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Transpiration and photosynthesis of holm oak trees in southern Spain

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ABSTRACT

"Dehesas", the Iberian semi-natural woodlands based on large holm oak (*Quercus ilex* L.) trees, represent a key agroforestry system in Spain and Portugal sustaining relevant economic activities (e.g. "iberico" pork feeding, truffle) and ecosystem services. Despite of their importance, scarce information is still available regarding both the transpiration (E_p) and photosynthesis of holm oaks growing in dehesas and the effects of irrigation on these fluxes. Two experiments were performed in Espiel and Hinojosa del Duque (southern Spain) where sap flow was measured in large trees between 2009 and 2011 and photosynthesis was measured with a transitory-closed chamber in smaller trees at different moments of 2009. In 2009, half of the trees equipped with sap flow sensors were drip irrigated and half were rainfed. However, the supply of irrigation barely affected E_p and shoot water potential. The seasonal course of E_p peaked in late spring and decreased gradually, reaching minimum values during winter. Vapor pressure deficit (VPD) exerted a strong control over canopy conductance and its seasonal variation was identified as a major driver for the observed patterns of E_p. Photosynthesis showed a similar course as that of E_p but there was a second smaller peak in autumn. Seasonal water use efficiency was remarkably high due to the reduced summer E_p. Our results also indicate that hyperbolic models of canopy conductance and water use efficiency in response to VPD may be used to estimate diurnal and seasonal E_p and net photosynthesis of holm oak if the tree size and leaf area density are known.

1. Introduction

Quercus ilex ssp. *rotundifolia* Lam. (holm oak) is a key species in Mediterranean forests and maquis vegetation. In several areas of the Mediterranean basin the main use of holm oak has traditionally been the production of charcoal and firewood from high density forests (de Rigo and Caudullo, 2016). Holm oak orchards and agroforestry systems are also important hosts of black truffles and, in recent decades, plantations of holm oak have been established to counter the reduction in natural abundance of this product in Europe (Büntgen et al., 2015). In the Iberian Peninsula, native forests of holm oak (and the related species *Quercus suber*) have been converted to a savannah-like agrosilvopastoral system ("dehesas" or "montados") with multiple productive functions and ecosystem services (Pinto-Correia et al., 2011). The highest quality Iberian ham is produced with free-range pigs feeding on acorns from holm oaks in Dehesas, but the low productivity of holm oaks limits the density of animals to less than 1 ha^{-1}. Dehesas and Montados occupy between 3.5 and 4.0 Mha (Pinto-Correia et al., 2011), with at least 2.1 Mha dominated exclusively by holm oaks (Vallejo Bombin, 2007). Hereafter we denote dehesas and montados dominated by holm oak simply as "dehesas".

Despite the importance of dehesas, little is known about the gap between realized and potential productivity in terms of wood or acorns, and to what extent this gap is the result of biotic or abiotic stresses. Regarding the production of acorns, Gea-Izquierdo et al. (2006) reported a range of yields of 87–286 g DW acorn m^{-2} crown area. Furthermore, tree size and density can vary substantially within dehesas and these would have an important effect on the productivity of both the trees and the associated pasture or crops. The ability to calculate the potential production of a dehesa is of interest to quantify the productivity gap and to guide management and resource allocation to reduce it.

Recently, the interest in irrigation of holm oak has arisen due to its possible positive effect on black truffle production (Büntgen et al., 2015) and to improve the survival of seedlings in the conversion of marginal agricultural lands to dehesas (Rey Benayas, 1998). Any strategy for irrigation management should be based on knowledge of tree transpiration (E_p) in the absence of water stress (i.e. potential E_p). Additionally, for the development of deficit irrigation strategies it is necessary also to

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evaluate the water use efficiency (WUE, ratio between net photosynthesis and E_p) of the trees during the whole season so we can select periods of lower WUE to reduce irrigation and thus, optimize water use (Fereres and Soriano, 2006).

Despite the importance of potential E_p and WUE, the number of studies on the carbon and water balance of this species is limited and most have concentrated on its tolerance to drought conditions during summer (e.g. Sanchez-Costa et al., 2015). Holm oak E_p has been measured with thermal dissipation sensors (Infante et al., 2003; Paço et al., 2009) and ecosystem carbon and water fluxes including the contribution of the understorey have been measured with eddy covariance (e.g. Reichstein et al., 2002; El-Madany et al., 2020). In the study of Unger et al. (2009) contributions of trees and the understorey to carbon fluxes were partitioned by additional measurements in a nearby location with no trees. Similarly, Joffre and Rambal (1993) characterized spatial heterogeneities in the water fluxes of a dehesa in southern Spain, comparing estimates of seasonal evapotranspiration among areas covered by tree canopies and those in the open with herbaceous cover.

Most literature on holm oak has dealt with high density mixed forests in Northwest Spain (Catalonia) and areas of France and Italy (e.g. Mayor and Rodà, 1994; Tognetti et al., 1998) while much less is known on the performance of larger holm oak trees scattered over dehesas despite their ecological and economic relevance. Most E_p data from large trees have been collected by Portuguese groups close to Evora (e.g. David et al., 2004; Paço et al., 2009) so our study covers a knowledge gap by adding data in a more extreme (colder, drier) environment in southern Spain. The objectives of this work are a) to assess the effect of irrigation in holm oak stands in southern Spain, b) to quantify seasonal and diurnal patterns of E_p and photosynthesis in the absence of significant water stress and c) to provide simple mathematical models to estimate potential E_p, photosynthesis and ecosystem productivity of holm oak trees in dehesas from knowledge of weather and tree size. This study should be seen as an exploration of observed patterns and their consequences rather than as an attempt to test a scientific hypothesis that would require a larger and more systematic study across sites.

