

**The effects of road dust on pollination and reproduction of the native
wildflower species, *Delphinium nuttallianum***

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Abstract: Roads have the potential to cause considerable changes in our environment. Roads can introduce invasive species into native lands as well as deposit particulates (dust). I investigated the effects of dust from a heavily traveled, unpaved road on pollination and seed production of the larkspur *Delphinium nuttallianum*, a native wildflower of western North America.

Road dust is likely to affect the reproduction of this wildflower both by direct and indirect mechanisms. Directly, the dust can inhibit pollen adhesion on stigmas and decrease pollen loads and seed set. Indirectly, the dust can dissuade pollinators and cause a decrease in pollen loads and seed set. The study system included potted plant arrays both near and far from the road as well as manually dusted arrays.

Deposition of dust declined significantly with distance from the road. Flowers positioned near the road accumulated fewer pollen grains, although this was not significant, and there was no significant difference between seed set of flowers growing near and far from the road. Flowers dusted manually had a significantly lower seed set than controls, but numbers of pollen grains deposited on these flowers did not differ significantly. Bumblebee pollinators visited plants far from the road significantly more often than those close to the road, but there was no detectable difference in visits to manually dusted flowers and controls.

My study provided evidence that dust can negatively affect aspects of plant reproduction both directly (lower seed set in the dusted arrays) and indirectly (difference in pollinator visitation rate in the roadside arrays). My study also suggests that another

abiotic factor associated with proximity to the road, such as vehicle traffic or pollution, may be causing decreased levels of pollinator visitation.

Key words: bumblebees, pollination, *Delphinium*, indirect effects, reproduction, plant biology, road dust.

Introduction:

In the last century, roads have become a dominant feature across the American landscape. In fact, an average 6.2 million kilometers of roads, both paved and unpaved, cover the United States (Forman, 2007). This anthropogenic development has the potential to affect the ecology of the flora and fauna close to these roads. It is already estimated that 15-20 % of land in the US is ecologically affected by roads (Forman and Alexander, 1998). However, it remains unclear the precise mechanisms of America's roads on the environment or if there is one particular abiotic factor that is more prevalent than the other. One road-associated factor that has been shown to have considerable ecological impact is dust. Dust has been shown to alter the ecology of roadside plant and animal communities, and this calls my interest to investigate the exact mechanisms by which roads could be affecting such communities (Lewis, 2013; Steltzer et al. 2009; Upekala *et al.* 2009; Ndibalema et al. 2008; Kalisz and Powell 2003; Sharifi et al. 1997; Walker and Everett, 1987; and Thompson et al., 1984).

Dust is generally defined as a particle that is light enough to be suspended in the atmosphere, but heavy enough to settle on the landscape. The final destination of dust is determined largely by the size and composition of the particulate (Field *et al.* 2009; Toy *et al.* 2002; Bagnold, 1941). More specifically, the size of dust ranges from 2-10

micrometers and can be carried long distances by wind, at times, traveling across continental scales (Field et al. 2009; Chadwick et al. 1999). Although dust is deposited in many ways, I am specifically interested in the anthropogenic movement of dust, as mediated by vehicle traffic on unpaved roads. As unpaved roads continue to increase across the country, I believe it is important to understand the mechanisms of road dust, especially in biologically sensitive areas (Lewis, 2013).

Dust is generally overlooked but is becoming increasingly important as it has the potential to affect many ecological processes. For example, dust layers on snow-covered peaks and vegetation have been shown to increase absorption of solar radiation, which accelerates snowmelt (Steltzer et al. 2009; Walker and Everett, 1987). Earlier snowmelt altered plant phenology and caused earlier exposure of vegetation, and as a result, grazers arrived earlier in the season. Moreover, dust has been shown to alter soil chemistry and soil invertebrates (e.g., Kalisz and Powell, 2003). This has the potential to change plant and animal communities.

In addition to its ecological effects, road dust affects our health and cultural heritage. For example, dust has been shown to aid in transportation of disease and increase respiratory problems (Sanders and Addo, 1993; United Nations, 1979). Road dust in particular is causing 10,000 images painted by the Fremont and Ute Indians to fade away along the nine-mile canyon in Utah. (Kloor, 2008). These examples illuminate how road dust has the potential to influence many processes, but the mechanisms are still unknown or poorly studied.

