

**FLORAL LARCENY:
IMPLICATIONS, RESISTANCE,
AND
THE POTENTIAL FOR TOLERANCE**

A DIVISION III THESIS

by

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PREFACE AND ACKNOWLEDGEMENTS

One year ago, this project did not yet exist for me. At that time it was merely a suggestion offered by my REU mentor, Becky Irwin, for an independent research project at the Rocky Mountain Biological Laboratory in Gothic, CO. When *Ipomopsis aggregata*, scarlet gilia, was just a plant I had never seen and nectar robbing was a phenomenon I had never before heard of, I had no idea exactly what this past year would become. I didn't know how hard the field work would be – or how rewarding. I didn't know how long and tedious counting seeds would be (and just when the field work had finally ended!). I didn't know the bite of a black fly or the taste of nectar or the crowd at ladies' night until I went to RMBL. And I certainly had no clue of what I was capable of. When I look at these two papers I feel so proud of what I have accomplished. I doubted myself many times – my strength, my intelligence, my creativity – but this work is a testament to what I have done and what I can do, and it's only the beginning.

First and foremost, I would like to thank my parents. Mom and Dad, without you I would be lost. Everywhere I have been, everything I have done, everything I have become is because of you. I love you. Becky Irwin, Larry Winship, and Charlene D'Avanzo – oh, how you have guided me through the murky waters of creating this, my monster, my masterpiece; it would not be what it is without you. Thank you for your knowledge, guidance, and patience. The Rocky Mountain Biological Laboratory and the National Science Foundation Research Experience for Undergraduates program – I had no idea what it meant to truly find a place that fits until RMBL. Thank you for providing the most gorgeous, stimulating working environment and for attracting such talented, exciting ecologists; I'm hooked. Mod 2 – you're the best I ever had; thanks for being the best family, keeping the best house, and not touching my computer. Emily Morris and Chris Muir – you're the greatest thing that RMBL has given me; I love you both so much. Nathan, my love – there's far too much to thank you for and not nearly enough with which to say it, so simple thanks will have to suffice. You know the rest and best never forget it. Lastly and vastly, I want to thank the thing that is Hampshire: this place and this idea. I came here merely by chance and I will never regret it. It was a perfect fit.

Part 1

Floral Larceny: Implications, Resistance, and the Potential for Tolerance

FLORAL LARCENY: IMPLICATIONS, RESISTANCE, AND THE POTENTIAL FOR TOLERANCE

INTRODUCTION

Plants and pollinators are abundant and complex: Buchmann and Nabhan (1996) estimate that Earth is home to 130,000-200,000 species of pollinators and some 200,000-300,000 types of flowering plants, many of which interact with numerous mutualists and antagonists (Strauss and Irwin 2004). Plants provide “rewards” to pollinators, mycorrhizae, seed dispersers and other mutualists in exchange for the services and rewards they offer. Plants have developed rewards such as nectar, Beltian bodies, and highly developed fruits to attract mutualists, but multi-species interactions are not two-dimensional. Antagonists are defined as those who obtain the benefits offered to mutualists without reciprocating (Bronstein 2001). Numerous mechanisms have been developed by plants to avoid damage and increase fitness in a world filled with mutualists and antagonists. Antagonists such as seed predators, florivores, and floral larcenists often place selective pressure on plants to reduce or modify rewards that may, in turn, affect the mutualisms that plants depend on. However, multi-species interactions are not black and white, and floral visitors do not always fit neatly into one category. As such, floral larcenists may have neutral or even positive effects on plant fitness, in contrast to the expected negative effects so commonly observed. In response to the selection pressures imposed by floral larcenists, plants have developed a wide range of resistance mechanisms, which will be reviewed here. In addition to mechanisms of resistance against floral larceny, the potential for tolerating floral larceny will also be explored.

FLORAL LARCENY

Floral larcenists are organisms that exploit plants by removing nectar from flowers without pollinating. Floral larcenists fit into two general categories: robbers and thieves (Inouye 1980). Nectar robbers remove nectar by piercing flowers and do not enter the flower directly. Nectar thieves enter flowers to retrieve nectar, but do not pollinate because of a morphological mismatch between the body shape of the nectar thief and the flower. Nectar robbing has been observed in at least 59 plant families (Irwin and Maloof 2002) and, along with nectar thievery, is likely to affect all nectar-producing angiosperms.

The effects of floral larceny are broad, ranging from negative to positive (Maloof and Inouye 2000). Positive effects can be direct, i.e. “accidental” pollination (Higashi et al. 1988, Waser 1979) or indirect, i.e. increased outcrossing (Maloof 2001, Maloof and Inouye 2000, Zimmerman and Cook 1985). Negative effects can also be direct, such as damage to reproductive structures (Galen 1983, McDade and Kinsman 1980, Traveset et al. 1998), or indirect, most notably by deterring pollinators either directly (Roubik 1982) or indirectly (Irwin and Brody 1998). The effects of pollinator avoidance of robbed flowers also range from negative to positive (Irwin et al. 2001). Irwin and Brody (1998, 1999, 2000) found that nectar robbing by the bumblebee *Bombus occidentalis* indirectly reduced male and female fitness of self-incompatible *Ipomopsis aggregata* by deterring the nectar-feeding hummingbird pollinators *Selasphorus platycercus* and *S. rufous*. Alternatively, nectar robbing may reduce geitonogamy (inter-plant pollen transfer) and increase pollen dispersal distance (Maloof 2001, Maloof and Inouye 2000). Although

floral larceny elicits a wide variety of plant responses, few of these have been studied in depth (Irwin et al. 2004).

Effects of Floral Larceny

Positive Effects

Positive effects of floral larceny can range from increased pollinator visitation of flowers (Richardson 2004, Wyatt 1980) and increased pollen dispersal distance (Maloof 2001, Maloof and Inouye 2000, Richardson 2004) to “accidental” pollination (Higashi et al. 1988, Waser 1979). In some cases floral larcenists may even act as primary pollinators, either as thieves or when they are not robbing (Graves 1982). Navarro (2000) examined the relationship between *Anthyllis vulneraria* subsp. *vulgaris* and its floral visitors. Both the primary pollinator, the long-tongued bee *Anthophora acervorum*, and two nectar-robbing bumblebees, *Bombus terrestris* and *B. jonellus*, comprised ~45% of floral visits. Although the incidence of robbing was high (66-77%), robbed flowers had a higher chance of setting fruit than unrobbed flowers (82% vs. 51%). Navarro concluded that higher fruit set in robbed flowers was the result of robbers making direct contact with the stigmas and anthers of flowers when they positioned themselves for robbing, thereby acting as successful pollinators. Such a classification might challenge the accepted role of nectar robbers; however, both nectar-robbing bees in this case did not enter the flower to remove nectar, thereby qualifying as nectar robbers.

