

**A Test of Sexual Dimorphism in *Valeriana edulis* Resistance and Induced Responses
to Herbivory**

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Advanced Independent Research

Summer 2010

Abstract

Dioecious plants have been observed to exhibit differences in growth rates and levels of herbivory defense between the sexes, with slower growing females investing more into constitutive defenses than fast-growing males. There are no predictions or empirical data, though, regarding differences in induced or indirect defense based upon plant sex. We tested for differences in levels of constitutive and inducible direct and indirect defense for male and female plants. We first measured the performance of an unidentified caterpillar species feeding on the inflorescences of male and female *Valeriana edulis* that either had or had not received previous damage by the same herbivore. After caterpillar pupation, we stocked damaged and undamaged plants with a constant density of the aphid *Aphis valerianae* and monitored population growth, rates of ant attendance and attack by natural enemies. Caterpillar pupa weights were lower on previously damaged plants than on previously undamaged plants, and the caterpillar mortality was significantly higher on male plants than on female plants. In addition, aphid colony sizes were larger on female plants, and ant tending occurred more frequently on female plants. Aphid predators visited damaged plants more frequently than undamaged plants. However, because there were no interactive effects of damaged and undamaged plants with male and female plants, *V. edulis* did not display any difference between males and females for induced or indirect defense.

Keywords: *Ant-aphid mutualism, plant dioecy, indirect defense, parasitoids, Valeriana edulis*

Introduction

Dioecious plants are species where individuals have separate sexes. Plant dioecy is common throughout angiosperms, occurring in 37 of 51 orders and composes over 10% of all angiosperm species (Renner and Feil 1993; Cornelissen and Stiling 2005). In dioecious species, male plants allocate less energy to reproduction than females that must produce flowers and seeds and, as a result, male plants tend to grow faster (Soule 1981; Cornelissen and Stiling 2005).

Plants with differing growth rates often have different constitutive defense levels. Slow-growing plants, such as females in dioecious species, are predicted to have greater investment in chemical and physical defenses against herbivores. In contrast, fast-growing plants, such as males, do not have as strong of defenses because they are able to re-grow and compensate for herbivore damage more easily (Coley *et al.* 1985). As a consequence of these trade-offs, male plants typically have more herbivore damage and are preferred by herbivores due to their higher nutrition (Coley *et al.* 1985; Mooney *et al.* 2010). As a result, there is often (but not always) male-biased herbivory in dioecious plant species (Cornelissen and Stiling 2005). As a result, dioecy results in plant sexual dimorphism due to different growth and herbivory rates (Cornelissen and Stiling 2005).

Because of the abovementioned differences in growth, indirect defense can be different for male and female plants. The plant's chemical resistance to herbivores may be different and it can therefore differentially influence the herbivore's susceptibility to predators and parasitoids (Mooney *et al.* 2010). Predators, in turn, can increase a plant's biomass indirectly by removing harmful herbivores from the plant (Mooney *et al.* 2010).

In addition to having direct and indirect defenses that are constitutive, such defenses can also be induced. An induced response is a plant's self-modification (phenotypic plasticity) after damage from an herbivore (Van Zandt and Agrawal 2004). These modifications can be plant traits that directly affect the herbivore, including physical changes like thorn production, or chemical changes such as increased toxin levels (Van Zandt and Agrawal 2004). Indirect defenses can also be induced (Van Zandt and Agrawal 2004). Accordingly, the performance of late season herbivores can be significantly influenced by early season feeders (Van Zandt and Agrawal 2004; Neuvonen *et al.* 2009). It is unknown if induced and indirect defenses, constitutive and induced, differ between the sexes of dioecious plants.

Direct and indirect plant defense can work in unison. The slow-growth / high-mortality hypothesis predicts that plant quality may mediate the interactions between predators and herbivores. Insects tend to grow more slowly on low nutritional quality plants, like females (Williams 1999). Slow growing herbivores are susceptible to greater rates of predation and parasitism because they are exposed to natural enemies for longer periods of time, resulting in high mortality (Williams 1999). At the same time, it is also possible that herbivores feeding on slow-growing plants might have fewer parasitoids because the herbivore has a low nutritional content for parasitoid eggs (Williams 1999). Herbivores would grow more slowly on female plants than on male plants, so the caterpillars on females should have low parasitoid rates (Damman 1987; Williams 1999).

