

Net photosynthesis-leaf temperature relations in plant species with different height along an altitudinal gradient

Rada F. (*), J. Gonzalez (**), A. Azocar (*),
B. Briceño (*) and R. Jaimez (***)

(*) *Centro de Investigaciones Ecologicas de los Andes Tropicales (CIELAT),
Facultad de Ciencias, Universidad de los Andes, Mérida, Venezuela*

(**) *Fundacion Miguel Lillo, Tucuman, Argentina.*

(***) *Centro de Simulacion y Modelos (CESIMO), Universidad de los Andes, Merida, Venezuela.*

Abstract

The relationship between net photosynthesis and leaf temperature was studied along a gradient in the tropical high Andes in order to determine if there were any differences in CO₂ assimilation parameters due to temperature changes along the soil-air continuum. Two acaulescent rosettes, *Calandrinia acaulis* and *Hypochoeris setosus*, a shrub, *Hypericum laricoides*, and a giant rosette, *Espeletia schultzii*, were chosen for this study at altitudes from 2 950 to 4 200 m. Optimum temperature for photosynthesis decreased from 11.5°C to 7.5°C for *E. schultzii* and from 13.9°C to 8.3°C for *H. laricoides* at 2 950 and 4 000 m, respectively. *H. setosus* showed an increase in optimum leaf temperature for photosynthesis from 3 550 to 4 200 m (8.8°C and 11.7°C, respectively), and *C. acaulis* showed no differences between altitudes (approximately 13.0°C). Net photosynthetic rates were also higher for the two ground level plants (6.5 to 8.0 μmol m⁻²s⁻¹) compared to the higher level plants (4.1 to 4.6 μmol m⁻²s⁻¹). All species showed positive assimilation rates at below 0°C. Optimum temperature for photosynthesis was coupled to air temperatures for the high stratum species, therefore decreasing at higher altitudes. The lower stratum species, strongly influenced by soil temperature, showed similar or even an increase in optimum leaf temperature for photosynthesis with an increase in altitude.

Keywords: Net photosynthesis, leaf temperature, life forms, high tropical mountains.

Résumé

Les relations entre la photosynthèse nette et la température des feuilles ont été étudiées le long d'un gradient dans les hautes Andes tropicales afin de déterminer s'il y a des différences dans les paramètres d'assimilation du CO₂ dues à des changements de température au long du continuum sol-air. Deux rosettes acaulescentes, *Calandrinia acaulis* et *Hypochoeris setosus*, un arbuste, *Hypericum laricoides* et une rosette géante, *Espeletia schultzii* ont été choisis pour cette étude, à des altitudes allant de 2 950 m à 4 200 m. La température optimum pour la photosynthèse décroît de 11,5°C à 7,5°C pour *E. schultzii* et de 13,9°C à 8,3°C pour *H. laricoides*, à 2 950 m et 4 000 m respectivement. *H. setosus* présente une augmentation de la température des feuilles optimum pour la photosynthèse, de 3 550 m à 4 200 m (8,8°C et 11,7°C respectivement) et *C. acaulis* ne présente pas de différence entre les altitudes (environ 13°C). Les taux nets de photosynthèse sont également plus

élevés pour les deux plantes basses ($6,5$ à $8,0 \mu\text{mol m}^{-2}\text{s}^{-1}$) que pour les deux plantes plus hautes ($4,1$ à $4,6 \mu\text{mol m}^{-2}\text{s}^{-1}$). Toutes les espèces présentent des taux d'assimilation positifs au-dessous de 0°C . La température optimum pour la photosynthèse est liée à la température de l'air pour les plantes hautes, elle décroît donc à des altitudes plus élevées. Les plantes basses, fortement influencées par la température du sol, présentent une température optimum pour la photosynthèse similaire ou même plus élevée avec une augmentation de l'altitude.

INTRODUCTION

Plant morphology strongly influences the day-time temperature regime of plants (NOBEL, 1988). Typical life-forms for tropical alpine environments have been described by many authors: giant rosettes are one of the most important ones (*Espeletia* in the Andes; CUATRECASAS, 1979; SMITH, 1981; *Dendrosenecio* and *Lobelia* in East Africa; HEDBERG, 1964; COE, 1967). Together with these giant rosettes, vascular plants may be included into four conspicuous growth forms: 1) tussock graminoids, 2) dwarf shrubs, 3) herbaceous, mostly perennial rosette plants and 4) cushion plants (SMITH & YOUNG, 1987; KORNER & LARCHER, 1988). The first two forms are plants that grow well above ground level, while the last two grow at most a couple of centimeters above ground.

