

Variability of water relations and photosynthesis in *Eucryphia cordifolia* Cav. (Cunoniaceae) over the range of its latitudinal and altitudinal distribution in Chile

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Summary The aim of this study was to investigate, during the summer of the year 2008, the variation in leaf water and photosynthetic characteristics of *Eucryphia cordifolia* Cav. (Cunoniaceae) along its broad latitudinal distribution in central south Chile (36° to 42° S). The latitudinal variation in water potential (Ψ_w), water potential at saturation ($\Psi\pi^{\text{sat}}$), water potential at the turgor lost point ($\Psi\pi^{\text{tlp}}$), stomatal density of the leaves, leaf nitrogen concentrations and photosynthetic light response were studied in eight populations. The populations located in the northern region of the distribution of *E. cordifolia* had the lowest leaf water potential. Osmotic potential at full turgor was highest in the two southernmost populations and gradually decreased towards the northernmost points. Similarly, osmotic potential at zero turgor was the lowest in the northern population. On the contrary, the symplastic water content was lower in the two southernmost populations. The highest net photosynthesis rate was recorded for plants in the populations of intermediate distributions, and it was dependent on the precipitation and temperature gradient. The northern populations, which are subject to the lowest precipitations, showed the lowest stomatal densities, which were tightly linked with stomatal conductance variation. Therefore, the variability of A_{max} was independent of stomatal density and conductance, so that the northern populations, subject to environments with less water availability, presented higher photosynthetic water use efficiency. Photosynthesis variations were also not associated with changes in leaf water potential or in nitrogen content in *E. cordifolia* leaves, which did not show any consistent latitudinal or altitudinal trend. In conclusion, the results support our hypothesis that the leaf water relations, stomatal characteristics and photosynthetic rates of the leaf would

vary along its latitudinal gradient, helping to explain the ability of *E. cordifolia* trees to inhabit a broad latitudinal and altitudinal range throughout the central south Chile.

Keywords: gas exchange, photosynthesis, stomata density, Valdivian forest, water potential, water use efficiency.

Introduction

The tree *Eucryphia cordifolia* Cav. (Cunoniaceae) is a canopy-emergent species of the Valdivian evergreen rainforest, usual in the wet sectors of the Chilean Coastal Range. It is distributed from 36° to 42° S and is found between the southern limit of the Mediterranean-type vegetation region and the northern limit of the North Patagonic forest region, up to 700 m a.s.l., generally in areas with low gradient slope, under warm conditions and humid soil but not in excess (Escobar et al. 2006). *E. cordifolia* is a long-lived early successional species that grows together with species of the Nothofagaceae and Myrtaceae families (Donoso 1993, Donoso and Lara 1998, Lusk and Del Pozo 2002). Additionally, in the pollen record, the thermophilous taxa *Eucryphia* suddenly expanded about 9600 ¹⁴C years BP, when the atmospheric conditions would have been warmer and drier than the current ones (Moreno and León 2003). The evidences indicate that the near-modern climate in central south Chile was established after ~3000–2000 years BP (Latorre et al. 2007).

In its current broad latitudinal distribution, *E. cordifolia* ranges from areas with Mediterranean-type climate, with mild winters and very dry and warm summers, to areas with tem-

perate climate, with more chilly winters and more mesic conditions during summer. Summer drought has been generally considered the primary environmental constraint to the productivity and distribution of the vegetation (Larcher 2000). Along the latitudinal gradient of *E. cordifolia*, summer drought increases towards the northern end of its distribution. It seems, therefore, that *E. cordifolia* shows the ability to inhabit in a broad range of habitats, which could be in part aided by variability in physiological traits, such as water relations and photosynthesis. Long-lived woody plant species must adjust to daily and seasonal fluctuations in environmental conditions, and such adjustment is usually initiated as an array of physiological responses to stress or changes in the environment that subsequently leads to stable long-term adjustments, reflecting a developmental response to the new environmental conditions (Huner et al. 1998, Lambers et al. 1998).

Summer droughts lead to a water deficit in the leaf tissue, which affects many physiological processes such as leaf water relations and photosynthesis (Flexas and Medrano 2002, Flexas et al. 2006). These traits are, therefore, those whose plasticity is more likely affecting the capacity of a species to acclimate to drought conditions. Differences in water potential (Ψ_w) among plants are often interpreted as indicating relative access to soil moisture. Thus, mean differences in leaf Ψ_w during the morning among populations of *E. cordifolia* could reflect differences in moisture along the tree's latitudinal distribution. Also, the minimum seasonal water potential that a plant experiences (Ψ_{\min}) provides an important measure of the plant water status, as it reflects the maximum water deficit that the leaves and xylem must tolerate to maintain physiological activity. Ψ_{\min} also acts as a selective pressure on xylem structure which, in turn, generates correlations between Ψ_{\min} and numerous hydraulic traits of the leaf (Bhaskar and Ackerly 2006). These aspects suggest that differences in water relation patterns between populations of the same species contribute to explain persistence within different habitats. However, it is necessary to have an understanding of ecophysiological implications between vulnerability to cavitation and the actual (Ψ_{\min}) water potentials experienced by plants under drought conditions in the field to explain that populations do not experience the same level of water stress (Valladares and Sánchez-Gómez 2005).

Also, photosynthesis responds to seasonal and diurnal changes in environmental variables. The optimum temperatures of photosynthesis are similar to the daytime temperature at which the plants normally grow, but the ability to acclimate to different temperatures has been demonstrated in several species (Cabrera et al. 1998). Air humidity and soil water status act on stomatal aperture and photosynthesis metabolism (Schulze 1986, Luoma 1997). Likewise, environmental conditions are associated to the stomatal characteristics of the leaf (Beerling and Kelly 1996, Galmés et al. 2007a, 2000b). A common response to water stress is a decrease in stomatal conductance, which decreases transpiration, photosynthesis and growth. Additionally, there is evidence of direct metabo-

lism inhibition of photosynthesis by water stress (Schulze 1986, Luoma 1997). Association among leaf water characteristics, stomatal density and CO₂ assimilation has mainly been studied on plants grown under laboratory or common garden conditions (Benowicz et al. 2000, Wang et al. 2003, Duan et al. 2005). Additionally, there are few studies on the variation of leaf water and CO₂ exchange characteristics of the plants in field conditions over all ranges of latitudinal distribution in plants with broad climate gradient (Luoma 1997, Palmroth et al. 1999). Likewise, there are scarce studies in tree species of the rainforest of Chile with this approach (Reyes-Díaz et al. 2005, Piper et al. 2007).

