Herbivory may modify functional responses to shade in seedlings of a light-demanding tree species

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Summary

1. The ability to display functional responses to shading, i.e. phenotypic changes that enhance plant performance in low light, is fundamental for plant success in forest communities. Herbivory may constrain plant functional responses to shading and this could explain the often observed lower plant tolerance of herbivore damage in the shade.

2. We carried out a field trial and a greenhouse experiment to evaluate whether simulated herbivory (i) causes greater fitness losses in the shade, and (ii) modifies plant functional responses to shading. Changes in functional traits were measured at the biochemical, leaf, shoot and whole-plant levels, in seedlings of the light-demanding evergreen tree *Aristotelia chilensis* (Elaeocarpaceae), which is endemic to South American austral forests.

3. We hypothesized that plant responses to shade would be constrained by herbivory due to within-plant limitations in resource availability and/or antagonistic interactions between responses to shade and herbivory. We further expected that this limitation would result in a greater field mortality of damaged plants in forest understory as compared to open sites.

4. Simulated herbivory (50% defoliation) on field seedlings decreased 8-month plant survival in the shade (forest understory) but not in the sun (canopy gaps) in a temperate rainforest. Likewise, simulated herbivory in the greenhouse decreased plant growth rate and final biomass in the shade treatment but not in the sun treatment.

5. The phenotypic responses to shading of seven traits were constrained by simulated herbivory: chlorophyll, leaf shape, leaf blade angle, petiole angle, SLA (specific leaf area), internodes length and total leaf area. A path-analysis showed that most of these constrained responses were uncorrelated to each other. Some of these results might be explained by antagonistic interactions between molecules or pathways involved in plant responses to shading and herbivory. Four functional traits exhibited greater responses to shading in damaged plants: A_{max} (Photosynthetic rate), foliar carbon, shoot : root ratio and LMR (leaf mass ratio); but most of them resulted from correlated changes in SLA.

6. The present study undertook a mechanistic approach to the often observed pattern of greater impact of herbivore damage on plant performance in low light environments. The central finding of this study is that the unraveling of herbivory-constrained plant functional responses to shading may explain the often observed greater fitness losses due to herbivory in the shade. It is also suggested that herbivory pressure is an underestimated factor in pioneer species distribution along the light gradient.

Key-words: *Aristotelia chilensis,* constraints, distribution, foliar damage, light gradient, low light, phenotypic plasticity, temperate rainforest

Introduction

Light is the most important resource for plant performance, and light availability is spatially and temporally heterogeneous in most natural ecosystems. Plants may modify their phenotypic traits in response to heterogeneity in the availability of light in such a way that plant performance is enhanced, i.e. plants display functional responses to light availability, and these responses occur at different levels of organization (Givnish 1988; Valladares & Niinemets 2008).

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At a biochemical level, there is evidence that plants in the shade reallocate nitrogen from carboxilase enzymes to chlorophyll (Evans & Poorter 2001), leading to lower photosynthetic and dark respiration rates that characterize a conservative strategy of resource use (Valladares & Niinemets 2008). At a leaf level, it has been observed that plants optimize light capture by increasing specific leaf area (SLA), thus minimizing the leaf mass used to display a given foliar area (Givnish 1988; Smith *et al.* 1997). Architectural responses to shade include elongation of internodes and petioles (Huber, Fijan & During 1998), modification of leaf blade angles, which are kept perpendicular to sunrays (Koller 1990), and reduced branching (Smith & Whitelam 1997). Plants also change biomass allocation patterns: in low light environments there is a greater investment of biomass in leaves and stems relative to roots (Givnish 1988).

Plants are commonly exposed to complex environments, where many biotic and abiotic factors interact. Co-occurrence of multiple stresses can alter the capacity of plants to respond effectively to a given ecological factor (Valladares, Gianoli & Gómez 2007; Gianoli, Quezada & Suárez 2009). Several studies have evaluated the effects of abiotic factors such as nutrients, water availability, temperature or photoperiod on the expression of plastic responses to shade (Weinig 2000; Gianoli 2004; Portsmuth & Niinemets 2007; Valladares & Niinemets 2008 and references therein). Less research has addressed the effects of biotic interactions such as herbivory on plant functional responses to reduced light availability (Kurashige & Agrawal 2005; Gianoli, Molina-Montenegro & Becerra 2007; González-Teuber & Gianoli 2008). Herbivores may have a negative impact on plant fitness, distribution and abundance (Maron & Crone 2006). Plants have evolved defensive mechanisms that prevent the attack of herbivores or reduce the fitness impact of damage caused by them (Núñez-Farfán, Fornoni & Valverde 2007). These defensive traits may be costly for plants because they can divert resources otherwise invested into maintenance, growth, reproduction and storage (Agrawal, Strauss & Stout 1999; Strauss *et al.* 2002). In a low-resource environment such as the shaded forest understory, it is expected that herbivory (or