2. Materials and methods

2.1. Experimental sites

Experimental data were collected in two holm oak stands located at “Villazulina” farm in Espiel (38.18° N, 5.03° W, 500 m) and the IFAPA center of Hinojosa del Duque (38.48° N, 5.12° W, 545 m), both in the north of Córdoba province, Spain. The climate in the area is Mediterranean with dry and hot summers and cool wet winters. Tree spacing and size in the Espiel stand was irregular, but the separation among holm oaks was large enough (> 18 m) to minimize competition for light, water or nutrients. In Hinojosa del Duque, measurements were performed in a stand planted in 1994 at a density of 100 trees ha^{-1} at a square planting pattern, and the soil was covered with natural grass grazed by sheep on a rotational schedule. According to Soil Taxonomy (Soil Survey Staff, 1999), the soils in Espiel and Hinojosa del Duque were classified as Haploxerept and Calcixerept, respectively.

Experimental measurements were conducted over three consecutive years (2009, 2010 and 2011) in Espiel and during 2009 in Hinojosa. Automated weather stations (Campbell Scientific Inc., Logan, UT, USA) located 50 m apart from the stands recorded the course of solar radiation, air temperature and humidity and wind speed at 10 min intervals throughout the experiments. Reference evapotranspiration (ET_0) was calculated with the Penman-Monteith-FAO equation (Allen et al., 1998). Mean daily temperatures typically ranged from 5–8 °C in winter to 25–30 °C in summer during the experimental period (Fig. 1a). ET_0 ranged from 0.3–1 mm d^{-1} in winter to 6.5–7.5 mm d^{-1} in July. In Espiel, annual ET_0 was 1250, 1141 and 1195 mm, respectively (Fig. 1b). The annual ET_0 in Hinojosa del Duque during 2009 was 1297 mm. Four trees (trees 1–4, Table 1) of different size were selected for sap flow measurements in Espiel. In 2009, trees 1 and 2 were drip irrigated from May 16 (DOY 136) to October 21 (DOY 274) with 2 L h^{-1} emitters distributed in a ring around the tree trunks (around 2 m apart). Because of their different crown size (Table 1), 20 emitters were installed for Tree 1 and 10 for Tree 2. The total water applied in the season was 13677 L for tree 1 and 6838 L for tree 2. Normalizing irrigation supply by crown projected area (see next section), the water applied was circa 150 mm for both trees, for a cumulative ET_0 of 782 mm during the same period. In subsequent years, no irrigation was applied, as there was no evidence of water stress in the non-irrigated trees in 2009 (trees 3 and 4, see Results for details). Besides, one individual at each location was chosen for performing measurements with a large canopy gas exchange chamber. A small tree in Espiel (tree 5) and one of average size in Hinojosa (tree 6) were selected for the purpose. Tree 5 was irrigated abundantly with a garden hose in the days preceding the measurements while Tree 6 was drip irrigated with 10 emitters of 2 L h^{-1}, applying 911 L from May 26 (DOY 146) to July 1 (DOY 182).

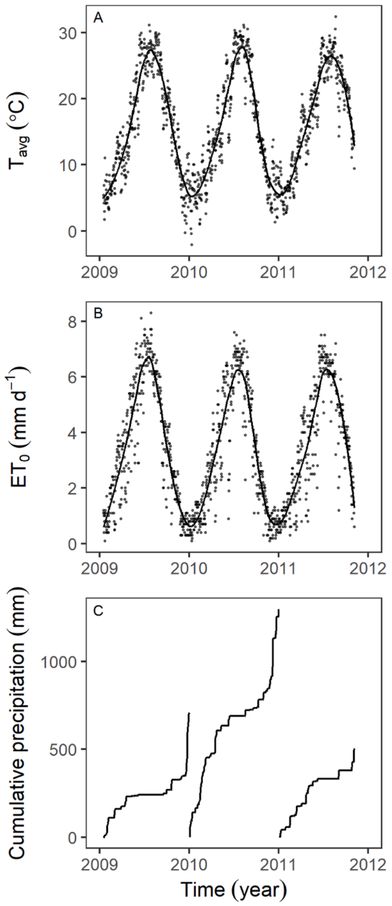

Fig. 1. Average daily temperature (T_{avg}, A), reference evapotranspiration (ET_0, B) and precipitation (C) in Espiel during the years 2009 – 2011. The curves in panels A and B are the result of fitting local polynomial regressions.

2.2. Plant leaf area and intercepted radiation

On April 22, 2009, the horizontal and vertical diameters of the canopy of the oak used in the Hinojosa del Duque experiment (tree 6) were measured. Radiation interception measurements were taken using a ceptometer (SunScan model, Delta-T Devices, Cambridge, UK). The volume of the crown of the tree was calculated, assuming a spheroid shape. Similar measurements were taken in the tree measured with the chamber in Espiel (tree 5), but with a single measurement for a zenith

Table 1

Main characteristics of the holm oak trees and their use in the experiments. V: crown volume, PLA: plant leaf area, PEA: mean projected envelope area, PA: vertically projected crown area, LAD: leaf area density. Crown radius is averaged from four orthogonal radii. Trunk radius and area refer to the cross-section where the sap flow sensors were inserted. The location of each tree (E = Espiel, H = Hinojosa del Duque) is indicated after each tree ID.

Tree ID	V (m^3)	PLA (m^2)	PEA (m^2)	PA (m^2)	PLA/PA	LAD (m^{-1})	Trunk area (dm^2)	Treatment	Measurement
1 (E)	456.6	310.5	75.6	107.1	2.9	0.7	10.7	Irrigated	Sap flow
2 (E)	127.3	114.6	32.3	46.0	2.5	0.9	5.7	Irrigated	Sap flow
3 (E)	273.0	166.5	52.3	66.5	2.5	0.6	13.2	Rainfed	Sap flow
4 (E)	113.2	88.3	29.8	41.3	2.1	0.8	8.5	Rainfed	Sap flow
5 (E)	4.8	7.6	3.9	3.1	2.4	1.6	-	Irrigated	Chamber
6 (H)	1.7	6.3	1.7	1.8	3.6	3.74	-	Irrigated	Chamber

angle of 1 radian. From these measurements, the total leaf area of each tree was calculated following the method of Lang (1987). This method estimates leaf area of sparse canopies by averaging the gap fraction detected with measurements of transmission of direct solar radiation for different zenith angles.