The aim of this study was to investigate the variation in leaf water relations, photosynthetic characteristics and stomatal density of *E. cordifolia* growth along its broad latitudinal distribution in central south Chile. The latitudinal variation in water potential (Ψ_w), water potential at saturation (Ψ_w^{sat}), water potential at the turgor lost point (Ψ_{π}^{tlp}), photosynthetic light response and stomatal density were studied. It is hypothesized that the leaf water characteristics, photosynthetic rate and stomatal density of the leaf would be linked to each other and that they would vary along its latitudinal gradient, helping to explain the ability of *E. cordifolia* trees to inhabit a broad latitudinal and altitudinal range throughout the central south Chile.

Materials and methods

Study sites and species

Measurements were conducted on juveniles of the evergreen tree *E. cordifolia* Cav. (Cunoniaceae) under field conditions in January 2008 (summer). This species of the Valdivian rainforest is dominant of the vegetation below 700 m a.s.l. both in the Coastal Range and in the Andes of southern Chile (Veblen et al. 1983). *E. cordifolia* grows between latitudes 36°47' S and 42°51' S (herbarium database of Universidad de Concepción, Chile), regenerating in large gaps of the canopy, like *Nothofagus dombeyi*, *Nothofagus nitida* and *Weinmannia trichosperma* (Donoso et al. 1985, Figueroa and Lusk 2001), and eight study sites were selected along the latitudinal distribution of species (Figure 1). In Chile, the Mediterranean-type ecosystems occupy a narrow band along the western margin of South America (30° to 36° S). These ecosystems represent the transition between the Atacama Desert, one of the driest deserts in the world (north of 28° S), and the mixed deciduous–evergreen temperate forests, south 36° S (Armesto et al. 2007). The temperate climate of the northern end is characterized by a rainfall of 950 mm/year and an annual temperature of 13.2 °C (Table 1). At the southern end, annual precipitation reaches nearly 1197 mm and an average annual temperature of 10.7 °C (Table 1). Since the sites had no meteorological station close-by, climate variable was obtained from an interpolated climate surface model,

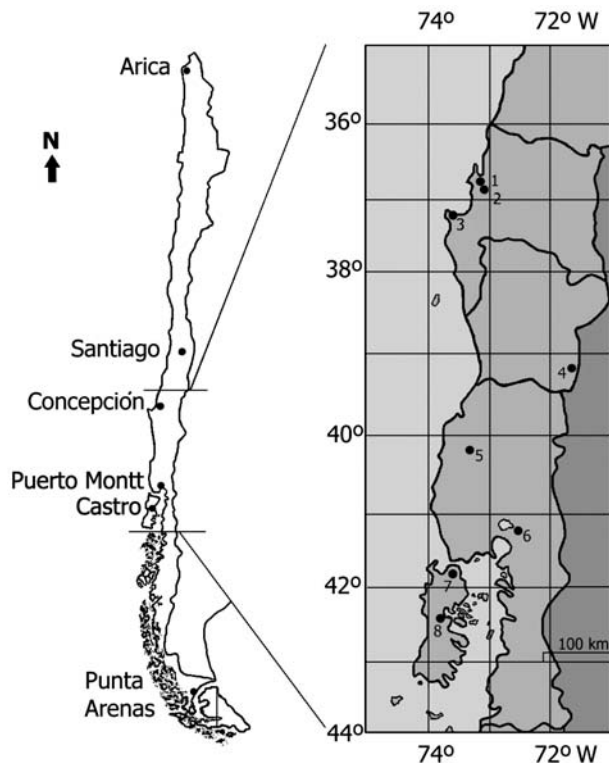


Figure 1. Distribution map of the eight study sites selected were located along the distribution ranges of *E. cordifolia*. 1, Hualpén; 2, Escuadrón; 3, Llico; 4, Huife; 5, Pelada; 6, Ensenada; 7, Quilar; 8, Queilen.

WORLDCLIM (Hijmans et al. 2005). The northern end is affected by the Mediterranean-type climate of central Chile and subject to a cool rainy winter and a summer drought period of 2–3 months. The southern end is affected by the southern westerly winds and high-precipitation regimes during the year. Additionally, southern end populations are subject to low-permeability soil. The Valdivian rainforest in the Coastal Range grew mainly on metamorphic and granitic material of the Palaeozoic age and in the Andes on soils originated from volcanic ash (Holdgate 1961, Mardones 2005).

Table 1. Location, nitrogen concentration in the leaves of *E. cordifolia*, altitude and climate variables of the eight study sites. N, total nitrogen concentration (% dry weight); Elev., altitude; T_{years} , average yearly temperature; TDQ, average temperature of driest quarter; PREC, average annual precipitation; PDQ, average precipitation of driest quarter. Content of N in Hualpén, Escuadrón and Llico is a measurement of combined material due to the scarce material.

