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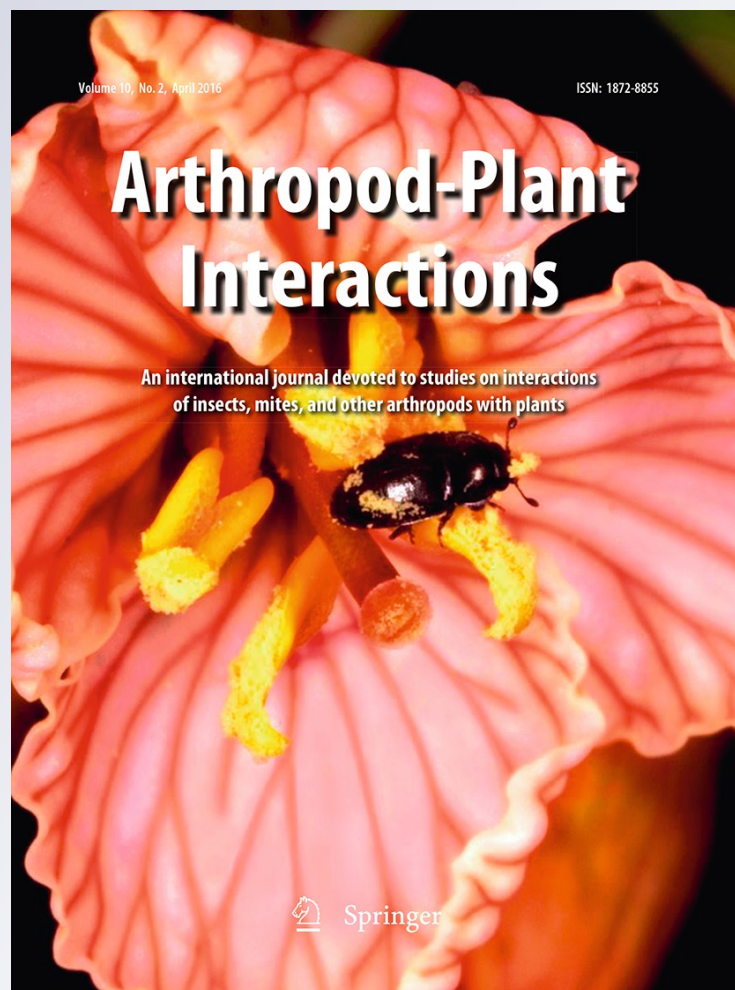
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# Floral damage induces resistance to florivory in *Impatiens capensis*

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**Abstract** Many plants produce defense chemicals that are induced in response to damage. In spite of the tight links between floral tissue and plant reproduction, very little is known about whether floral defenses are induced in response to floral damage. We manipulated *Impatiens capensis* flowers to determine whether floral damage reduces subsequent florivory, whether it induces anthocyanins or condensed tannins in floral tissues, and whether responses are localized or systemic. We damaged one flower per plant at one of three damage levels (0, 30, or 60 % tissue removal), collected subsequent flowers at set time intervals and branch locations, and measured whole-plant florivory for 3 weeks following damage. We also observed a flower color polymorphism and analyzed responses separately for red- and yellow-flowered plants. Moderate damage to a single flower reduced subsequent

whole-plant florivory, but heavy damage did not. Moderate damage to a focal flower also increased anthocyanins in subsequent flowers on the same branch of red-flowered plants, but decreased anthocyanins on parallel-branch flowers of yellow-flowered plants. Damage did not affect floral tannins. Because the reduction in florivory was systemic and induced anthocyanins were not consistently induced systemically, there may be other secondary compounds not measured in this study that were systemically induced, or effects of visual or olfactory cues of damage itself that reduced subsequent florivory. This is the first study demonstrating that damage to a single flower can reduce subsequent whole-plant florivory in the field, indicating that initial damage can have cascading effects on subsequent interactions.

**Keywords** Anthocyanins · Condensed tannins · Florivory · Induced defense · Optimal defense theory · Plant defense

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## Introduction

Plant antagonists can have large negative effects on plant fitness. For example, decades of studies demonstrate that leaf herbivores can reduce fruit or seed number (Breedlove and Ehrlich 1968; Cyr and Pace 1993; Marquis 1984). Florivory (herbivory on flowers) has received much less attention than leaf herbivory, although it occurs commonly and can reduce plant reproduction as much as leaf herbivory (McCall and Irwin 2006; Strauss et al. 2004). Florivores can disrupt flowering times (Kawagoe and Kudoh 2010), directly decrease plant fitness by damaging reproductive structures (Galen 1999; Legee and Wolfe 2002), and indirectly decrease plant fitness by reducing

pollinator preference (Adler 2000; Krupnick et al. 1999; Sober et al. 2010) and increasing pollen limitation (McCall 2010). The vast majority of research examining plant resistance and chemical defense mechanisms focuses on leaf herbivory, and thus relatively little is known about whether or how flowers are defended against floral herbivory (McCall and Irwin 2006). Considering the tight link between floral tissues and plant reproduction, florivory may play a significant role shaping the evolution of plant defenses (McCall and Irwin 2006).

Plants can defend tissues against damage via constitutive or induced defenses. Constitutive defenses are always produced and so require resource allocation regardless of antagonist presence (McCall and Forgyce 2010). Induced defenses are produced in response to damage, allowing plants to avoid physiological or ecological costs of defense production until such defenses are needed (Strauss and Agrawal 1999), but at the cost of leaving tissues undefended until damage occurs. Optimal defense theory suggests that tissues most valuable to the plant will be defended with constitutive rather than with inducible defenses (Schaffner et al. 2011; Strauss et al. 2004). Reproductive structures and young leaves may have greater constitutive defenses than tissues less likely to be attacked, such as roots and stems (Zangerl and Rutledge 1996). However, deterring florivory via constitutive defense may incur ecological costs to plants; while floral defenses may deter florivores, pollinators may also be deterred (Adler and Irwin 2005; Adler et al. 2012; Agrawal et al. 2000; Andrews et al. 2007; Kessler and Halitschke 2009; Kessler et al. 2004; McCall and Irwin 2006; Mooney et al. 2010). Induced defenses in floral tissues could mitigate potential ecological costs of defense due to reduced pollinator visitation.

A growing number of studies have demonstrated that vegetative damage can induce changes in floral secondary compounds (Marlin et al. 2014; McCall and Karban 2006; Schiestl et al. 2014; Wackers and Bezemer 2003). For example, leaf herbivory on *Nicotiana tabacum* by *Manduca sexta* increased floral nectar alkaloids (Adler et al. 2006). *Raphanus sativus* plants have a flower color polymorphism, and leaf damage induced changes in petal glucosinolates that differed with flower color morph (Strauss et al. 2004). However, very few studies have examined how floral interactions shape subsequent floral chemical defenses or interactions with floral antagonists. Many insect herbivores specialize on floral tissue, suggesting induced responses to floral damage would be adaptive (Hendrix 1984). For example, natural caterpillar florivory increased resistance against subsequent lepidopteran florivory in *Nemophila menziesii* (McCall 2006), but traits associated with resistance were not measured. *Nicotiana attenuata* flowers showed a twofold increase in nicotine

pools after damage (Euler and Baldwin 1996), but resistance to florivores was not measured. While these studies suggest that flowers are capable of inducing defenses, we are aware of no studies that simultaneously measure florivore-induced changes in subsequent natural florivory in the field and examine potential underlying chemical mechanisms.

