Leaf gas exchange in *Espeletia schultzii* Wedd, a giant caulescent rosette species, along an altitudinal gradient in the Venezuelan Andes.

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Abstract – Gas exchange studies along low altitudinal gradients (0-2 500 metres above sea level) suggest an increase in plant CO_2 assimilation with increasing altitude. The question arises however, will this hold true for higher altitudinal ranges? The purpose of this work was to characterize carbon and water relationships in *Espeletia schultzii* along an extreme altitudinal gradient. Gas exchange characteristics under field conditions during wet and dry seasons were documented for *Espeletia schultzii*, a giant caulescent rosette species, along an altitudinal gradient (2 950, 3 550 and 4 200 m) in the Venezuelan Andes. Significant differences in CO_2 assimilation rates between seasons were found for all studied sites. Low assimilation rates obtained during the dry season were a consequence of: low leaf conductances to counteract low leaf water potentials and/or high vapour pressure differences between leaf and air, and to high leaf temperatures measured during the dry season. Important differences in A/P_i curve parameters were obtained between wet and dry seasons. A decrease in A_{max} was observed for the two lower populations, while a similar value was obtained for both wet and dry seasons at the higher altitude. A decrease in the relation P_i/P_a, and an increase in stomatal limitation and leaf temperature were measured from wet to dry season. There was a differentiation in CO_2 assimilation with increasing altitude. At 2 950 m, maximum rates were above 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, at 3 550 m 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$, while at 4 200 m maximum CO_2 assimilation rate was below 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This decrease may be explained by: an increase in leaf pubescence, lower leaf conductances and/or lower leaf nitrogen content at higher altitude. © Elsevier, Paris

1. INTRODUCTION

Giant rosettes of the genus Espeletia (Asteraceae), are the dominant life form of the tropical high Andes above 2 600 m. Espeletia species consist of a terminal rosette, a stem protected from nocturnal freezing temperatures by a layer of marcescent leaves [9, 16, 24] and a central water storage made up of parenchymatous stem tissue [5, 6]. Leaves are lanceolate, succulent, with a thick pubescent layer that covers both upper and lower surfaces [14]. Within this genus, *Espeletia schultzii* is the species with the widest altitudinal distribution in the Venezuelan Andes, growing from 2 600 to 4 200 m. A series of morphological characteristics of *E. schultzii* change along this gradient. For instance, with increasing altitude, total plant height and pith volume per unit leaf area increases, resulting in a larger water storage capacity at higher elevations [6, 13]. Leaves become smaller and more erect with a thicker pubescent layer [14]. This increase in leaf pubescence leads to a greater leaf boundary layer resistance that results in higher leaf temperatures relative to air temperature and higher leaf to air vapour pressure gradients [14]. Leaf tissues of *E. schultzii*, as well as all other species belonging to this genus, endure nocturnal freezing temperatures in an unfrozen, supercooled state [7, 16, 17].

Gas exchange studies for this genus, and in general for the high tropical Andes are scarce. Baruch [1], using $^{14}CO_2$ measurements on mature leaves, reported low CO_2 assimilation rates (1-2 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for three different *E. schultzii* populations along an altitudinal gradient. Gas exchange studies on two other *Espeletia* species (*E. spicata* and *E. timotensis*) at 4 200 m showed low photosynthetic rates which occassionally reached values close to the compensation point. These rates were related to low stomatal con-

ductances and the vertical orientation of the rosette leaves [8]. *Espeletia schultzii* shows a decrease in optimum temperature for photosynthesis along a gradient, from 11.5 °C to 7.5 °C at 2 950 m and 4 200 m, respectively [18]. With respect to other high tropical mountains, Schulze et al. [23] describe carbon dioxide assimilation and stomatal response of four afroalpine rosette plants (*Dendrosenecio deniodendron, D. brassica, Lobelia telekii* and *L. keniensis*).

Most results found in the literature on CO_2 assimilation along altitudinal gradients come from low altitudinal gradients (0-2 500 masl) [3, 10, 26]. Their results indicate an increase in plant CO_2 assimilation with increasing altitude. We may ask ourselves: will the observations of an increase in photosynthetic potential with altitude hold true for higher altitudinal ranges where CO_2 partial pressures, together with other stresses, are even more limiting? The purpose of this work was to characterize carbon and water relations in *Espeletia schultzii* along an extreme altitudinal gradient. Wet and dry season studies were carried out since water availability and low temperatures also directly affect gas exchange characteristics of high mountain plants [11, 19, 21, 22].

