

Explaining differential herbivory in sun and shade: the case of *Aristotelia chilensis* saplings

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Abstract Differential herbivory in contrasting environments is commonly explained by differences in plant traits. When several plant traits are considered, separate correlation analyses between herbivory and candidate traits are typically conducted. This makes it difficult to discern which trait best explain the herbivory patterns, or to avoid spurious inferences due to correlated characters. *Aristotelia chilensis* saplings sustain greater herbivory in shaded environments than in open habitats. We measured alkaloids, phenolics, trichomes, leaf thickness and water content in the same plants sampled for herbivory. We conducted a multiple regression analysis to estimate the relationship between herbivory and each plant trait accounting for the effect of correlated traits, thus identifying which trait(s) better explain(s) the differential herbivory on *A. chilensis*. We also estimated insect abundance in both light environments. Palatability bioassays tested whether leaf consumption by the main herbivore on *A. chilensis* was consistent with field herbivory patterns. Overall insect abundance was similar in open and shaded environments. While saplings in open environments had thicker leaves, lower leaf water content, and higher concentration of alkaloids and phenolics, no difference in trichome

density was detected. The multiple regression analysis showed that leaf thickness was the only trait significantly associated with herbivory. Thicker leaves received less damage by herbivores. Sawfly larvae consumed more leaf tissue when fed on shade leaves. This result is consistent with field herbivory and, together with results of insect abundance, renders unlikely that the differential herbivory in *A. chilensis* was due to greater herbivory pressure in open habitats.

Keywords Herbivory · Leaf thickness · Light · Multiple regression · Plant defense · Sawfly

Introduction

Herbivory is a ubiquitous interaction in nature that may be influenced by several ecological factors, including plant defensive traits, herbivore counter-defenses, the abiotic environment in which the interaction takes place, and the associated communities of animals and plants (Zamora et al. 1999). Differences in herbivory rates within plant species are usually explained by either genetic or environmentally-mediated differences in plant resistance (Louda and Collinge 1992; Marquis 1992; Boege and Dirzo 2004; Johnson and Agrawal 2005), but abiotic constraints and natural enemies may also play an important role (Speight et al. 1999; Sipura et al. 2002).

Numerous studies have identified plant traits that are influenced by light availability and hence might explain differential herbivory in contrasting light environments (Lincoln and Mooney 1984; Larsson et al. 1986; Collinge and Louda 1988; Dudit and Shure 1994; Louda and Rodman 1996; Henriksson et al. 2003; Yamasaki and Kikuzawa 2003; Molina-Montenegro et al. 2006; Chacón and Armesto 2006; Muth et al. 2008). However, important issues are sometimes overlooked in this type of study. First, when several plant

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traits are considered, separate correlation (or regression) analyses between field herbivory estimates and each of the traits are sometimes conducted. This makes it difficult to discern which of the traits best explain the herbivory patterns or to avoid spurious inferences due to correlated characters. Both of these goals may be achieved using multiple regression analysis (e.g., Agrawal 2005; Agrawal and Fishbein 2006), a statistical tool that has proven useful to isolate the effects of selective agents on specific traits in natural selection studies (Brodie et al. 1995). Second, palatability studies that would corroborate field patterns of herbivory (e.g., Zangerl and Berenbaum 1993; Crone and Jones 1999; Elger et al. 2009) are not always included. Third, evaluations of the possibility that differential herbivory is due to contrasting herbivore abundance between habitats, and not to differential plant resistance, are often lacking.

Preliminary field observations in central Chile indicate that saplings of *Aristotelia chilensis* (Elaeocarpaceae), a small tree endemic to forests of southern South America, suffer greater herbivory in relatively shaded places than in open habitats. In this study we first confirmed this pattern of field herbivory in three different sites. This was done to assess the soundness of the herbivory pattern, thus ruling out the possibility of idiosyncratic differences between open and shaded places of a particular site. We then measured plant traits in the same individual plants sampled for herbivory patterns in the two contrasting light environments. These traits (alkaloids, phenols, trichome density, leaf thickness, leaf water content) have been reported to be associated with herbivore preference or performance in a number of plant-herbivore systems (Scriber and Slansky 1981; Rosenthal and Berenbaum 1991; Fritz and Simms 1992; Coley and Barone 1996; Lucas et al. 2000). We conducted a multiple regression analysis to estimate the relationship between herbivory and each of the plant traits measured, taking into account the effect of correlated traits also included in the analysis. Thus, this multivariate analysis would indicate which plant trait(s) better explain(s) the differential herbivory on *A. chilensis* in open and shaded environments. We tested in a palatability bioassay whether leaf consumption by the most common herbivore on *A. chilensis* was consistent with the field herbivory patterns. Finally, because differential herbivory damage could be a consequence of differential herbivory pressure instead of differential plant resistance, we estimated insect abundance in both light environments.

