

**DOES ROAD DUST AFFECT REPRODUCTION OF  
*DELPHINIUM NELSONII* AND *IPOMOPSIS*  
*AGGREGATA*?**

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## ABSTRACT

In the United States, the 6.2 million kilometers of paved and unpaved public roads and roadsides represent about nineteen percent of the total land area of over nine million Km<sup>2</sup>. These roadways have major ecological impacts, one of which is dust pollution. When it comes to the effect of dust on plants, research has mainly focused on population abundance as a function of increased pH or physiological effects on water balance or gas exchange. In this study, I looked at the effect of dust on plant reproduction, using *Ipomopsis aggregata* and *Delphinium nelsonii* as my study organisms and Gothic road as my site. The aim of my study was to find out whether road dust affects pollinator visitation, fruit production, and seed set. For my methods, I first set out 50-meter-long transects perpendicular to Gothic road and collected dust on filter paper to see whether there was more dust near the road than far away. Then, I recorded observations of hummingbirds visiting *Ipomopsis aggregata* flowers for six hours, counted pollen grains on stigmas of both species, and counted seeds of *Delphinium nelsonii* fruits. This was done along the existing transects, near the road and far away. I also experimentally dusted *Ipomopsis aggregata* plants that were far away from the road to control for other road effects. Results showed that there is more dust near the road than far away, but no difference in hummingbird visitation or pollen load for *Ipomopsis aggregata*. For *Delphinium nelsonii*, there were more seeds produced per number of pollen grains near the road than far away. Experimental dusting showed more pollen on non-dusted plants than dusted ones. However, analysis showed that these results were not very strong. This means that while dust can affect the reproduction of plant species and differently so, other road effects or biological factors, like hummingbird foraging behavior, may be stronger.

Key words: roads, dust, pollination, seed set, stigma

## INTRODUCTION

In the United States, the 6.2 million kilometers of paved and unpaved public roads and roadsides represent about 19% of the total land area of over nine million km<sup>2</sup> (Forman 2000). These roadways have major ecological impacts. They fragment habitat, provide corridors for invasion of exotic species, and disturb hydrology. They also generate dust pollution, which is not only a human safety and health hazard, but also has major ecological effects (Forman 1999). Specifically, the effects of atmospheric dust and dust deposition from roads (primarily paved) on adjacent ecosystems have been receiving attention from ecologists for at least thirty years.

In arctic environments, dust deposition can decrease the albedo of snow. This causes more rapid snow melt, which can also speed up the timing of soil thaw and flowering as well as migration and breeding of waterfowl, ptarmigan and their predators (Walker and Everett 1987). Road dust can also alter soil chemistry. For example, Kalisz and Powell (2003) reported an increase in soil pH with nearness to the road in a naturally acidic forest in Kentucky. Even at the biochemical level, dust particles can keep enzymes from breaking down organic matter and from mineralizing nitrogen in the soil. This affects ecosystem decomposition processes (Moorhead et al. 2006).

For plants, research has primarily focused on how proximity to paved roads affects physiological processes such as water balance or gas exchange (Fluckiger-Keller et al. 1978; Walker and Everett 1987). Little is known about how dust from unpaved roads affects plant reproduction. In my study, I explored how a dusty environment might compromise the health of plant populations by interfering with plant reproduction. Dust could influence all three sequential stages of reproduction—pollination, ovule fertilization, and seed maturation.

In the pollination stage, dust could inhibit the adherence of pollen to the flower stigma, a direct effect. Further, it could change the behavior of pollinators by making them less active in dusty areas, an indirect effect. The latter is a version of the dust aversion hypothesis that Ndbalema et al. (2007) tested on ungulates (hoofed animals) in the Serengeti. The authors hypothesized that grazers would feed farther from the road when there is high dust deposition. Though this was not supported by their data, results could be different for pollinators, especially insects whose flight or respiration during flight could be sensitive to high levels of airborne particulates. Moreover, dust deposition could lower plant visibility for all pollinators.

