Response of *Quercus pyrenaica* (melojo oak) to soil water deficit: a case study in Spain

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Abstract In the present study, carried out from 2004 to 2006, leaf and stem water potential and stem water content were measured in *Quercus pyrenaica* Willd. individuals in an experimental forested catchment located in Central Western Spain under Mediterranean subhumid conditions. These indicators of tree water status were compared with soil moisture contents measured in the same area from 0 to 1 m depth and from 0 to 2.5 m depth during the last year of the study. The objectives were to clarify the seasonal and year-to-year variations in tree water status, to examine applicability of stem water content as useful water stress indicator and to discuss how deep soil water and root uptake contribute to survival during the long dry summer. Seasonal variations in the tree variables measured revealed a typical pattern, with maximum values at the end of spring followed by a progressive decline during the summer drought in response to the decrease in soil water content (almost exhausted at 0–100 cm depth). The relatively high values and the non-significant variation in predawn leaf water potentials (except for 2005, which was exceptionally dry) indicate that no clear water stress situations occurred. This may be explained in terms of a progressive absorption of water from the deeper layers. The results also suggest

that the stem water content is a more sensitive indicator of long-term water limitation than the other variables measured.

Keywords Leaf water potential · Mediterranean oaks · Soil water content · Stem water content · Stem water potential

Introduction

Water-controlled ecosystems are complex and involve structures whose characteristics and dynamic properties depend on many interrelated links between climate, soil, and vegetation. Plants have a special role in this kind of ecosystem, playing an active role in water use that strongly affects the water balance. At the same time the soil water depletion, caused by plant water use, has negative consequences on tree water status (Rodríguez-Iturbe and Porporato 2005).

In Mediterranean areas, plant growth is constrained by the summer drought characteristic of the Mediterranean climate. High temperatures and low rainfall in summer lead to reduced availability of soil water and high evaporative atmospheric demand during a large part of the potential growth period. Under these conditions, the mechanisms by which different species respond to the summer drought largely determine their productivity, distribution and competitive relationships (Mediavilla and Escudero 2003). To assess the possible impacts of drought on forest ecohydrological processes due to climate change or inter-annual dry sequences, it is important to understand the plant water status in response to soil water stress (Kume et al. 2007).

In order to determine the influence of environmental conditions, a sensitive physiological indicator that integrates both soil and climatic conditions is required (Donovan et al.

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2003). The daily leaf water potential (Ψ_l) measured on a single leaf reflects a combination of many factors—local leaf water demand [vapour pressure deficit (D), leaf-intercepted radiation], soil water availability, internal plant hydraulic conductivity and stomatal regulation—and it is used widely (Gallego et al. 1994; Nardini et al. 1999; Stratton et al. 2000). The midday stem water potential ($\Psi_{St,m}$) can also be considered an indicator of water stress levels (Swiecki and Bernhardt 2001). The $\Psi_{St,m}$ is the result of whole-plant transpiration and soil and root/soil hydraulic conductivity (Choné et al. 2001).

Another method for estimating the plant water status is the measurement of the stem water content (θ_{St}), since there is evidence that stems undergo substantial seasonal variations in stem water contents as a consequence of use of this water in transpiration (Waring and Running 1978; Wullschlegel et al. 1996; Kravka et al. 1999). The water storage capacity of tissues may determine the ability of trees to survive long periods of drought (Tyree and Ewers 1991), but measuring water contents in standing trees still remains a challenging task, despite their importance as a water deficit indicator. The few tools available for measuring water contents in living trees lack many of the attributes associated with recent electronic instrumentation (Constantz and Murphy 1990). In addition, stem water content (θ_{St}) is difficult to determine because of tissue decay, high levels of hydration, and the geometrical constraints of the trunk (Raschi et al. 1995). Time domain reflectometry (TDR) can be used to estimate θ_{St} . Constantz and Murphy (1990) developed an equation relating θ_{St} to the apparent dielectric constant for *Pinus radiata* in order to monitor temporal variations in the stem water contents of different species. Using the same methodology in 10 evergreen and deciduous trees, the latter authors found that this method for monitoring the water stored in trees is rapid and that it affords accurate estimates of changes in the water stored to a given sampling depth. Since then, some authors have used TDR methodology with different species and aims: to address the contribution of stem water storage in transpiration (Holbrook and Sinclair 1992; Kobayashi and Tanaka 2001); to monitor the changes in stem water content over time (Wullschlegel et al. 1996; Irvine and Grace 1997); to determine the changes in ice fraction (Sparks et al. 2001); and more recently to test the possibility of using θ_{St} measured with TDR for monitoring irrigation (Nadler et al. 2003) and for water stress detection (Nadler et al. 2006). Nadler et al. (2003) found that water stress was reflected in changes in stem water content measured by TDR, but these changes were too small for routine irrigation control. Thus, to date, the experimental evidence has shown that TDR is capable of measuring differences in stem water content. However, only this methodology was initially tested as a water stress indicator (Nadler et al. 2006). To our knowledge no studies

have attempted to explore the possibility of using TDR to detect long-term water stress under natural conditions.

