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## SEXES SHOW DIFFERENTIAL TOLERANCE TO SPITTLEBUG DAMAGE AND CONSEQUENCES OF DAMAGE FOR MULTI-SPECIES INTERACTIONS<sup>1</sup>

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Antagonists can play a role in sexual system evolution if tolerance or resistance is sex-dependent. Our understanding of this role will be enhanced by consideration of the effects of antagonists on other plant–animal interactions. This study determined whether the sex morphs of a gynodioecious *Fragaria virginiana* differ in their susceptibility and response to damage by spittlebugs and whether damage altered pollinator attraction traits or interactions with other antagonists. Tolerance, but not resistance, to spittlebugs differed between the sexes. Generally, spittlebugs were more damaging to hermaphrodites than females, a finding in accord with the hypothesis that the pollen-bearing morph is less tolerant of source-damage than the pollen-sterile morph when damage is incurred during flowering. In both sex morphs, spittlebugs reduced inflorescence height, increased petal size, but did not affect the number of open flowers per day, suggesting that the net effect of damage may be to increase pollinator attraction. Spittlebug infestation modified interactions with other antagonists in a sex-dependent manner: spittlebugs reduced attack by bud-clipping weevils in hermaphrodites but increased infection by leaf fungi in females. The complex interactions between plant sex, antagonists, and pollinator attraction documented here emphasize the importance of considering sex-differential multi-species interactions in plant sexual evolution.

**Key words:** dioecy; *Fragaria virginiana*; gynodioecy; herbivory; plant–animal interactions; sexual system evolution.

Herbivore damage frequently varies with plant phenotype with the result that herbivores may mediate plant trait evolution. While it is well established that damage level is associated with resistance traits (e.g., trichomes, defense compounds; Kennedy and Barbour, 1992), it is also becoming clear that damage level can be associated with nontraditional resistance traits such as floral display (e.g., size, flower color), flowering phenology, pollen production, and sex expression (Marquis, 1992; Collin et al., 2002; Ashman et al., 2004; Strauss and Irwin, 2004). This suggests that herbivore-mediated selection may contribute to evolution of floral and sexual phenotype, as well as resistance. Even if damage level is independent of plant phenotype, the response to damage may vary with phenotype and can result in herbivore-mediated selection. For instance, plants with high investment in floral buds are more tolerant of damage than those with low investments (Ashman et al., 2004). Also, the sex morph with lower reproductive costs at the time of attack may be more tolerant than the one with greater costs (Ashman, 2002). In these scenarios, plants with high allocation to buds or low cost of reproduction will be favored by herbivore-mediated selection.

While numerous researchers have demonstrated that herbivore damage directly reduced components of plant fitness (e.g., Marquis, 1992), far fewer shown that herbivore damage indirectly affects fitness by depressing interactions with mutualists or by altering interactions with other antagonists (reviewed in Strauss and Irwin, 2004). Evidence is growing that herbivory can affect plant–pollinator interactions by altering floral display or reward (e.g., Mothershead and Marquis, 2000; Elle and Hare, 2002; Steets and Ashman, 2004). However, this

interaction may also be phenotype-dependent. For instance, plants that are more tolerant of herbivory may be less susceptible to trait-mediated negative effects of herbivory on pollination (Paige et al., 2001). In an analogous situation, susceptibility to one antagonist may lead to greater resistance to attack by another (e.g., Webber, 1981; Cronin and Abrahamson, 1999; Collin et al., 2002). Here, the net effect of the first antagonist may be positive if the second antagonist is the more damaging one. Alternatively, morphs that are heavily damaged by one antagonist may also be subject to high damage by others because either resistance to one antagonist also confers resistance to others or damage by an earlier herbivore leads to greater damage by later ones (Pilson, 1992). It is now becoming clear that in order to understand the role of herbivory in plant phenotypic evolution, we need to consider not only the direct effect of a focal herbivore on plant fitness but also its indirect effects on other interactions.

