

# Attracting pollinators and avoiding herbivores: insects influence plant traits within and across years

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**Abstract** Perennial plants interact with herbivores and pollinators across multiple growing seasons, and thus may respond to herbivores and pollinators both within and across years. Joint effects of herbivores and pollinators influence plant traits, but while some of the potential interactions among herbivory, pollination, plant size, and plant reproductive traits have been well studied, others are poorly understood. This is particularly true for perennial plants where effects of herbivores and pollinators may manifest across years. Here, we describe two experiments addressing the reciprocal interactions of plant traits with herbivore damage and pollination across 2 years using the perennial plant *Chamerion angustifolium*. We measured (1) plant responses to manipulation of damage and pollination in the year of treatment and the subsequent season, (2) damage and pollination responses to manipulation of plant size and flowering traits in the year of treatment, and (3) plant-mediated indirect interactions between herbivores and pollinators. We found that plant traits had little effect on damage and pollination, but damage and pollination affected plant traits in both the treatment year and the subsequent year. We found evidence of indirect effects between leaf herbivores and pollinators in both directions; indirect effects of pollinators on leaf herbivores have not been previously demonstrated. Our results indicate that

pollen receipt results in shorter plants with fewer stems but does not change flower number, while leaf herbivory results in taller plants with fewer flowers. Together, herbivory and pollination may contribute to intermediate plant height and plants with fewer stems and flowers in our system.

**Keywords** Plant–insect interactions · *Chamerion angustifolium* · Perennial · Indirect effects · Across-year effects

## Introduction

Pollinators and herbivores can influence and respond to plant traits. These interactions with traits of shared host plants can lead to indirect effects of herbivores on pollinators (Strauss 1997; Mothershead and Marquis 2000). There is much recent interest in how joint effects of herbivores and pollinators influence plant traits (Andrews et al. 2007; Kessler et al. 2010), and interest in improving our understanding of the interplay between antagonistic and mutualistic interactions more generally (Cariveau et al. 2004; Wolfe et al. 2005; Irwin 2006; van Dam 2009). Some of the potential pathways in the network of interactions among herbivore damage, pollination, plant size, and plant reproductive traits have been well studied. Other potential pathways are still poorly understood, and the full range of possible pathways has not been considered in a single system. This is particularly true for perennial plants, for which effects of herbivores and pollinators may be expressed both within the season in which the interaction occurs and in subsequent years. Understanding all these pathways will help us understand variation in plant traits as well as the potential for plant-mediated indirect effects

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between antagonistic and mutualistic insects with perennial plant hosts.

The direct effects of herbivores and pollinators on many plant traits have been well studied, as have herbivore and pollinator responses to many plant traits. Herbivores generally have negative effects on plant traits like height (Schat and Blossey 2005), flower number (Quesada et al. 1995), flower size (Steets and Ashman 2004), and seed number (Wise and Sacchi 1996). However, increased size or reproduction in response to herbivory (overcompensation) has also been demonstrated (Paige and Whitham 1987; Gadd et al. 2001). Pollination can reduce the number of open flowers (Clark and Husband 2007) and nectar production (Ladio and Aizen 1999), and has well-recognized positive effects on fruit and seed set (Campbell and Halama 1993). It is also known that plant traits influence herbivores and pollinators. For example, increased plant or flower size can attract pollinators (Carronero and Hamrick 2005; Glaetli and Barrett 2008) but can also make a plant more apparent to herbivores (e.g., Miller et al. 2008).

For perennial plants, effects of herbivores and pollinators can manifest in years following damage or pollination. We will call plant responses that occur in the year *following* damage or pollination “across-year effects” to distinguish them from effects that occur in the same year. Across-year responses to pollinators have been particularly well examined because costs of reproduction are of evolutionary and ecological interest (Obeso 2002). However, across-year effects of both herbivores and pollinators on plant traits, including traits that might mediate indirect effects between insects, have not been examined in a single system.

Because herbivores and pollinators both influence and respond to plant traits, they may also influence one another indirectly through a shared host plant. Damage has been shown to decrease pollinator attraction by reducing flower number or size (Lehtilä and Strauss 1997; Steets and Ashman 2004; Steets et al. 2006), making herbivores doubly detrimental. Positive indirect effects of pollinators on fruit (Herrera 2000) or seed-eating (Cariveau et al. 2004) herbivores have also been reported, but pollinator effects on leaf herbivores remain unexplored. For annual plants, pollinator effects on herbivores might be unlikely because pollinators generally interact with plants later in the growing season than herbivores. However, across-year pollinator effects on perennial plants (e.g., Lehtilä and Syrjanen 1995) could allow pollinators to indirectly affect herbivores. Perennial plants are common both in nature and as research subjects, and long-term studies are needed to characterize how herbivores and pollinators influence variation in perennial plant traits (e.g., Knight et al. 2006).