plant responses to herbivory) may limit plant functional responses to low light, moreover if it is considered that phenotypic plasticity also entails costs for plants (Auld, Agrawal & Reylea 2010). Furthermore, regardless of the resource availability scenario, plant functional responses to shade and herbivory may be in opposition for particular traits. For instance, whereas plants in the shade tend to display thinner – and more palatable – leaves (Givnish 1988), plants that have suffered herbivory often produce tougher leaves (Karban & Baldwin 1997). Importantly, if herbivore damage is proven to constrain plant functional responses to shading, then we will gain insights into mechanistic explanations for the often observed greater fitness losses due to herbivory in the shade (Pierson, Mack & Black 1990; Blundell & Peart 2001; Rogers & Siemann 2002; Norghauer, Malcolm & Zimmerman 2008).

Aristotelia chilensis (Elaeocarpaceae) is a small evergreen tree endemic to South American austral forests (Rodríguez, Matthei & Quezada 1983). *Aristotelia chilensis* is mainly distributed in canopy gaps and forest edges, where it is fairly common, and it is considered one of the few light-demanding species in these forests (Lusk, Chazdon & Hofmann 2006). However, seeds show a high percentage of germination in the shade (Figueroa & Lusk 2001) and seedlings and saplings can be found in shaded understories (Fig. 1; Lusk 2002; Lusk & Del Pozo 2002). Seedlings of *A. chilensis* respond to shading by an increase in specific leaf area and shoot biomass allocation (Lusk 2002; Lusk & Del Pozo 2002). Low shade tolerance is the main explanation for the under-representation of *A. chilensis* in mature forests (Lusk 2004), where individuals would eventually die of energy deficit because their intrinsically high foliage turnover cannot be matched by leaf production in low light (Lusk 2004). Plants suffer defoliation by insect herbivores (Vásquez *et al.* 2007; De La Vega & Grez 2008). Recent observations in a temperate rain forest showed that seedlings of *A. chilensis* had on average 32% of total leaf area removed, but the natural herbivory range was from 0% to 100% defoliation (Fig. 1; Salgado-Luarte and Gianoli, unpublished data). Severe defoliation has a detrimental effect on *A. chilensis* performance, particularly in the shade

Fig. 1. Seedlings of *Aristotelia chilensis* (Elaeocarpaceae) growing on the forest understory in a southern temperate rainforest. Left: Undamaged seedling. Dark bar = 1 cm. Right: Seedling showing foliar damage by insects and snails.

(Simonetti *et al.* 2007). Therefore, it could be suggested that herbivores may play an important role in either preventing seedling establishment or hastening plant exclusion from the shade.

In the present study with *A. chilensis* seedlings, we carried out a field trial and a greenhouse experiment to evaluate whether simulated herbivory (i) causes greater mortality in the shade, and (ii) modifies plant functional responses to shading. Changes in functional traits were measured at the biochemical, leaf, shoot and whole-plant levels. We expected that plant responses to shade would be constrained by herbivory due to within-plant limitations in resource availability and/or antagonistic interactions between responses to shade and herbivory. More importantly, we hypothesized that this limitation could explain the greater mortality of damaged plants in shaded habitats as compared to open sites.