To determine whether florivory induces floral defenses of a native annual plant, we conducted a manipulative field experiment to ask the following questions: (1) Does floral damage to a single flower affect subsequent natural florivory in the field? (2) Does florivory induce chemical defenses in floral tissues? (3) If induction is observed, is it localized or systemic?

## Methods and materials

### Study system

*Impatiens capensis* Meerb. (Balsaminaceae) is an herbaceous annual that grows in shady, moist areas across North America (Eastman 1995). *Impatiens capensis* has little to no seed bank, growing only from seed produced the prior year (Simpson et al. 1985). It has a mixed mating system with both cleistogamous and chasmogamous flowers. Cleistogamous flowers do not open and are self-pollinated, while chasmogamous flowers open to expose reproductive structures, allowing cross-pollination. We have not observed insect damage to cleistogamous flowers (MDHB, pers. obs.). Both flower types produce a capsule fruit that explosively dehisces upon maturation (Eastman 1995). The term ‘flower’ will be used to refer to chasmogamous flowers only hereafter. Flowers are open for 2–3 days, are cornucopia-shaped with a prominent nectar spur on the distal end, and are reliant on pollinators for reproduction (Rust 1977; Schemske 1978). Flowers are sequentially protandrous, with anthers dehiscing before carpel maturation to prevent selfing (Eastman 1995).

Flowers are visited by many beneficial and antagonistic insect species. Pollinators include *Bombus* spp., *Apis mellifera* (Apidae), and *Vespula maculifrons* (Vespidae) (Eastman 1995; Schmitt et al. 1985). More than 80 % of flowers experience some form of nectar larceny that varies spatially and temporally (Eastman 1995; Young 2008). Flowers are nectar-robbed by vespids and *Bombus* spp. that chew holes in the nectar spur to remove nectar without pollinating, and are visited by smaller insects (e.g., halictid bees, ants, and syrphid flies) that are nectar thieves, removing nectar without contacting the plant’s reproductive parts (Soper Gorden and Adler 2013). Flowers are regularly damaged by *Popillia japonica* (Scarabaeidae) and other generalist herbivores such as grasshoppers and

beetles (Soper Gorden and Adler 2013). In a previous study at our site, floral damage ranged from 0 to 100 % of flowers, but averaged 25 % of flowers damaged per plant (Soper Gorden and Adler 2013). Treatments were based on previous data from 391 flowers on 107 plants that had a mean of  $18.9 \pm 4.0$  % (mean  $\pm$  SE) of flowers damaged per plant, removing  $31.5 \pm 1.1$  % (mean  $\pm$  SE) floral tissue (range 0–95 %; data from Soper Gorden and Adler 2013).

*Impatiens capensis* flowers contain several secondary metabolites, including anthocyanins and condensed tannins (Aras et al. 2007; Waterman et al. 1983), but little is known concerning chemical traits associated with herbivore resistance in *I. capensis*. Anthocyanins are flavonoids largely responsible for the red pigmentation in plant tissues, including *I. capensis* flowers (Close and Beadle 2003). However, anthocyanins also serve a range of functions in plant tissues, including protecting against ultraviolet wavelengths and preventing photoinhibition in photosynthetic tissues (Close and Beadle 2003), and so increased anthocyanins may be associated with general stress rather than a direct mechanism of herbivore resistance. We analyzed anthocyanins due to their known occurrence in *I. capensis* tissues and their association with damage responses in other systems (Aras et al. 2007; Close and Beadle 2003; Johnson et al. 2008; Karageorgou and Manetas 2006). Condensed tannins are water-soluble phenolic compounds with a well-documented role in herbivore defense due to their ability to bind and precipitate proteins in insect herbivore digestive systems (Salminen and Karonen 2011), but their role affecting florivory is unknown.

### Study site and plant propagation

On May 4, 2010, 150 *I. capensis* seedlings ( $\sim$ 4–5 cm tall) were collected from a natural habitat on Hampshire Farm in Amherst, MA (N42°19' W72°31'). Seedlings were moved to the greenhouse at the University of Massachusetts at Amherst, and potted individually in 10-cm-diameter pots using Fafard #2 potting soil (Conrad Fafard, Inc., Agawam, MA). Plant positions on greenhouse benches were randomized weekly, and plants were kept under natural light and watered daily.

On June 8, plants were transplanted to a plot at Hampshire Farm bordering the northeast edge of a moist woodland habitat containing a wild population of *I. capensis*. Plants were spaced 1 m apart and arranged in four parallel rows at the woodland's edge. Natural soil nutrients were low (Soper Gorden and Adler 2013), and each plant was treated with 24 g of Osmocote Classic Controlled-release 14–14–14 Fertilizer (The Scotts Company, Maryville, OH) on July 7, applied to a 0.5-m-radius area surrounding each plant, to encourage flowering. The plants were watered

once immediately after fertilization and had only natural rainfall for the rest of the experiment.

### Experimental design

To determine whether floral damage induces defenses in subsequently produced flowers, we randomly assigned the 104 plants that flowered to one of three damage treatments (0, 30 %, or 60 % tissue removal) applied to one flower per plant on August 18, at the height of flowering. Only one flower was damaged to separate localized from systemic effects of damage. These damage levels were chosen to represent the average (30 %) and high value (60 %) of tissue loss due to natural florivory, as well as a control (0 %). Floral tissue was removed from corollas of one flower per plant with dissecting scissors, leaving the nectar spur and reproductive parts intact. We only applied treatments to flowers without natural damage. Flowers were not protected from natural florivory after treatments, and so our manipulation represents a conservative estimate of effects since subsequent interactions over the 2- to 3-day lifespan of a flower could add random variation to our manipulation. Each treatment flower was selected for its proximity to buds on the same and parallel branches (vascular-sharing branches directly above or below). A prior vasculature mapping study showed that branches both above and below a focal branch that were separated by an angle less than 50 degrees when viewed from above had shared vasculature (Online Resource 1). We considered parallel branches to be those connected to the same vasculature either above or below a focal branch. These locations were chosen to determine whether induction occurs locally or systemically, as a systemic response should be most evident in branches with shared vasculature (Viswanathan and Thaler 2004). Damaged and control flowers were marked with Wite Out (Bic USA, Shelton, CT) on the petiole to indicate treatment location after the damaged flower had senesced.

### Responses measured

To determine the effect of floral damage on subsequent florivory, surveys of whole-plant florivory were conducted 1 week prior to (as a baseline metric of damage), and 1, 2, and 3 weeks after treatment. Plants in the three damage treatments had similar levels of florivory before treatments were imposed (proportion flowers damaged, binomial generalized linear model,  $\chi^2 = 0.04$ ,  $df = 2$ ,  $P = 0.979$ ). Surveys recorded both the proportion of flowers damaged per plant and the percent tissue removed from each damaged flower.

