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Impact of two specialist insect herbivores on reproduction of horse nettle, *Solanum carolinense*

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Abstract The frequency of coevolution as a process of strong mutual interaction between a single plant and herbivore species has been questioned in light of more commonly observed, complex relationships between a plant and a suite of herbivore species. Despite recognition of the possibility of diffuse coevolution, relatively few studies have examined ecological responses of plants to herbivores in complex associations. We studied the impact of two specialist herbivores, the horse nettle beetle, *Leptinotarsa juncta*, and the eggplant flea beetle, *Epitrix fuscula*, on reproduction of their host, *Solanum carolinense*. Our study involved field and controlled-environment experimental tests of the impact on sexual and potential asexual reproduction of attack by individuals of the two herbivore species, individually and in combination. Field tests demonstrated that under normal levels of phytophagous insect attack, horse nettle plants experienced a reduction in fruit production of more than 75% compared with plants from which insects were excluded. In controlled-environment experiments using enclosure-exclosure cages, the horse nettle's two principal herbivores, the flea beetle and the horse nettle beetle, caused decreases in sexual reproduction similar to those observed in the field, and a reduction in potential asexual reproduction, represented by root biomass. Attack by each herbivore reduced the numbers of fruits produced, and root growth, when feeding in isolation. When both species were feeding together, fruit production, but not root growth, was lower than when either beetle species fed alone. Ecological interactions between horse nettle and its two primary herbivores necessary for diffuse coevolution to occur were evident from an overall analysis of the statistical interactions between the two herbivores for combined assessment of fruit and vegetative traits. For either of these traits alone, the interactions necessary to promote diffuse coevolution apparently were lacking.

Key words Herbivory · *Solanum carolinense* · Plant reproduction · Diffuse coevolution · Pairwise coevolution

Introduction

A primary assumption of plant-herbivore coevolutionary theory is that herbivores act as significant selective factors in the evolution of their host plants by reducing plant fitness (Strong et al. 1984, p. 210; Crawley 1987, 1989; Paige and Whitham 1987; Simms and Rausher 1987; Craig et al. 1988; Hendrix 1988; Berenbaum and Zangerl 1988; Bowers 1988; Sacchi et al. 1988; Maschinski and Whitham 1989). Debate concerning the negative effects of insect herbivores on natural populations of host plants persists (Jermy 1976, 1984; Belsky 1986; Paige and Whitham 1987; Maschinski and Whitham 1989). While some reviews of herbivore effects on host plants cite an increasing number of empirical studies that document the deleterious effects of herbivores on their host plants (Crawley 1983, 1989; Dirzo 1984; Hendrix 1988; Marquis 1992), others contend that insect herbivores rarely, if ever, act as selective forces on their host plants (Jermy 1976, 1984; Strong et al. 1984), or that insect or mammalian herbivory may increase the fitness of host plants (Owen and Weigert 1976; Simberloff et al. 1978; Stenseth 1978; Inouye 1982; McNaughton 1983, 1986; Maschinski and Whitham 1989). In the light of recent debate, there is a continued need to determine effects of insect herbivores in natural plant-insect communities other than

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those already studied (Janzen 1979; Dirzo 1984; Maschinski and Whitham 1989; Crawley 1989).

Beyond concern about the impact of single herbivore species on plant reproduction is the general failure to study the impacts of two or more herbivores on individual plant species in the light of recognition that the basic model of pairwise plant-herbivore coevolution is too simplistic given the prevalence of attack by several herbivore species on plants in nature (Futuyma 1983; Jermy 1984; Thompson 1986; Gould 1988). Examples of intense pairwise coevolution between host plants and herbivores have been called "small vortices in the mainstream of evolution" (Strong et al. 1984, p. 218). To reflect complexities of interactions in natural plant-herbivore associations, the concept of 'diffuse' coevolution was introduced (Janzen 1980; Fox 1981; Futuyma and Slatkin 1983).

Gould (1988) noted that most researchers have tended to avoid multi-herbivore systems to concentrate on simpler ones with the potential for pairwise interactions. Despite the concentration on simple systems, some authors have experimentally studied the impact of multiple herbivores on reproduction and/or allocation to growth in single plant species (Hartnett and Abrahamson 1979; Stamp 1984; Strauss 1991; Meyer 1993; Meyer and Root 1993; Hougen-Eitzman and Rausher 1994). Most multiherbivore studies have experimentally assessed the impact of either the community of herbivores or of each herbivore alone (Hare and Futuyma 1978; Hartnett and Abrahamson 1979; Winder and van Emden 1980; Winder and Harley 1982; Inouye 1982; Heithaus et al. 1982; Simms and Rausher 1989; Meyer and Root 1993). Notably, Strauss (1991) and Hougen-Eitzman and Rausher (1994) examined both individual and combined effects of herbivores on reproduction by a woody plant and an annual plant, respectively, providing information necessary to interpret the pairwise or diffuse nature of the plant-herbivore interaction.

Hougen-Eitzman and Rausher (1994) established one criterion to distinguish between "pairwise" and "diffuse" interactions between plants and two or more insect herbivores. They argued that non-additivity of effects of herbivores on plant fitness would engender diffuse coevolution because the effectiveness of resistance to one herbivore species would depend on the presence of the other. Non-additivity of effects of two herbivores is indicated by significant statistical interactions between herbivores in an analysis of variance model in which each herbivore species is considered a treatment factor. Hougen-Eitzman and Rausher (1994) argued that non-additivity of impacts by two or more herbivores on plant fitness is one ecological condition necessary to indicate potential for diffuse coevolution between a plant and its herbivores. Conditions promoting pairwise coevolution between a plant and its herbivores would be indicated in the absence of statistical interactions between herbivores on plant fitness.

