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# Cushion Plants as Microclimatic Shelters for Two Ladybird Beetle Species in Alpine Zone of Central Chile

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## Abstract

High mountain environments are highly stressful for insect survival. It has been suggested that small microtopographic variations generating less stressful microclimatic conditions than the surrounding environment would provide more suitable sites for insect development. Cushion plants represent one of the life forms best adapted to the extreme alpine habitats. Cushion plants can modify microclimatic conditions within and under their canopy, generating less severe microsites than the surrounding environment. In this study, we characterized the microclimatic modifications made by the cushion plants *Azorella monantha* and *Laretia acaulis* and examine their role as microclimatic shelters for two species of high Andean coleopterans (Coccinellidae): *Eriopis connexa* and *Hippodamia variegata* at 3200 m a.s.l. in the Andes of central Chile. Results showed that the cushion species create microhabitats with higher availability of water and less oscillating temperatures. However, the intensity of modifications was higher in *A. monantha* compared to *L. acaulis*. The abundance of the two ladybird beetle species was higher within cushions than outside, although *E. connexa* showed higher abundances compared to *H. variegata*. However, a habitat selection experiment in a greenhouse showed that under milder temperature conditions ladybird beetle species do not prefer cushions. This suggests that in the harsh alpine climate, cushion plants may act as microclimatic shelters since they reduce stressful environmental conditions, allowing greater abundances of coleopterans than in the surrounding environment.

## Introduction

High mountain environments are very stressful habitats for insect survival. The main climatic characteristics of these habitats are extreme temperatures, strong winds, nutrient and water shortage, and short growing seasons (Körner, 1999, 2000). Extreme temperatures and daily high thermal oscillation are the main factors determining insect reproduction success and survival (Matthews and Kitching, 1984; Jones et al., 2001). Other factors determining successful colonization by high-mountain insects are soil water availability and wind speed, since these factors affect oviposition rates and heat loss through evapotranspiration (Rockstein, 1964; Matthews and Kitching, 1984; Laub and Luna, 1992). It has been widely observed that many insects occurring in extreme environments are closely associated with plants, thus reducing the severe environmental conditions (Manley, 1996; Jones et al., 2001). Indeed, Matthews and Kitching (1984) and Manley (1996) have suggested that small microtopographic variations generate less stressful microclimatic conditions, which provide more suitable sites for insect development.

Cushion plants are one of the best-adapted growth forms in high mountain environments (Pysek and Lyska, 1991; Körner, 1999). Their small stature and compact architecture allow them to generate less severe microclimate conditions compared to their surrounding environment, mitigating unfavorable environmental effects (Cavieres et al., 1998; Körner, 1999). Microclimates created by cushion plants reduce extreme temperatures and enhance both water and nutrient availability (Cavieres et al., 1998; Nuñez et al., 1999; Körner, 1999). Considering that high mountain regions present very limited opportunities for insect development, and that cushion plants provide less stressful microclimatic conditions, it would be expected that the latter serve as microclimatic refuges for insects. We have observed that at least two ladybird beetle species inhabit the alpine zone of central Chile, with those species being

observed more frequently within cushion plants (Fig. 1). In order to test the hypothesis that cushions represent a shelter for insect species, we compared the abundance and richness of ladybird beetle species distributed on two cushion plants, *Azorella monantha* Clos (Apiaceae) and *Laretia acaulis* (Cav.) Gill. et Hook (Apiaceae), as well as in surrounding open areas, at 3200 m a.s.l. in the alpine zone of central Chile.

