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Attenuation of the Jasmonate Burst, Plant Defensive Traits, and Resistance to Specialist Monarch Caterpillars on Shaded Common Milkweed (*Asclepias syriaca*)

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Abstract Plant responses to herbivory and light competition are often in opposing directions, posing a potential conflict for plants experiencing both stresses. For sun-adapted species, growing in shade typically makes plants more constitutively susceptible to herbivores via reduced structural and chemical resistance traits. Nonetheless, the impact of light environment on induced resistance has been less well-studied, especially in field experiments that link physiological mechanisms to ecological outcomes. Accordingly, we studied induced resistance of common milkweed (*Asclepias syriaca,* a sun-adapted plant), and linked hormonal responses, resistance traits, and performance of specialist monarch caterpillars (*Danaus plexippus*) in varying light environments. In natural populations, plants growing under forest-edge shade showed reduced levels of resistance traits (lower leaf toughness, cardenolides, and trichomes) and enhanced light-capture traits (higher specific leaf area, larger leaves, and lower carbon-to-nitrogen ratio) compared to paired plants in full sun. In a field experiment repeated over two years, only milkweeds growing in full sun exhibited induced resistance to monarchs, whereas plants growing in shade were constitutively more susceptible and did not induce resistance. In a more controlled field experiment, plant hormones were higher in the sun (jasmonic acid, salicylic acid, abscisic acid, indole acidic acid) and were induced by herbivory (jasmonic acid and abscisic acid). In particular, the jasmonate burst following herbivory was halved in plants raised in shaded habitats, and this correspondingly reduced latex induction (but not cardenolide induction). Thus, we provide a mechanistic basis for the attenuation of induced plant resistance in low resource environments. Additionally, there appears to be specificity in these interactions, with light-mediated impacts on jasmonate-induction being stronger for latex exudation than cardenolides.

Keywords Cardenolide · Herbivory · Latex · Monarch butterfly · Plant defense · Plant-insect interactions · Shade-avoidance response · Signal cross-talk · Specialist herbivore

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Introduction

Because plant traits that maximize light capture and reduce herbivory are almost universally phenotypically plastic (i.e., induced by low light and herbivory, respectively), both have been the subject of intensive study as forms of adaptive plasticity (Dudley and Schmitt, 1996; Agrawal, 1998; Callaway et al., 2003; Auld et al., 2010; Salgado-Luarte and Gianoli, 2011). A relatively consistent aspect of these responses in sun-adapted species is that plasticity to shade causes greater susceptibility to herbivores, and conversely, induced responses to herbivory often make plants less competitive (Dudt and Shure, 1994; Jansen and Stamp, 1997; Kurashige and Agrawal, 2005; Van Dam and Baldwin, 2001). In their simplest form, such tradeoffs are driven by the dual impact of particular traits. For example, shaded leaves typically have reduced trichome densities and higher nitrogen content (Morgan and Smith, 1981; Rozendaal et al., 2006), which are responses thought to reduce self-shading and increase allocation to RuBisCO, respectively. These same trait changes are predicted to make plants more palatable for herbivores (Agrawal and Fishbein, 2006). Similarly, when plants exhibit induced resistance to herbivores,

allocation to resistance traits is often associated with plants having a reduced ability to capture resources (Van Dam and Baldwin, 2001; Salgado-Luarte and Gianoli, 2011). In such cases, resource limitation often has been invoked to explain the tradeoff.

Although most early models of plant responses to light availability and herbivory were based on the concept of resource allocation (Herms and Mattson, 1992; Dudt and Shure, 1994), more recent mechanistic approaches have sought to address the biochemical basis of interactions between these plant responses (Table 1, Cipollini, 2004; Roberts and Paul, 2006). Advances have come, in part, because of the recognition that plastic signaling pathways in plants may interact in multiple ways (i.e., at the level of precursors, receptors, hormones, etc.). In particular, it has been suggested that the inducibility of either response (to light or herbivores) may be impaired by the activation of the other. For example, a recent study found that the light environment impacted both the biosynthesis of, and plant responses to, jasmonates, which are critical hormones involved in induced responses to herbivory (Radhika et al., 2010). Nonetheless, much of the initial mechanistic work on the interaction between light availability and induced responses to herbivory was conducted in highly controlled environments. Thus, an important next step is to conduct field studies, which typically allow for more realistic light levels, natural patterns of insect attack, and involvement of other natural stressors. Although several field studies have emerged recently, (Boege, 2010; Salgado-Luarte and Gianoli, 2011), they have yet to take a mechanistic approach linking plant hormonal regulation with ecological outcomes.

