How do two *Lupinus* species respond to temperature along an altitudinal gradient in the Venezuelan Andes?

¿Cómo responden dos especies de *Lupinus* a la temperatura en un gradiente altitudinal en los Andes venezolanos?

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ABSTRACT

Temperature determines plant formations and species distribution along altitudinal gradients. Plants in the tropical high Andes, through different physiological and morphological characteristics, respond to freezing night temperatures and high daytime energy inputs which occur anytime of the year. The main objective of this study was to characterize day and night temperature related responses of two *Lupinus* species with different altitudinal ranges (*L. meridanus*, 1,800-3,600 and *L. eromonomos*, 3,700-4,300 m of altitude). Are there differences in night low temperature resistance mechanisms between the species along the gradient? How do these species respond, in terms of optimum temperature for photosynthesis, to increasing altitude? *Lupinus meridanus* shows frost avoidance, in contrast to *L. eromonomos*, which tolerates freezing at higher altitudes. Optimum temperature for photosynthesis decreases along the gradient for both species. Maximum CO_2 assimilation rates were higher in *L. meridanus*, while *L. eromonomos* showed decreasing CO_2 assimilation rates at the higher altitude. In most cases, measured daily leaf temperature is always within the 80 % of optimum for photosynthesis. *L. meridanus*' upper distribution limit seems to be restricted by cold resistance mechanisms, while *L. eromonomos*' to a combination of both cold resistance and to CO_2 assimilation responses at higher altitudes.

Key words: *Lupinus meridanus*, *Lupinus eromonomos*, frost avoidance, freezing tolerance, optimum temperature for photosynthesis.

RESUMEN

La temperatura determina las formaciones vegetales y la distribución de especies a lo largo de gradientes altitudinales. Las plantas en los altos Andes tropicales, a través de diferentes características morfológicas y fisiológicas, responden a temperaturas congelantes nocturnas y altas entradas energéticas durante el día en cualquier momento del año. El objetivo principal de este estudio fue caracterizar las respuestas relacionadas con temperaturas diurnas y nocturnas en dos especies de *Lupinus* con diferente distribución altitudinal (*L. meridanus*, 1.800-3.600 y *L. eromonomos*, 3.700-4.300 m de altitud). ¿Existen diferencias en los mecanismos de resistencia a bajas temperaturas entre las especies a lo largo del gradiente? ¿Cómo responden estas especies, en términos de temperatura optima para fotosíntesis, a incrementos en altitud? *Lupinus meridanus* evade el congelamiento, en contraste a *L. eromonomos*, que tolera el congelamiento a mayores altitudes. La temperatura óptima para fotosíntesis disminuye a lo largo del gradiente para ambas especies. Las máximas tasas de asimilación de CO_2 se obtuvieron en *L. meridanus*, mientras que *L. eromonomos* mostró una disminución en sus tasas de asimilación de CO_2 a mayor altitud. En la mayoría de los casos, la temperatura foliar medida siempre estuvo dentro del 80 % del óptimo fotosintético. El límite superior de distribución de *L. meridanus* parece estar restringido por mecanismos de resistencia a la temperatura, mientras que en *L. eromonomos* por una combinación de ambas resistencia al frío y respuestas en asimilación de CO_2 a mayores altitudes.

Palabras clave: *Lupinus meridanus*, *Lupinus eromonomos*, evasión al congelamiento, tolerancia al congelamiento, temperatura óptima para la fotosíntesis.

INTRODUCTION

The most evident altitudinal gradient in tropical high mountains, temperature, determines plant formations and species distribution (Sarmiento 1986). Plants in the high tropical Andes are confronted with a very unique environment and through different physiological and morphological characteristics have to adapt to low temperatures, which may occur anytime of the year. In contrast to higher latitudes, the high tropical Andes are subjected to very small annual differences in temperature, while daily differences are very pronounced. On the other hand, a high energy input during daytime hours also has its implications on leaf temperature. We may, therefore, differentiate day and night temperature effects on plant growth and survival.