On August 13, 2009 the dimensions of the crowns of trees 1–4 were obtained by digitizing photographs of the North-South and East-West profiles of the tree crown. A metric rod was used vertically for reference. From the envelope of the profiles, the vertical paths were determined. Then, the Leaf Area Density, crown volume and tree leaf area were obtained following the procedure of Iniesta et al. (2009) with a Plant Canopy Analyzer (LAI-2000, LI-COR Biosciences, Lincoln, Nebraska, USA) along four radii parallel to the photographed profiles. The measurements were taken under diffuse radiation conditions around sunrise and the values of the central measurement ring of the instrument were selected (i.e., the crown transmissivity was evaluated in the vertical direction only).

The mean Projected Envelope Area (PEA) of the crown was measured for all trees as the area of the shade at the time when solar zenith angle is 1 rad (Villalobos et al., 2017). The vertical projection was approximated as the area of the shade at solar noon in June.

2.3. *Sap flow measurements*

Four trees in Espiel were selected for measuring sap flow rates (trees 1–4) during the three years of the experiment (2009, 2010 and 2011). The measurements were based on the Compensation Heat Pulse technique using a system designed and manufactured at the IAS-CSIC laboratory in Cordoba (Testi and Villalobos, 2009). Sap flow probes contained four embedded type E (chromel-constantan wire) thermocouple junctions that were sampled separately to obtain heat pulse velocities at 5, 15, 25 and 35 mm below the cambium. Heat-pulse velocities were corrected for wounding reactions (Green et al., 2003) and sap flow was obtained integrating sap flux densities across the radius (Green et al., 2003). Two sensors per tree and a sampling interval of 15 min were used. More details on the measurement protocol may be found in (Testi and Villalobos, 2009).

2.4. *Canopy gas exchange measurements*

A large transitory-state gas exchange chamber was used to estimate CO_2 and water vapor fluxes of individual trees in Espiel (tree 5) and Hinojosa (tree 6). Measurements were performed on several dates of 2009: on September 11 (DOY 254) in Espiel, and on April 26 (DOY 116), April 30 (DOY 120), July 1 (DOY 182), July 8 (DOY 189) and July 22 (DOY 203) in Hinojosa del Duque. Each day, fluxes of CO_2 and water vapor were obtained at 20 min intervals, plus 2–3 measurements before sunrise and after sunset. Details on the meteorological conditions for the days of measurements are given in Table S1.

The chamber, similar to those used by Pérez-Priego et al. (2010) and Roccuzzo et al. (2014), was designed and built at IAS-CSIC with a cubic shape of 2 m side (8 m^3 total inner volume). The chamber contained two 10 W fans of 15 cm diameter, to mix the air inside when it was closed during the measurement period (typically around three minutes). A vacuum pump drew the air from several intake points distributed within the chamber and then returned it to the chamber in a closed circuit. A sample of 1 L min^{-1} of this airflow was diverted with a small pump to a CO_2/H_2O infrared gas analyzer (IRGA, model LI-840, LI-COR Biosciences, Lincoln, NE, USA). The output was stored by a data logger (model CR23X, Campbell Scientific, Logan, UT, USA) for further analysis. An infrared thermometer (model IRR-P, Apogee Instruments, Logan UT, USA) was mounted on the center of the chamber top, facing downwards, to measure the temperature of the canopy. The sensor had a 44° field of view and was adjusted in height to detect only the tree foliage. The canopy temperature variation was checked at post-processing time to ensure that the disturbance in canopy temperature was negligible. Air temperature and relative humidity were also measured inside the chamber with a combined probe (model HMP45AC, Vaisala, Finland) placed near the top of the chamber within a radiation shield.

At the time of measuring, the fans were turned on and the top and lateral windows were closed. The CO_2 and water vapor concentrations, measured by the IRGA, were recorded at a 1 Hz sampling frequency for 3 min. After that, the windows were opened, and the fans turned off. A second order polynomial was fitted to each time series of gas concentration after discarding an initial lag time of 15 s required for the air to mix. Each flux was then calculated as the first derivative of the polynomial at time zero, before any change in the concentrations of CO_2 and water vapor within the chamber (Wagner et al., 1997). The gas fluxes were transformed to mass basis per surface and time unit, simultaneously, following (Catsky et al., 1971) and corrected for air temperature and atmospheric pressure inside the chamber (Reicosky et al., 1990).

The chamber was sealed several centimeters above the ground, so soil respiration and evaporation fluxes were excluded in these measurements, Thus, the analysis of CO_2 and water vapor measurements resulted in calculations of aboveground net photosynthesis (P_n, g CO_2 h^{-1}) and E_p (L h^{-1}) rates. From these two fluxes, the water use efficiency (WUE, g CO_2 L^{-1}) was calculated as the ratio of P_n and E_p.

2.5. *Measurements of water status*

To assess the effect of irrigation on the water status of the two holm oak stands, shoot water potentials were measured in leaves (i.e. after covering the leaves for 30 min prior to the measurements to prevent transpiration and ensure equilibrium with shoot water potential) using a pressure chamber (Soil Moisture Equipment, Santa Barbara, California, USA). On August 15, 2009 in Espiel, measurements of shoot water potentials were performed throughout the day in trees 1 (irrigated) and 4 (non-irrigated). In Hinojosa, pre-dawn and noon shoot water potential were determined in the selected oak (tree 6), as well as in two adjacent trees that were not irrigated, on the same days when canopy gas exchange measurements were conducted. Additional pre-dawn and noon measurements of shoot water potential were conducted in Espiel in trees 1 and 5 on September 11, 2009 (the same date in which canopy gas exchange measurements were undertaken in tree 5). Four replicates (leaves) were used in all cases.

