Gas exchange and low temperature resistance in two tropical high mountain tree species from the Venezuelan Andes

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Abstract — Temperature may determine altitudinal tree distribution in different ways: affecting survival through freezing temperatures or by a negative carbon balance produced by lower photosynthetic rates. We studied gas exchange and supercooling capacity in a timberline and a treeline species (*Podocarpus oleifolius* and *Espeletia neriifolia*, respectively) in order to determine if their altitudinal limits are related to carbon balance, freezing temperature damage, or both. Leaf gas exchange, leaf temperature-net photosynthesis curves and leaf temperature at which ice formation occurred were measured at two sites along an altitudinal gradient. Mean CO_2 assimilation rates for *E. neriifolia* were 3.4 and 1.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$, at 2 400 and 3 200 m, respectively. Mean night respiration was 2.2 and 0.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for this species at 2 400 and 3 200 m, respectively. Mean assimilation rates for *P. oleifolius* were 3.8 and 2.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 2 550 and 3 200 m, respectively. Night respiration was 0.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for both altitudes. *E. neriifolia* showed similar optimum temperatures for photosynthesis at both altitudes, while a decrease was observed in *P. oleifolius*. *E. neriifolia* and *P. oleifolius* presented supercooling capacities of –6.5 and –3.0 °C, respectively. For *E. neriifolia*, freezing resistance mechanisms are sufficient to reach higher altitudes; however, other environmental factors such as cloudiness may be affecting its carbon balance. *P. oleifolius* does not reach higher elevations because it does not have the freezing resistance mechanisms. © 2000 Éditions scientifiques et médicales Elsevier SAS

Altitudinal gradient / CO_2 assimilation / *Espeletia neriifolia* / *Podocarpus oleifolius* / supercooling capacity

1. INTRODUCTION

The latitudinal and altitudinal limit where trees are found (treeline) is one of the most conspicuous of ecological boundaries [12]. Treeline environments are generally characterized by harsh climatic conditions, which restrict growth, reproduction, and many metabolic functions to short favorable periods [10]. Arctic and alpine treelines have seasonally harsh, long winter periods, characterized by strong winds and snow accumulation. Trees acclimate to this condition, however, by becoming dormant during winter. In contrast, tropical mountain environments are diurnally harsh, with freezing temperatures occurring any night of the year. Trees cannot become dormant under such conditions and must maintain their physiological activity on a daily basis all year round. In the case of timberlines, the upper limit of continuous forest (as opposed to treeline which corresponds to the extreme limits of individual or groups of trees), the most widely accepted explanation for higher latitudes, is that growing seasons are too short and too cool for shoots to fully develop and harden against winter conditions [2, 30, 34, 35]. However, the timberline in tropical mountains shows greater differences between day and night temperatures than between seasons, and timberline explanations that involve winter damage are, therefore, unlikely to be directly applicable here.

Temperature has long been recognized as one of the primary factors influencing global vegetation distribution. Tranquillini [31] in a complete review of the physiological ecology of trees in the alpine timberline, recognized that temperature can determine the altitudinal tree distribution in two main ways:
- First, by directly affecting the survival of trees trough the occurrence of freezing temperatures that could cause severe injury to plants, especially at the cellular

Table I. Environmental characteristics and studied species for each of the chosen sites.

Site	Elevation (m)	Mean temperature (°C)	Precipitation (mm)	Species
San José de Acequias	2 400	12	800–900	*E. neriifolia*
La Culata	2 550	13.6	1 655	*P. oleifolius*
San José de Acequias	3 200	8	1 000–1 100	*E. neriifolia*; *P. oleifolius*

level. This suggests that the limit extends to the altitude where the tissues can still survive ambient temperature (but see [12]). This last factor is not considered for timberlines in temperate climates due to the mentioned acclimation of trees to such condition by becoming dormant during winter. In tropical high mountains, however, due to low seasonal temperature variations, trees cannot avoid low nighttime temperatures by becoming dormant, and as a consequence, freezing temperatures could be involved in setting the altitudinal timberline. Studies have indicated that plants capable of withstanding freezing temperatures use at least one of three distinct survival strategies: deep supercooling, extracellular freezing and extraorgan freezing [3, 26, 28].

