Correlating road dust with reproduction of *Ipomopsis aggregata*

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Independent Research and Methods in Field Ecology Course
Summer 2014

Abstract

Roads potentially impact nearly one-fifth of the continental United States. Runoff, traffic disturbance, and maintenance associated with roads can affect both the abiotic and biotic features of roadside ecosystems. Species along unpaved roads are particularly susceptible to road dust effects. Plant physiology, such as gas exchange and overall productivity, is found to deteriorate in increased dust conditions. However, few studies have examined how dust influences plant reproduction. In this study, I examined how road dust impacts pollen delivery, pollen adhesion, and the translation of pollen to seed in Rocky Mountain populations of *Ipomopsis aggregata*. However, no stage of reproduction in *I. aggregata* was correlated with different dusting treatments, perhaps because visitation rates and pollen loads were high from high levels of pollinator activity. This study highlights the importance of temporal variation in future studies of road dust effects on plant reproduction.

Introduction

Public roads in the United States total over six million kilometers in length and cover about 1% of the country (Central Intelligence Agency, accessed 2014). In fact, at least half of the continental United States is within two kilometers of a public road (Central Intelligence Agency, accessed 2014). With this massive infrastructure, it is no surprise that Forman and Deblinger (2000) estimated the United States' ecological road-effect zone to cover 15-20% of the total land area. Therefore, research in this field of "road ecology" is crucial to understanding the total effect of human encroachment on the natural system.

Although some studies report benefits of properly managed roads and roadsides (e.g. increased species richness), roads often disturb surrounding ecosystems (Forman and Alexander 1998). For example, road runoff alters stream ecology (Forman and Alexander 1998) and leaches heavy metals (Niemela et. al 2004), traffic facilitates population fragmentation and decline (Forman and Alexander 1998), and road maintenance benefits invasive species establishment by disturbing the soil (Gelbard and Belnap 2002). These ecological effects are associated with both paved and unpaved roads. However, unpaved roads often present a unique arena for road ecology research: the ecological effects of road dust (Farmer 1993).

Generally, areas near the road (0-20m) experience the greatest dust effect (Forman et. al. 1997). Dust accumulation in this zone is correlated with shifts in both invertebrate (Farmer 1992; Kalisz and Powell 2003), and plant (Farmer 1993; Walker and Evertt 1987) communities. Furthermore, many studies report decreased photosynthetic rate, gas exchange, growth, and overall plant productivity in dustier environments (Wijayratne et. al 2009 and Farmer 1993). However, relatively few studies have examined how dust effects plant reproduction.

Angiosperm reproduction can be separated into three general steps: pollen delivery, pollen adhesion, and the translation of pollen to seed. For plants dependent on animal vectors for pollination, the first step is highly dependent on pollinator behavior. However, the latter two steps depend more on stigma receptivity, plant physiology, and resource allocation. Road dust could ultimately impact reproductive success through each of these stages. In fact, one study of the hummingbird and insect pollinated herb, scarlet gilia (*Ipomopsis aggregata*), suggests that pollen count and seed set decrease with increased proximity to an unpaved road (Waser and Price, unpublished data). However, sufficient visitation data were not collected in that study to investigate how dust impacts pollen delivery.

Through this research, I intended to expand road ecology by exploring the understudied relationship between road dust and plant reproduction. I examined how road dust impacts the three stages of reproduction in Rocky Mountain populations of *Ipomopsis aggregata* in two sites with different dust environments. At two distances from an unpaved road at each site, I quantified dust deposition rates, pollinator visitation rates, stigmatic pollen counts, and seed sets. In particular, I asked: Do increased dust loads correlate with pollinator visitation rate, pollen counts, and seed sets of *I. aggregata?*

Methods

Study Organism

Ipomopsis aggregata is a perennial native to Western North America. Between June and August, individuals display bright red tubular flowers on a racemose panicle. In the Rocky Mountains of Colorado, *I. aggregata* is predominately pollinated by two generalist hummingbird species, the Broad-Tailed Hummingbird (*Selasphorus platycerus*) and Rufous Hummingbird (*Selasphorus rufus*). *I. aggregata* is pre-zygotic self-incompatible and produces tricarpellate capsules vulnerable to pre-dispersal seed predation by *Hylemya* (Brody and Morita 2000). Importantly, this species is found along unpaved roads and has a well documented reproductive history.