How plants are affected by road dust, in particular, is of tremendous biological concern because of the vital role they play in terrestrial ecosystems. Dust has been

shown to reduce photosynthesis by shading the upper and lower surfaces of leaves (Thompson et al., 1984), which reduced plant growth. In Alaska, dust from unpaved roads negatively affected plant growth rate in tussock tundra ecosystems (Kalisz and Powell 2003). Some studies have shown that as dust affects the physiology of plants (Upekala et al. 2009; Sharifi et al. 1997), it not only reduces growth rate but influences reproduction (Lewis, 2013). Although there have been studies looking at physiological effects of road dust, little research has been done on the effects of road dust on plant reproduction.

My experiment added to the growing literature on road dust and will help conservation measures in biologically sensitive areas. Road dust deposition has the potential to impact angiosperm reproduction by altering pollinator behavior. For example, pollinators may avoid dusty flowers, just as African ungulates avoid eating dusty vegetation (Ndbalema et al. 2008). Road dust also has the potential to interfere with fertilization by inhibiting pollen adhesion and germination of pollen (e.g., Waser and Fugate, 1986). My study aimed (1) to uncover the effects of dust on reproduction of a native plant, as measured by the production of seeds, and if there is an effect, (2) to explore the mechanisms by counting pollen loads and observing pollinator behavior.

Materials and Methods:

Study system:

My study took place at the Rocky Mountain Biological Laboratory in Gothic, Colorado, a field station at 2900 meters elevation in west-central Colorado (Figure 1). I studied a native wildflower species Nuttall's larkspur, *Delphinium nuttallianum* Pritzel ex Walpers (= *D. nelsonii* Greene) (Ranunculaceae). Pollination and floral biology of *Delphinium*

nuttallianum have been studied extensively at the Rocky Mountain Biological Laboratory (e.g., Waser 1978, 1982, Waser and Fugate 1986, Waser and Price 1991a, Brody and Waser 1995, Price et al. 2005). Nectar-seeking hummingbirds and queen bumblebees pollinate the protandrous deep-blue flowers of *D. nuttallianum*, with contributions from other insects. Most reproductive plants produce up to ten flowers on a raceme inflorescence 20-25 cm tall. Flowering begins one-two weeks after snowmelt and ends about one month later. Flowers contain multiple carpels each of which produce up to ten seeds each. Seeds are about 1 mg in mass and disperse near the parent and experience little dormancy past the first winter. Because they are perennial, they grow slowly for several years before reaching reproductive maturity.

I studied plants that were originally growing along the east side of the US Forest Service Road north of County Road 317 in the East River valley. The US Forest Service Road does not experience MgCl treatment to control dust and therefore is exposed to dust continuously by heavy anthropogenic movement of particulates. The road is unpaved, composed of native soil, and is roughly six meters wide. It is visited by tourists and highly traveled by cars throughout the summer, once reaching a level of 39 cars in one half hour (Griffith, personal observation). Similarly, I had another field site just south of the RMBL in a research meadow, nestled far away from the roadside so as to not have any road dust influence the treatment.

Deposition of dust:

In early June I placed Whatman #1 filter paper discs at the East River Valley site at 0, 10, 20, 30, 40, and 50m from the road, attaching each disc to the soil surface with a small nail

pressed through the disc center. This was done when the potted plants (see transplanting) were put out to ensure an accurate account of what level of dust the native flora was being exposed to. Discs were left out for one week and then I quantified the amount of dust deposited during that time by counting number of dust particles within an area of 0.0576 mm^2 at each of ten haphazardly-chosen points on each disc, using a WILD M-5 stereo dissecting microscope at 50X magnification.