- Second, low temperatures diminish the rate of photosynthesis which may give rise to a negative carbon balance. This leads to the proposition that, in temperate zones, the altitudinal tree limit is found at the elevation where summer assimilation and growth is balanced by winter losses, or where trees can maintain a positive carbon balance throughout the entire year. In contrast, trees in tropical high mountains face the problem of adapting their carbon assimilation machinery in order to maintain a positive carbon balance in daily terms.

Practically all ecophysiological studies on timberline have been conducted in temperate and subpolar regions, where plants are subjected to winter stress [12, 34] and very few studies have addressed the problem of what could determine the altitudinal limit of trees in tropical environments. The main purpose of this work was to study gas exchange characteristics (as an approximation of plant carbon balance) and supercooling capacity (as a low temperature resistance mechanism) in a timberline and a treeline species of the tropical Venezuelan Andes. Are gas exchange characteristics and/or low temperature resistance mechanisms involved in setting the altitudinal limits of trees in tropical mountains?

2. MATERIALS AND METHODS

2.1. Study species

A tree species that grows and reproduces at timberline in the Venezuelan Andes was selected: *Podocarpus oleifolius* Buchh. et Gray (Podocarpaceae-Gymnospermae) is an evergreen tree with spiral-alternate leaves 3–12 cm long. This species grows between 2 200–3 200 m a.s.l., reaching no more than 4 m in height at the timberline. *Espeletia neriifolia* Sch. Bip. (Asteraceae), a treeline species belonging to the successful tropical high mountain genus of rosette plants, is an evergreen tree which grows 3–4 m in height. This species grows between 2 000–3 600 m a.s.l. [32].

2.2. Study sites

Two study sites at different elevations in the tropical Venezuelan Andes were selected for each species (*table I*). One site was below the average altitudinal limit of continuous forests in this area (approx. 3 000 m a.s.l.), and one site above this limit. The study sites present a bimodal precipitation regime [17, 18], characterized by a maximum between April and June and another between September and November. Cloudiness is one of the main climatic characteristics most of the year for the upper site. According to Azócar and Monasterio [5] at elevations above 3 000 m, incoming radiation during the wet months could be as low as 20 % of the maximum available.

2.3. Leaf gas exchange measurements

For each study site, two 24-h courses of gas exchange measurements were carried out during the wet season. At intervals of 2 h, six leaves were chosen from each of three to four different young individuals (< 2 m in height) of each species for the gas exchange measurements. A fully portable system, consisting of a leaf chamber, an air supply unit, and an infrared gas analyzer, operating in the differential mode was used to measure gas exchange in the field (LCA-2 System, The Analytical Development Co. Ltd., Hoddesdon, England).

Leaf temperatures ($n = 3$) were measured at hourly intervals with fine-wire copper-constantan thermocouples attached to the lower leaf surface. Air temperatures were measured with copper-constantan thermocouples placed at 1.5 m above the soil surface. Relative humidity was measured with a ventilated wet-dry bulb psychrometer. Leaf and air temperatures and relative humidity, were used to calculate vapor pressure difference between leaf and air (VPD). Leaf water potentials (Ψ_L, $n = 6$) were measured with a pressure chamber at 2-h intervals during the different daily courses.

2.4. Leaf temperature-net photosynthesis relationships

For net photosynthesis-leaf temperature curves, four saplings, < 1 m in height, were excavated with roots and surrounding soil from the study sites and transported immediately to the laboratory. Net photosynthesis curves were carried out in the laboratory using a gas exchange system with an infrared gas analyzer (LCA-2) and a plexiglass plant chamber (0.14 m^3) as described by Rada et al. [21]. The chamber was equipped with three 36-gauge copper-constantan thermocouples connected to a chart recorder to obtain air and leaf temperature. A photosynthetically active radiation (PAR) sensor was also fitted into the chamber for light measurements. 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 temperature and consequently leaf temperature within the chamber. CO$_2$ assimilation rates were measured through CO$_2$ depletion from the chamber making sure only photosynthetic tissue was exposed to the chamber environment. These measurements were done at 1-min intervals for 5 min 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 min at each temperature. Net photosynthesis (A, μmol·m^{-2}·s^{-1}) was calculated according to Field et al. [7]. 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 24 h.

