

# Effects of florivory on plant-pollinator interactions: Implications for male and female components of plant reproduction<sup>1</sup>

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**PREMISE OF THE STUDY:** Florivory could have direct negative effects on plant fitness due to consumption of floral organs, and indirect effects mediated through changes in traits important to pollination. These effects likely vary with plant sexual system, depending on sex- or morph-specific patterns of damage. We investigated the direct and indirect effects of simulated florivory on male and female components of reproduction in the native, distylous vine *Gelsemium sempervirens*.

**METHODS:** We crossed floral damage and supplemental pollination treatments in a common garden array and tracked pollinator behavioral responses. We also estimated male function using fluorescent dye as an analog for pollen transfer, and measured both fruit and seed production.

**KEY RESULTS:** The effects of floral damage varied by floral morph, the genus of floral visitor, and the component of reproduction measured. Damage reduced the number of pollinator visits to pin but not thrum plants, and increased the time some pollinators spent per flower in thrum but not pin plants. Flowers of damaged plants transferred more dye particles to recipient plants compared to undamaged plants, but only later in the season when the majority of dye transfer occurred. Damage had no effect on female reproduction.

**CONCLUSION:** These results suggest that florivory can have positive indirect effects on estimated male plant reproduction through changes in different pollinators' behavior at flowers, but the effects of floral damage vary with male vs. female function. These results underscore the importance of other species' interactions at flowers in driving pollinator behavior and pollen transfer dynamics.

**KEY WORDS** florivory; floral herbivory; pollination; indirect effects; pollen transfer

Flowers host a diverse web of species interactions with mutualist pollinators (Bascompte et al., 2003; Olesen et al., 2007) and antagonists that consume floral parts or rewards without providing pollination services (McCall and Irwin, 2006; Irwin et al., 2011). Many flowers receive damage from floral herbivores (hereafter florivores; McCall and Irwin, 2006), which, like foliar herbivores, can reduce seed production directly through the consumption of floral

reproductive parts and also indirectly via changes in pollination (Mothershead and Marquis, 2000; McCall and Irwin, 2006; Ashman and Penet, 2007). For example, bud herbivory in *Castilleja indivisa* Engelm. (Scrophulariaceae) has direct negative effects on seed production through reduced numbers of flowers and fruits, in addition to indirect negative effects on seed set through reduced pollinator visitation (Adler et al., 2001). While a growing number of studies have documented the effects of florivory on female components of plant reproduction, fewer have documented effects on male plant reproduction (reviewed in Schaeffer et al., 2013). However, given that male reproduction is often more sensitive to changes in pollinator behavior than female reproduction (e.g., Young and Stanton, 1990) and that florivory can affect traits important for pollinator attraction and behavior (Krupnick et al., 1999; Mothershead and Marquis, 2000), florivory has the potential to strongly affect male plant function.

The effects of florivory on male and female components of reproduction are likely to be influenced by plant sexual system, given

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that the sexual organs in flowers of dioecious (including gyno- and androdioecy) or heterostylous species may not be equally susceptible to florivory (Ashman, 2002; Ashman and Penet, 2007). For example, larvae of the tiger moth *Platyprepia virginialis* (Boisduval, 1852, Arctiidae) prefer hermaphroditic flowers of *Nemophila menziesii* Hook. & AM. (Boraginaceae) over entirely female flowers, leading to greater levels of floral damage to hermaphroditic plants (McCall and Barr, 2012). Moreover, even in cases where florivores target floral sexes or morphs equally, they may differentially damage male vs. female floral reproductive organs. For example, although the frequency of florivory on two morphs of distylous *Gelsemium sempervirens* (L.) J.St.-Hil. (Loganiaceae) did not differ, there were morph-specific differences in the pattern of damage to male and female structures, due to differences in the exertion of stigmas and anthers between morphs (Leege and Wolfe, 2002). Such morph-specific differences in florivore damage could lead to sex-specific effects of florivory between morphs. Florivory could directly reduce fruit and seed set in morphs where stigmas are consumed, and seeds sired in morphs where anthers are consumed. In addition, if floral damage decreases pollinator visitation or alters pollinator behavior differently between morphs (Krupnick and Weis, 1999), it could also lead to morph-specific indirect effects on female and male function via changes in pollination.

Although in most cases plant consumption reduces fitness (Louda and Potvin, 1995; Strauss et al., 2002), in some instances plants can compensate for, or even benefit from, being eaten. Positive effects of foliar herbivory have been documented via male and female components of plant reproduction (Paige, 1992; Gronemeyer et al., 1997; Agrawal, 1998). The mechanisms driving such overcompensation to herbivore damage are varied, but can include resource reallocation, increased photosynthetic rates, and increased growth (reviewed in Strauss and Agrawal, 1999). For example, pollen beetles, *Meligethes rufimanus* LeConte, 1857 (Nitidulidae), reduced pollinator attractiveness of cleome, *Isomeris arborea* Nutt. (Cleomaceae), resulting in lower pollen deposition, reduced seed set, and decreased pollen donation (Krupnick et al., 1999); however, plants partially compensated for the negative effects through increased flower production (Krupnick and Weis, 1999). In some cases, florivory can even benefit plants (reviewed in McCall and Irwin, 2006). For example, if pollinators probe a lower proportion of flowers on damaged plants, plants could benefit from reduced geitonogamous (within-plant) pollen transfer (Juenger and Bergelson, 2000; Irwin, 2003). Floral damage can also alter the expression of floral sexual characteristics in sexually labile species, altering the ratio of female-to-hermaphroditic flowers (Hendrix, 1984; Krupnick and Weis, 1998), which could subsequently alter maternal and paternal investment in reproduction to maximize reproductive output. Nonetheless, few studies to date have documented the degree to which florivores have negative vs. positive effects on both male and female reproductive success and the mechanisms involved.

We studied the effects of florivory on pollinator visitation and male and female components of plant reproduction in the distylous vine *Gelsemium sempervirens* (hereafter *Gelsemium*). *Gelsemium* is native to the southeastern United States, and its pollination success is highly variable across sites (Carper, 2013) where it interacts not only with mutualist pollinators, but also with florivores (Irwin et al., 2014). Florivory could be an important driver of variation in pollination success to *Gelsemium* (McCall and Irwin, 2006). To understand the role of florivory in driving pollinator behavior,

pollination, and *Gelsemium* reproduction, we manipulated both floral damage (simulated florivory) and pollination (supplemental pollination) in a common garden. Specifically, we asked (1) how does floral damage affect pollinator foraging behavior, and (2) what are the direct and pollinator-mediated indirect effects of floral damage on male and female components of plant reproduction?

