Tritrophic Interactions and Reproductive Fitness of the Prairie Perennial *Silphium laciniatum* Gillette (Asteraceae)

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ABSTRACT Recent studies have revealed that natural enemies can influence reproductive success of plants by eliminating their herbivores, thereby reducing damage to photosynthetic or reproductive tissues. Some plant species apparently have evolved "indirect defenses" in response to such top-down selective pressures, producing volatile compounds that are used as cues by natural enemies searching for their herbivorous hosts. The research summarized in this article evaluates the potential for such top-down influences on plant fitness in an endemic prairie system and the role of plant volatiles in location of hosts by parasitoids. The study system was comprised of the prairie perennial *Silphium laciniatum* L. (Asteraceae), the gall wasp *Antistrophus rufus* Gillette (Hymenoptera: Cynipidae), and its parasitoid *Eurytoma lutea* Bugbee (Hymenoptera: Eurytomidae). In common garden experiments, we assessed the impact of gall wasp herbivory on growth and reproduction of *S. laciniatum* and the mediating influence of the parasitoid using three treatments: plants caged with gall wasps, plants caged with gall wasps and parasitoids, and control plants caged without gall wasps. Despite technical difficulties in excluding wild gall wasps and parasitoids, plants caged with gall wasps flowered later than control plants and had reduced reproductive output, producing shorter flowering stems and fewer and smaller seeds of lower viability. The parasitoid apparently "rescued" plant reproduction by killing gall wasp larvae, resulting in larger seeds that were more likely to germinate. Parasitoid females responded more strongly to volatiles produced by galled plants compared with ungalled plants in field olfactometry bioassays. This seems to be the first evidence in an endemic community of a plant species gaining a fitness advantage by producing volatile compounds that attract natural enemies of herbivorous insects.

KEY WORDS gall wasp, indirect defenses, parasitoid, synomone, trophic cascade

PRICE ET AL. (1980) PROPOSED that natural enemies can indirectly influence plant fitness by killing herbivorous insects. This hypothesis has been supported by research on a diversity of predators, including birds, lizards, ants, and beetles (Marquis and Whelan 1994, de la Fuente and Marquis 1999, Pace et al. 1999, Van Bael et al. 2003). Parasitoids introduced into new regions as biological control agents also influence plant fitness by killing herbivores (DeBach and Rosen 1991). This effect rarely has been assessed for parasitoids in endemic systems, however, even though they often are important in regulating populations of herbivorous insects (DeBach and Rosen 1991, Gomez and Zamora 1994, Hawkins et al. 1999). Fitness benefits that some plant species accrue through parasitism of their herbivores presumably have selected for insect-induced release of plant volatiles that adult parasitoids exploit as cues for host location (Loughrin et al. 1995, van Loon et al. 2000, Gouinguene et al. 2001, Fritzsch-Hoballah and Turlings 2001). To our knowledge, attraction of natural enemies by such "indirect defenses" in plants have been documented in only one natural system, although the fitness benefit was not assessed (Kessler and Baldwin 2001).

In this article, we present field evidence that parasitoids of an endemic community enhance plant reproduction by killing larvae of gall wasps and that plants produce volatile cues that parasitoids use in locating hosts. Gall-forming insects severely alter host plant morphology and physiology and act as nutrient sinks, sapping resources that otherwise would be allocated to seed production (Abrahamson and Weis 1987, Bronner 1992). In fact, gall insects can have a greater negative impact on plant fitness per unit body mass than do foliage feeders (Abrahamson and Weis 1987), and so can be effective biological control agents of weeds (Harris and Shorthouse 1996, Hoffman et al. 2002). Parasitoids may improve plant fitness by retarding feeding rates of gallers or by killing them, which may arrest gall development and allow plant cells to revert to normal functioning (Rohfritsch and Shorthouse 1982, Bronner 1992).

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Larvae of the gall wasp *Antistrophus rufus* Gillette (Hymenoptera: Cynipidae) inhabit flowering stems of *Silphium laciniatum* L. (Asteraceae) (Tooker and Hanks 2004b, Tooker et al. 2004), a species endemic to tallgrass prairies of the Midwestern United States (Gleason and Cronquist 1991, Clevinger and Panero 2000). *S. laciniatum* bolts in mid- to late May in the area of our studies (east central Illinois). Each plant produces as many as 12 flowering stems that can reach 4.5 m tall (Weaver 1954, Tooker and Hanks 2004b). Tall stems are adaptive in the wind-swept prairie ecosystem because they maximize aerial dispersion of seeds (Pleasants and Jurik 1992). Female *A. rufus* (≈3 mm long) oviposit in young bolting stems (≈15–30 cm tall) of *S. laciniatum*, but late-emerging females will oviposit in taller stems (>1 m tall; for biology, see Tooker et al. 2002, 2004, 2005, Tooker and Hanks 2004a–c). Larvae develop in galls in the pith and cambium, and gall densities are highly variable within prairie sites, ranging from 0 to >600 galls per stem (Tooker and Hanks 2004b, Tooker et al. 2004, unpublished data).

