

RESEARCH ARTICLE

Partial Root-Zone Drying of Olive (*Olea europaea* var. 'Chetoui') Induces Reduced Yield under Field Conditions

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Abstract

The productivity of olive trees in arid and semi-arid environments is closely linked to irrigation. It is necessary to improve the efficiency of irrigation techniques to optimise the amount of olive fruit produced in relation to the volume of water used. Partial root-zone drying (PRD) is a water saving irrigation technique that theoretically allows the production of a root-to-shoot signal that modifies the physiology of the above-ground parts of the plant; specifically reducing stomatal conductance (g_s) and improving water use efficiency (WUE). Partial root-zone drying has been successfully applied under field conditions to woody and non-woody crops; yet the few previous trials with olive trees have produced contrasting results. Thirty year-old olive trees (*Olea europaea* 'var. Chetoui') in a Tunisian grove were exposed to four treatments from May to October for three-years: 'control' plants received 100% of the potential evapotranspirative demand (ET_c) applied to the whole root-zone; 'PRD₁₀₀' were supplied with an identical volume of water to the control plants alternated between halves of the root-zone every ten-days; 'PRD₅₀' were given 50% of ET_c to half of the root-system, and; 'rain-fed' plants received no supplementary irrigation. Allowing part of the root-zone to dry resulted in reduced vegetative growth and lower yield: PRD₁₀₀ decreased yield by ~47% during productive years. During the less productive years of the alternate bearing cycle, irrigation had no effect on yield; this suggests that withholding of water during 'off-years' may enhance the effectiveness of irrigation over a two-year cycle. The amount and quality of oil within the olive fruit was unaffected by the irrigation treatment. Photosynthesis declined in the PRD₅₀ and rain-fed trees due to greater diffusive limitations and reduced biochemical uptake of CO₂. Stomatal conductance and the foliar concentration of abscisic acid (ABA) were not altered by PRD₁₀₀ irrigation, which may indicate the absence of a hormonal root-to-shoot signal. Rain-fed and PRD₅₀ treatments induced increased stem water potential and increased foliar concentrations of ABA, proline and soluble sugars. The stomata of the olive trees were relatively insensitive to super-ambient increases in [CO₂] and higher [ABA]. These characteristics of 'hydro-passive' stomatal behaviour indicate that the 'Chetoui'

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variety of olive tree used in this study lacks the physiological responses required for the successful exploitation of PRD techniques to increase yield and water productivity. Alternative irrigation techniques such as partial deficit irrigation may be more suitable for 'Chetoui' olive production.

Introduction

The production of olives, and products derived from olives, is a major agro-industry in Mediterranean areas with the global market worth over €11 billion per annum [1]. The sustainability of this industry faces a number of converging pressures associated with climate change, population growth and unsuitable agricultural practices [2, 3]. The productivity of olive trees (*Olea europaea* L.) is largely constrained by the availability of water during the summer months when the fruit develops [4]. The majority of European olive groves are currently rain-fed without supplementary irrigation [5]. Global climate models predict that Mediterranean summers will likely become hotter, with an increased frequency and duration of drought events that will coincide with episodes of raised temperatures relative to the norm [6]. Olive trees possess a number of physiological adaptations to cope with drought [7–9]. Nevertheless, longer and more severe droughts may have significant implications for the production of olives [10, 11]. Supplementary irrigation increasing soil water content to field capacity dramatically increases the yield of olives per tree, but also promotes vegetative growth reducing the efficiency of irrigation when measured relative to crop production [12]. The effectiveness of irrigation is gauged by 'water productivity': the amount of yield produced per unit of water applied in irrigation [13]. Furthermore, in the future the availability of irrigation water will likely be constrained by increased population levels, industrialisation and urbanisation, combined with the possible effects of climate change on the temporal and spatial distribution of water [14]. It is therefore necessary to optimise the impact of irrigation on yield through development of irrigation technologies based on physiological studies of plant responses to water deficit [15, 16].

The partial root-zone drying (PRD) technique involves applying irrigation to one half of the root-zone whilst the remaining half is allowed to dry [15]. The PRD approach is based on laboratory split-root studies; whereby a plant experiences the physiological effects of water deficit due to the presence of root-to-shoot signals indicating soil drying, but as water uptake is sustained by the irrigated portion of the root-system the physical effects associated with drought, such as decreased water potential/content, do not occur [17]. As soil dries, the transport of abscisic acid (ABA) in the xylem increases [18], and this may also be associated with an alteration of pH [19]. These signals induce a number of physiological adaptations within the leaf such as stomatal closure [20], reduced mesophyll conductance (g_m) [21], lower respiration [22] and enhanced expression of antioxidants [22, 23] to conserve water and protect the photosynthetic physiology. The irrigated and drying portions of the root-zone are alternated every 2–4 weeks during PRD, as roots within a drying soil are only able to sustain an ABA 'drought' signal for 10 to 15 days [16]. Under field conditions PRD has been successfully utilised in grape (*Vitis vinifera* L.) vineyards, where plants subject to PRD exhibited reduced vegetative growth, no decline in yield and enhanced fruit quality in comparison to plants that received full irrigation to the entire root-system [15, 16]. Potato (*Solanum tuberosum*) when grown under PRD also exhibited lower vegetative growth, but identical tuber yield to plants grown under control conditions that received twice the amount of water [24]. Partial root-zone drying also maintained yield in field grown orange trees (*Citrus sinensis*) irrigated to 60% of the volume of water

used in control conditions (PRD₆₀) [25, 26], pomegranate (*Punica granatum*) at PRD₇₅ [27], apple (*Malus domestica*) at PRD₅₀₋₆₀ [28] and PRD₅₀ [29], mandarin (*Citrus reticulata*) at PRD₅₀₋₁₀₀ [30], mango (*Manifera indica*) at PRD₅₀ [31], cotton (*Gossypium hirsutum*) at PRD₅₀₋₁₀₀ [32], okra (*Abelmoschus esculentus*) at PRD₅₀ [33] and maize (*Zea mays*) at PRD₄₀₋₈₀ [32]. Field trials have also reported reductions in crop yield under PRD associated with lower total water availability (eg. [32, 34]). However, despite reduced yields, crops grown under PRD generally exhibited higher production relative to the total volume of water used in irrigation; possibly making PRD an acceptable technique in areas affected by limited water availability [35]. This may indicate that the PRD technique may improve the efficiency of irrigation by achieving a similar yield with less water.

The development of PRD techniques applicable to a high value crop that occurs in drought prone areas such as olives would confer significant economic and social benefits (eg. [30]). The yield and quality of olive fruit is closely related to water availability during the summer growing season, when precipitation is generally low and potential evapotranspiration is high [4, 36]. To reduce water-loss, the stomata of olive trees close as soils dry and evaporative demand increases [8, 37–39]. In response to water deficit, rates of stomatal (g_s) and mesophyll (g_m) conductance to CO₂ often decline in unison, these diffusive limitations to the uptake of CO₂ reduce the concentration of CO₂ at the site of carboxylation within the chloroplast envelope (C_c) causing a reduction in the rate of photosynthesis (A) [40, 41]. However, olive trees in a split-root experiment where one half of the root-system was exposed to a drying soil, while the remainder received the same volume of water as the control plants, exhibited enhanced g_m values. Increased g_m levels were not associated with any change in the carboxylation capacity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) ($V_{c_{max}}$), or the maximum rate of electron transport required for ribulose-1,5-bisphosphate (RuBP) regeneration (J_{max}) [22]. This may suggest that a root-to-shoot signal [18] induces increased transport of CO₂ across the mesophyll layer in drought stressed olives [22], thus enhancing the ratio of g_m to g_s [42]. Furthermore, olive trees grown in split-root pot experiments exhibited lower leaf water potentials and g_s when half of the root system was exposed to a drying soil [22, 23, 43, 44], but crucially did not have lower rates of A [22].

