

Are *Hylemya* avoiding dusted *Ipomopsis aggregata* for good reason?

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Abstract:

Waser et al. (2016) found that road dust consistently reduces the pollen received by *Ipomopsis aggregata* flowers but does not consistently reduce seed set. Over three years we tested the hypothesis that the reason undusted plants do not have higher seed sets is that more of their fruits are eaten by larvae of *Hylemya* sp. (Anthomyiidae), a predispersal seed predator. In all three years, *Hylemya* indeed oviposited on a greater fraction of the flowers of undusted than dusted plants, though the overall oviposition rate varied among years. In 2017 and 2018 we also compared the fates of *Hylemya* eggs laid on dusted and undusted flowers to determine whether the flies were choosing undusted plants because their eggs are more successful there. In 2017, 33.42% of eggs laid on dusted flowers and 55% of eggs laid on undusted flowers successfully hatched into a larva. In 2018, the success rate was 48.6% and 21.8% on dusted and undusted flowers, respectively. We conclude that *Hylemya* oviposition preferences are adaptive and likely contribute to the inconsistent effects of road dust on *Ipomopsis* reproduction.

Introduction:

Dust is small particulate material that can be lofted into the atmosphere by wind or road traffic (Bagnold 1941). A primary source of dust spreading into the atmosphere is traffic on unpaved roads. Road dust is easily dispersed by tire friction and can travel up to 200 meters from the road (Forman and Alexander 1998), bringing forth the question: does road dust have an ecological repercussion.

A study by Waser et al. (2016) discovered that in 19 of 20 independent tests wildflowers carried less pollen (up to six times less) on their stigmas in dusty conditions. Typically seed production of an angiosperm flower depends on the quantity and quality of pollen reaching its stigma (Waser and Price 1991). However, dust did not consistently lower the seed set of

undusted flowers (Waser et al. 2016). The authors listed four possible mechanisms that could cause consistently lower pollen loads to result in inconsistently lower seed set: pollen loads were high enough in all cases that seed set was not pollen limited; the excess pollen in undusted plants was of low quality; seed set was limited by resources other than pollen; or seed predators differentially reduced the seed set of undusted plants.

The last mechanism could act in the case of *Ipomopsis aggregata* because its reproduction is heavily impacted by the Anthomyiid fly, *Hylemya* sp., a pre-dispersal seed predator that oviposits between the sepal and the corolla of scarlet gilia flowers (Brody 1992b). Once the eggs hatch the larvae crawl into the maturing ovary and eat the developing seeds. After eating the seeds, they bore an exit hole and leave to pupate in the ground. Therefore, few or none of the seeds in an attacked ovary enter the seed pool (Zimmerman 1980). In his 2016 project, Danny Ramirez found evidence supporting the idea that *Hylemya* females were more prone to lay eggs in undusted individuals rather than dusted individuals (Ramirez, unpublished data). What this means is that measured seed sets of undusted flowers could be selectively reduced, thereby resembling seed sets of dusted flowers, even though the undusted flowers have more pollen.

This year I repeated the Ramirez study asking the question: do *Hylemya* avoid dusted plants for good reason? This question arose from reading a paper by Allison Brody and Nick Waser (1995), where they concluded that *Ipomopsis aggregata* flowers on which *Hylemya* lay eggs have a significantly higher probability to set fruits (and the larvae therefore have a higher probability of surviving) than flowers chosen at random; this probability did not decline if eggs were removed experimentally. These results suggest that female flies are choosing among *Ipomopsis aggregata* flowers in an adaptive manner or manipulating their quality on behalf of

offspring (Brody 1995). If so, then I hypothesize that *Hylemya* eggs have a lower probability of surviving if they are laid on dusted than on undusted flowers.

Methods:

This study was conducted in Upper Maxfield Meadow, 1 km south of the Rocky Mountain Biological Lab in Gothic, Colorado. Upper Maxfield Meadow (106°59' 26.5666" W, 38°56' 57.8" N) is a dry hillside where *I. aggregata* are commonly found. *Ipomopsis aggregata* is a monocarpic plant that has numerous red, tubular flowers and an average floral life span of 3-5 days (Campbell 1989).

Making pairs

In order to determine whether *Hylemya* flies prefer the dusted or undusted plants we conducted a series of egg censuses. Before *I. aggregata* bloomed we went to Upper Maxfield Meadow and picked out 23 pairs of single-stalked plants which were similar in height, location, and number of buds. We used a meter stick to measure the height of the plants from the base to the most distal part. We made pairs of plants that were like each other to control for variables other than dusting treatment, like plant size or location, that might influence fly oviposition choices or fruit production. For each pair we flipped a coin to assign treatment (Dusted or Undusted) to individual plants within the pair. Since the road near Upper Maxfield Meadow had magnesium chloride (MgCl_2) applied to suppress dust, the flowers needed to be dusted manually. To dust the plants, we filled a hand-duster (Spray doc Gilmour Gardening innovation) with dirt collected from the edge of an untreated, unpaved road at a single site, "Dusty Corner." Once the dirt was collected we sieved it through a USA standard testing sieve (No. 60) and dusted with the

fine fraction. We then gave each dusted plant 10 pumps of dust every other day. The undusted plants were exposed to 10 pumps from a clean duster to control for any possible effect of air alone. They were also kept undusted when nearby plants were dusted by placing a paper bag over them.