Zimmerman and Cook (1985) experimentally manipulated nectar standing crops of *Impatiens capensis* flowers to simulate nectar robbing and observed the resulting behavior of the pollinating bee *Bombus vagans*. Low nectar standing crops caused *B.*

vagans individuals to fly longer distances in search of nectar rewards, expanding “neighborhood size” and increasing the distance that pollen was exported without decreasing seed set of experimentally robbed plants. Increased pollen export distance should result in greater genetic variability due to increased outcrossing, which may increase seed set and lead to improved seedling-survival rates (Fenster 1991). Although *B. vagans* individuals quickly left areas with unrewarding flowers to forage on nearby plants, seed set was not reduced and pollen dispersal distance increased, leading Zimmerman and Cook to conclude that nectar robbing of *Impatiens capensis* may actually increase reproductive fitness.

Hummingbird flower mites (Ascidae) consume the nectar and pollen of nearly 100 hummingbird-pollinated plant species (Colwell 1985). Mites travel in the beaks of hummingbirds as “stowaways” (Colwell 1985) between plants, where they then spend several days traveling between the flowers of an inflorescence and feeding. Lara and Ornelas (2001a) explored the effects of nectar theft by mites on seed set in hummingbird-pollinated *Moussonia deppeana* and found that mites reduced per flower nectar availability by up to 56% without significantly reducing seed set. The authors suggest that the decrease in nectar availability might cause hummingbirds to visit more flowers, thereby increasing pollen export and preventing a decrease in seed production. In addition, mites may act as secondary pollinators of the self-compatible *M. deppeana*, transferring some pollen as they move between flowers on an inflorescence. As a result, the effects of mites on *M. deppeana* may be considered positive.

Negative Effects

As discussed earlier, the negative effects of floral larceny are varied and can be either direct or indirect (Maloof and Inouye 2000). Robbers and thieves can damage floral structures (Galen 1983, McDade and Kinsman 1980, Traveset et al. 1998) or directly or indirectly deter pollinators (Irwin and Brody 1998, 1999, 2000; Reddy et al. 1992, Roubik 1982). Three bee genera (*Bombus*, *Trigona*, and *Xylocopa*) are known to rob floral nectar (Barrows 1976, Inouye 1983). Of these three genera, carpenter bees (*Xylocopa*) alone rob nectar from flowers belonging to at least 22 families (Barrows 1980). Irwin and Brody (1998, 1999, 2000) explored the effects of the nectar-robbing bee *Bombus occidentalis* on the hummingbird-pollinated *Ipomopsis aggregata*. High levels of nectar robbing (>80% of flowers robbed per plant) by *B. occidentalis* resulted in a 50% loss in both male and female reproductive success in *I. aggregata*, and attributed to a 23% reduction in hummingbird visits due to decreased nectar rewards (Irwin and Brody 2000).

Roubik (1982) observed interactions between hummingbird pollinators (*Phaethornis superciliosus*) and the nectar-robbing bee *Trigona ferricauda* visiting the tropical shrub *Pavonia dasypetala*. When bees robbing flowers were approached by hummingbird visitors they flew aggressively towards the birds, possibly attempting to bite them. Hummingbirds then moved within the patch, but rarely left. Patches with high levels of robbing (32-94% of flowers robbed) received fewer hummingbird visits per flower per hour and produced fewer seeds per patch. Within these patches, robbed and unrobbed flowers did not vary in average seed set, suggesting that any reduction in seed

production was caused by the direct effects of robbers on hummingbird visitation to patches.

In addition to altering interactions between plants and their pollinators, floral larcenists can physically damage flowers, potentially rendering them incapable of reproducing (Galen 1983, McDade and Kinsman 1980, Traveset et al. 1998). Galen (1983) observed the effects of the nectar-thieving ant *Formica neorufibarbus gelida* on *Polemonium viscosum*. Ants chew through the style and/or ovaries to reach nectaries in *P. viscosum* flowers, damaging a total of 25% of *P. viscosum* plants examined. Styles were usually damaged prior to fertilization, preventing any seed set in damaged flowers, and resulting in a seed set of 0.08 seeds per flower, compared to 5.18 seeds per undamaged flower. Galen suggested that the effects of nectar-thievery on pollinator visitation were irrelevant because destruction of female reproductive structures in thieved flowers made fertilization impossible, but noted that male reproductive success may be unaffected.

Neutral Effects

A number of recent studies have challenged the assumption that floral larcenists, who characteristically fail to participate in a mutualism with their host plant, must have negative effects on plant fitness (Maloof 2001, Morris 1996). The absence of a negative effect, however, does not imply that floral larcenists must positively affect a plant's reproductive success. In fact, some cases of floral larceny appear to have little to no effect on plant fitness (Arizmendi et al. 1996, Lasso and Naranjo 2003, Maloof 2001, Richardson 2004), whereas others exhibit both positive and negative effects that cancel

each other out (Wyatt 1980). Moreover, some floral larcenists act as both legitimate pollinators and nectar robbers at separate times (Dedek and Delaplane 2004, Morris 1996). Morris (1996) found that the bumblebees *Bombus mixtus* and *B. frigidus* act, at different times, as both pollinators and robbers of *Mertensia paniculata*. More than 80% of bumblebee visits were nectar robbing visits; however, flowers that were bagged to exclude bumblebees rarely set fruit. Morris attributed this to the observation that bumblebees rob flowers that they have previously visited as legitimate pollinators in response to changing floral rewards. Young *M. paniculata* flowers offer pollen as a reward, while older flowers offer nectar. Upon removal of older, nectar-containing flowers, Morris found that visitation to young flowers on the same plant was significantly reduced. Because bumblebees forage simultaneously for nectar and pollen, nectar production in older flowers may serve to attract pollinating bumblebees to young flowers.

In contrast to the changing identity of bumblebee pollinators/nectar robbers of *Mertensia paniculata*, nectar thieving ants alter the behavior of butterflies pollinating *Asclepias curassavica* by reducing nectar availability, resulting in more frequent, shorter floral visits and reduced pollinia insertion (Wyatt 1980). In this system, a decrease in nectar standing crop causes butterflies to visit more flowers, and may benefit the plant by increasing pollen dispersal distance and pollen export (Maloof and Inouye 2000, Zimmerman and Cook 1985). Nevertheless, inflorescences subjected to nectar thievery had reduced levels of pollinia insertion relative to inflorescences without ants, thereby neutralizing any positive effects of increased outcrossing.

Three additional studies (Arizmendi et al. 1996, Maloof 2000, Richardson 2004) report results demonstrating few to no effects of floral larcenists on pollinator behavior

and no effects of floral larcenists on plant reproductive fitness. These, along with the aforementioned studies examining the neutral or neutralized effects of floral larcenists, show the range of pollinator and plant responses to floral larceny and the potential for these responses to cancel each other out. Furthermore, these results remind us that multi-species interactions are complicated and entangled, such that clear-cut classifications are not always possible. This is certainly true of floral larceny.

RESISTANCE AND DEFENSE

Resistance traits are those that reduce damage due to, in this case, floral larceny. Similarly, defense traits reduce damage while increasing plant fitness. Resistance and defense have been widely studied in response to herbivory, and a number of non-mutually exclusive hypotheses have been formulated to explain energy investments in defense and different methods of defense. The plant apparency hypothesis (Feeny 1976, Rhoades and Cates 1976) states that plants and plant tissues that are longer lived, highly visible, or occur frequently are more apparent to antagonists and should therefore be highly defended. Under the assumptions of this hypothesis, flowers, many of which last for only a few days but are highly visible, could be defined as both minimally and highly apparent. Defenses invested in flowers would last for only a brief period of time but would be protecting a very valuable, highly visible investment. The optimal defense theory (McKey 1974) takes consideration of not only apparency, but of fitness value to the plant as well. This theory assumes that defenses are costly in terms of resources and that their distribution will therefore be dependent on the fitness value to the plant and

vulnerability to damage. This theory suggests that floral tissues would be highly defended because they are of great fitness value to plants and highly vulnerable to damage.