In the fertilization stage (the union of plant male and female gametes) dust could cause damage when it is deposited on flower stigmas, the receptive female tissue that receives pollen. The damage could come about if dust prevents conspecific pollen from germinating properly, or if dust changes the ability of the stigma to receive additional pollen. Waser and Fugate (1986) found, for example, that heterospecific (foreign) pollen reaching the stigma of *Ipomopsis aggregata* caused the stigma to close, preventing subsequent deposition of conspecific pollen. Dust particles might have similar effects. Lastly, in the stage of seed maturation, dust-induced changes in a plant's resource status—through changes in leaf pH or by clogging stomates of leaves (the openings through which gases are exchanged with the outside)—might have negative effects.

The aim of my study was to find out whether road dust affected pollinator visitation, pollen deposition, and seed set. I tested five hypotheses: First, I believe that (1A) road dust deposition decreases with distance from the road, with (1B) heavier particles falling out near the road and lighter ones traveling further away. If so, plants near the road should (2) receive fewer visits by pollinators, (3) have less pollen on their stigmas, and (4) produce fewer seeds than plants far way

from the road. I also tested the possibility that (5), experimentally dusted plants should have lower pollen loads and/or seed production than nearby controls.

## METHODS

I chose *Ipomopsis aggregata* and *Delphinium nelsonii* as my study organisms. *Delphinium nelsonii* is a violet wildflower in the buttercup family (Ranunculaceae) that is mainly pollinated by hummingbirds and bumble bees, and *I. aggregata* is a red wildflower in the phlox family (Polemoniaceae) that is mainly pollinated by hummingbirds, (N. Waser and M. Price, personal communication). These particular species have been extensively studied at the RMBL and are also native to the Colorado Mountains.

To address my first hypothesis, that there is more dust near the road than far away, I ran 50-meter-long transects, which started one meter away from the road, and placed filter paper disks attached to nails at a distance of 0, 10, 20, 30, 40, and 50 meters from this point. The one meter separation was chosen to protect the filter paper from direct damage or disturbance from traffic (accidental trampling, blowing away). The exposure time of the filter paper was approximately four days. This took place in the *Delphinium nelsonii* site, by the Avery campground area on Gothic road, and the *Ipomopsis aggregata* site, also on Gothic road, by Ore House. Then, I counted the number of dust particles within a 0.096mm<sup>2</sup> area at five random points within each disk. The Avery site was sampled twice during the season and the Ore House site only once. The objective of this was to determine whether there is a gradient in dust deposition with distance from the road.

For testing the second part of this hypothesis, mainly, that dust particle size distribution should be different near versus far from the road; I chose the 0, 10 and 20m filter paper disks from July 9-13 at Avery West, the left side of the road at Avery. Each dust particle within a 0.048mm<sup>2</sup>

area was put in one of three size categories: small, medium, or large. These categories were reflective of the approximate size of the particle. Small particles had an area of approximately less than  $.0004\text{mm}^2$ , medium particles were between  $.0004$  and  $.0016\text{mm}^2$ , and large particles were greater than  $.0016\text{mm}^2$ . Disks were sampled at five random places.

Since initial counts of dust deposition at Avery showed a clear negative relationship between distance from the road and dust deposition, I went on to test hypotheses 1-4, that is, to monitor the reproductive stages of *D. nelsonii* and *I. aggregata* plants at sites near and far from the road. On July 9 and 13, 2008, the stigma and developing fruit of one flower of each of twenty *D. nelsonii* plants were collected. Half of the plants were zero to ten meters away from the road and the rest were forty to fifty meters away from the road. Then, from July 19 to 24, I collected the stigma of one flower of each of fifty *I. aggregata* plants. Half of these were zero to four meters away from the road and the rest were approximately forty meters away from the road. Stigmas were put on a microscope slide and stained with basic fuchsin gel. Pollen was then counted under 25 power with a compound microscope.