Pot based split-root studies confer high levels of temporal and spatial regulation of the distribution of water, allowing an in-depth analysis of the physiological responses of olive trees to drying of soil around a section of the root-zone (eg. [22]). However, it is not possible to achieve such a degree of control under field conditions, and as a result the observations of laboratory studies may not be fully replicated in the open field (eg. [9, 44]). Field grown olive trees (var. ‘Manzanilla de Sevilla’) where the root-zone was either totally irrigated or partially allowed to dry, exhibited broadly consistent values of g_s and A between the two treatments [45]. A two-year study into the effects of allowing half of the root-zone to dry (using PRD₁₀₀ and PRD₅₀ levels of irrigation) on 11 year-old olive trees (var. ‘Picholine marocaine’) in Morocco observed that under field conditions PRD₁₀₀ plants that received identical water levels to control increased yield, whilst trees receiving half of the amount of water supplied to the control plants (PRD₅₀) showed 15–20% decline in yield [46]. This marginally lower yield did not affect oil acidity or polyphenol content of the fruits, which determine the quality of olive oil [47]. The olive plants exposed to PRD₅₀ displayed the lowest leaf water potential values, while those of the PRD₁₀₀ plants did not statistically differ from the control. However, levels of A and g_s in the PRD₅₀ and PRD₁₀₀ plants were both ~33% lower than the control treatment. The photosynthetic capacity for CO₂ assimilation, expressed by $V_{c_{max}}$ and J_{max} , was not significantly reduced by PRD₅₀, suggesting that using 50% of the required volume of water to replace 100% of the potential evapotranspiration in a PRD system reduced A through stomatal closure and not via biochemical limitations [9]. In contrast, drying of a portion of the root-zone of 30 year-old

olive trees (var. 'Manzanilla de Sevilla') indicated that a PRD of 30% (raised to 100% during pit hardening and prior to harvest) of control water levels was relatively ineffective, inducing minor 5.2 and 11.4% declines in g_s and A , respectively, during June to August [48]. From 2007 to 2009 this PRD resulted in a 41.2% reduction in yield, but had no effect on the dimensions or quality of individual olive fruit [44]. However, field trials of PRD using low-quality saline water irrigation in Tunisian olive groves (var. 'Chemlali') to 30% of control irrigation levels induced a slight 11% reduction in olive yield with no effect on fruit oil content [49].

These field studies exposing part of the root-system to a drying soil indicate that PRD irrigation with reduced volumes of water do induce some reduction in the yield of olive trees (eg. [44, 46, 49]). However, the extent of any decline in yield and the underlying physiological causes are unclear. In drought affected plants, A and yield are often related to diffusive limitations to the transport of CO_2 [40, 41]; yet in the field grown olive trees subject to PRD there were contrasting observations of the strength of any relationship between g_s and yield (eg. [44, 48, 49]). The variation in these observations may be due to differences in the amount of water used in the PRD irrigation systems. For example PRD₁₀₀ treatment provides 100% of ET_c to one half of the root-system, thereby meeting all of the water requirements of the olive trees while simultaneously providing a root-to-shoot signal that may modify physiological and morphological growth responses of the olive trees. Whereas supplying a lower volume of water to the irrigated portion of the root-zone may induce a more pronounced drought response associated with lower overall water availability (eg. [9, 49]).

In this study we conducted a field based investigation into the effects of two different PRD irrigation levels (PRD₁₀₀ and PRD₅₀) in comparison to control (full ET_c irrigation to both sides of the root-zone) and rain-fed (no supplementary irrigation) growth conditions on 30 year-old olive trees (var. 'Chetoui') in Tunisia. The aims of this study were to: i) investigate the effect of PRD on both stomatal and mesophyll conductance to CO_2 and biochemical limitations to CO_2 uptake, and their relationship to A ; ii) characterise any potential relationships between g_s , g_m and A with the quality and quantity of olive fruit and oil produced by trees under different levels of PRD; iii) gauge the impact of differential PRD on the growth of olive trees, specifically whether enhanced vegetative growth may limit the effectiveness of supplementary irrigation in terms of fruit yield, and; iv) identify whether PRD is an effective irrigation technique in terms of the yield achieved on the basis of the amount of water supplied during irrigation, and the physiological and morphological mechanisms that underpin this response.

Materials and Methods

Experimental site and irrigation treatments

The study was conducted in the experimental farm of the National Agronomic Institute of Tunisia, located in the Mornag plain, 15 Km south east of Tunis (Latitude 32°7, Longitude 10° 14). The olive trees were 30 year-old trees belonging to the 'Chetoui' variety, which is the most important cultivar for olive oil production in the North of Tunisia. The olive grove had not previously been irrigated prior to the instigation of the study. The occurrence of alternate bearing of fruit in olive trees strongly affects production on a year-to-year basis [50]. The present study was conducted over a three-year period (2005 to 2007) consisting of two more-productive 'on-years' and one less-productive 'off-year'. Measurements of gas-exchange and biochemical analysis of leaves and olive fruit took place in the final on-year of the study in 2007. At the beginning of summer in May, four irrigation treatments were applied on the basis of potential evapotranspiration (ET_c) calculated using the formula:

$$ET_c = K_c * ET_o \quad (1)$$

where ET_o is the reference evapotranspiration calculated from the Penman-Monteith equation [51] and K_c is the crop factor (monthly values of 0.6 during June–September and 0.65 during October–November) [52]. Weather data was recorded each day at a weather station within the experimental farm and used to estimate ET_o [51]. Values of monthly rainfall are given in Table 1. The olive trees were subjected to four irrigation treatments: control trees received full irrigation with 100% of ET_c to both sides of the root system; PRD₁₀₀ irrigation supplied 100% of the volume of water required to meet ET_c to one half of the root system, with the irrigated and drying halves of the root-zone alternated every ten days; the PRD₅₀ irrigation treatment provided 50% of the volume of water equivalent to ET_c to one side of the root-system, alternated between sides every ten days, and; rain-fed plants received no supplementary irrigation. To provide the olive trees with water, a drip irrigation system was utilised. Emitters were placed at a distance of 0.5 m from the trunk. The discharge rate for each emitter was $8 \text{ dm}^3 \text{ h}^{-1}$, with a total of eight emitters used for each tree in the control and PRD₁₀₀ treatments (distributed according to whether water was applied to the whole or part of the root-zone), and four emitters per tree in the PRD₅₀ treatment. Water was provided to the olive trees from May until October. Trees were arranged in a randomised block design of twelve trees per block with three replicate blocks for each of the four irrigation treatments.

Stem water potential measurement

Midday stem water potential (Ψ_s) was measured using a Scholander pressure chamber (PMS Instrument Company, Albany, Oregon, USA) during October 2007. Stem water potential was determined on leaves enclosed in a black plastic bag covered with aluminium foil for two hours prior to measurement. Three stems of 15cm in length were analysed to produce a mean Ψ_s value for each plant, with the average of three replicates then taken to represent mean Ψ_s for each irrigation treatment. Measurements were performed between 12:00 and 13:00 hours.

