

Road Dust, *Ipomopsis aggregata* Seed Count, and attack
by *Hylemya* Flies—Are they Linked?

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Abstract:

At the Rocky Mountain Biological Laboratory (RMBL), road dust is easily dispersed by vehicle transportation and can travel 200 meters from the road. A previous study found that road dust had an ecological impact on several species of common wildflowers at the RMBL, including scarlet gilia, *Ipomopsis aggregata*. I studied the effects of road dust on the anthomyiid fly *Hylemya* sp., a pre-dispersal seed predator of *I. aggregata*. Experimental application of road dust affected the number of elongated flower buds produced by plants, but not the number of open flowers. *Hylemya* females laid eggs approximately twice as often on undusted flowers as on dusted flowers. The likelihood of finding *Hylemya* larvae or larval damage in fruits that came from flowers with eggs did not differ between treatments, nor did the estimated seed counts. The seed count remained relatively similar throughout the season regardless of the treatment. Results in this study suggest that *Hylemya* females are using cues from *I. aggregata* to select the best host, and additionally manipulating the hosts' condition to benefit their offspring.

Introduction:

Dust can be described as a dry powder or earth particle that arises from the ground. This particle can have different means of traveling. For instance, it's capable of traveling through wind dispersal, wildlife, agriculture, and vehicle transportation. Roads are perhaps a prime origin of dust. Considering 10 percent of road lengths in the U.S. are in national forest (Forman and Alexander 1998), the Rocky Mountain Biological Laboratory (RMBL) in western Colorado is constant victim of road dust. At the RMBL, vehicle transportation is perhaps the most common cause of dust dispersal. With constant vehicle traffic unpaved roads, road dust is easily dispersed by tire friction across meadows and is capable of traveling 200 meters from the road (Forman and Alexander 1998). In the U.S. alone, 15-20% of the continent is ecologically impacted by roads. Considering the different means dust can travel and the distances it can reach can bring concerns for ecologist and a question: does road dust have any ecological consequences?

One possible ecological consequence of dust is that it affects the pollination and reproduction of wildflowers. Waser et al. (2016) explored this possibility by exposing four common wildflowers to road dust at the RMBL. They found that stigmas (the receptive female structures of flowers) in dustier conditions carried less pollen (over six times less) for 19 of the 20 independent tests run on all the wildflowers. Generally, flowers that receive less pollen

produce fewer seeds. However, Waser et al. (2016) found inconsistent effects of road dust on seed set. One mechanism that could potentially explain this result is compensatory herbivory, which refers to a pre-dispersal seed predator reducing or offsetting the higher seed count that would occur in undusted flowers in the absence of the predator.

One particular angiosperm that may be victim of compensatory herbivory is *Ipomopsis aggregata* (see Brody and Waser 1995). The Anthomyiid fly (*Hylemya* sp.) is a pre-dispersal seed predator of *I. aggregata* that lays eggs under its sepals (Brody 1992b). The eggs hatch and the larvae burrow into the ovary, eating the developing seeds. Previous studies have found that flowers on which female flies oviposit are more likely to set seed than flowers without eggs (Brody 1992b; Brody and Waser 1995; Brody and Morita 2000). One plausible explanation is the use of floral traits or cues by female flies to make optimal host selections (Brody and Marita 2000). If female flies are more prone to oviposit on undusted plants, or if their larval mortality rate is lower in undusted plants, then average seed count could be reduced for undusted plants relative to dusted plants (Waser et al. 2016).

These studies and observations bring forth three questions that will be the focus of my study. (1) Do *Hylemya* females avoid dusty plants? (2) Does dust affect larval success? (3) Will this affect the seed count in *I. aggregata*? I hypothesized that road dust influences oviposition by *Hylemya* in *I. aggregata*, effecting seed count in the plant.