The relationship between leaf temperature and assimilation rate was obtained fitting the observed point to a second order polynomial function with the quasi-Newton method provided by STATISTICA [29]. Optimum temperature for photosynthesis, for each species, at each altitude was calculated through the first derivative of the polynomial curves. High and low compensation points, temperatures at which leaf carbon balance becomes zero, were obtained solving the polynomial equations.

2.5. Thermal analysis

To determine the temperature at which tissue freezing occurred [19], leaves were cut and immediately enclosed in small, tightly sealed test tubes, thus avoiding changes in tissue water content. Copper-constantan thermocouples were inserted in the tissue and temperatures were continuously monitored with a chart recorder. The tubes ($n = 3$) were placed in a refrigerated alcohol bath and temperature was lowered from 10 to –15 °C at a rate of approximately 10 °C·h^{-1}. Tissue freezing temperatures can be readily determined by a marked increase in temperature as a result of the exothermic process of ice formation.

3. RESULTS

Mean daily CO$_2$ assimilation rate for *Espeletia neriifolia* at 2 400 m was 3.4 μmol·m^{-2}·s^{-1}, with a maximum of 6.6 μmol·m^{-2}·s^{-1}. The highest assimilation rates were reached during the morning with values above 4 μmol·m^{-2}·s^{-1} until midday when photosynthetically active radiation (PAR) was high (*figure 1a*). CO$_2$ assimilation decreased after 13:00 hours due mainly to lower PAR values caused by cloud increase throughout the afternoon. Night respiration rate averaged 2 μmol·m^{-2}·s^{-1}, reaching a maximum of 2.8 μmol·m^{-2}·s^{-1} at 03:00 hours (*figure 1a*). Leaf water potential (Ψ_L) was relatively constant during the day, reaching a minimum of –0.9 MPa at around midday. At night, leaf water potential becomes slightly more positive reaching a maximum of –0.7 MPa. Leaf conductance was high during the morning hours decreasing after midday. As expected, stomata closed after 19:00 hours and remained closed until sunrise, when leaf conductance begins to increase.