In reference to low nighttime temperatures in tropical high mountains, plants have adapted through avoidance or tolerance mechanisms (Sakai & Larcher 1987). Plants growing closer to the ground or at higher altitudes, where more severe conditions are found, present tolerance as the main resistance mechanism to night freezing temperatures (Azócar et al. 1988, Squeo et al. 1991, 1996). While those plants growing well above ground level or lower altitudes show avoidance mechanisms, mainly through supercooling (Rada et al. 1985a, 1985b, Squeo et al. 1991). In relation to cold resistance mechanisms along altitudinal gradients, all species of the genus *Espeletia* growing from 2,600 to 4,400 m of altitude in the tropical high Andes show avoidance as the survival mechanism (Goldstein et al. 1985, Rada et al. 1987). On the other hand, for the subtropical high Andes, Squeo et al. (1996) have described avoidance mechanisms for species of the genus *Adesmia* growing at lower altitudes (up to 3,400 m of altitude), while three other species of the same genus growing above 3,400 m of altitude all present freezing tolerance.

The adjustment of the photosynthetic apparatus to temperature must be a prerequisite for plants in order to maintain a positive carbon balance in high-energy input environments of high altitudes. Larcher & Wagner (1976) have described a relationship between temperature limits for net photosynthesis of high mountain species and their temperature regime in their specific habitat. Microhabitat effects on optimum temperature for photosynthesis have been described in previous works. Plants growing closer to the ground, where temperatures may be higher due to radiant heating, show no difference or even an increase in optimum temperature for photosynthesis with increasing altitude (Körner & Diemer 1987, Rada et al. 1992, Cabrera et al. 1998). On the other hand, taller plants are directly influenced by air temperature and, therefore, optimum temperature for photosynthesis closely follows decreasing air temperature with altitude (Rada et al. 1992).

The main objective of this work was to characterize, in the laboratory, temperature related responses of two *Lupinus* species (Fabaceae) with different altitudinal ranges. *Lupinus meridanus*, an annual herb found at a lower and *L. eromonomos*, a perennial herb, at a higher portions of the range. Are there differences in cold resistance mechanisms between the two species along the gradient? One would expect *L. meridanus* to rely on avoidance, while *L. eromonomos* on tolerance mechanisms due to harsher temperature conditions with increasing altitude. How do these species respond in terms of optimum temperature for photosynthesis with increasing altitude? A decrease in the optimum temperature for photosynthesis with increasing altitude, additionally, as suggested by Goldstein et al. (1994), high CO_2 assimilation rates must be prerequisites in order to survive at such elevations. Answers to these questions may aid in understanding present plant distribution patterns under extreme environmental conditions of the high tropical mountains.

MATERIAL AND METHODS

Lupinus meridanus Moritz ex C. P. Smith distributed between 1,800 and 3,600 m of altitude; and *L. eromonomos* C.P. Smith from 3,700 to 4,300 m of altitude, were chosen for this study. Five sites along an altitudinal gradient (Table 1) in the Andean range (08°43'-08°52' N and 70°48'-70°59' W) were selected. *L. meridanus* at lower altitudes: Escaguey (2,200 m of altitude), Mucuchies (3,000 m of altitude) and Apartaderos (3,550 m of altitude)

as its upper most limit. While *L. eromonomos* is found at the two higher altitudes: Mifafí (3,750 m of altitude) and Piedras Blancas (4,200 m of altitude).

TABLE 1

Climatic data from meteorological stations located at or nearby our study sites along the altitudinal gradient. T_{MEAN} = mean annual temperature

Datos climáticos obtenidos de estaciones meteorológicas ubicadas en los sitios o cercanos a los sitios de estudio a lo largo del gradiente altitudinal. T_{MEAN} = temperatura media anual

Site*	Altitude (m)	Precipitation (mm)	T_{MEAN} (°C)
Escaguey	2,320	827.3	15.0
Mucuchíes	3,000	594.3	11.2
Llano El Hato	3,600	856.0	7.5
Pico El Águila	4,150	826.0	2.8

* Llano El Hato corresponds to a station between and close to both Apartaderos and Mifafí; Pico El Águila is the closest station to Piedras Blancas
* Llano El Hato corresponde a una estación entre y cercana a ambos Apartaderos y Mifafí; Pico El Águila es la estación más cercana a Piedras Blancas

