

**MYSTERIES OF ROAD DUST: DOES ROAD DUST INFLUENCE FLOWER
LIFESPAN IN SCARLET GILIA?**

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Abstract

This study aims to explore the exciting mysteries of road dust. In the beautiful Rocky Mountains, at the Rocky Mountain Biological Laboratory in western Colorado, there is an amazing diversity of plants, but one in particular near unpaved roads is *Ipomopsis aggregata*, Scarlet gilia, a perennial flowering plant. In this paper I unveil the effects that road dust has on the stigma closure rates on *Ipomopsis aggregata*. To find out this mystery, armed with a hand lens and a duster, I keep track of 216 individual flowers to see the influence of road dust. Interestingly, road dust decreases floral lifespan, and female longevity. The stigma position in these flowers also plays a role in the floral lifespan of this plant, with weather having surprising effect. With all these factors, the evidence suggests that road dust influences stigma closure rates, and therefore influence the pollen loads.

Introduction

Road dust is a common occurrence along unpaved roads. Most folks tend to just cover up their nose or mouth with their hands to avoid dust plumes when a vehicle passes by and think nothing of it. Without an overwhelming amount of dust or for a constant time period, the event usually goes unnoticed. With roads all over the United States, and 15-20 percent of the United States is ecologically impacted by roads, on top of the 10 percent of the road lengths in the United States that are in national forest, it is important to study those effects (Forman and Alexander. 1998). With 2,253,081.6 kilometers of those roads being unpaved in the United States, the opportunity for road dust to affect natural systems is gigantic. Including the fact that road dust is able to travel as far as 200 meters from the road, the ecological impacts can travel far from where dust was originally produced (Forman and Alexander. 1998). An overlooked factor is the heavy metals that are also in the mix with road dust and be deposited elsewhere (Apeagyei et al. 2011). Such events give room to explore more detailed effects of road dust.

Little is known about how road dust affects plants. Some studies have found that it can reduce photosynthesis and water use efficiency (Sharifi et al. 1997). But few studies have considered how road dust might influence pollination and reproduction. One study (Waser et al. submitted) found that wildflowers exposed to road dust had lower pollen loads. The wild flowers are also covered with more road dust when the wild flowers are closer to unpaved roads, but will have less road dust the further away from the roads (Waser et al. submitted). Stigma position also affects the amount of pollen that is received on a flower. Road dust thus interferes with Scarlet gilia reproduction by reducing the amount of pollen they receive (Waser et al. submitted).

We do not yet know how road dust lowers pollen receipt. One possibility is that road dust affects the length of time that stigmas are open to receive pollen. Also considering that an exerted stigma on *Ipomopsis aggregata* receives greater amounts of pollen than stigmas that are inserted in the corolla (Campbell et al. 1993). Previous work has demonstrated that the stigmas of Scarlet gilia, like those of some species in the Bignoniaceae, close in response to certain

stimuli (Waser and Fulgate. 1986; Milet-Pinheiro et al. 2007; Sritongchuay et al. 2012). Scarlet gilia plants also vary in the length of time that flowers spend in female phase, and plants whose flowers spend less time (those with inserted stigmas) receive less pollen (Campbell et al. 1994). If road dust causes stigmas to close or causes flowers to spend less time in female phase, then this effect could explain why flowers exposed to dust receive less pollen. Particularly, unlike the plants in the Bignoniace family, Scarlet gilia is not as sensitive enough to cause stigma closure within seconds, but instead closes within hours. (Campbell et al. 1994; Waser and Fulgate. 1986). Also, the stigmas on Scarlet gilia don't close by simple touch but close with foreign pollen (see Waser and Fulgate, 1986).

Which leads to the questions I want to answer; does road dust affect floral lifespan, or stigma closure in Scarlet gilia, *Ipomopsis aggregata*? If lifespan of flowers is reduced by dust, in particular the amount of time spent in female condition, then this might explain lower pollen numbers. Similarly if dust causes stigma lobes to close together this might explain lower pollen numbers in flowers exposed to dust.

Materials and Methods

This study was conducted near the Rocky Mountain Biological Laboratory (RMBL), in the Gunnison National Forest of Western Colorado, at an elevation of 9,500 feet. The specific locations of the study were in Upper Maxfield Meadow (106° 59' 57.8''W 38°56'57.8''N) and a site along the Gothic Road we call Road Bend (106°59'19.143''W 38° 57'10.668''N) (see figure 1).