Study Site

This study was conducted near the Rock Mountain Biological Laboratory, in Gothic, Gunnison County, Colorado (38.965^{0}N, 106.993^{0}W) I selected two research sites along County Road 317, an unpaved gravel road that traverses the town site (Fig. 1). The road along the southern site received a magnesium chloride dust suppression treatment the week of 22 June 2014. The road along the northern site was untreated. These two sites were used to inform the effect of different dust deposition rates on visitation rate, stigmatic pollen count, and seed set. *Ipomopsis aggregata* grew naturally at both sites.

Experimental Design

To assess the effects of road dust on *I. aggregata* reproduction, I studied plants at near (2.5m) and far (47.5m) distances from an unpaved road at both the northern and southern sites. I collected data on pollinator visitation, stigmatic pollen count, and seed set. During the study, I performed two experiments: the first used potted plants while the second used naturally growing plants.

Potted Array Experiment

Plant selection and pairing

Thirty plants from each site were potted on 25 June and 5 July. Plants at the northern site and southern site were selected from large patches 2.5m and 50m from the road, respectively. Individuals with more than one flowering stalk were not selected in order to reduce variance in life history and morphology. Plants were paired according to height within each site. Potting and pairing plants helps control for different growing conditions, such as soil composition and moisture content, and plant characteristics, including flower number and plant size.

Distance assignment and array design

Within each pair, I randomly assigned plants to near (2.5m, dusty) and far (47.5m, less dusty) distances from the road. Distances were selected based on data that described a negative, non-linear relationship between dust deposition and road proximity (Forman and Alexander 1998). Excess dust was gently rinsed from plants before assembling arrays. On 7 and 8 July, I assembled arrays at the northern site and southern site, respectively (Fig. 1). Each array was approximately 4.0 x 0.5m with two rows of offset plants parallel to the road. Similar vegetation surrounded the arrays within a site. Plants within an array were initially arranged by height. However, plants at both sites were randomly reordered within arrays on 10 July to account for larger visual cues that could results from larger plants in close proximity. Plants were collected from both sites on 12 July due to decreased flower counts at the northern site from deer herbivory.

Natural Plot Experiment

Plots of naturally growing *I. aggregata* were selected at near and far distances from the road in each site on 15 July (Fig. 1). Similarities in the number of flowering stalks, stage of flowering, and surrounding vegetation, were used to determine plot size (approximately 8.0 x 2.0m) and location. Plants with multiple flowering stalks were included within arrays.

Sampling Procedures

Dust Deposition

I characterized dust deposition rate to quantify the dust environment at each site and at increasing distances from the road. Three sets of filter paper discs (Whatman #1, 9cm diameter) were placed at six distances from the road in each site to determine dust deposition rate (2.5m, 10m, 20m, 30m, 40m and 47.5m) (Fig. 1). I placed two sets in transects near the potted arrays, and one set in transects near the natural plots. Discs were positioned parallel to the ground using nails and exposed for 11-28hr during the two-week period of pollinator observations. Within a set, date and time exposed were similar between sites. For each disc, I counted dust particles in ten, haphazardly selected 0.096mm^2 transects under dissecting microscope (50x). No transects were taken from a disc center or edge in order to decrease particle count variation due to handling.

Visitation

Potted array experiment

I observed pollinators to quantify pollen delivery. Pollinators were observed visiting the potted arrays between 8 and 12 July. Prior to each observation period, observers counted the number of open flowers on each plant. Morning, afternoon, and evening observations were conducted 8:30-12:00, 13:00-16:00, and 19:00-21:00, respectively. Near and far arrays were observed five times at the northern site (observation periods: two mornings, two afternoons and one evening) and six times at the southern site (observation periods: two mornings, three afternoons and one evening).

Two observers consecutively watched both arrays at a site during each observation period. An observer sat approximately 5m from an array so as not to disrupt pollinator behavior. I identified pollinators as hummingbirds, hawk moths, butterflies, or bees. The number of flowers a pollinator visited was recorded per plant. Each floral visit was recorded independently, regardless of whether a pollinator visited one flower multiple times. Total time observing per observation period did not exceed one hour.

Natural plot experiment

A similar protocol was followed for observing pollinator visitation to the natural plots. Observations were performed 15-18 July. For each observation period, both the total number of open flowers and the number of flowers visited per pollinator were recorded per plot. Each site was observed on three mornings, three afternoons, and one evening.