2.6. Calculation of tree transpiration, photosynthesis and conductance

Tree E_p (L h^{-1}) was estimated from sap flow rates (F, L h^{-1}) by considering the lag between them by explicit integration of the following differential equation (Tyree, 1988):

$$E_p = F + \tau_s \frac{dF}{dt} \quad (1)$$

Where τ_s (h) is the time constant of water transport in the tree. dF/dt was estimated as the first derivative of a smoothing spline fitted to the time series of F for each day and tree. The value of τ_s was calculated knowing that, if E_p is rapidly decreased to zero (e.g. during a strong rainfall event), F will decrease exponentially in time with an exponential coefficient equal to τ_s. To approximate this condition, we looked for rainfall events that started during daytime (period 900 – 1500 h GMT) and accumulated more than 1 mm in the first 15 min period. For those events, we took the sap flux density of the first and second period (F_0 and F_{15}, respectively) to calculate the coefficient of the exponential decay of sap flow as $\tau_s = -0.25/\log\left(\frac{F_{15}}{F_0}\right)$, where 0.25 h is the time interval between F_0 and F_{15}. The calculations were performed for each tree for 16 rainfall events (Table S2), yielding an average τ_s of 0.53 h.

Following a similar procedure to that of López-Bernal et al. (2015), we estimated net assimilation (P_n, g CO_2 m^{-2} leaf) during the daytime as:

$$P_n = WUE \cdot E_p \quad (2)$$

This equation was implemented for trees 1–4 in Espiel using our estimates of E_p obtained from sap flow records and expressed per unit of leaf area, while water use efficiency (WUE, g CO_2/g H_2O) was calculated for each sap flow measurement as a function of VPD using the following phenomenological model:

$$WUE = \frac{1}{a + b \cdot VPD} \quad (3)$$

Where a (L H_2O/g CO_2) and b (L H_2O/g CO_2/kPa) are empirical parameters that were estimated by fitting the equation to the measurements from the gas exchange chamber in trees 5 (Espiel) and 6 (Hinojosa) using non-linear least squares. Then, the calculated values of P_n and E_p were integrated over monthly periods and estimates of WUE were obtained for each month of the year.

Besides, the canopy conductance of the whole tree (G_c, mmol m^{-2} leaf s^{-1}) was calculated by inverting the Penman-Monteith equation (Monteith and Unsworth, 2013) assuming a negligible aerodynamic resistance relative to canopy resistance (i.e. imposed evaporation) and using E_p rates (obtained from sap flow records) and VPD as inputs:

$$G_c = \frac{P_a E_P}{VPD} \quad (4)$$

where P_a is the atmospheric pressure (kPa). The assumption of negligible aerodynamic resistance is reasonable for highly-coupled surfaces such as isolated trees (Raupach and Finnigan, 1988), and has been used for holm oak trees by David et al. (2007).

3. Results

3.1. Tree characteristics

We can group the trees in 2 distinct groups, large trees (1−4) with crown radii larger than 3.6 m and small trees (5–6) with crown radii smaller than 1.25 m (Table 1). The volume of the tree crowns (Table 1) ranged from 1.7 m^3 (tree 6 in Hinojosa) to 456.6 m^3 (tree 1 in Espiel). Leaf Area Density was small (0.61–0.90 m^2 m^{-3}) in large trees (1−4), intermediate in the small tree in Espiel (Tree 5, 1.6 m^2 m^{-3}) and large in the small tree of Hinojosa (Tree 6, 3.74 m^2 m^{-3}). Leaf area was highest in the large trees in Espiel (88−310 m^2) and smaller in trees 5 and 6 (7.6 and 6.3 m^2, respectively). The ratio of leaf area and horizontal projection was between 2.1 and 2.9 for all trees in Espiel and 3.6 for the tree in Hinojosa. Trunk diameters of large trees were between 28 cm and 42 cm (Table 1).

3.2. Water status of trees

In order to verify whether the large holm oak trees in Espiel (trees 1–4, Table 1) suffered from water stress during the experiment, average daily transpiration per unit of leaf area ($E_{p,d}$) of the irrigated and rainfed trees were compared during 2009. The $E_{p,d}$ of rainfed trees was 35% larger than that of irrigated trees throughout 2009 and irrigation barely altered this ratio over the season (Fig. 2).

Measurements of shoot water potentials conducted on August 15, 2009, revealed similar patterns for tree 1 (irrigated) and 4 (non-irrigated) during the early morning, both reaching a minimum value of -1.7 MPa at 9.00 GMT. After that, water potentials recovered slightly in tree 1 whereas they remained constant at around -1.7 MPa for most of the day in tree 4 (Fig. S1). Similar values were measured at noon on September 11, 2009, for trees 1 and 5 (Table 2). In Hinojosa, pre-dawn shoot water potentials in tree 6 (irrigated) were above −0.2 MPa throughout July (Table 2). Noon shoot water potentials during the same days were also moderate, with a minimum of −1.0 MPa (Table 2). The same parameters were measured on neighboring non-irrigated trees, resulting in similar pre-dawn values and 20% more negative at the time of solar noon (Table 2).

3.3. Transpiration

Sap flow varied concomitantly with the course of solar radiation; during sunny days (Fig. 3) sap flow peaked in the central hours of the daytime and was quite high after sunset, decreasing slowly throughout the night, which was more evident during the summer. Sap flow at sunset was between 32% and 40% of the maximum value (Fig. 3). The time of maximum sap flow occurred on average around 4 h after sunrise in summer, 3.5 in autumn, 5 in winter and 6 h in spring.

