UC Riverside

UC Riverside Previously Published Works

Title

Predispersal seed predation obscures the detrimental effect of dust on wildflower reproduction

Permalink

<https://escholarship.org/uc/item/9451v3jw>

Journal

International Journal of Plant Sciences, 182(4)

ISSN

1058-5893

Authors

Price, MV
Waser, NM
Lopez, DA
et al.

Publication Date

2021-05-01

DOI

10.1086/713440

Peer reviewed

2

3

4 **Pre-Dispersal Seed Predation Obscures the Detrimental Effect of Dust**
5 **on Wildflower Reproduction**

6

7

8 **Mary V. Price^{1,*†}, Nickolas M. Waser^{*,†}, David Lopez^{*}, Victor D. Ramírez^{*}, and**
9 **Carol E. Rosas^{*}**

10

11 ^{*}Rocky Mountain Biological Laboratory, Crested Butte, Colorado 81224; [†]School of
12 Natural Resources and the Environment, University of Arizona, Tucson, Arizona 85719

13

14

15

16

17

18 ¹Author for correspondence; email: mary.price@ucr.edu.

19

20 ORCIDs: Price, <http://orcid.org/0000-0002-2223-7792>; Waser, [http://orcid.org/0000-](http://orcid.org/0000-0001-8416-5037)
21 [0001-8416-5037](http://orcid.org/0001-8416-5037).

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

29 *Methodology.* Over three consecutive summers we scored the fates of over 4,000
30 flowers, comparing those on hand-dusted *I. aggregata* plants to those on clean control
31 plants in the same natural populations. We censused plants throughout each flowering
32 season, marking phenological cohorts of flowers and examining them for eggs of the
33 Anthomyiid fly *Hylemya* sp. We subsequently scored expanded fruits for seed loss to
34 *Hylemya* larvae.

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

42 *Conclusions.* Pre-dispersal seed predation likely contributed to the previous lack
43 of a negative effect of dust on seed set in *I. aggregata*. This study illustrates how
44 exploring unexpected experimental outcomes can reveal important ecological

45interactions, and how the mechanistic understanding so gained can improve the ability to
46generalize results to other systems.

47

48**Keywords:** Anthomyiid fly, indirect interaction, *Ipomopsis*, phenology, seed production

50

51 **Introduction**

52 A flowering plant can produce seeds only when it experiences tolerable physical
53 conditions, receives adequate high-quality pollen, has access to water and nutrient
54 resources, and avoids enemies. Ecologists have long considered how such factors limit
55 fruit and seed production, focusing especially on pollen and resources (e.g., Silander and
56 Primack 1978; Stephenson 1981; Udovic 1981; Knight et al. 2005; Aizen and Harder
57 2007) and on loss of reproductive effort to herbivores that eat flowers, inflorescences,
58 fruits, or developing seeds (e.g., Galen 1990; Louda and Potvin 1995; Sharaf and Price
59 2004; Maron and Crone 2006). How multiple constraints interact to affect fecundity is
60 less well studied. We know that pollen and resources may interact to mold patterns of
61 reproductive allocation in plants (Haig and Westoby 1988; Burd 1995, 2008), and that
62 both pollen and resources can limit seed production across different flowering episodes of
63 a perennial plant (Montalvo and Ackerman 1987) or even at different points within a
64 single flowering season (Campbell and Halama 1993). Less is understood about effects
65 on plant fecundity of herbivory, pollination, and resource availability acting together
66 (Sharaf and Price 2004; Brody and Irwin 2012).

67 Here we explore an interaction between herbivory and plant fecundity that varies
68 with one aspect of the abiotic environment—airborne dust. Dust is fine particulate matter
69 in the size range of microns to tens of microns that is generated primarily from wind-
70 scouring of dry soil surfaces. It is ubiquitous in the atmosphere of the Anthropocene,

71 fostered by increasing human soil disturbance and climate change. The study of dust's
72 ecological effects is in its infancy (Field et al. 2009).

73 Previous work on the fecundity of native wildflowers in the western USA (Waser
74 et al. 2017) suggested a surprising effect of dust that is generated by vehicular traffic on
75 unpaved roads. In 12 of 12 replicate experimental and observational studies of *Ipomopsis*
76 *aggregata*, samples of flowers from plants exposed to road dust (“dusty plants”) averaged
77 35% fewer pollen grains on their stigmas than did samples from clean control plants.
78 Pollen often limits per-fruit seed set in *I. aggregata* (e.g., Waser and Fugate 1986,
79 Campbell and Halama 1993), and we therefore expected fruits produced by the dusty
80 flowers to contain fewer seeds on average than those from clean flowers. But although
81 dusty plants fared worse than clean plants in 7 of the 12 studies, they fared better in 5; the
82 mean difference in seeds per fruit was a statistically insignificant deficit for dusty plants
83 of about 14%, less than half the pollen deficit.