Dust Calibrations

We did a dust calibration study in 2017 to ensure that we did not put an unnatural amount of dust on experimentally dusted plants. We collected five flowers and elongated buds from naturally dusted and undusted plants from the Dusty Corner site. The naturally dusted plants were found less than 2 meters from the road and naturally undusted plants were about 40 meters from the road. In order to count the dust particles, I placed the plant tissue onto a piece of clean wax paper and I then placed a microscope slide over the plant tissue and tapped for 10 seconds to transfer a layer of dust onto the slide. I marked the location of the top, middle, and end of the plant tissue on the slide and placed it under the microscope. Once under the microscope, using 50x magnification the dust particles in an area of 0.2023 mm^2 were counted, using the ocular micrometer as a guide, at the top of the flower (where the petals began to reflex) and again in the middle. I repeated the same process for four different experimental plants that had received five, ten, fifteen, and twenty dusting pumps. All the dust counts were done by one person to eliminate counting bias. The data were then analyzed to determine how dusted the experimentally dusted plants were in comparison to the naturally dusted plants.

Egg Census

To see whether *Hylemya* flies preferred the undusted or dusted flowers an egg census was conducted every 4 days, since the average floral lifespan is about 3-5 days for *I. aggregata* (Campbell 1989; Waser 1978). We would census every four days to minimize counting flowers multiple times but also minimize the number of uncounted flowers. For each census we counted and marked, with a census-specific color, the total number of elongated buds, reflexed flowers, and fresh stigmas that had not been counted in a previous census. A fresh stigma was classified as a corolla-less flower whose plump, red style and stigma indicated that the corolla had just fallen off. The egg count was categorized by flower type and number of eggs, whether the egg was on an elongated bud, reflexed flower, or fresh stigma as well as how many were eggs were found in the sepals. Since *Hylemya* flies oviposit between the sepal and the corolla of the flower, we used forceps and Opti Visors to look for eggs underneath the sepals. If an egg was found, we would mark the sepal with a yellow paint pen throughout all censuses (Brody 1992b; Brody and Morita 2000). Once marked the flower or sepal would no longer be checked for eggs in future censuses or included in the total flower counts. There was a total of eight censuses conducted beginning June 20, 2018 and they were all part of the analyses. The egg censuses method was taken from Waser-Price lab (Ramirez, unpublished). By continuing the same methods, we would be able to combine our data and analyze it across years.

Fruit Counts

After the censuses were completed, we began to collect fruits on July 24, 2018. We placed one ovary per envelope and let them dry for a few days. Once dried, we would open the fruits and count seeds, keep track of census color and parasitism. If the ovary did not expand,

then we would take note of the census color and mark it as an abort. We collected over 1,200 fruits for 2018 and over 2,500 in 2017.

Results

Assessment of Dusting Calibration from 2017

To see if our experimental dusting treatment mirrored natural dusting we conducted a series of dust calibrations. Figure 6 displays our experimental treatment in comparison to the naturally dusted and undusted plants. Our experimental treatment had more dust particles per transect than naturally undusted plants, but less dust particles per transect than naturally dusted plants. A Tukey HSD pairwise comparison of dust level means suggests that there is a significant difference ($P_D = 0.0027$, $P_U = 0.0001$; Figure 1) in the number of dust particles per transect between the experimentally dusted and naturally dusted plants. The naturally dusted plants had an average of 4.30 dust particles per transect, while the naturally undusted plants had an average of 1.40 dust particles per transect. The experimentally dusted plants with ten pumps had an average of 2.65 dust particles per transect and was significantly different in comparison to both naturally dusted and undusted treatments ($P_D = 0.0027$, $P_U = 0.0001$; Figure 1).

Flower and Oviposition Phenology 2018

Figure 1 shows that *Hylemya* oviposition peaked during the second census and flower production peaked during the fourth census. It also indicates that we were able to census throughout most of the flowering season. Early in the season the plants produced the most flowers, but after the fourth census flower production continuously dropped for the rest of the

sample period. Oviposition followed a similar decline, *Hylemya* oviposit the most during the second census and slowly tailed off.

Effect of Dust on Oviposition 2018

Figure 2 displays the percent of flowers with eggs on both dusted and undusted plants. The undusted flowers took a bigger hit at 7.2% versus 4.3% of dusted plants. *Hylemya* flies oviposited more often in undusted flowers *Ipomopsis aggregata* than in dusted *I. aggregata*. In 2018, on average there were 3.48 eggs per dusted plants versus 7.09 eggs per plant on undusted plants.

Comparison of Egg Loads across Years 2016-2018

Egg loads were higher on dusted than undusted plants in all three years (Figure 3). In 2016 there were on average 0.09 eggs per flower on dusted versus 0.18 in undusted plants. In 2017, there were 0.04 eggs per flower on dusted versus 0.07 on undusted plants. In 2018, there were on average 0.09 eggs per flower on dusted plants versus 0.19 on undusted. Furthermore, across all years 2018 had the most eggs with 243, then 2016 with 221, and 2017 with 192.

