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Leaf trichome density may explain herbivory patterns of *Actinote* sp. (Lepidoptera: Acraeidae) on *Liabum mandonii* (Asteraceae) in a montane humid forest (Nor Yungas, Bolivia)

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ABSTRACT

Increasing evidence shows that most insect herbivores of tropical forests show specific associations with their hosts. Environmental factors as well as foliar characters can modify insect preference. In the present work, we evaluated in a montane humid forest the preference and herbivory rate of *Actinote* sp. caterpillars (Lepidoptera: Acraeidae) on mature and young leaves of their specific host plant *Liabum mandonii* (Asteraceae) in two contrasting sites. Additionally, the density of non-glandular trichomes in young and mature leaves of plant at each site was evaluated. Analysis of variance of herbivory showed significant effects of site, leaf age, and the interaction of these factors. Higher herbivory levels were found on leaves from the site with lower levels of radiation and on mature leaves. On the other hand, trichome density was significantly higher in leaves from the site with higher levels of radiation and in young leaves. This suggests trichomes may explain the observed pattern of herbivory.

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1. Introduction

In tropical forests, patterns of spatial distribution and local abundance of herbivorous insects are related to light and humidity (Janzen, 1981; Coley, 1983; Coley and Aide, 1991; Marquis and Braker, 1994; Goehring et al., 2002). Environmental heterogeneity may affect the palatability of leaves to herbivorous insects (Louda and Collinge, 1992; Kennedy and Barbour, 1992). The nutritional quality of plant tissues also varies with ontogeny (Coley, 1980; Raupp and Denno, 1983; Clark et al., 2000; Boege and Marquis, 2005). Particularly, leaves developed in environments of high radiation tend to show thick waxy cuticles and/or high trichome density (Crawley, 1997). Trichome density has been related to the maintenance of water balance (Werker, 2000), reflection of excessive solar radiation (Levizou et al., 2004), and defense against herbivorous insects (Woodman and Fernandes, 1991; Ågren and Schemske, 1993; Molles and Westoby, 2000; Kennedy, 2003). Summarizing, leaf traits and environmental conditions may affect singly and jointly the preference and performance of herbivorous insects (Rausher, 1979; Gianoli and Hannunen, 2000).

In a montane humid forest (Nor Yungas, Bolivia), we addressed herbivory patterns of *Actinote* sp. (Lepidoptera: Acraeidae) on its specific host plant *Liabum mandonii* (Asteraceae). Particularly, we assessed the pattern of distribution of

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eggs and larvae of *Actinote* sp. on leaves of *L. mandonii* of different age (young and mature) in two sites with different solar exposure. In addition, we experimentally evaluated leaf palatability for caterpillars (no-choice tests) in leaves from those four groups (two sites and two leaf ages), and measured the density of non-glandular leaf trichomes.

2. Materials and methods

The study was carried out in the Tunquini Biological Station, Cotapata National Park (16°11'N; 67°52'E), located at 1550 m a. s.l., 80 km NW of La Paz city, Bolivia (Bach et al., 2003). This site is a montane humid forest with steep slopes and canopy species that reach 15 m. Vegetation is mostly of secondary origin (Paniagua-Zambrana et al., 2003). The climate is humid subtropical with annual precipitation of 2000 mm and average monthly temperatures between 17 and 20 °C (Ribera, 1995). For the present study, two sites with different microclimatic characteristics and separated by approximately 1.5 km were used. Site 1 is located in a north-facing slope, characterized by high solar radiation and showing an open canopy due to the predominance of shrubby vegetation. Site 2 is located in a south-facing slope, with low solar radiation and mainly arboreal vegetation with a semi-closed canopy and a relatively high humidity.

Actinote sp. is a butterfly species closely associated with its host plant *L. mandonii* (Asteraceae) in the study zone (Valdivia, unpubl. data). This neotropical genus has specific associations with its host plants, belonging to the Asteraceae family (Caldwell and Kluge, 1993). The *Actinote* sp. adult is black with broad red strips in both pairs of wings (Ledezma, 1998). The first-instar larvae are 0.3 cm long and cream colored with black hairs; the fifth-instar larvae are 8–9 cm long and black (Valdivia, unpubl. data). *L. mandonii* is a herbaceous species, characterized by the lack of latex, umbel-type inflorescence, and triangular opposite leaves with white abaxial surface and non-glandular trichomes (Cabrera, 1947).