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

90 We focus here on the fourth possibility. The red, tubular flowers of *I. aggregata*
91 are attacked by a fly, *Hylemya* sp. (Anthomyiidae), whose larvae consume developing
92 seeds within expanding fruits after pollination has occurred. If this pre-dispersal seed
93 predator attacks clean flowers more than dusty ones and destroys fruits that would have

94set more than the average number of seeds, its presence could obscure the fecundity
95advantage that higher pollen loads are expected to give clean flowers. In what follows we
96describe field experiments replicated over three years that were designed to evaluate this
97“compensatory herbivory” hypothesis. We began by affirming our previous observation
98that that the number of seeds produced by unparasitized fruits from clean control plants is
99no greater on average than the number for dusty plants. We then asked whether the
100conditions for pre-dispersal seed predation to counteract effects of low pollen loads are
101met. The answer is “yes”: clean flowers suffered a higher probability of *Hylemya* attack
102than did experimentally-dusted flowers, and flies preferentially attacked early-season
103flowers, which have lower than average probability of aborting and higher than average
104seed set if they do not abort. Finally, we asked whether the observed patterns of *Hylemya*
105oviposition can obscure the detrimental effects of dust on pollination. To answer this
106question, we simulated the absence of *Hylemya* by assuming that all parasitized flowers
107in our samples produced the average number of seeds for all unparasitized flowers open
108at the same time. Our calculations confirm that attack by this seed predator has the effect
109of disproportionally reducing the per-fruit fecundity of control plants.

110

111 **Methods**

112 *The Study System*

113 Scarlet gilia (*Ipomopsis aggregata* Pursh V. Grant, Polemoniaceae) is an
114herbaceous perennial plant native to mountains of the western USA. Below we describe
115the natural history of *I. aggregata* ssp. *aggregata* (sensu Grant and Wilken 1986) near the

116Rocky Mountain Biological Laboratory (hereafter RMBL; 2900 m a.s.l.; 38.96°N,
117106.99°W) in west-central Colorado, USA, where our previous road dust studies have
118taken place, and where nearly a half century of research has generated substantial
119knowledge of the pollination, life history, and population ecology of this wildflower (e.g.,
120Watt et al. 1974; Pyke 1978; Hainsworth et al. 1984; Caruso 1999; Campbell 2019).

121 Scarlet gilia plants flower once after growing as vegetative rosettes for several
122years (Price et al. 2008). Repeat flowering occurs rarely in the absence of damage during
123the vegetative stage (Brody et al. 2007). The flowers are most often produced on a single
124flowering stalk (Brody and Morita 2000), but plants may produce multiple inflorescences
125if the vegetative rosette is damaged or if ungulates browse elongating inflorescences
126during the summer of flowering (Sharaf and Price 2004). The main pollinators are
127Broad-tailed and Rufous Hummingbirds (*Selasphorus platycercus* and *S. rufus*), but long-
128tongued insects also contribute (Waser 1982; Mayfield et al. 2001; Price et al. 2005).
129Plants are highly self-sterile (Sage et al. 2006), so fruit and seed production depend on
130receipt of outcross pollen as well as on resource availability (Campbell and Halama
1311983). Expanded fruits mature about 5 seeds on average, each weighing about 1 mg (Fig.
1321A; a 1980 sample of 10 seeds from each of 5 plants yielded a mean of .92 mg (SD
133= .037 mg; N. M. Waser and M. V. Price unpublished data).

134 *Ipomopsis aggregata* and the confamilial *Polemonium foliosissimum* share a pre-
135dispersal seed predator, an undescribed species of fly in the genus *Hylemya*
136(Anthomyiidae; although the fly has also been placed in the genus *Delia*, we use *Hylemya*
137for consistency with previous publications). Interactions between the fly and its hosts are
138well studied at the RMBL (e.g., Zimmerman 1980; Hainsworth et al. 1984; Brody 1992,

1391997; Brody and Waser 1995; Brody and Morita 2000). Female *Hylemya* usually lay a
140single egg between the calyx and corolla of elongated flower buds and newly-opened
141flowers (Fig. 1B). Only 15 of 809 buds or flowers on which we detected eggs in the
142current study carried two eggs, and none carried more than two. If the egg hatches, its
143larva (Fig. 1C) burrows into the ovary and consumes the embryo and endosperm of
144developing seeds. If the larva survives, it generally destroys all seeds in its fruit before
145chewing an exit hole and falling to the soil to pupate.

146 The experiments described here took place in the summers of 2016, 2017, and
1472018, in a gently SE-sloping meadow 1 km south of the RMBL along an unpaved road.
148The road is treated with an aqueous solution of magnesium chloride each spring to
149suppress dust; we worked upslope of the road to avoid any impacts of runoff from the
150road surface. Input of water from melting of the winter snowpack—which strongly
151affects reproduction, growth, and survival of *I. aggregata* (e.g., Campbell and Halama
1521993; Price and Waser 1998; Campbell 2019)—varied among the three summers of
153study. Total water input from snow was highest in 2017 (785 mm) and lower in 2016 and
1542018 (536 mm and 424 mm respectively). All three values were lower than the 1976-
1552019 average of 970 mm (range 279 mm in 1977 to 1145 mm in 1995).