Antistrophus rufus larvae are attacked by at least seven species of parasitoids, but the specialist idiobiont ectoparasitoid *Eurytoma lutea* Bugbee (Hymenoptera: Eurytomidae) is strongly dominant (Tooker and Hanks 2004b). It can kill as many as 100% of gall wasp larvae in individual stems, but parasitism rates averaged 35 ± 17% (SD) in an earlier study (Tooker and Hanks 2004b). Larvae of *A. rufus* and *E. lutea* overwinter in senesced stems of *S. laciniatum*, adult gall wasps emerge in May to June, and adult parasitoids (≈3 mm long) emerge about 1 mo later.

The research reported here evaluates the relative influences of gall wasps and parasitoids on plant reproduction by testing the following hypotheses: (1) gall wasp larvae have a negative impact on plant reproduction; (2) by killing gall wasp larvae, parasitoids enhance plant reproduction; and (3) plants inhabited by gall wasps release volatile compounds that adult parasitoids use as cues for locating hosts. We tested hypotheses 1 and 2 by conducting two common garden experiments that manipulated exposure of plants to gall wasps and parasitoids and assessed plant reproductive output. We also conducted a greenhouse experiment to evaluate the influence of seed mass on viability. We tested hypothesis 3 by conducting olfactometry bioassays in the common garden to evaluate the response of parasitoids to volatiles emitted by galled and ungalled host plants.

Materials and Methods

Influence of Gall Wasps on Plant Reproduction. A common garden plot of *Silphium* species was established in spring of 2000 at the Landscape Horticulture Research Center on the campus of the University of Illinois at Urbana-Champaign. We planted 30 bare root plants each of *S. laciniatum* and two congeners (4–5 yr old; Missouri Wildflower Nursery, Jefferson City, MO) at 1-m centered spacing in a 30 by 3 array, species assigned randomly to position. In spring of 2001, the 10 *S. laciniatum* plants that produced single flowering stems were enclosed in individual wire tomato cages (107 cm tall, 29 cm diameter at top) covered with a sleeve of organdy gathered at the top and fastened to the ground with fencepost staples.

We released varying numbers of gall wasp adults in cages to establish different densities of galls: no gall wasps ($N = 3$ plants), five females ($N = 4$), and 30 females ($N = 3$). Treatments were assigned randomly to plants, and we released wasps into cages during 17–23 May 2001 when stems were the appropriate size for oviposition (≈15–30 cm tall) and *A. rufus* adults were ovipositing in *S. laciniatum* plants in nearby prairie sites, the closest of which was ≈500 m away. Adult gall wasps had been reared from *S. laciniatum* stems collected in late fall 2000 from Loda Cemetery Prairie (Iroquois Co., IL), and females had had the opportunity to mate with males in rearing cages. Flowering stems eventually grew out of the cages during summer, and we allowed them to protrude through cage tops, enclosing the basal ≈100 cm by gathering the organdy around the stem and securing it with twine. *A. rufus* females oviposit primarily in the basal 60 cm of stems (Tooker and Hanks 2004b), but gall wasps of the wild population nevertheless had an opportunity to oviposit in stem apices when stems emerged from cages. In October 2001, seeds already had begun to drop from some flower heads by the time we began collecting them, limiting assessment of treatment effects to average seed size.

Treatment effects on plant performance (average seed size) were tested by analysis of variance (ANOVA; PROC GLM; SAS Institute 2001) with plants as replicates. Differences between individual treatment means were tested as unplanned comparisons by the least significant difference (LSD) means separation test (Sokal and Rohlf 1995), and tests were "protected" (i.e., means-separation tests were contingent on a significant overall *F;* Day and Quinn 1989).

Seed Size and Germination Rate. We determined how viability of *S. laciniatum* seeds was influenced by their mass by planting seeds in flats in a greenhouse and evaluating germination success. We collected 119 seeds from plants used in the 2001 experiment to represent a broad range of seed sizes and recorded their individual mass. We followed standard procedures for propagating *Silphium* species (Rock 1977), packing seeds in moist sand and storing them in a 4°C refrigerator for 30 d. Seeds were planted in three flats (≈5 cm apart and 2 cm deep) with a sterilized potting mixture of equal proportions of soil, peat, and perlite, and slow-release fertilizer (15:9:12 N:P:K, Osmocote Plus; Scotts-Sierra Horticultural Products Co., Marysville, OH), irrigating as needed. We recorded germination success of each seed, defined as production of a viable seedling, and tested the influence of seed mass on germination success with the nonparametric Kruskal-Wallis test (PROC NPAR1WAY; SAS Institute 2001), comparing individual means with the LSD means separation test (Sokal and Rohlf 1995). We used the nonparametric test because a zero value for

one class mean violated assumptions of ANOVA (Sokal and Rohlf 1995).

