

Advertising to the enemy: enhanced floral fragrance increases beetle attraction and reduces plant reproduction

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Abstract. Many organisms face challenges in avoiding predation while searching for mates. For plants, emitting floral fragrances to advertise reproductive structures could increase the attraction of detrimental insects along with pollinators. Very few studies have experimentally evaluated the costs and benefits of fragrance emission with explicit consideration of how plant fitness is affected by both pollinators and florivores. To determine the reproductive consequences of increasing the apparency of reproductive parts, we manipulated fragrance, pollination, and florivores in the wild Texas gourd, *Cucurbita pepo* var. *texana*. With enhanced fragrance we found an increase in the attraction of florivores, rather than pollinators, and a decrease in seed production. This study is the first to demonstrate that enhanced floral fragrance can increase the attraction of detrimental florivores and decrease plant reproduction, suggesting that florivory as well as pollination has shaped the evolution of floral scent.

Key words: cucurbits; diabroctite beetles; floral volatiles; florivore; pollination; squash.

INTRODUCTION

Understanding how simultaneous selection pressures from mutualists and antagonists affect phenotypic traits remains a challenge to evolutionary ecologists. In plant–insect interactions, costs associated with attracting pollinators via inadvertent attraction of floral antagonists mean that increasing attractive traits may produce diminishing fitness returns (Charnov 1979, Charlesworth and Charlesworth 1987, Ashman 2002). Floral fragrance, for example, advertises reproductive structures to promote pollinator fidelity and efficiency (Dobson 1994). Heightened fragrance emissions could increase fitness for pollen limited plants by attracting pollinators, and recent efforts are underway to transform plants to make flowers more fragrant (Dudareva and Negre 2005) and identify more fragrant cultivars in order to enhance natural pollination (Mena Granero et al. 2004). Floral antagonists, however, are navigating within the same scent landscape as pollinators. If floral antagonists are also attracted to fragrance emissions, the fitness benefits of

attracting pollinators with increased fragrance emission may depend on the costs of attracting floral herbivores. Floral herbivores can reduce fitness directly and indirectly through seed predation (Thompson and Pellmyr 1991), damage to reproductive parts (Sowell and Wolfe 2010), and by reducing pollinator attraction (Lohman et al. 1996). Our goal was to assess the costs and benefits of enhanced fragrance for plant reproduction.

Foraging decisions by pollinators and florivores might impose opposing selection on floral fragrance (Galen 1983), but because of the logistical challenges few studies have tested this hypothesis. For example, studies that attempt to correlate the size of the olfactory display (concentration) with visitation by pollinators and florivores are problematic because measuring fragrance requires that the flower be enclosed, which prevents floral visitation (but see Schiestl et al. 2011). There is opposing selection pressure in sexually dimorphic species where staminate flowers are often more fragrant (Theis et al. 2007), and receive more pollinator visits (reviewed in Ågren et al. 1999), but also attract more florivores (Fenner et al. 2002, Theis et al. 2007) and tend to incur more herbivory (Ågren et al. 1999). However, these patterns could be due to flower size rather than fragrance since staminate flowers are also larger than their pistillate counterparts (Bawa and Opler 1975). While opposing selection has been documented for floral

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morphology (Kudoh and Whigham 1998), sex ratio (Wise and Hébert 2010), flowering phenology (Parachnowitsch and Caruso 2008), and flower number (Ohashi and Yahara 2000), relatively few studies have experimentally manipulated fragrance and even fewer have manipulated florivores because of the difficulty in controlling them without interfering with pollination. The net fitness consequences of the olfactory display will depend on the effects of floral traits on both pollinators and florivores, and the relative importance of each interaction for plant reproduction.

To ascertain the fitness consequences of the fragrance display we manipulated fragrance, pollination, and florivores on the Texas gourd, *Cucurbita pepo* var. *texana* (Scheele) D. Decker (Cucurbitaceae). We enhanced the fragrance of Texas gourd flowers using the dominant component of the fragrance blend, which was most attractive to the specialist pollinators. By also manipulating florivory and pollination, we could measure their direct fitness effects, indirect effects of florivores on pollination, and the costs and benefits of an enhanced fragrance display.