Table 1 Progress points over the last decade in understanding how a plant's light environment impacts induced resistance to herbivores

Study	Key Finding
Cipollini, 2004	Summarized the conceptual motivation and biochemical basis for why plant responses to shade and herbivory may interact
Kurashige and Agrawal, 2005	Directly found some evidence for reciprocal interactions between induced plant resistance to herbivory and the shade-avoidance response in *Chenopodium album*
Izaguirre et al., 2006	Showed that exposure of a wild tobacco (*Nicotiana longiflora*) to reflected far-red light impaired resistance to herbivores and some induced phenolic compounds. This effect was likely mediated by phytochrome B.
Mooney et al., 2009	A shade-adapted woody shrub (*Lindera benzoin*) showed stronger induction of peroxidase and resistance to caterpillars in shaded compared to sun leaves.
Moreno et al., 2009	Using *Arabidopsis* mutants, convincingly showed that attenuated induced responses in the shade are mediated by phytochrome and reduced sensitivity to jasmonates.
Radhika et al., 2010	Demonstrated that shade conditions reduce biosynthesis and responses to jasmonates, both of which modulate herbivore-induced extrafloral nectar in lima bean (*Phaseolus lunatus*).

Accordingly, our goal was to assess natural patterns of plant responses to light competition and herbivory, and then to experimentally assess the mechanistic basis of any potential conflict between these responses. We have been studying defense and competition in native common milkweed (*Asclepias syriaca* L), which typically occurs in full sun (open fields), although it also is found on forest edges, where it has a typical shade phenotype (Agrawal and Van Zandt, 2003; Agrawal, 2004; Mooney et al., 2008; Bingham and Agrawal, 2010). Shaded milkweeds appear to have larger, darker green, and floppy leaves compared to plants growing in the sun. Here, we addressed the impact of shade on the well-characterized induced responses to herbivory in milkweed. Specifically, we tested the following hypotheses: 1) Plants naturally growing in the shade will have lower levels of resistance traits and enhanced light capture traits compared to paired plants growing in full sun; 2) induced resistance to specialist monarch caterpillars will be impaired in naturally shaded plants; and 3) foliar hormones and resistance traits (i.e., cardenolides, latex) will show attenuated induction following herbivory in shaded compared to non-shaded plants.

Methods and Materials

Study System Asclepias syriaca is a native perennial plant that occurs throughout eastern North America, typically in open habitats but frequently extending to forest edges. When growing in competition, *A. syriaca* exhibits a shade-avoidance response, including stem elongation (Agrawal and Van Zandt, 2003). It reproduces both clonally and sexually, and is largely self-incompatible (Kephart, 1981). *Asclepias syriaca* employs a variety of heritable defense traits, including the production of toxic cardenolides, gummy latex, and non-glandular leaf trichomes (Agrawal, 2005). Cardenolide concentrations and latex exudation are both inducible following herbivory (Van Zandt and

Agrawal, 2004; Mooney et al., 2008; Bingham and Agrawal, 2010), the jasmonate pathway is involved in this induction (Rasmann et al., 2009; Agrawal, 2011), and specialist herbivores including monarch butterfly caterpillars (*Danaus plexippus*) are negatively impacted by induction (Van Zandt and Agrawal, 2004).

Natural Population Surveys To characterize the phenotypic differences between *A. syriaca* naturally growing in the sun and the shade that might be relevant to plant-herbivore interactions, we conducted a survey of natural populations. In July 2009, across four field sites we identified 24 pairs of naturally occurring mature *A. syriaca* plants in Tompkins County, NY (USA), with one individual occurring under natural shade at the forest edge and the other occurring in full sun in an open field (plants were typically≤10 m apart). Forests were mature, eastern deciduous forests (primary or secondary growth) next to open old-fields. We employed a paired design to minimize potential microclimatic, soil, and genetic differences. Although we have no information indicating that stems in the same pair were part of the same genet, this is certainly possible. Nonetheless, because microclimatic, soil, and genetic differences were not of direct interest in this study, all were considered part of the blocking factor (pair). Care was taken to include pairs where the shaded plant was facing each of the four compass directions. On average, the intensity of peak photosynthetically active radiation (PAR, 400-700 nm) was reduced by 91 % in the shade (mean±SE μmol/m^2/s, sun: 1784, shade: 168, $F_{1,23}$= 673, P< 0.001).

