The role of leaf traits and bird-mediated insect predation on patterns of herbivory in a semiarid environment in central Mexico

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Abstract

Background: In arid environments plants face aridity and herbivory, therefore it has been proposed that both are convergent selective forces. However the drivers of insect herbivory in these ecosystems remain poorly understood.

Question: Does insect herbivory vary in two plant associations subject to different levels of aridity? To what extent differences in herbivory are determined either by foliar traits, or predation by birds?

Study species: *Citharexylum tetramerum*, *Viguiera pinnatilobata*, and *Solanum tridynamum*.

Study site: We compared two-plant associations: the mezquital with a closed canopy and wettest conditions and the tetechera a dry place with an open canopy at the Zapotitlán Valley, México.

Methods: We evaluated leaf traits (N, C, water content, leaf strength and trichomes), herbivore insects abundance and the effects on herbivory when some predators are excluded.

Results: Herbivory was higher in the drier site (tetechera) than in the more humid one (mezquital) in one year but not in the second one. In both plant associations herbivory increased when predators of herbivores were excluded. Plants in mezquital had more water and nitrogen content than plants in tetechera. The later had higher carbon concentration and carbon:nitrogen ratio, leaf strength and density of trichomes. Abundance of herbivore insects and insect predation were higher in the most arid site.

Conclusions: Our results show that insect herbivory increases in the most arid site and that predation by birds plays a role as a driver of herbivory, while resistance to aridity seems to be the main driver of leaf structural characteristics.

Key words: herbivory, leaf traits, bird predation, Tehuacán-Cuicatlán, plant defence.

El papel de las características foliares y la depredación por aves en los patrones de herbivoría en un ambiente semiárido del centro de México

Resumen

Antecedentes: En ecosistemas áridos las plantas se enfrentan a la sequía y a la herbivoría, por lo cual se ha postulado que éstas pueden actuar como presiones de selección convergentes sobre los atributos foliares. Sin embargo, los determinantes de la herbivoría han sido poco explorados.

Pregunta: ¿Los niveles de herbivoría varían en sitios con condiciones de humedad contrastantes? ¿La herbivoría está determinada por las características nutritivas/defensivas de las plantas o por la depredación por aves?

Especies de estudio: *Citharexylum tetramerum*, *Viguiera pinnatilobata*, and *Solanum tridynamum*.

Sitio de estudio: Comparamos dos asociaciones vegetales: el mezquital con un dosel cerrado y húmedo y la tetechera un sitio seco con un dosel muy abierto, en el Valle de Zapotitlán, México.

Métodos: Evaluamos características foliares (N, C, contenido de agua, dureza y tricomas), la abundancia de insectos herbívoros y las consecuencias de la exclusión de algunos depredadores sobre los niveles de herbivoría.

Resultados: Los niveles de herbivoría fueron mayores en el sitio más árido (tetechera) comparado con el mezquital pero solo en un año. La herbivoría aumentó cuando los depredadores de los herbívoros fueron excluidos en las dos asociaciones vegetales. Las plantas del mezquital tuvieron un mayor contenido de agua y nitrógeno que las plantas de la tetechera. Éstas últimas, tuvieron una mayor concentración de carbono:nitrogeno, dureza y densidad de tricomas. La abundancia de insectos herbívoros y las tasas de depredación fueron mayores en la tetechera.

Conclusiones: La herbivoría aumenta en la asociación vegetal más árida. La depredación por aves influye en los niveles de herbivoría, mientras que la resistencia a la sequía parece ser el determinante de las características estructurales de las hojas.

Palabras clave: herbivoría, características foliares, depredación por aves, Tehuacán-Cuicatlán, defensa en plantas

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I t is generally acknowledged that plants of arid and semi arid ecosystems have evolved leaf traits that minimize water losses and heat gain, and maximize the protection against UV radiation. This includes trichomes, wax layers and leaf strength (Hanley *et al*. 2007). These leaf traits have also been recognized as anti-herbivore defences in some systems (Coley 1983, Adler *et al*. 2004). Because the leaf strength and the density of trichomes usually correlate negatively with herbivory and water losses (Quiroga *et al*. 2010) aridity and herbivory have been hypothesized as synergic selective pressures driving the evolution of these leaf traits (Adler *et al*. 2004, Quiroga *et al*. 2010). However, this evolutionary convergence model has been mainly focused in large herbivores and in grasses, and the role of the insect herbivory and its ecological consequences on other plant species in arid ecosystems remains poorly understood (Milchunas & Lauenroth 1993, Adler *et al*. 2004).

In general, aridity correlates positively with the availability of light, and negatively with soil water content and the availability of soil nutrients for the plants —all of which are factors that affect plant performance, plant resistance and tolerance to insect herbivory (Louda *et al*. 1987, Nichols-Orians 1991, Louda & Collinge 1992, Stowe *et al*. 1994, Richards & Coley, 2007). Plant defence theories predict that structural defences are more frequently encountered in resource-poor environments (Coley 1987, Lim & Turner 1996, Fine *et al*. 2004, 2006, Hanley *et al*. 2007). For instance, Grubb (1992) observed that the distribution of spinescence follows a general pattern of being more common in the drier, less fertile areas of the planet, and the density of trichomes and leaf strength also correlate negatively with the availability of resources (Grubb 1986, Turner 1994, Hoffland *et al*. 2000, Salleo & Nardini 2000, Fine *et al*. 2006, Wright *et al*. 2004). In this context, the carbon/nutrients balance hypothesis (Bryant *et al*. 1983) states that the resources acquired in excess are canalized into the production of defences. If soil nutrients (nitrogen) limit plant growth then excess carbon is canalized to carbon-based defences such as trichomes. In contrast, if growth is limited by light plants are expected to allocate any excess nitrogen to defensive nitrogen-based compounds. By the other hand, the growth-differentiation balance hypothesis (Herms & Mattson 1992), states that there is a physiological trade-off between growth and differentiation related processes (*e.g*. secondary metabolites) that change with the availability of resources. This hypothesis predicts that 1) plants growing in very low levels of resources should be limited in growth and photosynthetic capability and exhibit low levels of defence, 2) Plants facing intermediate resource availability will have high levels of differentiation and an moderate growth, and 3) plants in high resources sites will allocate resources to growth at the expense of defences (Wilkens 1997).