Feedbacks between plant traits, herbivory, and pollination form a network of potential pathways by which insects may influence plant size and flowering traits both in the

short term (e.g., plastic response of plant traits) and the long term (e.g., evolution of plant traits). While many potential pathways of interaction among herbivores, pollinators, and plant traits within a single growing season have been well explored, a smaller number of studies have considered more than a few of these pathways at once (but see Strauss et al. 1999). Additionally, studies of perennial plants often consider only within-year responses (but see Pratt et al. 2005; Sletvold and Ågren 2011). In this study, we quantify many of these pathways within a single perennial plant system to determine how they influence plant traits and observed variation in size and flowering among years. Because size and flowering can be closely related to fitness, the pathways we measured could influence the evolution of plant traits (Strauss et al. 1999), but we did not quantify selection in this study. We examined interactions among herbivores, pollinators, plant size, and flowering in two separate experiments to ask two questions:

- (1) What are the effects of plant size and flowering traits on insect herbivore damage and pollination?
- (2) What are the effects of herbivore damage and pollination on size and flowering traits within and across years?

## Materials and methods

### Study system

We conducted this research in the spring/summer of 2009 and 2010 at the Rocky Mountain Biological Laboratory (RMBL; Gunnison County, CO, USA; 38.9398°N, 106.9821°W). We used natural populations of *Chamerion angustifolium* (Onagraceae), a long-lived perennial with iteroparous sexual reproduction. *Chamerion angustifolium* produces many (hundreds per fruit) small wind-dispersed seeds as well as clonal stems from underground runners. Aboveground stems can be found singly or in clumps comprising one individual (genet) arising from a single rhizome. Each year all, some, or none of the stems of an individual may produce a multi-flowered inflorescence. While flowers are self-compatible, selfing rates are low (Myerscough 1980; Husband and Schemske 1997). Inbreeding depression in *C. angustifolium* is strong (Husband and Schemske 1997), so outcrossing provided by pollinators is important. *Chamerion angustifolium* at RMBL is pollinated by several bumblebee species, primarily *Bombus flavifrons* and *B. bifarius* (Apidae), and is damaged by both specialist and generalist leaf herbivores including caterpillars and adult chrysomelid beetles. Mule deer (*Odocoileus hemionus* (Cervidae)) are frequent herbivores, browsing the top several centimeters of stems and inflorescences of *C. angustifolium*.

## Effects of plant size and flowering traits on insect herbivore damage and pollination

### Experimental design

To determine the effects of *C. angustifolium* size and flowering traits on herbivore attack and pollination success, we compared insect herbivore damage and fruit number (as a proxy for pollen receipt) between control plants and plants with experimentally reduced stem number or flower number. Pollen receipt was measured by fruit number proxy rather than directly to increase sample sizes. Because *C. angustifolium* is distinctly protandrous and self-fertilization rates are low (Myerscough 1980; Husband and Schemske 1997), we consider fruit number to reflect an estimate of pollinator visitation. We interpret fruit number conservatively, acknowledging that fruit number is likely also affected by plant investment in fertilized ovules. Damage and fruit number were measured in the same year as treatments were imposed (2009).

In June 2009, we identified 105 *C. angustifolium* individuals in a single meadow; each individual had at least 10 stems and was ~20–30 cm in height. Each distinct clump of stems was considered an individual plant. Excavation of individuals not used in this experiment confirmed that clumps we visually identified as individuals each came from a single rhizome (A. Buchanan, unpublished data). We used a spade to trench to a depth of 20–25 cm around each individual to sever underground connections and reduce the influence of any vegetatively associated but unmanipulated ramets. We assigned each individual to receive either the stem removal treatment, flower removal treatment, or control (no removal) treatment randomly with respect to position in the meadow, but re-assigned plants if treatments were spatially aggregated. Stem removal consisted of removing 50 % of the stems at ground level with gardening shears; this treatment was imposed once in late June, after plants were ~30 cm tall ( $n = 35$ ). Flower removal consisted of removing 50 % of the flowers and large buds at the stem with small scissors; this treatment continued as new flowers matured in August ( $n = 12$ ). Control treatment consisted of only handling plants ( $n = 58$ ). Sample sizes were uneven because not all plants flowered. Plants that had been assigned to flower removal but then did not flower necessarily became controls.

We surveyed plants six times (every 2 weeks from June to August 2009). We visually estimated damage by insect herbivores as total percent leaf area missing per plant. This method produced results equivalent to averaging per-leaf damage estimates for all leaves (A. Buchanan, unpublished data). Because deer browsing was common, we also measured deer browsing as number of stems receiving any browsing damage, which was apparent as removal of the

top several centimeters of the stem. Fruit number (proxy for pollination success) was counted directly. We also quantified other plant responses to our treatments, measuring maximum height achieved during the season, total new stems produced after treatment (change in stem number between first and final surveys), total number of flowers produced after treatment, and maximum number of flowering stems over all surveys. None of these traits were significantly affected by removal treatments (data not shown).

