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Plant-mediated effects of host plant density on a specialist herbivore of *Solanum carolinense*

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Abstract. 1. Host plant density can affect insect herbivore oviposition behaviour, which can, in turn, affect both plant and herbivore populations. Because clear generalisations about density effects on oviposition remain elusive, a better understanding of underlying mechanisms is needed. One such mechanism is plant-mediated effects (i.e. changes in plant traits with density), which are often suggested but rarely isolated experimentally.

2. In an experiment directly manipulating host plant density (*Solanum carolinense*) in the field, oviposition by a specialist herbivore (*Leptinotarsa juncta*) declined as plant density increased.

3. A greenhouse cage experiment isolated the effects of plant-mediated traits by removing neighbour plants before introducing *L. juncta* females. Oviposition declined as host plant density increased, supporting the importance of plant-mediated effects in this system.

4. To determine whether food quality contributed to plant-mediated effects, larval growth rates were measured on leaves from both field- and greenhouse-grown plants. In both the field and the greenhouse experiments, larval growth rate was not significantly affected by plant density.

5. It is concluded that plant-mediated effects contribute to, but do not completely explain, the strong influence of density on oviposition in the field experiment. These results suggest that considering plant-mediated effects may help to explain variability in herbivore responses to plant density.

Key words. Bottom-up effects, density dependence, *Leptinotarsa juncta*, oviposition, plant–insect interactions, preference–performance hypothesis, resource concentration hypothesis.

Introduction

Determining how characteristics of host plant populations affect insect herbivore attack on plants is important both for our basic understanding of plant–herbivore interactions and for applications such as biological control. Host plant density is one characteristic known to influence herbivore loads on plants, but effects of host plant density vary (reviewed by Rhainds & English-Loeb, 2003) so that clear generalisations remain elusive (reviewed by Kareiva, 1983; Cook & Holt, 2006). The focus of this paper is on how host plant density influences oviposition. Because larvae often remain on their natal plant, oviposition has the potential to strongly influence the extent and distribution of herbivore damage to plants (e.g. McCauley, 1992). As a component of reproduction, oviposition also can affect insect populations and therefore subsequent damage. Oviposition behaviour can be affected by host plant density (Solomon, 1981; Rodriguez *et al.*, 2012 but see Horton & Capinera, 1987; zu Dohna, 2006), but studies have documented both fewer (Shea *et al.*, 2000) and more (Rhainds & English-Loeb, 2003) eggs laid per plant in high-density treatments. To improve our ability to predict patterns of oviposition, it is necessary to have a better understanding of the mechanisms that underlie changes in oviposition with host plant density.

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These mechanisms fall into two broad categories: plant density might directly influence herbivore loads on individual plants and thus oviposition patterns; or plant density might alter plant traits which then influence insect behaviour ('plant-mediated effects').

Plant density could directly influence oviposition in two ways. First, plant density can passively change oviposition even in the absence of an effect on herbivore behaviour. If immigration to a patch or oviposition rates (Rausher, 1983) are limited, then egg densities per plant will decrease with increasing plant density (a dilution effect, e.g. Otway *et al.*, 2005). Secondly, the resource concentration hypothesis (Root, 1973) predicts that density might affect oviposition through herbivore behavioural responses to plant spatial distribution; in this case the prediction is that increasing host plant density would reduce emigration from or increase immigration to patches, thereby increasing the local abundance of specialist herbivores and the total number of eggs they lay in the patch. Many studies have addressed this hypothesis, with varying findings (reviewed by Kareiva, 1983; Rhainds & English-Loeb, 2003); it is important to note, however, that studies often confound plant density with patch size or number of plants per patch. In addition to effects on immigration and emigration from patches of plants, density might also influence the number of eggs a female lays on a plant (e.g. Kunin, 1999; Shea *et al.*, 2000) if insects respond to inter-plant distance with changes in the propensity to leave or return to individual plants, or if plant density affects the probability that a female will rediscover and oviposit on the same plant repeatedly (Jones, 1977).

Many studies have focused on the influence of these direct effects, but changes in plant traits with plant density (i.e. plant-mediated effects) could also contribute to differences in oviposition with host plant density. Competition often affects plant characteristics that are important to herbivore behaviour and performance, such as size (reviewed by Bach, 1980; Kareiva, 1983), nutritional quality (reviewed by Kareiva, 1983) or induced responses to damage (Karban *et al.*, 1989; Karban, 1993; Cipollini & Bergelson, 2001; Agrawal, 2004). Density-induced changes in plant quality would be important for oviposition patterns when females preferentially lay eggs on plants that provide good food for their offspring (the preference–performance hypothesis, reviewed by Mayhew, 2001; Gripenberg *et al.*, 2010) and can move between areas with different host plant densities. The effects of density on plant quality may reinforce or counteract the spatial effects of plant density on oviposition. For example, induced resistance may be lower in plants growing at high density (Karban *et al.*, 1989; Karban, 1993; but see Kurashige & Agrawal, 2005), which could reinforce increases in eggs on plants at high density by reducing movement among plants (van Dam *et al.*, 2000), and thus emigration from the patch. Alternatively, reductions in plant nutritional quality due to competition could increase movement from plants (Bernays & Bright, 1991), leading to greater emigration rates from dense patches and thus fewer eggs on plants in the patch. Although the potential for plant-mediated effects to underlie patterns of herbivore and host plant density has been recognised repeatedly and for a long time (e.g. Kareiva, 1983; Kunin, 1999; Cook & Holt, 2006), they have rarely been isolated in experimental tests.