Influence of Parasitoids on Plant Reproduction. We conducted a second experiment in the common garden plot in spring of 2002 to assess again the impact of gall wasps on seed production and viability but also to evaluate the mediating influence of the parasitoid *E. lutea.* Of the 14 plants used for this experiment, 4 produced more than one stem. We caged bolting *S. laciniatum* as already described and released adult female gall wasps to establish varying densities of gall wasp larvae and female parasitoids to kill gall wasp larvae, randomly assigning the following treatments to plants: no insects (control, $N = 4$ plants), 50 gall wasps per stem and no parasitoids (gall wasps only, $N = 5$), and 50 gall wasps and 30 parasitoids per stem (gall wasps plus parasitoids, $N = 5$). We released recently emerged insects into cages when plant stems were the appropriate size for oviposition and *A. rufus* or *E. lutea* adults were active in the vicinity (gall wasps released 24 May to 9 June, parasitoids released 24 June to 8 July 2002). Insects were reared from *S. laciniatum* stems collected in late fall 2001 from Fithian Railroad Prairie (Vermilion Co., IL), and females had had the opportunity to mate in rearing cages. Stems were allowed to grow out of cage tops, and cages were adjusted, as in the previous experiment.

We evaluated plant growth and reproduction at the end of the season by quantifying flowering phenology as the number of flower heads per plant per week per developmental stage of flower (using a 10-point scale ranging from 1 for buds to 10 for petals withered). To compare treatment effects on flowering phenology, we regressed the proportion of flowers that had senesced (petals withered) on sampling date and modeled the relationship by fitting curves to optimize the correlation coefficient (SPSS 2000, Quinn and Keough 2002). We estimated with regression equations the Julian date on which 20, 40, 60, and 80% of flowers had senesced for each plant and used these percentages as covariates to test differences between treatment means in flowering phenology by analysis of covariance (ANCOVA; PROC GLM; SAS Institute 2001).

We examined plants at least every other day during September and November 2002 to collect mature flower heads before seeds began to drop. At that time we also recorded the length of senesced flowering stems and the number of flower heads per plant. We later dissected flower heads, recording the number of seeds per head and mass of each seed. We calculated means for these variables across flowering stems for the four plants that produced more than one stem. Each stem was cut into ≈20-cm sections and enclosed in a plastic storage bag, and bags were stored in an unheated outbuilding in Urbana, IL. Adult insects emerged from these stems in spring and summer of 2003. We calculated percent parasitism from the number of adult gall wasps and parasitoids that emerged per stem. We counted the number of emergence holes in the basal 20 cm of stems (hence referred to as "stem bases"), where gall densities typically are highest (Tooker and Hanks 2004b), to determine the number of wasps (gall wasps and parasitoids) that had successfully emerged. We also dissected stem bases and counted unemerged individuals to assess the relationship between gall density and survivorship of wasps, calculating percent survivorship by the number of emergence holes in stem bases divided by the total number of galls revealed by dissecting stem bases. It was not possible to determine whether dead larvae in galls had been parasitized or not. We estimated the total number of galls that developed within the entire stem by multiplying the number of adult wasps that emerged by the ratio of dead to surviving wasps in stem bases (to estimate the proportion that failed to complete development in the entire stem), adding that product to the number of wasps that emerged, and dividing by stem length to yield estimated density of galls per centimeter of stem.

We first compared mean gall densities in stem bases to assess how effective the treatments were in establishing different densities of gall wasps. We determined how percent survival of gall wasps (no. emergence holes/no. galls in stem bases) was influenced by treatments (by ANOVA) and two measures of gall density (by regression analysis): number of galls in the stem base and estimated gall density per centimeter of stem. We also determined how treatments influenced the two measures of gall density. We determined how percent parasitism (number of parasitoids/total number of emerged wasps for the entire plant) was influenced by treatments, by number of wasps completing development (no. emerging per plant), by number of galls in the stem base, and by estimated density of galls per cm of stem. Finally, we determined how the various measures of plant performance (flowering phenology, stem length, mass of individual seeds, etc.) were influenced by treatments, number of emerging gall wasps and parasitoids, percent parasitism, and the two measures of gall density. We include treatment as a categorical variable in this model because it provides the best assessment of the impact of oviposition by gall wasps, and parasitism of gall wasps, on plant performance. This analysis was conducted because we had no means of estimating the proportion of gall wasp ovipositions that failed to establish larvae or early mortality of gall wasp larvae caused by probing by parasitoids. We included gall density as a covariate because it provided an independent assessment of the impact of experimental treatments on plant performance that is more precise than using treatment as a categorical variable.

We used ANOVA or ANCOVA (PROC GLM; SAS Institute 2001) to test treatment effects on the number of emerging gall wasps and parasitoids, gall density, survival of wasps, stem length, number of seeds per flower head, number of seeds per plant, mass of individual seeds, seed mass per flower head, and seed mass per plant, with individual plants as replicates, as described above. Linear relationships between variables were tested by regression analysis (PROC REG; SAS Institute 2001). We confirmed that there were no patterns in the dispersion of residuals that would in-

dicate nonlinear relationships between variables (Sokal and Rohlf 1995).