Gas exchange and fluorescence measurements

Leaf gas exchange and fluorescence parameters of the central leaf section were simultaneously measured using a LI-6400-40 leaf chamber fluorometer (Li-Cor, Inc., Nebraska, USA) equipped with a 2 cm^2 cuvette during October 2007 (the most important period for olive fruit development prior to harvesting at the end of November). Measurements were performed on the youngest fully expanded leaf of at least two branches from each tree, with the mean of three trees taken to represent the value for a given treatment. The measurements were made between 10:00 and 15:00 hours at a saturating photon flux density (PPFD) of $1400 \mu\text{mol m}^{-2} \text{ s}^{-1}$, $[\text{CO}_2]$ of 380 ppm, leaf temperature of 25°C and relative humidity ranged between 45 and 55%.

Instantaneous transpiration efficiency was expressed as the ratio of A to g_s . Mesophyll conductance was calculated using the variable J method involving simultaneous measurements of gas-exchange and chlorophyll fluorescence parameters as described by Harley et al. [53]. The CO_2 compensation point to photorespiration (I^*) was measured by increasing C_i at four different levels of photosynthetically active radiation ($400, 300, 200$ and $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$) [54]. Levels of respiration in the light (R_d) were analysed using the Kok method [55]; and respiration in the dark (R_n) was measured by switching off the light in the cuvette, when CO_2 release from the leaf had become stable for approximately five to 10 minutes. This was recorded and considered to represent R_n [41]. Values of I^* and R_d used in the calculation of g_m utilising the variable J method are given in Table 2. Total conductance to CO_2 (g_{tot}) was calculated as:

$$g_{\text{tot}} = [g_s * g_m] / [g_s + g_m] \quad (2)$$

Table 1. Monthly rainfall in mm during the study. Irrigation was performed during May to October each year.

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
2005	55	103	38	39	8	10	3	28	42	30	19	105	480
2006	148	48	31	19	35	0	0	0	27	82	50	178	618
2007	10	55	120	20	32	12	0	0	4	122	56	73	504

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Photosynthetic response curves to increased [CO₂] were conducted in the field using the method of Centritto et al. [56]. These A/C_i curves were performed at a standard leaf temperature of 25°C and a higher temperature of 30°C. The Farquhar et al. [57] model of C3 photosynthesis was used to calculate values of V_{c,max} and J_{max} following Ethier and Livingston [58].

Leaf biochemical analysis

Leaves were collected from the olive trees at the same time as the leaf gas exchange measurements were conducted and immediately frozen in liquid nitrogen and then stored at -80°C prior to analysis. Total soluble sugars were quantified following the phenol-sulfuric acid method [59] using a spectrophotometer (Jenway 6505UV/VIS, Bibby Scientific, Staffordshire, UK) at 490 nm and D-glucose as standard. Proline was determined spectrophotometrically following the ninhydrin method of Bates et al. [60] at a wavelength of 520nm from the organic phase using toluene as a blank. The abscisic acid (ABA) content of leaves was measured using high-performance liquid chromatography (Alliance 2695, Waters Corporation, Milford, Massachusetts, USA). Hormone quantification was monitored with a mass spectrometer (Quattro LC, Micromass Ltd, UK) [61].

Olive yield, oil quality and olive tree growth parameters

Olive fruits were harvested by hand at the same phenological stage when the fruits had matured at the end of November. The Maturity Index was 5 according to Mailer et al. [62], indicating that the majority of the fruit had a colouring that was black with more than 50% purple flesh [63]. The yield of olive fruit of the nine trees monitored for each treatment was weighed using a field balance. Yield was then expressed as kg per hectare. The olives were crushed using a laboratory scale mill to extract their oil. To assess the quality of the olive oil: acidity was determined following Wolf [64]; polyphenols were measured spectrophotometrically at 727 nm using Folin-Denis reagent [65], and chlorophyll content of the oils was measured using a spectrophotometer at 630, 670 and 710 nm [66].

After the olive fruit harvest, vegetative growth was evaluated by measuring the shoot length and leaf surface area. Twenty vegetative and fruit bearing shoots evenly distributed around the

Table 2. Photon flux density saturated (1400 μmol m⁻²s⁻¹) photosynthesis (A), stomatal conductance (g_s), mesophyll conductance (g_m), total conductance (g_t), instantaneous transpiration rate (ITE), light respiration (R_L), dark respiration (R_D), maximal fluorescence yield (F_v/F_m) and stomatal density (SD) of control, PRD₁₀₀, PRD₅₀ and rain-fed *Olea europaea* 'Chetoui' trees. Values are means of eight to twelve plants per treatment. ± indicates one standard error. Means followed by different letters indicate significant difference (P < 0.05) using a one-way ANOVA with LSD *post-hoc* test.

	A (μmol m ⁻² s ⁻¹)	g _s (mmol m ⁻² s ⁻¹)	g _m (mmol m ⁻² s ⁻¹)	g _t (mmol m ⁻² s ⁻¹)	ITE (mmol mol ⁻¹)	R _d (μmol m ⁻² s ⁻¹)	R _n (μmol m ⁻² s ⁻¹)	F _v /F _m	SD (stomata / mm ²)
Control	11.44 ± 0.44 ^c	0.106 ± 0.005 ^b	0.198 ± 0.010 ^b	0.069 ± 0.003 ^b	4.70 ± 0.29 ^a	0.76 ± 0.07 ^b	1.35 ± 0.11 ^b	0.778 ± 0.006 ^a	608 ± 122 ^a
PRD ₁₀₀	12.22 ± 0.60 ^c	0.099 ± 0.005 ^b	0.194 ± 0.003 ^b	0.066 ± 0.002 ^b	6.95 ± 0.19 ^c	0.77 ± 0.03 ^b	1.42 ± 0.18 ^b	0.780 ± 0.013 ^a	576 ± 115 ^a
PRD ₅₀	9.45 ± 0.47 ^b	0.087 ± 0.003 ^a	0.157 ± 0.017 ^a	0.051 ± 0.003 ^a	5.53 ± 0.40 ^b	0.57 ± 0.05 ^a	1.10 ± 0.06 ^a	0.767 ± 0.006 ^a	620 ± 124 ^a
Rainfed	8.15 ± 0.54 ^a	0.080 ± 0.005 ^a	0.120 ± 0.022 ^a	0.045 ± 0.004 ^a	4.45 ± 0.33 ^a	0.46 ± 0.11 ^a	0.95 ± 0.09 ^a	0.768 ± 0.010 ^a	570 ± 114 ^a

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circumference of each tree were selected. Ten leaves were then chosen for area analysis from each shoot. Leaf surface area was measured using a digital planimeter (CID 203 LEASER). Measurements of stomatal density values of the mid-section of each leaf were performed by preparing nail varnish 'negatives' of the abaxial leaf surface. These were then placed onto glass microscope slides and the number of stomata per unit leaf area determined using an Olympus (B07, BH-2, Olympus, Tokyo, Japan) microscope equipped with an Olympus camera (B06, C-35AD-2, Olympus, Tokyo, Japan). Stomatal density was measured from 27 leaves per treatment (nine leaves for each tree), with the number of stomata being counted for three images for each leaf [67].