Materials and methods

FIELD TRIAL

A field experiment to evaluate the consequences of herbivory for seedling survival in sun and shade was carried out in Puyehue National Park (40°39′S, 72°11′W; 350–400 m), a temperate rainforest located in the western foothills of the Andes in southern Chile. The study site (Anticura) has a mean annual precipitation of 2800 mm and a mean temperature of 9·8 °C (Dorsch 2003). The mature lowland forest is composed of broad leaved evergreen tree species (Lusk 2002; Lusk & Del Pozo 2002). In September 2007, we chose 70 seedlings of *A. chilensis* growing in treefall canopy gaps (Sun: canopy openness = 26·3 ± 1·4%; PPFD = 11·35 ± 1·89 mol m^{-2} day^{-1}) and 70 seedlings growing in mature forest understory (Shade: canopy openness = 5·6 ± 0·6%; PPFD = 5·57 ± 0·55 mol m^{-2} day^{-1}). This classification of light environments was done using hemispherical canopy photographs taken directly above each plant. To estimate canopy openness and PPFD, images were analysed using WINPHOT 5.0 canopy analysis software (ter Steege, Tropenbos Foundation, Wageningen, the Netherlands). Sampling was carried out within an area of ~15 ha. Mean plant height was 21·9 ± 0·4 cm (range: 11·8–32·9 cm). Chosen seedlings were at least 5 m apart and sustained low levels of foliar damage (<0·85%). For the simulated herbivory treatment, we removed 50% of total leaf area using scissors, cutting 100% of leaf blades from half of the leaves on each seedling. Two additional simulated herbivory events were done in November 2007 and February 2008 to maintain the magnitude of leaf damage at 50%. This magnitude of damage corresponds to a high, but not extreme, defoliation level caused by small insects and gastropods in this temperate rainforest, where about 22% of seedlings show over 50% of foliar damage (Salgado-Luarte and Gianoli, unpublished data). Control plants were not damaged. To minimize the probability of additional damage due to natural herbivory, all experimental plants were treated monthly with systemic insecticide (Dimethoate, Fastac®) and molluscicide (Metarex®). This insecticide + molluscicide treatment is effective against herbivores (treated plants were almost undamaged in a field trial in the same forest; Salgado-Luarte & Gianoli, unpublished data) and has no effect on *A. chilensis* plants (greenhouse experiment conducted both in sun and shade conditions; Salgado-Luarte, unpublished data). Seedling mortality was recorded in May 2008.

GREENHOUSE EXPERIMENT

To evaluate the effect of simulated herbivory on plastic responses to shade, a greenhouse experiment was performed at Universidad de Concepción campus (36°49′S, 73°01′W) with *A. chilensis* seedlings collected from natural populations in forest remnants close to the northern limit of temperate rainforest. Seedlings were unearthed from sites of intermediate light availability between the dark mature forest understory and the sunlit treefall canopy gaps. Thus, plants were taken from secondary forest stands. This sampling procedure minimized the occurrence of bias in the results of the plasticity experiment. In July 2007, seedlings were transplanted into plastics bags with commercial potting soil. Two weeks later, seedlings were assigned to different light treatments and were kept there during 1 month before damage treatments were applied. Plants were watered to field capacity with tap water every other day (watering was skipped in cloudy days if substrate looked wet). Four experimental groups were assigned to a factorial array of two light environments (sun and shade) and two damage treatments (control and herbivory) (*n* = 40 seedlings per treatment). Seedlings were randomly assigned to one of the four groups and did not differ initially in height (mean: 21·6 ± 0·2 cm) or number of leaves (mean: 20 ± 0·4) among them (ANOVA, data not shown). Plants in the sun treatment were grown under full sunlight, or 100% light intensity (mean PAR at noon: 861·4 ± 85·7 μmol m^{-2} s^{-1}). Plants in the shade treatment were grown under a dark mesh, receiving ≈7% light intensity (mean PAR at noon: 60·8 ± 9·6 μmol m^{-2} s^{-1}). Herbivory treatment consisted in cutting with scissors 50% of the plant's total leaf area, as described above for the field trial. Simulated herbivory is useful to standardize the magnitude of damage inflicted among individuals (Gianoli, Quezada & Suárez 2009). Two additional damage events were applied to newly produced leaves, in November 2007 and February 2008, in order to maintain 50% of foliar area damaged. Control plants did not receive any damage and were not attacked by herbivores in the greenhouse. In February 2008, we measured biochemical and gas exchange traits and at the end of April 2008 we recorded most functional traits in the plants. This differential schedule was followed because biochemical/physiological responses are often short-term. A total of 21 functional traits were determined. The first 12 traits are defined at the biochemical or leaf level and the remaining nine are architecture or allocation traits (the list of traits appears in Table 1).