### Analysis

Separate models were used to ask how the fixed factor of treatment (stem removal, flower removal or control) affected insect damage, deer browsing, and pollination (fruit number). Insect herbivore damage was log transformed to make residuals normally distributed and analyzed with Type III SS analysis of variance (ANOVA). For flowering plants only, pollination (fruit number) was modeled as a function of both treatment and flower number (as a covariate) with a generalized linear model (GLM), assuming a negative binomial distribution. Deer browsing (number of browsed stems) was modeled as a function of treatment and stem number (as a covariate) with a GLM assuming a negative binomial distribution. We used planned contrasts to test for specific differences between the two removal treatments and the control treatment. All analyses were performed in R 2.13.0 (R Development Core Team 2011); ANOVA analyses used the package “car” (Fox and Weisberg 2011).

## Effects of herbivore damage and pollination on size and flowering traits within and across years

### Experimental design

To determine the effects of insect herbivory and pollen receipt on plant size and flowering traits, we crossed two damage treatments (natural damage and reduced damage) with two pollination treatments (natural pollination and reduced pollination) for four total treatment groups. We then measured number of new stems produced, height, flower number, and flowering stems as plant responses both in the treatment year (2009) and in the following year (2010). We also measured damage and pollination in the year of treatment and the following year to assess the effectiveness of our manipulations and examine indirect effects between herbivores and pollinators within and across years.

In June 2009, we identified 124 individual plants in a meadow near RMBL that was separated from the previous experiment by ~0.5 km of aspen stands, trenching around

individuals as in the first experiment. To reduce insect damage, we sprayed Carbaryl (Sevin<sup>®</sup> by GardenTech<sup>™</sup>; 22.5 % concentrate diluted to 5.85 mL/L water) every 2 weeks until flowering began for a total of three applications ( $n = 61$ ). Control plants were sprayed with water ( $n = 63$ ). Some damage accumulated after insecticide treatment stopped, but damage in insecticide-treated plants remained substantially lower than in control plants (Fig. 1).

We took a number of precautions to prevent harming bees with Carbaryl spray. We sprayed individual focal plants with a small hand sprayer and covered surrounding vegetation before application to prevent overspray onto surrounding plants, and ceased Carbaryl application when flower buds began to form, at least 1 week before any open flowers were observed. Because Carbaryl harms bees primarily when plants are sprayed during blooming, and because the toxic effects of Carbaryl to bees last 3–7 days (Mayer and Johnson 1999), we believe these precautions prevented bees from contacting, consuming, or storing Carbaryl. Several studies in other plant systems suggest no effects of Carbaryl on pollen viability or number of visits by pollinating bumblebees (N. Underwood, unpublished data), plant size (Lau and Strauss 2005), plant height (Geyer and Rink 1998), or seed set or survival (Stinchcombe and Rausher 2001), and effects of Carbaryl on photosynthetic rates have been found to be short-lived, lasting 7 days after a 60-day spray treatment (Abdelreheem et al. 1991). It is thus likely that effects of the Carbaryl treatment applied to our focal plants can be attributed to the intended effects on leaf-feeding herbivores.

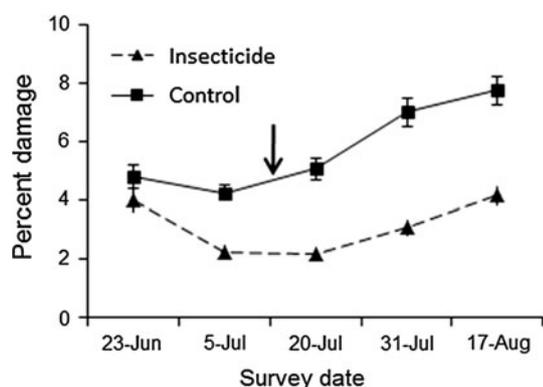
To reduce pollen receipt, we excised stigmas from all or nearly all flowers on half the flowering plants every 2–3 days as new flowers opened ( $n = 61$ ). Control plants were handled but stigmas were left intact ( $n = 63$ ). *Chamerion angustifolium* flowers are protandrous and stigmas are not receptive until a few days after flowers open (Clark and Husband 2007), so stigma removal within 3 days of

flower opening should prevent pollination. For a 1-week period in August, we were unable to access the plants and bagged inflorescences in mesh pollinator exclusion bags. Loss of some bags to wind during this period likely allowed some flowers to be pollinated. Stigma excision has been used to prevent pollination in other systems (e.g., Ladio and Aizen 1999), but can reduce flower lifespan in some species (e.g., Lovell et al. 1987). Although we did not measure floral life span, we noticed no reduction in flower life span following stigma excision. We chose this method instead of pollinator exclusion bags to minimize potential microclimatic effects of bags (e.g., Kearns and Inouye 1993) and to allow pollen and nectar removal by pollinators. Loss of plant biomass from stigma excision is likely negligible, and although flower damage can cause induced resistance in plants (McCall 2006), mechanical removal of plant parts usually does not elicit the same defense response as real herbivory (Walling 2000; Massey et al. 2007).