We used a combination of field and common garden experiments to investigate the impact of two species of insect herbivores on the herbaceous plant, *Solanum carolinense* (Solanaceae), horse nettle. In our study area, horse nettle had two common specialist leaf-feeding herbivores, the chrysomelid beetles *Leptinotarsa juncta*, the horse nettle beetle, and *Epitrix fuscula*, the eggplant flea beetle. We experimentally tested two hypotheses that elucidated the effects of these herbivores on their host plant within the context of a complex plant-herbivore association:

1. Phytophagous insects reduce the fitness of horse nettle, as measured by sexual reproductive output, in a natural population.
2 Feeding by either *L. juncta* or *E. fuscula* alone or in combination reduces the fitness, as measured by both sexual and potential asexual reproductive output of their host plant, in a common garden experiment.

Finally, using the results of herbivore impact on plant reproduction from the garden experiment, we examined statistical interactions between the two herbivores in an analysis of variance model to determine whether one necessary ecological condition is satisfied for diffuse convolution to take place between horse nettle and these specialist herbivores (Hougen-Eitzman and Rausher 1994).

Methods

Study site and species

This study was conducted in the summer and fall of 1990 at the University of Virginia's Blandy Experimental Farm, located in the northern Shenandoah River Valley in Clarke County, Virginia, United States. Blandy Farm is located at an altitude of 190 m at longitude 78°W, latitude 39°N, and receives an average annual precipitation of about 94 cm. The horse nettle population used for observations of phenology of herbivore attack and for the field experiment was located in an abandoned agricultural field adjacent to an ephemeral pond. Plants for a controlled-environment experiment were taken from two abandoned agricultural fields located in the northeastern and southwestern portions of Blandy Farm.

Solanum carolinense (Solanaceae), horse nettle, is a native perennial herb that grows along roadsides, in old fields, and in waste places (Duncan and Foote 1975). Horse nettle bears self-incompatible flowers (Hardin et al. 1972; Solomon 1981) that produce seeds in orange-yellow berries, 1–2 cm in diameter (Strausbaugh and Core 1978). *S. carolinense* reproduces vegetatively by sending up shoots from a thick perennial taproot or spreading lateral roots (Tisdell 1961). Horse nettle is apparently protected from feeding by most herbivores by spines on stems, leaf veins, and calyces (Solomon 1983), stellate trichomes on leaves (Radford et al. 1968), and alkaloids typical of solanaceous plants (Hsiao 1986).

Larvae and adults of *Leptinotarsa juncta* (Chrysomelidae: Chrysomelinae), the horse nettle beetle or false Colorado potato beetle, feed on leaves, flowers, or developing fruits of *S. carolinense*, their only native host (Hsiao 1986; Jacques 1988). Adults emerge in late spring and feed and lay small masses of eggs on horse nettle leaves and other substrates. The complete life cycle takes about 4–5 weeks. Insects were bivoltine at our study site. We observed larvae and adults of *Leptinotarsa decemlineata*, the Colorado potato beetle, in very small numbers at our site. Horse nettle beetle (HNB) refers to damage caused predominantly by *Leptinotarsa juncta*, with the possibility that some was caused by the rarer Colorado potato beetle which possesses a similar feeding mode (see Tower 1906; Hare and Kennedy 1986; Jacques 1988; Hare 1990 for natural history and feeding of *Leptinotarsa*).

The eggplant flea beetle, *Epitrix fuscula* (Chrysomelidae: Alticinae), feeds both on agricultural and native species of solanaceous plants (Berenbaum 1988; Campbell et al. 1989). The eggplant flea beetle (FB) was the only *Epitrix* observed at the study site and appeared to feed on *S. carolinense* almost exclusively. The 2-mm-long adults eat tiny holes in leaves of horse nettle while larvae feed on roots (Borror et al. 1989). Adults emerge in the spring and feed on young horse nettle plants. The beetle is bivoltine with development from eggs to adults taking about 4–5 weeks. Second-generation adults feed until frost when they enter into diapause in soil or leaf litter (Campbell et al. 1989).

Phenology – observations

We evaluated the phenology of herbivore attack on horse nettle in the field by setting up a 20-m transect through the horse nettle population every 2 weeks, beginning on 10 June. The location of the transect was haphazardly determined at each sampling date. We sampled 20 plants nearest to 1-m intervals along the transect. For each plant, we measured the phenological state of the main stem (height, total number of leaves), the number of leaves fed upon by FB and HNB, and the identity and abundance of insects present on horse nettle plants in the experimental field population. Herbivores caused characteristic damage that allowed quantification of leaves damaged by each. Horse nettle can produce many sub-shoots; however, we restricted surveys of damage to leaves on the main stem.

Field experiment

We experimentally tested the impact of insect herbivores on sexual reproduction of *S. carolinense* in the field population. On 21 June, we marked 45 ramets of heights between 26 cm and 45 cm. Fifteen plants were assigned at random to each of three treatments: (1) total herbivore removal by biweekly application of an aqueous solution of the insecticide carbaryl (Sevin), (2) hand-removal of all stages of HNB every 7–10 days, and (3) control, with natural levels of herbivory. Plants in treatments 2 and 3 were sprayed with water to control for water addition in the carbaryl treatment. We planned to differentiate the impact of HNB from that of other herbivores by assessing the difference between treatment 3 with all herbivores and treatment 2 with all herbivores except HNB. We were unable to unambiguously assess the impact of flea beetles on plant fitness through a flea beetle removal treatment because of their small size and great mobility.

