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# PREDISPERSAL SEED PREDATION OBSCURES THE DETRIMENTAL EFFECT OF DUST ON WILDFLOWER REPRODUCTION

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*Premise of research*. Seed production by flowering plants depends on abiotic and biotic factors whose interacting effects may be hidden. We previously reported that exposure to dust from unpaved roads reduced the average amount of pollen on flowers of *Ipomopsis aggregata* but did not consistently reduce mean seed set per fruit. Here we explore one possible explanation—that the expected detrimental effect of lower pollen loads on seed production was obscured because dust reduces not only pollination success but also attack by a predispersal seed predator.

*Methodology*. Over three consecutive summers, we scored the fates of more than 4000 flowers, comparing those on hand-dusted *I. aggregata* plants with those on clean control plants in the same natural populations. We censused plants throughout each flowering season, marking phenological cohorts of flowers and examining them for eggs of the anthomyiid fly *Hylemya* sp. We subsequently scored expanded fruits for seed loss to *Hylemya* larvae.

*Pivotal results*. Control plants consistently had higher egg loads and rates of seed loss to larvae than dusty plants. Furthermore, most eggs appeared early in the season, when flowers are most likely to set fruit and ovule numbers are highest. In agreement with earlier studies, unparasitized fruits from dusty plants did not contain fewer seeds than fruits from control plants. But when we simulated a system without *Hylemya* by assigning each parasitized fruit the average number of seeds for unparasitized flowers in the same phenological cohort, control plants outproduced dusty plants.

*Conclusions*. Predispersal seed predation likely contributed to the previous lack of a negative effect of dust on seed set in *I. aggregata*. This study illustrates how exploring unexpected experimental outcomes can reveal important ecological interactions and how the mechanistic understanding so gained can improve the ability to generalize results to other systems.

*Keywords:* anthomyiid fly, indirect interaction, *Ipomopsis*, phenology, seed production.

*Online enhancement:* appendix. *Dryad data:* https://doi.org/10.6086/D18D7B, https://doi.org/10.6086/D10X1R.

Dust, in the end, settles on everything. (Cornelia Parker)

## Introduction

A flowering plant can produce seeds only when it experiences tolerable physical conditions, receives adequate high-quality pollen, has access to water and nutrient resources, and avoids enemies. Ecologists have long considered how such factors limit fruit and seed production, focusing especially on pollen and resources (e.g., Silander and Primack 1978; Stephenson 1981; Udovic 1981; Knight et al. 2005; Aizen and Harder 2007) and on the loss of reproductive effort to herbivores that eat flowers, inflorescences, fruits, or developing seeds (e.g., Galen 1990; Louda and Potvin 1995; Sharaf and Price 2004; Maron and Crone 2006). How multiple constraints interact to affect fecundity is less well studied. We know that pollen and resources may interact to mold patterns of reproductive allocation in plants (Burd 1995, 2008) and that both pollen and resources can limit seed production across different flowering episodes of a perennial plant (Montalvo and Ackerman 1987) or even at different points within a single flowering season (Campbell and Halama 1993). Less is understood about the effects on plant fecundity from herbivory, pollination, and resource availability acting together (Sharaf and Price 2004; Brody and Irwin 2012).

Here we explore an interaction between herbivory and plant fecundity that varies with one aspect of the abiotic environment—airborne dust. Dust is fine particulate matter in the size range of microns to tens of microns that is generated primarily from wind scouring of dry soil surfaces. It is ubiquitous in the atmosphere of the Anthropocene, fostered by increasing human soil disturbance and climate change. The study of dust's ecological effects is in its infancy (Field et al. 2009).

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Previous work on the fecundity of native wildflowers in the western United States (Waser et al. 2017) suggested a surprising effect from dust that is generated by vehicular traffic on unpaved roads. In 12 of 12 replicate experimental and observational studies of *Ipomopsis aggregata*, samples of flowers from plants exposed to road dust ("dusty plants") averaged 35% fewer pollen grains on their stigmas than did samples from clean control plants. Pollen often limits per-fruit seed set in *I. aggregata* (e.g., Waser and Fugate 1986; Campbell and Halama 1993), and we therefore expected fruits produced by the dusty flowers to contain fewer seeds on average than those from clean flowers. But although dusty plants fared worse than clean plants in 7 of the 12 studies, they fared better in 5; the mean difference in seeds per fruit was a statistically insignificant deficit for dusty plants of about 14%, less than half the pollen deficit.

What might contribute to this quantitative mismatch between the effects of dust on pollen loads and on per-fruit seed set? Waser et al. (2017) discussed four possible causes, none mutually exclusive: (1) perhaps most pollen loads in sampled flowers were in excess of those needed for the full seed set, (2) perhaps flowers of clean plants received more low-quality self-pollen, (3) perhaps resources, not pollen, limited fecundity, and (4) perhaps herbivores preferred clean plants to dusty ones.

