

The effects of ungulate herbivory and nutrient variation on pollen receipt
in *Ipomopsis aggregata*

April Lynn Rogers

Mentor: Rebecca E. Irwin

Rocky Mountain Biological Laboratory

REU Program

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Abstract

The purpose of this study was to investigate the effects of ungulate herbivory (clipped, unclipped) and nutrient variation (ambient, water added, fertilizer and water added) on conspecific and heterospecific pollen receipt to *Ipomopsis aggregata*. I found that both clipping and adding nutrients influenced pollen receipt. Pollen receipt was highest in unclipped plants given fertilizer treatments. There was also a temporal effect on pollen receipt of conspecific pollen in unclipped plants. Pollen receipt decreased as the season progressed. As this paper is a study of the indirect effects of herbivory and nutrient variation on plant reproduction, it raises questions about the influence of herbivory and varied nutrients on plant reproduction, aside from seed production and other direct effects.

Keywords: *Ipomopsis aggregata*, ungulate herbivory, nutrient variation, pollen receipt, temporal effect

Introduction

Ipomopsis aggregata is an hermaphroditic monocarpic plant that is self-incompatible (Waser and Price 1989). Thus, a mutualistic relationship exists between *Ipomopsis aggregata* and two hummingbird species: *Selasphorus rufus* and *Selasphorus platycercus* (Mitchell and Waser 1992). The hummingbirds frequent the *Ipomopsis aggregata* to obtain nectar. While consuming the nectar, these hummingbirds also remove pollen. When they travel to the next flower to obtain nectar, they transfer this pollen to the new plant. Through this relationship, the *Ipomopsis* plant accomplishes pollen transport, while the hummingbird receives nutrients needed for survival.

Many different factors affect the relationship between *Ipomopsis* and its hummingbird pollinators, including herbivory by *Odocoileus hemionus*. The common mule deer found in Gothic, *O. hemionus*, are known to eat the tops of the *Ipomopsis* plants (Juenger & Bergelson 1997, Irwin and Brody 2000). Herbivory by mule deer breaks apical dominance, causing changes in plant growth and phenology (Juenger & Bergelson 1997). Aside from the factors imposed by herbivores, nutrient supply is also known to affect *Ipomopsis* reproduction. A previous investigation found that increased fertilizer increased flower production and seed production (Campbell and Halama 1993).

In years past, several investigations were conducted on the direct effects of herbivory and nutrient variation on reproductive fitness in *Ipomopsis aggregata*. In a study conducted by Paige and Whitham (1987), the amounts of inflorescences and fruits were measured to determine whether ungulate herbivory was beneficial or detrimental to *I. aggregata*'s reproductive fitness. They found that herbivory was beneficial and resulted in higher numbers of inflorescence and fruit production (Paige and Whitham 1987). Maschinski and Whitham (1989) also investigated

the direct effects of herbivory, plant association, nutrient availability, and timing on reproductive fitness. They found that the plant response to herbivory varied based on surrounding conditions (Maschinski and Whitham 1989).

Nonetheless, there has been little investigation of the indirect effects (e.g. pollen receipt, pollen export) of herbivory and nutrient variation. Instead of studying indirect effects, a great deal of investigations have been limited to the study of seed production. One disadvantage to this approach is the lack of information on male reproductive fitness (Strauss 1997). Strauss reports that there is little documentation on both male and female reproductive fitness (Strauss 1997). Juenger and Bergleson (1997), while studying direct effects of herbivory, pollination, and nutrient limitation, found that clipping exhibited the greatest overall effect (Juenger and Bergleson 1997). However, they do not explore whether or not clipped plants receive enough pollinator visitation later in the season, after compensation is completed.

In order to investigate these less commonly explored aspects of reproductive fitness in *Ipomopsis*, I have studied the effects of nutrient variation and ungulate herbivory on pollen receipt in *I. aggregata*. The following hypotheses were investigated: 1) Experimentally manipulated early-season herbivory and varied nutrient availability affect the level of both conspecific and heterospecific pollen receipt of *Ipomopsis aggregata*. 2) Unclipped, fertilized plants will exhibit the greatest amount of pollen across weeks. 3) While both clipped and unclipped plants are in bloom, unclipped plants will receive more conspecific and heterospecific pollen than clipped plants. 4) There will be a temporal effect on conspecific and heterospecific pollen receipt in unclipped plants.

Methods and Materials

A total of 312 plants were studied. Ungulate herbivory was simulated by clipping off the bolting stalks of half the total plants under investigation. Nutrient variation was achieved by using three nutrient types: 1) ambient control; 2) added fertilizer and water; 3) added water (Campbell and Halama 1993). There are a total of six plant groups (Irwin and Brody 2000):

- 1) HA- clipped to simulate herbivory, ambient
- 2) HW- clipped, water added
- 3) HF- clipped, water and fertilizer added
- 4) A- herbivory control (not clipped), ambient
- 5) W- herbivory control (not clipped), water added
- 6) F- herbivory control (not clipped), water and fertilizer added

Of the 312 plants studied, there were 52 plants per group, located in two different sites around the Rocky Mountain Biological Laboratory. The Ore House site had 30 groups (180 plants) and the Vera Falls site had 22 groups (132 plants).