Stigmas were only taken from old flowers, on which the corolla tubes were falling or had fallen off. Pollen tubes would have at that point formed inside the stigmas and the ovules been fertilized, therefore, this procedure did not interfere with fruit and seed production (M. Price and N. Waser, personal communication). Then, I counted the maturing seeds of the twenty *D. nelsonii* flowers. For *I. aggregata*, which flowered later in the season, timing only allowed for counting pollen on stigmas (my mentors, M. Price and N. Waser, will collect ripe fruits and count seeds later on). *D. nelsonii* flowers usually produce three carpels; therefore, I recorded three pollen and seed counts for each flower. *I. aggregata* flowers produce one carpel.

Hummingbird observations at the *I. aggregata* site coincided with the timing of stigma collection. That is, I observed hummingbirds and then collected the stigmas of flowers they had potentially visited. Before beginning an observation period, I first recorded the total number of *Ipomopsis aggregata* flowers per stalk within the patch. The objective was to observe a patch that had at least one hundred flowers, in order to increase the likelihood of visitation, and to observe a patch small enough that all the plants were visible from a distance of about three meters.

Then, I recorded the number of feeding bouts and flower visits during each one-hour observation period. Each patch was observed for a total of three hours between July 16 and 19, 2008. I did not distinguish between Broad-tailed and Rufous hummingbird visits. No pollinator observations were taken at the *D. nelsonii* site due to low visitation rates.

The final part of this project involved the experimental dusting of a patch of *I. aggregata* plants. These plants were located in the Gothic town site, a significant distance (more than fifty meters) away from Gothic road. Plants of similar size (based on stem width) were paired. One of each pair was randomly assigned the dust treatment, the other served as a control. The dust, taken from Gothic road, was reflective of the dust that roadside plants are exposed to. This was ensured by sifting road material through a soil sieve with 0.15 mm openings. Dusting was done using a bellows-style hand duster, which is ordinarily used for adding insecticide powders to cracks and crevices.

In total, there were ten control plants (treatment A) and ten dusted plants (treatment B). Plants were dusted twice a day (mid-morning and mid-afternoon), for a period of six days. Specifically, each time dusted plants received twenty to thirty puffs from the duster. It was not my objective to replicate the levels of dust achieved by a specific proximity to the road; rather, I simply tried to create a level of dust in the dusted plants that was visually distinguishable from the controls.

Stigmas from all the plants were collected on the fourth day of dusting. The goal was to collect two stigmas from each plant, though this was not always possible because plants varied in flower number. Also, the fourth day was chosen because at this point the corollas of flowers open on the first day would have freshly fallen off, so I captured the time the flower was open and receptive (to both pollen and the added dust). Again, stigmas were put on a microscope slide and stained with basic fuchsin gel. Pollen was counted under 25 power with a compound microscope.

## RESULTS

Dust deposition decreased significantly with distance from the road at both Avery and Ore House (Figure 1). A second-order polynomial regression indicated that the decrease was nonlinear, with a steep decline close to the road and shallower decrease further from the road (Table 1). At all sites sampled, there was a lot of dust very close to the road, zero meters, and then a close to equal low amount from ten to fifty meters (Figure 1). Thus, plants near the road and their pollinators are exposed to more dust. However, while dust loads decreased with distance from the road, Wilks' Lambda test of multi-variance found no significant effect of distance on particle size distribution (Approx.  $F = 1.4381$ ,  $P = .2495$ ). That is, there was an equal proportion of small, medium, and large particles at all distances from the road.

*Delphinium nelsonii* plants received on average 30% less pollen near the road than far away (Table 2). This difference was statistically significant ( $F = 10.9252$  d.f. = 1  $P = 0.0042$ ). However, the difference in pollen load was not reflected in seed set: plants near the road tended to produce more seeds per flower (Table 2). This means that plants near the road produced more seeds per pollen grain deposited. Also, plants near the road were significantly taller than plants far away from the road and also tended to produce more flowers (Table 2).

For *Ipomopsis aggregata*, I was able to observe hummingbird visitation. The average number of flowers and stalks was higher near vs. far from the road (Table 3). Mean number of flowers per stalk was also higher near the road (Near: mean = 6.693 Far: mean = 4.8). However, there was no significant difference in visits per flower near versus far from the road (Table 3). There was also no significant difference in pollen load (Table 3).

