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Light limitation reduces tolerance to leaf damage in *Datura stramonium*

Alejandro Cisneros-Silva, Guillermo Castillo[1], Mariana Chávez-Pesqueira[2],
Rafael Bello-Bedoy[3], Iván D. Camargo[4] and Juan Núñez-Farfán

Laboratorio de Ecología Genética y Ecológica, Instituto de Ecología,
Universidad Nacional Autónoma de México, México 04510, Distrito Federal, México

ABSTRACT

Question: Does light limitation reduce tolerance to leaf damage in the annual herb *Datura stramonium*?

Hypothesis: Tolerance to leaf damage should be lower under light limitation (shade) than under full sunlight, because a reduction in leaf area and less exposure to light will limit carbon photo-assimilation and plants' ability to maintain fitness.

Organism: Jimsonweed, *Datura stramonium* L. (Solanaceae).

Methods: Damaged and undamaged plants of ten full-sib families ($N = 471$ plants) were grown under two different light treatments, full sunlight (control) and 35% light reduction (shade). Total seed number produced per plant was used as an estimate of maternal plant fitness. To compare the effect of light reduction on tolerance, we used the index of tolerance (the difference in seed production between the damaged and undamaged treatment of each genetic family). We also evaluated the effect of defoliation and light limitation on photosynthetic activity (estimated as the chlorophyll content index) and leaf growth compensation (i.e. total leaf area, mean area per leaf, and leaf number), compensatory characters related to tolerance.

Conclusions: Light limitation exacerbates the negative effect of leaf damage on plant fitness, producing a two-fold average reduction in tolerance. Moreover, compensatory growth was lower in the light-limited environment. Under high damage and light limitation, the evolution of higher tolerance to damage can be highly constrained.

Keywords: index of tolerance, leaf damage, light limitation, Solanaceae, tolerance mechanisms.

Correspondence: J. Núñez-Farfán, Laboratorio de Ecología Genética y Ecológica, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, Circuito Exterior, Ciudad Universitaria, Coyoacán, México 04510, Distrito Federal, México. e-mail: farfan@unam.mx

[1] Present address: Escuela de Enología y Gastronomía, Universidad Autónoma de Baja California, Ensenada, Baja California, México.

[2] Present address: Unidad de Recursos Naturales, Centro de Investigación Científica de Yucatán, Mérida, México.

[3] Present address: Facultad de Ciencias, Universidad Autónoma de Baja California, Ensenada, Baja California, México.

[4] Present address: Departamento de Ecología y Territorio, Facultad de Estudios Ambientales y Rurales, Pontificia Universidad Javeriana, Bogotá, Colombia.

INTRODUCTION

Plants in their natural environment grow in a heterogeneous matrix of biotic interactions (e.g. herbivores and pollinators) and abiotic resources (e.g. minerals, water, and light) that determine their reproductive success. Resource availability can affect development and performance of plants if resource limitation compromises plant vital functions (Valladares *et al.*, 2007). Resource availability may also reduce plants' ability to respond to the biotic environment, modifying their interactions with other organisms (Fornoni *et al.*, 2003; Wise and Abrahamson, 2007; Sun and Ding, 2009). Such is the case in plant–herbivore interactions, where resource availability plays an important role in the evolution of defensive strategies (Coley *et al.*, 1985; Strauss and Agrawal, 1999; Agrawal and Fishbein, 2006; Núñez-Farfán *et al.*, 2007; Wise and Abrahamson, 2007).

Plant tolerance to damage by herbivores is a defensive strategy that buffers losses in individual fitness (Rosenthal and Kotanen, 1994; Strauss and Agrawal, 1999). When damage is experimentally imposed at a single level (e.g. damaged or undamaged plants), tolerance is defined as the difference in fitness between related damaged and undamaged plants or the proportional fitness of damaged individuals relative to undamaged ones (Strauss and Agrawal, 1999). In fact, for continuous damage environments, tolerance can be treated as the reaction norm of fitness as a function of leaf damage (Simms, 2000). Because individual plant fitness cannot be examined in both damaged and undamaged states, tolerance must be estimated from a group of related plants (genotypes) (Strauss and Agrawal, 1999; Simms, 2000; Stowe *et al.*, 2000).