Materials and methods

Study species

Aristotelia chilensis (Mol.) Stuntz (Elaeocarpaceae) is a small tree endemic to the temperate forests of southern

South America (Damascos and Prado 2001). *A. chilensis* is a light-demanding species of rapid growth that is able to colonize clearings and therefore persists after native forests are replaced by forestry plantations in central Chile (Bustamante et al. 2005). This species is mainly distributed in canopy gaps and forest (or plantation) edges (Repetto-Giavelli et al. 2007), but seedlings can be found in shaded places (Lusk 2002). Leaves of *A. chilensis* respond to shading with an increase in specific leaf area (Lusk 2002), which maximizes light harvest and may allow plant survival in a shaded understory. Leaves of *A. chilensis* contain monoterpenoid indole alkaloids, which are biologically active compounds (Silva et al. 1997) whose defensive properties have yet to be evaluated, and coumarins (Guerra et al. 2006, unpublished data), phenolic compounds with antifeedant properties (Berenbaum 1991).

Sampling

Field sampling was carried out at three sites located within *Eucalyptus* plantations in central Chile ($36^{\circ}49'41''$ to $36^{\circ}50'08''$ S; $73^{\circ}01'47''$ to $73^{\circ}02'55''$ W). Small temperate forest fragments where *A. chilensis* occurs remain embedded within the plantation matrix but sampling was entirely conducted in *Eucalyptus* plantations. The distance between sites was ca. 2 km. At each site we defined two light environments: sun and shade. In the sun environment, plants of *A. chilensis* were growing in open sites outside the *Eucalyptus* stand where PAR range was $630\text{--}730 \mu\text{mol s}^{-1} \text{m}^{-2}$ at noon on a cloudy day. In the shade environment, plants of *A. chilensis* were part of the edge vegetation of the *Eucalyptus* stand where PAR range was $270\text{--}360 \mu\text{mol s}^{-1} \text{m}^{-2}$ at noon on a cloudy day. In each light environment of each site we randomly chose 15 saplings 1–1.5 m tall (total $N = 90$) that were at least 5 m apart. In each sapling herbivory was visually estimated using four categories referring to percentage of leaf area damaged: 1 ($<25\%$), 2 (≥ 25 to $<50\%$), 3 (≥ 50 to $<75\%$) and 4 ($>75\%$). The most common herbivore on *A. chilensis* was a sawfly species (Hymenoptera: Tenthredinidae). The early instars were found feeding on leaf buds and mature sawfly larvae could be found all over the plant. We also found curculionid beetles and tortricid moths, as has been reported elsewhere (De la Vega and Grez 2008). Almost all types of leaf damage found could be attributed to insects.

We made a simple estimation of insect abundance in the study sites during early summer. Five saplings (1–1.5 m tall) were randomly chosen in each light environment of each study site (total $N = 15$ plants per light environment) and all insects on the plant were counted for 5 min. We also recorded the taxonomic order of the insects. This sampling was conducted during 2 weeks corresponding to the seasonal peak of insect activity in the study sites. Data from the different sites were pooled for the statistical analysis.

Leaf traits

Leaf thickness was measured in three leaves of the middle part of each plant with a digital caliper (0.01 mm resolution). In order to measure trichome density (number of trichomes per cm² of leaf) we collected one leaf per individual and preserved it in ethanol 30%. We then counted the number of trichomes in the adaxial surface of a 1 cm × 1 cm square of leaf under stereoscope (40×) after adding two drops of Congo red to facilitate the observation. Leaf water content was estimated as (fresh weight–dry weight)/(fresh weight) and expressed as percentage. Leaves were oven dried (60°C) after being weighed the first time, which was done within 1 h after leaf collection in the field.