In contrast to the two theories discussed above, the resource availability hypothesis (Coley et al. 1985) posits that plant allocation to defense depends solely on the availability of resources and that plants growing at different rates will have varied types and amounts of defense. This theory might explain regional differences (if there are any) in type, amount, and quality of defense but does not address intraplant allocation of defense (i.e. allocation to flowers and/or leaves). Given that plant reproductive success relies heavily on floral display and success, it seems that floral parts would be extremely important to defend as suggested by the optimal defense theory. Indeed, just as plants have developed numerous defenses against herbivore damage, there are a wide range of mechanisms tailored to defending against floral larceny (Irwin et al. 2004).

Escape in Time/Space

Escape in time describes any avoidance of floral larceny based on time of flowering or nectar production (i.e. time of day, time of season, etc.). This mechanism is only beneficial if periods of peak activity for pollinators and floral antagonists differ or do not overlap completely. *Ipomopsis aggregata* individuals that flower early in the summer may avoid robbing by *Bombus occidentalis*, which emerges in mid-summer (Irwin and Maloof 2002). Those individuals that display most or all of their flowers before *B. occidentalis* individuals emerge would presumably escape the negative effects of robbing. Similarly, nectar production in *Castilleja linariaefolia* peaks in the morning and

late afternoon when hummingbird pollinators are most active and nectar robbers are not (Carpenter 1979).

Escape in time does not appear to require increased energy inputs and may actually benefit the plant energetically by reducing nectar production. Both nectar production and flowering time could be heritable traits that could eventually shift plants towards earlier flowering or periods of peak nectar production. More research in this area is necessary to determine how many plants utilize escape in time as a defense mechanism and how changes such as peak nectar production developed.

Escape in space involves the separation of plants and their antagonists by association with other plants. Plants that grow in the presence of host plants preferred by antagonists may avoid nectar robbing (Irwin et al. 2004). *Ipomopsis aggregata* co-occurring in populations with *Linaria vulgaris*, an introduced species that is preferred by *B. occidentalis*, were robbed 68% less than in the absence of *L. vulgaris* (Irwin et al. 2001). By growing alongside *L. vulgaris*, a plant that has become the preferred host for *B. occidentalis*, *I. aggregata* individuals are subject to fewer robber visits and have increased seed set (Irwin et al. 2001). Escape in space could be considered an effective and simple method for deterring floral larcenists where fitness is not reduced by changes in pollinator behavior due to the presence of floral larcenists.

Unlike the other forms of resistance I will discuss, escape in space requires no energy input from the plant and avoids the resource-based and ecological tradeoffs that are thought to be associated with defenses like toxic nectar. This type of “free” protection might seem desirable from the plant’s perspective, but it relies heavily on the fitness of the associate host plant. The fitness of the preferred host will presumably be reduced as

robbing increases (assuming it is pollen-limited and reproduces solely by seed). This decrease in fitness should lead to a drop in the numbers of the preferred host and levels of robbing in the plant escaping floral larcenists might increase once again. In addition, plants have little control over where they grow and with whom they grow. Therefore, escape in space is difficult to control because it is not heritable.

Tough Floral Parts and Physical Barriers

A. Kerner von Marilaun (1878) described a number of physical barriers that plants can use to exclude floral larcenists: sticky or slippery stems and flowers, hairs, and moats (citation in Guerrant and Fiedler 1981). Kerner von Marilaun's observations give us a sampling of the potential for creative defense mechanisms. While some of these have been studied (such as bract liquid moats; Wootton and Sun 1989) others are purely anecdotal and have not been examined for potential effects on defense and plant fitness. Other mechanisms not discussed above have been explored and will be discussed below.

Lara and Ornelas (2001b) examined the likelihood of robbing by two hummingbird robbers (*Lampornis amethystinus* and *Colibri thalassinus*) in relation to corolla length in three hummingbird pollinated plants (*Salvia mexicana*, *S. iodantha*, and *Ipomoea hederifolia*). The authors evaluated whether *L. amethystinus* and *C. thalassinus*, both short-billed species, were more likely to rob flowers with corollas too long for them to legitimately pollinate. Results demonstrated that both birds were more likely to rob both natural and artificial flowers with long corolla tubes, entirely avoiding robbing nectar from short-corolla flowers of both *S. mexicana* and *S. iodantha*, instead opting to legitimately pollinate short-corolla flowers. These findings suggest that short corollas

may be beneficial in deterring nectar robbing by short-billed or short-tongued species, in contrast to opposing selection pressures by long-billed specialist pollinators (Lara and Ornelas 2001b).

The most specialized and distinct physical barriers to nectar robbing are the moat-like liquid-filled bracts of *Heliconia wagneria*. Wootton and Sun (1989) removed liquid from bracts of *Heliconia wagneria* flowers and found that liquid contained in bract “moats” was secreted by plants. More floral damage (inflicted by unknown antagonists) was observed on plants that had bract liquid experimentally removed than those with remaining bract liquid. Nectar was robbed only from drained bracts, which experienced a three-fold increase in ovary damage. Roubik (1982) observed nectar robbing bees of the genus *Trigona* spending up to 30 minutes making a 2-4 mm diameter robbing hole in flowers of *Pavonia dasypetala*. These two examples of physical barriers may deter at least some potential nectar robbers. As flowers vary widely in form, much more research can and should be done in a wide variety of species that experience nectar robbing to determine the frequency of robber deterrence via physical structures.

Toxic Nectar and Floral Parts

Floral nectar is a rich resource offered by plants to attract pollinators. Nectar constituents vary, but are primarily composed of water and sugar. Concentration of nectar sucrose equivalents range from 7.3% to as much as 77% and average between 23.4% and 25.4% (for honeyeater- and hummingbird-pollinated species, respectively; Pyke and Waser 1981). Although nectar is thought primarily to consist of sugar and water, many other chemical compounds are found in nectar including alkaloids, amino acids, and

phenolic compounds (Baker and Baker 1977, Rhoades and Bergdahl 1981). Some of these compounds can provide necessary nutrients to pollinators, but others could potentially deter both floral larcenists and pollinators. Nectar that is toxic or deterrent has been reported in sixteen plant families (Adler 2000).

Adler and Irwin (2005) manipulated alkaloid levels in nectar of *Gelsemium sempervirens* to examine the effects of nectar alkaloids on pollinator and nectar robber visitation and male and female fitness. Nectar containing high levels of alkaloids did not typically have a significant effect on pollinator and nectar robber visitation and female fitness. Male fitness (estimated by pollen analog dye donation) of plants with high nectar alkaloids, however, was reduced by 33% to 50%. These results suggest that high nectar alkaloid levels are not beneficial in *G. sempervirens*, failing to reduce nectar robbing while decreasing male fitness.