Sample sizes in each of the four treatments were: spray–excision = 32, spray–intact stigma = 31, water–excision = 31, water–intact stigma = 32. In the treatment year, data were collected every two weeks on the same schedule as in the previous experiment. Pollination (fruit number) and insect herbivory were measured as described in the previous experiment. We measured change in stem number, total flower number, and maximum number of flowering stems per plant over the season. We also measured maximum plant height achieved over the season, estimated as an average over all stems in a plant using a meter stick centered in the plant; this method produced results equivalent to averaging individual measurements for each stem (A. Buchanan, unpublished data). We measured deer browsing as total number of browsed stems over the season. In the year following treatment, we conducted two surveys and measured insect damage, fruit number, and flower number (determined by fruit number plus pedicel number) at the end of the season, maximum number of flowering stems achieved over the surveys, and difference between end of season stem number and stem number at the beginning of the experiment (in 2009).

### Analysis

We analyzed the effects of spray and excision treatments on plant traits in 2009 and 2010 separately. For each year, we used a separate model for each plant trait: change in stem number, height, flower number and number of flowering stems (flowering plants only). The model for number of flowering stems included stem number as a covariate, and used a negative binomial error distribution (glm function in R). All other plant trait models used ANOVA with Type III SS to assess significance. We analyzed the effect of



**Fig. 1** Temporal changes in percent damage to *Chamerion angustifolium* plants. Arrow indicates when insecticide treatments ended. Values are mean  $\pm$  SE

treatment on insect damage (log-transformed percent damage), pollination success (fruit number) with flower number as a covariate, and number of browsed stems with stem number as a covariate in both the treatment year and the following year. These models used negative binomial error distributions (glm). We dropped non-significant interactions from models, but retained all covariates regardless of their significance to account for effects of plants with more stems or flowers. Transformations resulted in approximately normal distributions of residuals.

Analyzing these responses within and across years resulted in 14 models, which tested for insecticide main effects using the full dataset, flowering and non-flowering plants alike. However, to test for insecticide, excision, and insecticide  $\times$  excision effects, we also ran the models using a smaller dataset that only included flowering plants since excision could necessarily only influence flowering plants. Where insecticide effects were found using the smaller dataset we did not repeat that model with the larger dataset, assuming that the effect would still be present using the full dataset. This resulted in a grand total of 23 models considered, of which we report those with significant treatment effects in Table 1.

## Results

In both experiments, approximately 50 % of the plants flowered in any given year; those that did flower produced approximately 25–90 flowers per plant. There was minimal plant mortality ( $\sim 2$  %) in either year, and plants that did not survive were not included in the analysis.

### Effects of plant size and flowering traits on insect herbivore damage and pollination

There was a significant treatment effect on fruit number ( $\chi^2_{2,42} = 10.46$ ,  $P = 0.005$ ). Contrasts showed that stem removal increased fruit number on remaining stems relative to control plants ( $P = 0.003$ ; Fig. 2a), while flower removal did not change fruit number (per flower remaining after flower removal treatment) relative to control plants. There were no significant treatment effects on insect damage or deer browsing, although there was a trend for both stem and flower removal treatments to reduce browsing (Fig. 2b). There were no treatment effects on stem production, plant height, flower production, or flowering stems.

### Effects of herbivore damage and pollination on size and flowering traits within and across years

Insecticide decreased insect herbivory in the treatment year by 57 % per plant (Table 1;  $P < 0.001$ ). There were no

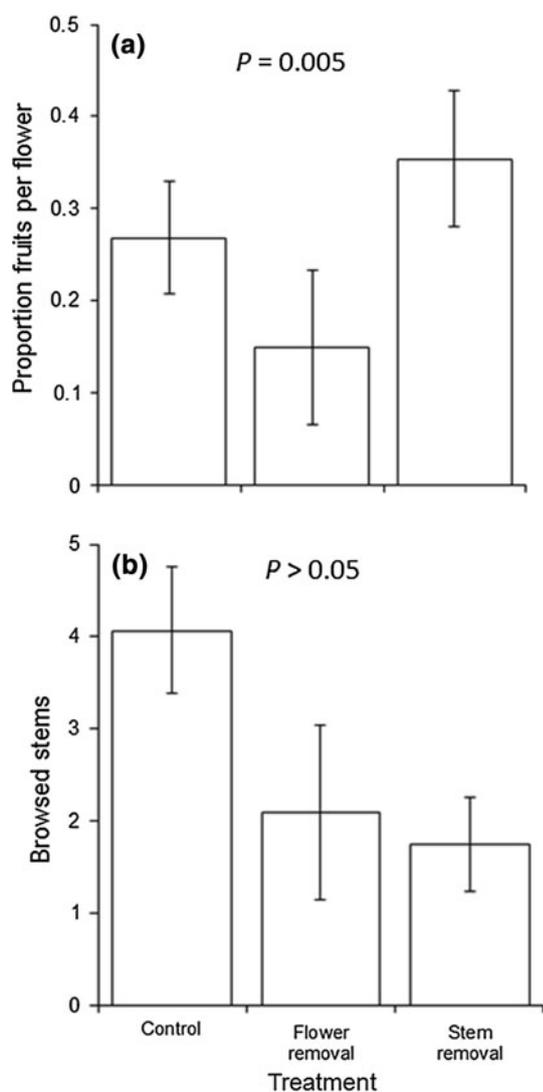
**Table 1** Effects of insecticide and excision treatments on *Chamerion angustifolium* plants and forager responses

