The effect of deficit irrigation on seasonal variations of plant water use in *Olea europaea* L.

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Abstract

A field experiment on olive trees (*Olea europaea* L.) was designed with the objective to search for an optimum irrigation scheduling by analyzing the possible effects of deficit irrigation. Treatments were: a non-irrigated control (rainfed) and three treatments that received seasonal water amount equivalent to 33 and 66% of crop evapotranspiration (ET_C) in the period August–September (respectively 33II and 66II), and 66% of ET_C from late May to early October (66I-II). Atmospheric evaporative demand and soil moisture conditions were regularly monitored. Irrigation effects on plant water relations were characterized throughout a growing season. Whole-plant water use, in deficit irrigated (66I-II) and rainfed olive trees, was determined using a xylem sap flow method (compensation heat-pulse technique). The magnitude of variations in water use and the seasonal dynamic of water relations varied among treatments, suggesting that olive trees were strongly responsive to both irrigation amount and time. Physiological parameters responded to variations in tree water status, soil moisture conditions and atmospheric evaporative demand. All measurements of tree water status were highly correlated with one another. There was a considerable degree of agreement between daily transpiration deduced from heat-pulse velocity and that determined by calibration using the water balance technique. Deficit irrigation during the whole summer (66I-II) resulted in improved plant water relations with respect to other watering regimes; while, severe regulated deficit irrigation differentiated only slightly 33II treatment from rainfed plants. Nevertheless, regulated deficit irrigation of olive trees after pit hardening (66II) could be recommended, at least in soil, cultivar and environmental conditions of this study.

Introduction

In Mediterranean-type agro-ecosystems olive tree (*Olea europaea* L.) has traditionally been cultivated in marginal areas with no irrigation, despite rainfall does not meet evapotranspirative demand (Fernández and Moreno, 1999). To improve yield and maintain low water consumption, modern olive plantations with relatively high number of trees per hectare require drip irrigation. Growth conditions that maximize crop growth in general optimize production, though a positive yield response to mild water deficits has been demonstrated for several fruit trees in Mediterranean environments (Girona et al., 1993; Marsal et al., 2002). Indeed, a sagacious irrigation approach is a major task in modern olive growing due to the limited water resources available in Mediterranean countries (Villalobos et al., 2000). Olive is a well-known drought avoidance species, though its degree of control over water

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loss under stressful conditions depends on structural and functional properties that have rarely quantified in field studies (Fernández et al., 1997; Moriana et al., 2002).

The efficient management of limited water resources in Mediterranean agriculture requires irrigation scheduling techniques based on deficit supply with respect to crop water losses. In particular, regulated deficit irrigation is applied by reducing irrigation rates only in those periods when fruit growth is less sensitive to water, and irrigation reductions are often defined as a percentage of an optimal irrigation rate (Marsal and Girona, 1997). The water balance technique developed by FAO (Doorembos and Pruitt, 1977) for scheduling irrigation is based on calculations of accumulated reference evapotranspiration and crop coefficients, which may fail in situations requiring accurate estimation of crop water consumption. A more comprehensive knowledge on the water movement in the soil–plant–atmosphere continuum for mature olive orchards may be gathered by directly monitoring whole plant transpiration (Fernández et al., 1997; Moreno et al., 1996).

Localized irrigation systems may limit transpiration in olive trees, thus increasing the water use efficiency but potentially decreasing crop performances (Fernández et al., 2003). During periods of moderate water stress, olive trees typically experience reductions in transpiration and gas exchange (Giorio et al., 1999). Under severe drought non-stomatal factors may further affect assimilation (Angelopoulos et al., 1996). Moriana et al. (2002) found that gas exchange responded diurnally and seasonally to variations in tree water status and evaporative demand. Though, in addition to these physiological and environmental variables, chemical and hydraulic signals are also involved in controlling stomatal conductance. Roots left in dry soil may drive stomatal closure through hydraulic and hormonal signals (Bongi and Palliotti, 1994; Fernández et al., 2003), thus influencing water uptake. Seasonal and diurnal transpiration efficiency of olive trees grown in the field is a complex character affected by plant water status. A combined analysis of relationships amongst transpiration, atmospheric evaporative demand and soil moisture, and between stomatal behavior and whole-tree hydraulic conductance, could provide a powerful tool to assess the sensitivity to deficit irrigation.

Regulated deficit irrigation strategies require a good knowledge of the phenological stages in which water stress can considerably reduce plant performances. In olive, the main body of irrigation research has dealt with season-long uniform irrigation regimes, and this novel approach needs to be calibrated for each cultivar–environment combination (Goldhamer et al., 1994). The growth curve of olive is double sigmoidal with two periods of increasing growth rate. The asynchronous growth of fruits and shoots reduces competition for resources at critical stages, providing a sound basis for the application of regulated deficit irrigation (Rapoport et al., 2004). Regulated deficit irrigation applied to olives over a 10-week period following pit hardening had no adverse effect on oil production (Alegra et al., 1999). Goldhamer (1999) reported water savings of 25% for regulated deficit irrigation applied to olives in California, with no yield reduction.

Irrigation effects on whole-plant sap flow, leaf-level water relations, and yield and oil composition were characterized in an experimental commercially like olive orchard, cv. Kalamata, nearby Benevento (southern Italy); atmospheric evaporative demand and soil moisture conditions were also monitored. Tognetti et al. (2004) monitored, throughout a growing season, fully irrigated (100% of crop evapotranspiration replaced) and non-irrigated olive trees, observing that physiological and environmental factors were involved in the control of water use in these olive trees during soil drying, by inducing feedback mechanisms and direct responses. Giorio et al. (1999) studied plants subjected to three irrigation treatments (0, 33 and 66% of crop evapotranspiration replaced) and invoked both hydraulic feedback and feed-forward mechanisms in the response of stomata to soil drying. Deficit irrigation decreased fruit growth during and after pit hardening, at peak water stress, (d'Andria et al., 2000); the highest yield was obtained from fully irrigated treatments but with modest gain as compared to trees with 66% of crop evapotranspiration replaced, while the oil quality did not vary consistently amongst watering regimes (Patumi et al., 2002). The higher oil yield obtained in the irrigated treatments resulted from the increase in the pulp–stone ratio, while the quantity of triglycerides

accumulating in fruits were similar for trees with 0, 33, 66 or 100% of crop evapotranspiration replaced (Patumi et al., 1999).

Overall, these olive trees were confirmed to be economical and sparing users of soil water, with an efficient xylem sap transport, maintenance of significant gas exchange and transpiration, even during drought stress. Moderate levels of water stress applied to olive trees, by withholding irrigation in a deep soil during the period of slow fruit growth, could maintain or even increase fruit and oil yields, while controlling excessive vegetative growth, without detrimental effects on olive oil quality or textural properties. The aim of the present study was to gain an understanding of temporal variations in water relations of olive trees as affected by regulated deficit irrigation treatments applied during different phenological stages, for optimum productivity of the cv. Kalamata in a typical Mediterranean environment of southern Italy characterized by relatively good late spring precipitation and deep soil profile.

