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IPOMOPSIS AGGREGATA: HIGH
NECTAR PRODUCTION DOES NOT
CONFER TOLERANCE**

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EFFECTS OF NECTAR ROBBING ON FEMALE FITNESS OF *IPOMOPSIS AGGREGATA*: HIGH NECTAR PRODUCTION DOES NOT CONFER TOLERANCE

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Abstract. Nectar robbing by the bee *Bombus occidentalis* has been shown to indirectly decrease estimates of both male and female fitness in the monocarpic herb *Ipomopsis aggregata* by decreasing pollinator visitation. I hypothesized that increased nectar availability would affect the degree to which *I. aggregata* plants could tolerate the effects of nectar robbers. Specifically, I hypothesized that plants with a higher nectar production rate (NPR) would have enough available nectar to satisfy both pollinators and nectar robbers, mitigating the negative effects of nectar robbing on plant fitness. I measured the effects of nectar addition, as a surrogate for high NPR, on female fitness estimates of both robbed and unrobbed plants in a natural population in the Rocky Mountains of Colorado, USA. Nectar was added to plants that were either artificially robbed or unrobbed and compared to robbed and unrobbed plants with normal nectar production. Pollen receipt, percent fruit set, seed set per fruit, and seed set per plant were measured as estimates of female fitness. Nectar robbing significantly reduced percent fruit set and seeds set per plant. Nectar addition did not significantly affect any estimates of female fitness, and there was no interaction between nectar addition and nectar robbing. These results suggest that high nectar production does not mitigate the negative effects of nectar robbing on female fitness of *I. aggregata*. Additionally, data presented here suggest that nectar robbing reduces the incidence of pre-dispersal seed predation by the dipteran fly *Hylemya* sp.

Introduction

Floral larceny, like all multi-species interactions, is varied and complex: effects can be positive, negative, or neutral (Maloof and Inouye 2000). Floral larcenists include nectar robbers, organisms that pierce flowers to obtain nectar without entering the flower directly and often without providing pollination service, and thieves, organisms that enter flowers but provide little or no pollen transfer due to a morphological mismatch between the flower and visitor body shape (Inouye 1980). Nectar robbing has been documented on

every continent except Antarctica and observed in at least 214 species in 59 plant families (Irwin and Maloof 2002). Nectar robbers can have a diverse array of effects on the plants they visit, ranging from direct negative effects such as damage to reproductive organs (McDade and Kinsman 1980, Galen 1983, Traveset et al. 1998) and deterrence of pollinators (Roubik 1982) to direct positive effects such as “accidental” pollination and increased pollinator visitation to more flowers (Lara and Ornelas 2001a). In addition to these direct effects, nectar robbing can indirectly affect plant fitness by altering interactions between plants and their pollinators, leading to a variety of plant-fitness consequences, including decreased plant fitness estimates (Irwin and Brody 1998, 1999, 2000) or increased pollen dispersal distance (Maloof 2001). The wide variety of direct and indirect effects induced by floral larcenists should provoke a range of plant responses.

Plants may deter nectar robbers by a variety of mechanisms, such as the production of toxins and chemical deterrents and physical barriers such as tough floral parts and short corollas. For example, Stephenson (1981) found that ants fell from trees after drinking *Catalpa speciosa* nectar, and skipper butterflies also suffered negative effects, ranging from inability to re-roll their proboscis to being rendered comatose. Similarly, Lara and Ornelas (2001b) found that several short-beaked hummingbirds selectively robbed flowers too long to be legitimately pollinated, completely avoiding robbing short-corolla flowers of two *Salvia* species. In flowers of *Heliconia wagneria*, Wootton and Sun (1989) found that bract liquid "moats" inhibited nectar robbing and ovary damage. Finally, *Ipomopsis aggregata*, a hummingbird-pollinated plant robbed by the bumblebee *Bombus occidentalis*, has been shown to use both time and space to avoid

nectar robbing (Irwin et al. 2004). For example, *I. aggregata* individuals that flower early in the summer may avoid robbing by *B. occidentalis*, which emerges in mid-summer (Irwin and Maloof 2002). Similarly, *I. aggregata* growing near *Linaria vulgaris*, a plant that is also robbed by *B. occidentalis*, were robbed 68% less than in the absence of *L. vulgaris* (Irwin et al. 2001).

In contrast to these mechanisms of resistance to nectar robbing, alternative strategies such as tolerance to nectar robbing have been left unexplored. Plants that undergo damage but incur little or no decrease in fitness relative to fitness in the undamaged state are considered tolerant (Strauss and Agrawal 1999). Although tolerance to herbivory is common and can occur by a variety of mechanisms (i.e. increased branching following release of apical dominance, increased carbon storage in roots; reviewed in Stowe et al. 2000, Tiffin 2000), tolerance to nectar robbing is virtually unexplored (Irwin et al. 2004). Plants with high nectar production rate (NPR) may be more tolerant of the negative effects of nectar robbing than plants producing less nectar (Barrows 1976, Lasso and Naranjo 2003, Irwin et al. 2004). Tolerance, in this case, implies that high rates of nectar production can satisfy both pollinators and nectar robbers while maintaining reproductive fitness at levels equivalent to unrobbed plants (Barrows 1976).

Ipomopsis aggregata provides an ideal study system for examining the effects of nectar robbing on plant fitness and potential mechanisms of tolerance to floral larceny. *Ipomopsis aggregata* is monocarpic, allowing lifetime reproductive success to be estimated in one flowering season (Waser and Price 1989). Pollen receipt of *I. aggregata* is positively correlated with pollinator visitation and plants with increased pollen receipt

produce more seeds (Engel and Irwin 2003). Additionally, plants with high nectar production have increased male fitness and receive longer, more frequent pollinator visits (Mitchell and Waser 1992, Mitchell 1993). However, frequent pollen limitation (Hainsworth et al. 1985) and the effects of a pre-dispersal seed predator, *Hylemya* sp. (Brody 1992a, 1992b; Brody and Morita 2000) can potentially complicate and obscure the effects of nectar robbing and tolerance to it. Levels of both pollen receipt and pre-dispersal seed predation are reported here.

Nectar robbing of *Ipomopsis aggregata* by *B. occidentalis* has been shown to decrease both male and female fitness estimates (Irwin and Brody 1998, 1999, 2000) and *I. aggregata* is known to employ two indirect forms of resistance against nectar robbing: escape in space and escape in time (Irwin et al. 2001, 2004; Irwin and Maloof 2002). Furthermore, a number of studies have demonstrated tolerance of herbivory and in some cases, overcompensation in response to herbivory, in two subspecies of *I. aggregata* (Paige and Whitham 1987, Juenger and Bergelson 1997, Paige 1999, Juenger and Bergelson 2000), although factors such as flowering phenology, resource availability, and pollen limitation may limit the extent of tolerance (Juenger and Bergelson 1997, Freeman et al. 2003, Sharaf and Price 2004).

Here I investigate the effects of high NPR and nectar robbing in *I. aggregata* to explore the potential for tolerance. I hypothesized that high NPR (simulated by nectar addition) would mitigate the negative effects of nectar robbing on female fitness estimates and increase plant fitness of unrobbed plants by satisfying both pollinators and nectar robbers. Specifically, I asked: (1) do nectar robbed plants with a high NPR have increased female fitness estimates relative to robbed plants with normal nectar production

and (2) is increased fitness of nectar-robbed plants with high NPR comparable to that of unrobbed plants?

