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Osmotic adjustment in two temperate oak species [*Quercus pyrenaica* Willd and *Quercus petraea* (Matt.) Liebl] of the Iberian Peninsula in response to drought

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Abstract

One-year-old seedlings of *Quercus pyrenaica* and *Quercus petraea* were subjected in a greenhouse to three consecutive water stress cycles. Watering was withdrawn in water stressed seedlings until 5% volumetric soil water content in the two first cycles and up to 3.5% in the third one. At the end of each cycle, predawn water potential (Ψ_{pd}) was measured by a pressure bomb. Furthermore, other water parameters: osmotic potential at full ($\Psi\pi_{100}$) and at turgor loss point ($\Psi\pi_0$) as well as bulk modulus of tissue elasticity at maximum turgor (E_{max}), were derived from the establishment of pressure-volume curves at the end of the second and third water stress cycles.

In the three drought periods there was a significant decrease of Ψ_{pd} in both species as compared to the controls which were maintained at field capacity. This decrease progressed from the first to the following cycles in *Quercus pyrenaica* up to -1.7 ± 0.31 MPa minimum value. In *Quercus petraea* the Ψ_{pd} value reached at the end of the first cycle ($\pm 1.8 \pm 0.52$ MPa), was no significantly different from those reached afterwards.

A large decrease in $\Psi_{\pi 100}$ and $\Psi_{\pi 0}$ together with an increase of E_{max} were recorded in both species after the second water stress cycle, without any new significant variation in the third one. When well-watered and water-stressed seedlings were compared, *Quercus pyrenaica* showed a decrease in $\Psi_{\pi 100}$ of 0.8 MPa with regard to 0.3 MPa in *Quercus petraea*. These results show the occurrence of osmotic adjustment in both species. However, the osmoregulation capacity was higher in *Quercus pyrenaica*, which pointed out to a higher ability to develop mechanisms of water stress tolerance. This could be recognized as one of the physiological traits that explain why *Quercus pyrenaica* is better adapted to xeric conditions than *Quercus petraea*.

Key words: osmotic adjustment, bulk modulus of elasticity, drought, *Quercus petraea, Quercus pyrenaica.*

Resumen

Ajuste osmótico en dos robles de clima templado [*Quercus pyrenaica* Willd y *Quercus petraea* (Matt.) Liebl] de la Península Ibérica en respuesta a la sequía

Brinzales de un año de melojo y roble albar se sometiron a tres ciclos consecutivos de estrés hídrico en un invernadero. El riego se retiró en los brinzales estresados hasta un 5% de contenido hídrico volumétrico del suelo en los dos primeros ciclos, y hasta el 3.5% en el tercero. Al final de cada ciclo, el potencial hídrico al amanecer (Ψ_{pd}) se midió con una cámara de presión. Además, otros parámetros hídricos fueron derivados a partir del establecimiento de las curvas presión volumen al final del segundo y tercer ciclo de estrés hídrico: potencial osmótico a plena turgencia ($\Psi\pi_{100}$) y en el punto de marchitez ($\Psi\pi_0$), así como el módulo de elasticidad a máxima turgencia (E_{max}).

En los tres períodos de sequía hubo una disminución significativa del Ψ_{pd} en ambas especies en comparación con las plantas control mantenidas a capacidad de campo. Esta disminución progresó desde el primer ciclo a los siguientes en el roble melojo hasta un valor mínimo de $-1{,}7 \pm 0{,}31$ MPa. En el roble albar el valor del Ψ_{pd} alcanzado al final del primer período de estrés hídrico ($-1{,}8 \pm 0{,}52$ MPa), no fue significativamente diferente de los alcanzados posteriormente.