Materials and methods

Study site and experimental design

The experiment was conducted in the summer 2003 on 12-year-old olive trees (*Olea europaea* L.) of the cv. Kalamata (used either for oil or picking) at the experimental farm of CNR-ISAFOM, located near Benevento ($41°06'$ N, $14°43'$ E; at an elevation of 250 m a.s.l.), a typical olive growing area of southern Italy. The site is flat and the trees are planted in rows 6 m apart, at a tree spacing of 3 m. The orchard was established in 1992 with one-year-old plants, grafted on DA 12 I clonal rootstock (patent CNR n. 1164/NV). Trees were clean cultivated and trained using the "monocone" system (Fontanazza, 1994). They have been pruned every year according to standard procedures for central leader training system. The climate is Mediterranean, with a mean annual rainfall of 714 mm (20-year average, 1984–2003); the amount of rainfall, however, is decreased in recent years (698 and 676 mm, respectively for the last 10- and 5-year average). The daily mean temperature generally increases from 10–12 °C in April

to 24–25 °C at the end of July, decreasing to 18–20 °C in September. The yearly mean reference evaporation is about 1240 mm and values increase from about 3 mm day^{-1} in April to about 8 mm day^{-1} in July, starting to decrease in August. The soil is sandy loam (organic matter 1.76%, $CaCO_3$ 1%, N 0.15% and pH 7.2), characterized by volumetric water content (m m^{-3}) of 35.6% at field capacity (soil matric potential of -0.03 MPa) and 21.2% at wilting point (soil matric potential of -1.5 MPa), whereas the apparent bulk density was 1.25 t m^{-3}. Formerly, soil water content at field capacity was measured soon after irrigation (nearby emitters) and wilting point was defined as the soil water content at which plants do not recover overnight from water stress inducing wilt (Taiz and Zeiger, 1991).

The groundwater used for irrigation has a pH of 7.4 and electrical conductivity equal to 0.68 dS m^{-1} (Na$^+$ 1.825 mM, K$^+$ 0.299 mM, Ca^{++} 3.3 mM, Mg^{++} 0.346 mM and Cl$^-$ 0.54 mM). During the first two years after planting, all trees were irrigated equally to guarantee the uniformity of plant development. In summer 1994, irrigation treatments were started based on crop evapotranspiration (ET$_C$, mm), subtracting useful rainfall. ET$_C$ was derived from Class A pan evaporation (Doorembos and Pruitt, 1977) placed in the proximity of a standard meteorological station adjacent to the experimental field, which recorded half-hourly averages of global radiation, air temperature, relative humidity, wind speed and rainfall. Pan evaporation (ET$_P$) data were corrected with a pan coefficient (K_P) of 0.8 (to obtain reference crop evapotranspiration, ET$_0$, mm), a crop coefficient (K_C) equal to 0.65 and a tree ground cover coefficient (K_R) of 0.85 (Fereres et al., 1981); a single K_C was adopted, since irrigation started relatively late in the season. The orchard trees were irrigated daily, 4 L h^{-1} tree^{-1}, using a system with four drip nozzles per plant, connected to a single drip line at a distance of 0.5 and 1 m from the trunk (two per side along the row). Olive trees were tested in a factorial combination with four irrigation levels (Table 1): a non-irrigated control (rainfed) and three treatments that received seasonal water amount equivalent to 33 and 66% of ET$_C$ in the period August–September (respectively 33II and 66II), and 66% of ET$_C$ from late May to early

Table 1. Applied irrigation and scheduling during main stages of fruit development in irrigated (33 and 66% of ET_C replaced) and non-irrigated (control) olive trees during the experimental period (See text for details)

Phenological phase	Bloom	Stage I	Stage II	Harvest	Irrigation volume
Treatment	May	June–July	August–September	October	mm
Control					0
33II			$\longrightarrow$		47.50
66II			$\longrightarrow$		94.67
66I-II			$\longrightarrow$		198.26

October (66I-II). The experimental design was a complete randomized block, replicated four times. Each plot consisted of seven adjacent trees, three of which (central) were selected for leaf level measurements and one for sap flow determination (only non-irrigated control and 66I-II treatment).

Soil water measurements

Volumetric soil water content (θ, $m^3 \, m^{-3}$) was determined by means of time domain reflectometer (TDR, 1502B, Tektronix, USA); self-constructed TDR probes of the balanced type (Clothier and Green, 1994) were used. A total of 21 wave-guides were installed vertically into the soil around each tree (three replicates) to record changes within 0–140 cm depth interval (with 20 cm increments), at 75 and 125 cm from the trunk within the row (measurements were averaged and reported as 100 cm) and at 150 cm between rows. Each TDR wave-guide comprised three parallel probes (5 mm in diameter stainless steel rods), which were manually connected in sequence to the TDR via a coaxial cable. Measurements of soil moisture were done periodically from May to October. Soil moisture deficit (SMD, mm) was calculated as the difference between cumulative ET_C and cumulative rainfall; it was assumed that by the end of April soil water reserves were replenished by winter and early spring precipitation (318 mm).

Leaf water potentials and stomatal conductance

Leaf water potentials (Ψ, MPa) were periodically measured on four leaves from three individual plants for each replicate plot, at predawn (Ψ_{PD}) and midday (Ψ_{MD}). Fully expanded leaves were detached from mid-canopy (shoot of the current year), wrapped in plastic envelopes and rapidly enclosed in a Scholander-type pressure chamber (SKPM 1400, Sky Instruments, UK). Stem water potential (Ψ_{STEM}) was monitored at midday on leaves previously enclosed in reflective envelopes to suppress leaf transpiration, allowing leaf water potential to equilibrate with stem water potential at the point of attachment; equilibration periods took 1–2 h. Since the pressure bomb was placed below the canopy, a few seconds were needed before pressurizing the chamber.

In the same days, measurements of stomatal conductance (g_s, mmol $m^{-2} \, s^{-1}$) were done with a transient porometer (AP4, Delta-T Devices Ltd., Cambridge, UK) on similar foliage material from the same replicates, in mid morning and at midday.

Sap flow measurements

Sap flow was monitored within the trunk of control and 66I-II trees (four replicates) using the compensation heat-pulse technique (Huber and Schmidt, 1937; Marshall, 1958; Swanson and Whitfield, 1981). Two sets of heat-pulse gauges (Environment and Risk Management Group, HortResearch Institute, Palmerston North, New Zealand) were installed into parallel holes drilled in radial positions (North and South side) into the semi-trunk of each tree, at a height of about 50 cm. The heat-pulse gauge consists of a heater of diameter 1.8 mm and two temperature-probes of the same diameter (one at 15 mm downstream and the other at 5 mm upstream of the heater). Each temperature probe has four (copper-constantan) thermocouple junctions spaced along the radius of the cross section. After the heat-pulse has been released, its 'ideal' velocity (v_h, mm s^{-1})

can be calculated by measuring the 'crossing' time (t_z, s) needed for the up- and down-pair of thermocouples to reach an equal temperature during the heating, according to the equation: $v_h = (x_d + x_u)/(2t_z)$, where x_d and x_u are the relative distances (x, mm) of the downstream- and the upstream-sensor, respectively, from the heater which is used to release the heat-pulse. Every half hour, after 1 s heat pulsing, t_z of all four pairs of thermocouples was monitored to calculate heat-pulse (ideal) velocity along the radial profile. Ideal velocity was then corrected by empirical coefficients as given by Green and Clothier (1988) for 2.0 mm wound width, to allow for the non-ideal water transport in the plant tissue and the intrusive nature of the gauges. Such coefficients were found to be correct for this species in calibration experiments carried out by Fernández et al. (2001) using the same system. From the radial profile of corrected heat-pulse velocity (v_c, mm h^{-1}), sap flux density is estimated and then integrated over all the cross section to obtain sap flow (Q, l h^{-1}) (see Fernández et al., 2001; Giorio and Giorio, 2003 for details; Green and Clothier, 1988). Heat-pulse gauges were powered by 12 V batteries and controlled by data-loggers CR 23X (Campbell Sci. Co., Logan, UT, USA). We used data of sap flow monitored at half hour intervals in the period June–September (2003). Trees were selected among those of similar size, basal area below cambium being 2147 ± 315 and 2322 ± 375 mm^2 in non-irrigated control and 66I-II treatment ($P > 0.05$), respectively. Values of sap flux density were expressed per unit sapwood area. Since the compensation heat-pulse technique does not distinguish low-flow from no-flow conditions, because of the inherent inaccuracy associated with integrating nightly sap-flow measurement (Burgess et al., 2001), all night-time sap flow velocities below the limit of detection were set to zero (Smith and Allen, 1996).