Study site	Location (S; W)	N (%)	Elev. (m)	T_{years} (°C)	TDQ (°C)	PREC (mm)	PDQ (mm)
Hualpén	36°5'; 73°1'	0.93	40	13.2	16.9	956	54
Escuadrón	36°6'; 73°1'	0.93	50	13.0	16.8	1161	65
Llico	37°1'; 73°3'	0.93	300	11.8	14.7	1150	77
Huife	39°1'; 71°4'	0.9	498	8.8	13.6	1590	142
Pelada	40°2'; 73°2'	0.74	354	10.4	14.8	2363	334
Ensenada	41°2'; 72°3'	1.28	533	10.6	13.2	2141	204
Quilar	41°5'; 73°5'	0.78	24	10.6	13.5	2319	316
Queilen	42°5'; 73°3'	1.04	30	10.7	13.9	1197	315

Pressure–volume curves

Leaf water potential was measured during the morning (between 09.00 and 11.00 h) to test the water status of leaves before the onset of photosynthesis measurements (see methodology for gas exchange). During the measurements in the field, rainfall was not present in the eight sites. January 2008 was a dry and warm month in central south Chile. Four plants from each of the eight sites (henceforth populations) were measured in branches with fully expanded leaves in a Scholander-type pressure chamber Model 1000 PMS (OR, USA). Immediately after the measurements of water potential, four to five shoots of *E. cordifolia* in the five southern populations were fully hydrated overnight with distilled water in plastic bags to bring leaves to full turgor. The next morning, the water potentials of the leaves were measured with a pressure pump and the tissue was weighed immediately after measuring their water potentials. The tissue was allowed to dry slightly at room temperature before being re-weighed; the water potential was re-measured and this process was repeated until the tissue reached constant weight. Generally, only short times (~5 min) of drying between measurements were necessary, although the time increased as the tissue dried. When there was no further decrease in weight, leaves (shoots) were dried for 48 h at 80 °C to determine dry weight.

Pressure–volume curves were prepared by plotting the reciprocal of the water potential (Ψ) versus the relative water deficit (RWD). Regression analysis was applied to find the best fit to the linear portion of the curve representing the osmotic potential term ($\Psi\pi$) at saturation ($\Psi\pi^{\text{sat}}$) and at turgor loss point ($\Psi\pi^{\text{tlp}}$). The bulk modulus of elasticity (ϵ) for the branches of the five populations was obtained describing the decrease of turgor potential (Ψ_p) with symplastic water (Tyree and Jarvis 1982).

Gas exchange and CO_2 assimilation

Photosynthesis (gas exchange and net CO_2 assimilation) was measured in five *E. cordifolia* plants in the eight populations with an open portable photosynthesis system LI-6400PF LI-COR (NE, USA) equipped with a LI-COR LI-6400-

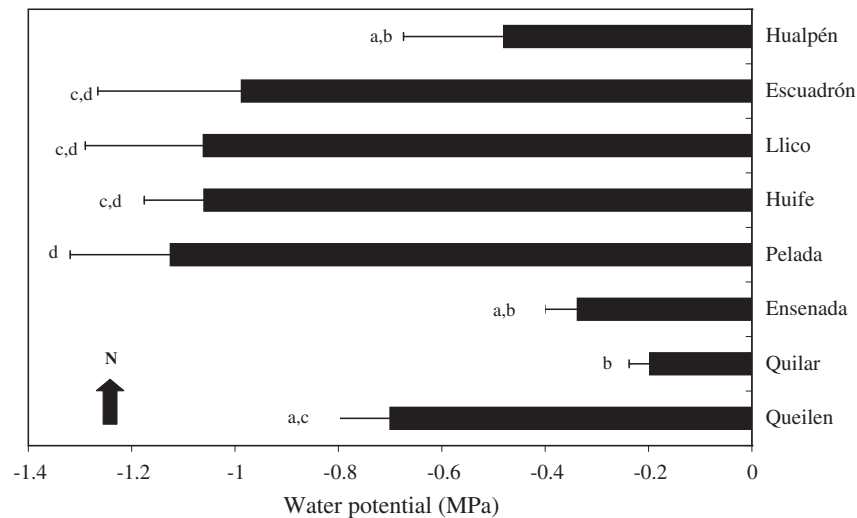


Figure 2. Water potential in branches with fully expanded leaves of the tree *E. cordifolia*. The measurements were performed in eight natural populations of the species in the central south of Chile. Hualpén corresponds to the northernmost end of the tree distribution and Queilen corresponds to the southernmost end of its distribution. Different letters indicate significant differences (a posteriori Tukey test, $P < 0.05$). The bars indicate ± 1 SD.

02B light source. This system permits the accurate control of cuvette temperature and irradiance at the leaf level to determined light-saturated CO_2 assimilation. Curves of net CO_2 assimilation at PPFs ranging from 0 to $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ were measured (every $\sim 50 \mu\text{mol m}^{-2} \text{s}^{-1}$) with CO_2 reference concentration at 360 ppm, 75% of relative humidity and temperature inside the leaf chamber maintained at $20 \pm 5 \text{ }^\circ\text{C}$, which is within the optimum temperature range for photosynthesis in temperate species. Maximum photosynthesis (A_{max}) was calculated with all measurements performed between 09.00 and 11.00 h. Photosynthesis in *E. cordifolia* was measured before or at midday because, after this time, gas exchange was lower. Photosynthesis measurements were done on fully expanded sun leaf, attached to the tree, between 1 and 1.5 m high and always in juvenile individuals to avoid any effect of the ontogeny (Coopman et al. 2008). We considered juvenile individuals < 10 cm DHB and > 2 m high (Donoso et al. 1985). Photo-

synthetic instantaneous water use efficiency was calculated as the ratio of A_{max} to g_s (Zhan et al. 1993).

Leaf nitrogen and stomatal density analyses

Nitrogen leaf concentrations were determined in hundreds of leaves collected in January 2008 in each the eight populations. The determinations were performed at the Soil Laboratory of Agronomy of the Pontificia Universidad Católica de Valparaíso (Chile). Additionally, five leaves of different individuals from all eight study populations were transported to the laboratory to determine the number and density of its sunken stomata. The hypostomatic leaves were kept in a Jeffrey solution (chromic acid at 10% and nitric acid at 10% in equal parts) for 24–48 h, until the epidermis could be easily separated from the mesophyll. Later, the epidermis was dyed in diluted safranin and examined under the microscope. The method used was a modification from Barrera and Meza (2006).