Statistical analyses

Statistical analyses were performed using SPSS 10 (IBM, New York, USA). To test the effect of irrigation treatment on physiological, biochemical and morphological parameters a one-way ANOVA with LSD *post-hoc* test was used to assess differences in variance between samples.

Results

Leaf gas-exchange

Partial root-zone drying reduced g_s values in both PRD₁₀₀ (-6.7%) and PRD₅₀ (-17.9%) treated trees compared to control olive trees (Table 2). Stomatal conductance in the PRD₁₀₀ plants was not statistically different to levels observed in the control plants, and the Ψ_s values of control and PRD₁₀₀ plants were also identical. Olive trees grown under PRD₅₀ (-115.4%) and rain-fed (-169.2%) conditions exhibited significantly reduced Ψ_s , that corresponded to the lowest values of g_s recorded in the study (Table 2 and Fig 1). Mesophyll and total conductance to CO₂ followed similar patterns to g_s ; being highest under control conditions and lowest under the PRD₅₀ and rain-fed treatments. Rates of photosynthesis were slightly, but not significantly, higher in the PRD₁₀₀ than control treatment; however, A declined alongside g_{tot} in the PRD₅₀ and rain-fed treatments (Table 2). The marginally higher values of A obtained under PRD₁₀₀ than the control treatment were not associated with biochemical capacity to assimilate CO₂

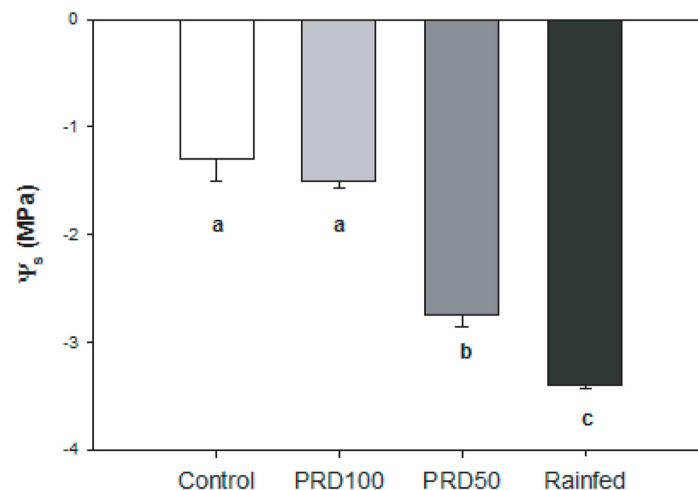


Fig 1. Midday stem water potential (Ψ_s) of olive trees (var. 'Chetoui') grown under control, PRD₁₀₀, PRD₅₀ and rain-fed irrigation treatments in October 2007. Error bars indicate one standard error either side of the mean. Letters indicate significant difference ($P < 0.05$) using a one-way ANOVA with LSD *post-hoc* test.

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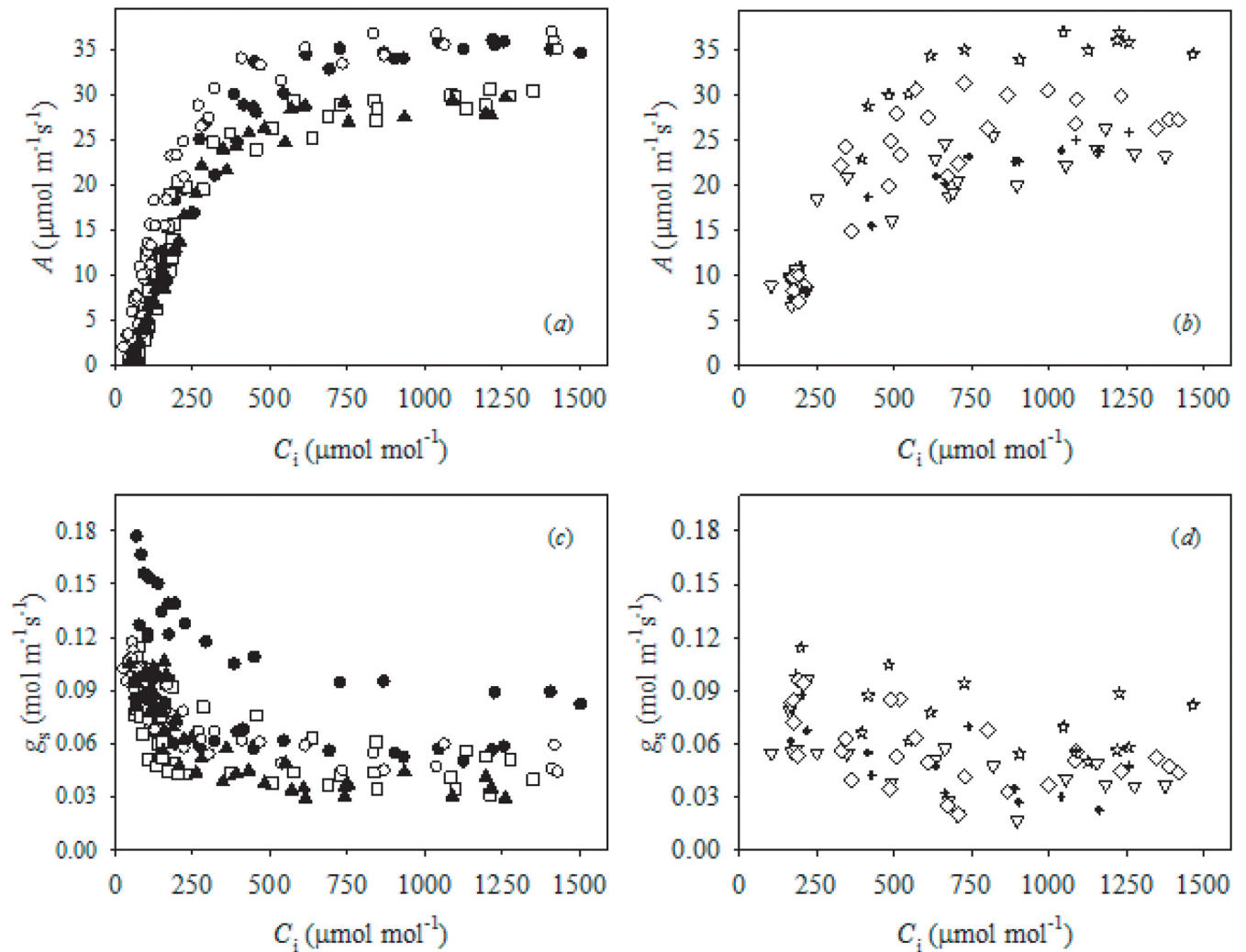


Fig 2. The relationship between (a, b) photosynthesis (A) and intercellular CO_2 (C_i), and (c, d) stomatal conductance (g_s) and C_i measured after exposing olive (var. ‘Chetoui’) leaves to a CO_2 of ~ 50 ppm for approximately 60 minutes to force stomatal opening [56] during the morning (between 900 and 1100 h), with relative humidity ranging between 45 and 55% and leaf temperature of 25°C (a, c), and during the early afternoon (between 1330 and 1430 h) with relative humidity ranging between 30 and 35% and leaf temperature of 30°C (b, d). The measurements were made on three to four plants per irrigation treatment in saturating PPFD ($1400 \mu\text{mol m}^{-2}\text{s}^{-1}$) in the control (\bullet, \star), PRD₅₀ (\square, ∇), PRD₁₀₀ (\circ, \diamond) and rain-fed ($\blacktriangle, \blackplus$) treatments during October 2007.

