

Does road dust affect growth rates in *Ipomopsis aggregata*?

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Abstract

The physiological effects of road dust on the wildflower rosettes *Ipomopsis aggregata*, scarlet gilia, are shown to non-significantly reduce their growth rate in two meadows near the Rocky Mountain Biological Laboratory. Growth rate was a proxy for the amount of photosynthesis that occurred in the rosettes during the summer. There were differences between the two site locations that suggest there are some unknown variables of microsite that were not explored. However, the overall trend does show that dusting treatments of rosettes reduced their growth over the course of the summer. Initial size of selected rosettes were bigger in Lower Maxfield Meadow than that of Upper Maxfield Meadow.

Introduction

Dust from off-road travel has been shown to reduce maximum net photosynthesis, leaf conductance, transpiration, instantaneous water-use efficiency in three types of desert shrubs in the Mojave desert (Sharifi et al., 1997). The same study concluded that dusted plants had an increase in leaf temperature of about 2-3 °C due to increased absorbance of infrared radiation. The decreased rates of photosynthesis in dusted desert shrubs makes one wonder what effects road dust has on montane wildflower species in southwestern Colorado.

Dirt roads of Southwestern Colorado circulate and deposit dust in large amounts during the peak vehicle traffic season of July, which also usually reflects peak density of all wildflower species (Waser et al., 2017). Dust is deposited in larger amounts on plants 1-2 m from the roadside than that of plants positioned 40-50 m away (Waser et al., 2017). Earlier studies have shown that dusty plants receive less pollen on their stigmas than non-dusty plants; whether photosynthetic rates and water use efficiency of wildflowers are likewise affected by varying

amounts of dust in the subalpine meadows of Gothic, Colorado, is only now being explored.

Road dust might have a possible effect on the amount of light energy being received. If dust coats the leaf surface there could be a decrease in the number of photons being captured by a plant. In the Mojave desert study, more infrared radiation might be absorbed, thus increasing leaf temperatures. Deposition of dust on leaves also might influence stomatal conductance in scarlet gilia. Dust may not only increase the temperature of the leaves, but also interfere with the opening and closing of the stomates. If the stomates remain open to reduce leaf temperatures, plants will lose more water in the attempt to cool down. Conversely, if the stomates remain closed to reduce transpiration, then the amount of photosynthesis the plant is able to engage in will decrease.

Because of these likely physiological effects of dust for scarlet gilia plants, I hypothesize that dust deposition on leaves of one Colorado wildflower, *Ipomopsis aggregata*, will slow the rate of growth in dusted rosettes compared to that of the undusted rosettes.

Study species

The selection of rosettes is based on rate of growth in rosettes compared to that in a flowering scarlet gilia plant. When scarlet gilia bolt and flower, they are entering the last stages of their life. According to a previous study conducted on scarlet gilia flower longevity, dusted scarlet gilia plants produced fewer flowers over time than undusted plants as well as a decrease in flower longevity (David Lopez. Student Paper. 2017). The previous study also took rates of photosynthesis using a Li-Cor as well as stomatal conductance between dusted and undusted bolting scarlet gilia plants, and discovered that rates of photosynthesis did not differ significantly but conductance of the stomates was lower in dusted plants, suggesting some physiological effects of dust on scarlet gilia.

Since the previous study was conducted on scarlet gilia plants that had bolted and flowered, the effects of dust on scarlet gilia in their earlier stages of life were not included. This is why the primary focus of my study was scarlet gilia rosettes. *Ipomopsis aggregata* plants remain in their rosette forms for most of their life until they bolt. Rosettes usually will grow for a number of years before bolting and flowering. The leaves of a rosette engage in photosynthesis and store energy for the future reproductive stage. By focusing on rosettes, I can track them as they progress toward their flowering stage and see if the physiological effects of dust have a greater impact on them in their early stages that are visible as flowering individuals. From there the hope is to determine if dust has long-term physiological and reproductive effects on *Ipomopsis aggregata*.

Methods

Location

The experiment was conducted near the Rocky Mountain Biological Laboratory (lat. 38°58'N, long. 106°59'W) in western Colorado. The two sites we primarily focused on were the upper Maxfield Meadow and the lower Maxfield Meadow (Figure 1). Upper Maxfield meadow is positioned closer to the unpaved Gothic Road (Gunnison County Road 317) than lower Maxfield Meadow. The lower meadow also is down slope of the upper meadow and is closer to the East River.

