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Contribution of physiological and morphological adjustments to drought resistance in two Mediterranean tree species

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Abstract

Plant water potential (ψ), its components, and gas exchange data of two Mediterranean co-occurring woody species (*Quercus ilex* L. and *Phillyrea latifolia* L.) were measured in response to seasonal changes in water availability over two consecutive years. The relative contribution of physiological and morphological adjustments to drought resistance was assessed through Principal Component Analyses. There were large adjustments in stomatal conductance (~36 % of accounted variance). Net photosynthetic rate and water use efficiency were closely tuned to water availability and accounted for ~17 % of variance. The slope of the water potential *vs.* relative water content ($d\psi/dRWC_0$) below zero pressure potential increased as a result of seasonal and ontogenic increases in apoplastic water fraction and accounted for ~20 % variance. This tolerance mechanism was accompanied by an increased range of positive pressure potential, suggesting a functional role of sclerophylly in these Mediterranean evergreens. Similarly, changes in the slope of $d\psi/dRWC$ in the range of positive pressure potential (~13 % of accounted variance) were associated to variations in cell wall elasticity and resulted in lower RWC at zero pressure potential. When considering the species studied separately, the results indicated the primary role of stomatal regulation in the drought resistance of *Q. ilex*, while increased apoplastic water fraction had a major contribution in the drought resistance of *P. latifolia*.

Additional key words: drought stress, *Phillyrea latifolia*, net photosynthetic rate, plant and tissue-water relations, *Quercus ilex*, stomatal conductance, water potential.

Introduction

When facing summer drought, perennial plants may postpone dehydration through a range of strategies which include alteration of water loss and/or water acquisition (Pereira and Chaves 1995). Stomatal regulation and structural adjustments in leaf area minimize water loss, while deep root systems and the development of a large gradient between soil and leaf water potential allow to maximize the potential for water uptake. In addition, periods of soil moisture deficit may be survived through pressure potential maintenance either by osmotic or elastic adjustment (Kozlowski *et al.* 1991). Pressure potential maintenance not only depends on the extent of osmotic and elastic adjustments but also on the interaction between these adjustments and apoplastic water fraction (AWF) (Maury *et al.* 2000). Additionally, characteristic attributes such as relative water content

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Abbreviations: AWF - apoplastic water fraction; $d\psi/dRWC$ slope of the water potential vs. relative water content above zero pressure potential; $d\psi/dRWC_0$ - slope of the water potential vs. relative water content below zero pressure potential; E - transpiration rate; g_s - stomatal conductance; HE - hydraulic efficiency; P_N - net photosynthetic rate; RWC_0 - relative water content at zero pressure potential; WUE - water use efficiency; $\Delta\psi$- diurnal amplitude of leaf water potential; ψ_{md} - midday leaf water potential; ψ_{pd} - predawn leaf water potential; ε - bulk modulus of elasticity; $(\pi_{100} - \pi_0)$ - range of positive pressure potential.

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at zero pressure potential (RWC_0), loss of hydraulic conductivity and hydraulic architecture are also major components of drought resistance that vary among tree species (Cochard 1992, Jackson *et al.* 1995, Tognetti *et al.* 1997).

Seasonal dynamics of plant and tissue water relations have been widely used to characterize the drought resistance of Mediterranean forest species (*e.g.* Rhizoupoulou and Mitrakos 1990, Nardini *et al.* 1996). Moreover, leaf water relations may provide an indication of the capacity of tree species to maintain gas exchange and growth during drought periods (White *et al.* 2000). However, to date the results obtained are not conclusive. For instance, gas exchange has been significantly related to tissue water relations (*i.e.* osmotic adjustment) in some studies (Yoon and Richter 1990, Ranney *et al.* 1991), but not in others (Hinckley *et al.* 1979, Kubiske and Abrams 1994). Conversely, relationships between gas exchange and leaf water potential seem more consistent than those of gas exchange vs. tissue-water relations (Bahari *et al.* 1985, Ellsworth and Reich 1992).

In this study we measured gas exchange and plant- and tissue-water relations in response to seasonal drought. The study was carried out over two consecutive years on two co-occurring Mediterranean evergreens (*Quercus ilex* and *Phillyrea latifolia*) with contrasting functional characteristics. *P. latifolia* has been reported to exhibit little stomatal regulation at low water potentials (De Lillis and Mirgone 1994, Peñuelas *et al.* 1998) while the opposite behaviour has been reported in *Q. ilex* (Savé *et al.* 1999, Infante *et al.* 2003, Pesoli *et al.* 2003). In addition, *Q. ilex* exhibits higher vulnerability to xylem embolism and larger declines in sap flow in response to drought stress than *P. latifolia* (Martínez-Vilalta *et al.* 2003). In order to identify the contribution of the several attributes of drought resistance to gas exchange regulation, we carried out a series of Principal Component Analyses (PCA) where we considered several attributes in conjunction: plant- and tissue-water relations, stomatal regulation, hydraulic efficiency, water use efficiency, photosynthetic rate, and range of turgor maintenance.