Cold resistance mechanisms, frost avoidance or freezing tolerance, were determined from temperatures at which (a) injury occurred and (b) ice nucleation initiated (Levitt 1972, Sakai & Larcher 1987, Squeo et al. 1991). Several plants from each altitude were excavated with roots and surrounding soil from each study site, transported to the laboratory and placed in a growth chamber for no more than 3 days prior to determinations. Chamber temperature was set at corresponding site daytime and nighttime mean temperatures. Chamber daytime light intensity was approximately 400 µmol m^{-2} s^{-1} obtained from combined incandescent / fluorescent sources. To determine injury of plant tissues, a triphenyl tetrazolium chloride (TTC) method (Steponkus & Lanphear 1967) was used. Leaf, stem and root tissues from plants (n = 5) for each site were placed in small test tubes and immersed in a refrigerated alcohol bath. Temperature was lowered from 10 °C to –25 °C (8 °C h^{-1}), samples removed at 5 °C intervals and incubated at 6 °C for 8 h. After this incubation period, the TTC solution was added,

infiltrated under vacuum for 30 minutes and left at 6 °C for 24 h. Afterwards, samples were extracted with ethanol and absorbance was measured at 530 nm. Cold injury was defined as the temperature at which a 50 % reduction in absorbance occurred when compared to the sample at 10 °C.

To determine the temperature at which ice nucleation began, samples of each tissue were enclosed in small, tightly sealed test tubes in order to avoid changes in tissue water content. Copper-constantan thermocouples were inserted in the tissue samples and temperature was continuously monitored with a data logger. The tubes were placed in a refrigerated bath and temperature was lowered from 10 °C to – 25 °C (8 °C h^{-1}). The temperature at which ice nucleation began was easily determined through the appearance of an exotherm due to heat released during the ice formation process.

Net photosynthesis-leaf temperature curves were obtained from plants brought to the laboratory (n = 4) from each altitude. These were kept in temperature/light controlled growth chambers (same as for the above mentioned plants) and determinations were carried out during the following two days. Curves were carried out with a gas exchange system with an infrared gas analyzer and a plant chamber. The plant chamber was equipped with three copper-constantan thermocouples connected to a chart recorder to obtain air and leaf temperatures. A 1,000 µmol m^{-2} s^{-1} light source was placed above the chamber. A refrigerated bath with hose connections to a radiator was used to increase or decrease air temperatures and, consequently, leaf temperatures within the chamber. CO_2 assimilation rates were measured through CO_2 depletion from the chamber. These measurements were done at 1 min intervals for five minutes at each set temperature (approximately 3 °C intervals at high and low extremes and 1 °C at temperatures near the optimum). Plants were left for 15 min at each temperature. Net photosynthesis (A; µmol m^{-2} s^{-1}) was calculated through:

$$A = V * \Delta CO_2 / L * \Delta \text{time},$$

where: V is the chamber volume (m^3), ΔCO_2 are changes in CO_2 concentration (µmol/m^{-3}), L is the leaf area (m^2), per time in seconds (Field et al. 1989).

Daily leaf temperature courses (n = 4) for each study site were carried out in order to compare with optimum temperatures for photosynthesis. Copper-constantan thermocouples were placed on the under surface of leaves of individuals and were measured at approximately one hour intervals during the cycles.

Mann-Whitney U-tests were used to determine significant differences between injury and ice nucleation temperatures in order to determine the corresponding resistance mechanism for both *Lupinus* species and to compare altitudinal differences between plants from the two *L. eromonomos* sites. Kruskal-Wallis and the posteriori Tukey's test were used to determine significant differences between altitudes for *L. meridanus* plants.