Una gran disminución en $\Psi_{\pi 100}$ y $\Psi_{\pi 0}$, junto con un aumento en E_{max}, fueron registrados en ambas especies tras el segundo ciclo de estrés hídrico, sin ninguna variación significativa en el tercero. Al comparar brinzales bien regados

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y estresados, *Quercus pyrenaica* mostró una disminución de 0.8 MPa en $\Psi_{\pi 100}$ respecto a 0.3 MPa en *Quercus petraea*. Estos resultados ponen de manifiesto la presencia de ajuste osmótico en ambas especies. Sin embargo, la capacidad de osmorregulación fue superior en *Quercus pyrenaica*, lo cual señala una mayor capacidad de puesta en marcha de mecanismos de tolerancia al estrés hídrico. Este podría ser reconocido como uno de los rasgos fisiológicos que explican porque *Quercus pyrenaica* está mejor adaptado que *Quercus petraea* a condiciones xerófitas.

Palabras clave: ajuste osmótico, módulo de elasticidad, sequía, *Quercus petraea, Quercus pyrenaica.*

Introduction

The climate of the Iberian Peninsula is characterised by dry summer months with a rainfall shortage. This affects the physiological response of sclerophyllous plants, typical of Mediterranean vegetation (Sala and Tenhunen, 1994) as well as of broad-leaved species growing at mid elevations (Aranda, 1998; Aranda *et al.,* 2000). In this context, European and American oak species, as a group, have showed to be quite drought tolerant (Bahari *et al.,* 1985; Abrams, 1990; Epron *et al.*, 1992; Bréda *et al.*, 1993; Corcuera *et al.*, 2002).

Tolerance to drought of *Quercus petraea* and *Quercus pyrenaica* has been pointed out (Gallego *et al.,* 1994; Epron and Dreyer, 1993). In the Iberian Peninsula *Quercus pyrenaica* is more abundant at xeric sites in the North-west (Jiménez *et al.*, 1998), while sessile oak is limited to the mountainous region of the north with a few populations at the centre (Díaz-Fernández *et al.*, 1995). In central Europe, where it is widespread, *Quercus petraea* is considered as rather drought tolerant (Lévy *et al.*, 1992; Bréda *et al.*, 1993). In a previous study, Aranda *et al.* (1996) found little variations in water relations between mature trees of the two species at the Montejo de la Sierra beech-oak forest in the centre of the Iberian Peninsula. They concluded that seasonal variations in leaf water status parameters derived from P-V curves were caused mainly by ontogenetic factors as it has been postulated by other authors. There was only a slightly higher drought tolerance of melojo oak than sessile oak when data were pooling for the overall growing season (Aranda *et al.,* 1996). Nevertheless, recent papers report the high capacity of osmotic adjustment in *Quercus petraea* submitted to drought (Collet and Guehl, 1997; Thomas and Gausling, 2000) as it has found for other oak species (Abrams, 1990; Gebre *et al.*, 1998). *Quercus petraea* has been profusely studied, so much in natural stands (Epron *et al.*, 1992; Epron and Dreyer, 1993; Breda *et al.*, 1993) as in controlled greenhouse conditions (Dreyer *et al.*, 1990; Collet *et al.*, 1996; Collet and Guehl, 1997). But at present, information about physiological responses to drought of *Quercus pyrenaica* is scarce (Gallego *et al.*, 1994). So, in the present study we analysed the water relations of these two deciduous oak species to test their drought adaptive behaviour. The specific objectives of the study were: i) to determine the degree of osmotic adjustment of both oaks in response to three water stress cycles, ii) to compare tolerance of both species settling the hypothesis of a higher ability by *Quercus pyrenaica* to support low soil moisture conditions.

Material and Methods

In 1997, *Quercus petraea* and *Quercus pyrenaica* acorns from Navarra and Salamanca provenances respectively (North of Spain), provided by ICONA, were sown in plastic bags filled with a sandy-peat mixture (2.7 kg of dry peat/10 l of sand). During the first year seedlings were grown in a greenhouse at the E.T.S.I. Montes (Madrid). Air temperature inside the greenhouse was kept over a minimum night temperature of 15ºC and a daily maximum of 30ºC. Seedlings were watered to field capacity three times week. In 1998, seedlings were transplanted to 3-l pots and fifteen plants per species with a similar size (average 30 cm height) were selected, randomly located on the greenhouse bench and well watered until beginning of drought imposition. Three water stress cycles were imposed by withdraw daily watering (figure 1). Only control seedlings were watered to maximum water holding capacity of pot soil. At the end of each water stress cycle, seedlings were rewatered. Every two days, soil moisture was measured with a TDR (Trase System I, Soil Moisture Equipment Corp., USA) provided with a 20 cm probe. In the same days, predawn water potential (Ψ_{pd}) was assessed with a pressure chamber (PMS 1000, PMS Instruments, Corvallis, Oregon) in four or five seedlings randomly selected for every species and irrigation treatment.