Materials and methods

Study site: The area of study was located at the Solana dels Torners, a south facing area located in Serra de Prades (Catalonia, Spain) (41°13’N, 0°55’E), at an altitude of 990 m a.s.l.. The soil was shallow with depths varying from 30 to 80 cm (mean 65 ± 3 cm), and classified as Typic Xerocrept (Soil Taxonomy System) with fractured schist.

The study was carried out in a holm oak forest dominated by *Q. ilex* L. with profuse presence of *P. latifolia* L. Tree height was about 5 to 7 m and mean stem diameters at 0.5 m were 6.03 ± 3.69 cm and 3.26 ± 0.95 cm for *Q. ilex* and *P. latifolia*, respectively.

Air temperature, relative air humidity, rainfall, wind speed and radiation were recorded every 30 min throughout the study period using a datalogger (*Campbell CRX10*, *Campbell Scientific Inc.*, Logan, UT, USA) located at the experimental site.

Soil volumetric water content was assessed using a Time Domain Reflectometry device (*Tektronix 1502B*, *Tektronix*, Beaverton, OR, USA) following the procedure described in Zegellin *et al.* (1989). Eight probes were randomly distributed at 15 cm and 4 probes at 40 cm depth within the area of study. Soil moisture was determined every 2 to 3 weeks throughout 1999 and 2000.

Plant water potential: Plant water potential was determined before dawn (ψ_{pd}) and at midday (ψ_{md}) in 8 terminal twigs from different trees for each species using a Scholander pressure chamber (*Soilmoisture 3005*, *Soil Moisture Corporation*, Santa Barbara, CA, USA). Plant water potential was measured on days close to the gas exchange and tissue-water relations sampling dates (see below) between February 1999 and November 2000. Diurnal amplitude of water potential ($\Delta\psi$) was calculated from differences between midday and predawn values and was associated with tree hydraulic architecture (Phillips *et al.* 2001).

Pressure-volume curves: On 27 May, 20 August, and 2 November 1999, and 7 July, 6 September and 11 November 2000 eight to twelve twigs with young, fully expanded leaves from different trees and from each species were collected. Samples were carried to the laboratory in an ice chest and allowed to rehydrate for 3 to 8 h by placing them in a beaker containing distilled water. Subsequently, twigs were placed on the laboratory bench and allowed to dehydrate by free transpiration, and were periodically reweighed and measured for xylem water potential (*Soilmoisture 3005*, *Soil Moisture Corp.*, Santa Barbara, USA). Shoot dry mass was determined after oven drying at 65 °C until constant mass. Pressure-volume data were analyzed according to Tyree and Richter (1981). The following parameters were derived: osmotic water potential at full turgor (π_{100}), osmotic water potential at the turgor loss point (π_0), RWC at turgor loss (RWC_0), and apoplastic water fraction (AWF). Bulk modulus of elasticity (ε) was determined according to Sobrado (1986). Since pressure potential at a given water content is determined by interactions between cell osmotic concentration, cell size and wall elasticity, we considered the slope of the ψ *vs.* RWC desorption isotherm (dψ/dRWC) in the range of positive pressure potential as an integrate measure of these above mentioned characteristics. Similarly, we considered the slope below zero pressure potential (dψ/dRWC$_0$) as an indicator of the resulting effects of changes in AWF and osmotic adjustment.

Gas exchange and hydraulic conductance: Stomatal conductance (g_s), transpiration rate (E) and net photosynthetic rate (P_N) were measured on days close to the pressure-volume measurements (see above) in the morning and midday. These gas exchange measurements were conducted with a portable gas exchange system (*ADC4*, configured with chamber model *PLC4B*, *ADC Inc*., Hoddeson, Hertfordshire, England) on 4 leaves from the upper third of the canopy for each species (4 trees/species). Daily maximum g_s and P_N values are reported for PPFD > 800 μmol m^{-2} s^{-1}.

Soil to leaf specific hydraulic conductivity (herein called hydraulic efficiency, HE) was calculated as g_s divided by $\Delta\psi$ [mol m^{-2} s^{-1} MPa^{-1}] (Savé and Serrano 1986) and was used as a surrogate of hydraulic conductivity (Stratton *et al.* 2000). Water use efficiency (WUE) was calculated as the ratio between P_N and g_s.