To quantify chemical defenses, ca. 20 g of leaves were harvested from the middle part of the plant and oven dried. Alkaloids were extracted from 7 g of dry leaves of each plant ($N = 90$) following the method described by Djilani et al. (2006). Leaves were ground in a mortar, suspended in 280 ml of the surfactant SDS and sonicated in an ultrasonic bath at 25°C for 2.5 h. This extract was filtered twice with distilled water and the solution of combined filtrates acidified with sulfuric acid to pH 3–4. The alkaloids were precipitated by adding 15 ml of Mayer reagent, dissolved in an alkaline solution of sodium carbonate (5%, m/m) and extracted with chlorhydric acid in a decantation funnel. The organic layer was then concentrated to dryness on a rotary vacuum evaporator in previously weighed 50 ml round bottom flasks. These flasks were weighed again (0.1 mg resolution scale) when the sample was completely dry and the content of alkaloids obtained by the weight difference. Total alkaloid concentration was expressed in mg per 100 g of dry mass. Total phenolic content was determined from 70 mg of dry leaves of each plant ($N = 90$) by colorimetry using Folin–Denis reagent. Leaves were suspended in 20 ml of methanol and sonicated at 25°C for 10 min. The sample volume was completed to 100 ml adding distilled water. Folin–Denis reagent (0.5 ml) was then added to a 5 ml aliquot of the aqueous extract. This blend was shaken, 2 ml of 2 M sodium carbonate solution were added, and sample volume was completed to 50 ml adding distilled water. The sample was allowed to react for 30 min and then absorbance was measured at 760 nm in a UV/Vis spectrometer. A standard curve was created by plotting absorbance values of known concentrations of tannic acid solution. Total phenolic content was expressed as tannic acid equivalents (mg) per gram of extract.

Palatability bioassay

A no-choice test was carried out to evaluate leaf palatability for sawfly larvae (Hymenoptera: Tenthredinidae), the main herbivore found on *A. chilensis*. Leaves from

saplings growing in open and shaded environments (sun leaves and shade leaves, hereafter) were collected in one of the study sites. Leaves of approximately the same size (5–6 cm long) were placed into individual Petri dishes with moistened filter paper (one leaf per Petri dish, 16 replicates per light environment). Thirty-two sawfly larvae (second–third instar) were collected on *A. chilensis* saplings of the same study site and starved for 24 h before the test was run. Larvae were put singly on Petri dishes, 16 on sun leaves and 16 on shade leaves. We took digital pictures of all 32 leaves three times: just before larvae were put into Petri dishes, 6 h later, and 18 h later. After 24 h the test was ended and we checked for larval mortality. We quantified leaf area removed by larvae (mm²) from the pictures using image analysis software (Sigma Scan Pro). Leaf consumption was estimated from the difference in leaf area between the first picture and those taken 6 and 18 h later.

Statistical analysis

Field herbivory patterns were analyzed with a two-way ANOVA with Site and Light environment as main effects. Because there were no significant effects of Site ($F_{2,84} = 0.09, P > 0.91$) or Site × Light environment ($F_{2,84} = 0.38, P > 0.68$) on herbivory, data from the three sites were pooled and leaf traits were compared between light environments using a one-way ANOVA. Insect abundance in the contrasting light environments was compared by a *t*-test. This was done for each taxonomic order and for overall insect abundance. In order to assess the relationship between herbivory (dependent variable) and each of the plant traits measured (leaf thickness, trichome density, water content, alkaloid and phenol concentration) we carried out a multiple regression analysis. This statistical procedure allowed us to test the relationship of each trait with herbivory taking into account the effect of correlated traits also included in the analysis. Differences in palatability between sun and shade leaves were evaluated by a *t*-test for independent samples.

Results

Data from the three field sites indicate that saplings of *A. chilensis* growing in shaded environments sustained greater herbivory damage than those growing in sunlit environments ($F_{1,88} = 80.02, P < 0.001$; one-way ANOVA) (Fig. 1). Our estimator of insect abundance showed similar values in open and shaded environments. This was true for overall insect abundance per plant (sun: 11.13 ± 0.72 ; shade: 10.53 ± 0.52 ; mean ± standard error [SE]; $t_{28} = 0.676, P > 0.50$) and for each of the taxonomic orders found (Fig. 2). Although we did not determine the feeding