PLANT TRAITS

The relative chlorophyll content (Chl) was estimated with a chlorophyll meter (CCM-200, ADC; OPTI-SCIENCES, Hudson, NH, USA). Measurements of CO_2 assimilation (A_{max}, μmol m^{-2} s^{-1}) and leaf transpiration (E, mmol m^{-2} s^{-1}) were done under 1000 PAR and 25 °C with a portable leaf chamber and infrared gas analyzer (LCi Portable Photosynthesis System; ADC BioScientific, Hoddesdon, Hertfordshire, UK). Instantaneous water use efficiency (WUE) was calculated as the ratio of A_{max} to E. All measurements were taken during the morning (9:30–12:30) in fully expanded leaves at mid shoot. Plant height (cm) and the length of internodes and petioles (cm) were determined with a ruler. Foliar blade and petiole angles with respect to horizon (°) were measured with a protractor. Leaf thickness (mm) was measured with a digital caliper (0·01 mm precision; Mitutoyo Corporation, Kanagawa, Japan). We also recorded the number of leaves and branches. After making all of these measurements, seedlings were harvested. Plant material was divided into leaves, stems and roots. We scanned the leaves with a flatbed scanner and obtained leaf size (cm^2) and leaf shape (4π area/

Table 1. Functional traits (Mean ± SE) of *Aristotelia chilensis* seedlings and corresponding two-way ANOVAS

	Sun		Shade		F-ratio		
	Control	Herbivory	Control	Herbivory	Light	Damage	Light × Damage
Foliar C (% leaf biomass)	49·20 ± 0·09	49·54 ± 0·08	46·01 ± 0·18	44·61 ± 0·16	887·3***	15·40***	41·40*** enhancement
Foliar N (% leaf biomass)	1·140 ± 0·032	1·142 ± 0·029	1·139 ± 0·022	1·106 ± 0·027	0·513	0·415	0·483 –
C : N (foliar C : N ratio)	44·54 ± 1·31	44·78 ± 1·44	40·95 ± 0·80	41·48 ± 1·10	8·437**	0·106	0·015 –
Chlorophyll (chlorophyll content index)	21·50 ± 0·09	21·70 ± 0·10	27·80 ± 0·18	26·98 ± 0·20	142·8***	4·100*	11·00** constraint
Chl : N (chlorophyll:foliar N)	19·43 ± 0·54	19·62 ± 0·67	24·75 ± 0·50	25·11 ± 0·71	80·14***	0·221	0·022 –
A_{max} (μmol m^{-2} s^{-1})	11·47 ± 0·24	11·96 ± 0·27	7·53 ± 0·21	6·11 ± 0·23	426·2***	3·912*	16·24*** enhancement
E (mmol m^{-2} s^{-1})	2·08 ± 0·03	2·31 ± 0·04	2·58 ± 0·08	2·74 ± 0·09	55·55***	9·347**	0·305 –
WUE (A_{max} : E ratio)	5·56 ± 0·13	5·23 ± 0·13	2·98 ± 0·10	2·30 ± 0·10	566·7***	19·16***	2·342
Leaf size (cm^2)	2·73 ± 0·06	2·12 ± 0·05	4·36 ± 0·34	3·96 ± 0·223	70·64***	5·939*	0·283 –
Leaf thickness (mm)	0·133 ± 0·009	0·144 ± 0·007	0·080 ± 0·005	0·087 ± 0·003	69·51***	1·745	0·096 –
Leaf shape (4π area perimeter^{-2})	0·620 ± 0·007	0·587 ± 0·008	0·508 ± 0·009	0·504 ± 0·007	134·6***	4·750*	3·25* constraint
SLA (cm^2 g^{-1})	187·4 ± 15·4	145·7 ± 8·4	358·0 ± 10·5	268·2 ± 4·4	197·1***	39·61***	5·310* constraint
Shoot : root (shoot : root biomass ratio)	0·64 ± 0·03	0·83 ± 0·04	1·88 ± 0·03	3·61 ± 0·02	908·7***	129·8***	46·03*** enhancement
LAR (leaf area per plant mass)	15·14 ± 1·25	14·73 ± 0·37	58·07 ± 1·89	63·76 ± 3·70	586·2***	1·08	3·840 –
LMR (leaf mass per plant mass)	0·088 ± 0·003	0·112 ± 0·010	0·152 ± 0·004	0·251 ± 0·012	144·3***	48·28***	16·67*** enhancement
Total leaf area (cm^2)	145·8 ± 5·17	110·4 ± 4·33	227·7 ± 6·46	162·3 ± 11·06	85·33***	48·46***	4·286* constraint
Petiole length (cm)	0·721 ± 0·044	0·649 ± 0·047	1·635 ± 0·044	1·632 ± 0·042	448·8***	0·714	0·598 –
Leaf blade angle (°, relative to the horizontal)	−61·03 ± 1·39	−49·51 ± 1·83	2·87 ± 0·45	0·69 ± 0·25	234·0***	15·66***	33·72*** constraint
Petiole angle (°, relative to the horizontal)	3·35 ± 1·09	9·96 ± 0·91	24·24 ± 0·52	19·73 ± 0·70	341·4***	1·602	44·94*** constraint
Relative internode length (internode : shoot length ratio)	0·051 ± 0·002	0·047 ± 0·001	0·151 ± 0·004	0·126 ± 0·004	761·5***	4·752*	21·66*** constraint
Branches (number of branches)	1·25 ± 0·19	1·38 ± 0·19	0·63 ± 0·14	0·73 ± 0·12	115·6***	0·486	0·006 –