Water treatments were administered four times per week: Monday, Wednesday, Thursday, and Saturday. Plants receiving added water received approximately 400 ml of water on each of the four treatment days. Plants receiving added fertilizer and water received 400 ml of water along with 4 ml/L Peters Professional General Purpose 20-20-20 NPK fertilizer once weekly (Monday). On the other three treatment days, they received water treatments. Treatments were given via 16 oz plastic cups connected by plastic tubing to a drip-o-meter placed at the base of each plant.

Pollen receipt data was collected weekly from all plants with elongated buds. On Mondays, up to three buds per plant were emasculated on plants bearing elongated buds. The anthers were removed from one of every two buds present. Using forceps, each of the five un-

dehisced anthers were removed in order to prevent self-pollination (as *I. aggregata* is not self-compatible). On Thursdays, stigmas of emasculated flowers were collected for analysis of pollen receipt once the flowers had fallen off. Stigmas were stained in a basic fuchsin dye and squashed for pollen identification and counting purposes. Pollen load on stigmas was counted using a compound microscope under 40x magnification. Pollen was identified as conspecific or heterospecific derived pollen. This process was repeated weekly for one month, from 6/17/02 until 7/14/02.

All statistical analysis were performed with done using JMP software. In order to determine which treatment received the most pollen. I calculated the mean pollen receipt per stigma per plant across weeks for conspecific and heterospecific pollen receipt. I then used a 2-way ANOVA with herbivory and nutrients as fixed effects, site as a random effect, and their interaction. To examine how pollen receipt varies across the season, I calculated mean pollen receipt per stigma per plant per week for unclipped plants for conspecific and heterospecific pollen. I then used an ANOVA with nutrient treatment and week as fixed effects and their interactions and site as a random factor. Finally, I examined pollen receipt to clipped and unclipped plants in week 4, as described above, because most plants were in bloom during this week.

Results

There was a significant effect of herbivory on mean *Ipomopsis* pollen receipt across all weeks ($F_{1,239} = 5.8978$, $P = 0.0159$), with clipped plants receiving, on average, 88.82 conspecific pollen grains and unclipped plants receiving 104.84 grains (Table 1, a & b, Fig.1). Unclipped plants also received significantly more heterospecific pollen, ($F_{1,240} = 19.1848$, $P = <0.0001$)

with clipped plants receiving, on average, 4.138 pollen grains and unclipped plants receiving 8.490 grains (Table 2, a & b, Fig. 2).

Nutrient variation also had a significant effect on mean conspecific pollen across all weeks ($F_{2,239} = 3.2693$, $P = 0.0397$), with ambient plants receiving an average of 85.51 conspecific pollen grains. Plants with added water received an average of 98.90 grains, and plants with added fertilizer and water received an average of 106.1 grains of conspecific (Table 1, a & b, Fig. 1). Also, nutrient variation had a significant effect on mean heterospecific pollen across all weeks ($F_{2,240} = 3.9302$, $P = 0.0209$), with ambient plants receiving an average of 4.059 conspecific pollen grains. Plants with added water received an average of 6.051 grains, and plants with added fertilizer and water received an average of 8.381 grains of *Ipomopsis* (Table 2, a & b, Fig. 2). The nutrient * herbivory effect was not statistically significant ($F_{2,239} = 2.4007$, $P = 0.0928$). Site had no effect on pollen receipt ($F_{1,239} = 0.0052$, $P = 0.9425$), or on other variables. Thus, for the rest of the data analyses site will not be discussed.

During week 4, when both clipped and unclipped plants were blooming, there was no significant effect of herbivory on mean conspecific (Table 3, a & b, Fig. 3) or heterospecific pollen receipt (Table 4, a & b, Fig. 4). Nutrient variation results were in the same direction as above but were not statistically significant for conspecific pollen receipt, ($F_{2,198} = 1.0211$, $P = 0.0738$), and heterospecific pollen receipt, ($F_{2,203} = 2.6078$, $P = 0.0762$). On average plants receiving fertilizer treatments received the most pollen, 96 conspecific grains. While plants receiving water treatments received 81 grains and ambient plants received 53 grains of conspecific pollen (Table 3,b). Heterospecific pollen receipt data also revealed greater pollen receipt in fertilized plants, 6 grains, than watered plants, 4 grains, and ambient plants, 3 grains (Table 4,b). Also, there was a significant nutrient*herbivory interaction, ($F_{2,203} = 5.5361$, $P =$

0.0343), with fertilization increasing other pollen receipt in clipped plants, but not in unclipped plants (Table 4,b).

