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Effects of low temperature acclimation on photosynthesis in three Chilean Proteaceae

Efectos de la aclimatación a baja temperatura sobre la fotosíntesis de tres proteáceas chilenas

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ABSTRACT

Embothrium coccineum, *Gevuina avellana*, and *Lomatia ferruginea* grow in habitats contrasting in temperature and light intensity. *Embothrium coccineum* is a pioneer species that establishes itself in open sites completely exposed to variable sunlight and temperature. *Gevuina avellana* is usually found in more protected sites. *Lomatia ferruginea* regenerates under the canopy in sites with low thermal oscillations and high humidity. In order to establish an association of their habitats with functional attributes of each species, we studied their photosynthetic responses to temperature and light intensity. We expected that *E. coccineum*, which grows at full sun, is able to acclimate much better its photosynthetic apparatus to different temperatures than the shade tolerant *L. ferruginea* and the semi-shade tolerant *G. avellana*. One group of plants of each species containing six individuals each was subjected to 4 ºC (cold-acclimated plants). Another group with the same number of individuals was subjected to 20 ºC (non-acclimated plants). In non-acclimated plants of *E. coccineum*, the photosynthetic rate as measured by O_2 evolution presented its maximum at 30 ºC (16.5 µmol O_2 m^{-2} s^{-1}) with an optimum between 20 and 35 °C, while in *G. avellana* and *L. ferruginea* the highest photosynthetic rate (~13 µmol O_2 m^{-2} s^{-}) was obtained at 25 ºC. Cold acclimation significantly reduced the photosynthetic rates of the investigated species. The Q_{10} for O_2 evolution decreased significantly in cold-acclimated *E. coccineum* and *G. avellana* but not in *L. ferruginea*. The fluorescence parameters of PSII showed that *E. coccineum* presents a higher effective quantum yield (ΦPSII) at both growth temperatures. Photochemical quenching (qP) was more affected by the photosynthetic photon flux density (PPFD) in *L. ferruginea* than in the other species. *Lomatia ferruginea* presented the highest non-photochemical quenching (NPQ) at lower PPFD. Thus, the photosynthetic apparatus of each species presents functional differences according to the characteristics of light availability and temperature changes of their habitats.

Key words: Proteaceae, cold acclimation, photosynthetic oxygen evolution, photochemical efficiency of PSII.

RESUMEN

Embothrium coccineum, *Gevuina avellana* y *Lomatia ferruginea* crecen en hábitat contrastantes en temperatura e intensidad lumínica. *Embothrium coccineum* es una especie pionera que se establece en sitios abiertos, completamente expuestos a luminosidad y temperatura variables. *Gevuina avellana* se encuentra usualmente en sitios más protegidos. *Lomatia ferruginea* regenera bajo el dosel en sitios con bajas oscilaciones térmicas y alta humedad. Con el objetivo de establecer una asociación de los hábitats que ocupan estas especies y los atributos funcionales de cada una de ellas, se estudió las respuestas fotosintéticas a la temperatura e intensidad lumínica. Un grupo de plantas de cada especie (seis individuos por especie) se aclimataron a 4 ºC (plantas aclimatadas al frío). Otro grupo con el mismo número de individuos fue mantenido a 20 ºC (plantas no aclimatadas al frío). Plantas no aclimatadas de *E. coccineum*, presentaron una tasa fotosintética máxima, medida como evolución de O_2, a 30 ºC (16,5 µmol O_2 $m^{-2}s^{-1}$) con un óptimo entre 20 y 35 °C, mientras que en *G. avellana* y *L. ferruginea* la mayor tasa fotosintética (~13 µmol O_2 m^{-2} s^{-1}) se obtuvo a 25 ºC. La aclimatación al frío redujo significativamente las tasas fotosintéticas de las especies. El Q_{10} para la evolución de O_2 fue similar en las tres especies en el estado no aclimatado al frío. La aclimatación al frío disminuyó Q_{10} en *E. coccineum* y *G. avellana*, pero permaneció invariable en *L. ferruginea*. Los

parámetros de fluorescencia del PSII mostraron que *E. coccineum* presentó un mayor rendimiento cuántico efectivo del PSII (ΦPSII) a ambas temperaturas crecimiento. El apagamiento fotoquímico (qP) fue más afectado por la densidad de flujo fotónico fotosintético (PPFD) en *L. ferruginea* que en las otras especies. *Lomatia ferruginea* presentó el mayor apagamiento no fotoquímico (NPQ) a una baja PPFD. Por lo tanto, el aparato fotosintético de cada especie presenta diferencias funcionales de acuerdo a las características de disponibilidad lumínica y cambios de temperatura de sus hábitat.

Palabras clave: Proteaceae, aclimatación al frío, evolución fotosintética de oxígeno, eficiencia fotoquímica del PSII.

INTRODUCTION

One of the most important factors that limit development and distribution of plants is temperature, because different physiological processes have different temperature optima for their functioning (Larcher 1995). Optimal temperature for gas exchange is known to vary among species (Berry & Björkman 1980, Badger et al. 1982, Ferrar et al. 1989, Makino et al. 1994, Hikosaka et al. 1999, Yamasaki et al. 2002). Low temperature decreases all biochemical processes including photosynthesis, respiration and protein synthesis (Hopkins 1999). This is due to the sensibility of cellular membranes to low temperature, independently of the damage that may occur due to intracellular formation of ice nuclei, which is usually lethal (Alberdi & Corcuera 1991). Plants growing in different environments have different optima for photosynthesis, with plants in colder environments having lower temperature optima than those in warmer climates (Ngugi et al. 2003). Furthermore, photosynthesis plasticity in response to temperature is decisive for the success of a species in different environments and microclimates (Cabrera 2002).