## MATERIALS AND METHODS

**Study system**—*Gelsemium sempervirens* is a native perennial, evergreen vine that occurs across the southeastern United States (Radford et al., 1968). Plants bloom for approximately six weeks in March and April. *Gelsemium* is distylous with tubular, yellow flowers that bloom for 5–7 days. Plants are either pin or thrum morphs. Pins have flowers with long styles and short stamens, whereas thrums have flowers with short styles and long stamens (Ornduff, 1970, 1979, 1980). *Gelsemium* is an obligate out-crosser and is intramorph incompatible, so that each morph is dependent on the opposite morph for fertilization. *Gelsemium* flowers are pollinated primarily by bees, including bumble bees, *Bombus bimaculatus* Cresson, 1863 (Apidae); honey bees, *Apis mellifera* Linnaeus, 1758 (Apidae); carpenter bees, *Xylocopa virginica* (Linnaeus, 1771, Apidae); blue orchard bees, *Osmia lignaria* Say 1837 (Megachilidae); and blueberry bees *Habropoda laboriosa* (Fabricius, 1804, Apidae), as well as by Lepidoptera and flies (Adler and Irwin, 2005). Carpenter bees visit flowers legitimately (usually transferring less pollen per visit than other bee visitors), but can also act as nectar robbers (Adler and Irwin, 2005, 2006).

In addition to pollinators and nectar robbers, *Gelsemium* interacts with the florivore *Amphipyra pyramidoides* Guenée, 1852 (Noctuidae), a common moth in forests of eastern North America. Larvae are generalist herbivores feeding on foliage of a variety of tree and shrub species (Wagner, 2005), as well as developing buds and flowers of *Gelsemium*. Florivores eat both petal tissue and exerted floral organs, typically consuming stigmas of pin flowers and anthers of thrum flowers (Leege and Wolfe, 2002). Florivory can be common on *Gelsemium*, with up to 60% of plants in some populations experiencing floral damage, and up to 90% of petal tissue removed from damaged flowers (Irwin et al., 2014).

**Experimental design**—To isolate the effects of floral damage on *Gelsemium* reproduction, we used horticulturally propagated *Gelsemium* in an experimental array (as in Adler and Irwin, 2005). Using horticultural plants in an array allowed us to reduce variation due to abiotic factors, such as water and nutrient availability, in a wild-growing population. Plants were purchased from a number of local distributors, garden centers, and nurseries around Raleigh, North Carolina, to obtain equal numbers of the two floral morphs and ensure adequate diversity of out-crossed pollen.

The array was located in the arboretum at Carl A. Schenck Memorial Forest, in Raleigh. The forest has a wild-growing *Gelsemium* population similar in density to those throughout the region (A. L. Carper, pers. obs.). Wild-growing *Gelsemium* occurred within 50 m of the array, ensuring that the array was placed in suitable habitat where associated pollinators would be found. We arranged 160 plants (80 pin and 80 thrum plants, each in 3.8 L pots) in a 10 m × 16 m array, leaving 1 m between plants (as in Adler and Irwin, 2005). Plants were fertilized once at the beginning of the flowering season with a 15-30-15 NPK fertilizer (Colorburst, Pursell

Industries, Sylacuaga, AL), and watered three times per week thereafter to minimize potential resource limitation. Any flowers or fruits produced prior to transplanting the plants into the array were counted and removed.

**Floral manipulations**—Study plants were randomly assigned to one of four treatments (20 plants per treatment per morph) representing a factorial combination of hand-pollination (supplemental vs. open) crossed with floral damage (control or damage) treatments. Crossing pollination and floral damage treatments allowed us to test for direct effects of florivory when pollen was not limiting (pollen supplementation) and the indirect effects when plants received ambient amounts of pollinator visitation (open pollination). We numbered each plant with a unique ID and visited all plants 2–3 times per week throughout the blooming season to perform pollination and damage treatments to ensure that all newly opening flowers were treated, and all treatments were performed at the whole-plant level.

Flowers of plants in the supplemental pollination treatment were hand-pollinated using camel-hair brushes to deposit pollen directly onto stigmas. To reduce possible effects of out-breeding depression between horticultural and local genotypes and to standardize supplemental pollen quality across treatments, we established a pollen-donor garden of approximately 40 horticultural *Gelsemium* adjacent to the array. Donor plants were spread around the array approximately 5 m apart and 5–10 m from any experimental plants. Pollen was collected from this donor population for supplemental pollination, combining pollen from at least three plants of the opposite morph for hand pollinations. This pollen donor garden was also available for free-flying pollinators to visit, so the open pollination treatment also had the potential to receive pollen from this donor garden. Plants in the open pollination treatment received no supplemental pollen, but their flowers were similarly handled during floral counts to mimic any effect of handling flowers during supplementation. All flowers in both treatments remained open to natural pollination.

Flowers on plants in the floral damage treatment were mechanically damaged using a hole-punch to remove approximately 50% of floral tissue most distal on the flower on 40–60% of open flowers. This is a common damage level and is a pattern of damage observed in the field (Irwin et al., 2014). In addition, given that morphs differ in the exertion of stigmas and anthers, which affects the probability of which floral organs will be damaged (Leege and Wolfe, 2002), we damaged exerted reproductive parts on damage treatments by removing either stigmas (in pin flowers) or anthers (in thrum flowers). Each day that we performed damage treatments, we also recorded the total number of open intact and damaged flowers. We did not actively deter natural florivores; however, natural florivory within the array was low (<1% of flowers). Because we relied on mechanical damage in this study, we could isolate the effects of damage on pollinator visitation and plant reproduction in the absence of any potential positive or negative responses that could be caused by the saliva of florivorous insects (Kessler et al., 2010), which could be assessed in future research.

**Pollinator visitation**—To determine how floral damage affected pollinator behavior, we observed floral visitors as they foraged throughout the array. Over nine days of observations spread across the flowering season, two observers used hand-held digital recorders (Olympus America, Center Valley, PA) to follow individual floral

visitors. On days of pollinator observation, we observed visitors for approximately one hour each in the morning and afternoon. We followed individual pollinators as they entered the garden—recording the identity of the visitor to the lowest taxonomic resolution possible (typically genus or species)—which plants each visited, the number of flowers probed per plant, and the time spent per flower. We also recorded the total number of open flowers on each plant on the day of observations. For each plant, we calculated the total number of pollinator visits throughout the blooming season, excluding nectar-robbing visits, as well as the mean proportion of flowers probed per plant visit and mean time spent per flower.