There were temporal changes throughout the data collection period in both conspecific and heterospecific pollen receipt. There was a significant effect of week on conspecific pollen receipt ($F_{2,265} = 11.5107$, $P < 0.0001$). Plants received an average of 115.8 grains of conspecific pollen in the first week, 122.6 grains in the second week, and 82.06 conspecific pollen grains during the fourth week (Table 5, a & b, Fig. 5). Data for week 3 was collected, but has not been analyzed for inclusion in this study due to time constraints. Post hoc tests indicate no significant difference between weeks 1 and 2, but significant difference was seen when these two weeks are compared to week 4. Similar results were found in the analysis of temporal effect on heterospecific pollen as well ($F_{2,265} = 16.1224$, $P < 0.0001$). All plants in bloom during the first week received 21.05 grains, 4.203 grains during week 2, and 4.358 grains during week 4 on average (Table 6, a & b, Fig. 6). Post hoc tests indicate no significant difference between weeks 2 and 4, but significant difference is seen when these two weeks are compared to week 1.

Table 1, a. 2-way ANOVA for the effects of herbivory and nutrient treatments on mean *Ipomopsis* pollen receipt across all weeks.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Herbivory	1	1	15345.279	5.8978	0.0159
Nutrients	2	2	17012.913	3.2693	0.0397
Nutrients*Herbivory	2	2	12492.856	2.4007	0.0928
Site	1	1	13.549	0.0052	0.9425
Error		239	621849.67		

Table 1, b. The effect of clipping and nutrient treatments on *Ipomopsis* pollen receipt across all weeks. Each value reported is a mean (± 1 SE).

Herbivory simulation	Ambient treatment	Water treatment	Fertilizer treatment
Clipped	87.93 $\pm$ 9.53	84.18 $\pm$ 8.28	94.36 $\pm$ 7.70
Unclipped	83.10 $\pm$ 7.53	113.62 $\pm$ 7.88	117.81 $\pm$ 7.44

Ipomopsis pollen means across weeks

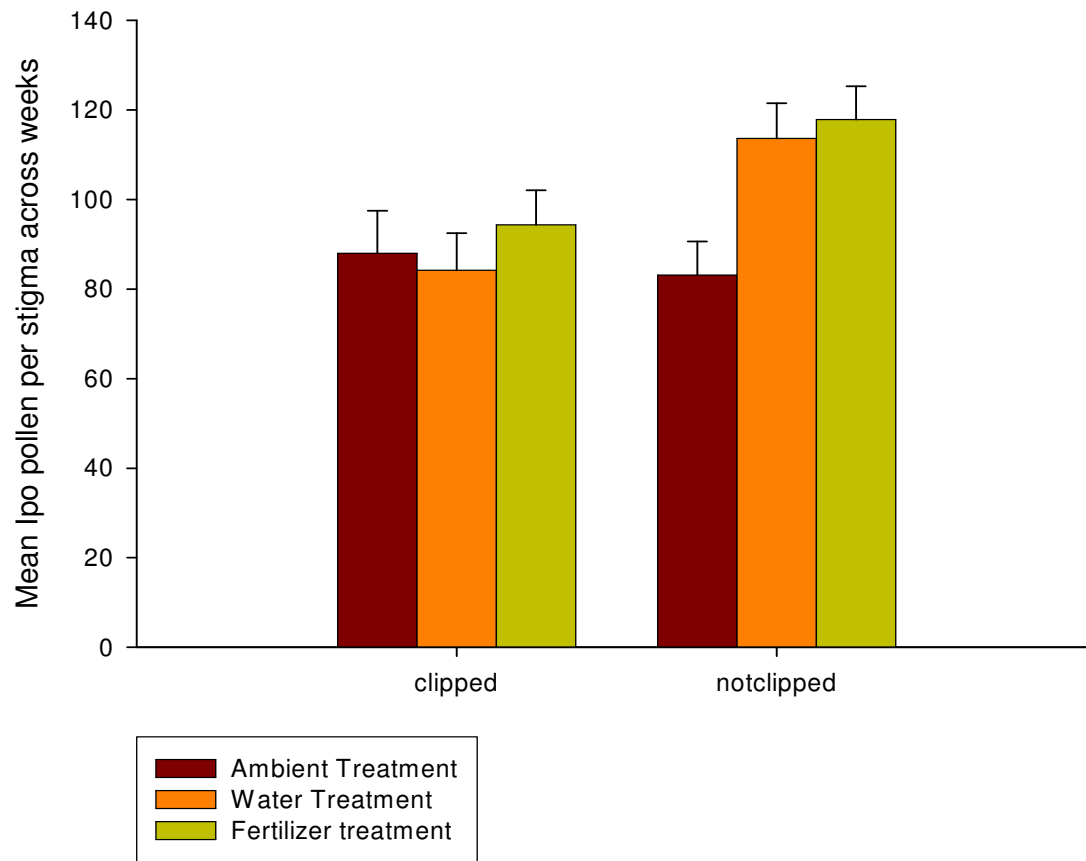


Figure 1. Mean number of *Ipomopsis* pollen grains deposited on the stigmas per nutrient treatment over all weeks. Error bars are 1 SE.

Table 2, a. 2-way ANOVA for the effects of herbivory and nutrient treatments on mean heterospecific pollen receipt across all weeks.