Here, we explore the effect of plant density on oviposition using the weedy perennial *Solanum carolinense* L. (Solanaceae) and a common specialist herbivore, *Leptinotarsa juncta* (Germar) (Coleoptera: Chrysomelidae). Our overarching hypothesis is that plant-mediated effects contribute to changes in oviposition with host plant density in this system. To address this hypothesis, we first document an effect of host plant density on oviposition in a field experiment. Secondly, we explicitly test the hypothesis that plant-mediated effects influence oviposition patterns by isolating plant characteristics in a greenhouse oviposition choice study. In this study, we predicted that *L. juncta* would lay fewer eggs on plants growing at low density, which are larger and may provide better-quality food for larvae. Finally, we test the hypothesis that the quality of plants as larval food (measured as larval growth rate) declines as host plant density increases in both the greenhouse and the field. If supported, these changes in food quality could underlie plant-mediated effects of density on oviposition.

Materials and methods

Study system

Solanum carolinense is a perennial herb that grows in disturbed habitats including pine-forest understorey, old fields and cultivated land, where it can be an important weed (Wehtje *et al.*, 1987; Nichols *et al.*, 1991). Reproduction occurs both sexually via flowers and asexually through stems off lateral roots (Hardin *et al.*, 1972; Miyazaki & Ito, 2004). *Solanum carolinense* is a host plant for more than 30 specialist and generalist insect herbivores (Imura, 2003; Wise, 2007), including the chrysomelid beetle *L. juncta* (Wise & Weinberg, 2002), which is a major herbivore in the southeastern U.S.A. (Wise, 2007; S. Halpern and N. Underwood, pers. obs.).

Leptinotarsa juncta specialises on host plants in the genus *Solanum* (Jacques & Fasulo, 2009); in northern Florida, they primarily feed on *S. carolinense*, with several generations occurring from early May through to late September (S. Halpern, pers. obs.). Both adults and larvae consume above-ground tissue, including leaves, stems, flowers and fruits (Wise & Weinberg, 2002; Wise, 2007); adults also consume eggs incidentally with leaf tissue (S. Halpern, pers. obs.). Clutches typically consist of five to 25 eggs (McCauley, 1992). Larval development lasts approximately 3 weeks and is often completed on a single stem (McCauley, 1992). Little is known about the effects of host plant density on *L. juncta* behaviour. There are some data about its congener *Leptinotarsa decimlineata* (Say), a major economic pest of Solanaceous crops. *Leptinotarsa decimlineata*'s colonisation of patches is affected by distance to another patch (Mena-Covarrubias *et al.*, 1996), but effects of crop density on *L. decimlineata* oviposition have not, to our knowledge, been examined.

Plants and *L. juncta* used in these experiments originated from field populations. For plants, source populations included several sites in northern Florida and a single location in Georgia. Collections occurred from agricultural fields, old

fields, roadsides, and disturbed areas in a managed pine forest. Plants were clonally propagated from root fragments and grown in the greenhouse in a 3 : 1 mixture of Fafard Professional 3 Mix (Conrad Fafard, Agawam, Massachusetts) and coarse sand. A laboratory colony of *L. juncta* was established from eggs and adults collected at various sites around Tallahassee, Florida. The colony was maintained in a growth chamber at 22–26 °C and LD 12 : 12 h. Larvae and adults ate potted plants of *S. carolinense*. Eggs were collected and allowed to hatch in Petri plates, and neonates were placed in larval cages until pupation. Pupae remained in the soil of pots containing larval food plants until eclosion.

Experiments

Experiment 1: Oviposition choice in the field. Oviposition in the field was observed in 20 plots that were part of a long-term demography experiment manipulating the density of *S. carolinense*, as described in Underwood and Halpern (2012). Briefly, clonally propagated and seedling *S. carolinense* plants were planted in plots in two spatial blocks at the North Florida Research & Education Center (Quincy, Forida). In each block, two plots were established at each of five densities (0.65, 2.8, 11.1, 22.7, and 30.9 plants m^{-2}, which was approximately 0.2–9× natural density at the site). To avoid completely confounding plot size and plant density, the two plots at each density in each block were of two different sizes (Table S1). Plots included 25 central plants (part of the long-term demography study) surrounded by a buffer of one to three rows of edge plants. Other species regrew in the plots after planting, but interspecific competitors were sparse during the first year (2007), when this study occurred.

Leptinotarsa juncta egg clutches were surveyed three times in summer 2007, on 21 June, 10 July, and 31 July. Randomly chosen buffer and central plants were searched for 20 min in each plot. For each plant observed, the number of *L. juncta* clutches and the number of eggs per clutch were recorded; clutch and egg numbers were then summed for each plot (the unit of replication). It was possible to count eggs clutches and to estimate clutch size even if eggs had hatched or been eaten, because distinctive egg fragments remained on leaves. Plants varied dramatically in size among plots (mean total stem length in plots ranged from 14 to 1288 cm), resulting in large differences in the number of plants observed per plot. To account for these differences, number of plants was included as a covariate in analyses; it was never significant. In a few cases in low-density plots, it was only possible to survey one (very large) plant in 20 min. In these cases, we observed two to three additional plants in the plot to avoid sampling just one plant; we reanalysed the data excluding these extra plants, and results did not differ, so here we present analyses of the full data set.

Egg and clutch count data were analysed with repeated-measures regression, using generalised linear models (PROC GENMOD in SAS 9.1, autoregressive correlation structure). Clutch number and egg number per plot were modelled (separately) as a function of the fixed effects of plant density in the plot (continuous), number of plants observed (continuous), mean plant size in the plot (continuous), and survey date (categorical); plot was treated as a subject to account for repeatedly surveying the same 20 plots. Because variances were larger than means for both clutch and total egg number, the negative binomial distribution was used. Significance of density and plant number were evaluated based on generalised score tests (SAS Institute, Inc., 2011); coefficients are also reported to show the direction of effects. Differences in mean clutch size were tested with analysis of covariance, using PROC GLM in SAS 9.3. Clutch sizes were averaged for each plot in each survey, and clutch size was modelled as a function of plant density and survey date. Untransformed data met model assumptions.