RESULTS

Temperatures at which injury occurred and at which ice nucleation began for the two *Lupinus* species are shown on Table 2. Even though differences were not significant, injury temperature showed a decreasing trend with increasing altitude for leaf tissues. Comparing injury and ice nucleation temperature, it was clear that both species have different cold resistance mechanisms. There were no significant differences between injury and ice

nucleation temperature for all *L. meridanus* populations along the gradient. This is an indication that this species was not able to resist freezing of any of its organs, i.e., injury occurred due to freezing. The only significant difference between ice nucleation and injury was observed for stems at 2,200 m of altitude. In this case injury occurred even before freezing began. On the other hand, *L. eromonomos* showed significant differences between injury and ice nucleation temperatures in most of the tissues, always being this latter one the most positive along the gradient, which suggests that it was able to survive higher altitudes through freezing tolerance. Root tissues, with no significant differences, were protected against freezing temperatures being underground.

In relation to daytime temperatures and its effects on CO_2 assimilation, there was a decrease in optimum temperature for photosynthesis (T_{opt}) along the gradient for both species (Fig. 1, Table 3). *Lupinus meridanus* at 2,200 m of altitude had a T_{opt} of 23.0 °C compared to 13.8 °C at 3,550 m of altitude. While *L. eromonomos* showed T_{opt} of 14.7 and 11.2 °C at 3750 and 4,200 m of altitude, respectively. In terms of maximum CO_2 assimilation (A_{max}), it is interesting to note that *L. meridanus*, at the intermediate altitude (3,000 m of altitude), presented the highest rates (9.9 μmol m^{-2} s^{-1}), decreasing towards low and high

TABLE 2

Plant height (PH in cm, n = 50), injury (IT in °C, n = 5) and freezing temperature (FT in °C, n = 5) for different *Lupinus* plant tissues along the gradient; * indicate significant differences between injury and freezing temperatures at P < 0.05 for each altitude; different letters correspond to significant altitudinal differences within each species at P < 0.05

Altura de planta (PH en cm, n = 50), temperatura de daño (IT en °C, n = 5) y de congelamiento (FT en °C, n = 5) para diferentes tejidos de plantas de *Lupinus* a lo largo del gradiente; * indica diferencias significativas entre temperaturas de daño y congelamiento a P < 0,05 para cada altiltud; letras distintas corresponden a diferencias significativas altitudinales dentro de cada especie a P < 0,05

Species and altitude (m)		Root		Stem		Leaf	
	PH	IT	FT	IT	FT	IT	FT
L. meridanus							
2,200	55 ± 3[a]	-1.3 ± 1.2[a]	-1.9 ± 0.5[a]	-1.7 ± 0.7*[a]	-3.3 ± 0.3[a]*	-2.7 ± 1.3[a]	-3.2 ± 0.3[a]
3,000	38 ± 2[b]	-1.8 ± 1.0[a]	-1.9 ± 0.2[a]	-3.4 ± 0.5[b]	-3.9 ± 0.3[a]	-3.5 ± 1.8[a]	-3.2 ± 0.3[a]
3,550	38 ± 3[b]	-1.9 ± 1.0[a]	-1.8 ± 0.2[a]	-2.9 ± 0.6[b]	-3.8 ± 0.4[a]	-4.7 ± 1.2[a]	-4.4 ± 0.2[b]
L. eromonomos							
3,750	33 ± 3[a]	-3.5 ± 0.5[a]*	-2.7 ± 0.3[a]*	-6.3 ± 0.6[a]*	-4.4 ± 0.3[a]*	-6.4 ± 1.3[a]*	-4.2 ± 0.2[a]*
4,200	26 ± 2[b]	-3.7 ± 0.3[a]*	-2.9 ± 0.2[a]*	-6.8 ± 1.1[a]*	-4.6 ± 0.3[a]*	-7.9 ± 1.4[a]*	

altitudinal limits, both with approximately 5 μmol m^{-2} s^{-1}. *L. eromonomos* showed an important decrease in A_{max} from low (5.4 μmol m^{-2} s^{-1}) to high (2.2 μmol m^{-2} s^{-1}) altitude. Figures 2 and 3 show how leaf temperatures through representative wet season daily courses, for each of the sites along the altitudinal gradient, remained close to the optimum