To measure the timing and systemic nature of induction, flower samples on the same branch and parallel branches in relation to the treated flower were harvested at 1 day,

2 days, 1 week, and 2 weeks after initial damage (number of flowers at each time point, *same branch*: 24, 34, 110, and 68, respectively; *parallel branch*: 28, 25, 52, and 38, respectively). Flowers were removed by cutting the petiole with dissecting scissors at the receptacle and stored individually in 30-mL plastic portion cups kept cold for transport. To analyze the percent red in corolla lips, all collected flowers were photographed prior to freezing using a digital camera with the entire corolla lip visible. Flowers were then frozen at  $-11\text{ }^{\circ}\text{C}$  for at least 24 h before being transferred to glassine envelopes and frozen at  $-80\text{ }^{\circ}\text{C}$  until chemical analyses.

To quantify red spotting, flower photographs were analyzed using ImageJ photo software (v1.43, National Institute of Health, 2010). Red spots were isolated using the threshold adjustment feature, measured as the number of pixels, divided by the total number of pixels in the lip, and multiplied by 100 to calculate the percentage of anthocyanin-rich areas (“percent redness”).

To measure anthocyanin concentration, frozen flowers were weighed, and then 1 mL of acidified methanol (1 % HCl) was added, and the flowers were wet-ground with the glass rods. Two milliliters of additional acidified methanol was added, after which the test tubes were covered with parafilm, vortexed, and kept dark at  $10\text{ }^{\circ}\text{C}$  for 48 h with occasional agitation. Samples were then filtered and measured with a spectrophotometer (Genesys 10S UV–Vis Spectrophotometer, Thermo Scientific) at 657- and 530-nm wavelengths. Anthocyanin concentration was quantified as  $[\text{Abs}_{657} - 0.25(\text{Abs}_{530})]/(\text{frozen flower mass})$  (Brussland 2007).

To measure condensed tannin concentration, frozen flowers were weighed and ground with tissue grinders in 3 mL of 70 % acetone and sonicated for 30 min. After 30 min of settling, 1 mL of the supernatant was pipetted out and transferred to a 15-mL centrifuge tube (Thermo Fisher Scientific, Waltham, MA) without disturbing the pellet. Six milliliters of acid butanol (5 % HCl) and 0.2 mL of iron reagent (2 % ferric ammonium sulfate in 2 N HCl) were added, and the resulting solution was left to react in a boiling water bath for 50 min. Samples were allowed to cool before spectrophotometric measurement (Genesys 10S UV–Vis Spectrophotometer, Thermo Scientific) at 550 nm. Condensed tannin levels were quantified by dividing absorbance by sample frozen mass.

Because determining chemical concentration required for destructive sampling, flowers were randomly assigned to either anthocyanin or condensed tannin analysis. When multiple samples were taken from a single branch position and time point, flowers were evenly divided between anthocyanin and condensed tannin analysis. Multiple samples of the same compounds from the same location and time point were averaged within plant.

## Statistical analysis

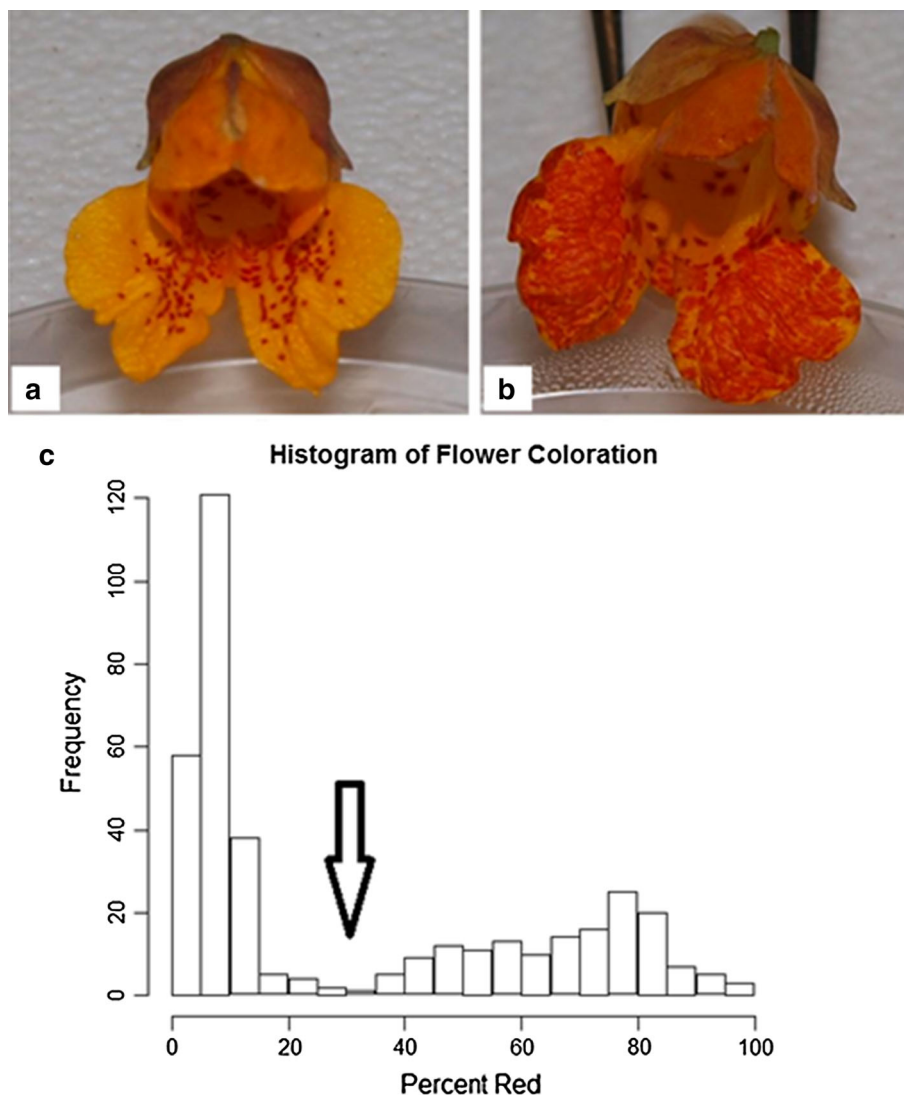
We analyzed the proportion of flowers damaged following treatments with a generalized linear mixed model (GLMM) using a binomial distribution and logit link function in the lme4 package (Bates et al. 2012) in R (R Development Core Team 2012). We analyzed mean percent flower tissue removed per damaged flower (log-transformed) with a GLMM using Gaussian distribution. For both responses, we treated plant as a random factor and treatment, date, and the treatment  $\times$  date interaction as fixed factors to determine whether the probability of florivory changed over time and whether treatment affected this, evaluating the interaction with a likelihood ratio test. The proportion of flowers damaged by florivores did not change with time, and this pattern was consistent across damage treatments (treatment  $\times$  date,  $\chi^2 = 0.57$ ,  $df = 2$ ,  $P = 0.753$ ), so the damage  $\times$  date interaction was removed from the model. Flower color (see below) was not included in this analysis because of limited sample size of yellow-flowered plants on some survey dates.

Plants consistently produced either predominantly red or predominantly yellow flowers (Fig. 1a, b) with clear bivariate distribution (Fig. 1c) with the valley between the peaks at 30 % red coloration. Because of this, plants were categorized as ‘yellow’ (having flowers with less than 30 % red coloration on the flower corolla lip,  $n = 42$  plants) or ‘red’ (greater than 30 % red coloration,  $n = 29$  plants) during analyses of chemical defenses. Plants could be easily categorized as yellow or red, with only 4 % of plants simultaneously producing both color morphs. These plants were excluded from the analysis.