On a typical cloudy day during the wet season, with PAR below 300 μmol·m^{-2}·s^{-1} throughout the day (*figure 1b*), mean and maximum CO$_2$ assimilation rates during the course were lower for *E. neriifolia* at 3 200 m (1.3 and 2.2 μmol·m^{-2}·s^{-1}, respectively). This represents a 62 % reduction compared to rates at 2 400 m. Nighttime respiration rate was lower compared to 2 400 m with an average of 0.9 μmol·m^{-2}·s^{-1}, and a maximum of 1.3 μmol·m^{-2}s^{-1} (*figure 1b*), representing a 53 % decrease compared to the average respiration rate obtained at 2 400 m. PAR levels were also lower than at 2 400 m and this could explain, in part, the lower assimilation rates found at 3 200 m. Ψ_L was maintained relatively constant during the day, reaching a minimum value of –0.8 MPa at midday. At night, Ψ_L becomes more positive reaching a maxi-
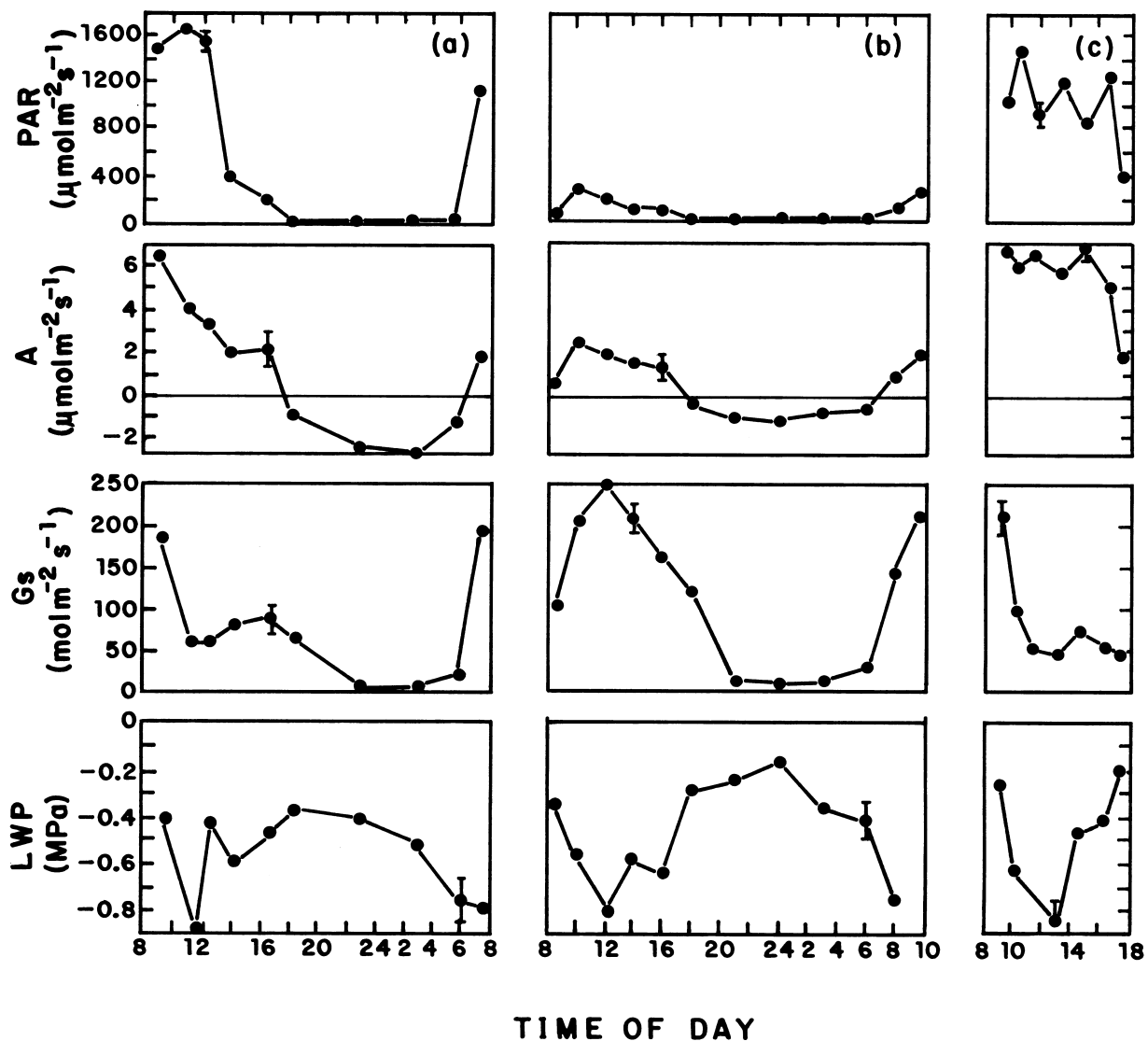
Figure 1. Representative daily courses of photosynthetically active radiation (PAR), CO_2 assimilation rate (A), leaf conductance (G_s) and leaf water potential (LWP) for *Espeletia neriifolia* at (a) 2 400 m, (b) 3 200 m on a typical cloudy day, (c) 3 200 m on an exceptional clear day. Vertical bars represent maximum standard error.

mum of –0.4 MPa. Leaf conductance was high during the morning hours, decreasing after midday. On an exceptionally clear day, mean CO_2 assimilation rate was higher for *E. neriifolia* at 3 200 m (5.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) compared to 2 400 m, due to higher PAR throughout the day (*figure 1c*). However, maximum CO_2 assimilation was similar at both altitudes (about 6.5 $\mu mol \cdot m^{-2} s^{-1}$).