Methods:

I. aggregata is a monocarpic perennial, commonly habituating roadsides of dry meadows (Brody 1992a). It is a semelparous plant and spends half of its time in the male phase (Brody and Waser 1995). The species produces many red tubular flowers that are protandrous, living for about 3-5 days (Campbell 1989; Waser 1978). *I. aggregata* is a self-incompatible and relies on pollinators (Waser and Price 1991). Broad tailed hummingbirds (*Selasphorus platycercus* Swainson) and rufous hummingbirds (*Selasphorus rufus* Gmelin) are prime pollinators for *I. aggregata*; small insects can also play roles as pollinators (Waser 1978; Brody 1992b).

The study was conducted in the summer of 2016 at two study sites south of the RMBL, in Gothic Colorado. Upper Maxfield Meadow (106°59' 26.5666"W, 38°56' 57.8" N) and Road Bend (106°59' 19.143"W, 38°57' 10.668"N) (see Figure 6). Maxfield and Road Bend are both dry hillsides where *I. aggregata* are commonly found (Brody 1992a).

To study the effects of road dust we began by selecting pairs of plants at each site that were matched by equal approximate height and base stem diameter. All pairs were about a meter apart at minimum. The height of the plant was measured using a meter stick from the base to the highest point of the plant. Using a caliper we measured the base of the stem in centimeters. In Maxfield we selected a total of 25 pairs and 11 pairs at Road Bend. Before carrying out the experimental procedures we removed all open flowers and any eggs laid by *Hylemya*. This created equal starting conditions and removed any potential bias in the sample size before beginning any treatments. Each pair was given their own ID such as 1D (D= dusted) and 1U (U= undusted). One individual from a pair was selected at random to be dusted by a flip of a coin. The dusting treatment began once every individual was given an ID. Considering the roads at RMBL contained magnesium chloride, dust had to be applied manually. All the dirt was collected south of the Judd Falls parking lot along the Gothic road. The dirt was sifted using a USA standard testing sieve (No. 35, opening in inches =.0197) until it was as fine powder. Using a hand-duster (Spray doc Gilmour Gardening innovation) each individual selected to be dusted was pumped with dust 15 times. While dusting any plants any nearby surrounding plants were covered briefly with a brown paper bag to maintain them as the control group. Dusting treatments were applied once a day, after flower and egg censuses had been completed.

To see the effects of road dust on floral production and *Hylemya* oviposition the following procedure was used. On each plant I recorded the number of elongated buds and open flowers. Open flowers had to have visible anthers and at minimum slightly refluxed petals. If the flower looked to be at its last stage of floral lifespan it would not be recorded. Open flowers on their last stage had lost colorization and had shriveled petals (pers. observ.). After recording the number of elongated buds and open flowers, I proceeded to look for *Hylemya* eggs in those individuals. Eggs are laid underneath the sepals of *I. aggregata*. To look for eggs I used forceps to look underneath the sepals. If an egg was found I marked the underside of the calyx with indelible ink (Brody 1992b; Brody and Morita 2000). I repeated this procedure every four days, to minimize recounting individual flowers. Every four days a different color was used to avoid any confusion between each census and any previous marked calyces were not looked at again. In all I conducted 5 censuses of buds and flowers beginning on July 6, 2016.

At the end of the season all marked calyces were collected, even if they were aborts. The fruits were examined under a dissecting microscope to look for evidence of *Hylemya* larvae and

to count seeds. They were categorized according to three fates: as expanded and unparasitized (no evidence of a larva), expanded and parasitized (evidence of a larva), or aborted (unexpanded ovary).

Results:

Floral production

It was evident that I was able to capture the majority flowering season of *I. Aggregata*. Early in the season there was a large mean value for number of elongated buds and open flowers per plant, but the values consistently dropped after the second census for the entire sample period (Figures 1 and 2). Although it appears that dusted and undusted plant didn't differ in floral production, the paired t-test showed otherwise. Undusted plants showed a marginally significant difference, producing a higher number of elongated buds compared to dusted plants (Table 1). However, there was no significant difference in the number of open flowers produced for dusted or undusted plants (Table 2).