Statistical analyses: Statistical analyses were carried out using a *SPSS 10.0* program package (*SPSS Inc.*, Chicago, IL, USA) and *Statistica 5.5* (*Statsoft Inc.*, Tulsa, OK, USA). The species and season effects, as well as their interactions, were assessed by analysis of variance (*ANOVA*). Principal component analyses (PCA) were conducted using the following attributes (variables): dψ/dRWC, dψ/dRWC$_0$, P_N, g_s, RWC$_0$, ψ_{pd}, $\Delta\psi$, HE, (π_{100} - π_0), WUE, and AWF. Although PCA approaches are mainly used as a descriptive tool to detect structure in a data set, PCA also permits to group attributes or subjacent factors in a syndrome and to estimate the range of variance (*i.e.*, the degree of contribution) of these attributes. PCA were based on correlation matrix and were forced to select five principal components, as we were interested in identifying the relative and overall contribution of the variables considered.

Results

Environmental conditions and soil water content: Over the time period of study precipitation in 1999 (610 mm) was near the 100-yr mean for a nearby weather station, while in 2000 precipitation (727 mm) was 18 % above the 100-yr mean (Fig. 1). Due to contrasted rainfall distribution between years, the length of the drought season was larger in 1999 than in 2000. However, while average soil water content in 1999 was lower than in 2000, the highest and lowest values were recorded in 2000.

Plant water relations: Both species showed a close correlation between ψ_{pd} and soil water content at 40 cm depth (the correlation coefficients were $r = 0.79$ ($P < 0.01$) and $r = 0.78$ ($P < 0.01$) for *P. latifolia* and *Q. ilex*, respectively). Differences in ψ_{pd} among dates were significant ($P < 0.001$) (Fig. 2*A*) and ψ_{pd} values in summer 2000 were significantly lower ($P < 0.01$) than in summer 1999. In summer, there were also significant differences ($P < 0.001$) between species, with lower ψ_{pd} and ψ_{md} values in *P. latifolia* than in *Q. ilex*. Despite

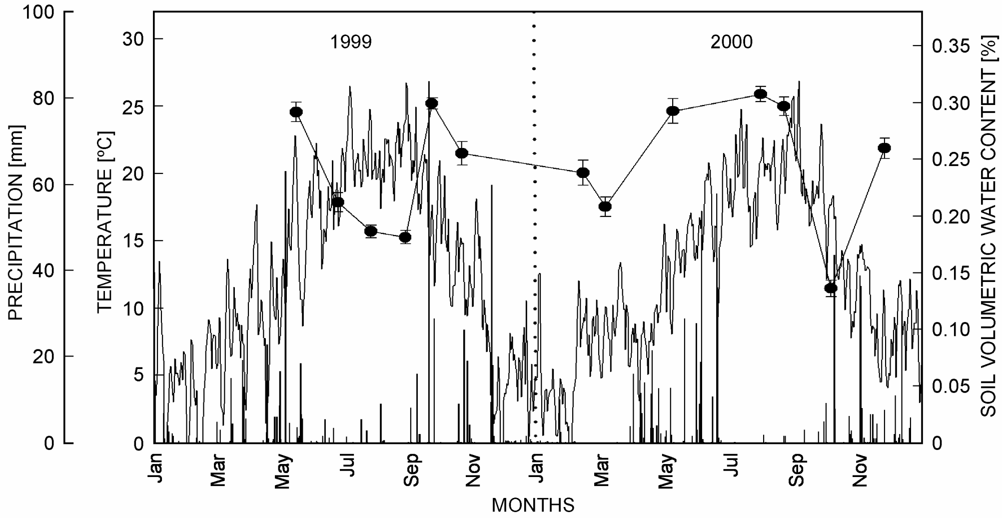

Fig. 1. Air temperature, precipitation (*vertical lines*) and soil volumetric water content (*circles*) at 40 cm depth (mean ± SE, $n = 4$) registered during 1999 and 2000 period of study at La Solana dels Torners (Prades, Catalonia).

significant changes in $\Delta\psi$ throughout the period of study ($P < 0.01$), differences between species in $\Delta\psi$ were not significant. In addition, $\Delta\psi$ reached minimum values during summer (*ca*. 0.5 MPa in *P. latifolia* and virtually zero in *Q. ilex*).

No significant relationship between $\Delta\psi$ and ψ_{pd} was found when all the data were taken into account. However, when only data for $\psi_{pd} > -1.5$ MPa were considered (*i.e.*, in spring and autumn) significant relationships emerged ($P < 0.05$). The regression equations were $\Delta\psi = 2.09 + 1.64 \times \psi_{pd}$ ($r^2 = 0.72$) and $\Delta\psi = 1.95 + 1.66 \times \psi_{pd}$ ($r^2 = 0.82$) for *P. latifolia* and *Q. ilex*, respectively. In summer, the slope of the $\Delta\psi$ *vs.* ψ_{pd} relationship significantly decreased in both species ($P < 0.01$) and the regression equations were $\Delta\psi = 2.23 + 0.27 \times \psi_{pd}$ ($r^2 = 0.70$) and $\Delta\psi = 1.73 + 0.38 \times \psi_{pd}$ ($r^2 = 0.63$) for *P. latifolia* and *Q. ilex*, respectively.