Guerrant and Fiedler (1981) offered ants nectar from 25 species alongside sugar solutions to see if ants preferred nectar lacking secondary compounds. Phenolic compounds, alkaloids, and non-protein amino acids were present in the nectar of two, seven, and thirteen species, respectively. Of 23 ant species observed visiting the nectar and sugar arrays, only one species preferred sugar solution to natural nectar. In response to these initial findings another trial was conducted, comparing sugar solution with macerated petal tissue from seventeen species and plain sugar solution. Results from this trial showed that macerated petal tissue of nine plant species made sugar solutions significantly less palatable to ant visitors. These results are in opposition to those found by Feinsinger and Swarm (1978), who found that ants avoided nectar of two (of four) species, and suggest that floral nectar does deter ant visitors. It is indicated that damaged

floral tissue, however, seems to contain compounds that repel ants and might act as a defense against nectar robbing ant species.

Stephenson (1981) explored the potential for toxic nectar as a deterrent to floral larceny in *Catalpa speciosa*. Despite large displays of flowers with large corolla tubes and containing copious amounts of nectar, Stephenson observed that *C. speciosa* attracted few nectar thieves and rendered comatose skipper butterflies probing for nectar. In response to these observations, Stephenson offered *C. speciosa* nectar alongside sucrose solution to two potential nectar thieves, skipper butterflies (*Paanes hobomok*) and various ant visitors, recording length of visit and behavior after consumption. Results showed that ants drank significantly longer at sucrose solution than nectar and 27% of ants that did consume *C. speciosa* nectar fell from the tree after drinking. Results for skipper butterfly visits showed that skippers drank significantly more sucrose solution than nectar. In addition, no skipper butterflies re-rolled their proboscis after feeding on nectar, seven began to shake after drinking nectar, and four could not walk or fly and went comatose. No skipper butterflies consuming sucrose solution showed these symptoms. Stephenson's results are in contrast to those presented by Guerrant and Fiedler (1981) and demonstrate that nectar toxicity does occur. The results of Guerrant and Fiedler (1981), Feinsinger and Swarm (1978) and Stephenson (1981) taken together suggest that there is immense variation in levels of nectar toxicity and tolerance to compounds in nectar (Adler 2000).

TOLERANCE

The mechanisms of plant resistance to floral larceny have been thoroughly investigated and delineated. Mechanisms of coping with, or tolerating, floral larceny,

however, have been left unexplored (Irwin et al. 2004). Whereas resistance describes a plant's ability to reduce the damage and/or likelihood of an attack, tolerance refers to a plant's ability to reduce the fitness consequences following an attack (Weinig et al. 2003). Juenger and Bergelson (2000) suggest that tolerance should be looked at in the context of phenotypic plasticity, i.e. operational tolerance, where the slope of the line representing plant fitness in related damaged and undamaged states is indicative of the degree of tolerance exhibited. Under this model, low tolerance is indicated by a negative slope, absolute tolerance by a slope of zero, and overcompensating tolerance by a positive slope (Strauss and Agrawal 1999). Tolerance to herbivory can occur by a variety of mechanisms (as reviewed in Stowe et al. 2000, Tiffin 2000) such as increased branching following the release of apical dominance (Paige 1999, Paige and Whitham 1987, Weinig et al. 2003), increased photosynthetic activity, reallocation of carbon stores, and phenological delays (reviewed in Stowe et al. 2000, Strauss and Agrawal 1999, Tiffin 2000). Although methods of tolerating herbivore damage have been thoroughly researched, the potential for tolerating floral larceny has been ignored (Irwin et al. 2004).

Plants with high nectar production rate (NPR) may be able to tolerate the negative effects of nectar robbing (Barrows 1976, Irwin et al. 2004). Tolerance conferred by high NPR 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). However, nectar production can be costly (4 to 37% of a plant's daily photosynthate assimilation in *Asclepias syriaca*; Southwick 1984), using as much as twice the energy allocated to seed set (Southwick 1984). Therefore, high nectar production might only be beneficial to plants in which the increased allocation to nectar

production does not significantly reduce allocation to reproductive structures such as seeds (Pyke 1980). Barrows (1976) studied the effects of the nectar-robbing bee *Trigona fulviventris* on butterfly-pollinated *Lantana camara* and concluded that nectar robbing of this self-incompatible plant had no effect on pollinator behavior. Barrows attributed this to the evolution of high nectar production, suggesting that nectar levels of *L. camara* satisfy both pollinators and nectar robbers.

As discussed earlier (see section Effects of Floral Larceny: Negative Effects), nectar robbing of *Ipomopsis aggregata* by *Bombus occidentalis* reduces male and female fitness by 50% and decreases pollinator visitation by 23% in response to reduced nectar rewards (Irwin and Brody 2000). Increased nectar standing crops of *I. aggregata* could potentially satisfy pollinators and nectar robbers (in concert with Barrows 1976) if decreased plant fitness and pollinator visitation is indeed the result of decreased nectar availability (as suggested by Irwin and Brody 2000). This possibility is further supported by studies of pollen export, pollen receipt, and nectar production of *I. aggregata* (Engel and Irwin 2003, Mitchell 1993, Mitchell and Waser 1992, Price et al. 2005).

Engel and Irwin (2003) and Price et al. (2005) found that increased pollinator visitation to flowers of *I. aggregata* resulted in increased pollen receipt, and plants with increased pollen receipt had higher seed set (Engel and Irwin 2003). *Ipomopsis aggregata* flowers with high NPR (5 μ L per day) exported significantly more pollen analogues (dye particles) than those producing only 1 μ L of nectar, and pollen removal was significantly correlated with pollinator visitation, with pollen removal increasing as pollinator visitation went up (Mitchell 1993, Mitchell and Waser 1992). Pollinator visits to plants with high NPR were longer and more frequent, and high NPR individuals had

increased male reproductive fitness (Mitchell 1993, Mitchell and Waser 1992). Lasso and Naranjo (2003) found similar results in the nectar-robbed plant *Hamelia patens*, reporting increased pollen receipt for flowers with artificially increased nectar standing crops.

These results taken together suggest that plants with high NPR may benefit from increased pollinator visitation and male and female reproductive success. The enhanced fitness benefits of increased nectar production could confer tolerance to nectar robbing by providing nectar resources able to satisfy both pollinators and robbers. While these results are suggestive, a direct relationship between high NPR and nectar robbing has not yet been demonstrated. Alternate mechanisms of tolerance such as evolution of self-compatibility and the reallocation of resources from damaged to undamaged flowers should be considered for their potential to help plants cope with the damage inflicted by floral antagonists.

CONCLUSIONS AND AVENUES FOR FUTURE RESEARCH

Floral larceny is a widespread phenomenon (Irwin and Maloof 2002) that is discouraged by numerous defense mechanisms. Because floral larceny continues to be shaped by the species affected by it, future research should further explore the mechanisms used to deter floral larcenists and the effects of floral larceny in more species to determine the costs and benefits of defense on a wider scale. As previous research has shown, potential defenses such as secondary compounds in nectar and dilute nectar do not always deter robbers and thieves (Adler and Irwin 2005, Feinsinger and Swarm 1978, Guerrant and Fiedler 1981, Pyke and Waser 1981). Results from these studies suggest that robbers and thieves may be able to tolerate defenses against floral larcenists.