Hylemya oviposition on elongated buds

There was a significant difference in the host selection of *Hylemya* females on elongated buds. At Maxfield, *Hylemya* females laid eggs on dusted flowers 17% of the time and on undusted flowers 57% of the time. At Road Bend, they laid eggs on dusted flowers 36% of the time on dusted plants and on undusted flowers 60% of the time. The mean eggs laid on undusted elongated buds (58%) was greater than for dusted elongated buds (50%). Overall the differences between the two treatments were significant ($p=.0031$) as were differences between the two sites ($p<.0001$; Figure 3), and there was a significant (effect of census date) on variation in egg numbers across both study sites ($p=.0126$).

Hylemya oviposition on open flowers

In oviposition of open flowers there was also a significant difference. At Maxfield, *Hylemya* females laid eggs on dusted flowers 40% of the time and on undusted flowers 48% of the time. At Road Bend, they laid eggs on dusted flowers 59% of the time and on undusted flowers 67% of the time. Overall the differences between the two treatments were significant

($p=.0278$) as were differences between the two sites ($p<.0001$; Figure 4), and there was a significant effect of census date on variation in egg numbers across both study sites ($p<.0001$).

Fate of fruits/larva

The fates of fruits that resulted from open flowers on which I had found fly eggs were significantly different by site and color (census), but not by treatment. In Maxfield (Table 3) the count of abortions increased through time as the number of unparasitized and parasitized decreased, and then vice versa on the third census (pink). The number of abortions at Road Bend (Table 4) consistently increased, all while the number of unparasitized and parasitized fruits decreased. At Maxfield and Road Bend the number of abortions, unparasitized, parasitized were relatively similar for dusted and undusted flowers that carried fly eggs (Tables 5 and 6). The larvae success varied across each census, but wasn't significantly different by treatment.

Seed sets

Mean seed set were significantly different between sites ($p=.0285$) and censuses ($p=.0070$). However, there was no significant difference between treatment ($p=.6143$) and fruit fates ($p=.6238$). In Maxfield the first census (green) displayed the greatest difference in mean set, while the remaining censuses were relatively similar (Figure 5). At Road Bend the mean seed set stayed relatively similar throughout the season (Figure 5).

Discussion:

Results from the dusting treatments suggest that *Hylemya* females prefer ovipositing on undusted plants. Interestingly, this result can potentially help explain the lack of consistent seed difference from the previous study by Waser (2016). Seeing that larger amount of eggs were found on undusted flowers, it can support the idea of compensatory herbivory. In the case of the Waser's study, it is conceivable that *Hylemya* females either reduced or were offsetting the seed count creating the inconsistent effects road dust on seed set.

Furthermore, the results also support the idea that *Hylemya* females use cues in selecting the best host available. A common uncertainty about *Hylemya* females is whether they're making "wise" decisions or manipulating their hosts by influencing plant hormones (Brody and Waser 1995). Given their response in this experiment, it seems *Hylemya* females are using cues to make

“wise” decisions. A possible explanation could be due to female flies’ dependency on flowers setting seed (Brody and Waser 1995). Since a female’s larva remains in the same flower she lays an egg on, it is crucial that pollinators visit the flower for it to likely produce fruit, otherwise the larva could perish (Brody 1992a). Therefore, it is possible that dusted plants are less likely to be visited by pollinators, which would then give those flowers a high probability of setting fruit, explaining one possible reason for female flies to avoid dusty plants.

Additionally, the floral production could have been another cue used by *Hylemya* females. In a study done by Brody (1992a), *Hylemya* eggs were more likely to be present in longer corollas. However, she found no significant correlation with floral display (number of open flowers). Despite floral display not being a factor, the sum display of elongated buds and open flowers can possibly show a different result in a future study. Although in this study the mean number of open flowers appeared to be similar between dusted and undusted, it is likely that I could have recounted open flowers that didn’t have eggs in my previous census. Another explanation could be due to newly developed elongated buds that weren’t recorded in between each census and later opened, creating a larger sum of open flowers. As a consequence, recounting and counting newly opened flowers could have given an unrealistic mean of opened flowers affected by the treatment. Therefore, it is possible that undusted flowers may have actually been producing a higher count of open flowers as they were elongated buds. In this case, it could be possible that dusting treatments affected overall floral production for dusted flowers giving *Hylemya* females less options to lay eggs on dusty flowers.