Sexually dimorphic plant species provide a unique opportunity to study the evolutionary consequences of phenotype-dependent herbivory, because the sex morphs can show differential resistance and tolerance to herbivores (Ågren et al., 1999; Ashman, 2002; Ashman et al., 2004). Because of greater apparency (e.g., floral display size or presence of pollen) and/or suitability (e.g., nutrient quantity), pollen-bearing morphs (i.e., males or hermaphrodites) suffer greater attack by herbivores than the pollen-sterile morphs (i.e., females) especially when reproductive structures (sinks) attract herbivores (reviewed in Ågren et al., 1999; Ashman, 2002; Ashman et al., 2004). Sex differences in reproductive costs at the time of damage are predicted to result in differential tolerance (Ågren et al., 1999; Ashman et al., 2004; Verdú et al., 2004). For instance, a higher investment in flowers by the pollen-bearing morph may lead to lower tolerance of damage to sources (foliage or vasculature) during flowering compared to the pollen-sterile morph (Ashman, 1994; Ashman et al., 2004). In contrast, the pollen-sterile morph may be less tolerant of source damage during fruiting (Ågren et al., 1999). Most studies,

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however, have approached the question of sex-differential response to herbivores using artificial defoliation (reviewed in Agren et al., 1999; but see Koskela et al., 2002; Ashman et al., 2004; Verdú et al., 2004), and it is not always known if the observed responses to artificial damage accurately reflect those induced by herbivores (Agrawal et al., 1999). Moreover, because many of these studies are conducted in greenhouses they eliminate the potential for other plant–animal interactions to mediate the net effect of herbivory, and thus may not accurately capture the natural dynamics of the plant–herbivore interaction.

We aimed to address this gap by determining whether the sex morphs of gynodioecious *Fragaria virginiana* Duch. (Rosaceae) differed in resistance and tolerance to damage by a generalist xylem-feeding herbivore (the meadow spittlebug, *Philaenus spumarius* [Homoptera: Cercopidae]), which attacks wild strawberry plants during their flowering period. Specifically, we asked the following questions: (1) Does spittlebug infestation (incidence, intensity) differ between the sex morphs in wild populations? (2) Does spittlebug damage affect growth, reproduction, or aspects of floral display known to be important in attracting mutualists (pollinators). Is the pollen-bearing morph (hermaphrodite) less tolerant of spittlebug damage than the pollen-sterile morph (female)? (3) Does the presence of, or damage by, spittlebugs affect plant susceptibility to contemporaneous antagonists (florivores, folivores, or fungal pathogens), and does this differ between the sexes?

## MATERIALS AND METHODS

**Study species**—*Fragaria virginiana*, the Virginian wild strawberry, is a perennial herb native to the meadows, wood edges, and old fields of eastern North America (Staudt, 1989). It has a gynodioecious sexual system, where females coexist with hermaphrodites, and sex is determined by nuclear genes (Ahmadi and Bringham, 1989). It reproduces asexually via creeping stolons (runners) and sexually via seeds. Flowering occurs from late April until mid June. Flowers of both morphs are borne on pliochasiol inflorescences (Ashman and Hitchens, 2000), and each contains a fleshy receptacle that supports numerous uniovulate carpels. Hermaphrodites set ~20% of their flowers into fruits, whereas females set ~90% of theirs (Ashman, 2003). Pollinators include native bees, flies, and ants (Ashman, 2000; Ashman and King, 2005). Antagonists include clipper weevils that damage sinks (flower buds), and grasshoppers, fungi, and spittlebugs that damage sources (foliage or vasculature).

The focal antagonist studied here is the generalist meadow spittlebug (*Philaenus spumarius*, [Homoptera: Cercopidae]) (Weaver and King, 1954). Spittlebug nymphs emerge during early spring (Ahmed and Davidson, 1950) and feed on xylem (Horsfield, 1978) that mixes with a surfactant in the gut to produce conspicuous “spittle” that protects the nymph from natural enemies and desiccation (Whittaker, 1970). Nymphs pass through five larval instars and emerge as adults and leave host plants within about a month. In early spring, spittlebugs are common on old field plants (*Solidago*, *Trifolium*) and have been observed on 24% of wild strawberry plants at a density of  $1.4 \pm 0.06$  spittlebugs/plant in populations in NW Pennsylvania (PA), USA (T.-L. Ashman, et al., unpublished data).