Using standardized methods detailed elsewhere (Agrawal, 2005), we measured leaf number, leaf size, leaf toughness, specific leaf area (SLA, area/dry mass), water content, trichome density, foliar carbon-to-nitrogen ratio, plant height, and latex exudation. Cardenolide concentrations were assessed by HPLC, following Bingham and Agrawal (2010). Briefly, 50 mg dried leaf tissue from each plant were ground to a fine powder and extracted with 1.8 ml methanol (MeOH), spiked them with 20 μg digitoxin as an internal standard, and sonicated for 20 min at 55°C in a water bath. After centrifugation, the supernatant was collected, dried, resuspended in 1 ml MeOH, and filtered through a 0.45 μm syringe driven filter unit. Fifteen μl of extract were then injected into an Agilent 1100 series HPLC, and compounds were separated on a Gemini C18 reversed phase column (3 μm, 150 x 4.6 mm, Phenomenex, Torrance, CA, USA). Cardenolides were eluted on a constant flow of 0.7 ml/min with an acetronile-0.25 % phosphoric acid in water gradient as follows: 0-5 min 20 % acetonitrile; 20 min 70 % acetonitrile; 20-25 min 70 % acetonitrile; 30 min 95 % acetonitrile; 30-35 min 95 % acetonitrile. UV absorbance spectra were recorded from 200 to 400 nm by diode array detector. Peaks with absorption maxima between 217 and 222 nm were recorded as cardenolides and quantified at 218 nm. Concentrations were calculated and standardized by peak areas of the known digitoxin concentration.

All measures were taken from the youngest fully expanded leaves (avoiding severely damaged leaves); total N=48. We measured internode length as the distance between the youngest fully expanded pair of leaves and the node below. Finally, we also recorded the number of aphids (*Myzocallis asclepiadis*) found on each plant, and estimated leaf herbivory as the fraction of the total number of leaves with clearly identifiable chewing damage (typically imposed by *D. plexippus* or *Tetraopes tetraophthalmus*). Analyses were conducted with two-way analysis of variance (with pair and light environment as the main effects). We first conducted a MANOVA, to assess effects across our 13 response variables, followed by univariate analyses.

Induced Resistance in Natural Populations In July of 2010 and 2011, we visited the same natural populations as above and selected clusters of four mature plants (two in the sun and two in the shade) to test for induced resistance. Again, these clusters were selected (plants≤10 m apart) as a blocking factor to minimize microclimatic, soil, and genetic differences. Plants with minimal natural herbivory were selected, and then the top portion of plants (8 leaf pairs in 2010, 4 pairs in 2011) was enclosed in a spun polyester sleeve. Our bagging treatment did not impact leaf temperatures ($F_{1,176}$=0.003, P=0.955), although shading reduced temperature by 18 % (mean sun temperature °C: 24.8 +/- 0.3; in the shade: 20.4 +/- 0.3, $F_{1,79}$=135.0, P< 0.001).

Plants in each light environment were assigned randomly to be either damaged (<5 % herbivory imposed by early instar *D. plexippus* larvae) or left as controls. Damage was controlled so as not to be different between the two light environments. After 3-5 d of feeding, the damaging caterpillars were removed, and pre-weighed bioassay caterpillars were introduced to all plants to assess their growth. In 2010, the bioassay consisted of two 1st instar caterpillars (20 plant clusters), whereas in 2011 we introduced a single 2nd instar caterpillar (25 plant clusters). We changed the procedure in 2011 to reduce mortality of the bioassay caterpillars (mortality was 18 % and 7 % in 2010 and 2011, respectively). We employed ANOVA to assess the impacts of shade, induction, shade-by-induction interaction, and block on the relative growth rate (final mass minus initial mass divided by initial mass) of the surviving caterpillars (total N=178). Block was each group of four plants, with one replicate in each treatment combination; in this way, we combined spatial variation (microclimatic, soil, and genetic differences) and variation across years in one blocking term. In this analysis, no interaction term between block and the other main effects is possible because a single replicate of each of the four treatment combinations is in each block. Mortality

was essentially random in 2010 and minimal in 2011. For the 2010 data, if two caterpillars were collected, their mass was averaged to produce a single datum. We took two approaches to assess differential induced resistance to caterpillars in the sun and shade: we first examined the statistical interaction between the shade and damage treatments, and second, we independently conducted analyses of the impact of damage treatment on monarch performance in the sun and shade environment.

Common Garden Study In a more controlled set of experiments, we exposed milkweed plants to natural sun and shade environments, moved them to a common "neutral" environment (see below), and then assessed traits associated with shade avoidance and induced resistance. In particular, our goal was to link plant hormonal responses to herbivory and the induction of defense traits in plants that had been grown in different light environment histories, but were assayed in a common environment. In 2009, we grew 10 full sibling families of *A. syriaca* (from a local natural population, all from a full sun habitat), randomized in a growth chamber in 500 ml pots filled with potting mix (mean of 15 plants per family, total N=149). Genetic families were used simply to control for variation. After 2 mo of growth, in October, the plants were moved out of doors, hardened in a lath house, clipped, and mulched to overwinter. In May 2010, we uncovered the plants and transplanted them into 4 l pots containing a 1:1:1 mixture of topsoil, compost, and sand. These second year plants were not fully mature (i.e., did not flower in 2010). Half of the plants from each family were randomized in a block under the canopy of a forest edge and the rest were placed 5 m away in a randomized block in the full sun. Both plots were fenced, and plants were watered as needed. This site was one of our original paired sun-shade sites and exhibited a 94 % reduction in PAR under the canopy. Our main goal in using this design was to have plants emerging from perennial root stocks in the contrasting light environments from the beginning of the growing season.