Embothrium coccineum J. R. et G. Forster, *Gevuina avellana* Mol. and *Lomatia ferruginea* (Cav.) R. Br. are woody species of Proteaceae that prosper in different biotopes along the latitudinal and altitudinal gradient in the forests of the southern tip of South America. *Embothrium coccineum* and *L. ferruginea* present the widest and most southerly latitudinal distribution (35 to 56º S and 35º30' to 52º S), respectively, whereas *G. avellana* has a narrower distribution (33 to 45º S) (Rodríguez et al. 1983). In addition to the differences in distribution, other important characteristics of ecological and/or physiological nature exist among these species. *Embothrium coccineum* is a pioneer sun plant that colonizes open areas after natural or anthropic disturbance events, where it grows quickly forming dense stands. *Gevuina avellana* grows in temperate habitats and although it can grow directly exposed to sun, it needs some protection under other trees in the coldest biotopes. The best biotope for *L. ferruginea* presents small thermal oscillations and high environmental humidity. These species differ in their pioneer or successional role because they occupy different habitats varying in their exposition to light and temperature.

In plants, high radiation levels may cause photoinhibition of photosynthesis and photooxidative destruction of the photosynthetic apparatus (Osmond 1994). The exposure of plants to low temperature produces adverse effects on metabolic functions and one of the sensitive processes is photosynthesis (Larcher 1995). Species that are sensitive to cool temperature have a tendency to exhibit photoinhibition of photosynthesis when they are exposed to low temperature, even at moderate light intensity. On the other hand, species that are able to acclimate to low temperature are much less sensitive to low temperature-induced photoinhibition (Alves et al. 2002). We studied the effects of temperature on the photosynthesis of three species of the Proteaceae that differ in their biotopes preferences. The study by Read & Hill (1985) about *Nothofagus* species and the present study are the first studies performed in species of the Chilean temperate rain forest on the relationship between temperature acclimation of photosynthesis and their habitats. It is postulated that *E. coccineum*, which grows at full sun, is able to acclimate its photosynthetic apparatus much better to different temperatures than the shade tolerant *L. ferruginea* and the semi-shade tolerant *G. avellana*.

MATERIAL AND METHODS

Experimental design

One year-old seedlings of the three species were collected from the field in Katalapi Park (Pichiquillaipe), Décima Región, Chile, in winter. Seedlings were transferred to pots filled with soil from their habitats and maintained in climatic chambers for six months. Then, one group of plants of each species was maintained for two months at 20 ºC (non-acclimated), 14:10 h day:night photoperiod, with a photosynthetic photon flux density (PPFD) of 200-250 mmol photons m^{-2} s^{-1} at the top of canopy and a relative humidity of 85 %. Another group of plants of the investigated species was maintained for the same period at 4 ºC (cold-acclimated) at the same photoperiod and PPFD. The light source consisted of cool-white fluorescent tubes F-40CW (General Electric, Charlotte, NC, USA). Seedlings were irrigated once a week and fertilized with Phostrogen (Solaris, Buckingghamshire, United Kingdom) using 0.2 g L^{-1} once every two weeks.

Photosynthesis (oxygen evolution)

Photosynthesis determined as oxygen evolution (Pn) was measured in leaf discs of each species with a gas phase oxygen electrode unit, using a Model LD2/3 oxygen electrode chamber and the PC operated oxygen electrode control unit, Oxylab (Hansatech Instruments Ltd. King's Lynn, Norfolk, England). Measurements were performed in non-acclimated (20 ºC) and cold-acclimated (4 ºC) plants at different temperatures between 5-40 ºC controlled by a circulating water bath (Thermo Haake K15, Electron Corporation, Germany) and at a light irradiance of 250 µmol photons m^{-2} s^{-1}, obtained from the light saturation curves, measured as mentioned below. A Leaf disc of 10 cm^{-2} was put inside of LD2/3 measuring chamber and adapted for 10 min to each temperature. Measurements were performed at saturating CO_2, obtained by adding a couple of drops of 1M carbonate/bicarbonate buffer at pH 9 into the measurement chamber. Light saturation levels for each species were obtained from photosynthesis light response curves made with nine irradiance values over the range of 0-800 µmol photons m^{-2} s^{-1} given by an array of red light emitting diodes (Model LH36/2R, Hansatech Instruments Ltd., King's Lynn, Norfolk, England).

The temperature dependence of photosynthesis, measured as the rate of oxygen evolution (P_n), was estimated by calculating the temperature coefficient (Q_{10}) for each species and treatments. Q_{10} is the empirically measured change in the rate of a reaction for an alteration in temperature of 10 ºC (Mohr & Schopfer 1995). The dependence of the reaction constant (k) upon temperature (T) could be described by the Arrhenius equation: $\ln k = \ln k_0 - A/R*T$, where k_0 and A, are temperature dependent. Arrhenius constants were empirically determined: R is the universal gas constant. There is a linear relation between ln k and T^{-1} following first equation. From the graphical presentation of the function $\ln k = - (AR^{-1}) T^{-1} + \ln k_0$ (which correspond to y = -ax + b), A and k_0 can be calculated. Substituting k for net photosynthesis rate (P_n) and considering that A/R is the slope of the curve $\ln P_n$ *versus* T^{-1}, Q_{10} was obtained in the following equation: $\ln Q_{10} = A/R*(1/T - 1/T + 10)$ (Mohr & Schopfer 1995).

Fluorescence parameters

Fluorescence signals were measured by a pulse-amplitude modulated fluorometer of attached non shaded leaves (FMS 2, Hansatech Instruments Ltd., United Kingdom). The protocol of Reyes-Díaz et al. (2005) was followed. Non-acclimated and cold-acclimated leaves of the three species were dark adapted for 30 min (to obtain open reaction centers) with leaf-clips provided with a mobile shutter plate. Then the fiber-optic and its fiber-optic adapter were fixed to a controlled temperature chamber (20 or 4 ºC), where the sample was placed and light pulses were applied. The minimal fluorescence (F_o) was determined by applying a weak modulated light (0.4 µmol photons m^{-2} s^{-1}) and maximal fluorescence (Fm) was induced by a short pulse (0.8 sec) of saturating light (9,000 µmol photons m^{-2} s^{-1}). Fluorescence signals were followed until steady state level of fluorescence (Fs) was reached at different actinic light levels. To obtain Fm' (maximal fluorescence during illumination) a pulse of 9,000 µmol photons m^{-2} s^{-1} was used. Fo', the minimal level of

fluorescence, was determined after turning the actinic light off and immediately a 2 sec far red (FR) pulse was applied. In this paper, the data of F_v/F_m = variable fluorescence/maximal fluorescence, where $F_v = (F_m-F_o)$ (maximal fluorescence – minimal fluorescence) and $\phi PSII = F_m' – (F_s/F_m')$ were used as indicators of the maximal and effective quantum yield of the PSII, respectively (Genty et al. 1989). Photochemical quenching (qP) was calculated as: $qP = (F_m' – F_s) / (F_m' – F_o')$, and non photochemical quenching was calculated as: $NPQ = (F_m – F_m') / F_m'$ (Maxwell & Johnson 2000).