Experiment 2: Oviposition choice in the greenhouse (plant-mediated effects). To determine if density-induced changes in plant characteristics alone can influence oviposition in *L. juncta*, a greenhouse experiment was conducted with potted plants. Plants grew in a 3 : 1 mixture of Fafard Professional 3 Mix and coarse sand in 4-litre pots (diameter 27.5 cm). Replicate sets of four pots each were established. Each replicate set included one pot at each of four plant densities: a focal plant with 0, one, three, or seven neighbours (approximately 17, 34, 67, and 134 plants m^{-2}, overlapping the upper range of densities in the field experiment). Within each set, the focal plants were the same genotype, and neighbour plants were a haphazard mix of other genotypes. All focal plants were started from root fragments with a wet weight of 1.4–2.8 g. Two temporal blocks were planted, one in late March 2006 and one in late May 2006, and allowed to grow under natural light in the greenhouse for 2–3 months. In total, the experiment initially included 34 sets of plants ($n = 136$ plants in total).

Oviposition preference was tested in greenhouse choice tests in July and August 2006. Immediately before each trial, stems of all neighbour plants were clipped so that each pot presented a single stem of the focal plant only. The density treatment thus consisted of the number of neighbours the focal plant grew with before the trial, not the number of plants in the pot during the trial. Cut stems were covered with Vaseline to reduce emission of volatiles, and plastic wrap with a layer of soil on top was spread between pots to eliminate gaps (females often walk between plants; S. Halpern, pers. obs.). For a subset of plants ($n = 78$), leaf lengths on the focal plant were measured and summed as an estimate of plant size. Trials consisted of introducing a single adult female *L. juncta* into the centre of a cage (collapsible field cage 1451D, BioQuip, Rancho Dominguez, California) containing one replicate set of potted plants. Females were removed from cages after 24 h and the number of clutches and the number of eggs per clutch were counted. Different plants and different beetles were used in each trial. In each temporal block, trials occurred over 5 days.

Data were analysed using generalised linear models (GLM) in R (R Core Development Team, 2010), including only trials where females laid at least one egg (i.e. made an oviposition choice, which occurred in 23 of 34 trials). All

four responses (clutch number, total egg number, clutch size, and total leaf length) were modelled as functions of temporal block (categorical) and plant density (continuous). For count responses, the error distributions that best fitted the data were Poisson for clutch number and negative binomial for total egg number. Regression coefficients were estimated for density and temporal block using the appropriate model. For clutch number, we evaluated whether coefficients differed significantly from 0 using 95% CIs calculated from robust SE estimates (using the sandwich package in R) to account for slight deviations from Poisson model assumptions. Clutch size (number of eggs per clutch) and total leaf length were modelled with a Gaussian error distribution and the significance of predictors was evaluated with F tests.

Experiment 3: Larval growth on field-grown leaves (plant-mediated effects). To test whether intraspecific plant density in the field affected the quality of plants as hosts for *L. juncta* larvae, leaves were collected from plants in the field experiment (Experiment 1 in this paper; Underwood & Halpern 2012) and fed to *L. juncta* larvae in the laboratory. Leaves were collected from four buffer (non-central) plants in each of the 20 plots from Experiment 1 (four plots at each of five densities) in July 2007. One to three of the most recent fully expanded leaves were removed, providing enough tissue to feed one *L. juncta* larva for 3 days. Leaves were placed in plastic bags and stored in a cooler until returning to the laboratory.

Larval growth rate was measured using late first- or early second-instar *L. juncta* larvae from the laboratory colony. Initial individual larval weights were measured on a microbalance (AX105 DeltaRange, Mettler-Toledo, Inc., Columbus, Ohio) after starving larvae for approximately 1 h to clear their guts. Each larva was then placed in a 160 ml plastic cup (Solo Cup Company, Lake Forest, IL, USA) with a damp piece of filter paper and the leaves from one plant (total number of larvae = 80). The cups containing larvae and leaves were stored in the laboratory at room temperature under natural light. After 3 days, all living larvae were starved for 1 h and reweighed. Larval relative growth rate (RGR) was calculated as [ln(final weight) − ln(initial weight)]/number of days.

Leaf characteristics of each plant were measured from two 3-mm circles of leaf tissue removed from one leaf using a cork borer. Using a dissecting microscope at 40×, the number of trichomes was counted on each side of each leaf circle. Leaf samples were dried at 60 °C, weighed, and the specific leaf area (SLA) was calculated [SLA = leaf area (cm^2)/dry mass (g)].

Effects of plant density on larvae and leaf characteristics were tested using GLM with a Gaussian error distribution in R. Larval RGR, larval survival, trichome count, and SLA were modelled as functions of host plant density (continuous) in separate analyses. Because density treatments occurred at the plot level, plot means were used for each response in analyses. For RGR, larvae that died were excluded, and data were log-transformed to meet model assumptions. Significance was evaluated using F tests. Additionally, Pearson correlation coefficients were calculated between larval RGR and the leaf traits, SLA and trichome count.

Experiment 4: Larval growth rates on greenhouse-grown leaves (plant-mediated effects). Larval growth rate was used to test whether plants grown at different densities in the greenhouse varied in food quality for *L. juncta* larvae. Five plant genotypes were clonally replicated and grown either alone (low density) or with four neighbours of different genotypes (high density); we chose four neighbours to create a very high-density treatment (approximately 280 plants m^{-2}). Plants grew in 1.68-litre pots filled with 3 : 1 mixture of Fafard Professional 3 Mix and coarse sand for 5–8 weeks in autumn 2006 under supplemental lights for 12 h day^{-1}. The experiment ran in two temporal blocks, each containing all treatments and equal replication. In addition to the density treatment, a damage treatment was included to test whether damage induced changes in host plant quality, and whether induction differed for plants growing at different densities.