Percent Aborted by Treatment and Egg Status 2017

Figure 4 shows in 2017, 11.9% of undusted flowers aborted, which was 1/3 less than those on dusted plants. It also demonstrates that the undusted flowers with an egg aborted less (11.9%) than the undusted flowers without an egg (26.6%). Overall, there wasn't much of a difference in abortion rate between dusted and undusted plants for flowers without an egg, but there was a difference between undusted and dusted flowers with an egg.

Parasite Success by Treatment 2017

Figure 5 demonstrates the success rate of *Hylemya* larva on undusted flowers 55%, versus 33.42% in dusted flowers. *Hylemyas* that oviposit in undusted plants had more of their eggs succeed in comparison to eggs that were laid on dusted plants.

Concluding Results Table from 2017 Fruit Counts

Table 1 concludes that overall in 2017, *Hylemya* oviposit more often on undusted flowers. The undusted flowers with an egg aborted less frequently than dusted flowers with an egg and undusted without an egg. The survival rate was higher in undusted flowers and the probability of an egg surviving was also higher in undusted flowers.

Discussion

Waser et. al (2016) discussed compensatory herbivory as a mechanism that produces inconsistent seed sets in undusted plants. Brody and Waser (1995) helps support Waser's compensatory herbivory hypothesis because they found that flowers in which *Hylemya* oviposit have a higher probability to set fruit. *Hylemya* oviposition peaked during the second census (beginning of the season) for both 2017 and 2018. Since the first flowers of a season are the more likely to set fruit (Price, unpublished) and *Hylemya* attacked more undusted plants throughout the season, including the second census. *Hylemya* were able to attack the flowers that were more likely to set fruit. Therefore, if the flowers that were attacked during the first censuses were more likely going to set fruit and were the most attacked. Then, *Hylemya* may be offsetting (lowering) the undusted seed set and thereby, resembling the dusted seed set.

This study has helped support that *Hylemya* oviposition in undusted plants is greater than in dusted plants. But, it has also raised questions about the cues that *Hylemya* may use to gauge a flower's probability of setting fruit. Brody and Waser (1995) claimed to be unsure of whether *Hylemya* chose flowers in an adaptive manner or manipulating their quality on behalf of offspring. *Hylemya* may not be doing as well on dusted plants, possibly because the dust may inhibit cues that may be easier to pick up on undusted flowers. To understand whether *Hylemya* use plant volatiles as a cue, future studies could compare the plant volatiles between dusted and undusted flowers and how their volatiles may change throughout the season. Comparing the plant volatile organic compounds (VOCs) and *Hylemya* oviposition may help support the idea that *Hylemya* use cues rather than manipulate the flower quality on behalf of offspring. Dust may affect more than just pollination in *Ipomopsis aggregata*, it could also make some plants less appealing to pre-dispersal seed predators. Dust may inhibit certain cues that pre-dispersal seed predators may use to attack flowers. Dust is playing a role in compensatory herbivory and future experiments can test for the extent of the effect.

Overall, *Hylemya* did oviposit more on undusted flowers for three consecutive years. The undusted flowers with an egg aborted less frequently than the undusted flowers without an egg. This follows the Brody and Waser (1995) conclusion that flowers with *Hylemya* eggs aborted less compared to flowers chosen at random. The survival rate was higher in undusted flowers and the probability of an egg surviving was also higher in undusted flowers. This helps support that *Hylemya* are choosing undusted plants for a good reason.