## Materials and Methods

### STUDY SITE

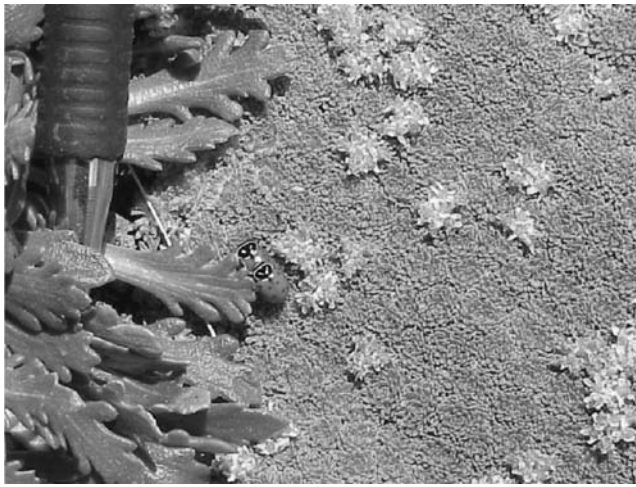
This study was conducted in the Andes of central Chile near the locality of Valle Nevado (33°20'S, 70°16'W), 50 km east of the city of Santiago. Climate is typically alpine, with strong influence of the Mediterranean-type climate that prevails in lowlands (di Castri and Hajek, 1976). In winter, mean annual air temperature is 1.7°C, with an absolute minimum of -15.0°C (Cavieres and Arroyo, 1999). In summer, mean annual air temperature is 6.8°C, and the absolute maximum is 17.0°C (Cavieres and Arroyo, 1999). Mean annual precipitation ranges from 500–900 m, occurring mainly as snow during winter months (Santibañez and Uribe, 1990).

At 3200 m elevation, on an east-facing slope, we selected an area of approximately 20,000 m<sup>2</sup>, where vegetation was dominated by cushions of *Azorella monantha* and *Laretia acaulis*.

### TEMPERATURE AND HUMIDITY

To characterize microclimate within cushions of *Azorella monantha* and *Laretia acaulis* and their surrounding environment, we measured substrate temperature and water availability on two consecutive sunny days in February 2003.

We randomly selected five cushions of each species, and five points in the surrounding environment. At each of these cushions and points



**FIGURE 1.** Individuals of ladybird beetles (*Eriopis connexa*) sheltered on *Azorella monantha* cushions in the high Andes of central Chile (3200 m a.s.l.).

in the bare ground, we measured substrate temperature at 1 cm depth with a digital thermometer (Digital Thermometer 871A, Tegam, HO, U.S.A.). Measurements were taken every 2 h between 08:00 and 20:00 h, and registrations on the two microhabitats (cushions and surrounding environment) were made simultaneously in order to obtain comparable data, which were analyzed with repeated measures using ANOVA.

To characterize water availability, soil matric water potential was measured with tensiometers (2725 Series Jet Fill, Soil Moisture, CO, U.S.A.) both beneath cushion plants and in surrounding open areas. These measurements were made at a depth of 10 cm on five cushions of *A. monantha*, five cushions of *L. acaulis*, and five random points in the surrounding environment, 2 m away from the edge of any cushion. Measurements within cushions and surrounding open areas were made simultaneously. These data were compared among the three microsites with one-way ANOVA, and *a posteriori* Tukey tests were used to assess differences between microsites.

#### RICHNESS AND ABUNDANCE

At the study site, 50 individuals of each cushion species were randomly selected. Previously, we constructed metallic-hoops of known diameter (20, 30, and 40 cm diameter). At each selected

cushion, a metallic hoop of similar size was placed over the cushion, and all individual ladybird beetles within the hoop were identified and counted. Sampling was conducted simultaneously in cushions and in the surrounding environment. For this, the metallic hoop used to sample each cushion was placed in the surrounding environment at a double diameter distance, and in a random direction, from the center of the respective cushion. Abundances of beetles (individuals  $\text{dm}^{-2}$ ) were compared among microsites (*Azorella monantha*, *Laretia acaulis*, and the surrounding environment) and beetle species with two-way ANOVA. Differences between mean abundances were assessed with *a posteriori* with Tukey tests.