**Spittlebug infestation in the wild**—To determine the intensity of spittlebug attack on wild strawberry and whether it varies between plant sex morphs, we conducted field surveys in a wild population (population HT; see Ashman, 1999 for details) in Crawford County, PA on two dates (7 and 16 May 2004). We recorded flowering status, sexual identity of flowering plants, and presence/absence of spittlebugs for plants located at meter intervals along several transects run through the population. A total of 291 plants were surveyed.

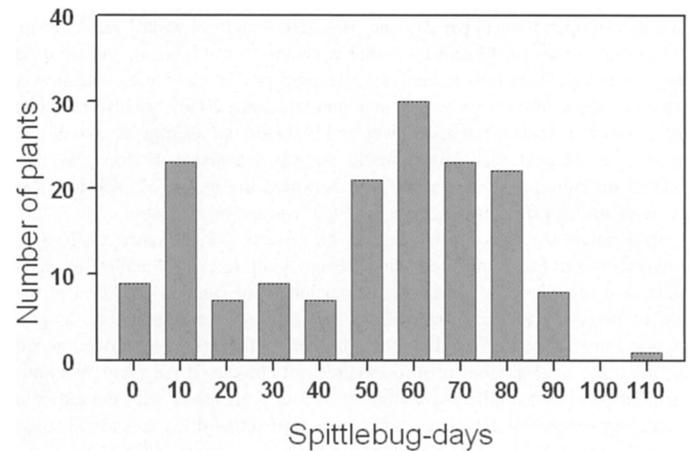


Fig. 1. Distribution of spittlebug-days among potted plants in the “tolerance to spittlebug infestation” experiment.

**Tolerance to spittlebug infestation in potted plants**—To determine whether the sexes of *F. virginiana* respond to spittlebug infestation differently, we experimentally manipulated spittlebugs on potted plants of both sex morphs under field conditions. Specifically, plants originally derived from a wild population (population PR; see Ashman, 1999 for details) were grown in 350 cc pots of a 2 : 1 mix of Fafard #2 (Fafard, Agawam, Massachusetts, USA) soil : sand in a greenhouse at the University of Pittsburgh and then over-wintered at the Pymatung Laboratory of Ecology (PLE) in Crawford County, PA. In the spring of 2004, 80 plants of each sex morph were fertilized with ~220 mg of Osmocote fertilizer (14-14-14 NPK; Scotts, Marysville, Ohio, USA) and organized into five spatial blocks in a “garden” located 200 m from the wild source population. Plants were protected with deer netting to allow access by insects but not deer. When rainfall was inadequate plants received supplemental water.

We collected spittlebug nymphs of all stages from two wild populations near PLE and placed on experimental *F. virginiana*. Originally, we planned to determine the effect of spittlebug presence or absence on plants and thus added three spittlebugs to each of 53 plants per sex morph and left 27 plants per sex morph as controls. However, spittlebug movement and losses obscured the integrity of the initial treatment categories. So, instead we treated spittlebug infestation as a continuous variable rather than a categorical one. To do this, we surveyed spittlebug infestation every 3 days and recorded the number of spittlebugs/plant. For plants originally assigned to the spittlebugs present group, at each survey we recorded the number of spittlebugs and added new spittlebugs to maintain a minimum of three spittlebugs per plant. If, however, the number of spittlebugs was above three, the number was recorded but the spittlebugs were not removed. For plants originally in the control treatment, we also recorded spittlebug presence but spittlebugs were neither removed nor were any new spittlebugs added. From these data, we calculated the number of “spittlebug-days” a plant experienced based on the number of spittlebugs and their tenure on the plant (i.e., for each plant, we summed the product of the number of spittlebugs and their residence time across all survey intervals). This value is a direct reflection of the intensity and duration of spittlebug infestation experienced by each plant. The average number of spittlebugs/plant was  $1.8 \pm 0.1$  (range 0–8), and the number of spittlebug-days ranged from 0–114 (mean  $\pm$  SE:  $44 \pm 2$ ), providing wide variation in spittlebug infestation among plants. However, because the distribution of spittlebug-days (Fig. 1) was non-normal (Kolmogorov-Smirnov  $D = 0.11$ ,  $P < 0.01$ ), we converted the raw values into ranks to improve normality (Kolmogorov-Smirnov  $D = 0.07$ ,  $P > 0.05$ ) and used ranked values in all subsequent analyses.