MATERIALS AND METHODS

Study system

Cucurbita pepo var. *texana* (Texas gourd hereafter), an annual monoecious vine native to Texas, USA and Mexico, is closely related to the domesticated *C. pepo*. Flowers of both sexes last one day; they open at dawn and wilt by late morning. Species in the genus *Cucurbita* are attacked at all stages of development by diabroticite beetles (squash and cucumber beetles; Metcalf and Metcalf 1992). The major diabroticite of cucurbit crops in Massachusetts is *Acalymma vittatum*, the striped cucumber beetle, whose native range encompasses both our field site and the native range of the Texas gourd. Diabroticite adults are attracted to floral fragrance (Metcalf et al. 1998). In addition to specialist herbivores, there are also specialist pollinators. The specialist squash bee, *Peponapsis pruinosa* (Hymenoptera: Apidae; Hurd et al. 1971) has a range that extends throughout Massachusetts and south and west to Texas and beyond. Generalists such as bumble bees (*Bombus* spp.; Apidae) and the introduced honey bee *Apis mellifera* (Hymenoptera: Apidae) also pollinate squash flowers (Shuler et al. 2005).

Fragrance addition experiment

Texas gourd plants were germinated from seed (USDA North Central Regional Plant Introduction Station [NCRPIS], Ames, Iowa). They were planted in the field at the Hampshire Farm (42°20'17" N, 72°32'17" E) at the four-leaf stage on 25 June 2008 in 14 rows of 12 plants each with 3 m between plants and 4 m separating each row. The 168 plants were randomly assigned to one of eight treatments in blocks (two rows of four to a

block, $n = 21$ plants/treatment) with a $2 \times 2 \times 2$ factorial combination of pollination treatments (natural pollination vs. hand pollination), florivory treatments (natural florivory vs. beetle removal from flowers), and fragrance (natural fragrance vs. fragrance addition). Kaolin (Surround WP, Engelhard, New Jersey, USA) at 11 kg/378 L water was sprayed onto the plants before transplantation to reduce early herbivory damage, which can affect fragrance emission (Theis et al. 2009). Organic fertilizer (3:4:4, N:P:K) was added twice to the soil (0.27 L per plant; Gardentone, the Espoma Company, Millville, New Jersey, USA). For all treatments, every pistillate flower was treated on every plant five to six days a week from the onset of flowering on 5 August 2008 through the end of the flowering on 29 August 2008. Every staminate flower was treated until 17 August 2008. After this date, a single focal staminate flower was chosen per plant per day because the high numbers of staminate flowers per plant made it prohibitively time consuming to maintain beetle removal and scent manipulation for every flower.

Pollination treatment.—All pistillate flowers in the hand-pollination treatments were pollinated using pollen from anthers collected at 07:00 from randomly selected staminate flowers planted in a distant field. Pollen was mixed and added with a paintbrush to cover the entire stigmatic surface. Flowers were also open to natural pollinator visits in both the hand and natural pollination treatments.

Fragrance treatment.—We chose the dominant compound of the Texas gourd blend, 1,4 dimethoxybenzene, for the fragrance enhancement treatment based on trapping experiments that established this to be the most attractive compound to the specialist squash bees (Appendix). This compound represents approximately 90% of the entire floral scent display of this species (Theis et al. 2009). At 05:30, all pistillate flowers received a cotton swab cut in half and treated with either 60 mg of 1,4 dimethoxybenzene dissolved in 100 μ L of acetone for treated plants or 100 μ L of acetone alone for the controls; these scent emitters matched the concentrations used in the trapping experiments (Appendix). Because of the large tubular nature of squash flowers, these fragrance emitters were sheltered visually, and from direct sun and wind, deep within the corolla. Throughout the experiment, emissions are likely to have varied somewhat depending on climatic conditions, however, based on analyses of emitter concentrations, 1,4 dimethoxybenzene was emitted at an average of ~ 45 -fold higher than naturally high levels of approximately 5.6 μ g/h in Texas gourd flowers (N. Theis, unpublished data), maximizing differences between the natural level and the fragrance-enhanced treatment.