Similarly, plants that are visited by floral larcenists may have mechanisms to tolerate robbing such as high nectar production (Irwin et al. 2004).

Continued research of floral larceny and the mechanisms used to resist and, potentially, tolerate it should expand its base to include more species of plants, larcenists, and pollinators while using a few model systems to delve deeper into the costs and benefits of resistance and tolerance. In addition, the effects of resistance and tolerance on pollinators need to be explored. This area of floral larceny research has, to my knowledge, been largely ignored under the assumption that pollinators have evolved to tolerate or specialize on dilute and/or toxic nectar. We know little about the possible effects that defenses such as bract liquid or tough, nontransparent floral tissue may have on pollinator visitation. It is possible that some of the effects believed to be caused by floral larceny may actually be the result of pollinator avoidance of plant traits. All of these possibilities should be explored to expand our knowledge of floral larceny, tolerance, and multi-species interactions

Floral larceny encompasses a broad range of interactions, from truly antagonistic to seemingly mutualistic. Although many plants have evolved mechanisms to resist and defend themselves against the negative effects of floral larceny, these effects persist. Plants and their floral visitors continue to change and adapt in response to environmental changes such as introduced plant and insect species and water and resource availability. As the environment changes, new interactions will surely arise and those that are time-tested may change drastically. Mechanisms of tolerance provide an interesting perspective on these changes, as tolerance is not species or interaction specific. Plants that can tolerate herbivory or floral larceny by one species will most likely be able to

tolerate damage inflicted by any species. These possibilities provide an intriguing scene in which to explore the mechanisms of tolerance.

Part 2

A Case Study:

Nectar Robbing in *Ipomopsis aggregata*:
Does High Nectar Production Confer Tolerance?

NECTAR ROBBING IN *IPOMOPSIS AGGREGATA*: DOES HIGH NECTAR PRODUCTION CONFER TOLERANCE?

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

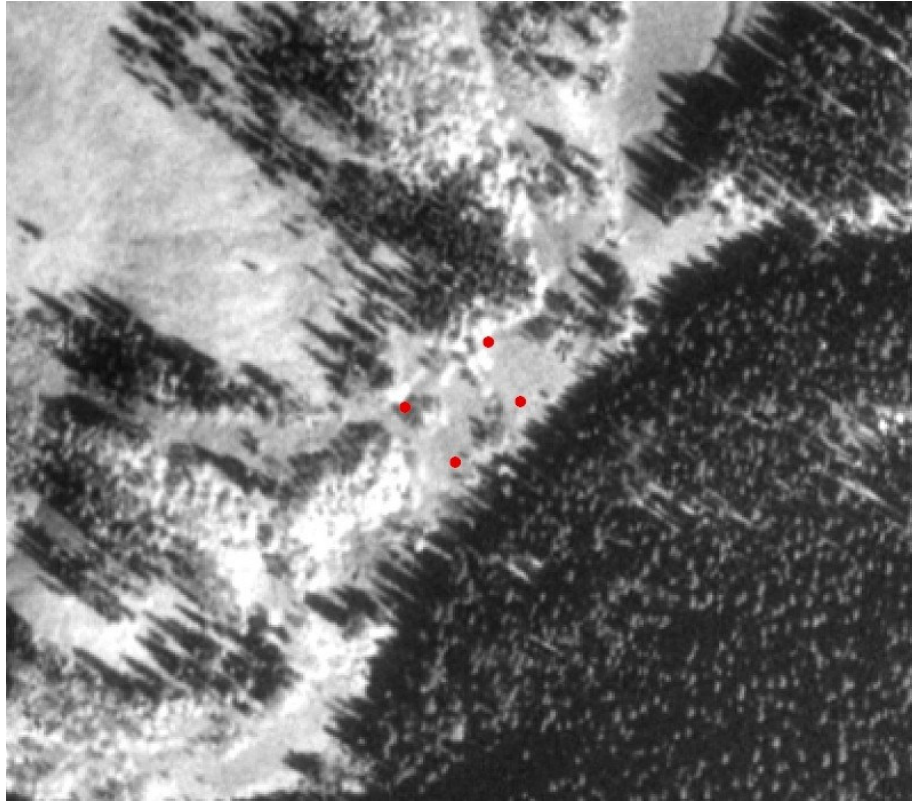


Figure 1: Copper Creek site.

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	<i>F</i>	<i>P</i>
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).