Materials and Methods

Study System

Research was conducted along the Copper Creek trail in the Maroon Bells Wilderness, approximately 1 mile east of the Rocky Mountain Biological Laboratory (RMBL), elevation 2800m, in the Elk Mountains of central Colorado, USA (Fig. 1). *Ipomopsis aggregata* (Pursh) V. Grant subsp. *aggregata* (Polemoniaceae) is a monocarpic perennial herb that grows as a rosette for 2-7 years, flowers once, and then dies (Waser and Price 1989). Plants produce an average of ~85 red, trumpet-shaped flowers (Grant and Wilken 1986, Campbell 1989b). Flowering lasts for 4-8 weeks and individual hermaphroditic flowers last approximately 3-5 days. Flowers are protandrous, but temporal separation between stigmas and anthers may be incomplete (Waser and Price 1991). *Ipomopsis aggregata* does not reproduce vegetatively and is self-incompatible, so all seeds are the product of pollen transfer between plants (Waser and Price 1983). Female fitness of *I. aggregata* is often pollen limited (Hainsworth et al. 1985, Campbell 1991, Campbell and Halama 1993).

Nectaries are located at the base of the corolla and nectar is produced at a rate of 1-5 μ l per flower per day with a concentration of 20-25% sucrose equivalents (Pleasants 1983, Mitchell and Waser 1992, Mitchell 1993). Nectar production does not decrease with flower age and occurs in both male and female phases, beginning as buds elongate (Pleasants 1983). There is a seasonal decline in nectar production rate; however, sugar concentration does not change (Pleasants 1983). Flowers are primarily pollinated by

broad-tailed hummingbirds (*Selasphorus platycercus*) and rufous hummingbirds (*S. rufous*) around the RMBL (Waser 1978). Pollinator visitation does not stimulate or inhibit nectar production (Pleasants 1983). Fruit capsules produce 3-20 arcuate seeds (Grant and Wilken 1986), which are subject to predation by larvae of the dipteran fly *Hylemya* sp. (Zimmerman 1980).

Flowers of *Ipomopsis aggregata* are robbed by the nectar robbing bee *Bombus occidentalis*, which uses its toothed mandibles to bite a hole in the base of the corolla and then remove nectar using its proboscis. *Bombus occidentalis* does not come in contact with the reproductive structures of *I. aggregata* while robbing its flowers (Irwin and Brody 1998). Nectar robbing in *I. aggregata* results in necrosis of petal tissue around robber holes, decreased volume of available nectar, and increased nectar sugar concentration by up to 10% due to evaporation of water through robber holes (Pleasants 1983, Irwin and Brody 1998). Rates of nectar robbing at the whole plant level range from 0-96% of available flowers robbed, with a seasonal mean (± 1 SD) of $51.3\% \pm 38.3\%$ (Irwin and Brody 1998).

High levels of nectar robbing (>80% of flowers robbed per plant) by *B. occidentalis* result in a 50% loss in both male and female reproductive success in *I. aggregata*, caused by a reduction in pollinator visitation at plants with high robbing levels (Irwin and Brody 2000). Plants with high levels of robbing receive 23% fewer hummingbird visits than plants with low robbing (Irwin and Brody 2000). Pollen remaining in *I. aggregata* flowers decreases as flowers are probed by pollinators, and standing crop of nectar affects the amount of pollen remaining per flower, with more pollen remaining in flowers with 1 μ L of nectar than those with 5 μ L (Mitchell and Waser 1992). Plants with high nectar

production rate (NPR) (5 μ L per day) have been found to disperse significantly more dye particles (pollen analogues) than those with lower NPR (Mitchell 1993), suggesting that high NPR increases pollinator visitation and pollen dispersal in accordance with Mitchell and Waser (1992). Plants with high NPR had 36 to 42% increased fitness through male function, demonstrating that high NPR improves pollination success (Mitchell 1993). Similarly, as per-flower visitation rate increases, pollen receipt increases significantly (Engel and Irwin 2003, Price et al. 2005) and plants with increased stigma pollen loads produce more seeds (Engel and Irwin 2003).

Field Methods

At the Copper Creek site (Fig. 1), 125 single-stalked, bolting *Ipomopsis aggregata* individuals were identified and assigned to one of five treatments. In a fully-crossed factorial design, four treatments were: nectar treatment (control, handled only, or addition of 2 μ l 25% nectar solution) crossed with robbing treatment (no simulated nectar robbing or high (>80% of flowers) simulated nectar robbing). The high simulated robbing treatment represents natural levels of high robbing commonly found in the field (Irwin and Brody 1998, 1999). Nectar production naturally varies between 1-5 μ l per flower per day with a sucrose concentration of 20-25% (Pleasants 1983, Mitchell and Waser 1992); therefore, an addition of 2 μ l of 25% sucrose solution is within the range of natural nectar production. In addition, a fifth supplemental hand pollination treatment was administered for comparison to the no robbing, no nectar addition treatment to test for pollen limitation of fruit and seed set. Testing for pollen limitation in this system is important because the primary effects of nectar robbing are indirect and are mediated through changes in pollinator behavior and pollen receipt. Flowers on all plants were handled to control for

researcher effects. Treatments were assigned at random to 25 plants per treatment for the total of 125 plants. Plants were assigned to treatments at random based on initial plant

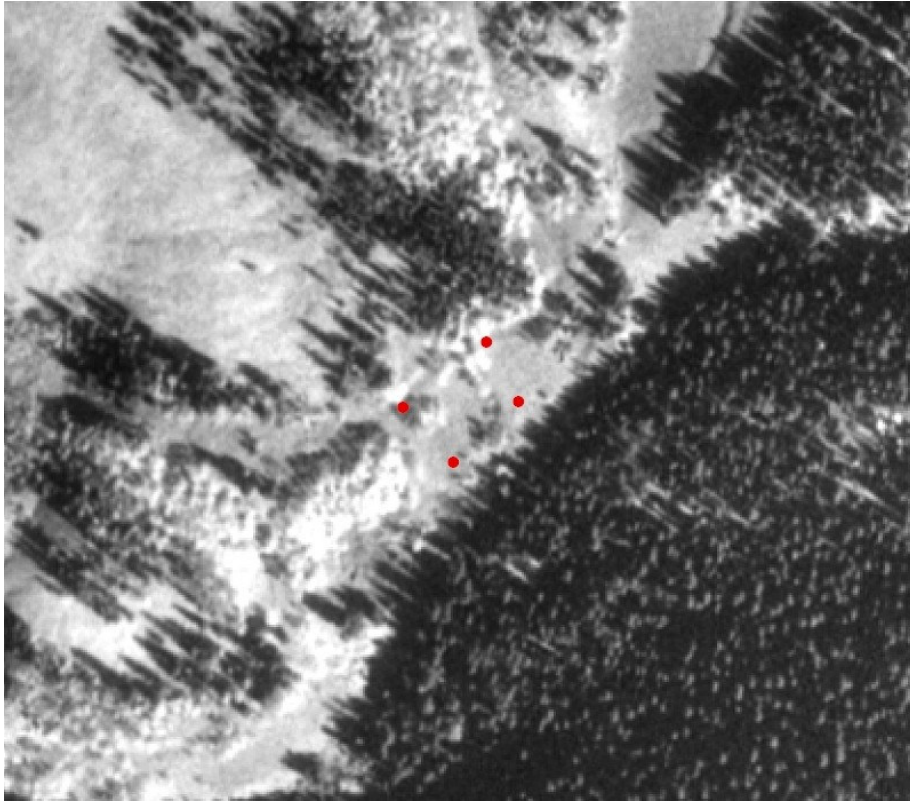


Figure 1: Copper Creek site.

size (root-stalk diameter, ANOVA: $F = 1.2010$, $P = 0.3140$). To prevent overhead herbivory by grazing animals, plants were loosely tied to flag stakes, leaving approximately 2 cm between each plant and flag stake. Staking plants in this way does not alter hummingbird-pollinator behavior.

