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EFFECTS OF ROAD DUST ON THE POLLINATION AND REPRODUCTION OF WILDFLOWERS

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Premise of research. Dust particles and pollen grains are similar in size. Dust deposition might therefore influence the pollination and reproduction of flowering plants. Little is known about such effects, however, despite more general interest in ecological effects of dust.

Methodology. We used observational and experimental methods to explore whether dust generated by traffic on unpaved roads affects the amounts of pollen received and numbers of seeds produced by four species of native wildflowers in the western United States.

Pivotal results. Flowers of Nuttall's larkspur (*Delphinium nuttallianum*), scarlet gilia (*Ipomopsis aggregata*), Lewis flax (*Linum lewisii*), and sulphur paintbrush (*Castilleja sulphurea*) growing 1–2 m from a road received substantially more dust and less pollen than those growing 40–50 m away. We observed the same pattern when we transplanted individuals of the first two species into pots and placed pots near to compared with far from a road. Experimental "hand dusting" of scarlet gilia and Lewis flax plants also reduced stigma pollen loads to a degree that resembled the average effect of road proximity for those species. On the other hand, numbers of seeds per flower ("seed set") did not vary consistently for any species as a function of road proximity or hand-dusting treatment.

Conclusions. Several mechanisms might contribute to the different effects of dust on pollen loads and seed set. We discuss four possible mechanisms, which we refer to as pollen excess, pollen quality, resource limitation, and compensatory herbivory. These mechanisms suggest avenues for further study of dust, pollination, and plant reproduction with this and other systems.

Keywords: *Castilleja sulphurea*, *Delphinium nuttallianum*, hand dusting, *Ipomopsis aggregata*, *Linum lewisii*, seed set.

Online enhancements: appendix tables.

Introduction

In German, the word *Staub* refers both to dust and to the pollen of flowering plants.[2] This dual definition is unsurprising. Pollen can resemble dust, which is defined as fine, dry, particulate matter with linear dimensions of microns to tens of microns, a size range that includes many pollen grains. These physical similarities between dust and pollen suggest that they might interact in more than a linguistic sense: dust might interfere with pollination (German *Bestäubung*) and thereby with plant reproduction.

Dust arises from natural sources, in particular from the scouring of soils in arid regions by wind, as well as from soil-disturbing human activities such as agriculture, livestock husbandry, and transportation along unpaved roads (Field et al. 2010). Most dust (including dust from roads) travels over short distances and is deposited locally. However, dust storms regularly cover large areas in dry regions such as the western United States (Steltzer et al. 2009), and some dust even reaches sufficient heights in the atmosphere to be carried over intercontinental distances (VanCuren and Cahill 2002; Prospero and Lamb 2003). Dust production is expected to increase with climate change and intensifying human land use (Field et al. 2010).

To date, research on ecological effects of dust has mostly focused on its consequences for ecosystem processes from local to global scales (Field et al. 2010). Much less is known about effects at the level of individual organisms. We do not know, for example, whether dust harms plant reproduction, even though there is ample reason to expect that it might. Consider possible direct effects related to the sequential phases of angiosperm reproduction: pollination, ovule fertilization, and seed maturation. Most angiosperm species rely on animals (primarily insects) for pollination (Ollerton et al. 2011), and pollinators might avoid dusty flowers, much as chewing herbivores avoid dusty or sandy forage

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[2] This Germanic introduction is not meant to slight Romance languages—the Latin root of "pollen" is a fine flour or meal, also implying dust.

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(Ndibalema et al. 2008; LoPresti and Karban 2016). Dust also could interfere with the culmination of the pollination phase and onset of the fertilization phase by inhibiting pollen adhesion or germination on the stigma, the receptive female floral organ, or through mechanical changes in the stigma that reduce subsequent pollen receipt (e.g., see Waser and Fugate 1986). Finally, dust-induced changes in plant resource status—e.g., via effects on leaf pH, light capture, or gas exchange through stomata—might reduce the number or mass of seeds that a plant can mature or the number of flowers it produces. To such direct effects might be added indirect ones. For example, airborne particulates generated by traffic on unpaved roads can alter soil chemistry (Kaliscz and Powell 2003), soil decomposition via effects on microbial biochemistry (Moorhead et al. 1996), and earliness of spring snowmelt (Walker and Everett 1987; Painter et al. 2007; Steltzer et al. 2009), all of which could influence the physiology of individual plant species and their reproduction.