Aphid colonies are often associated with ant species. Interactions between ants and aphids are often considered a mutualism because both partners are believed to benefit reciprocally (Ness *et al.* 2009). As aphids feed on their host plants, they secrete a

carbohydrate and amino acid-rich waste commonly called honeydew that ants feed on (Ness *et al.* 2009). The ants are believed to protect aphids from predators and parasitoids (Ness *et al.* 2009) as well as decrease fungal infection (Flatt and Weisser 2000). Several studies have established an increased aphid fecundity and growth in the presence of ant tending (Styrsky and Eubanks 2007). Because non-aphid herbivores sometimes have a stronger negative effect on the host plant than aphids, aphids may be less effective at recruiting ants if their performance is decreased by early-season herbivory on their host plants by the caterpillars (Van Zandt and Agrawal 2004; Styrsky and Eubanks 2007). Therefore, in plants affected by early-season herbivory, aphids may be less abundant because of their own lower growth rates and also due to less ant tending.

Because of differences between male and female plants and the resulting herbivory, the dioecious plant *Valeriana edulis* provides an excellent study system to compare induced and indirect defense for male and female plants. In addition there are early-season caterpillars and late-season aphids, tended by ants. Our study had several objectives. We tested for differences between male and female plants in constitutive defense against caterpillars. The rate of parasitoid emergence was used to determine if there is a difference in male and female indirect defense. Also, we investigated whether early-season caterpillar damage caused induced responses in the plant that affect late-season aphid performance and ant-aphid interactions.

Methods

Study site and organisms

The study was conducted in two open montane meadows near the Rocky Mountain Biological Laboratory in Gunnison County, Colorado (38° 57' 33" N, 106° 59' 21" W) at 2,900 m elevation. The study sites were adjacent to the Judd Falls parking area and just north of that meadow. Langenheim described the vegetation (1962).

The host plant, *V. edulis*, is a perennial herb with small yellow and white flowers on one or more open, branching inflorescences (Coulter 1909; Harrington 1967; Weber and Williams 2001). Each inflorescence has many flowers on it, and there can be several inflorescences per plant. *Valeriana edulis* is dioecious (Soule 1981). The *V. edulis* stems and leaves are the only host for large colonies of the aphid *Aphis valerianae* (Palmer 1952). *Aphis valerianae* has parthenogenic and wingless offspring in summer, and sexual winged offspring that disperse in fall (Palmer 1952). *Valeriana edulis* is also fed upon by several unidentified lepidopteran larvae (caterpillars) that range in size from about 5 mm to 20 mm and are light brown with a reddish-brown head. The caterpillars spin silk in the *V. edulis* inflorescences and leaves and leave light to dark brown frass near the silk.

Selection of experimental plants

A group of 19 male and 27 female *V. edulis* were selected that had no naturally occurring caterpillars in either the inflorescence or the basal leaves, designated as the caterpillar-free plants. We found an additional 64 similarly sized *V. edulis* that had 2-4 inflorescences with at least one caterpillar-free inflorescence (32 male, 32 female). None of the selected plants were colonized by aphids. Plant traits such as plant base diameter, length of longest leaf, width of widest leaf, and total number of leaves were measured as

co-variates. These traits did not affect the statistics significantly so they were not included in the results.

Experiment 1: Plant sex-based variation in caterpillar defense

The unidentified caterpillars were collected from the basal leaves and inflorescences of *V. edulis* in an adjacent field, weighed, and added to half of the caterpillar-free plants, and to half of the caterpillar-free inflorescences on plants with leaf damage and damage to other inflorescences. Caterpillars from male plants were placed on male plants, and caterpillars from female plants were placed on female plants. The caterpillars were placed within sealed mesh bags to prevent escape while also excluding ants, aphids, and enemies. The other plants were without caterpillars but the same bags were placed over inflorescences as a control. Therefore, there were eight treatments with a 3-way factorial design (Fig 1). For both males and females, there were previously damaged plants that received an experimental caterpillar (Male N= 16, Female N= 19) and those that did not (Male N= 16, Female N= 13), and for previously undamaged plants there were those that received caterpillars (Male N= 10, Female N= 13) and those that did not (Male N= 9, Female N= 14).

Because the caterpillars were collected after they had completed a significant portion of their development, there was sufficient time for some parasitoids to have laid eggs before the caterpillars were collected and bagged. All caterpillars were monitored in the field for parasitoids, pupation date, and mortality. As caterpillars pupated they were collected, weighed, and reared in the lab in Petri dishes by providing a moist paper towel, and monitoring their time until a moth or parasitoid emerged.