So, while there was a difference in dust load, there was no resulting difference in pollen load for *Ipomopsis aggregata*, which contrasts with results for *Delphinium nelsonii*. This result also contrasts those of experimental dusting on this species. A one-way analysis of variance of number of pollen grains by treatment indicated a trend toward lower pollen loads on experimentally dusted *Ipomopsis aggregata* flowers (Figure 2). In fact, dusted flowers had 44% less pollen than controls. Future collection and analysis of *I. aggregata* seeds should add more meaning to these opposing results.

## DISCUSSION

Road dust is mostly composed of particles of soil, tire material, brakes, organic material, and deposited motor vehicle exhaust (Miguel et. al 1999). Motor vehicle exhaust contains nitrogen oxides, ethylene, oil, and lead-containing solid particles (Thompson et. al 1984). All these substances can be harmful to plants. For example, metals like platinum and rhodium have the potential to accumulate in sensitive plant species and cause a significant decrease in their abundance (Niemela et al. 2004).

While this study did not conduct an analysis of the chemical composition of the dust from Gothic road, it would be safe to assume that it contains, to some level, the substances described above. It would then logical to think that plants near the road are less healthy than plants far away from the road. I did not measure the sizes of *Ipomopsis* plants near vs. far from the road.

However, *Delphinium nelsonii* plants were taller near the road versus far away. This result may be associated with hydrological effects of roads.

Road-induced increases in water runoff (Forman and Alexander 1998) could increase the water availability of roadside plants. Further, the chemicals carried by sediments in water runoff, most important of which are deicing salt and heavy metals, affect plant growth and chemistry (Forman and Alexander 1998). Since it is widely known that plant pollinators have a preference for tall plants, which are more visible and accessible, greater height caused by water runoff may explain why pollen loads of *Delphinium nelsonii* flowers near the road were only 30% lower than far from the road.

Had there been equal plant size, I could have detected a bigger dust effect. There may have also been a difference in flowering time near versus far from the road. Lastly, added nutrients from water runoff could increase a plant's resources, which can be allocated towards the maturation of fruits and seeds. This could explain the result of more seeds per pollen grain produced near the road. It would seem that hydrological effects may affect plant reproduction more than dust loads.

If we return to the dust-aversion hypothesis, which claims that animals will not feed close to the road, this study found no evidence to support it for hummingbirds, whose foraging behavior was not affected by distance. Not all bird species decrease in density near roads; some even show the opposite effect and preferentially use rights-of-way (Kaselloo 2005). High population density in some years may even cause birds to move into poorer-quality territory for breeding, which may include the roadside. Also, some species like to breed in exceptionally noisy environments like airports and roads (Kaselloo 2005).

This is supported by Develey and Stouffer (2001), who found that, in the case of flocks of insectivorous birds in the Amazon, roads are not an absolute barrier to movement. My

comparisons of distance to the road may have been affected by any of these factors that determine behavior, which occur at different gradients. Also, there was low traffic density in Gothic road and hummingbirds were able to time their visits between cars, often visiting flowers seconds after a car passed by (Katty Paulino, personal observation).

*Ipomopsis aggregata* patch density, height, and number of flowers per stalk greatly influence hummingbird visitation and may have confounded my results. Patch density and plant height were not recorded in this study. However, I did find more flowers per stalk near versus far from the road. Broad-tailed hummingbirds visit dense *Ipomopsis aggregata* patches and tall plants with many flowers in order to reduce foraging costs incurred from flight time. They also reduce flight time by returning to good patches, which would fit these characteristics (Wolf and Hainsworth 1990).

Male broad-tailed hummingbirds are very territorial. Their territories usually include willow trees, which were growing by my observation sites. This may have also confounded my results, increasing visitation near the road because visitation by males is much more dependent on territory than on the dust load on the plants within it. Both my far and near sites were both within a male hummingbird's territory (Katty Paulino, personal observation).