Table 2. Relationship between climate characteristics and water potential, osmotic potential at zero turgor, water content symplastic, elasticity modulus, stomatal density, maximum photosynthesis (A_{max}), stomatal conductance (g_s), photosynthetic water use efficiency (A_{max}/g_s) in *E. cordifolia*. Non-significant variables when P -value > 0.1 are not presented here.

Dependent variable	Independent variable	Coefficient	F	P -value
Water potential	PDQ	-0.68	4.30	0.093
Osmotic potential at zero turgor	PREC	-1.74	238.71	< 0.001
Water content symplastic	Elevation	0.29	111.22	0.002
Elasticity modulus	Elevation	-0.27	9.35	0.055
Stomatal density	PREC	0.82	104.25	< 0.001
A_{max}	Elevation	0.21	3953.11	< 0.001
	T_{years}	2.95	2938.31	0.002
	TDQ	-0.37	22.68	0.041
g_s	PDQ	0.33	1868.23	< 0.001
	PDQ	1.67	14.14	0.013
A_{max}/g_s	PDQ	-1.46	15.08	0.012

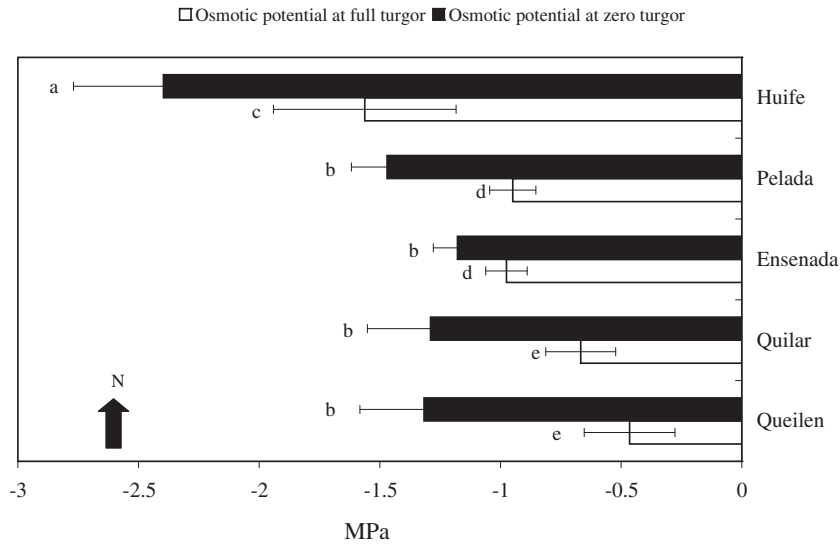


Figure 3. Osmotic potential at full turgor (white box) and osmotic potential at zero turgor (black box) of *E. cordifolia*, obtained from pressure–volume curves. The measurements were performed in five populations of the species in central south Chile. Different letters indicate significant differences (a posteriori Tukey test, $P < 0.05$). The bars indicate ± 1 SD.

Statistics

Significant differences among populations ($P < 0.05$) by leaf water potential, osmotic potential at full and zero turgor, symplastic water content at zero turgor, bulk modulus of elasticity, A_{max} and stomatal densities were evaluated using ANOVA and

Tukey tests (SYSTAT 1996). Significant differences among populations ($P < 0.05$) by g_s and A_{max}/g_s were evaluated using the Kruskal–Wallis test and the method of Dunn. Multiple linear regression analysis was used to determine site climate and orographic factors (see Table 1) that predict leaf water traits (water potential, osmotic potential at full and zero turgor, water

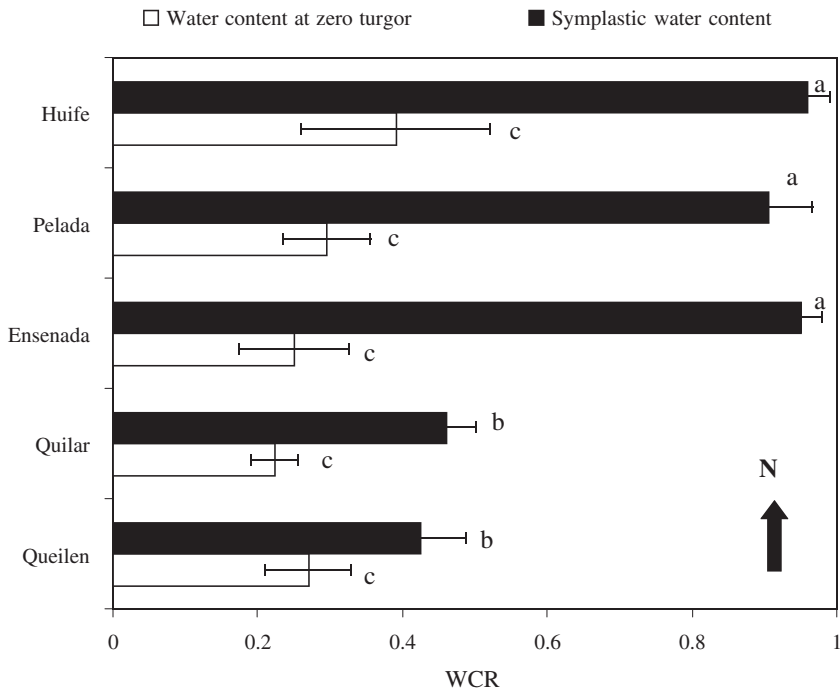


Figure 4. Water content relative to zero turgor and symplastic water volume of *E. cordifolia*, obtained from pressure–volume curves. The measurements were performed in five populations of the species in central south Chile. Different letters indicate significant differences (a posteriori Tukey test, $P < 0.05$). The bars indicate ± 1 SD.