The differences in constitutive and induced direct defense and in indirect defense by parasitoids were determined for male and female plants. We tested for the individual and interactive effects of plant sex and previous damage on pupal mass and days to pupation, including caterpillar initial mass as a covariate in an analysis of covariance. Model terms with $P > 0.15$ were removed, and we present the results for this reduced model. The rates of parasitoid infection and caterpillar mortality were analyzed by chi-square contingency test. Parasitoid infection was analyzed as a function of whether caterpillars were collected from male or female plants, and mortality was analyzed as a function of both plant sex and previous plant damage. All analyses were performed with SAS 9.2 (SAS Institute, 2008). A chi-square test of independence on a 3-way contingency table was used to compare mortality rates among treatments. In addition, a multi-factor ANOVA was performed, using the treatments of undamaged or damaged as well as male or female. Initial caterpillar mass was used as a co-variate, and interactions between treatment variables were included. Then the interaction and covariates greater than a significance of $P = 0.15$ were removed from the analysis to achieve a reduced statistical model using SAS. When comparing final pupa weight between damaged and undamaged plants we used a one-tailed t-test because we predicted that pupa weights would be lower on damaged plants.

Experiment 2: Plant sex-based variation in aphid defense

Experiment 2 was based upon the same set of plants used in Experiment 1. Once the caterpillars pupated, aphids were added to the bagged inflorescences on every plant at a density of 10-15 aphids and monitored every three days over an 8 to 12-day period.

The bags were removed and then any predators, parasitoids, or ants found on the plant were noted in the morning and afternoon for two days.

Aphid performance was analyzed as a function of plant sex and the presence previous caterpillar feeding. First, we tested for the individual and interactive effects of plant sex and previous damage on aphid population size at the conclusion of 10 days growth on bagged inflorescences, including the initial number of aphids as a covariate in an analysis of covariance. Second, after removing bags, we tested for the individual and interactive effects of plant sex and previous damage on recruitment of natural enemies and tending ants, using aphid abundance at the time of bag removal as a covariate. As described above, terms with $P > 0.15$ were removed and all analyses were performed with SAS 9.2 (SAS Institute, 2008). General linear model least square means were calculated for ant tending, accounting for aphid abundance.

Results

Experiment 1

There were 92 caterpillars initially weighed and bagged on plants. One caterpillar died during processing, and 11 escaped from the mesh bagging (3 male, 8 female) so they were not included in the data analysis.

Mortality among caterpillars on male and female plants was significantly different ($\chi^2_1 = 5.107$, $P = 0.0238$). Of 37 caterpillars on male plants, 8 pupated (21.6%) and 26 died (70.3%). Of 52 caterpillars on female plants, 20 pupated (38.5%) and 24 died (46.1%). The mortality among caterpillars on damaged and undamaged plants was not significantly different ($\chi^2_1 = 0.0363$, $P = 0.55$). There were 44 caterpillars on damaged

plants and 18 of those pupated (40.9%) and 26 died (59.1%). There were 33 caterpillars on undamaged plants; 10 pupated (30.3%) and 23 died (69.7%). Using a 3-way contingency table with log-linear analysis to compare the interaction between male/female mortality and damaged/undamaged mortality did not produce a significant interaction ($G_4 = 6.14$, $P = 0.19$) (FIG 2a). Therefore the effect of plant sex on caterpillar mortality did not differ between damaged and undamaged plants.

For pupa mass, the interaction between plant sex and previous damage was not significant ($F_{1,24} = 0.53$, $P = 0.47$) so it was removed from the analysis. Similarly, plant sex had no significant affect on pupa mass ($F_{1,25} = 0.00$, $P = 0.97$) and was also removed from the analysis. The effect of previous damage had a marginally significant effect on pupal mass with a two-tailed test ($F_{1,26} = 3.33$, $P = 0.08$), but the effect of previous damage becomes significant ($P = 0.04$) under a one-tailed test based on our hypothesis that previous damage should decrease caterpillar fitness. The final pupa weight on damaged plants was 0.025 ± 0.002 and the final pupa weight on undamaged plants was 0.031 ± 0.002 (FIG 2b).