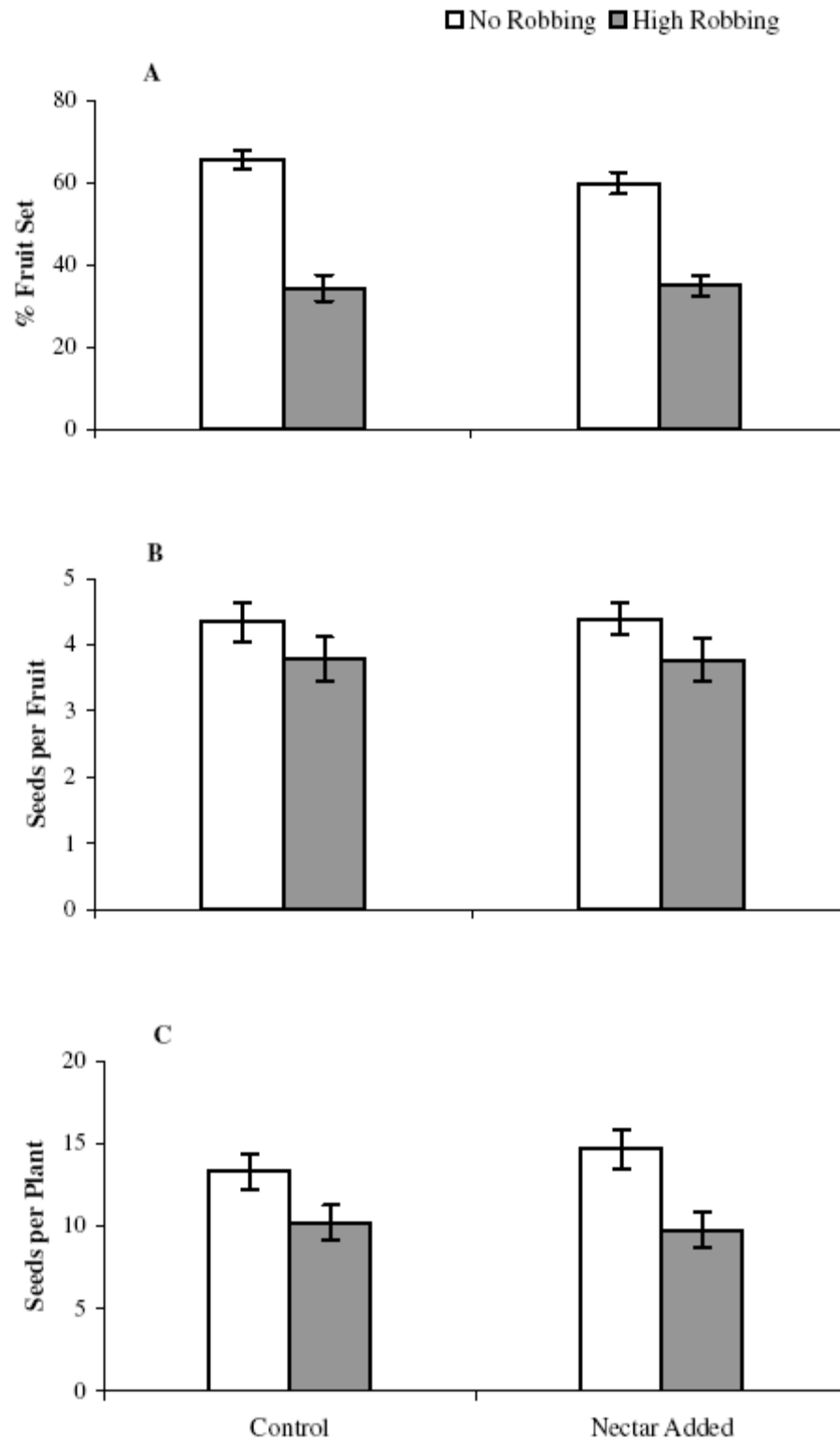


Figure 2: Effect of high robbing (>80%) on (a) percent fruit set, (b) seeds per fruit, and (c) seeds per plant (sq. rt. transformed). Bars are means ± 1 S.E.

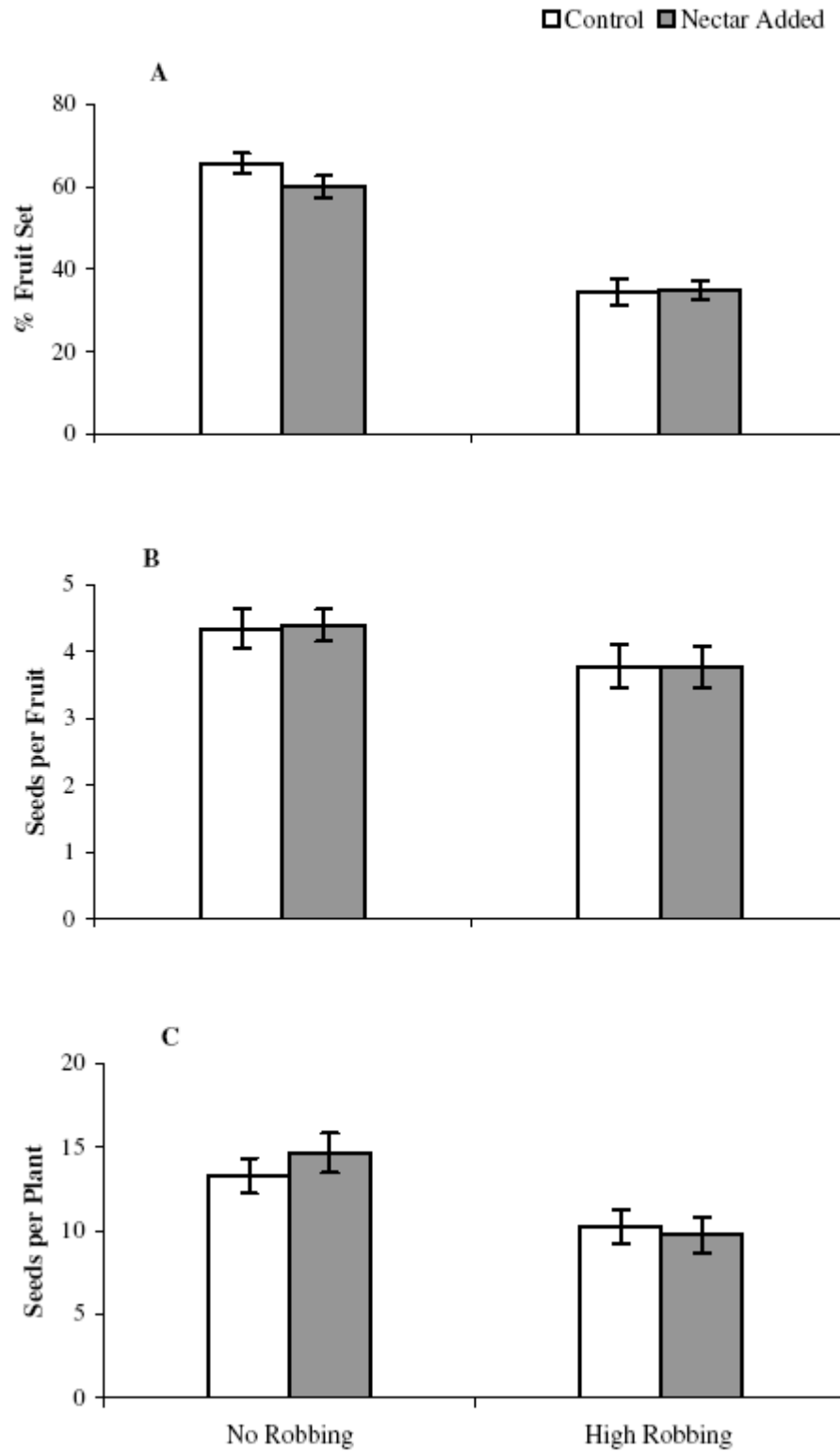


Figure 3: Effect of nectar addition on (a) percent fruit set, (b) seeds per fruit, and (c) seeds per plant (sq. rt. transformed). Bars are means $\pm$ 1 S.E.

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).