Here we estimated herbivory damage in two shrub species and one herbaceous species in two plant associations of the xerophytic shrubland of the Zapotitlán Valley. In addition to naturally occurring herbivory we excluded experimentally some of the main predators of herbivores (birds) in the two plant associations and assessed changes in herbivory. Also, we evaluated the abundance of herbivores, foliar traits (structural leaf defences, nutritional characteristics and concentration of soil nutrients) and predation by birds to gain insights on the mechanism that regulate herbivory in arid ecosystems, one of the most extend habitat on the land surface (over 33 %, Cooke *et al*. 2013) but where only 3 % of the herbivory studies have been conducted (R Guevara pers.com).

The study was conducted in two different plant associations (tetechera and mezquital) with different environmental characteristics. The mezquital is a dense plant association dominated by legumes that are associated to free leaving diazotrophs (Barness *et al*. 2009) increasing soil mineral content within this plant association (Perroni-Ventura *et al*. 2006). In contrast, the tetechera has an open canopy dominated by the columnar cacti *Neobuxbaumia tetetzo*. This plant association is located in sloping sites and in highly porous soils of low fertility (Valiente-Banuet *et al*. 2000). In this context we aimed to answer the following questions: a) does insect herbivory vary between plant associations (tetechera and mezquitera)? If so b) to what extent differences in herbivory are determined either by foliar traits, or predation by birds? Thus based on the potential resource availability at each plant associations we expect to find: 1) high levels of herbivory and herbivore abundance in the mezquital, associated with, 2) high soil mineral nutrient content and water availability, 3) high nutrient and water content of leaves, and 4) low levels of structural defences in the mezquital as compared with the tetechera.

Material and methods

Study area. The study was done in the xerophytic shrubland of the Zapotitlán Valley, Puebla, Mexico within the Tehucán-Cuicatlán Biosphere Reserve, one of the main reservoirs of biodiversity of arid and semiarid ecosystems in Mexico (Dávila *et al*. 2002). The study site is located in the mountainous region of south-eastern Puebla (18° 20' N, 97° 28' W; elevation 1,450-1,600 m). The mean annual temperature is 21 °C with rare freezing events, with an average accumulated precipitation of 380 mm. The rainy season extends from May to October and the dry season from November to April (Montaña & Valiente-Banuet, 1998). According to the FAO/UNESCO soils are xerosols derived from calcareous sedimentary marine rocks from the Cretaceous. Soils classes are calcaric fluvisol and calcaric regosol with sandy loam texture and pH ranging between 7 and 8.6 (López-Galindo *et al*. 2003). The predominant vegetation corresponds to the arid tropical scrub of Leopold (1950). Plant diversity in the Tehucán-Cuicatlán Biosphere Reserve is distributed in six vegetations types comprising 30 different plant associations (Valiente-Banuet *et al*. 2000) harbouring over 2,800 plant species, of which up to 13 % are endemic to the reserve (Dávila *et al*. 2002).

This study was restricted to two contrasting plant associations: mezquital and tetechera. The mezquital is found at 1,500 m asl, and is characterized by the predominance of mezquite trees (*Prosopis laevigata*). It is usually located on alluvial plains in the lower parts of the valley close to the rivers' banks with sandy soil and a 3-5 cm litter layer (López-Galindo *et al*. 2003, García-Chávez *et al*. 2010). Woody species in the mezquital are evergreen and reach up to 2-5 m in height (*P. laevigata*, *Cercidium praecox*, *Vallesia glabra*, *Celtis pallida* and *Castela tortuosa*). Columnar cacti such as *Myrtillocactus geometrizans*, *Pachycereus marginatus* and *Stenocereus pruinosus* are also common in the mezquital. In contrast, the tetechera in found between 1,600-1,800 m asl, and is dominated by the columnar cactus *Neobuxbaumia tetetzo*, cacti trunks reach up to 8 m in height and protrude from the shrub layer form by *Mimosa luisana*, *Agave karwinskii*, *A. marmorata*, *Bursera aloexylon*, *Ceiba aesculifolia Acacia coulteri* among others. The substrate is rocky with a very thin layer of soil (Valiente-Banuet *et al*. 2000, López-Galindo *et al*. 2003).

Study species. We selected species that were abundant in both plant associations (mezquital and tetechera), and from different families to minimize phylogenetic confounding factors. The selected species were the shrubs *Citharexylum tetramerum* Brandegee (Verbenaceae) and *Viguiera pinnatilobata* (Sch. Bip.) S.F. Blake (Asteraceae), and the annual herb *Solanum tridynamum* Dunal (Solanaceae).