Mean daily CO_2 assimilation rate for *P. oleifolius,* at 2 550 m, was 3.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with a maximum of

5.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (*figure 2a*). PAR reached a maximum of 1 330 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at midmorning and then decreased the rest of the day. Average nighttime respiration rate was 0.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with a maximum of 1.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at midnight (*figure 2a*). Ψ_L was maintained relatively constant during the day, reaching a minimum of –0.5 MPa at midday and slightly more positive values through the night. Leaf conductance was high during the afternoon hours until after 19:00 hours when stomata closed.
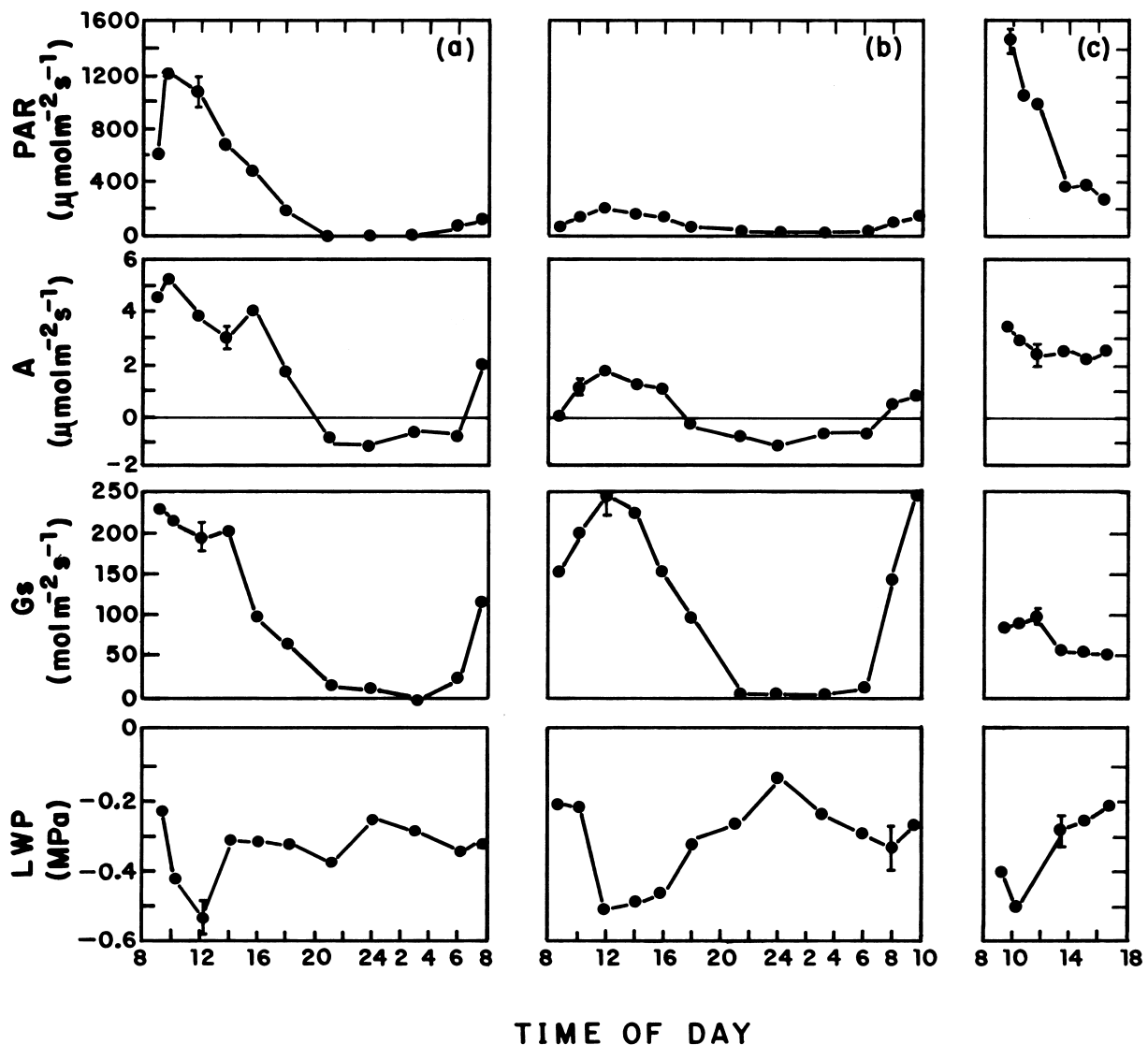
Figure 2. Representative daily courses of photosynthetically active radiation (PAR), CO_2 assimilation rate (A), leaf conductance (G_s) and leaf water potential (LWP) for *Podocarpus oleifolius* at (a) 2 400 m, (b) 3 200 m on a typical cloudy day, (c) 3 200 m on an exceptional clear day. Vertical bars represent maximum standard error.