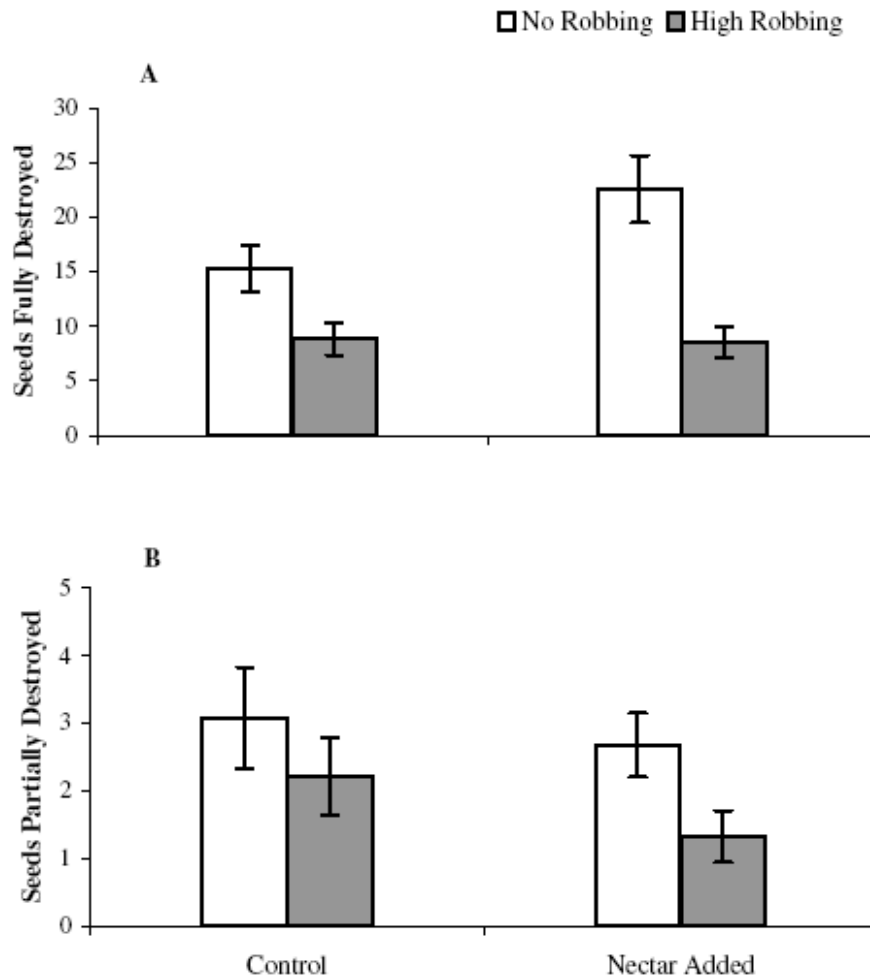


Figure 4: Effect of high robbing (>80%) on (a) fully destroyed seeds and (b) partially destroyed seeds. Bars are means $\pm$ 1 S.E.

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

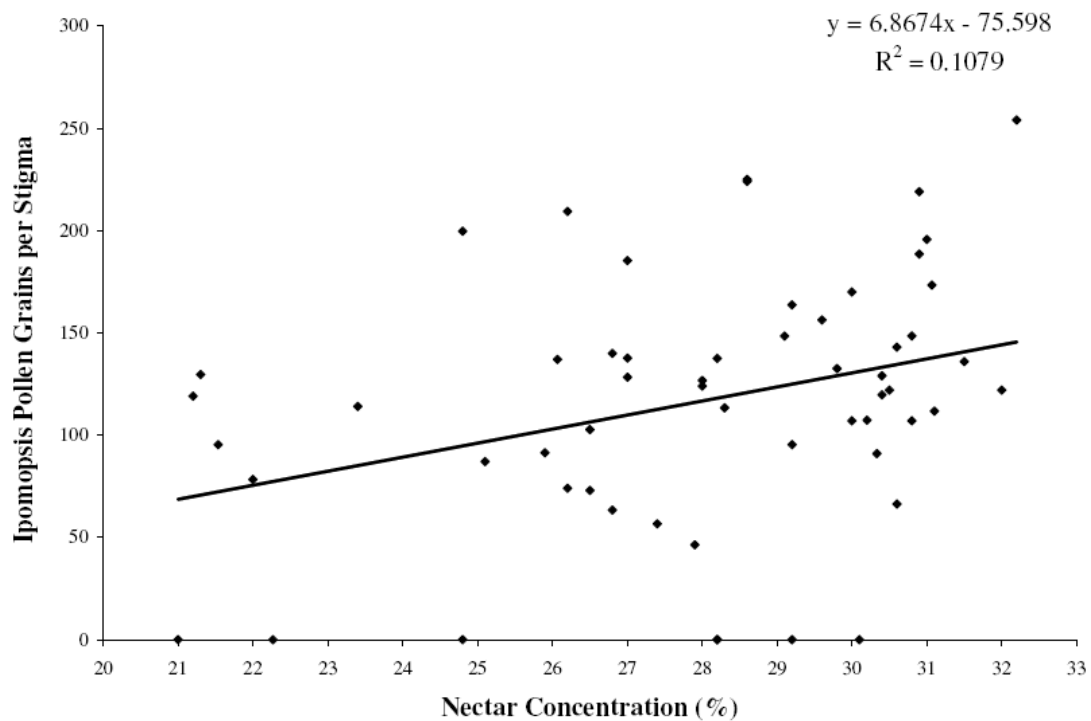


Figure 5: Effect of nectar concentration (%) on pollen receipt (*Ipomopsis* pollen grains per stigma). N = 57.

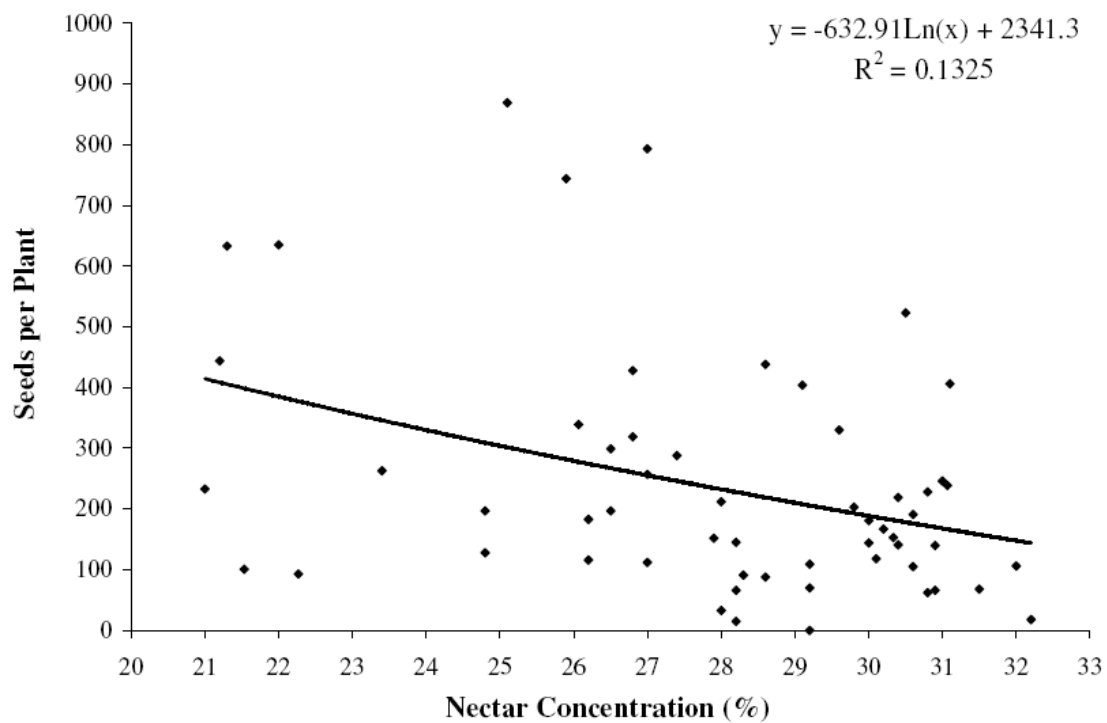


Figure 6: Effect of nectar concentration (%) on seed set per plant; N = 57.

