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THE EFFECT OF FLORAL HERBIVORY ON MALE AND FEMALE REPRODUCTIVE SUCCESS IN *ISOMERIS ARBOREA*

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Abstract. Flower-feeding herbivores can directly reduce plant reproduction by consuming gametes. They may have additional indirect effects if their damage disrupts pollinator service and causes uneaten gametes to go unused. In a two-year study we investigated direct and indirect effects of florivory by a pollen beetle, *Meligethes rufimanus,* on the male and female reproductive success of the andromonoecious shrub, *Isomeris arborea.* We measured pollen export, pollen receipt, fruit set, and outcrossing rates on plants exposed to herbivores (most flowers damaged) and protected from herbivores (few flowers damaged) by systemic insecticide. Pollen export per undamaged flower was reduced by one-half in exposed plants, as estimated by pollinator transfer of florescent dye, which demonstrated indirect negative effects on male reproductive success. Damaged flowers on exposed plants had a lower stigmatic pollen load than undamaged flowers on either exposed or protected plants. Furthermore, exposed plants produced fewer fruits and seeds during the year than protected plants. Although damage reduces pollen receipt, hand pollination experiments showed that neither exposed nor protected plants were pollen limited, which suggests that floral herbivores primarily affect female function through their direct destruction of gametes. Outcrossing rates did not differ between exposed plants (t_s = 0.920) and protected plants (t_s = 0.806), suggesting that herbivory does not indirectly reduce plant reproductive success by inducing autogamy and subsequent inbreeding depression. Overall, floral herbivory has direct consequences for both male and female reproductive success, but indirect effects are limited to male function.

Key words: autogamy; female reproductive success; fluorescent dye; geitonogamy; herbivory; Isomeris arborea; *male reproductive success;* Meligethes rufimanus; *outcrossing rates; pollen export; pollen limitation; stigmatic pollen load.*

INTRODUCTION

Flower-feeding herbivores may consume but a small fraction of their host plant's biomass, but then can have large effects on host reproductive success (Breedlove and Ehrlich 1968, Louda 1982, Zammit and Hood 1986, Schemske and Horvitz 1988, Wallace and O'Dowd 1989, Bertness and Shumway 1992, English-Loeb and Karban 1992, Cunningham 1995). Herbivore consumption of pistils, ovaries, or ovules will have an immediate and direct effect on reproduction through female function (Zammit and Hood 1986, Schemske and Horvitz 1988, Wallace and O'Dowd 1989, Bertness and Shumway 1992, English-Loeb and Karban 1992, Pellmyr and Huth 1994). Similarly, direct anther and pollen consumption may potentially diminish male function. But floral herbivores may have additional, *indirect* impacts on plant reproductive potential. Attack can degrade flower appearance, reduce the quantity and quality of floral rewards, and decrease the size of the floral display. Such changes in floral signals may affect pollinator visitation rates (Karban and Strauss 1993, Cunningham 1995, Krupnick et al. 1999), which in turn can alter patterns and rates of pollen donation (male function) and pollen receipt (female function). Thus, the total negative effect of herbivore damage can be realized through several channels.

The negative impact of general herbivory on male reproductive success has only recently been explored (Allison 1990*a*, Quesada et al. 1995, Mutikainen and Delph 1996, Strauss et al. 1996). Floral herbivory can have particularly negative impacts due to its potential effect on pollinator response to the plant. For instance, Karban and Strauss (1993) found a drop in bee visitation to *Erigeron glaucus* flowers with thrips damage. Lower visitation to damaged flowers or inflorescences will lower the probability that the surviving pollen reaches receptive stigmas. For every pollen grain destroyed, several others may go to waste.

Reduced visitation can also reduce female reproductive success if seed set is pollen limited. Pollen limitation is common among plants (Burd 1994), but may vary at several spatial or temporal scales (Schemske 1977, Snow 1986, Bertness et al. 1987, Campbell 1987, Lubbers and Lechowicz 1989, Ackerman and Montalvo 1990, Johnston 1991, Dudash 1993). When pollinator service falls, fertilization rates may fall below the level supportable by the plant's resource base.

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Finally, the herbivore's effect on pollinator behavior may alter plant reproductive success by changing the selfing rate, either by increasing autogamous pollen transfer or decreasing geitonogamous pollen transfer. Previous studies indicate that individuals that are more attractive to pollinators can have higher outcrossing rates (Brown and Clegg 1984, Epperson and Clegg 1987, Sun and Ganders 1990, Rausher et al. 1993) and that the lack of pollinator visits can increase autogamy in self-compatible plant species (Motten 1982, Schoen 1982, Kron et al. 1993, Rathcke and Real 1993). Hence, the reduced attractiveness of damaged plants may lead to higher selfing rates. Conversely, if an undamaged plant produces a large floral display, pollinators may visit more flowers per inflorescence per bout. This could result in greater geitonogamous pollen transfer (e.g., De Jong et al. 1992, Robertson 1992, Hodges 1995), causing outcrossing rates to drop (e.g., Barrett et al. 1994, Harder and Barrett 1995). In populations subject to inbreeding depression, such changes in selfing rate can influence offspring success.

We have explored the mechanisms through which a flower-feeding insect, *Meligethes rufimanus,* can affect components of male and female reproductive success in a perennial shrub, *Isomeris arborea,* both through direct destruction of gametes and indirect effects exerted through pollinator behavior. Previous work (Krupnick and Weis 1998) has shown that beetle damage increases flower bud abortion rate and changes sex allocation in *I. arborea.* A companion study of floral herbivory and pollinator behavior (Krupnick et al. 1999), finds that pollinator visitation rates decrease as a result of florivory.

This paper describes a two-year study that measured the effect of reduced pollinator services through effects on pollen export and seed production. Both naturally occurring and planted shrubs were subjected to one of two treatments: exposure to herbivore activity (most flowers damaged), and protection from herbivore activity (few flowers damaged) by application of a short-lived systemic insecticide. Various components of male and female reproductive function were analyzed by investigating the following questions: (1) Does floral herbivory indirectly reduce the export of undamaged pollen grains? (2) Do damaged plants receive fewer pollen grains than undamaged plants? (3) Does herbivory decrease fruit production? (4) Does reduced pollinator service to damaged plants result in pollen limitation? and (5) Do outcrossing rates change as a result of florivory?

Methods

Study system

I. arborea (Capparaceae) is an endemic southern California perennial shrub. This drought-deciduous plant typically flowers from January until November (Munz 1974:330). It is self-compatible and andromonoecious; hermaphroditic and male flowers are produced sequentially on the same terminal inflorescences. Male flowers produce six stamens and a nonfunctioning, undeveloped pistil, and hermaphroditic flowers produce six stamens and a pistil with a superior ovary extended on a gynophore. The shrub is visited by flying insects, including bumblebees (*Bombus* spp.), non-native honeybees (*Apis melifera*), and hummingbirds (*Calypte costae* and *C. anna*). While it is not known which visitor is the most effective pollinator, bumblebees are a likely candidate (Grant and Grant 1967), since they contact all reproductive parts during a visit to a flower and are more common than other pollinating species (G. A. Krupnick, *personal observation*).

M. rufimanus (Nitidulidae), a pollen beetle, feeds on the flowers of *I. arborea* in Southern California, as well as on capers and crucifers in other northern temperate regions. Between January and June, adult beetles feed on pollen from mature and developing flowers. Females oviposit into flower buds, where the larvae consume developing anthers.