To test the effect of floral damage on floral visitor behavior, we used three metrics: total number of visits per plant, mean proportion of flowers probed per plant visit, and mean time spent per flower. We restricted analyses to only legitimate floral visitors (i.e., no nectar robbing visits) and excluded any flower visits that were greater than 90 s, because these visitors only rested in flowers and we did not observe them collecting nectar or pollen. Nectar robbing was infrequent (<1% of floral visits) so it was excluded from all analyses. Pollination treatment was not significant in any analysis of visitation data and was therefore not included in the final models. We tested if floral damage affected the total number of plant visits using ANCOVA (PROC GLM, SAS 9.2, SAS Institute, Cary, NC), with floral morph, floral damage and their interaction as fixed factors, and the total number of flowers per plant as a covariate. We used the combined number of visits for all genera given that not all genera visited each plant, and  $\log_{10}$  transformed the number of visits to meet the assumptions of normality. We used separate generalized linear mixed models to test the effects of floral damage on the mean proportion of flowers probed per plant and mean time spent per flower by each visitor, specifying a binomial distribution for the proportion of flowers probed per plant and a gamma distribution for the time spent per flower. We excluded visits to plants with less than three open flowers to reduce outliers, and included damage treatment, floral morph, and visitor genus as fixed factors, all possible interactions between them, and the number of open flowers per plant as a covariate. We included a unique visitor ID as a random effect in the model to account for visits to different plants made by individual visitors and to avoid pseudoreplication. A significant effect of the floral damage treatment would suggest that florivory alters pollinator foraging behavior, while a significant interaction between floral morph and damage would indicate that there are morph-specific effects of florivory on pollinator behavior. Because some visitors were too difficult to identify to species on the wing, we combined visits within genera for all analyses. Effects of, or interactions with, visitor genus would indicate that floral visitors vary in their response to floral damage.

**Dye transfer**—To assess the effects of floral damage on male plant function, we used powdered fluorescent dyes (Series JST-300, Radiant Color, Richmond, CA) as an analog to estimate pollen transfer from donor to recipient plants. The number of fluorescent dye particles transferred to recipient flowers by bees is positively correlated with *Gelsemium* pollen grains, suggesting dye is a reliable analogue for pollen in this system (Adler and Irwin, 2006). Each floral damage treatment was assigned to one of two fluorescent dye colors (green or orange), with half of the plants in each treatment randomly assigned as donor (dyed) or recipient (undyed) plants. We conducted two rounds of dye application (29 March and 2 April 2012), reversing the donor and recipient plants in the second round

so that every plant in the array served as both a donor and a recipient. In the second round of dye application, we also switched the dye-color assignment of the treatments. At the start of each round, we applied dye particles to the anthers of all open flowers on donor plants using flathead toothpicks. After 24 h, we collected stigmas from 20% of the flowers with receptive stigmas on recipient plants (up to three per plant) and counted the numbers of each color dye particle under a dissecting microscope. We standardized the number of dye particles transferred per stigma by dividing by the total number of dyed flowers per treatment in each round. This method of counting dye particles transferred from donors to recipient stigmas to assess treatment effects is a commonly used protocol (e.g., Dudash et al., 2011).

We tested if damage altered dye transfer with a generalized linear mixed model (PROC GLIMMIX) with the round of dye application, damage treatment, and floral recipient morph as fixed factors, including all interactions. We used dye transfer to recipient flowers (the number of dye particles per stigma per flower dyed) as the replicate for analysis (similar to Dudash et al., 2011). We specified a Poisson distribution, and excluded stigmas that had no dye particles present, given that they likely had not been visited. Because we had up to three stigmas per recipient plant, and recipient plants in some cases received both dye colors within rounds, we included plant ID as a random effect in both analyses to avoid pseudoreplication and to account for paired observations on the same plant. An effect of floral damage on dye transfer would suggest an indirect effect mediated through altered pollinator behavior. We predicted that floral damage would decrease dye transfer; however, because distyly typically is associated with disassortative pollen transfer due to the spatial arrangement of anthers and stigmas, we assumed that the majority of pollen deposited on pin stigmas would come from thrum flowers and vice versa. Given this assumption, we also predicted that floral damage would have a stronger negative effect on dye transfer to pin plants, given that thrum donor plants are more likely to suffer damage to anthers (Leege and Wolfe, 2002). Thus, we predicted that there would be a significant interaction between floral recipient morph and donor damage, suggesting that florivory has morph-specific effects on dye transfer.

**Female function**—When all plants ceased flowering in May, they were moved to a shade house to allow fruit maturation in the absence of herbivory or other disturbances. We collected and froze fruit from all experimental plants in August, after the seeds had expanded and any aborted ovules were still recognizable. Fruits were thawed, dissected, and the number of seeds and aborted ovules per fruit were counted under a dissecting microscope. On plants that made over 50 fruits, we subsampled the number of fruits dissected and extrapolated this number to the whole plant; we haphazardly chose and dissected 50 fruits plus 20% of the remaining undissected fruit (up to 75 fruits). At the whole-plant level, we calculated proportion fruit set (number of seed-bearing fruits divided by the number of flowers produced per plant) and estimated the total seeds per plant by multiplying the mean seeds per fruit by the total number of seed-bearing fruits per plant.

To test how floral damage affected *Gelsemium* female reproduction, we used separate generalized linear models with pollination treatment, damage treatment, and floral morph as fixed factors (including all two-way interactions) and included the number of flowers removed prior to the start of the experiment and the total

number of flowers each plant produced during the experiment as covariates. On a per-plant basis, we modeled the proportion fruit set per plant with a binomial distribution, and total seed set per plant with an exponential distribution (PROC GLIMMIX), adding an over-dispersion parameter estimated from the residuals to adjust parameter estimates. On a per-flower basis, we similarly analyzed total seed set per fruit and the proportion seed set per fruit as responses in separate analyses with Poisson and binomial distributions, respectively. We included plant ID as a random effect to account for replicate measures of seed set in fruits from the same plant. A significant interaction between pollination and damage treatments would suggest florivory has indirect effects on female reproduction mediated through changes in pollination. A significant interaction between floral morph and the pollination or florivory treatments would suggest that the effect of pollination or florivory on female function varies between morphs.