2. MATERIALS AND METHODS

Three *Espeletia schultzii* Wedd populations were randomly chosen along an altitudinal gradient in the Venezuelan Andes (8° 52′ N, 70° 45′ W) (*table I*). Three daily courses of gas exchange were carried out during the wet season and two during the dry season, at each site. Measurements were carried out at 1 h intervals in four to six mature, fully expanded leaves (n = 12-18) from each of three different individuals at each site.

Table I. Climatic characteristics for the different study sites.

	altitude (m)	mean annual temp (°C)	precipitation (mm)
Los Plantíos	2 950	8.1	1 005
Mucubají	3 550	5.4	968
P. Blancas	4 200	2.8	798

A fully portable system, consisting of a leaf chamber, an air supply unit, and an infrared gas analyser unit operating in the open mode was used to measure gas exchange in the field (LCA-2 System, ADC Ltd., Hoddesdon, England). Leaf temperatures (n = 3) were measured with fine-wire copper-constantan thermocouples attached to the lower leaf surface. CO_2 assimilation/internal CO_2 concentration (A/P_i) curves were carried out in the field with the same gas exchange

system, with three modifications: (i) An 820 ppm CO_2 cylinder as the source, (ii) the CO_2 concentration in the air entering the system was modified through a CO_2 absorbing column (soda lime), and (iii) a constant light source (800 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was used for all measurements in order to standardize light conditions. The Michaelis Menten equation [$A=A_{max}(m+p_i)/(m+p_i+K)$] was fitted to the A-p_i relationships, with m and k as parameters.

Leaves from gas exchange measurements were used to determine leaf nitrogen content through micro-Kjeldahl. A composite sample from five adult leaves from three different plants were obtained at each site. Three replicas were then analysed from each of these samples.

Leaf water potential (Ψ_1, n = 3) was measured at approximately 2 h intervals during the different daily courses, using a pressure chamber. Air temperatures were measured with copper-constantan thermocouples placed at 1 m above the soil surface and relative humidity was measured with a ventilated wet and dry bulb psychrometer. These air temperatures and relative humidities, together with leaf temperatures were used to calculate vapour pressure difference between leaf and air (VPD).

3. RESULTS

CO_2 assimilation rates (A) decreased with increasing altitude during the wet season (*figure 1* A). At the lowest site, A reached maximum values between 5 and 6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ near midday. The maximum value at 3 550 m was approximately 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$, even though PFD was relatively high most of the day. At the highest site, CO_2 assimilation rates were below 1.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ throughout the complete daily cycle.