Transplanting:

In early June I potted 24 plants of *D. nuttallianum* at each of the East River valley and research meadow sites. Plants were paired as closely as possible by similarity in height, phenology stage, and bud number, after they had recovered from transplanting. At the East River site, one of each of the 12 pairs, chosen at random, was placed next to the road (0 meters) and the other of each pair placed far away (50 meters). At the research meadow site an identical approach gave me two arrays of 12 plants each separated by ten meters arranged in rows of 4 x 3. One array was manually dusted and the other was left as a control. The plants at the research meadow were dusted using on average two – five puffs from a Gilmour “Spray-Doc Gardening Hand Duster” that had dust collected from the East River valley to ensure the same dust was being analyzed in both treatments. The two research meadow arrays were rotated every day to control for confounding variables such as humidity, sun exposure, greater pollinator advantage, etc. The plants that were used for this experiment came from the research meadow for manually dusted experiments. Potted plant arrays were within 10 meters of the original location of the plants, and plants were returned to their original location once fruits had been collected. The plants from the road proximity experiments came from the East River valley and

were moved less than 50 meters from their home location. Again, plants were returned to their home site at the end of the experiment.

Pollinator Observations:

I recorded pollinators at each field site. I sat quietly more than three meters from the potted plants at each array and recorded visits by bumblebee queens, solitary bees, and small insects. I observed the near and far potted plants in the East River valley site as well as the dusted / non-dusted arrays at the research meadow site for a half hour at a time. This was done to ensure I was observing pollinators when they were experiencing the same abiotic conditions. The primary pollinators of *D. nuttallianum* were bumblebee queens.

Pollen loads and seed set:

Roughly three days after bringing the potted plants in from the field, stigma slides were prepared in order to count pollen grains and dust particles deposited. I collected stigmas from developed fruits (approximately two flowers per plant, ~ six stigmas per plant). Stigmas were squashed on glass microscope slides and I used basic fuchsin-tinted glycerin jelly to color the pollen grains and I used an alcohol burner to further melt stigmas as needed. Pollen loads and dust deposition were counted at X100 magnification with a Zeiss Standard compound microscope. One unit of the ocular micrometer scale under the microscope at X100 was ~ 9.5 micrometers. Based on the literature, dust is between 2-10 micrometers (Field et al. 2009; Toy et al. 2002). Therefore, I would only count dust particles that were one unit or less in diameter. Lastly, I counted maturing seeds within the same fruits whose stigmas were scored for pollen. Both the stigmas and

the carpels were scored in order to relate pollen count to seed set.

Statistical analysis:

I used JMP 9.0 to analyze my data. I used an ANOVA to test the effects of road dust on pollinator visitation, seed set, and pollen/ dust particles deposited. I used a linear regression to test how increasing proximity from the road affects deposition of particulates.

Results:

Deposition of dust:

Deposition of dust initially declined precipitously with distance from the road, with a mean of 118.8 particles per 0.0576 mm² of filter paper at zero meters and a mean of 51.5 at ten meters from the road. Then, there was more of gradual decline as the means are similar for distances 20 meters and greater from the road (Table 1). Linear regression shows that the number of dust particles per area of filter paper declined significantly with distance from the road ($P = 0.0001$; Figure 2).

Pollinator observations:

East River valley: The average visitation rate for *D. nuttallianum* arrays in total near the road was zero visits per total array per half hour, and the average visit far from the road was 0.023 visits per total array per half hour. There was a significant difference in pollinator visitations at near and far arrays ($P=0.0017$). The average open flowers in the near array were 25 open flowers, whereas the average number of open flowers in the far

array was 31.75. Twelve 30-minute observation periods were accumulated over the course of the study.

Research meadow: The average visitation rate for *D. nuttallianum* arrays in total at dusted arrays was 0.003 visits per total array per half hour, and the average visit for flowers at the non-dusted arrays was 0.017 visits per total array per half hour. There was no significant difference in pollinator observations at dusted and non-dusted arrays ($P=0.532$). The average number of open flowers in the dusted array was 37.6, whereas the average number of open flowers in the non-dusted array was 43. Eight 30-minute observation periods were accumulated over the course of the study.