Three weeks after emergence, we non-destructively measured the number of stems in each pot (because milkweeds are clonal, some pots had several stems), the height of the tallest stem, and internode length (2 nodes below the apex) on all plants. All plants then were moved and fully randomized in a common neutral enclosure. Pots were spaced at least 5 cm apart and had no leaf overlap or light competition. This enclosure made of Lumite insect screen fabric (Baldwin, GA, USA) reduced ambient light by≈50 %, and importantly served as a neutral filter (i.e., did not impose reflected far red light, as is the case under the canopy of leaves). We used this enclosure to provide intermediate (neutral) light levels between the two extremes experienced during the early part of the growing season. Tall, leaning plants were staked as needed. On the same day, we introduced a single freshly hatched monarch caterpillar to the apex of half of the plants, both to initiate induced responses to herbivory and to conduct a bioassay of performance on plants with different light environment histories. Critically, the bioassay was conducted in a common environment (neutral enclosure) where the plants had been fully randomized. After 4 d of feeding (<5 % damage), we measured latex exudation and destructively harvested the apical tissue (usually 2 pairs of leaves, 600-800 mg fresh mass) for measures of hormones (jasmonic acid (JA), salicylic acid, abscisic acid, indole acidic acid) and cardenolides (N=149). Nearly all of the monarch herbivory was on apical leaves, and thus all samples for chemistry contained local damage.

In addition to total HPLC-determined cardenolides (as above), we analyzed data individually for the five major cardenolide peaks (see Table 2b). We include one early, highly polar peak (eluting at 3.45 min) that was likely a cardenolide (given its absorbance spectra), but co-eluted or attached to a phenolic compound (again, based on the absorbance spectra). Hormones were quantified by using an established liquid chromatography – mass spectrometry procedure, modified from Thaler et al. (2010). Briefly, frozen samples were transferred into 2-ml screw cap tubes containing 900 mg zirconia/silica beads (BioSpec, Bartelsville, OK, USA) and 1 ml extraction buffer. d_4-SA, d_5-JA, d_6-ABA, d_5-IAA (CDN isotopes, Point-Claire, Canada) were added as internal standards, and samples were homogenized in a FastPrep homogenizer (MP Biomedicals, Solon, OH, USA) at 6 m/s for 45 s. Samples were dissolved in 200 μl methanol after extraction with dichloromethane and solvent evaporation and 15 μl were analyzed on a triple-quadrupole LC-MS/MS system (Quantum Access; Thermo Scientific, Waltham, MA, USA). Analytes were separated on a C18 reversed-phase HPLC column (Gemini-NX, 3 μ, 150 X 2.00 mm; Phenomenex, Torrance, CA, USA) using a gradient of 0.1 % formic acid in water (solvent A) and 0.1 % formic acid in acetonitrile (solvent B) at a flow rate of 300 μl/min. The initial condition of 10 % B was kept for 2 min and increased to 100 % solvent B at 20 min. Phytohormones were analyzed by negative electrospray ionization (spray voltage: 3.5 kV; sheat gas: 15; auxiliary gas: 15; capillary temperature: 350°C), collision-induced dissociation (argon CID gas pressure 1.3 mTorr [1.3 μm Hg], CID energy 16 V) and selected reaction monitoring (SRM) of compound-specific parent/product ion transitions: SA 137→93; d_4-SA 141→97; JA 209→59; d_5-JA 214→62; ABA 263→153; d_6-ABA 269→159; IAA 174→130; d_5-IAA 179→135.

Statistical analyses were conducted with analysis of variance (ANOVA) using light environment and monarch induction as main effects, their interaction term, and plant genetic family included as a blocking factor. Residuals were

Table 2 Analyses of variance (*F*-values) for effects of light environment, damage by monarch caterpillars, and full sibling genetic family of *Asclepias syriaca* on hormone and defense expression