Response	Predictor	df	F or $\chi^2$	P
Percent damage 2009	Insecticide	1	15.567	<0.001
	Excision	1	3.835	0.056
	Residual	52		
Fruit number 2009	Insecticide	1	30.003	<0.001
	Excision	1	0.928	0.335
	Flower number 2009	1	71.448	<0.001
	Insecticide $\times$ excision	1	9.667	0.002
	Insecticide $\times$ flower number 2009	1	36.857	<0.001
	Excision $\times$ flower number 2009	1	1.564	0.211
Flower number 2009	Insecticide	1	16.162	<0.001
	Excision	1	0.773	0.383
	Residual	52		
Flowering stems 2009	Insecticide	1	6.616	0.01
	Stem number 2009	1	1.09	0.3
	Residual	122		
Stem production 2010	Insecticide	1	4.140	0.044
	Residual	116		
Height 2010	Insecticide	1	6.366	0.013
	Residual	122		
Height 2010	Excision	1	4.065	0.049
	Residual	53		
Browsed stems 2010	Excision	1	5.439	0.02
	Stem number 2010	1	22.329	<0.001
	Excision $\times$ stem number 2010	1	3.271	0.071
	Residual	49		

Some response variables are given twice because analysis was done using all plants ( $df = 119$  or  $122$ ) and flowering plants ( $df = 48$ ,  $49$ ,  $52$ , or  $53$ ). Only models that explained significant variation in data are reported; see “Results” for non-significant models

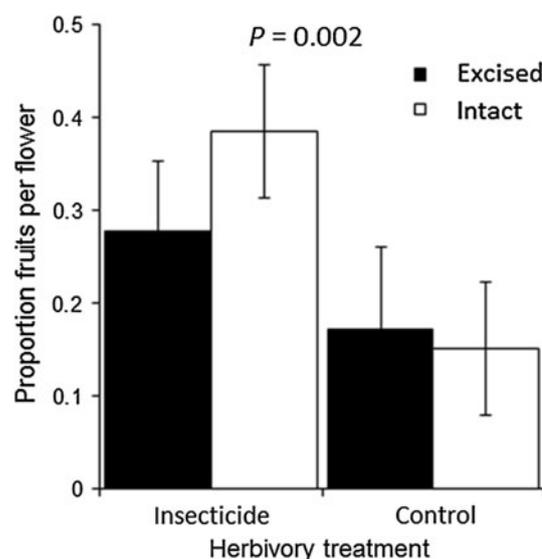
residual effects of insecticide on damage in the following year. Although the final insecticide application occurred on 13 July 2009, damage to insecticide-treated plants remained substantially reduced relative to control plants for the remainder of the season (Fig. 1). Stigma excision reduced fruit number in the treatment year only for plants that also received insecticide (interaction term  $P = 0.002$ ), while plants exposed to natural herbivory produced very few fruits regardless of excision treatment (Fig. 3).

Using the full dataset (i.e., flowering and non-flowering plants), insecticide increased flower number ( $P < 0.001$ ; Fig. 4a) and number of flowering stems ( $P = 0.01$ ; Fig. 4b) in the treatment year. Insecticide also revealed an indirect effect of herbivory on pollination by increasing



**Fig. 2** Effects of stem and flower removal on plant traits in 2009. **a** Fruits/flower and **b** browsed stems. Bars mean  $\pm$  SE

fruit number per flower, our proxy for pollination, in the treatment year ( $P < 0.0001$ ; Fig. 4c). Insecticide did not affect change in stem number in the treatment year but increased stem production in the following year ( $P = 0.04$ ) and decreased height in the following year ( $P = 0.013$ ; Fig. 5a, b). Including flowering plants only, there was a trend for stigma excision to increase leaf damage (by 40 %) in the treatment year relative to plants with intact stigmas ( $P = 0.056$ ; Fig. 6a), suggesting an indirect effect of pollination on herbivory. In the year following treatment, excision increased height by 11 % ( $P = 0.049$ ) and decreased browsed stems by 42 % ( $P = 0.02$ ; Fig. 6b, c). There were no significant effects of insecticide or stigma excision treatments on plant height or deer browsing in the year of treatment, or on flower number in the year after treatment. There was also no evidence of across-year