Many authors have studied the response to water stress of *Quercus* species through the leaf water potential and other variables, such as stomatal conductance, embolism, etc., mainly focusing on *Quercus ilex* (Infante et al. 1997; Rodá et al. 1999; Martínez-Vilalta et al. 2002; Ogaya and Peñuelas 2003; Cubera and Moreno 2007). Despite the importance of studying soil water content (θ_{Soil}) and plant water status together in water-controlled environments, only a few authors have taken this into account and most of them have measured the water content in the top layer of the soil. In particular, in studies addressing the response to water deficit of *Quercus pyrenaica* some investigators did not measure soil moisture (Mediavilla and Escudero 2003, 2004); others only in the topmost layer (Aranda et al. 1996 to a depth of 40 cm), and others from 0–100 cm depth, concluding that the best correlation between soil moisture and the plant water status was observed in the deepest soil layer (Gallego et al. 1994). However, many studies have highlighted the importance of a deep root as an adaptative trait in *Quercus* species. This point has been shown to be important in *Quercus ilex* (David et al. 2004; Cubera and Moreno 2007) but not demonstrated in other *Quercus* species, such as *Q. pyrenaica*, although some authors have recognised its importance in this latter species (Mediavilla and Escudero 2003; Lubczynski and Gurwin 2005). This deciduous species, *melojo* oak (*Quercus pyrenaica*), is distributed across mountainous sub-humid Mediterranean areas of the southwestern region of Europe. It has a short growing season, which may determine its distribution. The densest root system of this species reaches a depth of 50 cm. it has also a deep tap root which allow the trees to reach deep soil water content (Gómez Manzaneque et al. 1998; Silva et al. 2003). The species is well adapted to survive summer water deficits while maintaining photosynthetic production (Gallego et al. 1994; Rico et al. 1996) and therefore it often occupies transitional areas from sub-humid to semi-arid conditions. Despite its distribution and interesting ecology, *Q. pyrenaica* has been poorly studied in comparison with other Mediterranean *Quercus* species (Silla and Escudero 2006).

The aims of the present study were as follows:

To clarify the seasonal and year-to-year variations in tree water status in relation to soil water content in a sub-humid Mediterranean forest. We hypothesised that despite the high annual rainfall, the summer drought imposed by the Mediterranean climate and the high tree density derived from the abandonment of traditional forest practices would lead to a certain degree of tree water stress, especially in drier years.

To discuss how deep soil water and root contribute to survive long dry summer. We hypothesised that trees

must use water from layers deeper than 50 cm (where the densest root system of this species is located) to maintain a good water status.

To examine the applicability of stem water content as a useful water stress indicator. Our hypothesis was that since the stem water is in close hydraulic contact with the soil, the bole will reflect changes in soil water content very closely.

Methods

Study site

The research was conducted at four experimental plots distributed along a small experimental forested catchment (*Rinconada*) located 70 km south of the city of Salamanca, in the western sector of the *Sistema Central* range (Spain). Each plot was representative of the different forest stages. The maturity and size of the trees were different, although the differences were not very large (Table 1). The density and the leaf area index (LAI) were also different. Altitude ranges between 1,140 and 1,450 m a.s.l. with a SW–NE orientation and the soil has developed on a varied geological substrate (sandstones, limestones and quartzites). The climate is sub-humid Mediterranean, with a mean temperature of 10°C. Mean annual rainfall is around 1,000 mm, most of the rainfall is concentrated in the coldest part of the year and the dry periods coincide with the warmest season. November is the wettest month, with 129 mm precipitation, and August is the driest, with 15 mm precipitation (Martínez-Fernández et al. 2003). Annual potential evapotranspiration is 857 mm on average, calculated according to the Penman–Monteith method. There are no substantial differences in evapotranspiration among the studied years. The soils are mainly Leptosols, Cambisols and Regosols. On average, their texture is silty loam. The organic matter content is high (around 10%) between 0 and 15 cm depth, but is always less than 1% below a depth of 25 cm. The mean available soil water is 0.094 cm³ cm⁻³. Almost 70% of the surface of the basin is occupied by *melojo* oak forest (*Quercus pyrenaica* Willd.) and the basin is used for extensive livestock raising and forestry. Today, the forest is homogeneous, with a high tree density (2,300 tree ha⁻¹).

