

Insect herbivores, density dependence, and the performance of the perennial herb *Solanum carolinense*

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Abstract. How insect herbivores affect plant performance is of central importance to basic and applied ecology. A full understanding of herbivore effects on plant performance requires understanding interactions (if any) of herbivore effects with plant density and size because these interactions will be critical for determining how herbivores influence plant population size. However, few studies have considered these interactions, particularly over a wide enough range of densities to detect nonlinear effects. Here we ask whether plant density and herbivores influence plant performance linearly or nonlinearly, how plant density affects herbivore damage, and how herbivores alter density dependence in transitions between plant size classes. In a large field experiment, we manipulated the density of the herbaceous perennial plant *Solanum carolinense* and herbivore presence in a fully crossed design. We measured plant size, sexual reproduction, and damage to plants in two consecutive years, and asexual reproduction of new stems in the second year, allowing us to characterize both plant performance and rates of transition between plant size classes across years. We found nonlinear effects of plant density on damage. Damage by herbivores and plant density both influenced sexual and asexual reproduction of *S. carolinense*; these effects were mostly mediated via effects on plant size. Importantly, we found that herbivores altered the pattern of linear density dependence in some transition rates (including survival and asexual reproduction) between plant size classes. These results suggest that understanding the ecological or evolutionary effects of herbivores on plant populations requires consideration of plant density and plant size, because feedbacks between density, herbivores, and plant size may complicate longer-term dynamics.

Key words: asexual reproduction; clonal plants; density dependence; insect herbivores; plant–herbivore interactions; plant performance; plant size; sexual reproduction; structural equation models.

INTRODUCTION

Understanding the effects of insect herbivores on plant performance is central to basic questions about plant ecology and evolution, and to applied questions in both agriculture and biocontrol. While plant density is often considered the major influence on plant performance (reviewed by Silvertown and Doust 1993), herbivores also often affect plant growth, survival, and fecundity (reviewed by Crawley 1989). Plant density also can alter the effects of herbivores, so these two factors may not be independent. In particular, plant density and concomitant changes in resource availability can affect herbivore damage (Karban 1993, Izhaki et al. 1996), plant resistance (Karban et al. 1989, Cipollini and Bergelson 2001), the effect of damage on plant performance (i.e., tolerance; Hartnett 1989) and numbers of herbivores on plants (Root 1973, Kareiva 1985, Shea et al. 2000). Plant size also often strongly influences plant performance (e.g., Herrera 1991, Buckley et al.

2003) and both herbivore (Hodkinson et al. 2001, Arany et al. 2005) and density effects (Silva Matos et al. 1999, Horvitz and Schemske 2002) can vary with plant size. It is thus important to consider both how plant size might act as a mediator of density and herbivore effects, and how these effects may change with plant size. However, size dependence of interactions between density and herbivore effects has only been considered in one previous study (Horvitz and Schemske 2002) and never in an experimental setting.

The importance of effects of size on performance is reflected in the frequent use of size-structured models (or “matrix models”) for plant population dynamics (Ehrlén 1995, Parker 2000, Knight 2004) or fitness across the life cycle (Caswell 1989). Matrix models that group plants into discrete size classes and calculate rates of transition among these size classes as a function of survival, growth, and reproduction are common, and models like these are often used to assess the status of plant species of concern (those with threatened populations, or invasive species) and to make management decisions (Crone et al. 2011). Examining how herbivores or plant density influence transition rates in matrix models can provide valuable information about how these factors

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influence plant populations. Without data about every transition, one cannot predict population growth or fitness; nevertheless, matrices of transitions among size classes are an excellent tool for summarizing effects of herbivores and density on plant performance (Louda and Potvin 1995) while acknowledging the importance of these transitions for population dynamics or fitness.

For many species, plant densities vary greatly over time and space. Density dependence can be linear or nonlinear (Miller 2007); for example, nonlinear density dependence may occur if density effects become pronounced only with extreme crowding, or if Allee effects lead to positive density dependence at low densities. Linear and nonlinear density dependence can have quite different consequences for the dynamics of the growth of individual plants and populations, for example, nonlinear density dependence can create multiple attractors or drive population cycles (Turchin 2003). Understanding herbivore effects on plant performance thus requires considering multiple plant densities so that linear and nonlinear effects can be distinguished. However, previous studies of effects of herbivores and intraspecific plant density on plant performance (Rauscher and Feeny 1980, Parker and Salzman 1985, Fagan and Bishop 2000, Parmesan 2000) have typically only included two or three plant densities and have not accounted for differences in herbivore or density effects with plant size (but see Horvitz and Schemske 2002). Likewise, although reviews have pointed out that appropriate tests for effects of herbivores on plant population dynamics would include density dependence (Maron and Vilà 2001, Keane and Crawley 2002, Halpern and Underwood 2006, Maron and Crone 2006), previous tests of herbivore effects have mostly assumed density independent plant population growth (e.g., Fagan and Bishop 2000, Parker 2000). Manipulating herbivores over a wide range of plant densities and individual plant sizes makes it possible to detect nonlinear effects of density and herbivore damage, and to inform the development of models that consider long-term effects of herbivores on dynamic processes such as natural selection for resistance, plant population dynamics, or biological control.