The different actinic light were controlled by the light source of the FMSII apparatus and applied through an optic fiber. Light intensity at the leaf surface was calibrated using a LI-250 light meter (Li-Cor).

Determinations of total carotenoids and chlorophylls a and b in leaf extracts

Leaf extracts of non-acclimated (20 ºC) and cold acclimated (4 ºC) plants were made using 0.10 g FW with 5 ml of ethanol 96 % and 0.3 mg mL^{-1} of $CaCO_3$ in cold and dim light. Then the extracts were centrifuged for 3 min at 12000 rpm (about 5,000 g) and 4 ºC. Absorbance was measured in aliquots of the supernatant at 470, 649 and 665 nm (Shimadzu UV-1203) according to the method proposed by Lichtenthaler & Wellburn (1983).

Statistical analyses

Reported values correspond to the means of three replicates for pigment experiments, four replicates for fluorescence parameters and three replicates for photosynthesis. For determining the effect of temperature on P_{max} of each species, we performed a one way ANOVA, followed by a Tukey test to identify those values with significant differences. From these differences the optimum temperature for each species was established. The data were also subjected to a two-way ANOVA (where the factors were species and temperature of acclimation) and a Tukey test for multiple comparisons. The analyses were performed with the software STATISTICA (version 6, Stat Soft Inc.). Differences between the values were considered significant at $\alpha = 0.05$.

RESULTS

Effect of the temperature acclimation on photosynthesis rate (P_{max})

Photosynthesis determinations obtained by oxygen evolution showed differences in the temperature for maximum photosynthesis between the non-acclimated and cold-acclimated species (Fig. 1A and 1B). Non-acclimated plants of *E. coccineum* had their maximum photosynthesis (P_{max}) at 30 ºC (16.5 µmol O_2 m^{-2} s^{-1}), with an optimum between 20 ° and 35 °C while in *G. avellana* and *L. ferruginea* the highest photosynthetic rate (~13 µmol O_2 m^{-2} s^{-1}) was obtained at 25 ºC. Statistically significant differences among the P_{max} of non-acclimated *E. coccineum* and the other species were found ($P < 0.05$). Differences in oxygen evolution were found between non-acclimated and cold-acclimated plants of the three species being lower the photosynthesis in cold acclimated than in not acclimated ones ($P < 0.05$). Cold-acclimated *E. coccineum* showed two similar P_{max} at 10 °C and 35 ºC, whereas *G. avellana* P_{max} was at 30 ºC, and *L. ferruginea* at 25 ºC (Fig. 1B). At this temperature *L. ferruginea* species showed the highest P_{max} (around 2 fold-higher) with respect to the other species at the same temperature and 1.3- and 1.7-fold higher than P_{max} of *G. avellana* and *E. coccineum* respectively. Non-acclimated plants evidenced net oxygen consumption (respiration) at the lowest (5 °C) and at the highest (40 °C) temperature, whereas cold-acclimated plants showed net respiration only at 40 °C (Fig. 1A). In the non-acclimated state the highest rate of respiration at 5 °C was found in *L. ferruginea* (-2.6 µmol O_2 m^{-2} s^{-1}) and the lowest in *E. coccineum* (-0.6 µmol O_2 m^{-2} s^{-1}) ($P < 0.05$), while at 40 °C *E. coccineum* and *G. avellana* presented statistically significant higher respiration rates (around -3.3 µmol O_2 m^{-2} s^{-1}) than *L. ferruginea* (-0.5 µmol O_2 m^{-2} s^{-1}) ($P < 0.05$) (Fig. 1A). Nevertheless, cold acclimated *L. ferruginea* evidenced higher respiration rate (-2.5 µmol O_2 m^{-2} s^{-1}) than *E. coccineum* (around -0.67 µmol O_2 m^{-2} s^{-1}) at 40 °C ($P < 0.05$) (Fig. 1B). A statistically significant interaction between the acclimation treatment and temperature for oxygen evolution was found for each species ($P < 0.001$). Temperature coefficients (Q_{10}) of non-