$E_{p,d}$ of the large holm oaks (trees 1−4 of Table 1) followed a clear seasonal trend, present in all years of measurements (Fig. 4). Lowest values (circa 0.1 L m^{-2} leaf d^{-1}) occurred during winter and maximum values were found during May (1−1.2 L m^{-2} leaf d^{-1}). Important decreases in daily sap flow were associated to rainy days as wetting of the canopy suppressed E_p and decreased atmospheric demand (i.e. VPD). $E_{p,d}$ measured with the chamber in the small trees was larger, with a range of 0.9−4.1 L m^{-2} leaf d^{-1} (Table S1), but also peaking in late spring/

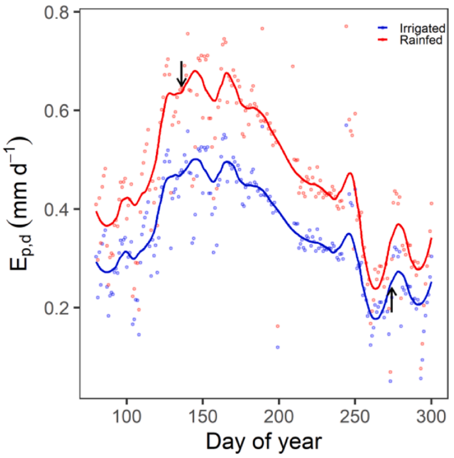

Fig. 2. Time course of daily transpiration per unit of leaf area ($E_{p,d}$) averaged over irrigated trees (1 and 2) and rainfed trees (3 and 4) in the year 2009. The arrows indicate the start (day of year 136) and end (day of year 274) of irrigation. The lines are the result of fitting local polynomial regressions.

Table 2
Shoot water potential before dawn (Ψ_p, MPa) and during solar noon (Ψ_n, MPa). “Others” refers to two unirrigated trees in the experiment of Hinojosa neighbouring the tree being measured. Tree numbers correspond to Table 1.

Hinojosa							Espiel		
	July 1		July 8		July 22			September 11	
	Ψ_p	Ψ_n	Ψ_p	Ψ_n	Ψ_p	Ψ_n		Ψ_p	Ψ_n
Tree 6	−0.1	−0.9	−0.2	−0.9	−0.2	−1.0	Tree 5	−0.8	−1.7
Others	−0.2	−1.2	−0.3	−1.2	−0.2	−1.2	Tree 1	−0.8	−1.6

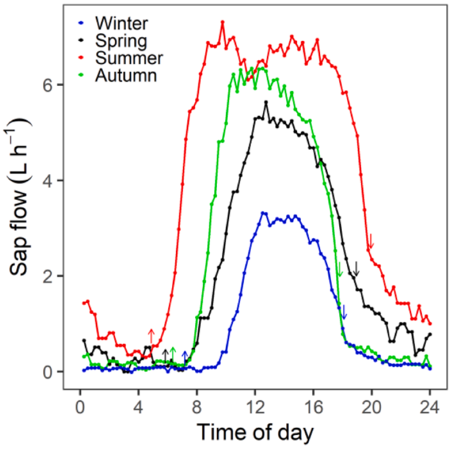

Fig. 3. Diurnal time series of sap flow for holm oak (tree 2, Espiel) in sunny days of spring (4 April 2009), summer (5 July 2010), fall (18 October 2010) and winter (6 February 2011). The arrows indicate the times of sunrise and sunset.

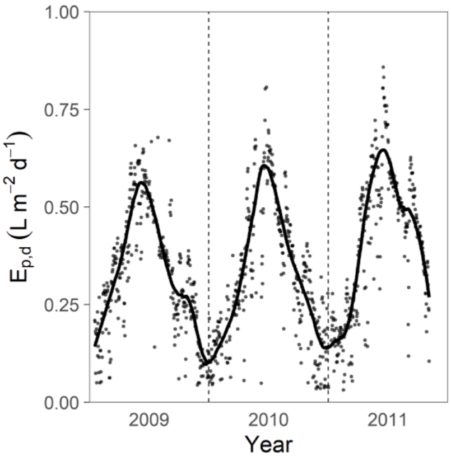

Fig. 4. Time course of daily transpiration per unit leaf area ($E_{p,d}$) in Espiel during 2009, 2010 and 2011 averaged across all four trees (trees 1 – 4, Table 1). The line is the result of fitting a local polynomial regression. No measurements were available for tree 2 after DOY 151 in 2011.

early summer. At the tree level, the average transpiration of the tree at Hinojosa del Duque during the measurement period was 15.5 L d^{-1}, compared to daily irrigation dose of 24.5 L d^{-1} during the same period.

There was an important variation in $E_{p,d}$ among the large holm oaks, with trees 2 and 4 being systematically larger than trees 1 and 3 (52% larger on average, Fig. S2). The difference in $E_{p,d}$ did not scale with the dimensions of the trees, as trees 1 and 3 were actually the largest (Table 1).

3.4. *Canopy conductance*

In sunny days, G_c peaked within 2−3 h after sunrise (except in summer, when it peaked 1 h after sunrise), decreasing throughout the rest of the day (Fig. 5). At the time of sunset, canopy conductance was between 9 and 37 mmol m^{-2} leaf s^{-1} which represent a range of 9−17% of the maximum daytime value. This diurnal pattern contrasts with sap flow (Fig. 3) and was the result of a strong effect of VPD on G_c (Fig. 6). The highest G_c values (above 300 mmol m^{-2} leaf s^{-1}) were observed in spring and the lowest happened during the summer with the peak value below 100 mmol m^{-2} leaf s^{-1} (Fig. 5).

3.5. *Water use efficiency*

WUE measured with the gas exchange chamber decreased with VPD (Fig. 7). The fitted hyperbola predicts a maximum WUE of 50 g CO_2 L^{-1} as VPD tends to zero and 4.6 g CO_2 L^{-1} at a VPD of 5 kPa which was the maximum recorded during the measurements. Monthly WUE varied from 7 g CO_2 L^{-1} (summer) to 25 g CO_2 L^{-1} (winter) with little variation among years (Table 3).

3.6. *Tree photosynthesis*

During sunny days, net photosynthesis calculated for the large trees peaked in the morning and decreased over the day (Fig. 8), with similar patterns to G_c (Fig. 5). Maximum values were in the range 0.9−1.6 g CO_2 m^{-2} leaf h^{-1} being larger for spring and autumn and lowest for winter.