We used GLMMs to test the effects of damage treatment and flower color on anthocyanin and condensed tannins in flowers collected from the same branches as and branches parallel to the focal flower. Within each location, damage treatment and flower color (red or yellow) were considered fixed factors. Because plants were sampled on multiple dates, we treated plant as a random factor and included date (days since damage treatment) as a continuous fixed factor. Models included all fixed factors, and the damage by color and damage by date interactions. Anthocyanin and condensed tannin absorbance were converted to integers and modeled with a Poisson error distribution and log-link function. All analyses were carried out in R (R Development Core Team 2011) using the function glmmPQL() in the MASS package (Venables and Ripley 2002), which corrects for overdispersion.

To test specific hypotheses about treatment effects on floral chemistry in red- and yellow-flowered plants, we used non-orthogonal contrasts: (1) control versus 30 % damage, red plants; (2) control versus 60 % damage, red plants; (3) control versus 30 % damage, yellow plants; (4)

**Fig. 1** Examples of *Impatiens capensis* flowers from **a** yellow-flowered and **b** red-flowered plants. **c** Histogram showing bimodal distribution of flower color. Flowers below 30 % red are considered yellow, and otherwise are considered red (arrow denotes delineation). Only 4 % of plants produced both color morphs



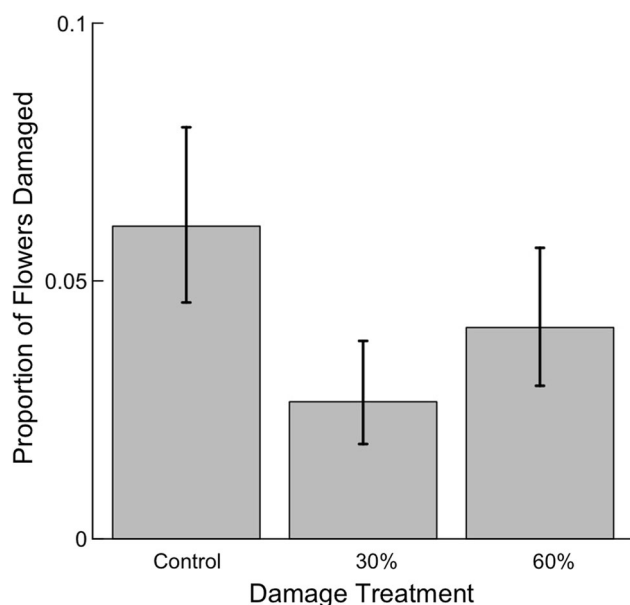
control versus 60 % damage, yellow plants; (5) red control versus yellow control. We also evaluated whether anthocyanin or condensed tannin concentration changed with collection date in control plants or damaged plants. These contrasts were carried out separately for same-branch and parallel-branch flowers because of limited sample size (some treatment/color/branch combinations were not present). For parallel-branch condensed tannin samples, the 30 and 60 % damage levels were combined due to low sample size (i.e., the contrast compared control to both damage treatments).

## Results

Floral damage treatment had a significant effect on the proportion of flowers subsequently damaged ( $\chi^2 = 25.52$ ,  $df = 2$ ,  $P < 0.001$ , Fig. 2). Planned contrasts show that

plants with 30 % damage had marginally less subsequent florivory than control plants (Wald  $z = 1.77$ ,  $P = 0.077$ ), with no significant difference between the control and 60 % damage in the proportion of flowers subsequently damaged (Wald  $z = 0.37$ ,  $P = 0.713$ ). Percent tissue removed per damaged flower was not affected by floral damage treatment ( $\chi^2 = 0.21$ ,  $df = 2$ ,  $P = 0.898$ ), or the treatment  $\times$  date interaction ( $\chi^2 = 4.80$ ,  $df = 2$ ,  $P = 0.091$ ).

Moderate (30 %) damage tended to increase subsequent anthocyanin production in flowers on the same branch, but only in red-flowered plants (Table 1; Fig. 3a). Neither the 60 % damage treatment in red morphs, nor any treatment in yellow morphs, changed the production of anthocyanins in same-branch flowers (Fig. 3a). By contrast, both moderate and heavy damage reduced anthocyanins compared to control treatments on parallel branches, but only in yellow-flowered plants (Table 1; Fig. 3b). Control group yellow



**Fig. 2** Effect of damage to a single flower on subsequent proportion of flowers damaged, averaged across 3 weekly censuses ( $\chi^2 = 25.52$ ,  $df = 2$ ,  $P < 0.001$ ). The 30 % treatment had marginally less subsequent florivory than control plants (Wald  $z = 1.77$ ,  $P = 0.077$ )

flowers had higher anthocyanin concentration than control red flowers, demonstrating an unexpected negative correlation between flower redness and anthocyanin content (Table 1). Anthocyanin concentration did not change with time since treatment (Table 1).

**Table 1** Generalized linear model assessing how 30 and 60 % floral damage treatments affected anthocyanin and condensed tannin concentration in same- and parallel-branch flowers in red and yellow *Impatiens capensis* flower morphs

Contrast	Anthocyanins			Condensed tannins		
	<i>df</i>	<i>t</i>	<i>P</i>	<i>df</i>	<i>t</i>	<i>P</i>
<i>Same-branch flowers</i>						
<i>Red-flowered plants</i>						
Control versus 30 %	38	2.000	0.053	40	0.597	0.554
Control versus 60 %	38	0.256	0.800	40	-0.060	0.952
<i>Yellow-flowered plants</i>						
Control versus 30 %	38	-0.454	0.652	40	1.029	0.310
Control versus 60 %	38	-1.261	0.215	40	-0.062	0.951
Yellow control versus red control	38	2.937	<b>0.006</b>	32	-1.510	0.831
Date	37	0.820	0.418	32	-2.660	<b>0.012</b>
<i>Parallel-branch flowers</i>						
<i>Red-flowered plants</i>						
Control versus 30 %	34	-0.241	0.811	29	0.404	0.689
Control versus 60 %	34	0.076	0.940			
<i>Yellow-flowered plants</i>						
Control versus 30 %	34	-2.045	<b>0.049</b>	40	0.159	0.875
Control versus 60 %	34	-2.047	<b>0.048</b>	40	-0.062	0.951
Yellow control versus red control	34	1.737	0.092	29	-0.219	0.829
Date	17	-1.129	0.275	20	-1.769	0.093

Bold indicates  $P < 0.05$ ; italics indicate  $P < 0.1$

Condensed tannins were not affected by damage in either red- or yellow-flowered plants, but tended to decline overall with date (Table 1; Fig. 3c, d).