Ψ_1 for the wet season were always above -0.5 MPa at all sites, while during the dry season it reached a minimum close to -1.0 MPa. A noticeable effect of water stress on leaf conductance (g_s), during the dry season, and consequently on CO_2 assimilation was observed (*figure 1* B). At all altitudes g_s was constantly low during the dry season daily courses, and its effect on A was also observed as none of the sites showed A_{max} above 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Mean and maximum values for gas exchange parameters obtained from all daily courses at each altitude support the results presented in the previous *figure*, showing a clear difference in seasonal response patterns (*table II*). For all three altitudes, low soil water availability and high vapour pressure differences between leaf and air translated into an important decrease in leaf conductance, with an 80 %, 77 % and 58 % decrease between seasons from low to high altitude, respectively. A decrease in CO_2 assimilation was
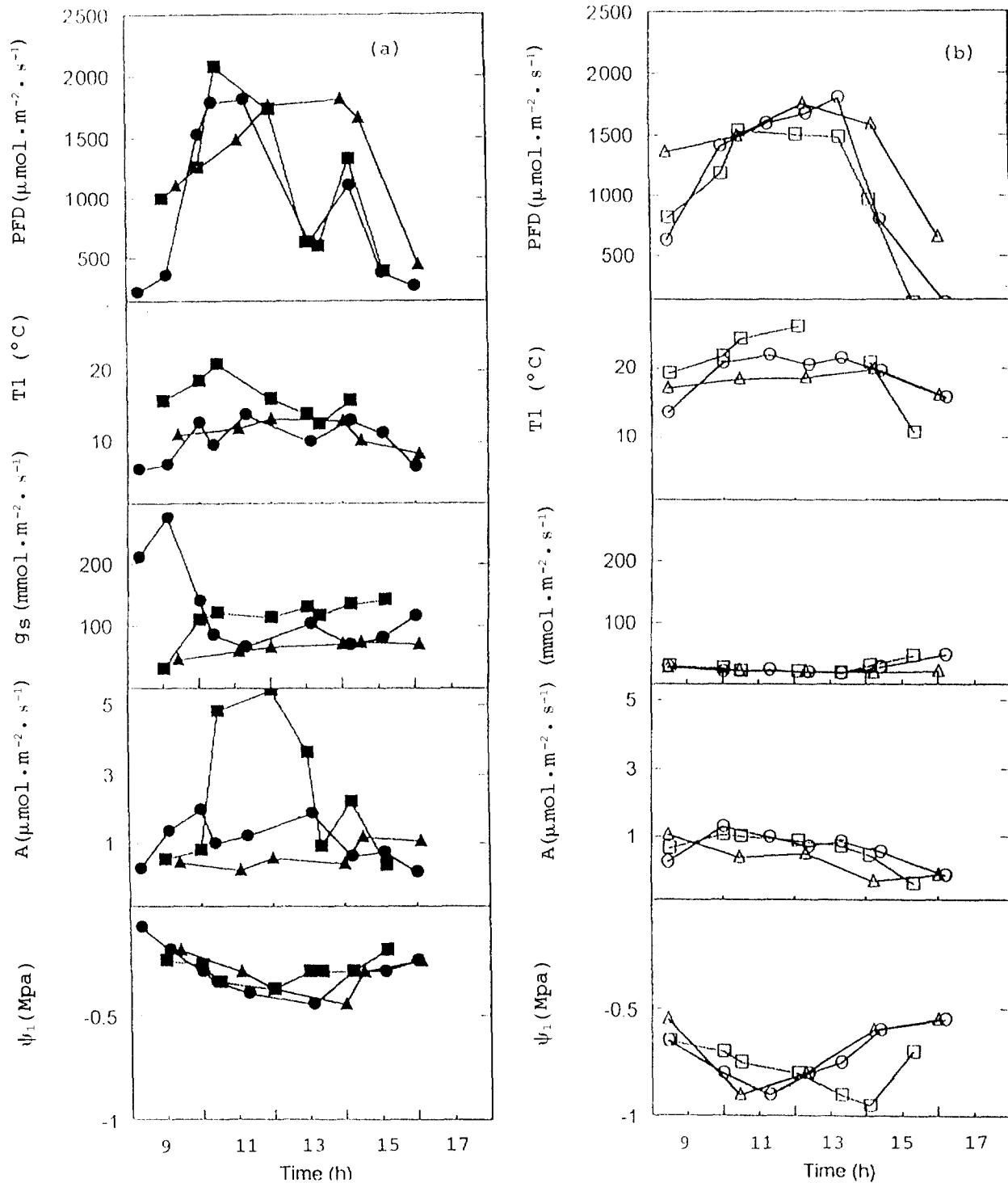
Figure 1. Leaf temperature (T$_1$), photon flux density (PFD), leaf conductance (g$_s$), CO$_2$ assimilation rate (A) and leaf water potential (Ψ_L) during (a) wet and (b) dry seasons for *E. schultzii* at Los Plantios (2 950 m, wet ■, dry ❑), Mucubaji (3 550 m, wet ●, dry ○) and Piedras Blancas (4 200 m, wet ▲, dry △).

Table II. Mean and maximum (in parenthesis) values of: leaf water potential (Ψ_L, MPa), vapour pressure difference between leaf and air (VPD, KPa), leaf temperature (T_L, °C), leaf conductance (g_s, mmol $\cdot$ m^{-2} $\cdot$ s^{-1}), transpiration rate (E, mmol $\cdot$ m^{-2} $\cdot$ s^{-1}), CO_2 assimilation rate (A, μmol $\cdot$ m^{-2} $\cdot$ s^{-1}) and leaf nitrogen content (%) for *Espeletia schultzii* along the altitudinal gradient during both wet and dry seasons. Means correspond to three wet season and two dry season courses ± standard error. Only those points where PFD was above 700 μmol $\cdot$ m^{-2} $\cdot$ s^{-1} were considered.