156

157Dusting Treatment

158 In each summer, before onset of the approximately one-month-long flowering
159season of scarlet gilia, we chose pairs of neighboring bolting plants (25 pairs in 2016; 32
160in 2017; 25 in 2018) of similar size. With a coin toss we chose one of each pair to be
161dusted, leaving the other as a clean control, and then measured stalk heights and basal

162stem diameters of marked plants. Paired t-tests confirmed that the coin-toss method
163indeed equalized plant sizes between treatments.

164 We obtained dust from a section of road north of the RMBL that has never been
165treated with magnesium chloride. Soil from this road was dried and sieved through a No.
16635 USA standard soil sieve (.5 mm mesh size), to produce a fine powder. We began to
167apply this powder with a hand duster (Gilmour Manufacturing, Somerset, PA) in the first
168week of flowering—on 3 July, 3 July, and 18 June in 2016, 2017, and 2018, respectively
169—and continued every 1-2 days, except during periods of rainfall, until plants ceased
170flowering. Either 10 (2016) or 15 (2017, 2018) pumps of the duster were applied to each
171dusted plant each time; plants in the control treatment were covered with a paper bag to
172shield them, and then received a sham dusting of 10-15 pumps of air alone from a clean,
173empty duster. Our treatment approximated natural roadside dust deposition: densities of
174dust particles on leaves of hand-dusted plants were intermediate between those of
175naturally dusted roadside plants and clean plants growing 50 m from the closest road (C.
176E. Rosas, unpublished data).

177

178*Assessing Seasonal Timing of Flowering and Hylemya Oviposition*

179 On the morning of every fourth day during each year's flowering season we
180counted open flowers and elongated buds that would open that afternoon and searched
181them for *Hylemya* eggs, using forceps to gently retract calyx lobes. The 4-day census
182interval minimized the probability of counting flowers more than once, because few *I.*
183*aggregata* flowers retain their corollas 4 days beyond the elongated bud stage (N. M.
184Waser and M. V. Price, unpublished data). We began censuses when most marked plants

185 had at least one open flower or elongated bud and few plants had old flowers or initiated
186 fruits; the first census took place on 6 July in 2016, 3 July in 2017, and 20 June in 2018.

187

188 *Measuring Flower Fate and Seed Set*

189 To evaluate any seasonal trends in flower fates and per-fruit seed set, we applied
190 an inconspicuous dot of non-toxic acrylic paint to calyces of elongated buds and flowers
191 after we examined them. In 2016 only those buds and flowers with *Hylemya* eggs were
192 marked with a census-specific color, whereas in 2017 and 2018 all buds and flowers were
193 so marked, and those with *Hylemya* eggs received an additional dot of yellow paint. At
194 the end of each summer we assessed fates of parasitized flowers (2016) or all flowers,
195 parasitized or not (2017, 2018). Flowers whose ovaries failed to expand after their
196 corollas had abscised were scored as aborted fruits (“aborts”) with zero seeds. Fruits that
197 had expanded were collected just before dehiscence and air-dried in the laboratory. Each
198 expanded fruit was gently opened under a WILD M-5 dissecting microscope (WILD
199 Heerbrugg AG, Switzerland). We counted mature, full-sized seeds of unparasitized
200 fruits. We scored fruits with evidence of *Hylemya* attack (living or dead larva, chewed
201 seeds, frass, or an exit hole) as “parasitized”, and their seed number as “missing”. This
202 fruit-scoring method is consistent with that used in the previous study (Waser et al.
203 2017). A single observer scored all fruits from a given year.

204

205 *Accuracy of Egg Censuses*

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

208difficult to examine (hence the species epithet *aggregata*), and flies continued to oviposit
209between censuses. Indeed, in 2017 and 2018, the summers in which we color-marked all
210flowers, we discovered many expanded fruits with evidence of *Hylemya* attack that
211lacked the yellow dot to indicate that we had detected an egg. An analysis from these
212two summers (Appendix S1) suggests that we detected 42% of all eggs on average, and
213that the probability of missing an egg did not differ significantly between dusty and
214control plants or among census dates. This lack of significant interactions suggests that
215egg counts are a useful indicator of oviposition patterns across treatments and flowering
216seasons, even though they underestimate true egg numbers.

217

218*Simulated Seed Production in the Absence of Hylemya*

219 To explore how *Hylemya* attack may have influenced observed patterns of
220fecundity, we simulated what seed sets per fruit would have been if the flies had been
221absent from the system and no fruits had been parasitized. We first calculated the mean
222of plant mean seed numbers for expanded, unparasitized fruits by year and census,
223pooling dusted and control treatments. Once again, 2016 values were only available from
224fruits known to have had an egg, whereas 2017 and 2018 values included all fruits.
225Replacing the “missing” values assigned to each parasitized fruit with these year- and
226census-specific mean values provided an estimate of what mean seed sets of dusty and
227control fruits would have been without *Hylemya* present.