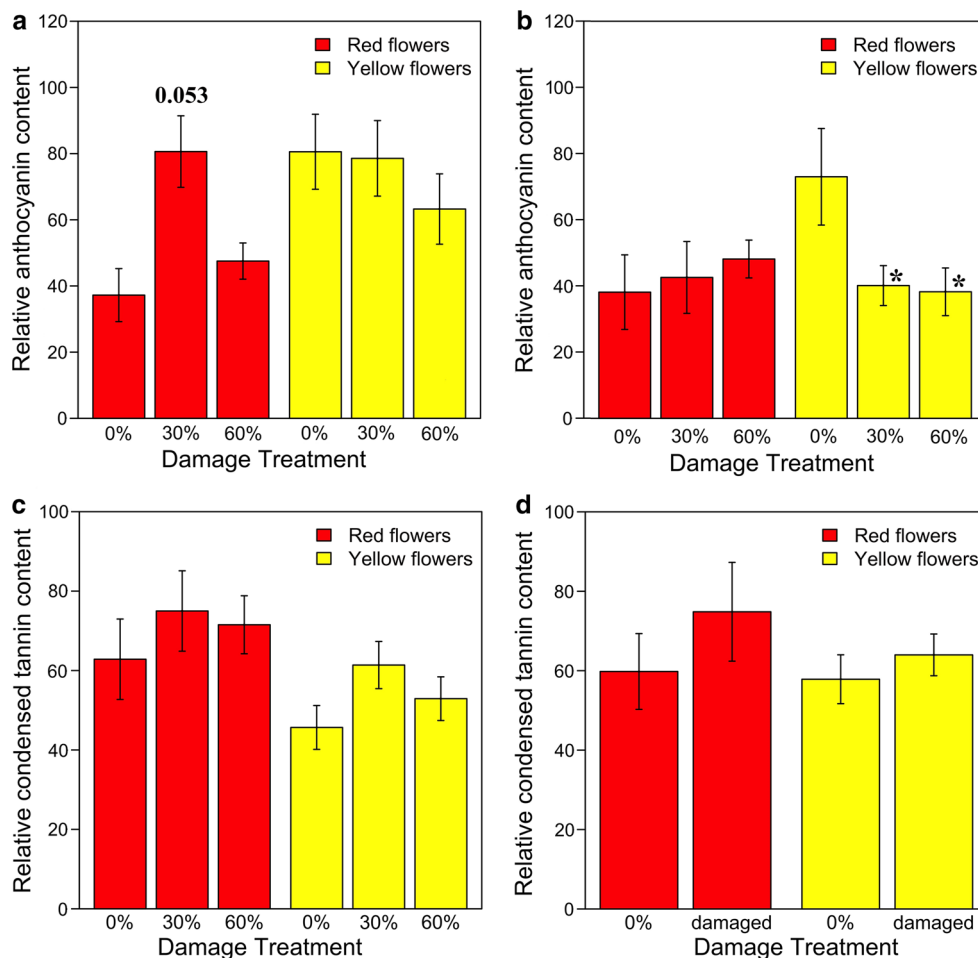
**Discussion**

We found that damage to a single flower significantly reduced subsequent whole-plant florivory over a 3-week period following damage. This result joins a growing number of studies demonstrating that small amounts of early damage to plants can have long-term consequences on subsequent interactions and potentially plant fitness (Van Zandt and Agrawal 2004). For example, leaf herbivory by the beetle *Popillia japonica* on *Oenothera biennis* induced chemical defenses in floral tissues that reduced Lepidopteran seed predation by more than half (McArt et al. 2013). Interestingly, leaf damage did not affect plant growth or reproductive phenology in isolation, but provided protection against floral damage that resulted in increased plant reproduction for leaf-damaged plants. Our study demonstrates that such an ‘inoculation’ effect of early damage may occur from damage to flowers as well as leaves.

Although anthocyanins were induced by floral damage in some circumstances, the patterns of induction suggest that these compounds were not the underlying mechanism responsible for the effect of floral damage on subsequent florivory. Floral damage induced anthocyanins in



**Fig. 3** Effect of damage treatments and flower color on **a** relative anthocyanin concentration of flowers [ $Abs_{657} - 0.25(Abs_{530}) / (\text{wet flower mass})$ ] from the same branch as the focal flower, **b** relative anthocyanin concentration of flowers from branches parallel to the focal flower, **c** relative floral condensed tannin content in flowers [ $Abs_{550} / (\text{wet flower mass})$ ] from the same branch as the focal flower, and **d** relative floral condensed tannin content in flowers from parallel branches of the focal flower. Asterisks over a damage treatment bar indicate a significant ( $P < 0.05$ ) difference, using a priori orthogonal contrasts, between that damage level and the control treatment within that color morph and branch location only. Error bars represent standard error



subsequent flowers, but in opposite directions in each color morph. In the red color morph, induction was a highly localized phenomenon that only occurred on the same branch as treated flowers. Interestingly, this localized induction only occurred in the medium-damage treatment, consistent with the result that medium but not heavy damage reduced subsequent florivory. By contrast, in yellow-flowered plants both moderate and heavy damage reduced anthocyanins, but only on parallel branches.

Because the effect of damage treatments on subsequent florivory was independent of color morph, and because we would expect decreased anthocyanins in yellow morphs to result in more rather than less florivory (Johnson et al. 2008), it does not appear that anthocyanins are responsible for the induced defense against florivores. Reduced florivory could be due to deterrence from the visual cue of initial damage (McCall and Irwin 2006), but is unlikely since the visual cue would only last approximately 2 days, the lifespan of an individual *I. capensis* flower. Additionally, a study on the closely related *Impatiens pallida* found that altering floral symmetry did not reduce seed set (Frey et al. 2005), suggesting that visual cues of damage did not

alter interactions that affect reproduction. Reduced florivory could also be due to induction of other chemical cues, including changes in volatile emissions (McCall and Irwin 2006; Zangerl and Berenbaum 2009), which merit future examination as mechanisms underlying the dramatic effect of early damage on subsequent florivory (Kessler et al. 2011; McCall and Irwin 2006).

Although the effects of florivory on subsequent plant-pollinator interactions have been examined in several systems (Botto-Mahan and Ojeda-Camacho 2000; Botto-Mahan et al. 2011; Cardel and Koptur 2010; Cares-Suarez et al. 2011; Leavitt and Robertson 2006; McCall 2008; Morris et al. 2007; Sanchez-Lafuente 2007; Sober et al. 2010), very few studies have examined whether florivory induces changes that affect subsequent floral damage. For example, Euler and Baldwin (1996) found that floral damage increased nicotine concentration in *Nicotiana attenuata* corollas. Although this induction resulted from prior damage to the same flower rather than subsequent flowers, it nonetheless demonstrates that damage to floral tissues can trigger a defense response. McCall (2006) found that artificial clipping of *Nemophila menziesii*

flowers reduced natural florivory over a 2-year period, and caterpillar damage increased resistance to florivory later in the season. These studies suggest that both local and systemic effects may result from floral damage.

To our knowledge, this is the first study testing whether the amount of floral damage affects the extent of induced defenses. Moderate levels of floral damage reduced subsequent herbivory, but heavy damage had no effect. This suggests there may be a range of damage that causes defense induction, with a threshold level beyond which less induction occurs. In general, induction can occur proportionally to the amount of damage, or abruptly as cues surpass some threshold value (Svenningsson et al. 2011). Dose-dependent thresholds were observed in two *Populus* species where, once a certain level of leaf damage was reached, leaves were more likely to abscise (Williams and Whitham 1986). In a classic example, yucca plants abort flowers that have received excessive oviposition from the yucca moth, but not when oviposition is moderate (Pellmyr and Huth 1994). Excessive tissue loss may hinder signaling to other parts of the plant (Das et al. 2013), making moderately damaged flowers more effective signalers than heavily damaged flowers. Further, beyond a certain level of damage the costs of producing or allocating defense chemicals may outweigh the benefits. At moderate levels of florivore attack, it may be advantageous to increase production of systemic defensive chemicals to ward off future attack. However, if the branch experiences severe attack, induced responses may not be effective, particularly in this system where *Popillia japonica* beetles often aggregate *en masse* (MDHB, pers. obs). Given that different levels of leaf herbivory vary widely in their effects on induced defenses and plant reproduction (McCall and Irwin 2006), future work should continue to examine how floral damage intensity affects induced defenses, subsequent interactions, and plant fitness.