Plant Volatiles as Synomones. We determined whether volatile compounds produced by *S. laciniatum* plants can be used by the parasitoid *E. lutea* to locate gall wasp hosts by conducting bioassays with a Y-tube olfactometer. The experiment was conducted in the common garden in 2002, 1 mo after gall wasps had oviposited. Parasitoids were reared from plant stems in the 2002 plant fitness experiment (see above), were not >5 d old, and were used only once in bioassays. They were held at 15°C in cardboard containers with water and honey for food. Volatiles from a galled and ungalled plant were sampled by enclosing the basal ≈30 cm of stems in a nylon oven cooking bag (Reynolds, Richmond, VA) supported by a cylindrical wire cage (≈30 cm tall, 20 cm in diameter). The bags were connected with Teflon tubing to arms of the Y-tube (6 cm diameter, main tube 26 cm long, arm length 22 cm, angle between arms 70°). Air was drawn through the system (≈2.2 liter/min; calibrated with an airflow meter) with a 1 hp vacuum cleaner (Shop-vac, Williamsport, PA), and air entering the bag was purified with a filter containing activated charcoal. The vacuum cleaner was run on a car battery through a power inverter, and air flow was adjusted with a variable autotransformer. Individual parasitoids were introduced into the Y-tube in a small vial. Parasitoids "responded" to volatiles in the Y-tube by walking upwind and crossing a decision line 11 cm up either arm and remaining for at least 30 s. Wasps not reaching a decision line within 5 min were recorded as "no response." We repeated this bioassay, switching treatments between Y-tube arms every five trials to control for position effects, rinsing the tube with acetone, and allowing it to air dry. We used three different pairs of galled and ungalled plants as sources of volatiles in these field bioassays, switching between pairs every 10 trials. Response of parasitoids to volatiles was analyzed with the χ^2 goodness-of-fit test with the null hypothesis being equal response to treatments (Sokal and Rohlf 1995). We conducted bioassays on 1 and 2 July 2002 from 1000 to 1400 hours. The weather was sunny, with air temperatures ≈30°C on both days.

We report means ± SE throughout unless stated otherwise.

Results

Influence of Gall Wasps on Plant Reproduction. We collected a mean of 20.3 ± 7.7 (SD) flower heads per *S. laciniatum* plant and a total of 5,452 seeds in the 2001 experiment. Mass of individual seeds was significantly influenced by exposure to gall wasp adults (Fig. 1; ANOVA $F_{2,9} = 10.3$, $P = 0.008$), averaging ≈60% greater for plants caged without gall wasp adults compared with those caged with 30 female gall wasps. Means for plants caged with only five gall wasps did not differ from controls (Fig. 1). Despite the significant treatment effect, seed mass was not significantly related to the number of gall wasps that emerged from stems (regression $P > 0.05$).

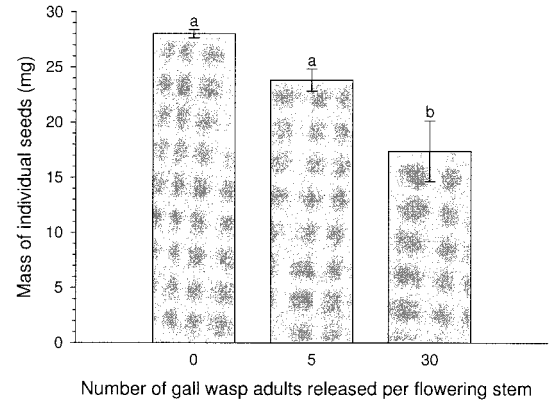

Fig. 1. Average mass of individual seeds produced by *S. laciniatum* plants that had been caged with three densities of adult gall wasps in the 2001 experiment. $N = 3, 4, 3$, left to right. Bars with different letters are significantly different (see text for ANOVA results; LSD $P < 0.05$).

Seed Size and Germination Rate. Germination of *S. laciniatum* seeds in the greenhouse was significantly influenced by their mass, with low germination success in seeds that weighed <15 mg (Fig. 2; Kruskal-Wallis $\chi^2 = 22.7$, $P < 0.0001$). Seeds in different mass classes appeared similar in length and width, but differed greatly in endosperm volume. All seeds produced by ungalled plants in the 2001 experiment exceeded this mass threshold, but 60% of the seeds produced by plants in the high gall density treatment had masses below 15 mg and probably were inviable. Thus, exposure to gall wasps was associated with a significant reduction in the number of viable seeds.

Influence of Parasitoids on Plant Reproduction. Dissections of stems in the 2002 experiment revealed that treatments had had a significant impact on number of galls in stem bases (ANOVA $F_{2,13} = 4.5$, $P = 0.04$), with significantly more galls in the gall wasps plus parasitoids treatment than in the control (means

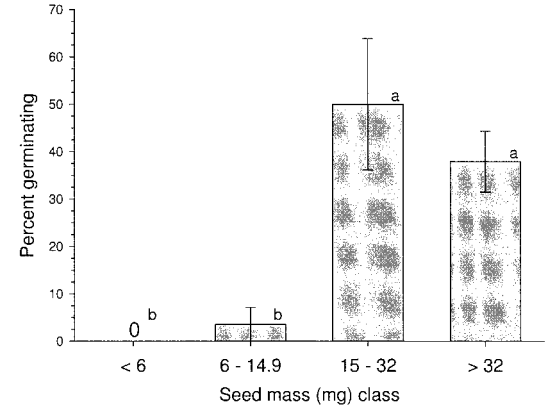

Fig. 2. Germination success versus mass of *S. laciniatum* seeds. $N = 19, 28, 14, 58$, left to right. Bars with different letters are significantly different (see text for ANOVA results; LSD $P < 0.05$).