Florivore removal.—Beetles were counted in all flowers and removed every 30 minutes with feather-weight forceps between 06:00 and whenever flowers

closed for the day. Beetles were removed and released singly and randomly within the field. Control flowers were handled to simulate conditions of the beetle removal treatment.

Insect observations.—Insect visits to flowers in each plot were observed for a two-minute period per flower each day. For every flower, the number of insect visits and time per visit was recorded. We also counted approaches that did not result in visits (honey bees and squash bees were not distinguished), hereafter described as rejections. The order of observations on individual plants was re-randomized daily to prevent bias. If time permitted, plants were observed more than once.

Reproduction.—We measured reproductive parameters similar to those used extensively with this species, including fruit mass, total seed number, and seed mass (e.g., Stephenson et al. 2004). Between three and eight mature fruits were harvested per plant approximately 4–7 weeks after anthesis to determine female fitness. Fruit quality was determined by measuring fruit mass; an important measure for farmers, but also one that is affected by biotic interactions (Poulton et al. 2002, Stephenson et al. 2004). Total seed number and average mature seed mass were determined for each fruit.

Statistics.—All data were analyzed using SAS software, version 9.2 (SAS Institute 2008) unless otherwise stated. We analyzed the number of visits by squash bees, honey bees, and striped cucumber beetles for each observation, averaged within day if more than one observation took place. To control for daily and seasonal variation, we first determined the residuals from a general linear model (GLM) on log transformed data for mean visitation at each flower using time and date as the independent factors. These residuals were then used in a general linear model with block as a random factor and fragrance enhancement, hand pollination, and beetle removal as fixed factors, including all of the interaction terms between treatments.

To determine whether insects prefer visiting staminate or pistillate flowers, visitation was coded by floral sex, and number of visits and time per visit were analyzed using a paired *t* test on log-transformed data for squash bees, honey bees, cucumber beetles, and pollinator rejections with plant as the unit of replication.

To determine whether beetles repel pollinators, Spearman's rank correlations were calculated on visitation data with a two tailed probability using Systat Software Version 12.0 (Systat Software 2007); visitation numbers were averaged per flower and then per plant.

To determine the direct and indirect effects of treatments on reproduction we analyzed fruit mass, total seeds, and average mature seed mass per fruit as the dependent factors in an ANOVA with fragrance enhancement, hand pollination, and beetle removal as fixed factors and block as a random factor, including all of the interaction terms between treatments.