Experimental Design

The experiment used thirty pairs of scarlet gilia rosettes. In June 2019, I chose pairs to be as similar as possible in size and location in the meadows. There were fifteen pairs located in upper Maxfield Meadow and fifteen pairs located in lower Maxfield Meadow. Rosette pairs also

had similar microsite conditions, such as (1) soil moisture, (2) substrate, (3) shading and (4) crowding of the neighborhood. Substrate was characterized as either having bare ground surrounding the rosette, leaf litter, or a combination of the two. The shading effect of other forbs and grasses on our rosettes was characterized by how much shade a rosette received throughout the course of the day. We counted the amount of shade a rosette receives for three parts of the day, 9:00 a.m., 12:00 p.m., and 3:00 p.m. We looked at their neighbors to the southeast, south, and southwest during these times of day and decided if they were partly shaded. If they were partly shaded only during one time of the day, we assigned them a (1), two times of the day (2), and three times of the day (3). Neighborhood crowding was determined by measuring the distance to the nearest scarlet gilia rosette, forb, rhizomatous grass, and bunch grass. The nearest scarlet gilia rosette was recorded only if it was of similar size or larger than our focal rosette. If there were no neighbors within a fifteen-centimeter radius, we did not record it and treated it as outside of the neighborhood.

The basic design was a randomized blocks design, meaning that within each pair of rosettes one was chosen *at random* to be dusted, and the other to be an undusted control. This meant that treatments did not differ in initial rosette size or microsite ($p > 0.05$). I expected that there would be a lot of variation from pair to pair (= block), but with this design I could look for treatment effects within each pair without being confounded by the variability between blocks.

To test my hypothesis that dust will reduce rosette growth, I measured the initial and final sizes of each of the 30 treated and 30 control rosettes over the course of the summer of 2019. I measured size by counting the number of green, current growth leaves in each rosette and taking the length of the longest leaf to the nearest 1 mm. I ignored overwintering leaves at the base of a rosette that had turned brown and were senescing. I also multiplied number of leaves by longest

leaf to serve as an overall index of size for each rosette. The growth rate of rosettes was then obtained by subtracting their final size measured on August 3, 2019, from their initial size measured on July 5, 2019. This growth rate serves as a proxy for how much photosynthesis is occurring in my rosettes. If there is less photosynthesis occurring in dusted plants, we expect rosettes to have a slower growth rate.

Hand Dusting

My dusted rosettes were dusted every other day. If there was rainfall, rosettes were not dusted until they were completely dry. If I had dusted them while wet, it would not have reflected realistic weather conditions. I applied dust using a Gilmour Hand Duster. Five pumps of dust were applied to our experimental rosettes using the dust applicator, and control rosettes received five pumps of air to simulate the conditions of the dusted rosettes. If rosette pairs were close in proximity and dust deposition on control rosettes was a concern, I covered the control rosette with a plastic container while dust was applied to the dusted rosette. It remained covered only for a short while as the dust settled.

Analysis

We will analyze our data using a 2 factor ANOVA test. To look at the effects of dusting treatments on our control and dusted rosettes in both upper and lower Maxfield Meadow.

Results

Effects of dusting on change in number of leaves

The change in leaf number from the initial count to the final count was greater for Upper Maxfield than Lower Maxfield Meadow [$F(1,56) = 7.9296$ $P = 0.0067$; Figure 2]. In both locations, dusted rosettes added fewer leaves than the control rosettes [$F(1, 56) = 2.6352$. $P =$

0.1101; Figure 2]. There was no significant interaction between location and treatment [$F(1, 56)=0.3962$, $P=0.5316$]; Figure 2]

Effect of dusting on change in length of longest leaves

The change in length of the longest leaf was greater in lower Maxfield Meadow compared to upper Maxfield Meadow [$F(1, 56)=20.6722$, $P<0.0001$; Figure 3]. Mean leaf length did not change significantly in upper Maxfield meadow. Dusting effect on change in leaf length. Leaf length increased non-significantly more in control rosettes than in dusted rosettes [$F(1,56)=0.1987$, $P=0.6575$; Figure 3]. There was no treatment by location interaction [$F(1,56)=0.0715$, $P=0.7901$; Figure 3].

Effect of dusting on the change in overall index of rosette size

Index of the change in rosette size between dusted and controlled rosettes in upper and lower Maxfield Meadow. Controlled rosettes increased in size more than dusted rosettes [$F(1, 56)=2.6638$, $P=0.1083$; Figure 4]. Rosettes in upper Maxfield grew non-significantly more than rosettes in lower Maxfield meadow [$F(1,56)=0.1611$, $P=0.6897$; Figure 4]. There was no significant interaction between treatment and location [$F(1,56)=0.0646$, $P=0.8003$; Figure 4]. In a subsequent ANCOVA, we found that change in rosette size (ln-transformed) increased with initial rosette size (ln-transformed; $t=2.66$, $p=0.0103$), was higher in controls than dusted ($t=1.73$, $p=0.0903$), and was higher in the upper Maxfield meadow ($t=-2.36$, $p=0.0219$).