Stigmatic Pollen Count

I counted stigmatic pollen loads to quantify pollen adhesion. A maximum of three stigmas were collected from each of the plants in the potted arrays on 12 and 13 July. Three stigmas were also collected from each of the fifteen plants in each natural plot on 19 July. Stigmas were optimally selected from recently abscised flowers at different positions on a plant. This protocol ensured that selected stigmas had been exposed during the experimental treatment and did not introduce a position effect on pollen count within plants. I marked calices of collected stigmas with permanent marker or acrylic fabric paint for subsequent determination of seed set. One slide of stigma squashes was created per plant. Basic Fucshin gel was used to dye the pollen in preparation for pollen counts. The number of pollen grains per stigma was determined using Köhler Illumination under a compound microscope (25x and 100x).

Seed Set

I counted mature, full-sized seeds to quantify the translation of pollen to seed. Fruits from potted plants were harvested from flowers marked during stigma collection between 30 July and 5 August. Fruits from natural plots were collected 7 August. All fruits were oven-dried prior to determination of seed set per flower to simplify the task of counting seeds.

Data Analysis

I performed all analyses using the JMP 11 (SW) statistical program. The same analyses were conducted for both experiments unless noted otherwise. Analyses of variance were two-way ANOVAs including the site, array, and the interaction term.

Dust Deposition

I averaged dust deposition rate (particles/cm^2*hr) over transects for each disc. The average dust deposition rate per disc was analyzed as a function of the distance from the road. Reduced two and three parameter exponential decay models of this relationship were compared using AICc. I used a sums of squares reduction test to determine if the pattern of dust deposition rate differed between sites. For discs placed at 2.5m and 47.5m from the road, I performed a one-way ANOVA and a Tukey's HSD test to determine if dust deposition rates significantly differed by site and distance.

Visitation

Visitation rate was calculated for each observation period at an array or plot as the total number of visited divided by the number of open flowers multiplied by the length of the observation period (visits/flower*hr). An ANOVA was performed to determined if site or distance from the road influenced visitation rate. Visitation rates in the potted experiment were square root transformed to improve the normality of the residuals.

Stigmatic Pollen Count

I averaged pollen counts (grains/stigma) for each plant. An ANOVA was performed to determine if site or distance from the road influenced pollen count. For each experiment, two outlying pollen counts were excluded from the analysis based on the residual scatter and the distribution of the raw data.

Seed Set

I averaged seed set (seeds/flower) for each plant. An ANOVA was performed to determine if site or distance from the road influenced seed set. Missing or parasitized fruits were excluded from the analysis. For each experiment, seed set was square root transformed to improve the normality of the residuals.

Results

Dust Deposition

Dust deposition rate decreased exponentially with increasing distance from the road at both sites, and was higher at the northern site at all distances (Fig. 2). A three parameter exponential decay model was a better fit (AICc = 477.7) for dust deposition rate compared to a two parameter model (AICc =

480.5). A sums of squares reduction test indicated that the exponential decay curves for dust deposition rate significantly differed between sites ($F_{3,30} = 48.520$, $p<0.0001$). Two of the three parameters, asymptote and scale, significantly differed between the site models.

Dust deposition rate was ranked by site and distance in order of decreasing dust loads (particles/cm^2*hr): near north (861), near south (226), far north (126), and far south (63). Dust deposition rate significantly differed between the near location at the northern site and all other locations (Tukey's HSD: Q = 3.20, alpha = 0.05). Visitation rate, stigmatic pollen counts, and seed sets were expected to inversely correlate with dust deposition rate (Table 1).

<u>Visitation</u>

<u>Potted array experiment</u>

Visitation rate was ranked by site and distance in order of increasing visitation rate (visits/flow*hr): south far (0.021), south near (0.032), north far (0.039), and north near (0.065) (Table 1). This ranking did not correlate with decreasing dust loads. Visitation rate did not significantly differ among the dusting treatments ($F_{3,7} = 0.170$, $p=0.916$).

<u>Natural array experiment</u>

Visitation rate was ranked by site and distance in order of increasing visitation rate (visits/flow*hr): south far (0.065), north near (0.073), south near (0.103), and north far (0.235) (Table 1). This ranking did not correlate with decreasing dust loads. Visitation rate did not significantly differ among the dusting treatments ($F_{3,24} = 2.516$, $p=0.082$).