Between 27 August and 3 October, we counted, collected, and measured the diameter of fruits to the nearest 0.05 mm with a dial caliper as they ripened. Less than 1% of fruits remained when the first frost occurred, at which time we collected these last fruits. Statistics testing for herbivore impact on horse nettle reproduction included values for response variables for all plants in the three treatments. About 14% of mature fruits were removed by vertebrates before we collected them. We assigned average fruit diameters for appropriate treatment categories to these lost fruits for estimation of the impact of herbivores on total seed production, for which no inferential statistics were run.

Enclosure-exclosure experiment

We established experimental plants for the enclosure-exclosure experiment from cuttings taken from taproots. In mid-May, from populations in each of two fields, we collected 60 *S. carolinense* taproots from individual ramets separated by a minimum of 1 m in an effort to collect distinct genetic individuals. Roots were washed, cut to 12-cm lengths, weighed, and one root section per taproot was planted in a commercial potting mix (WESCO growing media III; Wetsel Seed Company, Harrisonburg, Va.) in 15 cm diameter pots where they were allowed to grow in a greenhouse for about 3 weeks. On 8 June, 25 healthy plants from each source population were replanted into 19-l pots (nominal 5-gallon pots) in soil from a third, neutral site and were randomly placed in a grid in an open field. Potted plants were grown without competition in a sunny location, with regular watering, and addition of 14-14-14 Osmocote slow-release fertilizer. Each pot was covered with a 91 cm high×30 cm diameter, cylindrical galvanized-wire cage and fine nylon mesh bag to regulate insect access.

We created four treatments to measure the impact of FB and HNB when they attacked horse nettle alone and together. Ten plants, five from each of the two source populations, were randomly assigned to the following treatments: *Leptinotarsa* – feeding only (HNB), *Epitrix* – feeding only (FB), both *Leptinotarsa* and *Epitrix* – feeding (HNB+FB), and ten from each population were assigned to a control treatment with no herbivory. On 13 June, we placed insects on the plants as follows: four adult flea beetles on the FB-only plants and HNB+FB plants, two first-instar *Leptinotarsa* larvae on the HNB-only and the HNB+FB plants, and no insects on the control plants. Flea beetles were collected as adults while HNB eggs were collected and larvae reared from eggs in the laboratary. The numbers of insects of each species added in the first three treatments were based on surveys of abundance of these species on individual plants in the field.

We introduced new first-instar larvae of HNB onto experimental plants in the HNB and HNB+FB after the first two larvae pupated, with the number of larvae added based on plant size. Following the initial larval introductions, plant height was measured, the number of leaves were counted, and new larvae added in early July, late July, and late August–early September. The largest plants received the greatest number of larvae, with the maximum added to any plant being four, seven, and seven, respectively for the three dates. The number placed on smaller plants was scaled down with no larvae added to plants that were severely damaged by HNB larval feeding. Adults were removed from cages as they emerged because they do not remain on individual plants for extended periods in the field, instead moving among and feeding on many plants in natural populations (personal observations).

Similarly, adult flea beetles were added to enclosure plants according to plant size. The number of adult flea beetles was regularly monitored and individuals were added or removed as adults escaped or new ones emerged as a result of reproduction by adults added earlier. We could not control the numbers of larvae feeding on roots but rather monitored abundance of adults with the largest plant receiving no more than 15 FB adults.

Horse nettle plants in the enclosure experiment grew much larger than the vast majority of plants in natural populations because they were grown in controlled conditions with abundant light, water, and mineral nutrients. Field plants comparable in size to the large enclosure plants were observed to host as many as 12 potato beetle larvae or 66 flea beetles at a time. Thus, the numbers of insects added in this experiment should ensure that herbivory treatments were not extreme (D. Macauley, personal communication), and the results should be conservative with respect to herbivore impact.

To ensure seed set of caged plants, all open flowers were hand-pollinated every 2–3 days throughout the flowering season (27 June–2 October). We applied a mixture of pollen, taken from anthers of 50–100 plants, to receptive stigmas of enclosure plants with a paint brush. In late October, we collected and counted fruits from each plant and measured the diameter of each fruit to determine herbivore effects on fruit size.

We estimated the number of seeds produced on each plant by quantifying the relationship between fruit size and seed number. We collected 40 fruits representative of a wide range of diameters from both field and experimental plants from various treatments. Fruit diameter was measured and the number of seeds counted for each fruit. We used the regression equation defining the relationship between fruit diameter and seed number to estimate the number of seeds produced on both field and garden experimental plants.

The effect of herbivores on mean seed mass and proportion of seeds that germinated was also evaluated. We measured seed mass to the nearest 0.001 mg of eight seeds using a Cahn C-31 Electrobalance (Orion Research, Boston, Ma.). Seeds were taken from a sample of five fruits per treatment plant, unless fewer than five fruits were available. Mean seed mass per plant was used as the response variable in statistical analyses. We evaluated the proportion seed germination by planting each of ten seeds per treatment plant in an individual 5 cm×5 cm×10 cm pot containing commercial potting soil (WESCO growing media III; Wetsel Seed Company, Harrisonburg, Va.). We counted the number of seeds germinating over the next 4 weeks; all viable seeds germinated within 2–3 weeks.