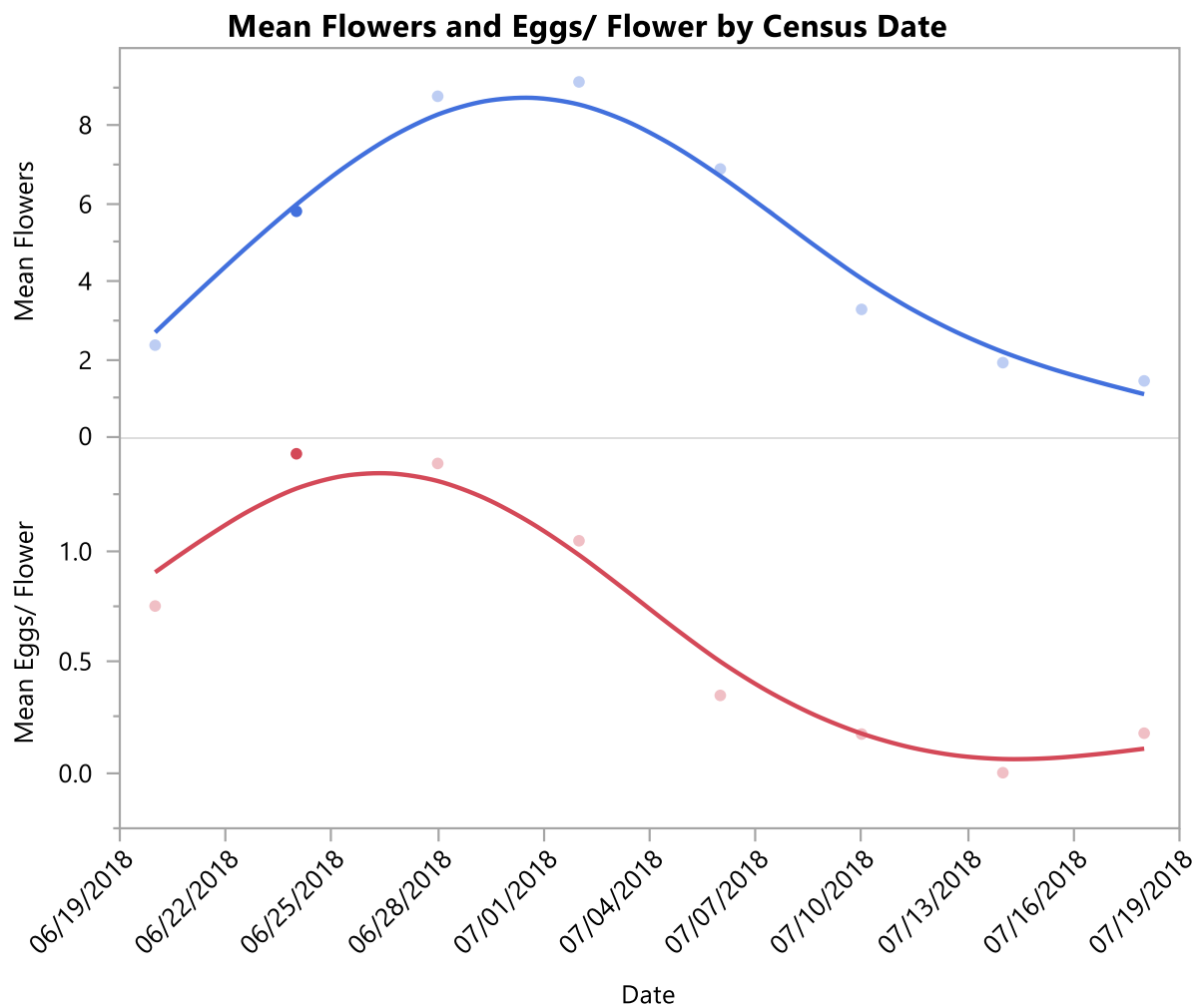


Figure 1. demonstrates mean flowers per plant by census and mean eggs/flower by census, this includes both dusted and undusted plants.