Considering the results presented here and those of Brody (1992a), it seems possible *Hylemya* females use various cues to make “wise” oviposition decisions. However, it is possible that while making “wise” decisions, they are still manipulating the host. In an experiment, fruit set was significantly higher for flowers that had an egg compared to those that didn’t (see Brody and Morita 2000). This intriguing result was thought to be due to a hormone released by the mother fly. *Hylemya* females are possibly releasing an auxin like hormone that can delay the abscission of a flower, and thus increase its possibility of being visited by pollinators (Brody and Morita 2000). Notice that the fruit fate in this study didn’t appear to be unaffected by the treatment, suggesting that female flies are manipulating their host to equalize the fate of the fruits despite the treatment applied. This can explain why flowers with eggs present are more likely to set fruit.

The results in the mean seed set of this study further supports that *Hylemya* females are manipulating their host, considering it was not affected by treatment and fruit fate. Overall the mean seed set was relatively equal throughout the season despite the conditions of *I. aggregata*. Therefore, it is plausible that *Hylemya* females are promoting seed production. The probability of setting seed is significantly greater for flowers with laid eggs compared to those that are not oviposited on by *Hylemya* females (see Brody 1992b), conceivably explaining why there was no significant difference in the mean seed set between dusted and undusted plants.

Altogether the results in this study and from previous studies appear to present that *Hylemya* females are making “wise” decisions and manipulating its host. Although, using cues seems unnecessary if they are ultimately capable of manipulating their host (Brody 1992b), but yet flies still seem to use them. Therefore, further experimental studies can help better understand the mechanisms that *Hylemya* females use to benefit their success.

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Figure 1. Average number of elongated buds throughout the season.

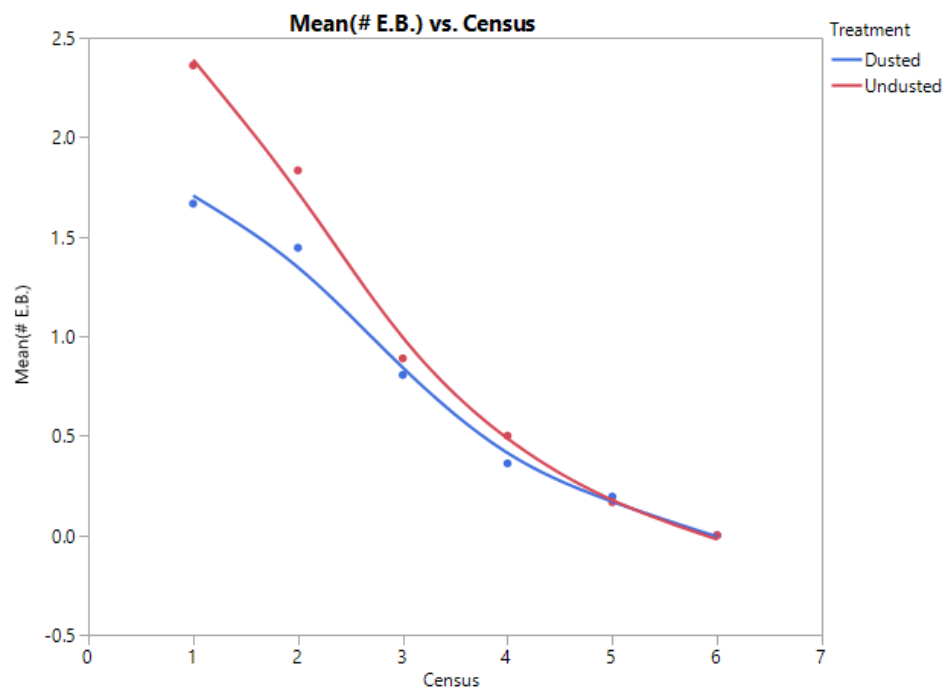


Figure 2. Average number of open flowers throughout the season.