Prior to exposure to spittlebugs, we estimated the vegetative size of each plant (hereafter, initial plant size) as the product of the number of leaves and the width of the central leaflet of the largest leaf (see Ashman, 1999) for use as a covariate in all subsequent analyses. To determine the effect of spittlebugs on plant attractiveness to pollinators, we recorded components of floral display

(number of open flowers per day and petal size [length  $\times$  width] see Ashman, 2000) three times (9, 12 and 16 d after exposure to spittlebugs), and we used the average for each trait in analyses. Because flower size varies with flower position on the inflorescence (Ashman and Hitchens, 2000), we also recorded the position of each measured flower and included the average as a covariate in analyses of petal size (discussed in Statistical analyses section). We calculated the duration of flowering. We measured the height of each inflorescence at the end of flowering and used the average in analyses.

To estimate the effect of spittlebugs on growth, we estimated final vegetative size from leaf number and the average width of central leaflets for each plant. We recorded the number and total length of runners produced at the end of flowering and used number and average runner length (total length/number) in analyses. To estimate the effect of spittlebugs on reproduction, we recorded the total number of flowers and fruits produced per plant. We enumerated filled and unfilled seeds on up to four fruits/plant with the aid of a dissecting scope. We calculated proportion seed set as filled seeds/total seeds and used the average value per plant in analyses.

To determine if spittlebugs alter plant interactions with other antagonists that initiate attack during wild strawberry's reproductive period, we recorded the number of flower buds clipped by weevils (*Anthonomus signatus*, Say; Coleoptera: Curculionidae), and the number of leaves damaged by fungi (leaf scorch, *Diplocarpon earliana* [Ell. and Ev.] Wolf or leaf spot, *Mycosphaerella fragariae* [Tul.] Lind) or by generalist folivores (grasshoppers or caterpillars) at the end of the experiment.

**Statistical analyses**—We determined if spittlebug infestation of *F. virginiana* in the wild population depended on plant flowering status (flowering or not) or sex morph of flowering plants (female or hermaphrodite) using log-likelihood *G* tests with the null hypothesis of equal infestation across the types (Zar, 1984). Data from two survey dates were analyzed separately, and then heterogeneity *G* tests were run to determine if there was heterogeneity between sampling dates (Sokal and Rohlf, 1981). We determined if spittlebug density varied with flowering status or sex morph using *t* tests.

We evaluated the effects of spittlebugs on plant growth (final vegetative size, number, and mean length of runners), reproduction (total flowers, total fruits, mean seed set), floral display (mean petal size, number of open flowers per day, mean inflorescence height, flowering duration), and damage by other antagonists (weevil clipped buds, fungal damage, folivory) using multiple regression for each sex morph separately. In each regression a given trait was the dependent variable, whereas ranked spittlebug-days, initial plant size, and flower position (for flower size only) were the independent variables. We removed the effects of block from dependent variables prior to analysis and calculated standardized regression coefficients (SAS, 1996) for all dependent variables for comparative purposes. We determined if the relationship between plant traits and spittlebug-days differed significantly between the sex morphs using ANCOVA with block, sex, spittlebug-days, initial plant size, and flower position (for flower size only) and the interactions of sex with spittlebug-days, initial plant size, and flower position (for flower size only) (SAS, 1996). We used the one-tailed significance of the spittlebug-by-sex morph interaction to test the directional hypothesis that spittlebug infestation was more damaging to hermaphrodites than females and used two-tailed significance of the spittlebug-by-sex morph interaction to test for differences between the sex morphs in interactions with other antagonists. In addition, considering only the traits that were reduced by spittlebugs, we performed a sign test to determine if damage had a greater effect on hermaphrodites than females more often than the reverse. To describe the overall effect of spittlebug infestation on *F. virginiana* traits, we also ran regressions on data pooled across sex morphs.