CO_2 assimilation rates for *P. oleifolius* were lower at 3 200 m compared to 2 550 m (*figure 2b*). Average CO_2 assimilation was 1.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a maximum of almost 1.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at midday. Nighttime respiration rates were also very similar to those obtained at the lower site with an average of 0.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and a maximum of 1.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Leaf conductance was high during the afternoon hours until around 19:00 hours when stomata closed until the next day (*figure 2b*). On a clear day (*figure 2c*), PAR levels were similar to those

at 2 400 m, with a maximum of 1 460 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at midmorning and then decreasing the rest of the day. Ψ_L, for *P. oleifolius* at 3 200 m, was relatively constant during the course, with a minimum value of –0.5 MPa at midday and slightly more positive values throughout the rest of the day. Mean daily assimilation rate was 2.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, while leaf conductance was lower compared to the other courses.

The CO_2 assimilation-leaf temperature relationship showed that optimum leaf temperature for photosyn-

Table II. Optimum temperature for photosynthesis (Opt. T°, °C), low temperature (LTCP, °C) and high temperature (HTCP, °C) compensation points; mean day (T°d) and night (T°n) leaf temperature for the studied species at the different altitudes. Mean day and night leaf temperatures at 3 200 m correspond to typical cloudy conditions for the wet season (see *figures 1b; 2b*).

Species	Altitude	Opt. T°	LTCP	HTCP	T°d	T°n
P. oleifolius	2 550 m	15.3	–2.6	33.0	19.1	12.1
	3 200 m	13.7	–1.4	28.9	13.4	7.8
E. neriifolia	2 400 m	19.8	2.7	36.9	18.8	12.5
	3 200 m	19.4	1.5	37.3	12.4	7.5

thesis for *P. oleifolius,* growing at 2 550 m, was 15.3 °C, decreasing to 13.7 °C at 3 200 m (*table II*; *figure 3*). Optimum leaf temperatures for photosynthesis in *E. neriifolia* were 19.8 and 19.4 °C at 2 400 and 3 200 m, respectively (*table II*; *figure 3*).

Thermal analysis showed that at 3 200 m, *P. oleifolius* had a supercooling capacity of –3.0 ± 0.8 °C while *E. neriifolia* supercooled down to –6.5 ± 1.3 °C.