228

229*Statistical Analysis*

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

240

241 **Results**

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

243 Waser et al. (2017) reported that flowers on dusty plants averaged fewer pollen
244 grains on their stigmas than flowers on clean control plants, but did not consistently
245 produce fewer seeds per unparasitized fruit. The present study confirms this seed set
246 result for three additional summers over which we scored the fates of 4575 flowers (Table
247 1). The mean seed set per unparasitized fruit was insignificantly higher for dusty than for
248 control plants (Treatment effects in Table 2), whether we analyzed all fruits (2017 and
249 2018), or only those on which we had detected *Hylemya* eggs (all three summers; mean
250 values could be obtained from fruits in which eggs failed to produce successful larvae).
251 Per-fruit seed set varied considerably among summers and was highest in 2017, the
252 wettest of the three. The year effect approached significance for the subset of fruits

253 known to have eggs (all three summers) and was highly significant for all fruits (2017
254 and 2018; Table 2). There were no significant Year $\times$ Treatment interactions, indicating
255 that treatment effects were similar across years.

256

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

258 Per-flower egg loads varied significantly among summers (Fig. 2; Year effect in
259 Table 3), being lowest following the wet winter of 2017 (least squares mean of plant
260 means = .09 eggs/flower) and higher in the drier years 2016 and 2018 (means = .17
261 and .23, respectively). Taking all summers together, oviposition was concentrated early
262 in the flowering season and decreased significantly overall by .03 eggs per flower with
263 each successive 4-day census period. The seasonal rate of decline varied significantly
264 among summers, however (Fig. 2; Year $\times$ Census effect in Table 3): oviposition
265 decreased significantly through time in 2016 and 2018, but not in 2017, the summer with
266 the lowest rate of *Hylemya* attack and thereby the least scope for a decline. In all
267 summers, flowers of clean control plants had significantly higher egg loads overall than
268 those of dusty plants (least squares means of plant means across all summers = .21 vs. .12
269 eggs/flower, respectively; Fig. 2; Treatment effect in Table 3).

270

271 *Oviposition and Flower Quality*

272 Judging from the different fates of flowers with and without eggs, *Hylemya*
273 oviposited preferentially on “high-quality” flowers that were more likely to set fruit with
274 many seeds. In data pooled across summers, censuses within summers, individual plants,
275 and treatments, fruits with *Hylemya* eggs were less likely to abort (33.9% of 4575 fruits)

276than fruits without eggs (42.1%; Likelihood Ratio Chi-square = 14.26, $P = .002$).
277Furthermore, the average number of seeds produced by expanded fruits with an egg but
278no sign of successful larval attack (5.46 seeds/fruit, $SE = .283$; data pooled across
279summers, censuses within summers, plants, and treatments) was higher than for expanded
280unparasitized fruits without an egg (4.91 seeds/fruit; $SE = .085$; $F_{1,2345} = 7.47$, $P = .0063$,
281based on square-root-transformed values).

282 How did *Hylemya* select high-quality flowers? First, they avoided dusty plants.
283Abortion rates were consistently higher for dusty than control plants (Table 4), whether
284the analysis included only fruits known to have carried an egg (all three summers), or all
285fruits (2017 and 2018). This pattern was significant in both comparisons (Treatment
286effects in Table 5). Second, most eggs were laid on flowers produced early in the season,
287which make a disproportionate contribution to overall seed production.

288 Two factors made early flowers the most fecund. First, the probability that a fruit
289aborted increased through the season in all three summers (likelihood ratio chi-square >
29013.89, $DF = 1$, $P < .0002$ in each of three separate analyses of 2016, 2017, and 2018
291data), albeit at different rates depending on the summer (chi-square = 28.93, $DF = 2$, P
292< .0001). The wettest year, 2017, had the lowest overall rate of abortion (Table 4), and
293was thereby constrained to have the smallest increase in abortion rate through the season.
294Second, the number of seeds produced per expanded, unparasitized fruit also decreased
295through the season by about .86 seeds per successive census overall (ANCOVA, square-
296root-transformed means of plant means; $F_{1,482} = 21.36$, $P < .0001$), albeit again at different
297rates (ANCOVA, square-root-transformed means of plant means; $F_{1,482} = 7.44$, $P = .0007$).
298The effect of census number on abortion rate or seed number did not differ between dusty

299and control treatments ($P = .969$ and $P = .983$ for abortion rate and seed number,
300respectively).

301 The seasonal decline in seed set per expanded, unparasitized fruit could be due to
302progressively lower allocation to female function by *I. aggregata* plants through the
303flowering season. In 1984, as part of an earlier unpublished study, we counted ovules
304from flowers that were open during the first and third week of the flowering season on 10
305untreated plants in the same meadow population as the present study, and found that
306ovule number decreased from a least-squares mean of 23.3 ovules in the first week to
30719.7 ovules in the third week. Maximum-likelihood ANOVA with plant treated as a
308random effect indicated that this decrease from week 1 to week 3 was significant
309(collection effect: $F_{1,186} = 93.89$, $P < .0001$; overall model $R^2_{\text{adj}} = .546$). We have no
310reason to suspect that plant allocation differed between the earlier sample and the current
311study period.