Canopy stomatal conductance and whole-plant specific hydraulic conductance

Sap flux is equivalent to transpiration on time-scales particular to the plant size of interest, as size-dependent time lags between sap flux at the stem, and water loss at the leaves occur as a result of water storage capacity. Although capacitance in large trees may cause time lags exceeding the 30-min averaging period used in the study, the effect has been found to be negligible in small trees (Pataki et al., 2000). Thus, sap flux was assumed to be equivalent to transpiration in order to calculate the average daytime stomatal conductance of the canopy (mmol m^{-2} s^{-1}): $G_C = (\gamma \lambda Q)/(\rho c_p \delta w)$, where γ is the psychrometric constant (Pa K^{-1}), λ is the latent heat of vaporization (J kg^{-1} K^{-1}), ρ is the density (kg m^{-3}) and c_p the heat capacity of air (J kg^{-1} K^{-1}), δw is the vapour pressure difference (Pa), and Q is the transpiration (kg m^{-2} s^{-1}). Daylight estimates of canopy conductance took into account treatment-specific leaf area index (from allometric relationships) and number of plants per hectare. When the canopy is well coupled to the atmosphere, and leaf and air temperatures are not different, the vapour pressure deficit may be substituted for δw. If boundary-layer conductance far exceeds the magnitude of stomatal conductance (during conditions of high wind, over rough canopies, where leaf dimensions are small), canopy stomatal conductance will be equivalent to average true stomatal conductance, and reflect stomatal behavior rather than physical processes governing boundary layer (Jones, 1992).

When possible, measurements of leaf water potentials were combined with those of sap flux (relative to sapwood cross-sectional area) to calculate whole-plant specific hydraulic conductance (K_{S-L}, kg m^{-2} MPa^{-1} s^{-1}) following the single point method ($K_{S-L} = Q_{MAX}/\Delta\Psi$; where $\Delta\Psi$ is the difference between Ψ_{PD} and Ψ_{MD}, and Q_{MAX} is sap flux density during peak transpiration at midday) (Wullschleger et al., 1998); analogously soil-to-stem hydraulic conductance ($K_{S-S} = Q_{MAX}/\Delta\Psi$, where $\Delta\Psi$ is the difference between Ψ_{PD} and Ψ_{STEM}).

Statistical analysis

Data were averaged on a plant basis and statistical analysis was conducted using GLZ (Generalized Linear Models) procedures of the statistical package Statistica (StatSoft Inc., Tulsa, OK, USA). Treatment means were compared with a least squares means procedure. Statistical comparisons were considered

significant at $P = 0.05$. Analysis of covariance (ANCOVA) was used to test the equality of regression coefficients.

Results

In the year 2003, the site was characterized by good precipitation in spring months, particularly June (74 mm) and scarce rainfall in the period July–October (37 mm per month). Scant precipitation during summer season (Table 2) did not balance the high seasonal ET_C (368.6 mm in the irrigation period). The climatic aridity index, calculated as precipitation/potential ET calculated by the method of Penman (Doorembos and Pruitt, 1977), averaged 0.30 for the summer period (June–September); this value sets the area among semiarid-sub humid zones. Total and useful precipitations (above 5 mm in a 24 h period) for the irrigation period were 164.2 and 68.4 mm, respectively. Total irrigation volume for 33II, 66II and 66I-II treatments is reported in Table 1.

The dynamics of θ showed the typical pattern, with decreasing values during the summer coinciding with higher temperature ($P < 0.0001$), evapotranspiration and soil moisture deficit (Figure 1). Soil moisture reduction throughout the growing season showed similar patterns for the different sample depths, so that the average of

these levels is reported. Soil moisture at the beginning of experimental period was alike either between irrigation treatments or sampling positions. In general, irrigation resulted in sorting treatments in two separate groups ($P < 0.01$), rainfed and 33II *versus* 66II and 66I-II. Within rows, volumetric soil water content decreased progressively near non-irrigated trees and remained below the wilting point threshold (about 21% as volume) throughout the period from mid August to late September. Values of q near continuously deficit-irrigated (66I-II) plants were constantly between field capacity threshold and 50% available moisture (respectively, about 36% and 29%) during the first half of the season, then gradually declined to about 50% available moisture by the end of the summer. Volumetric soil water content of plots deficit-irrigated only from early August with 66% of ET_C (66II) rapidly recovered to values observed for 66I-II treatment (probably boosted by summer showers), while 33II treatment remained somewhat above the wilting point. Between rows, differences in θ between treatments were less pronounced. Volumetric soil water content, from stage II to the end of irrigation, similarly kept on the wilting point threshold in rainfed and 33II plots, while ranged between 50% available moisture and the wilting point in 66I-II and 66II plots. At harvest time (late October), soil moisture in the vicinity of all plants (regardless of the

Table 2. Seasonal agroclimatic characteristics (precipitation, evapotranspiration and aridity index) at the study site; in italics are reported late May and early October periods (See text for details)

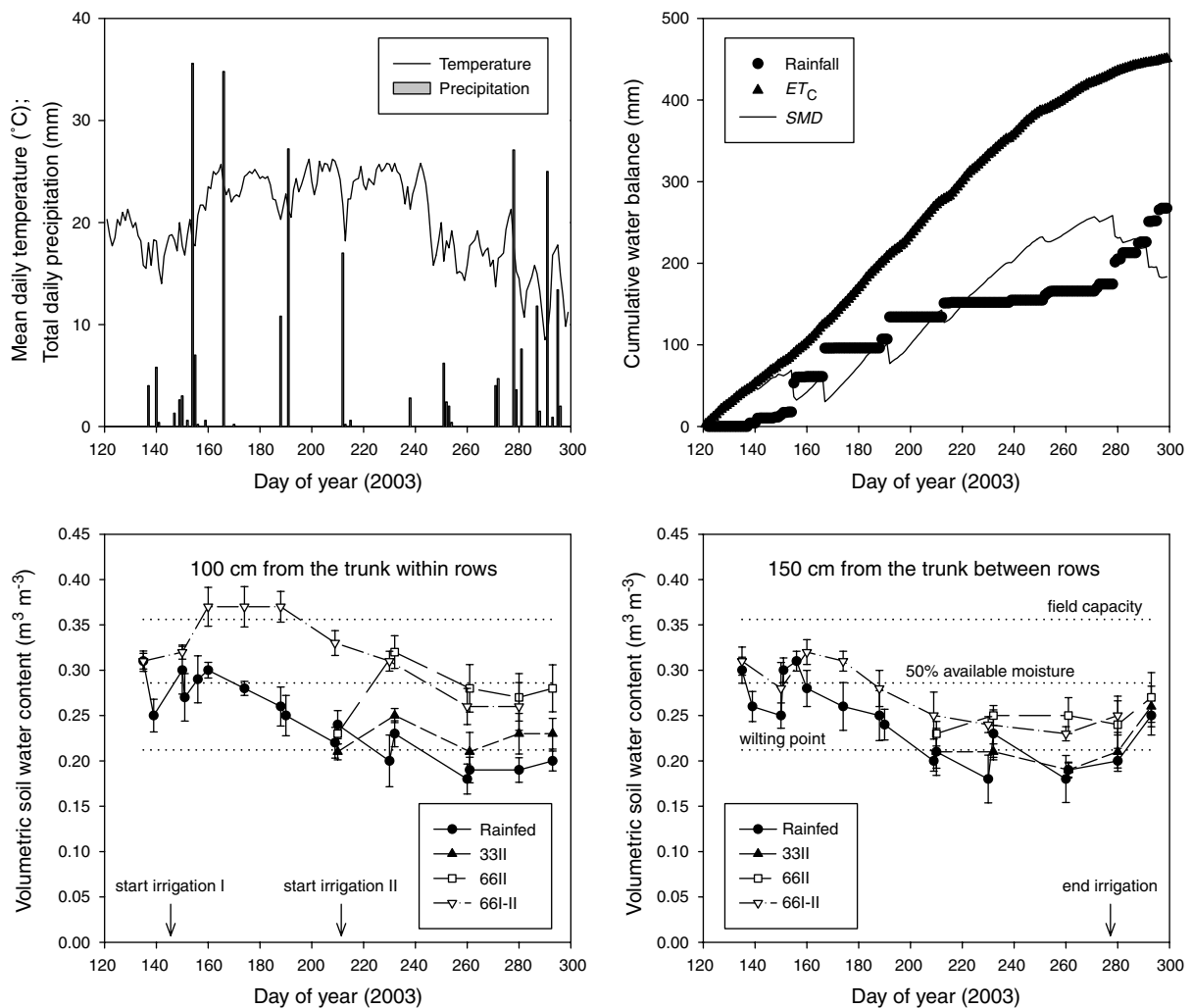| Month	Precipitation (mm)		Evapotranspiration (mm)		Aridity index
	Average 1984–2003	Total 2003	ET_C	ET Penman	
January	63.9	161.0			
February	57.5	42.6			
March	58.0	27.4			
April	73.7	54.4			
May	46.6	17.1	16.6		
June	33.4	79.0	94.2	142.58	0.55
July	38.3	55.0	103.3	150.54	0.37
August	31.3	3.6	91.5	129.6	0.03
September	54.3	19.7	57	83	0.24
October	64.0	9.1	5.9		
November	113.6	47.0			
December	79.8	77.6			
Annual	714.3	593.5			

