Florivory shapes both leaf and floral interactions

NICOLE L. SOPER GORDEN[1],† AND LYNN S. ADLER

Department of Biology and Graduate Program in Plant Biology, University of Massachusetts,
221 Morrill Science Center South, 611 North Pleasant Street, Amherst, Massachusetts 01003 USA

Abstract. Florivory, or the consumption of flowers, is a ubiquitous interaction that can reduce plant reproduction directly by damaging reproductive tissues and indirectly by deterring pollinators. However, we know surprisingly little about how florivory alters plant traits or the larger community of species interactions. Although leaf damage is known to affect floral traits and interactions in many systems, the consequences of floral damage for leaf traits and interactions are unknown. We manipulated floral damage in *Impatiens capensis* and measured effects on floral attractive traits and secondary chemicals, leaf secondary chemicals, floral interactions, leaf herbivory, and plant reproduction. We also examined relationships between early season floral traits and floral interactions, to explore which traits structure floral interactions. Moderate but not high florivory significantly increased relative selfed reproduction, leading to a shift in mating system away from outcrossing. Florivory increased leaf secondary compounds and decreased leaf herbivory, although mechanisms other than leaf chemistry may be responsible for some of the reduced leaf damage. Florivory altered four of seven measured interactions, including increased subsequent florivory and reduced flower spiders, although only leaf damage effects were significant after correcting for multiple tests. Pretreatment concentrations of floral anthocyanins and condensed tannins were associated with reduced levels of many floral antagonisms, including florivory, nectar larceny, and flower spider abundance, suggesting these traits play a role in floral resistance. Overall, our results indicate a broad range of community and potential evolutionary consequences of florivory through structuring subsequent floral interactions, altering leaf secondary chemicals, and shaping leaf herbivory.

[1] Present address: Department of Natural Sciences (Biology), Mars Hill University, 100 Athletic Street, Campus Box 6671, Mars Hill, North Carolina 28754 USA.
† **E-mail:** nsopergorden@mhu.edu

INTRODUCTION

While the role of pollinators in the evolution of floral diversity is well recognized (e.g., Fenster et al. 2004), there are many antagonists that are also attracted to flowers and can shape selection on floral traits. For example, florivores (herbivores that consume flowers) can be as or even more common than leaf herbivores (McCall and Irwin 2006). Florivores can directly reduce plant reproduction by damaging pollen or ovules (McCall and Irwin 2006), and, in severe cases, can cause the near collapse of plant populations (Washitani et al. 1996). Florivores can also have

indirect effects on plant reproduction by altering the preferences of other floral visitors, such as pollinators (McCall and Irwin 2006), or by changing traits including nectar production (Krupnick et al. 1999), flower size (Mothershead and Marquis 2000), and floral symmetry (McCall 2008).

While several studies have addressed how floral damage affects pollinator visitation (reviewed in McCall and Irwin 2006), the influence of florivory on other interactions is largely unknown. The net impact of florivory on plant reproduction could be strengthened or weakened if floral damage increases susceptibility to further damage, or induces defenses that reduce subsequent damage. In contrast, community consequences of leaf herbivory on subsequent antagonisms are well known (Strauss and Irwin 2004, Van Zandt and Agrawal 2004). The identity of the herbivore that first damages a plant can have cascading impacts on the entire community of subsequent consumers (e.g., Van Zandt and Agrawal 2004), and leaf herbivores can have strong indirect effects on plant reproduction by altering floral interactions. For example, early leaf damage by invasive *Popillia japonica* beetles to *Oenethera biennis* rosettes had little direct impact on reproduction but induced floral defenses that reduced seed predation, leading to a net increase in plant fitness (McArt et al. 2013). While the effect of leaf damage on floral interactions is relatively well-studied, the effect of floral damage on leaf interactions remains unknown.

Although florivory can have greater impacts on plant reproduction than leaf damage (McCall and Irwin 2006), we know surprisingly little about traits that mediate resistance to florivory relative to the wealth of information on resistance traits against leaf herbivory (e.g., Koricheva 2002). In dioecious or gynodioecious plants, florivores preferentially damage male (e.g., Ashman 2002) or hermaphrodite (Ashman 2002, McCall and Barr 2012) flowers over female flowers. In observational studies, reduced florivory can be associated with plants with smaller or less conspicuous flowers (Ashman et al. 2004, McCall and Barr 2012), or certain flower colors (e.g., pink vs. white flowers in *Raphanus sativus*; McCall et al. 2013). Manipulative studies found that corolla diameter explained florivore preference more than anther presence or corolla color (McCall and Barr 2012), and that facultative beetle florivores

preferred taller flowering plants (Held and Potter 2004), possibly due to more visible cues for flying insects. It has been hypothesized that many of the traits that attract pollinators will also attract florivores; some data support this for nectar robbing (e.g., Galen and Cuba 2001) but this prediction is largely untested for florivores (McCall and Irwin 2006).

In addition, although chemical defenses are most commonly studied in leaves, such defenses are also often present in flowers, sometimes at higher concentrations than in leaves (e.g., Euler and Baldwin 1996). Floral pigments can reduce florivore feeding and increase florivore mortality (Johnson et al. 2008, McCall et al. 2013). For example, transformed wild tobacco plants lacking nicotine had increased floral damage as well as nectar robbing (Kessler et al. 2008), and high furanocoumarin genotypes of *Pastinaca sativa* suffered less inflorescence damage (Zangerl and Berenbaum 1993). These studies suggest that chemical defenses that deter leaf herbivores should also be effective against florivores. Furthermore, because flowers are intimately related to plant fitness, optimal defense theory predicts that flowers should be well-defended, perhaps with constitutive defenses (McCall and Fordyce 2010). Floral chemical defenses can be induced following leaf damage (Euler and Baldwin 1996, Adler et al. 2006, McCall and Karban 2006, McArt et al. 2013), and one study has shown that floral damage induces resistance to subsequent florivores (McCall 2006). However, induction of chemical defenses in flowers following floral damage and the consequences of floral induction for interactions beyond pollination are unknown (but see Boyer et al. 2016). While flowers are often strong physiological sinks, which may reduce the likelihood of sending systemic vascular signals, floral damage could induce volatile signals that are detected by other plant tissues (Hopkins and Hüner 2004), or generalist herbivores that are deterred from feeding on flowers may switch to leaf tissue, structuring interactions beyond the floral sphere. Many generalist and even specialist Lepidopteran and Coleopteran herbivores feed on both floral and leaf tissue (Held and Potter 2004, McCall and Irwin 2006), suggesting that effects of florivory on leaf damage through changes in herbivore preference may be common.