We documented a flower color dimorphism in *I. capensis*, in which plants produced predominantly either red or yellow flowers (Fig. 1). To our knowledge, there has been no previous mention in the literature of a flower color dimorphism in this species, even though it has been a model system for pollination biologists (Hurlbert et al. 1996; Rust 1977; Temeles and Pan 2002; Travers et al. 2003; Wilson and Thomson 1991; Young et al. 2007). Although *I. capensis* often coexists with the congener *I. pallida*, which could explain color variation through hybridization, the two species have been shown not to hybridize (Randall and Hilu 1990). Yellow morphs had significantly higher levels of constitutive anthocyanins than red morphs (Table 1; Fig. 3a, b), which is surprising because the red spotting has previously been ascribed to anthocyanins (Close and Beadle 2003). We also found that induced responses varied with color morph, as has been

found previously for effects of leaf damage on petal glucosinolates in *Raphanus sativus* (Strauss et al. 2004). In *I. capensis*, damage induced higher anthocyanins in the red but lower anthocyanins in the yellow color morphs, while yellow morphs had higher constitutive anthocyanins. These results suggest that different defense strategies are used by each flower color morph (if anthocyanins serve a defensive function in this species), perhaps due to biochemical constraints underlying production of defense compounds that share precursors with pigment compounds, as has been found in other floral systems (Fineblum and Rausher 1997). Variation in floral pigmentation can affect interactions with both pollinators and herbivores (Chittka and Raine 2006; Doring and Chittka 2007). Therefore, the evolution of different defense strategies in each color morph could reflect selection by herbivores in addition to, or instead of, pollinators.

A caveat to our results is that we damaged flowers mechanically rather than with herbivores. Experimental manipulation of damage to floral tissue is often performed using dissecting scissors rather than herbivores (McCall 2010; McCall and Karban 2006) because flowers have a narrower window of time than leaves in which to apply damage, and it may be difficult to control natural herbivore damage to avoid damaging reproductive structures within flowers. Although saliva compounds can reduce or increase chemical induction after leaf herbivory (Musser et al. 2006; Strauss and Agrawal 1999; Weech et al. 2008), scissor damage in *Nemophila menziesii* was shown to be an accurate proxy for natural damage (McCall 2006). To our knowledge, prior research on the effect of herbivore saliva has focused on caterpillars and grasshoppers, whereas *I. capensis* is most often damaged by beetles. In addition, the timing of damage can affect induced responses (McCall 2006). The speed at which caterpillars consume tissue is often much slower than that of beetles (McCall 2006), and thus studies using caterpillars may not be analogous to beetle damage. If natural florivore damage induces greater defenses than mechanical damage, as has been found in leaf herbivory studies (McCall 2006), then our experiment may be a conservative test of the impacts of florivory on chemical induction and subsequent floral interactions.

The goal of our study was to examine the consequences of floral damage for subsequent florivory and chemical induction; examining consequences for plant reproduction was beyond the scope of this experiment. However, flowers can be tightly linked to plant fitness (Leege and Wolfe 2002), and optimal defense theory predicts that tissues that are most valuable to plant fitness should be more highly defended (Cirak et al. 2008; Frolich et al. 2007; McCall and Irwin 2006). Nonetheless, many plants, including *I. capensis*, are not solely reliant on outcrossing flowers to reproduce. *I. capensis* has both outcrossing

(chasmogamous) flowers that require pollinators and selfing (cleistogamous) flowers which produce seeds without pollinators (Rust 1977). In many systems, flowers can be produced in excess as a tolerance mechanism against damage (Wise et al. 2008), resulting in little relationship between floral production, damage, and reproduction. In *I. capensis*, production of selfing flowers can serve as a tolerance mechanism in times of stress, and selfing flowers are produced in response to leaf herbivory (Koslow and Clay 2007; Steets and Ashman 2004). Manipulations of florivory across the entire season are necessary to evaluate whether defenses that reduce florivory should be adaptive in this system.

While a growing number of studies have examined induction in floral tissues as a response to leaf damage, few have examined induction in flowers in response to floral damage (Euler and Baldwin 1996; Ohnmeiss and Baldwin 2000; Strauss et al. 2004), and fewer have incorporated different levels of damage or included both measures of florivory and changes in putative chemical defenses as responses (McCall 2006). Here, we demonstrate that damage to floral tissue induced changes in anthocyanins that vary with flower color morph. Furthermore, there was a systemic effect of damage on subsequent natural florivory over a period of 3 weeks, indicating that the first damage to a single flower can have cascading effects on long-term subsequent interactions.