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(Fig 2a and Table 3). Irrigation treatment did not affect $V_{c_{\max}}$ but declines in J_{\max} were observed in PRD₅₀ and rain-fed trees. Furthermore, the small differences in $V_{c_{\max}}$ and J_{\max} between olive trees receiving full ETc (control and PRD₁₀₀) and those receiving lower amounts (PRD₅₀ and rain-fed) became less apparent when the effect of g_m on movement of CO_2 was taken into consideration, and the relationship between A and C_c plotted (Fig 3). The increase in A with C_i and C_c is less pronounced in PRD₅₀ and rain-fed plants, suggesting that biochemical in addition to diffusive limitations to A occur under these conditions (Figs 2a and 3). An increase in cuvette temperature enhanced the apparent treatment effects on the biochemical efficiency of CO_2 assimilation. At the higher temperature, rain-fed and PRD₅₀ grown plants exhibited declines in $V_{c_{\max}}$ and J_{\max} . Furthermore, $V_{c_{\max}}$ and J_{\max} values of PRD₁₀₀ plants were also reduced in comparison to control levels, suggesting that PRD irrigation reduced the capacity for CO_2 -uptake at higher leaf temperatures (Fig 2b). The g_s values of olive trees under

Table 3. Analysis of A/C_i curves in Fig 2a based on the Farquhar et al. (1980) model of C3 photosynthesis following Ethier and Livingston (2004) to calculate the carboxylation capacity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) ($V_{c_{max}}$), the maximum rate of electron transport required for ribulose-1,5-bisphosphate (RuBP) regeneration (J_{max}) and their ratio. Values are the mean of three response curves. \pm indicates one standard error either side of the mean. Means followed by different letters indicate significant difference ($P < 0.05$) using a one-way ANOVA with LSD *post-hoc* test.

	$V_{c_{max}}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	$V_{c_{max}}: J_{max}$
Control	92.4 \pm 4.6 ^a	170.8 \pm 1.4 ^a	0.541 \pm 0.024 ^a
PRD ₁₀₀	91.0 \pm 15.6 ^a	159.9 \pm 5.9 ^a	0.566 \pm 0.082 ^a
PRD ₅₀	75.5 \pm 2.5 ^a	142.2 \pm 3.2 ^b	0.531 \pm 0.006 ^a
Rain-fed	66.8 \pm 1.3 ^a	136.2 \pm 5.0 ^b	0.491 \pm 0.011 ^a

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all irrigation treatments showed a decline as C_i was increased at sub-ambient concentrations. Increases of C_i to levels above ambient did not induce further reductions in g_s (Fig 2c). The efficiency of photosystem II (F_v/F_m) was broadly consistent in trees grown under all treatments, indicative of the adaptation of olive to environments characterised by high evaporative demand, high levels of PAR and low water availability (Table 2). Partial drying of the root-zone induced significant increases in leaf level instantaneous transpiration rate (ITE) relative to the control and rain-fed treatments (Table 2).

Water potential and biochemical effects of partial root-zone drying

The lower Ψ_s observed in olive plants grown under PRD₅₀ and rain-fed conditions may be the result of osmotic adjustment in the trees exposed to lower levels of water availability (Fig 4). The lower yield of the PRD100. The concentration of leaf soluble sugars was also elevated in the PRD₅₀ and rain-fed treatments relative to the control and PRD₁₀₀ (Fig 4b); replicating the

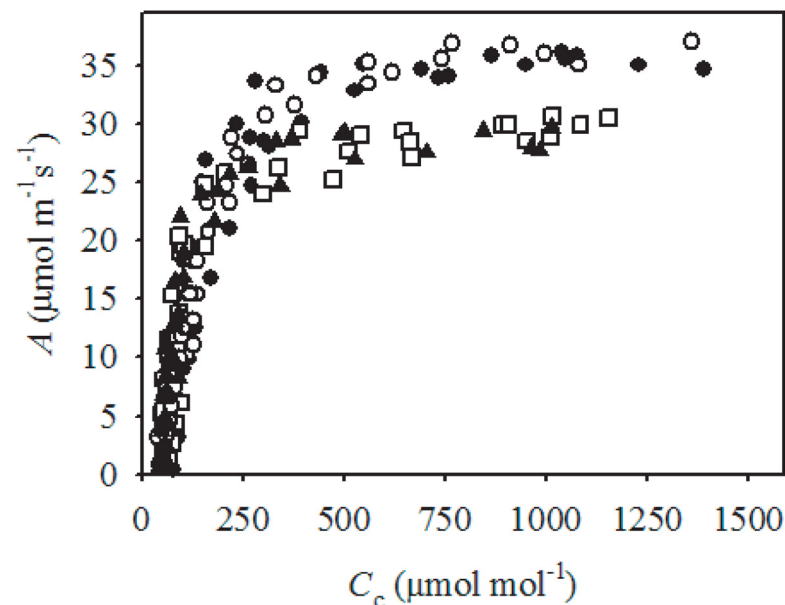


Fig 3. The relationship between photosynthesis (A) and chloroplastic $[CO_2]$ (C_c) measured after exposing olive (var. 'Chetoui') leaves to a $[CO_2]$ of ~ 50 ppm for approximately 60 minutes to force stomatal opening (Centritto et al., 2003) during the morning (between 9:00 and 11:00 h). The measurements were made on three to four plants per water treatment, in saturating PPFD ($> 1200 \mu\text{mol m}^{-2}\text{s}^{-1}$), with relative humidity ranging between 45 and 55%, and leaf temperature of 25°C in the control (\bullet), PRD₅₀ (\square), PRD₁₀₀ (\circ) and rain-fed (\blacktriangle) treatments.

doi:10.1371/journal.pone.0157089.g003

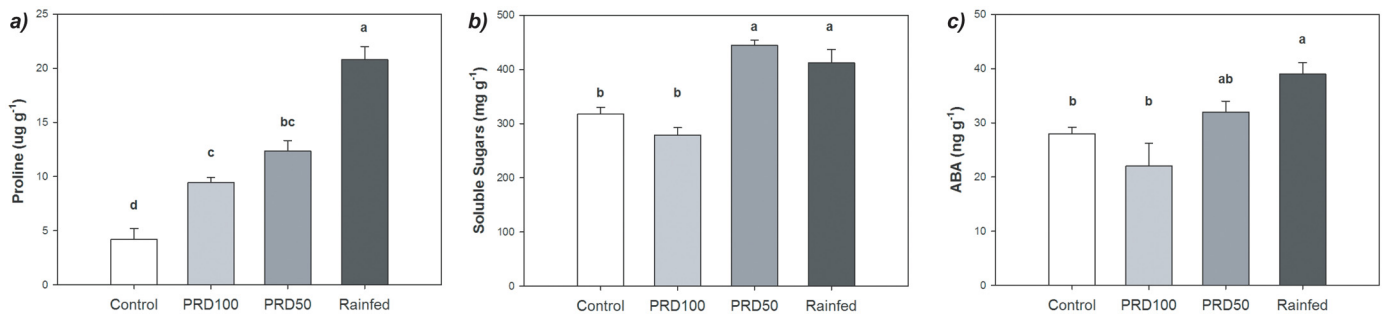


Fig 4. Regulators of leaf osmotic status in olive trees (var. ‘Chetoui’) exposed to control, PRD₁₀₀, PRD₅₀ and rain-fed irrigation treatments in October 2007: Foliar concentration of a) proline; b) soluble sugars, and c) abscisic acid (ABA). Error bars indicate one standard error either side of the mean. Letters indicate significant difference ($P < 0.05$) using a one-way ANOVA with LSD *post-hoc* test.