Here we use data from the first two years of a long-term field experiment to address density dependence in herbivore effects on plant performance. In particular, we ask: (1) How does plant density affect herbivore damage levels? (2) How do plant density and herbivores influence plant size and reproduction? and (3) Do herbivores alter density dependence in transitions among plant size classes? We use structural equation models to explore how plant size may mediate effects of density and herbivores on plant reproduction (see, e.g., Fig. 1) and present analyses of transitions among size classes to summarize size-specific herbivore and density effects. Our focus is on effects of herbivores and density on plant performance; in the future, we will use these

and additional demographic data to parameterize plant population dynamic models.

MATERIALS AND METHODS

Solanum carolinense is a perennial andromonoecious weed with sexual and asexual reproduction and plastic self-incompatibility (Travers et al. 2004). In north Florida where this study was conducted, *S. carolinense* grows frequently in old fields with grasses and other mostly perennial species such as *Sida spinosa* and *Rubus* sp. These *S. carolinense* populations naturally vary in density within (0–22 stems/m²) and among sites (mean densities 1.8–6.2 stems/m²; S. Halpern, unpublished data). *Solanum carolinense* has many specialized herbivores (e.g., *Leptinotarsa junta*, *Manduca sexta*, and *Epitrix fuscula*), which do most of the damage in our area. Herbivores influence *S. carolinense* fitness (Wise and Sacchi 1996), and *S. carolinense* has both constitutive and inducible herbivore resistance (Cipollini et al. 2002, Wise and Weinberg 2002). *Solanum carolinense* clones for this experiment were collected from five populations in north Florida and Georgia and greenhouse propagated for several asexual “generations.”

As part of a long-term study of the effects of insect herbivores on *S. carolinense* population dynamics, in 2007 we established a field experiment simultaneously manipulating *S. carolinense* density (five initial densities) and herbivores (present or absent). Each of these 10 treatment combinations was replicated four times for a total of 40 field plots, spaced a minimum of 10 m apart. Plot sizes varied by necessity with density to maintain logistical feasibility (ranging from 1.6 to 98.4 m²), but we used two different plot sizes within each density treatment to avoid confounding density and plot size; plot size was never significant in exploratory analysis and is not considered further here. Density treatments were randomly assigned positions within each of two spatial blocks, with sprayed and unsprayed plots of the same density and size paired spatially. The experiment was located at the North Florida Research and Education Center (NFREC) in Quincy, Florida, USA, in fields without *S. carolinense* but with natural populations of *S. carolinense* nearby. Plots were prepared by disking and application of glyphosate (Round-Up; Monsanto Corporation, St. Louis, Missouri, USA), planted March–April 2007, and watered until established (early June). After plant die-back in November, plots were mowed to prevent succession out of old field vegetation.

Initial *S. carolinense* densities (0.65, 2.8, 11.1, 22.7, and 30.9 per m²) ranged from about 0.2 to 9 times natural density, which averages 3.4 stems/m² when present at NFREC (S. Halpern, unpublished data). Plants were established in the greenhouse from root cuttings or seeds, then transplanted to the field in a regular grid and permanently tagged. Each plot contained at least 49 plants, but we collected data only on plants in the area surrounding the 25 central plants