The first 12 traits are defined at the biochemical or leaf level and the remaining nine are architecture or allocation traits. See Methods for a detailed definition of parameters. Main factors in the ANOVAS: Light (sun vs. shade conditions) and Damage (control vs. herbivory treatments). F-ratios (d.f. = 1, 156) are shown. The MANOVA, with all traits taken together, showed the following results. Light: Wilks' $\lambda = 0.012$; $P < 0.001$. Damage: Wilks' $\lambda = 0.274$; $P < 0.001$. Light × Damage: Wilks' $\lambda = 0.330$; $P < 0.001$. For each trait showing a significant Light × Damage interaction, the outcome in terms of constraint or enhancement of plasticity to shading following leaf damage is indicated.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

perimeter^2) using image analysis software (Sigmascan pro 5.0; SPSS Inc., Chicago, IL, USA). Plant parts were dried at 60 °C and their dry mass was determined with a digital scale. Foliar nitrogen and foliar carbon (% dry mass) were estimated using Kjeldahl method and Walkley-Black method respectively. From these data the following parameters were calculated: total dry mass, SLA (leaf area per unit leaf mass, cm^2 g^{-1}), leaf area ratio (LAR, leaf area per total plant mass), leaf mass ratio (LMR, leaf mass per total plant mass), total leaf

area, shoot : root biomass ratio, relative internodes length (internodes : shoot length), foliar carbon : nitrogen ratio (C : N) and chlorophyll : nitrogen ratio (Chl : N). Finally, we estimated relative growth rate (RGR) from the increase in the number of leaves as follows: RGR = Ln (final number of leaves) − Ln (initial number of leaves)/time (months). We chose number of leaves instead of plant height to estimate plant growth because the remarkable stem elongation of plants in the shade would confound results.

STATISTICAL ANALYSIS

Differences among treatments in seedling survival in the field were first detected using a binomial linear model with logit link (Factorial ANOVA – GLZ) that can predict responses for dependent variables with discrete binomial distribution. Then survival was compared pairwise using test of proportions.

In order to test the hypothesis that herbivory limits functional responses to shade, we first applied a factorial multivariate ANOVA (MANOVA; main factors: Light and Damage). This test would indicate that, in the case of a significant Light × Damage interaction, the global phenotypic response to shade was affected by the damage treatment. Afterwards we applied a series of univariate ANOVAs to identify those plant traits exhibiting significant Light × Damage interactions. In addition, to better interpret the interactive effects of herbivore damage and light availability on phenotypic traits, we examined the network of correlated changes in functional traits using path analysis, which tests for direct or indirect causal relationships between several variables. This analysis focused on the shade + damage treatment because it was in the phenotypic expression of this group of plants where the hypothetical constraints on functional responses would be evident. The correlation network was centred on SLA because it is considered the most important trait for carbon gain in low light environments (Evans & Poorter 2001). Standardized path coefficients were estimated using the structural equation modeling program SPSS/AMOS (SPSS Inc., Chicago, IL, USA). Finally, greenhouse data on plant biomass and RGR were also analysed using ANOVA. Data with non-normal distribution were Ln- or arcsine-transformed prior to analysis. All statistical analyses, with the exception of path analysis, were run with STATISTICA (StatSoft Inc., Tulsa, OK, USA).

Results

The field trial with *A. chilensis* seedlings in the southern temperate rainforest showed that, overall, 8-month seedling survival was reduced in the shade and after simulated herbivory (Fig. 2). More related to the study hypothesis, there was a significant interaction between main factors. Thus, simulated herbivory on *A. chilensis* decreased seedling survival in the shade (mature forest understory) but not in the sun (treefall canopy gaps) (Fig. 2).