temperature for photosynthesis. In most cases leaf temperature remained within 80 % of optimum temperatures for CO_2 assimilation rates throughout the day. Only for the highest site (4,200 m of altitude) did leaf temperatures reach values above this limit. This may be explained by the higher energy inputs at higher altitudes, even though air temperatures were lower.
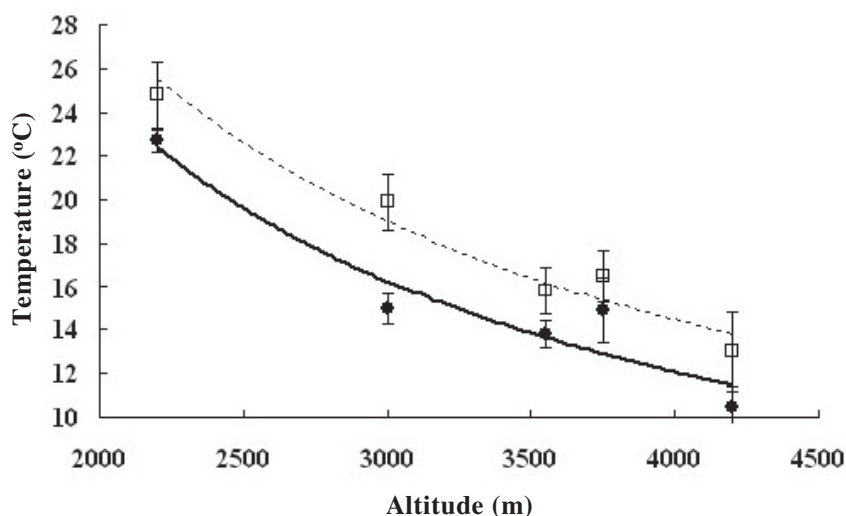

Fig. 1: Relationship between optimum temperature for photosynthesis ($\bullet$, y = 88413x$^{-1.0769}$, r^2 = 0.96) and measured mean leaf temperature during the wet season ($\square$, y = 38308x$^{-0.9502}$, r^2 = 0.95) versus altitude for *L. meridanus* (2,200, 3,000 and 3,550 m of altitude) and *L. eromonomos* (3,750 and 4,200 m of altitude). Bars are one standard error from the mean (n = 4).

Relación entre la temperatura óptima para la fotosíntesis ($\bullet$, y = 88413x$^{-1.0769}$, r^2 = 0,96) y la temperatura media medida durante la estación húmeda ($\square$, y = 38308x$^{-0.9502}$, r^2 = 0,95) versus altitud para *L. meridanus* (2.200, 3.000 y 3.550 m de altitud) y *L. eromonomos* (3.750 y 4.200 m de altitud). Las barras corresponden a un error estandar de la media (n = 4).

TABLE 3

Mean field leaf temperature (T_L), optimum temperature for photosynthesis (T_{opt}), maximum CO_2 assimilation rate (A_{max}, μmol m^{-2}s^{-1}) and low (LTCP) and high (HTCP) temperature CO_2 compensation points for the two species along the altitudinal gradient (n = 3). Different letters correspond to significant altitudinal differences within each species at P < 0.05

Temperatura foliar media en campo (T_L), temperatura óptima para fotosíntesis (T_{opt}), tasa máxima de asimilación de CO_2 (A_{max}, μmol m^{-2}s^{-1}) y puntos de compensación de CO_2 a bajas (LTCP) y altas (HTCP) temperaturas para las dos especies a lo largo del gradiente de altitud (n = 3). Letras distintas corresponden a diferencias significativas dentro de cada especie a P < 0,05