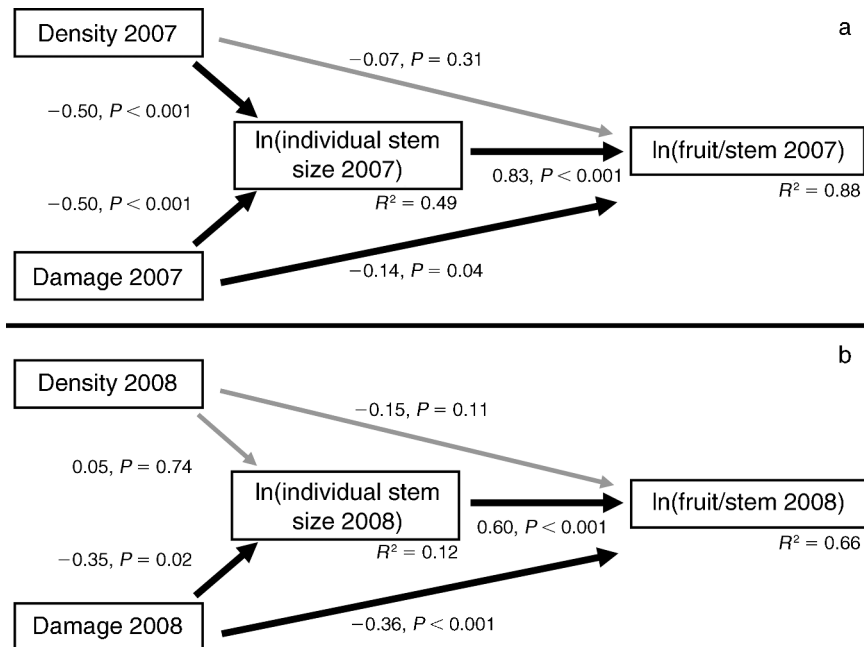


FIG. 1. Structural equation models for effects of damage and density on fruit production per stem in (a) 2007 and (b) 2008. Black arrows indicate significant relationships; gray arrows indicate nonsignificant relationships. Standardized path coefficients and P values are indicated near the arrows. Note that the direct effect of damage on fruit production was not significant with plot 6 excluded. There was no significant deviation of the model from the data in either year (2007, $\chi^2 < 0.002$, $df = 1$, $P = 0.98$, root mean square error of approximation [RMSEA] = 0; 2008, $\chi^2 = 0.001$, $df = 1$, $P = 0.93$, RMSEA = 0). Endogenous (dependent) variables have R^2 values below.

planted in 2007 (“central area”) in each plot, to control for edge effects. To allow collection of demographic data on plants of different sizes (necessary to determine transition rates), we planted each plot’s central area with five seedlings and 20 plants generated from root fragments of different sizes. Initial shoot sizes were all small (<20 cm stem length), but all plots received the same distribution of root fragment sizes. For the 20 root-propagated plants, each central area contained the same selection of greenhouse-propagated clones to control for possible genotype effects; seedlings originated from multiple maternal plants in our source populations.

We manipulated herbivores (including florivores) by spraying half the plots at each density level with 0.13% carbaryl insecticide (22 mL of Sevin Concentrate per 3.785 L; GardenTech, Lexington, Kentucky, USA) biweekly from April through October; we sprayed the other plots with water as a control. Carbaryl has been shown not to affect plant growth or seed production (Stinchcombe and Rausher 2001) and did not affect either number or duration of pollinator visits or pollen viability in *S. carolinense* in this experiment (K. Kilcourse and A. Winn, *personal communication*).

All stems emerging in 2008 were permanently tagged. For 2007, each tag was associated with an individual genet. While the vast majority of 2007 plants consisted of a single stem, a few plants may have had multiple

stems; because we knew such multiple stems had come from one root, we recorded data by genet. We measured plant size as the total of all stems, in the few cases where there were multiple stems. In 2008, we measured each stem emerging from the ground as a separate unit, as we did not know which came from which roots. For the remainder of this paper, we use “stem” when referring to data from both 2007 and 2008. In this study, our interest is the ecological effects of densities of stems, rather than densities of genets.

In June and July of 2007 and 2008, we measured the size of all stems in the central area of each plot (1000 stems in 2007 and 2464 stems in 2008). Stem size was measured as stem length, including all branches >5 cm; this measure correlates closely with total biomass in the greenhouse (Spearman’s $\rho = 0.935$, $N = 25$ samples, $P < 0.0001$) and aboveground biomass in the field (Spearman’s $\rho = 0.89$, $N = 16$ samples, $P < 0.0001$). We calculated densities of *S. carolinense* as number of stems per central area of the plot because herbivores typically respond to stem, not genet, density, and while physiologically integrated stems can compete for resources (Hellström et al. 2006, Matsushita et al. 2010), the extent of physiological integration among stems in *S. carolinense* is unknown. We visually estimated percentage of leaf area damaged on leaves on each stem (2007, 10 upper and 10 lower leaves; 2008, 5 upper and 5 lower leaves; all leaves for plants with <20 or <10 leaves,

respectively). In 2008, we also measured percentage of leaf area damaged in a separate survey in late July on 5 upper and 5 lower leaves on a random selection of 50 stems per central area of each plot. Each year we counted the number of ripe fruits per plant in September and October, when most fruiting occurs.