312

313*Simulated Seed Production in the Absence of Hylemya*

314 The mean seed sets per unparasitized fruit were slightly higher for dusty than for
315control plants (Table 1), even though dusty plants had higher abortion rates (Table 4).
316How might this pattern change in the absence of *Hylemya*? We simulated absence by
317replacing all the missing values that had been assigned to expanded, parasitized fruits
318with year- and census-specific means pooled across treatment (Table 6). When we did
319this, mean per-fruit seed sets for both dusty and control plants increased in all but one
320yearly sample (2018 fruits with eggs); in that year expanded, parasitized fruits (the ones
321with “missing” seed values) made up a very small fraction of all fruits, leaving little

scope for seeing an effect of the hypothetical absence of the seed predator. Control plants, which had higher parasitization rates, gained more than dusty plants from 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.

327

328 Discussion

329 The results we have presented suggest a possible reason that exposure to road dust
330 did not consistently reduce fecundity of *I. aggregata* plants in our earlier study, even
331 though it consistently reduced stigma pollen loads (Waser et al. 2017). The reason is that
332 *Hylemya* females avoid laying eggs on dusty plants. Compared to clean controls, flowers
333 on dusty plants had a smaller probability of carrying a *Hylemya* egg, had fewer
334 parasitized fruits that we scored as having missing values for seed number, and gained
335 relatively fewer seeds per fruit when we replaced missing values with year- and census-
336 specific means calculated from expanded, unparasitized fruits. The net effect of dust on
337 plant fecundity is therefore mediated through at least two causal pathways (Fig. 3). On the
338 one hand, dust reduces pollen receipt and therefore has a negative expected effect on
339 fecundity. On the other hand, dust also deters *Hylemya*, which preferentially attacks high-
340 quality early flowers on clean plants and thus lowers the average seed set of their
341 unparasitized fruits more than it does those of dusty plants.

342 Can we therefore say that attack by *Hylemya* by itself explains the unexpected
343 pollen load—seed set patterns that we reported previously? We cannot. For one thing,
344 we have improved our fruit scoring methods since the earlier study, making it difficult to

345compare seed sets quantitatively. Furthermore, time limitation kept us from measuring
346pollen loads in the present study, and we know too little about variation among sites and
347years in nonlinear pollen-seed relationships to set confidence limits on a quantitative
348expectation about the translation of pollen loads into seed set. Our understanding of
349natural variation in *Hylemya* attack rates is similarly incomplete at this point. Finally, we
350simulated absence of *Hylemya* by assigning a single value of seed number to parasitized
351fruits based on overall year- and census-specific means of unparasitized fruits. This is a
352reasonable first approach, but it is imprecise if mean values differ by treatment. For
353example, if female flies oviposit more on plants within treatments or flowers within plants
354that are less likely to abort (e.g., Brody and Waser 1995, Brody and Morita 2000), then
355our use of a single seed set value underestimates the degree to which controls would
356outperform dusty flowers without *Hylemya*.

357 That an abiotic factor like road dust influences plant reproduction through
358multiple pathways is consistent with the modern recognition that species interact with one
359another and with the abiotic environment through direct and indirect pathways, and on
360multiple scales of time and space (e.g., Wootton 1994; Estes et al. 2018). We propose
361that an effective way to confront such complexity is to follow up surprising, ambiguous,
362or serendipitous observations with further inquiry (Price and Billick 2010). This
363iterative, informal “Bayesian” approach can greatly extend our ability to generalize
364beyond the specific system being studied, to the extent that it reveals mechanisms that are
365known to occur in other ecological systems (Pulliam and Waser 2010).

366 In the example at hand, we might have been tempted to conclude from our earlier
367study of four species (Waser et al. 2017) that dust has a negligible effect on plant

368fecundity in general. But further sleuthing, informed by natural history, uncovers a more
369interesting picture in *Ipomopsis*: dust influences fecundity not only through pollination
370success, but also through pre-dispersal seed predation in such a way that one pathway at
371least partly counterbalances the other. This deeper appreciation of the *Ipomopsis* system
372suggests that dust could well affect plant reproduction more generally in complex,
373species-specific ways.

374 Our ability to generalize to other species depends as well on understanding more
375about the mechanisms by which dust affects *Ipomopsis* reproduction. Do eggs or pollen
376grains adhere poorly to dusty surfaces? If so, then stigma and epidermis properties may
377mediate dust effects. Does dust impose a physiological stress on the plant that reduces
378production of floral volatiles or nectar, or floral lifespan, thereby deterring pollinators or
379*Hylemya*? If so, then plant physiological responses to dust may dictate its effects. We
380are just beginning to explore such possibilities.

381 It certainly seems logical that dust could impose physiological costs on *I.*
382*aggregata* plants. Even “pristine” dust devoid of industrial toxins has been shown to
383influence gas exchange, photosynthesis, and water use efficiency in other plant species
384(Sharifi et al. 1997). Its deposition during the vegetative stage of the *I. aggregata* life
385cycle, which lasts several years in RMBL populations (e.g., Brody et al. 2007), might
386reduce individual growth rate and survival and increase the number of years until
387flowering, in addition to influencing fecundity. Dust might also influence biotic agents
388other than *Hylemya*—including other insect herbivores—that play roles we have not
389recognized, perhaps because they are active only in the vegetative stage. Finally, the
390observation that rates of fruit abortion were consistently higher for all plants in the dry

391summers of 2016 and 2018 suggests that dust might harm plants by reducing water use
392efficiency. This hint of a link between climate and dust suggests that it will be
393worthwhile to continue exploring effects of dust on pollination and plant reproduction in
394the changing climate of the Anthropocene.