	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Source					
Herbivory	1	1	16.267737	19.1848	<.0001
Nutrients	2	2	6.665236	3.9302	0.0209
Herbivory*Nutrients	2	2	3.705349	2.1849	0.1147
Error		240	203.5		

Table 2, b. The effect of clipping and nutrient treatments on heterospecific pollen receipt across all weeks. Each value reported is a mean (± 1 SE).

Herbivory simulation	Ambient treatment	Water treatment	Fertilizer treatment
Clipped	1.74 $\pm$ 2.98	4.08 $\pm$ 2.60	6.59 $\pm$ 2.42
Unclipped	6.38 $\pm$ 2.37	8.93 $\pm$ 2.48	10.17 $\pm$ 2.34

Other Pollen mean across weeks

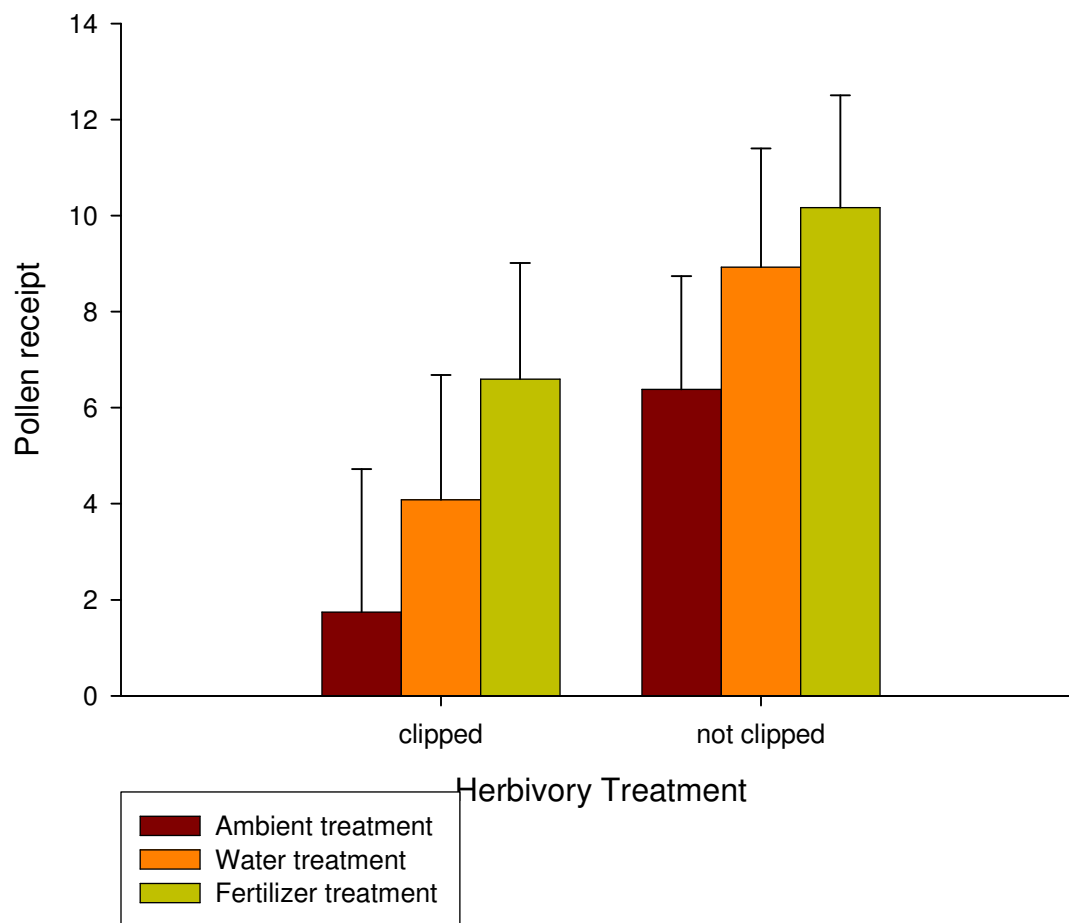


Figure 2. Mean number of heterospecific pollen grains deposited on the stigmas per nutrient treatment. Error bars are 1 SE.

Table 3, a. 2-way ANOVA for the effects of herbivory and nutrients on mean *Ipomopsis* pollen receipt in week 4.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Herbivory	1	1	2292.925	1.0211	0.3135
Nutrients	2	2	11862.719	2.6413	0.0738
Herbivory*Nutrients	2	2	3474.404	0.7736	0.4627
Error		198	444631.48		

Table 3, b. The effect of clipping and nutrient treatments on *Ipomopsis* pollen receipt during week 4. Each value reported is a mean (± 1 SE).