Table 1. ANOVA and linear regression results for common garden experiments in 2002, testing the influence of treatments (control, gall wasps only, and gall wasps plus parasitoids), two measures of gall density, and percent parasitism on measures of plant performance

Parameter	Treatments	Est. density of galls/cm of stem	No. galls in stem bases	Percent parasitism
Flowering phenology	$F_{2,55} = 18.6$ (<0.0001)[a]	$r^2 = 0.33$ (0.03)[b] $Y = 2.0X + 221$	$r^2 = 0.17$ (NS)	$r^2 = 0.56$ (0.001)[c] $Y = -51.7X + 251.4$
Stem length	$F_{2,13} = 10.7$ (0.003)	$r^2 = 0.25$ (0.07) $Y = -0.09X + 2.3$	$r^2 = 0.13$ (NS)	$r^2 = 0.19$ (NS)
No. flower heads/plant	$F_{2,13} = 0.77$ (NS)	$r^2 = 0.02$ (NS)	$r^2 = 0.16$ (NS)	$r^2 = 0.06$ (NS)
No. seeds/flower head	$F_{15,550} = 96.2$ (<0.0001)	$r^2 = 0.04$ (NS)	$r^2 = 0.04$ (NS)	$r^2 = 0.20$ (NS)
Mass of individual seeds	$F_{15,550} = 23.0$ (<0.0001)	$r^2 = 0.30$ (0.04) $Y = -0.001X + 0.03$	$r^2 = 0.14$ (NS)	$r^2 = 0.10$ (NS)
Seed mass/flower head	$F_{2,13} = 4.58$ (0.04)	$r^2 = 0.27$ (0.06) $Y = -0.03X + 0.74$	$r^2 = 0.15$ (NS)	$r^2 = 0.29$ (0.09) $Y = 0.97X + 0.52$
No. seeds/plant	$F_{2,13} = 3.20$ (0.08)	$r^2 = 0.08$ (NS)	$r^2 = 0.11$ (NS)	$r^2 = 0.01$ (NS)
Seed mass/plant	$F_{2,13} = 3.50$ (0.07)	$r^2 = 0.11$ (NS)	$r^2 = 0.12$ (NS)	$r^2 = 0.01$ (NS)

[a] See text for details on statistics (P values in parentheses; best fit regression line presented if $P < 0.1$; NS, $P > 0.10$).
[b] Regression statistics shown only for 20% flower senescence; 40, 60, and 80% flower senescence also statistically significant or marginally so ($r^2 \geq 0.25$, $P \leq 0.07$).
[c] Regression statistics shown only for 80% flower senescence; 20, 40, and 60% flower senescence also statistically significant ($r^2 \geq 0.46$, $P \leq 0.02$).

377 ± 108 and 11.5 ± 3.5 galls, respectively; LSD $P < 0.05$), and an intermediate value for the gall wasps only treatment (254 ± 84). The few galls in stems of control plants, from which we had hoped to exclude galls, suggests that some adult wasps of the wild population had entered cages, perhaps through gaps that opened as stems moved in the wind, or through small tears in the fabric caused by environmental degradation. A possible source of wild gall wasps and parasitoids was the nearby patch of prairie vegetation that included *S. laciniatum* (see above). Dissection of stem bases revealed that many gall wasps and parasitoids had failed to complete development. Survivorship was higher in the control treatment (95 ± 8.4%) than in treatments receiving gall wasps (means 73 ± 7.6 and 66 ± 7.6% for gall wasps only and gall wasps plus parasitoids, respectively), although these differences were only marginally significant (ANOVA $F_{2,13} = 3.5$, $P = 0.07$). These findings suggest that survivorship was favored under low -density conditions. In fact, survivorship declined with the number of galls in stem bases, but this relationship also was weak (best fit regression line: $Y = -0.04X + 82.3$; $r^2 = 0.26$, $P = 0.06$).

Numbers of insects emerging per centimeter of entire plant stems in the 2002 experiment also were consistent with our treatments, but large variance resulted in a lack of statistical significance (means 0.19 ± 1.7, 4.4 ± 1.5, and 4.0 ± 1.5 insects/cm for control, gall wasps only, and gall wasps plus parasitoid treatments, respectively; ANOVA $F_{2,13} = 1.85$, $P = 0.20$). Percent parasitism (no. emerged parasitoid adults/total number of emerged wasps) per stem was not significantly influenced by treatment (means 20.6 ± 6.3, 12.8 ± 5.5, and 12.1 ± 5.5% for control, gall wasps only, and gall wasps plus parasitoids treatments, respectively; ANOVA $F_{2,13} = 1.18$, $P = 0.34$), indicating that our cages also were not effective in excluding adult female parasitoids. The considerable amount of variation around means for both numbers of wasps and parasitism rates suggests that cages varied in their permeability to parasitoids from the wild population. Our failure to exclude adult parasitoids from stems may account for insignificant parasitoid effects in some measures of plant performance (see below). We nevertheless include the gall wasps plus parasitoids treatment in our analyses because parasitoid females released in cages may have had an impact on gall wasps beyond that indicated by parasitism rate, such as by killing gall wasp larvae by probing. Parasitism rate was not significantly influenced by the estimated density of galls per centimeter of entire stems ($r^2 = 0.06$, $P = 0.45$).