We focus here on the fourth possibility. The red tubular flowers of *I. aggregata* are attacked by a fly, *Hylemya* sp. (Anthomyiidae), whose larvae consume developing seeds within expanding fruits after pollination has occurred. If this predispersal seed predator attacks clean flowers more than dusty ones and destroys fruits that would have set more than the average number of seeds, its presence could obscure the fecundity advantage that higher pollen loads are expected to give clean flowers. In what follows, we describe field experiments replicated over 3 yr that were designed to evaluate this "compensatory herbivory" hypothesis. We began by affirming our previous observation that the number of seeds produced by unparasitized fruits from clean control plants is no greater on average than the number for dusty plants. We then asked whether the conditions for predispersal seed predation to counteract the effects of low pollen loads are met. The answer is "yes": clean flowers suffered a higher probability of *Hylemya* attack than did experimentally dusted flowers, and flies preferentially attacked early-season flowers, which have a lower than average probability of aborting and a higher than average seed set if they do not abort. Finally, we asked whether the observed patterns of *Hylemya* oviposition can obscure the detrimental effects of dust on pollination. To answer this question, we simulated the absence of *Hylemya* by assuming that all parasitized flowers in our samples produced the average number of seeds for all unparasitized flowers open at the same time. Our calculations confirm that attack by this seed predator has the effect of disproportionally reducing the per-fruit fecundity of control plants.

## Methods

### *Study System*

Scarlet gilia (*Ipomopsis aggregata* Pursh V. Grant, Polemoniaceae) is an herbaceous perennial plant native to mountains of the western United States. Below we describe the natural history of *I. aggregata* ssp. *aggregata* (sensu Grant and Wilken 1986) near the Rocky Mountain Biological Laboratory (RMBL; 2900 m asl; lat. 38.96°N, long. 106.99°W) in west-central Colorado, where our previous road dust studies have taken place and where nearly a half-century of research has generated substantial knowledge about the pollination, life history, and population ecology of this wildflower (e.g., Watt et al. 1974; Pyke 1978; Hainsworth et al. 1984; Caruso 1999; Campbell 2019).

Scarlet gilia plants flower once after growing as vegetative rosettes for several years (Price et al. 2008). Repeat flowering occurs rarely, in the absence of damage during the vegetative stage (Brody et al. 2007). The flowers are most often produced on a single flowering stalk (Brody and Morita 2000), but plants may produce multiple inflorescences if the vegetative rosette is damaged or if ungulates browse elongating inflorescences during the summer of flowering (Sharaf and Price 2004). The main pollinators are broad-tailed and rufous hummingbirds (*Selasphorus platycercus* and *Selasphorus rufus*), but long-tongued insects also contribute (Waser 1982; Mayfield et al. 2001; Price et al. 2005). Plants are highly self-sterile (Sage et al. 2006), so fruit and seed production depends on the receipt of outcross pollen as well as on resource availability (Campbell and Halama 1993). Expanded fruits mature about five seeds on average, each weighing about 1 mg (fig. 1*A*; a 1980 sample of 10 seeds from

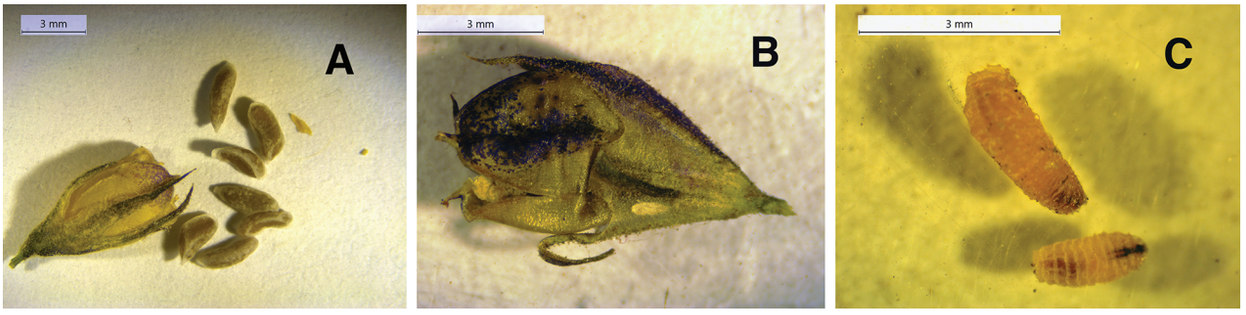


**Fig. 1** *A*, Expanded fruit and seven mature seeds of *Ipomopsis aggregata*; note also one aborted seed and one unexpanded ovule to the right. *B*, In the center of the image, an oval pale-colored *Hylemya* egg case is visible through the calyx of an expanded, parasitized fruit, and a pale larva is visible on the left emerging from the split fruit capsule. *C*, Two larval *Hylemya* that were extracted from expanded fruits; the dark sclerotized mouthparts are clearly visible in the smaller specimen.

each of five plants yielded a mean of 0.92 mg (SD = 0.037 mg; N. M. Waser and M. V. Price, unpublished data).