We mapped the location of all new and original stems based on the locations of their permanent tags. We used triangulation with a laser measurement system (Leica D3 Disto; Benchmark Measuring, Springfield, Virginia, USA) to get x, y coordinates for each tag (3.2 cm accuracy based on resampling). We did not observe any seedlings, so new stems were considered asexual progeny. Previous demographic studies of clonal plants have generally simply divided all new stems equally among possible parents from the previous year (Berg 2002, Ellis et al. 2007). However, we found a clear pattern of clumping of new 2008 stems near 2007 plants, suggesting that we could more accurately estimate asexual reproduction by assigning 2008 stems to the nearest 2007 stem rather than simply assuming equal reproduction for all individuals. Excavations of non-experimental plants in the field confirm that after a single year of growth most stems from a plant in the previous year emerge near the original plant (D. McNutt, *unpublished data*). Asexual reproduction was thus estimated by assigning each 2008 stem as offspring of the 2007 stem to which it was the closest.

Analyses

We treated density as continuous in all analyses; although discrete densities were planted in 2007, by 2008 those densities had shifted and could no longer be considered categorical. Herbivore effects were considered two ways. To test for effects of herbivores on transitions between size classes, and to test for an effect of spraying on damage, herbivores were considered a categorical variable (spray vs. no spray). For all other analyses, herbivore effects were represented as the continuous variable “damage” (field-measured damage); although spraying strongly reduced damage overall, there was some damage in some sprayed plots and unsprayed plots received a wide range of levels of damage. The response variables individual stem size and fruits per stem were natural-log-transformed to meet assumptions of our models; asexual reproduction and damage did not require transformation. As an alternative to density, we also examined effects of biomass per area (total stem length per plot/plot area) on damage, stem size, and reproduction; these results were similar to density effects and were not considered further. Structural equation models (SEM) were analyzed using AMOS 5.0.1 (Arbuckle 2003); other analyses used R 2.10.1 (R Core Development Team 2010).

We first tested for an effect of spray treatment on percentage of leaf area damaged in each year using t tests. Then, to examine the effects of density on damage (question 1), we used plot averages from only the

unsprayed plots because herbivore responses in sprayed plots could have been atypical (although results were qualitatively the same using all plots). We considered effects within each year and across years separately, in each case starting with a model including density, density², and average individual stem size, followed by stepwise model selection to arrive at a best model (lowest Akaike information criterion, AIC). For these analyses, we used June 2007 and July 2008 damage estimates; in 2007, we collected damage data only in June, although June estimates capture less of the total cumulative damage over the season and are thus a weaker measure of damage than July estimates. Results (not shown) are qualitatively similar using June instead of July 2008 data.

To address question 2, we used SEMs (Grace 2006) to explore multivariate relationships among factors that influenced plant reproduction. In particular, we asked whether average damage and density per plot had direct effects on sexual (fruit per stem) and asexual (change in the number of stems from 2007 to 2008) reproduction, indirect effects through stem size, or both. We tested three separate models containing all these effects for fruits per stem in 2007 and 2008 (sexual reproduction) and number of new stems in 2008 (asexual reproduction). All models were analyzed using maximum likelihood estimation and were considered a good fit to the data if the P value for the chi-square lack-of-fit test exceeded 0.05. We also used multiple regression to examine these same relationships and to test for nonlinear effects of density; results (see Appendix A) were consistent with results of the SEMs.

Finally, to address question 3, we examined effects of herbivores on transition rates between size classes for *S. carolinense* from one year to the next. We divided stems into three size classes to maximize evenness of representation across classes (there were no clear break points in survival or growth): class 1 (small, 0–20 cm), class 2 (medium, 20–100 cm), and class 3 (large, >100 cm). Transitions among size classes may be influenced by survival, growth, regression, stasis, and asexual reproduction. Because original plants often produced many new stems, we expect that asexual reproduction dominates many of these transitions, including backward transitions. Generalized linear models with Gaussian error were used to model transition rates (the fraction of plants in stage i in 2007 that transitioned to stage j in 2008 ($a_{ij} = N_{ij}/N_i$) as a function of density and density²; the best-fit model was selected based on Akaike information criterion for small sample sizes, AIC_c, scores. To determine support for density dependence of each transition rate, we calculated AIC_c weights for two models for each transition: a model with no density effects (intercept only) and the best possible model with any density effect (density, density², or both). AIC_c weights represent the relative likelihood of competing models; weights for models being compared sum to 100% of possible model support. Higher AIC_c weights

