

Plant water relations and edaphoclimatic conditions affecting acorn production in a holm oak (*Quercus ilex* L. ssp. *ballota*) open woodland

F. S. Carevic · M. Fernández · R. Alejano ·
J. Vázquez-Piqué · R. Tapias · E. Corral ·
J. Domingo

Received: 18 February 2009 / Accepted: 4 July 2009 / Published online: 18 July 2009
© Springer Science+Business Media B.V. 2009

Abstract Acorn production patterns and the annual evolution of water relations parameters of *Quercus ilex* ssp. *ballota* (Desf.) Samp. in a dehesa (an open woodland forest) subject to various soil treatments (ploughing, ploughing + sowing, control) were studied in southwest Spain from June 2006 to December 2008. The effects of soil water holding capacity and effective soil depth on soil and plant water status and acorn production were also investigated. Water parameters showed significant differences between the ploughing treatment and the control, and there were also significant temporal differences. Xylem water potential ranged from -3.4 ± 0.1 to -0.7 ± 0.2 MPa, and cuticular transpiration was 62.4–192.9 mmol H₂O kg⁻¹ s⁻¹. Acorn production did not differ significantly according to soil treatment, and showed large intra-specific variability. Individual values ranged from 0 to 1,220 g m⁻² (fresh weight). Positive relationships were found between xylem water potential, cuticular transpiration and relative water content measured at midsummer, and acorn production during the three studied years. These results suggest that climatic conditions and soil water availability have a strong influence on plant water

status, and therefore on acorn development during summer. The results also reflect the ability of this species to adapt to the Mediterranean climate by preserving water during dry periods, which to a large extent can be attributed to stomatal closure at high relative water content levels, and low cuticular transpiration during these periods.

Keywords Pressure-volume curves · Cuticular transpiration · Water potential · Acorn production · Soil treatments · Land use

Introduction

The dehesa (an open woodland forest) is the main agroforestry system in Mediterranean areas of southwest Spain. It is an artificial system used for livestock and other agro/silvi/pastoral purposes (Joffre et al. 1988; Olea and San Miguel-Ayanz 2006). Monospecific holm oak forests cover an area of about 820,000 ha in Spain. They are also found in mixed stands associated with other species such as *Q. faginea* Lam. or *Q. suber* L., covering a total area of about 2.5 Mha (Pulido et al. 2005; García-Mozo et al. 2007). Similar systems occur in Crete, Sardinia and northern Greece, but have almost entirely disappeared on the Italian mainland (Eichhorn et al. 2006). Moreover, dehesas dominated by holm oak are threatened by poor regeneration, inappropriate livestock management,

F. S. Carevic · M. Fernández (✉) · R. Alejano ·
J. Vázquez-Piqué · R. Tapias · E. Corral · J. Domingo
Departamento de Ciencias Agroforestales, Escuela
Politécnica Superior, University of Huelva, Campus de La
Rábida, 21819 Palos de la Frontera, Huelva, Spain
e-mail: manuel.fernandez@dcaf.uhu.es; nonoe@uhu.es

and the sometimes dramatic effect of the “oak decline” disease (Diaz et al. 1997).

In addition to their use for livestock, dehesas also have an important role as an ecological niche for various fauna species. Therefore, they are of great economic, ecological, social and aesthetic value to the Iberian Peninsula (Diaz et al. 1997; Moreno et al. 2007).

Acorns are a major feed resource for livestock and wild mammals, birds and insects in oak forests. Acorn development is very slow during spring and summer, and the fruits are still very small (less than 0.5 cm in diameter) in early September, when a fast growth period begins (Siscart et al. 1999). Individual trees show highly variable intra- and inter-annual production, depending on several environmental and endogenous factors, at least from late summer and autumn of the previous year until autumn in the dissemination year (Rodríguez-Estévez et al. 2007). Many studies related to acorn production by *Q. ilex* have focused on crop quantification (Martín Vicente et al. 1998; Álvarez et al. 2002; Carbonero et al. 2002; Gea-Izquierdo et al. 2006), but little research has focused on the influence of management practices (soil treatments and pruning) on tree physiological status and acorn production (Siscart et al. 1999; Moreno et al. 2007; Alejano et al. 2008; Moreno and Cubera 2008). Soil management techniques modify soil hydraulic functions and water infiltration into the soil (Pulido et al. 2001), so it is very important to determine the effect of these practices on water status of holm oaks. Special emphasis should be placed on the seasonal evolution of physiological water relations and its response to water stress, since there is no common oak strategy in response to dry periods (Abrams 1990).

The most important factor limiting the establishment, subsequent growth of tree stands, and plant distribution in Mediterranean ecosystems is the water deficit during summer (Di Castri 1981; Ogaya and Peñuelas 2006). Drought induces vegetation water stress, which is the result of complex interactions of the plant with soil and the climate (Baquedano and Castillo 2006). Strategies evolved in species to preserve water during periods of severe water deficit include changes in gas exchange, leaf area, rooting depth, stomatal opening and osmotic adjustment (Tognetti et al. 1998; Savé et al. 1999; Ogaya and Peñuelas 2003). For this reason the water status of

plants (e.g. xylem water potential) needs to be considered when assessing plant phytosanitary status and vigor (Sala and Tenhunen 1994; Knops and Koenig 2000), as does its influence on fruit production (Larcher 2003; Alejano et al. 2008). Water parameter studies carried out with *Q. ilex* under field conditions have particularly focused on the dry season or on *Q. ilex* ssp. *ilex*, the other form of the species (Rodà et al. 1999). *Q. ilex* ssp. *ballota* and *Q. ilex* ssp. *ilex* have morphologically different leaves but can interbreed (Terradas 1999); however, they have distinct main distribution ranges, with *Q. ilex* ssp. *ilex* occurring in wetter coastal or oceanic-influenced areas in the north and east of the Iberian Peninsula, and *Q. ilex* ssp. *ballota* occurring mainly in more continental and inland areas of southern, central and western Spain (Ruiz de la Torre 2006).