*Ipomopsis aggregata* and the confamilial *Polemonium foliosissimum* share a predispersal seed predator, an undescribed species of fly in the genus *Hylemya* (Anthomyiidae; although the fly has also been placed in the genus *Delia*, we use *Hylemya* for consistency with previous publications). Interactions between the fly and its hosts are well studied at the RMBL (e.g., Zimmerman 1980; Hainsworth et al. 1984; Brody 1992, 1997; Brody and Waser 1995; Brody and Morita 2000). Female *Hylemya* usually lay a single egg between the calyx and corolla of elongated flower buds and newly opened flowers (fig. 1*B*). Only 15 of 809 buds or flowers on which we detected eggs in the current study carried two eggs, and none carried more than two. If the egg hatches, its larva (fig. 1*C*) burrows into the ovary and consumes the embryo and endosperm of developing seeds. If the larva survives, it generally destroys all seeds in its fruit before chewing an exit hole and falling to the soil to pupate.

The experiments described here took place in the summers of 2016, 2017, and 2018 in a gently southeast-sloping meadow 1 km south of the RMBL along an unpaved road. The road is treated with an aqueous solution of magnesium chloride each spring to suppress dust; we worked upslope of the road to avoid any impacts of runoff from the road surface. Input of water from the melting of the winter snowpack—which strongly affects the reproduction, growth, and survival of *I. aggregata* (e.g., Campbell and Halama 1993; Price and Waser 1998; Campbell 2019)—varied among the three summers of study (data from http://www.gothicwx.org). Total water input from snow was highest in 2017 (785 mm) and lower in 2016 and 2018 (536 and 424 mm, respectively). All three values were lower than the 1976–2019 average of 970 mm (range, 279 mm in 1977 to 1145 mm in 1995).

### *Dusting Treatment*

In each summer, before the onset of the approximately 1-mo-long flowering season of scarlet gilia, we chose pairs of neighboring bolting plants (25 pairs in 2016; 32 in 2017; 25 in 2018) of similar size. With a coin toss, we chose one of each pair to be dusted, leaving the other as a clean control, and then measured the stalk heights and basal stem diameters of marked plants. Paired *t*-tests confirmed that the coin toss method indeed equalized plant sizes among treatments.

We obtained dust from a section of road north of the RMBL that has never been treated with magnesium chloride. Soil from this road was dried and sieved through a number 35 US standard soil sieve (0.5-mm mesh size) to produce a fine powder. We began to apply this powder with a hand duster (Gilmour Manufacturing, Somerset, PA) in the first week of flowering—on July 3, July 3, and June 18 in 2016, 2017, and 2018, respectively—and continued every 1 or 2 d, except during periods of rainfall, until plants ceased flowering. Either 10 (2016) or 15 (2017, 2018) pumps of the duster were applied to each dusted plant each time; plants in the control treatment were covered with a paper bag to shield them and then received a sham dusting of 10–15 pumps of air alone from a clean, empty duster. Our treatment approximated natural roadside dust deposition: densities of dust particles on leaves of hand-dusted plants were intermediate between those of naturally dusted roadside plants and clean plants growing 50 m from the closest road (C. E. Rosas, unpublished data).

**Table 1**

**Effects of Year and Treatment on Seeds per Fruit**

| Year, fruit egg status | Treatment | |
|---|---|---|
| | Dusty | Control |
| 2016, with eggs | 1.75 ± .59 (20) | 1.56 ± .52 (22) |
| 2017, with eggs | 4.48 ± 1.34 (19) | 3.37 ± .46 (24) |
| 2017, all fruits | 3.62 ± .33 (29) | 3.62 ± .32 (31) |
| 2018, with eggs | 4.21 ± 1.04 (19) | 1.81 ± .37 (23) |
| 2018, all fruits | 1.66 ± .24 (23) | 1.60 ± .22 (23) |

Note. Values are for unparasitized fruits and include aborts (zero seeds); they are means of individual plant means ± 1 SE, with sample sizes (number of plants sampled) given in parentheses. Fruit egg status: "with eggs" refers to the subset of fruits from flowers (2016, 2017, and 2018) on which we detected *Hylemya* eggs; "all fruits" includes fruits from all flowers (only 2017 and 2018), whether or not we detected an egg.