Pollen loads and seed set:

East River valley: *D. nuttallianum* flowers growing close to the road (0 meters) received an average of 40.1 pollen grains per stigma, whereas flowers farther from the road (50 meters) received 51.3 pollen grains per stigma. This is a slight difference in pollen deposition although not significant ($P = 0.28$; Table 3). Contrastingly, the average seed set per carpel for flowers growing near the road was 5.2, whereas the average seed set per carpel for flowers growing far from the road was 5.0. There was no significant difference in seed set for near and far arrays ($P=.79$; Table 5). Plants near the road had an average of 11.2 dust particles per stigma whereas plants far from the road had an average 2.5 dust particles per stigma. The amount of dust deposited per stigma was significantly different between near and far plants ($P=0.0002$; Table 6).

Research meadow: *D. nuttallianum* flowers that were manually dusted received an average of 123.3 pollen grains per stigma and flowers not exposed to dust received an

average of 103.4 pollen grains per stigma. There was not a significant difference between amounts of pollen grains on dusted or non-dusted plants ($P = 0.14$, Table 2).

Contrastingly, the average seed set for dusted flowers was 4.5 and the average seed set for non-dusted flowers was 6.8. There was significant difference between seed set in dusted and non-dusted treatments ($P = 0.0017$; Table 4). Plants that were dusted had an average of 11.2 dust particles per stigma and plants that were non-dusted had an average 3.8 dust particles per stigma. The amount of dust deposited per stigma was also significantly different between dusted and non-dusted plants ($P = 0.0001$; Table 7).

Discussion:

During the course of the study, I found that dust deposition decreased significantly as distance from the road increased (Fig. 2). This heavy anthropogenic movement of particulates near the road may explain why pollinators were dissuaded by the near arrays in the East River Valley. Pollinator observations showed that pollinators significantly preferred to forage on plants that were far from the road rather than near. This is supported by a similar mechanism to what Ndibalema et al. (2008) saw in African ungulates. As the food sources become dusty, animals will avoid those food sources. The pollinator visitation rates at the research meadow, where the same dust from the East River valley was applied to the larkspur, saw no significant difference in visitation from pollinators even though the level of dust on stigmas was similar to that observed at the East River site. This result leads me to believe that there is another abiotic factor that is more prevalent near the road than just dust itself. This abiotic factor could be increased level of traffic, pollution from car exhausts, or a combination of the two. Similarly, behavioral observations were different at both treatments. Many pollinators at the near

array in the East River valley site would forage on a single flower and then leave immediately whereas at the research meadow, the pollinators did not show behavioral differences between the two plant arrays. A possible explanation for the difference in pollinator behavior could be that the research meadow arrays did not receive enough dust, and perhaps it was enough dust to influence reproduction but not enough to dissuade pollinators. Likewise, there may have been not enough observations in the research meadow, which could explain an unclear pattern of visitation rates.

Dust deposition on stigmas was significantly different in both the research meadow and the east river valley treatment (Fig. 3). This is similar to what the filter paper discs revealed about the deposition pattern of dust as distance from the road increased. The difference in the manually dusted arrays confirms that there was adequate dust application but perhaps not enough to cause substantial changes in pollinator behavior. However, based on my results, it can be said that pollen receipt is not affected by dust itself (Fig. 4). For example, *D. nuttallianum* plants that were manually dusted had more pollen grains deposited, 123.3 vs. 103.4, but a significantly lower seed set, 4.5 vs. 6.8 (Fig. 5). This could be an example of how dust particles are not affecting the adhesion of pollen grains but are affecting the physiology, and ultimately, lowering reproductive rates. Perhaps, similar to what Thompson et al. (1984) saw when dust was placed on the surfaces of leaves, photosynthetic rate decreased, and the larkspur could not afford to allocate energy to developing seeds.

In contrast, plants that were in the East River valley displayed no significant difference in pollen grains deposited or seed set. The larkspur in the east river valley had an unclear pattern possibly due to a smaller sample set due to herbivore attacks during the

course of the experiment. The final sample size was nine plants for the east river valley after three attacks. I kept account of what plants were eaten, their flower stage, and their final destination. Although information about how long the plant was exposed to the treatment was recorded, the final numbers may not hold enough information to get an accurate picture of what is happening in nature. Future studies should demonstrate a similar pattern seen in the research meadow, and perhaps, a stronger correlation due to pollution from car exhausts in addition to dust being deposited.