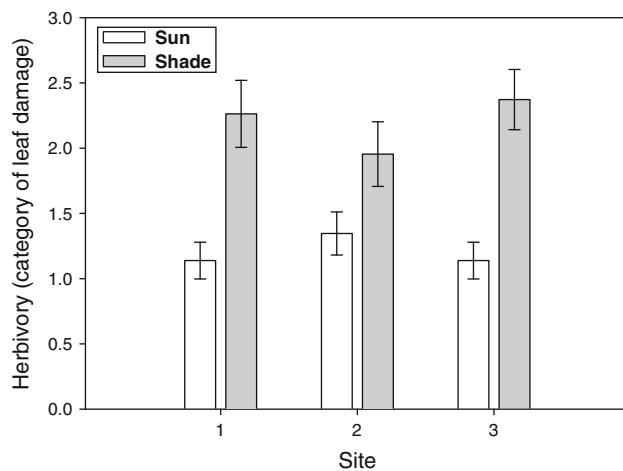


Fig. 1 Herbivory on *Aristotelia chilensis* saplings growing in open (*sun*) and shaded environments (*shade*) in three sites in central Chile. Herbivory was estimated visually as percentage of leaf area damaged and classified into five categories: 1 (<25%), 2 (≥25 to <50%), 3 (≥50 to <75%) and 4 (>75%). Means ± standard error (SE) are shown ($N = 45$ plants per light environment). See text for statistical analysis of pooled data

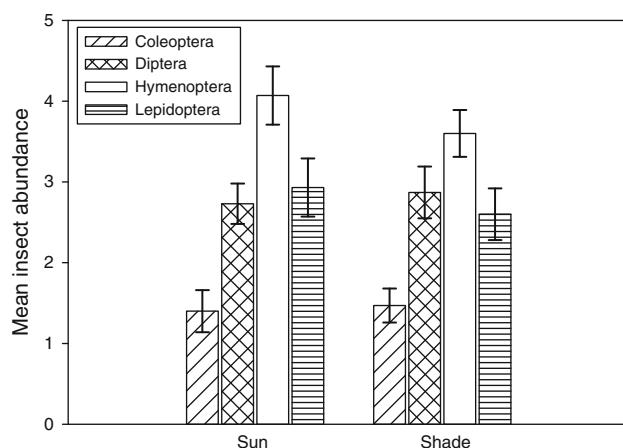


Fig. 2 Abundance of insects (number of insects per plant counted in 5 min) by taxonomic order on *Aristotelia chilensis* saplings growing in open (*sun*) and shaded environments (*shade*). There was no difference in abundance between light environments for any of the insect orders ($t_{28} < 1.02$ and $P > 0.32$ in all cases; t -test) nor for overall insect abundance ($t_{28} = 0.676$; $P > 0.50$). Means ± standard error (SE) are shown. $N = 15$ plants per light environment

habit of all individual insects found, it is reasonable to assume that the abundance of herbivorous insects did not differ between light environments.

While plants in open environments had thicker leaves and lower leaf water content than those in shaded environments, no difference in foliar trichome density was detected (Table 1). Both total alkaloids and total phenolics were in higher concentration in saplings growing in open environments (Table 1).

Table 1 Leaf traits (mean ± SE) of *Aristotelia chilensis* saplings growing in open (sun) and shaded environments (shade) in three sites in central Chile ($N = 45$ replicates per light environment)

Traits	Sun	Shade	$F_{1,88}$
Leaf thickness (mm)	0.37 ± 0.01	0.25 ± 0.01	161.2***
Trichome density (cm^2)	27.40 ± 1.54	28.67 ± 1.91	0.268 NS
Water content (%)	51.21 ± 1.03	60.32 ± 1.15	17.45***
Alkaloids (mg/100 g)	0.071 ± 0.003	0.045 ± 0.003	35.52***
Phenolics (mg/g)	3.68 ± 0.17	0.02 ± 0.10	70.87***

F-ratios after a one-way ANOVA

NS non-significant differences, SE standard error

*** $P < 0.001$

Table 2 Standardized regression coefficients (betas) of multiple regression of herbivory damage against leaf traits of *Aristotelia chilensis* saplings ($N = 90$)

Traits	Beta ± standard error (SE)	t (84)	<i>P</i> -level
Leaf thickness	-0.42 ± 0.11	-3.659	<0.001
Trichome density	0.05 ± 0.09	0.631	>0.53
Water content	0.17 ± 0.10	-0.686	>0.095
Alkaloids	-0.06 ± 0.10	-0.607	>0.54
Phenolics	-0.10 ± 0.11	-0.911	>0.36

Full model $R^2 = 0.40$; $F_{5,84} = 11.08$; $P < 0.001$. The only significant coefficient is shown in bold

The multiple regression analysis showed that, overall, field herbivory (dependent variable) and plant traits were significantly associated (Table 2). In particular, leaf thickness was the only trait showing a significant standardized regression coefficient in this analysis (Table 2). Thus, thicker leaves received less damage by herbivores. Interestingly, separated linear regressions between herbivory and each of the plant traits showed significant relationships in all cases ($P < 0.001$), with the exception of trichome density (data not shown).