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

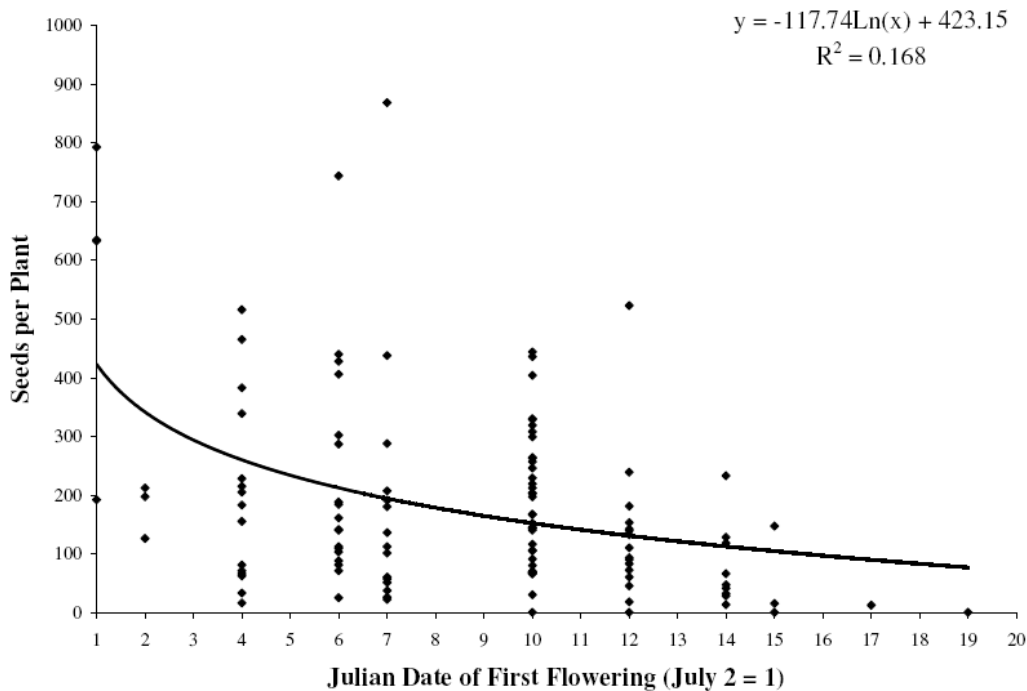


Figure 7: Effect of flowering date on seed set per plant. N = 125.

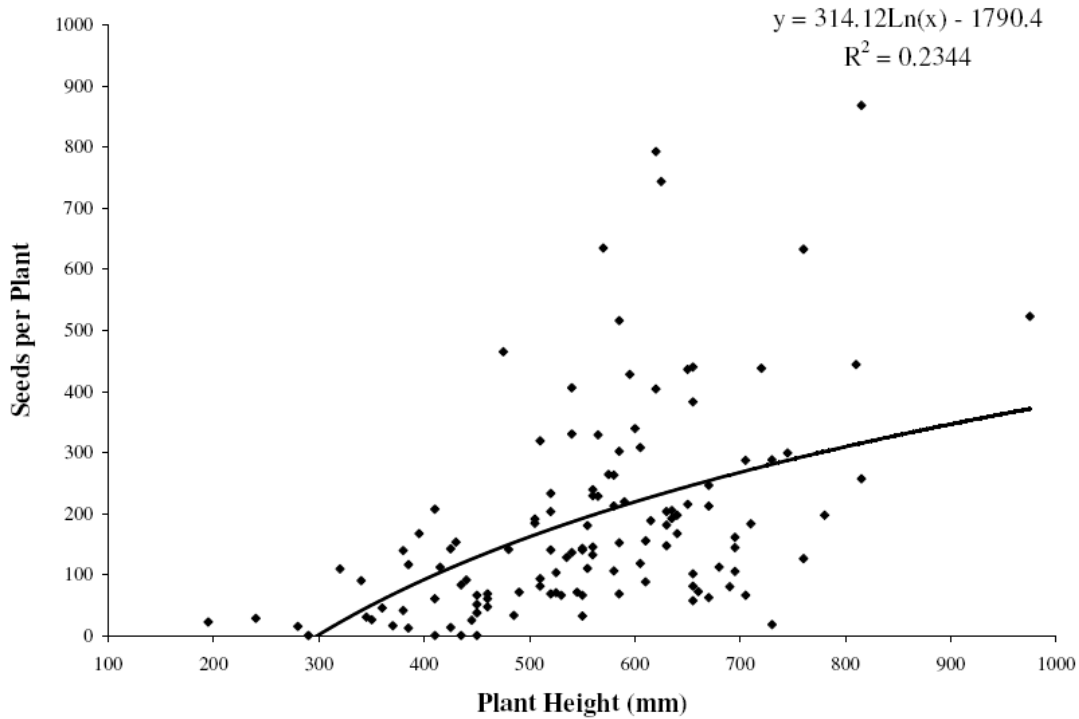


Figure 8: Effect of plant height on seed set per plant. N = 125.

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

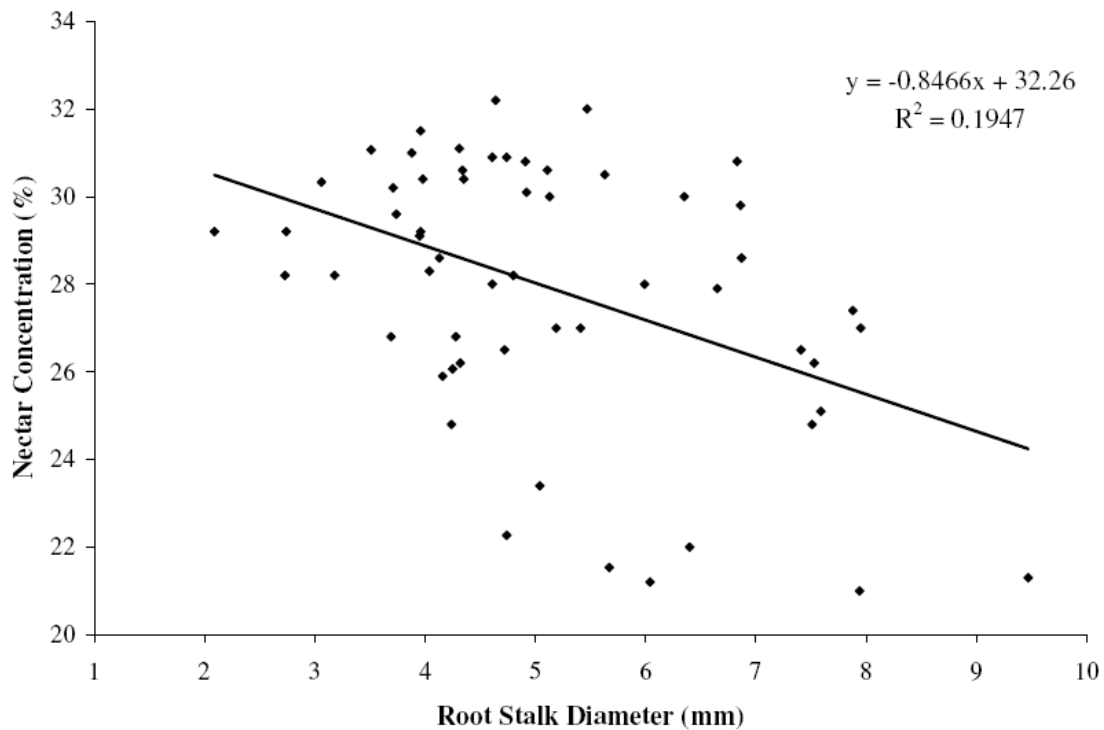


Figure 9: Effect of root stalk diameter (mm) on nectar concentration (%). N = 57.

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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