Flowering date and plant height at flowering were measured for each plant. Plant height was measured to the nearest 0.1 cm. Corolla length and width, sepal length and width, calyx length, and stigma position of up to three open, haphazardly chosen flowers per plant were measured once in the middle of the flowering season (21-27 July, 2005).

Root stalk diameter, corolla length and width, sepal length and width, and calyx length were all measured to the nearest 0.1 mm using a digital caliper. Nectar concentration and 48-hour nectar production of three haphazardly chosen flowers per plant were measured on 19 July, 2005. To determine nectar production, up to three elongate buds per plant were covered with folded drinking straws. Covering elongated buds prevented pollinator access while nectar accumulated in flowers. After 48 hours, nectar was extracted from the base of the removed corolla tube using a 10 μ l microcapillary tube. Nectar concentration of removed nectar was measured using a portable refractometer. Corolla length and width, flowering date, nectar production rate, and plant height were measured because they have all been shown to affect pollinator visitation, pollen export and/or pollen receipt (Campbell 1989a, Wolf and Hainsworth 1990, Campbell 1991, Campbell et al. 1991, Irwin 2000, Engel and Irwin 2003). Analysis of the above variables showed no significant difference between the five treatments (MANOVA: *Wilk's* $\lambda = 0.467$, $F_{40, 157} = 0.874$, $P = 0.684$).

Robbing and nectar-addition treatments were administered four days each week and the hand pollination treatment was administered three days each week beginning at first flowering on 2 July, and ending 4 August, 2005. At this time, all remaining flowers and buds were pinched and fruits were gathered when mature (3-24 August, 2005). There were no more than two back-to-back days of treatment each week (i.e. treatments on Monday, Wednesday, Friday, and Saturday). The nectar addition treatment was administered in the early morning and nectar robbing treatments were administered at midday, when *B. occidentalis* is most active (R. E. Irwin, *personal communication*). Pollen addition treatment was administered in either the morning or afternoon. Robbing

treatments were administered by cutting a hole ~1 mm in diameter near the base of the corolla using dissecting scissors and removing nectar using a 10 μ l micro-capillary tube inserted into the hole (Irwin 2003). There is no significant difference in fitness of plants that are robbed artificially vs. naturally (Irwin and Brody 1999). Flowers that had been previously robbed were robbed each successive day they were open by inserting a micro-capillary tube and removing all nectar from the existing hole. I injected 2 μ l of 25% sucrose solution into each open flower on plants assigned a nectar addition treatment using a 10- μ l glass syringe with a blunt-tipped needle and a PB600-1 repeating dispenser (Hamilton Co., Reno, Nevada). Care was taken not to move pollen among flowers and plants while adding nectar. Dehiscing anthers were gathered from untreated *Ipomopsis aggregata* individuals at the Copper Creek site on each pollination treatment day. Pollen was added to receptive stigmas of flowers on pollen addition treatment plants using a small paint brush.

Estimates of female fitness (pollen receipt, percent fruit set, seed set per plant, and seed set per fruit) were obtained to determine the effects of nectar robbing, nectar addition, and their interaction term. Once during the season, up to three stigmas from each flowering plant were collected after the corollas had abscised (for a total of 267 stigmas from 100 plants) to estimate pollen receipt per plant. Collecting stigmas at this stage does not affect pollen tube germination or fruit set (Waser and Fugate 1986, Waser and Price 1991). Stigmas were squashed in basic fuchsin dye and the numbers of conspecific and heterospecific pollen grains present on each stigma were counted using a compound microscope (Kearns and Inouye 1983).

Matured and aborted fruits were gathered from plants 3–24 August, 2006. Pre-dispersal seed predation by the dipteran fly *Hylemya* sp. was estimated by determining the number of fully or partially destroyed fruits. Fully destroyed fruits are characterized by the presence of *Hylemya* larvae and/or frass (Zimmerman 1980). If a *Hylemya* larva has exited a fruit capsule an exit hole is usually apparent (Zimmerman 1980). Similarly, partially destroyed fruits are identified by the same characteristics in the presence of some intact or partially destroyed seeds (R. E. Irwin, *personal communication*). All seeds from intact and partially destroyed fruits were counted.

Data Analysis

For each plant, I calculated percent fruit set, seed set per fruit, seed set per plant, and pollen receipt per stigma. Percent fruit set was calculated as $(100 \times ((\# \text{ expanded and aborted capsules produced} - \# \text{ aborted capsules produced}) / \# \text{ expanded and aborted capsules produced}))$. Seed set per plant was square-root transformed to improve normality. Pollen receipt was estimated as the average number of pollen grains received per collected stigma per plant (Engel and Irwin 2003, Irwin and Brody 1999). Heterospecific pollen receipt was not included in the analyses because levels of heterospecific pollen on collected stigmas were very low (less than 5% of total pollen receipt).

Results

Nectar robbing significantly lowered percent fruit set of *Ipomopsis aggregata* by 47% (Tables 1a and 2a, Figs. 2a and 3a) and seed set per plant by 23% (Tables 1c and 2c, Figs.

2c and 3c). In contrast, nectar robbing did significantly reduce seed set per fruit (Tables 1b and 2b, Figs. 2b and 3b).

Nectar addition did not significantly affect any measures of plant fitness reported here ($P > 0.05$ in all cases; Tables 1a-d and 2a-d, Figs. 2a-c and 3a-c). Additionally, supplemental hand pollination did not affect any reported measures of reproductive fitness ($P > 0.05$ in all cases; Tables 1d and 2d).

Nectar addition failed to mitigate the effects of nectar robbing, with a 42% reduction in percent fruit set and a 34% reduction in seed set per plant between the control and high robbing treatments (Tables 2a and 2c, Figs. 2a, 2c and 3a, 3c). Robbing did not, however, significantly reduce seed set per fruit in the presence of nectar addition (Tables 1b and 2b, Figs. 2b and 3b).

Table 1: Female reproductive success. Analysis examining the effects of high robbing (>80%) and nectar addition treatments on (a) percent fruit set, (b) seed set per fruit, (c) seed set per plant (square root transformed), and (d) pollen receipt.

Source of Variation	ss	df	F	P
a) Percent fruit set				
Robbing	1.942	1	112.8783	<0.0001
Nectar addition	0.017	1	0.9828	0.3240
Nectar addition x robbing	0.024	1	1.3892	0.2415
Error	1.634	95		
b) Seeds per fruit				
Robbing	4.224	1	2.0245	0.1581
Nectar addition	0.577	1	0.2766	0.6002
Nectar addition x robbing	0.941	1	0.4508	0.5036
Error	194.043	93		
c) Seeds per plant				
Robbing	394.512	1	13.778	0.0003
Nectar addition	4.915	1	0.1716	0.6796
Nectar addition x robbing	21.407	1	0.7475	0.3895
Error	2720.820	95		
d) Pollen grains per stigma				
Robbing	7028.445	1	2.0078	0.1607
Nectar addition	9474.962	1	2.7067	0.1042
Nectar addition x robbing	1926.515	1	0.5503	0.4605
Error	259040.27	74		

Table 2: Female reproductive success. Means and standard errors for (a) percent fruit set, (b) seed set per fruit, (c) seed set per plant (square root transformed), and (d) pollen receipt by control, nectar addition, nectar addition x control, and nectar addition x robbing treatments.