## RESULTS

**Spittlebug infestation in the wild**—Although nonflowering *F. virginiana* plants were more abundant than flowering plants (59% vs. 41%) in the wild population, spittlebug infestation was more common on flowering plants than nonflowering plants (77% vs. 22%;  $G_{\text{pooled}} = 38.48$ ;  $P < 0.001$ ,  $df = 1$ ), and this was the case at both sampling times ( $G_{\text{hetero}} = 3.72$ ;

$P > 0.05$ ,  $df = 1$ ). The sex morphs were attacked in proportion to their representation in the population of flowering plants (percentage hermaphrodites: 68% vs. 64%;  $G_{\text{pooled}} = 0.56$ ;  $P > 0.25$ ,  $df = 1$ ) at both sampling times ( $G_{\text{hetero}} = 2.47$ ;  $P > 0.10$ ,  $df = 1$ ), suggesting that spittlebugs do not discriminate between the sex morphs of flowering plants. Density of spittlebugs on infested plants was similar for flowering and nonflowering plants ( $1.4 \pm 0.1$  vs.  $1.4 \pm 0.1$  spittlebugs/plant;  $t = 1.97$ ,  $P = 0.66$ ,  $df = 163$ ), and for female and hermaphrodite plants ( $1.5 \pm 0.1$  vs.  $1.4 \pm 0.1$  spittlebugs/plant;  $t = 1.98$ ,  $P = 0.48$ ,  $df = 118$ ).

**Tolerance to spittlebug infestation in potted plants**—Despite spittlebug movement on potted plants, females (F) and hermaphrodites (H) of *F. virginiana* similarly were still likely to be infested (F vs. H: 92% vs. 96%;  $\chi^2 = 1.06$ ;  $P > 0.30$ ,  $df = 1$ ). In addition, spittlebugs were evenly distributed between infested female and hermaphrodite plants (F vs. H:  $1.7 \pm 0.1$  vs.  $1.9 \pm 0.1$  spittlebugs/plant;  $t = 1.98$ ;  $P = 0.30$ ;  $df = 149$ ), and average spittlebug-days did not differ between the sex morphs (F vs. H:  $42.6 \pm 2.9$  vs.  $46.9 \pm 3.0$ ;  $t = 1.02$ ;  $P = 0.31$ ;  $df = 158$ ).

Spittlebug infestation affected several, but not all, aspects of plant function, and for several traits the sex morphs differed in their response to spittlebug damage (Table 1). In particular, while neither vegetative growth (final size) nor number of runners were affected by spittlebug-days, average runner length decreased in both sex morphs with increasing spittlebug-days. Increasing spittlebug damage did not significantly affect total flowers, fruits, or proportion seed set. Spittlebug infestation had diverse effects on components of floral display: Increasing spittlebug-days reduced inflorescence height but increased petal size in both sex morphs. Spittlebug infestation also affected two of the three interactions with other antagonists. Increasing spittle-days reduced the numbers of buds clipped by weevils in hermaphrodites and increased the number of leaves damaged by fungal pathogens in females, but had no impact on general folivory in either sex morph.

Spittlebug infestation was more damaging to hermaphrodites than females in terms of all five plant traits reduced by spittlebug damage (Table 1). A sign test indicates that this pattern (5/5) represents a significant deviation from random ( $P_{1-\text{tail}} < 0.05$ ).

## DISCUSSION

We show here that while the sex morphs of *F. virginiana* are similarly susceptible to spittlebugs, they have differential tolerance to spittlebug infestation. We provide evidence that spittlebug infestation may alter interactions with pollinators via effects on floral display and also demonstrate that spittlebugs alter plant interactions with contemporary antagonists. We discuss each of these points in greater detail.

**Sex-differential tolerance to spittlebugs**—Our findings were in accord with the hypothesis that the pollen-bearing morph is less tolerant to damage during flowering: spittlebug infestation was more damaging to hermaphrodite than female *F. virginiana* in all traits reduced by damage. These results contribute significantly to our understanding of sex-differential tolerance to herbivores and also highlight the diversity of responses to be expected. Direction of sex-differential response is expected to be governed by (1) trade-offs between resistance

TABLE 1. Summary of the effects of spittlebug infestation on plant growth, reproduction, floral display, and damage by other antagonists. (A) Standardized regression coefficients from multiple regressions of the effects of spittlebug-days on plant traits of females, hermaphrodites, and across both sexes (both). (B) *F* statistics and *P* values for sex, sex-spittlebug-days interaction, whole model, and model *R*<sup>2</sup> from ANCOVAs. For plant traits, one-tailed significance of the spittlebug-days × sex interaction is reported as a test of whether the spittle-bugs are more damaging to hermaphrodites than females. For other antagonists, two-tailed significance of the spittlebug-days × sex interaction is reported as a test of whether the slopes differ between the sex morphs.