To explore the possible effects of road dust on floral lifespan and stigma closure rates, I studied one of the more common wild flowers around the RMBL, *Ipomopsis aggregata*, Scarlet gilia. When it flowers this plant varies in size with a thin green stem with multiple leaves extending out from the stem. Branching from the stem are flowers (sometimes in clusters). The flowers are mostly red with some variation with white reaching the center of the flower. The anthers are located within the flower, and have a light yellow coloration. The stigma with a purple color is inserted most of the time; however in some plants the style elongates beyond the corolla when the stigma matures.

In order to answer the questions of this study, the following procedures were followed. The Scarlet gilia in the study areas were examined visually to identify pairs that were similar in size (height, stem thickness) and in when they began to flower (the number of flowers that had opened). Once identified flags were assigned to every plant. For each pair, a coin was flipped to decide which plant in the pair was the designated dusted plant. Simultaneously, each flag was marked with a sharpie pen, with the plant's number and with a "D" for dusted or "U" for undusted. For Maxfield Meadow there were a total of 25 pairs, and in Road Bend there were a total of 22 pairs.

The size comparison for each plant was done by measuring the diameter of the stem at the root crown with a digital caliper base of the stem. The height was also measured with a metric ruler from the base of the plant to the maximum height without any manipulation of the plant's natural body position.

On every Monday morning from July 9th to July 23rd, 2016, I marked one elongated flower bud on every plant that would be ready to open in the afternoon (figure 2). If there were no elongated buds on any particular plant the Monday morning of tagging, then the plant was checked once more the following morning for elongated buds. If the plant had a qualifying elongated bud, the bud marked. But if no buds were present, then no other buds were added regardless of when an elongated was present. Only one flower per plant was used every week. To identify which flower was being observed for a given week, strings were used as identifying tags. The tags used were colored strings approximately 10 centimeters in length, and tied carefully but firmly around the pedicel. A different color of string was used each week. Blue string was used for buds that opened July 7th; green for buds that opened July 11th, and red for buds that opened July 18th. The study lasted 3 weeks and I tagged 131 flowers.

Dusting of the designated plants occurred every day during study. There was no particular time of day to dust, but most of the dusting occurred in the morning. The days that dusting did not occur were the days when it was raining and that was to mimic natural conditions since the roads do not produce dust on wet days. A Gilmore plant duster was used to dust experimental plants. The plants were dusted with "15" puffs of fine road dust. The road dust was collected from south of Judd Falls parking lot along the Gothic Road and sifted using Taylor soil sieves obtain fine dust with a diameter less than .0197 of an inch.

Marked buds were examined with a 10X hand lens three times per day, generally 8:00 AM, 12:00 PM, and 4:30 PM. At each census I scored characteristics of the corolla, anthers, and stigma that indicated phases in flower development. I recorded whether the corolla was closed (1=bud stage), beginning to open (2=start of anthesis), fully open with reflexed petal lobes (3=open flower), or absent (4=end of anthesis) (see figure 5a). Anthers were scored as immature (0=if the corolla was still closed, or if it was opening and the anthers had not started to dehisce), dehiscing (1=if the anthers had pollen on the outside), or stripped of pollen (2= if the anthers had no visible pollen). Stigmas were scored as immature (0) if the style had not fully elongated and the three stigma lobes had not separated, opening (1) if the stigma lobes were slightly separated, open (2) if the stigma lobes were reflexed, or partly closed (3) with pollen visible between the lobes (see figure 5a). Stigmas were also scored as inserted if they opened while still contained within the flower tube, or exerted if they extended beyond the flower tube when open. I defined floral lifespan as the time from the census when the corolla first opened until the census when either the corolla fell off or the stigma closed after opening. I defined the length of male phase as the time from first dehiscence until the census when the stigma first opened. The length of female phase was the time between the censuses when the stigma began to open until it closed again.

The resulting data were analyzed using the statistical program JMP version 12.0.1. We used step wise forward model selection to identify the model that best explained the variation in total floral longevity and female longevity. We used the minimum AICc criterion to determine the best model. Also we used a regression analysis to determine the contributions of variation in length male and female longevity to total variation in lifespan.

Results

Weather Conditions

Weather varied considerably during the study. The first week (July 7-July 10) was very windy with a maximum average wind speed of 20.53 mph, to the point that it had broken a couple of stems at both field sites, and also managed to blow away most of the white strings on the fourth day, right when I was expecting stigma closure. Needless to say, much of the female phase data was lost that week. The second week of the study (July 11- July 15) was not notably windy but still dry with no rain. Then the third week (July 18-July 23) it began to rain, with an average of .10 inches of rain for that week, compared to the last two weeks where there was no rain at all. The first two weeks were considered unusually dry because by then the time the summer monsoons begin.

