

Photosynthetic performance of *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) in a high-elevation site of the Andes of central Chile

Desempeño fotosintético de *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) en los Andes de Chile central

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ABSTRACT

Photosynthesis of *Colobanthus quitensis* and mesoclimatic conditions of air temperature and light intensity during the growing season were investigated at 2,650 m in the central Chilean Andes. On three typical days of the growing period (January, March and May), CO₂ exchange and chlorophyll fluorescence were measured. In addition, a series of fluorescence response curves with increasing light intensity at different temperatures were performed to estimate the propensity of Andean *C. quitensis* populations to be photoinhibited. Net Photosynthesis (Pn) was low (ca. 2.0 μmol CO₂ m⁻²s⁻¹) during the morning and noon in days with high photosynthetic active radiation (PAR, above 1,800 μmol photons m⁻²s⁻¹). Pn increased in the afternoon (3.5-4.8 μmol CO₂ m⁻²s⁻¹) when PAR decreased to ca. 1,400 μmol photons m⁻²s⁻¹ and leaf temperature were ca. 20 °C. F_v/F_m in the diurnal periods was between 0.7-0.75 without evidence of photoinhibition. Leaves at 15 and 22 °C exhibited a slow decrease of Φ_{PSII} with the increase in actinic light intensity, although the fraction of reaction centers open (expressed by q_p) remained higher at 22 °C. NPQ was saturated at light intensities close to 500 μmol photons m⁻²s⁻¹ in leaves at 22 °C and at higher intensities at 15 °C, suggesting that NPQ could be a mechanism of energy dissipation at high light intensity and high leaf temperature in the field. Our results indicated that *C. quitensis* is not photodamaged during the diurnal cycle and that the low Pn registered during some diurnal periods are likely to be related with photorespiration, which has been suggested as an efficient protective mechanism for photoinhibition in alpine plants. Our results are also compared with the photosynthetic performance of *C. quitensis* populations from the maritime Antarctic.

Key words: net photosynthesis, chlorophyll fluorescence, NPQ, alpine climate, alpine plants, Andes.

RESUMEN

Se estudió la fotosíntesis de *Colobanthus quitensis* y las condiciones mesoclimáticas de temperatura del aire e intensidad lumínica a 2.650 m en los Andes de Chile central. Se midió la tasa de intercambio gaseoso y la fluorescencia de la clorofila en tres días típicos durante la estación de crecimiento (enero, marzo y mayo). Adicionalmente, se realizaron una serie de curvas de respuesta a incrementos en la intensidad lumínica a diferentes temperaturas para estimar la propensión de *C. quitensis* a la fotoinhibición. La fotosíntesis neta (Pn) fue baja (ca. de 2,0 μmol CO₂ m⁻²s⁻¹) en la mañana y mediodía en días con alta radiación fotosintéticamente activa (PAR, sobre los 1.500 μmol fotones m⁻²s⁻¹). La tasa de fotosíntesis aumentó en la tarde (3,5-4,8 μmol CO₂ m⁻²s⁻¹) cuando la radiación PAR disminuyó a alrededor de 1.000 μmol fotones m⁻²s⁻¹. F_v/F_m osciló entre 0,7-0,75 en los registros de enero y marzo, decreciendo en mayo a 0,67. Hojas medidas a 15 y 22 °C mostraron un leve descenso en Φ_{PSII} con el aumento en la intensidad de luz actínica, aunque la fracción de centros de reacción abiertos (expresados por q_p) fue mayor a 22 °C. NPQ fue saturado a intensidades lumínicas cercanas a 500 μmol photons m⁻²s⁻¹ en hojas a 22 °C, y a intensidades mayores en hojas a 15 °C, sugiriendo que NPQ puede ser un mecanismo de disipación energética bajo condiciones de altas intensidades lumínicas y altas temperaturas en el campo. Nuestros resultados indican que *C. quitensis* no sufre fotodaño durante los ciclos diurnos, y probablemente, los bajos Pn registrados durante algunos periodos diurnos están relacionados con fotorrespiración, la cual ha sido sugerida como un mecanismo muy eficiente en la protección de plantas alpinas contra la fotoinhibición. Nuestros resultados también son comparados con el desempeño fotosintético de las poblaciones de *C. quitensis* en la Antártica marítima.

Palabras clave: fotosíntesis neta, fluorescencia de la clorofila, apagamiento no fotoquímico, clima andino, plantas andinas.

INTRODUCTION

High elevation habitats are well known for their severe climatic conditions for life. There, plants face low temperature, high diurnal temperature oscillations, short growing seasons, excessive radiation, soils with low nutrient availability, strong winds and unstable substrates as a result of local avalanching, cryoperturbation, and run-off associated with snow melt (Körner 1999). Like other alpine zones, the high-elevation habitats of the Andes of central Chile share all the above-mentioned climatic characteristics, although they are also characterized by scarce precipitations during the growing season, generating water shortage conditions that can last up to 5 months (Cavieres et al. 1998).

Colobanthus quitensis (Kunth) Bartl (Caryophyllaceae) is one of the species that grow under these severe environmental conditions of the central Chilean Andes. This species is a perennial plant that forms discrete compact cushions of small size. Its leaf anatomy is typical of xerophytes with thick mesophyll, small stomata and waxy cuticles (Mantovani & Vieira 2000). It is distributed along the Andes from the Ecuador to the Maritime Antarctic (Smith 2003). However, most of the ecophysiological information about this species and its adaptations to low temperature environments come from studies carried out in the Antarctic populations, under the intriguing question of why this species is the only dicot that has been able to colonize the Antarctic territories (Xiong et al. 1999, Xiong et al. 2000, Bravo et al. 2001, Alberdi et al. 2002). Antarctic populations of *C. quitensis* have been described as morphologically and physiologically adapted to succeed in this cold environment. For instance, Xiong et al. (1999) have shown that *C. quitensis* photosynthetic machinery is well adapted to the low temperature of the Antarctic, being able to maintain relatively high photosynthetic rates at low temperature (below 10 °C). However, plants under high temperature (above 20 °C) had negligible photosynthesis.