Here we describe the effects of dust from unpaved roads on pollination and reproduction of four species of montane wildflowers. In studies conducted over four summers, we did the following: (1) We characterized dust deposition at the soil surface and on stigmas of flowers as a function of distance from roads. (2) Having found substantial dust deposition near the roads, we used both observational and experimental methods to ask whether dust influences the pollination phase of reproduction, assessed as loads of pollen on stigmas. The answer is that lower pollen loads are consistently associated with dust deposition. (3) Using the same flowers from which we had collected stigmas (with one exception), we then asked whether dust influences overall female reproductive output of plants, assessed as numbers of seeds set per flower (hereafter seed set). Here the answer is that seed set is not consistently associated with dust deposition. We discuss four mechanisms that might possibly contribute to these seemingly discrepant results, as a means of suggesting avenues for further research. Finally, we consider general conditions under which dust is likely to have observable effects on seed production.

Material and Methods

Study System and Sites

To explore possible effects of dust on pollen loads and seed production, we chose four perennial herbaceous wildflowers that are native to the mountains of western Colorado. These are Nuttall's (=Nelson's) larkspur *Delphinium nuttallianum* (Pritzel ex Walpers) (Ranunculaceae), scarlet gilia *Ipomopsis aggregata* (Pursh) V. Grant (Polemoniaceae), Lewis flax *Linum lewisii* (Pursh) (Linaceae), and sulphur paintbrush *Castilleja sulphurea* (Rydberg) (Orobanchaceae). All four species are common in meadows around Gothic, Colorado, the site of the Rocky Mountain Biological Laboratory (RMBL; lat. 38°58′N, long. 106°59′W; elevation 2900 m), and in the Grand Mesa-Uncompahgre-Gunnison National Forest (US Forest Service [USFS]) near this field station. All four species are animal pollinated, and they exhibit a range of floral phenotypes (fig. 1) and of animal pollinators (table 1). Furthermore, all species, and especially the first two, have been studied previously at the RMBL, so substantial background knowledge was available to guide our study.

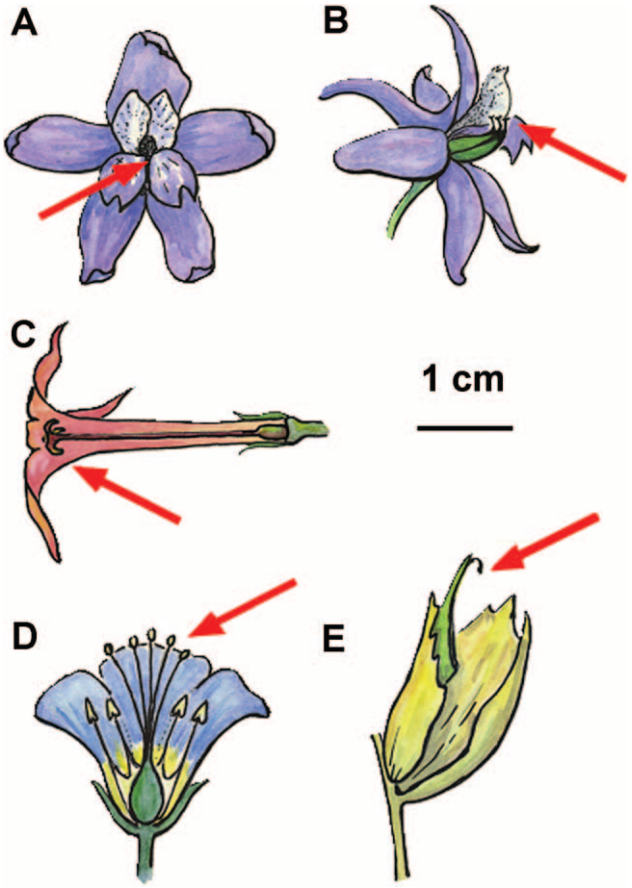

Fig. 1 Flower morphology of the study species. Arrows indicate stigma positions. *A*, *Delphinium nuttallianum*, frontal view showing five pigmented petaloid sepals, two whitish petals that contain the nectaries, and two pigmented "guard" petals that cover the sex parts. *B*, *Delphinium nuttallianum*, lateral view with right-hand nectar petal and guard petal removed to show positions of the three carpels, styles, and stigmas in female phase. *C*, *Ipomopsis aggregata*, lateral view with half of the corolla removed to reveal the pistil. The position of the three-lobed stigma ranges continuously across individuals from inserted within the corolla tube to exserted beyond the tube (Campbell et al. 1994). Illustrated is a common stigma position at the entrance to the tube. *D*, *Linum lewisii*, lateral view with two front petals and one front anther removed to show positions of the five stigmas. *E*, *Castilleja sulphurea*, lateral view of one flower and its subtending yellow bract, with neighboring bracts and flowers removed.