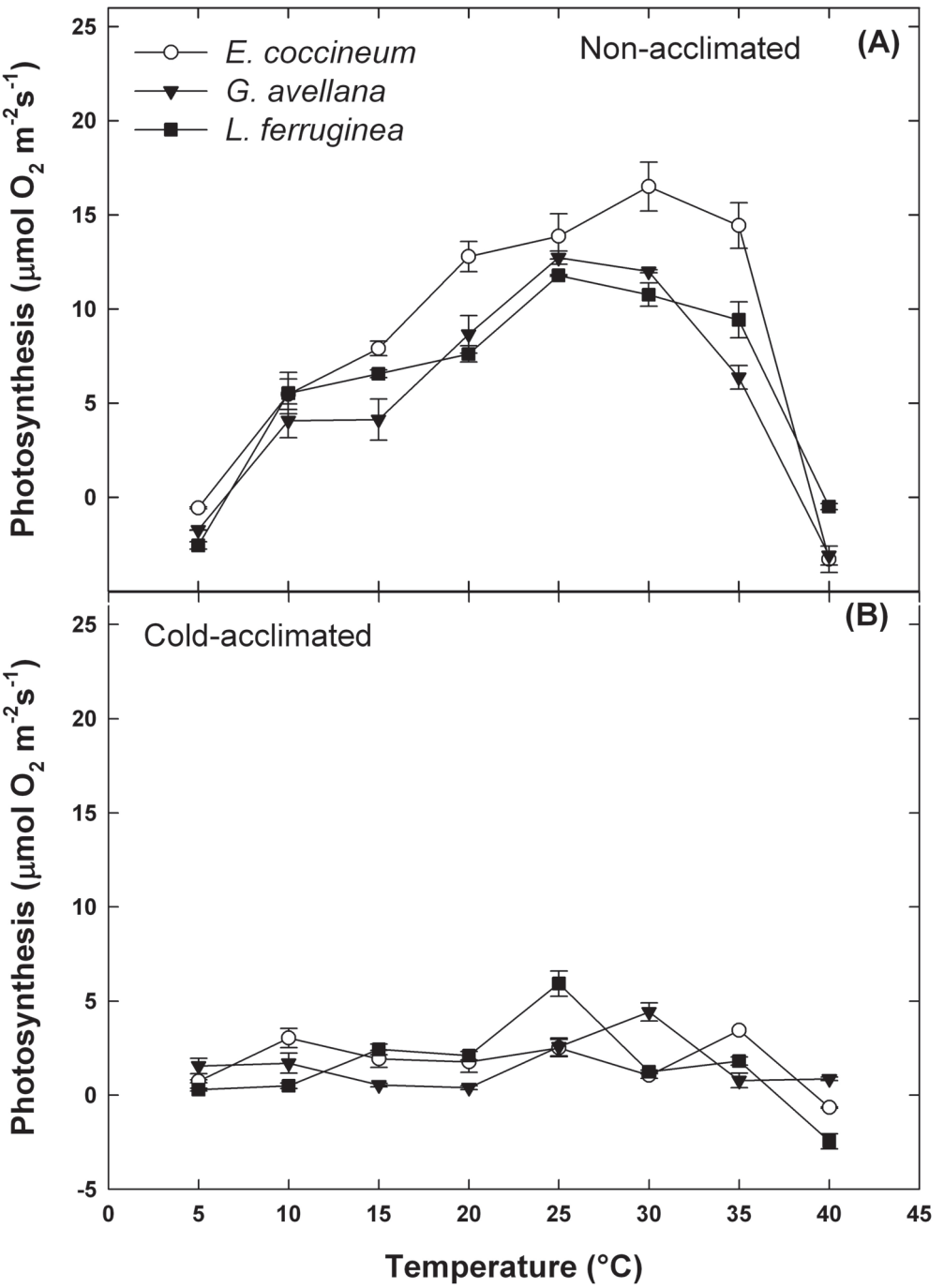

Fig. 1: Effect of cold acclimation on photosynthesis rate (P_{max}) at various temperatures, for the investigated species. Measurements of O_2 evolution were performed at saturating light and CO_2. (A) non-acclimated and (B) cold-acclimated. Values are means (n = 4) ± SE. Standard errors are not visible when their not exceeds the size of symbols.

Efecto de la aclimatación al frío sobre la tasa fotosintética (P_{max}) de las especies investigadas a varias temperaturas. Mediciones de la evolución de O_2 fueron realizadas a luz y CO_2 saturantes. (A) no aclimatadas y (B) aclimatadas al frío. Valores corresponden a promedios (n = 4) ± EE. Los errores estándar no se aprecian, cuando ellos no exceden el tamaño de los símbolos.

acclimated plants were very similar among them. In contrast, in cold-acclimated plants Q_{10} decreased more than 23 % with respect to the non-acclimated plants ($P < 0.05$), with the exception of the shade-tolerant *L. ferruginea* (Table 1).

Effect of cold acclimation on light response of net photosynthesis and fluorescence parameters of PSII

Light response of net photosynthesis was distinctive for the non-acclimated studied species and consistent with light levels observed in their natural environments. Thus, in the non- acclimated state, the shade species *L. ferruginea* exhibited the highest dark respiration (-5.4 μmol O_2 m^{-2} s^{-1} at 0 PPFD) and the lowest maximum rate of net photosynthesis (3.8 μmol O_2 m^{-2} s^{-1}) (Fig. 2A). *G. avellana*, the shade-semitolerant species, showed an intermediate value of maximum net photosynthesis (10.4 μmol O_2 m^{-2} s^{-1}) and *E. coccineum* the highest (16 μmol O_2 m^{-2} s^{-1}) (Fig. 2A). Cold acclimation reduced significantly (around in 80 %) the net photosynthesis of *E. coccineum* and *G. avellana* ($P < 0.05$), but did not significantly affect photosynthesis of *L. ferruginea* (Fig. 2B). Dark respiration rates remained similar in both cold-acclimated and non-acclimated plants (Fig. 2A, 2B at 0 PPFD). Maximal and effective quantum yield of PSII (Fv/Fm and ΦPSII, respectively), photochemical quenching (qP) and non-photochemical quenching (NPQ) in non-acclimated and cold-acclimated plants of the three studied species were measured (Fig. 3A-3H). Fv/Fm values of non-acclimated plants remained between 0.7 to 0.85, at increasing light intensities, indicating an optimal physiological state (Björkman & Demmig 1987, 1995), specially for *E. coccineum* and *G. avellana*, which had the highest values (Fig. 3A). Contrarily, in cold-acclimated plants the Fv/Fm values of *G. avellana*, decreased significantly above 500 μmol photons m^{-2} s^{-1} reaching photoinhibitory values (around 0.6) (Björkman & Demmig 1987, 1995), whereas *L. ferruginea* and *E. coccineum* showed normal values during the light treatment (Fig. 3B). In the three species, ΦPSII of non-acclimated plants decreased significantly at increased irradiances (Fig. 3A) Light response of ΦPSII of non-acclimated plants of *L. ferruginea* had a clear difference with respect to the other two species, showing the lowest light saturation and exhibiting the lowest values at high light indicating that this species would have the lowest electron transport rate (Fig. 3A). No major differences were observed in light response of ΦPSII between *G. avellana* and *E. coccineum*. Cold acclimated plants showed a decrease in light saturation of ΦPSII of all species, being *G. avellana* which exhibited the most conspicuous decrease with light intensity, being around 0.15 even at 200 mmol photons m^{-2} s^{-1} (Fig. 3B). Nonetheless, over 1,000 mmol photons m^{-2} s^{-1} similar values were reached in all species. Non-acclimated plants of *E. coccineum* maintained the highest fraction of open reaction centers (higher qP) at irradiances from 200-1,000