Tolerance to herbivory can be affected by the amount of genetic variance present in a given population, environmental deviations, potential trade-offs with resistance (defensive strategy that prevents herbivory through defensive traits) to herbivores (Tiffin and Rausher, 1999; Weinig *et al.*, 2003), and by genotype × environment interactions resulting from variation in resource availability and/or different type and abundance of herbivores (Rosenthal and Kotanen, 1994; Strauss and Agrawal, 1999; Stowe *et al.*, 2000; Hawkes and Sullivan, 2001; Núñez-Farfán *et al.*, 2007; Banta *et al.*, 2010). The Limiting Resources Model (LRM), proposed by Wise and Abrahamson (2005), states that tolerance will be limited if herbivory reduces plants' ability to acquire a limiting, focal resource in a given environment. A prediction of this model is that when herbivory reduces the acquisition of a limiting resource, such as minerals (by damaging roots) or CO_2 and light (by consuming leaves), plants will experience a drastic fitness reduction compared with conditions where these resources are not limited. Following this prediction, we hypothesized that plant tolerance should be reduced in light-limited environments because both leaf damage and reduced photosynthetic active radiation (PAR) decrease carbon sequestration (focal resource). Since a genotype's tolerance to damage is itself a reaction norm that can be expressed in different environments, say light and shade, we can ask if tolerance to damage has the potential to evolve (i.e. family × damage × light interaction). According to Falconer (1952), the interaction between genotype and environment can be regarded as a genetic correlation of the same character between two environments. Via and Lande (1985) considered that plasticity of a trait can evolve if selection acts in each environment. However, when character states are tightly genetically correlated, the evolution of new reaction norms may be limited. In this regard, the increase of tolerance to damage in two light environments would depend on the genetic variance within environments, and the genetic covariance between environments (Via and Lande, 1985; Via, 1987, 1994).

Here, we assess experimentally if light limitation reduces plant tolerance to leaf damage in the annual herb *Datura stramonium*. We hypothesized that the capability to maintain tolerance should be reduced under light limitation (shade treatment) because a reduction in leaf area and lower exposure to light will limit plants' ability to grow and reproduce.

To achieve this, we exposed artificially damaged and undamaged (control) plants of ten different genotypes (genetic families) to full sunlight and partial shade treatments. In order to evaluate the effect of light limitation on the tolerance response after damage, we measured total seed production in all treatment combinations. Then, we obtained average tolerance in each treatment by estimating the fitness differences between damaged and undamaged plants. We further assessed photosynthetic activity and leaf growth compensation (total leaf size, mean leaf size, and number of leaves per plant) in response to damage and light limitation, characters associated with tolerance (Strauss and Agrawal, 1999).

METHODS

Study system

Datura stramonium L. (Solanaceae) is an annual herb dispersed exclusively by seeds produced by selfing or outcrossing (Motten and Stone, 2000). This plant grows in old fields, roadsides, and disturbed areas in Mexico, USA, Canada, and Europe (Weaver and Warwick, 1984; Núñez-Farfán and Dirzo, 1994). The leaves of this plant are persistent, allowing insects to consume up to 100% of leaf area (J. Núñez-Farfán, personal observation). In Mexico, the main consumers of *D. stramonium* are the specialist folivores *Lema trilineat*a (syn. *Lema daturaphila*) and *Epitrix parvula* (Coleoptera: Chrysomelidae), and the generalist *Sphenarium purpurascens* (Orthoptera: Pyrgomorphidae). Leaf damage caused by these herbivores reduces seed production, imposing selection on traits of resistance and tolerance to leaf damage (Núñez-Farfán and Dirzo, 1994; Valverde *et al.*, 2001, 2003; Fornoni *et al.*, 2004). Studies both in greenhouse and in natural conditions have found genetic variation for tolerance within and between populations (Fornoni and Núñez-Farfán, 2000; Fornoni *et al.*, 2003). Increased growth rate of leaves and stems have been related to tolerance to herbivores (Valverde *et al.*, 2003; Bello-Bedoy and Núñez-Farfán, 2011). Furthermore, *D. stramonium* has shown genetic variation in resistance traits such as tropane alkaloids (hyoscyamine and scopolamine) and leaf trichomes (Shonle and Bergelson, 2000; Kariñho-Betancourt, 2009; Castillo *et al.*, 2013, 2014).