Gas exchange and hydraulic efficiency: The drought-induced decline in plant water potential was accompanied by large reductions in g_s and P_N. Although the decreases in P_N were associated to a reduction in g_s ($r = 0.60$ and $r = 0.64$, $P < 0.05$, for *P. latifolia* and *Q. ilex*, respectively), P_N was more closely linked to plant water potential than to g_s. The correlation coefficient between P_N and ψ_{md} was $r = 0.86$ ($P < 0.001$) in both species. The correlation between P_N and ψ_{pd} was even tighter, with $r = 0.93$ and $r = 0.95$ ($P < 0.001$) for *P. latifolia* and *Q. ilex*, respectively.

There were no significant differences in g_s, P_N, WUE and HE between species. However, both species showed significant reductions in g_s (Fig. 2*B*), P_N, WUE and HE (Fig. 2*C*) in response to drought. In *P. latifolia*, HE significantly decreased ($P < 0.01$) from spring to summer and increased in autumn (Fig. 2*C*). Similarly, in *Q. ilex*, HE decreased from spring to summer and significantly increased in autumn. The correlation between HE and $\Delta\psi$ was not significant, while HE and g_s were closely correlated ($r = 0.88$, $P < 0.001$ for both species).

Tissue-water relations: *Q. ilex* showed significantly ($P < 0.001$) lower values of ε, RWC_0 and AWF, and higher π_{100} than *P. latifolia*. On the other hand, π_0 did not show significant differences between species, while the range of maintenance of positive pressure potential ($\pi_{100} - \pi_0$) was significantly higher in *Q. ilex* than in *P. latifolia* ($P < 0.001$) (Table 1).

Across seasons, *P. latifolia* showed significantly higher ($P < 0.001$) $d\psi/dRWC$ than *Q. ilex* (average values were 13.96 MPa and 7.62 MPa for *P. latifolia* and *Q. ilex*, respectively) (Fig. 3*A*), while $d\psi/dRWC_0$ did not show significant differences between species (average value 18.24 MPa) (Fig 3*B*). Thus, water potential at any given RWC was lower in *P. latifolia* than in *Q. ilex*.

Within each year and species, seasonal changes in $d\psi/dRWC$ were not significant in *Q. ilex*, while $d\psi/dRWC$ increased significantly ($P < 0.001$) in autumn in *P. latifolia*. On the other hand, and for both species, $d\psi/dRWC_0$ significantly increased in summer and decreased in autumn ($P < 0.001$). For the two species altogether, the decreases in $d\psi/dRWC$ were related to decreases in ε ($r = 0.76$, $P < 0.001$) and were accompanied by reductions in RWC_0 ($r = 0.80$, $P < 0.001$) (data not shown). On the other hand, $d\psi/dRWC_0$ was correlated to AWF ($r = 0.73$, $P < 0.001$; and $r = 0.72$, $P < 0.001$, for *Q. ilex* and *P. latifolia*, respectively) and resulted in an enlarged range of positive pressure potential in both species ($r = 0.67$, $P < 0.001$).

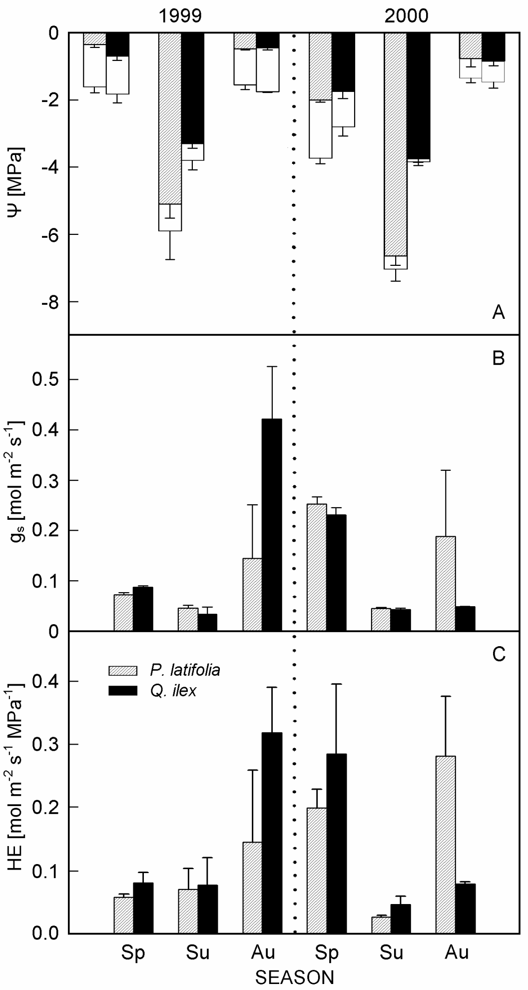

Fig. 2. Seasonal courses of predawn (filled portion of columns) and midday water potential (ψ) (white portion of the columns) in *P. latifolia* and *Q. ilex* (*A*) ($n = 8$). Stomatal conductance (g_s) (*B*) and hydraulic efficiency (HE) (*C*) calculated as $gs/\Delta\psi$. Gas exchange data are mean ± SE ($n = 4$).