Herbivory measurements. In October 2012 we selected hap-hazardously 20 individuals of the three study species within each plant association and collected at least 10 leaves of each selected plant. Plants were up to 50-100 cm tall and all leaves in each plant (< 50) were numbered from the lower position to the top and then 10 of them selected using random numbers from a uniform distribution. In the laboratory we selected randomly 100 leaves of each species within each plant association. Again all leaves were numbered and then selected following a sequence of random numbers. Percent leaf area eaten by herbivores was estimated visually including the following leaf damage types (missing parts of the leaf lamina): holes, scraped-off areas and incomplete leaf margin. Each selected leaf was scored following the protocol proposed by Dirzo & Domínguez (1995): zero (when the leaf was intact), one (a leaf with barely perceptible damage up to 6 % of the leaf area damaged); two (over 6 % and up to 12 % of damage), three (12-25 %), four (25-50 %) and five (over 50 % of damage). The score for each leaf was used to calculate an index of damage (*ID*) as

$$ID = \sum_{i=0}^{5} (X_i \ n_i)/N$$

where X_i is the i scores of damage (0 to 5), n_i is the frequency of leaves with score X_i, and N is the total number of scored leaves. Then, the index of damage was expressed as percentage of herbivory (hereafter herbivory) based on a simple cubic linear model with intercept set to zero *Herbivory* % = 5.6131 *ID*-2.4505 *ID*² + 0.8691*ID*³. This model is based on the premise that

the expected herbivory for each of the X_i score of damage is given by

$$EH(\%) = H_{i\,max} - (H_{i\,max} - H_{i\,min})/2$$

where $H_{i\,max}$ and $H_{i\,min}$ correspond to the upper and asymptotic lower bounds of herbivory, respectively, of each of the X_i score of damage based on this equation the expected percentages of herbivory are 0, 3, 6, 18.5, 37.5 and 75 % for scores 0 to 5 respectively.

Abundance of herbivores. We conducted censuses of herbivores in the same individuals selected to estimate herbivory. Each plant was scanned by necked eye for a period of three minutes and we recorded the taxonomic order (Coleoptera, Diptera, Lepidoptera, Homoptera and Orthoptera) of each herbivore that was observed. Censuses were repeated for three consecutive days in October 2012, and were done between 08:00 and 17:00 hours. For the purpose of analyses all counts made over the three days of each individual plant were added up.

Leaf defensive structural traits. Defensive traits, leaf strength and trichomes density, were quantified in each of the three species in each plant association. Leaf strength (the force needed to break the leaf lamina) was estimate in three randomly selected intact leaves of at least 10 individuals of each species in each plant association with a penetrometer (Silverado FHT 803). Each leaf was punched three times avoiding the main and secondary veins, and the average of the three measurements was used in the analysis.

Density of leaf trichomes was estimate in two randomly selected intact leaves of 10 individuals of each species in each plant association. All trichomes born from the leaf surface along a 20 × 1 mm transect drew on the adaxial leaf surface were counted at 40x magnification with a stereoscope.

Water and nutrient content of leaves. Foliage nutritional value was estimated as the content of nitrogen, carbon and water. For each species in each site we collected between three and five intact leaves of each of the marked individual plants. Leaves were weighed in fresh, then dried in the oven for 72 h at 60 °C and then weighed again. The percent of water content was estimated as the ratio of the differences between the fresh and dry weights divided by dry weight and multiplied by 100. The percent of nitrogen and carbon content were estimated with an automatic analyser (TruSpec CN, Leco Corporation 2002) in the laboratory of chemical analysis of soils, water and plants at INECOL.

Soil nutrient availability. We collected ten soil samples hap-hazardously distributed in each plant association. Water, carbon and nitrogen content were determined following the same protocols describe above for the nutrient content of leaves. For water content we used 10 g soil samples weighted immediately after collection and the oven dried at 100 °C for 48 h.

Exclusion experiment. In July 2014 we excluded birds and bats with experimental exclosures. While plants up to 50 cm height were fully excluded, for taller plants we installed the exclosure on a randomly chosen branch. We located 40 individuals of each of the three species in each plant association and excluded 20 of them while the other 20 remained unprotected, a total of 240 plants and half of them caged. Exclosures were constructed with agricultural netting (2 × 2 cm mesh size). The exclosures barred birds and bats, but allowed access to small lizards, mice and arthropods. Exclusions were in place for three months that is about the maximum life span of the leaves of the three studied species. Then in October 2014 we estimated herbivory in situ, we considered all the leaves in the plant or selected branches of large plants in all of the 240 plants.

Predation of artificial caterpillars: exclusion experiment. A caterpillar predation experiment was conducted in October 2014. We used the same 240 plants of the exclosure experiment. We built artificial caterpillars by putting together a 3 cm long brown plasticine segment with a 0.5 cm long red plasticine segment both of 4.1 mm in diameter simulating the body and head of an actual caterpillar respectively. Plasticine segments were obtained by squeezing the kneaded plasticine with a lemon squeezer. One end of a 10 cm long monofilament fishing line was embedded into each artificial caterpillar and the other end was used to tie up the artificial caterpillar to the host plants. Three artificial caterpillars were placed on the leaves and stems of each of the 240 focal plants (excluded and not excluded) for a total of 720 artificial caterpillars. All cages were kept in place still

through this experiment of predation; in other words, half of the caterpillars were inaccessible to predators (birds and bats). After three days we collected all the artificial caterpillars and recorded those attacked by birds. Bird attacks were inferred from the beak marks left on the plasticine caterpillars. Other types of predation (bats, ants, possible mice, etc) were ignoring because of their low frequency and difficulty in assigning them to a particular type of predator.