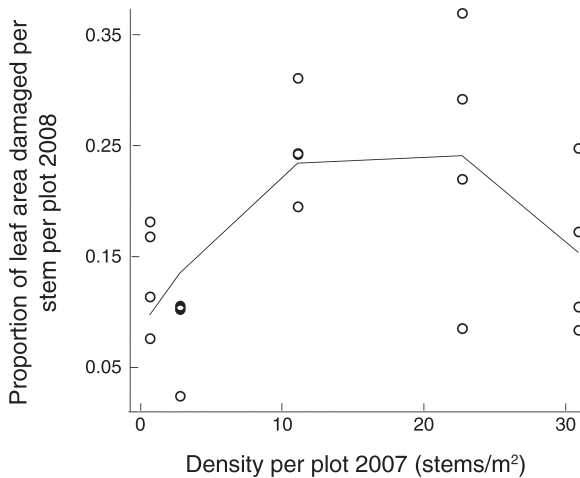


FIG. 2. Effect of plant density in 2007 on damage in 2008. Each point indicates the average percentage of damage per stem for a plot; only plots with herbivores (no insecticide spray) are included, $N = 20$ plots. The line indicates the best-fit model with linear ($P = 0.003$) and quadratic ($P = 0.004$) density terms.

indicate models with relatively higher support from the data. The parameter estimates for the model with the most support indicate (where density is included) the form and strength of density dependence for that transition rate.

RESULTS

Effects of spray treatment and density on damage (question 1)

Spray treatment significantly affected mean percentage of leaf area damaged in both years (2007, $t = 4.9$, $df = 19.5$, $P < 0.0001$; 2008, $t = 5.7$, $df = 20.7$, $P < 0.0001$); unsprayed plots received on average 4.5 (2007) and 3.5 (2008) times more damage than sprayed plots (Appendix B: Fig. B1). While the range of proportion leaf area damaged was similar between 2007 and 2008 (from a low of 0.02 in both years to highs of 0.33 and 0.37 in 2007 and 2008, respectively), in 2007 one unsprayed plot (plot 6) received twice as much damage as any other plot; with one exception, excluding plot 6 had no effect on results. The highest 2008 plant densities were twice as high as the highest plant density in 2007 (Appendix B: Fig. B2; see density axes) and average densities in 2008 did not differ between spray and no-spray plots (test not shown); no plot had an exceptional influence on results for density (based on Cook's distance). Average 2008 damage per plant per plot was influenced by plant density in 2007; there were significant linear ($t = 3.5$, $df = 16$, $P = 0.003$) and quadratic ($t = 3.3$, $P = 0.004$) effects of density (Fig. 2). The effect of stem size in 2007 on 2008 damage was marginally significant and negative ($P = 0.07$). Density and stem size did not affect damage within 2007 or 2008.

Effects of density and herbivores on plant reproduction (question 2)

Our structural equation models for effects of density and damage on sexual and asexual reproduction were all acceptable fits to the data (P values for lack-of-fit all > 0.9). These models indicate that sexual reproduction was affected directly and indirectly (via stem size) by damage, but only indirectly by density. Fruit production in 2007 increased with individual stem size (which was in turn decreased by density and damage; Appendix B: Fig. B2), and was also directly decreased by damage (Fig. 1a). The direct effect of damage in 2007 was not significant with plot 6 excluded, but the model was otherwise similar with or without plot 6. Fruit production in 2008 was similarly decreased directly and indirectly (via stem size) by damage; density had no effect on stem size or fruit production in 2008 (Fig. 1b; Appendix B: Fig. B2), despite a greater range of densities than in 2007. Asexual reproduction (number of new stems in 2008) was decreased indirectly by damage and density in 2007 through effects of those factors on stem size and a strong positive association of stem size in 2007 with number of new stems in 2008 (Fig. 3).

Effects of herbivores on rates of transition between plant size classes (question 3)

There was strong support for some transition rates being density dependent; AIC_c weights are much higher for models including density than for the intercept-only model for some transitions (bold values in Table 1 last two columns and Fig. 4). Density effects also differed between plots with and without herbivores. AIC_c weights suggest changes in the importance of density with herbivores for two transitions (Fig. 4). For the size class 2 to size class 1 transition (hereafter 2–1) without herbivores there was greater support (75%) for the best (linear) density model (the transition rate decreased with density) than for a density independent transition; with herbivores the support for density dependence was weak. For the class 1–2 transition, there was strong support (84%) for density effects with herbivores and weak support for density effects without herbivores. Note that weights for alternative models sum to 100% within herbivore treatments, rather than across herbivore treatments.