When it comes to the females, my sites were also in close proximity to aspen trees, which are used for nesting. Broad-tailed hummingbirds also reduce flight time by flying short distances (Wolf and Hainsworth 1990); therefore, it is possible that there was equal visitation by females at zero to four meters from their nest and at forty meters, which is still a relatively short distance for a bird. Again, visitation by females is much more dependent on proximity to nesting sites than on the dust load of the plants around it. Future research should control for hummingbird behavior by

including *Ipomopsis aggregata* sites that are located close to a road, but at least far away from hummingbird nesting sites.

Even so, the complication is that road effects on plants probably occur at short distances from the road, 0-200m, but birds, on the other hand, are affected up to about 1,000 meters from roads (Thompson et. al 1984, Forman and Deblinger 2000), primarily because birds are sensitive to traffic noise, which travels a great distance. So, the range of road effects on hummingbirds may be too large to be significantly different between zero and forty meters from the road. It may be that, at short distances, dust has a minor effect on hummingbird behavior.

Future work on the effect of road dust on plant reproduction in this system would benefit from including different sites and from adding a greater emphasis on experimental dusting. First of all, to control dust clouds during the summer, Gothic road is treated with magnesium chloride, which is very corrosive (Kloor 2008). Magnesium chloride had not been added to Gothic road during the time of this study; therefore, it would be interesting to study its effect.

Second, in conducting experimental dusting of *I. aggregata*, this study controlled for distance effects and found a significantly lower pollen load on dusted plants. Visually, it seems that the dust load of experimental plants was higher than the natural dust load. This result should be confirmed by a higher sample size, nonetheless, since dust levels are dependent on traffic density (Forman and Deblinger 2000), sites with higher traffic density (more dust) could yield natural pollen loads equal to the experimental ones.

Lastly, other variables like site topography and differences in habitat quality and competition from other plant species near versus far from the road were not addressed in this study. Taking them into account could help isolate dust effects.

The general conclusion of this study is that, while road dust may not be a key factor in determining the total distance affected by roads in a system (Forman and Deblinger 2000), it has the potential to affect the reproduction of plant species and, therefore, should continue to be studied. Also, experimental dusting treatments may be a very effective method for separating dust effects from numerous and sometimes greater road effects.

