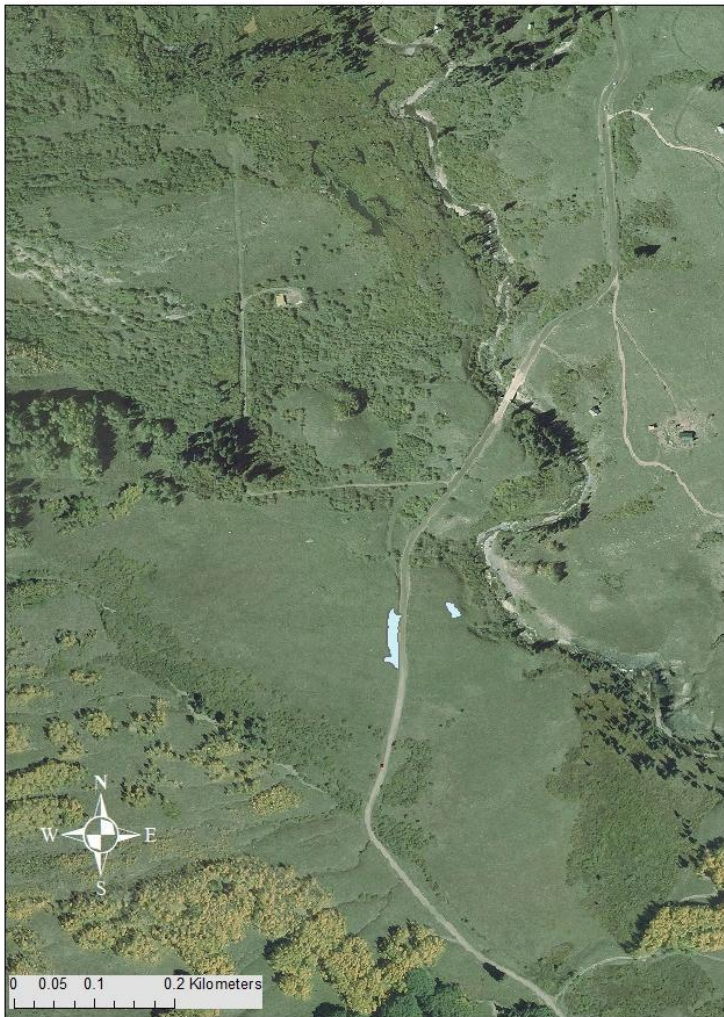
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**Tables and Figures:** (Figure 1) Map of Study Site

Maxfield Meadow Plot

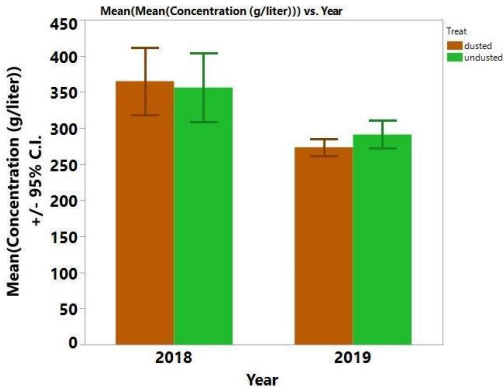


(Figure 2) Snow Fall data in inches

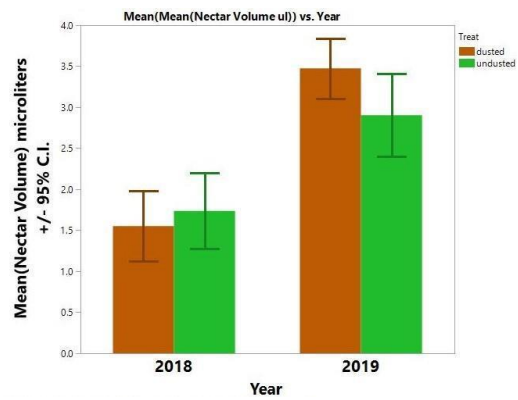
| Winter of | Sept. | Oct. | Nov. | Dec.  | Jan   | Feb  | Mar  | Apr  | May  | June | July | Total |
|-----------|-------|------|------|-------|-------|------|------|------|------|------|------|-------|
| 2010-11   | 0.0   | 42.1 | 45.3 | 146.9 | 37.4  | 87.0 | 81.9 | 99.2 | 47.6 | 0.0  | 0.0  | 587.4 |
| 2011-12   | 0.0   | 22.0 | 39.0 | 14.2  | 66.9  | 64.2 | 12.2 | 23.2 | 10.2 | 0.0  | 0.0  | 252.0 |
| 2012-13   | 0.4   | 9.4  | 19.7 | 72.8  | 35.0  | 22.4 | 52.4 | 64.2 | 33.9 | 0.0  | 0.0  | 310.2 |
| 2013-14   | 7.5   | 35.4 | 48.8 | 47.2  | 67.3  | 94.9 | 71.7 | 50.4 | 37.4 | 2.8  | 0.0  | 463.4 |
| 2014-15   | 4.3   | 14.6 | 62.6 | 50.4  | 26.8  | 36.2 | 40.6 | 36.2 | 63.8 | 0    | 0    | 335.4 |
| 2015-16   | 0.0   | 9.4  | 38.6 | 85.4  | 46.9  | 28.0 | 40.9 | 48.4 | 31.5 | 0.4  | 0    | 329.5 |
| 2016-17   | 6.3   | 3.5  | 37.0 | 67.3  | 147.6 | 65.4 | 14.6 | 31.5 | 11.8 | 0    | 0    | 389.8 |
| 2017-18   | 0.8   | 16.9 | 22.0 | 21.7  | 29.9  | 44.5 | 22.0 | 29.9 | 5.1  | 0    | 0    | 192.9 |
| 2018-19   | 0.0   | 24.4 | 41.3 | 35.8  | 57.9  | 46.1 | 95.3 | 34.3 | 42.5 | 3.5  |      | 380.7 |

**Commented [1]:** Re-order this table to match the order in which you refer to it in your narrativr.

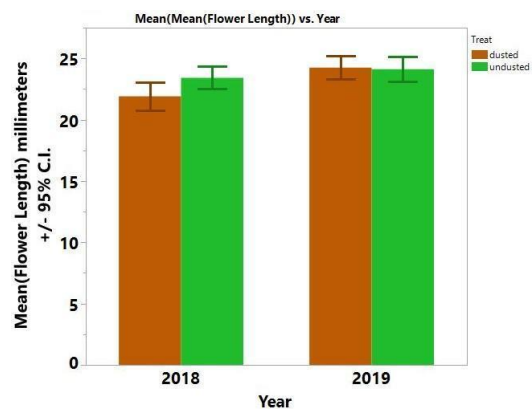
(Figure 3) Mean Nectar Concentration by Year and Treatment



(Figure 4) Mean Nectar Volume by Year and Treatment



(Figure 5) Mean Flower Length by Year and Treatment



(Figure 6) Mean Sucrose Mass by Year and Treatment