Data analyses. To analyse herbivory levels we used a bootstrap-based comparison of means *t*-test. First we resample with replacement the herbivory scores of the leaves of each species in each plant association, and we calculated the index of damage of each species in each plant association, and expressed it as percentage of herbivory as describe above. We repeated this process 1,000 times, and based on the collection of herbivory scores we calculated the mean and standard deviation (in this case corresponding to the standard error) of each sample. Then we used a mean comparison test between mean values of each species in each of the two plant associations. Similar procedures were followed to test differences in herbivory between vegetation associations and species. The abundance of different types of herbivores between plant associations was tested with a chi-square test of the contingence table.

Mixed effects models were used to analyse the abundance of herbivores, leaf strength, density of leaf trichomes and the content of nutrients in the leaves. The fixed effects were vegetation association (two levels) nested within species (three levels), and in the random component we modeled the overall mean as a function of the identity of the sampled individuals (random factor) of each species at each plant association. Also we modeled the variance with power function to meet model assumptions. We used one-way ANOVA models to analyse soil nutrient contents between plant associations (two levels).

To analyse the effect of the exclosures on herbivory levels and the proportion of attacks to artificial caterpillars we used a linear mixed effects model fixed factors included species, plant association, and the enclosure treatment as main factors and the three double interactions thereof. In the random component of the model we included the identity of the individual plants within each plant association. Additionally we modeled the variance with an exponential function to meet model assumptions. All analyses were performed in R 2.1.5.2 (R Core team, 2012).

Results

Herbivory. Most of the damaged leaves (63.1 %) had less than 6 % of herbivory, and 18 % and 10 % of the recorded leaves had damages ranging between 6 to 12 % and 12 to 25 % respective-

Figure 1. Herbivory (a) and herbivores abundance (b), in three plant species, *Citharexylum tetramerum* (C), *Viguiera pinnatilobata* (V) and *Solanum tridynamum* (S) in two plant associations: mezquital (black dots) and tetechera (grey dots) in the xerophytic shrubland of the Zapotitlán Valley in central Mexico.
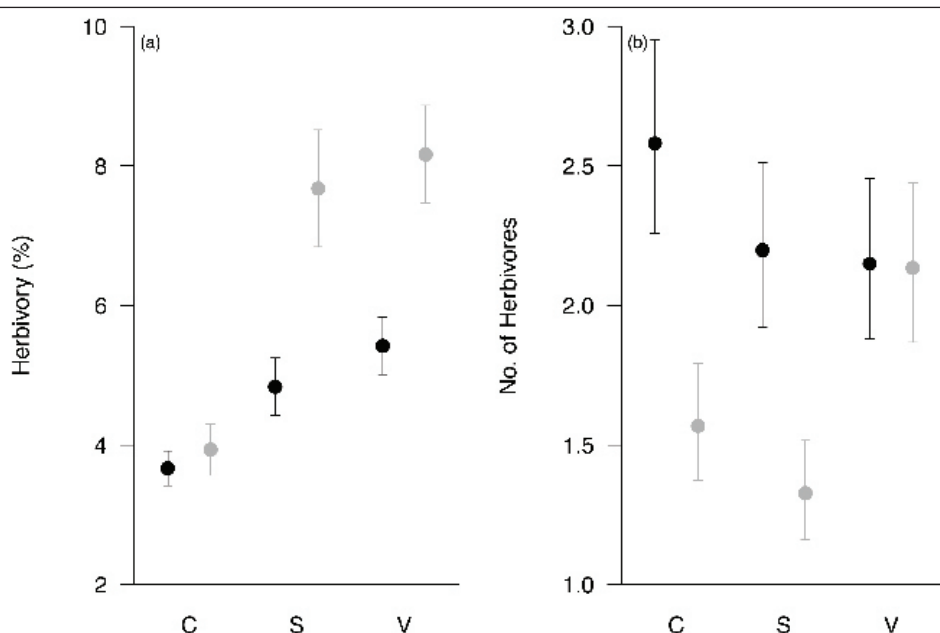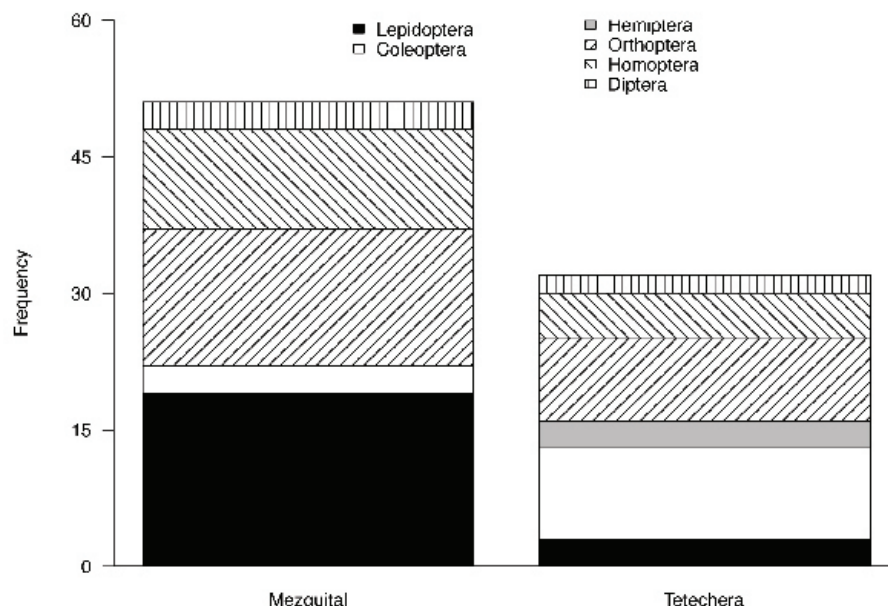
Figure 2. Frequency of herbivores in two plant associations: mezquital and tetechera in the xerophytic shrubland of the Zapotitlán Valley in central Mexico.