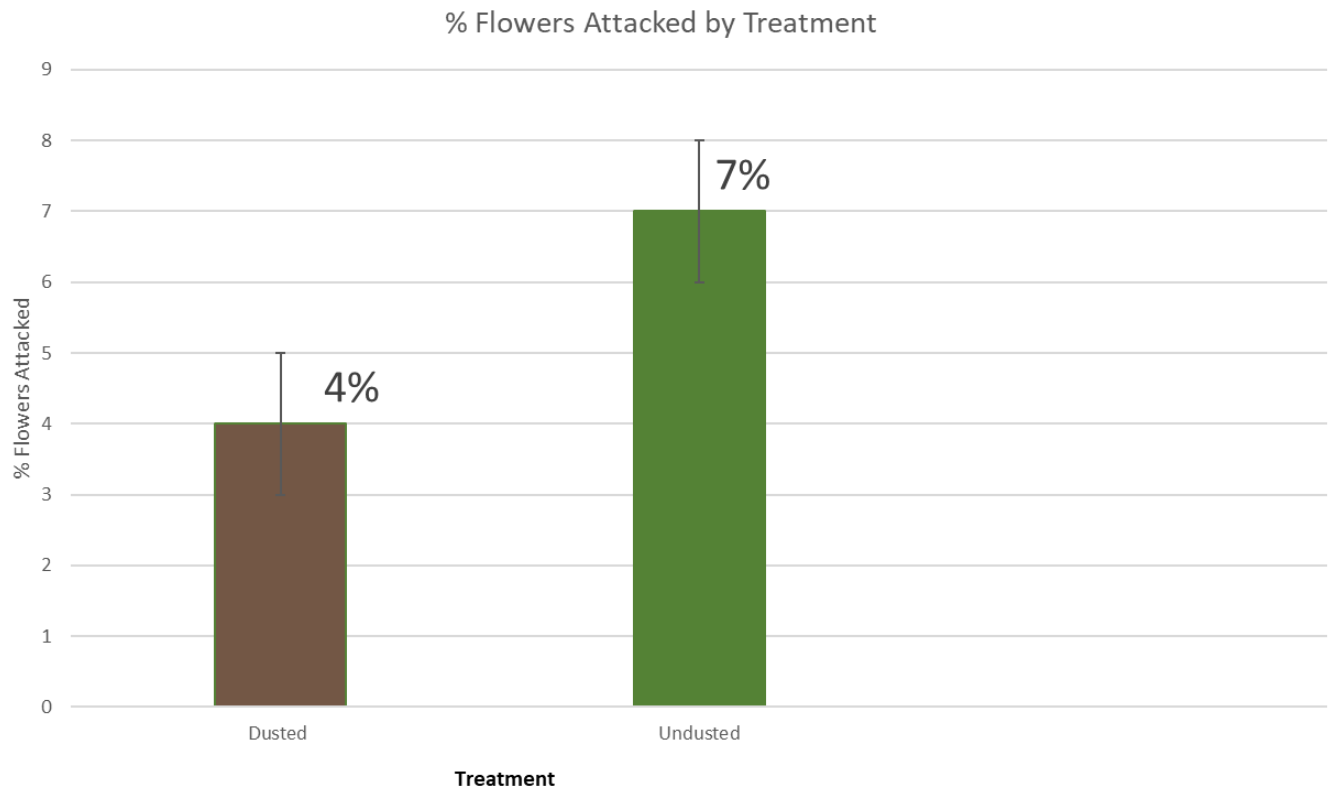


Figure 2. In 2018, the undusted plants had more of their flowers attacked, this was the mean total flower production divided by the fraction of flowers with yellow dots.

Mean Eggs per Flower by Year and Treatment

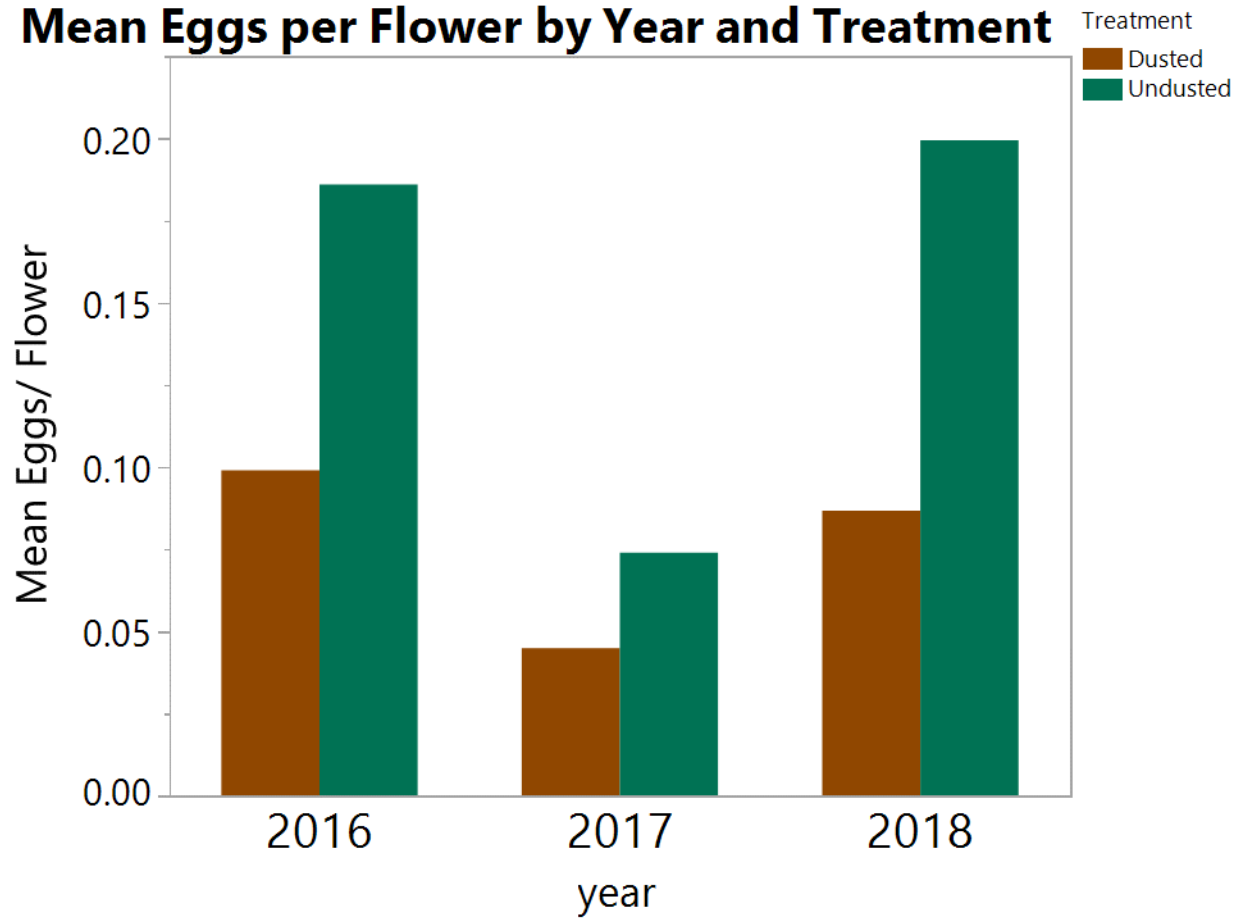


Figure 3. Across all years there were more eggs found on undusted plants.