<u>Stigmatic Pollen Count</u>

<u>Potted Array Experiment</u>

The average pollen count per stigma was ranked by site and distance in order of increasing pollen loads: south near (99), north far (130), north near (144), and south far (149) (Table 1). This ranking did not correlate with decreasing dust loads. Average pollen counts did not significantly differ among the dusting treatments ($F_{3,45} = 1.411$, $p=0.252$).

<u>Natural Plot Experiment</u>

The average pollen count per stigma was ranked by site and distance in order of increasing pollen loads: south near (175), south far (196), north far (222), and north near (233) (Table 1). This ranking did not correlate with decreasing dust loads. Average pollen counts did not significantly differ among the dusting treatments ($F_{3,54}$ = 1.150, p=0.337).

Seed set

Potted Array Experiment

The average seed set per flower was ranked by site and distance in order of increasing seed set: north near (1.84), south near (1.92), north far (2.05), and south far (2.22) (Table 1). This ranking correlated with decreasing dust loads. However, average seed set did not significantly differ among the dusting treatments ($F_{3,41}$ = 0.0302, p=0.993).

Natural Plot Experiment

The average seed set per flower was ranked by site and distance in order of increasing seed set: south near (3.93), north far (4.84), north near (5.12), and south far (5.69) (Table 1). This ranking did not correlate with decreasing dust loads. Seed set did not significantly differ among the dusting treatments ($F_{3,53}$ = 0.707, p=0.552).

Discussion

I hypothesized that road dust could influence the reproductive success of *I. aggregata* through three stages in reproduction: pollen deposition, pollen adhesion, and the translation of pollen to seed. If visitation rate, stigmatic pollen count, and seed set were influenced by road dust, I expected these variables to inversely correlate with increasing dust deposition rate. My results indicated that no stage of reproduction in *I. aggregata* was correlated with dusting treatment (Fig. 3-5). Three of the four dusting treatments had statistically similar dust deposition rates. Furthermore, visitation rates, stigmatic pollen counts, and seed sets were high compared to values recorded in previous studies (Price et al. 2005; Price and Waser, unpublished data). In general, this study suggests that the effects of road dust on plant reproduction may be sensitive to high levels of pollinator activity.

Visitation rates in this study approached maximum visitation rates reported in a seven-year study of *I. aggregata* pollination conducted near the Rocky Mountain Biological Laboratory (Fig. 3; Price et al. 2005). Migratory rufous hummingbird and hawk moth populations were high compared to previous summers (Nick Waser, personal communication). In addition, juvenile hummingbirds at the northern site were likely an indicator of increased hummingbird activity associated with a nearby nesting site.

However, high pollinator abundance would not necessarily attenuate the effects of road dust on pollination behavior. Hummingbirds, the primary pollinator of *I. aggregata*, use flower color as a visual cue while foraging. Depending on their spectral sensitivity, hummingbirds could be deterred from dusted flowers. However, Meléndez-Ackerman et al. (1997) showed that nectar reward is central hummingbird foraging behavior, where a red flower color is merely a morphological cue that hummingbirds learn to associate with higher nectar rewards. If dustier flowers did not differ in nectar reward compared to less dusted flowers, then it is unlikely that visitation rates would change with increased dust loads. Furthermore, even if nectar rewards differed across dusting treatments, juvenile hummingbirds and migratory rufous hummingbirds could be less attuned to the locations of prime foraging areas compared to adult broad-tailed hummingbirds that breed near the laboratory in summer. Cumulatively, the abundance, species, and age of pollinators appear to significantly influence visitation rates and visitation patterns.

This trend for high visitation rate was subsequently reflected in high stigmatic pollen counts (Fig. 4). Notably, pollen counts in this study were higher than those recorded for similar studies conducted near the Rocky Mountain Biological Laboratory in 2013 (60-160 pollen grains; Waser and Price, unpublished data). Harper (1979) suggests that "...dust can foul stigmatic surfaces and essentially eliminate pollination of obligate out crossers [*sic*]" for self-incompatible species. However, even though dust particles were observed on stigma lobes while counting pollen grains, overall pollen counts were still higher than anticipated.