Previous work has documented several direct effects of *M. rufimanus* on the reproductive success of bladderpod. Larval feeding causes flower-bud abortion rates approaching 80% on infested plants, while plants protected from attack by insecticide have abortion rates below 20% during the period of beetle attack (Krupnick 1996). Over the season, infested plants may produce only half as many flowers as protected ones. The sex ratio of the surviving flowers is also changed. In protected plants, the first few flowers produced are hermaphroditic. If these set fruit, the inflorescence switches to male flower production until the fruits approach maturity, when a second batch of hermaphroditic flowers is produced. When beetle damage prevents fruit set, the plant continues to produce hermaphroditic flowers. Krupnick and Weis (1998) have shown that over the season, highly damaged plants produce slightly more hermaphroditic flowers than protected plants, but many fewer male flowers. Approximately one-third of these hermaphroditic flowers, however, had damaged ovaries (Krupnick and Weis 1998).

I. arborea is host to two other herbivores: *Murgantia histrionica* (Pentatomidae) is a phloem feeder, attacking leaves, inflorescences, and capsules; and *Phyllotreta fulgida* (Chrysomelidae) is a leaf chewer. Both insects are found during the summer months after *M. rufimanus* activity.

Study area

We collected estimates of fecundity using natural and planted arrays of *I. arborea* within the University of California, Irvine, Ecological Reserve. This natural population of over 200 *I. arborea* shrubs grows along a hillside with other coastal sage scrub species (e.g., *Artemisia californica, Eriogonum fasciculatum,* and *Encelia californica*).

Some experiments used in situ plants. Sixty random-

ly selected individuals received one of three treatments: protection from natural levels of herbivory ("protected"), exposure to herbivory with a water sham-control application ("exposed"), or exposure to herbivory without a water spray ("exposed without spray"). Protected plants were sprayed once every two weeks between January and June from 1992 to 1995, with a systemic insecticide (Dimethoate CA267, obtained from Platte Chemical Company, Fremont, Nebraska) dissolved in water (29.5 mL/L); exposed plants were sprayed with water only. Both sprayed and watered treatments were applied at the rate of 40 mL per plant. A previous study showed that the insecticide has no effect on inflorescence or fruit production in a herbivore-free environment (Krupnick 1996). After 1992, the number of treatments in the experimental design for naturally growing plants and the experimental arrays was reduced to two: either protected or exposed.

Other experiments were conducted in two arrays planted during January 1993 ~50 m from the natural population. One-yr-old plants were first grown in a greenhouse from bulk seed collections made from 25 random *I. arborea* plants from the Ecological Reserve in 1992. Plants were then transplanted into the field in one of two arrays that were designed to study the effects of herbivore densities and neighborhood structure on pollinator behavior (Krupnick et al. 1999). Array 1 design was developed to study the effects of varying herbivore densities on pollinator choice and plant reproductive success. It contained nine 3 × 3 m plots of 16 plants each, for a total of 144 plants. The nine plots received one of three plot treatments: (1) In three plots, all plants were exposed to herbivory with the water application ("exposed plots"); (2) in three plots, all plants were protected from herbivory with the insecticide spray application ("protected plots"); (3) in the final three plots, half the plants were exposed (with water application) and the other half were protected (with spray application) from herbivory ("mixed plots"). Array 2 consisted of 90 plants arranged in a hexagonal grid in which half the plants were randomly assigned to the protected treatment and the others left exposed. Plants were separated by 1 m, and the edges of the hexagon measured 6 m.

Measuring pollen transfer

To examine pollen flow, we used fluorescent dye as an analogue to pollen. We tested the adequacy of the dye method by comparing the movement of pollen and dye particles in a controlled experiment (e.g., Waser and Price 1982, Campbell 1985, Thomson et al. 1986). In April 1993 and April 1995, nine individually captured bumblebees were each presented with one donor *I. arborea* flower in which the dehiscent anthers were dusted with fluorescent dye particles (obtained from Radiant Color Company, Richmond, California). Bees were captured, placed in an ice chest, and then allowed to warm and groom before visiting the donor flower. After it visited a donor flower, each bee was presented with 25 emasculated virgin hermaphroditic flowers within a 0.3 × 0.3 × 3 m flight cage. Each recipient flower was placed in a sterile glassine envelope directly after the visit to prevent any additional visits to that flower. Stigmas were then examined for both pollen grains (using incandescent light) and dye particles (using ultraviolet [UV] light) under a 50× dissection microscope.

The correlation coefficient was used to test for a relationship between the number of pollen grains and dye particles transferred onto the stigmas. We also compared regression slopes of the standardized mean number of pollen grains and dye particles deposited per flower visit in a sequence. To standardize we divided the numbers of dye particles and pollen grains on each visited stigma by the maximum number of grains deposited on any stigma during that run; this corrected for differences in the amount of dye and pollen carried in each run.

Measuring pollen flow in the field

During April and May in 1994 and 1995, we examined the indirect effect of herbivory on pollen export by comparing the export of dye from an equal number of undamaged flowers on exposed and protected plants. Our goal was to determine if the previously observed reduction in bee visitation to highly damaged plants (Krupnick et al. 1999) reduces pollen export. A protected plant can export more pollen than an exposed one simply because it produces more, but will pollen export differ between exposed and protected plants on a "per undamaged flower" basis? To answer this question, we dusted dye particles on the anthers of an equal number of undamaged donor flowers on each of two exposed plants and two protected plants in the natural population, and repeated the experiment with eight trials (four trials in 1994 and four in 1995), using a different set of four plants for each trial, for a total of 32 plants. Plants were selected based on the number of branches per plant, so that within a set of four plants, a damaged plant and an undamaged plant were matched for size. All four plants in each trial were within 5 m of each other. After 24 h, we collected stigmas from all 1–6 d old hermaphroditic flowers on all neighboring plants within a 25-m^2 neighborhood (ranging from 11 to 22 plants, depending upon the trial) and counted the number of dye particles on each. We also did the same with all nondonor hermaphroditic flowers ("self flowers") from each of the four donor plants. The four donor plants received a different dye color; to control for the possibility of pollinator bias for dye color, colors rotated among treatments in the eight trials. In 1994, each plant had 10–12 donor flowers dusted with dye (depending upon the trial); and in 1995, each plant had 5–7 donor flowers dusted with dye. Each pistil was excised at the base of the gynophore and collected within a sterile glassine envelope. Stigmas were examined

within 36 h using a 50× dissection microscope and a UV light.

The dye export was broken down into several components. We analyzed (1) the total number of dye particles per donor plant recovered from all outcrossed recipient stigmas; (2) the total number of dye particles per donor plant recovered from stigmas of self flowers on donor plants; (3) the proportion of neighboring flowers that received dye from target donor plants, which measures the number of potential sites for fertilization for each donor plant; and (4) the proportion of self flowers receiving dye from the donor selfing plant, which measures the level of geitonogamous pollen transfer. We used two-way, mixed-model ANOVAs for each component of dye export, with plant treatment as the fixed effect and date of trial as the random effect. Branch number was included as a covariate, to control for plant size. To satisfy the assumptions of ANOVA, proportional data were arcsin transformed before analyses. Because methods differed slightly from year to year (twice as many dye-dusted donor flowers per plant were used in 1994 as in 1995), each year was analyzed separately. All ANOVAs were conducted using the GLM procedure in PC SAS (SAS 1990).