While florivores are generally considered antagonists, their effects on plant reproduction can vary from neutral to negative (McCall and Irwin 2006). Although most studies have focused on plant female reproduction without considering seed quality, florivory may result in equivalent total reproduction but increased selfing (Penet et al. 2009). Selfing, through geitogamy or self-pollination, can have negative impacts on population dynamics and gene flow beyond reducing seed production. Selfed fruits can have more limited dispersal (Schmitt et al. 1985) and subsequent seedlings can be at a disadvantage during establishment (Waller 1985). Inbred plants often have fewer (Walisch et al. 2012), smaller flowers (Andersson 2012) and smaller leaves (Walisch et al. 2012), and may produce fewer or different volatiles (Ferrari et al. 2006). These changes may alter offspring attractiveness to pollinators or plant antagonists and so structure future animal as well as plant communities.

We manipulated florivory and assessed effects on floral attractive traits, floral and leaf secondary chemicals, floral and leaf interactions, and plant reproduction. We also measured pretreatment floral traits to shed light on their role structuring species interactions. Overall, we found that floral damage affected offspring quality and had far-reaching consequences, shaping traits and structuring interactions in both leaves and flowers.

METHODS

Study system

Impatiens capensis Meerb. (Balsaminaceae) is an annual native herb that grows in partial shade and moist soil (Leck 1979). It has a mixed-mating system with both selfing cleistogamous (CL) and open-pollinated chasmogamous (CH) flowers. CH flowers are protandrous, spending their first ~36 h in a male phase and their final ~12 h in a female phase. CH flowers are pollinated mostly by *Bombus* sp. and *Apis mellifera*, and are incapable of selfing due to floral anatomy and strong protandry (Rust 1977, Eastman 1995, Steets and Ashman 2004). Geitonogamy has been estimated at only 8.6% (Waller 1980). Both flower types produce capsule fruits with one to several seeds that dehisce explosively when mature. In Massachusetts, *I. capensis* generally germinates in late April or early May,

CL flowers appear in May, and CH flowers last from mid-July until mid-September. Seeds generally are not viable for more than 1 yr (Simpson et al. 1985), resulting in little to no seed bank. Unless otherwise indicated, "flowers" refers to CH flowers hereafter.

Impatiens capensis has many antagonists. Flowers are robbed by several insect species (including *Bombus* spp. and *Vespula maculifrons*), and visited commonly by nectar thieves such as ants and halictids that consume nectar without pollinating (Rust 1979, Eastman 1995, Young 2008). *Popillia japonica* (Scarabaeidae) beetles and other generalist herbivores consume petal and sepal tissue (N. L. Soper Gorden, *personal observation*). There is a species-specific Cecidomyiidae flower bud galler, *Schizomyia impatientis* (Hummel 1956). *Misumena vatia* crab spiders (Thomisidae) inhabit *I. capensis* flowers as pollinator predators (N. L. Soper Gorden, *personal observation*). Leaf herbivores include true bugs (Hemiptera), grasshoppers (Orthoptera), katydids (Tettigoniidae), aphids (Aphidoidea), and *P. japonica* (Eastman 1995, Steets and Ashman 2004).

Several floral traits could attract or deter floral visitors. Attractive traits may include the number or size of flowers, nectar or pollen production, or plant height, which alters flower visibility. *Impatiens capensis* flower color can vary from entirely yellow (no red spotting) to almost entirely red (extensive red spotting; Boyer et al. 2016). *Impatiens* spp. contain anthocyanins and condensed tannins (Clevenger 1971, Boyer et al. 2016). Anthocyanins are the most common flavonoid pigments, and can attract pollinators (Delpech 2000, Koes et al. 2005) and reduce florivore preference (Johnson et al. 2008). Anthocyanins are present in *Impatiens* spp. leaves, flowers, and stems, and cause the variable red spots on the lip petals of *I. capensis* (Aras et al. 2007). Condensed tannins are common in plant species that have anthocyanins, including *Impatiens* spp. (Waterman et al. 1983). Although condensed tannins are usually measured as vegetative defenses, they are also found in floral tissue and have the potential to deter florivores (Burggraaf et al. 2008).

Study location

The experiment took place at Hampshire Farm on Hampshire College, Amherst, MA (N 42°19′ W 72°31′). The site has a large

population of wild *I. capensis* plants. Study plots were located along the northwest edge of a swampy stand of trees. On 4 May 2010, we collected naturally growing *I. capensis* seedlings from the site and transplanted them into 10 cm diameter pots (Fafard #2 potting soil, Conrad Fafard, Inc, Agawam, MA). Seedlings were maintained in a greenhouse, with daily watering and weekly re-randomization of bench location.

On 1 June 2010, 200 plants were established at Hampshire Farm, in four rows of 50 plants closely following the contours of the forest edge to maintain shady conditions. Plants were 1 m apart. Wild growing *I. capensis* seedlings within a 25 cm diameter of experimental plants were removed to alleviate intraspecific competition, but all other wild plants were left in place. Transplant survival was high, and only four plants needed to be replaced in the first week due to mortality.