Because the main issue in this study was to study the *melojo* oak response to drought, and since the active growing period in this area for this species lasts from May/June to October, our analysis was restricted to measurements taken from late spring to late summer.

The data reported here correspond to those taken from June to September of 2004–2006, although θ_{Soil} and θ_{St} and atmospheric variables were measured throughout the year. Stem water potential was measured from June to September of 2005 and 2006, and $\theta_{\text{Soil } 200-250}$ from March 2006.

Water potential measurements

Leaf water potential, Ψ_l , was measured every 2–3 weeks during the growing period (June–September) in 2004–2006 ($n = 24$) with a pressure chamber (SKYE SKPM 1400, Skye Instruments Limited, Powys, UK) (Scholander et al. 1965). On each sampling date, leafy shoots from trees located on the experimental plots were measured at pre-dawn ($\Psi_{l,p}$) (just before sunrise) (0330–0500 h, solar time) and at midday ($\Psi_{l,m}$) (1130–1300 h), which is assumed to be when the minimal diurnal value is reached. Two current-year shoot tips were cut from two trees with a pruning pole at a height of about 5 m at each plot ($n = 16$ measurements). In addition to this, leaf water potentials were carried out every 2–3.5 h once a month (June–September) in 2004 from predawn to late afternoon (1800 h) to assess the variation in daily water potential.

Stem water potential, $\Psi_{\text{St},m}$, was estimated through leaf water potential measurements on branches at breast height in 2005 and 2006 during June–September. According to previous studies, the water status of leaves prevented from transpiring can reasonably be taken as equal to the water status of the stem around the base of the covered branches (Begg and Turner 1970). In order to avoid overheating during the bagging period, leaves were taken from the shaded side of the trees. The branches were artificially prevented from transpiring by covering them with clear plastic bags, which were then covered with opaque reflective aluminium ones. The shoot tips selected were those arising directly from the trunk or main branches near the trunk at DBH (Swiecki and Bernhardt 2001). The leaves were covered in this way for 1 or 2 h before measurements were taken to allow the leaf water potential to reach that of the subtending

Table 1 Characteristics of the experimental plots studied

Experimental plot	Tree ha ⁻¹	LAI (2005/2006)	Mean DBH (cm)	Mean height (m)	Altitude (m) orientation
1	1975	3.40/1.68	11.7	7.4	1310/100°E
2	1050	1.65/0.94	13.7	10.77	1286/15°NNE
3	1950	4.16/2.73	12.6	9.46	1244/0°N
4	4275	3.38/1.38	8.1	6.21	1216/14°NNE

LAI leaf area index, DBH diameter at breast height

stem at midday since leaves were artificially prevented from transpiring. Choné et al. (2001) reported that 1 h of “bagging” gave the same results as 2, 3, or 8 h. Two $\Psi_{\text{St,m}}$ determinations were made per tree in two trees from one of the plots previously described ($n = 4$). The measurements were carried out every 2–3 weeks between June and August/September of 2005 and 2006 ($n = 13$).

Stem water content

To study the seasonal variations in the stem water contents of the *melojo* oak, 4 representative trees at each plot were chosen for TDR monitoring (1502C, Tektronix, Beaverton, OR). Two different lengths of TDR probe rods were used, 10 and 12 cm, depending on the tree diameters. The probes were of the two-rod type. In June 2003, two pairs of parallel holes were drilled horizontally into the tree stems at two different heights (120 and 20 cm) before insertion of the TDR probe. The hole diameter was 3 mm and the separation between them was 3 cm. The two probes were inserted perpendicularly to each other and were similarly oriented in all the specimens. Measurements were taken every 2 weeks, starting in 2003, with less frequent readings during the winter. The data used here are those collected between June and September of 2004–2006 ($n = 24$). Measurements were taken at the same time in the morning (0700–0900 h). For the correlations between the soil and stem water content, measurements of 4 days were not used since they were taken on different days ($n = 20$). In addition, the trunk water content was determined at dawn and midday between June and September 2005 to assess maximum daily variation in θ_{St} (Nadler et al. 2003).