TABLE 1

Q_{10} for non-acclimated and cold-acclimated plants of three Proteaceae. Values of Q_{10} are means (n = 3) ± SE. Different lower case letters indicate statistically significant differences ($P < 0.05$) between the treatments for each species. Different upper case letters show statistically significant differences ($P < 0.05$) between species in the same treatment

Q_{10} de plantas no aclimatadas y aclimatadas al frío de las tres Proteáceas investigadas. Valores Q_{10} son promedios (n = 3) ± EE. Letras minúsculas diferentes muestran diferencias estadísticamente significativas ($P < 0,05$) entre tratamientos para una misma especie. Letras mayúsculas diferentes muestran diferencias estadísticamente significativas entre las especies en un mismo tratamiento

Treatment	Q_{10}		
	E. coccineum	*G. avellana*	*L. ferruginea*
Non-acclimated	2.26 ± 0.05 ^{aA}	2.10 ± 0.23 ^{aA}	2.23 ± 0.06^{aA}
Cold-acclimated	1.74 ± 0.07 ^{bBC}	1.42 ± 0.16 ^{bB}	2.31 ± 0.30^{aC}

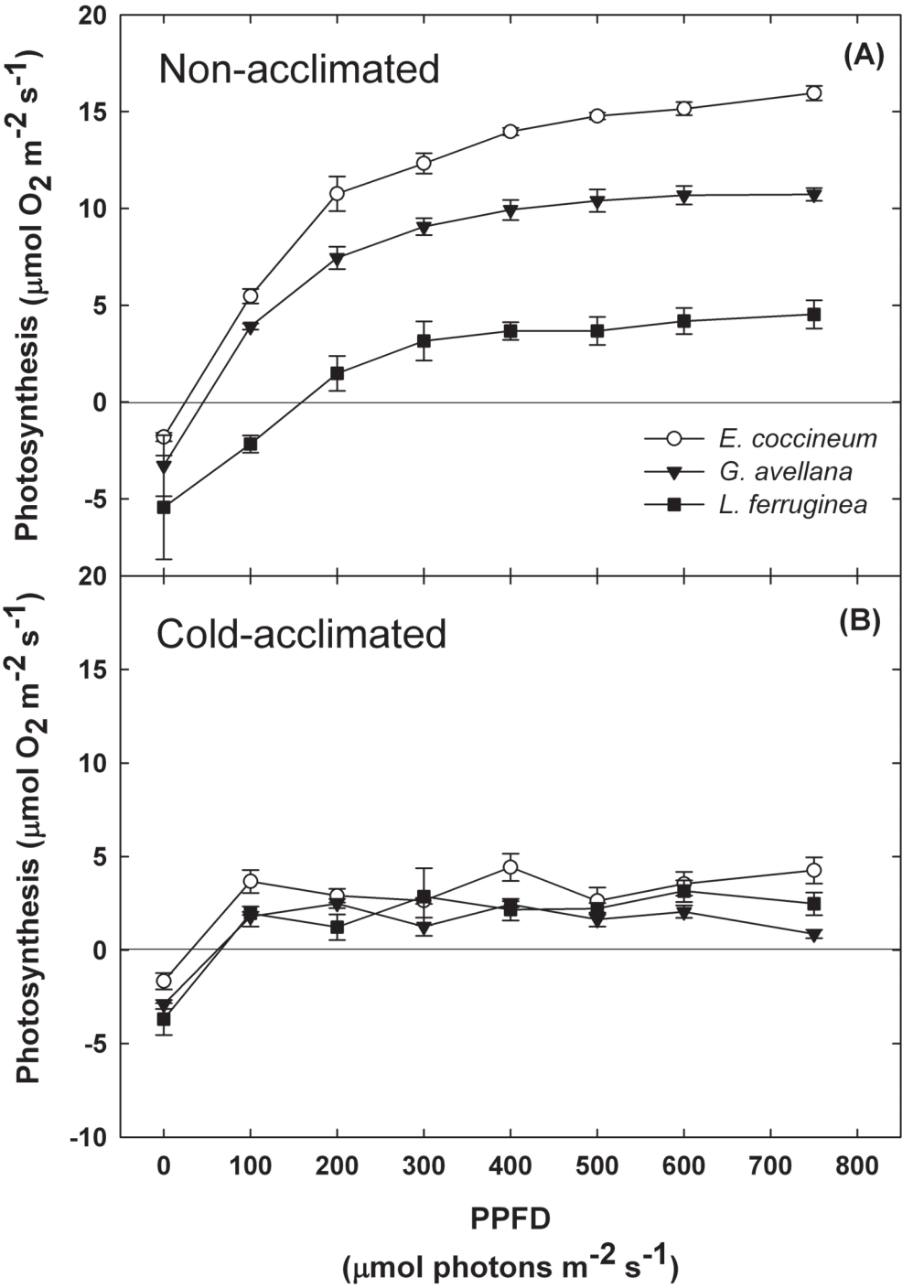

Fig 2: Light curves for oxygen evolution in non-acclimated (A) and cold-acclimated (B) plants of *E. coccineum*, *G avellana* and *L. ferruginea*. Measurements were performed at eight different levels of PPFD; (n = 3 ± SE). Standard errors are not visible when their magnitude does not exceed the size of symbols.

Curvas de luz para la evolución de Oxígeno en plantas no aclimatadas (A) y aclimatadas al frío (B) al aclimatadas de *E. coccineum, G avellana* and *L. ferruginea*. Mediciones fueron realizadas a ocho niveles diferentes de densidad de flujo de fotones (PPFD); (n = 3 ± EE). Los errores estándar no se aprecian, cuando ellos no exceden el tamaño de los símbolos.