Contributions of male and female longevity to overall lifespan

Dusted plants had fewer elongated buds on average during each census (0.89 buds) than did undusted plants (1.15 buds). This average difference of 0.25 buds was statistically significant by a paired t-test ($t=2.22$, d.f.=179, $P=0.028$). Not to mention that the dusted plants visually had more dust on them than those that were undusted (see figure 4a).

On average, male phase lasted 1.33 days, female phase 1.38 days, and total floral lifespan (approximately the sum of male and female phases) averaged 2.77 days. Length of the female phase contributed 73% of the variation in floral longevity ($R^2_{\text{adj}} = 0.74$), whereas male phase contributed 11% ($R^2_{\text{adj}} = 0.12$) (figure 1a). Because of this, we focused on length of the female phase in the remainder of our analyses.

The best ANOVA model, which explained 74% of the variation in female lifespan, included Treatment, Week, Site, and Stigma Position (see table 1). Site was not statistically significant, whereas the other factors were (see table 1).

The largest influence on female longevity was the variation in weather from week to week: female phase averaged 1.03 days the first week, 1.15 days the second week, but twice that long (2.54 days) in the third week, which was moist and cooler than the first two weeks (see figure 4a).

Stigma position also had a significant effect on female longevity: flowers that had exerted stigmas had longer female longevity (1.43 days) than did flowers with inserted stigmas (.90 days)

Regardless of treatment, flowers that had exerted stigmas averaged 1.43 (days) and flowers with inserted stigmas averaged .90 (days) (see figure 4c).

The dusting treatment consistently decreased female longevity regardless of site or stigma position. On average, the female longevity of dusted flowers was 1.2 days, whereas that of undusted flowers was 1.5 days.

Discussion

The results of our study support and confirm the hypothesis, that dusting reduces floral lifespan. We see that road dust does have influence on the time amount of time the stigma remains open and agrees with the findings and predictions of Waser et al (submitted), that pollen loads could be affected by stigma closure rates.

More surprisingly, our analysis showed that male and female phases were approximately equally long (about 1.3 days), but male phase did not contribute as much to variation in the total floral lifespan. Female phase accounted for almost 74 percent of the variation in the floral lifespan (see figure 3a).

For both dusted and undusted treatments, flowers with exerted stigmas lasted longer than those with stigmas that were inserted (figure 4c). This result mimics previous results of exerted stigmas being open for longer periods of time (Campbell et al. 1994). A possible reason for this pattern could be with pollinator visitations. Under natural conditions, Scarlet gilia receives about one visit per day by humming birds, and with exerted stigmas, not much pollen is transferred per visit (Price et al, 2005). But under road dust conditions those humming birds or other pollinators may be deterred by the road dust perhaps because the colors on Scarlet gilia are not as bright and colorful. It is possible that the stigmas remain open longer because there is no pollen on the stigmas to induce stigma closure (Waser and Fulgate. 1986).

Each week of the three-week study had somewhat different weather conditions. Female phase for both sites and the first two weeks together averaged about 1.03 days of longevity, but lasted an average 3 days in week 3 (Figure 4a). This week effect means the weather conditions had a significant impact on the female longevity.

Plants that were dusted looked browner (figure 4a) and drier and did not flower as much as those in the undusted treatment which is something that has been seen with the dusted shrubs in the Mojave Desert (Beatley. 1965). It is possible that dust also had a physical consequence to the entire plant. My prediction is that dust made the plant drier, possibly through diffusion,

because there would be more solutes outside the plant drawing out water. Another possibility is that dust could have clogged stomata, not allowing much gas exchange within the plant affecting its metabolism, just like in Sharifi et al. (1997).

Considering all the evidence collected from this study, it supports the idea that road dust does influence pollen loads by decreasing the amount of time stigmas remain open. If the stigmas remain open then there is a greater chance of pollen loads increasing, whereas if the stigmas close sooner, then there is less chance of pollen reaching the stigmas.

To build on this experiment, I feel there are important questions that would be interesting to study. One possible study could be on whether road dust directly causes the stigma to close the way pollen does? If so is it a physiological or chemical induced response? Also, is there a physiological signal when the stigma closes for the corolla to fall off? Does dust dry out the plant so that it drops flowers sooner? Another interesting question is if road dust on the plants deters the pollinators, thus extending the floral longevity? If Scarlet gilia is reduced of the normal pollen loads, this could affect how selection favors different traits such as the total amount of time stigmas remain receptive to pollen exposure or the effect on its ecological niche(s) (Campbell. 1989; Ashman et al. 2004). General improvement on the experiment could be made by using paint pens to mark the flowers instead of strings so the strings blow away in the event of strong winds. Also to measure the corolla in length and width as well as the length of the exerted stigmas to see if there may be a correlation with female longevity. Last recommendation is to begin with observations and dusting at the very start of the flowering season, to have more weeks and flowers for even more accurate statistical analysis.