## RESULTS

**Pollinator visitation**—We observed a total of 470 floral visitors, 1417 plant visits, and 2813 individual flower probes over 34 person-hours of observations. We observed a variety of floral visitors, including bees, butterflies, and flies. Carpenter bees (*Xylocopa*) were the most common floral visitor (Table 1). Mason bees (*Osmia* spp.) were the second most common visitor, followed by tiger swallowtail butterflies, *Papilio glaucus* Linnaeus, 1758 (Papilionidae, Table 1).

Floral damage reduced the number of pollinator visits to plants, but the effects of damage varied by floral morph (Fig. 1A). On average, floral damage decreased visitation to plants by 20% compared to undamaged controls ( $F_{1,148} = 5.75$ ,  $P = 0.018$ ). Thrum plants attracted more visitors overall than pin plants ( $F_{1,148} = 12.51$ ,  $P < 0.001$ ). However, the negative effect of damage on the number of pollinator visits was larger in pin morphs, decreasing the number of visits by 42% compared to an 11% decrease in thrums (morph\*damage:  $F_{1,148} = 4.88$ ,  $P < 0.029$ , Fig. 1A). Plants with larger floral displays also attracted more floral visitors (covariate:  $F_{1,148} = 51.94$ ,  $P < 0.001$ ).

On average, pollinators probed 9.8% more open flowers on damaged plants compared to undamaged plants; however, this difference was not statistically significant ( $F_{1,837} = 1.10$ ,  $P = 0.294$ ). The proportion of open flowers probed per plant decreased with increasing numbers of open flowers per plant ( $F_{1,837} = 271.03$ ,  $P < 0.001$ ). Floral visitors probed more than double the proportion of open flowers on pin plants compared to thrum plants ( $F_{1,837} = 24.74$ ,  $P < 0.001$ ), and the magnitude of this effect varied across different visitors (morph\*genus:  $F_{7,837} = 5.32$ ,  $P < 0.001$ ). *Xylocopa* and *Bombus* did not discriminate between morphs, while all other visitors probed a greater proportion of flowers on pin plants than on thrum plants.

Although damage was associated with a reduced number of plant visits, we were surprised to find that some visitors spent more time probing individual flowers on damaged plants compared to undamaged plants. While there was no main effect of damage on time spent per flower ( $F_{1,766} = 1.31$ ,  $P = 0.252$ ), time spent per flower varied by genus of visitor ( $F_{7,767} = 10.60$ ,  $P < 0.001$ ) and there was a significant interaction between visitor genus and damage treatment (genus\*damage:  $F_{7,767} = 3.06$ ,  $P = 0.004$ ). For example, damage resulted in an 18% reduction in the time spent per flower by *Bombus*, but a 24% and 111% increase in time spent per flower by *Osmia* and

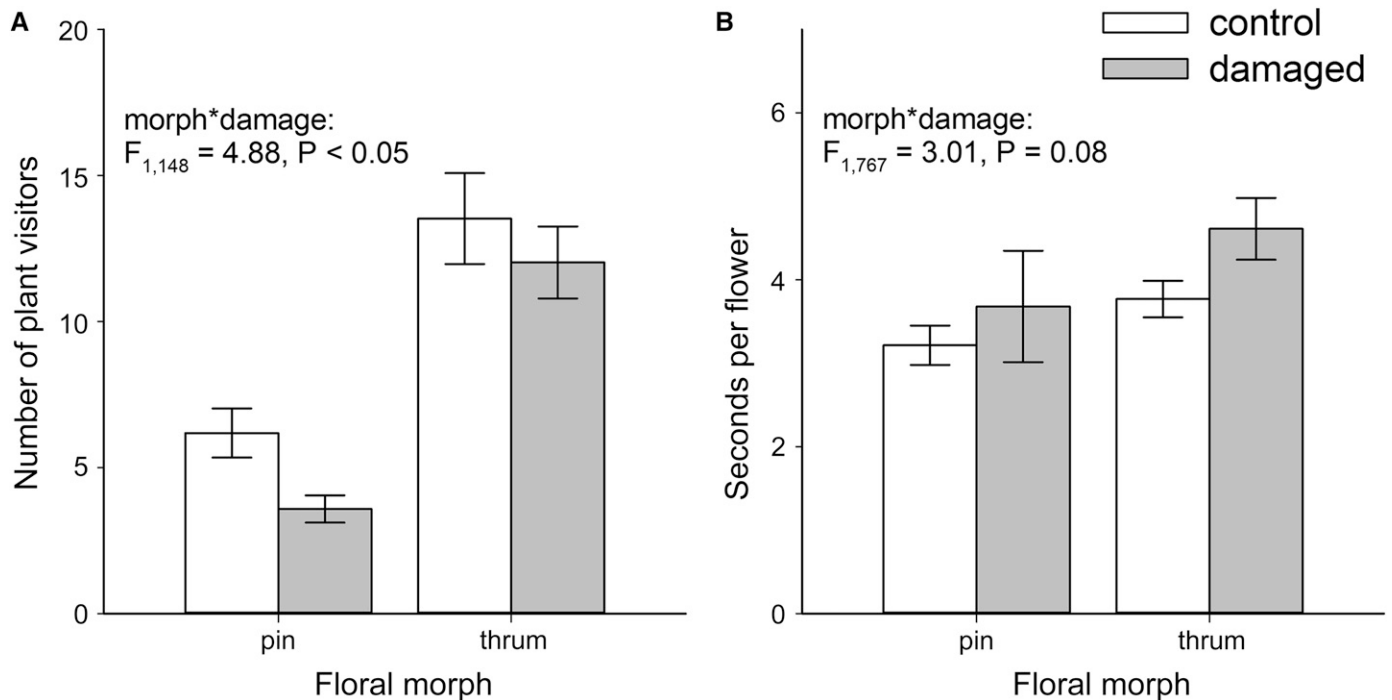
**TABLE 1.** The number of foraging bouts made by pollinators to *Gelsemium sempervirens* by pollinator genus, the total number of plants visited by each genus, and mean time spent per flower for control and floral damage treatments by floral morph. Visitation by *Xylocopa* excludes nectar-robbing visits. Statistics were not calculated for the least common visitors, because they were excluded from analyses.