Female reproductive success: pollen receipt

We examined the effects of floral damage on pollen receipt by counting the number of pollen grains on the stigmas of damaged and undamaged flowers from five exposed plants and five protected plants in Array 2 on 4 April 1995, when beetle infestation is at its peak (Krupnick et al. 1999). Pistils were collected from every 1–3 d old hermaphroditic flower on each plant, and placed in individual sterile glassine envelopes. We collected 65 damaged flowers and 28 undamaged flowers from five exposed plants, and 40 damaged flowers and 74 undamaged flowers from five protected plants. Flower age was estimated by the level of dehiscence among the anthers and flower position along the inflorescence. Collected flowers were recorded as being either damaged (visible damage to stamens, pistil, and/or corolla) or undamaged. In the laboratory, stigmas were excised from the pistils with a sterile knife, and then stained on glass slides with fuchsin within 4–7 h of collection. Stigmatic pollen load was estimated under a 400× compound microscope. The measure of stigmatic pollen load is conservative, as the pollen grains may either be outcrossed pollen or self pollen deposited from autogamous or geitonogamous transfer.

The number of pollen grains per stigma was first analyzed using a two-way nested ANOVA, with plant treatment and flower condition as the independent factors, with individual plant nested within plant treatment. Then, to compare damaged and undamaged flowers within each plant treatment, pollen loads from each plant treatment were analyzed separately using a two-way ANOVA, with individual plant and flower condition as the variables. Before analysis, all data were natural $\log(x + 1)$ transformed to obtain normality.

Female reproductive success: fruit and seed production

To examine the temporal effect of florivory on the female reproductive success of *I. arborea,* standing fruit crop size was censused once every two weeks on 30 naturally growing plants during the 1992 flowering season, weekly on all 144 plants in Array 1 during the 1994 flowering season, and once every two weeks on the surviving Array 1 plants during the 1995 flowering season. At each census we counted the number of active inflorescences and number of developing fruit per plant, and then collected all mature fruit. In 1994 all seeds were counted. Because fruit production and seed production were highly correlated ($r^2 = 0.9439$, $F_{1, 142} = 2389.40$, $P < 0.0001$), we only counted number of fruit in 1995. Seeds were scored as either viable (brown, thick seed coat) or aborted (pale, fragile seed coat and no endosperm).

We compared the standing crop of fruit of exposed and protected plants each year by repeated-measures ANOVAs. To obtain normality, data were $\log(x + 1)$ transformed before analysis. To remove the effect of plant size from the analysis, the number of branches per plant (counted at the beginning of each season) was used as a covariate. The initial number of branches did not differ between herbivory treatments in either year (1994: $F_{1, 143} = 1.81$, $P > 0.05$; 1995: $F_{1, 143} = 1.04$, $P > 0.05$). In addition, there was no significant interaction between herbivory treatment and branch number ($P > 0.05$), supporting the assumption of homogeneity of slopes on the covariate. To factor out plot effects from Array 1, we performed two additional repeated measures ANOVAs, with individual plots nested within herbivore treatment. We also compared exposed and protected plants within the mixed plots.

To measure the total effect of florivory on female reproductive success, seed production during the months of herbivore activity (January–June 1994) and during the entire year (January–October 1994) was analyzed by comparing the number of seeds per plant among the four plant treatments (exposed plants in exposed plots, exposed plants in mixed plots, protected plants in mixed plots, and protected plants in protected plots). Plant treatment was used as the independent factor, and the initial number of branches was used as a covariate to control for plant size. Pairwise comparisons on each of the variables were made using a Tukey's studentized range test in PC SAS (SAS 1990).

Pollen limitation

To determine if exposure to or protection from herbivory leads to pollen limitation, we hand-pollinated all hermaphroditic flowers on a random sample of 10 exposed and 10 protected plants ("hand-pollinated") from Array 2 every 5 d from 25 February 1994 through

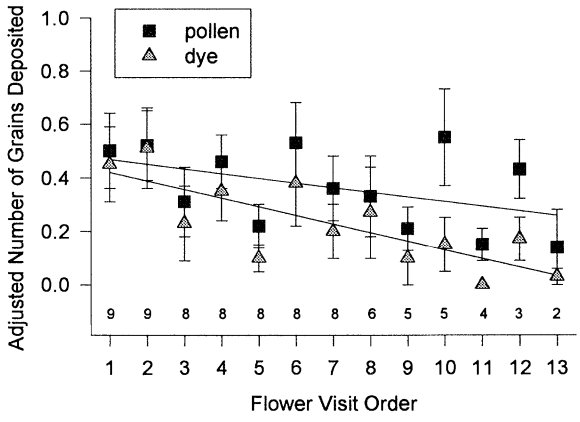

FIG. 1. Mean (±1 SE) adjusted values of the number of dye particles and pollen grains deposited on recipient flowers in a sequence. Solid squares = pollen grains; shaded triangles = dye particles. The number below each symbol represents flower sample size. The best fitting linear regression for pollen grains was $y = 0.48 - 0.0176x$; that for dye particles was $y = 0.45 - 0.032x$.

11 October 1995. Another 10 exposed and 10 protected plants ("open-pollinated") acted as controls and received natural visitations from animal pollinators. Pollen was collected from dehiscent anthers from 10 naturally growing *I. arborea* plants, and placed in microcentrifuge tubes. Mixed pollen loads were transferred to recipient stigmas with a plastic toothpick on the day of pollen collection. Every 5–6 d, we counted the number of hermaphroditic and male flowers, and the number of developing and mature fruit, and recorded whether the pistil of each hermaphroditic flower was undamaged or damaged (chew hole in ovary wall). All hermaphroditic flowers of each treatment were marked on the calyx with permanent ink to prevent recounting on subsequent days, and pollen was applied to stigmas of the hand-pollinated plants. All mature fruit were collected and seeds counted as either viable or aborted.

Using a repeated-measures two-way ANOVA with plant treatment and pollination type as the fixed factors, we analyzed the number of fruit set per flower, and the number of seeds produced per fruit over the two years of study, with year as the repeated factor. The initial number of branches was used as a covariate (the number of branches per plant in Array 2 did not differ between herbivory treatments [$F_{3,39} = 0.76$, $P > 0.05$]). Three plants that did not produce any hermaphroditic flowers and six plants that did not produce any fruit were excluded from the analyses of fruit set and seed set, respectively. Fruit set per flower was calculated as the total number of fruit produced per total number of hermaphroditic flowers. (Damaged hermaphroditic flowers with eaten ovaries were excluded in this calculation because of their inability to produce fruit.) Because floral herbivory takes place within a portion of the flowering season and damaged plants appear to compensate in flower production after the months of beetle activity (Krupnick et al. 1999), we analyzed separately the data collected during the months of herbivory and the entire year.

Outcrossing rates

To determine the effects of herbivory on the mating structure of this population, we compared outcrossing rates between 23 protected and 23 exposed plants. These were selected randomly from Array 1. To measure outcrossing rates, we compared maternal isozyme genotypes to their offspring. Mature fruit were collected from each plant on 20 June 1995. These fruit were most likely fertilized by pollinators in April or May, during high beetle infestation (Krupnick et al. 1999). Up to 100 seeds per plant were washed with 2% chlorine, rinsed, placed on moist filter paper in a petri dish, and then incubated at 27°C for a 12:12 (L:D) photoperiod. Cotyledons of the germinated seeds were collected in microcentrifuge tubes, frozen with liquid nitrogen, and stored at −70°C until electrophoresis. The proportions of seeds that germinated from the two herbivore treatments were compared by a t test.