Treatments

We randomly assigned each plant to one of three floral damage treatments: 0% (control), 30%, or 60% flower tissue removed. Floral damage treatments were applied to every fourth flower throughout the flowering season using dissecting scissors, removing lip and throat tissue without damaging the spur or reproductive parts (Appendix S1). Plants produced on average 113 flowers (range 0–634) throughout the season (average 1.4 flowers per day; range 0–98), with an average of 4.96 flowers treated over the season. Although florivores in many systems cause significant damage to flowers through direct damage to reproductive parts, in *I. capensis* we almost never observed damage to the stigma, androecium, or ovules (N. L. Soper Gorden, *personal observation*). Both damage treatments had asymmetrical flowers compared to the bilaterally symmetrical control flowers; natural insect florivory in this species also alters flower symmetry (N. L. Soper Gorden, *personal observation*). Natural florivory was allowed on all plants. Treatment damage levels were based on previous data from 391 flowers on 107 plants that had a mean of 18.9% ± 4.0% (mean ± SE) of flowers damaged per plant, removing 31.5% ± 1.1% (mean ± SE) floral tissue (range 0–95%; data from Soper Gorden and Adler 2013). Thus, our treatments were well within the range of natural damage levels.

Floral attractive traits

All floral traits were measured on CH flowers. We counted total flower production per plant. Flower size was measured on up to three flowers per plant seven times during the summer after treatments had begun, using five morphometric measurements (lip height and width, spur length, total flower length, and corolla opening height), which were highly correlated ($r > 0.39$, $P < 0.0001$ for all). Principal components analysis was used to reduce the five measures into one variable (prcomp() in R; R Development Core Team, 2.13.0, 2011, Vienna, Austria), with the first PC reflecting overall flower size and explaining 65% of the variance.

Nectar volume was measured in the middle of the summer, after treatments had begun, on up to two male phase flowers per plant that were bagged as buds to prevent pollinator visitation. Male and female phase flowers produce different amounts of nectar (Rust 1979, Young 2008, Soper Gorden and Adler 2013), so nectar was measured in male phase flowers for consistency. Nectar volume was measured during the first 8 h after flowers opened using microcapillary tubes by inserting the tube into the flower's throat, then snipping the end of the spur and squeezing remaining nectar into the tube.

Pollen production was estimated with anthers collected from the same flowers used for nectar measurements. Since the flowers were bagged as buds, no pollen could have been removed by pollinators. We collected the androecium upon dehiscence and excluded anthers that had shed pollen before collection. Pollen production was estimated by removing the entire androecium into a microcentrifuge tube, drying at 45°C for 48 h, suspending in 1.0 ml 70% ethanol, and counting 10 μL pollen samples six times per androecium on a hemacytometer; counts were averaged to produce one value per androecium.

Flower color was quantified from photographs, measured as the percent area of the flower lip that was red vs. yellow-orange using the threshold and measure features on ImageJ (v.1.43, National Institute of Health, 2010, Bethesda, Maryland, USA). We measured flower color on two flowers before and two flowers after treatments were applied. For the explanatory variable of flower color, plants were categorized as either "red" (>20% red on lip) or "yellow" (<20% red on lip) flow-

ered based on pretreatment measurements, using a naturally occurring cut-off in color data (Boyer et al. 2016). For the response variable of change in flower color, we subtracted the early average from the late average flower redness for each plant; no plant switched from red to yellow flowers (or vice versa) during the growing season.

Secondary chemicals

We collected flowers and leaves from each plant twice to measure anthocyanins and condensed tannins. Two flowers (one for anthocyanins and one for condensed tannins) were collected from the first flowers produced by each plant before treatments began ("early;" 16 July 2010–13 September 2010); a second set of two flowers was collected after 30 August 2010 (131 plants) or when the plant had at least one treated flower (22 plants; "late"), whichever came later. Because our damage treatments were imposed on every fourth flower for the entire season, sampling was constrained to happen while plants were still being damaged. By sampling once late in the season, our intent was to compare season-long changes in defenses due to recurring damage. All flowers were photographed with a digital camera for color analysis, then frozen at −80°C until defense extraction. Early season leaves were collected at the beginning of the season, before flowering started, whereas late season leaves were collected at the end of the season, after all other data had been collected. Leaves were stored at −80°C until defense extraction, at which point they were dried at 45°C for 48 h; drying the leaves after freezing had no effect on extracted defenses, as long as leaves were kept in the dark (NLSG, *unpublished data*). Anthocyanin and condensed tannin extraction and analysis methods are described in Appendix S2.

Insect interactions

Three times over the summer (July 6–7, 22 and 29), percent leaf damage was estimated on the four newest fully expanded leaves and crab spiders and flower bud galls were counted on the whole plant. Florivory was measured five times during the summer on all open flowers as flower tissue missing per flower, distinguishing between treatment and natural damage. This allowed us to test how our florivory manipulation affected natural insect florivory.

Pollinators, nectar robbers, and nectar thieves were observed during 15 min surveys of each plant with open flowers on 10 d throughout the flowering period. On each pollinator observation day, all plants with open flowers were observed. Due to differences in flowering, this resulted in plants having between one and three 15-min surveys. All floral visitors were identified to interaction type (pollinator, robber, or thief) and taxonomic group (*Bombus* sp., *A. mellifera*, or other insects to family), and their probe time recorded. Bumble bees and honey bees are both legitimate pollinators of *I. capensis* (Rust 1977, Eastman 1995, Steets and Ashman 2004). Smaller visitors (such as halictid bees and ants) were considered nectar thieves unless they were explicitly seen contacting pollen. Nectar robbers (mostly *V. maculifrons*) were observed chewing holes in nectar spurs and drinking.

Plant growth and reproduction

Plant growth was measured approximately once a month throughout the summer as plant height, the number of nodes, and average leaf size (leaf length × width for the three most apical fully expanded leaves). Aboveground tissues were harvested, dried, and weighed as each plant died or on 11 October 2010 after the first frost.

Approximately every 2 weeks, the number of CH and CL fruits on each plant was counted. Total fruit production can be counted from pedicel scars from dehisced fruits, but the process is extremely time-consuming. Previous work showed that the average number of fruits per day was highly correlated with the total number of fruits produced up to that point ($n = 40$, $r^2 = 0.91$, $P < 0.0001$; Soper Gorden and Adler 2013), so average CH and CL fruits per day were used to estimate total fruit production. Mature fruits were collected and stored at 4°C until seeds per fruit were counted and weighed. Seed mass is highly correlated with germination in this species (Waller 1985). Because *I. capensis* plants can respond to decreased CH reproduction with increased CL reproduction without changing total female reproduction (e.g., Steets and Ashman 2004), we also calculated the proportion of CH vs. CL fruits.