Sawfly larvae consumed more leaf tissue when fed on shade leaves. This was already evident 6 h after insects were allowed to feed ($t_{30} = -2.51$; $P < 0.05$) and the difference persisted 18 h after the onset of the test ($t_{30} = -2.16$; $P < 0.05$) (Fig. 3). Larval mortality was null at the end of the test.

Discussion

We found that *Aristotelia chilensis* saplings sustained greater insect herbivory when growing in shaded habitats. Similar patterns have been reported in a number of plant-herbivore systems (Dudt and Shure 1994; Yamasaki and Kikuzawa 2003; Molina-Montenegro et al. 2006;

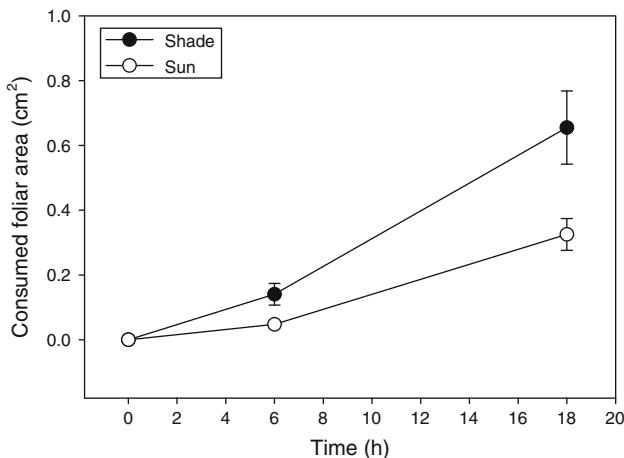


Fig. 3 Leaf area consumed by sawfly larvae (Hymenoptera: Tenthrenidae) in a no-choice test with leaves of *Aristotelia chilensis* saplings growing in open (sun) and shaded environments (shade). Means \pm standard error (SE) are shown ($N = 16$). Differences between groups are statistically significant both 6 and 18 h after the onset of the bioassay

Niesenbaum and Kluger 2006; Muth et al. 2008), but the opposite pattern, i.e., increased herbivory with solar irradiance, has also been reported (Lincoln and Mooney 1984; MacGarvin et al. 1986; Louda and Rodman 1996). When attempting to explain these patterns considering plant traits that covary with herbivory levels, authors have taken different approaches. We stress the usefulness of including multiple regression analysis in order to make inferences about the relationship between candidate plant traits and herbivory. For instance, in our study, had we conducted separate linear regressions (or correlations) between each plant trait measured and herbivory damage, or had we compared their mean values in each light environment, we would have concluded that increased leaf thickness, alkaloids and phenolics, and decreased leaf water content, could all be responsible for the lower attack rate on leaves of *A. chilensis* saplings in open habitats compared to shaded habitats. The multiple regression analysis, which accounted for correlated characters, indicated that leaf thickness was the only trait significantly associated with herbivory in the study system.

Variation in *A. chilensis* traits from sun to shade agrees with earlier work in other species which have shown an increase in phenolic content with light availability (Dudt and Shure 1994; Yamasaki and Kikuzawa 2003; Chacón and Armesto 2006) and reduced leaf water content in open habitats (Aide and Zimmerman 1990; Kyparassis et al. 1997), but results oppose the common pattern of increased alkaloids in shade conditions (Hoft et al. 1996; Salmore and Hunter 2001). However, only leaf thickness was significantly associated with herbivory damage in the field. Increased leaf thickness in *A. chilensis* in open environments

was expected from both ecophysiological considerations (Givnish 1988) and earlier work on the study species (Repetto-Giavelli et al. 2007) and other species (Gianoli et al. 2007; Muth et al. 2008). Studies at the intraspecific level indicate that leaf thickness and leaf toughness may be correlated (Gianoli and Hannunen 2000; Onoda et al. 2008) and hence the apparent protection against herbivory of leaf thickness in *A. chilensis* might be due to leaf toughness, which has been shown to be negatively associated with herbivory in the field (Coley and Barone 1996). We explored this possibility *a posteriori* with another set of leaves from one of the study sites. We made separate estimations for sun leaves and shade leaves because the correlation between leaf thickness and leaf toughness may vary with the environment (Gianoli and Hannunen 2000). Estimated leaf toughness (work to punch) and leaf thickness were positively correlated ($r_{\text{sun}} = 0.60$; $r_{\text{shade}} = 0.63$; $P < 0.001$ and $N = 70$ in both cases). Leaf toughness deters insect feeding by offering greater mechanical resistance to leaf lamina crack (Choong 1996) and by eroding mouthpieces (Raupp 1985). This might explain results of the palatability bioassay, where greater consumption of shade leaves by sawfly larvae was observed. This result is consistent with field herbivory patterns and, together with the outcome of estimations of insect abundance, renders unlikely that the observed differential herbivory in *A. chilensis* was due to greater herbivory pressure in shade habitats. It should be noted, however, that our estimates of insect abundance, which showed similar insect density on *A. chilensis* saplings in sun and shade, were not conducted throughout the whole season but during the peak of insect activity. The occurrence of differential variation in herbivore abundance during the season for sun and shade habitats cannot be ruled out. If this pattern is verified, it would be of interest to track parallel changes in the response variables herein considered in order to test whether the general explanations hold true.