To assess impact of herbivores on potential asexual reproduction of horse nettle, we measured the final root biomass of each enclosure plant as an indicator of the plant's potential to generate new ramets and/or flowers and fruits. In a related study, horse nettle plants derived from larger root segments yielded more fruits in a single year than plants from smaller segments suggesting that final root biomass is an indicator of future reproductive success (C.F. Sacchi and M. Priest, unpublished work). In mid-November, roots were removed from pots, washed, dried for 24 h at 60°C to constant mass, and weighed using a Mettler AE 63 balance (Mettler Instruments, Hightstown, N.J.).

Statistical analysis

Field experiment

The effect of three herbivory treatments on sexual reproduction of *S. carolinense* was assessed using one-factor analysis of variance (ANOVA) on each of two different response variables, fruit number and mean fruit size per plant. We corrected for heterogeneity of variance in fruit number per plant using a logarithmic transformation.

All possible pairwise contrasts were run on log-transformed mean fruit numbers to determine the specific effects of each level of the herbivory treatments using the contrast function in the SPSS/PC+ 4.0 statistical package (Norusis 1990). We maintained an experiment-wise α of 0.05 for contrasts using the Dunn-Sidak method (Sokal and Rohlf 1981).

Enclosure-exclosure experiment

We analyzed the plant response to herbivory using a three-factor multivariate analysis of covariance (MANCOVA), since we measured five response variables on each experimental unit. MANCOVA is the appropriate statistical procedure whenever more than one response variable is measured on individual experimental units, particularly when no correlations exist among response variables (Huitema 1980, pp. 237–238). Treatment factors were: (1) source field for plants, (2) FB treatment, and (3) HNB treatment. We ran one MANCOVA on two whole-plant response variables, fruit number and final root mass, using all plants for which both measures were available. A second MANCOVA was run on the reproductive traits mean fruit diameter, mean seed mass, and proportion seed germination from plants that produced fruit and for which seeds were available. We report the *F* for the Pillai-Bartlett trace (Huitema 1980).

We tested for two covariates, initial root mass for each plant and plant height at the experiment's outset and only adjusted for these variables when they were statistically significant. Treatments in the first MANCOVA were adjusted only for plant height, while no covariate was used for the second. Fruit number and root biomass were log-transformed to satisfy the assumption of homoscedasticity, and proportion germination data were arcsine-square root transformed.

Following multivariate analysis, we ran univariate tests using three-factor analysis of covariance (ANCOVA) on each of the five response variables to examine the response of individual reproductive traits to herbivory. It is appropriate to run univariate tests following MANCOVA given the necessary precaution of adjusting α levels of individual univariate tests to maintain an experiment-wise α of 0.05 (Huitema 1980). For the two whole-plant response variables, *F* statistics of individual univariate ANOVAs were compared to table *F* values for an α of 0.025 while for the three fruit and seed response variables *F* statistics were evaluated in relation to table *F* values for $\alpha<0.0167$. Experimental factors and covariates for ANCOVA were the same as for MANCOVA.

In higher-order MANCOVA and univariate ANOVA models, the existence of a significant statistical interaction indicates that the effect of a given experimental treatment "differs according to the levels of the second factor" (Kleinbaum and Kupper 1978, pp. 335–336). A significant statistical interaction between HNB and FB would suggest that the impact that one herbivore has on horse nettle reproduction changes in the presence or absence of the other.

Results

Phenology

Every *S. carolinense* individual we observed in the field was attacked by both flea beetles and horse nettle beetles on every sampling date. Moreover, the percentage of leaves that were damaged on each plant was high, exceeding 50% of leaves attacked both by flea beetles and horse nettle beetles throughout the growing season (Fig. 1). Flea beetles were observed on plants until the first severe frost killed all leaves (mid-October); the percentage of leaves with FB damage remained high through the end of the growing season. Levels of HNB damage also remained high throughout the season but decreased slightly after early September, when HNB began to disappear from the population (Fig. 1).

Field experiment

In the field experiment testing the impact of herbivores on horse nettle reproduction, the difference in numbers of fruits produced per plant among the treatment groups was highly significant ($F_{2,42}=20.753$; $P<0.001$, Fig. 2a).

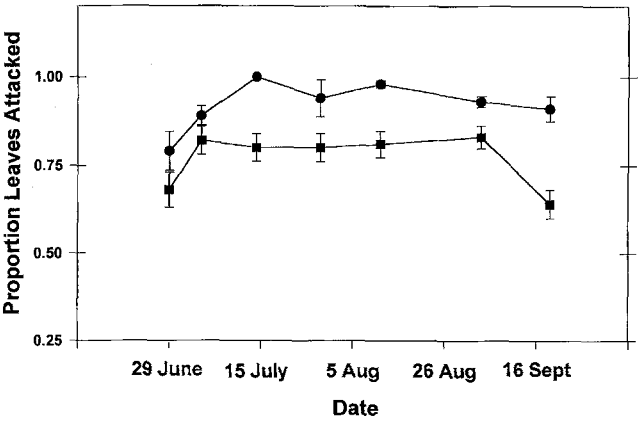

Fig. 1 Percentage of leaves exhibiting damage by flea beetles (*solid circles*) and by horse nettle beetles (*solid boxes*) on individuals in the field population of horse nettle at various sampling dates throughout the growing season. Twenty plants were sampled on each date. The *error bars* represent means±1 SE