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patterns observed in Ψ_s induced by the irrigation treatments (Fig 1). Levels of foliar [ABA] were 21.3% lower in the PRD₁₀₀ treatment than in the control. Leaf ABA concentration in PRD₅₀ plants was marginally 14.3% higher than their control counterparts; a significant increase in [ABA] (39.3%) was only observed in plants under the rain-fed treatment (Fig 4c).

Effect of partial root-zone drying on growth and yield

Allowing part of the root-zone to dry significantly altered the growth patterns of the 30 year-old olive trees. Shoot length was significantly reduced in the rain-fed and both of the PRD treatments (Fig 5a). Likewise, the leaf area of the fruit bearing shoots was also reduced in the PRD and rain-fed treated plants. However, alteration in the level and spatial distribution of irrigation did not alter leaf area on vegetative shoots; suggesting that PRD irrigation may affect reproductive tissues predominantly over vegetative growth (Fig 5b). Critically, this is borne out in the yield of the trees during the ‘on-years’ of 2005 and 2007; control irrigation to both sides of the root-zone to a level sufficient to replace potential evapotranspiration resulted in the highest yield of olive fruit of 45.3 Mg ha⁻¹; however, PRD₁₀₀ induced a significant 47.0% reduction in yield to 24.0 Mg ha⁻¹. Partial root-zone drying utilising 50% of the level of water applied to the control plants induced an -67.6% decline in yield to 14.7 Mg ha⁻¹; while the lowest yield of 2.4 Mg ha⁻¹ occurred in the rain-fed treatment, 5.3% of the yield achieved under full control irrigation (Fig 5c). During the ‘off-year’ of 2006, yield was reduced in all treatments and supplementary irrigation did not influence the production of olive fruit (Table 4). The amount of oil

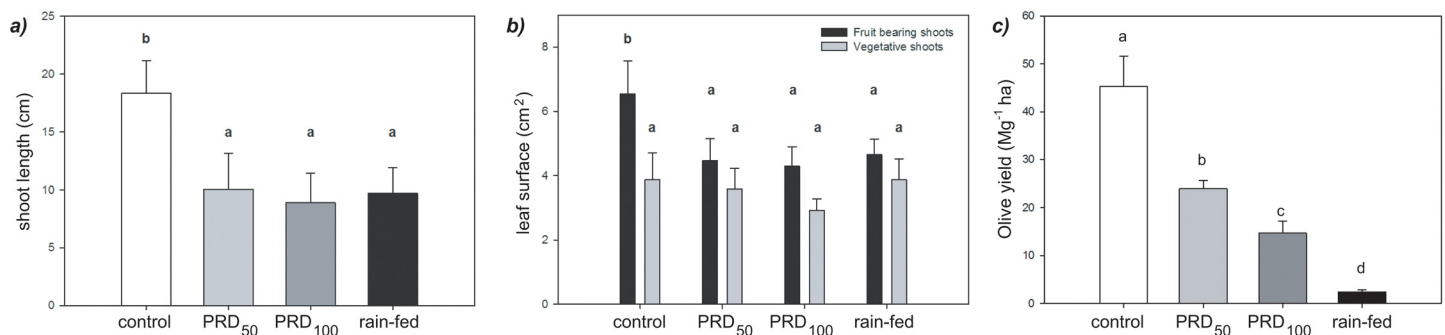


Fig 5. Growth effects of control, PRD₁₀₀, PRD₅₀ and rain-fed irrigation treatments on olive trees (var. ‘Chetoui’): a) shoot length in 2007; b) leaf surface area of vegetative and reproductive shoots in 2007, and; c) mean olive yield in the two productive ‘on-years’ 2005 and 2007. Error bars indicate one standard error either side of the mean. Letters indicate significant difference ($P < 0.05$) using a one-way ANOVA with LSD *post-hoc* test.

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Table 4. Olive fruit yield of olive trees (var. 'Chetoui) grown under control, PRD50, PRD100 and rain-fed irrigation treatments in the three years of this study. Olive trees generally alternate between productive 'on years' and less productive 'off years'; 2005 and 2007 were more productive 'on' years. Values indicate the mean of nine trees per treatment. ± indicates standard error. Letters after the value indicate significant difference using a one-way ANOVA with LSD *post-hoc* test.

Treatment	Yield (Mg ⁻¹ ha)		
	2005	2006	2007
rain-fed	2.700 ± 0.475 ^a	1.114 ± 0.102 ^a	2.067 ± 0.733 ^a
PRD ₅₀	9.443 ± 1.107 ^{ab}	1.943 ± 0.187 ^a	19.900 ± 1.790 ^{bc}
PRD ₁₀₀	25.650 ± 2.676 ^c	1.759 ± 0.540 ^a	22.333 ± 2.126 ^{bc}
control	47.167 ± 13.268 ^d	1.018 ± 0.073 ^a	43.422 ± 4.638 ^d

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produced per kg of olive fruit was unaffected by the irrigation treatment, as was the quality of the oil with the acidity occurring below the 0.8% level required to be classified as 'extra-virgin'. The total polyphenol content of the oils was relatively high [68], also consistent with extra-virgin standards of the International Olive Council [69] and suggestive of a high degree of oxidative stability [70]. The amount of chlorophyll in the oil is indicative of the maturity of the olives; the higher the concentration of chlorophyll, the less ripe the olives at the time of harvest [71]. The oil produced by plants subject to all treatments showed no significant effects of irrigation on chlorophyll concentration (Table 5). Nonetheless, values observed in this experiment were generally higher than values reported by other studies [44, 47, 71].

Discussion

The majority of olive groves are rain-fed, particularly those in hilly areas where water for irrigation is either expensive or impractical. Irrigation with relatively low volumes of water (70–200 mm³ ha⁻¹ per week) can increase yields to 80% of those of plants supplied with sufficient water to replace ETc [4, 72]. In 2010, within the EU ~40% of Spanish, 26% of Italian and ~36% of Greek olive groves were irrigated; with irrigated trees responsible for 52% of olive fruit production [5]. However, the availability of fresh-water for irrigation will likely be constrained by population growth, urbanisation and industrialisation, combined with the potential effects of climate change on precipitation patterns [73]. This necessitates the optimisation of water-use in irrigation techniques, often termed 'more crop per drop' [13]. Partial root-zone drying has been successfully applied to numerous crops (see summary in introduction) and to olives grown in split-pot experiments (eg. [43]). However, the results of this and previous studies (eg. [44, 48]) suggest that PRD may not be as effective in certain varieties of olive trees under field conditions, or it may not be possible to achieve sufficiently rigorous control of the distribution of water under field conditions.

Table 5. The percentage yield of olive oil per unit of olive fruit, and the quality of olive oil extracted from fruit grown under control, PRD100, PRD50 and rain-fed treatments. Olive oil quality was gauged by acidity (Kalua et al., 2007), polyphenol content (Tsimidou et al., 1992; Aparicio et al., 2013) and the amount of chlorophyll remaining within the oil from the skin of the oil fruit (Salvador et al., 2001). Values are means of eight to twelve plants per treatment. ± indicates one standard error. Means followed by different letters indicate significant difference (*P* < 0.05) using a one-way ANOVA with LSD *post-hoc* test.