395

396**Acknowledgements**

397We thank Nallely Centeno Armenta for help in the field, Alison. K. Brody, Amy M. Iler,
398William K. Petry, B. Adriaan Grobler, Jennifer J. Weber, James Ellis, and an anonymous
399reviewer for improvements to the paper; Jennie Reithel, RMBL Science Director, for
400logistical support; the RMBL Krakauer Endowment for support of CMR; and National
401Science Foundation grant DBI 1262713 for support of DL, VDR, and CMR.

402

403**References**

404Aizen MA, LD Harder 2007 Expanding the limits of the pollen-limitation concept: effects
405 of pollen quantity and quality. *Ecology* 88:271–281.

406Brody AK 1992 Oviposition choices by a pre-dispersal seed predator (*Hylemya* sp.) II. A
407 positive association between female choice and fruit set. *Oecologia* 91:63–67.

408Brody AK 1997 Effects of pollinators, herbivores, and seed predators on flowering
409 phenology. *Ecology* 78:1624–1631.

410Brody AK, RE Irwin 2012 When resources don't rescue: flowering phenology and species
411 interactions affect compensation to herbivory in *Ipomopsis aggregata*. *Oikos*
412 121:1424–1434.

413 Brody AK, SI Morita 2000 A positive association between oviposition and fruit set:
 414 female choice or manipulation? *Oecologia* 124:418–425.

415 Brody AK, MV Price, NM Waser 2007 Life-history consequences of vegetative damage
 416 in scarlet gilia, a monocarpic plant. *Oikos* 116:975–985.

417 Brody AK, NM Waser 1995 Oviposition patterns and larval success of a pre-dispersal
 418 seed predator attacking two confamilial host plants. *Oikos* 74:447–452.

419 Burd M 1995 Ovule packaging in stochastic pollination and fertilization environments.
 420 *Evolution* 49:100–109.

421 Burd M 2008 The Haig-Westoby model revisited. *American Naturalist* 171:400–404.

422 Campbell DR 2019 Early snowmelt projected to cause population decline in a subalpine
 423 plant. *Proceedings of the National Academy of Sciences (USA)* **116:12901–**
 424 **12906.**

425 Campbell DR, KJ Halama 1993 Resource and pollen limitations to lifetime seed
 426 production in a natural plant population. *Ecology* 74:1043–1051.

427 Caruso CM 1999 Pollination of *Ipomopsis aggregata* (Polemoniaceae): effects of intra-
 428 vs. interspecific competition. *American Journal of Botany* 86:663–668.

429 English-Loeb G, MJ Stout, SS Duffey 1997 Drought stress in tomatoes: changes in plant
 430 chemistry and potential nonlinear consequences for insect herbivores. ***Oikos***
 431 **79:456–468.**

432 Estes L, PR Elsen, T Treuer, L Ahmed, K Caylor, J Chang, JJ Choi, EC Ellis 2018 The
 433 spatial and temporal domains of modern ecology. *Nature Ecology & Evolution*
 434 **2:819–826.**

435Field JP, J Belnap, DD Breshears, JC Neff, GS Okin, JJ Whicker, TH Painter, R Sujith,
436 MC Reheis, RL Reynolds 2009 The ecology of dust. *Frontiers in Ecology and the*
437 *Environment* 8:423–430.

438Galen C 1990 Limits to the distributions of alpine tundra plants: herbivores and the alpine
439 skylipilot, *Polemonium viscosum*. **Oikos** 59:355–358.

440Grant V, DH Wilken 1986 Taxonomy of the *Ipomopsis aggregata* group
441 (Polemoniaceae). *Botanical Gazette* 147:359–371.

442Hainsworth FR, LL Wolf, T Mercier 1984 Pollination and pre-dispersal seed predation:
443 net effects on reproduction and inflorescence characteristics in *Ipomopsis*
444 *aggregata*. *Oecologia* 63:405–409.

445Knight TM, JA Steets, JC Vamosi, SJ Mazer, M Burd, DR Campbell, MR Dudash, MO
446 Johnston, RJ Mitchell, T-L Ashman 2005 Pollen limitation of plant reproduction:
447 pattern and process. **Annual Review of Ecology, Evolution, and Systematics**
448 36:467–497.

449Louda SM, MA Potvin 1995 Effect of inflorescence-feeding insects on the demography
450 and lifetime fitness of a native plant. *Ecology* 76:229–245.

451Maron JL, E Crone 2006 Herbivory: effects on plant abundance, distribution and
452 population growth. *Proceedings of the Royal Society of London B* 273:2575–
453 2584.

454Mayfield MM, NM Waser, MV Price 2001 Exploring the “most effective pollinator
455 principle” with complex flowers: bumblebees and *Ipomopsis aggregata*. *Annals*
456 *of Botany* 88:591–596.