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## References

- Adler LS (2000) Alkaloid uptake increases fitness in a hemiparasitic plant via reduced herbivory and increased pollination. *Am Nat* 156:92–99. doi:10.1086/303374
- Adler LS, Irwin RE (2005) Ecological costs and benefits of defenses in nectar. *Ecology* 86:2968–2978. doi:10.1890/05-0118
- Adler LS, Wink M, Distl M, Lentz AJ (2006) Leaf herbivory and nutrients increase nectar alkaloids. *Ecol Lett* 9:960–967. doi:10.1111/j.1461-0248.2006.00944.x
- Adler LS, Seifert MG, Wink M, Morse GE (2012) Reliance on pollinators predicts defensive chemistry across tobacco species. *Ecol Lett* 15:1140–1148. doi:10.1111/j.1461-0248.2012.01838.x
- Agrawal AA et al (2000) Benefits and constraints on plant defense against herbivores: spines influence the legitimate and illegitimate flower visitors of yellow star thistle, *Centaurea solstitialis* L. (Asteraceae). *Southwest Nat* 45:1–5. doi:10.2307/3672545
- Andrews ES, Theis N, Adler LS (2007) Pollinator and herbivore attraction to *Cucurbita* floral volatiles. *J Chem Ecol* 33:1682–1691. doi:10.1007/s10886-007-9337-7
- Aras A, Cevahir G, Yentur S, Eryilmaz F, Sarsag M, Cag S (2007) Investigation of anthocyanin localization in various parts of *Impatiens balsamina* L. *Biotechnol Biotechnol Equip* 21:69–73
- Bates D, Maechler M, Bolker B, Walker S (2012) lme4: linear mixed-effects models using Eigen and S4. R package version 0.999999-1. <http://cran.r-project.org/package=lme4>
- Botto-Mahan C, Ojeda-Camacho M (2000) The importance of floral damage for pollinator visitation in *Alstroemeria ligula* L. *Revista Chilena de Entomologia* 26:73–76
- Botto-Mahan C, Ramirez PA, Ossa CG, Medel R, Ojeda-Camacho M, Gonzalez AV (2011) Floral herbivory affects female reproductive success and pollinator visitation in the perennial herb *Alstroemeria ligula* (Alstroemeriaceae). *Int J Plant Sci* 172:1130–1136. doi:10.1086/662029
- Breedlove DE, Ehrlich PR (1968) Plant-herbivore coevolution—Lupines and Lycaenids. *Science* 162:671. doi:10.1126/science.162.3854.671
- Brussland J (2007) Anthocyanin extraction protocol. California State University, California
- Cardel YJ, Koptur S (2010) Effects of florivory on the pollination of flowers: an experimental field study with a perennial plant. *Int J Plant Sci* 171:283–292. doi:10.1086/650154
- Cares-Suarez R et al (2011) Do pollinators respond in a dose-dependent manner to flower herbivory? An experimental assessment in *Loasa tricolor* (Loasaceae) *Gayana. Botanica* 68:176–181
- Chittka L, Raine NE (2006) Recognition of flowers by pollinators. *Curr Opin Plant Biol* 9:428–435. doi:10.1016/j.pbi.2006.05.002
- Cirak C, Radusiene J, Janulis V, Ivanauskas L (2008) Pseudohypericin and hyperforin in *Hypericum perforatum* from northern turkey: variation among populations, plant parts and phenological stages. *J Integr Plant Biol* 50:575–580. doi:10.1111/j.1744-7909.2008.00657.x
- Close DC, Beadle CL (2003) The ecophysiology of foliar anthocyanin. *Bot Rev* 69:149–161
- Cyr H, Pace ML (1993) Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. *Nature* 361:148–150. doi:10.1038/361148a0
- Das A, Lee SH, Hyun TK, Kim SW, Kim JY (2013) Plant volatiles as method of communication. *Plant Biotechnol Rep* 7:9–26. doi:10.1007/s11816-012-0236-1
- Doring TF, Chittka L (2007) Visual ecology of aphids—a critical review on the role of colours in host finding *Arthropod-Plant Interactions* 1:3–16. doi:10.1007/s11829-006-9000-1
- Eastman J (1995) The book of swamp and bog: trees, shrubs, and wildflowers of eastern freshwater wetlands. Stackpole Books, Mechanicsburg
- Euler M, Baldwin IT (1996) The chemistry of defense and apparency in the corollas of *Nicotiana attenuata*. *Oecologia* 107:102–112
- Fineblum WL, Rausher MD (1997) Do floral pigmentation genes also influence resistance to enemies? The W locus in *Ipomoea purpurea*. *Ecology* 78:1646–1654. doi:10.1890/0012-9658(1997)078[1646:dfpgai]2.0.co;2
- Frey FM, Davis R, Delph LF (2005) Manipulation of floral symmetry does not affect seed production in *Impatiens pallida*. *Int J Plant Sci* 166:659–662. doi:10.1086/430335
- Frolich C, Ober D, Hartmann T (2007) Tissue distribution, core biosynthesis and diversification of pyrrolizidine alkaloids of the lycopsamine type in three Boraginaceae species. *Phytochemistry* 68:1026–1037. doi:10.1016/j.phytochem.2007.01.002

- Galen C (1999) Flowers and enemies: predation by nectar-thieving ants in relation to variation in floral form of an alpine wildflower. *Polemonium viscosum* Oikos 85:426–434. doi:10.2307/3546692
- Hendrix SD (1984) Reactions of *Heracleum lanatum* to floral herbivory by *Depressaria pastinacella*. *Ecology* 65:191–197. doi:10.2307/1939470
- Hurlbert AH, Hosoi SA, Temeles EJ, Ewald PW (1996) Mobility of *Impatiens capensis* flowers: Effect on pollen deposition and hummingbird foraging. *Oecologia* 105:243–246
- Johnson ET, Berhow MA, Dowd PF (2008) Colored and white sectors from star-patterned petunia flowers display differential resistance to corn earworm and cabbage looper larvae. *J Chem Ecol* 34:757–765. doi:10.1007/s10886-008-9444-0
- Karageorgou P, Manetas Y (2006) The importance of being red when young: anthocyanins and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light. *Tree Physiol* 26:613–621
- Kawagoe T, Kudoh H (2010) Escape from floral herbivory by early flowering in *Arabidopsis halleri* subsp. *gemmifera*. *Oecologia* 164:713–720. doi:10.1007/s00442-010-1709-y
- Kessler A, Halitschke R (2009) Testing the potential for conflicting selection on floral chemical traits by pollinators and herbivores: predictions and case study. *Funct Ecol* 23:901–912. doi:10.1111/j.1365-2435.2009.01639.x
- Kessler A, Halitschke R, Baldwin IT (2004) Silencing the jasmonate cascade: induced plant defenses and insect populations. *Science* 305:665–668. doi:10.1126/science.1096931
- Kessler A, Halitschke R, Poveda K (2011) Herbivory-mediated pollinator limitation: negative impacts of induced volatiles on plant-pollinator interactions. *Ecology* 92:1769–1780
- Koslow JM, Clay K (2007) The mixed mating system of *Impatiens capensis* and infection by a foliar rust pathogen: patterns of resistance and fitness consequences. *Evolution* 61:2643–2654. doi:10.1111/j.1558-5646.2007.00224.x
- Krupnick GA, Weis AE, Campbell DR (1999) The consequences of floral herbivory for pollinator service to *Isomeris arborea*. *Ecology* 80:125–134. doi:10.1890/0012-9658(1999)080[0125:tcofhf]2.0.co;2
- Leavitt H, Robertson IC (2006) Petal herbivory by chrysomelid beetles (*Phyllotreta* sp.) is detrimental to pollination and seed production in *Lepidium papilliferum* (Brassicaceae). *Ecol Entomol* 31:657–660. doi:10.1111/j.1365-2311.2006.00820.x
- Leege LM, Wolfe LM (2002) Do floral herbivores respond to variation in flower characteristics in *Gelsemium sempervirens* (Loganiaceae), a distylous vine? *Am J Bot* 89:1270–1274
- Marlin D, Nicolson SW, Yusuf AA, Stevenson PC, Heyman HM, Kruger K (2014) The only African Wild tobacco. *Nicotiana africana*: alkaloid content and the effect of herbivory. *Plos One* 9:e102661. doi:10.1371/journal.pone.0102661
- Marquis RJ (1984) Leaf herbivores decrease fitness of a tropical plant. *Science* 226:537–539. doi:10.1126/science.226.4674.537
- McArt SH, Halitschke R, Salminen J-P, Thaler JS (2013) Leaf herbivory increases plant fitness via induced resistance to seed predators. *Ecology* 94:966–975
- McCall AC (2006) Natural and artificial floral damage induces resistance in *Nemophila menziesii* (Hydrophyllaceae) flowers. *Oikos* 112:660–666
- McCall AC (2008) Florivory affects pollinator visitation and female fitness in *Nemophila menziesii*. *Oecologia* 155:729–737. doi:10.1007/s00442-007-0934-5
- McCall AC (2010) Does dose-dependent petal damage affect pollen limitation in an annual plant? *Botany* 88:601–606. doi:10.1139/b10-032
- McCall AC, Fordyce JA (2010) Can optimal defence theory be used to predict the distribution of plant chemical defences? *J Ecol* 98:985–992. doi:10.1111/j.1365-2745.2010.01693.x
- McCall AC, Irwin RE (2006) Florivory: the intersection of pollination and herbivory. *Ecol Lett* 9:1351–1365. doi:10.1111/j.1461-0248.2006.00975.x
- McCall AC, Karban R (2006) Induced defense in *Nicotiana attenuata* (Solanaceae) fruit and flowers. *Oecologia* 146:566–571. doi:10.1007/s00442-005-0284-0
- Mooney KA, Halitschke R, Kessler A, Agrawal AA (2010) Evolutionary trade-offs in plants mediate the strength of trophic cascades. *Science* 327:1642–1644. doi:10.1126/science.1184814
- Morris WF et al (2007) Direct and interactive effects of enemies and mutualists on plant performance: a meta-analysis. *Ecology* 88:1021–1029. doi:10.1890/06-0442
- Musser RO, Farmer E, Peiffer M, Williams SA, Felton GW (2006) Ablation of caterpillar labial salivary glands: technique for determining the role of saliva in insect-plant interactions. *J Chem Ecol* 32:981–992. doi:10.1007/s10886-006-9049-4
- Ohnmeiss TE, Baldwin IT (2000) Optimal defense theory predicts the ontogeny of an induced nicotine defense. *Ecology* 81:1765–1783. doi:10.1890/0012-9658(2000)081[1765:odtpto]2.0.co;2
- Pellmyr O, Huth CJ (1994) Evolutionary stability of mutualism between yuccas and yucca moths. *Nature* 372:257–260. doi:10.1038/372257a0
- R Development Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org>.
- Randall JL, Hilu KW (1990) Interference through improper pollen transfer in mixed stands of *Impatiens capensis* and *I. pallida* (Balsaminaceae). *Am J Bot* 77:939–944. doi:10.2307/2444509
- Rust RW (1977) Pollination in *Impatiens capensis* and *Impatiens pallida* (Balsaminaceae). *Bull Torrey Bot Club* 104:361–367
- Salminen JP, Karonen M (2011) Chemical ecology of tannins and other phenolics: we need a change in approach. *Funct Ecol* 25:325–338. doi:10.1111/j.1365-2435.2010.01826.x
- Sanchez-Lafuente AM (2007) Corolla herbivory, pollination success and fruit predation in complex flowers: an experimental study with *Linaria lilacina* (Scrophulariaceae). *Ann Bot* 99:355–364. doi:10.1093/aob/mcl267
- Schaffner U et al (2011) Plant invasions, generalist herbivores, and novel defense weapons. *Ecology* 92:829–835. doi:10.1890/10-1230.1
- Schemske DW (1978) Evolution of reproductive characteristics in *Impatiens* (Balsaminaceae)—Significance of cleistogamy and chasmogamy. *Ecology* 59:596–613
- Schiestl FP, Kirk H, Bigler L, Cozzolino S, Desurmont GA (2014) Herbivory and floral signaling: phenotypic plasticity and trade-offs between reproduction and indirect defense. *New Phytol* 203:257–266. doi:10.1111/nph.12783
- Schmitt J, Ehrhardt D, Swartz D (1985) Differential dispersal of self-fertilized and outcrossed progeny in jewelweed (*Impatiens capensis*). *Am Nat* 126:570–575
- Simpson RL, Leck MA, Parker VT (1985) The comparative ecology of *Impatiens capensis* Meerb (Balsaminaceae) in central New Jersey. *Bull Torrey Bot Club* 112:295–311. doi:10.2307/2996545
- Sober V, Moora M, Teder T (2010) Florivores decrease pollinator visitation in a self-incompatible plant. *Basic Appl Ecol* 11:669–675. doi:10.1016/j.baec.2010.09.006
- Soper Gordon N, Adler LS (2013) Abiotic conditions affect antagonists and mutualists in *Impatiens capensis*. *Am J Bot* 100:679–689. doi:10.3732/ajb.1200460
- Steets JA, Ashman TL (2004) Herbivory alters the expression of a mixed-mating system. *Am J Bot* 91:1046–1051. doi:10.3732/ajb.91.7.1046
- Strauss SY, Agrawal AA (1999) The ecology and evolution of plant tolerance to herbivory. *Trends Ecol Evol* 14:179–185