Soil moisture and atmospheric measurements

Measurements were taken every 2 weeks throughout the year. The data used here are those collected between June and September of years 2004–2005. At each study plot, soil moisture was measured from 0 to 50 cm depth ($\theta_{\text{Soil } 0-50}$) in the root zone of the trees chosen for trunk TDR monitoring. The densest root system of this species is located in this soil layer (Gómez Manzaneque et al. 1998). In addition to this, θ_{Soil} was measured at 12 measurement stations located on two transects perpendicular to the valley bottom. These transects are representative of the different exposures and stretches of the slopes of the basin. All the stations were in the forest and closed to the experimental plots. Each station has five double-wire probes in a horizontal position at depths of 5, 15, 25, 50 and 100 cm, respectively. The relationship between soil water content measured from 0 to 50 cm depth in the experimental plots and the stations close to them was very high (0.98) and the mean difference was low ($0.035 \text{ cm}^3 \text{ cm}^{-3}$). The coefficient of determination for the comparison of the soil water content at 50–100 cm depth and

the soil water content in the root zone of the trees was also high (0.89). The TDR probes used in the soils were longer (25 cm) than in θ_{St} but the tester used was the same used for trees monitoring. We analysed θ_{Soil} measured at 0–50 cm and at 50–100 cm depth separately. For the latter analysis θ_{Soil} averaged from a depth of 50 to 100 cm at the 12 stations, was used. For the water content measured at 0–50 cm, the mean of θ_{Soil} measured from the 16 soil–root zone trees and the aggregate of θ_{Soil} from 0 to 50 cm from the 12 stations, was used. The data used here correspond to the period between June and September 2004–2006 ($n = 24$). In March 2006, four probes of 10 cm in length were installed vertically at a depth of 200–250 cm near the trees in which θ_{St} was measured in plot 1, and only those measurements made between June and September, 2006 ($n = 8$) were used in the analysis.

At each point where soil moisture was measured unaltered soil monoliths were extracted for estimating the soil water retention curve. We measured nine experimental points of the soil retention curve points on the curve from 0 potential to -1.5 MPa , using the sand box method for potentials from 0 to -0.02 MPa and membrane pressure for potentials from -0.1 to -1.5 MPa . To these experimental points the Van Genuchten model was fitted to those points to obtain the entire curve. The goal of this procedure was to estimate the in situ soil water potential from the soil water retention curve and actual soil moisture data.

An automatic weather station (Campbell Scientific, Logan, UK) was set up at the study plot on a mast approximately at crown height. Relative humidity and temperature (HMP35C probe), rainfall (ARG 100 rainfall tipping bucket), solar radiation (R_s , SP-Lite Silicon pyranometer) and wind speed (RM Young 05103 anemometer) were recorded every 10 s and averaged or totalised every 10 min in a CR1000 datalogger (Campbell Scientific). Potential evaporation is calculated according to Penman–Monteith method.

Data analysis

Ψ_{I} , θ_{Soil} and θ_{St} were compared among plots, months and years with repeated ANOVA one-way measurements. Relationships between Ψ_{I} , θ_{St} and θ_{Soil} were evaluated by correlation analyses and simple and nonlinear regression procedures. The normality of the data was tested with the Kolmogorov–Smirnov test. All statistical analyses were conducted with SPSS (Version 11.0, SPSS, Chicago, IL).

Results

Seasonal water relationships

The dynamics of θ_{Soil} followed the typical trend found in the Mediterranean climate with the lowest values during the

summer months of the 3 years studied (Fig. 1) coinciding with periods with the highest temperatures, potential evapotranspiration and rainfall interception were recorded. A rapid depletion of $\theta_{\text{Soil } 0-100}$ occurred during the spring, followed by a rapid recharge during autumn. The drying process was not as marked for $\theta_{\text{Soil } 200-250}$. Regarding the moment at which the soil began to dry out, important differences were observed among the 3 years, depending on the rainfall in the wet season (October–May, 828 mm in 2003–2004; 508 mm in 2004–2005, and 725 mm in 2005–2006), although generally speaking the moisture started to decrease in April/May. Soil water content stored at 200–250 cm started to decrease at the end of June and the differences in $\theta_{\text{Soil } 0-100}$ were reduced throughout the summer. In each year θ_{Soil} profile reached similar values during the summer, although minimum values throughout the soil profile were reached in 2005 ($0.086 \text{ cm}^3 \text{ cm}^{-3}$ $\theta_{\text{Soil } 0-50}$ and $0.099 \text{ cm}^3 \text{ cm}^{-3}$ for $\theta_{\text{Soil } 50-100}$) due to the lower amount of rainfall that year. The period of soil water decline (i.e. lower soil water content period) showed large inter-annual variations, which ranged between 2 and 3 months in this study period.