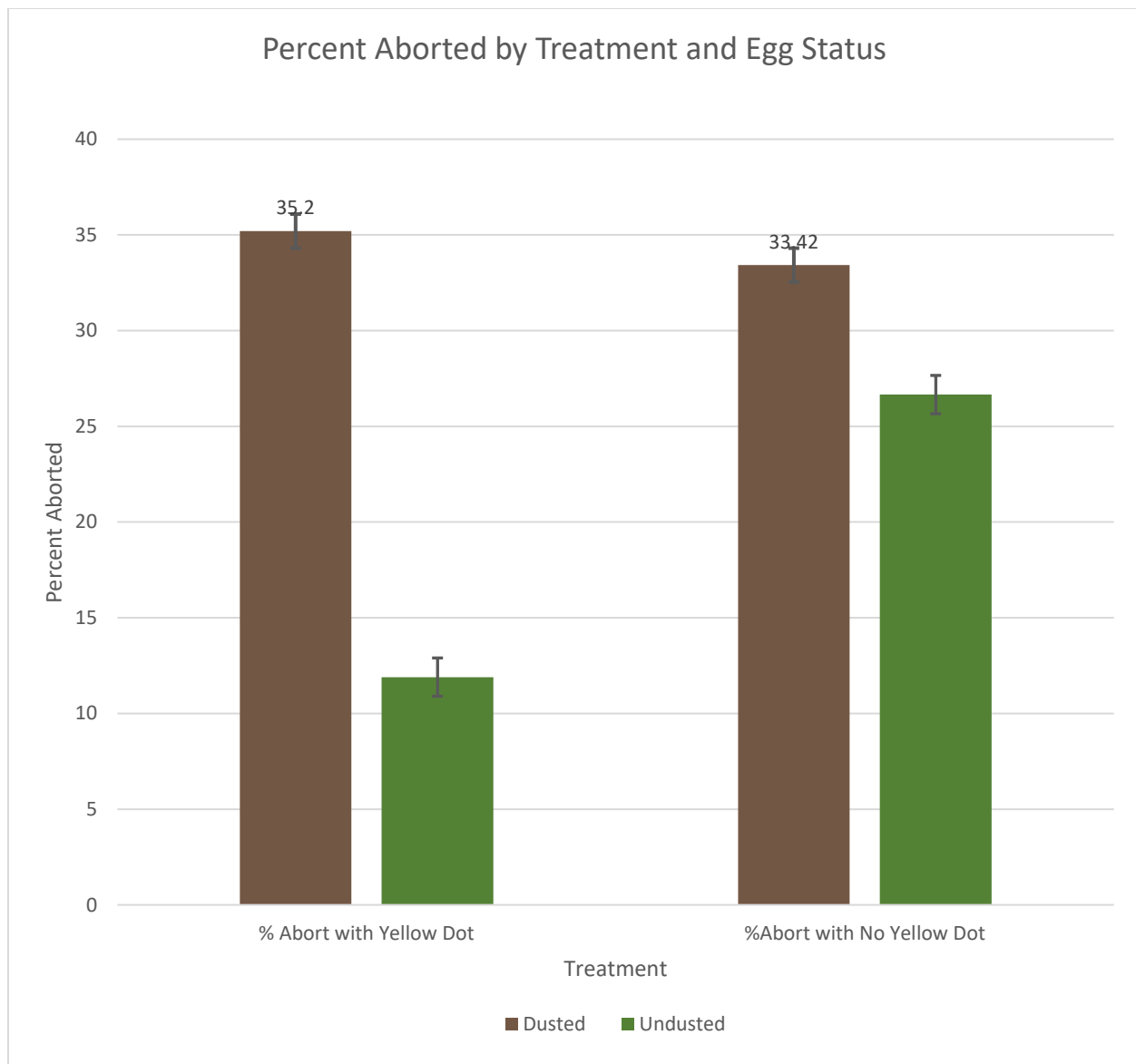


Figure 4 In 2017, 11% of undusted flowers with an egg aborted, which was about 1/3 the abortion rate of dusted flowers.