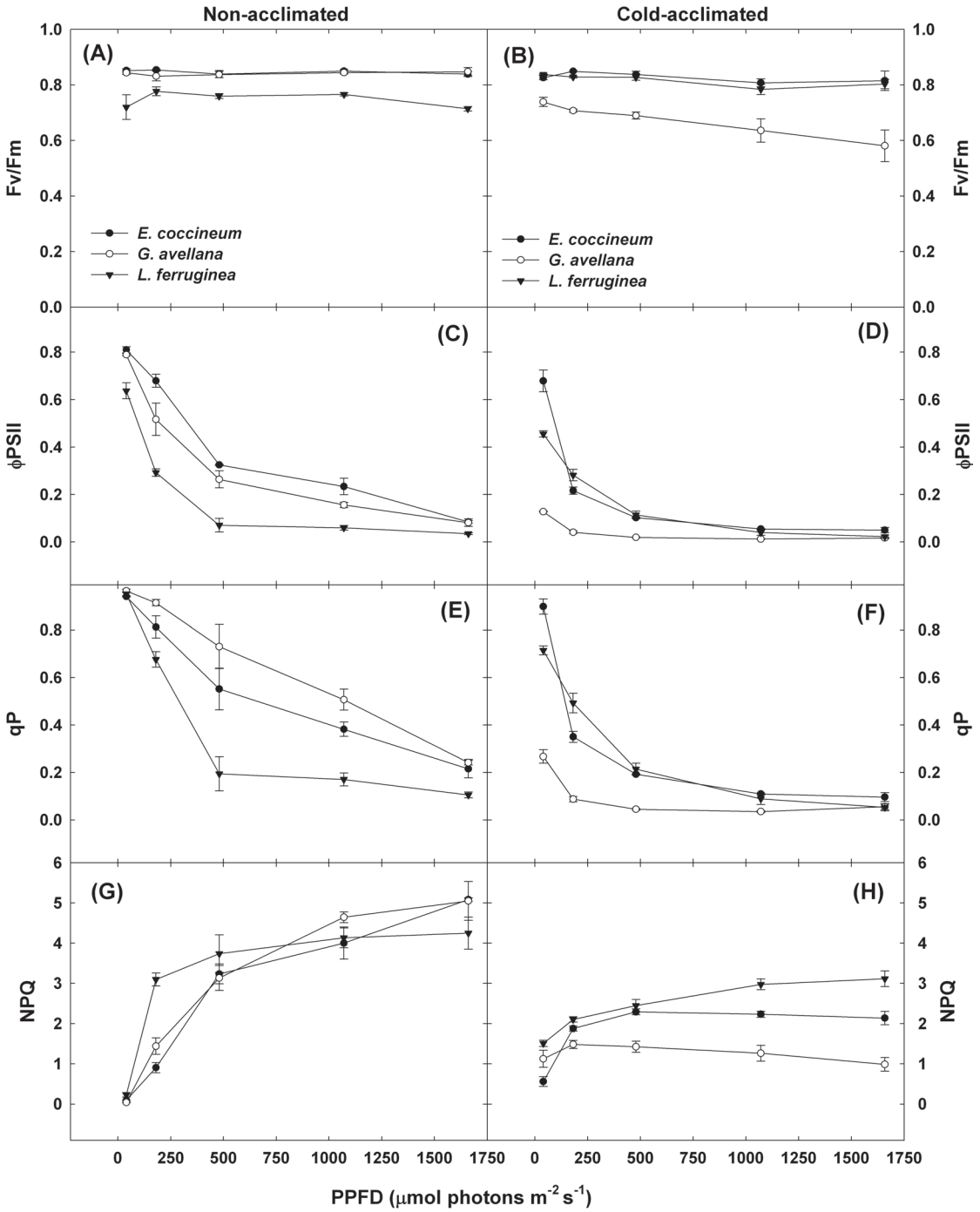

Fig. 3: Changes in the fluorescence parameters of the PSII in non-acclimated and cold-acclimated plants of *Proteaceae* at increasing light irradiances. PSII, photosystem II; Fv/Fm, maximum quantum yield of PSII (A, B); ΦPSII, effective quantum yield of PSII (C, D); qP, photochemical quenching (E, F); NPQ, non photochemical quenching (G, H). Values are means (n = 4 ± SE). Standard errors are not visible when their magnitude does not exceed the size of symbols.

Cambios en los parámetros de fluorescencia del PSII en plantas de *Proteaceae* no aclimatadas y aclimatadas al frío a intensidades crecientes de luz. PSII, photosystem II; Fv/Fm, rendimiento cuántico máximo del PSII (A, B); ΦPSII, rendimiento cuántico efectivo del PSII (C, D); qP, apagamiento fotoquímico (E, F); NPQ, apagamiento no fotoquímico (G, H). Valores son promedios (n = 4 ± EE). Los errores estándar no se aprecian, cuando ellos no exceden el tamaño de los símbolos.

mmol photons m^{-2} s^{-1}, exhibiting no differences with *G. avellana* at 1,600 mmol photons m^{-2} s^{-1} (Fig. 3C). *Lomatia ferruginea* was clearly the species that kept the lowest proportion of open reaction centers at the lowest irradiance (qP about 0.2 from 400 mmol photons m^{-2} s^{-1} ahead), which indicates that even a low irradiance caused a significant excitation pressure of PSII in this species. Cold acclimation sharply reduced the light intensity at which qP was saturated for all species (Fig. 3D), being more obvious in *G. avellana* where its qP decreased to one third of the value observed in the non-acclimated plants even at 200 mmol photons m^{-2} s^{-1}. Cold acclimated plants of *E. coccineum* also decreased qP with respect to non-acclimated ones reaching about 50 % of qP at about 200 mmol photons m^{-2} s^{-1}. Thermal dissipation of excess energy (NPQ) of non-acclimated plants was saturated at a lower irradiance (400 mmol photons m^{-2} s^{-1}) in *L. ferruginea* than in the other species (Fig. 2E). In this treatment, NPQ of *G. avellana* was saturated at about 1,100 mmol photons m^{-2} s^{-1}, while *E. coccineum* NPQ did not reach the saturation point. NPQ decreased in cold-acclimated plants of all species with respect to non-acclimated plants (Fig. 3E and 3F). Interestingly, cold acclimated *L. ferruginea* maintained the highest capacity for heat dissipation and a slightly higher light saturation of NPQ than in non-acclimated state (Fig. 3F). NPQ of cold treated *G avellana* and *E. coccineum* were readily saturated at 200 and 500 mmol photons m^{-2} s^{-1} respectively.