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Tables and Figures

Table 1

Summary of the ANOVA of female longevity with dusting treatment, Week, site, and stigma position.

Source	DF	SS	MS	F	P
Model	5	37.079	7.416	17.202	< 0.0001
Treatment	1	1.728	1.728	4.008	0.0483
Week	2	28.700	14.349	33.283	<.0001
Site	1	0.208	0.208	.482	0.4895
Stigma Position	1	4.190	4.190	9.717	0.0025

Error	90	38.800	0.431		
C. Total	95	75.878		<.0001	

Figure 1a

Aerial view of the Rocky Mountain Biological Laboratory. South of RMBL is Road Bend and Upper Maxfield.



Figure 2a

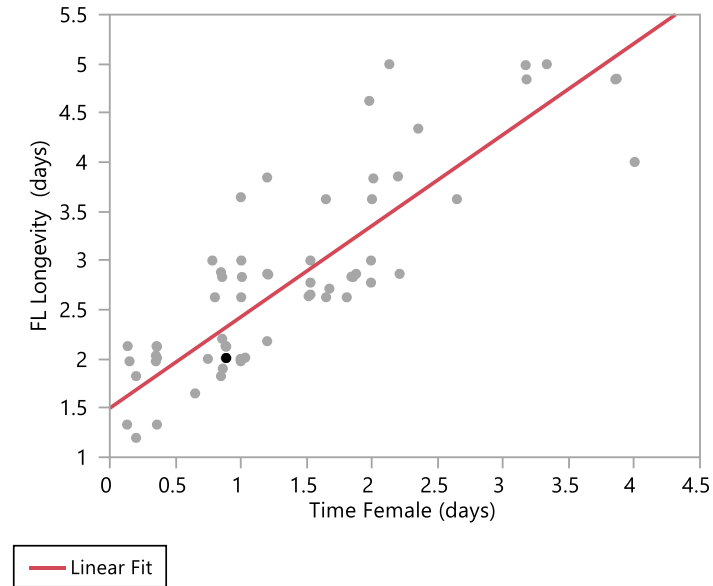
Pictures of Scarlet gilia. (Right) Undusted plant, and tagged with blue string. (Left) Dusted plant with almost no elongated buds or open flowers.



Figure 3a.

Graph and tables to describe the variation in floral lifespan that is female phase.

Bivariate Fit of FL Longevity (days) By Time Female (days)



Linear Fit

FL Longevity (days) = 1.4992574 + 0.9275103*Time Female (days)

Summary of Fit

RSquare	0.738777
RSquare Adj	0.735999
Root Mean Square Error	0.495521
Mean of Response	2.781293
Observations (or Sum Wgts)	96

Analysis of Variance

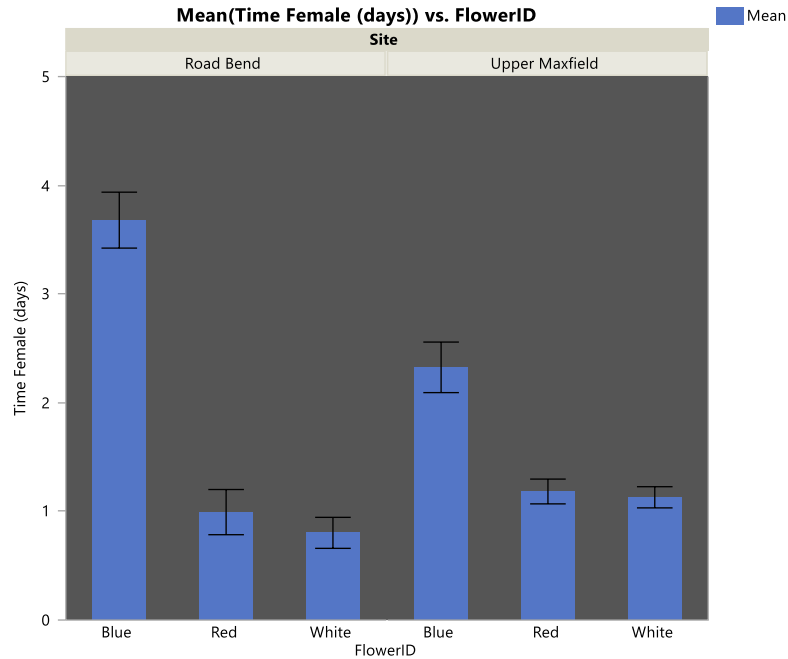
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	65.276167	65.2762	265.8464
Error	94	23.080842	0.2455	Prob > F
C. Total	95	88.357009		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	1.4992574	0.09349	16.04	<.0001*
Time Female (days)	0.9275103	0.056886	16.30	<.0001*