Considerable less attention has been paid to the Andean populations of *C. quitensis* and their particular adaptations to the local environment. Recently, Gianoli et al. (2004) reported that individuals of *C. quitensis*,

collected from La Parva in the Andes of central Chile and from the Antarctic, were morphologically different when growing in a common garden experiment, suggesting local differentiation or ecotypes. Furthermore, after cold acclimation at 4 °C, plants from the Antarctic were more tolerant to freezing than those from La Parva, indicating that Andean populations also attain a different physiological performance compared to Antarctic populations (Gianoli et al. 2004).

Although there are no studies detailing temperature and irradiance conditions during a entire growing season in the Andes of Central Chile, clear days with high irradiance (above 2,000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) and high diurnal temperature seems to be frequent. For instance, (Cavieres et al. 2006) reported that soil surface temperature may reach up to 45 °C several times during the growing season, indicating that heat stress could be experienced by Andean plants (Körner 1999). The high irradiance and the high temperature conditions of the central Chilean Andes may determine special adaptations in the photosynthetic apparatus of *Colobanthus quitensis* populations in order to cope with the constrains imposed to photosynthesis performance by higher excitation pressure (Huner et al. 2002).

The aims of this study were to describe the prevailing conditions of temperature and irradiance in the Andes of central Chile during the snow-free period, and characterize the diurnal photosynthetic response of an Andean population of *Colobanthus quitensis* during typical days of the summer-autumn period. In addition, we performed a series of fluorescence response curves with increasing light intensity at different temperatures to estimate the propensity of this *C. quitensis* population to be photoinhibited. This study is the first step for a comprehensive understanding of the ecophysiology of this plant species in the high elevation habitats of the Andes.

MATERIAL AND METHODS

Study site

Work was conducted at 2,650 m elevation (33°19' S, 70°17' W), on a west-facing slope nearby La Parva ski resort, distanced 50 km

east of the city of Santiago. The dominant vegetation is comprised by dwarf shrubs, cushion-forming species, rosette forming perennial herbs, and some annual species (Cavieres et al. 2000). *Colobanthus quitensis* grows as discrete very small tussocks of < 5 cm diameter and 15 mm height in the vegetative state. The climate has been regarded as alpine with influence of the Mediterranean-type climate that prevails in the lowlands of central Chile (Cavieres & Arroyo 1999).

Mesoclimatic characterization

To have a detailed characterization of the mesoclimatic conditions of temperature and irradiance that prevail during the growing season in the study site, a weather station provided with a datalogger (Delta-T, DL2e, Cambridge, United Kingdom) was installed in November 2001. The datalogger was fitted with a sensor for air temperature (1.5 m above soil level) and a quantum sensor (LI 190, LICOR Lincoln USA) for photosynthetically active radiation (PAR, $\mu\text{mol photons m}^{-2}\text{s}^{-1}$). The system was set to continuously register temperature and PAR conditions every hour. Daily maximum and minimum air temperatures, as well as absolute maximum PAR, were directly obtained from the hourly registered values. Daily mean temperature was estimated as the average of the hourly registered temperatures. We analyzed the data for the periods between December 2001-April 2002 and November 2002-May 2003.

Gas exchange and fluorescence measurements of plants in the field

Gas exchange and fluorescence measurements were carried out on three different days during the growing season of 2002: 22 January, 21 March and 10 May. In each of these days, three individuals of *Colobanthus quitensis* were selected, and measurements of CO_2 exchange and fluorescence were done at three intervals during the day: in the morning (9:00-10:00 h), noon (12:00-13:00 h), and afternoon (17:00-18:00 h). It must be emphasized here that this species is not abundant in the Andes, and given that gas exchange measurements usually involve the destruction of several individuals we opted for a conservative number of

replicates. Moreover, the stabilization period of the equipment with a single individual took several minutes, increasing the chance that measurements with more individuals does not constitutes real replicates due to the changes in the environmental conditions.

Net-photosynthesis rate (P_n , $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) and leaf conductance (g_s , $\text{mmol m}^{-2}\text{s}^{-1}$) were measured through gas exchanges with a portable photosynthesis system (CIRAS-1, PP-Systems Haverhill, Massachusetts, USA). Measurements were carried out directly on individual plants, where both PAR ($\mu\text{mol photons m}^{-2}\text{s}^{-1}$) and leaf temperature ($^{\circ}\text{C}$) were also registered.

Fluorescence signals were generated by a pulse-amplitude modulated fluorometer (FMS 2, Hansatech, Instruments Ltd. Norfolk, England) according to Schreiber & Bilger (1987). Fully developed leaves were dark adapted (to obtain open PSII centers) for 30 min using a black cloth cover to ensure maximum photochemical efficiency. The fiber-optic was located at about 10 mm from the sample with a holder, and the different light pulses (see below) were applied following the standard routines programmed in the instrument. Minimum fluorescence (F_0) with all PSII reaction centers in the open state was determined by applying a weak modulated light ($0.4 \mu\text{mol m}^{-2}\text{s}^{-1}$). Maximum fluorescence (F_M) with all PSII reaction centers in the closed state was induced by a 0.8 s saturating pulse of white light ($9,000 \mu\text{mol m}^{-2}\text{s}^{-1}$). The maximum quantum yield of PSII was calculated as F_V/F_M (F_V = variable fluorescence = $F_M - F_0$).

Light responses curves at different temperatures

On January 2004, tussocks of *Colobanthus quitensis* were collected from La Parva and transported to a laboratory next to the field. Immediately, individual leaf rosettes ($n = 4$) were carefully separated from the tussocks, and fluorescence measurements at different light intensities were performed. Light response curves were done with those plants at three different temperatures: 4, 15 and 22 $^{\circ}\text{C}$ (room temperature). To maintain a constant temperature during the measurements, plants were placed in a chamber (LD 2/3, Hansatech, Instruments Ltd. Norfolk, England) connected to a thermoregulated water bath at 4 or 15 $^{\circ}\text{C}$

(Haake DC10). Measurements at 22 °C were directly performed using the dark adaptation leaf clips (Hansatech, Instruments Ltd. Norfolk, England).