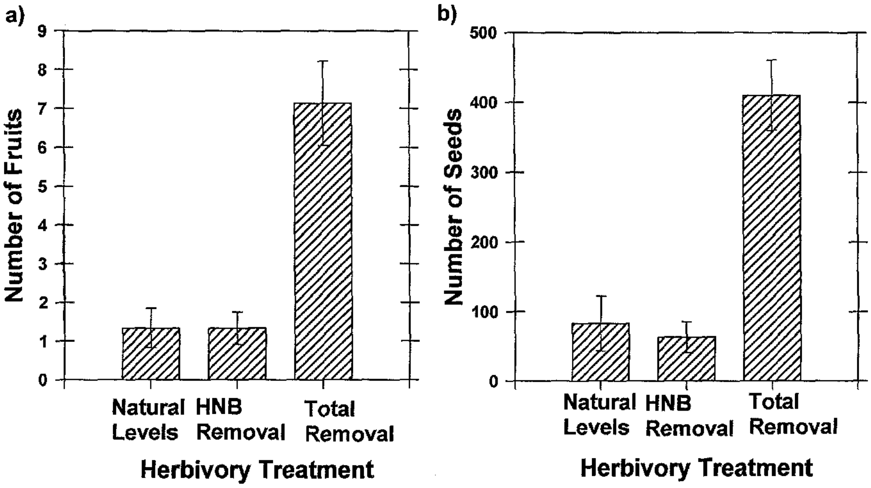

Fig. 2a, b Sexual reproductive output of field plants (n=15 plants per treatment): **a** mean number of fruits produced by each plant, **b** mean number of seeds produced by each plant. The *error bars* represent means±1 SE

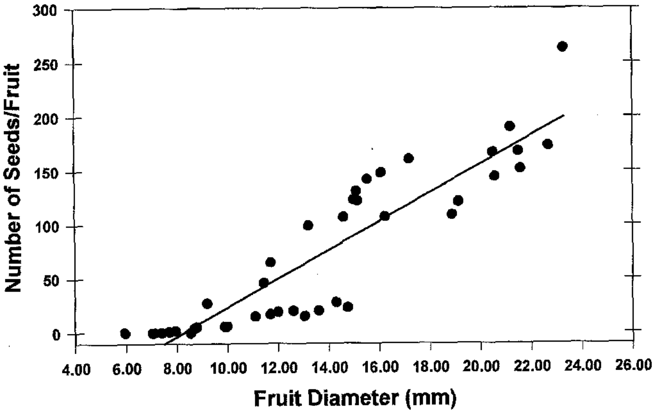

Fig. 3 Correlation between the diameter of *Solanum carolinense* fruits and the number of seeds they contain (r^2=0.819, n=40, P<0.001; seed number=[13.38×fruit diameter (mm)] −109.58

Pairwise contrasts reveal that the fruit number per plant was significantly higher in the herbivore-free treatment than in either the control group, where natural levels of herbivory occurred (t_{42}=5.448, P<0.001), or in the group where only HNB were removed (t_{42}=5.271, P<0.001). Fruit number did not differ between the control and the HNB-removal treatment (t_{42}=0.175, P>0.80).

In addition to reducing the number of fruits per plant, insect herbivores reduced the proportion of plants that reproduced. While 93% of insecticide treated plants matured fruits, only 53% of controls and HNB-removal plants matured any (χ^2=7.2, df=1, P<0.01).

Herbivores did not significantly affect the average diameter of the fruits produced by each horse nettle individual ($F_{2,27}$=1.8379, P<0.15: herbivore-free=11.8 mm±0.8; HNB Removal=11.9 mm±0.5; control=12.9 mm±0.3).

The impact of herbivory on total seed production was predicted by converting the fruit diameters to seed umbers using the relationship between these variables (Fig. 3). Herbivores decreased the average seed output of each plant by greater than 75% compared to the total herbivore-exclusion plants (Fig. 2b). We ran no statistics on estimated seed numbers but report them since they provide meaningful estimates of herbivore effects on plant reproduction.

Enclosure-exclosure experiment

Overall effects on reproduction

In the garden experiment, both FB and HNB caused a significant overall reduction in both sexual reproductive measures and root biomass. For whole-plant reproductive measures, there were significant differences among the herbivore-free-control plants and treatment plants fed on by either FB or HNB (Table 1A). We detected statistically significant interactions between FB and HNB and FB by source field (Table 1a).

Overall, there was no significant multivariate response to herbivory in fruit size, seed mass, and germination success (Table 1b). Effects of source field, herbivory treatment by source field, and FB by HNB interactions were not significant.

Sexual reproduction – univariate responses

The univariate analysis of fruit number indicated that both herbivores significantly affected reproductive output of *S. carolinense* (Table 1A, Fig. 4A). Initial plant height was the only significant covariate. We detected no significant statistical FB×HNB interaction on fruit number but found a significant field by FB interaction (Table 1A).

Estimates of seed numbers in the different treatments indicated that flea beetles and horse nettle beetles feeding alone reduced seed output by 65% and 31%, respectively (Fig. 4b). The combined herbivory of flea beetles and horse nettle beetles decreased seed output by 89% compared to controls.