The MANOVA of functional traits measured in the greenhouse revealed a significant Light × Damage interaction (Table 1), indicating that simulated herbivory modifies the global phenotypic responses to light availability. Univariate ANOVAs showed significant Light × Damage interactions for 11 functional traits. Five out of 12 traits at the biochemical or leaf level and six out of nine architectural or allocation traits showed a significant Light × Damage interaction. Thus, trait responsiveness to the interaction of main factors did not differ between organization levels (5/12 vs. 6/9, $\chi^2 = 0.40$, $P > 0.52$; 2 × 2 table of contingency).

Whereas the phenotypic responses to shading of seven traits were constrained by herbivory (chlorophyll, leaf shape, leaf blade angle, petiole angle, SLA, internodes length and total leaf area), four traits exhibited greater responses to shading in damaged plants (A_{max}, foliar C, shoot : root ratio and LMR) (Table 1).
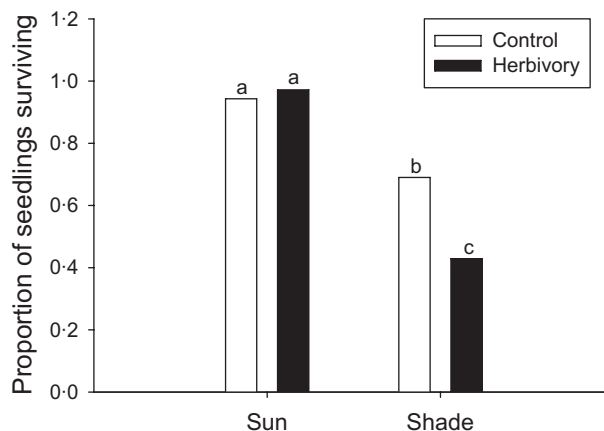

Fig. 2. Effects of simulated herbivory (50% defoliation) on *Aristotelia chilensis* seedlings in sun (treefall canopy gaps) and shade (mature forest understory) in a southern temperate rainforest. Bars show 8-month seedling survival (proportions, ±SE) after inflicting leaf damage. Bars sharing a letter are not significantly different ($P > 0.05$, paired proportion tests). Results of the binomial linear model with logit link were as follows. Light: log-likelihood = −67·84; $\chi^2 (1) = 42.02$; $P < 0.001$. Damage: log-likelihood = −61·26; $\chi^2 (1) = 13.16$; $P < 0.001$. Light × Damage: log-likelihood = −59·16; $\chi^2 (1) = 4.187$; $P < 0.05$.

The path analysis showed that six out of 11 traits showing modification in their responses to shade after foliar damage were directly or indirectly linked to SLA (Fig. 3). However, in only four traits the particular pattern of either reduced or increased slopes of reaction norms to shade in damaged plants (Table 1) could be accounted for by the herbivory-constrained display of SLA: reduced plasticity in total leaf area, and increased plasticity in A_{max}, LMR, and shoot/root (Fig. 3).

In general, greenhouse seedlings showed several of the typical plant responses to shading (Table 1). Plants exposed to experimental shade increased their chlorophyll content and the allocation of N to chlorophyll, and showed lower A_{max} and WUE than sun plants (Table 1). Both foliar C and C : N decreased in the shade, but foliar N did not change with light availability (Table 1). Plants in low light produced larger leaves, with greater SLA, thus having greater total leaf area, and allocated more biomass to the shoot, thus exhibiting higher LMR and LAR (Table 1). Shade plants had fewer branches and longer internodes and petioles than sun plants, and they oriented leaf blades more horizontally (Table 1). Damaged plants had smaller leaves, lower specific leaf area and WUE, and higher E, shoot : root ratio and LMR (Table 1). While responses of shoot : root ratio and LMR to herbivory and to shading were in the same direction, plants showed counteractive responses of SLA and total leaf area to the experimental factors.