Statistical analyses

The effect of florivory on logical sets of trait response variables (plant growth, floral secondary chemicals, leaf secondary chemicals, floral attractiveness, and nectar/pollen production) was tested using five separate MANCOVAs (v 9.2, SAS Institute, 2008, Cary, North Carolina, USA); individual ANCOVAs were investigated when MANCOVA results were significant, and Tukey's Studentized Range Tests were used to test for differences between individual treatments. In each MANCOVA, the independent variables were treatment, initial flower color (categorical – red or yellow), early season floral anthocyanins and condensed tannins, early season leaf anthocyanins and condensed tannins, initial plant height, and Julian date of first flower. Plant growth was analyzed using plant height, number of nodes, leaf size, and final dry biomass, with biomass log transformed to improve normality. Floral secondary chemicals were analyzed using floral anthocyanins and floral condensed tannins from late season flowers, both square root transformed to improve normality. Leaf secondary chemicals were analyzed as late season leaf anthocyanins and leaf condensed tannins, with leaf anthocyanins log transformed to improve normality. Floral attractiveness traits were analyzed using the total number of CH flowers, flower size (using PC1 from the PCA on flower morphology), and flower color, measured as the change in redness over the season to assess whether treatments altered color; both the number of flowers and the change in flower redness were log transformed to improve normality. Because of a limited number of samples, nectar and pollen production were tested in their own MANCOVA instead of being included as traits in our floral attractiveness test; nectar volume was square root transformed to improve normality.

Many floral interactions and plant reproduction measurements were highly non-normal, and were therefore tested using generalized linear models (GLIMs): number of pollinator, nectar robber, and nectar thief visits per hour (multiplying the number per 15 min observation by 4 and rounding to the nearest whole number); percent leaf herbivory and subsequent florivory; number of flower bud gallers and crab spiders; number of CH and CL fruits; number of CH and CL seeds

per fruit; seed mass for CH and CL fruits; and proportion of CH fruits. All GLIMs were run in R (R Development Core Team, 2.13.0, 2011, Vienna, Austria) using glm() and Tukey's post hoc means comparisons using the multcomp() package (Hothorn et al. 2008). Count data (pollinators, robbers, thieves, gallers, spiders, fruits, seeds per fruit) were analyzed using a Poisson distribution with a log link function with the exception of number of fruits for both fruit types, which used a quasipoisson distribution with a log link function due to overdispersion; seed mass measures were analyzed using a Gaussian distribution with an identity link function; proportional variables (percent herbivory, percent florivory, and proportion of CH fruits) used a binomial distribution with a logit link function (Tables 1 and 2). As in the MANCOVAs, each GLIM used early season traits as covariates in the analysis in addition to treatment. For response traits measured more than once during the summer, the average value per plot was used (rounded to the nearest integer for counts). Because we conducted 14 separate GLIM analyses, we used Bonferroni corrections to set our alpha at $P = 0.004$.

RESULTS

Effects of florivory treatments

Experimental florivory treatments had no significant effect on any measure of plant growth, floral secondary chemicals, floral attractive traits, or nectar and pollen production (MANCOVA: $F \leq 0.95$, $P \geq 0.47$ for all; Appendix S3). Florivory did, however, significantly affect leaf secondary chemicals (MANCOVA: $F_{4,164} = 4.12$, $P = 0.003$), with higher leaf anthocyanins in plants with high compared to medium florivory (ANCOVA: $F_{1,92} = 6.60$, $P = 0.002$), although control levels were intermediate (Fig. 1). High florivory also significantly reduced the percent leaf herbivory relative to control plants, with a nonsignificant trend for plants with moderate florivory (Table 1, Fig. 2G). In total, florivory influenced four of seven measured interactions, including increasing subsequent florivory (Fig. 2F) and reducing flower spiders (Fig. 2D) and nectar thief visits (Fig. 2E), but none except leaf damage were significant after Bonferroni corrections with alpha at $P = 0.004$ (Table 1). Florivory did not

Table 1. Generalized linear model results of florivory treatments (post hoc comparisons of control, moderate, and high damage) and early season floral trait covariates on insect interactions.

Explanatory variable	Pollinator visits	Nectar robbers	Nectar thieves	Flower galls	Flower spiders	Florivory	Leaf herbivory
Distribution	P	P	P	P	P	B	B
Control vs. high damage	−2.031	0.618	−2.902	−1.181	−2.133	1.650	**−4.066*****
Control vs. moderate damage	−0.507	0.347	−0.318	−0.635	*−2.860*	*2.434*	*−2.901*
Moderate vs. high damage	1.561	−0.258	*2.631*	−0.588	−0.737	0.812	1.210
Flower color	−0.386	**−4.562*****	**−3.206***	0.133	1.056	**4.139*****	−0.315
Date of first flower	1.594	*2.471*	**−3.699****	−0.629	*−2.584*	**3.017***	0.749
Initial plant height	**4.769*****	**7.710*****	1.865	0.524	−1.097	**3.242***	−0.825
Floral anthocyanins	**3.38***	1.475	**3.179***	−1.749	−2.422	**−4.945*****	1.632
Floral condensed tannins	−1.568	**−4.906*****	**−4.476*****	1.389	**−3.491****	*−2.441*	**3.394***
Leaf anthocyanins	*2.740*	**3.992*****	−2.033	1.444	**−3.865****	−1.057	**2.854***
Leaf condensed tannins	**−3.219***	−0.244	*2.653*	0.849	−0.169	*−2.695*	−1.208

Notes: Explanatory variables are listed in the far left column and the column headings are response variables. Positive values indicate an increase, and negative values a decrease; for flower color, positive values indicate increased redness. Distribution indicates which distribution was used for the analysis (P, Poisson; B, Binomial). Bonferroni corrections set α = 0.004. Bold indicates significant results; italics indicate results that were significant before Bonferroni corrections. *<0.004, **<0.001, ***<0.0001.

affect pollinator or nectar robber visits or flower galls (Table 1, Fig. 2). Florivory did not affect fruit or seed production or seed weight (Table 2, Fig. 3) but did alter the proportion of CH vs. CL fruits; plants with moderate florivory had proportionally fewer CH fruits than plants with control or high florivory (Table 2, Fig. 3G).