Principal component analysis: In order to rank the contribution (*i.e.* variation) of the studied attributes (Table 1) to seasonal drought acclimation, we carried out

Table 1. Descriptive statistics for several ecophysiological variables. Pressure-volume derived parameters [modulus of elasticity (ε), osmotic water potential at full (π_{100}) and zero pressure potential (π_0), relative water content at zero turgor (RWC_0), apoplastic water fraction (AWF), and saturated to dry mass ratio (TM/DM)] were measured at least on 8 twigs at each season and year. Net photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration rate (E) were measured on 4 leaves of each species at each season and year. Hydraulic efficiency (HE) is derived from the ratio between stomatal conductance and plant water potential amplitude for a given day. SD and CV indicate standard deviation and coefficient of variation, respectively.

Parameters	*Phillyrea latifolia*					*Quercus ilex*				
	minumum	maximum	mean	SD	CV [%]	minumum	maximum	mean	SD	CV [%]
ε [MPa]	2.92	16.38	7.27	5.163	71.1	2.92	16.38	7.27	5.163	71.1
π_0 [MPa]	-3.11	-1.70	-2.23	0.430	19.3	-3.11	-1.70	-2.23	0.430	19.3
π_{100} [MPa]	-2.40	-0.61	-1.26	0.524	41.6	-2.40	-0.61	-1.26	0.524	41.6
RWC_0 [%]	77.7	90.8	84.0	3.54	4.2	77.7	90.8	84.0	3.54	4.2
TM/DM	2.00	3.45	2.28	0.449	19.7	2.00	3.45	2.28	0.449	19.7
AWF	0.201	0.754	0.583	0.196	33.6	0.201	0.754	0.583	0.196	33.6
P_N [μmol m^{-2} s^{-1}]	-2.386	5.129	2.694	2.311	85.8	-2.386	5.129	2.694	2.311	85.8
g_s [mol m^{-2} s^{-1}]	0.038	0.320	0.125	0.109	87.4	0.038	0.320	0.125	0.109	87.4
E [mmol m^{-2} s^{-1}]	0.381	3.687	1.82	0.982	53.9	0.381	3.687	1.82	0.982	53.9
HE [mol m^{-2} s^{-1} MPa^{-1}]	0.023	0.376	0.130	0.114	87.9	0.023	0.376	0.130	0.114	87.9

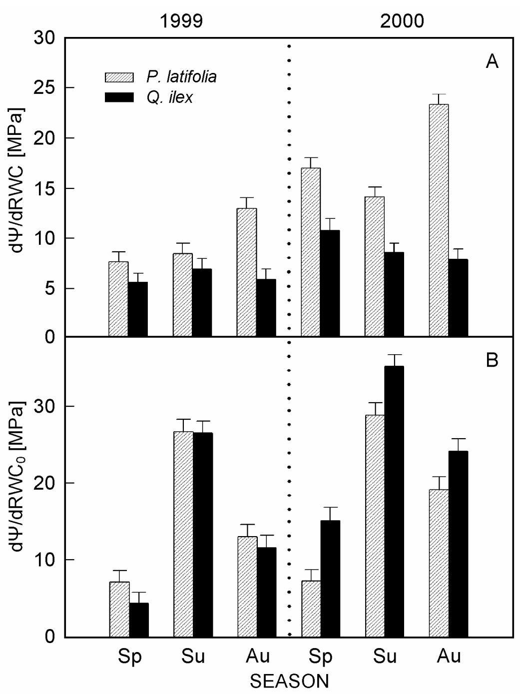

Fig. 3. Seasonal course of the slope of the water potential *vs.* RWC relationship above (*A*, dψ/dRWC) and below (*B*, dψ/dRWC_0) zero pressure potential in *P. latifolia* and *Q. ilex*. Means ± SE (n = 8).

PCA with the whole data set as well as for each species separately.

When the overall data set (two species over two years) was considered, we obtained the following results: the first component (PC1) was related to changes in stomatal and hydraulic conductance (g_s and HE) (35.8 % of the accounted variance) (Table 2), and the second component (PC2) was related to the following variables: dψ/dRWC_0, π_{100} - π_0, and AWF, and accounted for *ca.* 20.3 % of the variance. A third component (PC3) was related to P_N, WUE and water availability (ψ_{pd}) (17.6 % of the accounted variance). The fourth principal component (PC4) was related to dψ/dRWC and RWC_0 (12.9 % of the accounted variance), and the fifth component was related to the diurnal amplitude of water potentials ($\Delta\psi$) (8 % of the accounted variance).