For the large holm oaks, daytime net photosynthesis (i.e., excluding nighttime respiration) estimated from Eq. 2 increased from the winter to a maximum in late spring, then decreased during the summer, a second peak appeared in the autumn followed by a drop to minimum values during winter (Fig. 9). Maximum daily values typically exceeded 12 g CO_2 m^{-2} leaf d^{-1} in the spring while minimum winter values could drop below 5 g CO_2 m^{-2} leaf d^{-1}. Comparatively, estimates of net

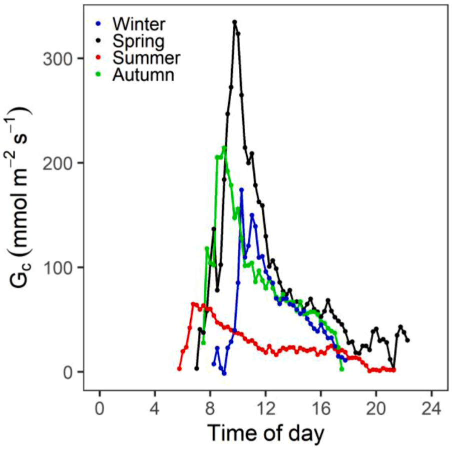

Fig. 5. Diurnal variation of canopy conductance for holm oak (tree 2, Espiel) in sunny days of spring (4 April 2009), summer (5 July 2010), fall (18 October 2010) and winter (6 February 2011).

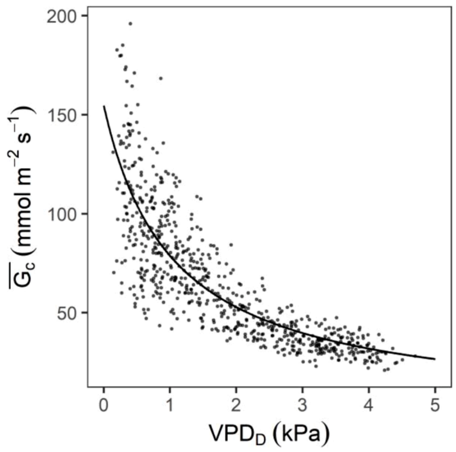

Fig. 6. Relationship between average daytime canopy conductance and mean daytime vapor pressure deficit (VPD_D) in Espiel during 2009, 2010 and 2011 averaged across all four trees (trees 1 – 4, Table 1). The fitted equation is $\overline{G_C} = 1000/(6.5 + 6.2\ VPD_D)$ ($R^2 = 0.69$, $n = 688$).

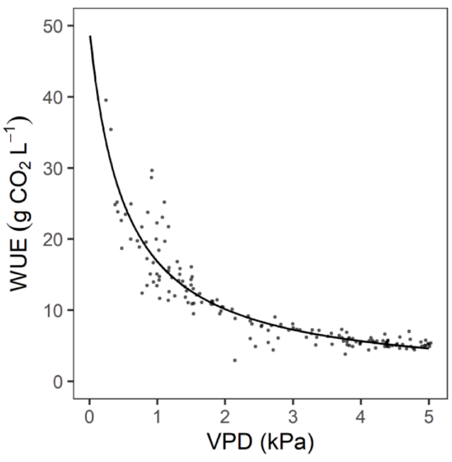

Fig. 7. Relationship between water use efficiency and vapor pressure deficit (VPD), of holm oaks (trees 5 and 6) in Espiel and Hinojosa, Spain. The line represents the fit WUE = 1/(0.020 + 0.039 VPD).

Table 3
Mean monthly water use efficiency (g CO_2 L^{-1}) of holm oak trees in Espiel. No measurements were available for tree 2 after DOY 151 in 2011.

Month	2009	2010	2011
January	24.1	23.9	23.0
February	19.9	21.7	18.7
March	15.3	18.9	18.5
April	15.9	14.8	12.9
May	11.8	13.4	12.9
June	8.8	10.4	8.8
July	7.1	6.7	7.5
August	6.8	7.1	7.6
September	10.9	10.7	9.3
October	12.0	14.8	10.7
November	17.3	20.5	19.6
December	24.9	22.2	

photosynthesis from the canopy gas exchange measurements for tree 6 had a range of total measured daytime net photosynthesis of 9.2–24.7 g CO_2 m^{-2} leaf d^{-1} while those for tree 5 in September yielded 8.9 g CO_2 m^{-2} leaf d^{-1} on September 11, 2009 (Table S1).

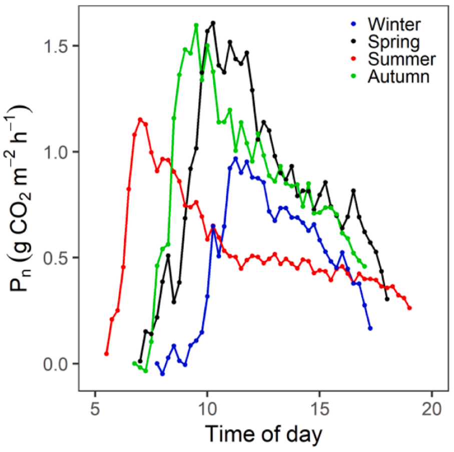

Fig. 8. Diurnal time series of tree net photosynthesis per unit leaf area for holm oak (tree 2, Espiel) in sunny days of spring (4 April 2009), summer (5 July 2010), fall (18 October 2010) and winter (6 February 2011).

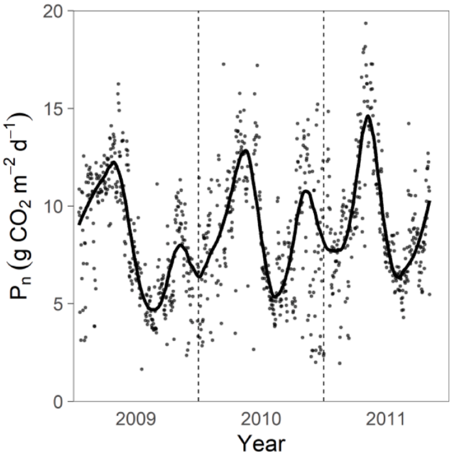

Fig. 9. Time course of calculated daily net photosynthesis per unit of leaf area in Espiel during 2009, 2010 and 2011 averaged across all four trees (trees 1 – 4, Table 1). The line is the result of fitting a local polynomial regression.

