

Does Road Dust Affect Hylemya Oviposition in Scarlet Gilia (*Ipomopsis aggregata*)?

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Abstract:

A previous study conducted at RMBL has found that road dust affects wildflower pollination and reproduction, this study included *Ipomopsis aggregata*. In 19 of 20 independent tests wildflowers carried less pollen on their stigmas in dusty conditions, but their seed sets were not consistently affected. The study lists compensatory herbivory as a mechanism that could contribute to the inconsistent seed set, therefore for this study we will be looking at the pre-dispersal seed predator the Anthomyiid fly *Hylemya* spp. We studied whether road dust affects *Hylemya* oviposition. We found more *Hylemya* eggs in experimentally undusted than in dusted *Ipomopsis aggregata* plants, both on a per-flower and whole-plant basis. On average there were 4.3 eggs per undusted plant and 2.2 eggs per dusted plant. This study also compared the results of a similar 2016 study. In both years undusted plants had higher egg loads, but egg loads were overall higher in 2016. Overall, between both years, 11.43% of undusted elongated buds had an egg and 5.22% of dusted elongated buds had an egg with a significant difference.

Introduction:

Dust is small particulate material that is removed from the land surface by wind erosion and can be lofted in the atmosphere (Bagnold 1941). One of the primary causes of dust spreading into the atmosphere is unpaved roads. Road dust is easily dispersed by tire friction and is capable of traveling up to 200 meters from the road (Forman and Alexander 1998), bringing forth the question: does road dust have an ecological repercussion? Dust responses can become interdependent with changes in climate and land use (Field et al 2009). For instance, off-road vehicle use leads to decreases in plant biomass and cover, as a result of direct impacts to vegetation and dusting of nearby plants (Sharifi et al 1999). When these impacts occur during times of reduced soil moisture, the reduction in plant cover is even greater, allowing for increased erosion (Field et al 2009).

Road dust lofted into the air by vehicles affects more than just plant cover and biomass. 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. Most of these tests included *Ipomopsis aggregata*. 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) 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 to result in high seed sets; the excess pollen in undusted plants was of low quality; seed set was limited by resources other than pollen; or compensatory herbivory differentially reduced the seed set of undusted plants.

One of the mechanisms that could contribute to these previous findings in *Ipomopsis aggregata* is compensatory herbivory. This study will focus specifically on the Anthomyiid fly *Hylemya* sp., a pre-dispersal seed predator, which oviposit 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 supports 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 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* females prefer undusted individuals?

Methods:

This study was conducted in Upper Maxfield Meadow which is 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 (Brody 1992a). *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 31 pairs of single-plants, avoiding plants with multiple stalks, which were similar in height, stem diameter, 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 measured the stem width using a caliper at the base of each plant. Each pair was then given their own ID by flipping a coin which also determined their treatment. For example, a plant ID of 1D/1U indicated the pair number as well as the dusting treatment, D for dusted and U for undusted. Since the road near Upper Maxfield Meadow had magnesium chloride (MgCl₂), which is used as a dust suppressant for unpaved roads, the flowers needed to be dusted manually. In order to dust the plants by hand we used a hand-duster (Spray doc Gilmour Gardening innovation), all the dirt collected was from the Dusty Corner site. Once the dirt was collected we used a USA standard testing sieve (No. 60) until it was a fine powder. We then gave each plant 10 pumps of dust once a day, undusted plants were kept undusted by placing a paper bag over the entire plant during the dusting treatment.

Dust Calibrations

We did a series of dust calibrations 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. Then I removed the slide and labeled the top, middle, and end of the plant tissue and placed it under the microscope. Once under the microscope the dust particles were counted in an area of 0.2023 mm², using the ocular micrometer as a guide, at the top of the flower (where the petals began to reflex) and in the middle. I repeated the same process for four different experimental plants all with different number of dust pumps five, ten, fifteen, and twenty pumps. All of the dust calibrations were done by one person to eliminate counting bias. The data was then analyzed to determine how dusted the experimentally dusted plants were in comparison to the naturally dusted plants.

Egg Census

In order 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). For the census we counted and marked, with a census-specific color, the total number of elongated buds, reflexed flowers, and fresh stigmas. 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 OptiVisors 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. In all seven censuses were conducted beginning July 3, 2017, but only the first six were used in the analysis.