Regarding the differences between months, in 2004 and 2006 the analysis revealed the same pattern (Table 2), showing that the water shortage started in July or in August, depending on soil depth. In 2005, the trend was different owing to the scarcity of rainfall episodes in the previous months and the θ_{Soil} reached lower values earlier (Table 2). The value of $\theta_{\text{Soil } 50-100}$ was on an average higher (9.8%) than $\theta_{\text{Soil } 0-50}$ throughout the year (Fig. 1). This difference was greater (ca. 20%) when only the months between June and September were considered. Soil moisture measured at 200–250 cm depth for the June–September period in 2006 was on average 24% higher than in the 50–100 cm layer. The statistical analysis revealed that only $\theta_{\text{Soil } 200-250}$ differed significantly from $\theta_{\text{Soil } 0-50}$. The maximum

Fig. 1 Stem water content and soil moisture measured at different depths, 0–50 cm (2004–2006), 50–100 (2004–2006) and 200–250 cm (2006). Each point represents the mean of the measurements taken that day

Table 2 Average soil water content measured at different depths

Soil depths	June	July	August	September
2004				
0–50 cm	0.225a	0.139b	0.128b	0.114b
50–100 cm	0.233a	0.169a	0.127b	0.119b
2005				
0–50 cm	0.161a	0.112a	0.103a	0.097a
50–100 cm	0.199a	0.134b	0.109b	0.101b
2006				
0–50 cm	0.171a	0.116b	0.100b	0.104b
50–100 cm	0.203a	0.145a	0.113b	0.105b
200–250 cm	0.253a	0.253a	0.199b	0.151b

Values within a row followed by the same letter are not significantly different

difference between $\theta_{\text{Soil } 0-50}$ and $\theta_{\text{Soil } 200-250}$ was recorded during June–July, and the differences were minimal at the end of the summer.

The values of $\Psi_{l,p}$ were relatively high in the three consecutive summers (Fig. 2). Although a decrease was observed from spring to the end of summer, these values always remained above -1 MPa , except for some days in August and September. This was especially the case in 2005, when the values for some trees reached absolute minimum values, ranging from about -1.1 to -1.8 MPa . The highest values were recorded at the beginning of June (-0.3 MPa in 2004 and 2006 and -0.4 MPa in 2005). The lowest mean obtained was -1.5 MPa in 2005, whereas in 2004 and 2006 the lowest average measurement was about -0.8 MPa . During 2005, $\Psi_{l,m}$ decreased from June to September, reaching an absolute minimum of -3.1 MPa . The mean midday minimum values were similar along the three consecutive summer periods (about -2.5 MPa).

Fig. 2 Evolution of leaf water potential measured at predawn and midday and stem water potential. Each point of leaf water potential is a mean of 16 leaves measured that day (16 at predawn and 16 at midday) and stem water potential represents the mean of the four shoot tips with leaves measured that day

Regarding the measurements carried out in 2004 (Fig. 3), the daily Ψ_l fell from a high value in the early morning (the highest in June -0.3 MPa) to a low one in the middle of the day (the lowest value, -2.2 MPa, was seen in July but this was very similar to the values found in August and September) and rose again in the afternoon.

$\Psi_{St,m}$ followed a similar temporal trend over the 2 years studied (Fig. 2). In both years, maximum values were reached during June, when D started to increase and θ_{Soil} was still relatively high. $\Psi_{St,m}$ decreased progressively from -0.4 to -1.3 MPa in 2005, and from -0.6 to -1.2 MPa in 2006. When $\Psi_{St,m}$ was at its minimum, -1.3 MPa, $\Psi_{l,m}$ was -2.4 MPa. These two variables are strongly linked ($R = 0.88$, $P < 0.0001$).

Ψ_l and $\Psi_{St,m}$, especially those measured before dawn, tended to be slightly lower in 2005 than in the other years (Fig. 2). This result is consistent with the lower θ_{Soil} found in 2005. Moreover, only in this year the statistical analysis revealed that there were differences in $\Psi_{l,p}$ and $\Psi_{l,m}$ between June and September. During 2005, $\Psi_{St,m}$ decreased significantly between June and July/August (September not measured).

The average θ_{St} paralleled θ_{Soil} (Table 3). Seasonal differences in θ_{St} were observed during the 3 years studied (Fig. 1). In all years, maximum values were reached in May ($0.654 \text{ cm}^3 \text{ cm}^{-3}$ in 2004; $0.648 \text{ cm}^3 \text{ cm}^{-3}$ in 2005; $0.626 \text{ cm}^3 \text{ cm}^{-3}$ in 2006) and were associated with new leaf growth (leaf sprouting takes place at the end of May and leaf growth occurs mainly in June) and absence of a soil moisture deficit. Thereafter, the stem water content gradually decreased throughout the summer. The minimum value ($0.520 \text{ cm}^3 \text{ cm}^{-3}$ in 2005) was reached at the end of September, when θ_{Soil} values were lowest. Similar trends were observed for the other 2 years. Once the soil had been rewatered due to the autumn rainfall, the stems were partially recharged. The variation in mean stem water values ranged between 17% for 2005 and 10% for 2006. The year 2004 followed an intermediate trend (15%). There were significant