**Table 2**

**ANOVAs of the Effects of Year and Treatment on Seeds per Fruit**

| Variable, source | df | *F* | *P* |
|---|---|---|---|
| With eggs: | | | |
| Model: | 5 | 2.036 | .080 |
| Year | 2 | 3.013 | .054 |
| Treatment | 1 | .899 | .345 |
| Year × treatment | 2 | 1.463 | .236 |
| Error | 102 | | |
| All fruits: | | | |
| Model: | 3 | 16.698 | <.0001 |
| Year | 1 | 50.089 | <.0001 |
| Treatment | 1 | .011 | .916 |
| Year × treatment | 1 | .0001 | .982 |
| Error | 102 | | |

Note. Analyses are based on square-root-transformed plant means of seeds per unparasitized fruit to normalize residuals. "With eggs" and "all fruits" are as defined in table 1. Model $R^2_{adj} = 0.046$ (with eggs) and 0.310 (all fruits).

### *Assessing the Seasonal Timing of Flowering and* Hylemya *Oviposition*

On the morning of every fourth day during each year's flowering season, we counted open flowers and elongated buds that would open that afternoon and searched them for *Hylemya* eggs, using forceps to gently retract calyx lobes. The 4-d census interval minimized the probability of counting flowers more than once because few *I. aggregata* flowers retain their corollas 4 d beyond the elongated bud stage (N. M. Waser and M. V. Price, unpublished data). We began censuses when most marked plants had at least one open flower or elongated bud and few plants had old flowers or initiated fruits; the first censuses took place on July 6 in 2016, July 3 in 2017, and June 20 in 2018.

### *Measuring Flower Fate and Seed Set*

To evaluate any seasonal trends in flower fates and per-fruit seed set, we applied an inconspicuous dot of nontoxic acrylic

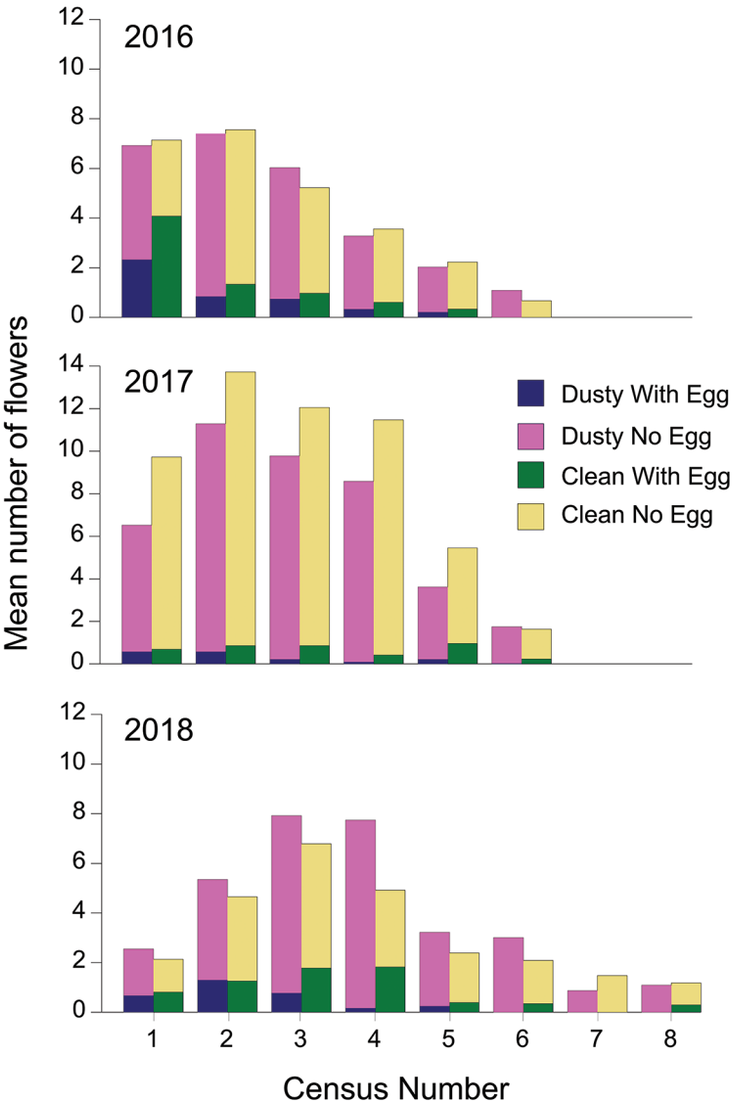


**Fig. 2** Flowering and *Hylemya* oviposition phenology for dusted and clean plants during three summers of study. The total heights of the stacked bars indicate mean per-plant numbers of elongated flower buds and open flowers counted in successive censuses, which occurred every 4 d. The darker bottom portion of each bar indicates the mean number of elongated buds and flowers on which we detected *Hylemya* eggs during a census; the lighter upper portion indicates the mean number of buds and flowers without eggs. Bars for dusty plants are to the left of those for clean plants in each census.