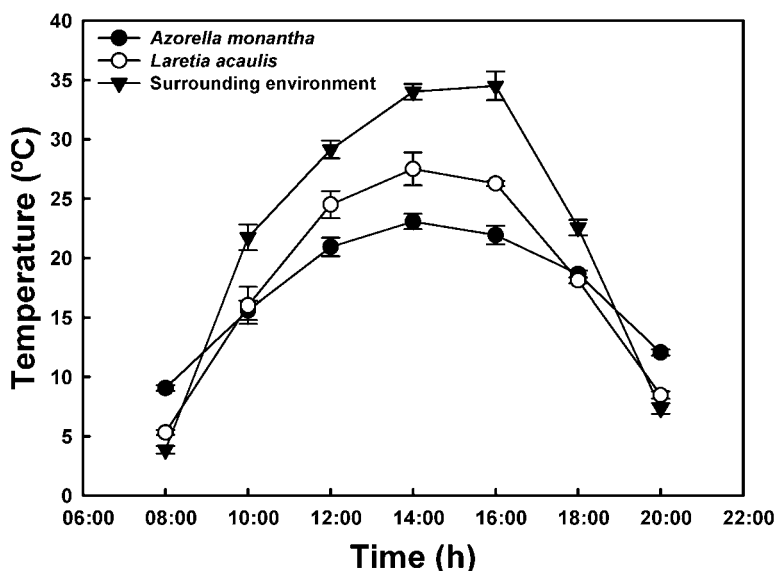
#### EXPERIMENT OF HABITAT SELECTION

To test whether cushion plants serve as microclimatic shelters for high-Andean ladybird beetles, 30 individuals of each beetle species detected in field were collected alive and maintained in glass jars. Additionally, three individual cushions of *Azorella monantha* and other three of *Laretia acaulis* (40–50 cm diameter) were taken to a greenhouse with controlled conditions of temperature and relative humidity of air (25°C, 75% RH). Three further plots (40 cm diameter, 10 cm depth) filled with soil from open areas surrounding cushions were placed in the greenhouse. In the greenhouse, both cushion plants and plots with soil were disposed on a white cloth sheet following a circular arrangement (2 m diameter). In this circular arrangement, cushion species and plots with soil were regularly alternated. At the center of this system, we placed 20 individuals of each beetle species collected in field and set a photoperiod 16/8 h day-night. After 48 h, we examined cushion plants and plots with soil looking for ladybird beetles. All individuals beetles detected at each experimental unit were identified and counted. These data were compared among microsites and beetle species with two-way ANOVA, and Tukey tests were used to assess differences *a posteriori*.

## Results

#### TEMPERATURE AND HUMIDITY

Averaging substrate temperatures at each hour, maximum substrate temperatures within both cushion species, as well as in the surrounding environment, were recorded between 12:00 and 16:00 h, whereas minimum temperatures were detected at 8:00 and 20:00 h (Fig. 2). Repeated measures with ANOVA indicated differences in substrate temperatures between microsites ( $F_{2,6} = 99.51$ ;  $P < 0.001$ )



**FIGURE 2.** Average temperature ( $^{\circ}\text{C} \pm 1 \text{ SE}$ ) detected at each hour within cushions of *Azorella monantha* and *Laretia acaulis*, as well as in their surrounding environment in the high Andes of central Chile (3200 m a.s.l.).

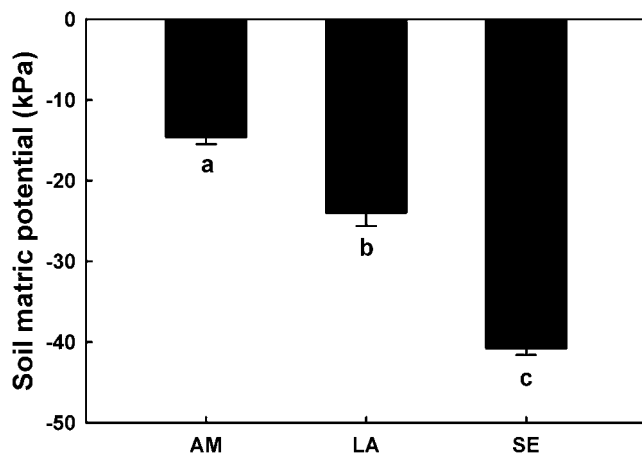


FIGURE 3. Average soil matric potential (kPa  $\pm$  1 SE) measured beneath cushions of *Azorella monantha* (AM) and *Laretia acaulis* (LA), as well as in their surrounding environment (SE), in the high Andes of central Chile (3200 m a.s.l.). Different letters indicate significant differences (*a posteriori* Tukey test,  $\alpha = 0.05$ ).

as well as across time ( $F_{6,36} = 757.23$ ;  $P < 0.001$ ). Most of the time, both cushion species maintained lower mean temperatures than the surrounding environment, but cushions of *Azorella monantha* maintained higher temperatures than *Laretia acaulis* and open areas at 8:00 and 20:00 h (Fig. 2).

Soil matric potentials within *A. monantha* and *L. acaulis* were higher than in the surrounding environment ( $F_{2,11} = 663.51$ ;  $P < 0.001$ ), indicating that both cushion species contain higher soil moisture than surrounding open areas (Fig. 3). Differences between cushion species were also found; *A. monantha* had higher soil moisture than *L. acaulis* (Fig. 3).