A temperature gradient exists along soil-air profiles, day-time air temperature closer to the ground are always significantly higher than those further away from the ground (MONASTERIO, 1979; AZOCAR & MONASTERIO, 1980) which means that those plants growing closest to the ground will be subjected to greater daily leaf temperatures. Low plant canopies in alpine regions are substantially warmer during the day but cooler during the night with respect to air temperature (KÖRNER & LARCHER, 1988). With respect to the colder nights near the ground for the tropical Andes, AZOCAR *et al.* (1988) and SQUEO *et al.* (1991) have found that plants near the ground use mainly tolerance mechanisms to survive under these more extreme low temperature conditions, while those plants that grow above ground level use avoidance mechanisms, mainly through supercooling, to resist the less rigorous night-time conditions (RADA *et al.*, 1985; GOLDSTEIN *et al.*, 1985). On the other hand, the higher day-time temperatures must have different effects on the ground vegetation with respect to the taller growing plants.

Since leaf temperature is one of the main determinants of the photosynthetic capacity of plants we would expect to find differences in certain parameters such as optimum leaf temperature for photosynthesis and temperature compensation points for plants growing in different strata. Therefore, the purpose of this work was to study how this difference in leaf temperature influences the photosynthetic capacity of plants with different height, comparing on one hand two acaulescent rosettes, and on the other a small shrub and a giant rosette.

MATERIALS AND METHODS

Four different species from two different altitudes were chosen for this study: *Calandrinia acaulis* (Portulacaceae) and *Hypochoeris setosus* (Asteraceae), two acaulescent rosettes growing at 3 550 m (Paramo de Muecubaji) and 4 200 m (Paramo de Piedras Blancas) were selected as the ground level plants. Both species have thin membranaceous leaves, the former 3 to 6 cm long and 0.4 to 0.7 cm wide and the latter 3 to 6 cm long and 1 to 1.5 cm wide. *Espeletia schultzei* (Asteraceae), a giant caulescent

rosette, and *Hypericum laricoides* (Hypericaceae), a small shrub; growing at 2 950 m (Los Plantios) and 4 000 m (Pico El Aguila) were the two higher stratum plants. *E. schultzii* has very thick pubescent leaves 20 to 40 cm long and 3 to 5 cm wide; while *H. laricoides* has membranaceous scale-like leaves approximately 0.5 cm long. Additional information on site descriptions and climatic conditions are described in MONASTERIO & REYES (1980), GOLDSTEIN *et al.* (1985) and RADA *et al.* (1987).

Several plants (n=4-5) of each species at each altitude were excavated with roots and surrounding soil from the study site, transported to the laboratory and placed in a growth chamber equipped with light (12 h photoperiod, $600 \mu\text{mol m}^{-2}\text{s}^{-1}$) and temperature control simulating field conditions (15°C light period, 5°C dark period at 2 950 m; 12°C light period, 3°C dark period at 3 550 m; 7°C light period, 0°C dark period at 4 000 and 4 200 m). Net photosynthesis-leaf temperature curves were carried out in the laboratory using a gas exchange system with an infrared gas analyzer (LCA-2, ADC Ltd.) and a plant chamber (014 m³). The curves were started immediately after the plants were brought to the laboratory completing all measurements for a species at a given altitude in a period no longer than 30 hours. The chamber was equipped with three 36-gauge copper-constantan thermocouples connected to a chart recorder to obtain air and leaf temperatures. A Photosynthetically Active Radiation sensor was also fitted into the chamber for light measurements. A 1 000 $\mu\text{mol m}^{-2}\text{s}^{-1}$ 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. CO₂ assimilation rates were measured through CO₂ depletion from the chamber making sure only photosynthetic tissue was exposed to the chamber environment. These measurements were done at 1 minute intervals for five minutes at each chosen temperature (approximately 3°C intervals at high and low extremes and 0.5 to 1°C at temperatures near the optimum). Plants were left from 10 to 15 minutes at each set temperature. Net photosynthesis (A; $\mu\text{mol m}^{-2}\text{s}^{-1}$) was calculated through:

$$A = V * \Delta\text{CO}_2 / L * \Delta\text{time}$$

where: V is the chamber volume (m³), L is the leaf area (m²), ΔCO_2 are changes in CO₂ concentrations ($\mu\text{mol m}^{-3}$) per time (seconds) (FIELD *et al.*, 1989).