Fig. 3 Daily evolution of leaf water potential in 4 days in 16 leaflets from four trees (2004) distributed along the Experimental Catchment of Rinconada (Salamanca, Spain)

Table 3 Correlation values and significance between soil water content and the all the plant water status variables

	Soil water content		
	0–50 cm	50–100 cm	200–250 cm
Predawn leaf water potential	0.56 ($P < 0.01$)	0.69 ($P < 0.001$)	0.90 ($P < 0.05$)
Midday leaf water potential	0.72 ($P < 0.0001$)	0.84 ($P < 0.0001$)	0.77 ($P < 0.05$)
Stem water potential	0.70 ($P < 0.001$)	0.77 ($P < 0.001$)	0.88 ($P < 0.05$)
Stem water content	0.86 ($P < 0.0001$)	0.93 ($P < 0.0001$)	0.97 ($P < 0.01$)

differences in the decrease in θ_{St} during the summer of the 3 years. In 2004, there were significant differences in June and September; in 2005 1 month before, and in 2006 between June and the rest of the months. The correlations between θ_{St} and Ψ_l measured at predawn and midday were very high (0.82 and 0.94, respectively, $P < 0.0001$, $n = 8$).

The statistical analyses of θ_{St} and Ψ_l revealed no significant differences between the experimental plots. Thus, it seems that differences in density and tree size among the plots are not sufficiently pronounced to produce variations. However, a general trend was observed in the measurements taken. The $\Psi_{l,p}$ and $\Psi_{l,m}$ values were lower in plot 4 than in the other plots. The trees in plot 1 had lower θ_{St} values while in plot 3 θ_{St} was almost always higher than in the other plots, which is in accordance with the fact that Ψ_l values in plot 3 were usually higher than in the other plots, especially at midday.

Tree water status in relation to soil water content

The highest soil water contents of the period under consideration were observed in June, after the winter and spring precipitation. When the water deficit became more severe, all the indicators used to determine tree water status exhibited differences.

During summer, the continuous increase in the soil moisture deficit resulted in lower $\Psi_{l,p}$ and $\Psi_{l,m}$ values. The correlation analyses (Table 3) revealed that surprisingly relationship between θ_{Soil} and Ψ_l for the data from the 3 years was unexpectedly low and even lower for $\Psi_{l,p}$ than for $\Psi_{l,m}$ when θ_{Soil} 0–50 was considered. The same was observed for the correlation between $\Psi_{l,p}$ and $\Psi_{l,m}$, and θ_{Soil} 50–100, but the correlation between the two variables was higher (Table 3). However, this pattern changed when θ_{Soil} 200–250 was compared with the measurements of water potential. At this depth, the best indicator of θ_{Soil} was $\Psi_{l,p}$, and the worst was $\Psi_{l,m}$. These latter results should be considered with caution, since θ_{Soil} was measured at this depth only during 2006. Soil moisture values were transformed to

soil water potentials based on water retention curves in order to repeat all the correlation analyses with the soil water potential. The correlations thus calculated (Table 4) followed a very similar trend and were higher than those estimated with θ_{Soil} , although the values were not very different. Only the correlations calculated with the soil water potential at 0–50 cm were not significant in the case of $\Psi_{\text{l,p}}$ and lower for $\Psi_{\text{l,m}}$ and $\Psi_{\text{St,m}}$.

A correlation analysis was also carried out between the θ_{St} and the θ_{Soil} , (Table 3) measured at a depth of 0–50 cm and 50–100 cm (Fig. 4) for the period when the Ψ_{l} was measured (June–September). The correlation was higher than when comparing θ_{Soil} with any of the water potentials measured.

θ_{St} was significantly correlated with the seasonal dynamics of $\Psi_{\text{l,p}}$, $\Psi_{\text{l,m}}$ and $\Psi_{\text{St,m}}$, showing that these variables are strongly linked ($R = 0.77$, $P < 0.0001$ for $\Psi_{\text{l,m}}$ and $\Psi_{\text{St,m}}$ and slightly higher for $\Psi_{\text{l,p}}$ 0.80, $P < 0.0001$).