paint to the calyxes of elongated buds and flowers after we examined them. In 2016, only those buds and flowers with *Hylemya* eggs were marked with a census-specific color, whereas in 2017 and 2018, all buds and flowers were so marked, and those with *Hylemya* eggs received an additional dot of yellow paint. At the end of each summer, we assessed the fates of parasitized flowers (2016) or all flowers, parasitized or not (2017, 2018). Flowers with ovaries that failed to expand after their corollas had abscised were scored as aborted fruits ("aborts") with zero seeds. Fruits that had expanded were collected just before dehiscence and air-dried in the laboratory. Each expanded fruit was gently opened under a WILD M-5 dissecting microscope (WILD Heerbrugg, Switzerland). We counted the mature full-size seeds of unparasitized fruits. We scored fruits with evidence of *Hylemya* attack (living or dead larvae, chewed seeds, frass, or an exit hole) as "parasitized" and their seed number as "missing." This fruit-scoring method is consistent with that used in the previous study (Waser et al. 2017). A single observer scored all fruits from a given year.

### *Accuracy of Egg Censuses*

We expected that our censuses of *Hylemya* eggs underestimated true oviposition rates because eggs are small (<1 mm; fig. 1*B*), flowers are usually tightly clustered and difficult to

examine (hence the species epithet *aggregata*), and flies continued to oviposit between censuses. Indeed, in 2017 and 2018, the summers in which we color marked all flowers, we discovered many expanded fruits with evidence of *Hylemya* attack that lacked the yellow dot to indicate that we had detected an egg. An analysis from these two summers (app. A1, available online) suggests that we detected 42% of all eggs on average and that the probability of missing an egg did not differ significantly between dusty and control plants or among census dates. This lack of significant interactions suggests that egg counts are a useful indicator of oviposition patterns across treatments and flowering seasons, even though they underestimate true egg numbers.

### *Simulated Seed Production in the Absence of* Hylemya

To explore how *Hylemya* attack may have influenced observed patterns of fecundity, we simulated what the seed sets per fruit would have been if the flies had been absent from the system and no fruits had been parasitized. We first calculated the mean of plant mean seed numbers for expanded, unparasitized fruits by year and census, pooling dusted and control treatments. Once again, 2016 values were available only for fruits known to have had an egg, whereas 2017 and 2018 values included all fruits. Replacing the "missing" values assigned to each parasitized fruit with these year- and census-specific mean values provided an estimate of what the mean seed sets of dusty and control fruits would have been without *Hylemya* present.

### *Statistical Analysis*

Fruits with incomplete information (e.g., those whose collection envelope lacked source plant or treatment information or were missing the color-coded calyx or that began to shed seeds before being collected) were excluded from analyses. All analyses were done with JMP Pro 11 (SAS Institute, Cary, NC). We used logistic regression to analyze categorical response variables and ANOVA for continuous response variables. Residuals were normalized as necessary (judging from visual inspection of residual vs. predicted output graphs) by analyzing plant means, transforming variables, or treating plant as a random effect, depending on the analysis. We did not need to remove outliers. Details of each analysis are indicated below as results are reported.

## Results

### *Effects of Dust on Seed Set per Unparasitized Fruit*

Waser et al. (2017) reported that flowers on dusty plants averaged fewer pollen grains on their stigmas than flowers on clean control plants but did not consistently produce fewer seeds per unparasitized fruit. The present study confirms this seed set result for three additional summers over which we scored the fates of 4575 flowers (table 1). The mean seed set per unparasitized fruit was insignificantly higher for dusty than for control plants (treatment effects in table 2), whether we analyzed all fruits (2017 and 2018) or only those on which we had detected *Hylemya* eggs (all three summers; mean values could be obtained from fruits in which eggs failed to produce successful larvae). Per-fruit seed set varied considerably among summers and was highest in 2017, the wettest of the three. The year effect approached significance for the subset of fruits known to have eggs (all three summers) and was highly significant for all fruits (2017 and 2018; table 2). There were no significant year × treatment interactions, indicating that treatment effects were similar across years. Fruit fate data are deposited in the Dryad Digital Depository (https://doi.org/10.6086/D10X1R; Price et al. 2021).

### *Effects of Year, Census, and Treatment on the* Hylemya *Oviposition Rate*

Per-flower egg loads varied significantly among summers (fig. 2; year effect in table 3); they were the lowest following the wet winter of 2017 (least squares mean of plant means = 0.09 eggs per flower) and higher in the drier years, 2016 and 2018 (means = 0.17 and 0.23, respectively). Taking all summers together, oviposition was concentrated early in the flowering season and decreased significantly overall by 0.03 eggs per flower with each successive 4-d census period. The seasonal rate of decline varied significantly among summers, however (fig. 2; year × census effect in table 3): oviposition decreased significantly through time in 2016 and 2018 but not in 2017, the summer with the lowest rate of *Hylemya* attack and thereby the least scope for a decline. In all summers, the flowers of clean control plants had significantly higher egg loads overall than those of dusty plants (least squares means of plant means across all summers = 0.21 vs. 0.12 eggs per flower, respectively; fig. 2; treatment effect in table 3). Egg census data are deposited in the Dryad Digital Depository (https://doi.org/10.6086/D18D7B; Price et al. 2021).