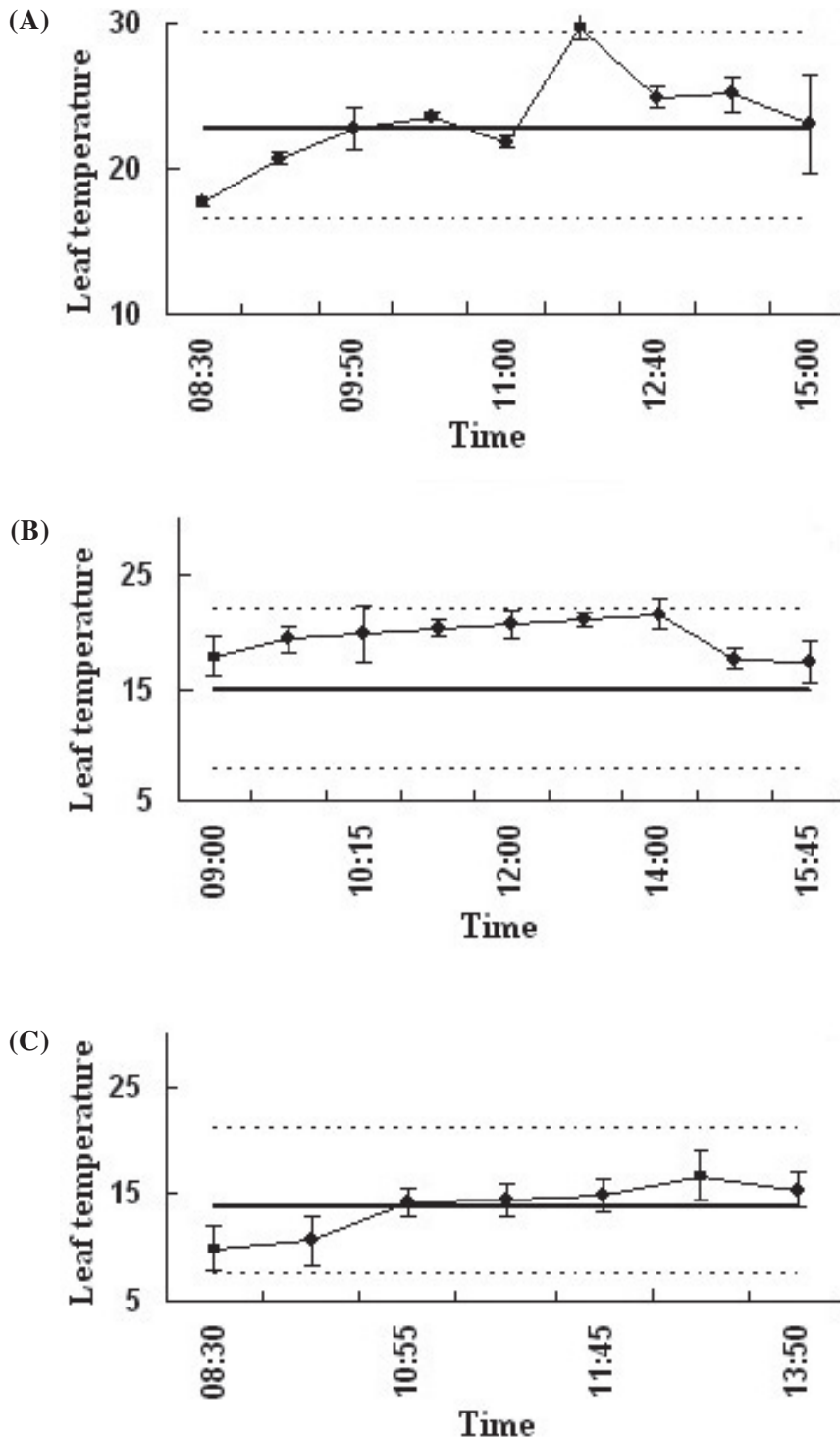| Species	Altitude of site (m)	T_L (°C)	T_{opt} (°C)	A_{max}	LTCP (°C)	HTCP (°C)
L. meridanus	2,200	24.8 ± 1.5[a]	22.7 ± 0.5[a]	5.2 ± 1.2[b]	2.7 ± 1.2[a]	42.2 ± 1.6[a]
	3,000	19.9 ± 1.3[b]	15.0 ± 0.7[b]	9.9 ± 1.3[a]	-0.8 ± 2.3[ab]	34.5 ± 2.5[b]
	3,550	15.8 ± 1.1[c]	13.8 ± 0.6[c]	5.1 ± 1.2[b]	-1.6 ± 0.3[b]	29.1 ± 1.2[c]
L. eromonomos	3,750	16.5 ± 1.2[a]	14.9 ± 1.5[a]	5.4 ± 0.5[a]	-1.0 ± 1.1[a]	30.9 ± 2.8[a]
	4,200	13.0 ± 1.8[b]	10.5 ± 0.9[b]	2.2 ± 0.4[b]	-0.2 ± 2.5[a]	18.9 ± 0.5[b]

Fig. 2: A representative daily course of leaf temperature (-•-) in relation to optimum temperature for CO_2 assimilation rate (—), and temperatures corresponding to 80 % of optimum photosynthetic rate (……) for *L. meridanus* at the three altitudes: (A) Escaguey, 2,200 m of altitude; (B) Mucuchíes, 3,000 m of altitude and (C) Apartaderos, 3,550 m of altitude.