Herbivory simulation	Ambient treatment	Water treatment	Fertilizer treatment
Clipped	87.86 $\pm$ 8.80	84.77 $\pm$ 7.79	93.96 $\pm$ 7.22
Unclipped	71.43 $\pm$ 9.48	76.84 $\pm$ 8.25	97.90 $\pm$ 7.79

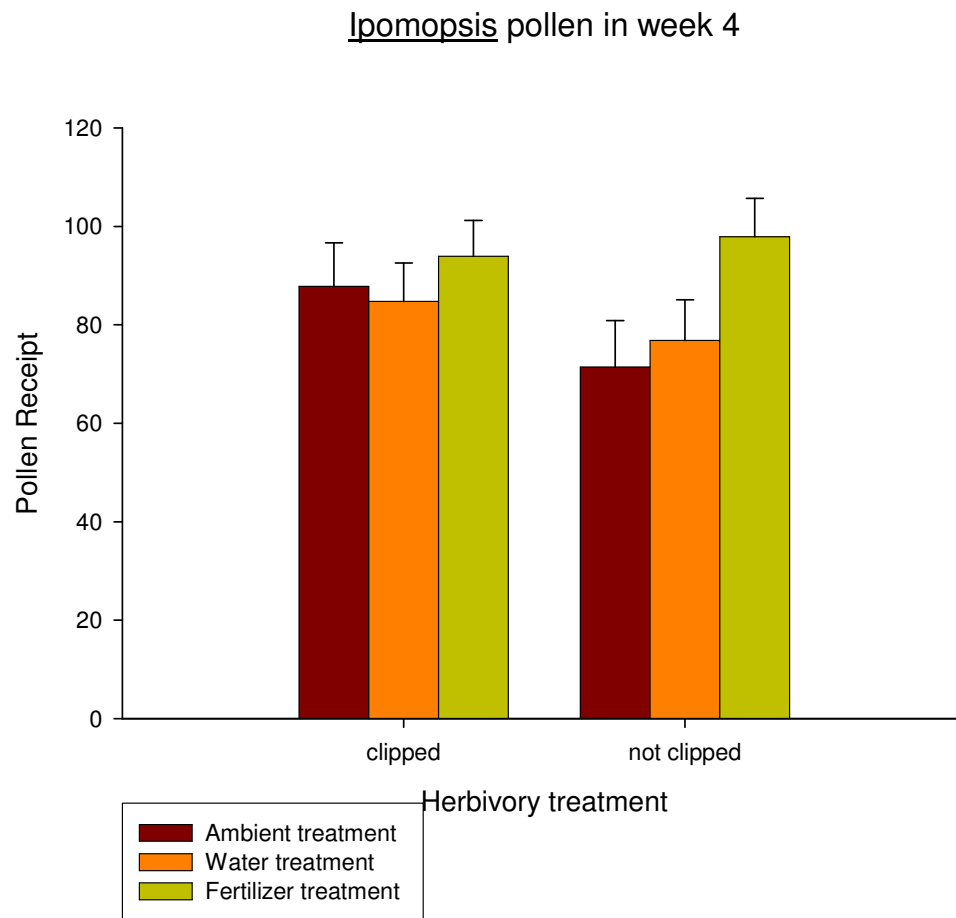


Figure 3. Mean number of *Ipomopsis* pollen grains deposited on the stigmas per nutrient treatment. Error bars are 1 SE.

Table 4, a. 2-way ANOVA for the effects of herbivory and nutrients on mean heterospecific pollen receipt during week 4.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Herbivory	1	1	1.1151255	1.3826	0.2411
Nutrients	2	2	4.2065317	2.6078	0.0762
Herbivory*Nutrients	2	2	5.5360650	3.4320	0.0343
Error		203	170.47		

Table 4, b. The effect of clipping and nutrient treatments on *Ipomopsis* pollen receipt during week 4. Each value reported is a mean (± 1 SE).

Herbivory simulation	Ambient treatment	Water treatment	Fertilizer treatment
Clipped	1.74 $\pm$ 1.60	3.94 $\pm$ 1.42	6.72 $\pm$ 1.31
Unclipped	4.74 $\pm$ 1.72	4.01 $\pm$ 1.50	4.32 $\pm$ 1.42