We prepared cotyledons for electrophoresis by grinding them in a Tris-HCL extraction buffer with polyvinyl pyrrolidone (PVP) and 1% mercaptoethanol (Gottlieb 1981, Soltis et al. 1983). We absorbed the extract on paper wicks, and then ran the wicks on starch gels

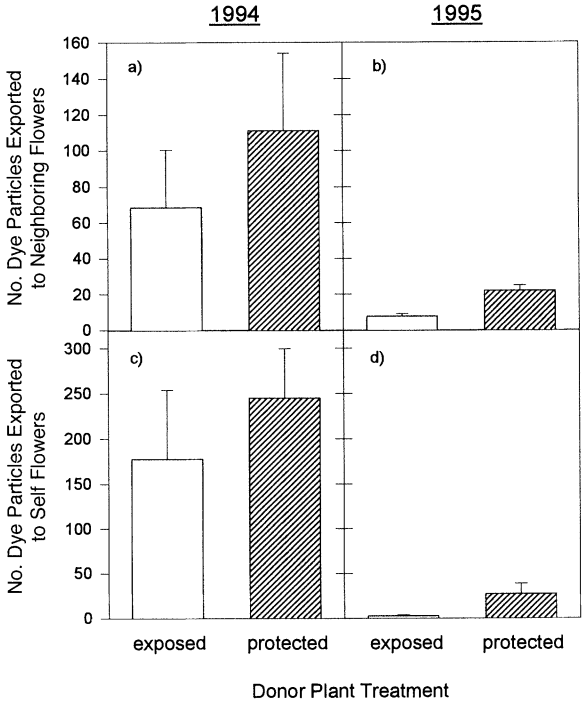

FIG. 2. Mean number (+1 SE) of dye particles received by (a, b) neighboring flower stigmas and (c, d) self flower stigmas donated by the exposed and protected donor plants in (a, c) 1994 and (b, d) 1995. (a) $F_{1,0.20} = 26.54$, $P = 0.5387$; (b) $F_{1,3.77} = 19.52$, $P = 0.0131$; (c) $F_{1,2.66} = 3.22$, $P = 0.1821$; (d) $F_{1,3.79} = 1.06$, $P = 0.3635$.

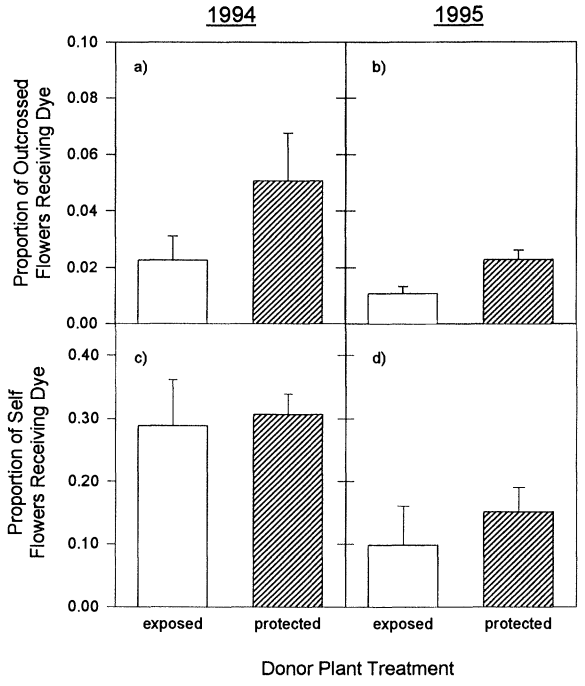

FIG. 3. Mean proportion (+1 SE) of (a, b) neighboring flowers and (c, d) self flowers that received dye from the exposed and protected donor plants in (a, c) 1994 and (b, d) 1995. (a) $F_{1,2.33} = 12.65$, $P = 0.0562$; (b) $F_{1,4.48} = 6.98$, $P = 0.0512$; (c) $F_{1,2.34} = 1.3881$, $P = 0.3448$; (d) $F_{1,9.85} = 0.8733$, $P = 0.3724$.

containing 12.3% starch (Soltis et al. 1983). In a preliminary study, we examined 21 different isozymes for polymorphism among the flower buds from 40 shrubs and found only one, isocitrate dehydrogenase (IDH), to be sufficiently polymorphic. IDH was resolved on a Tris-citrate pH 8.0 gel (Soltis et al. 1983) run for 4 h at 150 V and 75 mA. The IDH locus was stained using procedures from Ranker et al. (1989).

We determined outcrossing rates using Ritland and Jain's (1981) maximum likelihood estimation program (Ritland 1990). We excluded families with fewer than eight progeny from the analysis, leaving a total of 33 families and 570 progeny. Standard errors were estimated from 500 bootstraps. The outcrossing rates of exposed and protected plants were compared by a two-tailed z test (K. Ritland, *personal communication*). We also examined the effect of plant size by comparing the outcrossing rates of small plants (<30 inflorescences) to those of large plants (>40 inflorescences) in both plant treatments using t tests, with the critical level adjusted for multiple comparisons according to the Dunn-Sidák method (Sokal and Rohlf 1981).

RESULTS

Male reproductive success: pollen export

Dye particle movement mimicked pollen grain movement. In the experimental flight cages, the number of dye particles and pollen grains deposited on each stigma was highly correlated ($n = 83$, $r = 0.79$, $P > 0.0001$). The number of pollen grains and dye particles deposited diminished with each successive visit (Fig. 1); this relationship was not statistically significant for pollen grains ($F_{1,12} = 2.993$, $P > 0.10$, $r^2 = 0.21$), but it was significant for dye particles ($F_{1,12} = 18.037$, $P < 0.01$, $r^2 = 0.62$). The confidence intervals around the slopes of each function overlap, and thus do not differ significantly from each other. We concluded that dye particles are an adequate analogue for pollen grains in this case.

Overall, floral damage by the pollen beetle reduced the export of surviving pollen. Neighboring flowers received more dye particles from protected donor plants than from exposed donor plants; this trend, however, was statistically significant in 1995 only (Fig. 2a, b). Furthermore, undamaged flowers on protected plants exported dye particles to twice as many neighboring flowers as undamaged flowers on exposed plants in both 1994 and 1995 (Fig. 3a, b).

Geitonogamous pollen transfer did not appear to differ between the two treatments. Dye particles from protected donor plants did not reach self flowers with any greater frequency than did dye particles from exposed plants (Fig. 2c, d). In addition, we found no significant difference between exposed and protected plants in the proportion of self flowers that received self dye particles (Fig. 3c, d).

Female reproductive success: pollen receipt

Damaged flowers received fewer visits than undamaged ones (Krupnick et al. 1999) and so we expected them also to receive less pollen. Damage did reduce pollen receipt, as measured by stigmatic pollen loads, but only when the damaged flower occurred on a highly damaged (i.e., exposed) plant (Fig. 4, Table 1). When damaged flowers occurred in the "low damage" context of protected plants, stigmatic pollen loads were

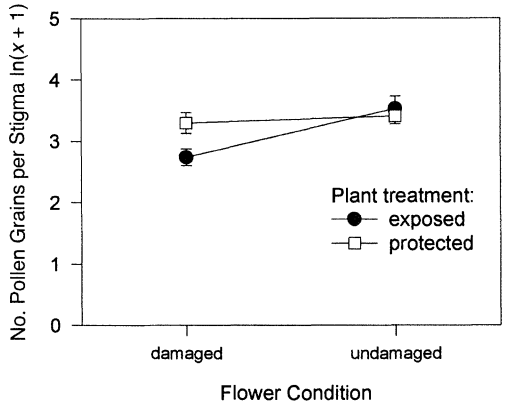

FIG. 4. Mean number (±1 SE) of pollen grains found per stigma from damaged and undamaged flowers of exposed plants (solid circles) and protected plants (open squares).

TABLE 1. Results of ANOVA on the number of pollen grains deposited on *I. arborea* stigmas.