Modifications

In comparison to the Ramirez study that was conducted in 2016 there were a few modifications in the methods. First, in the Ramirez study flowers were only marked if there was an egg in the sepal. In this study we would mark all new elongated buds, open flowers, and fresh stigmas with the census color in which they were tallied, regardless of egg load. Also, once flowers were tallied, whether they were elongated buds, open flowers, or fresh stigmas, they would no longer be checked for eggs. In the 2016 study, flowers could have been tallied and checked for eggs multiple times. For example, in census one an elongated bud could have been tallied and then tallied once more as an open flower in census two if an egg had not been found during the first census. Both studies did not check for eggs in flowers that had already been marked with an egg color. Therefore, since open flowers were counted in different ways we only compared egg loads in elongated buds because the elongated buds were tracked in a similar way.

Results:

Assessment of Dusting Treatment

In order to see if our experimental dusting treatment mirrored natural dusting we conducted a series of dust calibrations. Figure 1 displays our 10-pump 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 fewer dust particles per transect than naturally dusted plants. A Tukey HSD pairwise comparison of 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 from both naturally dusted and undusted treatments ($P_D = 0.0027$, $P_U = 0.0001$; Figure 1).

Phenology and Oviposition

Figure 2 shows that both the total number of new flowers and egg loads peaked during the second census. It also indicates that we were able to census throughout most of the flowering

season. Early in the season plants produced the most flowers, but after the third census flower production continuously dropped for the rest of the sample period. Oviposition followed a similar decline, except for in census five, the first census with clear skies after a rainy week that affected censuses three and four. Overall, we were able to capture most of the flowering and oviposition season and see that oviposition and new flower production followed a similar seasonal pattern (Figure 2).

Effect of Dust on Egg Loads

Figure 3a displays the average total new flowers produced in undusted plants was significantly ($P=0.015$) greater than in dusted plants. *Hylemya* flies oviposit more often in undusted *Ipomopsis aggregata* than in dusted *I. aggregata*. The number of flowers on a plant affects egg loads therefore, on average there were 4.3 eggs per undusted plant and 2.2 eggs per dusted plant, with a significant difference ($P=0.019$; Figure 3a) between the two treatments. Also, 9.77% of undusted flowers had an egg in comparison to 6.4% of dusted flowers, but the difference was not significant ($P=0.179$; Figure 3b).

Effect of Flower Stage on Egg Loads- Elongated Bud

Figure 4 displayed both flower stages, elongated buds and open flowers, had more mean eggs per plant for the undusted treatment. *Hylemya* females laid eggs more often in undusted elongated buds than in dusted elongated buds, but the difference between the treatments was not significant ($P=0.645$; Figure 4).

Effect of Flower Stage on Egg Loads- Open Flower

Continuing the pattern, Figure 4 displayed undusted open flowers also had more eggs per flower in comparison to dusted open flowers. Although 9.9% of undusted open flowers had an egg in comparison to 6.5% of dusted open flowers there was no significant difference ($P=0.14$; Figure 4) between the two.

Comparison of Egg Loads across Years

Figure 5 displayed higher egg loads in undusted elongated buds for both years, with 2016 having higher egg loads. There was a significant difference ($P=0.0027$; Figure 5) in the fraction of parasitized elongated buds between years, in total 11.81% of elongated buds had an egg in 2016 in comparison to 4.96% in 2017. Overall, between both years, 11.43% of undusted elongated buds had an egg and 5.22% of dusted elongated buds had an egg with a significant difference ($P=0.0073$; Figure 5).