Week 4 other Pollen

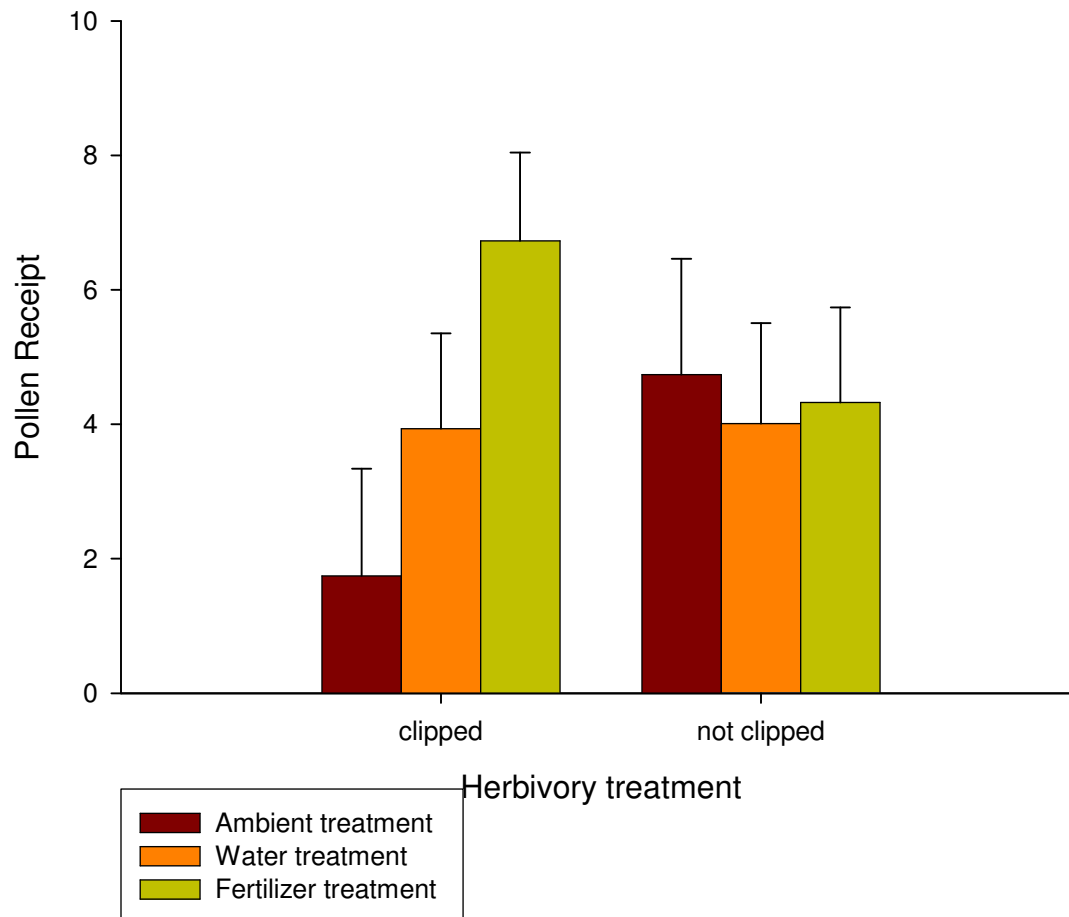


Figure 4. Mean number of heterospecific pollen grains deposited on the stigmas per nutrient treatment. Error bars are 1 SE.

Table 5, a. 2-way ANOVA for the effects of week and nutrients on mean *Ipomopsis* pollen receipt.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Nutrients	2	2	47763.248	6.2260	0.0023
Week#	2	2	88306.202	11.5107	<.0001
Week#*Nutrients	4	4	24330.039	1.5857	0.1784
Error		265	1016492.3		

Table 5, b. The effect of week and nutrients on *Ipomopsis* pollen receipt. Each value reported is a mean (± 1 SE).

Week Number	Ambient treatment	Water treatment	Fertilizer treatment
1	85.90 $\pm$ 11.50	144.95 $\pm$ 13.52	116.60 $\pm$ 12.39
2	105.89 $\pm$ 10.78	133.53 $\pm$ 11.12	128.41 $\pm$ 9.79
4	71.43 $\pm$ 12.39	76.84 $\pm$ 10.78	97.90 $\pm$ 10.81