The evaluation of photochemical performance was done at five different actinic light intensities (150-700 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$). The optimal photochemical efficiency of plant under the desired temperature (4, 15 or 22 °C), was obtained measuring F_V/F_M with a saturating actinic light pulse, after 30 min of dark adaptation as described above. Plants were then exposed to 10 s of dark, and for each of the light intensity evaluated, actinic light was turned on and saturating pulses (9,000 $\mu\text{mol m}^{-2}\text{s}^{-1}$) were applied every 20 s. F_S and F_M' were registered after steady-state photosynthesis was reached. Finally, F_0' was measured immediately after turning off the actinic light followed by a 2 s far-red light pulse. The following fluorescence parameters were obtained: photochemical quenching (q_p), non-photochemical quenching (NPQ) and quantum yield of PS II ($\Phi_{PS II}$), were done according to Van Kooten & Snel (1990), whereas relative electron transport rate (ETR) was calculated according to Schreiber et al. (1995).

Data analyses

Two way-ANOVAs were applied to test for significant differences in the diurnal cycles of gas exchange and fluorescence measurements, where month and hour (morning, noon, afternoon) were considered as independent factors. When ANOVA indicated significant effect of one or more factors ($P < 0.05$), a LSD a posteriori test was applied. For light responses curves, two way ANOVAs were applied to test for the effect of temperature (three levels) and actinic light intensity (five levels) on each modulated fluorescence parameters.

RESULTS

Mesoclimate

The recorded mesoclimatic conditions cover the snow free period between December 2001 to April 2002, and from November 2002 to May 2003. However, due to a technical failure of the

weather station, no climatic conditions were registered during February and March 2002. Hence, analyses of frequency of temperatures (Fig. 1) were only carried out for the growing season of December 2002/May 2003. Nonetheless, similar trends on temperature and PAR levels during the growing season can be observed between the two recording periods (Fig. 1). In both registered growing seasons, January was the month with the highest mean temperature, and the highest maximum temperature (Table 1). In contrast, the months with the lower mean temperatures, as well as the lower minima, were April 2002 and May 2003 (Table 1). During the middle of the growing season (December to January), maximum air temperatures usually were between 10-15 °C, in both periods (Fig. 1A and 1B). However, maximum temperatures between 0-5 °C were registered during the beginning (December 2001 and November 2002) and at the end of the growing season (March and April 2002 and April and May 2003 (Fig. 1A, B and 2A). The most frequent values for minimum temperature were between 0-5 °C (Fig. 1A, 1B and 2B). During the warmest months (December-January 2001/2002 and January to March 2003) minimum temperature fluctuated between 5-10 °C (Fig. 1A, 1B and 2B). In February and March 2003 no freezing air temperature occurred, whereas in November 2002 and April-May 2003 ca. 20 % of the days reached freezing condition (Fig. 1, 2B, and Table 1).

Sharp decreases in PAR levels were observed toward the fall, both in 2002 and 2003 (Fig. 1C and 1D). The daily maximum values of PAR occurred both in December with 2,327 and 2,206 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ in 2001 and 2002, respectively (Fig. 1C and 1D). Monthly averages of maximum PAR levels indicated that January 2002 and December 2003 were the months with the highest values (2,062 and 2,015 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, respectively, Table 1). At the end of summer, PAR decreased continually with maximum values above 1000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ in both periods (Fig. 1C and 1D).

Net photosynthesis and maximum quantum yield of PSII of plants in the field

Colobanthus quitensis leaves did not show significant differences in net photosynthesis

rate (P_n) among the evaluated months ($F_{2,18} = 2.63$, $P = 0.098$). However, significant differences were observed among the different periods of the diurnal cycle ($F_{2,18} = 5.36$, $P = 0.015$). Overall, P_n was low during the morning and noon hours, becoming higher in the afternoon (Fig. 3A). While P_n fluctuated between 2-4.5 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ on the measurements taken in January and March, P_n values of May were lower than 2.0 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$, irrespective of diurnal cycle (Fig. 3A).

The stomatal conductance was high, with measurements taken in January being significantly higher than that measured during the other months ($F = 2.18$, $P = 0.05$, Fig. 3B). While in January the lower stomatal conductance was registered at noon, in March, it was found in the morning (Fig. 3B). There were no differences in stomatal conductance within the diurnal cycle in May. Thus, the patterns of variation between P_n and g_s during the growing season were different