For the damage treatment, *L. juncta* larvae were enclosed on leaves using mesh bags. Larvae were allowed to consume one full leaf per plant (approximately 10% of total leaf area per plant) over a 1- to 2-day period; this damage level is similar to median damage levels we have measured in the field (8–16% leaf area removed, Underwood & Halpern, 2012). The damaged leaf was located two to four leaves from the top of the plant, leaving younger leaves to be used in feeding trials. A similarly located leaf was bagged on undamaged plants to control for handling. Leaves for feeding trials were removed with a clean razor blade. Leaves were fed to larvae 2 days after damage treatments ended. The experiment initially included two replicate plants per treatment combination (two temporal blocks × two damage treatments × two densities × five genotypes), but neighbours did not sprout in several pots in the high-density treatment. Thus, final sample sizes differed slightly for low-density ($n = 40$) and high-density ($n = 32$) treatments.

In these feeding trials, second- or third-instar larvae were placed individually in plastic cups with excised leaves and damp filter paper, as in Experiment 3. Initial larval weights were measured after starving larvae for approximately 1 h to clear their guts. Larvae fed for 2–3 days and were weighed again. Larval RGR was calculated, excluding any larvae that ran out of food before the end of the trial. Two cups per plant were set up (except for a few cases where plants were too small to supply food for two larvae) and mean larval growth rate of surviving larvae for that plant was used as our unit of observation.

To test for density effects on food quality as measured by larval RGR, data were analysed in R using GLM with a Gaussian error distribution. Larval RGR was modelled as a function of plant genotype, temporal block, plant density, damage treatment, and the damage × density interaction. All factors were treated as fixed and categorical, and F tests were used to evaluate significance. Untransformed data met the assumptions of the model. Analyses included larvae with negative RGR (i.e. weight loss) but not those that died, as RGR is not biologically meaningful if the individual dies during the trial; contingency tables were analysed to test for differences in survival among densities. After mortality, the two temporal blocks included 69 and 62 larvae on 38 and 34 plants, respectively.

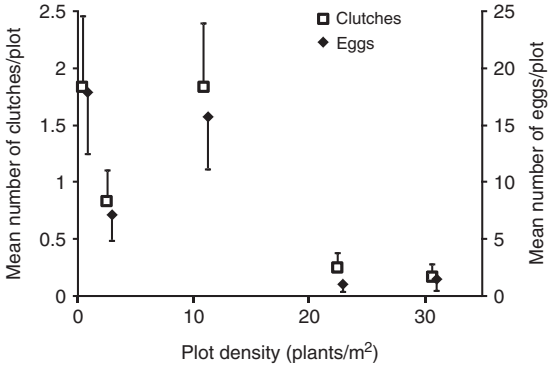

Fig. 1. Number of *Leptinotarsa juncta* clutches and eggs on *Solanum carolinense* plants grown at five different densities in field plots ($n = 20$). Each plot was surveyed three times in June and July 2007. Natural *S. carolinense* density nearby averaged 3.4 stems m^{-2}. Error bars $\pm$ 1 SE. Points are jiggered on x-axis to avoid overlap.

Results

Experiment 1: Field oviposition

In three surveys, *L. juncta* clutches were observed in plots 26 times (out of 60 visits) and on 40 individual plants (out of 463 total plants observed). Plots contained zero to seven clutches, each ranging from two to 24 eggs (mean = 9.1 eggs per clutch). Across the three surveys, one plant had six clutches, but most individual plants had one to two clutches. On average, plots with at least one clutch present had 1.5 clutches per plant surveyed.

Plant density affected both clutch number and total egg number observed in field plots. Clutch ($X^2 = 6.07$, d.f. = 1, $P = 0.014$; coefficient = -0.068 ± 0.02) and total egg number ($X^2 = 5.20$, d.f. = 2, $P = 0.02$; coefficient = -0.11 ± 0.02) declined as density increased, with much lower counts at the two highest densities than at the three lower densities (Fig. 1). Egg and clutch number were approximately 90% reduced in the highest density plots compared with the lowest density plots. Survey date influenced clutch number ($X^2 = 6.84$, d.f. = 2, $P = 0.03$) but not egg number ($X^2 = 2.02$, d.f. = 2, $P = 0.36$); the number of plants observed and mean plant size in the plot affected neither ($P > 0.06$). Mean clutch size did not vary with density ($F_{1,22} = 3.95$, $P = 0.06$) or among survey dates ($F_{2,22} = 1.23$, $P = 0.31$).

Experiment 2: Greenhouse oviposition

Females laid zero to three clutches for a total of 37 clutches in the 34 choice tests; in 12 cases, no clutches were laid on any plants in the cage, and these trials were excluded from further analysis. In the 22 trials where any eggs were laid, clutch size ranged from two to 26 eggs (mean = 11.1), and females laid a total of two to 39 eggs (mean = 14.7).

Density influenced clutch number (Table 1) but not mean clutch size ($F_{1,25} = 0.002$, $P = 0.97$) or total egg number (Table 1). Beetles laid more clutches on plants that had been grown at lower density (Table 1); for example, females laid twice as many clutches on plants grown alone as they did on plants grown with one neighbour (Fig. 2). Temporal block did not affect clutch number (Table 1), clutch size ($F_{1,25} = 1.17$, $P = 0.29$), or total egg number (Table 1). For the subset of plants where we measured plant size, density significantly affected plant size ($F_{3,77} = 23$, $P < 0.0001$, Fig. 2). Total leaf length declined approximately 37 cm with each additional neighbour.