Alt.	Ψ_L	VPD	T_L	g_s	E	A	N
Wet season							
2 950 m	– 0.31	0.76 ± .07	19.3 ± 0.5	170.3	1.66	2.53	1.69
	± .03	a	a	± 19.6	± 0.27 a	± 0.27 a	± .03 a
	(– 0.55)	(1.18)	(21.8)	(323)	(2.68)	(5.27)	
3 550 m	– 0.30	0.79 ± .08	14.6 ± 0.6	202.1	1.26	1.76	0.93
	± .03	a	b	± 26.6 a	± 0.21	± 0.31 b	± .04 b
	(– 0.45)	(1.16)	(17.1)	(327)	(2.34)	(3.11)	
4 200 m	– 0.29	1.04 ± .11	13.5 ± 0.6	122.3	1.06	1.28	1.05
	± .04	b	b	± 22.4 b	± 0.16 b	± 0.22 b	± .07 b
	(– 0.45)	(1.85)	(16.2)	(284)	(1.87)	(2.57)	
Dry season							
2 950 m	– 0.63	1.28 ± .17	22.9 ± 0.9	34.5	0.68 ± .09	0.78	
	± .08		a	± 4.2	a	± 0.10	
	(– 0.90)	(2.10)	(26.6)	(53)	(1.06)	(1.37)	
3 550 m	– 0.84	1.47 ± .30	19.8 ± 0.7	46.8	0.59 ± .12	0.80	
	± .05		b	± 11.3		± 0.16	
	(– 1.0)	(2.80)	(23.0)	(105)	(1.26)	(1.79	
4 200 m	– 0.67	1.46 ± .22	16.8 ± 1.1	40.4	0.33 ± .06	0.91	
	± .06		c	± 7.6	b	± 0.28	
	(– 0.95)	(2.60)	(21.1)	(71)	(0.66)	(2.20)	

Significant differences at $P < .05$.

also observed between seasons, with a greater impact on the lowest site (70 % reduction from wet to dry), compared to the highest site (29 % reduction).

In relation to the altitudinal gradient (wet season), important differences were observed in most of the parameters measured (*table II*). Ψ_L was constant for all three altitudes, while VPD was higher at 4 200 m compared to the other two altitudes. g_s was significantly lower at the highest site and E decreased with altitude. CO_2 assimilation decreased with altitude: by 30 % at 3 550 m and by 50 % at 4 200 m, compared to the lowest site. Leaf nitrogen content was significantly higher at the lowest altitude compared to the other two sites.

Important differences in A/P_i curve parameters were obtained between wet and dry seasons for the three sites (*figure 2, table III*). A decrease in A_{max} was observed for the two lower populations, while a similar value was obtained for both wet and dry season at the highest altitude. A decrease in the relation P_i/P_a.

and an increase in stomatal limitation were also found from wet to dry season.

4. DISCUSSION

These CO_2 assimilation rates are low when compared to those for *E. spicata* (6-8 μmol $\cdot$ m^{-2} $\cdot$ s^{-1}) and *E. timotensis* (4-5 μmol $\cdot$ m^{-2} ¡ s^{-1}) reported by Goldstein et al. [8] at 4 200 m, and those described by Schulze et al. [23] for the Afroalpine *Dendrosenecio keniodendron, D. brassica, Lobelia telekii* and *L. keniodendron* (8-11 μmol $\cdot$ m^{-2} $\cdot$ s^{-1}), equivalent life-form to the Andean *Espeletias*.

Goldstein et al. [6] describe a well developed water storing pith tissue in the stem of *Espeletia* species which may be essential during periods of seasonal drought. Low leaf conductances during the dry season are sufficient to maintain a positive balance in the assimilation of CO_2 throughout the daily courses. This water storing capacity plays an important role

in *E. schultzii*, maintaining leaf water potentials above -1.0 MPa, compared to other species without this capacity which show minimum Ψ_L below -1.5 MPa (*Hypericum laricifolium*, a shrub) and -2.0 MPa (*Hypochoeris setosus* and *Calandrinia acaulis*, acaulescent rosettes) at the same study sites [F. Rada and A. Azócar, unpublished data].