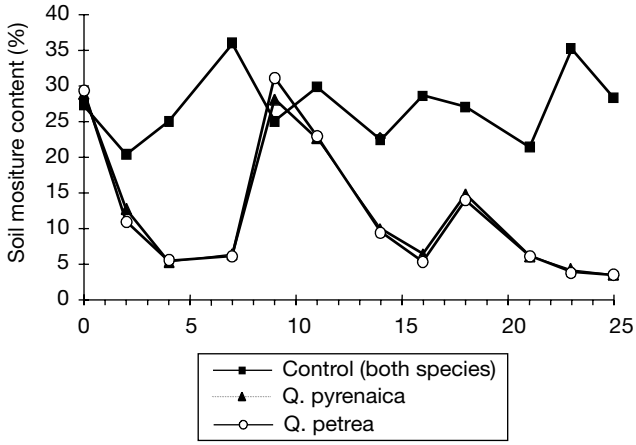

Figure 1. Volumetric soil moisture content measured in pots along three water stress cycles. Values for control plants of both species were grouped because not significant differences.

Because of a failure in the greenhouse cooling system just at the beginning of the experiment, temperature increased to maximum values of 40-42 ºC so VPD was very high on the first five days of the experiment. This brought about a fast depletion of soil water in the pots. After the technical problem was solved, temperature and relative humidity were controlled and VPD was never higher than 3.5 kPa.

At the end of the second and third water stress cycles, one leaf was excised from each one of the five well-watered and stressed seedlings for pressure-volume curves establishment. The previous day, all seedlings were irrigated to full water holding-capacity of soil. This procedure of natural rehydration of the plants during the night previous to the construction of P-V curves avoided the problems of leaf oversaturation and the errors associated with the estimate of leaf water parameters derived from the curves (Dreyer *et al.,* 1990; Kubiske and Abrams 1991; Abrams and Menges 1992). The experimental protocol for curves construction was the same as in Aranda *et al.* (1996).

Analysis of data were performed using ANOVA methods, taking species and watering regime as main factors followed by Duncan´s Multiple Range Test to estimate differences between average values. The BMDP package was used for all the statistical analysis.

Results

In the first two stress cycles, volumetric soil moisture content of drought stressed plants decreased to 5% and it was allowed to diminish up to 3.5 % in the last one. Average leaf predawn water potential in both species felt down below –0.3 MPa for water contents of the substrate lower than 5% (figure 2 a, b). *Quercus petraea* and *Quercus pyrenaica* showed a similar pattern of Ψ_{pd} decrease as soil moisture content diminished up to practical water exhaustion in pots. Both species showed a high decrease of Ψ_{pd} during the water stress cycles and a fast recover after watering. A very acute drop in the first cycle was recorded in *Quercus petraea*, the minimum Ψ_{pd} was reached four days after irrigation withdrawal and no significant additional decrease was recorded in the two subsequent water stress cycles. Minimum values at the end of the three cycles were respectively -1.80 ± 0.52, -1.52 ± 0.49 and -1.83 ± 0.36 MPa (figure 3).

The decrease in water potential was more gradual in *Quercus pyrenaica*. The minimum Ψ_{pd} values measured in the three water stress cycles were respectively -0.60 ± 0.16, -0.79 ± 0.29 and -1.70 ± 0.31 MPa. In control plants Ψ_{pd} was ranging in the interval –0.15 to –0.38 MPa along all the experiment for both species.