Genus	Number of bouts	Number of plant visits	Pin plants				Thrum plants			
			Control		Damaged		Control		Damaged	
			N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
<i>Xylocopa</i>	65	332	32	2.3 ± 0.4	21	2.8 ± 0.6	156	2.5 ± 0.3	123	2.2 ± 0.2
<i>Osmia</i>	223	308	38	5.2 ± 0.8	17	8.2 ± 3.3	122	7.1 ± 0.7	131	8.3 ± 0.8
<i>Papilio</i>	47	282	60	2.1 ± 0.3	34	2.2 ± 0.2	109	1.5 ± 0.1	79	1.9 ± 0.2
<i>Habropoda</i>	36	109	20	2.7 ± 0.9	20	5.4 ± 1.9	43	4.1 ± 0.8	26	9.5 ± 3.6
<i>Bombylius</i>	41	104	28	4.2 ± 0.6	10	1.3 ± 0.3	41	4.4 ± 0.6	25	5.1 ± 1.7
Pierid	8	102	30	2.6 ± 0.3	13	4.0 ± 0.8	28	2.4 ± 0.4	31	1.9 ± 0.2
<i>Vanessa</i>	14	72	19	3.2 ± 0.4	8	4.2 ± 1.0	22	3.6 ± 0.6	23	3.0 ± 0.4
<i>Bombus</i>	9	48	7	4.4 ± 2.8	9	2.7 ± 0.6	17	5.7 ± 1.4	15	5.6 ± 1.4
<i>Hemaris</i>	2	16	—	—	—	—	—	—	—	—
<i>Apis</i>	4	14	—	—	—	—	—	—	—	—
<i>Megachile</i>	7	12	—	—	—	—	—	—	—	—
Hesperiid	5	7	—	—	—	—	—	—	—	—
Halictid	6	6	—	—	—	—	—	—	—	—
Total	467	1417	234	—	132	—	538	—	453	—

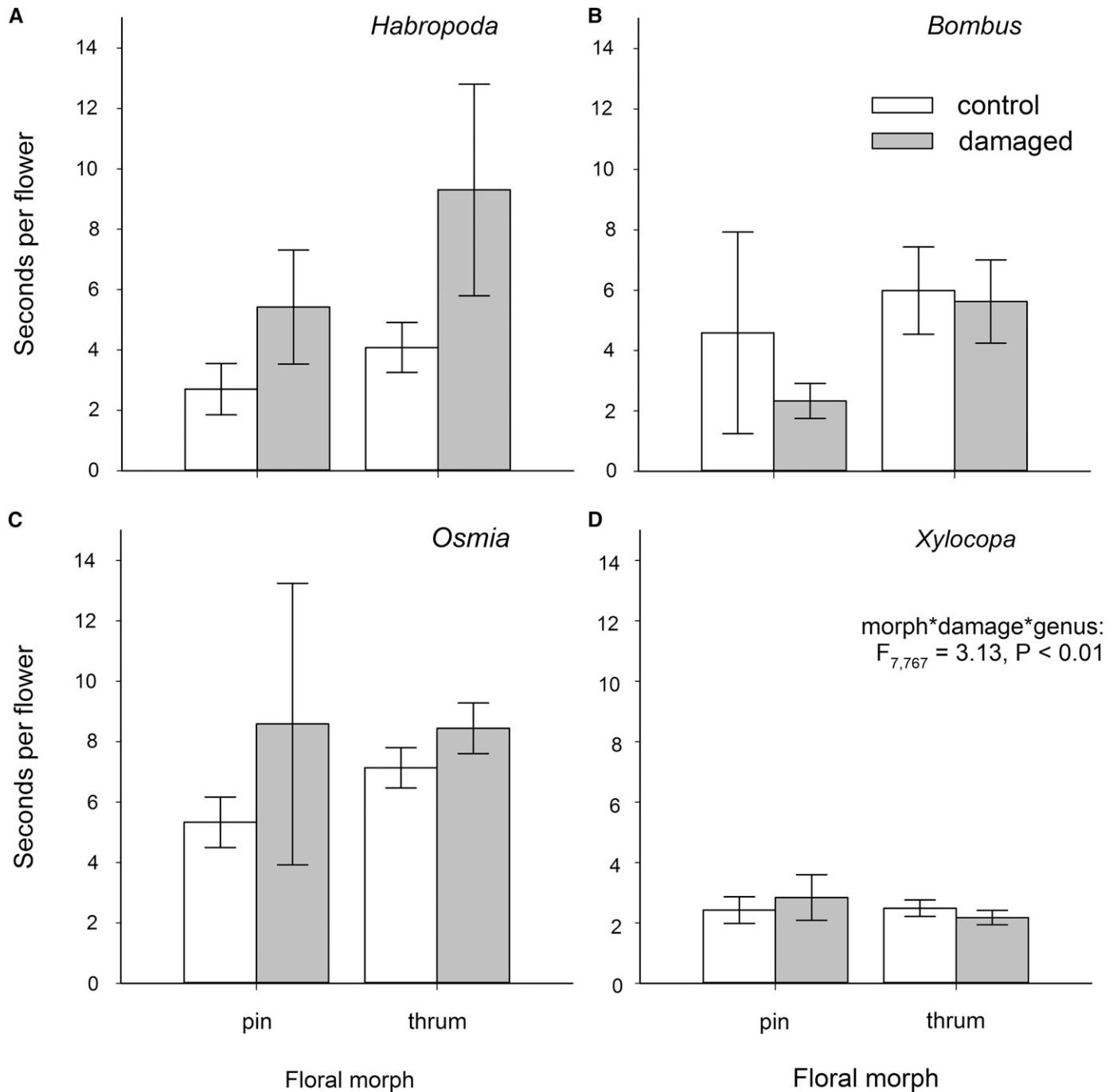
*Habropoda*, respectively (Table 1). There was no main effect of floral morph on time spent per flower ( $F_{1,767} = 3.62, P = 0.058$ ), but some floral visitors foraged longer on flowers of thrum plants than pin plants (morph\*genus:  $F_{7,767} = 2.11, P = 0.041$ ). The effect of damage on time spent per flower did not vary by floral morph (morph\*damage:  $F_{1,767} = 3.01, P = 0.083$ , Fig. 1B). However, a 3-way interaction (morph\*damage\*genus:  $F_{7,767} = 3.13, P < 0.003$ , Fig. 2) suggested that the effects of damage on time spent per flower depended on both floral morph and the genus of visitor, with damage leading to longer times per flower for Pierids, *Vanessa*, and *Xylocopa* on pin plants, but shorter times for all three on thrum plants.

The effect of damage was more consistent between morphs for other visiting genera (Table 1). There was no effect of the number of open flowers per plant on time spent per flower ( $F_{1,766} = 0.10, P = 0.752$ ).