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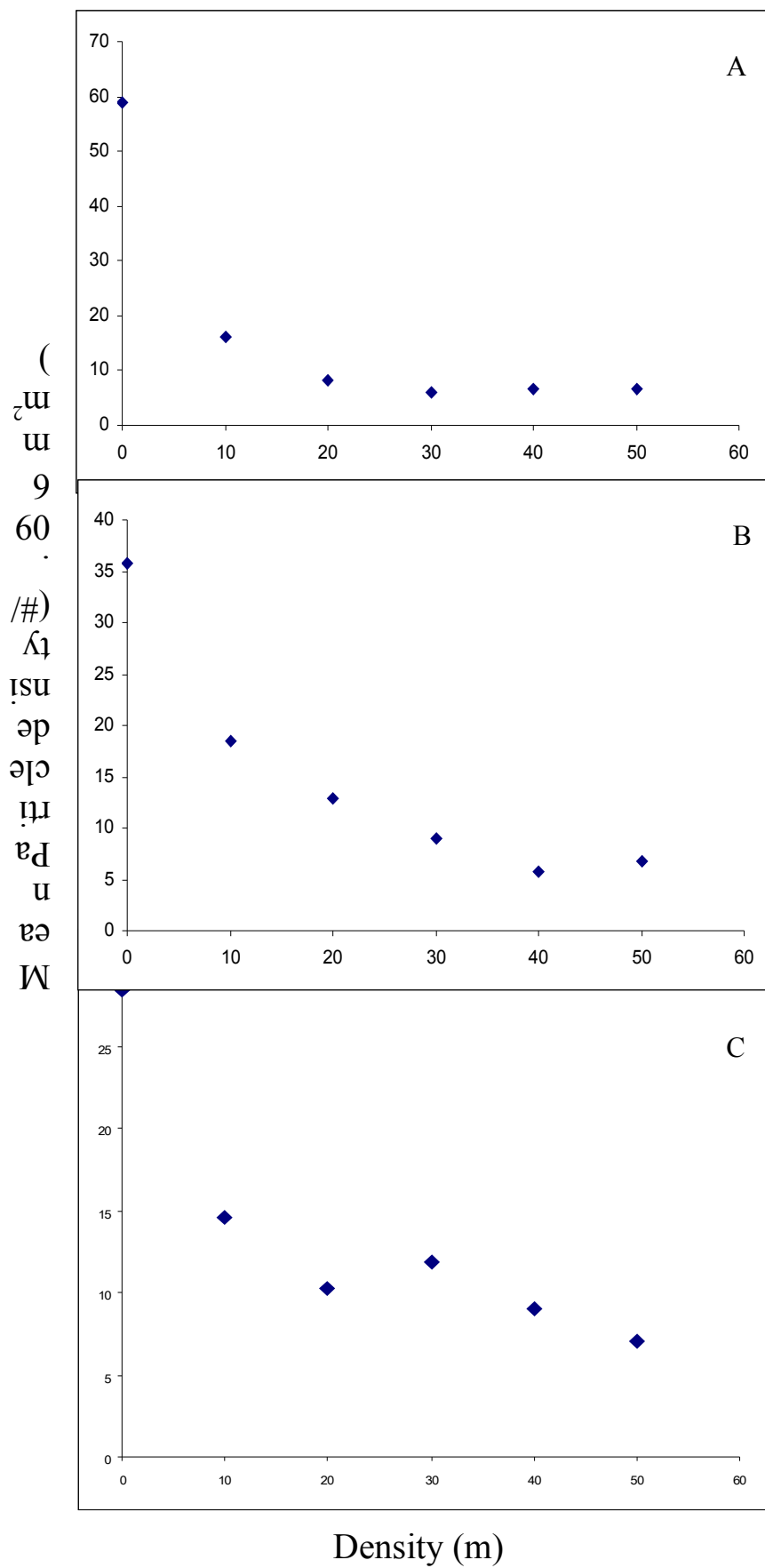


Figure 1. Dust deposition vs. distance from the road.  
 A = Avery East. B = Avery West. C = Ore house.

Table 1. Polynomial regression analysis of dust deposition as a function of distance from road at two sites. Two transects were established at the Avery site, and one at the Ore House site. Distance is in meters. Analyses were conducted in JMP.

| Transect   | Variable                | Coefficient | t     | P      |
|------------|-------------------------|-------------|-------|--------|
| Avery East | Intercept               | 22.342992   | 4.43  | 0.0013 |
|            | Distance                | -0.721358   | -5.84 | 0.0002 |
|            | (Distance) <sup>2</sup> | 0.0414078   | 4.84  | 0.0007 |
| Avery West | Intercept               | 21.371261   | 7.81  | <.0001 |
|            | Distance                | -0.474567   | -7.07 | <.0001 |
|            | (Distance) <sup>2</sup> | 0.0175063   | 3.77  | 0.0037 |
| Ore house  | Intercept               | 18.822321   | 6.33  | 0.0080 |
|            | Distance                | -0.349143   | -4.26 | 0.0237 |
|            | (Distance) <sup>2</sup> | 0.0116786   | 2.08  | 0.1288 |

Table 2. *Delphinium nelsonii*: Analysis of variance (ANOVA) of plant height, flower number, pollen load, and seed set as a function of distance from the road. Height is in cm. Flower number is per plant. Pollen load and seed set are per carpel. Plant was nested within distance in the analysis for seed set.