ly— only 8.5 % of the leaves showed damage higher than 25 %. Overall, herbivory was 36 % higher ($t = 4.1, p = 0.027$) in the tetechera (herbivory = 6.3 %) than in the mezquital (4.6 %), and this difference between plant associations was driven by the herbivory in *Solanum tridynamum* and *Viguiera pinnatilobata* that showed higher herbivory in the tetechera than in the mezquital ($t = 2.91, p = 0.05$, and $t = 3.38, p = 0.038$ respectively) while *Citharexylum tetramerum* showed no significant differences between plant associations ($t = 0.58, p = 0.3102$, Figure 1a).

Overall *Citharexylum tetramerum* showed the lowest herbivory (3.8 %) and it was significantly different from *Solanum tridynamum* (6.1 %, t = 4.5, p = 0.0224) and *Viguiera pinnatilobata* (6.6 %, $t = 6.3, p = 0.0122$), and there was no significant difference between the herbivory of the latter two species ($t = 0.95, p = 0.2222$).

Abundance of herbivores. Overall the abundance of herbivores was 59 % higher in the mezquital than in the tetechera ($t = 7.0, p = 0.0162$). In *Citharexylum tetramerum* and *Solanum tridyna-*

Figure 3. Leaf structural defenses, leaf strength (a) and density of trichomes (b), of three plant species, *Citharexylum tetramerum* (C), *Viguiera pinnatilobata* (V) and *Solanum tridynamum* (S) in two plant associations: mezquital (black dots) and tetechera (grey dots) in the xerophytic shrubland of the Zapotitlán Valley in central Mexico.
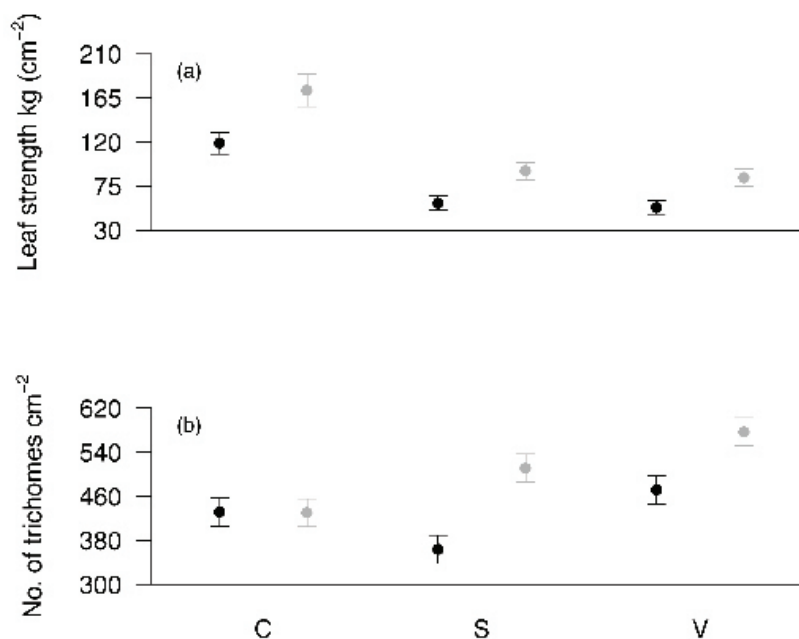
Figure 4. Foliar nutritional traits: water content (a), nitrogen content (b), carbon content (c) and carbon/nitrogen ratio (d), of three plant species *Citharexylum tetramerum* (C), *Viguiera pinnatilobata* (V) and *Solanum tridynamum* (S) in two plant associations: mezquital (black dots) and tetechera (grey dots) in the xerophytic shrubland of the Zapotitlán Valley in central Mexico.
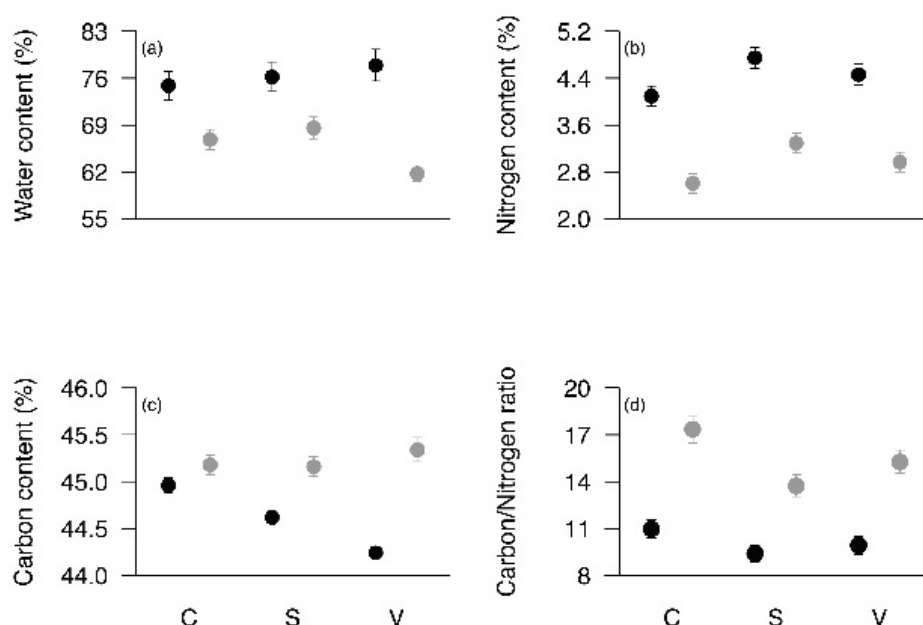