Treatments	Oil concentration (%)	Acidity (%)	Polyphenol (ppm)	Chlorophyll (ppm)
Control	16 ± 0.20 ^a	0.10 ± 0.05 ^a	472.43 ± 37.64 ^b	1.86 ± 0.60 ^a
PRD100	16 ± 0.20 ^a	0.10 ± 0.05 ^a	416.56 ± 54.38 ^b	1.33 ± 0.67 ^a
PRD50	15 ± 1.41 ^a	0.30 ± 0.07 ^a	462.84 ± 13.70 ^b	1.80 ± 0.29 ^a
Rain-fed	16 ± 0.20 ^a	0.35 ± 0.07 ^a	540.03 ± 24.99 ^a	1.12 ± 0.51 ^a

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Photosynthesis and diffusive conductance to CO₂

Leaf area photosynthetic rates were unaffected by the PRD₁₀₀ treatment relative to control irrigation; however, halving of the volume of water supplied to the plant in the PRD₅₀ treatment reduced A by 17.4%, with levels of A 28% lower in rain-fed than control plants (Table 2). In contrast to previous studies, this reduction in A induced by PRD was not solely the result of diffusive limitations (eg. [9, 22]), but a combination of reduced g_{tot} and decreased biochemical uptake of CO₂, as indicated by lower values of $V_{\text{c,max}}$ and J_{max} (Table 3). These declines in $V_{\text{c,max}}$ and J_{max} become more pronounced at higher temperatures, exacerbating the effect of drought on the carbon-uptake of olive trees through increased photorespiration (Fig 2b) [74]. The higher yield of the control plants may be related to their greater photosynthetic area (Fig 5), as allometric relationships have been observed between leaf biomass and yield [75], possibly due to correlations between whole plant photosynthetic rates, total leaf area and yield [76]. Higher vegetation growth is often associated with increased levels of respiration to fulfil the energetic requirements of metabolic processes [77]. Olive trees grown under rain-fed and PRD₅₀ conditions exhibited respective ~31 and ~26% lower levels of respiration in the light and dark than their counterparts receiving the full volume of water required to meet ETc; potentially accounting for their lower vegetative growth and fruit production (Fig 5) (eg. [78]).

As the availability of water in soil declines, stomatal closure occurs to reduce transpiration and limit water-loss from the plant [38]. In a split-root experiment involving bean (*Phaseolus vulgaris*) stomatal closure occurred as a result of a root-to-shoot ABA signal indicating soil drying prior to any reduction in leaf water potential [79]. A reduction in g_s did not occur in the olive tree subject to PRD₁₀₀ irrigation (Table 2), possibly indicating that a hormonal root-to-shoot signal did not occur or was not inducing stomatal closure (eg. [80–82]). A degree of stomatal closure occurred in the PRD₅₀ and rain-fed treatments; but this was a relatively minor reduction in g_s of 20–25% (Table 2). The lower values of g_s in the PRD₅₀ and rain-fed olive trees corresponded to lower Ψ_s ; possibly indicating that leaf water content and not a hormonal root-to-shoot signal of soil drying affected g_s values of olive trees under field conditions (eg. [48]). The osmotic adjustment responsible for the lower Ψ_s in PRD₅₀ and rain-fed olive trees may be the result of increased concentration of proline [83] and soluble sugars [84]. In addition to the regulation of osmotic potential, proline may play a protective role in the response of olive trees to drought and temperature stress [85].

Stomatal and mesophyll conductance concomitantly decline following drought stress (eg. [41]). The purpose of stomatal closure is to reduce the loss of water from the leaf to the external environment; however, the functional significance of a reduction in the rate of transport of CO₂ across the mesophyll is less clear [86]. An increase in the ratio of g_m to g_s would theoretically improve plant photosynthetic performance under drought conditions [42]. Indeed, under PRD₁₀₀ conditions in a split-root pot experiment, olive trees exhibited a 63% increase in g_m relative to g_s ; potentially indicative of a root-to-shoot signal altering the biochemical properties of the mesophyll layer to the transport of CO₂ [22]. However, in this study under field conditions g_m and g_s were unaffected under PRD₁₀₀, and the g_m to g_s ratio remained constant under both PRD treatments. The ratio of g_m to g_s did decline by ~20% in rain-fed olive plants, suggesting that the overall lower level of water availability in the rain-fed treatment reduced CO₂ transport to the chloroplast envelope (Table 2).

The relatively constrained reductions in g_s values observed in the PRD₅₀ and rain-fed plants (Table 2) may be somewhat surprising given the well-documented adaptations of olive trees to drought stress [7]; in particular evidence of stomatal responsiveness to drought [8, 36]. However, PRD₅₀ resulted in a mean 5.2% reduction in g_s values of 35 year-old olive trees [48], suggesting that physiological stomatal closure may not be the result of a root-to-shoot signal of

soil drying. Stomatal conductance of water vapour showed similar patterns to increased C_i in olive trees under all irrigation treatments (Fig 2c). Stomatal conductance declined markedly to sub-ambient increases in C_i , but remained constant as C_i was increased above ambient levels; contrasting to the hypothesised evolutionary response of angiosperm stomata to above ambient increases in $[CO_2]$ (cf. [87]), and further evidence to support the lack of a phylogenetic pattern in stomatal responses to CO_2 [67, 88]. Furthermore, increased foliar [ABA] in the PRD₅₀ and rain-fed olive plants did not alter stomatal sensitivity to $[CO_2]$, despite being considered a defining characteristic of angiosperm stomatal physiology (cf. [89]) and being observed in rose (*Rosa hybrid*) [90]. Not all angiosperms may possess the physiological responses required for PRD to be successful. A split-root study of bell pepper (*Capsicum annuum* L.) found that g_s was not regulated by a root-to-shoot chemical signal, but stomatal closure occurred in a 'hydro-passive' fashion (ie. where guard cell turgor and stomatal opening follow the water status of the whole leaf) related to soil water potential in both root compartments [80]. The absence of evidence indicative of a hormonal root-to-shoot signal of soil drying, or stomatal response to super-ambient $[CO_2]$ and increased foliar [ABA] may suggest that the varieties of olive used in this study (var. 'Chetoui') and others (eg. [44, 48]) lack the physiological capacity to rapidly alter g_s in response to environmental signals through 'hydro-active' stomatal control (ie. where guard cell turgor and stomatal opening are rapidly modified by an influx/efflux of ions/metabolites) (eg. [88, 91]). In essence, the physiological mechanisms required for the successful implementation of a PRD irrigation strategy may not be present in these olive varieties.

Analysis of the most widely grown Tunisian olive cultivars found that Chemlali exhibited greater stomatal control and was more tolerant of drought than Chetoui [37]. The results of the present study indicate that PRD was an ineffective irrigation method in the Chetoui variety; whereas, the yield of the more drought resistant Chemlali variety was only reduced 11% during PRD₃₀ irrigation [49]. This may suggest that physiological differences between olive varieties may account for differential responses to PRD irrigation treatments. Stomatal physiological behaviour may vary in olive varieties (eg. [37, 92]) between those that are dominated by 'hydro-passive' and 'hydro-active' stomatal physiology [93]. This may also offer a mechanistic basis to account for the contrasting results achieved in PRD studies involving olive trees and other angiosperm crops (see summary in [introduction](#)), and the comparative success of partial deficit irrigation techniques when applied to olive groves [72].