457 Montalvo AM, JD Ackerman 1987 Limitation to fruit production in *Ionopsis*
 458 *utricularioides* (Orchidae). *Biotropica* 19:24–31.
 459 Price MV, I Billick 2010 Building an understanding of place. Pages 177–183 in I Billick,
 460 MV Price, eds. The ecology of place: contributions of place-based research to
 461 ecological understanding. University of Chicago Press, Chicago.
 462 Price MV, NM Waser 1998 Effects of experimental warming on plant reproductive
 463 phenology in a subalpine meadow. *Ecology* 79:1261–1271.
 464 Price MV, DR Campbell, NM Waser, AK Brody 2008 Bridging the generation gap in
 465 plants: from parental fecundity to offspring demography. *Ecology* 89:1596–1604.
 466 Pulliam HR, NM Waser 2010 Ecological invariance and the search for generality in
 467 ecology. Pages 69–90 in I Billick, MV Price, eds. The ecology of place:
 468 contributions of place-based research to ecological understanding. University of
 469 Chicago Press, Chicago.
 470 Pyke GH 1978 Optimal foraging in hummingbirds: testing the marginal value theorem.
 471 *American Zoologist* 18:739–752.
 472 Sage TL, MV Price, NM Waser 2006 Self-sterility in *Ipomopsis aggregata*
 473 (Polemoniaceae) is due to pre-zygotic ovule degeneration. *American Journal of*
 474 *Botany* 93:254–262.
 475 Sharaf KE, MV Price 2004 Does pollination limit tolerance to browsing in *Ipomopsis*
 476 *aggregata*? *Oecologia* 138:396–404.
 477 Sharifi MR, AC Gibson, PW Rundel 1997 Surface dust impacts on gas exchange in
 478 Mojave Desert shrubs. *Journal of Applied Ecology* 34:837–846.

479 Silander JA, RB Primack 1978 Pollination intensity and seed set in the evening primrose
 480 (*Oenothera fruticosa*). American Midland Naturalist 100:213–216.
 481 Stephenson AG 1981 Flower and fruit abortion: proximate causes and ultimate functions.
 482 Annual Review of Ecology and Systematics 12:253–279.
 483 Udovic D 1981 Determinants of fruit set in *Yucca whipplei*: reproductive expenditure vs.
 484 pollinator availability. Oecologia 48:389–399.
 485 Waser NM 1982 A comparison of distances flown by different visitors to flowers of the
 486 same species. Oecologia 55:251–257.
 487 Waser NM, ML Fugate. 1986. Pollen precedence and stigma closure: a mechanism of
 488 competition for pollination between *Delphinium nelsonii* and *Ipomopsis*
 489 *aggregata*. Oecologia 70:573–577.
 490 Waser NM, MV Price 2016 Drought, pollen and nectar availability, and pollination
 491 success. Ecology 97:1400–1409.
 492 Waser NM, MV Price, G Casco, M Diaz, A-L Morales, J Solverson 2017 Effects of road
 493 dust on the pollination and reproduction of wildflowers. International Journal of
 494 Plant Sciences 178:85–93.
 495 Watt, WB, PC Hoch, SG Mills 1974 Nectar resource use by *Colias* butterflies. Oecologia
 496 14:353–374.
 497 Wootton JT 1994 The nature and consequences of indirect effects in ecological
 498 communities. Annual Review of Evolution, Ecology, and Systematics 25:443–
 499 466.

500Zimmerman M 1980 Reproduction in *Polemonium*: pre-dispersal seed predation. Ecology
501 61: 502–506.

502

504**Figure 1. A:** Expanded fruit and 7 mature seeds of *I. aggregata*; note also one aborted seed and one unexpanded ovule to the right.

505**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

506a pale larva is visible on the left emerging from the split fruit capsule. **C:** two larval *Hylemya* that were extracted from expanded

507fruits; the dark sclerotized mouthparts are clearly visible in the smaller specimen.

Figure 2. Flowering and *Hylemya* oviposition phenology for dusted and clean plants during three summers of study. Total heights of stacked bars indicate mean per-plant

512 numbers of elongated flower buds plus open flowers counted in successive censuses,
513 which occurred every 4 days. The darker bottom portion of each bar indicates the mean
514 number of elongated buds and flowers on which we detected *Hylemya* eggs during a
515 census; the lighter upper portion indicates the mean number of buds and flowers without
516 eggs. Bars for dusty plants are to the left of those for clean plants in each census.

517

518

519

520**Figure 3.** A conceptual path diagram of direct and indirect causal relationships between
 521dust deposition and seed set per unparasitized fruit in the *I. aggregata* system. Paths with
 522strong support from this and our previous studies are shown with solid arrows, and two
 523possible additional paths are shown with dashed arrows. Each path is shown as negative
 524or positive (or with a question mark if both seem possible) to indicate whether an
 525increase in the upstream factor increases or decreases the level or activity of the
 526downstream factor. The final sign of a path (negative or positive) is the product of the
 527signs of the component parts of that path. Thus, dust has a positive net effect on seed set
 528via *Hylemya* attack, and a negative net effect via stigma pollen loads and plant
 529physiological status. Notice that the precise mechanisms for many effects remain
 530unknown. For example, dust might decrease stigma pollen loads by deterring pollinators

531or changing their behavior, or because it reduces adhesion of pollen to the stigma surface,
532or by some other mechanism.