Figure 1. Daily values of mean air temperature and total precipitation, cumulative water balance (rainfall, crop evapotranspiration and soil moisture deficit) and seasonal evolution of volumetric soil water content during the experimental period at the study site (see text for details). Vertical bars for θ represent standard errors ($n = 3$ replicates); error bars not visible indicate SE smaller than the symbol. Watering regimes are referred to by symbols in the legend.

treatment) was recovering to pre-summer values between rows, while lagging behind within rows.

Irrigation treatments resulted in a wide range of leaf water status (Figure 2). Predawn and midday leaf water potentials decreased progressively and concomitantly with increasing seasonal drought ($P < 0.0001$), reaching the lowest values between the end of August and mid September, when θ was at its minimum. By this time, the four treatments were clearly ranked ($P < 0.0001$) according to the level of stress reached; rainfed > 33II > 66II > 66I-II. However, minimum values of Ψ_{PD} and Ψ_{MD} never exceeded −1.5 and

−3.5 MPa, respectively, regardless of the treatment. In early fall, Ψ_{PD} and Ψ_{MD} were not completely recovered yet to spring values. Maximum and midday stomatal conductance followed similar seasonal patterns (Figure 2). Stomatal conductance reached invariably the highest daily values in mid June ($P < 0.0001$). Daily maximum g_s stabilized on 300 mmol m^{-2} s^{-1} in 66II and 66I-II treatments, while decreased down to 200 mmol m^{-2} s^{-1} in 33II and dropped to about 100 mmol m^{-2} s^{-1} in rainfed plants. By the end of the summer, irrigation treatments differed significantly ($P < 0.0001$); 66II and 66I-II > 33II > rainfed. Midday g_s
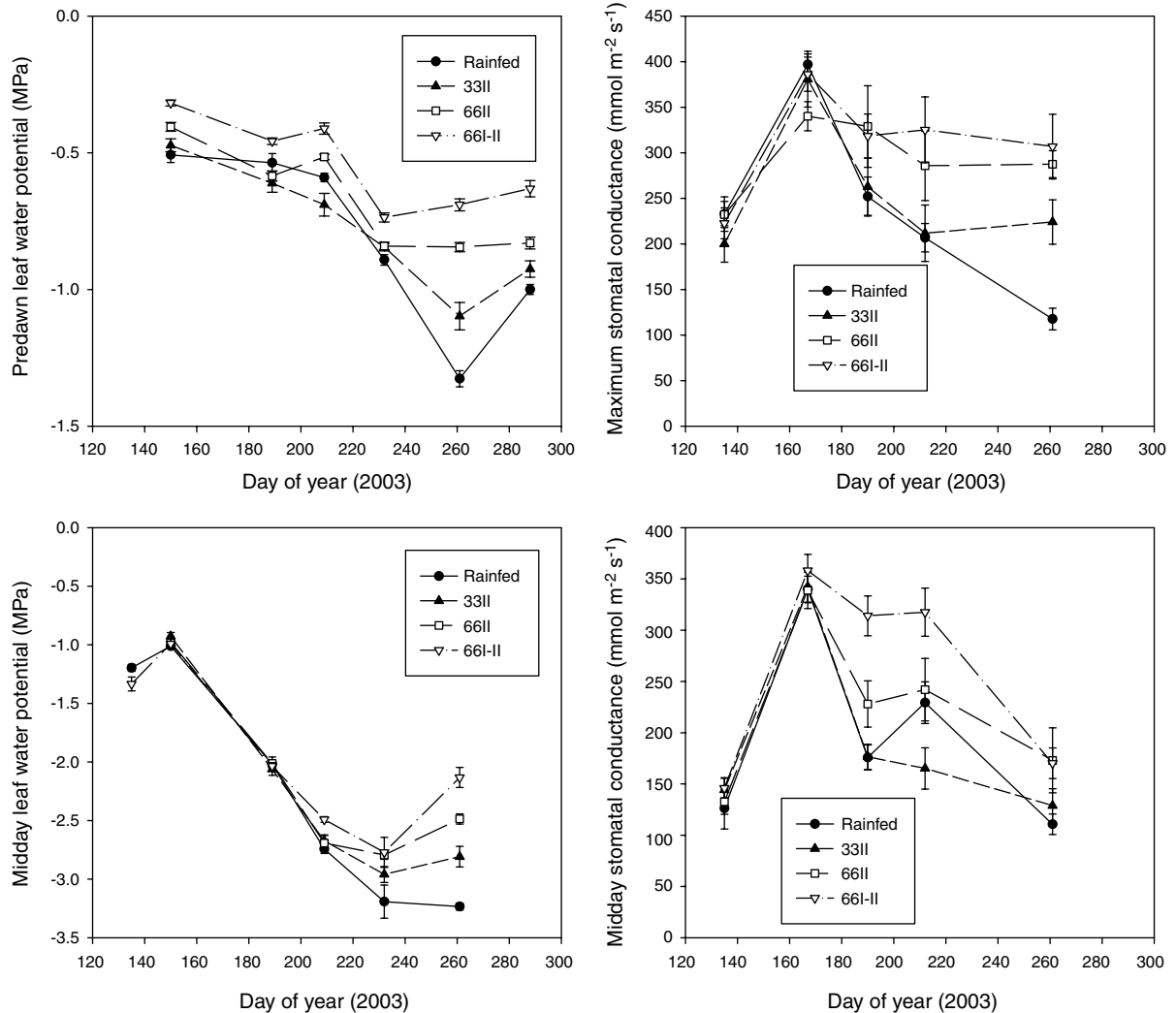
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Figure 2. Seasonal fluctuations in predawn and midday leaf water potential, and in maximum and midday stomatal conductance of olive trees during the experimental period. Vertical bars represent standard errors ($n = 4$ replicates) error bars not visible indicate SE smaller than the symbol. Watering regimes are referred to by symbols in the legend.

showed a similar treatment ranking, though less clear with respect to maximum g_s.