Variation in the content of pigments

Because pigments are strongly associated with the photochemical performance and photosynthesis of plants, pigments contents of non-acclimated and cold-acclimated plants of the investigated species were studied. Non-acclimated plants of *E. coccineum* and *L. ferruginea* showed similar content in total chlorophylls and carotenoids, both in higher amounts than in *G. avellana* ($P < 0.05$), (Table 2). These species showed a similar Chl *a*/*b* ratio. A decrease in total chlorophyll contents (around 2-fold) was found in the cold-acclimated individuals with respect the non-acclimated ones of the three species (Table 2). Total chlorophyll contents of cold acclimated species presented slightly differences between them. *Embothrium coccineum* and *G. avellana* ($P < 0.05$) decreased noticeably their carotenoids contents with respect to non-acclimated plants, exhibiting a lower carotenoids/chlorophyll ratio and also a low Chl *a*/*b* ratio than non-acclimated plants. Interestingly, cold-acclimated plants of *L. ferruginea* had a similar Chl *a*/*b* ratio and carotenoids/chlorophyll ratio than non-acclimated ones (Table 2). Independently of the treatment, carotenoid contents of *L. ferruginea* were the highest among the species. Statistically significant interactions between treatments and species were obtained for chlorophyll *a* ($P = 0.016$), total chlorophyll ($P = 0.028$) and total carotenoids ($P = 0.041$).

DISCUSSION

Photosynthesis and respiration are strongly dependent on leaf temperature (Larcher 1995). These rates generally increase exponentially with temperature, although it has been demonstrated that after the photosynthetic parameters reach their maximum level, they decrease at higher temperature. The quantization of the photosynthetic response of individual species to temperature and light is important for comparing the plasticity or adaptability of different species to environmental conditions (Dungan et al. 2003). According to Krause (1994) photosynthetic rates diminished in cold-acclimated plants at 4 ºC due to the highest proportion of inactive PSII centers caused by low temperature. This assumption is consistent with the lower proportion of open reaction centers (qP) and lower light saturation of qP and ΦPSII exhibited by cold acclimated plants of *G. avellana* and *E. coccineum* respect to non-acclimated ones (Fig. 3B and 3D). In addition, it has been reported that a higher dark respiration level in cold-acclimated plants may be associated with low levels of net photosynthesis (Graham & Patterson 1982). However, in our study, this appears not to be the case because a slightly decreased or similar dark respiration was observed in cold acclimated and non-acclimated plants of the studied species (Fig. 2A and 2B). It has been reported that low growth temperature may

decrease the activation energy of enzymatic reactions (Pereira 1995, Hikosaka et al. 2006) leading to a lower temperature requirement to reach maximum net photosynthesis. This was the case only for *L. ferruginea* and *E. coccineum*. Furthermore cold acclimated plants exhibited a higher photosynthesis stability at low temperature, preserving higher positive net photosynthesis at the lowest temperature than non-acclimated plants (Fig.1 A and 1B). Non-acclimated plants of *E. coccineum* presented a high photosynthesis rate accompanied by a wide optimum temperature range for photosynthesis. This is consistent with its typical character of sun plant (Weinberger 1974) and their widest latitudinal and altitudinal distribution. On the other hand, *G. avellana* and *L. ferruginea* present a more restricted optimum range of photosynthesis than *E. coccineum*, which is consistent with their more shade tolerance features and their lower latitudinal distribution for *G. avellana* but not for *L. ferruginea* which has a wider latitudinal distribution than *G. avellana*. Non-acclimated plants of *E. coccineum* showed significant differences with respect to the other species in photosynthesis level, but not in the cold-acclimated state, indicating that its photosynthetic apparatus is more sensitive to variations of temperature (Fig. 1A and 1B). Nonetheless, *E. coccineum* seems to be more adapted to low temperature than the other two species because it presents the highest photosynthesis rate, together with a high ΦPSII even at high light intensity, which demonstrated that it possesses a more efficient

TABLE 2

Effect of cold acclimation on pigments contents of the investigated Proteaceae. Chlorophyll *a* (Chl*a*), chlorophyll *b* (Chl*b*), total chlorophylls (Chl*a*+Chl*b*), relationship Chl*a* and *b* (Chl*a*/Chl*b*), total carotenoids (Car). Values are means (n = 3) ± SE. Different lower case letters indicate statistically significant differences ($P < 0.05$) between the treatments for each species and each pigment. Different upper case letters show statistically significant differences ($P < 0.05$) between species in the same treatment for each pigment. Asterisks* indicate statistically significant differences between the treatments for the pigments or their ratios in each species

Efecto de la aclimatación al frío sobre el contenido de pigmentos en las Proteaceae investigadas. Chlorofila *a* (Chl*a*), chlorofila *b* (Chl*b*), clorofilas totales (Chl*a*+Chl*b*), relación Chl*a* y *b* (Chl*a*/Chl*b*), carotenoides totales (Car). Valores representan promedios (n = 3) ± EE. Letras minúsculas differentes muestran diferencias estadísticamente significativas ($P < 0,05$) entre tratamientos para cada pigmento. Letras mayúsculas diferentes muestran diferencias estadísticamente significativas entre las especies en un mismo tratamiento para cada pigmento. Asterisco* indica diferencias estadísticamente significativas para los pigmentos o sus radios entre los tratamientos, en cada especie