A.	MANOVA	JA	SA	ABA	IAA		Latex
Light (L)	10.038***	4.575*	7.093**	32.681***	10.771**		94.132***
Induction (I)	24.856***	98.650***	0.251	6.078*	0.504		1.665
LxI	1.674	4.915*	0.295	0.175	0.317		2.878†
Genetic family	1.511*	0.651	1.845†	1.647†	1.646		2.465*
B.	MANOVA	Cardenolide 3.45	Cardenolide 5.1	Cardenolide 13.8	Cardenolide 14.6	Cardenolide 18.4	Total cardenolide
Light (L)	4.574***	8.885**	6.060*	3.619†	7.366**	0.346	10.650**
Induction (I)	2.181†	7.095**	0.002	0.004	2.597	<0.001	3.901*
LxI	0.341	0.216	0.046	0.025	1.027	0.466	0.013
Genetic family	1.802**	1.036	1.687†	2.154*	1.991*	2.753**	1.146

a) Analysis of four plant hormones (*ja* jasmonic acid, *sa* salicylic acid, *aba* abscisic acid, and *iaa* indole acidic acid). Latex exudation was tested separately. b) A similar analysis on the five cardenolide peaks that were present in most samples (represented by their hplc retention time, the first two peaks made up 83 % of the total), with a MANOVA followed by univariate analyses. Total cardenolide concentration was tested separately. All measures were taken on a fresh mass basis. ***$P<0.001$, **$P<0.01$, *$P<0.05$, AND †$P<0.1$

distributed normally unless otherwise noted. In addition, to address the role of jasmonic acid in driving the patterns of induction in other traits, we conducted an additional set of analyses that examined the impacts of three factors (light environment, monarch damage, and JA concentrations) on the other hormone concentrations as well as latex and cardenolide values using analysis of covariance (ANCOVA). In other words, in this analysis, JA shifts from being a response variable to being a predictor as a means to address its role in the induction process. Indeed, the impact of JA on hormones and defensive end-products is well-established, and thus considered an *a priori* expectation. Although we started with the fully factorial model, all interaction terms with JA (which were never significant) were removed from this analysis to simplify and maximize power. Genetic family was included as a blocking factor in all models. We interpret a significant effect of JA in this set of analyses as an indication of a physiological correlation between JA and the traits, irrespective of treatment effects on JA or the response variable.

Results

Natural Population Surveys In our assessment of plant traits and leaf damage in natural populations, most of the 13 variables measured showed differences between the sun and shade, as well as some site differences between the 24 pairs (MANOVA: light environment: exact $F_{13,11}=24.301$, $P<0.001$; pair: Wilks' *lambda*<0.001, $F_{299,164}=1.478$, $P=0.003$; Fig. 1). Shaded plants produced less tough, larger leaves that were less dense, had fewer trichomes, a lower carbon-to-nitrogen ratio, and less concentrated cardenolides than their paired plants from the sun (statistics provided in Fig. 1). Plants from the shade also had about half the natural leaf herbivory of sun plants (although there was no difference in latex exudation or aphid abundance). Finally, although shaded plants had internode lengths almost 10 % longer than sun plants, this effect was not significant (Fig. 1H). We repeated the survey of insect damage on 20 of the pairs in 2010 and found no difference in leaf damage between plants in the sun and shade ($F_{1,19}=0.068$, $P=0.797$).

Induced Resistance in Natural Populations Across the two years of the experiment to examine induced resistance to monarch caterpillars in the sun and shade, we found that caterpillars grew 27 % faster in the shade ($F_{1,135}=10.821$, $P<0.001$) (Fig. 2). We did not detect an overall effect of previous monarch damage (induction, $F_{1,135}=0.611$, $P=0.441$), and the interaction between light environment and induction was suggestive, but not significant ($F_{1,135}=2.256$, $P=0.139$). Nonetheless, inspection of the means indicated a 16 % impact of induction (decrease in relative caterpillar growth) in the sun, with a reversal in the direction (3 % increase in caterpillar mass) caused by induction on shaded plants. To further contrast induction effects in the sun vs. shade, we conducted separate analyses for the two light environments. Indeed, in the sun, previous monarch damage significantly reduced monarch mass ($F_{1,49}=7.870$, $P=0.007$), but there was no effect in the shade ($F_{1,49}=0.179$, $P=0.674$) (Fig. 2).

Common Garden Study Our experimentally shaded plants differed substantially from sun plants, with a strong induction of the shade-avoidance response (Fig. 3). Shaded plants were taller, with longer internode lengths, and produced fewer stems compared to sun plants (Figs. 3, 4). Consistent with results from the natural populations, monarch caterpillars grew≈10 % faster on shade plants ($F_{1,69}=4.248$, $P=0.043$);