Stem water potential behaved similarly to Ψ_{MD} (Figure 3), with separation among irrigation treatments ($P < 0.01$) increasing with proceeding of summer drought ($P < 0.0001$) and depletion of θ. All three methods of estimating olive tree water status were highly correlated with one another (Figure 3). The best correlation was between Ψ_{MD} and Ψ_{STEM}.

Diurnal patterns of sap flow showed, for rainfed and 66I-II plants (antipode watering regimes), a steep morning increase leading to maximum rates achieved in the central hours of

the day (when VPD and irradiance were at their maximum), followed by a sustained gradual decrease until late in the afternoon (Figure 4). Rainfed plants showed invariably lower ($P < 0.0001$) daytime sap flow rates than 66I-II plants, particularly in mid and late summer. Plots of sap flow *versus* irradiance and VPD showed, in general, lower hysteresis effects in the latter case (data not shown). Hysteresis in Mediterranean environment may be a common response because stomatal conductance is always higher in the morning than in the afternoon. Sap flow lagged behind irradiance in 66I-II plants. The sap flow-activity period increased in early
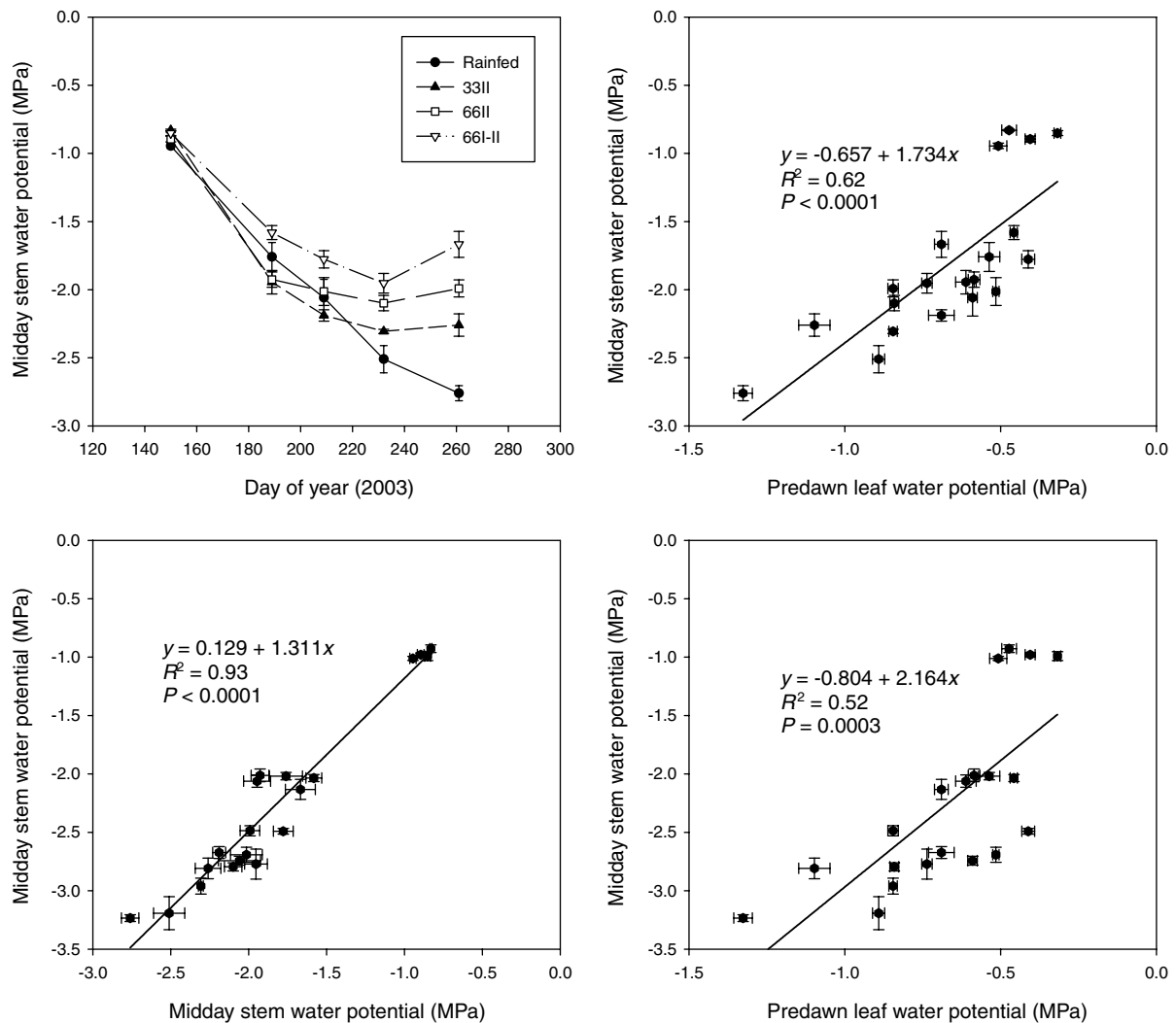
Figure 3. Seasonal variation in stem water potential of olive trees during the experimental period, and relationships among predawn, midday and stem water potentials. Vertical and horizontal bars represent standard errors ($n = 4$ replicates) error bars not visible indicate SE smaller than the symbol. Regression parameters of linear functions fitted to data are reported in the figure. Watering regimes are referred to by symbols in the legend.

season, peaked in late July for 66I-II plants and then showed a progressive reduction until late summer; rainfed plants showed a seasonal plateau and smoother seasonal variations.

Environmental conditions typical of Mediterranean climate were reflected in the seasonal pattern of daytime mean sap flux density (Figure 5), for both rainfed and 66I-II plants. In all cases, maximum values were reached during early summer ($P < 0.0001$), when θ was relatively high, VPD started to increase and global radiation was at the uppermost level. Daytime mean sap flux densities showed a progressive