We examined dust effects on one or more of these wildflower species during four summers (2008, 2013, 2014, and 2015; see table 2 for details of years, species, and sites). We worked mostly in the East River Valley, either in Gothic or north of Gothic along USFS Road 317. In 2015 we added sites along USFS Road 811 in Washington Gulch, the parallel valley just to the west of the East River Valley, and along USFS Road 734 in the Slate River Valley, the parallel valley just to the west of Washington Gulch. All of these roads are unpaved and ca. 6 m wide. During the snow-free season (approximately June–September) the roads experience heavy use by private automobiles and pickup trucks (two axles), motorcycles, and larger vehicles (more than two axles). For example, Road 317 just north of Gothic averaged about 400–600 vehicles per day in the snow-free season from 2001 to 2013 and

Table 1

Characteristics of the Study Species

Species	Stigmas	Approximate stigma area (mm^2)	Pollen shape	Pollen diameter (μm)	Main pollinators around the RMBL
Delphinium nuttallianum	Usually 3; 2-lobed, dry	.40	Tricolpate	20–27	Hummingbirds, long-tongued bumble bee queens[a]
Ipomopsis aggregata	Single; 3-lobed, dry	.85	Spherical; 7–9 apertures	45–65	Hummingbirds, long-tongued bumble bee queens[b]
Linum lewisii	Usually 5; knob, dry	.55	Tricolpate	65–85	Anthomyiid, muscid, empidid, syrphid flies[c]
Castilleja sulphurea	Single; knob, wet	.40	Tricolpate	20–23	Bumble bees and hummingbirds[d]

Note. Receptive stigma areas were estimated at × 50 and pollen diameters at × 100. RMBL = Rocky Mountain Biological Laboratory.

[a] Broad-tailed hummingbirds (*Selasphorus platycercus*) and bumble bees (*Bombus appositus*, *Bombus nevadensis*, *Bombus californicus*, and *Bombus flavifrons*) in roughly equal proportions, with minor contributions from other insects (Waser 1978; Waser and Price 1981).

[b] Broad-tailed and rufous hummingbirds (*S. platycercus* and *Selasphorus rufus*) with minor contributions from bumble bees (primarily *B. appositus*) and other insects (Mayfield et al. 2001; Price et al. 2005).

[c] Kearns and Inouye (1994).

[d] Bumble bees (primarily *B. appositus*) and occasional broad-tailed and rufous hummingbirds (Pyke 1982; Waser 1983).

more than 700 per day in 2014 (Gunnison County Public Works Department, personal communication)—almost one vehicle per minute on average during daylight hours, when most use occurs. During the peak summer season in July, which corresponds roughly to the peak in density of wildflowers of all species (Cara-Donna et al. 2014), the counts must greatly exceed these averages, since the averages include periods of much lower use in spring and autumn. A noticeable plume of dust trails each vehicle (fig. 2), except during periods of rain, which suppresses dust. Dust-free periods are relatively rare during summer months in western Colorado, because rain comes largely from brief convective thunderstorms and ample sunshine dries road surfaces between storms.

Estimating Dust Deposition

To characterize how dust from the unpaved roads listed above is deposited on plants growing in adjacent subalpine meadows, we placed filter-paper disks (Qualitative; 9 cm diameter) on the soil surface along transects running at right angles from road verges. Disks were fastened to the soil using small steel nails. They were left in place for 1–4 rain-free days, after which we counted

Table 2

Features of 20 Separate Tests of Dust Effects on Pollen Loads and Seed Set, Arranged by Species and within Species by Year

Test	Species	Year	Valley	Site	Method
1	*Delphinium nuttallianum*	2008	East River	Avery	Natural near vs. far
2	*D. nuttallianum*	2014	East River	Carpenter	Potted near vs. far
3	*Ipomopsis aggregata*	2008	East River	Gothic	Potted dusted
4	*I. aggregata*	2013	East River	Gothic North	Potted near vs. far
5	*I. aggregata*	2013	East River	Gothic North	Potted dusted
6	*I. aggregata*	2014	East River	Gothic North	Potted near vs. far
7	*I. aggregata*	2014	East River	Gothic North	Natural near vs. far
8	*I. aggregata*	2015	East River	Gothic North	Natural near vs. far
9	*I. aggregata*	2015	East River	Friends' Pond	Natural near vs. far
10	*I. aggregata*	2015	East River	Gothic	Natural dusted
11	*I. aggregata*	2015	Washington Gulch	Low site	Natural near vs. far
12	*I. aggregata*	2015	Washington Gulch	High site	Natural near vs. far
13	*I. aggregata*	2015	Slate River	Low site	Natural near vs. far
14	*I. aggregata*	2015	Slate River	High site	Natural near vs. far
15	*Linum lewisii*	2015	East River	Gothic North	Natural near vs. far
16	*L. lewisii*	2015	East River	Gothic	Natural dusted
17	*L. lewisii*	2015	Washington Gulch	Low site	Natural near vs. far
18	*L. lewisii*	2015	Washington Gulch	High site	Natural near vs. far
19	*Castilleja sulphurea*	2015	East River	Avery	Natural near vs. far
20	*C. sulphurea*	2015	East River	Avery	Natural dusted