Relationships between floral traits and species interactions

We found several effects of early season plant traits on subsequent interactions (Table 1). Nectar robbers and nectar thieves visited red flowered plants more, whereas florivores damaged yellow flowered plants more. Higher early floral anthocyanins were associated with more visitation by pollinators and nectar thieves, and less florivory. Early floral condensed tannins were associated with lower visitation by nectar robbers, nectar thieves, and flower spiders, and higher levels of leaf herbivory (Table 1), suggesting that floral secondary chemicals shape leaf interactions. Similarly, early leaf secondary chemicals shaped floral interactions. Plants with high levels of

Table 2. Generalized linear models results of florivory treatments (post hoc comparisons of control, moderate, and high damage) and early season floral trait covariates on plant reproduction.

Explanatory variable	Number of CH fruits	Number of CL fruits	Seeds per CH fruit	Seeds per CL fruit	CH seed mass	CL seed mass	CH to CL fruit ratio
Distribution	QP	QP	P	P	G	G	B
Control vs. high damage	−0.423	−0.056	0.440	−0.989	−0.447	−0.550	−0.767
Control vs. moderate damage	−1.711	−0.648	*2.688*	1.091	−0.782	*−2.448*	**−5.302***
Moderate vs. high damage	−1.293	−0.594	2.180	2.102	−0.346	−1.897	**−4.507***
Flower color	0.311	−0.719	0.685	0.583	*−2.183*	−1.166	**3.315***
Date of first flower	**−4.337*****	**−3.730****	**−4.059*****	−1.248	0.851	−0.169	**−14.257*****
Initial plant height	*2.251*	1.765	**3.207***	1.689	1.192	1.413	**6.193*****
Floral anthocyanins	1.226	*1.992*	**3.668***	0.524	0.308	*2.131*	0.238
Floral condensed tannins	−0.813	−0.836	−0.731	−0.748	1.609	**3.011***	**−6.449*****
Leaf anthocyanins	0.187	1.079	*2.538*	0.909	−0.116	−0.264	0.063
Leaf condensed tannins	0.554	0.656	*−2.838*	−1.211	1.285	−0.826	1.055

Notes: Explanatory variables are listed in the far left column and the column headings are response variables. Positive values indicate an increase, and negative values a decrease; for flower color, positive values indicate increased redness. Distribution indicates which distribution was used for the analysis (P = Poisson, B = Binomial, G = Gaussian, QP = Quasipoisson). Bonferroni corrections set α = 0.004. Bold indicates significant results; italics indicate results that were significant before Bonferroni corrections. *<0.004, **<0.001, ***<0.0001.
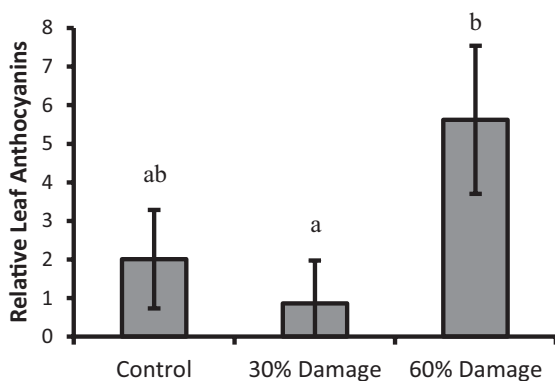
Fig. 1. Effect of artificial florivory (no damage, 30% flower tissue removed, or 60% flower tissue removed) on relative leaf anthocyanins. This value was calculated based on absorbance with a UV–Vis spectrophotometer as abs(530 nm)–0.25abs(657 nm), as per Mancinelli (1990), and then scaled by initial dry weight leaf mass, providing relative anthocyanin concentration per g dry weight. See Appendix S2 for further method details. Error bars show standard error. Lower case letters indicate significant differences between treatments, using Tukey's Studentized Range Test.

early leaf anthocyanins had more leaf herbivory and nectar robbing, but fewer flower spiders, whereas plants with high levels of early leaf condensed tannins had fewer pollinator visits (Table 1). Early season traits were also correlated with late season traits (Appendix S3) and several measures of plant reproduction (Table 2; discussion of relationships between early season traits and reproduction in Appendix S3).

Discussion

Effects of florivory treatments

Although previous studies have demonstrated that florivory can deter pollinators (reviewed in McCall and Irwin 2006), the community effects of florivory on floral as well as leaf interactions are largely unexplored. Through an experimental manipulation, we found that florivory had surprising consequences, increasing leaf defenses and reducing leaf damage. Florivory also tended to influence a wide range of other floral interactions, although pollinators were unaffected. This suggests that floral damage may have consequences beyond direct damage to flowers or even pollinator deterrence

by altering the community of subsequent interactions on both flowers and leaves.

Surprisingly, floral damage decreased leaf herbivory (Fig. 2G), indicating that the consequences of floral damage extend well beyond the floral interface. Given of the importance of leaf damage for belowground interactions (van Dam and Heil 2011) and nutrient cycling (Frost and Hunter 2004), our results suggest that, by decreasing leaf damage, florivory may have wide-ranging community and ecosystem-level consequences. While previous studies have shown that leaf damage can induce resistance in flowers (Euler and Baldwin 1996, Adler et al. 2006, McCall and Karban 2006), we are unaware of any previous work examining how floral damage affects leaf damage or chemical defenses.