The study is a crucial step in discovering how roads affect the reproductive ecology of plants. During the summer months where the climate is driest, road dust on unpaved roads increases at alarming rates, dusting the vegetation and releasing pollutants from car exhausts. Based on my findings I can say that pollinators, namely bumble bee queens, are affected by another abiotic factor, other than dust, which causes them to avoid roads. The significantly different visitation rates in the east river valley suggest that they prefer carbohydrate sources away from the road, however, the unclear pattern in the research meadow in conjunction with a lack of significant difference in pollen loads in near and far arrays suggests that it is not the dust affecting bumble bee queens, but perhaps pollution from car exhausts or increased mortality rates or disturbance near road sides. Ultimately, the change in pollinator behavior is negatively affecting reproduction in my study, supporting the hypothesis that plant reproduction could be reduced indirectly by changing pollinator behavior, and ultimately, affecting reproduction.

Implementing policies to protect roadside vegetation and their pollinators will become increasingly important in the coming years. Successful completion of this task will require a better understanding of how dust affects all aspects of reproduction, and of

course a species-specific population model to predict the most sensitive stage in development. Perhaps creating a population matrix for *Delphinium nuttallianum* will give insights in the future to how to best protect and preserve native road side populations in alpine environments.

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Graphs and Tables:

Table 1: This displays the mean values of dust deposited per unit area (0.0576 mm^2) at each distance from the road as represented by level ($n=10$).

Least Squares Means Table – how distance from the road affects dust deposition				
Distance (m)	Least Sq. Mean	N	Std. Error	Mean
0	118.80000	10	9.72	118.800
10	51.50000	10	6.01	51.500
20	13.50000	10	2.21	13.500
30	9.60000	10	2.34	9.600
40	7.60000	10	1.12	7.600
50	7.20000	10	1.27	7.200

Table 2: Least squares means table and ANOVA of pollen grains per carpel at the research meadow at dusted (D) and non-dusted (ND) treatments.

Least Squares Means Table – pollens grains per carpel				
Level	Least Sq. Mean	N	Std. Error	Mean
D	123.32692	104	9.6649940	123.327
ND	103.41284	108	9.4407176	103.413

Analysis of Variance – pollen grains per carpel					
Source	DF	Sum of Sq.	Mean Sq.	F ratio	Prob.> F
Model	1	21105.7	21105.7	2.1725	0.1420
Error	211	2049835.3	9714.9		
C. Total	212	2070941.1			

Table 3: Least squares means table and ANOVA of pollen grains per carpel at the East River Valley in far (F) and near (N) treatments.

<u>Least Squares Means Table – pollen grains per carpel</u>					
Level	Least Sq. Mean	N	Std. Error	Mean	
F	51.276923	64	6.6354339	51.2769	
N	40.111111	45	7.9747990	40.1111	
<u>Analysis of Variance – pollen grains per carpel</u>					
Source	DF	Sum of Sq.	Mean Sq.	F Ratio	Prob. > F
Model	1	3315.23	3315.23	1.1584	0.2842
Error	108	309083.46	2861.88		
C. Total	109	312398.69			

Table 4: Least squares means table and ANOVA of number of seeds per carpel at the research meadow in dusted (D) and non-dusted (ND) treatments. Significant difference in seed set of both treatments with P = 0.0017.

<u>Least Squares Means Table – mean seeds per carpel</u>					
Level	Least Sq. Mean	N	Std. Error	Mean	
D	4.5000000	104	0.52073005	4.50000	
ND	6.8165138	108	0.50864650	6.81651	
<u>Analysis of Variance – mean seeds per carpel</u>					
Source	DF	Sum of Sq.	Mean Sq.	F Ratio	Prob. > F
Model	1	285.5946	285.595	10.1272	0.0017
Error	211	5950.3303	28.201		
C. Total	212	6235.9249			

Table 5: Least square means and ANOVA of number of seeds per carpel at the East River Valley in far (F) and near (N) treatments,

<u>Least Squares Means Table – mean seeds per carpel</u>					
Level	Least Sq. Mean	N	Std. Error	Mean	
F	4.9076923	64	0.58996321	4.90769	
N	5.1555556	45	0.70904754	5.15556	
<u>Analysis of Variance – mean seeds per carpel</u>					
Source	DF	Sum of Sq.	Mean Sq.	F Ratio	Prob. > F
Model	1	1.6336	1.6336	0.0722	0.7887
Error	108	2443.3573	22.6237		
C. Total	109	2444.9909			

Table 6: Least square means and ANOVA of dust deposited on stigmas at the East River Valley in near (N) and far (F) treatments.