Table 2*A*. Pattern matrix for oblique rotations reporting the factor loadings (partial correlation) for each variable on the components after rotation. Data are for *P. latifolia* and *Q. ilex*. PC denotes Principal Component. The studied variables were the slope of the water potential *vs.* relative water content desorption isotherm above and below zero pressure potential (dψ/dRWC and dψ/dRWC_0, respectively), net photosynthetic rate (P_N), stomatal conductance (g_s), RWC at zero pressure potential (RWC_0), predawn water potential (ψ_{pd}), diurnal amplitude of water potentials ($\Delta\psi$), hydraulic efficiency (HE) calculated as the ratio between g_s and $\Delta\psi$, range of positive ψ_p (π_{100} - π_0), water use efficiency (WUE) calculated as the ratio between P_N and g_s, and apoplastic water fraction (AWF).

Parameters	PC1	PC2	PC3	PC4	PC5
dψ/dRWC	0.203	0.045	0.105	0.918	-0.015
dψ/dRWC_0	-0.281	0.881	-0.087	-0.066	-0.093
P_N	0.542	-0.249	0.754	-0.086	0.211
g_s	0.959	-0.138	0.037	-0.060	0.179
RWC_0	-0.175	-0.048	-0.077	0.966	0.014
ψ_{pd}	0.378	-0.459	0.759	0.064	0.058
$\Delta\psi$	0.003	-0.137	0.184	0.017	0.971
HE	0.962	-0.053	0.025	0.076	-0.191
π_{100} - π_0	0.142	0.796	-0.319	-0.424	-0.014
WUE	-0.256	0.021	0.951	0.049	0.119
AWF	-0.071	0.838	-0.041	0.390	-0.123

Table 2*B*. Eigenvalues, variance (V) and cumulative variance (CV) accounted by the Principal Component Analysis. The initial solution as well as the rotated solution are provided. Data are for both *P. latifolia* and *Q. ilex.* PC denotes Principal Component. Further explanation is provided in Table 2*A*.

	Initial eigenvalues			Rotation sums of squared loadings		
PC	Total	V [%]	CV [%]	Total	V [%]	CV [%]
1	3.943	35.85	35.85	2.522	22.93	22.93
2	2.233	20.30	56.14	2.429	22.09	45.01
3	1.934	17.58	73.72	2.214	20.13	65.14
4	1.418	12.89	86.61	2.135	19.41	84.55
5	0.872	7.93	94.54	1.099	9.99	94.54

When splitting the data set by species, the principal components related to the studied variables in an analogous way in *Q. ilex* (Table 3). PC1 accounted for 39.1 % of the variance, and PC2 accounted for 23.3 % of the variance. The PC3 and PC4 accounted for 17.5 % and 11 % of the variance, respectively, while PC5 accounted for 6 % of the variance. In *P. latifolia* (Table 3), the first principal component related to $d\psi/dRWC_0$, π_{100} - π_0, and AWF and accounted for 43.5 % of the variance, while the second principal component related to P_N, pressure potential and WUE (25.6 % of the accounted variance). In *P. latifolia*, PC3 was related to g_s and HE for (16.8 % of the accounted variance). The subsequent principal components (*i.e.* PC4 and PC5) were related to the same variables reported above for *Q. ilex* and accounted for 8.1 % and 3.4 % of the variance, respectively.

Discussion

Plant and tissue water relations: The seasonal changes in ψ_{pd} and ψ_{md} could be mainly attributed to variation in soil moisture as confirmed by the close relationship with soil water content in both species. Although the ψ_{md} experienced were far below the ψ threshold for increased embolism initiation (Martínez-Vilalta *et al.* 2002), the water deficit did not surpass the lethal level as we did not observe any significant leaf dessication damage. In agreement with previous results (De Lillis and Fontanella 1992, Rhizoupoulou and Mitrakos 1990), *P. latifolia* showed a lower water potential operative range than *Q. ilex* as indicated by the *ca*. 3.0 MPa difference in ψ_{pd} between species during summer drought. However, in contrast with previous studies (Gucci *et al.* 1999, De Lillis and Fontanella 1992), both species showed similar $\Delta\psi$. throughout the seasons. Moreover, although *P. latifolia* has been reported to maintain large $\Delta\psi$even in summer (Gucci *et al.* 1999), under our experimental conditions a strong reduction in $\Delta\psi$. was observed. This might be attributed to the thin soil in our experimental plots, and, therefore, reduced soil moisture availability. Further evidence of reduced soil moisture availability is given by the $\Delta\psi$ increase experienced in response to autumn rains, which might also indicate relatively shallow root systems.