Long-term rainfall analysis

An analysis was carried out with the data from the automatic weather station and the climate data measured at a nearby long-term weather station at 998 m. The relationship between the data from the two stations was very high ($R^2 = 0.97$). Thus, the analysis was carried out with the data

Table 4 Correlation values and significance between soil water potential and plant water potential

	Soil water potential		
	0–50 cm	50–100 cm	200–250 cm
Predawn leaf water potential	NS	0.85 ($P < 0.001$)	0.96 ($P < 0.01$)
Midday leaf water potential	0.59 ($P < 0.01$)	0.91 ($P < 0.0001$)	0.87 ($P < 0.05$)
Stem water potential	0.61 ($P < 0.001$)	0.77 ($P < 0.01$)	0.91 ($P < 0.01$)

NS no significant

Fig. 4 Relationships between stem water content and soil water content measured at different depths (0–50 cm, 50–100 cm and 200–250 cm). Soil water content at a depth of 200–250 cm was only measured in 2006

from the latter station (1951–2006). The objective was to assess whether 2005 was an exceptionally dry year, and whether years with similar amounts of rainfall are frequent in this area. The analysis revealed that there were only 4 years drier than 2005. A percentile analysis (Fig. 5) showed that from April to September 2005 only August was at percentile 50 the rest at percentile 20. This shows that the growing season in 2005 was very dry. A decadal analysis of the rainfall over the last 55 years pointed to a progressive decreasing trend (a rainfall decrease of 17.7%). This trend was more marked when the June–September period was analysed (decrease of 26.1%). If this trend continues in forthcoming years, it is likely that the water stress detected in 2005 will be more pronounced.

Discussion

Available soil water was almost exhausted in summer at a depth of 0–100 cm, a situation of water deficit arose. Therefore, the amount of water stored in the soil during the wet season cannot prevent the existence of a pronounced soil water deficit during the active period of the trees. However, unvarying levels of stored soil water do not mean that the trees do not consume water, because of the possible existence of capillary rise, providing the trees with access to water (David et al. 2004), and hydraulic lift, all of which could also play an important role.

Seasonal variations in $\Psi_{\text{l,p}}$ followed a typical trend, with maximum values being observed at the end of spring, followed by a progressive decline during the summer drought and a recovery in response to autumn rainfall (Fig. 2). The decrease in Ψ_{l} in the summer resulted from decreases in θ_{Soil} (Fig. 1). It was found that during the summer, the Ψ_{l} and $\Psi_{\text{St,m}}$, and θ_{St} values decreased in all monitored trees (Figs. 1, 2), consistent with an increase in the soil moisture deficit (Table 2). However, $\Psi_{\text{l,p}}$ values were not very low, compared to other values found for this species (−3.2 MPa,

Fig. 5 Percentile analysis of rainfall of the Rinconada forest area since 1951 to 2006 and comparison with 2005 data

Mediavilla and Escudero 2003). This also suggests that these oaks are able to explore the soil adequately in their search for water resources, extending tap roots deeper in the soil. Similar values of absolute maximum measurements at predawn (-0.2 and -0.3 MPa) and minimum values at midday (-3.0 and -3.3 MPa) were reported by Gallego et al. (1994) also in Salamanca (Spain). Those authors stated that a certain degree of stress may occur beyond -2 MPa, since stomatal conductance decreased rapidly as the water potential declined, according to Rico et al. (1996). Those authors reported a range of leaf water potential values above which stomatal conductance showed little response but remained at the maximum level. However, different types of behaviour were also detected at different sites characterised by different climatic conditions, highlighting the notion that these species show an adaptation to drier conditions and employ a more conservative strategy (Rico et al. 1996).

In 2005, the variation in $\Psi_{l,p}$ and $\Psi_{l,m}$ suggested that the trees had undergone more acute water stress (Fig. 2). Moreover, low $\Psi_{l,p}$ values were only measured in this year; some close to the threshold value of -2 MPa (Rico et al. 1996). This situation would have been caused by an incomplete recharge of the soil, owing to the very low rainfall throughout the year. Unfortunately, we do not have data from 200–250 cm depth.

The relatively small variation in $\Psi_{l,p}$ during 2004 and 2006 is consistent with the access of roots to deeper stored water, since deep-rooted species typically have higher predawn shoot water potentials than shallow-rooted species, because soil water availability increases with soil depth access (Abrams 1990). All the correlations became higher when deeper soil layers were considered (Table 3). This means that although the highest density of roots is located in the first 50 cm, the roots have to explore deeper layers to access water in summer, thereby avoiding competition with grasses and bushes. Thus, the tree water status is highly dependant on the water stored deeper. The relationship between the θ_{Soil} and θ_{St} changed during the study period, indicating that θ_{St} extraction increased as the summer drought progressed (Fig. 4). The non-significant variation in $\Psi_{l,p}$ (except for 2005) indicates that no clear water stress situations had occurred, which is not concordant with the decreasing rates of $\theta_{\text{Soil } 0-100}$, which at the end of the growing season were close to or equal to the wilting point (Table 2). Accordingly, water would have to be pumped from greater depths by capillary rise, or absorbed by a deep tap root. Although a denser root system of this species is found in the first 50 cm, a deep pivoting system enabling transpiration rates to be maintained throughout the summer (Moreno et al. 1996) seems to be very important in months with a soil water deficit (Mediavilla and Escudero 2003). During these months, the deepest layers are wetter than the