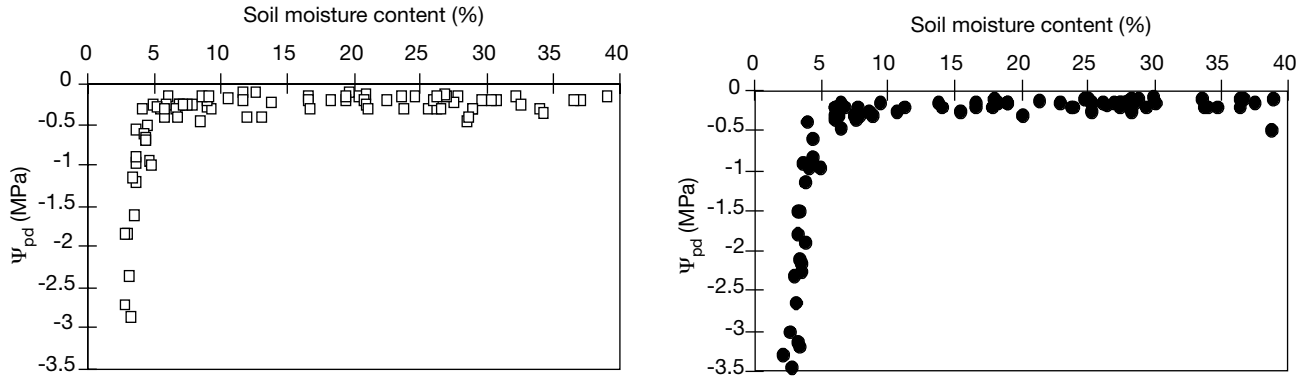

Figure 2. Relation between Ψ_{pd} (MPa) and volumetric soil moisture content (%) in pots for *Quercus pyrenaica* (white squares) and *Quercus petraea* (black circles) seedlings.

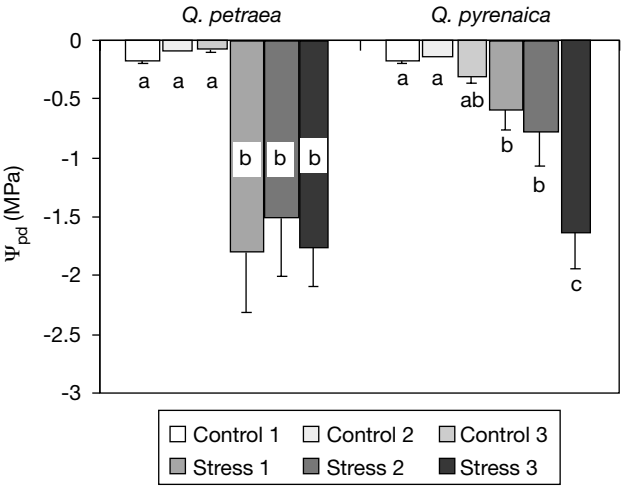

Figure 3. Predawn leaf water potential (Ψ_{pd}-MPa) measured at the end of every water stress cycle. Means with the same letter are not significantly different using Duncan´s multiple rang test ($P > 0.05$).

Osmotic potential at full turgor ($\Psi\pi_{100}$) at the end of the second cycle decreased significantly in both species in response to water stress ($P < 0.05$), the lowest value was reached in *Quercus pyrenaica* seedlings. Afterwards, in the last cycle, significant variations in $\Psi\pi_{100}$ were not observed (figure 4). $\Psi\pi_0$ showed a similar pattern of variation than $\Psi\pi_{100}$ in both species.

The bulk modulus of tissue elasticity at maximum turgor (E_{max}) increased for the two species in the second cycle of water stress (figure 5) and there were not significant variations in the third one. Only a significant decrease in E_{max} for *Quercus pyrenaica* in the well-watered seedlings was noticeable from the second to the third cycle of stress ($P < 0.05$). E_{max} was always higher in *Quercus pyrenaica* than in *Quercus petraea*.