The main herbivore on *A. chilensis*, a sawfly species (Hymenoptera: Tenthrenidae), was seemingly not deterred by chemical defenses but by a general feature of the leaf (thickness or toughness). Likewise, larval performance of four sawfly species was strongly associated with leaf water content of *Betula pubescens* but phenolics explained only a small part of the variance in leaf consumption (Riipi et al. 2005). The Tenthrenidae larvae showed the particular behavior of covering their body with their own feces, a feature that is typical of rather specialized insect species. Moreover, this species was only found on *A. chilensis* in the three study sites (Guerra 2006, personal observations). These observations suggest that this Tenthrenidae is probably a specialist insect. However, in view of the similar herbivore density in habitats with contrasting plant quality, it may be inferred that *A. chilensis* herbivores do not discriminate between sun and shade plants.

Our study showed that herbivory load on the light-demanding species *A. chilensis* is greater in the shade. Besides its intrinsic physiological constraints to cope with reduced light availability, herbivory may further limit the shade distribution of *A. chilensis*. Louda and Rodman (1996) concluded that greater insect herbivory in the sun is a major factor in the shade habitat restriction of a perennial herb. Among several traits evaluated, we identified leaf thickness (or the associated leaf toughness) as the plant trait most likely explaining the differential herbivory on *A. chilensis*. This was possible by the use of multiple regression analysis, a statistical tool that has boosted the explanatory power of natural selection studies (Brodie et al. 1995) and that should be used more often to explain patterns of herbivory in the field when candidate plant traits have been measured. Plant defensive traits may be positively correlated (Koricheva et al. 2004). A recent study showed a positive correlation between leaf toughness and phenolics across species (Read et al. 2009). Therefore, to make valid inferences about the relationship between herbivory damage in the field and plant traits, the analysis must take into account character correlations (e.g., Agrawal 2005). Although the main conclusion of this study is grounded on several lines of evidence, ecological factors other than plant quality may also be involved in the field herbivory pattern. For instance, increased pressure by natural enemies or reduced performance due to larval dehydration in sun sites as compared to shade environments (see Connor 2006) might contribute to explain the observed greater insect herbivory on *A. chilensis* in shaded habitats. Further research should also consider the role of these biotic and abiotic factors on the pattern herein reported.