RESULTS

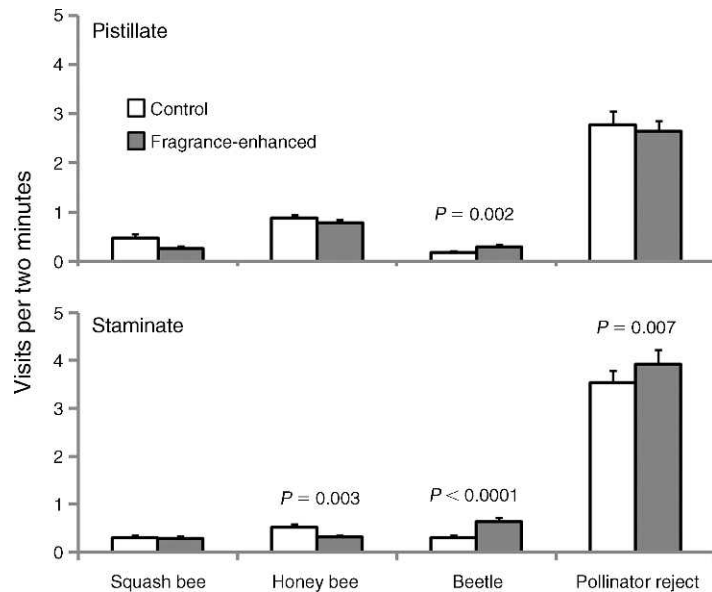
Fragrance treatment.—Striped cucumber beetles were attracted to flowers with enhanced fragrance in both staminate ($F_{1,113} = 19.17$, $P < 0.0001$) and pistillate flowers ($F_{1,98} = 10.59$, $P = 0.002$; Fig. 1). Enhanced fragrance roughly doubled the number of beetles per flower (staminate 2.1-fold, pistillate 1.7-fold; Figs. 1 and 2). The specialist squash bee was not attracted to fragrance-enhanced flowers (all $P > 0.1$; Fig. 1). The generalist honey bee visited fewer staminate flowers if fragrance was enhanced ($F_{1,113} = 9.22$, $P = 0.003$) but did not discriminate pistillate flowers by fragrance treatment ($F_{1,124} = 0.82$, $P = 0.37$). Similarly, visit length by honey bees was lower on staminate flowers with added fragrance than on control flowers (11 ± 0.05 vs. 19 ± 0.06 s, $F_{1,117} = 13.42$, $P = 0.0004$; all values presented as mean \pm SE). Additionally, pollinators were significantly more likely to reject staminate (but not pistillate) flowers that had added fragrance ($F_{1,128} = 7.51$, $P = 0.007$; Fig. 1).

Florivore treatment.—The florivore removal treatment significantly reduced the presence of cucumber beetles on staminate and pistillate flowers (staminate, 0.69 ± 0.08 beetles vs. 0.21 ± 0.03 beetles, $F_{1,113} = 36.97$, $P < 0.0001$; pistillate, 0.35 ± 0.05 beetles vs. 0.12 ± 0.03 beetles, $F_{1,98} = 13.64$, $P = 0.0004$) but did not affect visitation or rejection by squash bees or honey bees (all $P > 0.32$). The florivores spent significantly more time at staminate flowers that had florivores removed (107 ± 0.08 s vs. 113 ± 0.03 s, $F_{1,72} = 4.90$, $P = 0.03$). However, visit length was not affected by florivore treatment for any insect on pistillate flowers (all $P > 0.1$), nor for pollinators on staminate flowers (all $P > 0.1$).

Pollination treatment.—Over the season, the pollinator assemblage at squash flowers was composed almost entirely of squash bees (59%) and honey bees (40%). Pollination treatments had no significant effect on whether flowers were visited (all $P > 0.3$), but there was a significant pollination treatment \times beetle removal interaction for honey bee visit length (natural pollination with beetle control, 21 ± 3 s; natural pollination with beetle removal, 20 ± 3 s; hand pollination with beetle control, 18 ± 3 s; hand pollination with beetle removal, 20 ± 3 s; $F_{1,90} = 5.90$, $P = 0.02$), and a pollination \times fragrance interaction for cucumber beetle visit length (with natural pollination and natural fragrance, 114 ± 6 s; with natural pollination and enhanced fragrance, 112 ± 7 s; with hand pollination and natural fragrance, 120 ± 0 s; with hand pollination and enhanced fragrance, 117 ± 3 s; $F_{1,72} = 4.15$, $P = 0.05$).

Attraction to staminate vs. pistillate flowers.—Squash bees did not distinguish between staminate and pistillate flowers ($df = 121$, $t = 1.70$, $P = 0.09$). Honey bees, however, were 66% more likely to visit pistillate than staminate flowers ($df = 121$, $t = 7.45$, $P < 0.0001$), while striped cucumber beetles were 66% more likely to visit staminate than pistillate flowers ($df = 121$, $t = -3.84$, $P = 0.0002$).

FIG. 1. Average number of visits by insects at control and fragrance-enhanced pistillate and staminate flowers. Error bars indicate \pm SE. P values are displayed above significantly different means.



Pollinator rejections were 58% more likely at staminate than pistillate flowers ($df = 93$, $t = -2.75$, $P = 0.007$).