	mean	S.E.
a) Percent fruit set		
Control	65.617	2.454
Robbing	34.495	3.157
Nectar addition x control	59.895	2.614
Nectar addition x robbing	34.988	2.283
b) Seeds per fruit		
Control	4.352	0.291
Robbing	3.786	0.336
Nectar addition x control	4.393	0.237
Nectar addition x robbing	3.778	0.312
c) Seeds per plant		
Control	13.294	1.045

Robbing	10.231	1.021
Nectar addition x control	14.760	1.172
Nectar addition x robbing	9.746	1.054
d) Pollen grains per stigma		
Control	142.873	14.879
Robbing	133.788	14.098
Nectar addition x control	130.717	9.937
Nectar addition x robbing	101.667	14.254

Nectar robbing significantly reduced the number of fruits per plant fully destroyed by *Hylemya* sp. seed predation by 42% and 62% in the control and nectar addition treatments, respectively ($F = 22.5286$, $P < 0.0001$; Table 3a, Fig. 4). The number of fruits partially destroyed by seed predation was also reduced in the high robbing treatments, by 28% and 51% in both the absence and presence of nectar addition, respectively, although these results were not as strongly significant ($F = 3.9090$, $P = 0.0509$; Table 3b, Fig. 4).

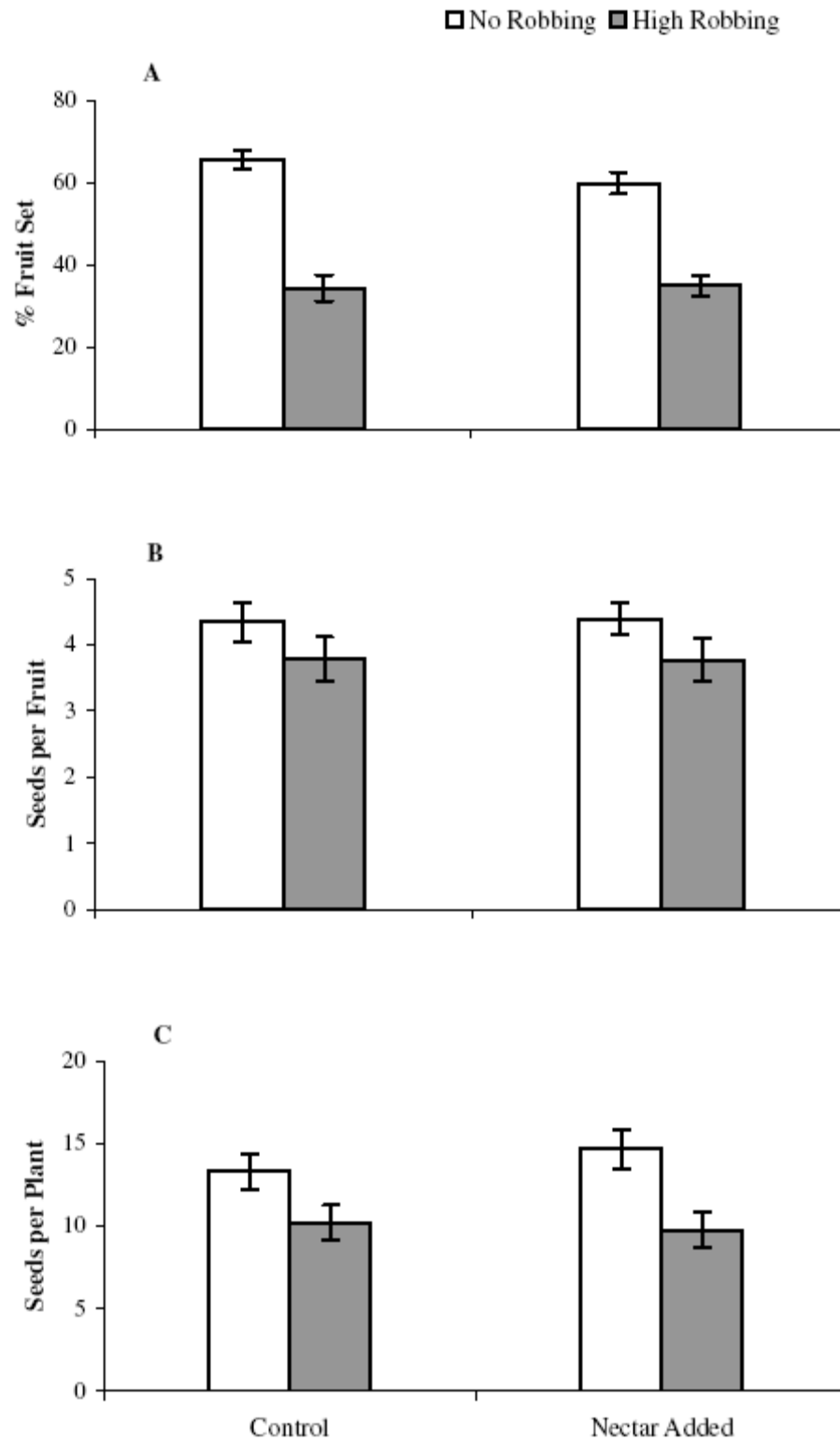


Figure 2: Effect of high robbing (>80%) on (a) percent fruit set, (b) seeds per fruit, and (c) seeds per plant (sq. rt. transformed). Bars are means ± 1 S.E.