### *Oviposition and Flower Quality*

Judging from the different fates of flowers with and without eggs, *Hylemya* oviposited preferentially on "high-quality" flowers that were more likely to set fruit with many seeds. In data pooled across summers, censuses within summers, individual plants, and treatments, fruits with *Hylemya* eggs were less likely to abort (33.9% of 4575 fruits) than fruits without eggs (42.1%; likelihood ratio $\chi^2 = 14.26$, $P = 0.002$). Furthermore, the average number of seeds produced by expanded fruits with an

**Table 3**

**ANOVA of the Effects of Year, Census, and Treatment on Mean *Hylemya* Eggs per Flower**

| Source | df | *F* | *P* |
|---|---|---|---|
| Model: | 11 | 5.505 | .0002 |
| Year | 2 | 7.255 | .003 |
| Census | 1 | 13.374 | .001 |
| Year × census | 1 | 4.900 | .016 |
| Treatment | 1 | 7.710 | .010 |
| Year × treatment | 2 | 1.108 | .345 |
| Census × treatment | 1 | .076 | .785 |
| Year × census × treatment | 2 | 1.004 | .380 |
| Error | 26 | | |

Note. Analyses are based on the number of eggs detected per flower, averaged across plants within a treatment and census date. Census date ("census") is treated as a continuous covariate. Residuals were normally distributed. The overall model $R^2_{adj} = 0.573$.

egg but no sign of successful larval attack (5.46 seeds per fruit, SE = 0.283; data pooled across summers, censuses within summers, plants, and treatments) was higher than for expanded, unparasitized fruits without an egg (4.91 seeds per fruit, SE = 0.085, $F_{1,2345} = 7.47$, $P = 0.0063$, based on square-root-transformed values).

How did *Hylemya* select high-quality flowers? First, they avoided dusty plants. Abortion rates were consistently higher for dusty than control plants (table 4), whether the analysis included only fruits known to have carried an egg (all three summers) or all fruits (2017 and 2018). This pattern was significant in both comparisons (treatment effects in table 5). Second, most eggs were laid on flowers produced early in the season, which make a disproportionate contribution to overall seed production.

Two factors made early flowers the most fecund. First, the probability that a fruit aborted increased through the season in all three summers (likelihood ratio $\chi^2 > 13.89$, df = 1, $P < 0.0002$ in each of three separate analyses of 2016, 2017, and 2018 data), albeit at different rates depending on the summer ($\chi^2 = 28.93$, df = 2, $P < 0.0001$). The wettest year, 2017, had the lowest overall rate of abortion (table 4) and was thereby constrained to have the smallest increase in abortion rate through the season. Second, the number of seeds produced per expanded, unparasitized fruit also decreased through the season by about 0.86 seeds per successive census overall (analysis of covariance [ANCOVA], square-root-transformed means of plant means; $F_{1,482} = 21.36$, $P < 0.0001$), albeit again at different rates (ANCOVA, square-root-transformed means of plant means; $F_{1,482} = 7.44$, $P = 0.0007$). The effect of the census number on the abortion rate or seed number did not differ between dusty and control treatments ($P = 0.969$ and $P = 0.983$ for abortion rate and seed number, respectively).

The seasonal decline in seed set per expanded, unparasitized fruit could be due to progressively lower allocation to female function by *Ipomopsis aggregata* plants through the flowering season. In 1984, as part of an earlier unpublished study, we counted ovules from flowers on 10 untreated plants in the same meadow population as the present study that were open during the first and third week of the flowering season and found that ovule number decreased from a least squares mean of 23.3 ovules in the first week to 19.7 ovules in the third week. Maximum likelihood ANOVA with plant treated as a random effect indicated that this decrease from week 1 to week 3 was significant (collection effect: $F_{1,186} = 93.89$, $P < 0.0001$; overall model $R^2_{adj} = 0.546$). We have no reason to suspect that plant allocation differed between the earlier sample and the current study period.

**Table 4**

**Effects of Year and Treatment on Fruit Abortion Rates**

| | Treatment | | |
|---|---|---|---|
| Year, fruit egg status | Dusty | Control | Pooled |
| 2016, with eggs | .566 (106) | .515 (167) | .535 (273) |
| 2017, with eggs | .320 (51) | .085 (94) | .166 (145) |
| 2017, all fruits | .322 (1254) | .288 (1220) | .305 (2474) |
| 2018, with eggs | .442 (77) | .420 (150) | .427 (227) |
| 2018, all fruits | .570 (1086) | .533 (815) | .554 (1901) |

Note. Values are proportions of fruits with ovaries that did not expand and that were scored as aborts, with the total number of fruits given in parentheses. "Fruit egg status" is defined as in table 1. "Dusty" and "control" refer to the fruits for a given year and fruit egg status pooled across censuses and plants within the dusty or control treatments, respectively. "Pooled" refers to all fruits for a given year and fruit egg status, pooled across censuses, treatments, and plants within a treatment.