Correlations between visitors.—There was a significant positive correlation between the per-plant average number of cucumber beetles per flower and rejections by pollinators ($n = 143$, $r = 0.42$, $P < 0.001$; staminate and pistillate flowers analyzed separately showed comparable results). Similarly, honey bee visitation was negatively correlated with the number of floral beetles ($n = 143$, $r = -0.22$, $P = 0.009$). This pattern persisted in staminate flowers alone ($n = 141$, $r = -0.17$, $P = 0.04$), but not pistillate flowers ($n = 126$, $r = -0.03$, $P = 0.7$). Squash bee visits were positively correlated with beetles ($n = 143$, $r = 0.23$, $P = 0.006$; staminate and pistillate flowers analyzed separately showed comparable results).

Treatment effects on reproduction.—Fragrance addition significantly reduced measures of reproduction including fruit mass (natural fragrance, 124 ± 4 g, enhanced fragrance, 113 ± 5 g; $F_{1,96} = 5.35$, $P = 0.02$) and total seed production (natural fragrance, 240 ± 6 seeds, enhanced fragrance, 220 ± 6 seeds; $F_{1,96} = 5.02$, $P = 0.03$). For fruit mass, there was a significant interaction between pollination and fragrance addition ($F_{1,96} = 5.27$, $P = 0.02$), such that hand pollination recovered mass lost due to fragrance addition. Seed mass and fruit mass varied with block ($F_{19,96} > 2.0$, $P < 0.02$ for both), but there were no other main effects of either the pollination treatment or the florivore treatment (all $P > 0.1$).

DISCUSSION

By advertising reproductive structures with floral fragrances, plants risk attracting detrimental insects along with pollinators. This negative fitness consequence of a fragrance display has long been proposed (Galen

1983, Dobson 1994), but so far no study has simultaneously manipulated both scent and interactions to determine the mechanisms underlying the effects of scent on plant reproduction. We manipulated fragrance, pollination, and florivores in the Texas gourd to determine the effects of increasing fragrance emission and provide the first evidence of a reproductive cost of fragrance that is likely driven by the attraction of florivores to fragrance-enhanced flowers.

We hypothesize that the increased abundance of florivores is driving the detrimental effect of fragrance enhancement. Fragrance enhancement increased beetle abundance by 65% compared to control pistillate flowers, and more than doubled beetles in staminate flowers. This increase in beetle abundance may have reduced reproduction directly, suggested by the reduc-

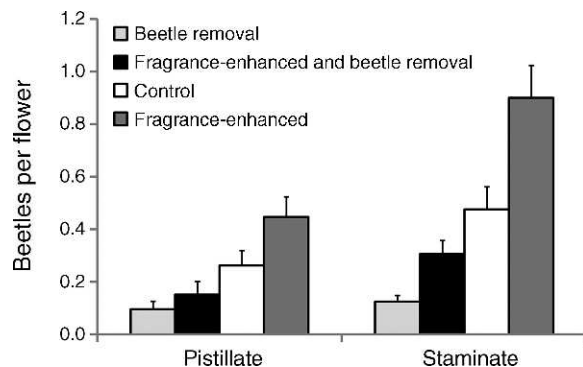


FIG. 2. The average number of striped cucumber beetles observed at flowers of each treatment during a 2-minute observation (averaged over pollen addition treatment); presented in order of beetle abundance. Error bars indicate one standard error.

tion in seed number that occurred regardless of hand pollination treatment. However, scent could also have indirect effects on reproduction by reducing pollinator attraction, as evidenced by the fact that hand pollination recovered the reduction in fruit mass due to enhanced fragrance, and the significant increase in pollinator rejections as beetle abundance increased. Although pollinators were not identified unless they landed on the flower, our data suggest these rejections may have been driven by honey bees, since only honey bee visits were negatively correlated with beetle abundance. Staminate flowers harbored twice as many beetles as pistillate flowers, which would explain why honey bees avoided fragrance-enhanced staminate flowers but did not discriminate in pistillate flowers (Fig. 2).