DISCUSSION

We found evidence for feedbacks between plant density, herbivores, and plant size that are likely to influence plant performance in complex ways. Plant density had a nonlinear effect on herbivore damage. In turn, damage by herbivores and plant density both influenced sexual and asexual reproduction of *S. carolinense*. The effects on reproduction were mostly, although not entirely, mediated by effects on plant size. Critically, we found that herbivore damage could alter the pattern of density dependence in transitions between

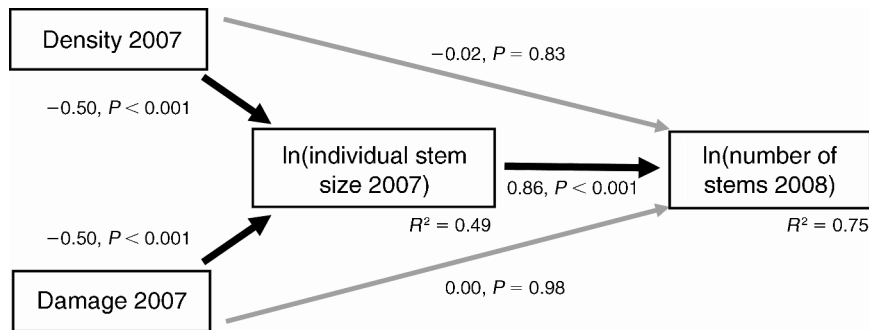


FIG. 3. Structural equation model for effects of damage and density on asexual reproduction. Asexual reproduction was measured as the number of stems per plot in 2008 (all plots contained the same number of plants in 2007; $N = 40$ plots). Shading and presentation are as in Fig. 1. There was no significant deviation of the model from the data ($\chi^2 < 0.002$, $df = 1$, $P = 0.98$, RMSEA = 0).

plant size classes, and this effect differed among transitions. Together, these results suggest that understanding long-term ecological or evolutionary effects of herbivores on plant populations will require explicitly considering both plant density and plant size.

Effects of damage and density on reproduction

The net negative effects of density and damage on reproduction that we observed are consistent with results of previous studies. Reduced reproduction at higher density is commonly observed for both total sexual reproduction (Ågren et al. 2008) and asexual reproduction

(Bishop and Davy 1985); these patterns are consistent with density dependence in population growth. Our finding that herbivores depressed sexual reproduction is also consistent with many previous studies showing reductions in seed or fruit production due to herbivores (Marquis 1992, Wise and Sacchi 1996). Few studies have considered effects of herbivores on asexual reproduction. Some have found effects of herbivores on potential asexual reproduction (root mass; Meyer and Root 1993, Wise and Sacchi 1996) or reproduction via fragmentation (Dyer et al. 2004) but only a handful (Cain et al. 1991, Brathen and Juntilla 2006) have directly measured herbivore effects on asexual

TABLE 1. Best-fit models for transitions rates between plant size classes from 2007 to 2008.

Transition (2007–2008)	Plots with this transition (N)	Parameter estimates from best-fit model	AIC _c weight	
			Best model with density	Intercept-only model
Herbivores present (no spray)				
1–1	16	intercept = 1.62***	28	72
1–2	13	intercept = 1.33***; density = -0.04*	84	16
1–3	0	NE	NE	NE
2–1	19	intercept = 1.19	37	63
2–2	18	intercept = 0.75***	29	71
2–3	1	intercept = 0.2 (1 plot)	NE	NE
3–1	18	intercept = 1.99***; density = -0.17*; density ² = 0.005	56	44
3–2	19	intercept = 1.7***; density = -0.04**	98	2
3–3	6	intercept = 0.12***	24	76
Herbivores absent (spray)				
1–1	16	intercept = 4.18**; density = -0.12*	54	46
1–2	9	intercept = 2.66	39	61
1–3	0	NE	NE	NE
2–1	20	intercept = 5.34**; density = -0.55*; density ² = 0.01	75	25
2–2	18	intercept = 4.51*; density = -0.16	61	39
2–3	4	intercept = 0.26	13	87
3–1	19	intercept = 2.12***; density = -0.17*; density ² = 0.004	79	21
3–2	18	intercept = 2.27***; density = -0.06*	75	25
3–3	8	intercept = 0.16*	21	79

Notes: Transitions are between size classes. Akaike information criterion for small sample sizes (AIC_c) weights for alternative models sum to 100. “NE” indicates transitions that were not estimable (these transitions occurred in only one or no plots). AIC_c weights in boldface type indicate models with significant density effects, as in Fig. 4. Significant density parameters indicate density dependence, and the sign of the term indicates the type of density.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

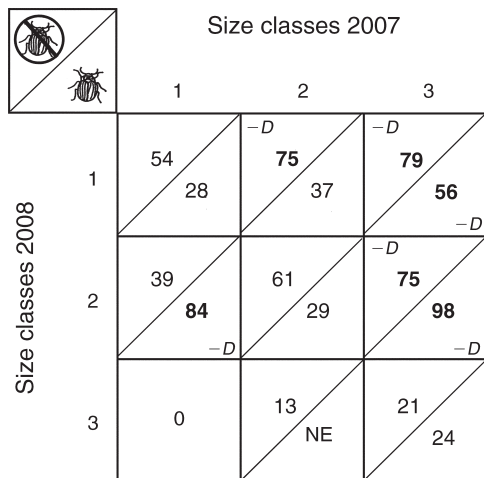


FIG. 4. Support for density dependence of transition rates for plots with herbivores (values below diagonal lines) and without herbivores (i.e., sprayed with insecticide; values above the diagonal lines). Values are Akaike information criterion adjusted for small sample sizes (AIC_c) weights for the best model containing density, and values in boldface type indicate significant density effects. Symbols in corners (D) indicate significant linear effects of density in the best-fit models. "NE" indicates that the transition was not estimable because it occurred in only one plot.