To evaluate the distribution of eggs and larvae of *Actinote* sp. on *L. mandonii*, we searched on all leaves of 30 individuals randomly chosen at each site (range of height: 60–90 cm; range of number of leaves per plant: 16–26). We collected both young and mature undamaged leaves (young leaves: fully expanded leaves of the upper third of the plant; old leaves: non-senescent leaves of the lower third of the plant) for herbivory bioassays. The distribution pattern was evaluated by a contingency analysis (Chi-square). On the other hand, in laboratory conditions, we estimated the herbivory rate of *Actinote* sp. caterpillars on young and mature leaves of *L. mandonii*. A leaf disc (5.6 cm diameter) was placed individually in a Petri dish with moistened filter paper (10 replicates per each of the four treatments). Five larvae of *Actinote* sp. (three first-instars and two second-instars) were added to each Petri dish. After 72 h we quantified the leaf area consumed by caterpillars using image analysis software (Sigma-Scan).

We counted the number of trichomes per cm^2 in the abaxial surface of leaves by observation under stereoscope. This was done in 10 leaves of each of the four experimental groups (total: 40 leaves). Results of both the herbivory bioassay and the leaf trichome evaluation were analyzed using a two-way analysis of variance (ANOVA), with leaf area consumed (cm^2) and number of trichomes per cm^2 as dependent variables, respectively, and leaf age and site as main factors.

Table 1 – Distribution pattern of *Actinote* sp on *L. mandonii* individuals. The number of individuals with eggs and/or larvae in young and mature leaves from site 1 (open habitat) and site 2 (shaded habitat) are shown

	Site 1	Site 2
Young leaves	1	13
Mature leaves	0	1

A Yates-corrected Chi-square indicated marginally significant differences between sites and leaves ($\chi^2 = 3.23$; $P = 0.07$).

3. Results

Of the 30 plants of *L. mandonii* evaluated in the field, 15 had either eggs or caterpillars of *Actinote* sp. Nearly all of these plants (14 out of 15) were located in site 2, where the environment is relatively shaded and humid. In this site most eggs and caterpillars were found on young leaves (13 out of 14 cases) (Table 1). Likewise, the single plant where *Actinote* sp. was found on site 1 had the caterpillars on young leaves. These patterns suggest an oviposition preference for shaded sites and young leaves (Table 1).

The factors site, leaf age, and their interaction were significant in the ANOVA of herbivory rate of caterpillars (Table 2). A greater consumption of leaf tissue was observed in samples from site 2, and—overall—herbivory was higher on mature leaves than on young leaves. However, the latter was clearly observed only in leaves from site 2 (Fig. 1AB). Likewise, leaf trichome density was affected by the site of origin, leaf age, and their interaction (Table 2). A greater density of trichomes was found on leaves from site 1 (the sunny site), and young leaves showed more trichomes per cm^2 than mature leaves, being this effect greater in leaves from site 2 (Fig. 1AB).

4. Discussion

Plant species occurring in different microhabitats often show some degree of phenotypic variation functional to the prevailing environmental conditions (Sultan, 1995; Gianoli, 2004). Consequently, insect herbivores associated to such plant species must deal not only with environmental chal-

Table 2 – Two-way ANOVAs of herbivory by caterpillars of *Actinote* sp. (consumed leaf area) and density of foliar trichomes in young and mature leaves of *L. mandonii* from sites 1 and 2

	Herbivory		Trichomes	
Factor	$F_{(1, 36)}$	P	$F_{(1, 36)}$	P
Site (S)	5.28	0.0275	41.58	0.0001
Leaf age (LA)	5.86	0.0207	10.60	0.0020
S × LA	5.23	0.0283	5.55	0.0024