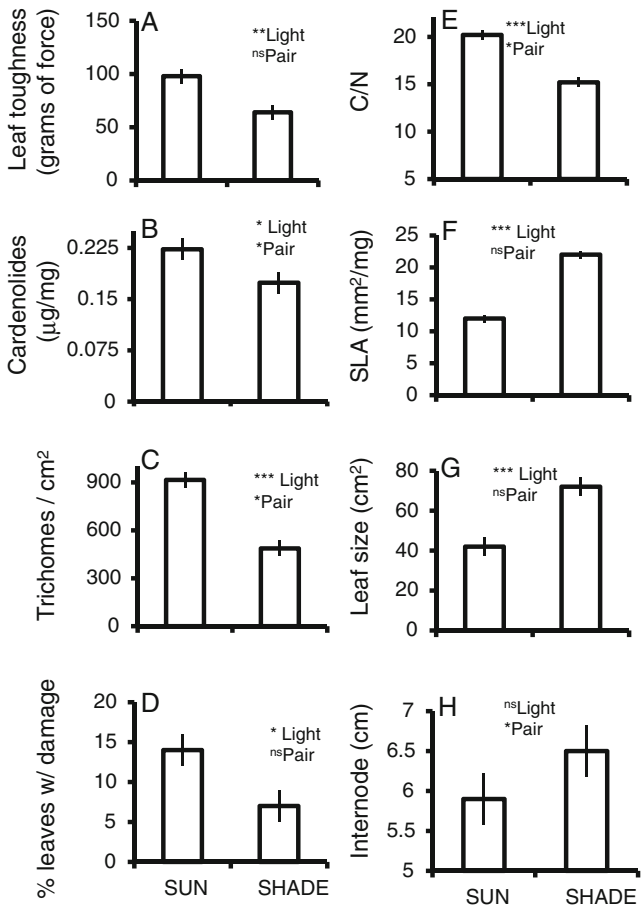

Fig. 1 A summary of phenotypic differences (mean ± SE) between paired *Asclepias syriaca* in full sun and shade (91 % reduction in photosynthetically active radiation at mid-day, N=24 pairs) from natural populations around Ithaca, NY (USA). Traits primarily involved in defense are on the left (panels A-D), while functional and structural traits are on the right (panels E-H). Significance of light environment and plant pair is indicated by * $P<0.05$, ** $P<0.01$, *** $P<0.001$, and ns $P>0.1$. C/N is the ratio of leaf carbon to nitrogen on a dry mass basis, SLA is specific leaf area (area per dry mass) and cardenolides are reported on a fresh mass basis. Data not shown for aphids per plant, plant height, number of leaves, latex exudation, and foliar water content (all factors not significant except pair for latex, and light environment for water content: $F_{1,23}=15.15$, $P<0.001$)

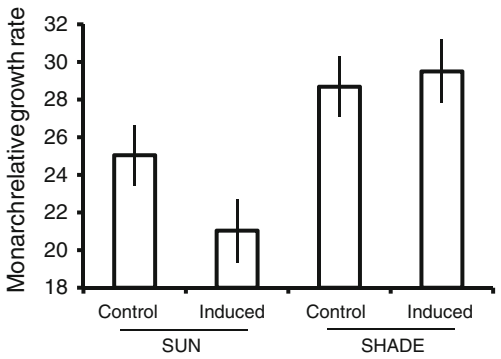

Fig. 2 Effects of experimentally imposed caterpillar damage (induced resistance) on relative growth rate (final minus initial mass divided by initial mass) of subsequently feeding monarch caterpillars (*Danaus plexippus*) on naturally occurring common milkweed (*Asclepias syriaca*) in the sun and shade. Shown are mean ± SE from experiments conducted over two years

because this bioassay was conducted in a common environment, this result was due clearly to differences in plant quality (Fig. 4D).

Induction of hormones and resistance traits in the neutral environment was impacted by the plants' light environment history. Shaded plants showed a nearly 50 % attenuated jasmonate burst following monarch herbivory (see interaction term in Table 2 , Fig. 5A), and this was concordant with latex induction, which showed a 17 % increase in sun plants, but no effect in shade plants (see marginally significant interaction in Table 2, Fig. 5B). All other hormones (salicylic acid, abscisic acid, and indole acidic acid) were higher in sun compared to shade plants, and only abscisic acid showed a main effect of induced response (20 % increase) following herbivory (Table 2). For cardenolides, sun plants had 27 % higher concentrations than shaded plants, which again was consistent with our results from natural populations. Total cardenolide concentrations were inducible by herbivory, and the earliest peak to elute appeared to drive this pattern (a significant 14 % increase following damage, Table 2); light environment did not impact this induction. Nearly all hormones and defenses measured showed variation among the ten genetic families tested (Table 2).

In follow-up analyses, we treated JA as a predictor variable (alongside light environment and monarch damage treatment), to address directly the *a priori* prediction that JA impacts other hormones and the production of defensive end-products. A significant effect of JA in this set of analyses would indicate a linear correlation between JA and the traits, irrespective of treatment effects on JA or the response

Fig. 3 Representative full-sibling common milkweed (*Asclepias syriaca*), germinated and grown under the same conditions in the first year of life. Just before stem emergence in their second growing season, the plant on the left was placed at the forest edge under a tree canopy, whereas the plant on the right (note 3 stems) was placed in full sun, 5 m away