Source of variation	df	MS	*F*
Among-plant Treatments			
Insecticide treatment	1	1.94	0.09
Plant (insecticide treatment)	8	23.34	19.81****
Flower condition	1	8.74	1.74
Insecticide treatment × flower condition	1	5.03	4.27*
Within-plant Treatments			
Exposed plants			
Plant	4	3.25	5.30‡
Flower condition	1	6.41	9.27*
Flower condition × plant	4	0.61	0.51
Protected plants			
Plant	4	39.04	17.60**
Flower condition	1	0.29	0.14
Flower condition × plant	4	2.22	1.94

Note: Data were $\ln(x + 1)$-transformed before analysis.
‡ $P = 0.0675$; * $P < 0.05$; ** $P < 0.01$; **** $P < 0.0001$.

not significantly different from their undamaged counterparts (Fig. 4). This lack of difference in protected plants was a surprise, since damaged flowers were less frequently visited than undamaged flowers, regardless of plant treatment (Krupnick et al. 1999).

Female reproductive success: fruit and seed production

Herbivore damage directly reduced fruit production in *I. arborea* in the three years of study. Standing crops of fruit were far larger on protected plants than on either of the controls in the 1992 study of the natural population (Fig. 5a, Table 2). Fruit production was also increased by protection in Array 1 during both 1994 and 1995 (Fig. 5b, c, Table 2). To factor out plot effect, we analyzed individual plots nested within herbivore treatments. Plants in fully protected plots had more fruit than plants in fully exposed plots in 1994 (herbivore treatment: $F_{1,4} = 8.62$, $P < 0.05$; plot [treatment]: $F_{4,89} = 4.17$, $P < 0.005$; date × treatment: $F_{32,2848} = 33.18$, $P < 0.0001$), but we only found a significant date × treatment effect in 1995 (herbivore treatment: $F_{1,2} = 0.54$, $P > 0.05$; plot [treatment]: $F_{2,76} = 9.41$, $P < 0.0005$; date × treatment: $F_{7,532} = 14.39$, $P < 0.0001$). In 1994, exposed plants in mixed plots were marginally statistically different in their fruit crop than protected plants in mixed plots (during herbivore treatment: $F_{1,45} = 3.09$, $P = 0.08$), but not in 1995 (herbivore treatment: $F_{1,28} = 1.46$, $P > 0.10$). After the period of beetle attack, exposed plants have an apparently compensatory production of fruit (Fig. 5), which follows a postherbivore increase in flower production (see Krupnick et al. 1999).

We found significant neighborhood effects on seed set. Plants in protected neighborhoods had greater female reproductive success than those in exposed neighborhoods (Fig. 6), as would be expected, since exposed plants in mixed plots received moderate damage due to the experimentally reduced beetle density (Krupnick and Weis 1998). However, seed production of plants in the mixed plots (whether the plants were themselves exposed or protected) was no lower than that of plants in protected plots (Fig. 6). Because the levels of damage are not as great as those for exposed plants in exposed plots, these plants were able to compensate for damage. In comparison, when damage is high (i.e., exposed plants in exposed plots), compensation is incomplete (Fig. 6).

Pollen limitation

Floral damage to *I. arborea* reduces pollinator visitation (Krupnick et al. 1999) and can reduce pollen receipt (see *Female reproductive success: pollen receipt*). Therefore, part of the reduced seed set in damaged plants is potentially caused by pollen limitation. If so, we predicted that supplemental pollination would increase seed set in exposed (damaged) plants by a greater amount than in protected plants. This differential response to supplemental pollen would be reflected in a significant "insecticide treatment × pollination treatment" interaction effect. We hand-pollinated exposed and protected plants in Array 2 and compared their female performance to open-pollinated counterparts. The initial measure of hermaphroditic flower production showed no difference between hand- and open-pollinated plants within either plant treatment (Krupnick 1996).

Responses to supplemental pollinations were not always significant, and when they were, the difference was in a direction opposite to our prediction. We present the results for the months of beetle activity and for the total season separately. During the months of herbivory in 1994 and 1995, no significant treatment × pollination effect was found for the number of fruits per flower nor for the number of seeds per fruit (Fig. 7, Table 3). When female reproductive success is totaled over the entire year, however, we find significant interaction effects for seed set, but not for fruit set (Fig. 8, Table 4).

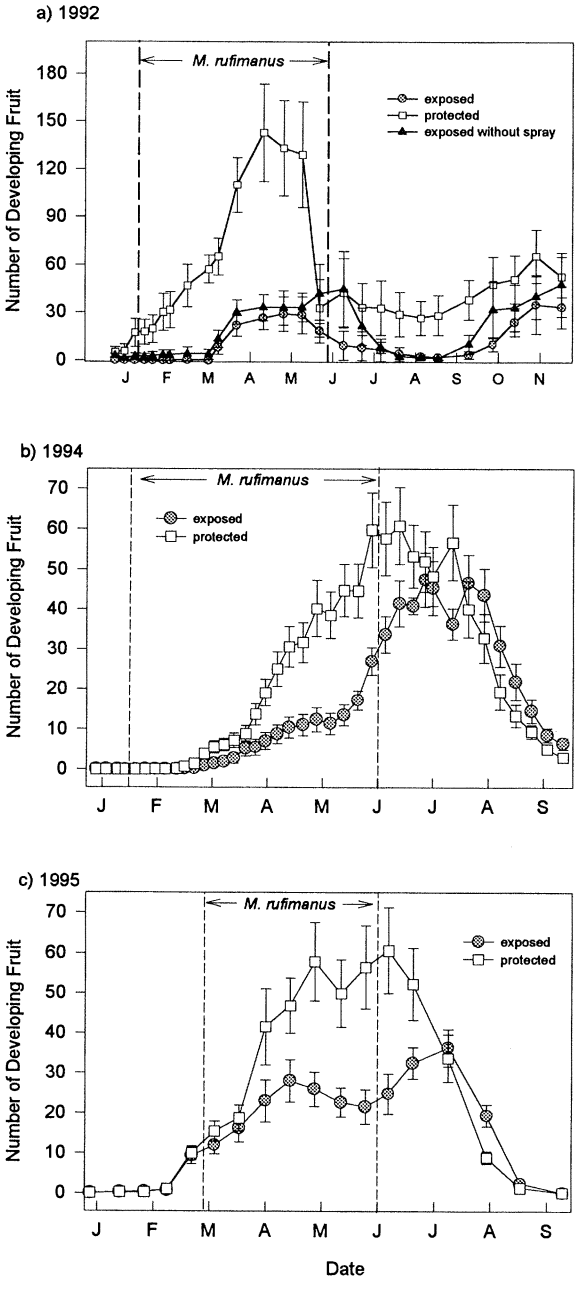

FIG. 5. The standing crop of fruit per plant treatment during the (a) 1992, (b) 1994, and (c) 1995 flowering seasons for various herbivory treatments of *I. arborea*. Shaded circles = plants exposed to herbivory with water spray; open squares = plants protected from herbivory with insecticide spray; solid triangles = plants exposed to herbivory without water spray. Errors bars indicate ± 1 SE. The time between dashed lines indicates feeding activity of *M. rufimanus* on *I. arborea*.

Outcrossing rates

Changes in pollinator visitation rates could influence the degree of selfing. The reduced visitation rates caused by damage could increase the frequency of autogamy. On the other hand, extended visits to lush inflorescences on protected plants could increase geitonogamous pollen transfer. Seeds were collected from exposed and protected plants in Array 2, and their isozyme phenotype examined to determine if outcrossing rate differed with herbivory treatment. The proportions of seeds that germinated from exposed and protected plants were not significantly different (proportion of seeds germinating: exposed = 0.191 ± 0.025 [mean ± 1 SE]; protected = 0.229 ± 0.030; $t = 0.9579$, df = 44, NS).