reduction throughout summer season in both treatments. Such a reduction was rather similar between treatments, yet variation in response to irrigation was not as pronounced, though again consistent ($P < 0.0001$). The agreement between measured daytime tree water use and predicted daily reference evapotranspiration (calculated following Penman equation) was good for both treatments (Figure 5), though coefficients of the regression (not shown, $P < 0.0001$) were higher in 66I-II plants compared to rainfed trees (slope and elevation, $P < 0.001$, were 0.589 and 0.4, and 0.209 and 0.053, with R^2 equals to 0.72 and
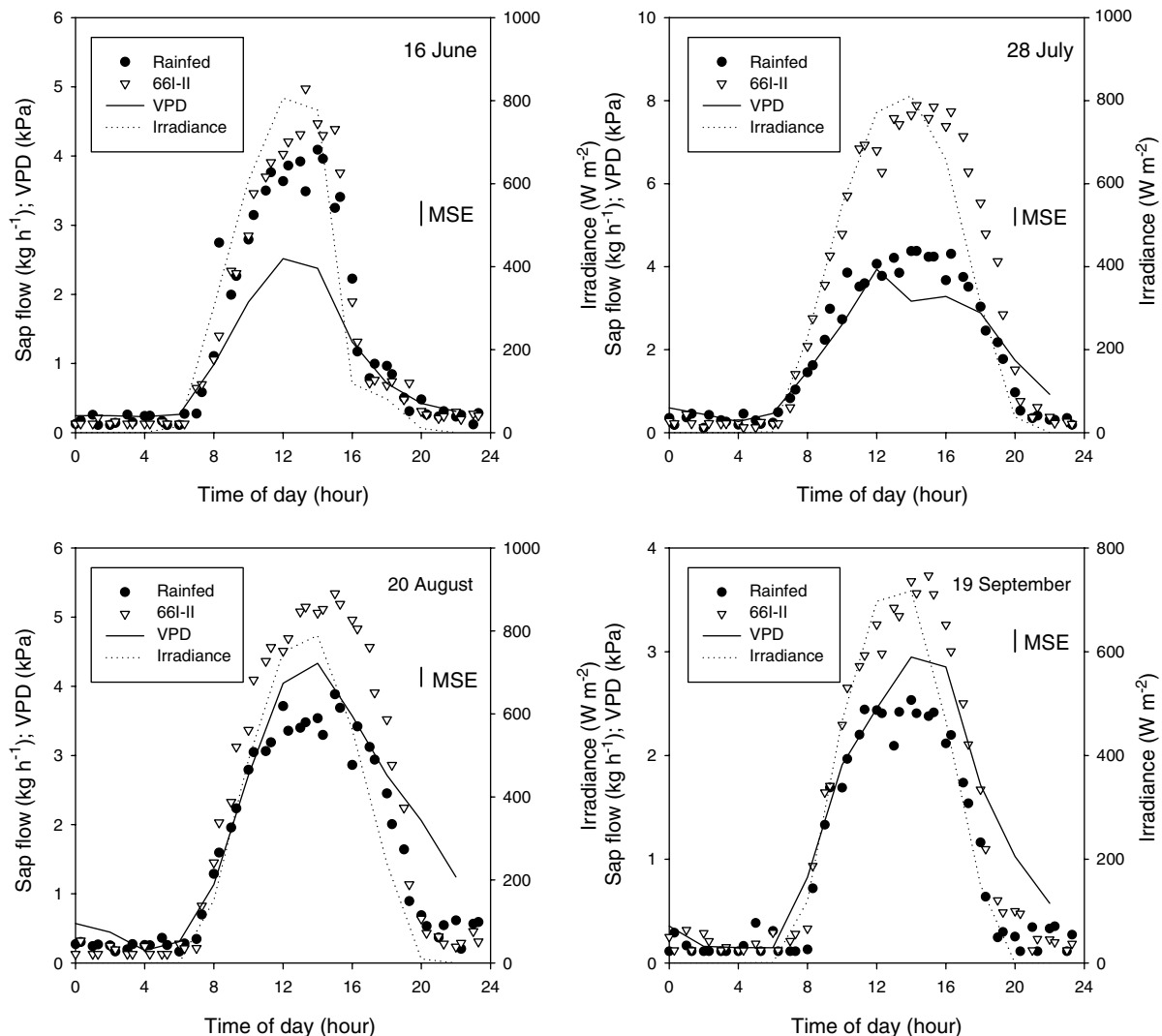
Figure 4. Representative diurnal patterns of sap flow, VPD and irradiance at the olive plantation, during the experimental period. Mean standard error is presented for the sake of clarity (MSE; $n = 4$ replicates). Watering regimes are referred to by symbols in the legend.

0.86, respectively for irrigated and rainfed plants). Daytime mean sap flux density was positively correlated with VPD and global radiation in both treatments (not shown, $P < 0.0001$), logarithmically (with R^2 equals to 0.59 and 0.73, respectively for irrigated and rainfed plants) in the former case and linearly in the second (with R^2 equals to 0.60 and 0.72, respectively for irrigated and rainfed plants). Cumulative daily stand transpiration differed consistently ($P < 0.0001$) between rainfed and 66I-II, starting from late June, with water consumption constantly higher in irrigated than rainfed trees,

differences increasing as the season progressed (Figure 5).

Estimated K_{S-L} and K_{S-S} decreased sharply from high pre summer values to a mid summer minimum ($P < 0.0001$), which did not differ between rainfed and 66I-II plants (Figure 6). In correspondence to periods of relatively low evaporative demand and when water use was still relatively high K_{S-L} and K_{S-S} values were consistently ($P < 0.0001$) higher in deficit irrigated than rainfed trees.

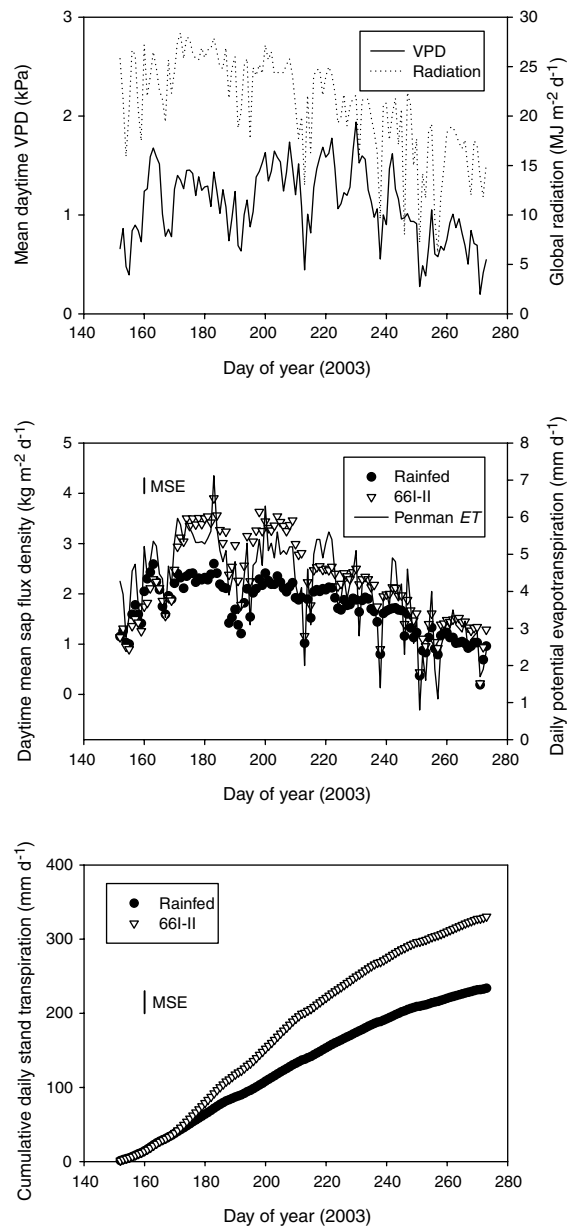Mean daytime canopy conductance followed a scattered seasonal pattern with values highest in

Figure 6. Seasonal trends of whole-plant and soil-to-stem specific hydraulic conductance of olive trees during the experimental period. Vertical bars represent standard errors ($n = 4$ replicates); error bars not visible indicate SE smaller than the symbol. Watering regimes are referred to by symbols in the legend.

order to separate the effects of light and evaporative demand, we divided mean daytime VPD by daily global radiation, then we plotted mean daytime G_C to this ratio (Figure 7), showing an exponential decay function. The linear relationship between G_C and mean daytime VPD also differed between 66I-II and rainfed trees (not shown; $P < 0.05$ and 0.0001, respectively for slope and elevation coefficients), indicating increased stomatal closure as drought progressed; however, the relation was quite shallow and poor, particularly for irrigated plants ($R^2 = 0.04$ and 0.22, $P = 0.0273$ and < 0.0001, respectively for 66I-II and rainfed plants).