Larval Survival Rate

The larval survival rate was affected by
Treatment
Census

Fruit Fate

Percent of fruits that were parasitized by
Treatment
Census

Seed Set

Seed set was found to be different between
Treatment
Census

Discussion

Literature Cited

Figures, Tables

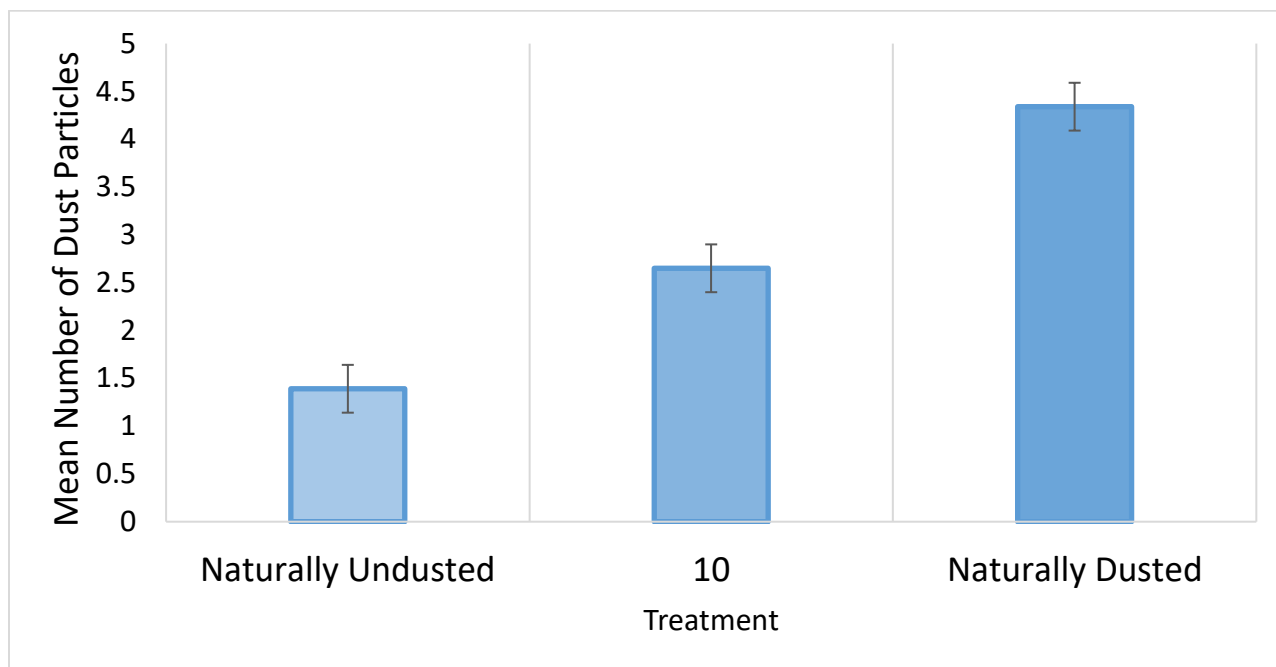


Figure 1. Mean number of dust particles by treatment for 2017 with error bars indicating the 95% confidence intervals. The naturally dusted/undusted plants were collected from Dusty Corner and the 10 represents the experimentally dusted plants with ten pumps.