4. Discussion

In this study we have explored the response of tree conductance of holm oak to environmental conditions. Auxiliary irrigation was used to ensure good water status but no apparent effects on conductance were observed. This is at odds with the general response of trees to irrigation, namely increased growth, and higher photosynthesis and transpiration (e.g. Iniesta et al., 2009, for olive trees, Roccuzzo et al. 2014 for orange) and the observation that across a wide range of tree species (apricot, apple, citrus, olive, peach pistachio and walnut) tree potential conductance will be attained under irrigation (Villalobos et al., 2013).

Trees 1 and 2 received about 20% of ET_0 (calculated over projected crown area) as irrigation for 138 days centered on the drought period (Fig. 2), but in practice this irrigation had no relative effect on $E_{p,d}$ with respect to trees 3 and 4 that received no irrigation. There are two possible explanations: (i) that trees 1 and 2 did not make use of the water from irrigation and therefore suffered from the same level of water stress as trees 3 and 4 or (ii) that none of the trees suffered from water stress (e.g. because of access to deep soil water) and therefore our measurements in trees 1 – 4 correspond to potential transpiration.

As a complement, measurements of shoot water potential were taken on trees 1, 4 and 5 (Table 2, Fig. S1). These measurements indicate that pre-dawn and minimum shoot water potential during the day were similar in the three trees. Since tree 5 is much smaller than tree 1 or 4, it is more unlikely that it would not make use of the irrigation water. Also, published pre-dawn water potentials for holm oaks during the summer vary from −0.8 MPa (David et al., 2004) to −2 MPa (Infante et al., 1997), so our lowest values are in the upper end of the range. It is also possible that the trees in Espiel were accessing the water table, though its depth in this site was between 13 and 15 m, as measured in a nearby well. On the other hand, the analysis of water potentials in the unirrigated neighbors of tree 6 in Hinojosa indicates that irrigation in July did not improve water status either (Table 2). In an experiment in a cork oak stand in Portugal, Besson et al. (2014) also found a lack of response of predawn and midday shoot water potentials to irrigation, but they did observe a slight increase in seasonal potential transpiration in relation to non-irrigated trees.

Barbeta et al. (2015) showed that holm oak can develop a dimorphic root system with both superficial and deep roots that would then be able to make use of irrigation water during the summer. However, if that had been the case in our study, we would have expected a difference in the temporal evolution of sap flow between irrigated and non-irrigated trees during the summer of 2009 (Fig. 2), as well as significant differences in the leaf water potentials (Table 2). However, we cannot discard that the hydraulic characteristics of the trees may had changed over time if the irrigation treatment had continued over consecutive years, perhaps by adjusting the spatial distribution of roots or by adjusting other hydraulic limitations in the system. Testing such hypothesis over the long term dynamics requires further research.

The seasonal variation of $E_{p,d}$ in large holm oaks shows a clear decline from late spring onwards (Fig. 4). The maximum values per unit of leaf area were in the range from 1.0 to 1.2 L m^{-2} leaf d^{-1} (equivalent to 2.5–3.0 L m^{-2} crown area d^{-1}) and occurred at the end of May when ET_0 is around 5 mm d^{-1}. Although estimates of transpiration from sap flow without calibration may be biased (López-Bernal et al., 2015), our calculations are similar to reported values in the literature. For example, Paço et al. (2009) found a maximum of 1.15 L m^{-2} leaf d^{-1} (3.0 L m^{-2} crown area d^{-1}) at the end of May for large holm oak trees in Portugal whereas David et al. (2004) found slightly lower values (2.5 L m^{-2} crown area d^{-1}) occurring at the end of June in the same area.

Despite uncertainties about absolute values of transpiration, the seasonal and diurnal variations are reasonably accurate because of the use of the compensated heat pulse technique (Steppe et al., 2010). The aforementioned Portuguese researchers used the Granier heat dissipation method which is known to show a time lag that depends on sap velocity and sapwood properties and is prone to errors difficult to predict (Hölttä et al., 2015). Annual transpiration was 485 L m^{-2} crown area yr^{-1} for the large trees in Espiel, which is higher than the value of 414 L m^{-2} crown area yr^{-1} found by David et al. (2004) but much smaller than the results of Paço et al. (2009) which are close to 600 L m^{-2} crown area yr^{-1} in Evora. These authors recognized that their transpiration measurements were uncertain due to methodological problems inherent to the heat dissipation method. On an ecosystem area basis, the transpiration measured by the Portuguese researchers was between 120 and 160 mm yr^{-1} while in Espiel it was 121 mm yr^{-1} (25% ground cover). In any case, seasonal holm oak potential transpiration seems to be far below values reported for olive trees (612 mm yr^{-1} with 45% ground cover; (Orgaz et al., 2006), another Mediterranean sclerophyll adapted to the same environment.

Maximum transpiration per unit of leaf area of small trees was larger than that of large trees. Tree 5 in September 2009 had an average $E_{p,d}$ of 1.7 L m^{-2} leaf d^{-1} while tree 6 in Hinojosa had a range from 0.8 (early spring) to 4.1 L leaf m^{-2} d^{-1} (end of June) (Table S1). The smaller $E_{p,d}$ of large (older) trees may be explained by the acclimation of stomatal conductance in shaded leaves (e.g. Niinemets and Kull,1995) as the fraction of sunlit leaf area decreases with crown size of trees. However, when comparing among the large trees (trees 1–4), the higher $E_{p,d}$ occurred in the larger trees that also had higher leaf area densities (Table 1, Fig. S2), so we cannot discard a reduction of stomatal conductance with tree age like in other species (e.g. Baret et al., 2018).

The conservative behavior of holm oak stomata is clear in Fig. 5 that shows strong stomatal closure soon after sunrise on a summer day resulting in almost constant potential transpiration during most of the daytime (Fig. 3). Also, the time of the day at which conductance peaks shifts seasonally. This would suggest an isohydric behavior confirming the results of Quero et al. (2011) and Garcia-Forner et al. (2017) in North-East Spain although Aguade et al. (2015) found a more anisohydric pattern of holm oak when compared to *Pinus sylvestris* in that same area. Dewar (2002) showed that isohydric and anisohydric behaviors are the two extremes of a continuum that can be explained by a model relating stomatal conductance to photosynthesis, water potential and ABA. Some species are more conservative (isohydric), restricting the fall in water potential by a strong reduction in stomatal conductance as evaporative demand increases. This occurs at the expense of lower photosynthesis.