**Table 5**

**Logistic Regression of the Effects of Year and Treatment on Fruit Abortion Rates**

| Variable, source | df | $\chi^2$ | $P$ |
|---|---|---|---|
| With eggs: | | | |
| Model: | 5 | 70.560 | <.0001 |
| Year | 2 | 50.648 | <.0001 |
| Treatment | 1 | 9.971 | .0016 |
| Year × treatment | 2 | 8.424 | .015 |
| Error | 645 | | |
| All fruits: | | | |
| Model: | 3 | 281.674 | <0.0001 |
| Year | 1 | 287.445 | <0.0001 |
| Treatment | 1 | 6.049 | .014 |
| Year × treatment | 1 | .008 | .930 |
| Error | 4375 | | |

Note. "With eggs" and "all fruits" are as defined in table 1. Generalized $R^2 = 0.140$ (with eggs) and 0.084 (all fruits).

### *Simulated Seed Production in the Absence of* Hylemya

The mean seed sets per unparasitized fruit were slightly higher for dusty than for control plants (table 1), even though dusty plants had higher abortion rates (table 4). How might this pattern change in the absence of *Hylemya*? We simulated absence by replacing all the missing values that had been assigned to expanded, parasitized fruits with year- and census-specific means pooled across treatment (table 6). When we did this, mean per-fruit seed sets for both dusty and control plants increased in all but one yearly sample (2018 fruits with eggs); in that year, expanded, parasitized fruits (the ones with "missing" seed values) made up a very small fraction of all fruits, leaving little scope for seeing an effect from the hypothetical absence of the seed predator. Control plants, which had higher parasitization rates, gained more than dusty plants from the simulated absence of *Hylemya* in all comparisons (table 6). Under this simulated scenario, dusty plants would have produced overall about 7% fewer seeds per fruit than clean controls if *Hylemya* had not been present.

## Discussion

The results we have presented suggest a possible reason that exposure to road dust did not consistently reduce the fecundity of *Ipomopsis aggregata* plants in our earlier study, even though it consistently reduced stigma pollen loads (Waser et al. 2017). The reason is that *Hylemya* females avoid laying eggs on dusty plants. Compared with clean controls, flowers on dusty plants had a smaller probability of carrying a *Hylemya* egg, had fewer parasitized fruits that we scored as having missing values for

**Table 6**

**Simulated Seed Production in the Absence of *Hylemya***

| | Treatment | | | |
|---|---|---|---|---|
| | Dusty | | Control | |
| Year, fruit egg status | Seed set | Gain | Seed set | Gain |
| 2016, with eggs | 2.59 ± .46 (20) | .84 | 2.99 ± .42 (22) | 1.43 |
| 2017, with eggs | 4.29 ± .74 (19) | .19 | 4.44 ± .25 (24) | 1.07 |
| 2017, all fruits | 3.71 ± .30 (29) | .09 | 3.78 ± .29 (31) | .16 |
| 2018, with eggs | 4.19 ± .87 (19) | −.02 | 2.24 ± .87 (23) | .43 |
| 2018, all fruits | 1.72 ± .23 (23) | .06 | 1.81 ± .23 (23) | .21 |

Note. Values are means of plant means ± 1 SE, with sample sizes (number of plants sampled) given in parentheses and with all parasitized fruits being assigned the mean seed numbers found in expanded, unparasitized fruits from the same year and census. "Gain" is the difference between the hypothetical values that result from assigning these seed set values to parasitized fruits and the observed values reported in table 1. "With eggs" and "all fruits" are defined as in table 1.

seed number, and gained relatively fewer seeds per fruit when we replaced missing values with year- and census-specific means calculated from expanded, unparasitized fruits. The net effect of dust on plant fecundity is therefore mediated through at least two causal pathways (fig. 3). On the one hand, dust reduces pollen receipt and therefore has a negative expected effect on fecundity. On the other hand, dust also deters *Hylemya*, which preferentially attacks high-quality early flowers on clean plants and thus lowers the average seed set of their unparasitized fruits more than it does those of dusty plants.