Greenhouse experiment

A greenhouse experiment was performed from June to December 2007. The plants belonged to ten genetic families of *D. stramonium* previously obtained from an autogamous cross-design experiment. Therefore, individual plants of each family shared at least 50% additive genetic variance (full-sibs) (Falconer and Mackay, 1996). Parental plants were collected from a natural population located in Patria Nueva, State of Hidalgo, in central Mexico (20°22′12.46″W, 99°3′1.98″N). To assess the effect of light limitation on plant tolerance to damage, we randomly assigned 48 plants from each of the ten genetic families to the following damage and light treatments (12 plants per combination):

1. Plants without foliar damage + full sunlight (control; $n = 118$)
2. Plants without foliar damage + shade ($n = 115$)
3. Plants with 50% foliar damage + full sunlight ($n = 119$)
4. Plants with 50% foliar damage + shade ($n = 119$).

Nine seedlings died during the experiment, leaving a total sample size of 471 plants. We distributed plants in a randomized block design to control environmental variation within the greenhouse (Steel and Torrie, 1980).

Leaf damage and light treatments

Using scissors, we removed 50% of the foliar tissue from plants assigned to the damage treatment by cutting along their main veins without damaging the veins. To assess tolerance at the same phenotypic stage, the leaf damage treatment was applied when the first floral bud appeared (*c.* 6–7 leaves) (Fornoni and Núñez-Farfán, 2000). Any new leaves that appeared during the experiment were damaged in the same way. The shade treatment consisted in covering the plants with shade cloth that reduced photosynthetic active radiation (PAR) by about 35%.

Data collection

To obtain the total number of seeds produced by each plant, all fruits were individually collected into paper bags; fruits were then opened to count the seeds. To minimize variation in seed number between plants due to differences in herkogamy, all flowers were manually self-pollinated at dusk when the stigmas of *D. stramonium* are fully receptive (Motten and Antonovics, 1992). Total seed number has been shown to be an important component of fitness in annual plant species that possess a high degree of self-fertilization; therefore, seed number was used to estimate differences in tolerance between families and treatments (Fornoni and Núñez-Farfán, 2000; Valverde *et al.*, 2003; Mostafa *et al.*, 2011). Following Simms and Triplett (1994), we defined the index of tolerance (T) of each family (i) as

$$T_i = \frac{\bar{S}_{damage} - \bar{S}_{no\text{-}damage}}{\bar{S}_{no\text{-}damage}} = \left(\frac{\bar{S}_{damage}}{\bar{S}_{no\text{-}damage}} - 1\right),$$

where $\bar{S}_{damage}$ and $\bar{S}_{no\text{-}damage}$ are the mean seed number of a family in the damaged and undamaged (control) treatment respectively. When calculating the average index of tolerance ($\bar{T}_E$) in a light environment, all ten families were included, i.e.

$$\bar{T}_E = \frac{1}{n} \sum_{i=1}^{n} T_i.$$

Thus, $\bar{T} = 0$ indicates complete tolerance, $\bar{T} > 0$ over-tolerance, and $\bar{T} < 0$ under-tolerance.

We estimated the relationship between tolerance of genotypes (families) in the two light environments (Via and Lande, 1987) by means of a Pearson correlation of family mean values [i.e. a genetic correlation between environments (Falconer, 1952)]. We compared the average tolerance between light environments using Student's t-test.

To evaluate leaf responses to artificial damage and shade, we measured chlorophyll content on five fully expanded leaves per plant using a portable chlorophyll meter (CCM-200 model, Opti-Science). The chlorophyll meter measures the chlorophyll content index (CCI), which indicates the amount of total chlorophyll for a given unit area. Previous studies have shown that the CCI is a good indirect proxy of photosynthetic activity (Buttery and Buzzell, 1977; Naumann *et al.*, 2008; Hu *et al.*, 2009; Mostafa *et al.*, 2011). We counted leaf number and collected all the leaves to obtain total leaf area and mean leaf size per plant. To do this we used the image analyser software Image Pro Plus v.6.0.0.260 (Media Cybernetics, Inc.).

Statistical analyses

To assess the effect of light limitation and leaf damage on relative seed number, we performed a mixed-model analysis of variance (ANOVA). The model included the random effect *family*, and the fixed effects *damage* and *light availability*, as well as the interactions between them. We compared mean tolerance of plants in full light and partial shade using a *t*-test.