Low assimilation rates during the dry season were a consequence of low leaf conductances and high leaf temperatures. Rada et al. [18] have documented CO_2 assimilation-leaf temperature relationships (A-LT) for *E. schultzii* at the two extreme sites. Our results show an average leaf temperature of approximately 17 °C for the dry season and 13 °C for the wet season at
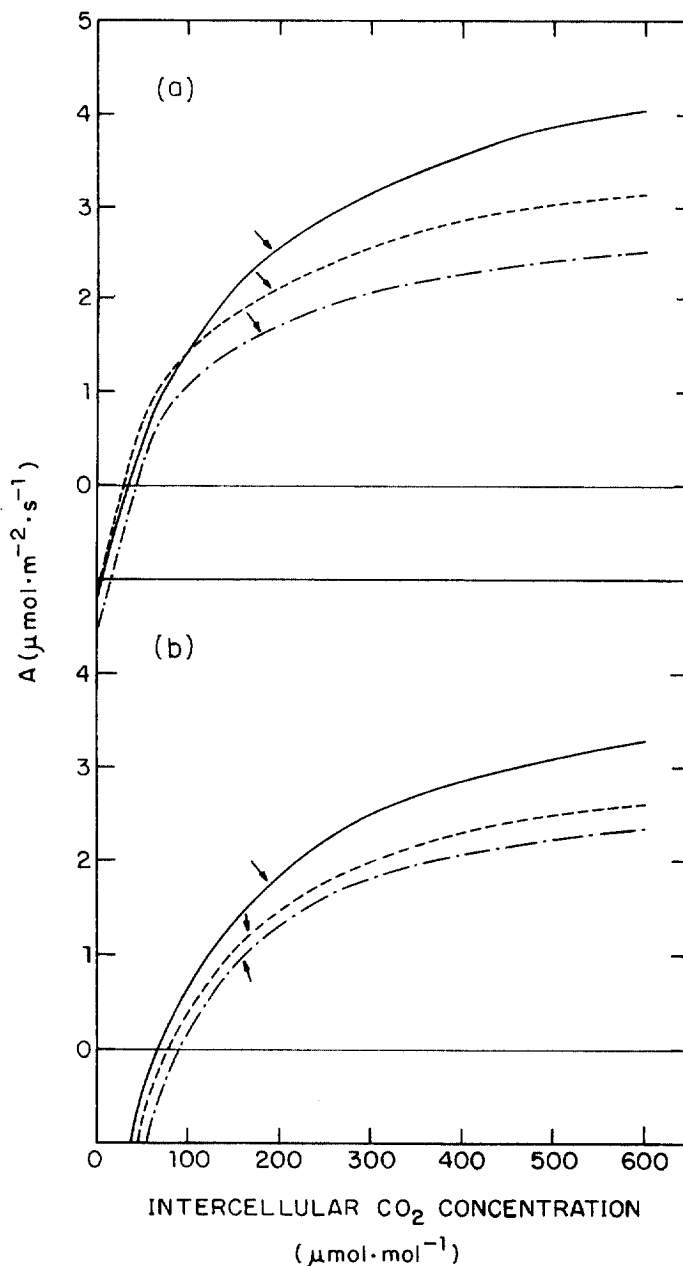

Figure 2. CO_2 assimilation rate as a function of intercellular CO_2 concentration for *E. schultzii* during (a) wet: 2 950 m, r^2 = .78 (—), 3 550 m, r^2 = .76 (...) and 4 200 m, r^2 = .81 (---); and (b) dry: 2 950 m, r^2 = .82 (—), 3 550 m, r^2 = .78 (...) and 4 200 m, r^2 = .87 (---) seasons. The arrows indicate the intercellular CO_2 concentration and assimilation rate at ambient CO_2.

Table III. Maximum CO_2 assimilation rate (A_{max}, µmol · m^{-2} · s^{-1}), internal CO_2/ambient CO_2 relation (P_i/P_a), carboxylation efficiency (dA/dP_i), stomatal limitation (SL, %) and leaf temperature (T_L, °C) obtained from A/P_i curves for *Espeletia schultzii* along the gradient during both wet (n = 4) and dry (n = 4) seasons. Values are means ± standard error.