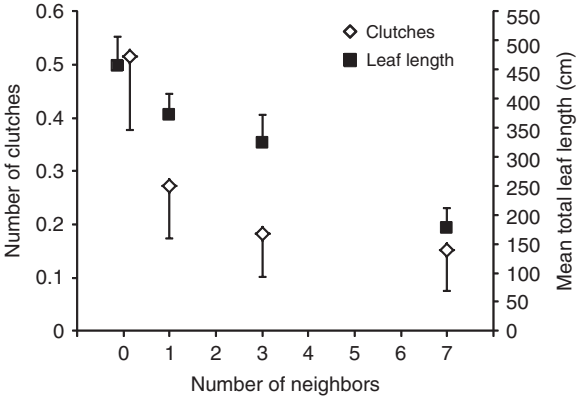

Fig. 2. Mean plant size (measured as total leaf length) and number of *Leptinotarsa juncta* clutches on *Solanum carolinense* plants grown at four different densities in the greenhouse. For clutch number, replicates ($n = 33$) consisted of single females caged for 48 h with one potted plant from each density treatment; neighbour plants were clipped prior to introducing the female to the cage, and replicates were excluded from analysis if the female did not lay any eggs. For leaf length, all leaf lengths were measured and summed for a subset of plants from each density ($n = 18$–20). Error bars $\pm$ 1 SE. Some points were jiggered on the x-axis to avoid overlap.

Table 1. Coefficients with 95% CIs from generalised linear models of the effects of density (0, one, three, or seven neighbours) and temporal block on clutch number and egg number in greenhouse oviposition choice tests. Analyses only included trials in which eggs were laid ($n = 23$).

	Clutch number*		Egg number†	
	Coefficient (95% CI‡)	*P*	Coefficient (95% CI)	*P*
Intercept	−0.27 (−0.85 to 0.31)	0.36	2.06 (1.38–2.74)	**<0.001**
Density	−0.18 (−0.36 to −0.005)	**0.04**	−0.15 (−0.35 to 0.06)	0.16
Temporal block	−0.06 (−0.85 to 0.72)	0.87	−0.21 (−1.23 to 0.81)	0.68

*Model fitted with Poisson distribution.
†Model fitted with negative binomial distribution.
‡Ninety-five per cent CIs were calculated using robust SEs to account for small deviations from Poisson assumptions.

Experiment 3: Larval growth on field-grown leaves

Of 80 larvae in the growth trial, 58 (72%) survived, with a mean growth rate of 0.41 mg mg^{-1} day^{-1} (range −0.04 to 0.69 for individual surviving larvae). Neither survival ($F_{1,17} = 0.38$, $P = 0.55$) nor larval RGR ($F_{1,17} = 0.10$, $P = 0.76$) differed significantly among densities. Leaves had a mean (range) density of 1108 (396–2100) trichomes cm^{-2} and a mean (range) SLA of 153 (60–283) cm^2 g^{-1}. Neither trichome number ($F_{1,17} = 2.52$, $P = 0.13$) nor SLA ($F_{1,17} = 0.06$, $P = 0.82$) varied among densities. There was no correlation between larval RGR and trichome number ($r = -0.1$, $P = 0.48$, $N = 57$) or SLA ($r = 0.16$, $P = 0.25$, $N = 56$).

Experiment 4: Larval growth on greenhouse-grown leaves

Overall mean larval RGR was 0.34 ± 0.02 g g^{-1} day^{-1}. Feeding on high-density plants reduced mean larval RGR by 13% compared with feeding on low-density plants (from 0.36 ± 0.02 to 0.31 ± 0.03 g g^{-1} day^{-1}), but this difference was not statistically significant ($F_{1,63} = 2.72$, $P = 0.10$). Larval RGR also was not affected by plant genotype ($F_{1,63} = 1.91$, $P = 0.12$), damage ($F_{1,63} = 0.34$, $P = 0.56$) or the damage × density interaction ($F_{1,63} = 0.25$, $P = 0.61$). Larval mortality was high in the first temporal block (29%) and low in the second temporal block (2%). Mortality did not differ for larvae eating high- versus low-density plants ($X^2 = 2.12$, d.f. $= 1$, $P = 0.15$).

Discussion

In these studies, we document both a negative effect of host plant density on oviposition in the field (Fig. 1) and, most importantly, clear plant-mediated effects of density on oviposition in the greenhouse (Fig. 2), suggesting that plant-mediated effects could contribute to oviposition patterns in the *S. carolinense–L. juncta* system. However, none of the specific plant traits that we tested clearly account for the plant-mediated effect that was observed. Plant-mediated effects of density on oviposition could be driven by several plant traits, including nutritional quality (reviewed by Kareiva, 1983), size (reviewed by Bach, 1980; Kareiva, 1983), and induced resistance (Karban *et al.*, 1989; Karban, 1993; Cipollini & Bergelson, 2001; Agrawal, 2004). In particular, density effects on plant quality as food for herbivores have been suggested as sources of variation in oviposition behaviour or herbivore densities (Horton & Capinera, 1987; Bach, 1988; Shea *et al.*, 2000; Rodriguez *et al.*, 2012). This study, however, found little evidence of effects of host plant density on food quality for *L. juncta* larvae, whether plants were grown in the field or in the greenhouse. Although lower larval growth rates have been observed for *L. juncta* feeding on *S. carolinense* grown at higher density in other studies (Kim, 2012), the absence of such effects in our studies indicates that, at a minimum, other plant-mediated effects of density contribute to *L. juncta* oviposition choices.