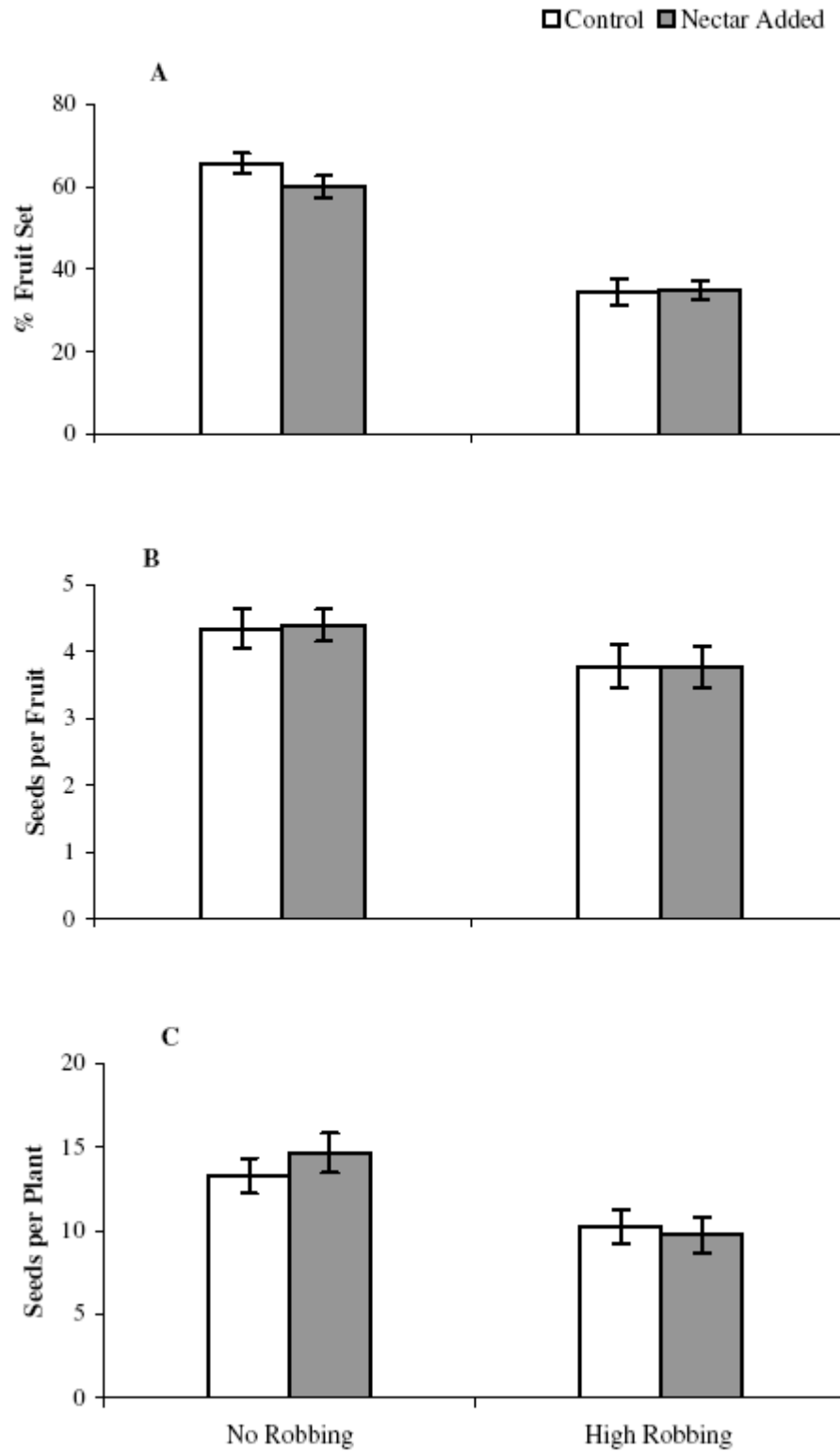


Figure 3: Effect of nectar addition on (a) percent fruit set, (b) seeds per fruit, and (c) seeds per plant (sq. rt. transformed). Bars are means $\pm$ 1 S.E.

Table 3: *Hylemya* sp. seed predation. Means and standard errors for (a) seeds fully destroyed and (b) seeds partially destroyed by control, nectar addition, nectar addition x control, and nectar addition x robbing treatments.

	mean	S.E.
a) Seeds fully destroyed		
Control	15.280	2.129
Robbing	8.833	1.465
Nectar addition x control	22.560	3.076
Nectar addition x robbing	8.560	1.484
b) Seeds partially destroyed		
Control	3.080	0.755
Robbing	2.208	0.580
Nectar addition x control	2.680	0.472
Nectar addition x robbing	1.320	0.382

Pollen receipt was not significantly affected by robbing, nectar addition, or the interaction of the two terms (Table 1d). Stigmas received a range of 0-299 pollen grains, with a mean ± 1 SE of 129.42 ± 5.63 pollen grains. All treatments received similar numbers of pollen grains per stigma (Table 2d).

Natural variation in nectar concentration was positively associated with pollen receipt ($R^2 = 0.1079$, $F = 5.4108$, $P = 0.0250$; Fig. 5), but was negatively associated with seed set per plant ($R^2 = 0.1325$, $F = 4.7626$, $P = 0.0340$; Fig. 6). Seed set per plant was also higher in plants that began blooming earlier in the season ($R^2 = 0.168$, $F = 10.0212$, $P = 0.0027$; Fig. 7) and in taller plants ($R^2 = 0.2344$, $F = 12.7337$, $P = 0.0008$; Fig. 8). Additionally, nectar concentration decreased as root stalk diameter increased ($R^2 = 0.1947$, $F = 13.2953$, $P = 0.0006$; Fig. 9).

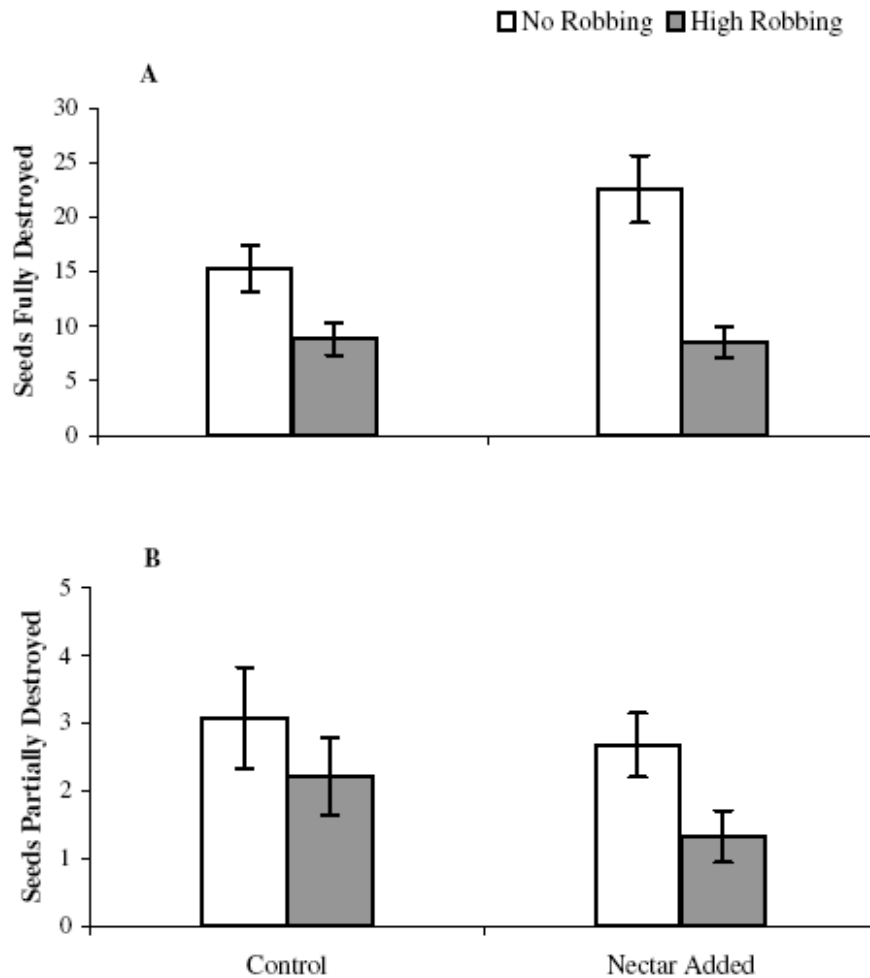


Figure 4: Effect of high robbing (>80%) on (a) fully destroyed seeds and (b) partially destroyed seeds. Bars are means $\pm$ 1 S.E.