Although many factors affect acorn production, plant water status during summer is likely to be a major factor affecting seed growth and acorn production. Therefore, the main objective of this study was to assess how, and to what degree, is annual holm oak acorn production influenced by plant water status, edaphoclimatic conditions and land use. The detailed steps of this study were: (1) to characterize the evolution of plant water relations parameters in a dehesa of *Q. ilex* ssp. *ballota*; (2) to analyze the possible relationship between the water relations parameters and acorn production; and (3) to identify the edaphoclimatic parameters influencing plant water status and acorn production, as a basic tool for managing such agroforestry systems.

Materials and methods

Description of the experimental plots

The study was carried out at the Huerto Ramírez farm (Villanueva de los Castillejos, Huelva province, Spain UTM, zone 29: X, 644288: Y, 4161376). The total plot area involved was approximately 2.9 ha, and the average density of trees was 69 trees ha⁻¹. Average size characteristics (mean ± SD) of trees involved are 6.15 ± 1.68 m (height); 32.56 ± 10.79 cm (diameter) and 3.86 ± 1.36 m (crown radius). The main use of this farm is for Iberian pig and sheep breeding, so the plot was surrounded by an electric fence to prevent animal entry. The climate of

the area is Mediterranean, with rainfall (annual precipitation = 633.4 mm) occurring mainly in autumn and winter, and the average annual temperature is 18.6°C. The vegetation of the area is dominated by holm oaks (*Q. ilex* ssp. *ballota* with an understorey mainly composed of *Cistus ladanifer* L. The latter has changed substantially as a result of frequent ploughing of the soil. A weather station that registered rainfall and temperature data on a daily basis was established in the area.

Two soil treatments (ploughing, *P*; ploughing + sowing, *PS*) were carried out on the plot in winter 2005, leaving an untreated area as control (*C*). Each treatment (*P*, *PS*, *C*) was applied in three replicate subplots (0.325 ha per subplot), covering a total area per treatment of about 1.0 ha (3 treatments × 3 subplots × 0.325 ha/subplot ≈ 2.9 ha). For *PS* treatment, 300 kg of superphosphate was spread as fertilizer and *Lupinus luteus* L. was sown using an oscillating tube. In addition, 11 ditches (3 ditches in *C*; 4 in *P*, and 4 in *PS*) were dug in the whole plot to facilitate description of the soil profiles, and to determine the soil water holding capacity (WHC_u) and effective soil depth (ESD), according to Domingo et al. (2006). Soil profiles in the plot revealed a parent rock of slates and quartzites, a stoniness of $31.9 \pm 2.9\%$, while the fine earth was comprised of $24.5 \pm 1.5\%$ sand, $42.6 \pm 1.1\%$ silt and $32.9 \pm 1.8\%$ clay. The pH was 4.3 ± 0.1 , the ESD ranged from 42 to 100 cm (75.6 ± 5.4 cm), and WHC_u ranged from 29.0 to 128.4 mm (90.9 ± 7.8 mm).

Assessment of acorn production

Acorn production (AP) was assessed in 18 trees (six per treatment/control). The trees used to estimate acorn production were selected by stratified sampling considering stem diameter and treatment. To harvest acorns, four containers (0.45 m diameter at the top) were placed under the crown of each tree to the north, south, east and west of the trunk, respectively, at a distance of 0.75 of the crown radius, and fixed to the ground with long nails. The bottom of each container had small holes to facilitate the evacuation of rainwater. Acorns were collected from each container on a fortnightly basis during three dissemination periods (2006/2007 to 2008/2009) from October to January. The acorns were transferred to the laboratory in polyethylene bags for counting and

determination of fresh weight. The moisture content was also determined by drying in an oven (65°C) until a constant weight was reached. AP was calculated as grams fresh weight of acorns per m² of the orthogonal projection of the crown on the ground (g FM m⁻²).

Water relations parameters

Water parameters for nine trees (three per soil treatment/control) chosen at random within the 18 trees selected for AP were measured every 6–7 weeks during three consecutive years (from June 2006 to December 2008). Two humidity digital probes (ECHO[®]) were installed at a distance of approximately 1–1.5 m from the selected trees, one monitoring the mean soil water content from 5 to 25 cm depth and another monitoring from 25 to 45 cm depth. Xylem water potentials (Ψ) were measured using a pressure chamber (Model 1000, PMS Instruments, Corvallis, OR, USA). On each sampling occasion and no later than 1 h after dawn two current-year shoots per tree containing fully expanded leaves were collected from the east and west sides of the external part of the crown at the middle of its height. These samples were immediately refrigerated in the dark prior to transfer to the laboratory in a plastic bag within 2 h. We checked in advance that there were no significant differences between the water potential measurements made in the field immediately after the shoots were cut, and those made in the laboratory after transfer of the shoots (Myers and Craig 1988). The selected shoots were 10 cm long and each had a minimum of five attached leaves. In addition to the above, a further two shoots (15–20 cm in length) were cut per tree and sampling occasion. These were kept refrigerated in the dark with their basal ends immersed in distilled water until transferred to the laboratory. The latter shoots were subsequently used for cuticular transpiration measurements and to construct isothermal pressure-volume curves.

To construct the pressure-volume curves, a total of nine shoots (one per tree) were used on each sampling occasion, taken from among the shoots refrigerated in distilled water; they were covered with a polyethylene bag for 24 h to improve hydration. Further details related to the methodology for pressure-volume curves are described in Corcuera

et al. (2002) and Dreyer et al. (1990). Data were obtained using the free transpiration method (Robichaux 1984), which involved making Ψ and fresh weight measurements of the shoots over short periods at a constant temperature ($25 \pm 2^\circ\text{C}$) until they were dehydrated. The shoots were oven dried at 70°C to determine dry weight (DW) and relative water content (RWC). Following construction of the pressure-volume curves (no over-saturation points were detected), the following parameters were determined: osmotic potential at full turgor ($\Psi\pi_{100}$); osmotic potential at the turgor loss point ($\Psi\pi_0$); relative water content at the turgor loss point (RWC_0); and apoplastic relative water content (RWC_a) (for further information about the parameters mentioned in this paragraph see Koide et al. 1989).