A variety of nonexclusive mechanisms may explain the effect of florivory on leaf damage. First, induced leaf chemical defenses could play a role; high floral damage resulted in concentrations of leaf anthocyanins that were nearly six times higher than in plants with moderate damage and nearly twice as high as control plants, although the latter comparison was not significant (Fig. 1). However, induced leaf anthocyanins are unlikely to be the sole mechanism reducing leaf damage, since leaf anthocyanins were induced only with high florivory, and the impacts of florivory on leaf damage were similar at medium and high florivory levels (compare Figs. 1 and 2G). It is also possible that the induction of other leaf resistance traits, such as volatiles that deter leaf herbivores or changes in nutritional value due to resource reallocation, could be the underlying mechanism behind changes in leaf damage, or that plants with damaged flowers are less visually appealing to herbivores. If florivory accurately predicts the probability of subsequent leaf damage, induced vegetative defenses in response to floral damage could be adaptive (Karban et al. 1999).

It is also possible that reduced damage to leaves is due to increased attractiveness of damaged flowers, rather than changes in leaf quality (reviewed in Lucas-Barbosa et al. 2015). All of the florivores on *I. capensis* are generalists that consume leaves as well as flowers; *P. japonica* is considered a "facultative florivore" that often consumes a mixed diet of floral and leaf tissue of many hosts, but preferred and performed well on floral tissue (Held and Potter 2004).
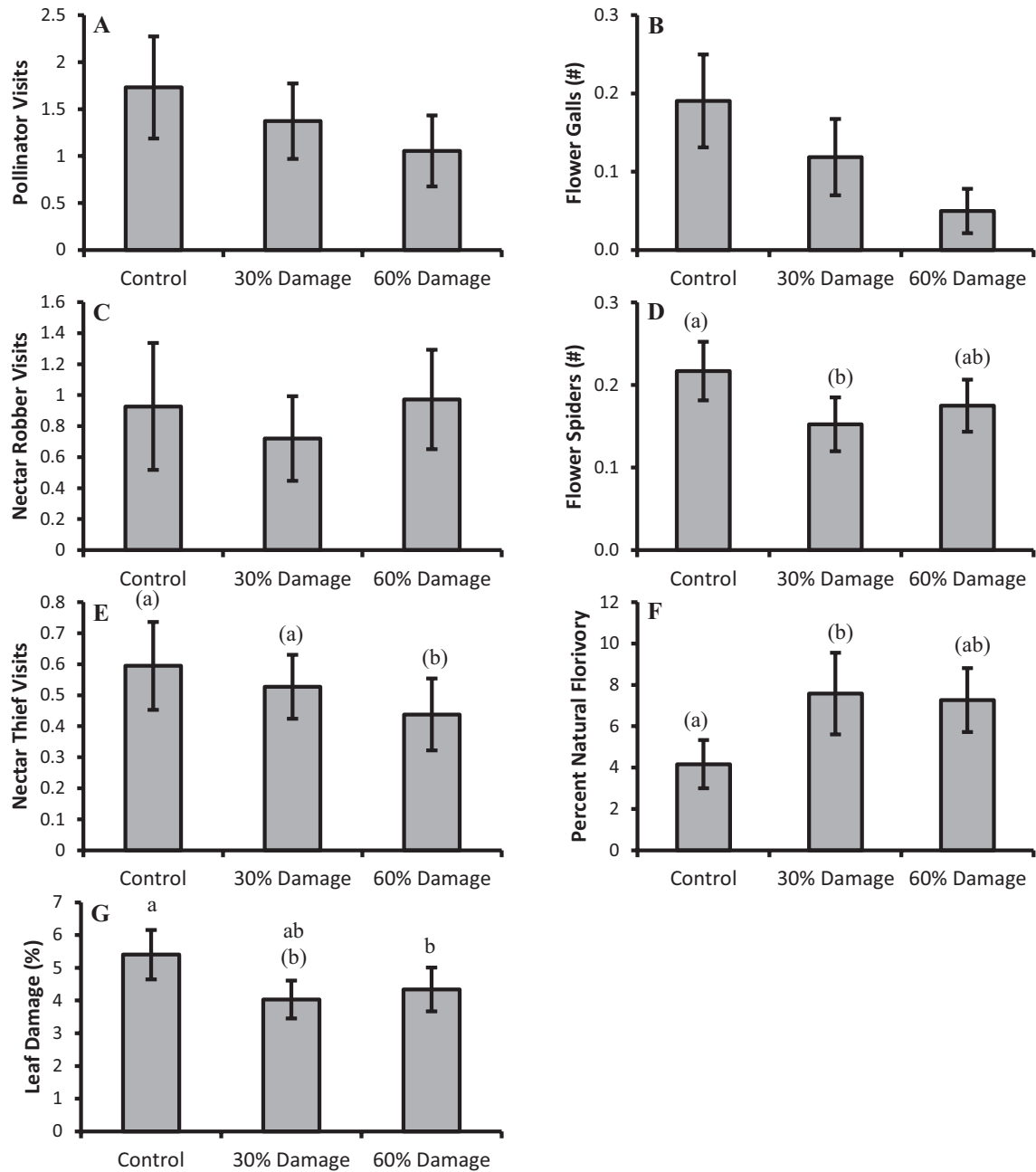
Fig. 2.   Effect of artificial florivory (no damage, 30% flower tissue removed, or 60% flower tissue removed) on floral and leaf interactions. (A) Total pollinator visits per hour, (B) Flower galls per plant over the season, (C) Nectar robber visits per hour, (D) Number of flower spiders per flower per census, (E) Nectar thief visits per hour, (F) Percent florivory per flower averaged across censuses, (G) Percent leaf herbivory per leaf averaged across censuses. Error bars show standard error. Lower case letters indicate significant differences between treatments at $\alpha = 0.004$ (with Bonferroni correction), using Tukey's Studentized Range Test. Letters in parentheses indicate treatments that were significantly different before but not after Bonferroni correction.
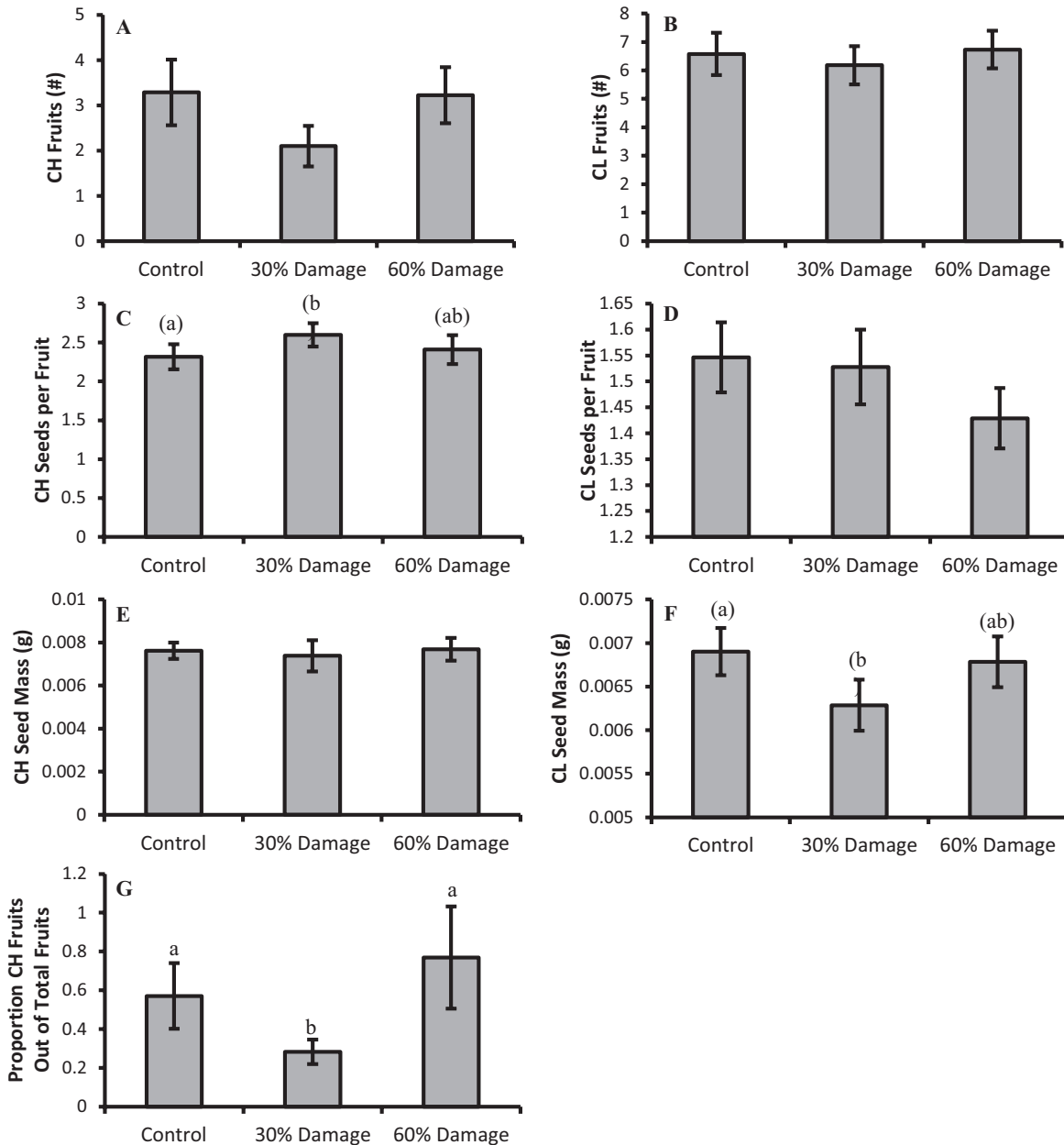
Fig. 3. Effect of artificial florivory (no damage, 30% flower tissue removed, or 60% flower tissue removed) on measures of plant reproduction. (A) Mean number of CH fruits per weekly census, (B) Mean number of CL fruits per weekly census, (C) mean CH seeds per fruit, (D) mean CL seeds per fruit, (E) mean CH seed mass, (F) mean CL seed mass, (G) proportion of CH (chasmogamous, outcrossing fruits) relative to total fruit number. Error bars show standard error. Lower case letters indicate significant differences between treatments at α = 0.004 (with Bonferroni correction), using Tukey's Studentized Range Test. Letters in parentheses indicate treatments that were significantly different before but not after Bonferroni correction.