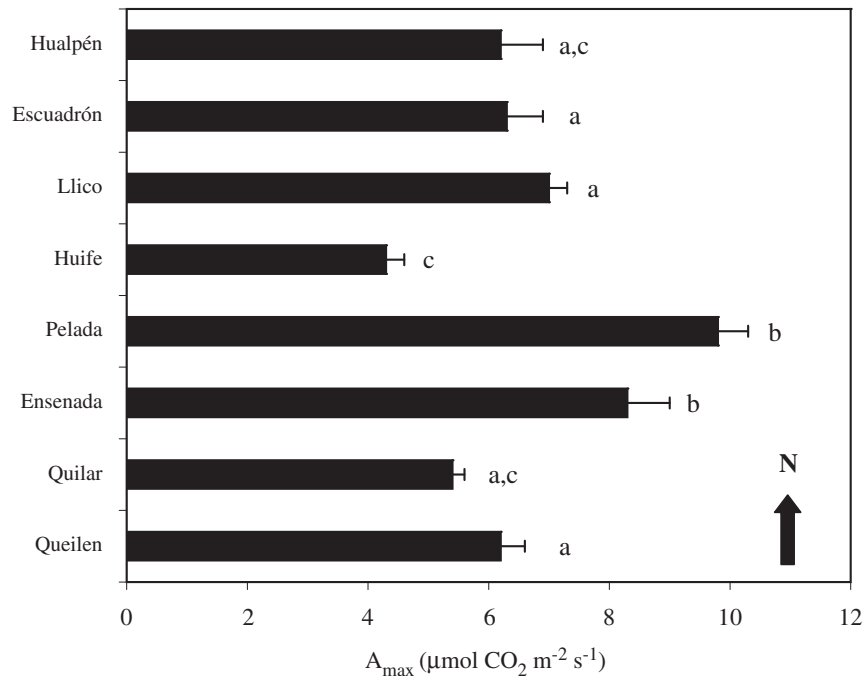


Figure 5. Maximum photosynthesis rate (A_{max}) in the leaves of *E. cordifolia*. The measurements were performed in eight populations of the species in central south Chile. Hualpén corresponds to the northernmost point of the species distribution and Queilen corresponds to the southernmost point of its distribution. Different letters indicate significant differences (a posteriori Tukey test, $P < 0.05$). The bars indicate ± 1 SD.

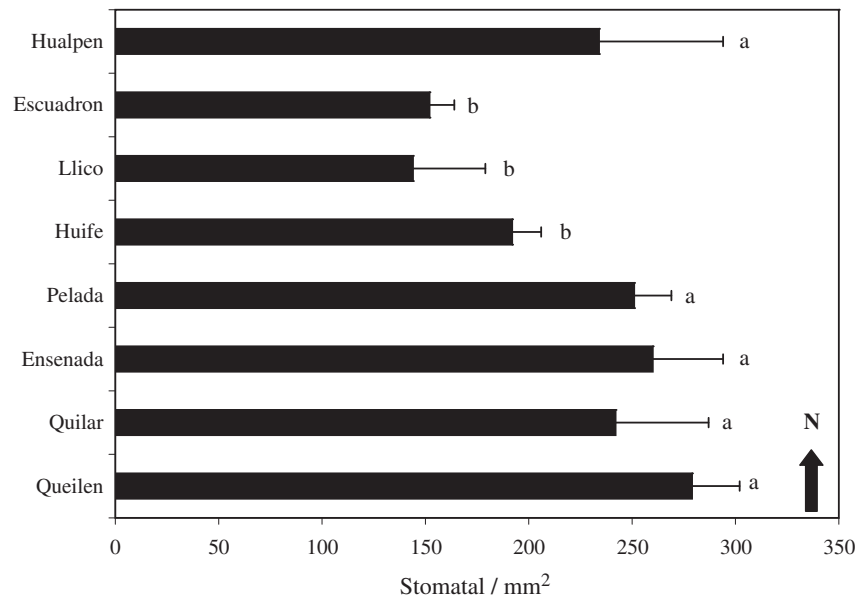


Figure 6. Stomatal density in the leaves of *E. cordifolia*. The measurements were performed in eight natural populations in central south Chile. Different letters indicate significant differences (a posteriori Tukey test, $P < 0.05$). The bars indicate ± 1 SD.

content to zero turgor, symplastic water volume, bulk modulus of elasticity), photosynthetic light response (A_{max}), water use (g_s , A/g_s) and stomatal density of *E. cordifolia*. Likely, multiple linear regression was used to determine stomatal density and conductance that predict A_{max} . Predictors were removed with a backwards procedure when P -values were < 0.1 . A transformation to natural logs occurred when the data did not have a normal distribution.

Results

Water potentials and water relations

Water potentials significantly varied among the eight populations under study ($F_{(7,24)} = 17.7$; $P < 0.0001$) (Figure 2) and

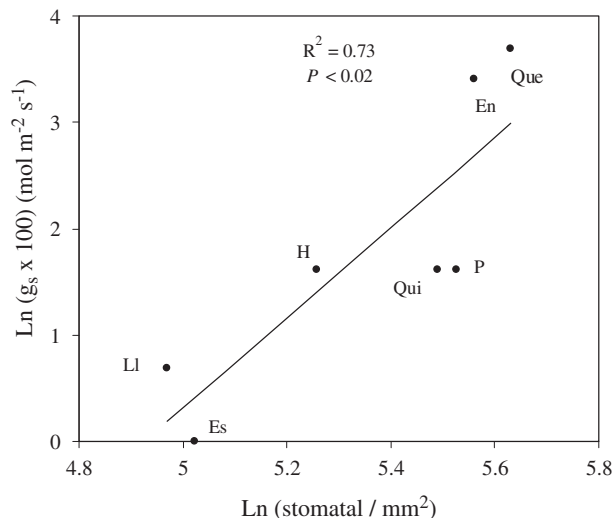


Figure 7. Stomatal conductance versus stomatal density in seven *E. cordifolia* populations located throughout their latitudinal distribution. Es, Escuadrón; Ll, Llico; H, Huife; P, Pelada; En, Ensenada; Qui, Quilar; Qu, Queilen.