Fruit size and seed measures were not significantly affected by herbivores (Tables 1b and 2). In agreement with results of the field experiment, the mean size of

Table 1 a, b Summary of *F* statistics from MANCOVA and ANCOVA for plant reproductive response to insect herbivory, source field, and initial plant height as a covariate. **a** *F* values for the overall MANCOVA for two whole-plant response variables and the univariate ANCOVA for these variables that include log-transformed values for number of fruits and final root mass (g). **b** *F* values for overall MANCOVA for fruit and seed attributes and univariate ANCOVA for these variables that include fruit diameter, seed size per plant, and arcsine square-root transformed proportion seed germination. Univariate ANOVA results are statistically significant when $P<0.025$ for reproductive response variables in **a** and for $P<0.016$ for fruit and seed variables in **b**, based on the Bonferroni adjustment. No significant covariate was found for the MANCOVA for fruit and seed attributes

	Fruit number	Root mass (g)	Overall	
a Whole-plant response variables				
Field	$F_{1,40}=0.009$	$F_{1,40}=3.101$	$F_{2,39}=1.989$	
FB	$F_{1,40}=6.132^{2*}$	$F_{1,40}=5.840^{2*}$	$F_{2,39}=3.415^{1*}$	
HNB	$F_{1,40}=13.285^{4*}$	$F_{1,40}=11.613^{3*}$	$F_{2,39}=6.815^{3*}$	
Field×FB	$F_{1,40}=8.042^{3*}$	$F_{1,40}=2.803$	$F_{2,39}=4.052^{2*}$	
Field×HNB	$F_{1,40}=0.370$	$F_{1,40}=0.868$	$F_{2,39}=0.234$	
FB×HNB	$F_{1,40}=0.003$	$F_{1,40}=4.325^{*NS}$	$F_{2,39}=3.448^{1*}$	
FdxFBxHNB	$F_{1,40}=0.464$	$F_{1,40}=2.325$	$F_{2,39}=1.209$	
HEIGHT 1	$F_{1,40}=21.650^{4*}$	$F_{1,40}=9.551^{3*}$	$F_{2,39}=4.019^{1*}$	
	Fruit size (mm)	**Seed mass (g)**	**Arcsin germ**	**Fruit and seed**
b Fruit and seed response variables				
Field	$F_{1,33}=1.418$	$F_{1,28}=0.307$	$F_{1,28}=4.779^{*NS}$	$F_{3,26}=1.716$
FB	$F_{1,33}=0.004$	$F_{1,28}=0.241$	$F_{1,28}=0.113$	$F_{3,26}=0.769$
HNB	$F_{1,33}=0.318$	$F_{1,28}=1.872$	$F_{1,28}=1.652$	$F_{3,26}=1.317$
Fld×FB	$F_{1,33}=0.181$	$F_{1,28}=0.426$	$F_{1,28}=0.010$	$F_{3,26}=0.156$
Fld×HNB	$F_{1,33}=0.122$	$F_{1,28}=0.380$	$F_{1,28}=0.035$	$F_{3,26}=0.197$
FBxHNB	$F_{1,33}=0.171$	$F_{1,28}=0.418$	$F_{1,28}=4.604^{*NS}$	$F_{3,26}=1.552$
FdxHNBxFB	$F_{1,33}=1.040$	$F_{1,28}=0.043$	$F_{1,28}=0.042$	$F_{3,26}=0.474$

$^{1*}P<0.05$, $^{2*}P<0.025$, $^{3*}P<0.01$, $^{4*}P<0.001$; ^{*NS} indicates non-significance for the test statistic following a Bonferroni adjustment

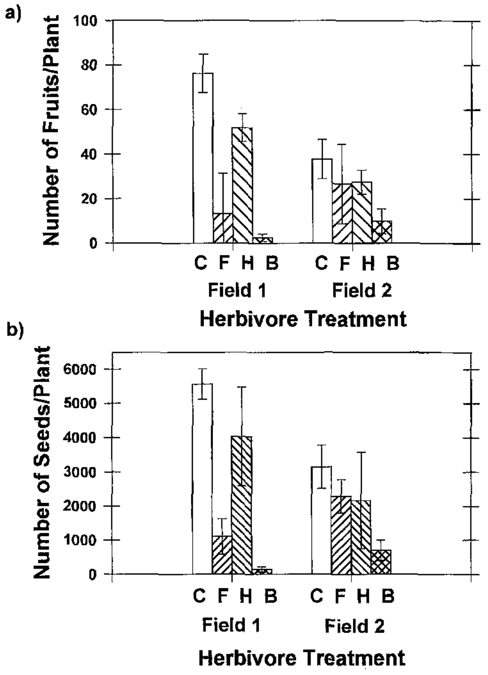

Fig. 4a, b Sexual reproductive output of enclosure-exclosure plants (n=10 plants for the control treatment in each of the fields, and n=5 per field for each of the treatments where herbivores are present): **a** mean number of fruits produced per plant, **b** mean number of seeds produced per plant.The *error bars* represent means±1 SE. (Treatments: *C* unattacked controls, *F* flea beetles alone, *H* horse nettle beetles alone, *B* both herbivores)

fruits produced by horse nettle was not affected by herbivory (Tables 1b and 2). Neither seed mass nor proportion seed germination was affected significantly (Tables 1B and 2). No statistically significant FB by HNB interactions were detected for measures of fruit size, proportion seed germination, or seed mass. We detected no field or field by herbivore interactions (Table 1B).

Asexual reproduction

In addition to significantly reducing sexual reproduction, herbivores led to a significant decrease in potential vegetative reproduction of *S. carolinense* individuals, as estimated by final root biomass (Table 1A and Fig. 5). Source field and source field by herbivore interaction effects on root biomass were not significant. After Bonferroni adjustment, we detected a non-significant FB×HNB interaction (Table 1A). Initial plant height was a significant covariate.