Discussion

Patterns of seasonal water use, with maximum stand transpiration in the early summer and gradual reductions as the summer drought progressed

Figure 5. Seasonal patterns of mean daytime VPD and global radiation, daytime mean sap flux density and daily potential evapotranspiration (Penman), and cumulative daily stand transpiration at the olive plantation, during the experimental period (see text for details). Mean standard error is presented for the sake of clarity (MSE; $n = 4$ replicates). Watering regimes are referred to by symbols in the legend.

early summer (Figure 7). Mean daytime canopy conductance decreased further into summer as well as differences between 66I-II and rainfed trees, the former showing higher values ($P < 0.0001$), noticeable in the early season. In
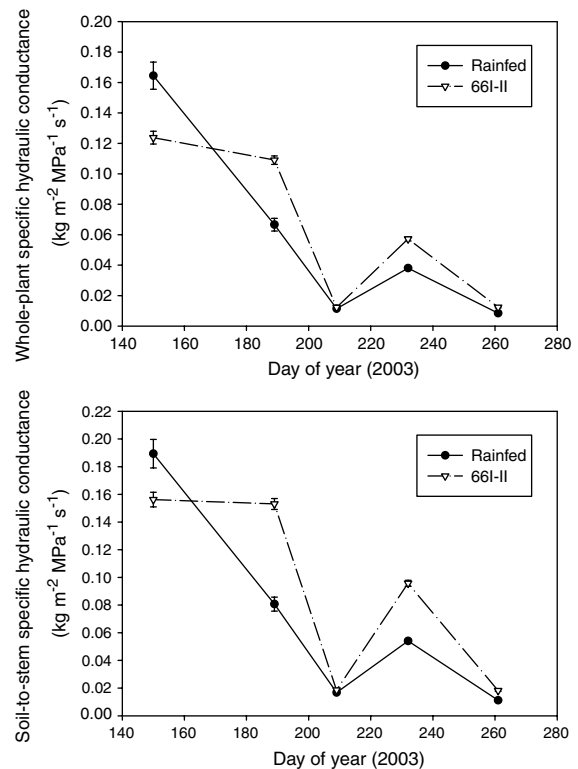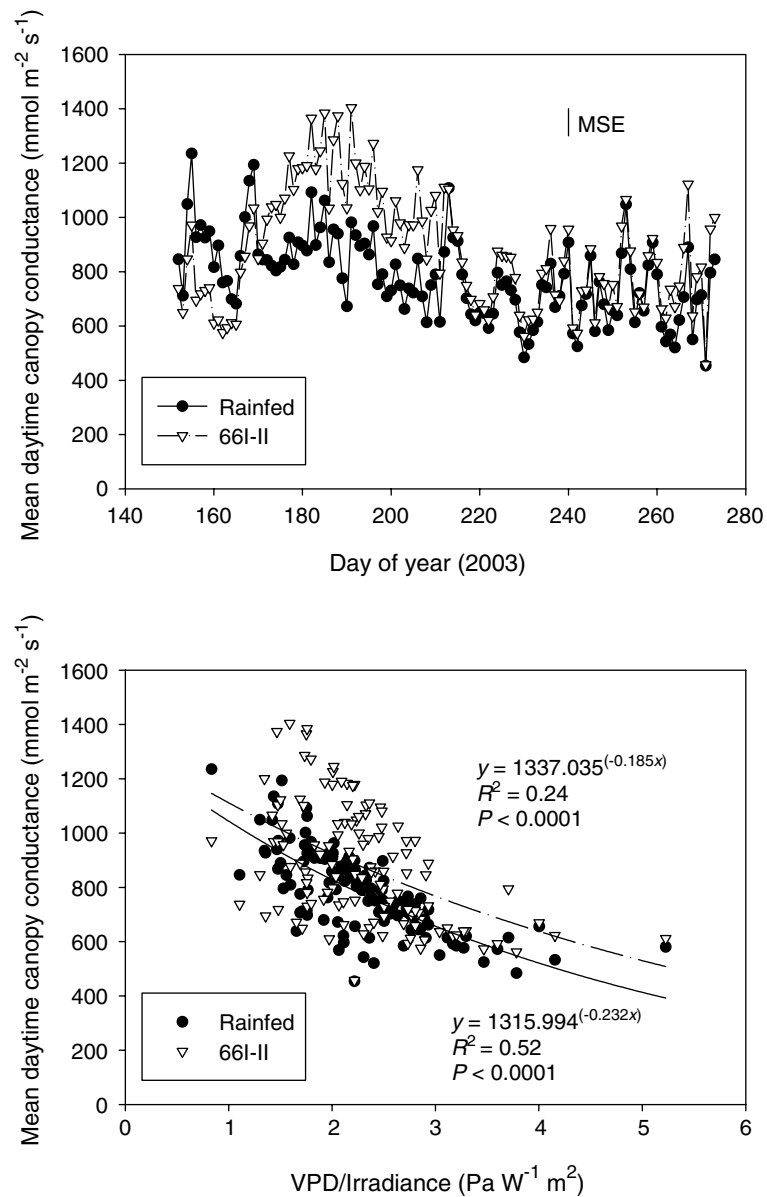
Figure 7. Seasonal variation of mean daytime canopy conductance of olive trees during the experimental period, and its relationship with VPD/irradiance ratio (see text for details). Mean standard error is presented for the sake of clarity (MSE; $n = 4$ replicates). Regression parameters of exponential functions fitted to pooled data are reported in the figure. Watering regimes are referred to by symbols in the legend.

regardless of the irrigation treatment, were typical of Mediterranean conditions. Meteorological conditions during the experiment were representative of the study area, within the average for summer season, but with relatively high precipitation in early summer. A high evaporative demand was, however, observed in this period that caused a progressive depletion of soil moisture reserves around rainfed trees since the beginning of watering treatment, until the wilting point. Plots irrigated with 66% of ET_C throughout the season (stage I and II) also showed a gradual reduction of soil moisture within rows; yet, taking away from the field capacity was buffered by deficit watering

and soil was maintained around 50% of available moisture level. Plots irrigated with 66% of ET_C only in the second part of the season (stage II) beneficed of watering, promptly recovering soil moisture levels close to those observed for 66I-II plots. Instead, plots irrigated with 33% of ET_C in the stage II were merely able to oscillate above the wilting point threshold. Deficit irrigation was less effective between rows, though ranking of irrigation treatments was maintained. Water uptake strategies adopted by olive trees to tolerate summer drought would include tuning extraction over the soil profile and from orchard areas with different moisture levels (Michelakis et al., 1995; Moreno et al., 1996).

Water potentials showed similar values across all irrigation treatments at the beginning of the season, when relative extractable water was still high enough to prevent induction of water stress (Fernández et al., 1997), then diverged progressively from one another through summer drought, ranking treatments in order of amount of water supplied. Water demand in terms of climate and foliage area remained the same, thus the decrease in leaf water potentials may have contributed to maintain sap flow during soil water shortage. Treatment-related differences were also observed in g_s, showing that olive trees were able to restrict water loss by modulating stomatal closure at different levels of drought stress. Stomata are integrators of all environmental factors affecting plant growth (Morison, 1998), resulting in a wider scattering of stomatal conductance than water potentials. The values of stomatal conductance remained fairly constant throughout morning hours (similar maximum and midday values); thus, plants restricted water loss under high evaporative demand by closing stomata early in the morning, regardless of the irrigation treatment, preventing an excessive drop in water potential. Hypotheses considering the effect of soil water deficits on stomatal behavior via hydraulic and chemical signals, may provide useful explanation to soil drying responses in this anysoidric species (Jones, 1998; Moriana et al., 2002). Indeed, olive trees did not show a quick recovery in g_s when water became available, probably because the low hydraulic conductance of the xylem, which may be responsible for the tree to prevent at least, in part, water loss under high atmospheric evaporative demand (Rieger,

1985). Extremes of stomatal conductance and water potentials measured in this study were similar in range to that reported on other olive cultivars (Fernández et al., 1997; Moriana et al., 2002)

The highest correlation of the comparisons among Ψ_{PD}, Ψ_{MD} and Ψ_{STEM} was between Ψ_{MD} and Ψ_{STEM}. Only a few studies have actually compared one of the three methods of measuring water potential with one another for determination of plant water status. A high correlation between Ψ_{MD} and Ψ_{STEM} was found in Mediterranean fruit woody plants, such as grapevine (Stevens et al., 1995; Williams and Araujo, 2002), though this may not hold true for other fruit trees of temperate environments, such as apple (Naor et al., 1995). Measurements of Ψ_{PD} on olive provided a good estimate of the soil moisture status within the orchard, being responsive to water applications in early stages of the experiment; however, also Ψ_{STEM} was reflective of the amount of water in the soil profile under the environmental and soil conditions of this study, being a sensitive indicator of water stress earlier than Ψ_{MD} (see Shackel et al., 1997). Experimental evidences suggest that Ψ_{PD} may come into equilibrium with the wettest portion of the soil in the plant's root zone (see Ameglio et al., 1999). In this sense, the soil moisture a tree responds to at midday may differ from that at predawn due to the flux of water occurring while the plant is actively transpiring (uncovered leaves).