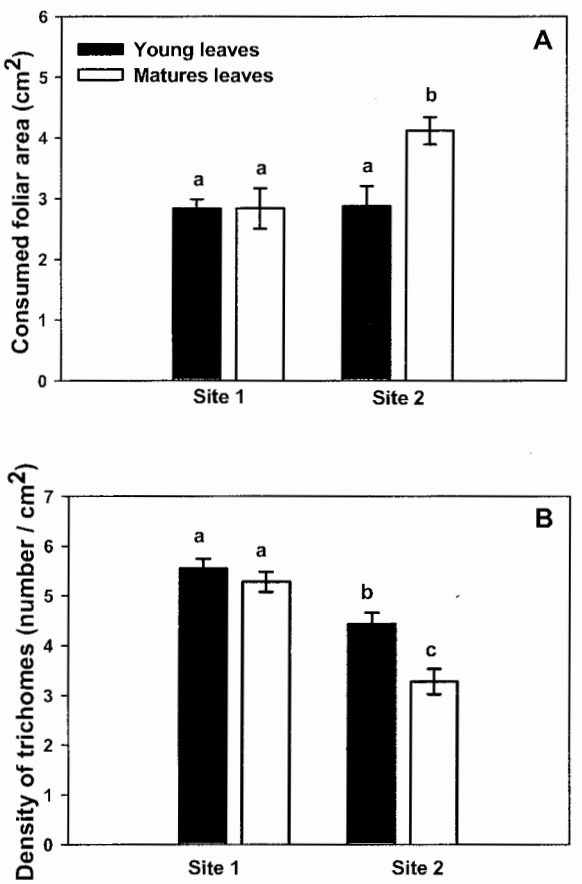

Fig. 1 – A. Leaf area consumed by *Actinote* sp. caterpillars and B. Density of foliar trichomes in young leaves (black bars) and mature leaves (gray bars) of *L. mandonii* from sites 1 and 2 (see details in the text). Different letters indicate significant differences ($P < 0.05$, Tukey test).

lenges, but also with environment-mediated changes in the traits of their host plants (Weis, 1992; Miller and Weis, 1999). In the present study, the lepidopteran species *Actinote* sp. seemingly showed preference for individuals of its specific host plant growing in a shaded microhabitat. This field pattern was reflected in the results of the herbivory experiments, where a greater consumption by caterpillars of leaves of L. *mandonii* from the shaded site was found. Furthermore, the observed site-dependent greater consumption of mature leaves was paralleled by the recorded pattern of trichome density (Fig. 1). Therefore, it might be concluded that leaf trichome density plays a role in the herbivory patterns herein described. Density of trichomes has been associated with reduced insect herbivory in other systems (Baur et al., 1991; Fernandes, 1994; Van Dam and Hare, 1998; Letourneau, 1999; Molles and Westoby, 2000; Gassmann and Hare, 2005). On the other hand, leaves developed under shaded habitats exhibit greater specific leaf area (SLA) than those grown in sunny habitats, which would result in softer leaves (Crawley, 1997). Thus, both mechanical (less trichomes) and morphological (thinner leaves) plant traits associated with relatively shaded habitats might contribute to make leaves more palatable to herbivorous insects. In addition, the role in the observed herbivory pattern of secondary metabolites that may covary with leaf trichomes cannot be ruled out.

Young leaves from site 2 were less consumed, and had more trichomes, than mature leaves from the same site. This pattern is opposite to earlier work in tropical habitats reporting higher herbivory rates on young leaves than on mature leaves, which was associated with higher contents of water and nitrogen and lower concentrations of chemical defenses (Coley, 1980; Dirzo, 1984). From an optimal defense perspective (McKey, 1979; Rhoades, 1979; Zangerl and Bazzaz, 1992), the greater endowment of mechanical defenses to young leaves observed in site 2 might reflect a higher value of these leaves for the plant. However, we currently lack data to support such hypothesis.

Although the present study has found some evidence of intrinsic leaf factors explaining the distribution pattern of *Actinote* sp. on *L. mandonii* observed in the field, other ecological factors affecting habitat selection of insect herbivores such as risk of parasitism (Gonzáles et al., 2001) or avoidance of extreme temperatures (Blanford and Thomas, 2000) should also be considered.

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