The response of holm oak stomata to evaporative demand shown in Fig. 6 is similar to that of other fruit trees grown in the Mediterranean area (apricot, apple, citrus, olive, peach pistachio and walnut), with conductance being inversely related to VPD (Villalobos et al., 2013). David et al. (2004) presented a similar relationship for holm oak canopy conductance though based on noon values only. This type of relationship, along with Eq. (4) may be useful for estimating maximum potential transpiration of holm oak stands as a function of VPD and tree size. This is simpler than trying to apply the approach of FAO which requires knowing ET_0 (rarely available locally and difficult to interpolate between existing stations in hilly agroforestry areas). We may use either ground cover or LAI for scaling up as the ratio of leaf area/ground cover was fairly conservative (average 2.49, standard deviation of 0.24, Table 1). This method is also more suited to semi-natural vegetation stands with intrinsic variability in tree size and density.

Despite the low seasonal conductance and potential transpiration, the monthly values of WUE were remarkably high (Table 3), always above 6.8 g CO_2 L^{-1} in summer and above 12 g CO_2 L^{-1} in spring. Interestingly, although the function WUE = f(VPD) was similar to that of olive trees (López-Bernal et al., 2015), the mean annual WUE was higher in holm oak (1115 g DM m^{-2} / 485 L m^{-2} = 2.3 g DM L^{-1}) than in olive trees (2000 g DM m^{-2} / 1200 L m^{-2} = 1.7 g DM L^{-1}). This is the result of the strong control of stomata that deeply restricts transpiration during the summer when the carbon gains per unit water lost is minimal. Therefore, chances of increasing productivity by irrigation in summer are scant, due to the limitations imposed by the strongly isohydric stomatal control. Further research should focus on managing supplementary irrigation for spring and autumn, which would result in enhanced vegetative growth and acorn productivity.

In the large trees, estimated net photosynthesis averaged 7.2 g CO_2 m^{-2} leaf d^{-1} (equivalent to 18.8 g CO_2 m^{-2} crown area d^{-1}) with mean solar radiation of 17.8 MJ PAR m^{-2} d^{-1}. In order to convert photosynthesis to net ecosystem exchange (NEE) we have to discount soil (root + heterotrophic) respiration and night respiration of aboveground organs. We may assume 25% of NEE for the former as found by López-Bernal et al. (2015) for olives and 10% NEE for the latter, which is the average measured with the chambers in small trees. With these numbers a net ecosystem productivity of 779 g CO_2 m^{--} leaf d^{-1} (2031 g CO_2 m^{-2} crown area yr^{-1}) can be estimated. On an ecosystem area basis, such an estimate is translated into 5.1 t CO_2 ha^{-1} yr^{-1} (25% ground cover), which is in the upper end of the range (1.0–5.1 t CO_2 ha^{-1} yr^{-1}) of net ecosystem productivity reported by Pereira et al. (2007) for a Portuguese dehesa including both cork and holm oaks.

We can also set an approximate upper limit for biomass and acorn productivities. Taking a ratio CO_2/dry matter of 0.5 and assuming that 50% of NEE goes to aerial biomass, annual biomass production results 428 g DM m^{-2} leaf yr^{-1} (equivalent to 1115 g DM m^{-2} crown area yr^{-1}). Assuming a harvest index of 0.3, maximum acorn productivity results 132 g DM acorn m^{-2} leaf yr^{-1} (334 g DM acorn m^{-2} crown area yr^{-1}), which is close to the maximum values reported by Gea-Izquierdo et al.

(2006) (286 g DM acorn m^{-2} crown area yr^{-1}). The results of the present and the aforementioned study suggest that improving acorn productivity of dehesas should be based on increased radiation interception by keeping a higher tree density.

Although the patterns in the reported data are clear and consistent with the hypothesis of a strong isohydric behavior, our observations were only performed on a relatively small number of trees at only two sites and irrigation was only used for one year. Furthermore, we did not collect information on the root system or spatial distribution of soil water, so there maybe alternative hypotheses that could explain the observations but cannot be tested with the data. Further uncertainties affect our estimates of net ecosystem, biomass and acorn productivities as they might be biased by some of the assumptions undertaken in the calculations. Hence, conclusions from this study should be applied with caution to other environments and further verification of these results would be needed in additional sites and environments.

5. Conclusions

In this experiment the summer irrigation of holm oaks has produced marginal or null effect over the trees' water status, a fact confirmed even during the very dry and water demanding conditions of summer 2009, when irrigated and non-irrigated trees showed similar shoot water potentials and transpiration rates. This somewhat unexpected behavior implies that canopy conductance of holm oaks is weakly dependent on water source availability but strongly on atmospheric water vapor demand (also demonstrated in this work). All these observations depict the holm oak as a species characterized by a strong isohydric behavior which exerts an intense stomatal control to avoid excess of transpiration at high VPD - and therefore assumes the associated cost in terms of lower carbon assimilation - almost regardless the water available to the roots. The isohydric pattern may be seen in holm oak as a conservative adaptive trait favored in dry environments where high WUE is more rewarding than fast growth in terms of survival and propagation chances. In light of our results, it is unlikely that summer irrigation of holm oak plantations in southern Spain will significantly increase the stand productivity due to the high VPD and the species isohydric response, though further verification of these results would be needed in additional sites to ensure generality.

A general corollary that may be drawn from this research is that we can expect that the response to irrigation of Mediterranean species is inversely proportional to their level of isohydricity. Last but not least, the empirical hyperbolic models of canopy conductance and water use efficiency as a function of VPD developed in this study may serve for estimating diurnal and seasonal courses of potential transpiration and net photosynthesis of holm oaks if tree size and leaf area density are known.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tfp.2021.100115.

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