Discussion

This study suggests that high nectar production (in the form of nectar addition) does not significantly mitigate the negative effects of nectar robbing on female fitness estimates of *Ipomopsis aggregata*. Nectar addition did not significantly affect percent fruit set, seed set per fruit, seed set per plant, or pollen receipt. Previous studies (Mitchell and Waser 1992, Mitchell 1993) found that *Ipomopsis aggregata* individuals with high nectar standing crops received longer pollinator visits, had more pollen removed from

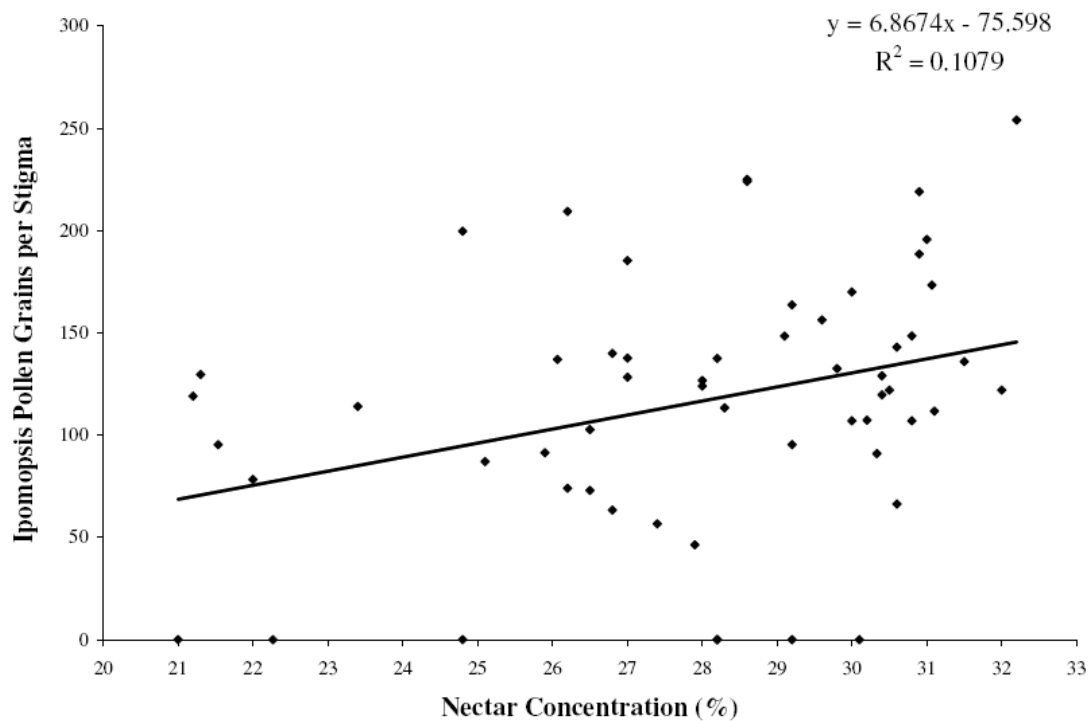


Figure 5: Effect of nectar concentration (%) on pollen receipt (*Ipomopsis* pollen grains per stigma). N = 57.

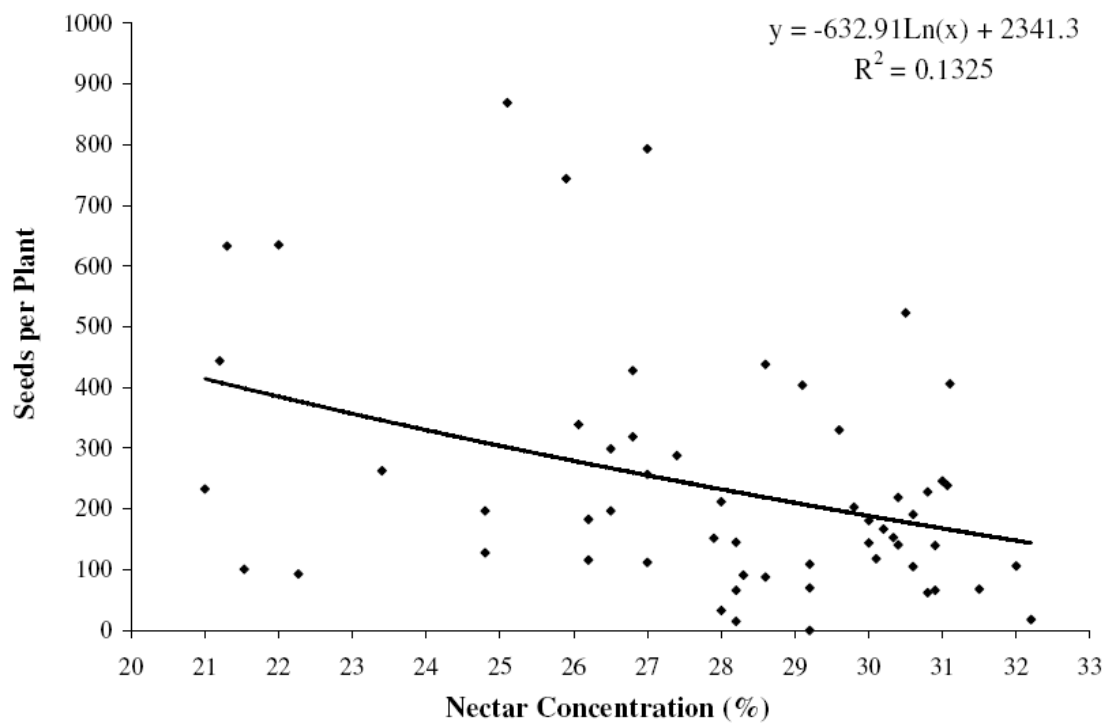


Figure 6: Effect of nectar concentration (%) on seed set per plant; N = 57.

anthers, and donated more dye particles (pollen analogues) than plants with lower nectar standing crops. Despite these increases in male fitness estimates, nectar standing crop had no effect on per visit pollen receipt (Mitchell and Waser 1992), as seen here (and in Lasso and Naranjo 2003). I did not examine the effects of nectar addition on pollinator visitation or male fitness in this study, so the effects of nectar addition on these components and their relationship to nectar robbing are unknown. Future studies of the relationship between nectar production rate and nectar robbing should consider the effects of nectar addition on male fitness and pollinator visitation in addition to the female fitness estimates seen here. The importance of measuring male fitness has been clearly demonstrated by previous studies (Campbell 1989a, 1991; Mitchell and Waser 1992; Mitchell 1993), wherein factors affecting pollinator visitation had significant

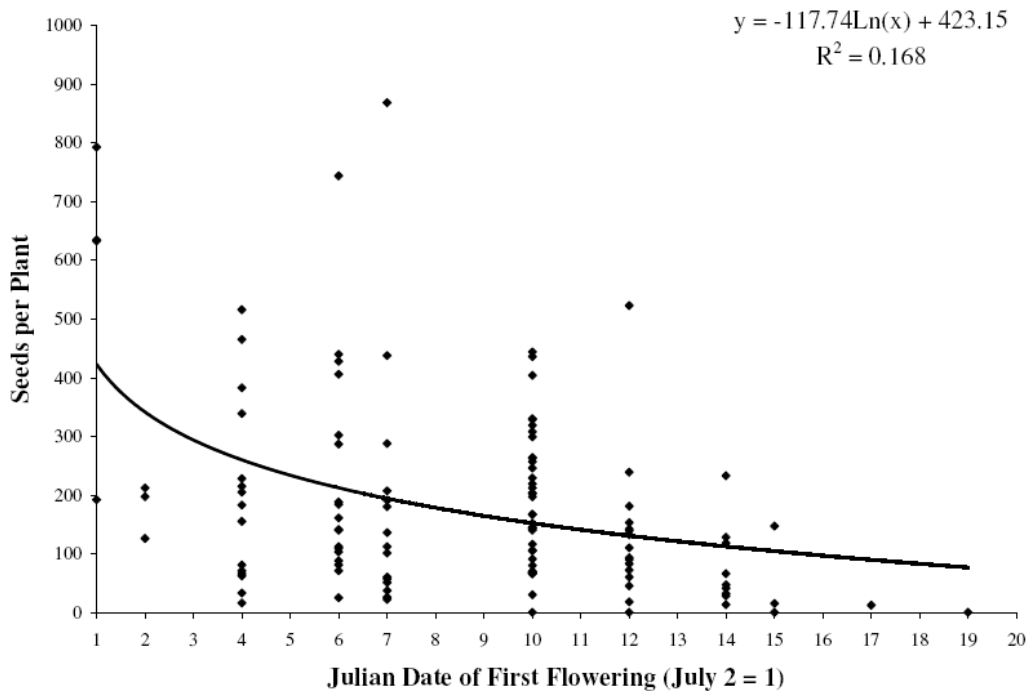


Figure 7: Effect of flowering date on seed set per plant. N = 125.