reproduction in seasons following damage. Determining how herbivores affect asexual reproduction is important for understanding whether herbivores will strongly affect plant populations because asexual reproduction can be an important means of spread for many plants, including many weedy or invasive species. It is important to note that in our study, spray treatments manipulated herbivores including florivores, but our data are on leaf damage only. Thus our damage estimates may underestimate total damage, and do not allow separation of effects of leaf vs. flower feeding insects.

Structural equation models showed that effects of damage and density on *S. carolinense* reproduction were almost entirely indirect and mediated by stem size (Figs. 1 and 3). Although few previous studies have explicitly measured the contribution of plant size to net effects of damage or density on plant reproduction, plant size likely often mediates these effects; damage and density are known to affect plant size, and plant size is known to be associated with reproduction, as we also observed. This result is consistent with many previous reports that larger plants produce more seeds, fruit or flowers (Herrera 1991, Aarssen and Taylor 1992; but for a contrary example with *S. carolinense*, see Elle 1999) and more clonal offspring (Buckley et al. 2003). Stem size was in turn strongly and negatively influenced by both herbivore damage and plant density (Fig. 1; Appendix B: Fig. B2), as one would expect based on previous work (Harper 1977, Louda 1984, Silvertown and Doust 1993). Over the ranges of density and damage in our study, the strengths of effects of damage and density on stem size

were about equal in 2007 but damage had a stronger influence than density in 2008 (c.f. Figs. 1 and 3). The stronger density effect in 2007 was not due to convergence of densities in 2008; in fact the range of densities was larger in 2008 than 2007. Instead, the stronger density effect in 2007 may have occurred because *S. carolinense* was nearly the only plant in the plots for a short time in 2007, creating a brief window during which *S. carolinense* was subject mostly to intraspecific competition, as opposed to both intra- and interspecific competition. Damage effects may have been larger in 2008 because damage was on average higher in 2008, and/or because by 2008 plants in no-spray plots had been subject to herbivores for two years rather than only one year.

While the effects of herbivores in our experiment were mostly indirect, in both years damage also had negative direct effects on fruit production (Fig. 1). This direct effect could arise because some of the major herbivores we observed damaging leaves also damaged reproductive parts (e.g., *Leptinotarsa juncta* and grasshoppers), thus stems with high leaf damage might also have had high flower or fruit damage. Stems with more damage may also receive fewer visits from pollinators and thus set fewer fruit; leaf damage reduces pollinator visits in some systems (e.g., Strauss 1997). If florivory (which we did not measure) was positively correlated with leaf damage, flower damage could also contribute to lower reproduction in unsprayed plots; one florivore that can substantially reduce *S. carolinense* fitness is the potato bud weevil, *Anthonomus nigrinus* (Wise et al. 2008). Finally, increased fruit abortion in response to leaf damage (Wise and Cummins 2006) could also contribute to direct effects of herbivores on sexual reproduction.

Interannual, nonlinear effects of density on damage

We found that stem density influenced herbivore damage (Fig. 2); in conjunction with the effects of damage on plant reproduction our results show the possibility of dynamic feedback between plant density and herbivore damage. Previous studies have found both positive (Turchin 1988, Karban 1993) and negative (Izhaki et al. 1996) effects of plant density on damage within a year, but have not extended across years, so the prevalence of cross-season effects is unknown. It is possible that our methods of sampling were not adequate to detect effects of density on damage within years; increased herbivore density in denser plant patches (Turchin 1988) might only manifest late in the season (when we did not sample) as insect populations build up. Unfortunately it was not possible to exhaustively sample herbivore populations without excessive disturbance to the plots. Alternatively, the effects of 2007 stem density on 2008 herbivore damage might be due to insects that overwinter in plots, or to cross-year effects on plant quality. For herbivores that may overwinter within plots (e.g., *Epitrix fuscula* and *Leptinotarsa juncta* both overwinter in the soil), larger densities built up by the end of one year could result in

larger initial populations and thus higher early season damage in plots with higher stem density. In fact, damage was on average higher across all plots in 2008 than in 2007. Interannual effects of density on damage might also be due to changes in plant quality or induced resistance with density (Karban et al. 1989) that only manifest in newly emerging stems. The cross-season effect we observed is unlikely to arise from effects of density on stem size as damage was not affected by stem size within 2007 or 2008.