IDH has three common alleles in this population of *I. arborea* (allele frequencies = 0.099/0.748/0.153). The single locus outcrossing rates of exposed and protected plants are 0.920 ± 0.108 and 0.806 ± 0.067, respectively, and are not significantly different from each other ($t = 1.26$, $P > 0.10$). The effect of damage on bee visitation could interact with plant size, and so plants were sorted into small and large categories. This analysis, however, also failed to find significant differences in outcrossing rate (Table 5). But this result should be interpreted with caution, since Array 2 received a mixed insecticide treatment (half the population sprayed, the other half not). The generally lower beetle populations in mixed neighborhoods may not inflict enough damage to cause a shift in selfing.

DISCUSSION

Floral herbivory can directly reduce plant fecundity by reducing the number of gametes, both ovules and pollen grains. However, floral herbivores can also indirectly reduce the success of the surviving flowers through effects on pollinator behavior. When herbivory reduces flower number or otherwise changes floral display or rewards, it can inhibit visitation rates and thereby diminish pollen export and pollen receipt. Pollinator foraging responses to damage can also affect the rate of selfing by either changing the proportion of geitonogamous visits or of autogamous pollinations. In this paper and others (Krupnick and Weis 1998, Krupnick et al. 1999) we have shown that feeding by the pollen beetle *M. rufimanus* imposes many, but not all, of these potential reductions in reproductive success on its host plant, *I. arborea*. In this discussion we integrate the results of this study of pollen export, pollen receipt, pollen limitation, and outcrossing rates with our previous findings concerning the beetle's impact on flower production, sex allocation, and pollinator visitation. We present these findings for both male and female function, comparing the relative strengths of direct and indirect herbivore effects (Table 6). We end with a discussion of the relative impacts of floral vs. vegetative herbivory, and how these will tend to be divided between direct and indirect effects on reproduction.

Male reproductive success

The most accurate measure of paternal reproductive success is the number of seeds sired by a plant. Male reproduction can be divided, however, into several se-

TABLE 2. Results of repeated measures ANOVA on fruit production in *I. arborea*.†

Year	Source of variation	df	MS	*F*
1992	Between plants			
	Insecticide treatment	2	225.98	21.18***
	Number of branches	1	104.67	9.81**
	Error	26	10.67	
	Within plants			
	Date	25	17.59	13.74***
	Date × insecticide treatment	50	2.00	1.56**
	Date × number of branches	25	2.92	2.28***
	Error (date)	650	1.28	
1994	Between plants			
	Insecticide treatment	1	211.35	10.47**
	Number of branches	1	1331.70	65.97***
	Error	141	20.19	
	Within plants			
	Date	32	19.77	32.23***
	Date × insecticide treatment	32	13.29	21.67***
	Date × number of branches	32	8.08	13.16***
	Error (date)	4512	0.61	
1995	Between plants			
	Insecticide treatment	1	53.67	7.38**
	Number of branches	1	757.97	104.16***
	Error	108	7.28	
	Within plants			
	Date	13	12.26	18.31***
	Date × insecticide treatment	13	6.73	10.05***
	Date × number of branches	13	7.30	10.90***
	Error (date)	1404	0.67	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
† Data were $\log(x + 1)$-transformed.

quential components of various contributing measures (Weis and Campbell 1992): (1) the number of pollen grains available for export, (2) the frequency of pollinator visitations, and (3) the number of pollen grains exported to and received by neighboring flowers.

Pollen grains available for export.—Previous work shows that beetle attack severely reduces pollen production in this system (Krupnick et al. 1999). Plants exposed to herbivory have higher flower bud abortion rates, and thus fewer buds mature into open flowers. The surviving flowers tend to be hermaphroditic, but still develop the same basic complement of six anthers, as do males. But of the buds that do survive to anthesis, beetle larvae on average destroy four of the six anthers. Pollen production in the surviving anthers is probably not affected. Other studies show that paternal success generally increases with flower production (Broyles and Wyatt 1990, Devlin et al. 1992, Conner et al. 1996;

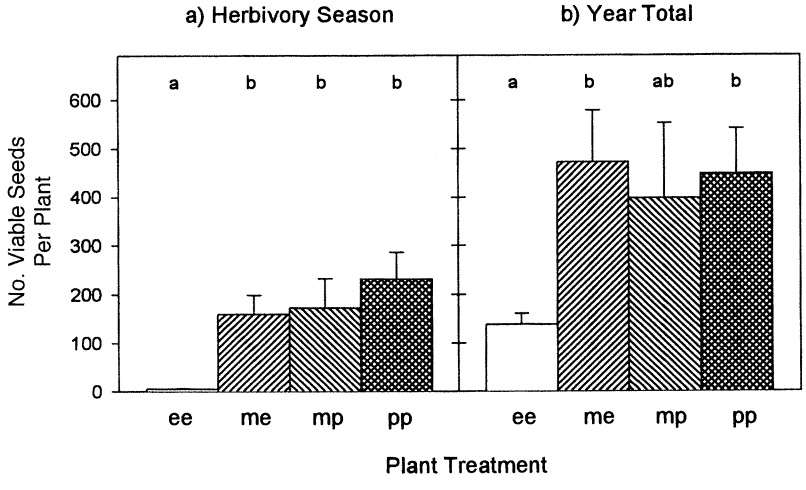

FIG. 6. The least squares means (+1 SE), adjusted for plant size, of the number of seeds per plant produced across four plant treatments during (a) the months of herbivore infestation and (b) the entire year. Plant treatments: ee = exposed plants in exposed plots; me = exposed plants in mixed plots; mp = protected plants in mixed plots; and pp = protected plants in protected plots. (a) $F_{3, 138} = 8.60$, $P = 0.0001$; (b) $F_{3, 137} = 5.32$, $P = 0.0017$.

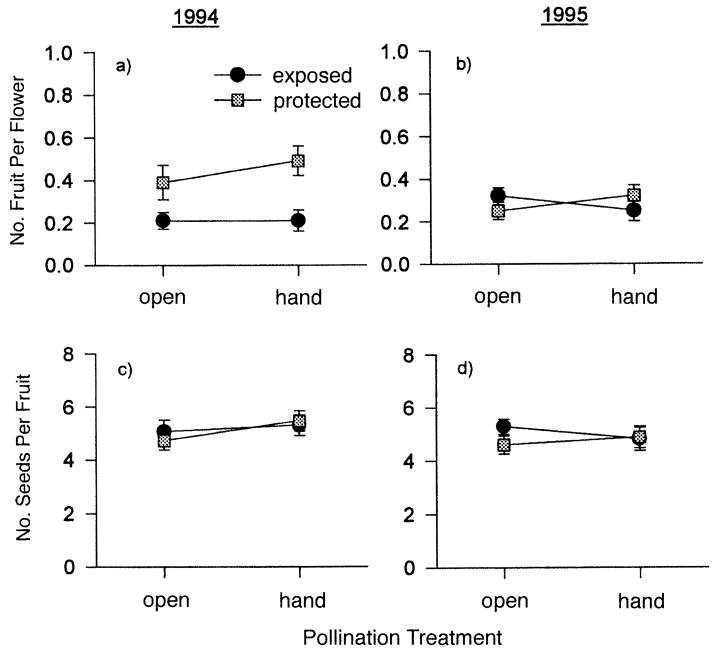

FIG. 7. Fecundity measures during the months of herbivory on *I. arborea*. Mean (±1 SE) fruit set (a, b) and seed set (c, d) of open-pollinated and hand-pollinated plants in (a, c) 1994 and (b, d) 1995. Solid circles = exposed plants; shaded squares = protected plants.

but see Meagher 1991), and thus a reduction in flower number due to herbivory can affect male reproductive success.