Simulated herbivory in the greenhouse decreased plant RGR and final biomass in the shade treatment but there were no differences in the sun treatment (Fig. 4). Plant mortality in the greenhouse experiment was very low (< 5%) and there were no differences among treatments (data not shown).
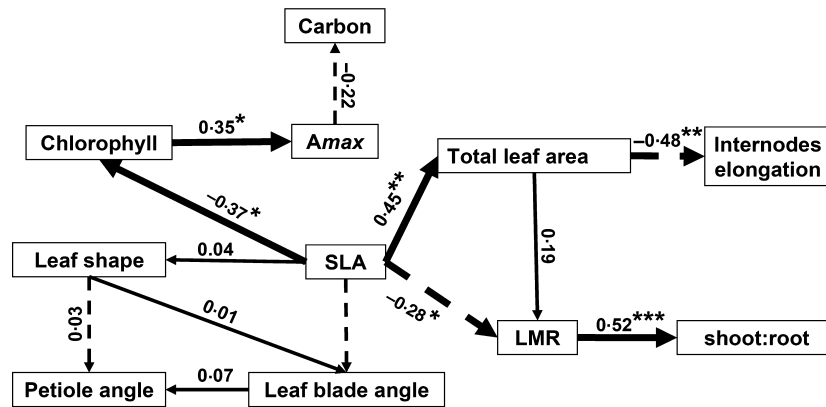
Fig. 3. Path diagram showing the relationships between functional traits that modified their response to shade after simulated herbivory in *Aristotelia chilensis* seedlings in the greenhouse (see Results section). Data are from the Shade + Herbivory treatment. Arrows represent the hypothetical causal relationship between variables; solid and dashed arrows indicate positive and negative relationships, respectively. Standardized path coefficients are shown over arrows and their significance is: *$P < 0.05$, **$P > 0.01$ and ***$P < 0.001$.
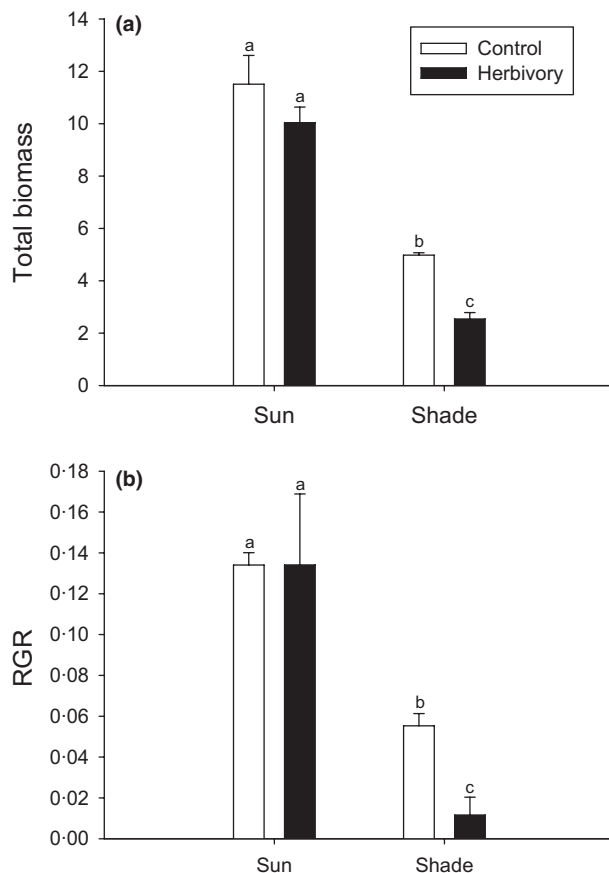

Fig. 4. Performance of *Aristotelia chilensis* seedlings in the greenhouse under different treatments of light intensity (sun and shade) and damage (control and herbivory). Means and SE are shown. Bars sharing a letter are not significantly different ($P > 0.05$, Tukey test). (a) Total biomass (g). Two-way ANOVA, Light: $F (1, 156) = 263.9$; $P < 0.001$. Damage: $F (1, 156) = 36.52; P < 0.001$. Light × Damage: $F (1, 156) = 3.639; P < 0.05$. (b) Relative growth rate (RGR, leaves month^{-1}). Two-way ANOVA, Light: $F (1, 156) = 258.9$; $P < 0.001$. Damage: $F (1, 156) = 13.68; P < 0.001$. Light × Damage: $F (1, 156) = 10.08; P < 0.05$.