Curso diario representativo de temperatura foliar (-•-) en relación a la temperatura óptima para la asimilación de CO_2 (—), y temperaturas correspondientes a 80 % de la tasa fotosintética óptima (……) para *L. meridanus* en las tres altitudes: (a) Escaguey, 2.200 m de altitud; (b) Mucuchíes, 3.000 m de altitud y (c) Apartaderos, 3.550 m de altitud.
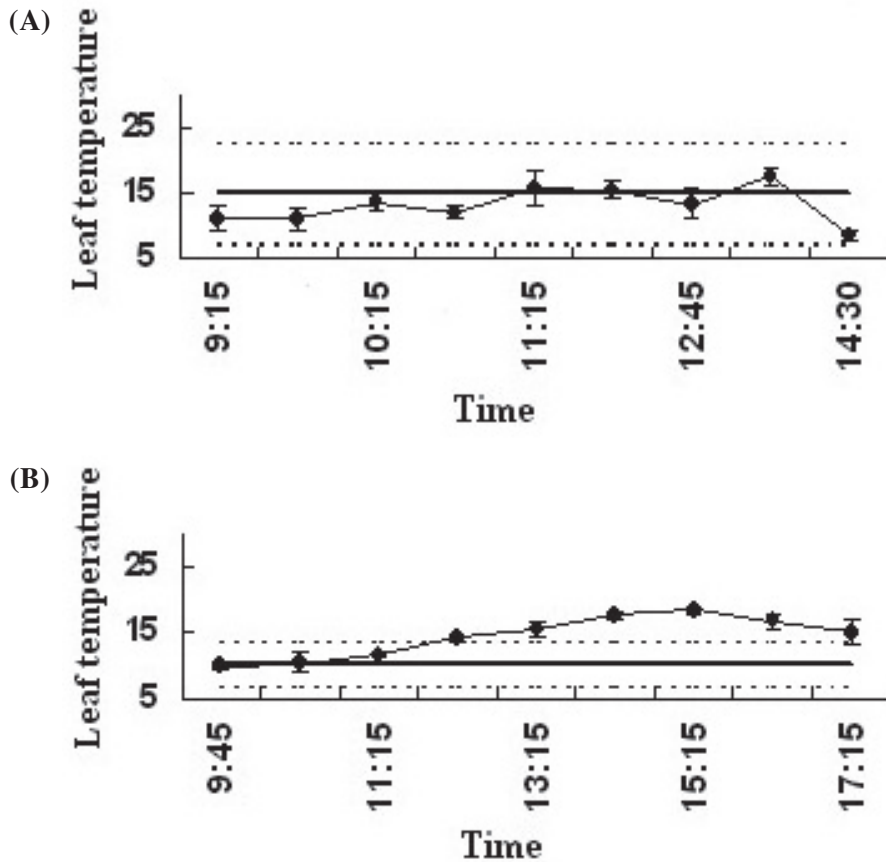
(A)

(B)

Fig. 3: A representative daily course of leaf temperature (-•-) in relation to optimum temperature for CO_2 assimilation rate (—), and temperatures corresponding to 80 % of optimum CO_2 assimilation rate (......) for *L. eromonomos* at the two altitudes: (A) Mifafí, 3,750 m of altitude and (B) Piedras Blancas, 4,200 m of altitude.

Curso diario representativo de temperatura foliar (-•-) en relación a la temperatura óptima para la asimilación de CO_2 (—), y temperatures correspondientes a 80 % de la tasa fotosintética óptima (......) para *L. eromonomos* en las dos altitudes: (A) Mifafí, 3.750 m de altitud y (B) Piedras Blancas, 4.200 m de altitud.