Plant size is a key plant-mediated effect that could influence selection of plants by ovipositing *L. juncta* females. Density reduced individual plant size in the greenhouse (Fig. 2) and in the field (Underwood & Halpern, 2012). Females may select larger plants because they are more likely to provide sufficient food for larvae to complete development. *Leptinotarsa juncta* larvae typically remain on their natal plant until pupation (McCauley, 1992) and larvae may starve if the female selects a small plant for oviposition. Plant size alone, however, is unlikely to drive the observed decline in oviposition with *S. carolinense* density, because mean plant size in a plot did not significantly affect either clutch number ($X^2 = 2.18$, $P = 0.14$) or egg number ($X^2 = 3.46$, $P = 0.06$) in the field experiment.

Another plant-mediated trait that could influence oviposition is induced resistance. We found no evidence for induction in the greenhouse, but *S. carolinense* can have induced responses to damage (Wise & Weinberg, 2002, McNutt and Underwood, unpublished), and plant density has been shown to influence induced responses to damage in this (Kim, 2012) and other systems (Karban *et al.*, 1989; Karban, 1993; Kurashige & Agrawal, 2005). We might not have observed induction in the greenhouse either because damage levels were too low or the time between damage and bioassays was too short. Induced resistance could have affected oviposition in the field, because damage varied (non-linearly) with plant density (Underwood & Halpern, 2012). Unfortunately, damage levels on the plants used for field feeding trials were not measured, so it was not possible to test for a correlation between prior damage and larval RGR. However, there can clearly be plant-mediated density effects that do not involve induction because plants in our greenhouse oviposition study did not experience damage prior to trials, so induced responses could not have influenced oviposition choice in that experiment.

While our greenhouse experiment shows plant-mediated effects of density on oviposition, and our field experiment confirms that real density effects occur in the field, our studies do not allow us to infer relative contributions of plant-mediated versus direct effects of density in the field. Direct effects of density could have contributed to oviposition patterns in the field in several ways. Higher densities could have diluted herbivore effects among individual plants (Otway *et al.*, 2005). Alternatively, host plant density could affect herbivore behaviour in ways that increase oviposition in low-density plots; for example, isolated plants at low density might receive more eggs (Jones, 1977; Solomon, 1981), because females may be less likely to leave or more likely to rediscover an individual plant in the absence of other nearby hosts. This study did not include formal observations of *L. juncta* behaviour, so these mechanisms remain untested possibilities to explain the oviposition patterns we observed in the field experiment.

Although patch size could have contributed to the patterns of oviposition observed in the field, our study suggests this effect does not account for all the observed effects of density. Most previous studies of plant density effects on herbivores completely confound patch size and plant number or density (but see Shea & Chesson, 2002; Rhainds & English-Loeb, 2003), and herbivore densities can be strongly influenced by changes in immigration or emigration rates with patch area and perimeter (reviewed by Kareiva, 1983; Hambäck & Englund, 2005). We were able to partially disentangle patch size and

host plant density because two patch sizes per density were planted in the field experiment (Table S1, details in Underwood & Halpern, 2012). Patch size still necessarily decreased with increasing plant density, so if females selected patches first (on a landscape scale), and then plants within patches, patch size could have contributed to the observed effect of density on oviposition in the field. Within each density, however, patch size had a significant effect only at high densities where patches were small (clutch number affected at only density $= 22.7$ plants m^{-2}, $X^2 = 4.16$, d.f. $= 1$, $P = 0.04$; egg number affected at both density $= 22.7$ plants m^{-2}, $X^2 = 16.6$, d.f. $= 1$, $P < 0.0001$ and density $= 30.9$ plants m^{-2}, $X^2 = 25$, d.f. $= 1$, $P < 0.0001$). In addition, density effects on oviposition remained important even in patch size ranges where patch size did not have an effect on oviposition. Moreover, patch size did not influence plant size or total damage levels in this field experiment (Underwood & Halpern, 2012). Thus, patch size is unlikely to be driving the observed relationship between density and oviposition.

The results of our studies are also relevant to the preference–performance hypothesis (PPH, reviewed by Thompson & Pellmyr, 1991; Mayhew, 1997; Gripenberg *et al.*, 2010). Under the PPH, females prefer host plants for oviposition on which their larvae will have the highest performance, thus enhancing the fitness of offspring (but see Mayhew, 2001). Based on a recent meta-analysis, there is general support for the PPH (Gripenberg *et al.*, 2010), including an example in our system (Wise & Weinberg, 2002). In our experiments, however, we observed an effect of density on oviposition preference, but not larval performance. Disconnections between larval performance and oviposition preference are not uncommon (e.g. Facknath, 2005; Brodbeck *et al.*, 2007; Eben & Lopez-Carretero, 2008) and may occur, among other reasons, if females choose plants that increase their survivorship or fecundity rather than ones that improve individual larval performance (e.g. Scheirs *et al.*, 2000; Brodbeck *et al.*, 2007). An important caveat to interpreting our results in a PPH framework is that we measured only a small component of larval performance, growth rates over a couple days in enemy-free space in the laboratory. Components of larval fitness (such as development time, pupal weight, and survivorship) are sometimes uncorrelated (e.g. Mayhew, 1998), so we might have detected effects of host plant density on one or more of these later measures of fitness. It is also possible that small differences in larval growth rate (such as the non-significant trend detected in the greenhouse experiment) could affect susceptibility to natural enemies, which have been observed to cause substantial mortality in the congener *L. decimlineata* (Mena-Covarrubias *et al.*, 1996).