mum (Table 1a and Figure 1b) the average abundance of herbivores in the mezquital was 65 % higher than in the tetechera ($t = 2.6, p = 0.0105$; and $t = 2.7, p = 0.0008$, respectively). In *Viguiera pinnatilobata* there were no significant differences in the abundance of herbivores between the two plant associations ($t < 0.1, p = 0.9723$). In general, there were more caterpillars in the mezquital while beetles and bugs were more abundant in the tetechera ($\chi^2 = 19.0, p = 0.0019$, Figure 2).

Leaf defensive structural traits. Leaf strength varied significantly among the three species and between the plant associations (Table 1b, Figure 3a). The leaves of *C. tetramerum* were over two-fold stronger than the leaves of *S. tridynamum* and *V. pinnatilobata* ($t > 6.2, p < 0.0001$). Also, the leaves of the three species were stronger in the tetechera than in the mezquital (*S. tridynamum*: $t = 2.8, p = 0.009$; *V. pinnatilobata*: $t = 2.7, p = 0.011$; *C. tetramerum*: $t = 2.8, p = 0.011$).

The density of leaf trichomes varied significantly among the three species and between the interaction species*plant association (Table 1c and Figure 3b). The density of leaf trichomes in *V. pinnatilobata* was on average 20 % higher than in *C. tetramerum* and *S. tridynamum*. The density of leaf trichomes in *S. tridynamum* and *V. pinnatilobata* was higher in the tetechera than in the mezquital ($t = 4.4, p < 0.001$; $t = 3.1, p = 0.004$; respectively), whereas the density of trichomes in *C. tetramerum* did no vary between sites ($t = 0.5, p = 0.620$).

Water and nutrient content of leaves. Most of the nutrient content of the leaves varied significantly between plant associations and among species (Table 2, Figure 4). The water content of

Table 1. Linear mixed effects models' table for a) abundance of herbivores, b) leaf strength, and c) density of leaf trichomes, in three plant species growing in two plant associations (mezquital and tetechera) in the xerophytic shrubland of the Zapotitlán Valley in central Mexico.

	d.f.	F	p
a) Number of herbivores			
Species	2, 75	0.5	0.5950
Species (Plant Assoc.)	3, 75	4.8	0.0043
b) Leaf leaf strength			
Species	2, 47	8.25	< 0.0001
Species (Plant Assoc.)	3, 47	6.69	< 0.0001
c) Density of leaf trichomes			
Species	2, 10	8.9	< 0.0001
Species (Plant Assoc.)	3, 10	9.5	< 0.0001

Table 2. Linear mixed effects models' table for water content and foliar nutrients in three species growing in two plant associations (mezquital and tetechera) in the xerophytic shrubland of the Zapotitlán Valley in central Mexico

	d.f.	F	p
a) Water content			
Species	2, 75	13.3	< 0.0001
Species (Plant Assoc.)	3, 75	30.5	< 0.0001
b) Nitrogen content			
Species	2, 15	245.0	< 0.0001
Species (Plant Assoc.)	3, 15	878.9	< 0.0001
c) Carbon content			
Species	2, 15	83.0	< 0.0001
Species (Plant Assoc.)	3, 15	43.0	< 0.0001
d) Carbon/nitrogen ratio			
Species	2, 15	213.9	< 0.0001
Species (Plant Assoc.)	3, 15	503.9	< 0.0001

Table 3. Anova tables for the content of soil nutrients between two plant associations (mezquital and tetechera) in the xerophytic shrubland of the Zapotitlán Valley in central Mexico. In all cases there was 1 and 18 degrees of freedom in the numerator and denominator respectively.

	F	P
a) Water content	16.77	0.0007
b) Carbon content	16.23	0.0008
c) Nitrogen content	22	0.0002
d) Carbon/Nitrogen ratio	0.08	0.7805

Citharexylum tetramerum, *Solanum tridynamum* and *Viguiera pinnatilobata* were 12, 11 and 26 %, respectively, higher in the mezquital than in the tetechera ($t > 3.6, p < 0.0001$, Figure 4a). Also, the nitrogen contents of *C. tetramerum*, *S. tridynamum* and *V. pinnatilobata* were 57, 44 and 50 %, respectively, higher in the mezquital than in the tetechera ($t > 31.7, p < 0.0001$, Figure 4b). In contrast, the carbon contents of *S. tridynamum* and *V. pinnatilobata* were 10 and 20 %, respectively, higher in the tetechera than in the mezquital ($t > 5.5, p < 0.0001$) while the carbon content of the leaves of *C. tetramerum* did not differ significantly between plant associations ($t = 1.94, p = 0.071$, Figure 4c). The carbon/nitrogen ratio of *C. tetramerum*, *S. tridynamum* and *V. pinnatilobata* were 58 %, 46 % and 54 %, respectively, higher in the tetechera than in the mezquital ($t > 25.9, p < 0.0001$, Figure 4d).