Although the effect of florivory on floral volatiles has rarely been examined, in two systems floral damage increased components of floral volatile emissions (Rose and Tumlinson 2004, Zangerl and Berenbaum 2009). If damaged flowers emit volatiles or other cues that attract herbivores, then decreased leaf herbivory may be due to herbivores switching from leaf to floral tissue. Experimental florivory tended to increase natural florivory as well as decrease leaf herbivory (Fig. 2F), consistent with this hypothesis. Many other Lepidopteran and Coleopteran herbivores, even those that are specialists on particular plant taxa, can consume both leaves and flowers (e.g., Lucas-Barbosa et al. 2014). Thus, our results suggest that florivory could affect leaf interactions in many systems due to changes in herbivore preference for tissue types.

Although floral damage affected leaf secondary chemicals, it had no effect on the floral secondary chemicals measured. Optimal defense theory predicts that flowers should be well-defended against damage because of their close association with fitness, with constitutive rather than induced defenses to protect flowers before damage occurs (McCall and Irwin 2006, McCall and Fordyce 2010). Our observational results suggest that floral anthocyanins may have a defensive function against florivory; there was a negative correlation between early season floral anthocyanins and florivory (Table 1), and anthocyanins have been implicated as defenses against florivores in petunias (Johnson et al. 2008) and radishes (McCall et al. 2013). However, natural levels of florivory are high despite anthocyanins, reaching 95% on some flowers (data from Soper Gorden and Adler 2013). Taken together, the lack of induction of floral defenses and the continued high levels of florivory suggest that *I. capensis* may rely on tolerance mechanisms, such as increased selfing, instead of resistance.

In addition to reduced leaf herbivory, experimental florivory also influenced several other interactions (Table 1, Fig. 2), including increasing subsequent florivory, reducing the number of flower spiders, and reducing nectar thief visits. Although none of these interactions except leaf damage were significant after Bonferroni corrections with alpha at $P = 0.004$, we note that four of seven measured interactions were significant before such a correction (Table 1, Fig. 2).