Frequency of pollinator visitations.—Pollen transfer is mediated by pollinator visitation. Our previous work shows that damaged flowers receive fewer bee visits than undamaged flowers (Krupnick et al. 1999). Not only do pollinators respond negatively to individual damaged flowers; service is further depressed by pollinator aversion to whole plants and to plant neighborhoods with high levels of beetle damage (Krupnick et al. 1999). Greater pollinator visitation rates have been shown to increase pollen removal (Young and Stanton 1990, Galen 1992, Rush et al. 1995) and increase the probability of pollen grains' reaching mates (Galen 1992), further showing the indirect impact that floral herbivory may have on paternity.

Pollen grain export to and receipt by neighboring flowers.—The reduced visitation by pollinators results in a reduction in pollen export. Here, we show that highly damaged plants have a lower *per flower* rate of pollen transfer to other plants and to other flowers. Thus, the herbivore in this system indirectly reduces the number of potential offspring that the host plant sires, and the extent to which the pollen grains are spread to neighboring flowers within the population. Pollen use may also be reduced during geitonogamous transfer (De Jong et al. 1992), but herbivory does not further reduce or increase levels of geitonogamy in this system.

Additional, unmeasured effects could further reduce

TABLE 3. Results of repeated-measures ANOVA examining the effects of insecticide treatment (exposed vs. protected) and pollination (hand vs. open) on various fecundity measures in *I. arborea* during the months of herbivore activity.

Source of variation	df	MS	*F*
a) Fruit Set			
Between plants			
Insecticide treatment	1	0.215	5.07*
Pollination	1	0.011	0.26
Treatment × pollination	1	0.082	1.92
Number of branches	1	0.000	0.00
Error	32	0.042	
Within plants			
Year	1	0.009	0.37
Year × insecticide treatment	1	0.207	8.36**
Year × pollination	1	0.008	0.33
Year × number of branches	1	0.002	0.11
Year × treatment × pollination	1	0.000	0.00
Error (year)	32	0.024	
b) Seed Set			
Between plants			
Insecticide treatment	1	3.61	2.57
Pollination	1	2.84	2.02
Treatment × pollination	1	2.99	2.13
Number of branches	1	0.75	0.53
Error	29	1.41	
Within plants			
Year	1	0.59	0.67
Year × insecticide treatment	1	4.04	4.59*
Year × pollination	1	1.22	1.38
Year × number of branches	1	0.07	0.08
Year × treatment × pollination	1	0.02	0.03
Error (year)	29	0.88	

* $P < 0.05$; ** $P < 0.01$.

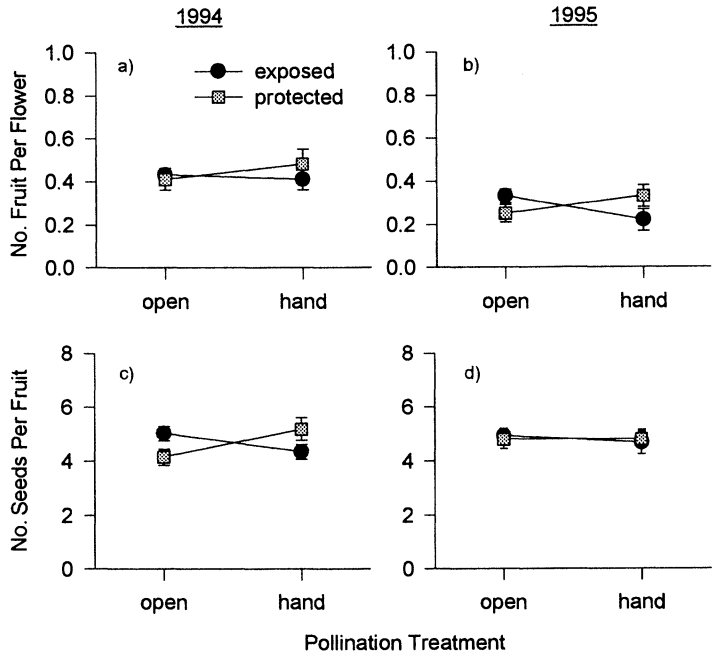

FIG. 8. Fecundity measures during the entire year on *I. arborea*. Mean (±1 SE) fruit set (a, b) and seed set (c, d) of open-pollinated and hand-pollinated plants in (a) 1994 and (b) 1995. Solid circles = exposed plants; shaded squares = protected plants.

TABLE 4. Results of repeated-measures ANOVA examining the effects of insecticide treatment (exposed vs. protected) and pollination (hand vs. open) on various fecundity measures in *I. arborea* during the entire year.

Source of variation	df	MS	*F*
a) Fruit Set			
Between plants			
Insecticide treatment	1	0.005	0.15
Pollination	1	0.003	0.09
Treatment × pollination	1	0.068	2.02
Number of branches	1	0.001	0.02
Error	32	0.034	
Within plants			
Year	1	0.062	5.43*
Year × insecticide treatment	1	0.004	0.42
Year × pollination	1	0.001	0.12
Year × number of branches	1	0.000	0.01
Year × treatment × pollination	1	0.003	0.23
Error (year)	32	0.011	
b) Seed Set			
Between plants			
Insecticide treatment	1	1.40	1.32
Pollination	1	0.52	0.49
Treatment × pollination	1	5.33	5.00*
Number of branches	1	0.50	0.47
Error	29	1.07	
Within plants			
Year	1	1.51	5.45*
Year × insecticide treatment	1	0.35	1.27
Year × pollination	1	0.21	0.75
Year × number of branches	1	0.04	0.16
Year × treatment × pollination	1	0.44	1.61
Error (year)	29	0.27	

* $P < 0.05$.

male reproductive success. Lowered pollen viability in damaged plants is possible (e.g., Quesada et al. 1995, Mutikainen and Delph 1996). A reduction in the quality of pollen could put damaged plants at a competitive disadvantage during fertilization, assuming mixed pollen loads. The strength of this effect, however, could vary with interplant variation in damage levels: If all plants are damaged, all competitors will have the same lowered abilities, and so competitive outcomes will not be influenced by damage.

When all known factors are considered (floral bud abortion, anther damage, and reduced pollen export), herbivore attack reduces the number of pollen grains reaching stigmas per starting flower bud by 95.1% (Table 6). Of this reduction, 86.1% is due to the initial reduction in pollen availability. This then leaves 9% caused by the failure of pollen to be dispersed. Thus, the damaging effects of a floral herbivore on male reproductive success can go beyond direct pollen consumption, though these effects may be minor compared to the tremendous direct effect of flower consumption.

Female reproductive success

The indirect effect of herbivory on male reproduction is clearly a loss in gamete export. The indirect effects on female reproduction are less apparent. Previously, we showed a moderate effect of beetle damage on ovule production (Krupnick et al. 1999). Although most buds on damaged plants abort, shifts in sex allocation allow female function to be conserved. Without damage, *I. arborea* inflorescences produce her-

TABLE 5. The single-locus outcrossing rates (t_s) of small and large plants in exposed and protected treatments of *I. arborea*. Numbers in parentheses are each one standard error based on 500 bootstraps. Fam = number of maternal families; Prog = number of progeny.