Soil nutrient availability. The content of soil nutrients was significantly higher in the mezquital than in the tetechera while the carbon nitrogen ratio did not differ between the plant associations (Table 3). There was 23 %, 73 % and 79 % more water, carbon and nitrogen content respectively in the mezquital soils than in the tetechera soils (Table 4).

Herbivory: exclusion experiment. In the tetechera exclusion of predators resulted in significant increases in herbivory in the three plant species (*Citharexylum tetramerum* : $t = 2.78, p = 0.007$;

Table 4. Soil nutrient availability in two plant association (mezquital and tetechera) in the xerophytic shrubland of the Zapotitlán Valley in central Mexico. Different letters between plant association indicate statistical significant differences ($p < 0.05$)

	Mezquital	Tetechera
Water content	32.46 ± 1.24a	26.33 ± 0.84b
Carbon content	6.54 ± 0.51a	4.84 ± 0.47b
Nitrogen content	0.67 ± 0.05a	0.38 ± 0.03b
Carbon/Nitrogen ratio	9.70 ± 0.37a	9.90 ± 0.61a

Figure 5. Probability of attacks by birds on caged and exposed artificial larvae in two plant associations: mezquital (black dots) and tetechera (grey dots) in the xerophytic shrubland of the Zapotitlán Valley in central Mexico.
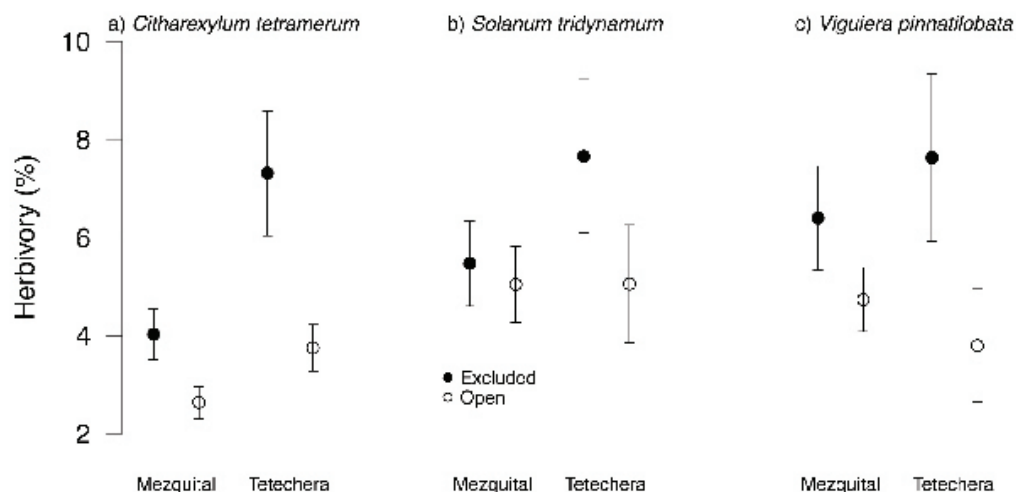

Table 5. Linear mixed effects models' table for a) herbivory and b) predation or artificial caterpillars in an enclosure experiment of three plant species growing in two contrasting plant associations (mezquital and tetechera) in Zapotitlán, Mexico.

	d.f.	F	P
a) Herbivory			
Species	2, 36	2.4	0.105
Plant Assoc.	1, 9	2.3	0.164
Exclosure	1, 50	5.4	0.024
Species* Plant Assoc.	2, 36	2.0	0.153
Species*Exclosures	2, 50	1.4	0.266
Plant Assoc.*Exclosure	1, 50	0.8	0.371
b) Predation of artificial caterpillars			
Species	2, 63	0.1	0.883
Plant Assoc.	1, 19	0.2	0.680
Exclosure	1, 13	30.9	< 0.001
Species* Plant Assoc.	2, 63	0.7	0.507
Species*Exclosures	2, 13	0.4	0.691
Plant Assoc.*Exclosure	1, 13	11.4	0.001

Solanum tridynamum: $t = 2.12$, $p = 0.038$; *Viguiera pinnatilobata*: $t = 2.08$, $p = 0.043$), whereas in the mezquital herbivory changed with exclusion only in *C. tetramerum* ($t = 2.32$, $p = 0.024$, Figure 5). None of the other main factor (species, and plant association) and interactions (species×exclusion treatment, species×plant association and plant association×exclusion

Figure 6. Figure 6. Probability of attacks by birds on open and excluded artificial larvae in two contrasting plant associations: mezquital and tetechera in the xerophytic shrubland of the Zapotitlán Valley in central Mexico.
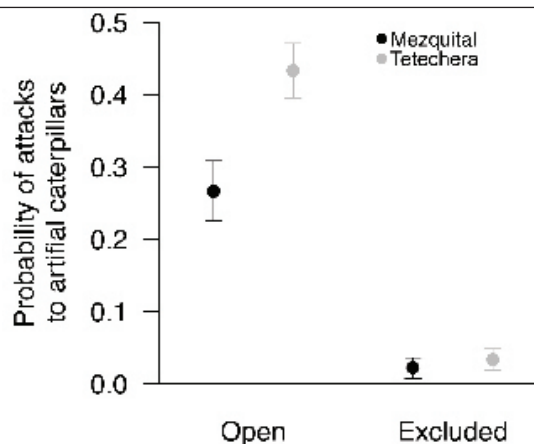
treatment) showed significant effects (Table 5a). Also the experiment revealed that herbivory changes annually. While in 2012 we detected an overall higher herbivory in the tetechera compared to the mezquital (see above), when we compared not excluded plants in 2014 we observed no significant differences between sites ($t < 1.94$, $p > 0.0593$). The overall herbivory in the tetechera was 57 % higher in 2012 than in 2014 while in the mezquital it was 11 % higher in 2012 than in 2014.