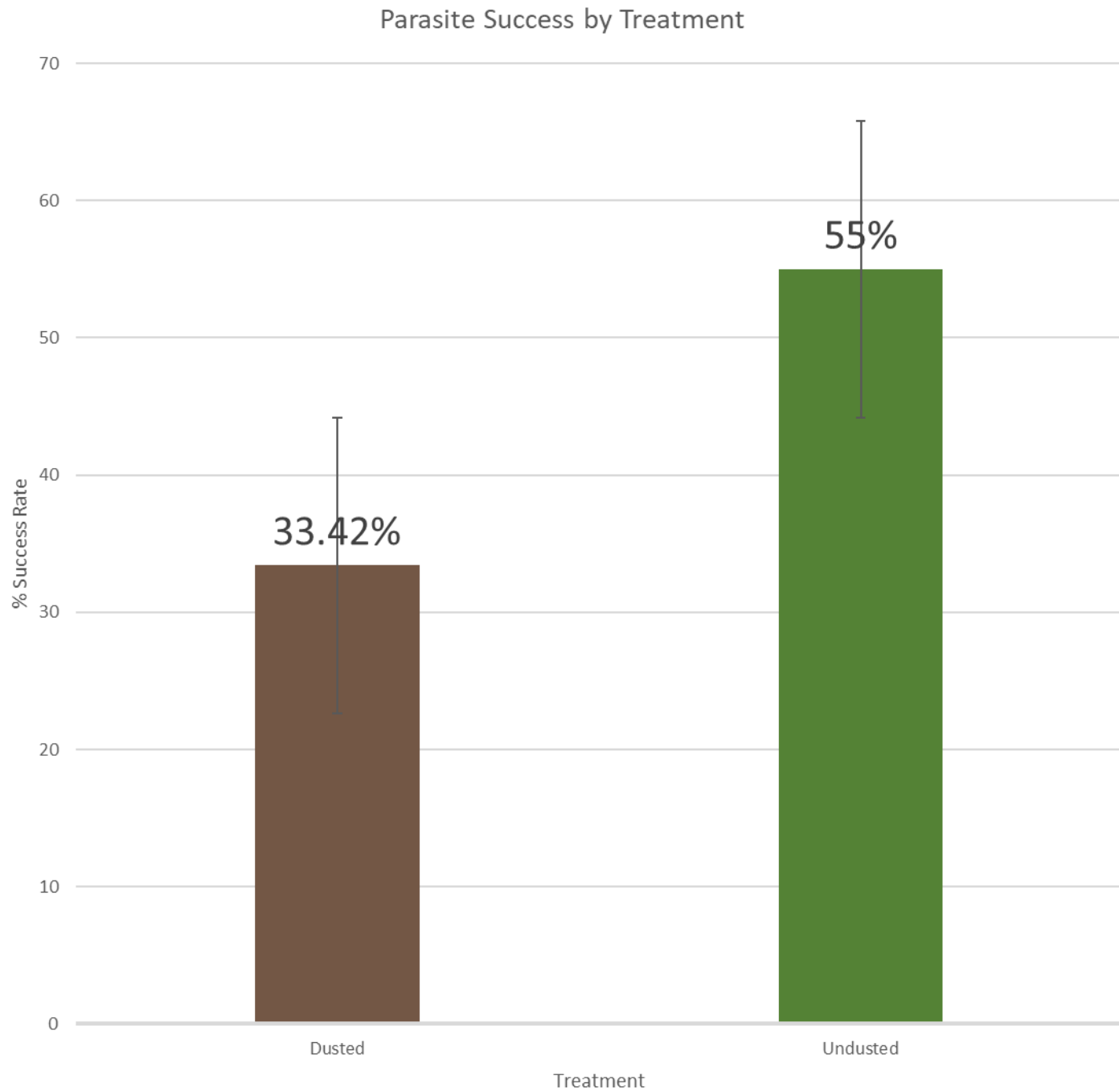


Figure 5 The parasites were more successful on dusted plants.

2017 Fruit Counts Table- Parasitism and survival rate

Treatment	Flowers with eggs	Expansion rate with an egg	Expansion rate without an egg	Parasite Survival Rate	Probability Egg Survives
Dusted	4.2%	64.8%	70%	33.42%	21.66%
Undusted	7.3%	88.1%	73.4%	55%	48.46%

Table 1 Overall, the Hylemas are choosing undusted plants and for good reason; higher probability of survival in 2017.