Ipomopsis pollen temporal effect

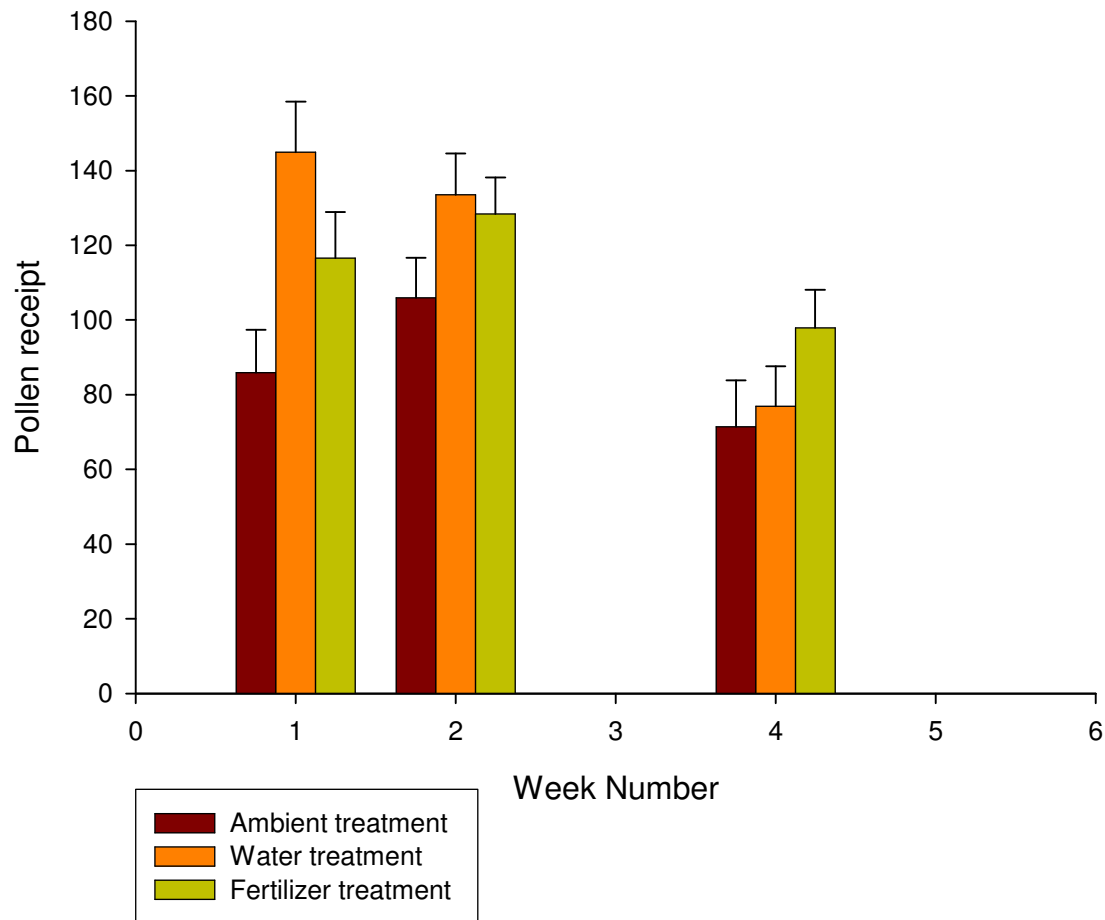


Figure 5. Mean number of *Ipomopsis* pollen grains deposited on the stigmas per week. Values are means $\pm$ SE throughout all weeks.

Table 6, a. 2-way ANOVA for the effects of week and nutrients on mean heterospecific pollen receipt.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Nutrients	2	2	1.964906	1.0145	0.3640
Week#	2	2	31.227200	16.1224	<.0001
Week#*Nutrients	4	4	11.516183	2.9729	0.0199
Error		265	256.64		

Table 6, b. The effect of week and nutrients on heterospecific pollen receipt. Each value reported is a mean (± 1 SE).

Week Number	Ambient treatment	Water treatment	Fertilizer treatment
1	11.91 $\pm$ 5.10	25.81 $\pm$ 5.99	26.25 $\pm$ 5.49
2	2.81 $\pm$ 4.78	4.04 $\pm$ 4.93	5.75 $\pm$ 4.34
4	4.74 $\pm$ 5.49	4.01 $\pm$ 4.78	4.32 $\pm$ 4.77