Cuticular transpiration was assessed using the method of Quisberry et al. (1982). Two healthy and fully developed leaves were picked at random from each of the nine trees (one leaf per shoot), rehydrated in the dark for 24 h, then placed abaxial surface down on a plastic mesh in a lighted laboratory. The leaf fresh weights (FW) were measured at intervals of 5, 10 and 30 min for a minimum of 7 h (with 5 min intervals during the 1st h; 10 min intervals during the 2nd h; and 30 min intervals during the 3rd h and until the end). Precision scales (0.1 mg) and a chronometer were used for weight measurements. Before the measurements were taken the leaves were exposed to light with the base of the shoot immersed in water, and were covered with a translucent polyethylene bag for at least 60 min to facilitate stomatal opening and temperature equilibration (Cape and Percy 1996; Burghardt and Riederer 2003). This process enabled construction of descending transpiration curves which were used to estimate the relative water content at the point of stomatal closure (RWC_c) and the cuticular transpiration (E_c , $\text{mmol H}_2\text{O kg}^{-1} \text{s}^{-1}$). Following measurement of the dry weight of each leaf the specific leaf area (SLA , $\text{m}^2 \text{kg}^{-1}$) was calculated.

Data analysis

Plant characteristics (SLA and water relations parameters) measured on 24 sampling dates, and AP measured on three sampling dates, were analyzed by repeated measures analysis of variance, soil treatment being considered a fixed factor. Normality

and equality of variances were checked using the Kolmogorov-Smirnov test. When differences among treatments were significant ($P \leq 0.05$), Tukey's HSD test was used to check them. Bonferroni test was used to check differences among dates. Possible links between AP and physiological and edaphical parameters were estimated by regression analysis (AP as dependent variable) and Pearson's correlations. All statistical analyses were carried out using SPSS[®] 17.0 statistical software. The criteria used to perform and interpret the statistical analyses were based on Dillon and Goldstein (1984) and Sokal and Rohlf (1995).

Results

Water relations parameters

Differences between soil treatments during the study period were only significant for $\Psi\pi_{100}$ ($P < 0.001$, $F = 192.50$) between P and C, with lower values for the latter (-1.8 ± 0.1 and -1.3 ± 0.1 MPa, respectively). Therefore, we focused on analyzing the differences found between measurement dates and trees, as well as the possible effect of environmental parameters on water status and AP.

The seasonal evolution of air temperature, soil humidity and rainfall during the study period are shown in Figs. 1 and 2. Xylem water potential ranged from -3.4 ± 0.1 to -0.7 ± 0.2 MPa (Fig. 3), and there were significant variations depending on measurement date ($P < 0.001$, $F = 84.19$) and a significant relationship with precipitation in the 30 days period prior to the water potential measurements ($r^2 = 0.26$; $P = 0.010$). The seasonal variations of the parameters derived from the pressure-volume curves are shown in Fig. 4. The analysis revealed significant differences among dates, although not for the parameter $\Psi\pi_{100}$ ($P = 0.253$, $F = 8.74$), where values ranged from -1.9 ± 0.4 to -1.2 ± 0.3 MPa in midsummer 2006 and April 2007, respectively. Moreover, RWC_a and RWC_0 were strongly correlated ($r^2 = 0.86$; $P < 0.001$). Values of RWC_a ranged from $33.0 \pm 4.8\%$ (April 2007) to $76.9 \pm 4.0\%$ (July 2008).

Figure 5 shows the seasonal course of relative water content at RWC_c and E_c during the study. Average RWC_c values for the nine holm oaks ranged from a minimum of $62.0 \pm 3.9\%$ (March 2008) to

Fig. 1 Absolute maximum (T_{max}) and minimum (T_{min}) temperatures, and monthly average temperatures (T) and rainfall (P) at the Huerto Ramírez plot from January 2006 to December 2008

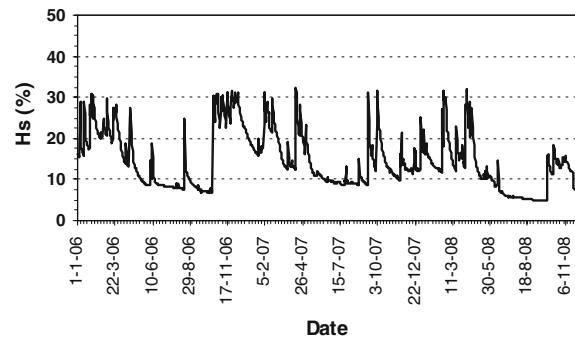
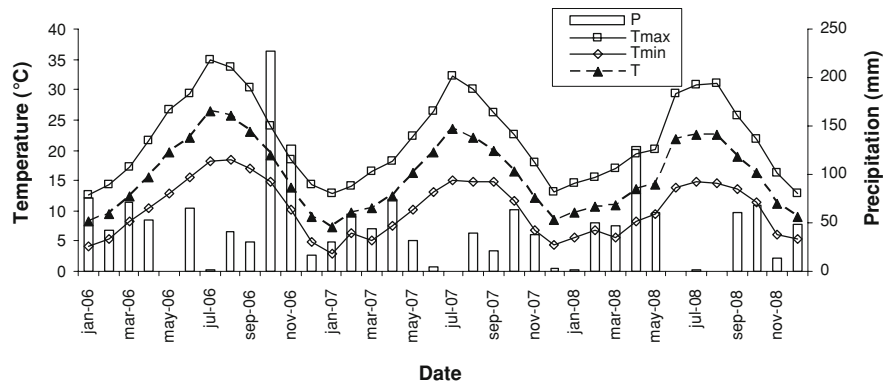


Fig. 2 Seasonal evolution of the mean daily values of soil water content (H_s , 0–45 cm depth) at the Huerto Ramírez plot for all probes