While the data suggest that high numbers of beetles may have reduced pollinator visits, the low beetle numbers achieved by the beetle removal treatment had no effect on pollinators. Taken together these data suggest that there may be a threshold below which plants can tolerate beetles with no fitness reduction. Beetle removal from pistillate flowers decreased beetle abundance by 27% compared to controls, with no significant increase in either pollinator attraction or plant reproduction (Fig. 2). This contrasts with the effect of enhanced fragrance, which increased beetles and reduced reproduction. Thus, traits that enhance beetle attraction may reduce reproduction while traits decreasing beetles below a threshold may have little effect.

The negative fitness consequences of beetle attraction may be greater than recognized in this study due to other, unmeasured costs. First, while fragrance attracted beetles to pistillate flowers and reduced maternal reproduction, beetle attraction was even higher to staminate flowers where fitness was not measured. Because these beetles feed on pollen (Metcalf and Metcalf 1992), the detrimental effects on staminate reproduction may be even higher than for pistillate reproduction. Few studies have measured the effect of florivory on paternal fitness; when it has been measured, costs have been documented (Krupnick and Weis 1999). Second, while bacterial wilt was not present in our fields, it can be transmitted when *Acalymma vitatum* defecate in flowers and is lethal for the plant (Sasu et al. 2010). Thus, traits that attract beetles to flowers, such as scent emission, may have more negative consequences than our data show.

Pollinators were not attracted to fragrance-enhanced flowers. This was anticipated for honey bees since our trapping experiment found that 1,4 dimethoxybenzene was no more attractive to these bees than controls, but unexpected for squash bees who showed a strong preference for this compound (Appendix). These specialist pollinators may respond to compound presence but not concentration. There may be a trade-off between accurately choosing rewarding flowers and flower visitation rate (Chittka and Raine 2006), and concentration

might be irrelevant for specialist squash bees in a field of flowers. For cucumber beetles who visit fewer flowers, flower choice may be more important. Striped cucumber beetle attraction to fragrance-enhanced flowers was surprising, however, since they were not attracted to 1,4 dimethoxybenzene in trapping experiments (Lewis et al. 1990, Appendix). Insects use multisensory input to identify flowers, including odor, color, shape, and even texture (Raguso 2004). Fragrance-enhanced flowers are therefore a more ecologically relevant way to assess preferences than scent-emitting insect traps.

Fragrance can attract floral antagonists and thus may be the result of a compromise between selection by pollinators and floral antagonists (Galen 1983, Euler and Baldwin 1996, Baldwin et al. 1997). Galen and colleagues (2011) found a component of the bouquet of *Polemonium viscosum* fragrance that acts defensively, repelling florivores but also pollinators at high concentrations. Although other manipulations of scent in field conditions have shown that scent is important for pollinator attraction and effectiveness (Ashman et al. 2005, Kessler et al. 2008, Waelti et al. 2008, Galen et al. 2011), in our study we found that fragrance enhancement had no significant effects on pollinators. While specialist pollinators were attracted to 1,4 dimethoxybenzene at high concentrations in trapping experiments, they did not discriminate between high and natural levels in a field of flowers. Our work demonstrates that there may be fitness costs due to beetle attraction when scent is high, but that below a threshold beetle reduction does not further benefit plants. Taken together, our results suggest that there may be selection against high emission rates due to the detrimental effects of beetle attraction. Our study is the first to demonstrate that enhanced fragrance from flowers can increase the attraction of detrimental florivores and have significant negative effects on plant reproduction. Thus, this work adds to a growing body of evidence that florivores as well as pollinators may have shaped the evolution of the characteristic fragrances we associate with flowers.

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LITERATURE CITED

- Ågren, J., K. Danell, E. Elmqvist, L. Ericson, and J. Hjalten. 1999. Sexual dimorphism and biotic interactions. Pages 217–246 in M. A. Geber, T. E. Dawson, and L. F. Delph, editors. Gender and sexual dimorphism in flowering plants. Springer, Berlin, Germany.
- Ashman, T.-L. 2002. The role of herbivores in the evolution of separate sexes from hermaphroditism. *Ecology* 83:1175–1184.