| Variable     | Distance | Mean    | S.E        | d.f.<br>(num,den) | F       | P      |
|--------------|----------|---------|------------|-------------------|---------|--------|
| Plant height | Near     | 26.0000 | 2.1713624  | 1, 14             | 6.6932  | 0.0238 |
|              | Far      | 16.6000 | 2.9131884  |                   |         |        |
| # Flowers    | Near     | 5.22222 | 0.67189309 | 1, 12             | 2.6269  | 0.1310 |
|              | Far      | 3.40000 | 0.90143918 |                   |         |        |
| Pollen load  | Near     | 86.742  | 44.147609  | 1, 17             | 10.9252 | 0.0042 |
|              | Far      | 173.111 | 44.147609  |                   |         |        |
| Seed set     | Near     | 10.3871 | 0.70872538 | 18, 39            | 6.8913  | <.0001 |
|              | Far      | 8.6667  | 0.75657925 |                   |         |        |

Table 3. *Ipomopsis aggregata*. Analysis of variance (ANOVA) of flower number, number of visits per flower, and pollen load as a function of distance from the road.

| Variable        | Distance | Mean     | S.E       | d.f.<br>(num,den) | F      | P      |
|-----------------|----------|----------|-----------|-------------------|--------|--------|
| # Flowers       | Near     | 408.333  | 33.859    | 1, 4              | 2.7680 | 0.1715 |
|                 | Far      | 328.667  | 33.859    |                   |        |        |
| # Visits/Flower | Near     | 0.163280 | 0.09435   | 1, 4              | 0.0434 | 0.8451 |
|                 | Far      | 0.191085 | 0.09435   |                   |        |        |
| Pollen load     | Near     | 140.400  | 15.367743 | 1, 58             | 1.1892 | 0.2800 |
|                 | Far      | 116.700  | 15.367743 |                   |        |        |

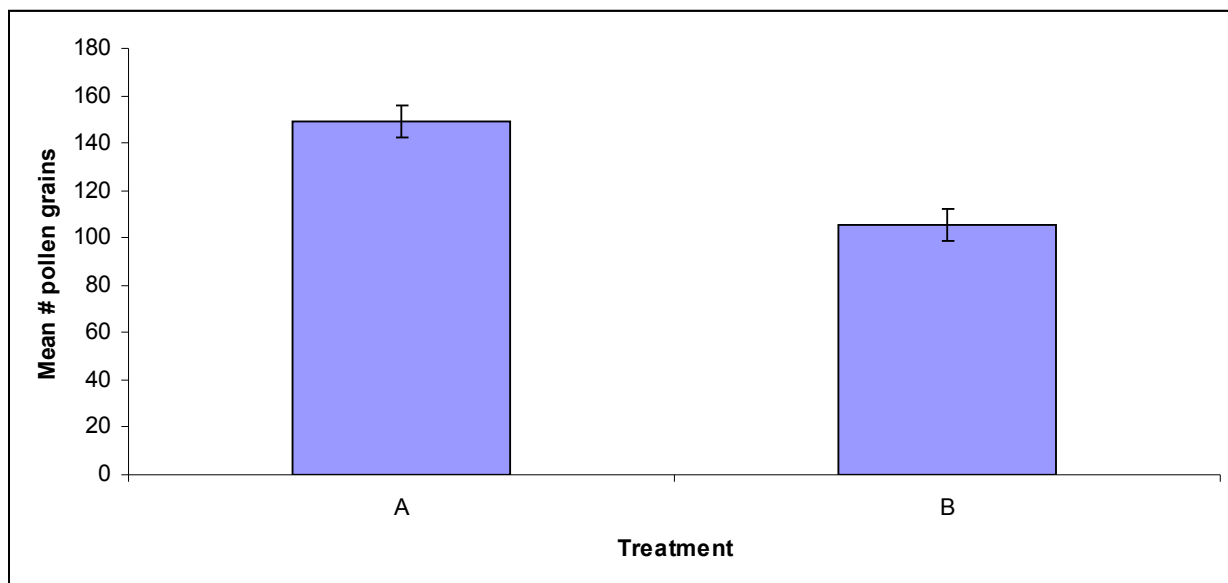


Figure 2. *Ipomopsis aggregata*. Experimental dusting ANOVA. Mean pollen grains per stigma. A = Control treatment. N = 10. Mean = 149.07. SE = 19.785. B = Dusted treatment. N = 9 Mean = 105.333. SE = 20.855. F = 2.3147. P = .0733.

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