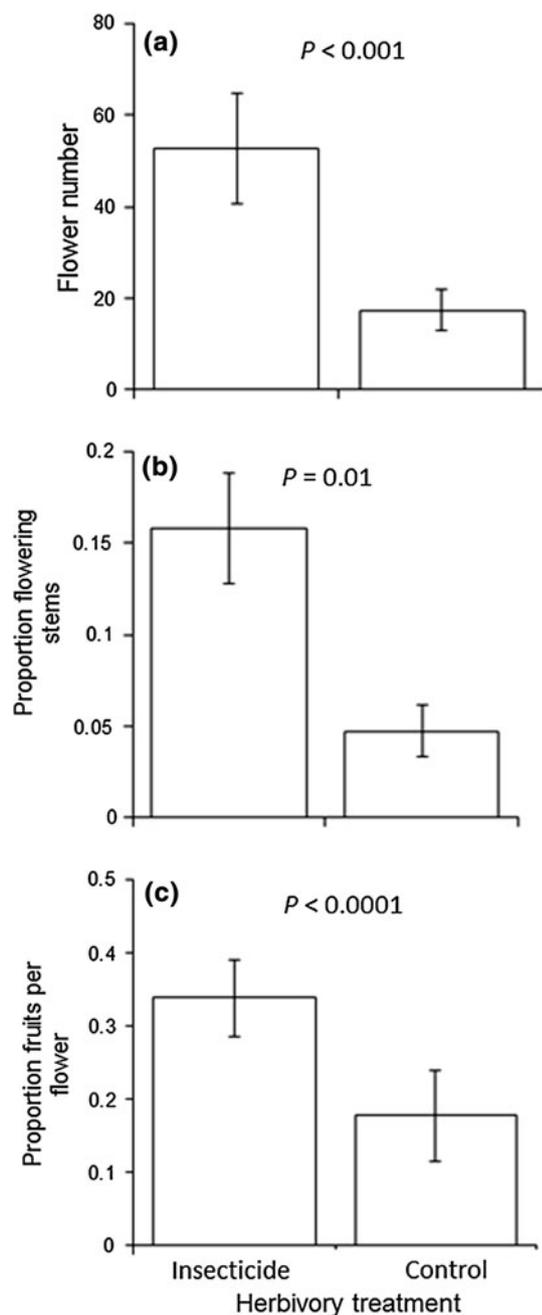
**Fig. 3** Effect of insecticide and pollination on fruit number in 2009. Bars mean  $\pm$  SE

indirect effects; there was no effect of insecticide on fruit number or of stigma excision on insect damage in the year following treatment.

## Discussion

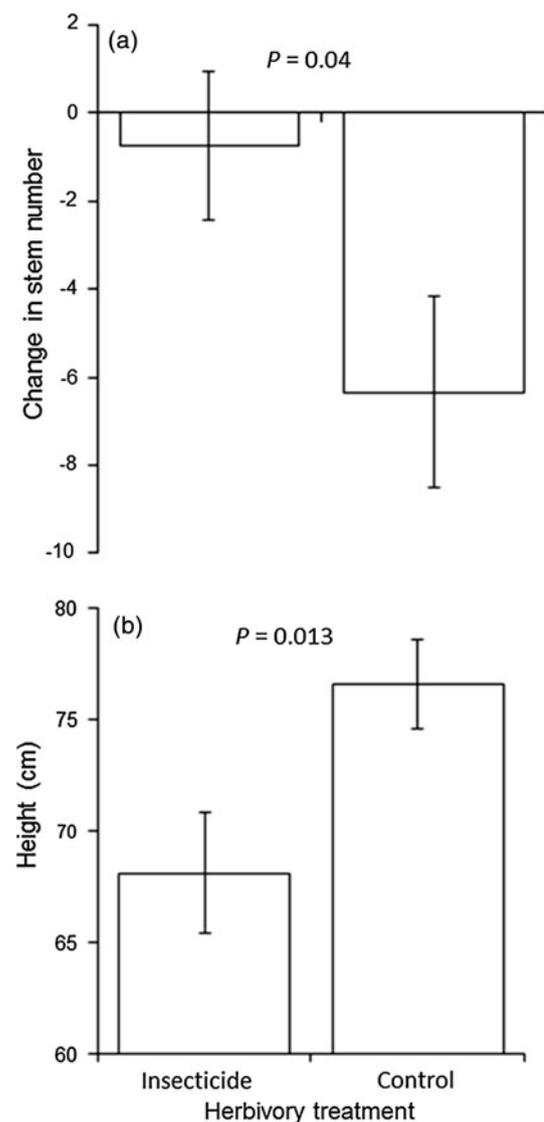
Our results support a number of pathways of influence among herbivores, pollinators, and plant traits within and between years. Taken together, these results suggest insect-mediated limitation of the expression of some plant traits. While some of these pathways have been well explored in other systems, others have not, and few studies have considered multiple pathways simultaneously. We found that in general plant traits had little effect on damage and pollination, but insect damage and reduction in pollen receipt both affected plant traits. Importantly, some effects only manifested in the year after treatments were imposed. The data also suggest that insect damage and pollen receipt may have indirectly affected each other.