**Dye transfer**—The effects of floral damage on dye transfer (an estimate of male plant reproduction) varied between rounds of dye application. Overall, dye transfer per flower was more than six times greater in the second round of application ( $F_{1,236} = 56.28, P < 0.001$ ). While there was no primary effect of floral damage on dye transfer ( $F_{1,236} = 0.43, P = 0.512$ ), there was a significant interaction between



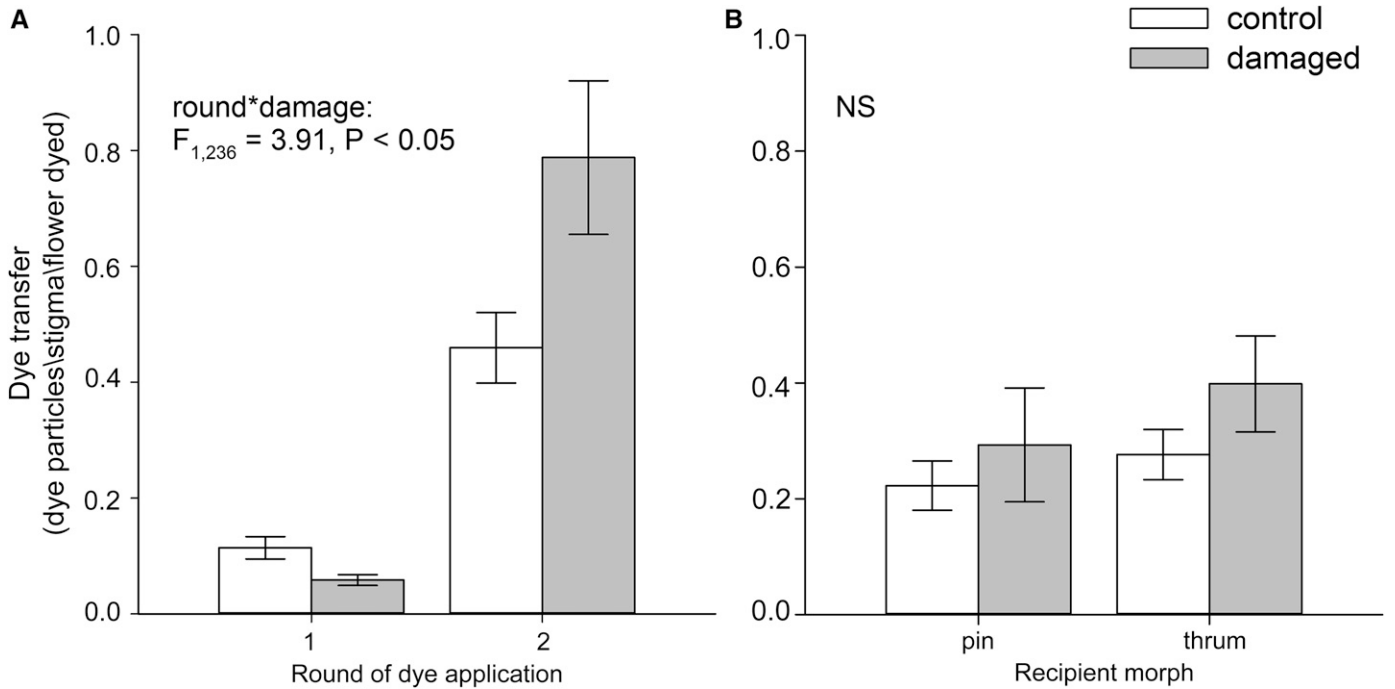
**FIGURE 1** Floral damage decreased pollinator visitation to pin but not thrum plants (A), but tended to increase the time spent per flower in thrum but not pin plants (B). Bars are means ± SE.



**FIGURE 2** The effects of floral damage on time spent per flower varied by morph and the genus of floral visitor (morph\*damage\*genus interaction). For example, damage increased the time spent per flower in *Habropoda* (A) and *Osmia* (C), but not in other visitors like *Bombus* (B) and *Xylocopa* (D). Bars are means  $\pm$  SE.

the round of dye application and damage treatment ( $F_{1,236} = 3.91, P = 0.049$ ). In the first round of dye application, floral damage decreased dye transfer by 72%; while in the second round of dye application when the majority of dye transfer occurred, contrary to predictions, floral damage increased dye transfer by 69% (Fig. 3A). There was no effect of floral morph on dye transfer ( $F_{1,236} = 0.04, P = 0.833$ ) and no interaction between floral morph and damage ( $F_{1,236} = 0.01, P = 0.941$ , Fig. 3B).