- Ashman, T.-L., M. Bradburn, D. H. Cole, B. H. Blaney, and R. A. Raguso. 2005. The scent of a male: the role of floral volatiles in pollination of a gender dimorphic plant. *Ecology* 86:2099–2105.
- Baldwin, I. T., C. Preston, M. Euler, and D. Gorham. 1997. Patterns and consequences of benzyl acetone floral emissions from *Nicotiana attenuata* plants. *Journal of Chemical Ecology* 23:2327–2343.
- Bawa, K. S., and P. A. Opler. 1975. Dioecism in tropical forest trees. *Evolution* 29:167–179.
- Charlesworth, D., and B. Charlesworth. 1987. The effect of investment in attractive structures on allocation to male and female functions in plants. *Evolution* 41:948–968.
- Charnov, E. L. 1979. Simultaneous hermaphroditism and sexual selection. *Proceedings of the National Academy of Sciences USA* 76:2480–2484.
- Chittka, L., and N. E. Raine. 2006. Recognition of flowers by pollinators. *Current Opinion in Plant Biology* 9:428–435.
- Dobson, H. 1994. Floral volatiles in insect biology. Pages 47–81 in E. Bernays, editor. *Insect-plant interactions*. CRC Press, Boca Raton, Florida, USA.
- Dudareva, N., and F. Negre. 2005. Practical applications of research into the regulation of plant volatile emission. *Current Opinion in Plant Biology* 8:113–118.
- Euler, M., and I. T. Baldwin. 1996. The chemistry of defense and apparency in the corollas of *Nicotiana attenuata*. *Oecologia* 107:102–112.
- Fenner, M., J. E. Cresswell, R. A. Hurley, and T. Baldwin. 2002. Relationship between capitulum size and pre-dispersal seed predation by insect larvae in common Asteraceae. *Oecologia* 130:72–77.
- Galen, C. 1983. The effects of nectar thieving ants on seed set in floral scent morphs of *Polemonium viscosum*. *Oikos* 41:245–249.
- Galen, C., R. Kaczorowski, S. L. Todd, J. Geib, and R. A. Raguso. 2011. Dosage-dependent impacts of a floral volatile compound on pollinators, larcenists, and the potential for floral evolution in the Alpine Skypilot *Polemonium viscosum*. *American Naturalist* 177:258–272.
- Hurd, P. D., E. G. Linsley, and T. W. Whitaker. 1971. Squash and gourd bees (*Peponapis*, *Xenoglossa*) and origin of cultivated *Cucurbita*. *Evolution* 25:218–234.
- Kessler, D., K. Gase, and I. T. Baldwin. 2008. Field experiments with transformed plants reveal the sense of floral scents. *Science* 321:1200–1202.
- Krupnick, G. A., and A. E. Weis. 1999. The effect of floral herbivory on male and female reproductive success in *Isomeris arborea*. *Ecology* 80:135–149.
- Kudoh, H., and D. F. Whigham. 1998. The effect of petal size manipulation on pollinator/seed-predator mediated female reproductive success of *Hibiscus moscheutos*. *Oecologia* 117:70–79.
- Lewis, P. A., R. L. Lampman, and R. L. Metcalf. 1990. Kairomonal attractants for *Acalymma vittatum* (Coleoptera, Chrysomelidae). *Environmental Entomology* 19:8–14.
- Lohman, D. J., A. R. Zangerl, and M. R. Berenbaum. 1996. Impact of floral herbivory by parsnip webworm (Oecophoridae: *Depressaria pastinacella* Duponchel) on pollination and fitness of wild parsnip (Apiaceae: *Pastinaca sativa* L.). *American Midland Naturalist* 136:407–412.
- Mena Granero A., F. J. E. Gonzalez, A. G. Frenich, J. M. G. Sanz, and J. L. M. Vidal. 2004. Single step determination of fragrances in *Cucurbita* flowers by coupling headspace solid-phase microextraction low-pressure gas chromatography-tandem mass spectrometry. *Journal of Chromatography A* 1045:173–179.