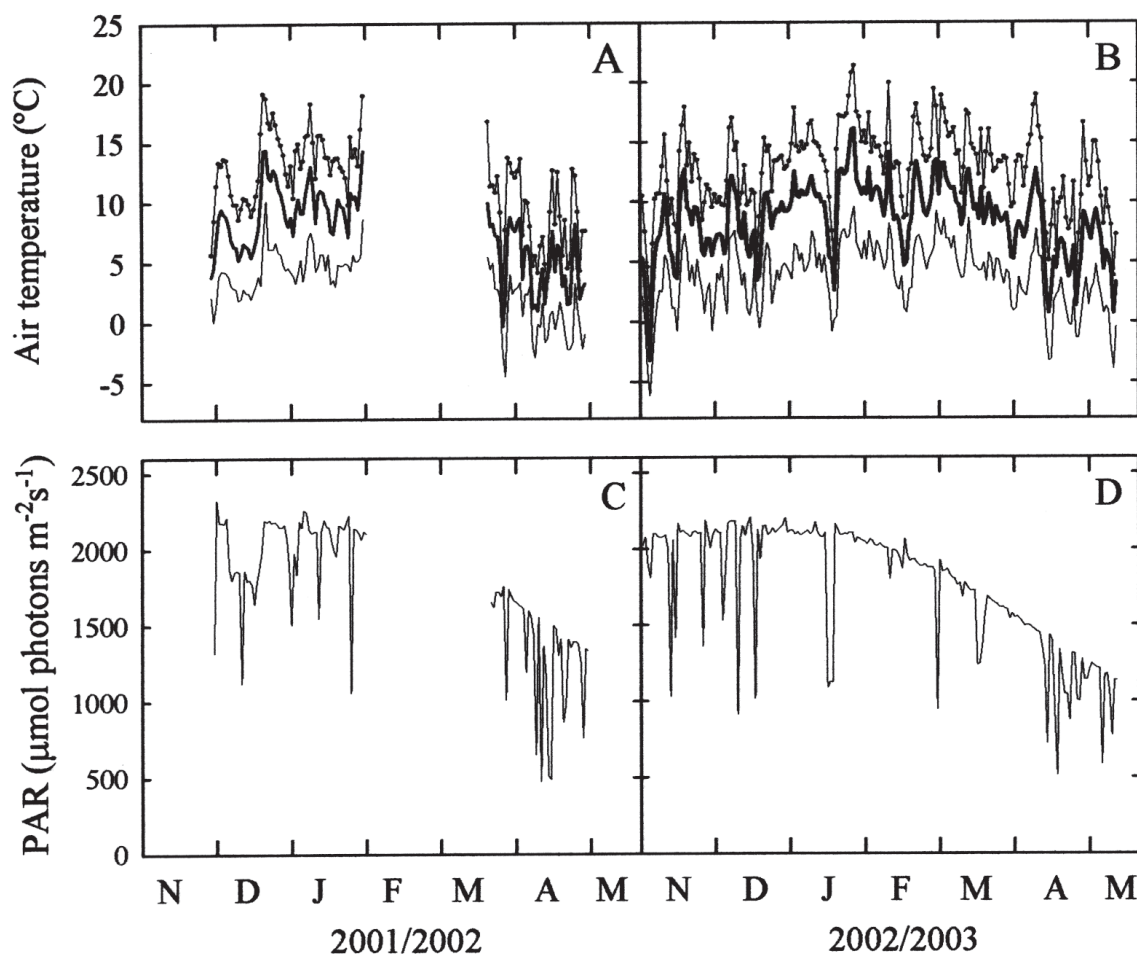


Fig. 1: Climatic conditions at La Parva (2,650 m of altitude, 33°19' S, 70°17' W) in the central Chilean Andes. Measurements were made during December 01, 2001 to April 30, 2002 (A, C) and November 4, 2002 to May 15, 2003 (B, D). (A) and (B): daily maximum, minimum and mean air temperature; (C) and (D): daily means of photosynthetically active radiation (PAR, $\mu\text{mol photons m}^{-2}\text{s}^{-1}$).

Registro climático en La Parva (2.650 m de altitud 33°19' S, 70°17' O) en los Andes de Chile central. Las mediciones fueron realizadas desde el 1 de diciembre de 2001 hasta el 30 de abril de 2002 (A, C) y desde el 4 de noviembre de 2002 al 15 de mayo de 2003 (B,D). (A) y (B): valores diarios de temperatura máxima, mínima y promedio del aire (°C); (C) y (D): promedios diarios de radiación fotosintéticamente activa (PAR, $\mu\text{mol photons m}^{-2}\text{s}^{-1}$).

TABLE 1

Monthly averages of mean, maximum and minimum daily temperatures of the air (°C) and photosynthetic active radiation (PAR, $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) registered during two growing seasons (2001/2002 and 2002/2003) in the Andes of central Chile. Absolute minimum and maximum temperature correspond to the highest or lowest value registered at each month, respectively. Values are means \pm SE

Promedios mensuales de los valores diarios de temperatura media, máxima y mínima del aire (°C) y radiación fotosintéticamente activa (PAR, $\mu\text{mol photons m}^{-2}\text{s}^{-1}$), registrada durante dos estaciones de crecimiento en los Andes de Chile central. Las temperaturas máximas y mínimas absolutas corresponden al valor más alto y más bajo registrado en cada mes, respectivamente. Los valores corresponden a medias \pm EE

Year	Month	Absolute minimum	Absolute maximum	Minimum	Maximum	Average	Maximum PAR	Average PAR
2001	December	0.1	19.1	4.0 \pm 0.4	12.7 \pm 0.6	8.7 \pm 0.5	1,968 \pm 46	1,037 \pm 54
2002	January	3.0	18.9	5.1 \pm 0.3	14.2 \pm 0.4	10.0 \pm 0.3	2,062 \pm 41	1,165 \pm 42
2002	April	-3.0	13.6	0.2 \pm 0.3	8.2 \pm 0.6	4.4 \pm 0.4	1,246 \pm 64	588 \pm 36
2002	November	-6.1	17.9	2.2 \pm 0.7	10.9 \pm 0.8	6.8 \pm 0.7	1,973 \pm 56	1,122 \pm 54
2002	December	-0.8	17.0	3.2 \pm 0.4	11.7 \pm 0.4	7.8 \pm 0.4	2,015 \pm 56	1,110 \pm 51
2003	January	-0.9	21.4	5.1 \pm 0.4	14.8 \pm 0.6	10.5 \pm 0.5	2,014 \pm 54	1,176 \pm 53
2003	February	0.8	19.9	5.1 \pm 0.4	14.2 \pm 0.5	9.9 \pm 0.4	1,973 \pm 14	1,126 \pm 21
2003	March	3.1	19.4	5.5 \pm 0.3	14.7 \pm 0.4	10.3 \pm 0.3	1,676 \pm 39	933 \pm 25
2003	April	-3.3	18.9	2.1 \pm 0.5	11.1 \pm 0.7	6.5 \pm 0.5	1,296 \pm 52	630 \pm 32
2003	May	-3.9	16.5	1.3 \pm 0.6	10.9 \pm 0.9	6.3 \pm 0.6	1,114 \pm 49	497 \pm 26

The maximum photochemical yield of PSII (F_v/F_m) did not change during the active growth season ($F_{2,18} = 1.51$, $P = 0.25$) or during the diurnal cycle ($F_{2,18} = 0.37$, $P = 0.70$). The lowest value was registered on May, during the morning (0.68).