Note. "Valley" and "method" are described in the text. Gothic North (2920 m), Carpenter (2920 m), Avery (2930 m), and Friends' Pond (3005 m) are, respectively, 0.7, 1.4, 2.1, and 5.5 km up valley from the Rocky Mountain Biological Laboratory field station (Gothic, 2900 m) along US Forest Service Road 317. Low site (3020 m) and high site (3035 m) in Slate River Valley and Washington Gulch are along USFS Roads 811 and 734, respectively.



Fig. 2 A noticeable dust plume follows vehicles traveling on the unpaved roads used in this study, except during periods of rain.

dust particles within an area of 0.096 mm^2 at each of 5–10 haphazardly chosen points on each disk, using a dissecting microscope at × 50 magnification. In early years of the study, we sampled next to the road and at 10, 20, 30, 40, and 50 m from the road. Because this approach always yielded comparable results, we sometimes sampled only next to the road and at 50 m in later years of the study, simply to confirm differences in dust deposition next to study plants. Disks next to the road were placed as close as feasible (generally 1–2 m from the edge of the road) without risking disturbance.

To verify that more dust reached the sexual parts of flowers in dustier conditions, we scored dust on a subset of the stigmas that were collected and prepared for pollen counts. It proved impractical to count numbers of dust particles precisely, so we assigned qualitative scores for dust loads. We always assigned a score of 0 to stigmas with no dust, and scores of 1 and 2 to higher values (table A1 gives details; tables A1, A2 available online).

Exposing Plants to Dust

We treated our exploration of dust effects as a set of 20 separate tests of the underlying hypothesis that dust would influence pollen loads and seed set (see table 2 for details of tests and table 3 for sample sizes in each test). Exposure to dust was varied using four methods, three of them experimental. (1) In the nonexperimental "natural near versus far" method, we located natural populations near (1–2 m) versus far (40–50 m) from roads before plants had begun to flower. We matched individuals between populations as closely as possible for numbers of inflorescences and phenological stages, but exact matches were not always possible because of interpopulation variation. (2) In the "potted near versus far" method, we located single populations >40 m from the nearest road. We chose matched pairs of plants and transplanted each plant into a fiber flower pot (8 cm diameter), again while flowers were in bud stage. Potted plants were hand watered for several days until they recovered from transplanting; survival was near 100%. We then placed one of each pair chosen at random into a linear array 1–2 m from the nearest road, with interplant spacing of ca. 1 m, and the other into an equivalent array 40–50 m from the road. Arrays were watered every other day. This method removed the possibility that differences between populations near versus far from the road are confounded with dust effects. (3) In the "potted dusted" method, we paired and potted plants as described above. We placed one member of each pair chosen at random into an array that was dusted every other day by hand and the other into an equivalent control array. Arrays contained three rows of four plants each with interplant spacing and watering as above. They were situated >40 m from the nearest road and 10 m apart from each other, and we switched their locations every fourth day to minimize position effects. This