We found only one effect of a plant trait on damage or pollination: pollination (as measured by fruit set) increased on plants with fewer stems (stem removal treatment; Fig. 2a). These results could be explained by reproductive compensation, that is, an increase in flowering following damage (e.g., Paige and Whitham 1987). In our experiment, plants in the stem removal treatment increased in proportion of flowering stems by 13 % and in number of total flowers by 55 % relative to control plants, although these changes were not statistically significant. Pollinator visitation is known to increase with the size of floral display (Conner and Rush 1996; Hegland and Totland 2005;



**Fig. 4** Effects of damage on plant traits and pollination in 2009. **a** Flower number, **b** flowering stems/total stems, and **c** fruits/flower. Bars mean  $\pm$  SE

Glaetli and Barrett 2008), so more flowering stems and total flowers might have attracted more pollinators. It is surprising that we did not find other effects of plant traits on herbivores and pollinators because these effects have commonly been seen in other systems. Herbivores and pollinators both can be attracted to floral display size (e.g., Miller et al. 2008) and plant size (e.g., Ehrlén 1995; Hodkinson et al. 2001; Carronero and Hamrick 2005). It is possible that the alterations we made to plant size and



**Fig. 5** Effects of damage on plant traits in 2010. **a** Change in stem number and **b** height. Bars mean  $\pm$  SE

flowering phenotype missed the traits that herbivores and pollinators use to choose host plants in this system. *Chamerion angustifolium* grows in a dense mix of other vegetation at our study sites, so number of stems or flowers per plant might not be as important as other traits such as plant height or volatile production, which our study did not address.

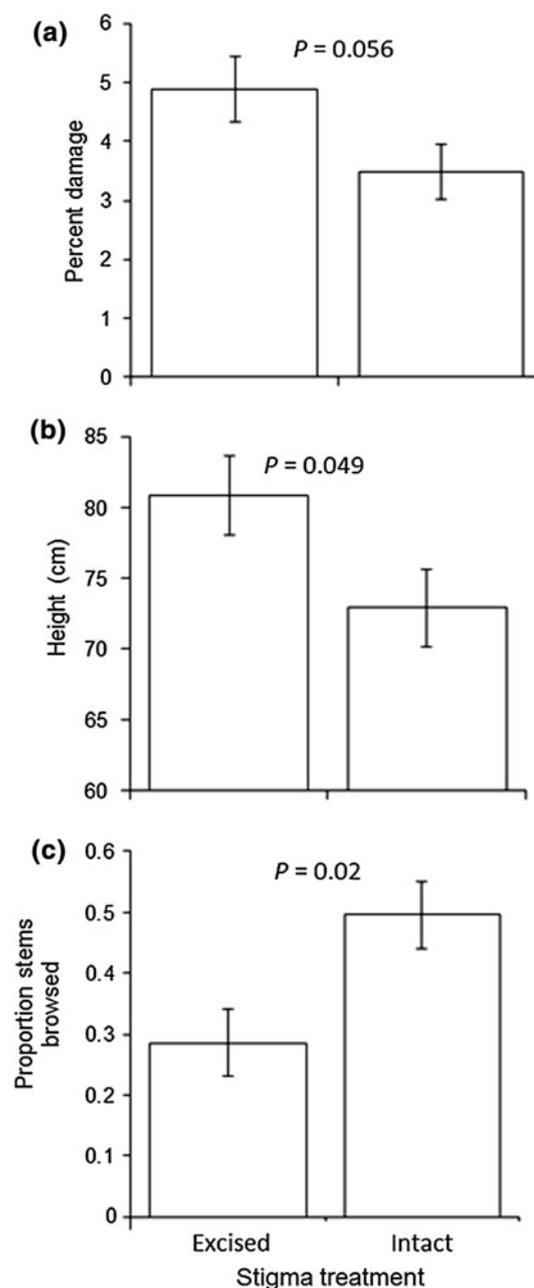
Herbivores and pollinators had a range of effects on plant traits in our system, as well as indirect effects on one another. Insect damage decreased flower number, number of flowering stems, and fruit number (our proxy for pollination) in the treatment year (Fig. 3a–c). Because our analysis for fruit number accounts for flower number, reduced fruits could represent reduced pollen receipt per flower, suggesting fewer or less effective pollinator visits.

It is worth noting that insecticide application increased fruits produced per flower, indicating that insecticide spray did not adversely affect pollinators. Indirect effects of damage on pollination are not surprising: leaf herbivores have been shown to decrease pollinator visitation per flower within years by reducing flower number (as in this study) and size (Lehtilä and Strauss 1997; Steets and Ashman 2004). It is also possible that leaf damage decreased fruit number by reducing resources available for maturing seeds (e.g., Mothershead and Marquis 2000). However, we found no effect of damage on plant size in the treatment year, suggesting that resource limitation by herbivores was not severe. Positive correlations between size and flowering traits in control plants pooled across both experiments (data not shown) suggest that plant responses were not influenced by trade-offs between size and flowering. Other plant systems have also demonstrated a positive relationship between size and flowering (e.g., Herrera 2004).