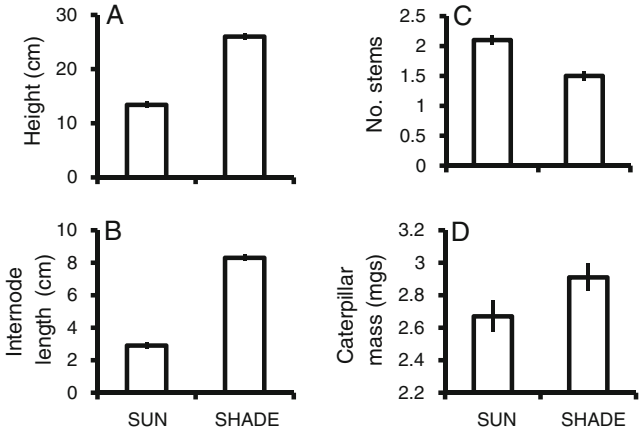

Fig. 4 A summary of phenotypic differences (mean ± SE) of *Asclepias syriaca* grown in full sun or shade from an experimental population: A height, B internode length, C number of stems, D and mass of a monarch caterpillar used as a bioassay. Caterpillar mass was assessed after plants were moved and randomized into a neutral environment (see Methods). All traits were significantly different in the two environments, ANOVAs, all *P*s<0.05

variable. Across treatments, jasmonate levels were positively correlated with latex, salicylic acid, and abscisic acid (Table 3). Light environment had an additional impact on all response variables (Table 3), potentially indicating a resource effect independent of JA. Despite the jasmonate burst following damage being qualitatively concordant with cardenolide induction (Fig. 5), there was no quantitative relationship between the two. In other words, the cardenolide induction effect was not impacted by the inclusion of JA in the model (Table 3). Although we do not interpret this result to mean that cardenolide induction is independent of JA, there was not a linear relationship between JA and cardenolides.

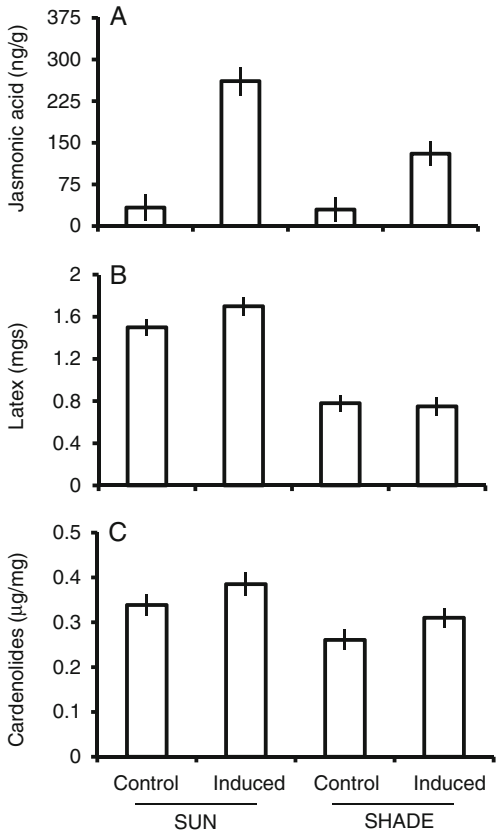

Fig. 5 Effects of previous damage (induced responses) on common milkweed (*Asclepias syriaca*) grown in the sun or shade, but then transported to a neutral environment prior to the experiment (see Methods for details). Shown are means ± SEs for A jasmonic acid, B latex exudation, and C total cardenolides, all taken on a fresh mass basis

Discussion

In this study we demonstrated that common milkweed, a plant that typically grows in full sun, has substantially altered resistance traits and hormonally-mediated interactions when growing in shaded habitats. Although the enhanced susceptibility of shaded plants has been reported in a wide array of species (Table 1), the means by which these changes occur have only recently been studied. For *A. syriaca*, naturally shaded plants produced leaves that were less defended by mechanical traits (less tough, fewer trichomes) and leaf chemistry (lower carbon-to-nitrogen ratio, reduced cardenolides) traits than plants from full sun. We further evaluated induced resistance in shaded and full sun habitats. For both *Arabidopsis* and lima bean, laboratory experiments have demonstrated that shading effects on induced resistance likely occur through phytochrome pigments, and can affect both the production of jasmonates as well as endogenous plant responsiveness to these jasmonates (Moreno et al., 2009; Radhika et al., 2010). For milkweeds, not only did monarch caterpillars grow better on shaded plants, but a strong attenuation of the jasmonate burst was concordant with reduced induction of latex and reduced induced resistance to caterpillars. Despite overall reductions of cardenolides in shaded plants, their induction was proportional in plants from both light environments (Fig. 5C). These results have two major implications. As

Table 3 Partial coefficients for the effects of jasmonic acid levels on five plant traits