Days until pupation were not affected by the interaction between plant sex and previous damage ($F_{1,23} = 1.31$, $p = 0.26$) so it was removed from the analysis. We then found that plant sex alone did not have a significant affect on development time ($F_{1,24} = 0.81$, $p = 0.38$), and neither did the previous damage ($F_{1,24} = 0.25$, $p = 0.62$). The initial caterpillar mass was significant as a co-variate ($F_{1,24} = 20.58$, $p = 0.0001$). Across all caterpillars, the mean $\pm$ 1SE development time was 13.32 ± 1.36 (FIG 2c).

No parasitoids emerged from caterpillars in the field, and moths and parasitoids began to emerge when the collected pupa were stored in Petri dishes. Only 5.0% of

caterpillars from male plants had parasitoids, and 9.7% of caterpillars from female plants had parasitoids. There was no significant difference ($\chi^2_1 = 1.622$, $P = 0.80$)

Experiment 2

The aphid colony size per stem was not affected by plant damage ($F_{1,117} = 0.01$, $P = 0.93$), or the interaction between plant sex and previous damage ($F_{1,117} = 0.67$, $P = 0.42$) but initial aphid colony size was significant as a co-variate ($F_{1,117} = 24.13$, $p < 0.0001$). There was a significant difference in male and female aphid colony sizes just before removing the bagging ($F_{4,117} = 7.06$, $p < 0.0001$) (FIG 3a).

The number of ants per stem was not affected by previous damage ($F_{1,118} = 0.96$, $P = 0.33$) or the interaction between plant sex and the damage treatment ($F_{1,118} = 0.38$, $P = 0.54$), but the aphid colony size was significant as a co-variate ($F_{1,118} = 10.89$, $P = 0.0013$). There was a significant difference in ants per stem between male and female plants ($F_{4,118} = 6.38$, $P = 0.0001$) (FIG 3b). There were 116% more ants on female stems than on male stems, and without using the least square means to account for aphid abundance there were 167% more ants on female stems than male stems.

The number of predators per plant was not affected by plant sex ($F_{1,119} = 0.82$, $P = 0.37$), or the interaction between plant sex and the damage treatment ($F_{1,119} = 0.55$, $P = 0.46$), although there were significantly different numbers of predators on damaged and undamaged plants ($F_{3,119} = 3.68$, $P = 0.014$) (FIG 3c).

After exposure to predators and ants, aphid colony sizes changed. The number of aphids per stem was not affected by previous damage ($F_{1,118} = 1.65$, $P = 0.2016$) or the interaction between plant sex and previous damage ($F_{1,118} = 0.14$, $P = 0.71$) but the

previous colony size was a significant co-variate ($F_{1,118} = 14.77$, $P = 0.0002$). There was a significant difference between male and female colony sizes ($F_{4,118} = 5.11$, $P = 0.0008$).

Discussion

Overall, we found several differences between previously damaged plants and undamaged plants, as well as differences between male and female plants. Where effects of plant sex and previous damage occurred, there were no interactive effects so male and female plants do not have different induced or indirect responses to caterpillar damage or aphid damage. For caterpillars, pupa masses on damaged plants were lower than pupa masses on undamaged plants. Also, predator recruitment to aphids was significantly more common on damaged plants than on undamaged plants. These differences were uniform across all damaged and undamaged plants. There was significantly higher caterpillar mortality on male plants than on female plants. Parasitoid emergence from pupa was low, but there were slightly more parasitoids on female plants. In addition, aphid abundance, and ant tending corrected for aphid abundance, were both significantly higher on female plants than on male plants. Again, these differences were uniform across all male plants and all female plants. Some, but not all, measurements of caterpillar fitness and aphid performance were affected by plant sex or damage.

There was a difference in pupa masses on damaged and undamaged plants, with previously undamaged plants having larger pupa than damaged ones. Smaller pupa on damaged plants may be because of a difference in induced chemical responses from earlier herbivore damage, causing larva on previously damaged plants to grow more slowly (Van Zandt and Agrawal 2004; Neuvonen *et al.* 2009). Damaged plants may

allocate more energy into chemical defense and less energy into vegetative growth, so herbivores feeding on damaged plants for the same approximate time as herbivores feeding on undamaged plants would grow less.

In addition, there was a significant difference in mortality rates on male and female plants. There was higher mortality on male plants, contrary to our prediction that female plants would have higher herbivore mortality from their slow growth time with lower plant nutrition (Williams 1999). Although not statistically significant, there were a greater proportion of deaths from parasitoids on female plants than on male plants. The plant quality, nutritionally or structurally, may vary enough to cause differences in mortality rates. The male plants have a more open inflorescence structure, which may have left the caterpillars more exposed as they attempted to pupate, leaving them open to the elements and sub-freezing temperatures at night. In addition, *V. edulis* plants may be an exception to theory and previous empirical studies because male plants may be more toxic or less nutritious than female plants.