A mixed-model ANOVA (as described above) was also performed to calculate the CCI, mean leaf size, total leaf area, and number of leaves produced (in independent models). Whenever an ANOVA was significant, we performed a Tukey-Kramer LSD *post hoc* test to highlight significant differences between treatments. All analyses were performed in JMP v.9 (SAS Institute, 2007). Following Garrido *et al.* (2010), prior to analyses, seed production was relativized per plant genotype to control for differences in vigour among genetic families. Thus we expressed average seeds produced by a family relative to the average seed production across environments.

RESULTS

Effect of light limitation and leaf damage on seed production and tolerance

Seed production in *D. stramonium* was significantly reduced in the leaf damage and light limitation treatments (Table 1a). In both treatments, seed production was reduced relative to the control treatment (Fig. 1a). Plants in the leaf damage and light limitation treatments showed the lowest seed production (Fig. 1a). Moreover, the relative seed number of undamaged plants exceeded that of damaged plants (Fig. 1a). The shade × damage interaction was also significant; plants with no damage and full light attained the highest mean seed production, whereas plants in the damage + shade treatment showed the lowest mean seed production (Fig. 1a). The family × damage interaction was significant, indicating between-family variation in tolerance to damage (Fig. 2, Table 1a). However, the interaction family × shade × damage was not significant, indicating that the combination light level plus damage affected all families equally (Table 1).

The mean index of tolerance of plants grown in full sunlight was 2.1-fold higher than plants grown in low light ($T_{FL} = -0.3260 \pm 0.022$ vs. $T_{LL} = -0.1550 \pm 0.022$, respectively; $t = -3.54$, $P = 0.0022$, $N = 10$) (Fig. 1b). The genetic correlation between family tolerance in the two light environments was high ($r = 0.685$, $N = 10$, $P = 0.028$). The level of tolerance in one environment (full light) predicts well tolerance in the other (shade).

Chlorophyll content index

Chlorophyll content was significantly affected by leaf damage and light reduction (Table 1b). Shade reduced mean CCI in relation to control plants (Fig. 3a). The interaction shade × damage was significant; in full sunlight damaged plants had higher CCI values than undamaged plants, whereas both groups of plants had similar and lower CCI values in the shade (Fig. 3a, Table 1b). Finally, we detected genetic variation in CCI expression in response to the shade treatment (family × shade interaction; Table 1b).

Table 1. Results of ANOVA of (a) relative seed number, (b) chlorophyll content index (CCI), (c) mean leaf area, (d) total leaf area, and (e) number of leaves in response to family, damage, and shade treatments, in a greenhouse experiment with *Datura stramonium*

	(a) Relative seed number				(b) CCI				(c) Mean leaf area			
Source of variation	d.f	SS	*F*	*P*	d.f	SS	*F*	*P*	d.f	SS	*F*	*P*
Family	9	3.8771	1.6506	0.2097	9	2452.25	2.6115	0.0810	9	24925.2	1.5877	0.4278
Damage	1	8.8471	44.9669	**<0.0001**	1	1042.96	24.5397	**0.0008**	1	119790	99.5462	**<0.0001**
Shade	1	5.7129	51.5161	**<0.0001**	1	21660.8	243.58	**<0.0001**	1	498144	193.855	**<0.0001**
Family × Damage	9	1.7713	4.2148	**0.0217**	9	382.564	1.5658	0.2573	9	10822.7	0.5928	0.7760
Family × Shade	9	0.9979	2.3746	0.1069	9	800.771	3.2775	**0.0459**	9	23134.3	1.2671	0.3651
Damage × Shade	1	0.7110	15.2030	**0.0036**	1	257.083	9.4676	**0.0132**	1	3426.76	1.6893	0.2259
Family × Damage × Shade	9	0.4202	0.3659	0.9508	9	244.321	0.7674	0.6468	9	18258.3	1.0692	0.3844
Error	430	54.8684			430	15211.9			424	804463.2		

	(d) Total leaf area				(e) Number of leaves			
Source of variation	d.f	SS	*F*	*P*	d.f	SS	*F*	*P*
Family	9	184.5444	3.0726	**0.0014**	9	271.36291	3.6577	**0.0002**
Damage	1	2277.2492	341.2437	**<0.0001**	1	740.55597	89.8378	**<0.0001**
Shade	1	2642.4132	395.9632	**<0.0001**	1	127.62005	15.4817	**<0.0001**
Family × Damage	9	43.9205	0.7313	0.6803	9	65.31586	0.8804	0.5427
Family × Shade	9	105.9168	1.7635	0.0732	9	63.3194	0.8535	0.5672
Damage × Shade	1	65.5005	9.8152	**0.0019**	1	42.9622	5.2118	**0.0229**
Family × Damage × Shade	9	49.0037	0.8159	0.6017	9	56.11856	0.7564	0.6570
Error	424	2829.5134			430	3544.601		