Predation of artificial caterpillars: exclusion experiment. Overall 19 % of the artificial caterpillars were attacked by birds as evidenced from the beak marks on the plasticine. Ninety-three percent of the attacks occurred in the unprotected plants (Table 5b). There was 63 % more attacks in the tetechera than in the mezquital ($t = 3.0$, $p = 0.007$, Figure 6).

Discussion and conclusions

We detected significant differences in herbivory among species and plant associations, but not all species showed the same responses to plant associations. Differences in herbivory between plant-associations were only observed in 2012 when the overall herbivory was higher. Overall, our findings oppose our initial predictions based on the Resource Availability Hypothesis (Coley 1983). Herbivory levels were higher in the resource poor and more arid site (tetechera), where leaves had also low nutritional content (water and nitrogen) and presented high levels of structural defences (leaf strength and trichomes). The exclusion of predators resulted in more herbivory levels in both plant associations.

The low nutrient content of leaves at the tetechera may be a determinant factor leading insect herbivores to consume more leaf tissue to satisfy their nutritional needs and therefore inflicting high levels of damage to the plants. In sunny environments the increase in carbon content in plant tissues and the consequent changes in the carbon to nitrogen ratio correlates negatively with the rate of acquisition of nutrients (nitrogen) by herbivores; more leaf tissue has to be consumed to assimilate enough nutrients due to the nitrogen dilution effect (Mattson Jr. 1980). In agreement with our findings it has been reported that herbivory correlates positively with sunlight exposition (Louda & Rodman 1996, Dicke 2000) although the opposite pattern (*i.e* low herbivory in sunny microhabitats) has also been documented in some systems (Guerra *et al*. 2010; Barber & Marquis 2011). Explanations for the negative relationship between herbivory and sunlight invoke three main issues: 1) Predation rates on herbivores is high in open sites and low in shaded sites. This is consistent with the higher predation of artificial caterpillars observed in the tetechera than in the mezquital. 2) Adult female herbivores avoided edges as oviposition sites. Selection against edges may be partially driven by the higher predation risk of larvae in open sites. Also we found some evidence supporting this statement since there were more caterpillars in the shade site as compared with the open site. 3) Growth rate of herbivores in sunny and warm habitats may be higher than in shaded and fresher sites, leading to lower levels of herbivory especially if leaves are nutrient rich. This is also concordant with our observations since we found less herbivory, despite a high abundance of herbivores in the site where leaves had higher content of nutrients. Then, our evidence agree with three of the most likely process that should lead to low levels of herbivory in sunny and warm habitats (Guerra *et al*. 2010, Barber & Marquis 2011). However, it was in the open canopy site where we found higher herbivory as compared with the more shaded site. Unlike those studies, this was conducted in an arid ecosystem where light may not be a limiting factor for plant growth and some other factors like water availability may limit plant growth in the open canopy site. Thus increase in water content in plant leaves may lead to high growth rates of herbivores in the mezquital, especially since leaves are rich in water and nitrogen (Huberty & Denno 2004), shortening the development time of herbivores and reducing herbivory levels. To overcome the speculative nature of the arguments, experiments comparing growth rates of herbivores between plant associations and estimating the amount of leaf tissue consumed to complete development are needed.

In agreement with predictions based on morphological responses to xeric environments (Crawley 1997), and consistent with predictions of the carbon nutrient balance hypothesis and the balance growth differentiation hypothesis, leaves of two out of the three species had more defences in the low resource habitat, and these defences were carbon based (trichomes and leaf

strength). However, these traits did not limit consumption by insect herbivores in our study site. Thus phenotypic changes in leaf traits seem to be driven by aridity (Valladares *et al*. 2007). The high rate of attack to artificial caterpillars in the more arid site with an open canopy may be related with the higher visibility of artificial caterpillar for birds. In the closed canopy site visibility ranges are shortened since light penetration to the understory is reduced and also the vegetation at this stratum is denser than in the open canopy site. Abundance of natural enemies and environmental conditions are likely factors regulating the abundance of herbivorous insects. Here we observed more herbivores in the nutrient rich site than in the nutrient poor site. Counts of herbivores where conducted during daylight hours, and in the tetechera many herbivores may have been inactive and hiding to avoid exposition to the intense solar radiation (see Holm & Edney 1973) while in the mezquital, a canopy protected plant association, activities of insect herbivores may be extended during daylight hours.

In conclusion in 2012 the overall herbivory was high and more damage was observed in the driest plant association but in 2014 the overall herbivory was low and no differences in herbivory between plant-associations were detected. Despite that, in 2014 when predators were excluded herbivory increased by 28 % in the mezquital and 80 % in the tetechera. This result combined with the fact that there were a higher rate of attacks of artificial caterpillars in the tetechera indicates that bird predation of herbivores is one of the main drivers of plant-herbivore interaction. Because plants with stronger leaves and with a higher density of trichomes were more consumed, the phenotypic changes observed in leaf traits seem to be driven by aridity rather than a selective pressure of herbivores. Given that less than 5 % of the studies on herbivory have been conducted in arid and semiarid ecosystems, it is clear that more studies on herbivory are needed in these ecosystems that cover up to 33 % of the terrestrial land surface.

Acknowledgements

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