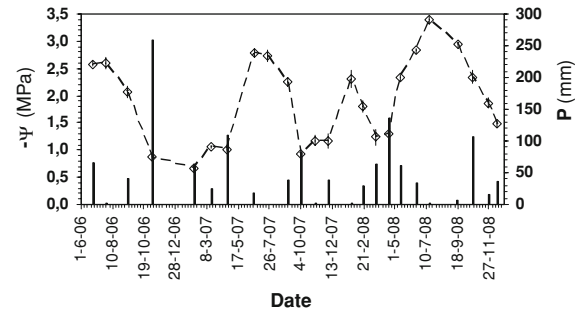


Fig. 3 Seasonal evolution of xylem water potential (Ψ , mean \pm SE). For each date, bars represent the accumulated rainfall in the 30 days prior to the water potential measurements

91.2 \pm 5.9% (June 2006). The E_c showed a sudden increase in autumn and a decline at the end of winter. There were no significant differences for SLA among the study dates. Monthly average values ranged from 26.2 \pm 9.7 m² kg⁻¹ in October 2008 to 39.3 \pm 3.4 m² kg⁻¹ in February 2007.

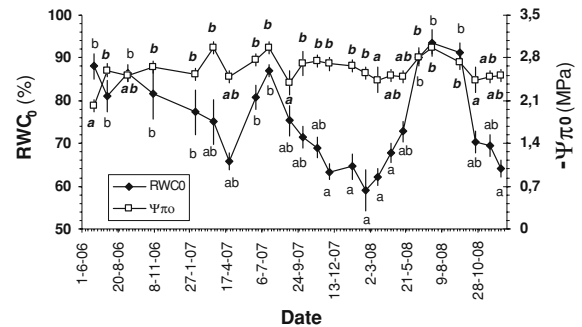


Fig. 4 Seasonal pattern of mean values (\pm SE) of osmotic potential at the turgor loss point ($\Psi\pi_0$), and relative water content at the turgor loss point (RWC_0). Significant differences between dates are denoted by different letters ($P < 0.05$)

Acorn production

AP varied significantly during the study period, ranging from 0 to 1,220 g m⁻² per tree and year. Average AP per treatment for the study period (2006–2008) was 339.7 \pm 98.8 g FM m⁻² for *C*, 265.4 \pm 40.6 g FM m⁻² for *P*, and 233.4 \pm 20.7 g FM m⁻² for *PS*. Average AP per year was 338.8 \pm 83.1 (2006); 313.7 \pm 60.9 (2007) and 186.1 \pm 28.4 (2008). The differences were not significant ($P > 0.05$) neither among years nor among treatments. The annual average of moisture content of acorns was 40.3% (2006), 44.3% (2007) and 48.1% (2008).

The regression analysis of the studied parameters (both edaphoclimatic and physiological) revealed a significant positive relationship between AP and xylem water potential at midsummer in the three summers of the study (Fig. 6). Moreover, a positive relationship was also found between E_c (July) and AP

Fig. 5 Seasonal evolution of mean values (\pm SE) of cuticular transpiration (E_c , $\text{mmol H}_2\text{O kg}^{-1} \text{s}^{-1}$) and relative water content at the point of stomatal closure (RWCC, %). Significant differences among dates are denoted with different letters ($P < 0.05$)

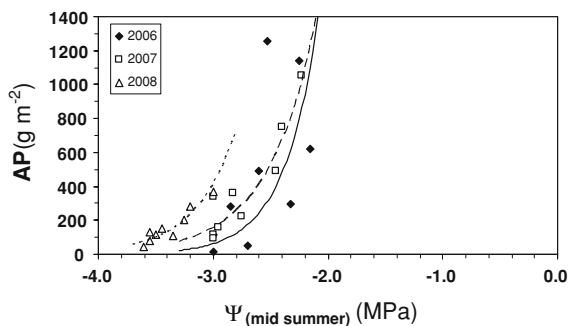
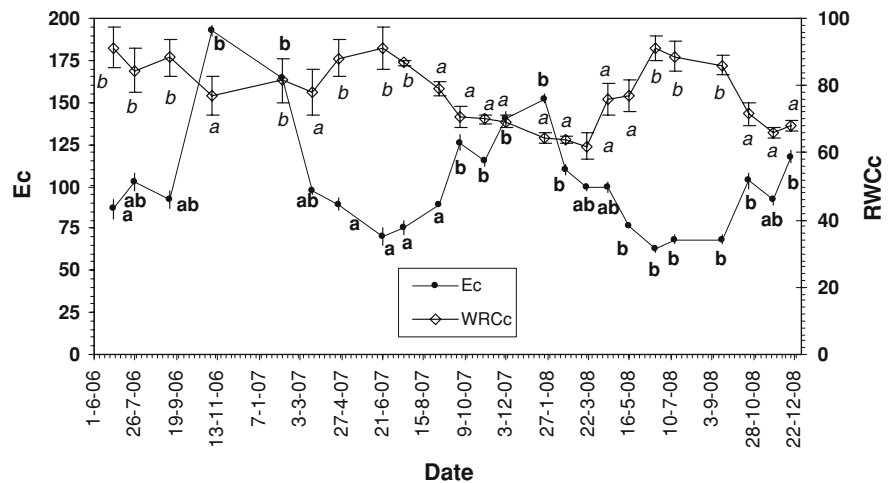


Fig. 6 Relationship between acorn production (AP, g FM m^{-2}) and xylem water potential (Ψ) measured at midsummer. Each year is showed by the average value per tree ($n = 9$): 2006 ($\text{AP} = 2.0 \cdot 10^6 e^{3.393x}$, $r^2 = 0.52$, $P = 0.028$, continuous line), 2007 ($\text{AP} = 2.2 \cdot 10^5 e^{2.423x}$, $r^2 = 0.78$, $P = 0.002$, dashed line), 2008 ($\text{AP} = 2 \cdot 10^6 e^{2.870x}$, $r^2 = 0.77$, $P = 0.002$, dotted line)