Higher seed sets in the natural experiment compared to the 2013 study (2-5 seeds) reflected high visitation rates and pollen counts (Fig. 5; Waser and Price, unpublished data). Interestingly, recent research on the endangered shrub, *Hesperidanthus suffrtescens,* showed that decreasing distance from an unpaved road resulted in lower fruit set per plant, but not lower seed set (Lewis 2013, master's thesis). The same study also reported significantly decreased stomatal conductance in leaves. Leaves allocate resources to fruits during maturation and, if leaf productivity were reduced by dust, then fewer fruits would probably develop (Stephenson 1981). *I. aggregata* individuals naturally growing near the unpaved road had visibly dusty leaf surfaces (personal observation). Therefore, fruit set per plant could be reduced in *I. aggregata* through a decrease in leaf productivity. However, as Lewis (2013) showed, lowered fruit set is not necessarily related to decreased seed set. Especially in years of high visitation, it appears that seed set in *I. aggregata* is neither resource nor pollen limited by dust under natural growing conditions.

High pollinator abundance and the pollinator community structure may offset the anticipated consequences of road dust in each stage of *I. aggregata* reproduction. This conclusion underlies the importance of temporal variation in studies examining pollinator behavior (Price et al. 2005). Future research examining road dust effects on plant reproduction should include multiple research sites and seasons in the experimental designs. I also encourage future studies to focus on endangered plant species, or species pollinated by endangered pollinators that are found along heavily travelled unpaved roads. The effect of road dust effect on plant reproduction is sensitive to species, location, and time. However, untangling this facet of road ecology is crucial to understanding the deleterious effects of human activity on the environment.