surface layers (Table 2), which are depleted by evaporation and the transpiration of herbaceous and shrub species. Thus, faced with a progressive decrease in θ_{Soil} , trees would tend to use up the water reserve and progressively absorb water from deeper layers (Moreno et al. 1996). In fact, Mediavilla and Escudero (2003) found that this species was better able to supply water to its foliage as compared with evergreen oaks, which would be attained by the development of deep roots to tap soil water reserves.

The trees must take up water from layers deeper than 50 cm, because according to our results $\theta_{\text{Soil } 0-50}$ was below the wilting point ($0.097 \text{ cm}^3 \text{ cm}^{-3}$ for these soils Martínez-Fernández et al. 2003) for 2 weeks (2006) or more than 2 months (2005) (Fig. 1). Soil water content measured from 50 to 100 cm never reached the wilting point ($0.096 \text{ cm}^3 \text{ cm}^{-3}$), although a value close to this was attained during the 3 years of the study. Soil moisture stored at 200–250 cm started to decrease about 1 month later than $\theta_{\text{Soil } 0-100}$, and this depletion was more progressive and the wilting point was not reached (Table 2). These observations show that the trees started to use the water at this depth later, once $\theta_{\text{Soil } 0-100}$ has been almost depleted. Therefore, trees do not undergo very acute water stress thanks to soil water storage at 200–250 cm or deeper.

The θ_{St} values revealed differences in the temporal water status throughout the 3 years, whereas the other indicators did not. This suggests that θ_{St} is a more sensitive indicator of water limitation. This is important because θ_{Soil} undergoes a significant decrease during the summer. When the soil water deficit became more severe (2005), all the plant water status indicators exhibited significant differences. The correlation analysis revealed that θ_{St} was the variable most strongly related to the soil water status at all depths. As pointed out by Tyree and Ewers (1991), most of the mass of a tree is contained in the bole, which is in close hydraulic contact with the soil, so the bole will reflect changes in soil water potential more closely than Ψ_l . However, Ψ_l and $\Psi_{\text{St},m}$ exhibited a larger amplitude than θ_{St} , quantitatively more similar to θ_{Soil} . The mean variation for θ_{Soil} was 51%, which is more similar to $\Psi_{l,p}$ (48%), followed by $\Psi_{\text{St},m}$ (55%), and finally $\Psi_{l,m}$ (45%), compared to only 14% of θ_{St} . Several authors suggested that water stored in stems might be used in transpiration to overcome lack of water during summer (Waring and Running 1978; Wullschlegel et al. 1996; Kravka et al. 1999). In extreme conditions, up to 30–50% of the transpired water can be derived from stem capacitance, as found in arborescent palm (Holbrook and Sinclair 1992).

Although, according to current measurements, this species seems to be relatively well watered through a deep root, only one exceptionally dry year resulted in severe water stress. Drier conditions will be more frequent in the near future as predicted by climate changes scenarios and

corroborated through long-term rainfall analysis of the study area. Thus, more severe drought due to climate change or inter-annual variations may have negative implications for trees in terms of water stress.

Conclusions

In this study carried out between 2004 and 2006, leaf and stem water potential and stem water content were measured in the species *Quercus pyrenaica*. Soil water content was also determined from 0 to 250 cm depth. In response to progressive decreases in soil water, no very remarkable changes were found in $\Psi_{l,p}$ or in the other variables, indicating a tendency for the trees to use water reserves from progressively deeper soil layers. This is consistent with the data obtained in the analysis of θ_{soil} measured at different depths, and the correlations calculated between θ_{soil} measured at different depths and the plant water status indicators.

Stem water content was demonstrated to be a comprehensive indicator of early water deficit in plants and a soil water deficit, while Ψ_l was not such a good indicator. In general, the two variables measured in the trunk appeared to be better indicators of soil water deficit better.

The analysis of the rainfall revealed a decreasing trend in this variable in the study area. This is consistent with the climate change predictions. Recurrent dry years, such as 2005, may represent a risk for this type forest because, as demonstrated here, the trees were under a certain degree of water stress. Climate change and the abandonment of traditional practices in rural areas such as this, leading to a denser forest, could further weaken this kind of ecosystem.

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