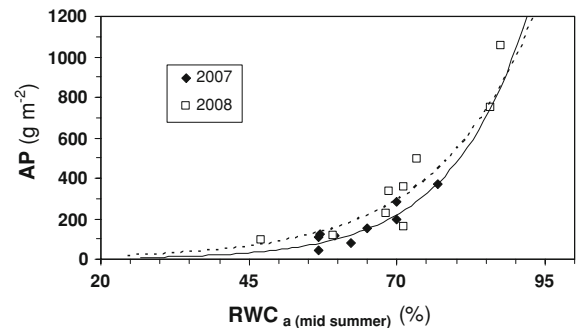


Fig. 7 Relationship of acorn production (AP) to apoplastic relative water content (RWC_a) measured at midsummer. Each year is showed by the average value per tree ($n = 9$): 2007 ($\text{AP} = y = 4.0278 e^{0.0612x}$, $r^2 = 0.84$, $P < 0.001$, continuous line) and 2008 $\text{AP} = 0.9982 e^{0.0771x}$, $r^2 = 0.71$, $P = 0.004$, dashed line)

($P = 0.015$, $F = 6.68$, $r^2 = 0.21$, $\text{AP} = 19.112 e^{0.0369E_c}$). Parameters derived from the pressure-volume curves (RWC₀ and RWC_a) were positively related to AP (Figs. 7, 8), except for RWC_a measured in 2006, that was not correlated with AP ($P = 0.952$, $F = 0.00$, $r^2 < 0.001$).

There was a positive correlation between ESD and WHC_u ($r^2 = 0.76$; $P < 0.001$). These two parameters were positively correlated to soil water content during summer ($r^2 = 0.63$; $P = 0.020$). In contrast, the relationship between WRC_u and AP was negative but significant for the 2006 data ($P = 0.048$, $F = 4.61$, $r^2 = 0.15$; $n = 18$), whereas the trends for 2007 and 2008 showed negative correlations but were not significant ($r^2 < 0.05$, $P > 0.10$).

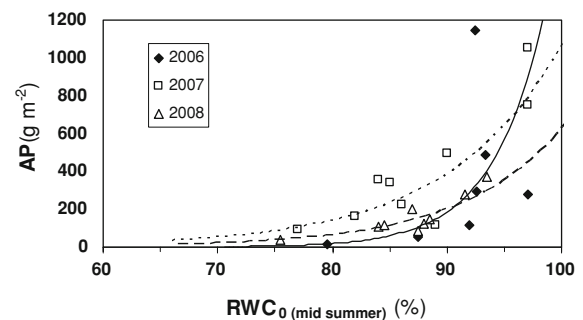


Fig. 8 Relationship between acorn production (AP, g FM m^{-2}) and relative water content at the turgor loss point (RWC₀). Each point represents the average value per tree: 2006 ($\text{AP} = 3E-07 e^{0.2235x}$, $r^2 = 0.56$, $P = 0.031$, continuous line), 2007 ($\text{AP} = 0.042 e^{0.1014x}$, $r^2 = 0.66$, $P = 0.008$, dotted line), 2008 ($\text{AP} = 0.0072 e^{0.1136x}$, $r^2 = 0.80$, $P = 0.001$, dashed line)

Discussion

Xylem water potential showed marked seasonal variations that reflected seasonal changes in rainfall and temperature. The lowest values were recorded during the summer months despite the fact that unusually rainy summers (over 100 mm) occurred in the area during 2006 and 2007. These results are similar to those obtained in other surveys involving *Q. ilex* (Martinez-Vilalta et al. 2002; Cubera and Moreno 2007) and other Mediterranean sclerophyll species of the same genus (Oliveira et al. 1992; Knops and Koenig 1994; Nardini et al. 1999; Otiendo et al. 2006). Ψ values during summer 2008 (-3.4 MPa), the least favourable period, indicated marginally severe water stress during the year concerned. Typical values indicating onset of severe water stress, i.e. between -3.0 and -3.5 MPa (Aussenac and Velette 1982; David et al. 2002) occurred during summer 2008, and the level recorded in that year was substantially lower than in 2006 and 2007, reflecting a lack of rainfall. In a recent study in the province of Huelva, Alejano et al. (2008) recorded average xylem water potential values of approximately -4.0 MPa during the summer months of 2004 and 2005, as a consequence of the severe drought in that area in those years.

We observed high intraspecific variability in AP, consistent with the findings of Olea and San Miguel-Ayaz (2006), and individual tree production was similar to that obtained in other studies (Cañellas et al. 2007; Alejano et al. 2008). The positive relationship between Ψ (measured in midsummer) and AP could indicate that trees require adequate water levels for acorn development during summer and the beginning of autumn. Alejano et al. (2008) made similar observations and reported water potentials ranging from -2.5 to -4.1 MPa, a much greater range than that which occurred in our study. Resource deficiency, such as low water availability, is a main reason for the premature abortion of fruits (Larcher 2003), and typically occurs at the beginning of seed development. AP is likely to fail with summer water potentials below -4.0 MPa, similar to the minimum limit determined by Alejano et al. (2008). This lower limit is probably associated with total stomatal closure and the loss of hydraulic conductivity, although production would be seriously limited with values below -3.0 MPa (Aussenac and Velette 1982; David et al. 2002; Pesoli et al. 2003).