#### RICHNESS AND ABUNDANCE

Only two ladybird beetle species were recorded in the study site, *Eriopis connexa* and *Hippodamia variegata*. Irrespective of species, a total of 250 individual beetles were detected during sampling, where 86% were found on cushions of *Azorella monantha*, 13% on cushions of *Laretia acaulis*, and 1% in the surrounding environment. Of a total of 182 individuals of *E. connexa*, 82% were found on *A. monantha*,

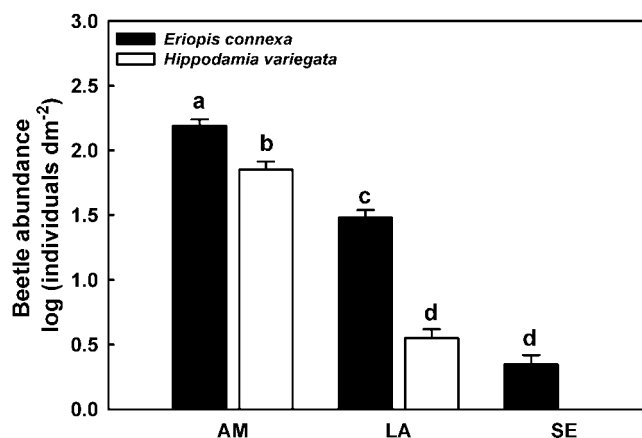


FIGURE 4. Average abundance (log individuals  $\text{dm}^{-2} \pm 1$  SE) of *Eriopis connexa* and *Hippodamia variegata* detected on cushions of *Azorella monantha* (AM) and *Laretia acaulis* (LA), as well as in open areas surrounding cushion plants (SE), in a high alpine zone of central Chile (3200 m a.s.l.). Different letters indicate significant differences between microsites and beetle species (*a posteriori* Tukey test,  $\alpha = 0.05$ ).

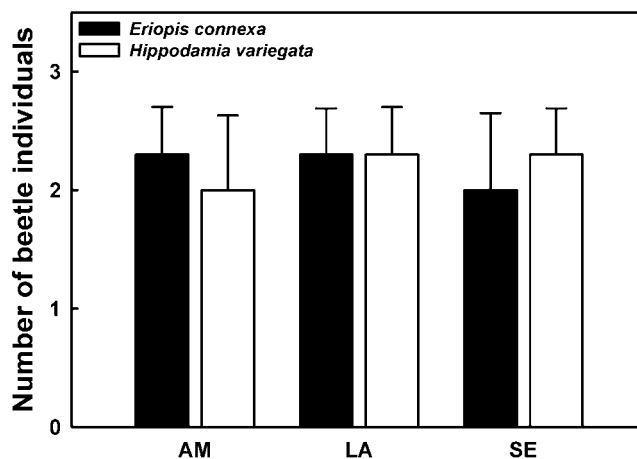


FIGURE 5. Average number of individuals ( $\pm 1$  SE) of *Eriopis connexa* and *Hippodamia variegata* detected on cushions of *Azorella monantha* (AM) and *Laretia acaulis* (LA), as well as in plots filled with soil in open areas surrounding cushions (SE), in the greenhouse experiment of habitat selection. Different letters indicate significant differences between microsites and beetle species (*a posteriori* Tukey test,  $\alpha = 0.05$ ).

17% on *L. acaulis*, and 1% in the surrounding environment. On the other hand, of a total of 68 individuals of *H. variegata*, 96% were detected on *A. monantha* and 4% on *L. acaulis*. No *H. variegata* individuals were recorded in the surrounding environment.

Results of the two-way ANOVA indicated that beetle abundances differed significantly between microsites ( $F_{2,12} = 2858.9$ ;  $P < 0.001$ ), as well as between beetle species ( $F_{1,12} = 772.6$ ;  $P < 0.001$ ). Analyses *a posteriori* indicated that *A. monantha* was the microsite with the higher abundance of beetles, followed by *L. acaulis* and the surrounding environment, respectively (Fig. 4). Additionally, these analyses indicated that *E. connexa* was significantly more abundant than *H. variegata* in all microsites (Fig. 4).