Figure 4a

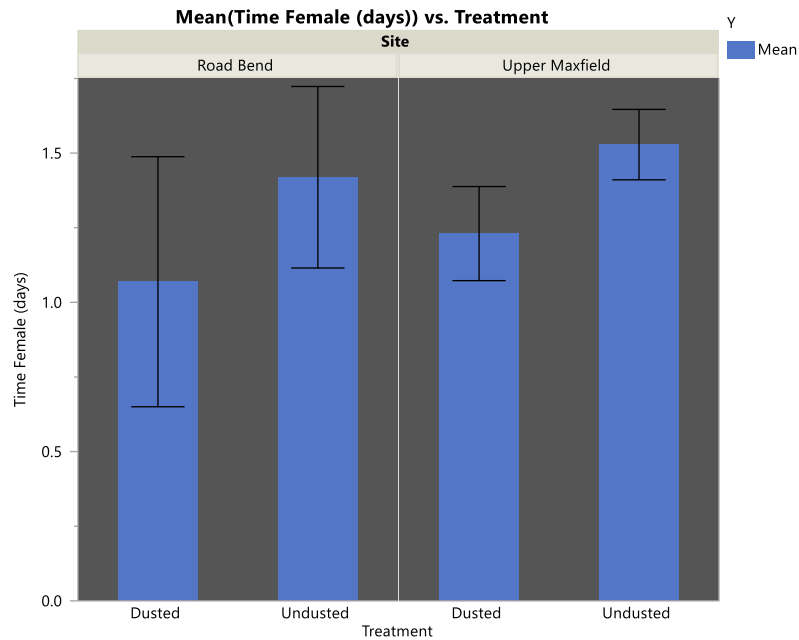
Graph shows the time in female phase vs. site, and flower ID (White= Week 1, Red= Week 2, Blue= Week 3).



Each error bar is constructed using 1 standard error from the mean.

Figure 4b

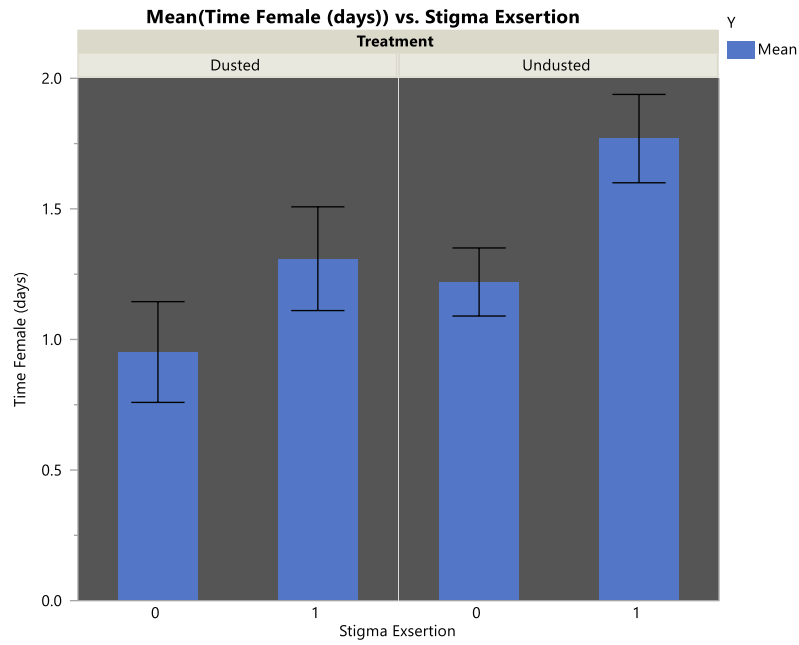
Graph shows time in female phase vs. site and treatment.



Each error bar is constructed using 1 standard error from the mean.

Figure 4c

Graph shows time in female phase vs. treatment and stigma exertion (0=inserted, 1=exserted).



Each error bar is constructed using 1 standard error from the mean.

Figure 5a

This is a drawing to represent the different states in the corolla on the top half of the picture (1=closed, 2=partly open, 3=reflexed). The bottom half represents the stigma states (1=closed, 2=partly open, 3= reflexed).