Discussion

When field seedlings of *A. chilensis* were experimentally subjected to natural levels of defoliation, they showed decreased survival in the shade but not in the sun. Likewise, the greenhouse experiment showed a differential effect of simulated herbivory on seedling growth rate and biomass. A similar result was found when the same experimental approach was undertaken with field plants of *Embothrium coccineum* (Proteaceae) (Salgado-Luarte & Gianoli 2010), the other common light-demanding tree species in this forest (Lusk 2002). It is often found that plant tolerance to herbivory is lower in shaded habitats than in open sites (Pierson, Mack & Black 1990; Blundell & Peart 2001; Rogers & Siemann 2002; Norghauer, Malcolm & Zimmerman 2008). Herbivory pressure could be a factor contributing to the observed distribution of plants along the light gradient (Louda & Rodman 1996; Pearson *et al.* 2003; Dalling *et al.* 2009). This should be particularly relevant for light-demanding species, which are not able to replace leaf tissue lost to herbivores in the shade because of limited resource availability in the forest understory. Moreover, herbivore damage may constrain functional responses to low light, thus causing plants to deviate from the 'target phenotype' that allows a better performance in the shade as shown by plant ecophysiological studies. In this scenario, plant performance would be severely impaired, ultimately leading to negative carbon balance and poor chances of survival.

Results showed that undamaged seedlings exhibited a remarkable plasticity to shading at several integration levels. Most of these phenotypic changes are consistent with the shade-avoidance syndrome, which refers to the set of architectural, morphological and physiological responses that contribute to enhance light capture (Smith & Whitelam 1997; Franklin 2008). More related to our research questions, we found that foliar damage constrained plasticity to shading in *A. chilensis*. This was verified for nearly two-thirds of the traits

that showed significant Damage × Light interactions, namely chlorophyll content, leaf shape, leaf blade angle, petiole angle, SLA, internodes elongation and total leaf area. A similar result was reported for a twining vine that showed reduced plasticity to shading in leaf shape, internodes and petiole length after being subjected to 25% defoliation (Gianoli, Molina-Montenegro & Becerra 2007). The path analysis showed that increased total leaf area was the only constrained response to shade that was a consequence of correlated changes in another trait, SLA. The latter, defined at the leaf level, may explain the former when it scales up to whole-plant level. Summarizing, the general outcome of reduced plasticity in functional traits due to herbivore damage holds true, moreover if it is considered that three of the four cases of traits showing the opposite trend, i.e. greater plasticity in damaged plants (see below), could result from correlated responses in SLA.

Explanations for some of the constrained plasticity cases could be suggested in view of evidence showing antagonistic interactions between molecules or pathways involved in plant responses to low light and herbivory (Cipollini 2004; Ballaré 2009). The biosynthesis of indole alkaloids, which are present in *A. chilensis* leaves (Silva *et al.* 1997) and may increase upon herbivory (Karban & Baldwin 1997), includes indole-acet-aldoxime. This metabolite is a precursor of the phytohormone indole-acetic acid (Hansen & Halkier 2005), which mediates stem elongation in response to shade (Morelli & Ruberti 2000). In general, folivory induces cell wall peroxidase activity that increases leaf toughness but constrains leaf expansion (Moore *et al.* 2003), which is a typical plant response to shading (Givnish 1988).

Four traits showed greater plasticity to shading in damaged plants: A_{max}, foliar C, shoot : root ratio and LMR. The path analysis revealed direct or indirect relationships with SLA for all of them except for C, and a strong positive correlation between shoot : root and LMR. Therefore, the cases of greater plasticity after herbivore damage are somewhat inflated. Nevertheless, some of these results may be interpreted in terms of synergism between plant responses to shading and herbivory. For instance, increased biomass allocation to leaves or the shoot in low light allows the plant to enhance light capture (Givnish 1988), and these responses coincide with plant compensatory responses to herbivory: increased biomass allocation to aboveground tissues consumed by herbivores (Strauss & Agrawal 1999).

Plants can hardly display simultaneous optimal responses to both shading and herbivory because of their associated costs and the antagonistic interactions between their underlying metabolic pathways (Valladares, Gianoli & Gómez 2007). We found evidence that plants of *A. chilensis* subjected to a defoliation level comparable to field herbivory decreased their functional responses to the shade, including changes in resource allocation to chlorophyll, leaf area and shape, leaf blade and petiole orientation, and internode elongation. A reduction in these responses could compromise the carbon balance of this early successional species of a temperate rain-forest, and therefore result in decreased performance in low light environments, as was found in the greenhouse, and greater mortality in the shaded understory, as was detected in the field. The present study undertook a mechanistic approach to the often observed pattern of greater impact of herbivore damage on plant performance in low light environments. The central finding of this study is that the unraveling of herbivory-constrained plant functional responses to shading may explain the often observed greater fitness losses due to herbivory in the shade. It is also suggested that herbivory pressure is an underestimated factor in pioneer species distribution along the light gradient.

Acknowledgements

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