There was a strong similarity between diurnal patterns of sap flow, VPD and irradiance for the two-antipode irrigation treatments (rainfed and 66I-II). Olive transpiration appeared strongly coupled with the surrounding air and the radiation environment, despite their opposite effects on stomatal conductance. Elucidation of specific factors driving transpiration of individual tress is typically hampered by covariance and interaction among the environmental drivers. Sap flow showed a step morning increase, but lagged behind irradiance in 66I-II plants that somewhat could be related to higher water capacitance of woody tissues with respect to rainfed plants. The progressive reduction of diurnal sap flow time-courses during the season reflected soil water depletion around rainfed trees, as well as increasing water deficit experienced by deficit-irrigated trees. Water depletion of non-wetted areas between rows explored by roots

might limit diurnal water use patterns in this species with a shallow root system, through hydraulic or chemical signal. Decreasing diurnal sap flow rates in late season, as VPD recovered, might suggest that soil moisture and soil/root hydraulic resistance limited transpiration as well. Reduction in stomatal conductance was sufficient to decrease transpiration in response to increasing VPD; it is likely, that the basis of some hysteresis in the relationship between sap flow and VPD or irradiance observed in the present study lies in the stomatal response to VPD and resistance flow within the plant and soil.

Daily transpiration rates were only partially reduced in rainfed with respect to deficit irrigated trees (66I-II). Small differences between fully irrigated and unirrigated olive trees of the same cultivar were found by Tognetti et al. (2004). Olive trees might limit their water use in response to signaling from portions of the root system remained dry during water application, even in deficit regime, while rainfed olive trees might benefit of water extracted from partially moist soil layers. The relationships between daily water consumption and Penman evapotranspiration resembled those obtained by Fernández et al. (2001) and Tognetti et al. (2004). Sap flux measured in the field, under different conditions of atmospheric evaporative demand and soil water content, led to consistent results across the whole range, validating the heat-pulse technique as a sustainable method for irrigation scheduling in olive orchards. Consistent separation between rainfed and deficit irrigated trees in terms of estimated cumulative orchard transpiration derived from sap flow measurements revealed an overall coherency of the method for monitoring both daily and seasonal dynamics of water use in olives.

Seasonal patterns and differences between deficit-irrigated and rainfed trees in specific hydraulic conductance reflected those in water use, decreasing during peak drought stress because changes in the liquid–water path from soil to leaves. Differences, in $K_{S–L}$ and $K_{S–S}$, however, were not strong, indicating that the conductivity of the wood was only in part impaired by water stress. Olive xylem has intrinsic low hydraulic conductivity and can withstand water potential values below turgor loss point with minor seasonal xylem embolism (see Salleo and Lo Gullo, 1993). Nevertheless, stem and root hydraulic con-

ductivity in olive can be reduced under drought stress conditions due to cavitation (see Rieger, 1995). Moreno et al. (1996) discovered cavitated vessels after restoring irrigation; the intrinsic high resistance to flow of narrow conduits in olive may account for the observed incomplete recovery of xylem functionality in late summer. The low hydraulic conductance of drying soil volume has been found to decrease the hydraulic conductance of citrus trees subjected to partial irrigation and drought, by interrupting water transport in a large portion of the root system (Cohen et al., 1987). Indeed, the response of whole-tree hydraulic conductance to deficit irrigation may be mainly attributable to the progressive decline of soil moisture in upper horizons, inducing changes in hydraulic properties of soil compartments, whereas the embolism of apical twigs, resulting in decreases of leaf specific conductivity, should have a minor effect on tree hydraulic resistance (see Tognetti et al., 1998). When atmospheric evaporative demand was high, a lower hydraulic conductance led to decreased xylem water potential as well as lower leaf conductance, which ought to control plant water deficit within a safety range for the hydraulic system.

Differences in irrigation during canopy development may result in differences in the relationship between G_C and VPD (Ewers et al., 2000). The relationship between G_C and mean daytime VPD differed between irrigation treatments, showing increased stomatal closure as drought progressed. At any given VPD, a reduction in stomatal or canopy conductance was expected as the soil dried out, because seasonal drought-induced xylem cavitation should reduce $K_{S–L}$ and $K_{S–S}$. However, there did appear to be significant intervals in which VPD did not decrease consistently canopy conductance, if this was the major factor driving transpiration during the initial increase in sap flow seen in the early summer. The differences between treatments probably reflected both differences in conducting area (active sapwood), but also in the crown characteristics. When the effect of light on the behavior of stomata was controlled, G_C decreased exponentially with increasing VPD/irradiance ratio; though the linear relationship between G_C and VPD was less reliable. Bongi and Loreto (1989) found low responsiveness of stomatal conductance to VPD in olive seedlings. Giorio et al.

(1999) did not find any correlation between these two parameters, studying irrigated and non-irrigated olive trees in the field. Fernández et al. (1993) presented a number of field observations that suggested an upper-bound relationship between stomatal conductance and VPD. Limitations to sap flow under limited soil water deficiency might be induced by concurrent high atmospheric VPD. Transpiration of olive trees diverged from Penman evapotranspiration, suggesting restrictions of canopy conductance by stomatal closure induced by high VPD (Bréda et al., 1993). We hypothesize that the limitation to water use in olive trees lies in the conductance pathway from soil to leaves to air, with contributions of canopy or meteorological factors and soil water availability. The effect of soil moisture on stomatal behavior might superimpose to that of VPD as soil continues drying in the proximity of active roots.

Despite qualitatively similar patterns of seasonal water use, the magnitude of the decrease in sap flow during the summer and the seasonal dynamics of water potentials varied among treatments, as also observed in a previous experiment (Tognetti et al., 2004), suggesting that olive trees followed closely decreasing amount of applied water and irrigation scheduling. Deficit irrigation during stage I and II (66I-II) affected plant water relations, ranking this treatment between rainfed and fully irrigated plants (see Tognetti et al., 2004). Severe regulated deficit irrigation differentiated to some extent 33II treatment from rainfed plants. Tuning regulated deficit irrigation might have a major impact on water saving in areas with relatively good late spring precipitation. Regulated deficit irrigation after pit hardening could be recommended, at least at 66% of crop evapotranspiration replaced, and in soil, cultivar and environmental conditions of the experimental orchard. Further studies warrant exploring the possibility to use moderate deficit irrigation with temporary deprivation (66II) in environmental conditions that allow for replenished soil water reserves before summer drought. In this sense, current research efforts aim to evaluate the effect of regulated deficit irrigation on olive yield and oil quality (Patumi et al., 2002). Future researches will enable relationships between hydraulic (sufficiency, safety), canopy (structure, conductance) and atmospheric (irradiance, VPD) parameters to be used for irrigation management.

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