	Estimate	t	Other factors that remain significant
Latex	0.001	2.261*	Light***, Family**
Cardenolides	<-0.001	-1.76	Light***, Induction*
Salicylic acid	0.063	2.620**	Light***, Family*
Abscisic acid	0.031	3.207**	Light***
Indole acidic acid	<0.001	0.122	Light**

These results are from five separate ANOVA models that each additionally included light, induction treatment, light-by-induction interaction, and genetic family as predictors. ***P< 0.001, **P< 0.01, *P< 0.05

with previous studies, both latex and cardenolides likely contribute to resistance to specialist monarchs, although their relative importance appears to vary (Zalucki et al., 2001; Agrawal, 2005), and negative effects of latex are more consistent. Second, the two traits appear to be somewhat uncoupled in their regulation among genotypes and in their jasmonate-mediated phenotypic responses to the environment (Bingham and Agrawal, 2010).

The linkage of plant hormones, resistance traits, and insect performance may be impacted by the way in which they are measured. Here, we considered two aspects: the environment in which they are measured and whether plant traits are reported on a fresh or dry mass basis. First, because the light environment impacts the temperature, resource availability, and potentially other local interactions, common environment assays are critical for assessing the impacts of shading (Sipura and Tahvanainen, 2000). We achieved this by moving our plants, which were under divergent light conditions, to a common (neutral) environment the day before induction treatments and caterpillar bioassays were conducted. Our results confirmed that the jasmonate burst, latex exudation, and caterpillar performance were impacted by past light conditions, even when intermixed and identically treated for the duration of the experiment. Although we did not see this effect for cardenolides, it is conceivable that cardenolide induction would also be attenuated in shaded plants when plants are maintained in the shade during the induction process.

Because light environments often influence plant water content, or said another way, leaves in the shade often have reduced dry mass (Morgan and Smith, 1981), calculations of the impacts of induction treatments may be sensitive to whether they are calculated on a fresh or dry mass basis (Koricheva, 1999; Agrawal et al., 2012). In our study, milkweed leaves in the shade were 81.5 % water, vs. 79.3 % in the sun, a small but statistically significant effect (Fig. 1 legend). This potentially could be problematic because the change in water content is confounded with light environment, and it is unclear whether plant hormones or resistance traits are functionally impacted by water content. For our study, we calculated cardenolide values on both a fresh- and dry mass basis, yet the results were qualitatively the same (data not shown). Nonetheless, future work, especially in systems where there is a strong impact of environment on plant tissue water content, should consider drawing conclusions based on both types of calculations.

In this study, we focused on sun-loving plants and the impact of shading or growing on forest edges. Ultimately, the impacts of shading on herbivory will be the sum of changes in plant resistance, the probability that herbivores attack plants in the shade, and the per capita damage imposed by herbivores in the different environments. For example, Guerra et al. (2010) reported that *Aristotelia chilensis* (Elaeocarpaceae) saplings received more damage in the shade than the sun. This effect was concordant with laboratory bioassays of tissue quality, and was driven by leaf thickness (not secondary chemistry, water content, or insect abundance in the respective habitats). Similarly, Muth et al. (2008) report greater levels of plant damage in shaded habitats despite equal herbivore abundances in both habitats. Nonetheless, many herbivores are known to avoid entering shaded habitats, which could leave higher quality foliage in the shade unattacked. In particular, we found that despite lower mechanical and chemical resistance traits in shaded plants, levels of herbivore damage in the field were either equal between habitats or reduced in the shade. We speculate that milkweed herbivores are less abundant and behaviorally avoid shaded habitats. Future work on *A. syriaca* would benefit from a focus on herbivore behavior and more precise measures of plant damage.

Does the fact that shaded leaf tissues are often of higher quality represent a tradeoff for plants, or an adaptive strategy (Agrawal et al. 2010)? A few studies suggest that that there may be no ecological tradeoff realized in the field. For example, beetles on willows were primarily found in the sun in the field, although their performance was enhanced on leaves of shaded plants in laboratory experiments (Sipura and Tahvanainen, 2000). Similar results were found for South American *Embothrium coccineum* (Proteaceae), where herbivory levels in the field were highest in the sun, but palatability in the laboratory was highest for shaded leaves (Salgado-Luarte and Gianoli, 2010). Again, our results for milkweed also showed this pattern. Despite bioassays consistently showing greater susceptibility to monarch caterpillars in the shade over the sun, over two years of field observations, damage was never higher in the shade.

Thus, we hypothesize that impaired constitutive and induced resistance in the shade could actually be an adaptive strategy of common milkweed plants. In shaded plants, not only are some plant responses likely to enhance light capture (few trichomes, higher nitrogen, larger thinner leaves), but investment in induced defense is also less necessary given the low probability of attack. In our experiments and those using mutant lines (Moreno et al., 2009), it appears that limiting resource availability is not the sole cause of higher susceptibility in shaded plants.

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