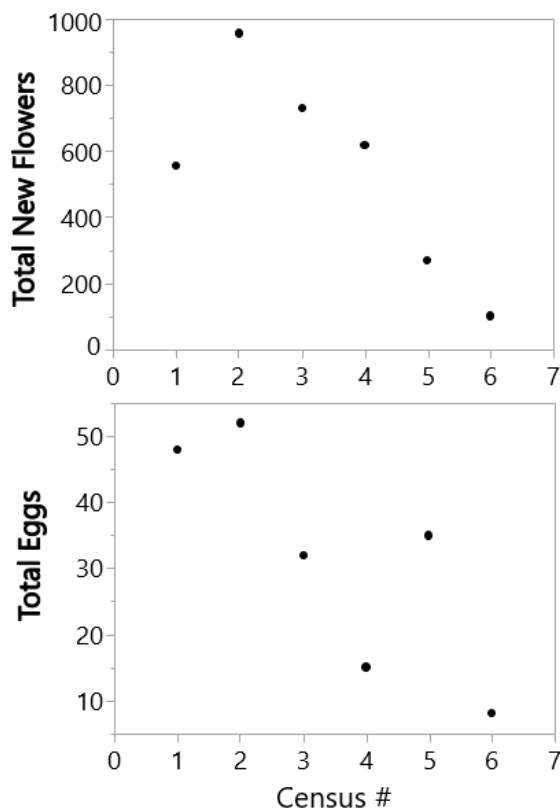


Figure 2. Total eggs and new flowers throughout the censuses for 2017

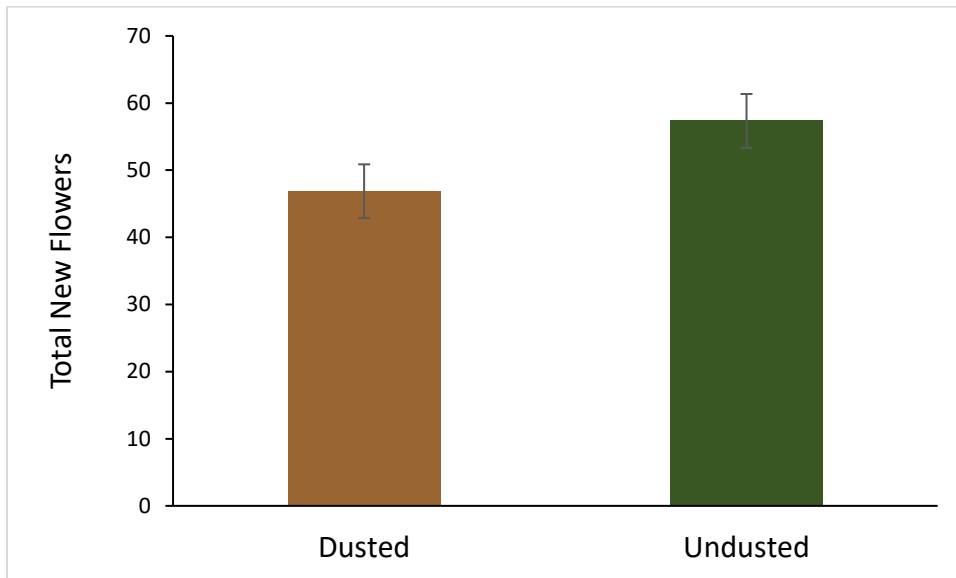


Figure 3a. Total new flowers by treatment for 2017 with error bars indicating 95% confidence intervals

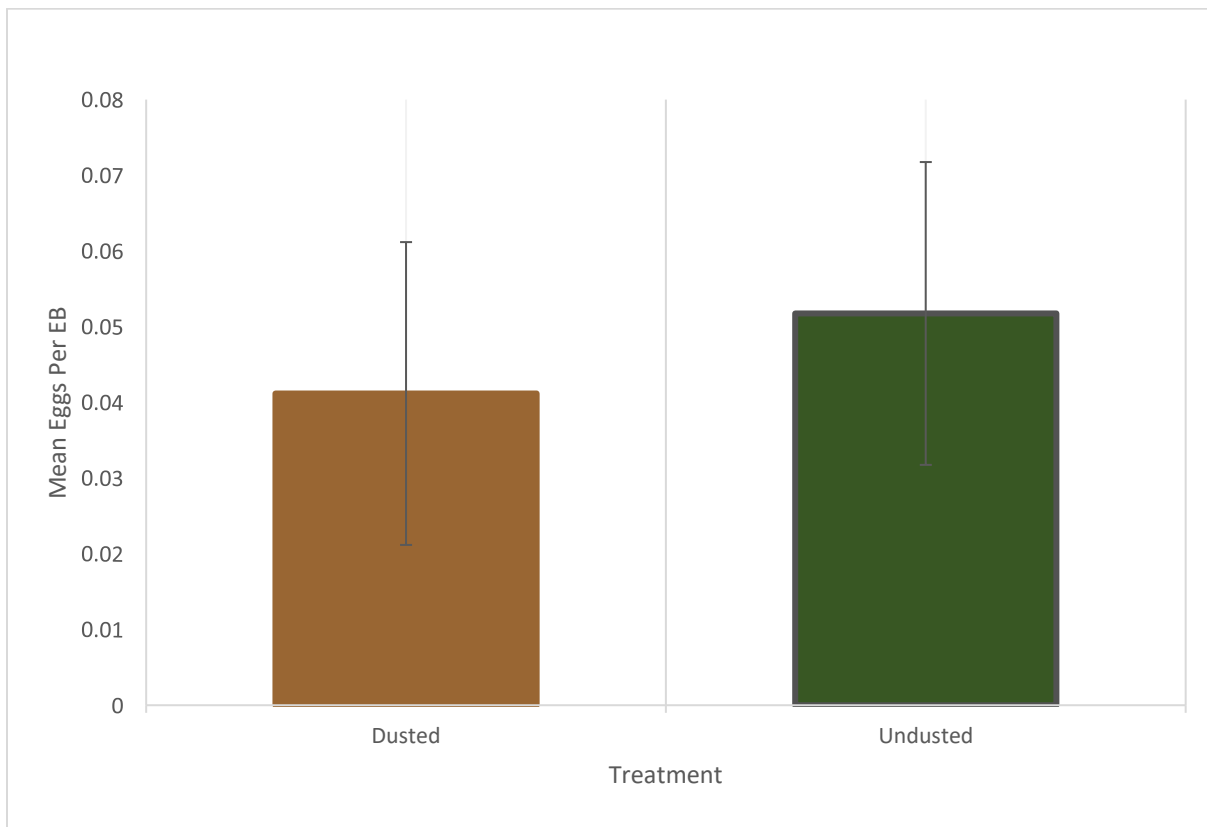


Figure 3b. Mean eggs per elongated bud by treatment for 2017 with error bars indicating 95% confidence intervals around the mean