Alt.	A_{max}	P_i/P_a	dA/dP_i	SL	T_L
Wet season					
2 950 m	3.06	.823	.018	10.6	16.4
	±.19 a	±.032 a	±.0019	±1.1 a	±.6
3 550 m	3.17	.894	.018	4.5	15.1
	±.10 b	±.031	±.0017	±2.0 b	±.4
4 200 m	2.53	.917	.019	2.9	15.8
	±.22 c	±.22 b	±.0023	±1.6 b	±.5
Dry season					
2 950 m	3.08 a	.757	.016	28.1	18.7
	±.27	±.041	±.0024	±2.1	±.3 a
3 550 m	2.59 b	.667	.014	33.1	18.5
	±.39	±.038	±.0017	±3.4	±.2 a
4 200 m	2.33 b	.729	.015	31.0	16.4
	±.19	±.047	±.0026	±1.4	±.2 b

Significant differences at $P < .05$.

4 200 m. According to the A-LT curves for this altitude, there is a reduction of 35 % and 8 % in the assimilation rate for the dry and wet seasons, respectively. At 2 950 m, we obtained average leaf temperatures of 23 °C and 19 °C for dry and wet seasons, respectively. These temperatures represent a 42 % reduction for the dry and 20 % reduction for the wet season in terms of A.

There is also a differentiation in CO_2 assimilation along the gradient. For this comparison, we concentrate on wet season results (*figure 1* A, *table II*), since negative effects on CO_2 assimilation are produced by factors such as water stress or high leaf temperatures during the dry season, as was described previously. Mean and maximum CO_2 assimilation rates decreased from 2 950 m to 4 200 m. These differences in assimilation are maintained in all daily courses carried out, even though plants were not under water stress (Ψ_L above -0.5 MPa), and leaf temperatures for photosynthesis were within the 80 % range of optimum temperature for photosynthesis for this species [18].

The lower assimilation rates at higher altitudes may be explained by a combination of different factors: (i) A significant increase in thickness of leaf pubescence with altitude [14], which may decrease light absorption [8], and/or add a resistance to CO_2 diffu-

sion from ambient to leaf intercellular spaces. Although some authors [4, 10, 11] suggest that the decrease in CO_2 partial pressure with altitude is compensated by the increase in the diffusion rate from ambient to the inner leaf spaces, the significant increase in leaf pubescence with increasing altitude may play an important role in terms of decreasing carbon assimilation. (ii) Lower stomatal conductances due to a higher vapour pressure difference between leaf and air at the highest site. (iii) A decrease in leaf nitrogen content at the higher sites which affects photosynthetic capacity. A clear relationship has been established between leaf nitrogen content and assimilation rate in many species from different habitats [2]. The difference in leaf nitrogen content between the lowest site and the other two sites also coincides with the differences in CO_2 assimilation observed between 2 950 m and the higher altitudes. A pronounced environmental oligotrophism exists in these high mountains. Malagon [12], studying different tropical mountain soils, found significantly poorer soils at the highest altitude (Piedras Blancas) compared to lower sites. Sarmiento [20] indicates that low nutrient availability together with low turnover rates are limiting productivity in Piedras Blancas.

The increase in plant CO_2 assimilation with increasing altitude [3, 10, 26] is explained by an increase in carboxylation efficiency, leaf nitrogen content and leaf conductance with altitude. On the other hand, Vitousek et al. [25] report a decrease in CO_2 assimilation with elevation for *Metrosideros polymorpha* growing on wet sites. These authors suggest that this decrease is due mainly to a decrease in leaf nitrogen concentration. Furthermore, their results do not support an increase in carboxylation efficiency in higher elevation plants. As mentioned in the introduction, most results found in the literature on CO_2 assimilation along altitudinal gradients come from low altitudinal gradients (0-2 500 masl). The gradient we have described begins at higher altitudes and therefore the different stresses discussed (low CO_2 partial pressures, low temperatures, water stress and low nitrogen availability) produce a greater pressure on plant survival.

E. schultzii may be considered a classic example of a species which has been succesful at integrating different morphological and physiological features in order to maintain a favourable water status and a positive carbon balance, thereby establishing along a wide altitudinal gradient in the high Andes.

Acknowledgments

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