We collected an average of 39.4 ± 7.4 flower heads per *S. laciniatum* plant, which yielded a total of 15,944 seeds. Flower development was strongly correlated with time, as expected, and linear relationships between the percentage of flower heads that had senesced and time for individual plants were well described by sigmoid curves ($r^2 \geq 0.96$, $P < 0.0001$). The gall wasp treatment significantly delayed flowering phenology (Table 1), with flowers senescing 2–3 wk later in plants with gall wasps than in control plants

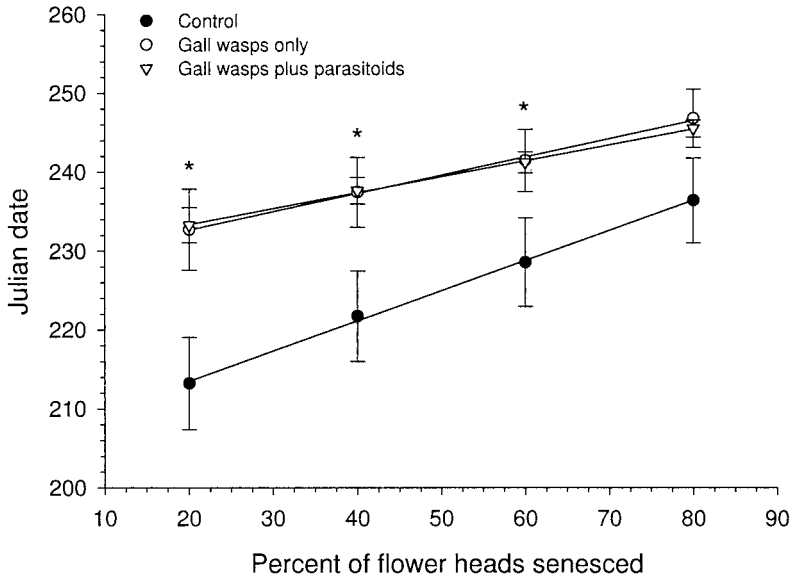

Fig. 3. Relationship between Julian date and the percentage of flower heads that had senesced on stems of *S. laciniatum* that were caged with different densities of adult gall wasps and parasitoids. Best fit regression equations ($r^2 > 0.99$ and $P < 0.0001$ for all): control, Y = 0.38X + 206; gall-wasps-only, Y = 0.23X + 228; gall-wasps-plus-parasitoids, Y = 0.20X + 229. Asterisks indicate significant differences between means (see text for ANOVA results; LSD $P < 0.05$).

(Fig. 3). Presence or absence of parasitoids did not affect the timing of senescence (Fig. 3). The Julian date on which 20% of flower heads had senesced was positively associated with the estimated density of galls per centimeter of stem (Table 1). This finding is consistent with significant treatment effects on both flowering phenology and gall density (see above). Flowering phenology was not correlated with the number of galls in stem bases, but was significantly and negatively influenced by percent parasitism, with flowers senescing earlier on plants with higher rates of parasitism (Table 1).

Stem length was significantly influenced by treatment, being greater in control plants than in either of the treatments in which plants were caged with gall wasps (means 285.3 ± 15.1, 166.0 ± 18.3, and 183.6 ± 21.7 cm for control, gall wasps only, and gall wasps plus parasitoids treatments, respectively; Table 1; LSD $P < 0.05$). Although plants caged with parasitoids had longer stems than those caged only with gall wasps, the means were not significantly different (LSD $P > 0.05$). Stem length also was inversely related to estimated density of galls per centimeter of stem, but the regression was only marginally significant (Table 1). Stem length was not significantly associated with the number of galls in stem bases or percent parasitism (Table 1).

Number of flower heads per plant was not significantly influenced by treatment, estimated density of galls per centimeter of stem, the number of galls in stem bases, or percent parasitism (Table 1). Number of seeds per flower head was significantly lower in plants that had been caged with gall wasps than in control plants and even lower in plants caged with both gall wasps and parasitoids (Table 1; Fig. 4), an unexpected finding. Seed production was not influenced by the estimated gall density per centimeter of stem, number of galls in stem bases, or percent parasitism (Table 1).