Since the slope of the $\Delta\psi$ vs. ψ_{pd} resembles the flow conductance from soil to leaf expressed on a leaf area basis (Rambal and Debussche 1995), the results obtained indicate that both species experienced large reductions in hydraulic conductance in response to summer drought. This is also supported by the strong reduction in sap flow measured in both species under summer drought (Martínez-Vilalta *et al.* 2003).

Plants can maximize gas exchange either by developing high hydraulic conductance or maintaining large $\Delta\psi$. As both species did not differ in the time course of $\Delta\psi$ and HE, both species might be able to sustain growth in a similar extent. However, given that elongation growth is sensitive to changes in pressure potential (Bradford and Hsiao 1982), maintenance of positive pressure potential over a wider range in *Q. ilex* than in *P. latifolia* might result in higher growth rates in *Q. ilex* or survival as well. In fact, in our study area, a *ca*. 15 % reduction in soil water availability resulted in larger growth decreases in *P. latifolia* than in *Q. ilex* as measured by stem diameter increment (47 and 30 %, respectively when compared to control plots) (Ogaya, personal communication).

No significant differences between species in $d\psi/dRWC_0$ imply a similar degree of drought tolerance (Radin 1983). Nonetheless, higher $d\psi/dRWC$ in *P. latifolia* than in *Q. ilex*, indicated that ψ will drop considerably as leaves loose water in the former species, which might result in an increased water uptake capacity (Nardini *et al.* 1999). On the other hand, low $d\psi/dRWC$ allows *Q. ilex* to maintain pressure potential over a wider range of RWC (Savé *et al.* 1999). Overall, these differences result in a greater drought resistance in *P. latifolia* than in *Q. ilex* when exposed to severe water stress.

In our study, seasonal adjustments in $d\psi/dRWC_0$ relied on structural adaptations (*i.e.* increased AWF) as evidenced by the association of the variables AWF, π_{100}-π_0, and $d\psi/dRWC_0$ in the PC2. Increases in AWF would lead to a passive osmotic adjustment (Tognetti *et al.* 2000) which, in turn, could result in lower π_0, widening the range of positive pressure potential. Since large volumes of apoplastic water are typically associated to small cell volume and thick cell walls (a characteristic feature of sclerophyllous leaves), our results suggest that sclerophylly in Mediterranean vegetation may play an important role in drought tolerance (Salleo *et al.* 1997).

Table 3. Pattern matrix for oblique rotations reporting the factor loadings (partial correlation) for each variable on the components after rotation. Data are for *P. latifolia* (A) and *Q. ilex* (B). PC denotes Principal Component. The studied variables were the slope of the water potential *vs.* relative water content desorption isotherm above and below zero pressure potential ($d\psi/dRWC$ and $d\psi/dRWC_0$, respectively), net photosynthetic rate (P_N), stomatal conductance (g_s), RWC at zero turgor (RWC_0), predawn water potential (ψ_{pd}), diurnal amplitude of water potentials ($\Delta\psi$), hydraulic efficiency (HE) calculated as the ratio between g_s and $\Delta\psi$, range of maintenance of ψ_p (π_{100} - π_0), water use efficiency (WUE) calculated as the ratio between P_N and g_s, and apoplastic water fraction (AWF).

Species	Parameters	PC1	PC2	PC3	PC4	PC5
Phyllirea latifolia	$d\psi/dRWC$	-0.044	0.201	0.277	0.921	-0.045
	$d\psi/dRWC_0$	0.884	-0.329	-0.060	0.039	0.171
	P_N	-0.404	0.697	0.437	0.243	0.299
	g_s	-0.261	0.036	0.937	0.194	0.050
	RWC_0	0.260	0.126	0.190	0.896	-0.187
	ψ_{pd}	-0.475	0.819	0.278	0.151	0.027
	$\Delta\psi$	0.001	0.258	-0.129	-0.191	0.926
	HE	-0.087	0.040	0.877	0.346	-0.314
	π_{100} - π_0	0.771	-0.512	-0.305	-0.050	0.085
	WUE	-0.068	0.880	-0.310	0.189	0.281
	AWF	0.905	0.008	-0.163	0.244	-0.260
Quercus ilex	$d\psi/dRWC$	0.041	0.271	0.200	0.946	0.031
	$d\psi/dRWC_0$	-0.437	0.854	-0.039	0.038	-0.125
	P_N	0.574	-0.168	0.750	-0.039	0.258
	g_s	0.920	-0.034	0.044	-0.150	0.308
	RWC_0	-0.406	-0.203	0.108	0.759	-0.422
	ψ_{pd}	0.485	-0.373	0.749	0.011	0.206
	$\Delta\psi$	0.188	-0.258	0.246	-0.119	0.901
	HE	0.979	-0.005	-0.001	-0.034	-0.017
	π_{100} - π_0	0.293	0.834	-0.427	-0.086	0.074
	WUE	-0.260	0.057	0.956	0.082	0.042
	AWF	0.015	0.881	0.061	0.256	-0.249

More evidence of the role of sclerophylly is provided by the fact that (π_{100} - π_0) was correlated with increased AWF. The correlation coefficients were $r = 0.68$ ($P < 0.02$) and $r = 0.71$ ($P < 0.01$) for *Q. ilex* and *P. latifolia*, respectively.