Parasitoids are common in many insect larvae. Male plants had only 5.0% parasitoids emerging from pupa, and female plants had a 9.7% parasitoid emergence rate. The effects of bagging caterpillars on plants before they pupated may contribute to the low parasitoid rate. In nature, there may be more parasitoids that lay eggs during the later stages of the caterpillar's development so we may have missed these later parasitoids.

Female plants had significantly larger aphid colonies than male plants in opposition to the pattern shown from past work on chewing-herbivores (Cornelissen and Stiling 2005). However, the jasmonic acid pathway may have influenced our results. Jasmonic acid is a plant hormone induced in plants by damage from chewing herbivores

like caterpillars. In contrast, sap-sucking herbivores like aphids induce the plant hormone salicylic acid (Traw and Bergelson 2003). These pathways can interfere with one another, such that when the jasmonic acid pathway is induced (e.g. from caterpillar feeding) a plant cannot easily induce defense to sap-feeding herbivores (e.g. aphids) mediated by salicylic acid (Traw and Bergelson 2003). Because there was greater caterpillar mortality on male plants, possibly from lower nutrition or stronger chemical defense, it is possible that female plants had greater amounts of damage from the caterpillars. Therefore, female plants may have a strong response to the caterpillars and begin producing mass quantities of jasmonic acid, and may not be able to switch as easily as male plants to deter aphids with salicylic acid.

High ant attendance indicates a healthy aphid population, able to attract their mutualists by producing quality and quantity honeydew, which in turn indicates a plant with fewer chemical defenses and high nutrition. There were significantly more ants on female plants, which would indicate a faster growing plant with less chemical defense, opposite of our prediction.

Plants with higher predator visitation may have more effective indirect responses to herbivory by recruiting predators for the herbivores. Although there were no differences between male and female plants, predator visitation increased as plant damage increased. Research by Turlings *et al.* suggests that corn seedlings produce large amounts of terpenoid volatiles when fed upon by leaf-chewing caterpillars, and these volatiles attract parasitic wasps (1990). Therefore, greater caterpillar herbivory may produce greater quantities of volatiles and attract more predators once the aphid colonies

are established. Therefore, damaged inflorescences from chewing herbivores may have stronger chemical cues that recruit predators to sapsuckers.

There are many factors involved in herbivore mortality, herbivore growth, herbivore abundance and performance, as well as predator and parasitoid recruitment. Plant sex and the plant's damage both influence some of these factors, but not all. In all metrics of caterpillar fitness and aphid performance, because there were no interactive effects, male and female plants did not have different induced or indirect responses to either chewing or sapsucker herbivory.

Acknowledgements

Special thanks to Dr. Kailen Mooney for support and guidance throughout this project. Field assistance was provided by Mitchell Lopez and William K. Petry. This research was supported by an NSF REU Supplemental Grant. Laboratory space and housing were provided at the Rocky Mountain Biological Laboratory, Gothic, Colorado, USA.

Literature Cited

- Coley, P. D., J. P. Bryant, F. S. Chapin III. 1985. Resource availability and plant antiherbivore defense. *Science* 230(4728): 895-899.
- Cornelissen, T. and P. Stiling. 2005. Sex-biased herbivory: a meta-analysis of the effects of gender on plant-herbivore interactions. *Oikos* 111: 488-500.
- Coulter, J. M. 1909. *Valerianaceae* (Valerian Family). Page 475 in A. Nelson, editor. *New Manual of Botany of the Central Rocky Mountains (Vascular Plants)*. American Book Company, New York City, New York, U.S.A.