was not significantly affected by average precipitations of the driest quarter of the year or summer (Table 2). The populations located in the northern region of the distribution of *E. cordifolia* had the lowest xylematic potential (~ -1 MPa). The exception was the population in Hualpén, located at the northern end, which showed water potential values similar to those of populations at the southernmost points of Ensenada, Senda Darwin and Queilen (Figure 2). Osmotic potential at full

turgor was highest in the two southernmost populations of *E. cordifolia* and gradually decreased towards the northernmost points (Figure 3). Similarly, osmotic potential at zero turgor was lowest in the northern population located at Huife (Figure 3) and significantly increased in populations subject to higher precipitation (Table 2). Noticeably, the symplastic water content was lower in populations of the two southernmost points (Figure 4) and significantly increased in the higher altitude populations (Table 2). Nevertheless, the water content at zero turgor did not significantly vary in the studied populations (Figure 4). The bulk modulus of elasticity did not significantly vary among populations of *E. cordifolia* (Huife, 7.1 ± 5.2 MPa; Pelada, 8.9 ± 5.5 MPa; Ensenada, 9.5 ± 5.4 MPa; Quilar, 15.3 ± 5.2 MPa; Queilen: 20.4 ± 8.7 MPa) ($F_{(4,15)} = 0.41$; $P > 0.05$) and was not associated to its altitudinal and latitudinal gradient (Table 2).

Gas exchange and CO_2 assimilation

The maximum photosynthesis significantly varied among the eight studied populations ($F_{(7,121)} = 9.1$; $P = 0.001$). The populations of *E. cordifolia* located at the northernmost and southernmost points of their distribution showed similar maximum photosynthesis rates (Figure 5). However, the populations of intermediate distributions located at the Pelada and Ensenada showed the highest net photosynthesis rate with $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (9.8 and $8.3 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$, respectively). Also, the population of intermediate distribution located at Huife showed the lowest net photosynthesis with $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR ($4.3 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), a

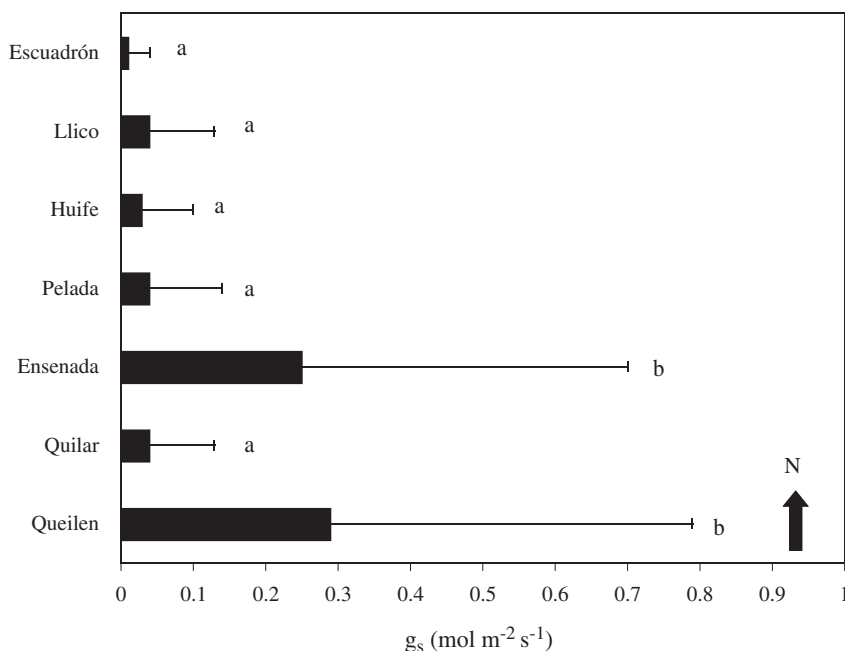


Figure 8. Stomatal conductance in the leaves of *E. cordifolia* in seven natural populations in central south Chile. Different letters indicate significant differences (method of Dunn, $P < 0.05$). The boxes indicate the median and the bars indicate percentile 75%.

decrease of ~40% with respect to Pelada (Figure 5). According to multiple regressions, A_{\max} was significantly associated to four independent variables combined: altitude, average yearly temperature, average temperature of the driest quarter of the year and average precipitation of the driest quarter (Table 2). Maximum photosynthesis significantly increased in populations of higher altitude and subject to higher temperature precipitation (Table 2).

Leaf analysis

The northern populations showed the lowest stomatal densities ($F_{(7,28)} = 8.6$; $P < 0.001$), with the exception of the population located in Hualpén (Figure 6). It must be borne in mind that this population is located at the shore of Lago Hualpén with water available during the entire year. When the Hualpén population in the northern end is removed, we see that the higher stomatal density of the leaves was significantly associated to the average annual precipitation (Table 2). The Queilen population had the highest significant stomatal density and was similar to the other populations located in Pelada at the southern populations, which are subject to higher precipitations than the northern populations (Table 1). The nitrogen content in *E. cordifolia* leaves varied among populations, between 0.74% (Pelada) and 1.28% (Ensenada) (Table 1); but the pattern was not associated to the latitudinal and altitudinal gradient of the populations.

Relationship between water use and photosynthesis

Along its distribution range, the stomatal density variation of leaves was significantly associated with stomatal conductance variation (Figure 7), which significantly varied among populations (Figure 8). However, A_{\max} variation was independent of stomatal density variation ($R^2 = 0.07$; $P > 0.05$). Likewise, the maximum photosynthesis was not associated to stomatal conductance ($R^2 = 0.02$; $P > 0.05$) nor to xylematic potential ($R^2 = 0.01$; $P > 0.05$). However, the stomatal conductance and water use efficiency were significantly associated to the average precipitation of the driest quarter (Table 2), and we found that the population of *E. cordifolia* subjected to higher precipitation during summer significantly showed greater stomatal conductance and lower water use efficiency (Table 2).