Table 3

Pollen Loads and Seed Sets in the 20 Tests Shown in Table 2

Test	Species	Pollen near or dusted	Pollen far or undusted	Pollen load effect size	Seeds near or dusted	Seeds far or undusted	Seed set effect size
1	*Delphinium nuttallianum*	86.01 ± 20.91 (5)*	169.67 ± 21.83 (5)*	.51	10.52 ± 2.10 (5)	9.15 ± 2.15 (5)	1.15
2	*D. nuttallianum*	157.49 ± 19.70 (10)	178.97 ± 19.60 (10)	.88	11.03 ± 1.66 (10)	14.49 ± 1.66 (10)	.76
3	*Ipomopsis aggregata*	111.74 ± 121.26 (10)	161.11 ± 19.40 (10)	.69	2.10 ± .90 (10)	3.28 ± .90 (10)	.64
4	*I. aggregata*	59.31 ± 17.84 (11)*	160.04 ± 17.58 (11)*	.37	2.10 ± .92 (11)†	4.78 ± 1.12 (11)†	.44
5	*I. aggregata*	94.93 ± 20.93 (11)	141.82 ± 20.93 (11)	.67	3.03 ± 1.07 (11)	4.05 ± 1.10 (11)	.75
6	*I. aggregata*	150.03 ± 24.84 (14)	155.47 ± 31.07 (9)	.97	2.87 ± .95 (14)	3.48 ± 1.17 (9)	.82
7	*I. aggregata*	233.07 ± 35.96 (15)	274.82 ± 356.96 (15)	.85	5.73 ± .79 (15)	5.10 ± .83 (15)	1.12
8	*I. aggregata*	98.90 ± 23.11 (10)†	158.96 ± 23.49 (10)†	.62	4.21 ± 1.55 (10)	4.22 ± 1.34 (10)	1.00
9	*I. aggregata*	64.57 ± 19.56 (10)*	139.77 ± 19.56 (10)*	.46	.90 ± .44 (10)	1.29 ± .45 (10)	.70
10	*I. aggregata*	74.05 ± 12.98 (10)*	157.56 ± 12.28 (10)*	.47	1.81 ± .57 (10)	1.18 ± .57 (10)	1.53
11	*I. aggregata*	121.30 ± 19.10 (10)†	170.60 ± 19.10 (10)†	.71	1.33 ± .86 (10)	2.10 ± .82 (10)	.63
12	*I. aggregata*	107.80 ± 23.40 (10)	153.40 ± 23.40 (10)	.70	2.67 ± .71 (10)	2.50 ± .87 (10)	1.07
13	*I. aggregata*	115.10 ± 26.61 (10)†	188.40 ± 26.61 (10)†	.61	1.88 ± 1.41 (10)	5.00 ± 1.37 (10)	.37
14	*I. aggregata*	123.60 ± 21.86 (10)*	200.50 ± 21.86 (10)*	.62	3.75 ± 1.12 (10)	3.11 ± 1.06 (10)	1.21
15	*Linum lewisii*	17.70 ± 12.82 (10)*	63.42 ± 12.82 (10)*	.28	5.70 ± 1.28 (10)	7.22 ± 1.35 (10)	.79
16	*L. lewisii*	4.81 ± 4.10 (10)*	30.12 ± 4.36 (10)*	.16	.60 ± .44 (10)	.58 ± .46 (10)	1.03
17	*L. lewisii*	22.40 ± 19.19 (10)	51.08 ± 19.19 (10)	.44	3.60 ± 1.38 (10)	3.60 ± 1.38 (10)	1.00
18	*L. lewisii*	32.38 ± 14.25 (10)	39.38 ± 14.25 (10)	.82	5.90 ± 1.25 (10)	3.67 ± 1.32 (10)	1.61
19	*Castilleja sulphurea*	574.65 ± 83.96 (10)	529.90 ± 83.96 (10)	1.08	91.29 ± 18.08 (10)	69.10 ± 15.12 (7)	1.32
20	*C. sulphurea*	253.44 ± 73.39 (9)*	645.75 ± 65.64 (10)*	.39	39.44 ± 14.69 (9)	50.70 ± 13.93 (10)	.78

Note. Values are means of individual plant means ± 1 SE; sample sizes (no. plants sampled) are given in parentheses. Significance level of individual comparisons is coded as in the footnotes. Effect size is the value for near or dusted divided by the value for far or undusted.

* $P < 0.05$.

† $0.05 < P < 0.10$; all others are $P \geq 0.10$ (see table A2).

method removed any possible effects of road proximity other than dust level. (4) In the "natural dusted" method, we chose plants growing in single natural populations >40 m from the road. Without potting them, we paired nearby plants as described above and chose one of each pair at random to be dusted by hand and the other to serve as a control. We covered control plants momentarily with paper bags to protect them while we dusted neighboring individuals. As with method 3, this method removed any possible effects of road proximity other than dust exposure. For both the potted dusted and natural dusted methods, we collected soil from the nearby road, sieved it to fine powder, and applied it to flowers daily with 30 pump strokes of a garden duster (either a Puffer Eaton 530 Bellows Duster [J. T. Eaton, Twinsburg, OH] or a Gilmour Hand Duster [Gilmour Manufacturing, Somerset, PA]).

Measuring Pollen Loads and Seed Set

To assess whether dust influenced pollen loads and seed set, we marked multiple flowers per plant (the number varied with the test) that were open and female receptive during the treatments just described (see table 3 for sample sizes expressed as number of plants). After these flowers had begun to shed petals or petaloid sepals, we excised their stigmas. By this stage, pollen tubes have long since fertilized ovules in both *D. nuttallianum* and *I. aggregata* (Waser and Price 1991*a*, 1991*b*), and we assumed that the same was true for the other two species. Stigmas were immediately placed on glass microscope slides with a drop of heated basic-fuchsin gel and squashed under cover slips. We counted pollen loads (and scored dust particles) on the stigmas at × 100 magnification with a compound microscope. Finally, we counted maturing seeds within the same fruits whose stigmas were scored for pollen, except for *I. aggregata* flowers in 2008, which were not marked. In that case, we collected fruits from the same plants but could not match individual fruits and stigmas. Counts did not include fruits damaged by predispersal insect seed predators but did include aborts (zero seeds)—fruits that failed to expand (*I. aggregata, D. nuttallianum, C. sulphurea*) or that detached from the pedicel before maturity (*L. lewisii*).