Plant trait	(A) Regression						(B) ANCOVA			
	Female		Hermaphrodite		Both		Sex	Sex-spittlebug-days	Model	R <sup>2</sup>
	Spittlebug-days	Initial plant size	Spittlebug-days	Initial plant size	Spittlebug-days	Initial plant size				
<b>Growth</b>										
Final plant size	0.061	0.574***	0.111	0.647***	0.085	0.613***	0.02	0.06	10.25***	38.7
Runner length	-0.150	0.033	-0.301*	-0.217	-0.256*	-0.040	5.34*	0.97	1.49	15.4
Runner number	0.002	0.010	0.037	-0.093	0.027	-0.058	1.79	0.09	0.57	3.4
<b>Reproduction</b>										
Total flowers	0.133	0.412***	0.077	0.496***	0.033	0.453***	0.00	2.38	4.63***	22.3
Total fruits	0.118	0.455***	0.147	-0.104	0.015	0.284***	220.6***	0.03	30.71***	65.4
Proportion seed set	-0.082	-0.051	-0.192	-0.074	-0.196	-0.021	51.15***	1.83†	7.81***	44.1
<b>Floral display</b>										
Petal size <sup>b</sup>	0.128	0.048	0.241*	0.035	0.238**	-0.044	127.01***	1.82	14.97***	56.1
Number of open flowers/day	0.030	0.472***	-0.034	0.443***	-0.006	0.464***	0.74	0.17	5.05***	23.6
Inflorescence height	-0.252*	0.144	-0.315**	0.420**	-0.270***	0.268***	3.01†	0.46	4.55***	22.2
Flowering duration	0.047	0.307**	-0.023	0.112	0.006	0.224**	1.31	0.07	1.35	7.7
<b>Other antagonists</b>										
Weevil clipped buds	-0.042	0.040	-0.246*	0.279*	-0.135†	0.135†	24.7***	5.65**	5.06***	23.9
Foliivore damage	-0.013	0.047	0.032	0.126	0.0002	0.100	1.94	0.05	0.55	3.3
Leaf fungal damage	0.246*	0.462***	0.044	0.399***	0.150*	0.432***	0.00	1.96†	4.81***	22.9

Note: Significance of individual coefficients is denoted as: † 0.10 < *P* < 0.05; \* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001.  
<sup>a</sup> Degrees of freedom (numerator, denominator) are 9, 146–143 for all except runner length (9, 74), proportion seed set (9, 89), and petal size (9, 129).  
<sup>b</sup> Partial regression coefficient for flower position is -0.349\*\*\*, -0.241\*, -0.165\* for multiple regressions on petal size in females, hermaphrodites, and both, respectively.

and tolerance (reviewed in Weinig et al., 2003), (2) whether the attacked organs are sources or sinks (Ashman, 2002), and (3) the magnitude of reproductive investment at the time of attack (Ågren et al., 1999; Ashman, 2002). Our finding that hermaphrodites are generally less tolerant of spittlebug damage than females contrasts with the pattern of tolerance to a flower-clipping weevil (Ashman et al., 2004). In the latter study, hermaphrodites were more tolerant (in terms of seed production), but less resistant than females. With respect to spittlebugs, the sexes were similarly resistant, so the differences in the direction of tolerance may reflect the difference in the organs damaged (flower clipping removes sinks while spittlebugs reduce sources). The impact of source damage to hermaphrodites may be greater because of their larger reproductive expenditure during flowering (e.g., investment in larger flowers and pollen) compared to females. It is clear that more studies are needed to confirm these predictive patterns, especially those that include information on both tolerance and resistance (e.g., Verdú et al., 2004; Ashman et al., 2004). These data also highlight the usefulness of sexually dimorphic species in discerning the factors underlying variation in tolerance.