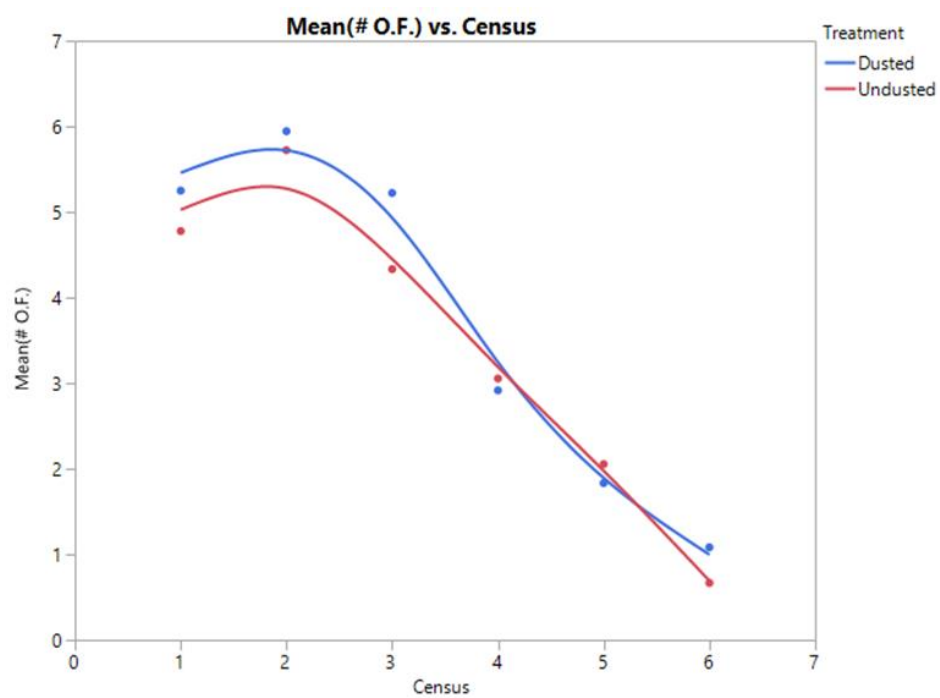


Table 1. Paired t-test correlation analysis of mean number of elongated buds produced in *I. aggregata*. Plants matched as dusted and undusted pairs.

Number of elongated buds

Mean difference	0.255
DF	179
t-ratio	2.216
P	.0279

Table 2. Paired t-test correlation analysis of mean number of open flowers produced in *I. aggregata*. Plants matched as dusted and undusted pairs.

Number of open flowers

Mean difference	-0.244
DF	179
t-ratio	-0.965
P	0.336

Figure 3. A histogram with 95% confidence intervals representing the average number of eggs per elongated bud by site and treatment. RB = Road Bend site; UPMF = Upper Maxfield Meadow site.