- Strauss SY, Irwin RE, Lambrix VM (2004) Optimal defence theory and flower petal colour predict variation in the secondary chemistry of wild radish. *J Ecol* 92:132–141
- Svenningsen TO, Holen OH, Leimar O (2011) Inducible defenses: continuous reaction norms or threshold traits? *Am Nat* 178:397–410. doi:10.1086/661250
- Temeles EJ, Pan IL (2002) Effect of nectar robbery on phase duration, nectar volume, and pollination in a protandrous plant. *Int J Plant Sci* 163:803–808. doi:10.1086/342033
- Travers SE, Temeles EJ, Pan I (2003) The relationship between nectar spur curvature in jewelweed (*Impatiens capensis*) and pollen removal by hummingbird pollinators. *Can J Bot Rev Can Bot* 81:164–170. doi:10.1139/b03-014
- Van Zandt PA, Agrawal AA (2004) Community-wide impacts of herbivore-induced plant responses in milkweed (*Asclepias syriaca*). *Ecology* 85:2616–2629. doi:10.1890/03-0622
- Venables WN & Ripley BD (2002) Modern applied statistics with S. 4th edn. Springer, New York
- Viswanathan DV, Thaler JS (2004) Plant vascular architecture and within-plant spatial patterns in resource quality following herbivory. *J Chem Ecol* 30:531–543. doi:10.1023/B:JOEC.0000018627.26420.e0
- Wackers FL, Bezemer TM (2003) Root herbivory induces an above-ground indirect defence. *Ecol Lett* 6:9–12
- Waterman PG, Choo GM, Vedder AL, Watts D (1983) Digestibility, digestion-inhibitors and nutrients of herbaceous foliage and green stems from an African montane flora and comparison with other tropical flora. *Oecologia* 60:244–249
- Weech MH, Chapleau M, Pan L, Ide C, Bede JC (2008) Caterpillar saliva interferes with induced *Arabidopsis thaliana* defence responses via the systemic acquired resistance pathway. *J Exp Bot* 59:2437–2448. doi:10.1093/jxb/ern108
- Williams AG, Whitham TG (1986) Premature leaf abscission—an induced plant defense against gall aphids. *Ecology* 67:1619–1627. doi:10.2307/1939093
- Wilson P, Thomson JD (1991) Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. *Ecology* 72:1503–1507. doi:10.2307/1941124
- Wise MJ, Cummins JJ, De Young C (2008) Compensation for floral herbivory in *Solanum carolinense*: identifying mechanisms of tolerance. *Evol Ecol* 22:19–37. doi:10.1007/s10682-007-9156-x
- Young HJ (2008) Selection on spur shape in *Impatiens capensis*. *Oecologia* 156:535–543. doi:10.1007/s00442-008-1014-1
- Young HJ, Dunning DW, von Hasseln KW (2007) Foraging behavior affects pollen removal and deposition in *Impatiens capensis* (Balsaminaceae). *Am J Bot* 94:1267–1271. doi:10.3732/ajb.94.7.1267
- Zangerl AR, Berenbaum MR (2009) Effects of florivory on floral volatile emissions and pollination success in the wild parsnip *Arthropod-Plant Interactions* 3:181–191. doi:10.1007/s11829-009-9071-x
- Zangerl AR, Rutledge CE (1996) The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. *Am Nat* 147:599–608