Leaf temperature recorded on January and March, during Pn and F_v/F_m measurements, ranged from 19 to 22 °C (Fig. 3D). In May, however, leaf temperature during measurements varied between 11-14 °C (Fig. 3D). High PAR levels during measurements were registered in January, with values in the morning and at noon increasing between 1880 to 2200 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, but decreasing in the afternoon to values close to 1300 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (Fig. 3E). In March, a similar trend in PAR values was observed during the daily cycle, although values were not as high as in January. In contrast, PAR values registered on May oscillated between 300 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ in the morning to 550 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ at noon (Fig. 3E).

Light response curves at different temperatures

Photochemical efficiency of PSII (F_v/F_M) did not show marked changes after measurements at different temperatures and actinic light intensities (Fig. 4A), being the average value of the control leaves 0.83 at 22 °C and 0.85 at 15 °C (Fig. 4A). A slight decrease in F_v/F_M , (0.82 to 0.75), was observed at 4 °C and 580 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (Fig. 4A). At all the temperatures tested, the quantum yield of PSII (F_{PSII}) significantly decreased with increasing actinic light intensity ($F_{2,30} = 955.1$, $P < 0.001$). This decrease was higher in leaves at 4 °C, where decreases were close to ca. 67 % at 148 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (Fig. 4B). Similarly, significant decreases in photochemical quenching (q_p) were observed both for actinic light intensity ($F_{2,30} = 373.4$, $P < 0.001$) and temperature ($F_{2,30} = 387.3$, $P < 0.001$, Fig. 4C). The decrease in q_p was high in plants at 4 °C (ca. 86 %), followed by plants measured at 15 and 22 °C (72 %).

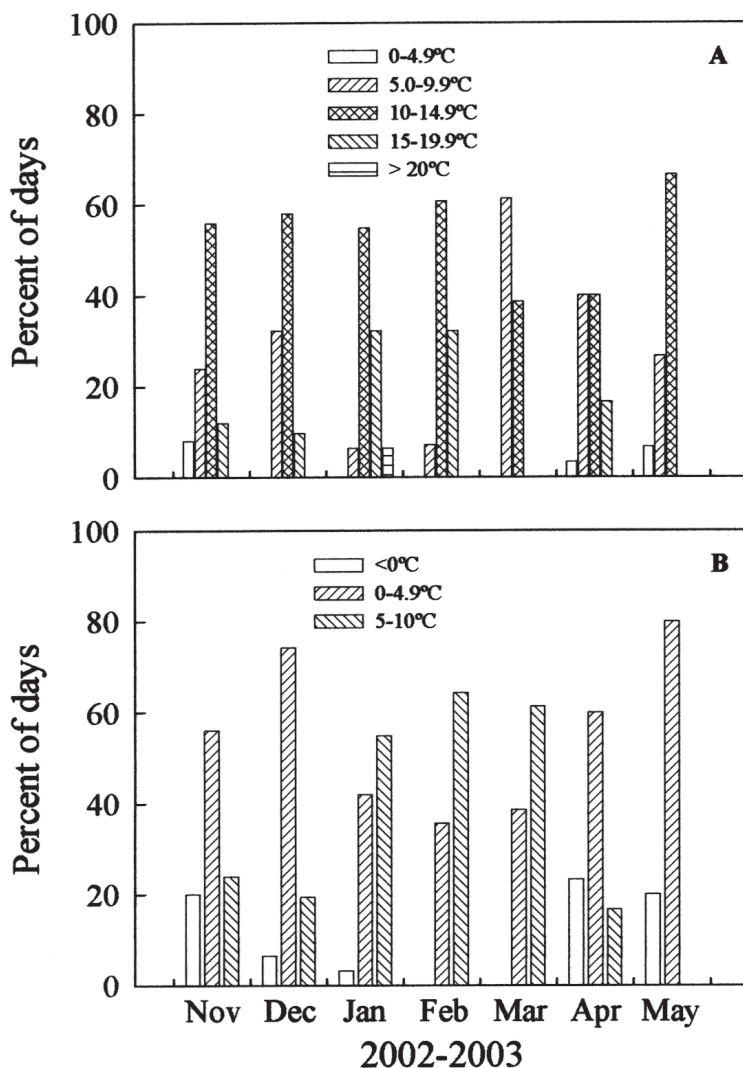


Fig. 2: Monthly distribution of maximum and minimum air temperatures during the growing season of December 2002/May 2003. Bars indicate the percentage of days for each range of maximum (A) and minimum (B) daily air temperatures. For November and May the sum of all days were 25 and 15, respectively.

Distribución mensual de temperaturas máximas y mínimas del aire de la estación de crecimiento diciembre 2002/mayo 2003. Las barras indican el porcentaje de días para cada rango de temperaturas del aire máxima (A) y mínima (B) diaria. Para noviembre y mayo la suma de todos los días fue 25 y 15, respectivamente.

Different responses were observed for the increase in non-photochemical quenching (NPQ) where the effects of both factors, temperature ($F_{2,30} = 9.86$, $P < 0.001$) and actinic light intensity ($F_{2,30} = 101.2$, $P < 0.001$), were significant. At 4 °C a 3.6 fold increase was observed in NPQ after exposure to 148 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, remaining high with increasing actinic light intensities (Fig. 4D). At 15 and 22 °C, NPQ reached higher values than those observed at 4 °C. While at

15 °C NPQ did not reach saturation until ca. 600 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ of actinic light intensity, at 22 °C NPQ saturation was reached ca. 480 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (Fig. 4D). Furthermore, in leaves at 4 °C, and exposed to the different actinic light intensities, relative electron transport rate (ETR) was significantly lower, with similar values for all the light intensities tested ($F_{2,30} = 434.7$, $P < 0.001$) (Fig. 4E). At 22 °C, ETR was significantly higher than that at 15 °C ($F_{2,30} = 330.86$, $P < 0.001$).