Partitioning variation of ecophysiological parameters: The first component of the PCA for the overall data set indicated adjustments related to water loss and acquisition (stomatal regulation and hydraulic efficiency) in response to seasonal drought. Thus, stomatal regulation, an avoidance mechanism, contributed to drought resistance in these two co-occurring evergreen species. The simultaneous reduction in g_s with decreasing water potentials is a characteristic response of Mediterranean species to diurnal and seasonal water deficits and is an indicator of conservative water use (Archer and Rambal 1992, Castell *et al.* 1994). Despite similar g_s between species, the decline in g_s with decreasing ψ_{pd} was larger in *Q. ilex* than in *P. latifolia* which indicates a greater conservative water use in the former species (Turner 1986). Moreover, given that HE presented consistent relationships with g_s ($r = 0.86$, $P < 0.001$) regardless of soil water availability and no significant relationship with $\Delta\psi$, it is likely that maximum g_s was determined by hydraulic conductance (Nardini and Salleo 2000) in these species.

The second component highlighted important morphological/anatomical adjustments with positive factor loadings related to AWF, $d\psi/dRWC_0$, and range of maintenance positive pressure potential. Thus, increases in apoplastic water fraction, a mechanism linked to drought tolerance as mentioned above, also contributed to drought resistance by increasing the range of positive pressure potential.

The PC3 evidenced a strong association between WUE, P_N and water availability (ψ_{pd}). This is in agreement with previous findings where the relationships between P_N and plant water potential appear to be more consistent than those of P_N with tissue-water relations derived parameters (Ellsworth and Reich 1992, Kubiske and Abrams 1994). Moreover, *Q. ilex* showed higher PC3 factor scores than *P. latifolia* which might suggest that *Q. ilex* is well-adapted to drought conditions (Epron and Dreyer 1990). It also shows that WUE declines with increasing drought, probably as a result of increased temperatures during drought periods and the subsequent increases in photorespiration, in agreement with recent findings (Reichstein *et al.* 2002).

The PC4 was related to changes in $d\psi/dRWC$ which, in turn, reflected changes in cell wall elasticity and osmotic potential. In our study, increased cell wall elasticity allowed maintenance of positive pressure potential at lower water potentials which confers a high tolerance to water deficits (Grammatikopoulous 1999).

In addition, the principal components PC2 and PC4 differentiated species and seasons, thus, confirming that these species presented different drought resistance strategies. *P. latifolia* showed positive factor scores in PC4 and *Q. ilex* negative factor scores, while PC2 showed positive factor scores in spring and negative factor scores in summer and autumn (data not shown). Moreover, in fully developed leaves (*i.e.* summer and autumn) this second component slightly separated seasons with less negative factor scoring in autumn when compared to summer. Thus, PC2 accounted for adjustments in AWF in response to both drought and ontogenic effects, while elastic adjustment (*i.e.* PC4) allowed to distinguish between species.

When considering each species separately, the PCA permitted to rank the contribution of the above mentioned strategies to drought resistance. Drought resistance in *Q. ilex* relied primarily in avoidance mechanisms based on stomatal regulation (~39 % variation). In contrast, drought resistance in *P. latifolia* largely relied in tolerance mechanisms associated to changes in apoplastic water fraction (~44 % variation). However, and for both species, stomatal regulation and changes in apoplastic water fraction together accounted for *ca.* 60 % of the variance.

Conclusions

In summary, the PCA allowed to identify the different proportions of regulatory and adaptive mechanisms used by these two co-occurring Mediterranean species. Drought avoidance mechanisms based on adjustments in stomatal conductance showed the largest contribution to drought resistance in these two co-occurring evergreens. In addition, assimilation rates were adjusted to water availability indicating that these Mediterranean species have an intrinsic growth strategy that prioritizes water saving over carbon uptake. Tolerance mechanisms through elastic adjustment ($d\psi/dRWC$) allowed to distinguish between species (*P. latifolia* showed a higher and wider range in ε values than *Q. ilex*), while drought tolerance through changes in $d\psi/dRWC_0$ allowed to distinguish among seasons. Furthermore, changes in $d\psi/dRWC_0$ relied on structural adjustments (*i.e.*, the apoplastic water fraction substantially increased during summer) which might be interpreted as an adaptive role of sclerophylly in these Mediterranean evergreens.

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