533

534

Table 1

535

Effects of year and treatment on seeds per fruit

536

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)

537

538NOTE. — Values are for unparasitized fruits and include abortions (zero seeds); they are

539means of individual plant means ± 1 SE, with sample sizes (number of plants sampled)

540given in parentheses. “Fruit egg status”: “With eggs” refers to the subset of fruits from

541flowers (2016, 2017, and 2018) on which we detected *Hylemya* eggs; “All fruits”

542includes fruits from all flowers (2017 and 2018 only), whether or not we detected an egg.

543

544

Table 2

545

ANOVAs of effects of year and treatment on seeds per fruit

546

Variable	Source	DF	F	P
With eggs	Model	5	2.036	.080
	Year	2	3.013	.054
	Treatment	1	.899	.345
	Year $\times$ Treatment	2	1.463	.236
	Error	102		
All fruits	Model	3	16.698	<.0001
	Year	1	50.089	<.0001
	Treatment	1	.011	.916
	Year $\times$ Treatment	1	.0001	.982
	Error	102		

547

548NOTE. – Analyses are based on square-root-transformed plant means of seeds per

549unparasitized fruit, to normalize residuals. “With eggs” and “All fruits” are as defined in

550Table 1. Model $R^2_{\text{adj}} = .046$ (with eggs), and .310 (all fruits).

551

552

Table 3553 ANOVA of effects of year, census, and treatment on mean *Hylemya* eggs per flower

554

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		

555

556NOTE. – Analyses are based on number of eggs detected per flower, averaged across

557plants within a treatment and census date. Census date (“Census”) is treated as a

558continuous covariate. Residuals were normally distributed. The overall model R^2_{adj}

559= .573.

560

561

562

563

564

565

Table 4

566

Effects of year and treatment on fruit abortion rates

Year	Fruit egg status	Treatment		
		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)

567

568NOTE. — Values are proportions of fruits whose ovaries did not expand and were scored

569as abortions, with total number of fruits given in parentheses. “Fruit egg status” is defined as

570in Table 1. “Dusty” and “Control” refer to fruits for a given year and fruit egg status

571pooled across censuses and plants within the Dusty or Control treatment, respectively.

572“Pooled” refers to all fruits for a given year and fruit egg status, pooled across censuses,

573treatments, and plants within treatment.

574

575

Table 5576 **Logistic regression of effects of year and treatment on fruit abortion rates**

577

Variable	Source	DF	Chi-square	P
With eggs	Model	5	70.560	<.0001
	Year	2	50.648	<.0001
	Treatment	1	9.971	.0016
	Year $\times$ 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 $\times$ Treatment	1	.008	.930
	Error	4375		

578

579NOTE. — Generalized $R^2 = .140$ (with eggs) and $.084$ (all fruits).

580

Table 6

581

Simulated seed production in the absence of *Hylemya*

Year	Fruit egg status	Treatment			
		Dusty	Gain	Control	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

583

584NOTE. – Values are means of plant means ± 1 SE, with sample sizes (number of plants

585sampled) given in parentheses, and with all parasitized fruits being assigned the mean

586seed numbers found in expanded, unparasitized fruits from their same year and census.

587“Gain” is the difference between the hypothetical values that result from assigning these

588seed set values to parasitized fruits, and the observed values reported in Table 1. “With

589eggs” and “All fruits” are defined as in Table 1.

590

591

592

593

594

595

596

597**Appendix S1.** Calculation of the degree to which actual *Hylemya* egg numbers were

598underestimated in field censuses of elongated buds and newly-opened flowers.

599

600 In 2017 and 2018, the summers in which we collected all fruits, we discovered
601evidence of larvae in 259 expanded fruits that did not have a yellow paint dot on the
602calyx to indicate that we had detected an egg. But the flowers that produced these fruits
603must have carried an egg, since there is no evidence that *Hylemya* larvae move between
604flowers after they hatch. By how much did our censuses underestimate oviposition rates?
605Of the 378 fruits with a yellow dot that we knew had eggs, 49.2% contained evidence of
606larval attack. If this success rate held more generally, then $259/.492 = 526$ flowers
607without yellow dots actually carried eggs. The 378 flowers on which we detected eggs,
608then, were 42% of the estimated total of $378 + 526 = 904$ flowers with eggs. Does this
609underestimation affect our assessment of seasonal and treatment oviposition patterns? In
610both 2017 and 2018 the probability of finding evidence of a larva was significantly higher
611for fruits with a yellow dot, which we knew had an egg (46.6%), than for fruits without a
612yellow dot (10.7%; Logistic regression: Chi-Square = 91.15, DF = 1, $P < .0001$). But this
613probability did not obviously differ by census date or treatment (in all cases chi-square <
6140.715, DF = 1, $P > .318$). This lack of significant interactions indicates that even though
615our egg counts underestimate total oviposition rates, they remain useful indicators of
616treatment and seasonal patterns of oviposition.
617