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References

- Agrawal AA (2005) Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evol Ecol Res* 7:651–667
- Agrawal AA, Fishbein M (2006) Plant defense syndromes. *Ecology* 87:S132–S149
- Aide TM, Zimmerman JK (1990) Patterns of insect herbivory, growth, and survivorship in juveniles of a neotropical liana. *Ecology* 71:1412–1421
- Berenbaum MR (1991) Coumarins. In: Rosenthal GA, Berenbaum MR (eds) *Herbivores: their interactions with secondary plant metabolites*. Academic Press, San Diego, pp 221–249
- Boege K, Dirzo R (2004) Intraspecific variation in growth, defense and herbivory in *Dialium guianense* (Caesalpiniaceae) mediated by edaphic heterogeneity. *Plant Ecol* 175:59–69
- Brodie ED, Moore AJ, Janzen FJ (1995) Visualizing and quantifying natural selection. *Trends Ecol Evol* 10:313–318
- Bustamante RO, Simonetti JA, Grez AA, San Martín J (2005) Fragmentación y dinámica de regeneración del bosque maulino: diagnóstico actual y perspectivas futuras. In: Smith-Ramírez C, Armesto JJ, Valdovinos C (eds) *Historia, biodiversidad y ecología de los bosques costeros de Chile*. Editorial Universitaria, Santiago, pp 555–564
- Chacón P, Armesto JJ (2006) Do carbon-based defences reduce foliar damage? Habitat-related effects on tree seedling performance in a temperate rainforest of Chiloé Island, Chile. *Oecologia* 146: 555–565
- Choong MF (1996) What makes a leaf tough and how this affects the pattern of *Castanopsis fissa* leaf consumption by caterpillars. *Funct Ecol* 10:668–674
- Coley PD, Barone JA (1996) Herbivory and plant defenses in tropical forest. *Annu Rev Ecol Syst* 21:305–335
- Collinge SK, Louda SM (1988) Herbivory by leaf miners in response to experimental shading of a native crucifer. *Oecologia* 75: 559–566
- Connor EF (2006) Effects of the light environment on oviposition preference and survival of a leaf-mining moth, *Cameraria hamadryadella* (Lepidoptera: Gracillariidae), on *Quercus alba* L. *Ecol Entomol* 31:179–184
- Crone EE, Jones CG (1999) The dynamics of carbon: nutrient balance: effects of cottonwood acclimation to short- and long-term shade on beetle feeding preferences. *J Chem Ecol* 25: 635–656
- Damascos MA, Prado CHBA (2001) Fenología foliar y sus caracteres asociados en la especie invierno-verde *Aristotelia chilensis* (Mol.) Stuntz (Elaeocarpaceae). *Rev Chil Hist Nat* 74: 805–815
- De la Vega X, Grez AA (2008) Composición, riqueza de especies y abundancia de insectos defoliadores de actividad nocturna asociados a *Aristotelia chilensis* (maqui) en el bosque maulino fragmentado. *Rev Chil Hist Nat* 81:221–238
- Djilani A, Legseir B, Soulaimani R, Dickob A, Younos C (2006) New extraction technique for alkaloids. *J Braz Chem Soc* 17:518–520
- Dudt JF, Shure DJ (1994) The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology* 75:86–98
- Elger A, Lemoine DG, Fenner M, Hanley ME (2009) Plant ontogeny and chemical defence: older seedlings are better defended. *Oikos* 118:767–773
- Fritz RS, Simms EL (1992) *Plant resistance to herbivores and pathogens*. University Chicago Press, Chicago
- Gianoli E, Hannunen S (2000) Plasticity of leaf traits and insect herbivory in *Solanum incanum* L. (Solanaceae) in Nguruman, SW Kenya. *Afr J Ecol* 38:183–187
- Gianoli E, Molina-Montenegro MA, Becerra J (2007) Interactive effects of leaf damage, light intensity and support availability on chemical defenses and morphology of a twining vine. *J Chem Ecol* 33:95–103
- Givnish TJ (1988) Adaptation to sun and shade: a whole-plant perspective. *Aust J Plant Physiol* 15:63–92
- Henriksson J, Haukioja E, Ossipov V, Sillanpää S, Kapari L, Pihlaja K (2003) Effects of host shading on consumption and growth of the geometrid *Epirlita autumnata*: interactive roles of water, primary and secondary compounds. *Oikos* 103:3–16
- Hoft M, Verpoorte R, Beck E (1996) Growth and alkaloid contents in leaves of *Tabernaemontana pachysiphon* Stapf (Apocynaceae) as influenced by light intensity, water and nutrient supply. *Oecologia* 107:160–169
- Johnson MTJ, Agrawal AA (2005) Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology* 86:874–888