RESULTS

Leaf temperature-net photosynthesis relations for the two taller plants, *Espeletia schultzii* and *Hypericum laricoides*, are shown in figure 1. For *E. schultzii* optimum temperature for photosynthesis decreased from 11.5°C at 2 950 m to 7.9°C at 4 000 m, and for *Hypericum laricoides* it decreased from 13.9°C at 2 950 m to 8.3°C at 4 000 m. Maximum photosynthetic rates for both species were between 4.0 and 5.0 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Figure 2 shows the leaf temperature-net photosynthesis relations in the lower stratum species, *Calandrinia acaulis* and *Hypochoeris setosus*. As opposed to the higher stratum species, *H. setosus* showed an increase in leaf temperature optimum with increase in altitude from 3 550 m to 4 200 m (8.8°C and 11.7°C, respectively). In the case of *C. acaulis*, there were no differences in leaf temperature optimum between the two altitudes (approximately 13°C). Net photosynthetic rates for these lower stratum species were significantly higher compared to the high stratum species (between 6 and 8.5 $\mu\text{mol m}^{-2}\text{s}^{-1}$). Table I includes mean values and standard errors for the different measured parameters for each species at each altitude. There are no differences in term of net photosynthesis comparing different altitudes for each species. With respect to the other parameters, *C. acaulis* does not show any significant differences in any of them, while the other three species show differences in optimum temperature for photosynthesis and high temperature compensation point. In relation to the low temperature compensation

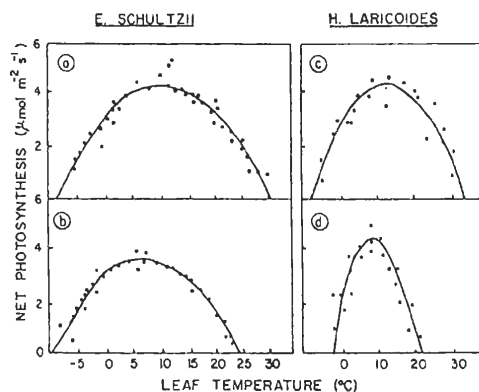


FIG. 1. - Net photosynthesis-leaf temperature curves for the two high stratum plants at two different altitudes. *Espeletia schultzei*: a) 2 950 m, $y = -0.012x^2 + 0.257x + 2.878$, $r^2 = .89$; and b) 4 000 m, $y = -0.012x^2 + 0.180x + 2.950$, $r^2 = .91$. *Hypericum laricoides*: c) 2 950 m, $y = -0.001x^2 + 0.243x + 2.60$, $r^2 = .81$; and d) 4 000 m, $y = -0.024x^2 + 0.397x + 2.60$, $r^2 = .78$.

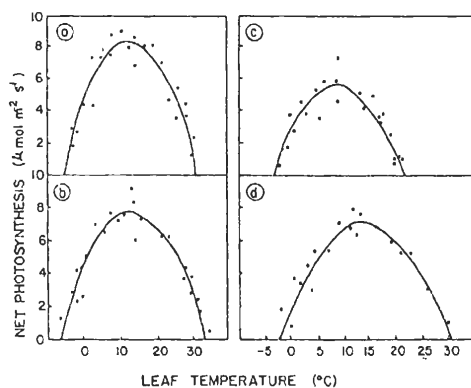


FIG. 2. - Net photosynthesis-leaf temperature curves for the two high stratum plants at two different altitudes. *Calandrinia acaulis*: a) 3 550 m, $y = -0.021x^2 + 0.590x + 4.10$, $r^2 = .78$; and b) 4 200 m, $y = 0.017x^2 + 0.465x + 4.49$, $r^2 = .81$. *Hypochoeris setosus*: c) 3 550 m, $y = -0.032x^2 + 0.580x + 2.83$, $r^2 = .84$; and d) 4 200 m, $y = -0.025x^2 + 0.689x + 2.15$, $r^2 = .82$.

point only *H. laricoides* shows significant differences. Two important results stand out: in the first place, optimum temperature for photosynthesis are much lower in the higher stratum plants as compared to the ground level ones. And secondly, net photosynthetic rates calculated on a leaf area basis are much higher in the ground level plants.

TABLE I. – Low temperature compensation point (LTCP), high temperature compensation point (HTCP), optimum temperature for photosynthesis (P_n (opt)) and maximum net photosynthesis (P_n (max)) for the four studied species at different altitudes.