- Damman, H. 1987. Leaf quality and enemy avoidance by the larvae of a pyralid moth. *Ecology* 68(1): 88-97.
- Flatt, T., and W. W. Weisser. 2000. The effects of mutualistic ants on aphid life history traits. *Ecology* 81(12): 3522-3529.
- Harrington, H. D. 1967. Roots and Underground Parts: *Valeriana edulis*. Pages 225-227 in H. D. Harrington, editor. *Edible Native Plants of the Rocky Mountains*. The University of New Mexico Press, Albuquerque, New Mexico, U.S.A.
- Langenheim, J. H. 1962. Vegetation and environmental patterns in the Crested Butte area, Gunnison County, Colorado. *Ecological Monograph* 32: 249-285.
- Mooney, K. A., R. Halitschke, A. Kessler, and A. A. Agrawal. 2010. Evolutionary trade-offs in plants mediate the strength of trophic cascades. *Science* 327: 1642-1644.
- Ness, J., K. Mooney, L. Lach. 2009. Ants as mutualists. Pages 97-114 in L. Lach, C. L. Parr, and K. L. Abbott, editors. *Ant Ecology*. Oxford University Press, Oxford, England.
- Neuvonen, S., S. Hanhmarki, J. Suomela, and E. Haukioja. 2009. Early season damage to birch foliage affects the performance of a late season herbivore. *Journal of Applied Entomology* 105(1-5): 182-189.
- Palmer, M. A. 1952. Subfamily *Aphinae*: Tribe *Aphini*: *Macrosiphum valerianae* (Clarke). Pages 329-330 in M. A. Palmer, editor. *Aphids of the Rocky Mountain Region* (vol. 5). The A. B. Hirschfeld Press, Denver, Colorado, U.S.A.
- Renner, S.S., and J. P. Feil. 1993. Pollinators of tropical dioecious angiosperms. *American Journal of Botany* 80(9): 1100-1107.

- Soule, J. D. 1981. Ecological consequences of dioecism in plants: A case study of sex differences, sex ratios and population dynamics of *Valeriana edulis* Nutt. Ph.D. dissertation, Michigan State University, East Lansing.
- Styrsky, J. D., and M. D. Eubanks. 2007. Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of the Royal Society B* 274: 151-164.
- Traw, M. B., and J. Bergelson. 2003. Interactive effects of jasmonic acid, salicylic acid, and gibberellin on induction of trichomes in *Arabidopsis*. *Plant Physiology* 133: 1367-1375.
- Turlings, T. C. J., J. H. Tumlinson, and W. J. Lewis. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250: 1251-1253.
- Van Zandt, P. A., and A. A. Agrawal. 2004. Community-wide impacts of herbivore-induced plant responses in milkweed (*Asclepias syriaca*). *Ecology* 85(9): 2616-2629.
- Weber, W. A., and R. C. Wittmann. 2001. Valerianaceae. Page 339 in W. A. Weber and R. C. Wittmann. *Colorado Flora: Western Slope* (3rd ed.). The University Press of Colorado, Boulder, Colorado, U.S.A.
- Williams, I. S. 1999. Slow-growth, high-mortality—a general hypothesis, or is it? *Ecological Entomology* 24: 490-495.

Figures

		Males and Females	
		Addition of caterpillars?	
		Y	N
Pre-existing damage?	Y	35	29
	N	23	23

FIGURE 1. Experimental design. There were 8 treatments, based on pre-existing damage, the addition of caterpillars, and the plant sex. After the caterpillars pupated, aphids were added to all four treatments.