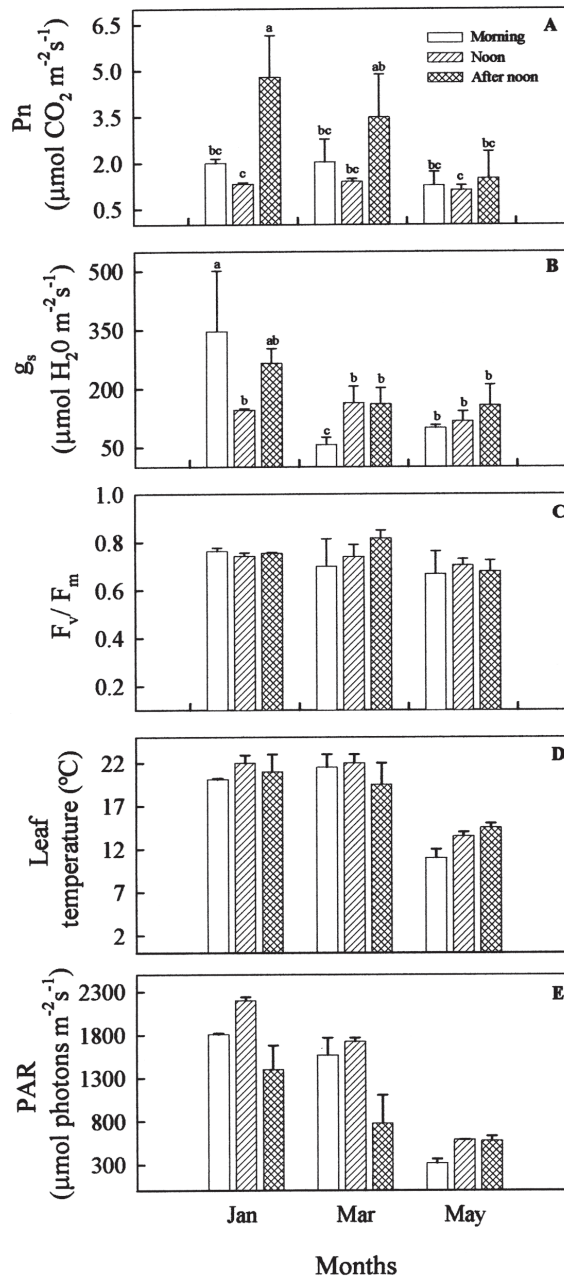


Fig. 3: Gas exchange measurements in *Colobanthus quitensis* on diurnal periods at La Parva in the central Chilean Andes. Time ranges for morning, noon and afternoon are 9:00-10:00, 12:00-13:00 and 17:00-18:00 h, respectively. Measurements were made on January (Jan), March (Mar) and May (May). (A) Net photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); (B) stomatal conductance ($\mu\text{mol m}^{-2} \text{ s}^{-1}$), (C) maximum quantum yield of PS II (F_v/F_m); (D) leaf temperature ($^{\circ}\text{C}$) and (E) PAR ($\mu\text{mol m}^{-2} \text{ s}^{-1}$). Bars are means \pm SE ($n = 3$). Different letters indicate significant statistical significance ($P < 0.05$) according to LSD-test. No statistic was carried out for microclimatic conditions.

Mediciones de intercambio gaseoso en *Colobanthus quitensis* durante períodos diurnos en La Parva, Andes de Chile central. Los horarios para las mediciones de mañana, mediodía y tarde son 9:00-10:00, 12:00-13:00 y 17:00-18:00 h, respectivamente. La mediciones fueron hechas en enero (Jan), marzo (Mar) y mayo (May). (A) tasa de fotosíntesis neta ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); (B) conductancia estomática ($\mu\text{mol m}^{-2} \text{ s}^{-1}$); (C) rendimiento cuántico máximo del PS II (F_v/F_m); (D) temperatura foliar ($^{\circ}\text{C}$) y (E) PAR ($\mu\text{mol m}^{-2} \text{ s}^{-1}$). Las barras muestran medias \pm EE ($n = 3$). Letras diferentes indican diferencias estadísticas significativas ($P < 0,05$) de acuerdo a la prueba de LSD. No se realizó análisis estadísticos con las condiciones microclimáticas.