Effect of PRD on olive yield and fruit quality

The aim of PRD techniques is to increase the yield of olive fruit per unit of water used in irrigation. Partial root-zone drying has been successfully applied to other crops under field conditions; however, the results of this trial indicate that the PRD approach may be less effective in the Chetoui variety of olive trees. Critically, the yield of olive fruit grown under PRD₁₀₀ during the two 'on-years' analysed in this study was ~47% lower than the trees subject to the same level of irrigation under control conditions; while PRD₅₀ resulted in a 67.6% reduction in yield, suggesting that the volume of water received by olive trees and the spatial distribution of water determine yield [48]. The reduction in yield induced by PRD₁₀₀ found in this study appears to be at the upper end of decreases in yield observed in previous investigations utilising identical levels of irrigation between the control and PRD treatments that recorded declines of ~51% [44], ~20% [46] and ~11% [49]. The lower yield of the PRD₁₀₀ grown olive trees may be the result of lower xylem flux acting as a hydraulic signal of soil drying (eg. [80]). The increase in foliar concentrations of soluble sugars that occurs during drought has been associated with reduced yield, as the export of photosynthate from the leaf is reduced, thus reducing Ψ_s [94]. However, Ψ_s (Fig 1) and the concentration of soluble sugars (Fig 4b) were identical under

control and PRD₁₀₀ irrigation treatments, suggesting that impaired transport of sugars from the leaf were not responsible for the reduced yield observed under PRD₁₀₀ in this study. Supplementary irrigation during the lower productivity 'off-year' did not affect yield; raising the possibility that the effectiveness of water application may be improved over a two-year cycle by withholding irrigation during the non-productive phases of the alternate bearing cycle. This would also decrease vegetative growth during the 'off-year', reducing the requirements for pruning or water to sustain the additional leaf area during the following productive 'on-year'. Nevertheless, reducing irrigation levels during the 'off-year' may potentially adversely affect production in the 'on-year' if the plants experienced stress that subsequently impaired growth; an aspect that should be determined in future studies of irrigation efficiency.

Despite the lack of evidence of a root-to-shoot signal affecting g_s in the PRD₁₀₀ olive trees, the reduction in shoot length and leaf area indicate that exposing a portion of the roots to drying soil did effect plant growth (Fig 5a and 5b), and possibly suppressing investment in reproductive tissues [95]. However, increased root-to-shoot ABA signals in grapevine promoted reproductive growth, resulting in enhanced yield [96]. Nonetheless, different selective pressures may have resulted in a dissimilar response in olive, where as a comparatively long-lived woody tree, allocation of photosynthate to reproductive growth is reduced under water deficit [97].

The quality and quantity of the oil produced from olive fruit was unaffected by the irrigation treatment (Tables 4 and 5). The olive oil was of a comparatively high standard with low acidity and high levels of polyphenols required for classification as 'extra-virgin' [68, 69, 98]. The lack of effect on the characteristics of the oil [44, 49] and the amount of oil produced for a given amount of fruit [44, 47, 49] under PRD irrigation in comparison to control irrigation found in this study is consistent with previous reports. Olive fruit grown under rain-fed conditions has been observed to contain a higher proportion of oil than their irrigated counterparts [44, 49]; a similar response was not observed in the present study, where the percentage oil content of olive fruit was identical under all treatments (Table 4).

The results of this study indicate that PRD irrigation was relatively ineffective in enhancing the yield of olive fruit relative to the volume of water utilised in irrigation (Fig 5c). This may be due to a comparative lack of control afforded under field conditions in isolating part of the root-zone to allow the soil to dry; nonetheless, field trials of PRD have been successful in other woody trees (eg. [25, 27–30]). The results of this study suggests that the Chetoui variety of olive used may lack the necessary physiological responses [37] fundamental to a successful PRD irrigation strategy; whereby a root-to-shoot signal of soil drying affects photosynthetic, leaf gas exchange and osmotic behaviour to improve WUE [16, 17]. The absence of pronounced stomatal closure (Table 2) or active physiological stomatal behaviour to [CO₂] (Fig 2c) or [ABA] (Fig 4c) may indicate that stomatal control in this variety of olive is largely hydro-passive [88], and the signalling network required for a split-root system to induce stomatal closure and increased water use efficiency is not present (eg. [80]). These findings may suggest that PRD is not suitable for Chetoui variety olive groves; irrigation of the entire root-zone may be more effective in maximising yield through the optimisation of water productivity (eg. [44, 48]). Regulated deficit irrigation to the whole root-zone may be a more effective approach when applied to olive groves, as small volumes of supplementary irrigation have been shown to produce significantly enhanced yield [72].

Conclusions

Partial root-zone drying has been utilised to improve the water productivity of numerous crops. The successful application of PRD to olives would permit the optimisation of yield relative to water-use in a crop grown in drought prone areas. However, while the results of laboratory

based split-root studies of olive trees have been promising; the efficacy of PRD irrigation in the field has been equivocal. In this study, during productive ‘on-years’, yield was significantly reduced by 47% in the PRD₁₀₀ treatment relative to the control, despite receiving the same volume of water. Yield was 68 and 95% lower in the PRD₅₀ and rain-fed treatments. The yield of fruit relative to the amount of water used was significantly lower under PRD in comparison to application of water to the whole root-zone. Supplementary irrigation did not enhance olive fruit yield during the less productive ‘off-year’, suggesting that co-ordination of the supply of water with the alternate bearing cycle may enhance water-productivity on a two-year basis. The quality and quantity of oil produced by equal amounts of olive fruit from each irrigation treatment was identical. Lower *A* was observed in the PRD₅₀ and rain-fed treatments due to higher diffusive (Table 2) and biochemical (Fig 3) constraints to CO₂-uptake. A similar pattern was not observed in the PRD₁₀₀ treatment, possibly indicating that a root-to-shoot signal inducing stomatal closure was not present. Stomatal conductance was identical in the control and PRD₁₀₀ treatments, as were Ψ_s and foliar [ABA]. Stomatal closure occurred in the PRD₅₀ and rain-fed olive trees, with a relatively small reduction in g_s of 19–29%, which corresponded to lower Ψ_s and higher concentrations of the osmotic regulators ABA, proline and soluble sugars (Fig 4). The lack of clear active physiological stomatal behaviour to [CO₂] (Fig 2c) and [ABA] (Fig 4c) may indicate that the dominant component of stomatal control in the Chetoui variety of olive trees is hydro-passive. The physiological mechanisms required to produce a root-to-shoot signal of soil drying and then induce stomatal closure to enhance the WUE of photosynthesis, may be absent in the Chetoui variety of olive tree; thus constraining the effectiveness of PRD in optimising the water productivity of irrigation. Nonetheless, the required physiological mechanisms for successful application of the PRD technique may be present in other olive varieties. The apparent absence of physiological mechanisms required for PRD in Chetoui olive may negate the effectiveness of PRD in Chetoui olive groves. Periodic deficit irrigation of the entire root-zone may be a more successful approach in optimising crop yield and water productivity in olive trees than applying water to part of the root-system.

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Author Contributions

Conceived and designed the experiments: MBM MC AGC. Performed the experiments: SD MBM MC. Analyzed the data: MH MC. Contributed reagents/materials/analysis tools: MC GE. Wrote the paper: MH MC.

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