Discussion

Variation of water status in *E. cordifolia*

The results showed that the water status of *E. cordifolia* populations varied with the latitudinal distribution (Figures 2, 3 and 4) and would be dependent on the local water availability (Table 2). These tree populations should be adapted and acclimated mainly to variable water availability during the summer in their respective environments. Low water supply has a direct

effect on the physiological processes of the plants, especially transpiration, photosynthesis, respiration and rooting pattern (Lloret et al. 1999, Cabrera 2002, Duan et al. 2005, Valladares and Sánchez-Gómez 2006).

The northern populations showed lower water content in their tissues during the morning, excepting the individuals at the northernmost end, located on the shore of Lake Hualpén (Figure 2). This latitudinal trend was expected since water availability for precipitation in Chile gradually increases towards the south (see Table 1). Additionally, these northern populations of *E. cordifolia* are subject to an annual pattern of precipitations characterized by several weeks of summer drought. On the contrary, the southern populations are subject to the hyper-humid coastal climate of southern Chile, with abundant rains even during the summer, which are enough to maintain water accumulated in the low-permeability soil the whole year and air in high-humidity regimens due to strong Pacific winds (Almeyda and Sáez 1958, Di Castri and Hajek 1976).

Our results suggest that, in *E. cordifolia*, there would be a compensatory physiologic mechanism that counteracts the lower water availability in the soil during the summer. Populations further north showed a more negative osmotic potential at full turgor and zero turgor (Figure 3). However, the water content within cells was higher for these boreal populations (Figure 4), which would indicate acclimation and/or adaption at lower water availability (Palmroth et al. 1999). Probably, the involved mechanism is osmotic because the modulus of elasticity did not consistently vary among *E. cordifolia* populations. Compensatory osmotic mechanisms have been described in central Chile for angiosperm of the genus *Kageneckia* growing in a Mediterranean-type climate, which can bear strong water stress during the summer (Cabrera 2002). Nevertheless, compensatory mechanism presence in *E. cordifolia* must be re-evaluated in populations covering all of the northern distribution area, since the material to estimate the pressure–volume curves was misleading from Llico at Hualpén (northern end).

The populations studied presented a broad range of altitudinal distribution, nearly 500 m. However, water potential of *E. cordifolia* was not correlated to altitude, and populations located at 500 m high (Huife) presented xylematic potential as low as populations near sea level (Escuadrón) (Table 1, Figure 2). However, other water variables were more sensitive to altitudinal gradient than water potential. Water content within cells was greater in populations located at higher altitude, and osmotic potential at zero turgor increase in populations subject to higher annual precipitation (Table 2). It is likely that measurements of water status were affected by climate conditions, as there were 3 weeks without precipitation along the transect during January 2008, and by the dissimilar environmental conditions between the Coastal (more humid) and Andes Ranges (colder). There are several studies suggesting that drought stress would be the determinant variable in the distribution of evergreen trees at low or intermediate altitudes (Mooney et al. 1977, Cabrera 2002, Aranda et al.

2005, Piper et al. 2007). Our results support the idea that the summer drought should be constraining the altitudinal and latitudinal distribution of *E. cordifolia* in central south Chile. Although the compensatory osmotic mechanisms mentioned above should allow, at least partly, the displacement of *E. cordifolia* towards a habitat of low water availability. Other studies will be necessary to determine the effects of altitudinal distribution on water use and availability in trees with broad distribution areas (Wang et al. 2003, Duan et al. 2005, Reyes-Díaz et al. 2005).

Variation of photosynthesis in E. cordifolia

The results of this investigation showed that maximum photosynthesis rate significantly varied between the populations of *E. cordifolia*, reaching the highest values in intermediate locations of its latitudinal distribution area and decreasing towards both ends of the latitudinal transect (Figure 5). As expected, photosynthetic rate varied along its latitudinal and altitudinal gradient associated to precipitation and temperature variation during several weeks of summer drought (Table 2). The populations subject to higher average precipitation and lower average temperature during the summer presented the higher photosynthetic rate.

Photosynthesis is adapted to the physical environment of the plants, and the rate at which it occurs is regulated by temperature, light, water and soil nutrients (Jones 1985, Cabrera 2002, Galmés et al. 2007a, 2007b). According to the trade-off between photosynthesis and water cost, northern populations could be limiting their maximum photosynthetic rates due to lesser water availability. For trees of seasonal regions, it has been commonly recorded that the water deficit during the summer drought decreases the photosynthetic capacity, among other effects, such as water relationships, transpiration and stomatal behaviour (Tognetti et al. 1998, Bernacchi et al. 2002, Cabrera 2002, Duan et al. 2005). Similarly, in the case of trees from temperate regions in the south of Chile, it has been suggested that drought tolerance in photosynthesis may be involved in the distribution patterns of the closely related species *N. nitida* (Phil.) Krasser and *N. dombeyi* (Mirb.) Blume on the Coastal Range (Piper et al. 2007). The authors suggest that the lower drought tolerance of *N. nitida* could explain this species' virtual exclusion from the rain shadow created by the Chilean Coastal Range.

In contrast, at the southern end, photosynthesis may be limited by lower maximum temperatures during the day and is commonly reflected in lower biomass accumulation in populations from colder regions (Morgenstern 1996, Benowicz et al. 2000) or a clear dependence of photosynthetic rate on temperature during the summer (Beadle et al. 1985, Battalgia et al. 1996). *E. cordifolia* has previously shown that resistance to cold was related to their geographical occurrence, while temperature variation affects its optimal photosynthesis (Hill et al. 1988, Read and Hill 1989).