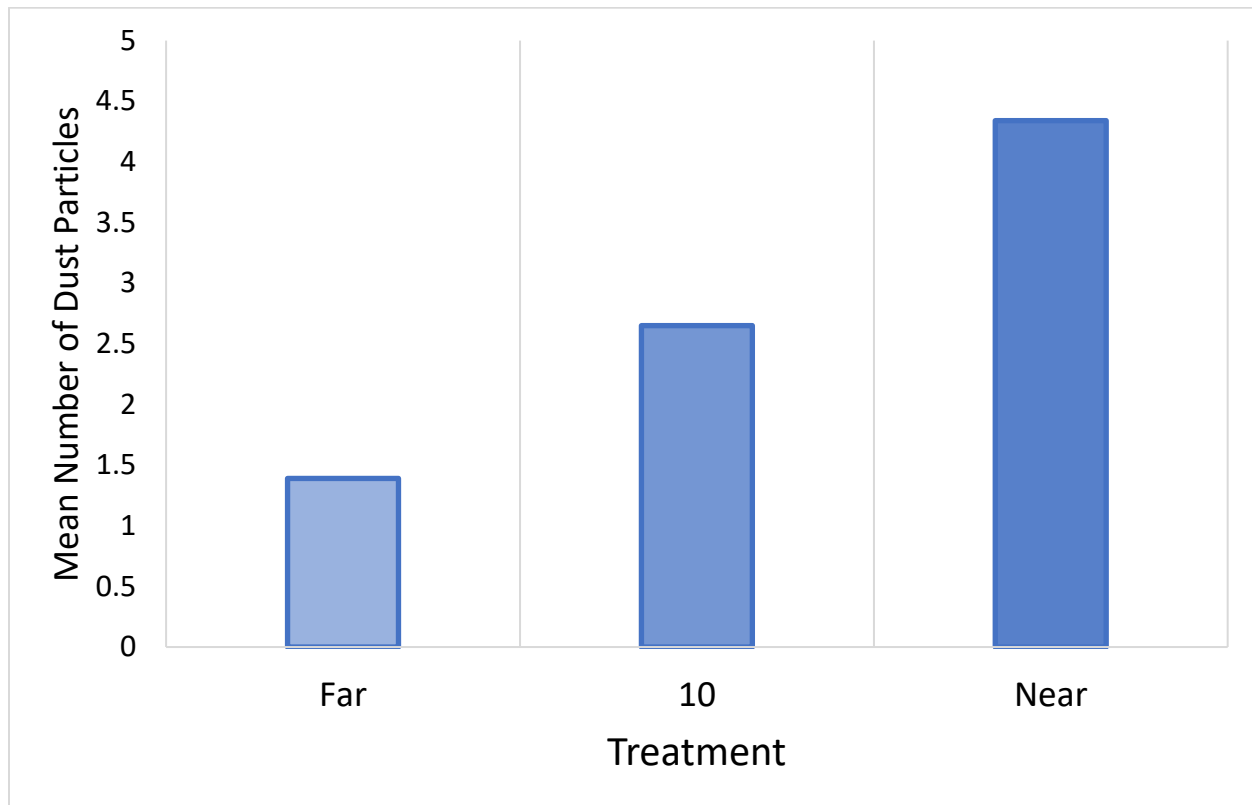


Figure 6 Demonstrates dust calibration with 10 pumps compared to near and far natural dust.

Maxfield Meadow

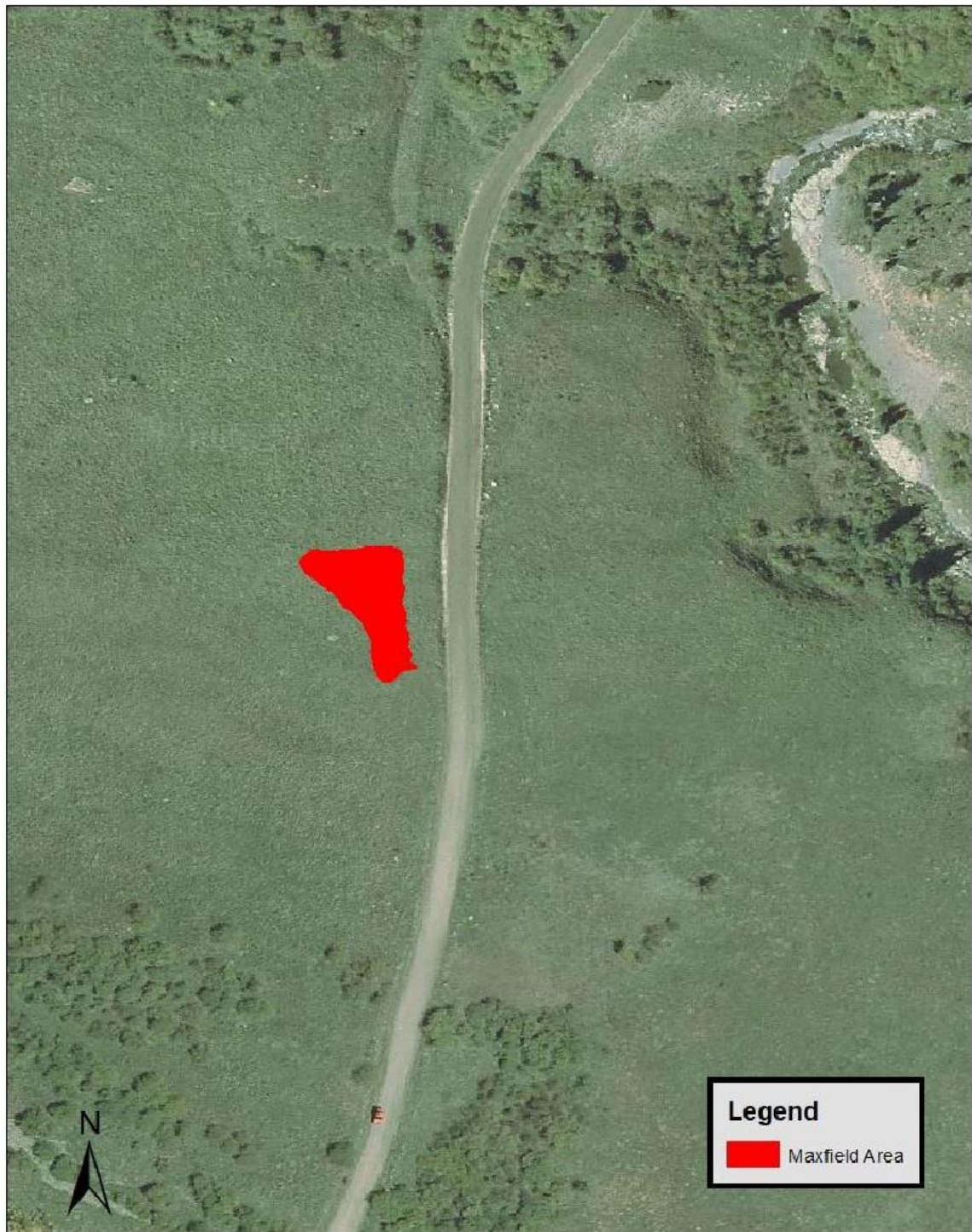


Figure 7 displays an aerial view of Maxfield Meadow.