**Spittlebug effects on floral display**—Spittlebug damage reduced inflorescence height, but did not affect the number of open flowers per day, and unexpectedly increased petal size in both sex morphs of *F. virginiana*. This contrasts with other studies that generally found that source herbivory led to reductions in the number of open flowers (Strauss et al., 1996; Elle and Hare, 2002; J. A. Steets and T.-L. Ashman, unpublished data) and in flower size (Strauss et al., 1996; Mothershead and Marquis, 2000; Steets and Ashman, 2004). However, in the only other study to record the effect of xylem-feeding herbivores on attraction traits, spittlebug density had only a minimal effect on corolla diameter but a large negative effect on inflorescence height (Hamback, 2001). Reductions in stem (inflorescence) elongation may be characteristic of spittlebug damage (Meyer and Whitlow, 1992; Cronin and Abrahamson, 1999), so the potential effect on pollination will depend on whether the pollinating fauna is sensitive to height. For instance, Hamback (2001) found that large bees reduced visitation in response to reduced inflorescence height in *Rudbeckia hirta*, but this did not lead to reduced seed production. In *F. virginiana*, bee and fly pollinators increase visitation with increasing petal size (Ashman, 2000), but respond significantly to variation in height (Ashman et al., 2000). If there are no other effects of spittlebugs on attractive traits, i.e., nectar production or floral scent (Ashman et al., 2000, 2005), then the net effect of spittlebug damage in this system may be to increase pollinator visitation rather than to decrease it. Data on pollinator visitation to damaged and undamaged plants is required to confirm this.

**Spittlebug effects on interactions with other antagonists**—Spittlebug infestation had effects on two other plant–antagonist interactions. First, increasing spittlebug-days increased the number of leaves infected by fungal pathogens in females, although not in hermaphrodites. Spittlebugs serve as vectors for fungal and bacterial pathogens in other systems (Storer and Wood, 1998; Almeida and Purcell, 2003), so it is possible that the combination of spittlebugs and the high resource demand of fruit production borne by females made them more susceptible to fungal infection. Because leaf fungi reduce vegetative

growth rate (T.-L. Ashman, unpublished data), spittlebug-mediated enhanced fungal damage may have a more lasting effect on females than the transient and relatively minor direct effects of spittlebugs on growth. Second, increasing spittlebug-days resulted in reduced numbers of buds clipped by weevils in hermaphrodites, the weevils' preferred host (Ashman et al., 2004). This modification of the plant–weevil interaction has the potential to positively affect male fitness. Specifically, while hermaphrodites can compensate for weevil clipping in terms of female fitness, they are unable to fully compensate for pollen lost in clipped buds (Ashman et al., 2004). Consequently, by reducing weevil-clipping spittlebugs would reduce this direct loss of male gametes. Moreover, spittlebugs could lead to increased pollen export by hermaphrodites because spittlebug infestation leads to an increase in petal size (Table 1) which in turn, increases pollinator visitation and pollen export (Ashman and Diefenderfer, 2001). We should note, however, that it is not known whether spittlebug infestation has negative effects on pollen quantity or quality. If so, such a detriment could offset the seemingly positive effects on male fitness. In the only other study of multi-species interactions in a sexually dimorphic plant, Collin and colleagues (2002) also found that one antagonist could reduce a plant's interaction with another more injurious one. Specifically, while hermaphrodites of *Dianthus sylvestris* suffered higher predation by a predispersal seed predator than females, the net effect of seed predators on hermaphrodite fitness was concluded to be positive because larval herbivory prevented infection by an anther smut fungus that sterilizes its host.

In conclusion, our work joins others in highlighting the importance of considering multi-species interactions in our quest to understand the role of herbivory in plant phenotypic evolution (e.g., Pilson, 1996; Inouye and Stinchcombe, 2001; Strauss and Irwin, 2004), but also emphasizes the importance of examining morph-differential interactions and outcomes of multi-species interactions, especially if one wants to understand plant sexual evolution. We still need to determine whether the interaction modifications elucidated here are direct (i.e., weevil avoidance of spittlebug damaged plants, or spittlebug vectored disease) or trait-mediated (i.e., via changes in plant morphology or physiology), and whether they significantly alter fitness parameters important to sexual system evolution (Ashman, 2002).

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