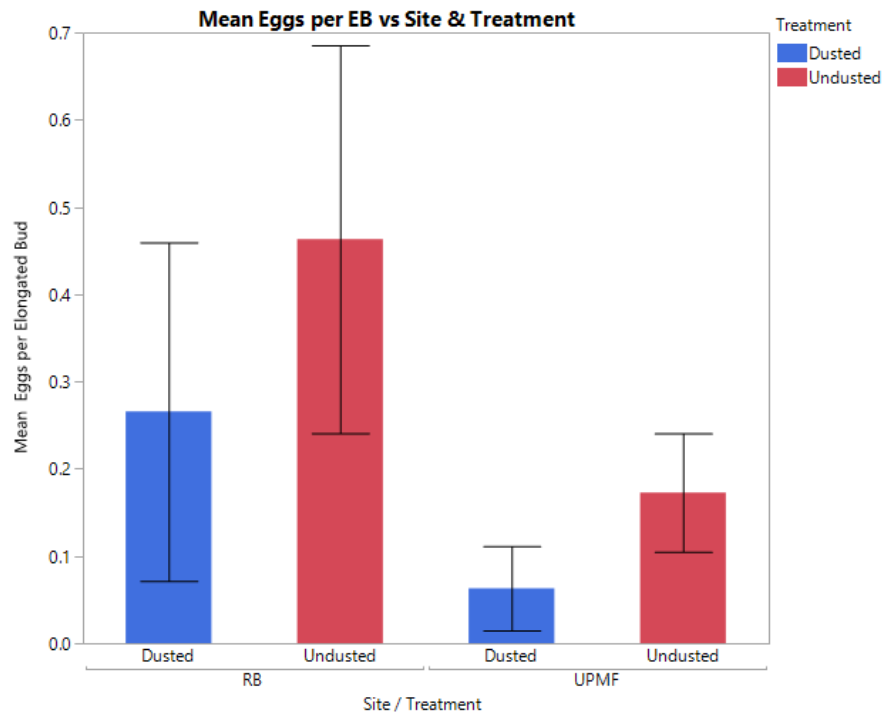


Figure 4. A histogram with 95% confidence intervals representing the average number of eggs per open flowers by site and treatment. RB = Road Bend site; UPMF = Upper Maxfield Meadow site.

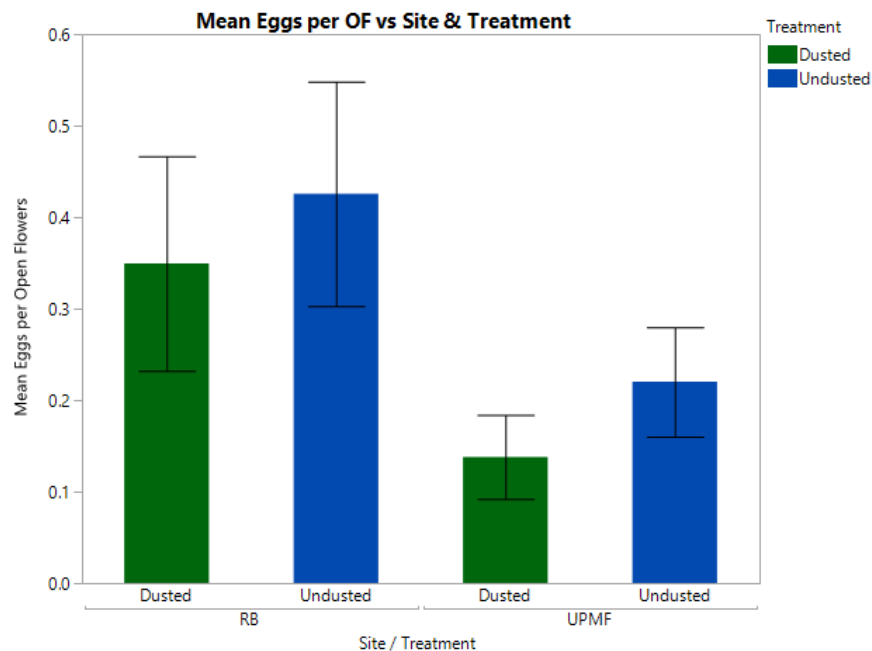


Table 3. Percent of fruits that were abortions, unparasitized, and parasitized by census in the Upper Maxfield Meadow site.

Color (census)	Aborts	Unparasitized	Parasitized
Green	17.48%	40.78%	39.81%
Blue	51.11%	17.78%	22.22%
Pink	36.36%	21.21%	39.39%
Yellow	55%	5%	25%
Black	100%	0%	0%

Table 4. Percent of fruits that were abortions, unparasitized, and parasitized in Road Bend site. There was no fruits from census number five at this site.

Color (census)	Aborts	Unparasitized	Parasitized
Green	40.35%	24.56%	35.09%
Blue	69.70%	9.09%	21.21%
Pink	70%	5%	20%
Yellow	85.71%	0%	0%
Black	0%	0%	0%