<u>Least Squares Means Table – mean dust deposited on stigmas</u>					
Level	Least Sq. Mean	N	Std. Error	Mean	
F	2.485714	69	1.3848383	2.4857	
N	11.159091	44	1.7467138	11.1591	
<u>Analysis of Variance – mean dust deposited on stigmas</u>					
Source	DF	Sum of Sq.	Mean Sq.	F Ratio	Prob. > F
Model	1	2032.461	2032.46	15.1400	0.0002
Error	112	15035.372	134.24		
C. Total	113	17067.833			

Table 7: Least square means and ANOVA of dust deposited on stigmas at the research meadow in dusted (D) and non-dusted (ND) treatments.

<u>Least Square Means Table – mean dust deposited on stigmas</u>					
Level	Least Sq. Mean	N	Std. Error	Mean	
D	10.711538	104	0.76295699	10.7115	
ND	3.816514	108			
<u>Analysis of Variance – mean dust deposited on stigmas</u>					
Source	DF	Sum of Sq.	Mean Sq.	F Ratio	Prob. > F
Model	1	2530.183	2530.18	41.7944	<.0001
Error	211	12773.676	60.54		
C. Total	212	15303.859			

Figure 1: *Delphinium nuttallianum* field sites near The RMBL. The northern two points represent the East River valley plant arrays that were separated by 50 meters near the US Forest Service Road. The southeast two points represent the plant arrays located in the research meadow separated by ten meters and dusted manually/ left as controls.



Figure 2: Mean dust particles deposited per area of 0.0576 mm^2 as a function of distance from the road (0-50 meters). There was a significant difference between dust deposited and distance from the road with $P = 0.001$. The error bars represent the 95 % confidence interval for each point.

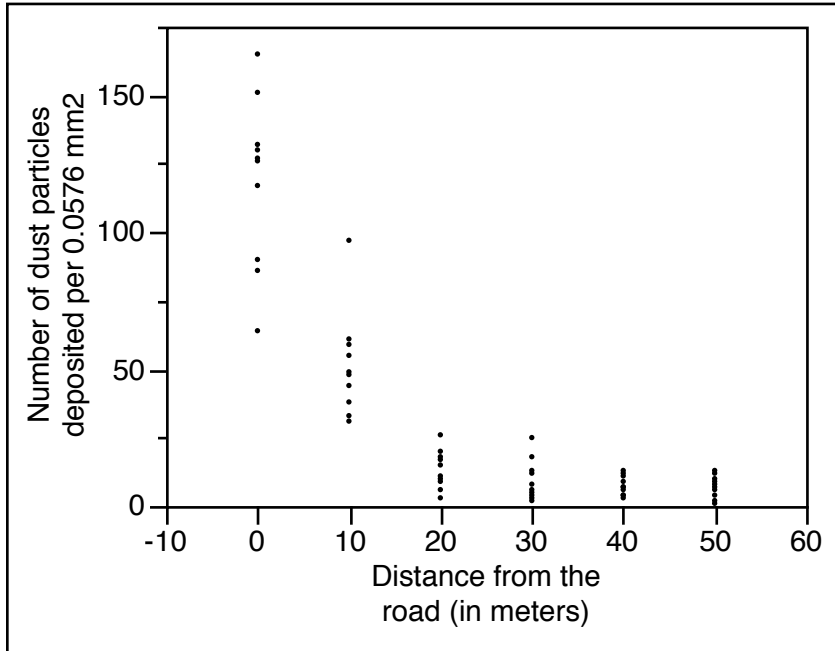


Figure 3A and B: Mean values $\pm$ SER of dust particles deposited on stigmas in the East River Valley (Fig 3A) and research meadow (Fig 3B). Asterisks represent significant difference between the treatments in dust deposited. $P=0.002$ for Fig 2A, and $P=0.0001$ for Fig 2B.