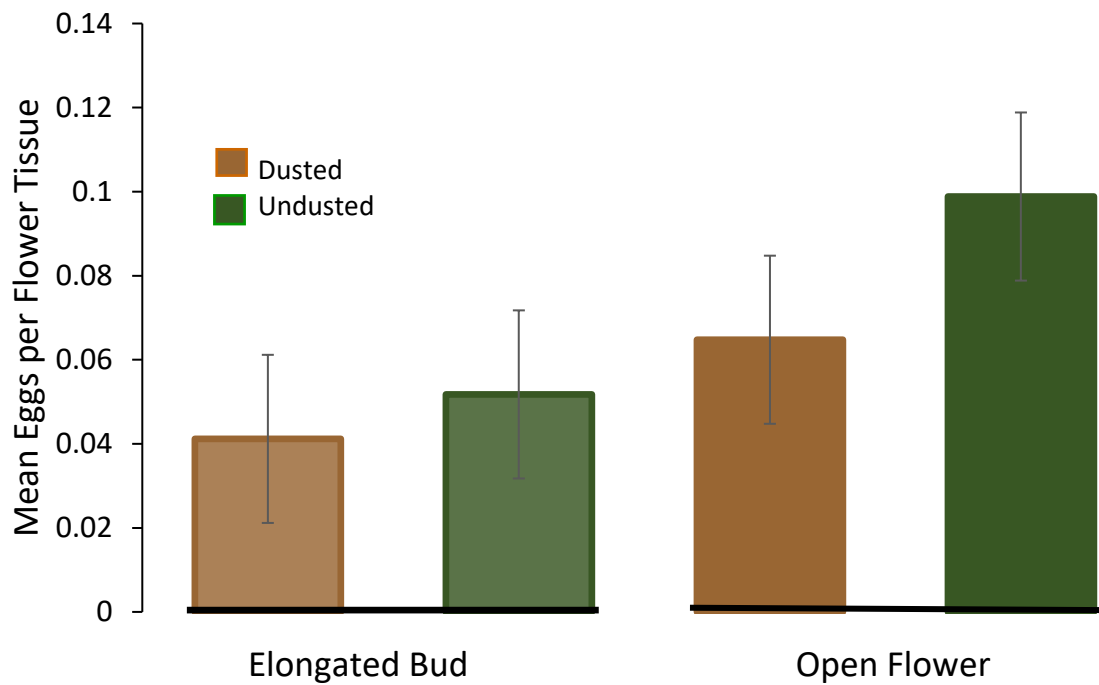


Figure 4. Histogram with 95% confidence intervals displaying mean eggs per flower tissue, either open flower or elongated bud, by treatment for 2017.