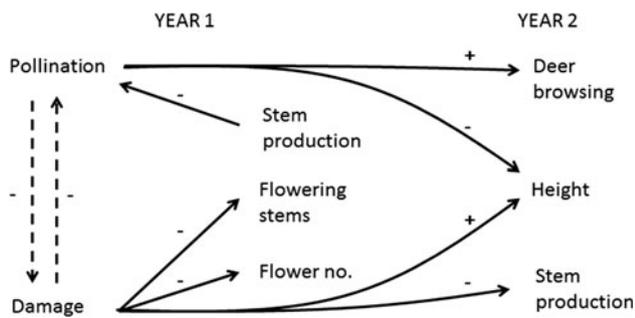
Although indirect effects of herbivores on pollination are fairly common, the trend of pollination decreasing damage in our system (Fig. 6a;  $P = 0.056$ ) was surprising. It is possible that the act of excising stigmas induced plant defense, but the tiny amount of mechanical damage from stigma excision is unlikely to have greatly altered plant defenses (e.g., Underwood 2000; Massey et al. 2007), and defense induction following stigma excision should decrease, rather than increase, subsequent damage. Likewise, diversion of resources from defenses to fruit production would have increased leaf herbivory for the plants receiving pollen, but this is not what we found. Plant nutrient quality may have been reduced following allocation to fruits, but our study was not able to test that possibility. Indirect effects of pollination on leaf herbivores have not been previously explored, and while our power to detect effects may have been reduced due to low flower number, these suggestive results call for more data.

Our results suggest that herbivore and pollinator effects on plants can manifest across growing seasons. Herbivores affected plant traits in the year of treatment and the following season (Figs. 4, 5). Pollination, in contrast, only affected traits in the following year (Fig. 6). These across-year effects are probably due to effects of our treatments on allocation since the treatments themselves did not carry over, i.e., insecticide treatments did not affect leaf damage in the following year, and stigma excision did not prevent stigma formation in the following year. Across-year effects of herbivory and pollination have been found in other systems. For example, leaf herbivory increased stem number but decreased reproduction across years in *Melaleuca quinquenervia* (Pratt et al. 2005), and, after an initial negative effect, leaf herbivory increased both stem length and flower production across years in *Cornus florida*

(Sacchi and Connor 1999). Pollination decreased next-year fruit production in *Gymnadenia conopsea* (Sletvold and Ågren 2011) and decreased next-year plant size in *Tipularia discolor* (Snow and Whigham 1989). Although we saw no across-year indirect effects in this study, our results suggest that future studies should consider the potential for these interactions to manifest at longer time-scales than a single growing season. Our results suggest more long-term



**Fig. 6** Effects of stigma excision on plant traits and damage in 2009 and 2010. **a** Percent leaf damage in 2009, **b** height in 2010, and **c** proportion browsed stems in 2010. Data are from flowering plants only. Bars mean  $\pm$  SE



**Fig. 7** Summary of relationships found in both experiments. Signs (+, -) indicate direction of effect. Dashed arrows show an indirect effect

studies will be needed to understand effects of herbivores and pollinators on perennial plant traits.

It is worth noting herbivore damage in this study had positive (Fig. 5b) as well as negative (Fig. 4) effects on plant traits. Compensatory responses to damage have been found in response to apical damage (Paige and Whitham 1987; Pilson and Decker 2002), but overcompensation in response to leaf damage is less common, and reports of overcompensation typically address only within-year effects (but see Brody et al. 2007). The long-term (lifetime) consequences of overcompensation in perennial plants are unclear, but our data suggest that future research should address whether long-term compensatory effects occur.

Our results suggest that the joint effects of herbivores and pollinators on plant size and flowering traits contribute to the expression of intermediate values of some plant traits. The general effect of herbivores in this study was to decrease flowering traits (Fig. 4a–c) and increase one size trait while decreasing another (Fig. 5a, b). Overall, herbivory should result in taller plants with fewer stems and flowers (Fig. 7). Whether these effects would result in long-term selection pressures on plants is unclear from these experiments, given the lack of herbivore response to manipulated plant phenotype. Pollen receipt (as measured by fruit number) decreased height (Fig. 6c), so pollination should result in expression of smaller (shorter) plants. However, because pollination appeared to respond negatively to plant size (manipulated stem number) and size and height are positively correlated, it is possible that the strength of this effect would diminish as plant size decreases. If pollinators do respond negatively to plant size (Fig. 2a) they should select for smaller plants. Because herbivory results in tall plants with few stems and flowers, while pollination results in short plants, the joint effects of herbivores and pollinators in this system may contribute to intermediate values of plant height. Studies quantifying plant fitness and plant–herbivore–pollinator interaction strengths, ideally across plant lifetimes, are necessary to

determine the net outcome of herbivore and pollinator effects on plant traits.

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