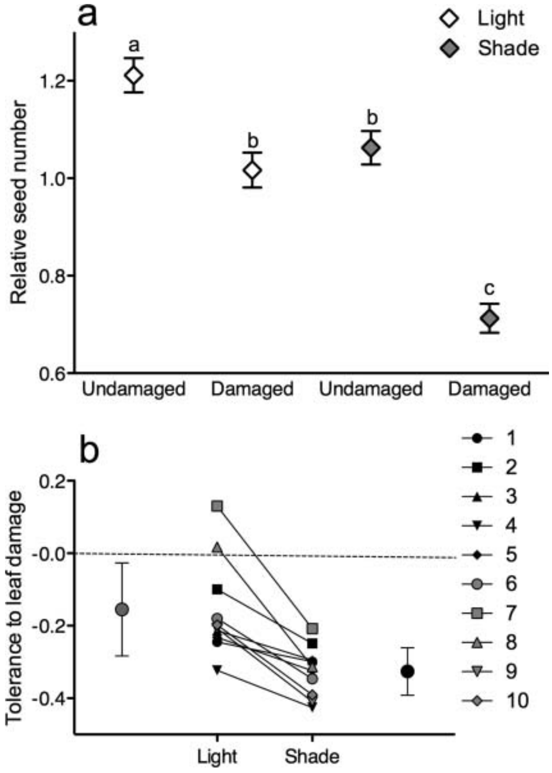

Fig. 1. (a) Average relative seed number (± 1 SE) produced per plant of *Datura stramonium* grown under two light conditions (light and shade) and with or without foliar damage. Averages with different letters differ significantly after a Tukey-Kramer LSD *post hoc* test; $N = 471$. (b) Index of tolerance (see Methods) of ten genetic families of *Datura stramonium* in control and light-limited environments. Circles represent the average index of tolerance (± 1 SE) in light environment.

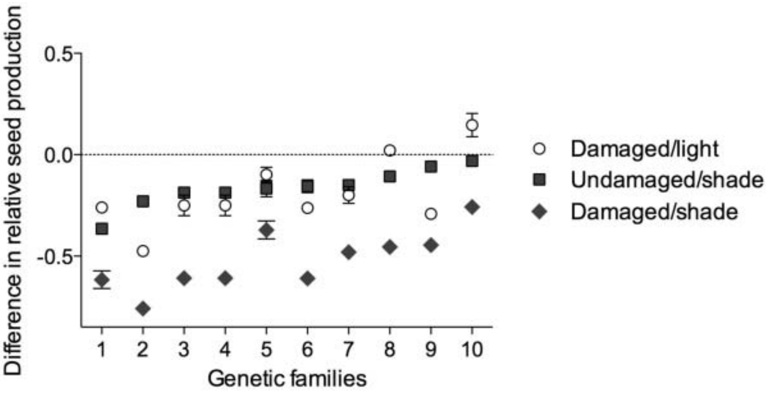

Fig. 2. Differences in relative seed production of ten genotypes of *Datura stramonium* exposed to a combination of leaf damage (0 and 50%) and light limitation (0 and 35%) treatments. Dotted line represents relative seed production of plants (a genotype) in the control treatment (undamaged/ natural light). Whiskers represent ± 1 SE.