4. DISCUSSION

At high altitudes, the zone where forest extension terminates represents the most abrupt change in plant dominance on a large geographical scale [12, 27]. The lack of coincidence between timberline and treeline in general, suggests that it is strongly determined by the ability of tree species, which grow above timberline, to
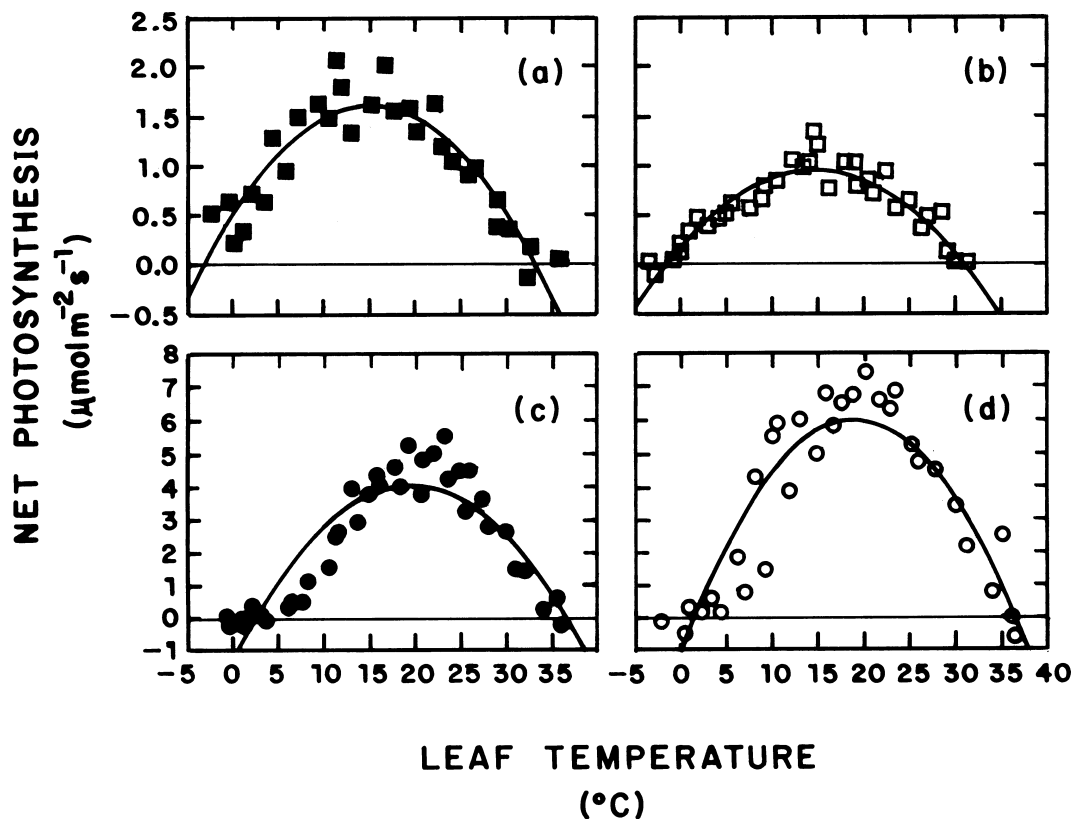

Figure 3. Net-photosynthesis-leaf temperature curves for *Podocarpus oleifolius*, (a) 2 550 m ($y = -0.0049x^2 + 0.151x + 0.43$, $r^2 = 0.79$) and (b) 3 200 m ($y = -0.0036x^2 + 0.106x + 0.16$, $r^2 = 0.87$); and *Espeletia neriifolia*, (c) 2 400 m ($y = -0.0133x^2 + 0.527x - 1.32$, $r^2 = 0.79$) and (d) 3 200 m ($y = -0.0186x^2 + 0.722x - 1.05$, $r^2 = 0.81$).

adapt to the increasing severity of natural climatic conditions encountered at higher altitudes.

The results of this study on gas exchange and supercooling capacity in *P. oleifolius* and *E. neriifolia* at two different altitudes, show that the first of these species has the capacity to adjust its photosynthetic machinery as altitude increases. Optimum leaf temperature for photosynthesis decreased from 15.3 °C at 2 550 m to 13.7 °C at 3 200 m. Average CO_2 assimilation rate decreased with increasing altitude suggesting that *P. oleifolius* could be limited, to a certain degree, in terms of carbon gains. Assimilation/respiration ratios from integration of daily courses for both altitudes were 4.8 at 2 550 m and 1.4 at 3 200 m. On the other hand, with a supercooling capacity of –3 °C, this species does not have cold resistance mechanisms that enable it to have a higher altitudinal distribution. Goldstein et al. [9] have described an injury temperature of –1.8 °C for this species. Freezing temperatures of this magnitude are frequent at 3 200 m, especially at night during the dry season [17].

On the contrary, for *E. neriifolia*, a supercooling capacity of –6.5 °C at 3 200 m a.s.l. indicates that cold resistance mechanisms are sufficient to reach its present altitudinal limit (3 600 m). However, our data suggest that the altitudinal limit for this species is likely to be linked to carbon balance. Daytime energy input is relatively low above 3 000 m in tropical environments, especially during the wet season, due to enhanced cloudiness. Only with very particular conditions during the wet season, with a high energy input during the whole day, which meant higher PAR and leaf temperatures, did we observe a much greater assimilation to respiration ratio. On typical cloudy days, which account for most of the year, this ratio could not be maintained. Therefore, with no adjustment in optimum temperature for photosynthesis and with lower PAR values, this assimilation to respiration ratio must surely decrease. Even though *E. neriifolia* has been reported up to 3 600 m, at this altitude, it is found near massive rock outcroppings considered true thermal refuges [9, 33].