Discussion

Field experiment

The field experiment demonstrated that insect herbivores have a negative impact on reproduction by *S. carolinense* plants in a natural population. This study adds to a grow-

Table 2 Summary statistics (means±SE) for fruit and seed attributes in response to herbivore attack and source field. Germination is represented by proportion germination but ANCOVA was run on arcsine square-root transformed data

Source Herbivory		Fruit Diameter (mm)	*n*	Seed Mass (mg)	Proportion Germination	*n*
Field	Category					
NE	Control	13.8±0.5	10	1.886±0.400	0.96±0.02	10
	FB	14.8±0.8	3	2.231±0.248	1.00±0.00	2
	HNB	14.0±0.3	4	1.843±0.391	0.98±0.03	4
	Both	13.3±2.0	3	1.853±0.596	0.80±0.10	2
SW	Control	14.7±0.4	10	1.937±0.682	0.81±0.04	9
	FB	14.4±0.7	5	1.985±0.262	0.92±0.04	5
	HNB	14.2±0.1	2	1.604±0.174	0.90±0.00	2
	Both	14.5±1.2	4	1.480±0.267	0.50±0.30	2

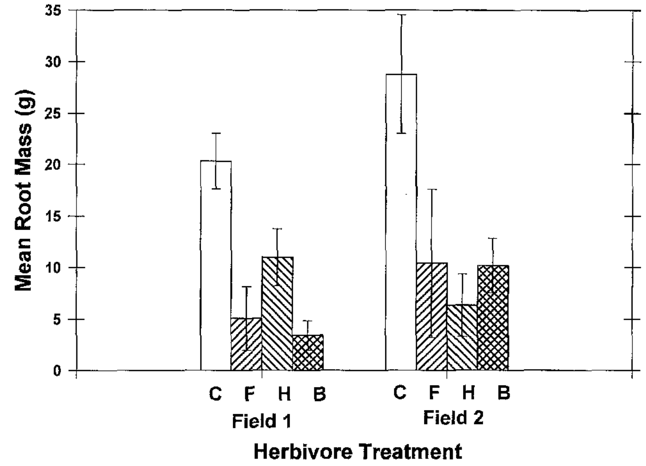

Fig. 5 Final root biomass for the plants in the enclosure-exclosure experiment (n=10 for the control treatment in each field, and n=5 for each of the treatments where herbivores are present). The *error bars* represent means±1 SE. (Treatments: *C* unattacked controls, *F* flea beetles alone, *H* horse nettle beetles alone, *B* both herbivores)

ing number of studies in which fitness was higher in plants from which herbivores were excluded by the use of insecticides in natural populations (e.g., Waloff and Richards 1977; Louda 1984; Simms and Rausher 1987, 1989). The large reductions in fruit and seed number, about 5 to 6-fold, brought about by insect herbivory suggest that herbivores can exert the kind of pressure that must exist in order for herbivore resistance to evolve.

Methods we used for limiting herbivore abundance were not likely to influence interpretation of results for either the field or enclosure study. Carbaryl has been shown to have little or no effect on growth or reproduction of plants to which it is applied (Jones et al. 1986; M. Bucher and E.L. Simms, unpublished work, cited in Simms and Rausher 1987). Results of the enclosure-exclosure experiments confirmed that insect herbivory itself reduced the reproductive output of horse nettle. Lastly, use of herbivores in the enclosure experiment avoided the many problems associated with studies that measure plant response following artificial defoliation (see Baldwin 1990).

We anticipated that the difference in fruit production between the HNB-removal treatment and the herbivore-attack control would allow us to estimate the independent impact of horse nettle on plant reproduction in the field. Removing horse nettle beetles did not significantly affect reproduction of horse nettle plants compared to controls. While this suggests that HNB had no impact on the fitness of its host, we offer two alternative explanations. Removal of beetles at weekly intervals did not prevent feeding by larvae or adults that moved to plants between removals; in fact, there was abundant evidence of continued feeding between removals. Thus, the control (all herbivores present) and the HNB-removal treatments may not have differed in insect attack, despite efforts to limit HNB damage. Another possibility is that HNB had little or no impact on plants growing in the field while HNB herbivory reduced fruit production by plants growing in resource-rich conditions in pots. This interpretation is inconsistent with predictions of other studies that suggest that plants grown in resource-rich conditions would be less likely to suffer negative effects from herbivore feeding than resource-deprived plants (Maschinski and Whitham 1989).

We have demonstrated that the community of herbivores, with eggplant flea beetles and horse nettle beetles being the most abundant members of this community, significantly reduces horse nettle fruit and seed production. Methodological difficulties prevented us from isolating the effects of the two specialist herbivores on plant fitness in the field.

Enclosure-exclosure experiment

Herbivore impact on fitness

The results of the enclosure-exclosure experiment demonstrate that insect herbivores can significantly reduce the potential fitness of their host plants by reducing current sexual reproduction as well as investment in asexual reproduction, as represented by root biomass. Control plants produced nearly ten times as many seeds as plants that experienced combined herbivory of HNB and FB. For different plant-herbivore systems, Hartnett and Abrahamson (1979), Strauss (1991), Meyer and Root (1993), and Hougen-Eitzman and Rausher (1994) found within the context of multi-herbivore studies that each herbivore might have unique impacts on reproduction by their host plant, with effects ranging from negative to compensatory. However, we found that the two most common herbivores on horse nettle at our site each had a large, significant negative impact on plant reproduction.