Analyses

Because we have multiple independent tests of the hypothesis that dust harms pollination and reproduction, a straightforward way to draw inferences is to use a standard nonparametric analysis based on the binomial distribution (e.g., Hoel 1971, pp. 58–60). The logic is to calculate the probability across N tests of obtaining x outcomes in one direction (e.g., lower stigma pollen loads in dustier conditions) and $N - x$ outcomes in the opposite direction (e.g., higher stigma pollen loads in dustier conditions), under the null assumption that the two outcomes are equally likely. This approach suffices because we are not inherently interested in differences among sites and years (there is ample evidence that pollen loads and seed sets vary in space and time for several of these species) or in formally testing differences among species. In any case, replication across sites, years, and species was insufficient for a more detailed analysis of their main effects and interactions. However, we did also analyze each of the 20 inde-

pendent tests of the hypothesis individually, using mixed-model ANOVA with REML estimation to assess effects of treatment (either distance from the road or hand dusting) on stigma pollen loads and seed sets (see table A2 for details). Whenever multiple flowers were sampled per plant and data were not badly unbalanced, we tested treatment (fixed effect) over plant nested within treatment (random effect). Otherwise, we calculated plant means and tested treatment over the error. In most cases, pollen loads were square-root transformed to improve normality of model residuals; seed sets did not require transformation. ANOVAs were performed with JMP Pro 11 (SAS Institute, Cary, NC).

Results

Deposition of Road Dust

The particles of road dust deposited on filter-paper disks ranged in linear dimension from less than 1 μm to approximately 40 μm, within the range of pollen grain sizes of all species except *Linum lewisii*, which has somewhat larger pollen (table 1). Relative rates of deposition decreased rapidly and in nonlinear fashion with distance from the road (fig. 3), reaching an average asymptotic value of 21% of the roadside deposition rate at a distance of 50 m (SE = 2.4%, range = 8.7%–35.1%, $N = 13$). Roadside deposition rates averaged 657.5 particles per square centimeter per hour (SE = 54.07, range = 308–975, $N = 13$).

The amount of dust on stigmas in natural near versus far and potted near versus far tests reflected ambient dust levels. In all 13 such tests for which we had quantitative dust scores, mean scores were higher near than far from the road, as they also were for dusted plants in three potted dusted and natural dusted studies (table A1). The probability that all 16 comparisons differed in the same direction by chance alone is vanishingly small ($P = 2^{-16}$, two-sided binomial test). We conclude that our near-road and hand-dusting treatments succeeded in exposing plants to higher levels of dust than did the far and undusted treatments.

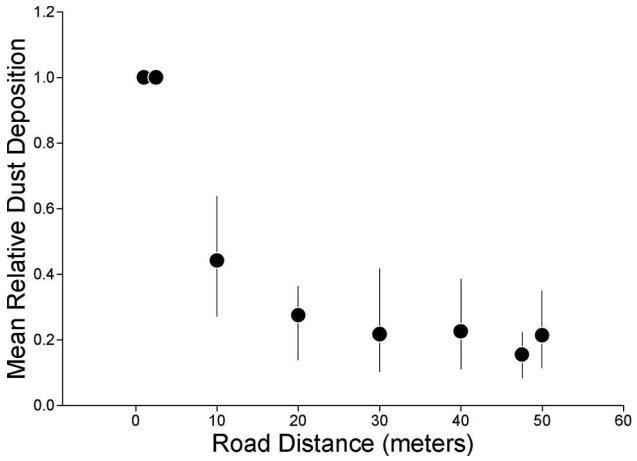

Fig. 3 Relative dust deposition rate on filter-paper disks as a function of distance from the nearest unpaved road. Relative deposition rate is expressed as mean number of dust particles per square centimeter per hour of exposure, divided by the value for the disk next to (1–2 m from) the road. Values are means across all studies that used a given distance, with bars indicating the range. Because values are expressed relative to the maximum rate next to the road, there is no variation around the roadside values.

Pollen Receipt and Seed Set

In 19 of 20 independent tests using the four plant species, stigmas in dusty conditions carried less pollen than those in less dusty treatments (table 3). The effect sizes, expressed as ratios of mean stigma pollen loads of dusted versus undusted plants or of those near to versus far from a road, ranged from 0.16 to 1.08, with a mean of 0.62. In other words, exposure to dust caused an overall average decrease of 38% in pollen loads across all tests. Eight of 20 tests were individually significant at the 5% level of critical α and two others at the 10% level (tables 3, A2). The more telling consideration, however, is the vanishingly small chance of 19 of 20 tests going in the same direction ($P = 20 \times 2^{-20}$, two-sided binomial test) if pollen loads were in fact equivalent for dusty and less dusty treatments.