We observed a nonlinear effect of density on damage, with the highest level of damage at intermediate densities (Fig. 2). Damage may initially increase with density because denser patches are easier for herbivores to find or remain in (Kareiva 1982); as plants become very dense, local herbivore populations may be too small to respond numerically to such high plant densities, or plant quality for herbivores may decrease (e.g., oviposition can decline with an increase in *S. carolinense* density; S. Halpern, unpublished data). The humped form of the relationship that we found might cause herbivores to slow plant population growth over lower plant densities, but fail to control plant populations as densities increase. Previous studies have generally examined only two or three plant densities, making assessment of the form of the relationship between plant density and herbivore damage impossible.

Conditional density dependence in transitions between size classes

To integrate the effects of density and damage on plant performance across plant sizes, we examined effects of herbivores on transition rates between three stem size classes. Although these transitions do not include sexual reproduction (data on seed viability and seedling performance are still being collected), transitions based on growth and survival are often more important to population growth than recruitment from seeds in perennial plants (Silvertown et al. 1993). Four transitions were density dependent under some conditions (see boldface values in Fig. 4 and Table 1), which reinforces our conclusion that density affects *S. carolinense* performance, but also indicates that density effects are more important for transitions between some size classes than others, suggesting that simply measuring overall density dependence may not give an accurate picture of how density influences these populations. Whether and how the magnitude and form of density dependence in individual transitions ultimately influences population dynamics will depend on the structure of the full demographic matrix (including sexual reproduction), and on the importance of each transition (measured as the elasticity or sensitivity of the matrix to each element). The few previous studies that have looked for it have found that transitions can be density dependent for plant populations (Silva Matos et al. 1999, Pardini et al. 2009), but very few plant demographic models include density dependence. Although for some applications including

density effects is not critical (Crone et al. 2011), density dependence in size transitions changes the long-term behavior of populations.

Importantly, we found that the density dependence of stem size transitions is influenced by herbivores (Fig. 4). When herbivores affect density dependence in size transitions, they will influence not just growth but also regulation of plant populations because regulation results from density dependence, and the form of density dependence will determine equilibrium population size (Turchin 2003). Previous studies that have shown that herbivores can influence plant demography (Ehrlén 1995, Shea and Kelly 1998, Fagan and Bishop 2000, Parker 2000, Horvitz and Schemske 2002, Rooney and Gross 2003, Knight 2004) have not looked specifically for effects of herbivores on density dependence in demographic transitions. Thus these studies thus cannot address whether herbivores contribute to regulation of plant populations, a critical question both for our understanding of the role of herbivores in natural systems and for our understanding of biocontrol and whether release from herbivores may contribute to the invasiveness of some plants (Maron and Vilà 2001, Halpern and Underwood 2006). Results of our study highlight the need for analysis of density dependent demographic models; we plan to explore such analyses once full demographic models are built for this system.

Summary

Our results show that herbivores influence density dependence in plant performance, and depress both sexual and asexual reproduction. Because we also found that plant density influences herbivore damage, there is likely to be feedback between these two factors; the net outcome of this feedback will require mathematical modeling to understand. For example, the fact that damage is highest at intermediate densities might suggest that herbivores may slow population growth over lower plant densities but fail to control plant populations as densities increase. However the fact that this effect of density on herbivores is coupled with effects of herbivores on density dependence in transition rates creates a type of feedback that is too complex to interpret intuitively. Although our results do not by themselves allow us to describe how herbivores will affect *Solanum* populations, they do suggest that effects of herbivores and density can be interdependent and should be considered jointly.

Many reviews have pointed out the absence of appropriate tests of effects of herbivores on plant population dynamics (Maron and Vilà 2001, Keane and Crawley 2002, Halpern and Underwood 2006, Maron and Crone 2006). In particular, previous tests of herbivore effects on plant population growth have mostly assumed density-independent plant population growth (Fagan and Bishop 2000, Parker 2000). While previous studies have documented separate effects of both herbivores and density dependence on plant

performance, none that we know of consider a sufficient range of densities to capture potential nonlinearities in density or herbivore effects, or examine how these effects might change with plant size. Our results show that herbivores may have complex effects on plant population density dependence; future tests for herbivore effects on plant populations should thus measure density effects when possible (Halpern and Underwood 2006, Maron and Crone 2006).

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SUPPLEMENTAL MATERIAL

Appendix A

Multiple regressions for effects of damage and density on reproduction (*Ecological Archives* E093-089-A1).

Appendix B

Figures showing the effect of insecticide treatment on damage and effects of damage and density on stem size (*Ecological Archives* E093-089-A2).