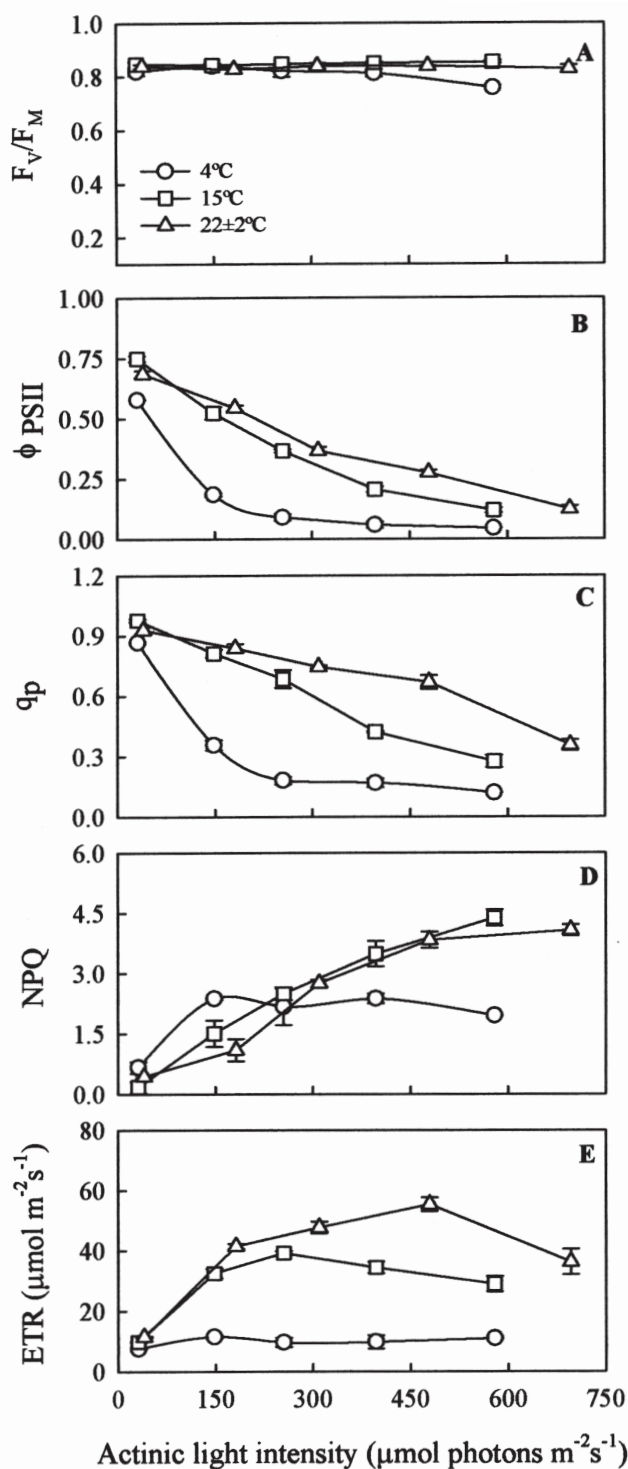


Fig. 4: Light responses curves of *Colobanthus quitensis* at different temperature and actinic light intensities: (A) maximum quantum yield of primary photochemistry of PSII (F_v/F_M); (B) quantum yield of PSII (ϕ_{PSII}) (C) photochemical quenching (qp); (D) non-photochemical quenching (NPQ) and (E) electron transport rate (ETR).

Curvas de respuesta a la luz de *Colobanthus quitensis* a diferentes temperaturas y niveles de luz actínica: (A) rendimiento cuántico máximo de la fotoquímica primaria del fotosistema II (F_v/F_M); (B) rendimiento cuántico del Fotosistema II (ϕ_{PSII}); (C) apagamiento fotoquímico (qp); (D) apagamiento no fotoquímico (NPQ); (E) tasa de transporte de electrones (ETR).

DISCUSSION

The climate context

Climatic information for high-elevation sites is scarce (Körner 1999), specially for mountains in areas with influence of the Mediterranean-type climate. Our climatic register for the 2002 and 2003 growing season shows that maximum air temperature during the summer-autumn months at 2650 m elevation in the Andes of central Chile fluctuated between 10–15 °C, with January being the month with the highest averages of maximum air temperature. Cavieres & Arroyo (1999) reported that mean monthly temperature for the summer-autumn months ranges from 12 to 7 °C at 2,600 m elevation in a south-facing slope near our study site. It is well known that in the south hemisphere temperatures in north-facing slopes tend to be higher than those in south-facing slopes because of the direct incidence of solar beams during the summer (Rozzi et al. 1989). In the study of Rozzi et al. (1989), performed in the same area of our study site, but at 2,500 m, they reported that monthly maximum air temperatures fluctuated between 21 °C in January to ca. 10 °C in April. Although it is well known that lapse rate is higher during summer months (ca. 1 °C km⁻¹ elevation), our results (see Table 1) compared to that of Rozzi et al. (1989), suggest large climatic variations between years, which is a well known phenomenon in other alpine sites of the north hemisphere (Walker et al. 1994).

Temperatures registered in the central Chilean Andes are similar to those reported for some locations of Sierra Nevada of California, which is also influenced by a Mediterranean-type climate. For instance, Chabot & Billings (1972) reported that in the Bishop Creek area (37° N) at 2,440 m, mean air temperature during the growing season ranged from 15–20 °C. However, it is important to note that Bishop Creek is located 4° latitude degrees further poleward than Farellones-La Parva area, which suggest that at similar latitudes the central Chilean Andes have lower temperatures than the Sierra Nevada. The lower temperatures during the growing season in the Andes of central Chile compared to California are likely to be related with the higher influence of the maritime climate and lower aridity that occur in central Chile (Arroyo et al. 1995).

Physiological responses

High diurnal fluctuations in net photosynthetic rate were found in *Colobanthus quitensis* growing in the central Chilean Andes. For example, in two diurnal cycles (January and March), photosynthesis was low, both during the morning and noon (1–2 μmol CO₂ m⁻²s⁻¹). These days were characterized by high PAR and high leaf temperature (Fig. 3D,E), conditions that not affected the maximum quantum yield of PSII (F_V/F_M) (see below) (Fig. 3C). In the afternoon, when PAR decreased and leaf temperature was close to 20 °C, Pn reached the highest values (ca. 3.5–4.8 μmol CO₂ m⁻²s⁻¹). The low values of photosynthesis found in May were probably due to the low PAR levels and cool temperatures (air temperature between 8–10 °C and leaf temperature between 11–15 °C) registered during the diurnal cycle. The net photosynthetic rates of *Colobanthus quitensis* found in La Parva were in the range of those reported by Xiong et al. (1999) in the Antarctic, who found that this species had positive photosynthetic rates of ca. 1.8 μmol CO₂ m⁻²s⁻¹ at temperatures below 10 °C and PAR intensities between 500 and 800 μmol photons m⁻²s⁻¹. Nevertheless, while Xiong et al. (1999) reported negative photosynthetic rates (ca. -0.3 μmol CO₂ m⁻²s⁻¹) at higher temperatures (above 20 °C) and higher PAR levels (above 1500 μmol photons m⁻²s⁻¹), in La Parva, Pn reached the highest values when leaf temperatures were ca. 20–22 °C and PAR intensities between 800–1,400 μmol photons m⁻²s⁻¹. From Pn measurements at different leaf temperatures in the laboratory, Xiong et al. (1999) reported maximal Pn (8.2 μmol CO₂ m⁻²s⁻¹) at 14 °C and PAR of 700–800 μmol photons m⁻²s⁻¹. In general our results suggests that for Andean populations, the high PAR intensity that prevails in the field during the summer (1,600–2,200 μmol photons m⁻²s⁻¹) could be considered a stress factor, as it has been shown for other high elevation plants (Körner 1999).