Microsites variation among rosettes

Preliminary analyses indicated that the major axis in microsite variables was an indicator of neighborhood crowding. Weighted on mean neighbor distance, which was correlated with shading and litter index. Locations and treatment did not differ in the score of the first principal component. But initial size of rosettes was bigger in less crowded neighborhoods and in lower

Maxfield Meadow.

Discussion

Our results suggest that our hypothesis was supported: that the rate of growth in *Ipomopsis aggregata* rosettes would be decreased due to dusting treatments. Although the results suggest the dusting treatments have no significant effect on the growth of rosettes, we do see a trend of dusted rosettes growing less than rosettes that were untreated. During the analysis, we saw that leaf number was the dominant variable when calculating the overall size index. Since we multiplied the number of leaves by the longest leaf length, Figure 4 is similar to that of Figure 2.

Figure 3, we see that the overall mean of leaf length decreased in upper Maxfield. This suggests that rosette leaves in upper Maxfield shrank, possibly due to unknown plant stress that is occurring in upper Maxfield Meadow. It is possible that there is an unknown effect of soil moisture or some other variable that we did not analyze. It would be worthy of noting that future studies should look in depth at the abiotic conditions of the different locations.

In addition, the two meadows might differ in the amount of dust deposited on the meadow naturally. The upper Maxfield Meadow is much closer to the Gothic Road than the lower Maxfield Meadow, which is located down slope near the river. If there was any additional dust deposited on our rosettes in the upper meadow, that may have influenced the rosettes' responses to treatment. It would be wise to conduct future studies with this thought in mind.

Part of our long-term goals for this study, is to track these rosettes into next summer. We would like to see if dusting has long term effects on growth, physiology, and reproduction. We have tagged these individuals with identified aluminum tags to locate them in the following summer. Our goal is to see which of the rosettes will bolt and flower, and which remain in

rosette form. The ones that do bolt and flower, will be studied further. We would like to at their reproductive output. As well as measure flower length, flower longevity, and nectar production to see if the dusting of rosettes has a long-term consequence in their final stage of life. We would also gather photosynthetic rates, stomatal conductance, and leaf temperatures of both rosettes and flowering scarlet gilia.

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Figure 1: Upper and Lower Maxfield Meadow pictured below. Blue is upper Maxfield Meadow and Red is Lower Maxfield Meadow.



Figure 2: Effects of dusting on number of leaves. The effects of treatment (dusted vs. control) and location (upper vs. lower Maxfield) on number of leaves added to rosettes over the course of the summer (5 July - 4 August 2019). Green bars indicate control rosettes and brown bars indicate dusted rosettes. Bar height indicates mean values and error bars are $\pm$ 95% confidence interval of the mean.