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Appendix

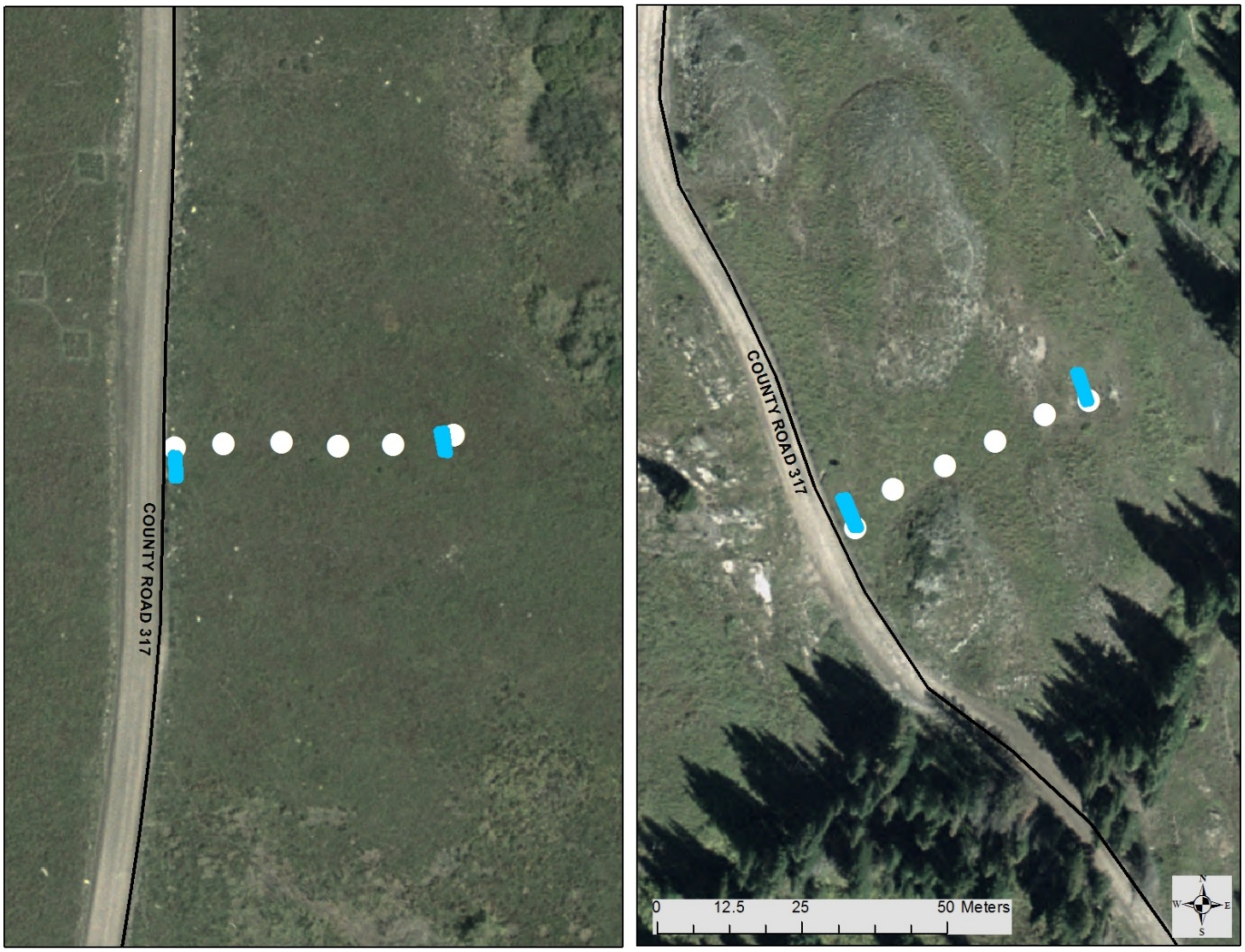

Figure 1. Research design for potted array experiment at southern (left pane) and northern (right pane) sites. The natural experiment was located across the road at the southern site and about 100m north along County Road 317 at the northern site. Blue polygons are arrays or plots. White circles are filter paper discs. Data collected using Trimble® GeoXT™ and mapped in ArcMap 10.2.1.

Table 1. Summary of response variable measurements by site and distance from the road for both experiments. Ranks from 1 to 4 indicate an increase in the response variable. Expected ranks across locations are based on decreasing dust deposition rates (see Fig. 2).

Variable	**Experiment**	**North Near**	**South Near**	**North Far**	**South Far**
Expected	NA	1	2	3	4
Visitation rate	Potted	4	2	3	1
	Natural	2	3	4	1
Pollen count	Potted	3	1	2	4
	Natural	4	1	3	2
Seed set	Potted	1	2	3	4
	Natural	3	1	2	4

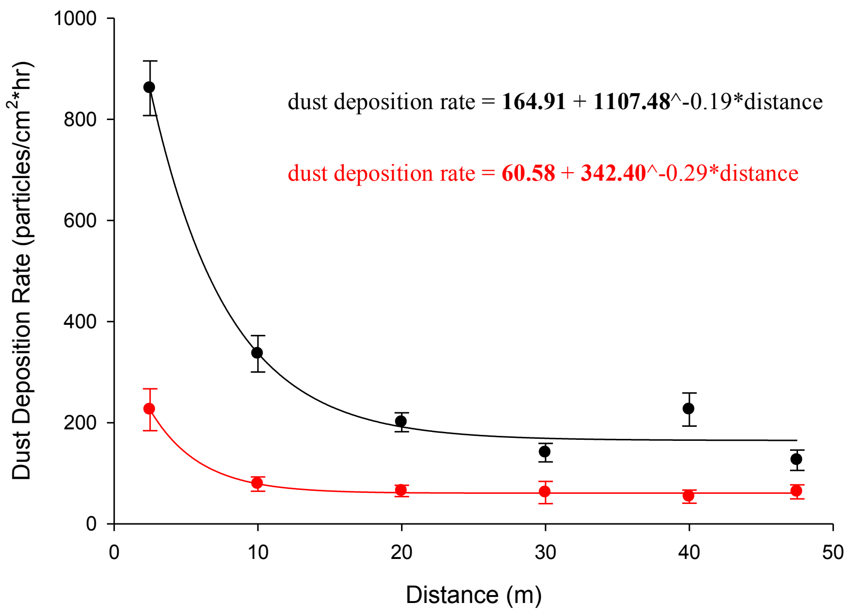

Figure 2. Pattern of dust deposition rate with increasing distance from the unpaved road at the southern (color) and northern (color) sites. Means ± SE calculated over the average dust deposition rate on filter paper discs (N=3, all distances). Fit lines are three parameter exponential decay models. Bolded equation terms indicate significantly different model parameters.