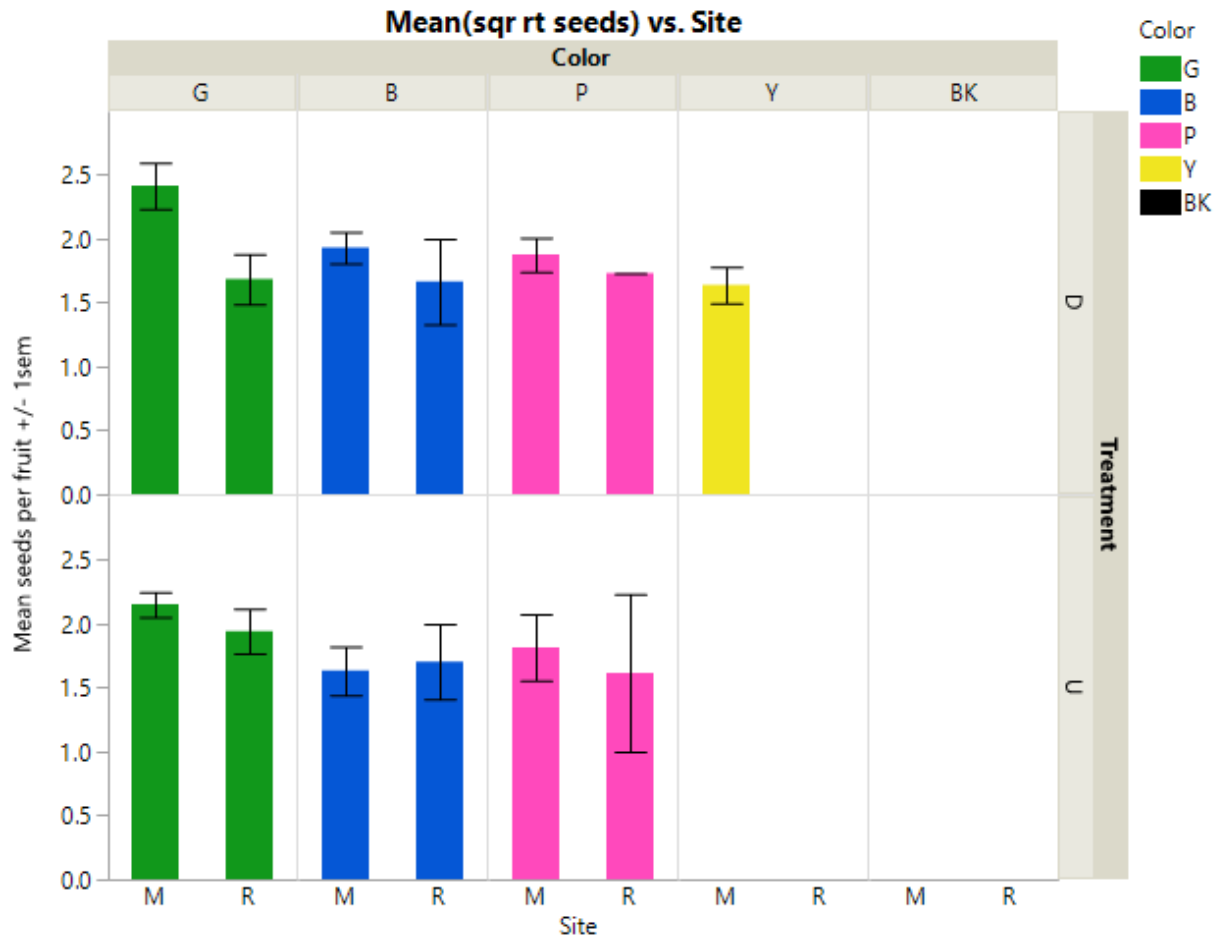
Table 5. Percent of fruits that were abortions, unparasitized, and parasitized by treatment in the Upper Maxfield Meadow site.

Treatment	Aborts	Unparasitized	Parasitized
Dusted	37.5%	27.5%	30%
Undusted	33.08%	27.69%	34.62%

Table 6. Percent of fruits that were abortions, unparasitized, and parasitized by treatment in Road Bend site.

Treatment	Aborts	Unparasitized	Parasitized
Dusted	55%	12.5%	30%
Undusted	57.14%	16.88%	24.68%

Figure 5. Average (of square root) number of seed per fruit (+/- standard error mean) by color (census) and treatment. There was no seeds for the 4th census on the dusted flowers in Road Bend, nor was there any for undusted flowers in both sites. The 5th census had no seeds. R = Road Bend site; M = Upper Maxfield Meadow site.



Map: Displays both field sites located in South Gothic.