Can we therefore say that attack by *Hylemya* by itself explains the unexpected pollen load and seed set patterns that we reported previously? We cannot. For one thing, we have improved our fruit-scoring methods since the earlier study, making it difficult to compare seed sets quantitatively. Furthermore, time limitations kept us from measuring pollen loads in the present study, and we know too little about variation in nonlinear pollen-seed relationships among sites and years to set confidence limits on a quantitative expectation about the translation of pollen loads into seed set. Our understanding of natural variation in *Hylemya* attack rates is similarly incomplete at this point. Finally, we simulated the absence of *Hylemya* by assigning a single value of seed number to parasitized fruits on the basis of overall year- and census-specific means of unparasitized fruits. This is a reasonable first approach, but it is imprecise if mean values differ by treatment. For example, if female flies oviposit more on plants within treatments or flowers within plants that are less likely to abort (e.g., Brody and Waser 1995; Brody and Morita 2000), then our use of a single seed set value underestimates the degree to which controls would outperform dusty flowers without *Hylemya*.

That an abiotic factor like road dust influences plant reproduction through multiple pathways is consistent with the modern recognition that species interact with one another and with the abiotic environment through direct and indirect pathways and on multiple scales of time and space (e.g., Wootton 1994; Estes et al. 2018). We propose that an effective way to confront such complexity is to follow up on surprising, ambiguous, or serendipitous observations with further inquiry (Price and Billick 2010). This iterative, informal "Bayesian" approach can greatly extend our ability to generalize beyond the specific system being studied, to the extent that it reveals mechanisms that are known to occur in other ecological systems (Pulliam and Waser 2010).

In the example at hand, we might have been tempted to conclude from our earlier study of four species (Waser et al. 2017) that dust has a negligible effect on plant fecundity in general. But further sleuthing, informed by natural history, uncovers a more interesting picture of *Ipomopsis*: dust influences fecundity not only through pollination success but also through predispersal seed predation, in such a way that one pathway at least partly counterbalances the other. This deeper appreciation of the *Ipomopsis* system suggests that dust could well affect plant reproduction more generally in complex, species-specific ways.

Our ability to generalize to other species depends as well on understanding more about the mechanisms by which dust affects *Ipomopsis* reproduction. Do eggs or pollen grains adhere poorly to dusty surfaces? If so, then stigma and epidermis properties may mediate dust effects. Does dust impose a physiological stress on the plant that reduces the production of floral volatiles or nectar or the floral life span, thereby deterring pollinators or *Hylemya*? If so, then plant physiological responses to dust may dictate its effects. We are just beginning to explore such possibilities.

It certainly seems logical that dust could impose physiological costs on *I. aggregata* plants. Even "pristine" dust devoid of industrial toxins has been shown to influence gas exchange,

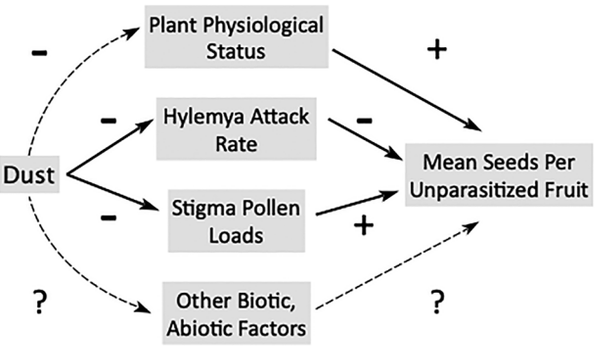


**Fig. 3** Conceptual path diagram of direct and indirect causal relationships between dust deposition and seed set per unparasitized fruit in the *Ipomopsis aggregata* system. Paths with strong support from this and our previous studies are shown with solid arrows, and two possible additional paths are shown with dashed arrows. Each path is shown as negative or positive (or with a question mark if both seem possible) to indicate whether an increase in the upstream factor increases or decreases the level or activity of the downstream factor. The final sign of a path (negative or positive) is the product of the signs of the component parts of that path. Thus, dust has a positive net effect on seed set via *Hylemya* attack and a negative net effect via stigma pollen loads and plant physiological status. Notice that the precise mechanisms for many effects remain unknown. For example, dust might decrease stigma pollen loads by deterring pollinators or changing their behavior, because it reduces the adhesion of pollen to the stigma surface, or by some other mechanism.

photosynthesis, and water use efficiency in other plant species (Sharifi et al. 1997). Its deposition during the vegetative stage of the *I. aggregata* life cycle, which lasts several years in RMBL populations (e.g., Brody et al. 2007), might reduce individual growth rate and survival and increase the number of years until flowering, in addition to influencing fecundity. Dust might also influence biotic agents other than *Hylemya*—including other insect herbivores—that play roles we have not recognized, perhaps because they are active only in the vegetative stage. Finally, the observation that rates of fruit abortion were consistently higher for all plants in the dry summers of 2016 and 2018 suggests that dust might harm plants by reducing water use efficiency. This hint of a link between climate and dust suggests that it will be worthwhile to continue exploring the effects of dust on pollination and plant reproduction in the changing climate of the Anthropocene.

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