Conclusions

Host plant density in the field strongly influenced oviposition by a specialist herbivore in this system. The greenhouse oviposition study indicated that plant-mediated effects could contribute to density effects on oviposition, although the specific traits involved are not yet clear. It is possible that density alters plant traits such that the suite of changes affects oviposition, even if each trait alone does not have a statistically significant effect. This could occur if changes in plant traits with density reinforce each other or act synergistically on oviposition; for example, as plant density increased, both plant size and plant quality (measured in larval bioassays) decreased. Explicitly testing this possibility would require a factorial experiment that independently manipulated each trait. More broadly, additional studies that can fully disentangle direct and plant-mediated effects will be important for moving beyond the current lack of consensus about how host plant density influences herbivore populations (reviewed by Cook & Holt, 2006).

More generally, density effects on oviposition are important because they could be a mechanism by which herbivores influence density dependence in the host plant population. We have found that herbivores change density dependence in some of *S. carolinense*'s demographic transitions (Underwood & Halpern, 2012). Because larvae typically complete their development on the stem where they were laid (McCauley, 1992) and can completely defoliate a stem (S. Halpern, pers. obs.), higher levels of oviposition in low-density populations, resulting in more feeding damage from larvae, could reduce the benefits to the plant of growing with fewer competitors. A similar process seems to occur in another early successional species, *Lupinus lepidus* Douglas ex. Lindl. var. *lobbii*, where more herbivores (and higher damage levels) had greater effects on population growth of low-density edge populations than high-density core populations (Bishop, 2002). How extensively such density effects occur will determine how often herbivores generally contribute to host plant population regulation and plant invasions (reviewed by Maron & Vilá, 2001; Halpern & Underwood, 2006; Maron & Crone, 2006).

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12088

Table S1. Size of experimental plots (= patch sizes) at each density in the field experiment.

References

Agrawal, A.A. (2004) Resistance and susceptibility of milkweed: competition, root herbivory, and plant genetic variation. *Ecology*, **85**, 2118–2133.

Bach, C.E. (1980) Effects of plant density and diversity on the population dynamics of a specialist herbivore, the striped cucumber beetle, *Acalymma vittata* (Fab). *Ecology*, **61**, 1515–1530.

Bach, C.E. (1988) Effects of host plant patch size on herbivore density: patterns. *Ecology*, **69**, 1090–1103.

Bernays, E.A. & Bright, K.L. (1991) Dietary mixing in grasshoppers: switching induced by nutritional imbalances in foods. *Entomologia Experimentalis et Applicata*, **61**, 247–253.

Bishop, J.G. (2002) Early primary succession on Mount St. Helens: impact of insect herbivores on colonizing lupines. *Ecology*, **83**, 191–202.

Brodbeck, B.V., Andersen, P.C., Oden, S. & Mizell, R.F. III (2007) Preference-performance linkage of the xylem feeding leafhopper, Homalodisca vitripennis (Hemiptera cicadellidae). *Environmental Entomology*, **36**, 1512–1522.

Cipollini, D.F. & Bergelson, J. (2001) Plant density and nutrient availability constrain constitutive and wound-induced expression of trypsin inhibitors in Brassica napus. *Journal of Chemical Ecology*, **27**, 593–610.

Cook, W.M. & Holt, R.D. (2006) Influence of multiple factors on insect colonization of heterogeneous landscapes: a review and case study with periodical cicadas (Homoptera: Cicadidae). *Annals of the Entomological Society of America*, **99**, 809–820.

van Dam, N.M., Hadwich, K. & Baldwin, I.T. (2000) Induced responses in *Nicotiana attenuata* affect behavior and growth of the specialist herbivore *Manduca sexta*. *Oecologia*, **122**, 371–379.

zu Dohna, H. (2006) The distribution of eggs per host in a herbivorous insect – intersection of oviposition, dispersal and population dynamics. *Journal of Animal Ecology*, **75**, 387–398.

Eben, A. & Lopez-Carretero, A. (2008) Asymmetry of larval diet breadth and oviposition preference in *Leptinotarsa undecimlineata*. *Entomologia Experimentalis et Applicata*, **128**, 27–33.

Facknath, S. (2005) Leaf age and life history variables of a leafminer: the case of *Liriomyza trifolii* on potato leaves. *Entomologia Experimentalis et Applicata*, **115**, 79–87.

Gripenberg, S., Mayhew, P.J., Parnel, M. & Roslin, T. (2010) A meta-analysis of preference–performance relationships in phytophagous insects. *Ecology Letters*, **13**, 383–393.

Halpern, S.L. & Underwood, N. (2006) Approaches for testing herbivore effects on plant population dynamics. *Journal of Applied Ecology*, **43**, 922–929.

Hambäck, P.A. & Englund, G. (2005) Patch area, population density and the scaling of migration rates: the resource concentration hypothesis revisted. *Ecology Letters*, **8**, 1057–1065.

Hardin, J.W., Doerksen, G., Herndon, H., Hobson, M. & Thomas, F. (1972) Pollination ecology and floral biology of four weedy genera in southern Oklahoma. *Southwestern Naturalist*, **13**, 185–202.

Horton, D.R. & Capinera, J.L. (1987) Effects of plant diversity, host density, and host size on the population ecology of the Colorado potato beetle (Coleoptera: Chrysomelidae). *Environmental Entomology*, **16**, 1019–1026.

Imura, O. (2003) Herbivorous arthropod community of an alien weed *Solanum carolinense* L. *Applied Entomology and Zoology*, **38**, 293–300.

Jacques, R.L. & Fasulo, T.R. (2009) *The Potato Beetles of Florida* [WWW document]. URL http://entnemdept.ifas.ufl.edu/creatures/veg/leaf/potato_beetles.htm [accessed on 2 December 2013].

Jones, R.E. (1977) Movement patterns and egg distribution in cabbage butterflies. *Journal of Animal Ecology*, **46**, 195–212.