Water parameters derived from the pressure-volume curves were similar to those obtained in other surveys involving *Q. ilex* (Corcuera et al. 2002; Corcuera 2003; Villar-Salvador et al. 2004). Our results revealed significant variations in RWC_0 and RWC_a values throughout the year. The lower values occurred in late winter and early spring, which may have been due to the age of the leaves; during this period leaves from the previous year still remained on the trees. However, in summer RWC_0 and RWC_a values tended to remain high. Seasonal RWC patterns are usually associated with the ability to resist water stress and freezing temperatures (Nardini et al. 2000). Maintenance of high of RWC_0 and RWC_a values during summer may be a strategy for preserving symplastic water. These two parameters showed positive relationships with AP, probably due to the acorn growth process, as the absorption of water is necessary to sustain cell expansion and solute accumulation in leaf apoplast (Nonami and Boyer 1990), and is likely to increase AP. The differences between the high $\Psi\pi_0$ values in late spring and early summer and those that occurred in the following months could be due to osmotic adjustment. This, with stomatal regulation, would enhance the formation of water potential gradients, which would in turn enable growth in dry soils (Infante et al. 2003; Mediavilla and Escudero 2004; Serrano and Peñuelas 2005; Fernández et al. 2008). The extremely high $\Psi\pi_0$ value in June 2006 may be related to slow recovery of the holm oaks from the severe water stress that occurred in 2005, in addition to the high temperatures and low rainfall during the previous month (May 2006). Annual rainfall in 2005 was only 55% of the long term local average, and some authors have reported the effect of this drought period on the water status of holm oak in the neighborhoods of our plot (Alejano et al. 2008; Paço et al. 2009). Water stress decreases transpiration and photosynthesis rates in holm oak (Sala and Tenhunen 1996), and the carbon fixed by photosynthesis may register a 40% fall when soil moisture decreases 15% (Ogaya and Peñuelas 2003; Ogaya et al. 2003). This means the reduction of growth rate and energetic reserves storage; the latter playing a main role in the osmotic adjustment for the species (Serrano and Peñuelas 2005; Fernández et al. 2008).

Parameters related to cuticular transpiration indicated a clear strategy for preserving water. *Q. ilex* has

morpho-physiological foliar adaptations for reducing water loss, enabling survival in arid soils and during drought periods (Bussotti et al. 2002; Pena-Rojas et al. 2005). This was evident in our study through the decrease of E_c during drier periods, in addition to the higher values of RWC_c . During periods of greater water availability, this species tends not to control water loss to the same degree, and hence is termed a “water-spender species” because it preserves water during dry periods but not during wet ones (Infante et al. 2003; Pena-Rojas et al. 2005; Baquedano and Castillo 2006). SLA is an important leaf morphological trait affected by environmental conditions and leaf age (Ackerly and Reich 1999). We did not find significant differences among dates or soil treatments for SLA. This was probably due to the use of fully expanded leaves for all measurements; in June these were 1-month-old, whereas in April of the following year they were approximately 1-year-old.

Analysis of the relationships between soil treatments and water relations parameters for *Q. ilex* indicated that ploughing affected $\Psi\pi_{100}$. An explanation for this is that water infiltration in ploughed soils reduces water stress on the trees, and reduces the need to make osmotic adjustments. Several authors have described positive effects of dehesa silvicultural treatments on AP (Pulido et al. 2001) and photosynthetic activity (Montero et al. 2004).

Although not significant in 2007 and 2008, the negative relationship between WRC_u and AP could be associated with the presence of *oak decline* that affects *Quercus* forests in the SW of Iberian Peninsula. This disease is caused by different biotic and abiotic factors. One of them is *Phytophthora cinnamomi*, a pathogenic fungus that causes serious damage to *Q. ilex* in the dehesas of southern Spain (Rodríguez-Molina et al. 2002). *Phytophthora* infection causes roots to die and decreases Ψ , stomatal conductance and above-ground biomass; declines in these parameters are directly related to decreased AP (Maurel et al. 2001). The symptoms of oak decline were evident in our study during 2006, when tree defoliation ranged between 5 and 20% without great differences among trees and no trees have died. It was probably due to the rainy and warmer summer period stimulating a major effect of *P. cinnamomi* on AP in areas associated with higher WRC_u . Various factors influence acorn production in *Q. ilex*, including genetic variability, tree density, spring rain and

average temperatures in June (García-Mozo et al. 2007; Rodríguez-Estévez et al. 2007), or water stress during the midsummer months (Alejano et al. 2008). Future research in dehesas should consider long-term capacity of response of *Q. ilex* to long summer drought periods and its effects on acorn production, consistent with the requirements of conservation of this important natural ecosystem.

Acknowledgments This study was sponsored by the Department of Innovation, Science and Business of the Regional Government of Andalucía (ref: C03-192) and by MEC-INIA (ref: SUM2006-00026-00-00). The authors also wish to thank the Provincial Government of Huelva and AEIC. Thanks also go to our collaborators Daniel Martín and Enrique Andivia. We would like to thank Mrs Esther Martínez-Fernández for translating the English version of this document.

References

- Abrams MD (1990) Adaptations and responses to drought in *Quercus* species of North America. *Tree Physiol* 7:227–238
- Ackerly DD, Reich PB (1999) Convergence and correlations among leaf size and function in seed plants: a comparative test using independent contrasts. *Am J Bot* 86:1272–1281
- Alejano R, Tapias R, Fernández M, Torres E, Alaejos J, Domingo J (2008) The influence of pruning and climatic conditions on acorn production in holm oak (*Quercus ilex* L.) dehesas in SW Spain. *Ann For Sci* 65(2):209–215
- Álvarez S, Morales R, Bejarano L, Durán A (2002) Producción de bellota en la dehesa salmantina. XLII Reunión científica de la Sociedad Española para el Estudio de los Pastos, pp 645–649
- Aussenac G, Velette JC (1982) Comportement hydrique estival de *Cedrus atlantica* Manetti, *Quercus ilex* L. et *Quercus pubescens* Willd. et de divers pins dans le Mont Ventoux. *Ann Sci For* 39:41–62
- Baquedano F, Castillo J (2006) Comparative ecophysiological effects of drought on seedlings of the Mediterranean water-saver *Pinus halepensis* and water-spenders *Quercus coccifera* and *Quercus ilex*. *Trees* 20:689–700
- Burghardt M, Riederer M (2003) Ecophysiological relevance of cuticular transpiration of deciduous and evergreen plants in relation to stomatal closure and leaf water potential. *J Exp Bot* 54:1941–1949
- Bussotti F, Bettini D, Grossini P, Ansuino S, Nibbi R, Soda C, Tani C (2002) Structural and functional traits if *Quercus ilex* in response to water availability. *Env Exp Bot* 47:11–23
- Cañellas I, Roig S, Poblaciones MJ, Gea-Izquierdo G, Olea L (2007) An approach to acorn production in Iberian dehesas. *Agroforest Syst* 70:3–9
- Cape J, Percy K (1996) The interpretation of leaf-drying curves. *Plant Cell Environ* 19:356–361
- Carbonero MD, Fernández P, Navarro R (2002) Evaluación de la producción y del calibre de bellotas de *Quercus ilex* L.