Additionally, there was a pattern in the average values of maximum photosynthetic rate along the altitudinal gradient (Table 2). The highest maximum photosynthesis was found in the middle to high altitudes, except in Huife, located in the highest altitude and associated to the lowest maximum photosynthesis in our study. Huife, located in the Andes Range at ~500 m high, is subject to the lowest temperatures of the eight sites (see Table 1). It is known that photosynthetic performance may be constrained due to low temperatures occurring during the winter in the Andes Range (Reyes-Díaz et al. 2005, Zúñiga et al. 2006). To photosynthesize, the Huife population must be located near the minimum point of the temperature tolerance range during the winter.

However, the effects of nutrients in the soil, irradiance on canopy and leaf morphoanatomy and genotypes on photosynthesis in populations of different locations should not be ignored (Giertych 1979, Molotkov and Patlaj 1991, Luoma 1997, Benowicz et al. 2000, Bernacchi et al. 2002, Lawlor and Cornic 2002, Brodribb et al. 2007). These were not specifically evaluated in this study. However, according to the variation pattern of N content of leaves in the studied populations, there is no sufficient evidence that suggests that maximum photosynthetic variation is due to variations in the concentrations of RuBisCo or in the levels of chlorophyll contents, as generally accepted (Duan et al. 2005). Further research, considering the leaf mass per area, is necessary to elucidate factors that may be limiting photosynthesis rate at altitudinal and latitudinal ends of the distribution area in trees of broad range. However, the relationship between water use and maximum photosynthesis will be discussed below.

Stomatal traits and photosynthetic water use efficiency

E. cordifolia leaves present sunken stomata. Sunken stomata are associated with a xerophytic habitat (Larcher 2003), which is characterized by low water availability supply for plants; this should allow *E. cordifolia*, among other factors, to be under drier and warmer conditions than other trees of the Valdivian rainforest (Donoso et al. 1985, Moreno and León 2003).

We found that the average value of stomatal conductance varied among *E. cordifolia* populations (Figure 8). The highest average conductance was measured in the southern populations, which show a latitudinal tendency associated to water availability during the summer as well (Table 2). Likewise, stomatal density increases toward the southern end due to the highest precipitation during summer (Table 2) and explains, in part, the variation of stomatal conductance (Figure 7). Also, literature shows that the number of open stomata affects stomatal conductance (Lushnikov et al. 1994), and it predicts a decrease in conductance with the increase of the drought (Palmroth et al. 1999). For conductance regulation and its optimal adjustment, the stomatal and leaf morphoanatomy have a key function (Woodward and Kelly 1995, Zwieniecki et al. 2001). There is agreement that stomatal aperture is the

result of a trade-off between the needs to conserve water and CO₂ exchange, and the conductance regulation should allow an optimal adjustment to minimize transpiration and maximize assimilation simultaneously (Berninger et al. 1996, Luoma 1997, Cabrera 2002). However, we did not find a significant relationship between stomatal conductance and maximum photosynthesis rate, which has been recorded in other studies (Field et al. 1983, Wong et al. 1985, Zhan and Marshall 1995, Luoma 1997, Palmroth et al. 1999; Lawson 2009).

Photosynthetic water use efficiency, expressed as maximum photosynthesis over stomatal conductance (A_{\max}/g_s ; see Zhan et al. 1993), varied between populations and increased towards the northern end of the distribution area of *E. cordifolia* (Table 2). High water use efficiency in plants from dry environments has been postulated to be a consequence of a non-linear relationship between g_s and A_{\max} (Cowan 1982, Palmroth et al. 1999). While transpiration increases linearly with g_s (Palmroth et al. 1999), A_{\max} saturates at high g_s . Our results showed low A_{\max} associated to low g_s because saturation by CO₂ was not achieved. The lack of association could be due to the methods used where the environmental variables (temperature, drought, etc.) could not be controlled as in common garden experiments.

The literature is contradictory in relation to the association between climate and photosynthetic water use efficiency. Studies in water use efficiency over a climate gradient conclude that high water use efficiency is typical in plants of dry habitats (Field et al. 1983, Comstock and Ehleringer 1992, Anderson et al. 1996). Moreover, there are studies in plants that report a decrease in photosynthetic water use efficiency in drought climates (Körner et al. 1991, Lloyd and Farquhar 1994, Palmroth et al. 1999). Our results in *E. cordifolia* showed that the northern populations, subject to environments with less water availability, present higher photosynthetic water use efficiency (Figure 7). Additionally, it has adjusted a physiologic mechanism, since water content within cells was higher for these boreal populations (Figure 4).

Conclusion

In summary, there are not many studies in angiosperm trees recording variation in photosynthetic and leaf water characteristics along its broad latitudinal distribution (Palmroth et al. 1999), highlighting our findings in *E. cordifolia* in central south Chile. Our results support the hypothesis that the photosynthetic rate and leaf water characteristic variation are associated to the latitudinal and altitudinal climate gradient in central south Chile. The populations located in the northern region of the distribution of *E. cordifolia*, which are subject to lowest precipitations, had lowest water potential, lowest stomatal densities and higher photosynthetic water use efficiency than the southern populations. Also, the intermediate distribution populations presented the highest net photosyn-

thesis rate, and A_{\max} was significantly dependent on the summer precipitation and temperature along its distribution range. The idea that the summer drought should be limiting the altitudinal and latitudinal distribution of *E. cordifolia* in central south Chile is support for our results due to the water use strategy that the plant presented.

We suggest that, in order to identify other determinants on the variations of the traits analysed, studies of genetic adaptation and environmentally determined acclimation in both common garden and original geographical location experiments are necessary. The results in our experiments suggest that intraspecific variations of water relations, water use patterns, photosynthesis and stomatal density to drought and temperature that occur between *E. cordifolia* populations considered in our study could be key in helping to explain the ability of *E. cordifolia* trees to inhabit a broad latitudinal and altitudinal range throughout the central south Chile. Finally, as the measurements were obtained in one season, it is recommended for additional investigation to extend it over inter-annual and intra-annual variation (Benowicz et al. 2000, Palmroth et al. 1999).

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