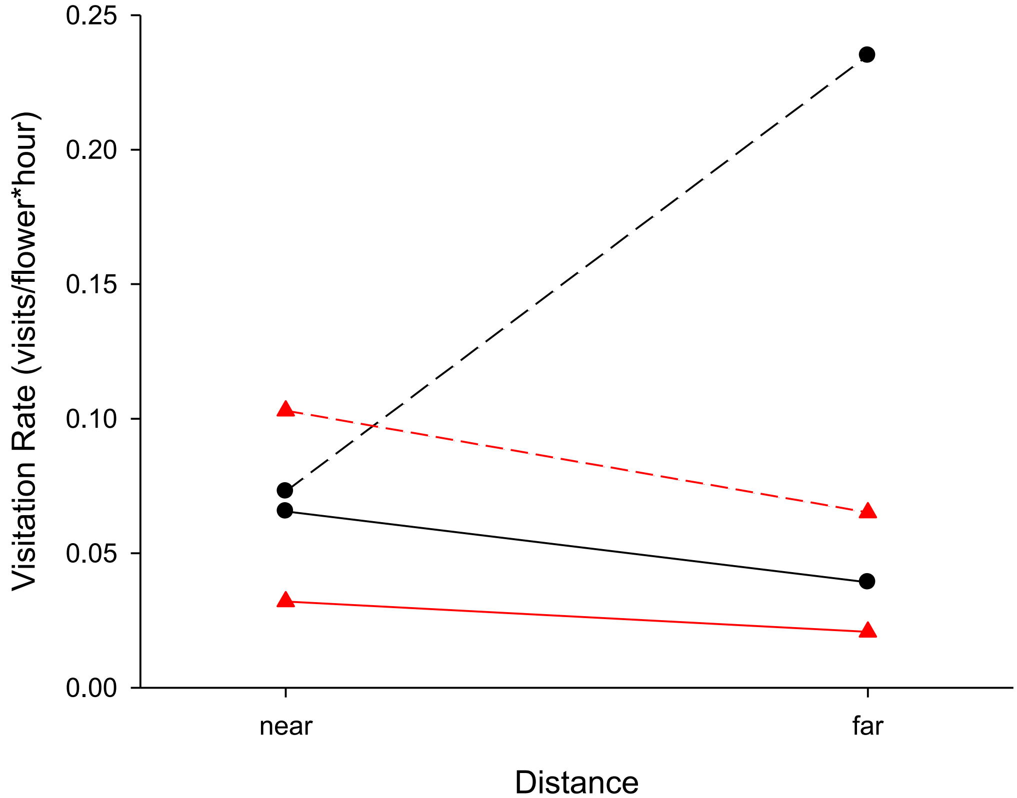

Figure 3. Visitation rates to potted arrays (solid line) and natural plots (dashed line) near and far from the unpaved road at the southern (red triangle) and northern (black circle) sites. Least squares means calculated over observation periods per array or plot (N_{potted} = 11; $N_{natural}$ = 22). Least squares means back transformed from square-root values for the potted experiment.