- Koricheva J, Nykänen H, Gianoli E (2004) Meta-analysis of trade-offs among plant antiherbivore defenses: are plants Jacks-of-all-trades, masters of all? *Am Nat* 163:E64–E75
- Kyparissis A, Grammatikopoulos G, Manetas Y (1997) Leaf demography and photosynthesis as affected by the environment in the drought semi-deciduous Mediterranean shrub *Phlomis fruticosa* L. *Acta Oecol* 18:543–555
- Larsson S, Wiren A, Lundgren L, Ericsson T (1986) Effects of light and nutrient stress on leaf phenolic chemistry in *Salix dasyclados* and susceptibility to *Galerucella lineola* (Coleoptera). *Oikos* 47:205–210
- Lincoln DE, Mooney HA (1984) Herbivory on *Diplacus aurantiacus* shrubs in sun and shade. *Oecologia* 64:173–176
- Louda SM, Collinge SK (1992) Plant-resistance to insect herbivores—a field-test of the environmental-stress hypothesis. *Ecolgy* 73:153–169
- Louda SM, Rodman JE (1996) Insect herbivory as a major factor in the shade distribution of a native crucifer (*Cardamine cordifolia*, A. Grey, bittercress). *J Ecol* 84:229–238
- Lucas WP, Turner MI, Dominy NJ, Yamashita N (2000) Mechanical defenses to herbivory. *Ann Bot* 86:913–920
- Lusk CH (2002) Leaf area accumulation helps juvenile evergreen trees tolerate shade in a temperate rainforest. *Oecologia* 132:188–196
- MacGarvin M, Lawton JH, Heads PJ (1986) The herbivorous insect communities of open and woodland bracken: observations, experiments, and habitat manipulations. *Oikos* 47:135–148
- Marquis RJ (1992) Selective impact of herbivores. In: Fritz RS, Simms EL (eds) *Plant resistance to herbivores and pathogens*. Univ Chicago Press, Chicago, pp 301–325
- Molina-Montenegro MA, Avila P, Hurtado R, Valdivia AL, Gianoli E (2006) Leaf trichome density may explain herbivory patterns of *Actinote* sp. (Lepidoptera: Acraeidae) on *Liabum mandonii* (Asteraceae) in montane humid forest (Nor Yungas, Bolivia). *Acta Oecol* 30:147–150
- Muth NZ, Kluger EC, Levy JH, Edwards MJ, Niesenbaum RA (2008) Increased per capita herbivory in the shade: necessity, feedback, or luxury consumption? *Ecoscience* 15:182–188
- Niesenbaum RA, Kluger EC (2006) When studying the effects of light on herbivory, should one consider temperature? The case of *Epimecis hortaria* F. (Lepidoptera: Geometridae) feeding on *Lindera benzoin* L. (Lauraceae). *Environ Entomol* 35:600–606
- Onoda Y, Schieving F, Anten NPR (2008) Effects of light and nutrient availability on leaf mechanical properties of *Plantago major*: a conceptual approach. *Ann Bot* 101:727–737
- Raupp MJ (1985) Effects of leaf toughness on mandibular wear of the leaf beetle *Plagiodesma versicolora*. *Ecol Entomol* 10:73–79
- Read J, Sanson GD, Caldwell E, Clissold FJ, Chatain A, Peeters P, Lamont BB, De Garine-Wichatitsky M, Jaffre T, Kerr S (2009) Correlations between leaf toughness and phenolics among species in contrasting environments of Australia and New Caledonia. *Ann Bot* 103:757–767
- Repetto-Giavelli FR, Cavieres LA, Simonetti JA (2007) Respuestas foliares de *Aristotelia chilensis* (Molina) Stuntz (Elaeocarpaceae) a la fragmentación del bosque maulino. *Rev Chil Hist Nat* 80:469–479
- Riipi M, Kause A, Haukioja E, Ossipov V, Ossipova S, Pihlaja K (2005) Variable responses of folivorous sawflies to leaf quality of mountain birch. *Can J Forest Res* 35:189–198
- Rosenthal GA, Berenbaum MR (1991) *Herbivores: their interactions with secondary plant metabolites*. Academic Press, San Diego
- Salmore AK, Hunter MD (2001) Environmental and genotypic influences on isoquinoline alkaloid content in *Sanguinaria canadensis*. *J Chem Ecol* 27:1729–1747
- Scriber JM, Slansky F (1981) The nutritional ecology of immature insects. *Annu Rev Entomol* 26:183–211
- Silva M, Bittner M, Céspedes C, Jakupovic J (1997) The alkaloids of the genus *Aristotelia*. *Aristotelia chilensis* (MOL.) Stuntz. *Bol Soc Chil Quim* 42:39–47
- Sipura M, Ikonen A, Tahvanainen J, Roininen H (2002) Why does the leaf beetle *Galerucella lineola* F. attack wetland willows? *Ecology* 83:3393–3407
- Speight MR, Hunter MD, Watt AD (1999) *Ecology of insects*. Blackwell, Oxford
- Yamasaki M, Kikuzawa K (2003) Temporal and spatial variations in leaf herbivory within a canopy of *Fagus crenata*. *Oecologia* 137:226–232
- Zamora R, Hódar JA, Gómez JM (1999) Plant-herbivore interaction: beyond a binary vision. In: Pugnaire FA, Valladares F (eds) *Handbook of functional plant ecology*. Marcel Dekker, New York, pp 677–718
- Zangerl AR, Berenbaum MR (1993) Plant chemistry, insect adaptations to plant chemistry, and host plant utilization patterns. *Ecology* 74:47–54