Treatment	Size	Fam	Prog	t_s
Exposed	Small	9	121	0.881 (0.224)
Exposed	Large	5	101	0.970 (0.187)
Protected	Small	8	140	0.937 (0.242)
Protected	Large	11	208	0.753 (0.089)

maphroditic flowers first, then male flowers. Since many hermaphroditic flowers are damaged (Krupnick and Weis 1998), these plants set fewer fruit, and thus hermaphroditic flowers continue to be produced. These extra hermaphroditic flowers represent a potential compensatory response. It appears, however, that the additional flowers produced during the months after herbivore activity by exposed plants in fully exposed patches (Krupnick et al. 1999) were not numerous enough to compensate in terms of fruit or seed set (Figs. 5 and 6). Therefore, the direct reduction in gamete production is primarily caused by a reduction in the number of maturing flowers.

Once fertilization occurs, further effects of damage are either absent or too weak to detect in these experiments. Although observed visits by bees are diminished by moderate damage, pollen receipt during the peak of beetle infestation is reduced only when damage is widespread within an inflorescence or within a whole plant. To judge by the pollen-limitation experiments, these reduced pollen loads may be insufficient to reduce fruit set; still, interpretive caution should be exercised, because we did not quantify stigmatic pollen loads during months of low or absent beetle activity.

We had the paradoxical result that, when supplemental pollination had any differential effect on damaged and undamaged plants, it was the undamaged plants that benefited. Pollen limitation can result from different processes: (1) low pollen availability (e.g., Allison 1990*b*, Bertness and Shumway 1992), (2) low pollinator visitation rates (e.g., Zimmerman 1980, Gross and Werner 1983, Sih and Baltus 1987), or (3) an interaction between pollen and resource availability (e.g., Campbell and Halama 1983). Herbivores may reduce pollen levels through preferential feeding on male flowers (Bawa and Opler 1978, Muenchow and Delesalle 1992) or by reducing the frequency of male flower production (Bertness et al. 1987, Allison 1990*a*, Krupnick 1996). Herbivory could have reduced the pollen pool in our experiment, but we saw no evidence for overall pollen shortages. We did find a drop in pollinator service rates to damaged flowers and plants (Krupnick et al. 1999), and thus we would expect damaged plants to be pollen limited. Supplemental pollen did not, however, substantially increase the fruit and seed set of these exposed plants. Either these plants are not pollen limited, or they are pollen limited and supplemental pollen was not applied in sufficient quantities (Young and Young 1992). Furthermore, we would not predict any decrease in the resource pool of exposed plants, because the floral herbivores do not appear to affect resource acquisition (see *Floral vs. vegetative herbivory,* below). Thus, the exposed plants should not be any more resource limited than protected plants.

Exposed plants may not have been sufficiently damaged to induce pollen limitation, since this experiment was performed in a mixed treatment neighborhood. Furthermore, damaged plants in highly damaged environments receive fewer visits than damaged plants in mixed treatment patches (Krupnick et al. 1999). Moreover, the local pollen supply is much lower when all plants are highly damaged than it is when only half the plants are damaged, as in a mixed treatment patch. Sih and Baltus (1987), for example, show that patch size explains a significant proportion of the variation in pollinator limitation in *Nepeta cataria.* Patch size and patch quality may thus be significant components in pollen-limitation dynamics.

Female reproduction can be reduced further through selfing. We have some evidence for inbreeding depression in *I. arborea* (Krupnick 1996), which would reduce seed quality. Selfing rates could thus influence reproductive success. We found no evidence for differences in geitonogamous pollen transfer between protected and exposed plants. Other studies show that an increase in flower number leads to higher geitonogamous pollen transfer (De Jong et al. 1992, Robertson 1992, Hodges 1995; but see Geber 1985) and selfing rates (Barrett et al. 1994, Harder and Barrett 1995). The greater flower number of protected plants, however, did not lead to greater levels of geitonogamy. We also found no evidence for higher levels of selfing in damaged or undamaged plants. Our isozyme study measured the secondary selfing rate (the selfing rate after all components of inbreeding depression have acted; Lande et al. 1994). Differential success of embryo fertilization in protected and exposed plants could have occurred during pollen germination, pollen tube growth, or during embryo development. Thus, early effects could be masked. If such effects occur in *I. arborea,* they are more likely to hide autogamous matings. Exposed plants had higher seed-abortion rates than protected plants (Krupnick 1996), which may provide evidence at an early reproductive stage for higher selfing in damaged plants.

In the end, it appears that the consequences of floral herbivory are far stronger for male than female reproduction. Damaged plants not only mature less pollen; that which is produced is spread to fewer flowers on fewer plants. Conversely, nearly all negative effects of floral herbivory on female reproductive success occur between bud initiation and flower maturation.

Floral vs. vegetative herbivory

No matter what plant part a herbivore eats, its effects on plant reproductive success can have direct and in-

TABLE 6. Summary of direct and indirect effects of floral herbivory of the reproductive success of *I. arborea*. Most figures contrast the fate of flowers and their gametes on plants growing in plots protected from herbivory by insecticide application (the protected/protected treatments in Array 1) to those in untreated plots (exposed/exposed).

Plant reproduction state	Effect on male function	Effect on female function
Direct effects		
Bud development†	58% decrease in maturation of developing flower buds	same
Sex determination†	66% decrease in number of male flowers per inflorescence	slight increase in number of hermaphroditic flowers per inflorescence
Gamete survival†	a) 67% reduction in number of functional anthers per flower that reached anthesis (damaged and undamaged flowers) b) no reduction of pollen initially produced in surviving anthers in damaged flowers c) unknown amount of pollen consumed in surviving anthers	30% decrease in number of hermaphroditic flowers with functional ovaries
Indirect effects		
Pollinator visitation‡	a) 53% decrease in pollinator visits to plants b) 68% decrease in pollinator visits per surviving flower	same
Pollen transfer§	a) 65% decrease in pollen reaching other flowers b) no change in geitonogamous pollen transfer	a) 70% decrease in stigmatic pollen loads on damaged flowers on highly infested plants (compared to undamaged flowers in lightly infested plants) b) *but* no detectable pollen limitation
Outcrossing§	no detectable change in selfing	same
Summed effects†,‡,§	95.1% decrease in pollen transferred per flower bud Calculated as (no. flowers/bud) × (no. pollen grains/flower) × (no. pollen exported/total pollen available), where pollen production was estimated from anther number	81.7% decrease in number of seeds matured per flower bud Calculated as (no. flowers/bud) × (no. fruit/flower) × (no. seeds/fruit)

† Krupnick and Weis 1998.
‡ Krupnick et al. 1999.
§ This paper.

direct components. Leaf, root, and stem feeders can reduce floral production (Quesada et al. 1995, Strauss et al. 1996). These effects will immediately reduce gamete production, but can also reduce the attractiveness of the floral display, with attendant consequences (Quesada et al. 1995, Aizen and Raffaele 1996, Mutikainen and Delph 1996, Strauss et al. 1996). In this respect, floral herbivores are similar.

Damage to leaf, root, or stem tissue, however, has a different effect on the source–sink relationships of flowers and developing seeds than damage to floral structures. Increased vegetative herbivory will reduce the resources available to all flowers on the attacked plant module; each sink gets less resource. The quality of each potential fruit can be maintained by decreasing the number of flowers and fruit initiated. Destruction to the flower, embryo, or fruit, on the other hand, reduces the number of sinks, which increases the resource level per surviving sink. As bet-hedging theory would predict, plant resistance to floral herbivory should include initiation of more flowers than can be matured (Stephenson 1981). Thus, as flowers are damaged, resources get channeled to the healthy ones. This occurs in *Yucca,* for example, where fruit with low levels of yucca moth attack are retained, while those with higher levels are aborted (Pellmyr and Huth 1994, Richter and Weis 1995). Floral herbivores, by promoting selection for increases in initial numbers of flowers, may, in turn, promote flexibility in plant reproductive ecology. Vegetative herbivores are less likely to have these effects.

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