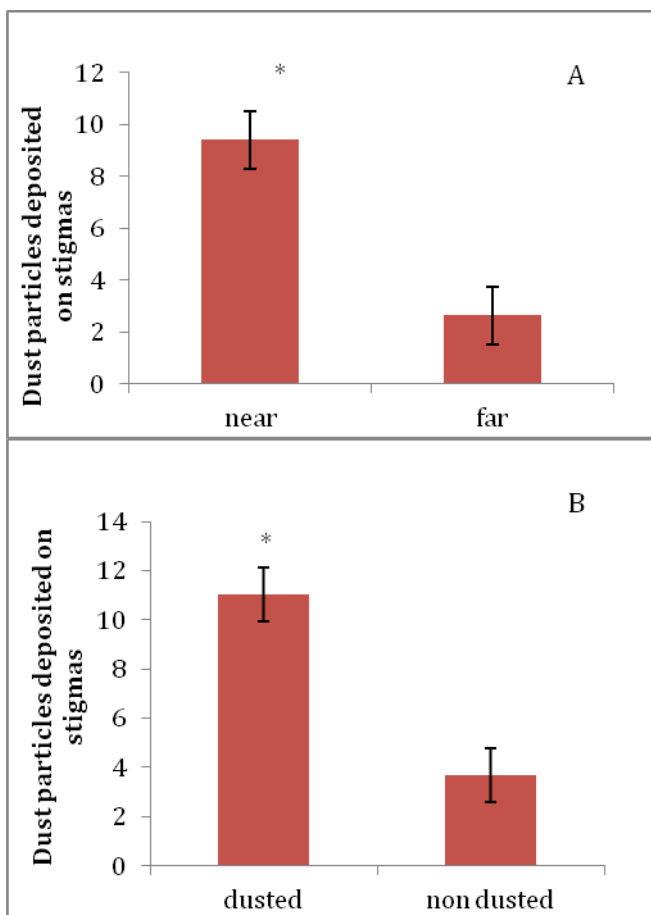


Figure 4: Mean values $\pm$ SER of pollen grains per carpel at each treatment. Near (N) and far (F) represent the two treatments at the East River Valley. Dusted (D) and non-dusted (ND) represent the two treatments at field site research meadow.

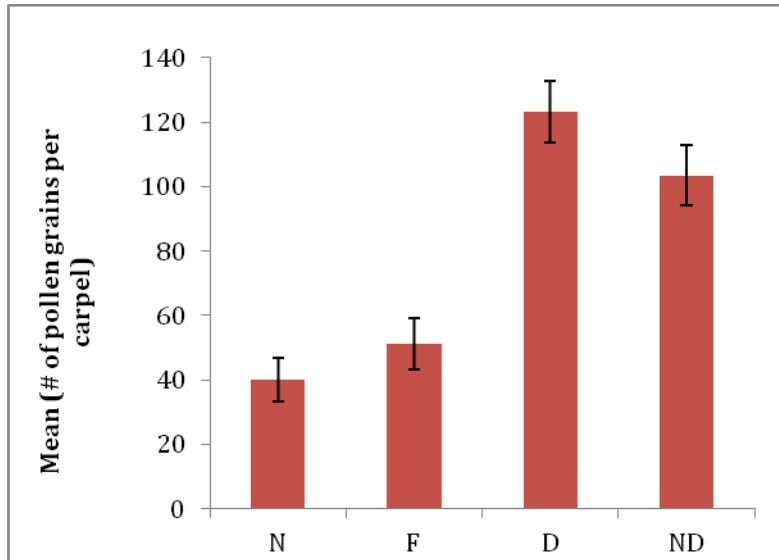


Figure 5: Mean values $\pm$ SER of number of seeds per fruit at each treatment. Near (N) and far (F) represent the two treatments at the East River Valley. Dusted (D) and non-dusted (ND) represent the two treatments at the research meadow, asterisks represent a significant difference between seed set in D and ND with a P- value of 0.017.