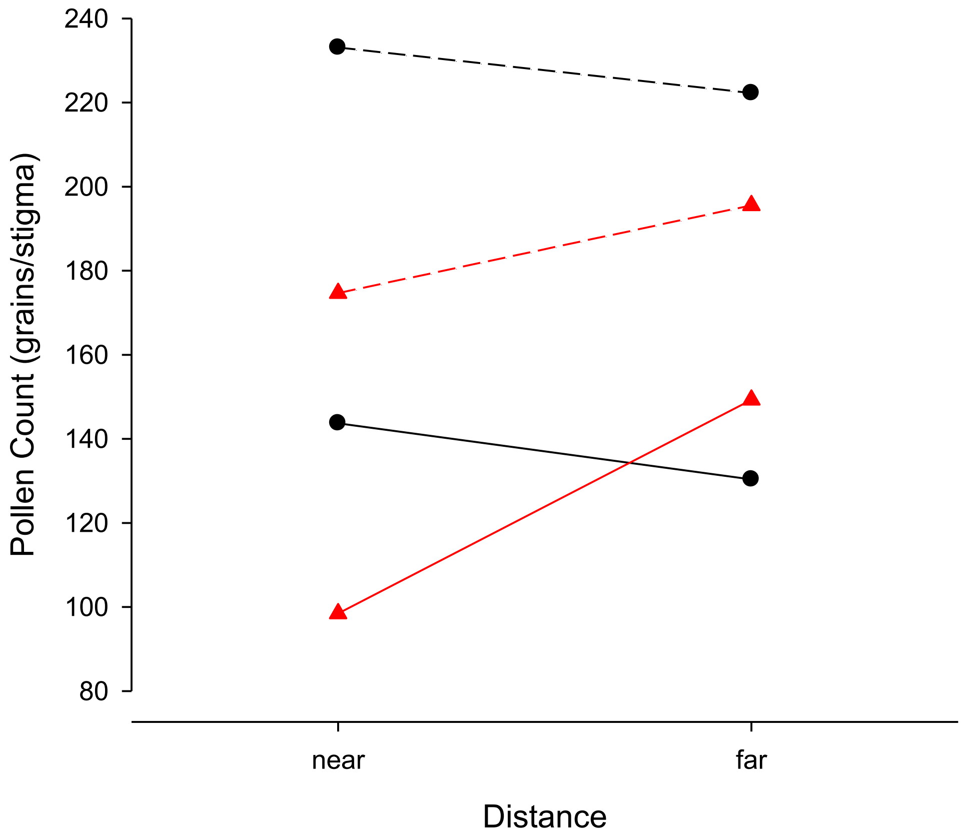

Figure 4. Stigmatic pollen counts for potted arrays (solid line) and natural plots (dashed line) near and far from the unpaved road at the southern (red triangle) and northern (black circle) sites. Least squares means calculated over the average pollen count per plant in an array or plot (N_{potted} = 49; $N_{natural}$ = 58).

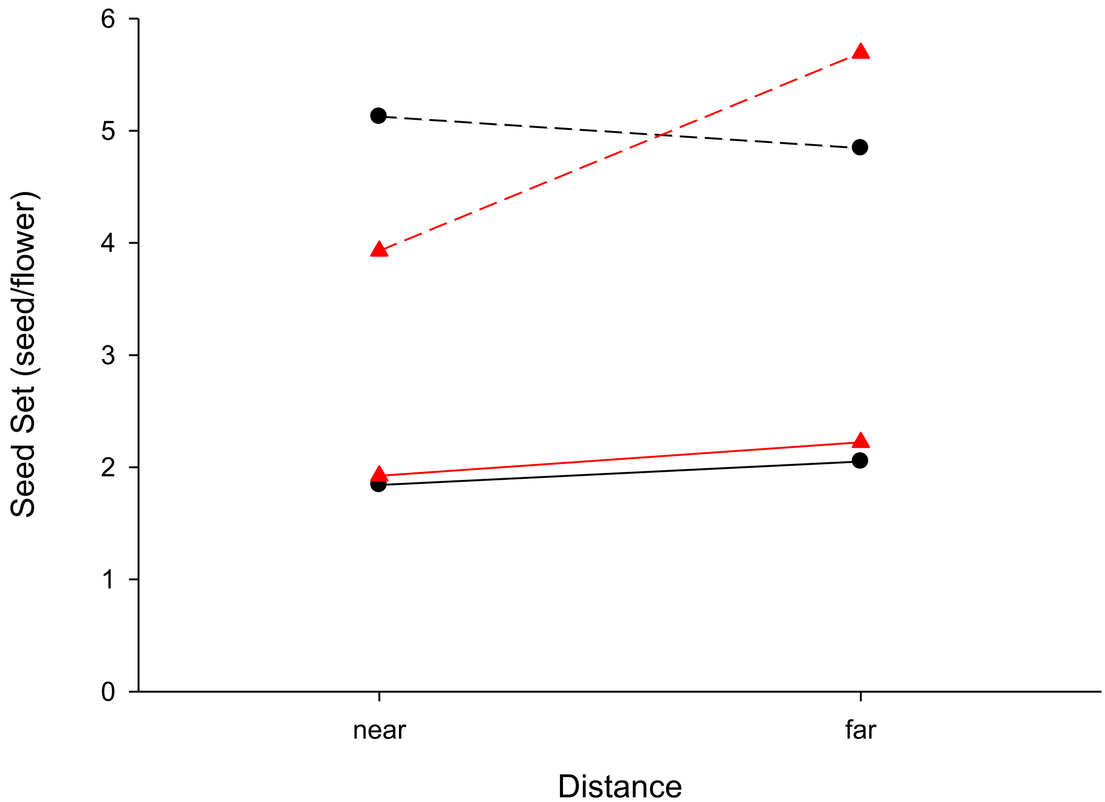

Figure 5. Seed sets for potted arrays (solid line) and natural plots (dashed line) near and far from the unpaved road at the southern (red triangle) and northern (black circle) sites. Least squares means calculated over the average seed set per plant in an array or plot (N_{potted} = 34; $N_{natural}$ = 54). Least squares means back transformed from square-root values for both experiments.