Discussion

Water stress caused osmotic adjustment in seedlings of *Quercus pyrenaica* and *Quercus petraea*. This is in accordance with data from literature where oak species are characterised by a high capacity of osmoregulation in response to water stress (Bahari *et al.*, 1985; Abrams, 1990). However, since ontogenetic changes could give rise to shifts in the osmotic potential (Tyree *et al.*, 1978; Abrams and Kubiske, 1994), it is difficult to attribute osmotic adjustment to drought because control well watered seedlings are usually not available below natural conditions of drought (Aranda *et al.*, 1996; Backes and Leuschner, 2000).

The use of well-watered seedlings allows to link osmotic adjustment to water stress imposition. The adjustment reached 0.3 MPa and 0.8 MPa for *Quercus petraea* and *Quercus pyrenaica* respectively, even though Ψ_{pd} recorded in *Quercus petraea* was lower in the first and second water stress cycles. The lower osmotic potentials for *Quercus pyrenaica* were in accordance with its higher drought tolerance as it has been noted previously (Aranda *et al.*, 1996). Osmotic adjustment has been reported in *Quercus petraea* in

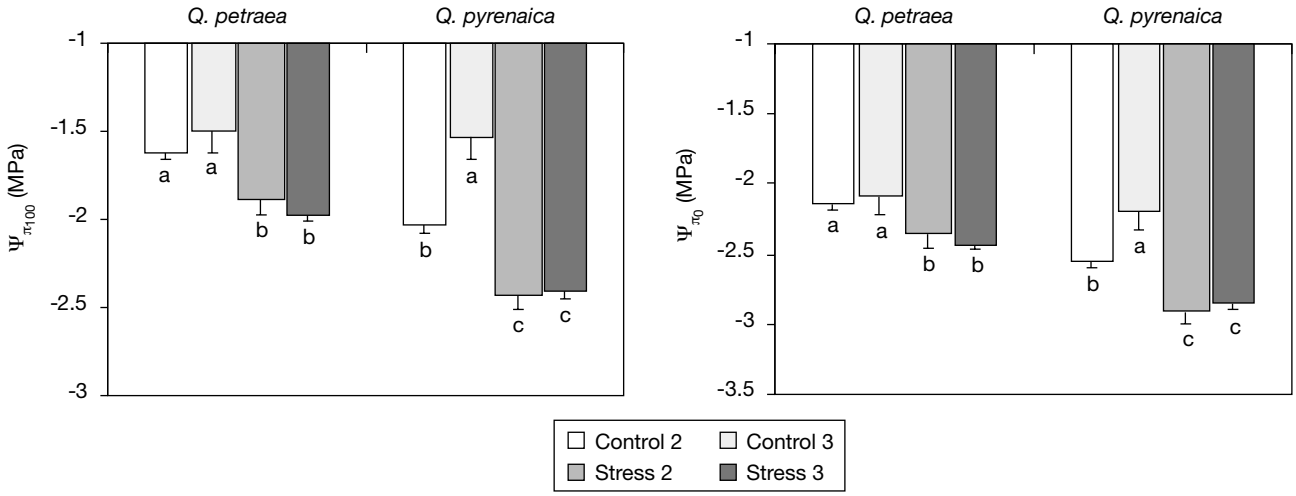

Figure 4. Osmotic potentials at full ($\Psi_{\pi_{100}}$-upper) and zero (Ψ_{π_0}-lower) turgor (MPa) on *Quercus petraea* and *Quercus pyrenaica* seedlings derived from P-V curves establishement after two or three water stress cycles. Each bar represents average values (n = 5) ± s.e. Differences between means on stress and control treatments for a species ($P < 0.05$) are indicated with different letter.