Plants that were caged with adult gall wasps produced seeds of significantly lower mass than seeds of control plants, a finding consistent with the 2001 study (see Fig. 1), but seed mass was significantly higher in plant that also were caged with parasitoids (Table 1; Fig. 5). Mass of individual seeds also decreased sig-

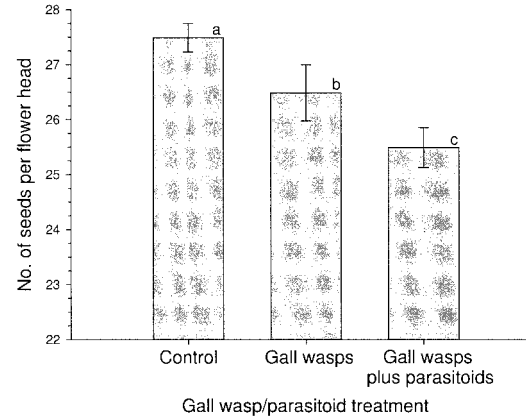

Fig. 4. Number of seeds per flower head of *S. laciniatum* plants caged with different densities of adult gall wasps and parasitoids. $N = 4, 5, 5$, left to right. Bars with different letters are significantly different (see text for ANOVA results; LSD $P < 0.05$).

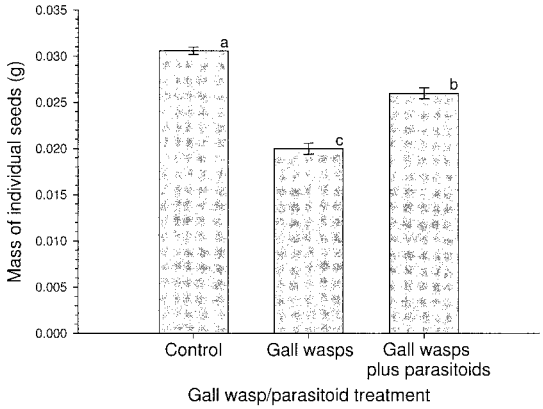

Fig. 5. Mass of individual seeds of *S. laciniatum* plants caged with different densities of adult gall wasps and parasitoids. $N = 4, 5, 5$, left to right. Bars with different letters are significantly different (see text for ANOVA results; LSD $P < 0.05$).

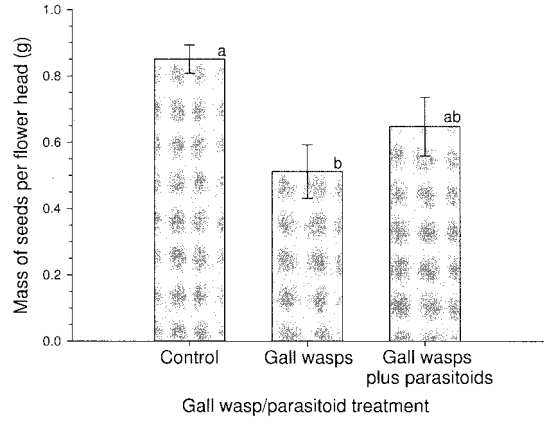

Fig. 6. Total mass of seeds produced per flower head of *S. laciniatum* plants caged with different densities of adult gall wasps and parasitoids. $N = 4, 5, 5$, left to right. Bars with different letters are significantly different (see text for ANOVA results; LSD $P < 0.05$).

nificantly with the estimated density of galls per centimeter of stem, but was not significantly influenced by the number of galls in stem bases or percent parasitism (Table 1). The estimated number of viable seeds produced per plant also was marginally influenced by treatment (ANOVA $F_{2,13} = 4.02$, $P = 0.05$), being highest in control plants, which produced significantly more viable seeds than the gall wasps only treatment (LSD $P < 0.05$), whereas the number of viable seeds produced by the gall wasps plus parasitoids treatment was intermediate and not significantly different from the other two (LSD $P > 0.05$; means 2662 ± 1015, 1061 ± 110.9, 689.2 ± 130.9, control, gall wasps plus parasitoids, and gall wasps only treatments, respectively; percent of viable seeds of total produced for all plants per treatment, means 99.6, 91, and 74%; $\chi^2 = 7.6$, df = 2, $P < 0.05$).

Total seed mass per flower head was significantly lower in plants caged with gall wasps than in control plants (Table 1; Fig. 6). The insignificant parasitoid effect (Fig. 6) apparently was caused by opposing trends in numbers of seeds per flower head (Fig. 4) and mass of individual seeds (Fig. 5). Total seed mass per flower head declined with the estimated density of galls per centimeter of stem and increased with percent parasitism, although the regressions were only marginally significant and was not significantly correlated with the number of galls in stem bases (Table 1).

Total number and mass of seeds per plant were lower in plants that had been caged with gall wasp adults, but differences were only marginally significant (Table 1; numbers of seeds 2673 ± 1019; 931.3 ± 176.9, and 1166 ± 121.8 for control, gall wasps only, and gall wasps plus parasitoids treatments, respectively; seed mass 80.9 ± 34.1, 18.6 ± 4.3, 30.3 ± 6.2 g for control, gall wasps only, and gall wasps plus parasitoids treatments, respectively). When we combined data for the two treatments that included gall wasps (gall wasps only and gall wasps plus parasitoids), however, the mean total seed number averaged 1,049 ± 109, and seed mass averaged 24.4 ± 4.0 g, and they were significantly different from controls (seed number: ANOVA $F_{1,13} = 6.7$, $P = 0.02$; seed mass: ANOVA $F_{1,13} = 7.1$, $P = 0.02$). Neither total number of seeds nor mass of seeds per plant were significantly influenced by the estimated density of galls per centimeter of stem, the number of galls in the basal 20 cm of plants, or percent parasitism (Table 1).