	Non-acclimated		
Pigment (μg g^{-1} FW)	*E. coccineum*	*G. avellana*	*L. ferruginea*
Chl*a*	1,075 ±30^{aA*}	595 ± 68^{aB*}	972 ± 53^{aA*}
Chl*b*	681 ± 57^{aA*}	297 ± 60^{aB}	594 ± 60 ^{aA*}
Chl*a*+Chl*b*	1,756 ± 58^{aA*}	892 ± 110^{aB}	1,567 ± 105^{aA*}
Chl*a*/Chl*b*	1.6 ± 0.16^{aA}	2.1 ±0.34^{aA}	1.7 ± 0.13 ^{aA}
Car	159 ± 20^{aA*}	113 ±6 9^{aB*}	188 ± 3 ^{aA*}
Car)/ Chl*a*+Chl*b*	0.091 ± 0.01^{aA}	0.129 ± 0.01^{aAB*}	0.121 ± 0.01^{aB}
	Cold-acclimated		
Pigment (μg g^{-1} FW)	*E. coccineum*	*G. avellana*	*L. ferruginea*
Chl*a*	338 ± 70^{bA}	242 ± 78^{bA}	467 ± 37^{bA}
Chl*b*	284 ± 74^{bA}	208 ± 94^{aA}	274 ± 47^{bA}
Chl*a*+Chl*b*	622 ± 138^{bA}	450 ± 171^{bA}	741 ± 83^{bA}
Chl*a*/Chl*b*	1.3 ± 0.2^{aA}	1.3 ± 0.1^{bA}	1.8 ± 0.2^{aA}
Car	28 ± 14^{bA}	43 ± 5^{bA}	108 ± 13^{bB}
Car)/ Chl*a*+Chl*b*	0.061 ± 0.03^{aA}	0.055 ± 0.04^{bA}	0.151 ± 0.03^{bB}

photosynthetic apparatus than the other species. The high values of photosynthesis are consistent with the behavior of species that are often among the first to become established in mesic successional sequences (Dungan et al. 2003) as it occurs with *E. coccineum*. Moreover, this species can colonize highly unfavorable open habitats exposed to low temperature and high irradiance (Weinberger 1974, Alberdi & Donoso 2004). Sun plants possess efficient energy dissipation mechanisms, including the xanthophylls cycle (carotenoids) (Loggini et al. 1999) and other physiological processes such as photorespiration and Mehler reaction (Niyogi et al. 1998, Pérez-Torres et al. 2004). During sunny days, the most abundant pigment of the xanthophyll cycle is zeaxanthin. Most plants require a higher photoprotective potential during the summer (Niyogi et al. 1998, Loggini et al. 1999). Also during the summer some plants present the lowest chlorophyll concentration, being capable of reducing the overexcitement, together with maximizing the xanthophyll cycle function (Björkman & Deming-Adams 1995, Kyparissis et al. 2000). Shade species present frequently smaller total carotenoids and higher chlorophylls contents than sun plants (Adams et al. 2004). However, non-acclimated plants of *L. ferruginea* and *E. coccineum* showed higher chlorophyll content than the other investigated species. In cold-acclimated plants, however, their contents decreased with respect to non-acclimated plants. This is most likely due to an acclimation response before the decrease in the enzymatic activity of the photosynthesis process (Pérez-Torres et al. 2004). *Embothrium coccineum*, *G. avellana,* and *L. ferruginea* decreased their total carotenoids content in cold-acclimated plants. However, *L. ferruginea* maintained a higher content of total carotenoids than the other species under the same condition. This could indicate that the exposition of this species to low temperature induced a major photosensitivity of its photosynthetic apparatus, and thereby a higher demand for photoprotection, with a higher NPQ, contrary to the other species (Fig. 3F). The decrease in pigments contents occurs as a consequence of cold-acclimation (Alves et al. 2002). It is notorious that *G. avellana*, in non-acclimated state, presents an intermediate behavior in the measured pigments parameters, while under cold-acclimation conditions it was more affected. A higher acclimation capacity would be advantageous when radiation or temperature suddenly increase having important effects on forest composition and regeneration (Oguchi et al. 2003). NPQ in cold-acclimated *E. coccineum* and *L. ferruginea* showed a strong increase from 0 to 200 μmol photons m^{-2} s^{-1}. This agrees with a decrease in qP and ΦPSII, indicating a high saturation of the reaction centers at relatively low light intensities (Fig. 3). This was probably related to PSII photoprotective thermal energy dissipation of excess absorbed light (Colom et al. 2003). The saturation of ΦPSII, qP and NPQ at low irradiance in *L. ferruginea* explains why this plant is a shade-tolerant species. *E. coccineum* shows a light response in heat dissipation (NPQ) similar to the semi-shade tolerant species (Fig. 3E) and a higher level of photosynthesis (Fig. 2), thus confirming the higher capacity of *E. coccineum* to respond to environmental variations. Two different strategies for light utilization in relation to growth and cold acclimation have been described (Savitch et al. 2002). The first strategy consists in maintaining high levels of photosynthetic rates, no chlorophyll losses, and minimal NPQ. The second strategy shows general depression of photosynthesis, pigments losses, and high and sustained capacity for NPQ. In our case, the three species appear to belong to the second strategy, but with lower NPQ with respect to the non-acclimated species. It is also important to mention that *G. avellana* seems to be more affected by cold acclimation than the other species, which is corroborated by the low Fv/Fm values.

It is well known that temperature decreases with increasing latitude and altitude (Bannister 2007). Cabrera et al. (1998) reported lower photosynthesis in species of the tropical high Andes distributed at higher altitudes with respect those located at lower ones. A similar behavior was found by Pipper et al. (2006) in *Kageneckia angustifolia* growing in the Chilean Andes at different altitudes.

In general, it appears that the photosynthetic apparatus of *L. ferruginea* is less plastic than the other two species. This is consistent with their habitat preferences, in which *L. ferruginea*, a shade tolerant plant with more restricted

latitudinal distribution than *E. coccineum*, is usually protected from drastic environmental variations. In contrast, *E. coccineum* shows a much higher plasticity in its photosynthetic performance, which would be required by the greater changes in radiation and temperature of its habitats, located at the most southerly easterly, altitudinal and latitudinal distribution (Escobar et al. 2006). *G. avellana* and *L. ferruginea* are distributed at lower altitudes in the occidental sites of the Andes, with more equilibrated thermal regimes (Donoso et al. 2006, Donoso & Utreras 2006). In general, the distribution of these species is concordant with their lower photosynthetic performance.

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