Karban, R. (1993) Induced resistance and plant density of a native shrub, *Gossypium thurberi*, affect its herbivores. *Ecology*, **74**, 1–8.

Karban, R., Brody, A.K. & Schnathorst, W.C. (1989) Crowding and a plant's ability to defend itself against herbivores and diseases. *American Naturalist*, **134**, 749–760.

Kareiva, P. (1983) Influence of vegetation texture on herbivore populations: resource concentration and herbivore movement. *Variable Plants and Herbivores in Natural and Managed Systems* (ed. by R. F. Denno and M. S. McClure), pp. 259–289. Academic Press, New York, New York.

Kim, T.N. (2012) *Community-level consequences of plant-herbivore interactions*. PhD, Florida State University, Tallahassee.

Kunin, W.E. (1999) Patterns of herbivore incidence on experimental arrays and field populations of ragwort, *Senecio jacobaea*. *Oikos*, **84**, 515–525.

Kurashige, N.S. & Agrawal, A.A. (2005) Phenotypic plasticity to light competition and herbivory in *Chenopodium album* (Chenopodiaceae). *American Journal of Botany*, **92**, 21–26.

Maron, J.L. & Crone, E. (2006) Herbivory: effects on plant abundance, distribution, and population growth. *Proceedings of the Royal Society of London*, **273**, 2575–2584.

Maron, J.L. & Vilá, M. (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos*, **95**, 361–373.

Mayhew, P.J. (1997) Adaptive patterns of host-plant selection by phytophagous insects. *Oikos*, **79**, 417–428.

Mayhew, P.J. (1998) Testing the preference-performance hypothesis in phytophagous insects: lessons from chrysanthemum leafminer (Diptera: Agromyzidae). *Environmental Entomology*, **27**, 45–52.

Mayhew, P.J. (2001) Herbivore host choice and optimal bad motherhood. *Trends in Ecology & Evolution*, **16**, 165–167.

McCauley, D.E. (1992) Family structured patterns of mortality in the false Colorado potato beetle. *Ecological Entomology*, **17**, 142–148.

Mena-Covarrubias, J., Drummond, F.A. & Haynes, D.L. (1996) Population dynamics of the Colorado potato beetle (Coleoptera: Chrysomelidae) on horsenettle in Michigan. *Environmental Entomology*, **25**, 68–77.

Miyazaki, K. & Ito, M. (2004) Root system structure and shoot arrangement of 1-year-old *Solanum carolinense* L. *Weed Biology and Management*, **4**, 122–125.

Nichols, R.L., Cardina, J. & Gaines, T.P. (1991) Growth, reproduction and chemical composition of horsenettle (*Solanum carolinense*). *Weed Technology*, **5**, 513–520.

Otway, S.J., Hector, A. & Lawton, J.H. (2005) Resource dilution effects on specialist insect herbivores in a grassland biodiversity experiment. *Journal of Animal Ecology*, **74**, 234–240.

Rausher, M.D. (1983) Alteration of oviposition behavior by *Battus philenor* butterflies in response to variation in host-plant density. *Ecology*, **64**, 1028–1034.

R Core Development Team (2010) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rhainds, M. & English-Loeb, G. (2003) Testing the resource concentration hypothesis with tarnished plant bug on strawberry: density of hosts and patch size influence the interaction between abundance of nymphs and incidence of damage. *Ecological Entomology*, **28**, 348–358.

Rodriguez, E., Peco, B. & Gurrea, M.P. (2012) Effect of Scotch broom, *Cytisus scoparius*, pod size and patch density on *Exapion fuscirostre* (Coleoptera, Apionidae) seed weevil oviposition. *Australian Journal of Entomology*, **51**, 127–132.

Root, R.B. (1973) Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs*, **43**, 95–123.

SAS Institute, Inc (2011) *SAS/STAT 9.3 Users Guide, Version 9.3*. SAS Institute, Inc.

Scheirs, J., de Bruyn, L. & Verhagen, R. (2000) Optimization of adult performance determines host choice in a grass miner. *Proceedings of the Royal Society of London - Series B: Biological Sciences*, **267**, 2065–2069.

Shea, K. & Chesson, P. (2002) Community ecology theory as a famework for biological invasions. *Trends in Ecology & Evolution*, **17**, 170–176.

Shea, K., Smyth, M., Sheppard, A., Morton, R. & Chalimbaud, J. (2000) Effect of patch size and plant density of Paterson's curse (*Echium plantagineum*) on the oviposition of a specialist weevil, *Mogulones larvatus*. *Oecologia*, **124**, 615–621.

Solomon, B.P. (1981) Response of a host-specific herbivore to resource density, relative abundance, and phenology. *Ecology*, **62**, 1205–1214.

Thompson, J.N. & Pellmyr, O. (1991) Evolution of oviposition behavior and host preference in Lepidoptera. *Annual Review of Entomology*, **36**, 65–89.

Underwood, N. & Halpern, S.L. (2012) Insect herbivores, density dependence, and the performance of the perennial herb *Solanum carolinense*. *Ecology*, **93**, 1026–1035.

Wehtje, G., Wilcut, J.W., Hicks, T.V. & Sims, G.R. (1987) Reproductive biology and control of *Solanum dimidiatum* and *Solanum carolinense*. *Weed Science*, **35**, 356–359.

Wise, M.J. (2007) The herbivores of *Solanum carolinense* (Horsenettle) in northern Virginia: natural history and damage assessment. *Southeastern Naturalist*, **6**, 505–522.

Wise, M.J. & Weinberg, A.M. (2002) Prior flea beetle herbivory affects oviposition preference and larval performance of a potato beetle on their shared host plant. *Ecological Entomology*, **27**, 115–122.

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