Plant Volatiles as Synomones. Fifteen of 23 parasitoid females responded to volatiles in field olfactometry bioassays by moving upwind, and 100% of these respondents choose volatiles from galled stems over ungalled controls ($\chi^2 = 15$, $P < 0.0001$). Most responding wasps walked directly up the appropriate arm of the Y-tube, but on five occasions they ventured up the side with volatiles of ungalled plants and immediately turned and walked up the arm with volatiles of galled plants and remained there.

Discussion

The experiments strongly supported the first hypothesis: gall wasps have a detrimental impact on plant reproduction. The fact that seed mass was influenced by the gall wasp treatment in the 2001 experiment but was not correlated with the number of adult gall wasps that emerged from plant stems suggests that plant reproduction was influenced by galls of wasps that did not complete development. Low survivorship of gall wasps in the 2002 study also suggests that oviposition by gall wasps females and/or early development of larvae may have important consequences for plant reproduction beyond that indicated by production of adult progeny. Density-dependent mortality factors may have been responsible for the lack of correlation between numbers of galls wasps emerging from stems and plant reproduction.

The 2002 study further supported the first hypothesis by showing that the gall wasp treatment resulted

in delayed blooming phenology and reductions in stem length, seed number per flower head, mass of individual seeds, proportion of viable seeds, total seed mass per flower head, and total seed mass per plant. Most of these variables also were negatively correlated with the estimated density of galls per centimeter of plant stem but were not significantly correlated with gall density in stem bases. The congener *Antistrophus silphii* Gillette also delays flowering phenology of its host plant *Silphium integrifolium* Michaux, but its larvae would seem especially likely to influence stem development because they inhabit apical galls in proximity to the meristem (Fay and Hartnett 1991). Delayed flowering phenology in other plant species results in reduced rates of visitation by pollinators and lower seed set (Jennersten et al. 1988), but we did not detect a difference between galled and ungalled plants in numbers of floral visitors during our experiments (unpublished data). Because the distance that seeds are dispersed from parent *S. laciniatum* plants is strongly and positively correlated with stem length (Pleasants and Jurik 1992), seeds of galled plants would travel a shorter distance than those of ungalled plants, possibly resulting in increased competition among seedlings and between seedlings and their perennial parents (Venable and Levin 1985). Reduced seed mass and number would result in decreased recruitment in subsequent generations (Crawley and Nachapong 1985). The apical galler *A. silphii* also diminishes reproductive output of *Silphium* hosts that are competing with other plant species, but in the absence of competition, galled plants produced axillary growth that compensated for damage to the apical meristem (Fay et al. 1996). Galling by *A. rufus* may cause even greater reductions in reproductive output of *S. laciniatum* that are competing with other species of prairie plants.

Our test of the second hypothesis, that parasitoids enhance plant reproduction by killing gall wasp larvae, was flawed by inadequate cage design. Perhaps as a result of movement of adult parasitoids into and out of cages, the parasitoid treatment did not influence blooming phenology or stem length of plants. Despite the problem with cage design, plants caged with parasitoids as well as gall wasps produced a greater number of seeds per plant (Fig. 5), larger seeds, and a greater percentage of viable seeds than plants that were caged with gall wasps. These significant treatment effects suggest that parasitoids had an impact on plant fitness greater than that indicated by percent parasitism alone. Nevertheless, percent parasitism was negatively correlated with flowering phenology and positively correlated with seed mass per flower head (Table 1), further suggesting that parasitoids can mitigate the negative impact of gall wasps on host plants. We may not have fully assessed the total impact of parasitoids on gall wasps densities because parasitoid females may have killed gall wasp larvae by probing or parasitoid larvae may have failed to complete development. Galling by *A. silphii* reduces growth and development of *S. integrifolium* even in the presence of parasitoids because the galls irreversibly damage the apical meristem (Fay et al. 1996).

Olfactometry bioassays of adult female *E. lutea* supported the third hypothesis: plants inhabited by gall wasps release volatile compounds that provide cues that parasitoids use to locate hosts. Galled and ungalled *S. laciniatum* plants seem to produce the same five volatile monoterpenes (α-pinene, camphene, β-pinene, β-myrcene, and +-limonene), although there are significant differences between galled and ungalled plants in relative quantities of volatiles (Tooker et al. 2005, unpublished data). Parasitoids may exploit differences in volatile profiles to discriminate between plants in their search for gall wasp hosts. Moreover, parasitoids also may exploit differences between galled and ungalled *S. laciniatum* in enantiomeric ratios of α-pinene and β-pinene that serve as mate location cues for male gall wasps (Tooker et al. 2002). The possibility remains, however, that parasitoids are responding to volatile cues emitted by the gall wasps themselves.

To our knowledge, only one other field study of a natural system has provided evidence that parasitoids improve fitness of host plants by killing their herbivores (Gomez and Zamora 1994). The present study, however, is apparently the first to show in an endemic system a link between improved fitness in host plants through parasitism of herbivores and production of plant volatiles that serve as host location cues for parasitoids.

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