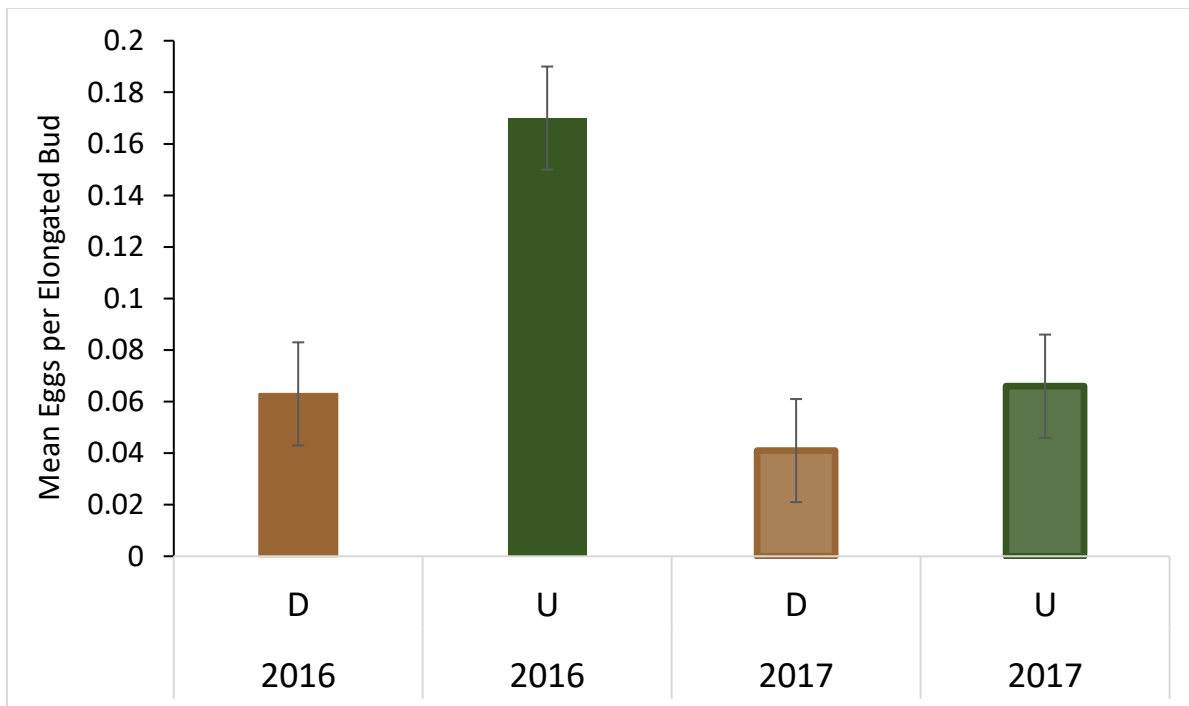


Figure 5. Mean eggs per elongated bud by year and treatment with error bars indicating the 95% confidence intervals. D= dusted; U= Undusted.