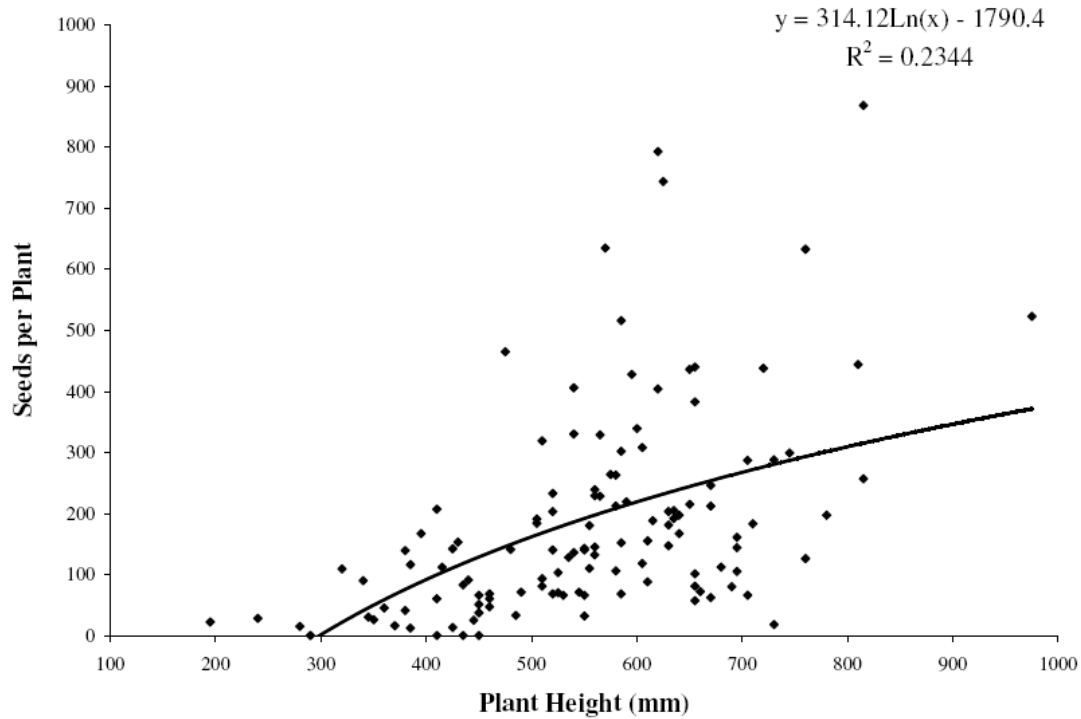


Figure 8: Effect of plant height on seed set per plant. N = 125.

effects on male fitness, but no effects on female fitness. Factors associated with increasing pollinator visitation may be more likely to affect male rather than female reproduction because male fitness is often pollinator limited, whereas female fitness is often both pollen and resource limited (Burd 1994). Furthermore, pollen limitation has been previously shown to limit tolerance to herbivory in *I. aggregata* (Juenger and Bergelson 1997, Sharaf and Price 2004), and may limit tolerance to nectar robbing via high nectar production as well.

As has been previously demonstrated (Irwin and Brody 1998, 1999, 2000), nectar robbing significantly reduced seed set per plant in *I. aggregata*. Robbing also reduced percent fruit set, which has been previously shown to decrease in response to nectar robbing (Irwin and Brody 1998, 1999; Irwin 2003). Robbing did not, however, reduce the

number of seeds produced per fruit (as in Irwin and Brody 1999, Irwin 2003). It is possible that a reduction in percent fruit set but not in seeds per fruit could be caused by “tasting” of robbed plants by hummingbird pollinators, in which hummingbirds visit flowers, realize they are unrewarding, and then move on (R. E. Irwin, *personal communication*). In this situation, hummingbirds may deposit enough pollen to saturate the stigma, either as a result of a single visit by one hummingbird or numerous visits by one or more hummingbirds. If stigmas are saturated on “tasted” flowers there would be little to no discernible difference in per flower seed set, but an overall decrease in flowers visited per plant in response to unrewarding flowers could result in a reduced percent fruit set. My results demonstrate that nectar robbing decreased the number of successful fruits and seeds produced, as both percent fruit set and seed set per plant were negatively

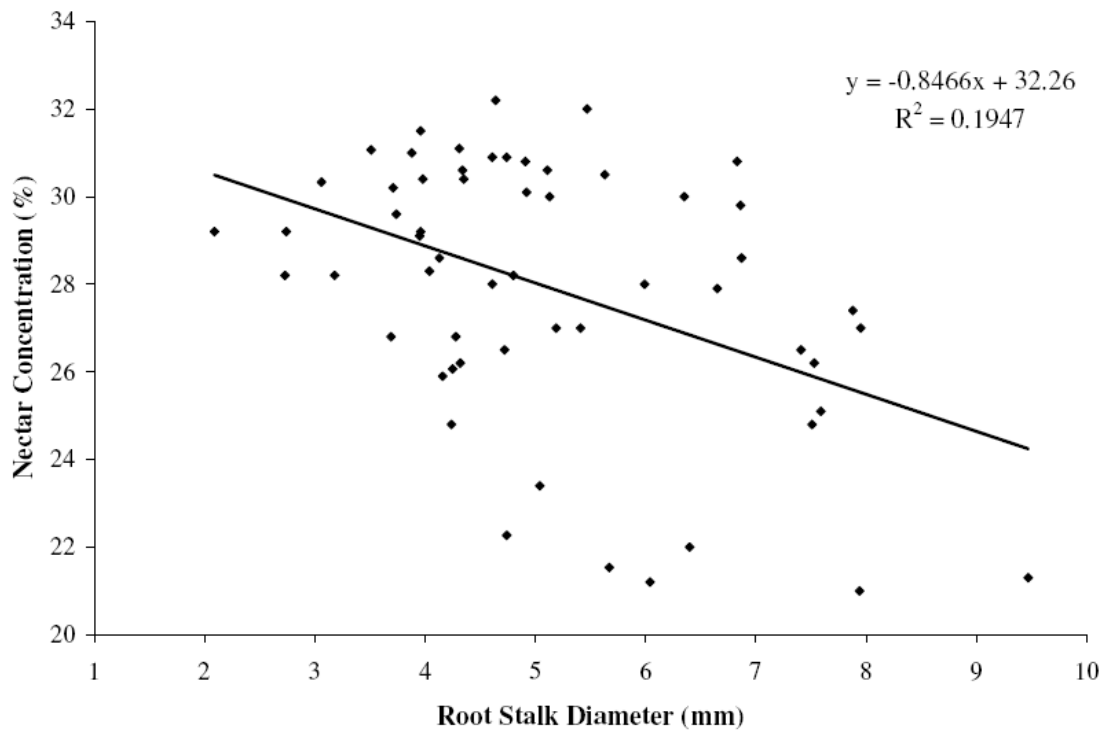


Figure 9: Effect of root stalk diameter (mm) on nectar concentration (%). N = 57.

correlated with robbing. Despite reductions of percent fruit set and seed set per plant, the number of seeds per fruit did not significantly vary between treatments, suggesting that the observed decrease in seed set per plant was directly caused by a reduction in percent fruit set and not by a reduction in the number of seeds per fruit.