The probability of having this many significant tests at alpha = 0.05 is 0.0019 (calculated based on Moran 2003). The use of Bonferroni corrections may result in under-estimating the effects of manipulations in field studies, since effects may not be highly statistically significant due to substantial environmental variation (Moran 2003). Thus, florivory may have substantial effects on the community of floral as well as leaf interactions. The net effect of florivory on plant reproduction via changes in species interactions will ultimately depend on the balance of changes in negative and positive floral interactions. In our experiment, the net effect of florivory on female reproduction was negligible; this may be due to tolerance mechanisms, or due to a cancelling out of consequences for subsequent interactions, including negative effects (e.g., increased subsequent florivory) and positive effects (e.g., decreased visits by nectar thieves and flower spiders that consume pollinators).

Surprisingly, experimental florivory did not affect interactions with pollinators or nectar robbers (Table 1; Fig. 2A, C). This contrasts with previous studies, which have typically found that florivory reduces subsequent pollination (reviewed in McCall and Irwin 2006). Our florivory treatments altered flower size and symmetry (Appendix S1: Figure S1), but did not affect nectar or pollen rewards. This suggests that visitors to *I. capensis* flowers were driven more by rewards such as nectar and pollen than by visual cues. This result is consistent with a study showing that altered symmetry had no effect on pollinator visitation or plant reproduction in *Impatiens pallida*, a close congener of *I. capensis* (Frey et al. 2005).

In addition to influencing interactions, moderate florivory altered the mating system by reducing the proportion of total fruits that were from CH flowers without changing total fruit number, although we did not observe this effect with high florivory (Table 2, Fig. 3G). Thus, studies that focus on total fruit number without considering mating system may miss important consequences of floral damage that does not remove reproductive parts. Because CL flowers are inconspicuous and therefore difficult to count, we do not have the data to assess whether the different effects of moderate and high florivory on mating system are due to changes in production of CH vs. CL flowers, or differences in fruit maturation from

each flower type. In other systems, a shift toward a greater reliance on self-pollination after florivory has also been documented (Ashman and Penet 2007, Penet et al. 2009), and *I. capensis* has been previously shown to respond to other antagonisms, including leaf herbivory and competition, by increasing selfing CL reproduction (Steets 2005, Steets et al. 2006*a*,*b*). Making flowers less apparent (e.g., with small corollas or inserted anthers) may provide resistance to florivores (Ashman et al. 2004, McCall and Irwin 2006); increased allocation to inconspicuous CL over showy CH flowers could be a mechanism of induced resistance to florivores. Alternatively, in plants with a mixed mating system such as *I. capensis*, selfing may be a mechanism of tolerating antagonists. Compared to leaves and CH flowers, CL flowers require fewer resources to produce (Waller 1979) and inbreeding depression for most traits in *I. capensis* is low (Heschel et al. 2005). Therefore, tolerance via increased selfing may be a more effective strategy than investing in chemical defenses against florivory.

Relationships between floral traits and species interactions

We observed correlations between early season floral secondary chemicals and subsequent visitation by flower insects; generally, nectar consumers preferred flowers with high floral anthocyanins, whereas floral antagonists preferred flowers with low floral condensed tannins (Table 1). Floral condensed tannins were negatively correlated with nectar robbing, nectar thieving, and florivory (although the latter was not significant after Bonferroni correction) but not with pollination (Table 1). Thus, both anthocyanins and condensed tannins may function as defenses against florivory, and condensed tannins may provide more broad-spectrum floral defense. Interestingly, leaf herbivores preferred plants with higher levels of floral condensed tannins, suggesting there may be a tradeoff between protecting flowers and protecting leaves. Alternatively, by affecting floral interactions including deterring predatory spiders (Table 1), floral condensed tannins may increase leaf herbivory.

Early season leaf defense levels were also related to floral visitation rates. For example, pollinators preferred plants with lower leaf condensed tannins, whereas nectar robbers preferred plants with higher leaf anthocyanins (Table 1). This suggests that not only do interactions with flowers affect leaf defenses and herbivory, but leaf defenses could also affect interactions with flowers, either through reducing damage that could increase available resources for flowering or through changes in resource or defense allocation. Future research should focus on teasing apart the direct and indirect effects of leaf interactions and floral interactions.

Our data show a pattern that florivores tended to prefer the opposite traits of those preferred by nectar consumers. While nectar consumers preferred plants that flowered earlier, had red flowers, and had higher levels of floral anthocyanins, florivores preferred late flowering plants with yellow flowers and less floral anthocyanins (Table 1). This may highlight the difference between which floral resource (nectar vs. petal tissue) is being used. Alternately, the difference in preference may be due to which insect orders consume nectar vs. eat flowers, with mostly bees and flies in the former category and mostly beetles, caterpillars, and grasshoppers in the latter. For example, while bees tend to focus on flower color as a cue for visitation (Willmer 2011), beetles might ignore flower color and instead avoid anthocyanin defenses (Johnson et al. 2008, McCall et al. 2013). The only trait nectar consumers and florivores preferred in common was taller plants (Table 1); this could simply be a function of plant resource availability, where plants with higher resources grow larger and therefore attract more interactions (e.g., Soper Gorden and Adler 2013).

CONCLUSIONS

Florivory significantly reduced leaf herbivory and increased leaf anthocyanins, suggesting broad community consequences of floral damage for whole-plant interactions. Florivory also influenced several other plant-insect interactions, including a tendency to cause increased subsequent florivory. However, florivores did not induce changes in floral anthocyanins or condensed tannins. Moderate florivory altered mating system expression, leading to a greater proportion of selfed reproduction. Decreasing allocation to outcrossing reproduction could

provide a mechanism of tolerating florivory, or of resistance through reduced floral display. Overall, our results indicate that florivory may shape the community of species that interact with plants, alter interactions such as leaf herbivory that occur outside the realm of flowers, and alter mating systems.

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Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1326/supinfo