- subsp. *ballota* (Desf.) a lo largo de un ciclo de poda. Resultados de la campaña 2000–2001. Actas de la XLII Reunión Científica de la Sociedad Española para el Estudio de los Pastos, pp 633–638
- Corcuera L (2003) Comparison between two methods of generating pressure-volume curves in *Quercus* species. Investigación Agraria, Sistemas y Recursos Forestales 12:111–121
- Corcuera L, Camarero J, Gil-Pelegrín E (2002) Functional groups in *Quercus* species derived from the analysis of pressure-volume curves. Trees 16:465–472
- Cubera E, Moreno G (2007) Effect of single *Quercus ilex* trees upon spatial and seasonal changes in soil water content in dehesas of central western Spain. Ann For Sci 64:355–364
- David TS, Ferreira I, Pereira JS, Cohen S, David JS (2002) Transpiração em arvoredos isoladas de um montado de azhino. Evolução sazonal e condicionantes hidráulicas. Silva Lusitana 10:133–149
- Di Castri F (1981) Mediterranean-type shrublands of the world. In: Di Castri F, Goodall DW, Specht RL (eds) Mediterranean-type shrublands. Elsevier, Amsterdam, pp 1–52
- Díaz M, Campos P, Pulido FJ (1997) The Spanish dehesas: a diversity in land-use and wildlife. In: Pain DJ, Pienkowski MW (eds) Farming and birds in Europe. The common agricultural policy and its implications for bird conservation. Academic Press, San Diego, pp 178–209
- Dillon W, Goldstein M (1984) Multivariate analysis. Methods and applications. Wiley, New York
- Domingo JM, Fernández de Villarán R, Corral E, Rapp I (2006) Estimación de la capacidad de retención de agua en el suelo: revisión del parámetro CRA. Invest Agrar: Sist Recur For 15:14–23
- Dreyer E, Bousquet F, Ducrey M (1990) Use of pressure volume curves in water relation analysis on woody shoots: influence of rehydration and comparison of four European oak species. Ann Sci For 47:285–297
- Eichhorn MP, Paris P, Herzog F, Incoll LD, Liagre F, Mantzanas K, Mayus M, Moreno G, Pilbeam DJ, Dupraz C (2006) Silvoarable agriculture in Europe—past, present and future. Agroforest Syst 67:29–50
- Fernández M, Alejano R, Domínguez L, Tapias R (2008) Temperature controls cold hardening more effectively than photoperiod in four Mediterranean broadleaf evergreen species. Tree For Sci Biotechnol 2(1):43–49
- García-Mozo H, Gómez-Casero M, Domínguez E, Galán C (2007) Influence of pollen emission and weather-related factors on variations in holm-oak (*Quercus ilex* ssp. *ballota*) acorn production. Environ Exp Bot 61:35–40
- Gea-Izquierdo G, Cañellas I, Montero G (2006) Acorn production in Spanish holm oak woodlands. Investigación Agraria, Sistemas y Recursos Forestales 15(3):339–354
- Infante JM, Domingo M, Fernández R, Joffre R, Rambla S (2003) *Quercus ilex* transpiration as affected by a prolonged drought period. Biol Plantarum 46:49–55
- Joffre F, Vahar J, De las Llamas G, Long G (1988) The dehesa: an agrosilvopastoral system of the Mediterranean region with special reference to the Sierra Morena of Spain. Agroforest Syst 6:71–96
- Knops MH, Koenig WD (1994) Water strategies of five sympatric species of *Quercus* in central coastal California. Madroño 4:290–301
- Knops MH, Koenig WD (2000) Annual variation in xylem water potential in California oaks. Madroño 47:106–108
- Koide R, Robichaux R, Morse S, Smith C (1989) Plant water status, hydraulic resistance and capacitance. In: Pearcy RW, Ehleringer JR, Mooney HA, Rundel PW (eds) Plant physiological ecology: field methods and instrumentation. Chapman & Hall, New York, pp 161–182
- Larcher W (2003) Physiological plant ecology, 4th edn. Springer, Berlin 513 p
- Martín Vicente A, Infante J, García Gordo J, Merino J, Fernández Alés R (1998) Producción de bellotas en montes y dehesas del suroeste español. Pastos 28:237–248
- Martínez-Vilalta J, Prat E, Oliveras I, Piñol J (2002) Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. Oecol 133:19–29
- Maurel M, Robin C, Capron G, Desprez-Loustau ML (2001) Effects of root damage associated with *Phytophthora cinnamomi* on water relations, biomass accumulation, mineral nutrition and vulnerability to water deficit of five oak and chestnut species. For Path 31:353–369
- Mediavilla S, Escudero A (2004) Stomatal responses to drought of mature trees and seedlings of two co-occurring Mediterranean oaks. For Ecol Manag 187:281–294
- Montero M, Obrador J, Cubera E, Moreno G (2004) The role of Dehesa land use on tree water status in Central-Western Spain. In: Schnabel S, Ferreira A (eds) Sustainability of agrosilvopastoral systems—dehesas, montados-, Catena Verlag, Reiskirchen, Germany. Advances in GeoEcology vol 37, pp 125–136
- Moreno G, Cubera E (2008) Impact of stand density on water status and leaf gas exchange in *Quercus ilex*. For Ecol Manag 250:74–84
- Moreno G, Obrador J, García E, Cubera E, Montero M, Pulido F, Dupraz C (2007) Driving competitive and facilitative interactions in oak dehesas through management practices. Agroforest Syst 70:25–40
- Myers B, Craig I (1988) Changes in water potential of *Pinus radiata* fascicles during temporary storage. J For Sci 18:3–8
- Nardini A, Lo Gullo MA, Salleo S (1999) Competitive strategies for water availability in two Mediterranean *Quercus* species. Plant Cell Environ 22:109–116
- Nardini A, Salleo S, Lo Gullo MA, Pitt F (2000) Different responses to drought and freeze stress of *Quercus ilex* L. growing along a latitudinal gradient. Plant Ecol 148:139–147
- Nonami H, Boyer J (1990) Wall extensibility and cell hydraulic conductivity decrease in enlarging stem tissues at low water potentials. Plant Physiol 93:1610–1619
- Ogaya R, Peñuelas J (2003) Comparative field study of *Quercus ilex* and *Phillyrea latifolia*: photosynthetic response to experimental drought conditions. Environ Exp Bot 50:137–148
- Ogaya R, Peñuelas J (2006) Contrasting foliar responses to drought in *Quercus ilex* and *Phillyrea latifolia*. Biol Plant 50:373–382
- Ogaya R, Peñuelas J, Martínez-Vilalta J, Mangirón M (2003) Effect of drought on diameter increment of *Quercus ilex*, *Phillyrea latifolia*, and *Arbutus unedo* in a holm oak forest of NE Spain. For Ecol Manag 180:175–184