#### EXPERIMENT OF HABITAT SELECTION

Since both, *Eriopis connexa* and *Hippodamia variegata* were the only beetle species detected in the study site, these ladybird beetles were used to conduct the greenhouse experiment to assess differences in their habitat preferences. Results of this experiment showed no differences in the number of individual beetles neither among microsites ( $F_{2,12} = 0.25$ ;  $P = 0.78$ ) nor between beetle species ( $F_{1,12} = 0.11$ ;  $P = 0.98$ ) (Fig. 5).

#### Discussion

Physiological adaptations and the use of microclimatic shelters by insects as a survival strategy in high-mountain environments have been observed worldwide (Schmoller, 1970; Matthews and Kitching, 1984; Somme, 1993; Jones et al., 2001). According to our results, in the high Andes of central Chile, cushions of *Azorella monantha* and *Laretia acaulis* may provide microhabitats that could act as thermal refuges, maintaining milder temperatures when the surrounding environment reaches either extremely low or extremely high temperatures, as well as reduce daily thermal oscillation (see Fig. 2). Furthermore, cushion plants maintain higher soil moisture than their surrounding environment, suggesting that both *A. monantha* and *L. acaulis* provide habitat patches in which beetle species can have conditions more suitable for survival and development of different life stages in their life cycle.

For example, Acar et al. (2004) and Simmons and Legaspi (2004) have shown that high temperatures ( $>35^{\circ}\text{C}$ ) decrease the survival both of larval and adults in Coccinellidae species. In our study, while soil in

open areas surrounding cushions can easily reach that temperature at midday, cushions never exceeded 30°C (Fig. 2). Regarding soil moisture, Fujiyama et al. (2003) showed that adults of Coccinellidae species are more abundant in soils with high moisture. This, added to the lower larval mortality suggested by Venette et al. (2000) for habitat with low oscillating thermal and hydric conditions, could explain the observed patterns reported here for two ladybird beetle species. Hence, the microclimatic modifications induced by cushion plants could generate microsites more favorable for oviposition, egg development, larval and adult survival, and permanence of *Eriopsis connexa* and *Hippodamia variegata* in the Andes of central Chile.

The lower beetle abundance recorded in areas surrounding cushions suggest that this microenvironment could be highly stressful for development and survival of the ladybird beetle species inhabiting the high Andes. Conversely, the higher beetle abundance detected within cushion plants reinforces the suggestion that habitat patches created by cushions may act as microclimatic shelters for those beetle species. Nevertheless, abundances of both beetle species detected in the study site (*E. connexa* and *H. variegata*) differed between the two cushion species. Such differences may be related to different effects of *A. monantha* and *L. acaulis* on microclimatic conditions. For instance, our data indicate that *A. monantha* mitigates temperature and soil moisture conditions with higher intensity than *L. acaulis* (see Figs. 1 and 2). Thus, such differences could help to explain why both *E. connexa* and *H. variegata* had higher abundances on the former cushion species. This follows the suggestions of Rockstein (1964) and Laub and Luna, (1992), who have found that there is a positive correlation between insects' density and soil hydric content, which could also explain the greater number of individuals of ladybird beetle species on *A. monantha* than on *L. acaulis*, and on both cushions than in the surrounding environment.

Nevertheless, the distribution of ladybird beetle species among microhabitats could be determined by differences in the availability of prey (e.g., mites, aphids). However, mites of the genera *Pherioldes* and *Novonthrus* were found within both cushion species as well as in soil from the surrounding environment (Torres, data unpublished), suggesting that the observed pattern is unlikely to be related only with differential availability of prey.

Notwithstanding, patterns of beetle abundances obtained in the greenhouse experiment contrasted with those patterns observed in field. In this experiment, ladybird beetle abundance showed no differences between cushions species or between cushions and soil from surrounding environment. This lack of response to the presence of cushion plants might eventually indicate that, under greenhouse-controlled conditions, microclimatic differences between cushions and soil from surrounding environment were reduced, so that distribution patterns of ladybird beetles disappeared.

As occur with many other taxa, the diversity and abundance of Coleoptera fauna in high mountain areas decrease with elevation (McCoy, 1990; Lobo and Halffter, 2000). Nevertheless, the presence of more benign microhabitats (e.g., cushion plants) may extend the altitudinal limits of certain species, maintaining greater diversity and abundance in alpine environments than would be expected without the presence of this shelter. Hence, cushion plants can be considered key elements for the maintenance of diversity of alpine habitats of the central Chilean Andes.

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