Nectar robbing did not, as has been previously demonstrated (Irwin and Brody 1999), decrease pollen receipt. There was no significant difference in pollen grains received per stigma between pollen-supplemented plants and naturally pollinated controls. Although this data suggests that plants were not pollen limited at this site, there are two possibilities why this might not be a reasonable interpretation, given other data presented here. Stigmas were collected in the middle of the flowering season (mid-July), when both hummingbird pollinators are present (Waser 1978, Wolf and Hainsworth 1990) and may be at peak activity. High pollinator activity may cause flowers blooming in the middle of the season to experience little to no pollen limitation because increased pollinator visitation leads to increased pollen receipt (Engel and Irwin 2003, Price et al. 2005). This does not, however, mean that plants did not experience pollen limitation over the course of the flowering season. Additionally, I found that 81% of stigmas measured were either inserted (52%) or level with anthers (29%) in the corolla tube. Only 19% of stigmas were exerted, a floral characteristic that increases hummingbird efficiency of pollen deposition on *Ipomopsis aggregata* stigmas (Campbell 1989a, 1989b; Campbell et al. 1994). Due to observed high levels of inserted and level stigmas, and because these stigmas may receive more self-pollen (Price and Waser 1982, Waser and Price 1991), the results for pollen receipt may not represent an accurate estimation of between-plant pollen flow and may not reflect observed seed set.

Observed variation in stigma pollen loads was related to natural variation in nectar sugar concentration (Fig. 5). Stigma pollen loads were positively correlated with nectar sugar concentration, suggesting that pollinators were attracted to flowers containing nectar with higher concentrations of sucrose equivalents. These results, however, are in opposition to those presented by Irwin (2000), who found that increased nectar concentration had no significant affect on hummingbird visitation rate to *Ipomopsis aggregata*. While these results do suggest a connection between nectar and pollinator visitation, there is no demonstrable link between artificially increased nectar production and the female fitness estimates measured here (but see Lasso and Naranjo 2003). Mitchell (1993) found that pollinator visitation and success through male function increased significantly for *Ipomopsis aggregata* plants with both supplemented and naturally high nectar production rates, whereas female reproductive success had no relation to nectar production rate. I did not measure male fitness estimates in this study; therefore, possible significant effects of nectar addition may have been ignored. Mitchell's (1993) results demonstrate the importance of measuring both male and female fitness to obtain an accurate measure of total plant reproductive success (Zimmerman 1988).

Floral characteristics have been strongly correlated with pollinator visitation and reproductive fitness in previous studies (Campbell 1989b, 1991; Mitchell and Waser 1992; Mitchell 1993; Engel and Irwin 2003); some of these correlations are observed in this study as well. Taller plants and plants that began flowering earlier in the season had increased seed set per plant, perhaps due to larger, longer lasting floral displays (Figs. 7 and 8). Taller *I. aggregata* plants have been previously shown to receive significantly

more pollinator visits (Wolf and Hainsworth 1990, Engel and Irwin 2003) and flower number has been both positively and negatively associated with nectar concentration (Pleasants and Chaplin 1983, Campbell et al. 1991), a floral trait that significantly influences both pollinator visitation and pollen export (Mitchell and Waser 1992, Mitchell 1993). Flower number has also been positively correlated directly with pollinator visitation (Wolf and Hainsworth 1990) and pollen export and receipt (Campbell 1989b).

Nectar concentration was negatively correlated with per plant seed set, indicating that plants with higher nectar concentrations produced fewer seeds per plant (Fig. 6). Nectar concentration in *I. aggregata* has been previously shown to increase by as much as 10% in response to nectar robbing, due to evaporation of water from robber holes (Pleasants 1983, Irwin and Brody 1998). Robbing treatment did not, however, significantly affect nectar concentration in this study ($F = 0.1007$, $P = 0.7524$). A decrease in seed set in plants with high nectar concentration could be caused by a change in resource allocation to nectar rather than seed production or be the result of association. Nectar concentration was negatively correlated with root stalk diameter, suggesting that smaller plants produce flowers with the highest nectar concentrations (Fig. 9). Smaller plants will, by nature, produce fewer seeds per plant because they produce fewer flowers; therefore, the observed negative correlation between nectar concentration and plant size may inadvertently result in a negative correlation between nectar concentration and seeds per plant. The high concentration of nectar in smaller plants may be a method of compensation for smaller floral displays and/or low pollinator visibility. By producing more concentrated nectar, smaller plants may ensure that they are visited by pollinators,

which can be particularly important when plants are pollen limited. Alternatively, nectar concentration may increase as a result of water limitation (Kenoyer 1917). This idea is further supported by the positive correlation between nectar concentration and pollen receipt (Fig. 5). In years of severe pollen limitation, small plants may have equal or greater reproductive success compared to larger plants with lower nectar concentrations. To my knowledge, no studies have examined this relationship directly. Previous studies (Pleasants and Chaplin 1983, Zimmerman and Pyke 1986, Campbell et al. 1991) have found negative (in *Asclepias quadrifolia*), insignificant (in *Ipomopsis aggregata*), and positive (in *Ipomopsis aggregata*), correlations, respectively, between nectar production and both flower number (Pleasants and Chaplin 1983, Zimmerman and Pyke 1986, Campbell et al. 1991) and plant height (Pleasants and Chaplin 1983, Zimmerman and Pyke 1986), but nectar concentration was not measured in any of these studies.

The results presented here show that high nectar robbing was significantly related to a decrease in full and partial seed predation by *Hylemya* sp. larvae. As far as I know, this relationship has not been previously observed. It is unclear why robbing would decrease the prevalence of seed predation by *Hylemya* sp.; however, *Hylemya* may be more likely to oviposit on flowers with a higher probability of being pollinated, or may manipulate flowers in some way that ensures they set fruit or that ensures fruits acquire enough resources to set seed (Brody 1992b, Brody and Morita 2000). Egg-laying females may avoid plants with robbed flowers because they are less likely to set fruit or *Hylemya* eggs and/or larvae may be less successful on robbed plants. Alternatively, *Hylemya* sp. females may be directly or indirectly deterred by *Bombus occidentalis* in years when *I.*

aggregata is subject to robbing. The relationship between nectar robbing and pre-dispersal seed predation should be further explored in future studies.

This study reasserts the negative effects of nectar robbing on female fitness of *Ipomopsis aggregata*. The results presented here also suggest that nectar addition (as an analogue for high nectar production rate) does not mitigate the negative effects of robbing. One caveat of this study is the limited data for pollen receipt and the lack of data concerning the effects of nectar addition on male fitness and pollinator visitation. Future studies should expand their scope to examine the effects of nectar addition on both male and female fitness estimates and pollinator visitation at numerous sites and/or over several years. Tiffin (2000) suggests that simply comparing two groups (damaged and undamaged) is not sufficient to measure the expression of tolerance. Rather, the level of damage and tolerance must be measured on a scale, comparing varying levels of damage and tolerance traits (Juenger et al. 2000, Tiffin 2000). Future research should take into account varying levels of both damage and tolerance in order to consider the adaptive plasticity of tolerance traits (Juenger et al. 2000). Although the results presented here suggest that high nectar production rate does not confer tolerance to nectar robbing in *Ipomopsis aggregata*, there may be other unexplored pathways by which plants can mitigate, or even reverse, the negative effects of nectar robbing such as reallocation of resources to increase attractiveness of undamaged flowers, and the evolution of self-compatibility as a means of circumventing the indirect effects of nectar robbing on pollinator visitation.

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