- Olea L, San Miguel-Ayaz A (2006) The Spanish dehesa. A traditional Mediterranean silvopastoral system linking production and nature conservation. *Grassland Sci Eur* 11:3–13
- Oliveira G, Correia AO, Martins-Louscao MA, Catarino FM (1992) Water relations of cork oak (*Quercus suber* L.) under natural conditions. *Vegetatio* 99/100:199–208
- Otiendo DO, Kurz-Besson C, Liu J, Schmidt M, Vale-Lobo R, David TS, Siegwolf R, Pereira JS, Tenhunen JD (2006) Seasonal variations in soil and plant water status in a *Quercus suber* L. stand: roots as determinants of tree productivity and survival in the Mediterranean-type ecosystem. *Plant Soil* 283:119–135
- Paço T, David T, Henriques M, Pereira J, Valente F, Banza J, Pereira F, Pinto C, David J (2009) Evapotranspiration from a Mediterranean evergreen oak savannah: the role of trees and pasture. *J Hydrol* 369:98–106
- Pena-Rojas K, Aranda X, Joffre R, Fleck I (2005) Leaf morphology, photochemistry and water status changes in resprouting *Quercus ilex* during drought. *Funct Plant Biol* 32:117–130
- Pesoli P, Gratani L, Larcher W (2003) Response of *Quercus ilex* from different provenances to experimentally imposed water stress. *Biol Plantarum* 46:577–581
- Pulido FJ, Díaz M, Hidalgo S (2001) Size structure and regeneration of Spanish holm oak *Quercus ilex* forests and dehesas: effects of agroforestry use on their long-term sustainability. *For Ecol Manag* 146:1–13
- Pulido FJ, García E, Obrador J, Montero M (2005) Effects of management on acorn production and viability in holm-oak dehesas. In: *Silvopastoralism and Sustainable Management International Congress*. Lugo 18–21/4. CAB International, pp 180–181
- Quisenberry J, Roark B, McMichael B (1982) Use of transpiration decline curves to identify drought-tolerant cotton germplasm. *Crop Sci* 22:918–922
- Robichaux RH (1984) Variation in the tissue water relations of two sympatric Hawaiian *Dubautia* species and their natural hybrid. *Oecol* 65:75–81
- Rodà F, Mayor X, Sabaté S, Diego V (1999) Water and nutrient limitations to primary production. In: Rodà F, Gracia C, Retana y J, Bellot J (eds) *The ecology of Mediterranean evergreen oak forests*. Springer, Berlin, pp 183–194
- Rodríguez-Estévez V, García A, Perea J, Mata C, Gómez A (2007) Producción de bellota en la dehesa: factores influyentes. *Arch Zootec* 56:25–43
- Rodríguez-Molina M, Torres-Vila C, Blanco L, Palo A, Torres-Álvarez E (2002) Viability of holm and cork oak seedlings from acorns sown in soils naturally infected with *Phytophthora cinnamomi*. *For Path* 32:365–372
- Ruiz de la Torre J (2006) *Flora Mayor*. Organismo Autónomo de Parques Nacionales. Dirección General para la Biodiversidad. Ministerio de Medio Ambiente, Madrid. 1756 p
- Sala A, Tenhunen JD (1994) Site-specific water relations and stomatal response of *Quercus ilex* L. in a Mediterranean watershed. *Tree Physiol* 14:601–617
- Sala A, Tenhunen J (1996) Simulations of canopy net photosynthesis and transpiration in *Quercus ilex* L. under the influence of seasonal drought. *Agr For Meteorol* 78:203–222
- Savé R, Terradas J, Castell C (1999) Gas exchange and water relations. In: Rodà F, Gracia C, Retana y J, Bellot J (eds) *The ecology of Mediterranean evergreen oak forests*. Springer, Berlin, pp 135–147
- Serrano L, Peñuelas J (2005) Contribution of physiological and morphological adjustments to drought resistance in two Mediterranean tree species. *Biol Plantarum* 49:551–559
- Siscart D, Diego V, Lloret F (1999) Acorn ecology. In: Rodà F, Gracia C, Retana y J, Bellot J (eds) *The ecology of Mediterranean evergreen oak forests*. Springer, Berlin, pp 75–87
- Sokal R, Rohlf F (1995) *Biometry: the principles and practice of statistics in biological research*, 3rd edn. W. H. Freeman and Co, New York
- Terradas J (1999) Holm oak and holm oak forests: an introduction. In: Rodà F, Gracia C, Retana J, Bellot J (eds) *The ecology of Mediterranean evergreen oak forests*. Springer-Verlag, Berlin, pp 3–14
- Tognetti R, Longobucco A, Miglietta F, Raschi A (1998) Transpiration and stomatal behaviour of *Quercus ilex* plants during the summer in a Mediterranean carbon dioxide spring. *Plant Cell Environ* 21:613–622
- Villar-Salvador P, Planelles R, Olliet J, Peñuelas J, Jacobs D, González M (2004) Drought tolerance and transplanting performance of Holm Oak (*Quercus ilex*) seedlings after drought hardening in the nursery. *Tree Physiol* 24:1147–1155