The pattern for seed set stands in contrast to the clear effect of dust treatment on pollen receipt, both for individual plant species and overall. Here there was no consistent difference between plants exposed to more dust versus those less exposed (tables 3, A2). Two tests were ties with effect size of unity, 10 were in the direction of lower seed set for plants exposed to more dust, and eight were in the direction of higher seed set for such plants ($P = 0.81$, two-sided binomial test). The effect sizes ranged from 0.38 to 1.61, with a mean of 0.94, close to unity.

Discussion

Our measurements confirm what is visually obvious: traffic on unpaved roads can produce substantial amounts of dust. Along the roads we studied, most of this dust was deposited within 30 m of the road verge, although some traveled longer distances. The dust particles we examined varied greatly in size and shape, but their linear dimensions overlap most pollen, including pollen of three of the four species we studied. We were therefore not surprised to find more dust particles on stigmas that were exposed to more road dust either naturally or experimentally.

This increased dust load was associated with lower stigma pollen loads. In all but one of 20 independent tests, stigmas of plants exposed to more dust received less pollen—sometimes more than six times less (i.e., study 16 of tables 2, 3). This consistent result is highly improbable if dust exposure had no effect on pollen receipt. Furthermore, the fact that we observed the same pattern with experimental hand dusting implicates dust, rather than other effects of roads or features correlated with roads (e.g., Geerts and Pauw 2011; Suárez-Esteban et al. 2014), as a major (perhaps sole) cause of reduced pollen receipt.

The negative relationship between dust and pollen loads did not extend to subsequent seed set. Over the same 20 independent tests, there was no consistent effect on the number of seeds that a flower produced based on having more dust and less pollen on its stigma. This is a surprising result, because seed set is often pollen limited in angiosperms in general (Knight et al. 2005) and for at least two of the species included in this study (*Delphinium nuttallianum* and *Ipomopsis aggregata*). In what follows,

we list four possible mechanisms for the inconsistent translation of pollen loads into seed set. These mechanisms are neither mutually exclusive nor exhaustive, and they bear further scrutiny. We refer to them as pollen excess, pollen quality, resource limitation, and compensatory herbivory.

One possibility is that numbers of pollen grains on many stigmas were in excess of those needed for maximum seed set. Because each flower has a finite number of ovules, seed set increases with pollen load in a decelerating fashion, reaching an asymptote. Asymptotes for *D. nuttallianum* and *I. aggregata* sometimes are reached at about 100 pollen grains (Kohn and Waser 1985; fig. 1 of Waser and Price 1991*a*), although the value sometimes may be higher (Waser and Fugate 1986; Waser and Price 1990). In this study, one of two tests with *D. nuttallianum* and nine of 12 with *I. aggregata* involved average stigma loads ≥100 grains for both dusted and undusted treatments (total 10 of 14 tests; table 3; $P = 0.18$, two-sided binomial test). Thus, there is a hint that pollen may not have been limiting even for dusted flowers in some tests, at least for the two species that we have studied extensively.

Another explanation involves an aspect of pollen quality. The studies of pollen-seed relationships just cited involved hand pollination with outcrossed pollen, whereas natural pollen loads may include self-pollen from transfer within flowers and among flowers within the same plant (i.e., geitonogamy). Geitonogamy in the self-sterile *I. aggregata* can average half of all pollen delivery, depressing seed set by 25% (de Jong et al. 1992), presumably through maternal abortion of ovules (Sage et al. 2006). For this species, undusted flowers might average seed sets close to those of dusted flowers if pollinators tend to visit more flowers (and therefore effect more geitonogamy) on undusted plants. Indeed, hummingbirds visited 5.8 flowers on average per undusted *I. aggregata* plant in 2014 versus 4.3 per dusted plant (N. M. Waser and M. V. Price, unpublished data). We also note that bumble bees visited 3.6 flowers per undusted *D. nuttallianum* plant in 2014 versus 2.3 per dusted plant (N. M. Waser and M. V. Price, unpublished data). These values suggest a possible geitonogamy effect on seed set for these two species.

Consider next that resources rather than pollen loads may limit the ability of plants to fill seeds (Stephenson 1981). Campbell and Halama (1993) showed that addition of NPK fertilizer did increase seed set of *I. aggregata* flowers. Addition of pollen in their study also increased seed set (this and other results show that the two limiting factors need not be mutually exclusive). It remains possible that seed set in some of our tests with *I. aggregata* were constrained by resources, and this is not logically excluded for the other plant species.