Abrahamson and McCrea (1986) and Cain et al. (1991) claimed that decreased vegetative reproduction should lead to reduction in the sum of future sexual reproductive output. Once a horse nettle seedling is established, vegetative reproduction is thought to be its main mode of propagation within a population (Tisdell 1961; Solomon 1981). A horse nettle's horizontal roots can spread rapidly throughout a field (Kiltz 1930; Tisdell 1961). While root mass alone does not equal asexual reproduction, we would argue that an individual which can spread its roots over a larger area will have a better chance of producing a larger number of successful new ramets than will an individual with a smaller root system. Therefore, we consider root biomass to represent allocation to future reproduction. Furthermore, experimental manipulation of root fragment size indicated that both longer or heavier root segments yielded plants that produced more fruits in a single growing season (C.F. Sacchi and M. Priest, unpublished work). The threefold reductions in root growth resulting from herbivory suggests that insect herbivores can have a strong influence on plant fitness through reduced allocation to asexual reproduction.

Ideally, herbivore impact should be measured by evaluation of effects on plant fitness (Hendrix 1988). Actual measurement of impact on lifetime fitness in long-lived plants is difficult if not impossible, but lifetime impacts have been modelled (Doak 1992). Measurement of loss in fruit and seed production in a single year is satisfactory in the absence of evidence that the plant has allocated resources in a way that will lead to reproductive overcompensation in future years. Among studies that have evaluated herbivore impacts on allocation to flowers and below-ground structures in long-lived plants, there is no necessary correlation between decreased seed production and rhizome or root production (Hartnett and Abrahamson 1979; Abrahamson and McCrea 1986; Fay and Hartnett 1991; Meyer and Root 1993; Cain et al. 1991). Our results strongly suggest that lifetime reproduction will be reduced and overcompensation is unlikely in horse nettle attacked by horse nettle beetles and flea beetles. Significant reductions in both sexual and asexual reproduction indicate that the plant may suffer reduced fitness following herbivory by either or both specialist herbivores.

Maschinski and Whitham (1989) predicted that herbivore effects on plant reproduction may depend on availability of resources to the plant and suggested that under conditions of high nutrient availability, a plant may be able to overcompensate for the damage herbivores cause. Plants in the enclosure-exclosure experiment were treated with slow-release fertilizer and were watered regularly. Plants in pots grew vigorously and produced an average of 8 times as many fruits as field plants. Despite the abundance of resources available to plants, they still suffered a decrease in reproductive output in the presence of herbivores feeding either in isolation or together. Since we did not experimentally vary nutrient or water availability, our study did not experimentally test the hypothesis of Maschinski and Whitham (1989). However, since resources were uniformly high for plants used in the enclosure-exclosure experiment, our study offers strong support to the caution of Meyer and Root (1993) concerning the generality of the expectation that a plant with access to abundant nutrients will be able to overcompensate for herbivore damage.

Interactions of two herbivores: ecological impacts

Despite widespread recognition that most plants are fed on by more than one species of herbivore, studies that permit assessment of the independent and combined impact of two or more herbivores on plant reproduction are rare (but see Strauss 1991; Hougen-Eitzman and Rausher 1994). Hougen-Eitzman and Rausher (1994) argued that ecological interactions between herbivores and their host plant that might presage pairwise coevolution would be suggested in the absence of statistical interactions between herbivores in their effects on plant reproduction. Alternatively, they hypothesized that the existence of statistical interactions between the herbivores provides evidence for ecological interactions that could lead to diffuse coevolution.

These predictions are based on the assumption that pairwise coevolution is likely only when no ecological interactions exist whereby one herbivore affects evolution of resistance to other species of herbivores (Hougen-Eitzman and Rausher 1994). The nature and effectiveness of plant defenses against specialized and generalist herbivores that feed on individual plant species has been the subject of study and speculation (e.g., DaCosta and Jones 1971; Hare and Futuyma 1978; Futuyma 1983; Futuyma and Slatkin 1983; Maddox and Root 1990; Simms and Fritz 1990). Our objective is to focus on ecological impacts of HNB and FB on horse nettle fitness that may influence the evolution of resistance. We have not documented the evolution of herbivore resistance nor, in this study, have we examined the extent of genetic variation among horse nettle plants in susceptibility to attack by these two specialist herbivores.

Our results provide an independent test of the prediction that examination of statistical interactions between herbivores can be used to characterize the ecological conditions that may influence plant evolution of herbivore defenses. Overall, we found modest and possibly ambiguous evidence for diffuse ecological interactions because statistical interactions between HNB and FB were significant overall for the MANCOVA using both fruit number and final root mass but were non-significant for each of these same variables treated in a univariate ANOVA . Statistical interactions were non-significant for all other reproductive measures based on fruit size and two seed attributes. Pairwise effects were suggested by non-significant FB by HNB interactions for all univariate analyses of all reproductive response variables.

Gould (1988) suggested that the processes involved in the coevolution of multispecies plant-herbivore systems may be complex, but they need not be "diffuse" in the sense of being vague or obscure. The potential for coevolution between chrysomelids and solanaceous plants has been questioned since chrysomelids are generally small and "their impact on Solanaceae may not be sufficiently severe for them to be considered as a major natural selection agent of the plants" (Hsiao 1986, p. 361). On the contrary, our study has shown that natural levels of damage by specialist chrysomelids can have a large negative impact on the fitness of their solanaceous host plant. The reciprocal nature of the evolutionary relationships, at least for the system of *S. carolinense* and its principal herbivores, *E. fuscula*, and *L. juncta*, may have been underestimated. We have clearly documented the potential for selection by these specialist herbivores when feeding alone or together on their solanaceous host. Though tentatively, we offer evidence based on overall reproductive measures for interactions between horse nettle and its herbivores suggestive of the potential for diffuse coevolution to follow.

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