Other pollen temporal effect

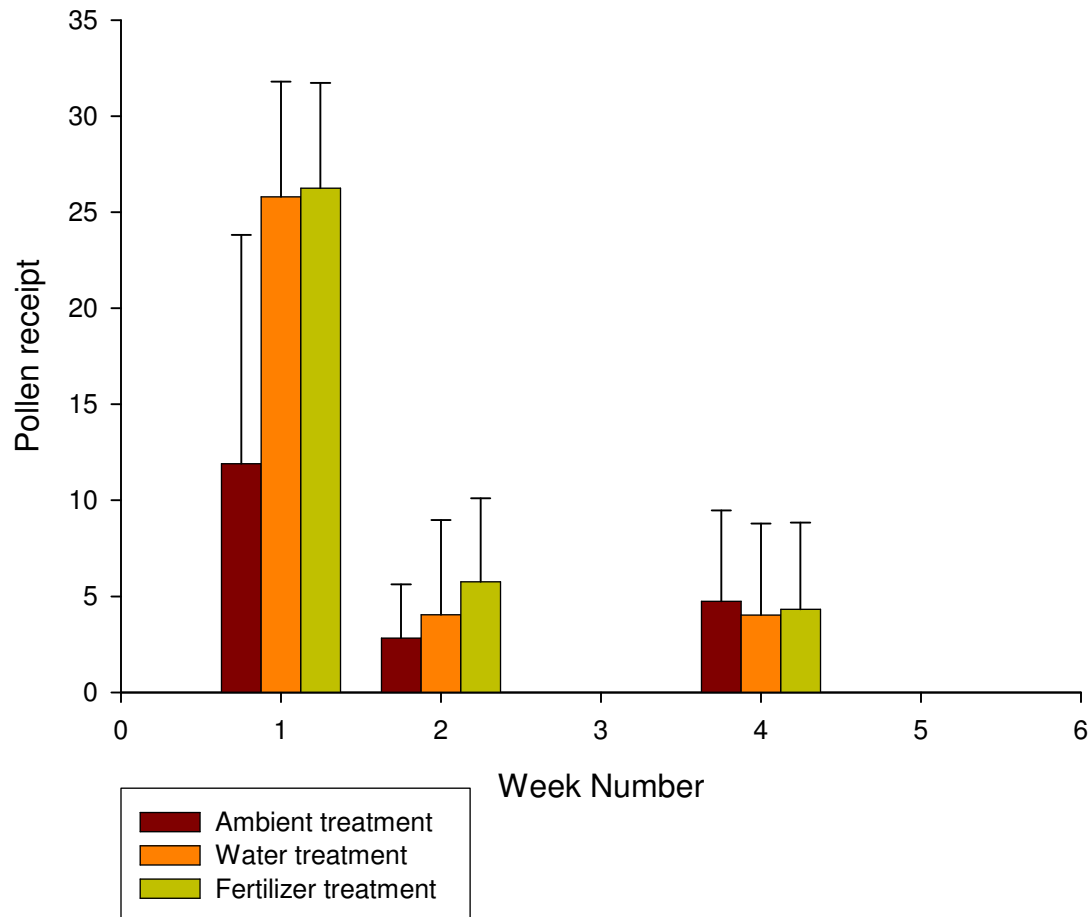


Figure 6. Number of heterospecific pollen grains deposited on the stigmas per week. Values are means $\pm$ SE throughout all weeks.

Discussion

Experimentally simulated ungulate herbivory had a significant detrimental effect on the mean receipt of both conspecific and heterospecific pollen across weeks. Thus, unclipped plants received more pollen per stigma than clipped plants. One possible explanation for this result is that clipped plants were in bloom for less days of the data collection period than unclipped plants. Also, nutrient variation had a significant effect on the receipt of both conspecific and heterospecific pollen. Overall, unclipped plants receiving water treatments and those receiving fertilizer treatments received significantly more pollen than all clipped plants as well as the ambient unclipped plants. However, in a previous study of nutrient variation, it was found that fertilizer treatment, alone, caused increased reproductive success (Campbell and Halama 1993). I speculate that, due to this season's very dry climate, the addition of water was much more significant than in years past. Thus, both fertilizer and water treatments significantly increased pollen receipt.

In the analysis of week 4 pollen receipt data, herbivory played no significant role, most likely, because by week 4, many clipped plants were in bloom. Thus, unclipped plants were in competition with clipped plants for visitation unlike in previous weeks before clipped plants bloomed. Also, some pollinators from weeks 1 and 2 may have migrated by this time. Thus, pollen receipt was low for both clipped and non-clipped plants.

The significant week effect on conspecific pollen of unclipped plants leaves room for much investigation. This temporal effect could result from several different factors. Perhaps this data is due to the decrease of pollinator visitation over time, as the hummingbirds migrate south. Another cause for this lower rate of pollen receipt in week 4 could be competition from clipped plants in bloom. As clipped plants, in previous studies, were found to produce many

inflorescences (Paige & Whitham 1987), and Brody and Mitchell (1997) found that larger floral displays attracted more pollinators, in an effort to compensate for herbivory.

Along with nutrient variation and ungulate herbivory, other factors affect the mutualistic relationship between hummingbirds and *Ipomopsis aggregata*. These other factors include seed predation by *Hylemya* insect species (Brody & Mitchell 1997), and nectar-robbing by *Bombus occidentalis* (Irwin & Brody 1999). These factors may have affected results determined in this study. For instance, assuming there has been corolla damage due to nectar robbing, pollinators may have been deterred from otherwise attractive blooms. Clearly, further investigation of factors alongside herbivory and nutrient variation should be done. Also, an area of focus equally important as pollen receipt, the effect of different factors on pollen export would also give more insight into the factors affecting reproductive success of *Ipomopsis aggregata*.

Works Cited

- Brody, A. K. and R. J. Mitchell. 1997. Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant *Ipomopsis aggregata*. Oecologia 110: 86-93
- Campbell, D. R. and K. J. Halama. 1993. Resource and pollen limitations to lifetime seed production in a natural plant population. Ecology 74: 1043-1051
- Irwin, R. E. and A. K. Brody. 1999. Nectar-robbing bees reduce fitness of *Ipomopsis aggregata* (polemoniaceae). Ecology 80: 1703-1712
- Irwin, R. E. and A. K. Brody. 2000. Consequences of nectar robbing for realized male function in a hummingbird-pollinated plant. Ecology 81: 2643-2673
- Irwin, R. E. and A. K. Brody. 2000. Proposed Research.
- Juenger, T and J. Bergelson. 1997. Pollen and resource limitation of compensation to herbivory in scarlet gilia, *Ipomopsis aggregata*. Ecology 78: 1684-1695
- Maschinski, J. and T. G. Whitham. 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. The American Naturalist 134: 1-19
- Mitchell, R. J. and N. M. Waser. 1992. Adaptive significance of *Ipomopsis aggregata* nectar production: pollination success of single flowers. Ecology 73: 633-638
- Paige, K. N. and T. G. Whitham. 1987. Overcompensation in response to mammalian herbivory: the advantage of being eaten. The American Naturalist 129: 407-416
- Strauss, Sharon Y. 1997. Floral characters link herbivores, pollinators, and plant fitness. Ecology 78: 1640-1645
- Waser, N. M. and Price. 1989. Optimal outcrossing in *Ipomopsis aggregata*: seed set and offspring fitness. Evolution 43: 1097-1109