Species	Altitude	LTCP	HTCP	P_n (opt temp)	P_n (max)
<i>E. schultzi</i>	2950 m	-8±1.1	30.5±2.2	11.5±0.6	4.2±0.8
	4000 m	-9.5±0.8	24.7±1.1	7.9±1.0	3.9±0.4
<i>H. laricoides</i>	2950 m	-8.0±1.6	33.0±1.6	13.9±2.4	4.3±0.6
	4000 m	-2.5±0.8	22.0±2.4	8.3±1.8	4.4±0.8
<i>C. acaulis</i>	3550 m	-5.4±0.3	31.6±0.3	13.1±1.3	8.2±1.4
	4200 m	-6.4±1.0	33.3±1.6	12.8±1.8	7.9±0.8
<i>H. setosus</i>	3550 m	-2.9±1.4	24.4±1.6	8.8±1.7	6.0±1.6
	4200 m	-2.5±1.8	30.8±1.0	11.7±0.5	7.4±1.2

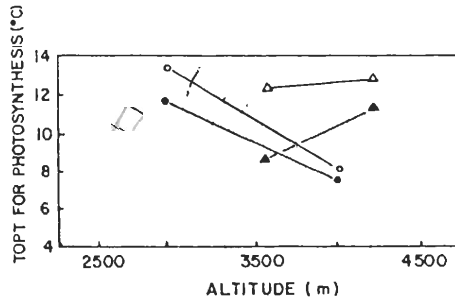


FIG. 3. – Optimum temperature for photosynthesis at different altitudes for species in both strata. *Espeletia schultzi* (●), *Hypericum laricoides* (○), *Calandrinia acaulis* (△) and *Hypochaeris setosus* (▲).

The high temperature compensation points also show interesting results. At 4 000 m, this temperature for the high level plants is 22°C for *H. laricoides* and 25°C for *E. schultzi*. While at 4 200 m the two acaulescent rosettes show compensation points at much higher temperatures, 31°C and 33°C for *H. setosus* and *C. acaulis*, respectively (figures 1 and 2, table I). With respect to low temperature compensation points there are no clear trends between strata. *E. schultzi* shows a low temperature compensation point of approximately -10°C for both altitudes while the other three species have much higher points, *H. setosus* has the highest points with approximately -3.0°C for the two altitudes.

The differences in optimum temperature for photosynthesis and altitude for the chosen species are therefore well differentiated if we compare the different strata (fig. 3). The two higher strata species show an expected decrease in leaf temperature optimum for photosynthesis with increase in altitude. Surprisingly enough, one of the acaulescent species, *H. setosus* shows an opposite trend, while the other, *C. acaulis* maintains similar values along the gradient.

DISCUSSION

Many authors have described how leaves of alpine plants reach impressively high temperatures compared to air temperatures. GATES & JANKE (1966) describe leaf temperatures of 8 to 10°C above air temperature for *Polygonum histortoides* in the Rocky mountains. SALISBURY & SPOMER (1964) find leaf temperatures 10 to 15°C

above air temperature for different Rocky mountain plants at 3 800 m. KÖRNER & COCHRANE (1983) working with different life forms at 2 040 m in the Snowy Mountains of Australia find that the smallest differences between daytime leaf and air temperatures occur in trees and shrubs (between 0 and 10°C); sessile rosettes have mean differences of 14.5°C and maximum differences of up to 24°C and cushion plants have the greatest differences with a mean of 27.4°C and maximum of 30°C. These last authors suggest that these differences nearly compensate for the altitudinal drop of maximum air temperature. Although we did not measure field leaf-air temperature differences in this study, we have found for other species quite similar results as those of KÖRNER and COCHRANE (1983). These differences range from 0 to 10°C in *Espeletia spicata* and *Polylepis sericea*, a giant rosette and a small tree, respectively (RADA, 1983); from 15 to 25°C in *Hinterhubera lanuginosa* and *Arenaria jahnii*, a dwarf shrub and a cushion plant, respectively (unpublished data); all of them growing at 4 200 m in the Paramo de Piedras Blancas. We may then suppose that the plants chosen for this study behave in a similar manner in terms of daytime leaf temperature.