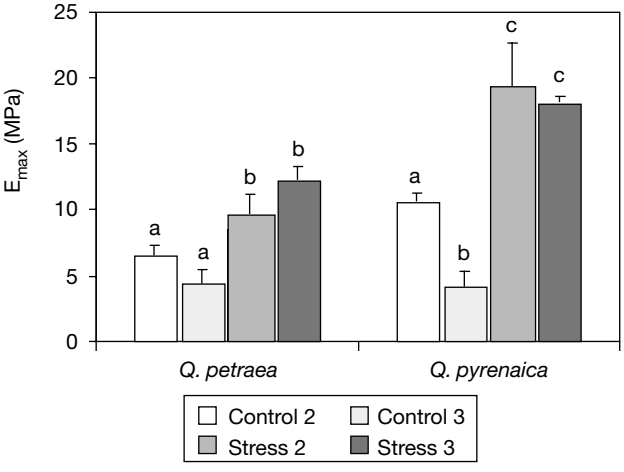

Figure 5. Modulus of elasticity at maximum turgor (E_{max}-MPa) for control and water stressed oak seedlings after the three drought cycles imposition. Average values (± s.e., n = 5) statistically different are indicated with a different letter.

response to moderate (Thomas and Gausling, 2000) or severe drought (Vivin *et al.*, 1996; Collet and Guehl, 1997). Values of $\Psi\pi_{100}$ and $\Psi\pi_0$ for *Quercus petraea* were higher than those found by Collet and Guhel (1997) who recorded a decrease in $\Psi\pi_{100}$ around 0.9 MPa in stressed seedlings, with minimum value of –2.24 MPa. In the present study minimum $\Psi\pi_{100}$ in *Quercus petraea* was –2.02 MPa at the end of the experiment. The rate of soil dehydration has been indicated as an important factor for the decrease of $\Psi\pi_{100}$. The higher rate of water stress imposition in the present study than the one reported by Collet and Guehl (1997), may have been the cause of the lower osmotic adjustment.

There was not a new decrease of $\Psi\pi_{100}$ after the two first water stress periods. However, the minimum Ψ_{pd} was reached in *Quercus pyrenaica* in the third water stress cycle. Therefore seedlings conditioned by moderate and severe drought showed the same degree of osmotic adjustment. This may be linked to the limitation of osmoregulation in situations of drought after water stress relief, as much as that carry-over effects of drought on gas exchange capacity limit net photosynthesis and accumulation of osmotically active solutes (Kwon and Pallardy, 1989, Edwards and Dixon, 1995). Another explanation may be that both species reached the limit to their capacity of osmotic adjustment after two water stress cycles.

Changes in the modulus of elasticity have been reported in response to drought, but there are not conclusive explanations about their role in an ecological context (Kloeppel *et al.*, 1994). In general, more xeric species or plants growing in more sunny environments have the highest E_{max}. There is a general pattern of increase during the growing season linked to phenological events during leaf expansion (Salleo and Lo Gullo, 1990) or leaf maturation (Tyree *et al.*, 1978; Davis and Mooney, 1986; Abrams and Kubiske, 1994). However, as consequence of water stress after leaf maturation, an increase (Augé *et al.*, 1990; Ellsworth and Reich, 1992) a decrease (Joly and Zaerr, 1987; Blake *et al.*, 1990; Saliendra and Meinzer, 1991) or absence of variation (Thomas, 2000) in E_{max} have been observed. Shifts in tissue water parameters without any change related to phenological events or drought occurrence have also been described (Abrams and Kubiske, 1994). In this study there was an increase in E_{max} in both species as result of drought imposition. More inelastic tissues will result in a more rapid decrease of turgor with loss of water, this will generate a steeper soil to leaf water potential gradient and consequently less dehydration of leaves. This may improve water uptake from dry soils avoiding a severe water deficit in leaf tissues. This explanation could be important for species with deep rooting as oaks. Their capacity of prospecting deep soil levels may improve soil water extraction by maintaining a soil to leaf Ψ gradient without leaves become too much dehydrated.

In conclusion, both oak species showed osmotic adjustment capacity in response to drought. *Quercus pyrenaica* showed a typical pattern of submediterranean and mediterranean oaks with osmoregulation as an strategy of drought tolerance. A similar pattern was observed for *Quercus petraea* but the degree of osmotic adjustment was lower. This species lost turgor at higher water potential than *Quercus pyrenaica*. These differences are in consonance with the occurrence of *Quercus pyrenaica* in more xeric habitats characteristic of the mediterranean mid-mountain.

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