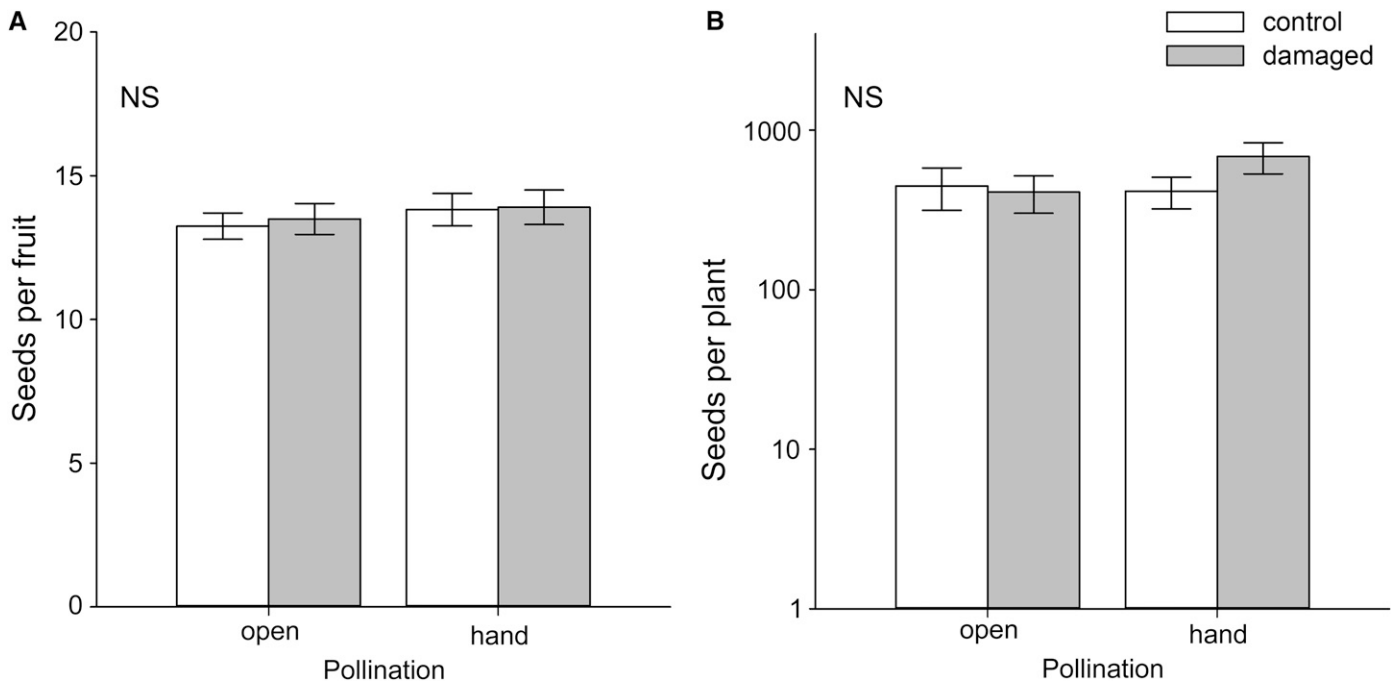
**Female function**—Floral damage had little effect on female reproduction. There were no main effects of either hand pollination ( $F_{1,3517} = 0.39, P = 0.531$ ) or floral damage ( $F_{1,3517} = 0.70, P = 0.403$ ) on the number of seeds per fruit. However, thrum morphs set 3.7 more seeds per fruit (31.3%) on average than pin morphs ( $F_{1,3517} = 17.22, P < 0.001$ ). Moreover, there was no interaction between hand-pollination and floral damage ( $F_{1,3517} = 0.10, P = 0.747$ , Fig. 4A), although there was a significant interaction between hand-pollination



**FIGURE 3** The effect of damage on fluorescent dye transfer varied between rounds (A), but not between floral morphs (B); NS indicates nonsignificant interactions. Bars are means  $\pm$  SE.

and floral morph ( $F_{1,3517} = 5.39, P = 0.0203$ ). Hand pollination increased seeds per fruit by 12.2% in thrum plants, compared to a 5.9% reduction in pin plants. In general, the number of seeds per fruit also increased with the total number of flowers plants produced ( $F_{1,3517} = 52.21, P < 0.001$ ). We found no relationships between floral morph, hand pollination, or floral damage on the

proportion of fruit set per plant ( $F_{8,150} = 0.61, P = 0.766$ ). There was also no significant interaction between floral morph and damage treatment on total seed set per plant or any other effects of floral morph, hand pollination, or floral damage on total seeds produced per plant ( $F < 3.0, P > 0.09$  in all cases, Fig. 4B). The only significant relationship we observed was a positive relationship between number



**FIGURE 4** No evidence was found that floral damage affected seed set per fruit (A), or total seed set per plant (B). “Open” and “hand” refer to open- and hand-pollinated treatments, respectively, and NS indicates nonsignificant interactions. Bars are means  $\pm$  SE.

of flowers produced per plant and number of seeds produced per plant ( $F_{1,138} = 37.80, P < 0.001$ ).

## DISCUSSION

In hermaphroditic plant species, understanding how species interactions affect plant reproduction requires measuring both male and female components (Schaeffer et al., 2013), because male and female function may not respond in the same way or with equal magnitude to mutualist and antagonist species interactions. We found that floral damage decreased pollinator visitation almost four times more in pin plants compared to thrum plants. Contrary to predictions, damage increased the time some floral visitors spent at individual flowers. Subsequently, although damage had little effect in the first round of dye application, it increased dye transfer in the second round when most of the dye transfer occurred, suggesting that florivory can have positive effects on male function through increased per-flower pollen transfer when highly effective pollinators are present. However, we found little evidence of direct or indirect effects of florivory on any measure of female plant reproduction, suggesting that the effects of floral damage were dependent on the component (male vs. female) of reproduction measured.

The reduction in floral visitors, especially to pin plants, due to floral damage in our study adds to the growing evidence that florivory can have negative indirect effects on pollinator visitation (reviewed in McCall and Irwin, 2006). For example, simulated florivory decreased visitation to flowers of *Nemophila menziesii* (McCall, 2008), and natural florivory reduced pollinator visitation to both wild parsnip, *Pastinaca sativa* L. (Apiaceae) (Lohman et al., 1996), and *Centrosema virginianum* (L.) Benth. (Fabaceae) flowers (Cardel and Koptur, 2010). Reductions in pollinator visitation to damaged flowers and plants are likely due to reduced visual attractiveness (e.g., fewer flowers per inflorescence, changes in floral symmetry), as well as changes in the rewards provided to pollinators (Krupnick et al., 1999). For example, the removal of tepal tissue from *Alstroemeria ligtu* Curtis (Alstroemeriaceae) changed the pattern of UV reflectance of nectar guides and subsequently decreased pollinator visitation (Botto-Mahan et al., 2011). Although we did not measure floral traits in our study, we hypothesize that flower size and shape were important for pollinators in making the initial decision to forage on plants.

Few studies have documented the effects of florivory on multiple aspects of pollinator behavior during visits to individual plants. In doing so, this study provides evidence of positive indirect effects of floral damage on pollinator activity at individual flowers. Contrary to our initial predictions, floral damage increased the time spent per flower for two visiting bee species, *Osmia* and *Habropoda*. While damage decreased the time spent per flower by the other visitors, the magnitude of the effect was smaller. Interspecific variation in response to floral damage likely depends on how floral damage affects floral rewards, how visiting genera or species perceive and respond to those potential changes, and also how floral damage affects the handling time of pollinators. Few studies have documented how different floral visitors respond to changes in the same floral traits. Adler and Irwin (2005) found variable responses to high nectar alkaloids in *Gelsemium* across a number of visiting bee species. Bees' responses included positive, negative, and no effects, suggesting bee species respond differently to changes in nectar

traits. We did not quantify floral rewards in this study, but hypothesize that floral damage may have increased nectar production to remaining flowers, as plants reallocate resources to undamaged flowers. Visit duration is often positively associated with nectar quantity (Hodges and Wolf, 1981; Zimmerman, 1983), and increased nectar production could help compensate for reduced pollinator visits at the whole-plant level. Smaller bodied bees (*Osmia* and *Habropoda*) exhibited the greatest responses to floral damage, and even minor changes in nectar could modify the extraction time these pollinators need relative to larger bodied bees. An additional hypothesis is that floral damage may structurally affect flowers or the pattern of nectar guides, increasing handling time, and subsequently the duration of probes by some visitors. However, why handling time in this scenario would be more strongly affected in *Osmia* and *Habropoda* is unknown.