We now may ask ourselves: how do these leaf-air temperature differences influence on the process of photosynthesis for each of these life-forms? Our results show how each of the species adapts to the microenvironment where it is found. Having in mind that *E. schultzi* and *H. laricoides* grow at 50 to 120 cm above ground level, the lowering of the leaf temperature optimum for photosynthesis is coupled to the lowering in air temperature along the gradient. On the other hand, *H. setosus* and *C. acaulis*, growing between 0 and 2 cm are greatly influenced by soil temperature and as a consequence optimum temperature for photosynthesis is similar or it even increases with altitude since bare soil at the higher altitude warms up much more than at the other altitude. Similar results have been described for other species in other regions. SLATYER (1978) shows that for *Eucalyptus pauciflora*, in the Australian Snowy Mountains, the optimum temperature for photosynthesis follows the normal air temperature decrease while other authors have found different results for low growing plants. KÖRNER & DIEMER (1987) find similar optimum temperature for photosynthesis in different herbaceous plants from the Alps along an altitudinal gradient.

With respect to low limits for net photosynthesis, it is interesting to note that all four species show positive assimilation rates at below 0°C for all altitudes. Together with these four species, *Lucilia venezuelensis*, a cushion plant, growing at 4 200 m showed positive net photosynthesis at temperatures of -5 to -7°C (unpublished data). Other authors (PISEK *et al.*, 1967; TIESZEN *et al.*, 1981; taken from KÖRNER & LARCHER, 1988) obtain CO₂ gains in high alpine forbs down to -5°C. *Espeletia schultzi*, a freezing intolerant species (RADA *et al.*, 1987) shows carbon gain down to approximately -10°C. This supports KÖRNER & LARCHER's (1988) observation on net photosynthetic CO₂ uptake being very close to the low temperature limit of leaf survival in species which avoid freezing. Cold resistance mechanisms would have to be studied for the other three species. In the case of high temperature limits for photosynthesis, our results are below those found in the literature. We obtain carbon gains at temperatures of up to 30-34°C for all species, contrasting those results of LARCHER and WAGNER (1976) who report values from 38 to 47°C for different species; including 42°C for *Espeletia semiglobulata* compared to 31°C at 2 950 m and 25°C at 4 000 m for *Espeletia schultzi* in our study.

A final consideration would have to be with respect to maximum photosynthetic rates. At optimum temperatures for photosynthesis, the two acaulescent rosettes show higher assimilation rates ($6-8 \mu\text{mol m}^{-2}\text{s}^{-1}$) compared to the taller plants ($3-5 \mu\text{mol m}^{-2}\text{s}^{-1}$) in this study and other field studies with giant rosettes (GOLDSTEIN *et al.*, 1989). A possible explanation could be a greater concentration of CO_2 from soil respiration for the ground level plants although more detailed studies are required to confirm this hypothesis.

We may conclude this work quoting KÖRNER & LARCHER (1988): “an analysis of the temperature dependence of plant processes will yield different answers dependent on the type of life process (daytime or nighttime activity) considered” and more important “areas typically classified as cold climates are not necessarily ‘cold’ for all plants and their respective functions”.

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REFERENCES

- AZOCAR A. & MONASTERIO M., 1980. – Estudio de la variabilidad meso y microclimática en el Paramo de Mucubaji. In: MONASTERIO M., ed., *Estudios Ecológicos de los Paramos Andinos*. Universidad de los Andes, Merida, 255-262.
- AZOCAR A., RADA F. & GOLDSTEIN G., 1988. – Freezing tolerance in *Draba chionophila*, a “miniature” caulescent rosette species. *Oecologia*, **75**, 156-160.
- COE M. J., 1967. – *The ecology of the alpine zone of Mt. Kenya. Monogr. Biol.*, **17**. The Hague: W. Junk.
- CUATRECASAS J., 1979. – Growth forms of the Espeletinae and the correlation to vegetation types of the high tropical Andes. In: LARSON K. & NIELSON L. B., eds., *Tropical Botany*. New York: Academic Press, 397-410.
- FIELD C. B., BALL J. T. & BERRY J. A., 1989. – Photosynthesis: principles and field techniques. In: PIERCY R. W., EHLERINGER J., MOONEY H. A. & RUNDEL P. W., eds. *Plant Physiological Ecology. Field methods and instrumentation*. Chapman and Hall, 209-253.
- GATES D. M. & JANKE R., 1966. – The energy environment of the alpine tundra. *Oecol. Plant.*, **1**, 39-62.
- GOLDSTEIN G., RADA F. & AZOCAR A., 1985. – Cold hardiness and supercooling along an altitudinal gradient in andean giant rosette species. *Oecologia*, **68**, 147-152.
- GOLDSTEIN G., RADA F., CANALES M. & ZABALA O., 1989. – Photosynthetic gas exchange of two giant caulescent rosette species. *Oecol. Plant*, **10**, 359-370.
- HEDBERG O., 1964. – Features of Afroalpine plant ecology. *Acta Phytogeographica Suecica*, **49**, 1-44.
- KÖRNER C. & COCHRANE P. M., 1983. – Stomatal responses and water relations of *Eucalyptus pauciflora* in summer along an elevational gradient. *Oecologia*, **66**, 443-455.
- KÖRNER C. & DIEMER M., 1987. – In situ photosynthetic responses to light, temperature and carbon dioxide in herbaceous plants from low and high altitude. *Function. Ecol.*, **1**, 179-194.
- KÖRNER C. & LARCHER W., 1988. – Plant life in cold climates. In: LONG S. P. & WOODWARD F. I., eds., *Plants and Temperature. Symp. Soc. Exp. Biol.*, vol. 42, Cambridge: The Company of Biologists Limited, 25-27.
- LARCHER W. & WAGNER J., 1976. – Temperaturgrenzen der CO_2 - Aufnahme und Temperaturresistenz der Blätter von Gebirgspflanzen im vegetationsaktiven Zustand. *Oecol. Plant.*, **11**, 361-374.
- MONASTERIO M., 1979. – El Paramo Desertico en el Altandino de Venezuela. In: SALGADO-LABOUR M. L., ed., *El Medio Ambiente Paramo*. UNESCO-IVIC, Caracas, 117-146.