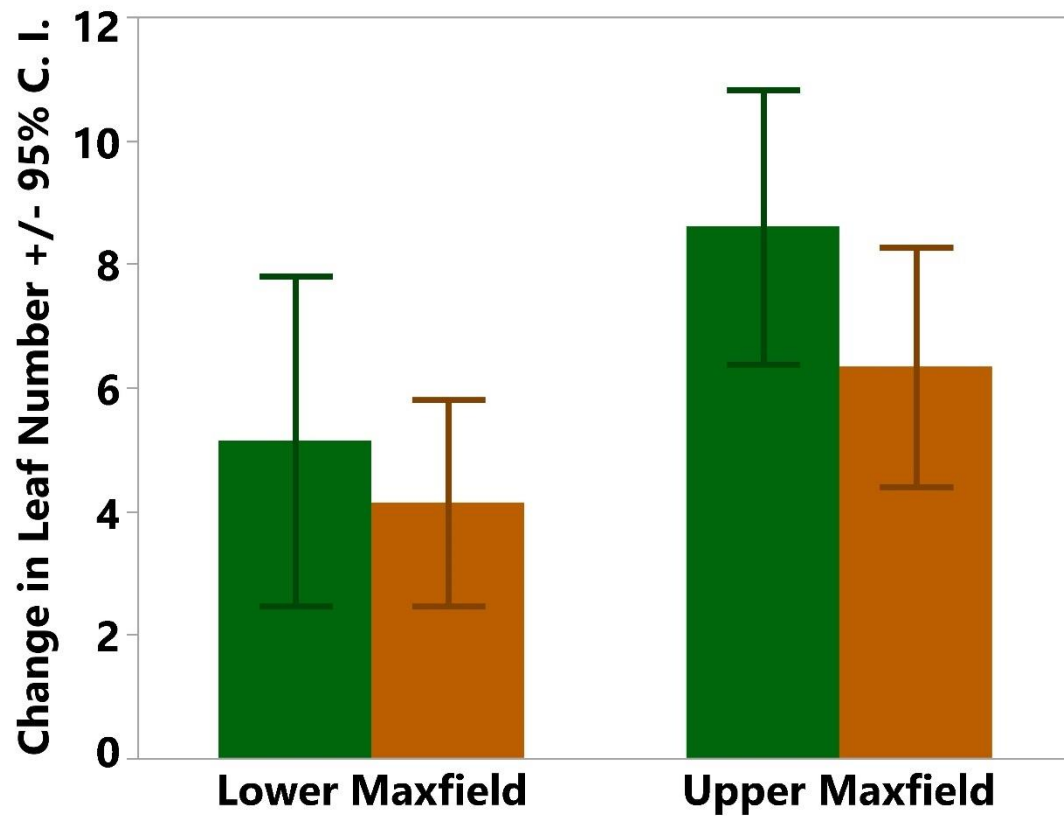


Figure 3: Effect of dusting on length of longest leaf. The effects of treatment (dusted vs. control) and location (upper vs. lower Maxfield) on the length of the longest leaf in rosettes over the course of the summer (5 July - 4 August 2019). Green bars indicate control rosettes and brown bars indicate dusted rosettes. Bar height indicates mean values and error bars are $\pm$ 95% confidence interval of the mean.