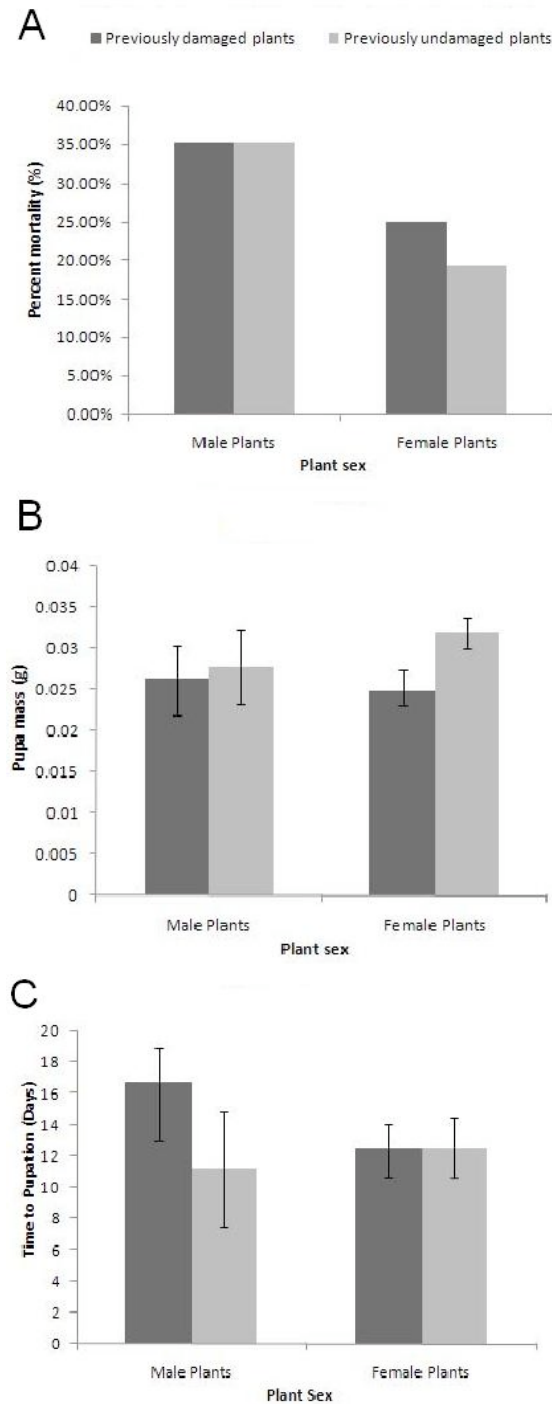


FIGURE 2. Bars represent 1SE. (a) Percent mortality of caterpillars for all treatments. There was a significant difference between male and female plants. (b) The final pupa masses for all treatments. There was a significant difference between all previously undamaged plants and all previously damaged plants. (c) The time to pupation for all treatments. The times are based on the general linear model least squared means, accounting for initial caterpillar mass. There was no significant difference.

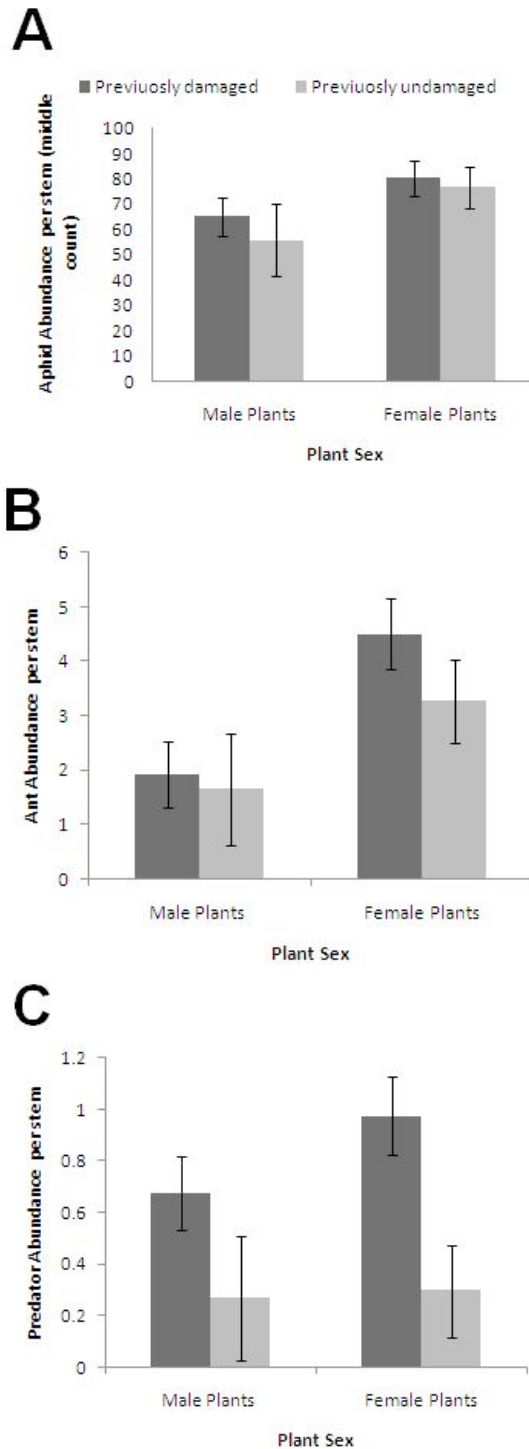


FIGURE 3. Bars represent 1SE. (a) Average aphid abundance per stem. There was a significant difference between male and female plants but not between damaged and undamaged plants. (b) General linear model least square mean ant abundance per stem. There was a significant difference between male and female plants but not between damaged and undamaged plants. (c) Average predator visitation per stem. There was a significant difference between damaged and undamaged plants but not between male and female plants.