DISCUSSION

There is a clear differentiation in cold resistance mechanisms between these *Lupinus* species. As described by some authors (Azócar et al. 1988, Squeo et al. 1991), freezing tolerance is a less expensive and more secure mechanism than frost avoidance mechanisms in cold environments. Furthermore, avoidance mechanisms such as supercooling, in tropical high mountains, are only safe under conditions of mild freezing temperatures that last short periods of time (Larcher 1982, Rada et al. 1985a). *Lupinus meridanus* relies on avoidance mechanisms through a slight supercooling capacity in order to survive the lower less harsh conditions in terms of nighttime temperatures.

It is interesting to note that at the lower altitude (2,200 m of altitude), this species has no need of freezing resistance mechanisms since it is well below the frost limit that occurs at approximately 2,900 m of altitude. The low supercooling capacity observed at all altitudes for this species explains its capacity to survive along its gradient, but may also define this species' altitudinal limit due to the inability to increase its supercooling capacity as temperatures become lower and more frequent at higher altitudes. We have recorded minimum temperatures of -3 °C and 59 % occurrence of frost events during the wet season for Mucubaji, a nearby site at 3,550 m of altitude (Rangel 2004). Melcher et al. (2000) find that supercooling capacity increases from sea level

to treeline (2,500 m of altitude) in a Hawaiian tree species, *Metrosideros polymorpha*. These authors suggest that limited supercooling capacity in this species restricts its higher altitudinal establishment and may be partially responsible for the occurrence of the treeline at lower elevations in Hawaii. *Lupinus eromonomos*, on the other hand, has adapted to higher altitudes through its capacity to tolerate ice formation in its tissues. Injury temperatures for this species are similar to measured minimum air temperatures for the 4,200 m of altitude site. Azócar (2006) reports absolute minimum air temperatures of - 7.8 ºC at ground level and a 92 % occurrence of frost events during the dry season for the 4,200 m of altitude site where *L. eromonomos* reaches its altitudinal limit. Even though injury temperatures for both species, at their respective altitudes, seem rather high, they are sufficient since, *L. meridanus*, as an annual herb, completes its life cycle before lowest freezing temperatures occur during the dry season. On the other hand, the perennial *L. eromonomos* is associated with rocks, which act like thermal refuges (Walter & Medina 1969, Rada et al. 1985b, Goldstein et al. 1994) at higher altitudes. Similar results have been reported by Squeo et al. (1996) who describe avoidance mechanisms through supercooling for *Adesmia hystrix*, a shrub, growing up to 3,400 m of altitude. While other three *Adesmia* woody species which grow above 3,500 m of altitude are all freezing tolerant. As described in the literature, woody species, trees and shrubs, have relied on either frost avoidance or freezing tolerance. With respect to non-woody species, more recently, Márquez et al. (2006) have found only tolerance strategies in different grass species along these tropical Andean altitudinal gradients.

Phenotypic plasticity in woody species is common in cold environments (Tranquillini 1979) and results in lower optimum temperatures for photosynthesis in plants at the colder higher compared to lower altitudes. Both *Lupinus* species, growing well above ground respond directly to decreases in air temperature with altitude. As stated previously, similar results have been reported (Körner & Diemer 1987, Rada et al. 1992, Cabrera et al. 1998). In the case of *L. meridanus*, even though it shows maximum assimilation rates at the intermediate

altitude, high CO_2 assimilation rates are still maintained at its highest altitude. These values are within the range reported by Körner (1999) in a review of alpine forbs, shrubs and rosettes. On the other hand, *L. eromonomos*' significant decrease in maximum CO_2 assimilation rates towards the higher altitudes may have an influence on its carbon balance and, therefore, on its survival. These values are much lower compared to other plants from the high tropical Andes (Azócar & Rada 2006) or high mountain environments in general (Körner 1999).

Therefore, the lack of a lower temperature resistance mechanism is the probable constraint to *L. meridanus*' upper limit distribution, while it seems that a combination of low temperature resistance mechanisms and CO_2 assimilation restrictions at higher altitudes may be responsible for *L. eromonomos*' present distribution.

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