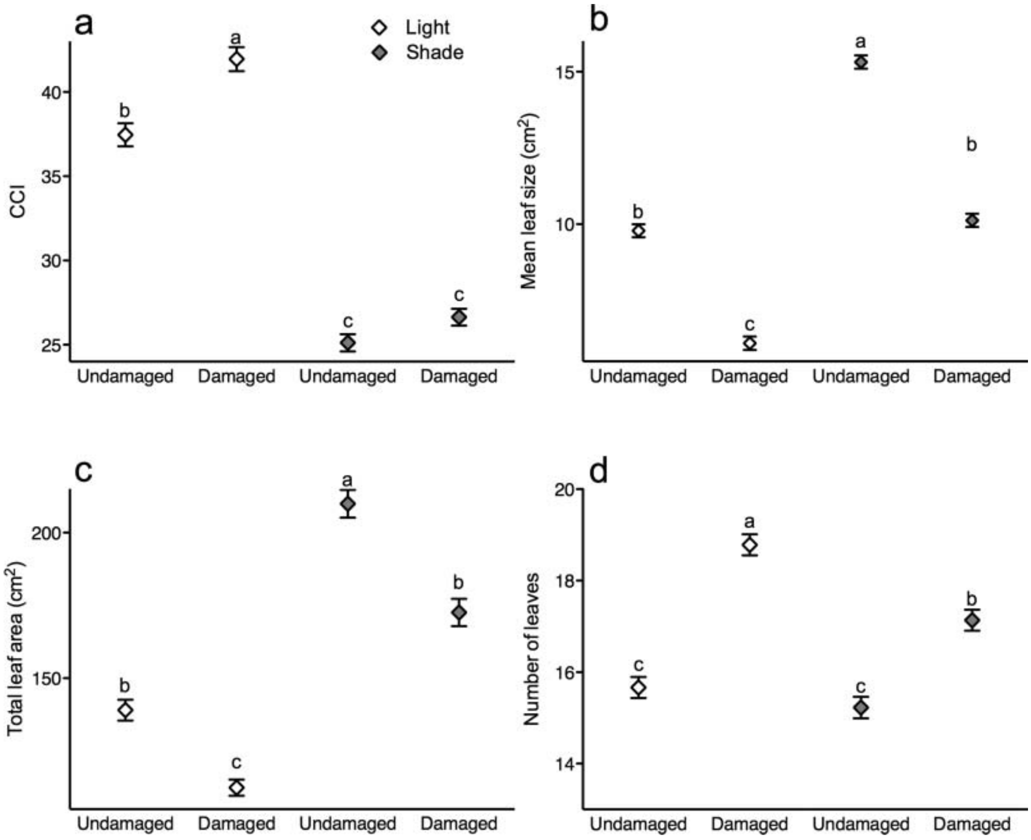

Fig. 3. Averages (± 1 SE) of (a) chlorophyll content index, (b) mean leaf size, (c) total leaf area, and (d) number of leaves produced by plants of *Datura stramonium*, in a greenhouse experiment under damage and light availability treatments. Averages with letters differ significantly after a Tukey-Kramer LSD *post hoc* test; $N = 471$.

Leaf traits

Leaf damage reduced both mean leaf size and total leaf area, both of which were larger under light limitation (Table 1c, d and Fig. 3b, c). Furthermore, the analyses detected a significant damage × shade interaction and genetic variation for total leaf area and number of leaves (Table 1d, e). Within each light environment, number of leaves was higher for damaged plants, and plants in the shade produced a lower number of leaves than plants in full sunlight (Fig. 3d, Table 1e).

DISCUSSION

Foliar damage negatively affected seed production in *D. stramonium*. Likewise, plants growing in partial shade showed reduced seed production compared with plants in full sunlight. The tolerance response is expected to buffer fitness losses due to foliar damage. In the present study, we found that damaged plants – both in full sunlight and partial shade –

showed lower fitness compared with undamaged plants. Furthermore, the ability of plants to tolerate leaf damage was on average two-fold lower in plants grown in partial shade than plants grown in full sunlight. Thus, light-limited environments constrain tolerance.

In line with our expectations, damaged plants growing under light limitation showed the lowest seed production, whereas undamaged plants in full sunlight showed the highest seed production, and although genetic variance for tolerance occurs across light environments, the interaction family × damage × light was not significant. Our results show that the light-limiting condition exacerbates the negative effects of leaf damage on seed production in *Datura stramonium*, resulting in a reduced tolerance to damage. Moreover, the strong negative genetic correlation of tolerance in the two light environments suggests limits to the evolution of tolerance in light-limited environments (see Via, 1994).