- Metcalf, R. L., R. L. Lampman, and P. A. Lewis. 1998. Comparative kairomonal chemical ecology of diabroticite beetles (Coleoptera: Chrysomelidae: Galerucinae: Luperini: Diabroticina) in a reconstituted tallgrass prairie ecosystem. *Journal of Economic Entomology* 91:881–890.
- Metcalf, R. L., and E. R. Metcalf. 1992. *Plant kairomones in insect ecology and control*. Chapman and Hall, New York, New York, USA.
- Ohashi, K., and T. Yahara. 2000. Effects of flower production and predispersal seed predation on reproduction in *Cirsium purpuratum*. *Canadian Journal of Botany* 78:230–236.
- Parachnowitsch, A. L., and C. M. Caruso. 2008. Predispersal seed herbivores, not pollinators, exert selection on floral traits via female fitness. *Ecology* 89:1802–1810.
- Poulton, J., D. Bryla, R. Koide, and A. Stephenson. 2002. Mycorrhizal infection and high soil phosphorus improve vegetative growth and the female and male functions in tomato. *New Phytologist* 154:255–264.
- Raguso, R. A. 2004. Flowers as sensory billboards: progress towards an integrated understanding of floral advertisement. *Current Opinion in Plant Biology* 7:434–440.
- SAS Institute. 2008. *Statistical analysis system*. SAS Institute, Cary, North Carolina, USA.
- Sasu, M. A., K. L. Wall, and A. G. Stephenson. 2010. Antimicrobial nectar inhibits a florally transmitted pathogen of a wild *Cucurbita pepo* (Cucurbitaceae). *American Journal of Botany* 97:1025–1030.
- Schiestl, F. P., F. K. Huber, and J. M. Gomez. 2011. Phenotypic selection on floral scent: trade-off between attraction and deterrence? *Evolutionary Ecology* 25:237–248.
- Shuler, R. E., T. H. Roulston, and G. E. Farris. 2005. Farming practices influence wild pollinator populations on squash and pumpkin. *Journal of Economic Entomology* 98:790–795.
- Sowell, D. R., and L. M. Wolfe. 2010. Pattern and consequences of floral herbivory in four sympatric *Ipomoea* species. *American Midland Naturalist* 163:173–185.
- Stephenson, A. G., B. Leyshon, S. E. Travers, C. N. Hayes, and J. A. Winsor. 2004. Interrelationships among inbreeding, herbivory, and disease on reproduction in a wild gourd. *Ecology* 85:3023–3034.
- Systat Software. 2007. *SYSTAT for Windows, Version 12.0*. Systat Software, San Jose, California, USA.
- Theis, N., K. Kesler, and L. S. Adler. 2009. Leaf herbivory increases floral fragrance in male but not female *Cucurbita pepo* subsp. *texana* (Cucurbitaceae) flowers. *American Journal of Botany* 96:897–903.
- Theis, N., M. Lerchau, and R. A. Raguso. 2007. The challenge of attracting pollinators while evading floral herbivores: patterns of fragrance emission in *Cirsium arvense* and *Cirsium repandum* (Asteraceae). *International Journal of Plant Sciences* 168:587–601.
- Thompson, J. N., and O. Pellmyr. 1991. Evolution of oviposition behavior and host preference in Lepidoptera. *Annual Review of Entomology* 36:65–89.
- Waelti, M. O., J. K. Muhlemann, A. Widmer, and F. P. Schiestl. 2008. Floral odour and reproductive isolation in two species of *Silene*. *Journal of Evolutionary Biology* 21:111–121.
- Wise, M. J., and J. B. Hébert. 2010. Herbivores affect natural selection for floral-sex ratio in a field population of horse-nettle, *Solanum carolinense*. *Ecology* 91:937–943.

SUPPLEMENTAL MATERIAL

Appendix

Methods, results, and figure for trapping experiments (*Ecological Archives* E093-039-A1).