Male function, estimated as dye transfer per flower dyed from donor treatments to recipient plants, varied between rounds of application. During the second round of application when dye transfer was highest, per-flower dye transfer was 70% higher from damaged plants compared to undamaged plants. This positive indirect effect of damage on dye transfer was the opposite of our prediction and contrary to what other studies have reported. For example, *Isomeris arborea* exposed to flower feeding beetles exported half as many dye particles as plants protected from beetles, indicating a negative indirect effect of floral herbivory on male function (Krupnick and Weis, 1999). Similarly, in gynodioecious wild strawberries, *Fragaria virginiana* Mill. (Rosaceae), damage to developing buds by a flower-feeding weevil had strong negative direct effects on male fertility, although the indirect effects on male fertility were relatively small and were positive and negative in different years (Ashman and Penet, 2007). One possible explanation for the positive indirect effect of damage on dye transfer in our study could be the longer time spent per flower by two of the three most common bee visitors, *Osmia* and *Habropoda*. *Osmia* and *Habropoda* both transfer more *Gelsemium* pollen per visit than the other common bee visitor in our study, *Xylocopa* (Adler and Irwin, 2006). The increased foraging time by these two highly effective pollinators could have offset any negative effects of floral damage on pollen (dye) transfer by less effective floral visitors. Increased time spent foraging per flower can increase both the pollen removed from flowers and deposited on stigmas (Kudo, 2003). Floral visitors also vary in foraging strategies. For example, *Osmia* actively collected pollen from *Gelsemium* while other visitors were primarily observed foraging for nectar (A. Carper, pers. obs.). Such differences may help explain differences in handling time and pollinator responses to florivory. One caveat to the interpretation of the dye transfer results is that they were measured on a per-flower basis. It remains unknown how florivory affects per-plant estimates of male reproduction, and in particular whether the per-flower benefits of florivory can compensate for anther removal and pollinator visitation at the plant level. Measuring per-plant estimates of dye transfer was beyond the scope of this study, but will yield unique insights in future research. Finally, the different magnitude of dye transfer between the two rounds of application was not surprising. We observed 25% more floral visitors in the second round, and nearly four times the number of *Osmia* visits. Within-season variation in pollinator activity and/or dye transfer has been observed in other systems (Palmer et al., 1988; Campbell and Waser, 1989), suggesting that the effects of florivory likely vary with the availability of alternative pollinators.



We did not detect any direct or indirect effects of florivory on female reproduction in this study. Other studies documented a range of effects, from negative to positive, due to direct consumptive effects as well as indirect effects mediated through changes in pollinator visitation (reviewed in McCall and Irwin, 2006). For example, McCall (2008) detected primarily direct negative effects of both natural and simulated floral damage on pollinator visitation, fruit and seed set in *Nemophila menziesii*, and showed that pollen-limitation generally increased with increasing levels of floral damage (McCall, 2010). However, plants can also compensate for floral damage through resource reallocation or selective abortion (Stephenson, 1981; Ashman et al., 2004) or increases in flower production (Wise et al., 2008). For example, increased flower production after inflorescence consumption by deer resulted in positive effects on both male and female reproduction in *Ipomopsis aggregata* (Pursh) V. E. Grant (Polemoniaceae) (Gronemeyer et al., 1997). In our study, damage had no effect on fruit set. Future work should track the fate of individual flowers following the damage or consumption of floral parts to explore mechanisms of potential compensation to florivory.

Manipulative studies often find varying effects of species interactions on male vs. female components of reproduction (reviewed in Schaeffer et al., 2013). Theory predicts that male reproduction should be more limited by the number of matings than female reproduction, suggesting male plant reproduction should be more susceptible to changes in pollination than female plant reproduction (Burd, 1994). Correspondingly, estimates of male reproduction typically show greater responses to changes in floral traits than female reproduction. For example, higher alkaloid concentrations in *Gelsemium* nectar reduced pollinator visitation and male plant reproduction, but had no effect on female reproduction (Adler and Irwin, 2005). The lack of effects on female function in our study suggests that pollen receipt did not limit female reproduction within the array and that any effects of damage on pollinator behavior did not translate into differences in female function, although this may not hold in pollen- and resource-limited natural populations. It is also possible that the effects of florivory vary with the intensity of damage. We did not vary levels of damage in this study, but *Gelsemium* flowers can have almost 100% of petal area removed from damaged flowers (A. Carper, personal observation), and increasing intensities of damage could have greater effects on female and male reproduction. Future research should evaluate variation in the intensity of floral damage and the subsequent effects on both male and female reproduction.

Given the capacity of florivory to alter the functional gender of *Gelsemium* flowers (Leege and Wolfe, 2002), it is surprising we found so few interactions between floral damage and floral morph. While damage did have morph-specific effects on aspects of pollinator behavior, we found no morph-specific effects of floral damage on any estimates of male or female components of plant reproduction. Morph-specific florivory and florivore-induced changes in sexual allocation have been reported in other studies (Ashman et al., 2004; Tsuji and Sota, 2010). Sex-specific tolerance to florivory in sexually dimorphic species could help plants compensate for damage. For example, *Solanum carolinense* L. (Solanaceae) damaged by beetles initiated more inflorescences, aborted fewer buds prior to anthesis and fewer ovaries after fertilization, and increased the ratio of male-to-female flowers (Wise et al., 2008). Subsequently, florivory resulted in directional selection on floral sex ratio away from male to perfect flowers (Wise and Hébert,

2010). Further study on changes in maternal vs. paternal investment in different morphs is needed to determine whether sex- or morph-specific compensation to florivory occurs in *Gelsemium*.

Taken together, these results suggest that floral damage can have contrasting effects on plant reproduction, driven by changes in different pollinators' behavior at flowers and depending on the component of plant reproduction measured. Given the consequences for the ecology and evolution of flowering species (Mitchell and Ashman, 2008) and breeding systems (Wise and Hébert, 2010), future studies addressing spatial and temporal variation in pollination, floral herbivory, and the relationship between the two could shed light on the ecological and evolutionary implications of multi-species relationships occurring at flowers.

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