- MONASTERIO M. & REYES S., 1980. – Diversidad ambiental y variación de la vegetación en los paramos de los Andes venezolanos. In: MONASTERIO M., ed., *Estudios Ecológicos de los Paramos Andinos*, Universidad de los Andes, Mérida, 47-91.
- NOBEL P. S., 1988. – Principles underlying the prediction of temperature in plants with special references to desert succulents. In: LONG S. P. & WOODWARD F. I., eds., *Plants and Temperature. Symp. Soc. Exp. Biol.*, vol. 42, Cambridge: The Company of Biologists Limited, 1-23.
- PISEK A., LARCHER W. & UNTERHOLZNER R., 1967. – Kardinale Temperaturbereiche der Photosynthese und Grenztemperaturen des Lebens der Blätter verschiedener Spermatophyten. I. Temperaturminimum der Nettoassimilation, Gefrier- und Frostschadensbereiche der Blätter. *Flora Abt B*, 157, 239-264.
- RADA F., 1983. – Mecanismos de resistencia a temperaturas congelantes en *Espeletia spicata* and *Polytepsis sericca*. Unpublished MS Thesis, Universidad de los Andes, Mérida, Venezuela.
- RADA F., GOLDSTEIN G., AZOCAR A. & MEINZER F., 1985. – Freezing avoidance in Andean giant rosette plants. *Plant, Cell & Environment*, 8, 501-507.
- RADA F., GOLDSTEIN G., AZOCAR A. & TORRES F., 1987. – Supercooling along an altitudinal gradient in *Espeletia schultzii*, a caulescent giant rosette species. *J. Experiment. Bot.*, 38, 491-497.
- SALISBURY F. B. & SPOMER G. G., 1964. – Leaf temperatures of alpine plants in the field. *Planta*, 60, 497-505.
- SLATYER R. O., 1978. – Altitudinal variation in the photosynthetic characteristics of snow gum, *Eucalyptus pauciflora* sieb ex Spreng. VII. Relationship between gradients of field temperature and photosynthetic temperature optima in the Snowy Mountains area. *Austr. J. of Bot.*, 26, 111-121.
- SMITH A. P., 1981. – Growth and population dynamics of *Espeletia* (Compositae) of the Venezuelan Andes. *Smithsonian Contrib. Bot.*, 48, 1-45.
- SMITH A. P. & YOUNG T. P., 1987. – Tropical alpine plant ecology. *Ann. Rev. Ecol. Syst.*, 18, 137-158.
- SQUEO F., RADA F. & AZOCAR A., 1991. – Freezing tolerance and avoidance in high tropical andean plants: Is it equally represented in species with different plant height? *Oecologia*, 86, 378-382.
- TIESZEN L. L., LEWIS M. C., MILLER P. C., MAYO J., CHAPIN III F. S. & OECHEL W., 1981. – An analysis of processes of primary production in tundra growth forms. In: BLISS L. C., CROGG J. B., HEAL D. W. & MOORE J. J., eds., *A Comparative Analysis of Tundra Ecosystems*. International Biological Programme, 25, Cambridge: Cambridge University Press, 285-356.