Finally, consider compensatory herbivory. In *I. aggregata*, a predispersal seed predator (the anthomyiid fly *Hylemya* sp.) preferentially lays eggs on flowers that ultimately would produce more seeds if they were not parasitized (e.g., Brody and Waser 1995). If flies attack more fruits on undusted plants, or if larval survival is higher there than on dusted plants, mean seed counts from undusted plants (which exclude counts from parasitized fruits) would be reduced relative to dusted plants when flies are abundant. Examining our 12 studies with *I. aggregata* does reveal a lower number of parasitized fruits from dusted relative to undusted plants (respectively, 24 of 344 total fruits attacked, or ca. 7%, vs. 42 of 303, or ca. 14%). These parasitized fruits come almost entirely from studies 3, 4, and 5 of table 2 (i.e., studies of 2008 and 2013), and we note that in those same studies undusted plants had higher, not lower, seed sets (table 3). Thus, *Hylemya* attack by itself seems unlikely to explain the variable seed set results reported here for scarlet gilia, but this example does illustrate the more general possibility that dust could influence plant reproduction by affecting herbivory.

A strength of our study is that it spanned multiple years and sites, rather than being a single sample of each. The results therefore suggest that any effects of road dust are not anomalies related to an unrepresentative choice of a single time or place. We also find it interesting that the results did not obviously vary with floral morphology. We initially hypothesized that dust might depress pollen loads more strongly in flowers with more exposed stigmas, here represented by *Linum lewisii*, than in those with more protected stigmas, here represented by *I. aggregata* and *Castilleja sulphurea*, and those with fully protected stigmas, here represented by *D. nuttallianum*. There is no obvious hint of such a difference, or of effects of wet versus dry stigmas, here represented by *C. sulphurea* versus the other species. The sample of morphologies is small, but the results suggest that dusty environments can compromise pollination in a diversity of flowers.

We stress (e.g., Price et al. 2008; Waser et al. 2010) that seed set is equivalent neither to plant fitness nor to finite rate of increase and so is a shaky basis for conclusions about natural selection or demography. Nonetheless, fecundity is one component of fitness, and so the lack of a consistent seed set response to dust in our studies might lead some to conclude that any effect of dust on reproduction is inconsequential. As hinted above, however, there are several ways in which our sample of species and systems might underestimate dust effects on fitness, thus leaving open the potential for evolutionary or demographic consequences. It is possible that pollen is generally more limiting to seed set than appears to have been the case in our study. If pollen is limiting—e.g., if seed set is not constrained by ovule number, resources, compensatory herbivory, or other mechanisms—then differences in pollen deposition due to dust may well translate into consistent seed set differences. We also note that dust might influence the total number of flowers that plants produce, and thus overall seed set, even if it does not reduce seed set per flower. Furthermore, dust levels depend on traffic density (Forman and Deblinger 2000) and vehicle size and may often exceed the level experienced in our study. In such situations, seed set might be measurably and consistently affected, and even if not, success through male function (i.e., through the siring of seeds) might suffer.

How important might such effects on reproductive success be on a larger scale? One consideration is the areal coverage of unpaved roads. For example, the United States supports some 6.2 million kilometers of roads, covering about 1% of total land surface (Forman 2000), of which about one-third are unpaved (https://www.fhwa.dot.gov/policyinformation/statistics/2013/hm12.cfm). Adding informal dirt tracks also used by motor vehicles leads to an estimate of 0.5% of land surface, and if we imagine that dusty verges on both sides of roads are equal to twice the road width, we arrive at about 2.5% of total land area potentially affected by road dust. This value will underestimate the coverage in geographic regions where unpaved roads are more common. And even if the value for a given region is small, it is not neces-

sarily inconsequential, given the special importance of road verges as refugia for plants and pollinators. The margins of roads often represent remnants of natural and seminatural habitat within more heavily human-altered landscapes, e.g., agricultural landscapes (Forman and Deblinger 2000). Flowering plants, as well as bees and other pollinators, may depend heavily on these remnants (e.g., Ouin et al. 2004; Hopwood 2008; Noordijk et al. 2009), and they are precisely the areas most likely to be affected by road dust.

Previous studies such as those cited in the Introduction, along with a consideration of possible effects based on first principles, suggest many ways in which dust might directly or indirectly influence plant physiology, pollination, and, ultimately, reproduction. Our study has surprised us with the consistency of dust effects on pollen loads and puzzled us with the apparent failure of lower pollen loads to lead consistently to lower seed production. Whether such results are representative of other systems remains to be seen. There is much to learn, and we hope that the exploration of dust effects on plant reproduction is just beginning.

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