Maxfield Meadow

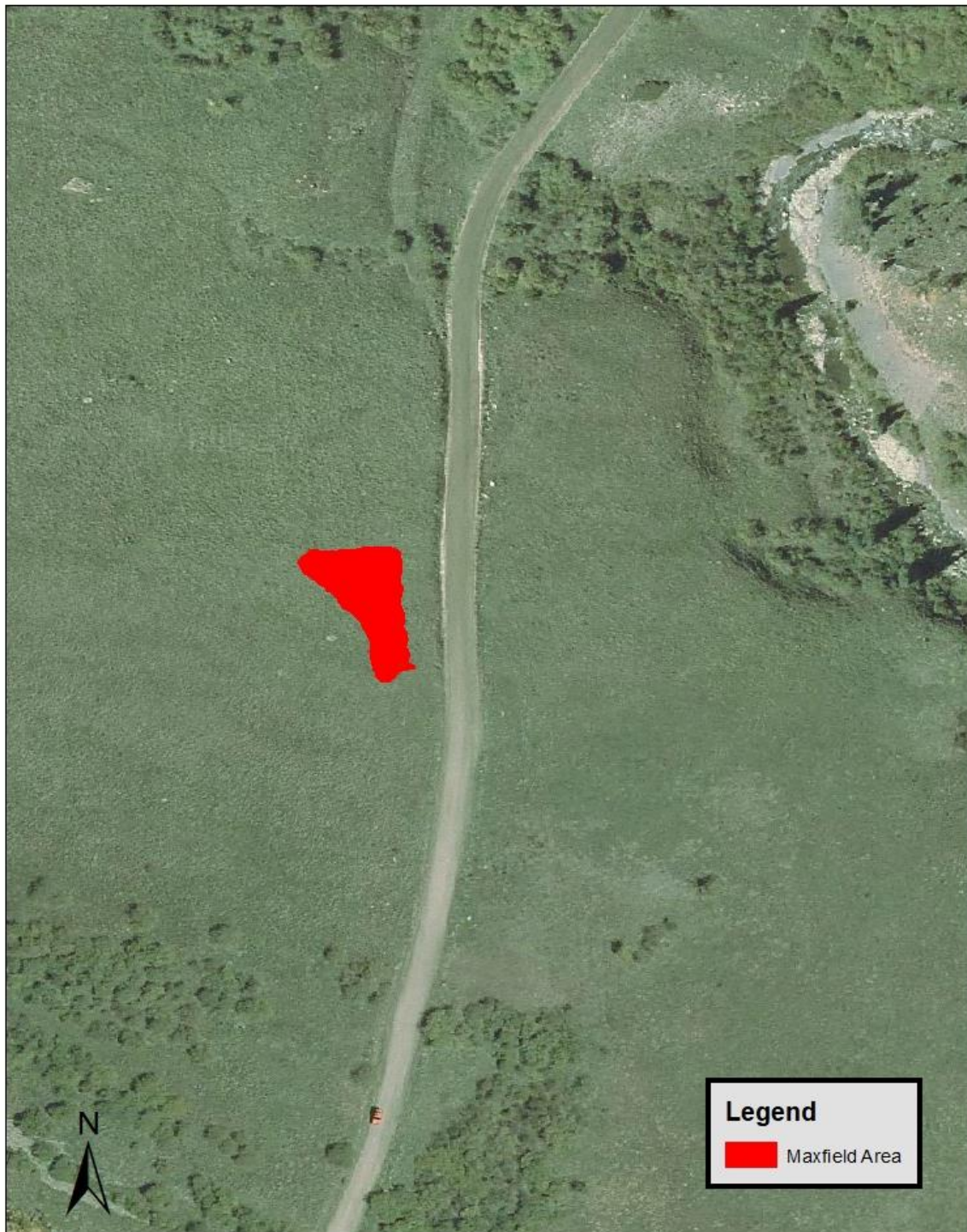


Figure 6 Aerial view of Maxfield Meadow Site

Discussion

In an effort to see whether *Hylemya* flies preferred to oviposit on undusted *Ipomopsis aggregata* plants we performed a series of egg censuses and the results suggested that they laid more eggs in undusted plants. Although the difference was not significant, the results the egg censuses suggest that *Hylemya* females prefer undusted plants to dusted plants. A study by Waser, et al. (2017) discovered that in 19 of 20 independent tests wildflowers carried less pollen (up to six times less) on their stigmas in dusty conditions, most of these tests included *Ipomopsis aggregata*. However, dust did not consistently affect the seed set between dusted and undusted flowers. Waser (2017) suggested four mechanisms that could contribute to the inconsistent seed set one being compensatory herbivory. Since the *Hylemya* females laid more eggs in undusted plants, this may help explain why the undusted plants have an inconsistent seed set.

A series of dust calibrations were performed to see how our manual dusting compared to a naturally dusted environment. We compared our experimentally dusted plants to the naturally dusted and undusted plants, the naturally dusted plants were less than two meters from the road and naturally undusted plants were forty meters from the road. In comparison to the natural dusting, the experimentally dusted plants were significantly ($P_b = 0.0027$, $P_v = 0.0001$) between the two natural treatments. The naturally dusted plants had an average of 4.3 dust particles, naturally undusted had 1.40 dust particles, and the experimentally dusted plants had an average of 2.65 dust particles. The experimental plants were significantly different ($P_b = 0.0027$, $P_v = 0.0001$) to those that were naturally dusted and undusted, they had more dust than the naturally undusted plants and less dust than the naturally dusted plants.

Hylemya oviposition was recorded in open flowers and elongated buds for a series of seven censuses (only six were analyzed) to test whether *Hylemyas* preferred undusted plants. The average total new flowers produced in undusted plants was significantly ($P = 0.015$) greater than in dusted plants. *Hylemya* flies oviposit more often in undusted *Ipomopsis aggregata* than in dusted *I. aggregata*. The number of flowers on a plant affects egg loads therefore, on average there were 4.3 eggs per undusted plant and 2.2 eggs per dusted plant, with a significant difference ($P = 0.019$) between the two treatments and number of eggs per plant. Interestingly, there was no significant difference between undusted elongated buds and open flowers in comparison to dusted elongated buds and open flowers. Although females did oviposit more often in undusted flowers and buds the difference was not significant for either flower tissue. Females laid eggs in 5.2% of undusted elongated buds and 4.2% of dusted elongated buds, but there was no significant difference ($P = 0.645$). There was also no significant difference ($p = 0.14$) in the number of eggs laid in open flowers, whether they were undusted with 9.9% or dusted with 6.5%.

Higher egg loads were found in undusted elongated buds for both years, with 2016 having higher egg loads. Overall, between both years, 11.43% of undusted elongated buds had an egg and 5.22% of dusted elongated buds had an egg with a significant difference ($P = 0.0073$; Figure 5). The year 2016 had higher egg loads than 2017, there could be multiple variables influencing fly populations.

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