Several authors reported that the excess of excitation energy due to high PAR levels above photosynthetic saturation may result in the inactivation of PSII, oxidative damage, and the photoinhibition of photosynthesis (Huner et al. 1993, Oquist & Huner 1993). In the Andes,

however, under high PAR intensity and under different mean air temperatures, *Colobanthus quitensis* showed small decreases of the dark-adapted F_v/F_m ratios (0.7-0.75 on January and 0.7-0.82 on March) compared to control values (0.83) obtained in the laboratory. This suggests a small effect of high light intensity on PSII photochemistry, without evidence of photodamage (Fig. 3C). These results are similar to those reported by Streb et al. (1998) who showed that the diurnal increase in light intensity was not accompanied by significant declines of F_v/F_m ratios in three alpine plants growing between 2,400-2,700 m of altitude in the French Alps. According to these authors, F_v/F_m ratios were lower in *Homogyne alpina* (0.66) and *Soldanella alpina* (0.71), whereas values of 0.8 were recorded in *Ranunculus glacialis*. Similarly, individuals of *Bouteloua gracilis* growing at 1,500 and 3,000 m of altitude in the Rocky Mountains in Colorado showed F_v/F_m ratios of 0.74 and 0.76, respectively (Pittermann & Sage 2000), suggesting that the high irradiance of high elevation habitats does not necessarily lead to photoinhibition. Nonetheless, recent reports showed that in Antarctic populations of *C. quitensis*, after 24 hours of high light intensity ($1,600 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) and low temperature (4°C), F_v/F_m was reduced to 38 %, indicating a decrease in the photochemical efficiency (Pérez-Torres et al. 2004). These authors also showed that in plants exposed to high light intensity and temperature close to the photosynthetic maximum (15°C), the photochemical efficiency (F_v/F_m) also decreased to 21 %. Hence, differences between Andean and Antarctic populations of *C. quitensis* can also be observed in their propensity to photoinhibition. In this sense, considering the relaxation times, two classes of photoinhibition are recognized: dynamic photoinhibition, which occurs rapidly and is reversible. Dynamic photoinhibition has been suggested to be a photoprotective mechanism (Long et al. 1994, Osmond 1994), whereas chronic photoinhibition (photodamage) is slowly reversible and comprises a sustained decrease of Pn (Osmond 1994). According to our results, it seems likely that *C. quitensis* growing in the field experiences dynamic photoinhibition given the F_v/F_m values obtained.

Light response curves at 15 and 22°C showed that while F_v/F_m was constant at all of PAR irradiances applied, Φ_{PSII} decreased with increasing PAR. Moreover, the small decrease in q_p indicated that the fraction of reaction centers that remained open (oxidized) was high, and that the reduction of this reaction centers only began after the application of about $300 \mu\text{mol photons m}^{-2}\text{s}^{-1}$. Moreover, electron transport rates (ETR) were higher in leaves at 22 and 15°C than at 4°C , indicating that at these temperatures (15 and 22°C), *Colobanthus quitensis* leaves were able to maintain a high de-excitation capacity (see also NPQ results). These experimental results suggest that the observed decrease of Pn in the field, under high PAR intensities and temperatures close to their photosynthetic optimum (ca. 14°C) may be due to an excess of PAR, which decreased Φ_{PSII} but maintained high ETR values, suggesting that the absorbed energy was used in alternative ways. It has been shown that in some plant species (e.g., tropical trees), CO_2 assimilation (Pn) is highly correlated with the electron transport rate (Krause et al. 1995). However, it has also been suggested that the observed deviation from a linear relationship between Φ_{CO_2} and Φ_{PSII} in plants under stress is due to a redirection of the electrons to alternative sinks, such as photorespiration or the Mehler reaction (Krause et al. 1995, Franco & Lüttge 2002, Fracheboud & Leipner 2003). For alpine plants, it has been observed that photorespiration is an efficient protector of their photosynthetic apparatus against photodamage under high intensities of sun light (Heber et al. 1996), which could be the case of *C. quitensis*.

Finally, at 4°C , leaf showed a sharp decrease in Φ_{PSII} at low light intensities (ca. $150 \mu\text{mol photons m}^{-2}\text{s}^{-1}$), which is parallel with a decrease in the fraction of open reaction center as indicated by q_p . This could indicate a reduction of photochemical enzymatic processes at 4°C , with a concomitant higher excitation pressure of PSII caused by actinic light (between $150\text{-}600 \mu\text{mol photons m}^{-2}\text{s}^{-1}$). Furthermore, leaves at this temperature were not able to increase their dissipation of excess energy (see NPQ results, Fig. 4), which helps to protect PSII against photodamage (Adams & Demmig-Adams 1995, Müller et al. 2001). Probably, field plants growing under warm

summer conditions are sensible to the short-time low temperature episode used in the laboratory measurements.

In conclusion, although our experiments were performed on a low number of replicates, our results clearly show that *Colobanthus quitensis* in the high alpine zone of the Central Chilean Andes do not experienced photodamage under field conditions during the three diurnal cycles measured in this study. This photosynthetic response is different from that observed in the Antarctic (Xiong et al. 1999) where photoinhibition occurred. However, it should be emphasized that unlike the Andes, cold temperatures is a constant condition in the Antarctic during the growing season. This different response of *C. quitensis* in the Andes of central Chile is consistent with the existence of ecotypic differentiation in this species, as it has been shown by Gianoli et al. (2004). Clearly, more research is needed to clarify the mechanisms involved in the photobiology of this species, as well as a characterization of its optimum temperature and PAR responses under field conditions.

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