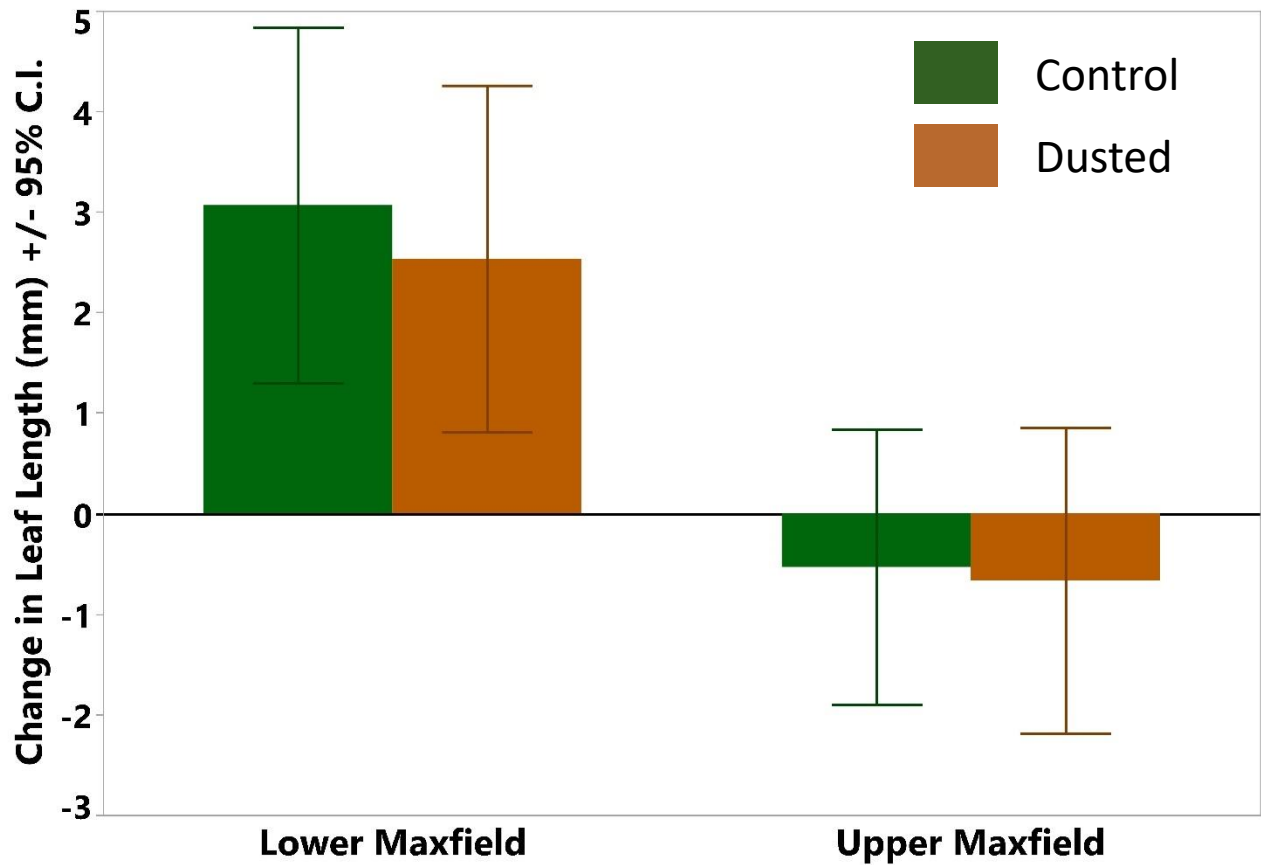
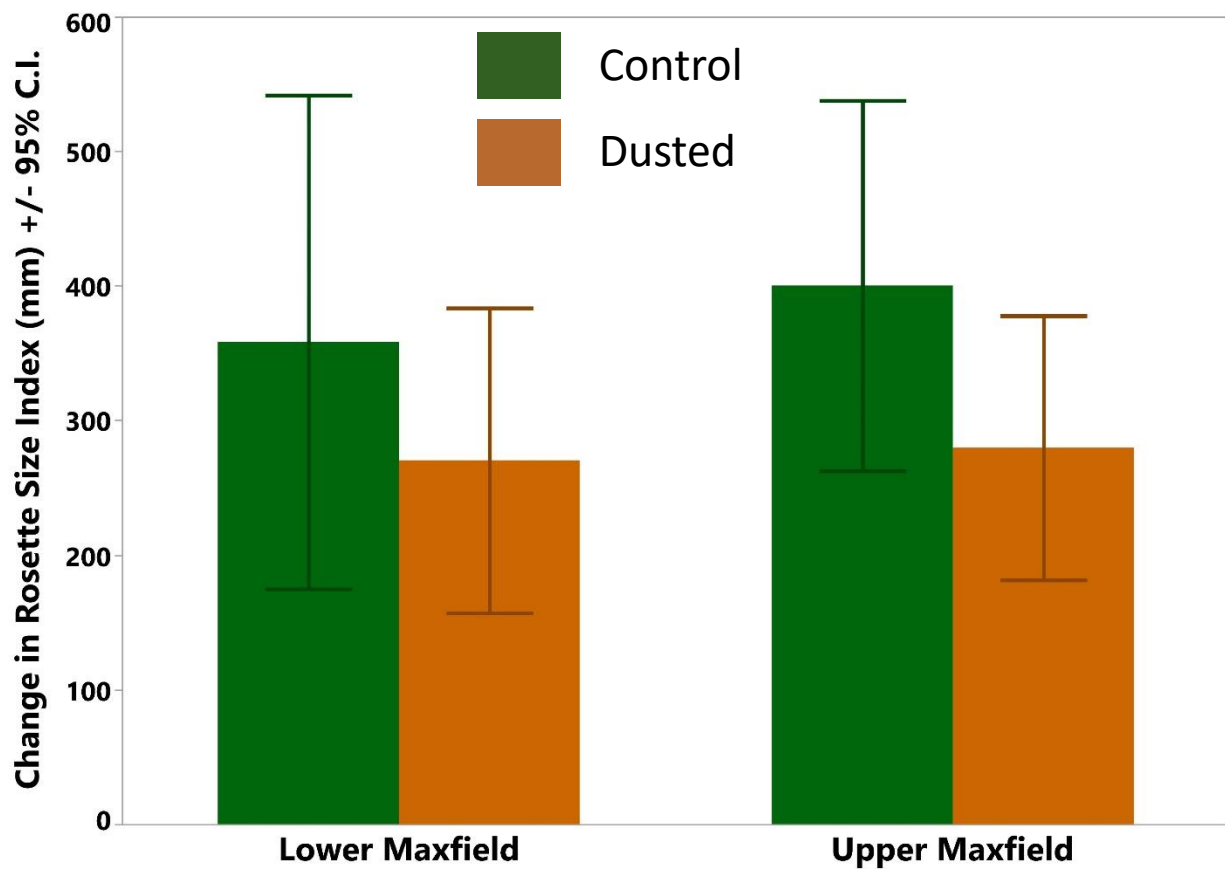


Figure 4: Effect of dusting on overall index of rosette size. The effects of treatment (dusted vs. control) and location (upper vs. lower Maxfield) on the overall index of size of rosettes over the course of the summer (5 July - 4 August 2019). Green bars indicate control rosettes and brown bars indicate dusted rosettes. Bar height indicates mean values and error bars are $\pm 95\%$ confidence interval of the mean.



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