High elevation trees from temperate regions have higher leaf dark respiration rates compared to those of lowland species [14, 31]. In the case of tropical high mountains, Goldstein et al. [9] have reported even higher leaf respiration rates for tree species compared to temperate mountain species and to tropical cloud forest species. These authors also conclude that "high respiration rates coupled with a relatively high photosynthetic capacity may be a prerequisite for tree survival at high elevations in the tropics".

Dark respiration of foliage consumes carbon fixed in photosynthesis for the construction of new tissue and maintenance of existing tissue [24, 25]. In general, root and shoot respiration (growth and maintenance), account for approximately 43 % of the total carbon assimilated each day in photosynthesis [14]. Thus, the rest (ca. 57 %) corresponds to carbon invested in growth. High leaf respiration rates may be needed to increase osmotically active solutes at night [20] or to repair cellular damage produced by low temperatures [15, 31], both of which imply significantly greater energy expenditure. This determines that carbon assimilated by plants under such conditions may be used mainly for metabolic processes of maintenance and repair of structures and organs, decreasing the carbon availability for other processes such as accumulation of dry matter. This restriction in the quantity of dry matter that can be accumulated by plants could determine that arborescent forms become poor competitors in these high mountain ecosystems.

In general, gymnosperm trees show lower assimilation rates than angiosperm trees [13]. According to the existing literature, photosynthesis values range between 2–25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for angiosperm trees (mainly deciduous broad-leafed trees) and between 1–10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for gymnosperm trees (mainly coniferous) [6]. As expected, the gymnosperm *P. oleifolius* displays, independently of elevation, lower assimilation rates than the angiosperm *E. neriifolia*. For *E. neriifolia*, our results show a maximum CO_2 assimilation rate of 6.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at both altitudes. *Espeletia schultzii*, a giant rosette, presents maximum CO_2 assimilation rates of 1.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 4 200 m and it was only at 2 950 m that maximum rates of 5–6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were obtained [23]. Maximum assimilation rates of approximately 4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ have been described for *P. oleifolius* in previous studies [9, 16], similar to our results (5.7 and 2.9 $\mu mol \cdot m^{-2} s^{-1}$ at 2 550 and 3 200 m, respectively). In juveniles of two cloud forest tree species, *Decussocarpus rospigliosii* and *Alchornea triplinervia*, maximum photosynthetic rates in forest gaps were around 4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [8]. For three upper montane cloud forest tree species along a successional gradient, Añez [1] reports maximum assimilation rates of 7.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *Heliocarpus americanus*; 5.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *Tetrarchidium rubrivenium* and 3.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *Aspidosperma fendleri*. Three tree species of the upper montane rain forest of Jamaica, *Clethra occidentalis*, *Lyrilla racemiflora* and *Hedyosmum arborescens*, showed maximum photosynthetic rates of 6.6, 5.6 and 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively [4]. These results show

that independently of differences due to species, the assimilation rates of trees at the timberline are low. Nevertheless, *Polylepis sericea* can grow up to 4 600 m, with an average CO_2 assimilation rate of approximately 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the wet season and maximum rates of 7.4 in the field and 8.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the laboratory [22].

In summary, our study shows that for *E. neriifolia*, cold resistance mechanisms should be sufficient to reach higher altitudes; however, other environmental factors, such as cloudiness, may be affecting its carbon balance. On the other hand, *P. oleifolius* does not have the cold resistance mechanisms that enable it to reach higher altitudes. These results suggest that, at least in tropical high elevation environments, there is no unique mechanism that determines the altitudinal limits in trees, rather this limit depends on both intrinsic characteristics of the species and particular environmental conditions. Körner [11, 12] suggests that the tree life form is limited at treeline by the potential investment rather than production of assimilates, in other words, a growth limitation instead of a photosynthesis or carbon balance limitation. Even though we do not have data on carbon allocation and growth, our results suggest that, in the case of these species, carbon balance may be affected by environmental factors such as a low quantum input due to cloud cover most of the year.

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