Resource availability, light exposure (full or partial), and soil nutrients help explain the level of tolerance of plants when damaged (Coley *et al.*, 1985; Núñez-Farfán *et al.*, 2007). It has been hypothesized that the tolerance response to damage by herbivores should decrease if a resource relevant for tolerance to damage is not freely available, and if damage by herbivores to plant tissues exacerbates such limitation (Wise and Abrahamson, 2007). Thus, individuals would incur an extra fitness cost. In this study, tolerance was lower in light-limited conditions compared with natural light (see Fig. 1b). A reduction in leaf area owing to damage and light limitation could diminish CO_2 fixation and photosynthetic rate, and thus the compensatory response (Mabry and Wayne, 1997; Rand, 2004; Salgado-Luarte and Gianoli, 2011).

Although a previous experiment in *D. stramonium* found no changes in tolerance at similar levels of defoliation in different light environments (Aguilar-Chama and Guevara, 2012), our results indicate that light availability *per se* has a negative effect on the tolerance of plants. Differences between experiments might be due to differences in experimental conditions or studied populations. For instance, we measured the index of tolerance by family to assess genetic variation in tolerance (cf. Table 1a) because it has been documented for different populations of *D. stramonium* (Fornoni and Núñez-Farfán, 2000; Valverde *et al.*, 2003; Fornoni *et al.*, 2004). In contrast, Aguilar-Chama and Guevara (2012) used different plants ($n = 6$) from different populations ($n = 8$), so tolerance could not be assigned to a given family or genotype.

Understanding changes in plant physiology and leaf traits induced by herbivore damage may help predict the potential for tolerance to evolve in heterogeneous environments (Fornoni *et al.*, 2003; Núñez-Farfán *et al.*, 2007). Chlorophyll content is a good indirect proxy of photosynthetic activity (Buttery and Buzzell, 1977; Naumann *et al.*, 2008; Hu *et al.*, 2009; Mostafa *et al.*, 2011), which has been suggested to be an important mechanism of plant tolerance to herbivore damage (Strauss and Agrawal, 1999; Fornoni, 2011). However, it is unclear whether plants should increase or maintain chlorophyll content as an adequate response to herbivore damage. We found evidence of genetic variation in the chlorophyll content index in shade conditions, indicating the potential for this trait to evolve in light-limited environments (Coley *et al.*, 1985; Wise and Abrahamson, 2005; Stevens and Kruger, 2008). Furthermore, the increment in chlorophyll content in damaged plants in full light indicates that plants responded to leaf damage by increasing chlorophyll content. This increase was small in this as in previous studies (Huang *et al.*, 2013) and limited by shade (low light negatively affected chlorophyll content). Both increased and constant leaf chlorophyll content can form part of the compensatory response to tolerate damage, since remaining leaf tissue is photosynthetically active after damage (Zangerl *et al.*, 2002), contributing to plant growth and reproduction. Thus, increasing the level of chlorophyll in damaged plants in full light could contribute to tolerance of damage, whereas this response is absent when light is limited.

Variation in tolerance can also be explained by differences in total leaf area between plants growing under full light compared with plants growing in shade. Our previous studies in *D. stramonium* have found that plants with large leaves are more tolerant than plants with small leaves (Fornoni *et al.*, 2003; Valverde *et al.*, 2003; Bello-Bedoy and Núñez-Farfán, 2011). These results showed that shaded plants had both higher total leaf area and mean leaf size than plants exposed to natural light levels. This response, however, did not increase their tolerance. Instead, plants in shaded conditions with larger leaves produced fewer leaves and less seeds, indicating that the increase in leaf area did not buffer the negative impact on seed number in *D. stramonium*. Thus, there is evidence to suggest that light limitation reduces tolerance persistence in populations under strong and constant pressure by herbivores. The plastic responses of plants to shade are not always adaptive and can even be maladaptive when negatively related to other fitness components (Valladares and Niinemets, 2008). Our results show a contrasting pattern of total and mean leaf area with seed number in undamaged and damaged plants, suggesting a potential trade-off between these traits. This trade-off may arise from the costs associated with the production of new leaf area to capture light, reducing the resources available for reproduction (Valladares and Niinemets, 2008). The fitness cost associated with the plastic response to light limitation could partially explain the difference in tolerance to defoliation between damaged and undamaged plants growing under different conditions of light availability.

The lower tolerance observed in shaded plants was related to the lower amount of compensation in the putative traits measured, compared with plants in full light. Thus, this study highlights the importance of measuring not only the pattern of tolerance but the mechanisms associated with the tolerance response (Camargo *et al.*, 2015). Finally, these results offer insights to better understand the evolution of tolerance to herbivory in natural environments.

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