Photosynthetic limitations by water deficit: effect on fruit and olive oil yield, leaf area and trunk diameter and its potential use to control vegetative growth of super-high density olive orchards

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ABSTRACT

Regulated deficit irrigation (RDI) reduces leaf area, which is advantageous for fruit tree orchards with high plant densities to increase their long-term productive life. However, RDI also decreases fruit yield. To establish an optimum irrigation level to control tree vegetative growth without severely penalizing fruit yield it is necessary to analyze the effect of the limited photosynthesis produced by RDI on the carbon allocation patterns between yield and tree vegetative growth, which are not fully established in olive. Thus, our main objective was to unravel the relationships between limited photosynthesis and tree growth as well as yield to establish an optimum level of deficit irrigation. We conducted the research during four irrigation seasons in a super-high density olive orchard using four irrigation treatments: a full irrigation treatment (control) and three RDI treatments with increasing levels of water reduction scaled to replacing 60%, 45% and 30% of the irrigation needs. The plant water stress produced by RDI reduced photosynthesis, which resulted in a significant decline of leaf area. In contrast, neither single fruit weight nor total fruit yield normalized by leaf area was adversely affected by RDI. We found significant and direct relationships between photosynthesis and leaf area ($r^2 = 0.90$, $p < 0.0001$) as well as between leaf area and yield ($r^2 = 0.55$, $p < 0.05$). Thus, we conclude that while leaf area is determined mainly by photosynthesis, fruit yield is largely determined by leaf area, and thus, photosynthesis and leaf area are the main variables to control tree growth without curtailing the yield. The lowest RDI levels (30% and 45%) lead to greater water savings than 60%, with a similar effect on leaf area and fruit yield, and thus, any of these lowest irrigation strategies is preferred to achieve the best balance between crop water consumption and fruit yield.

1. INTRODUCTION

One of the major causes of yield reduction in water-limited environments is stomatal limitation of photosynthesis controlled by water stress (Flexas and Medrano, 2002; Grassi and Magnani, 2005; Flexas et al., 2014). Indeed, the most immediate response of plants to water stress is to limit leaf transpiration by stomatal closure, which allows to avoid harmful hydraulic failure of the plant (Sperry et al., 1998; McDowell et al., 2008). However, this also causes a decline in leaf intercellular CO₂ concentration, thereby limiting photosynthesis (Diaz-Espejo et al., 2007). These limitations can be produced either by low supply (soil water deficit) (Caruso et al., 2013; Rallo and Provenzano, 2013) or by high atmosphere demand (high vapor pressure deficit) (Fernández, 2014), being the effects of the interaction between both variables on photosynthesis not fully assessed (Giorio et al., 1999; Moriana et al., 2002; Perez-Martin et al., 2009). Water stress produced by soil water deficit can be ameliorated with irrigation which stabilizes the economic return and increases crop yield in comparison to rainfed yield crops (Ali et al., 2008).

Uncontrolled tree vigor is a major problem in super-high density (SHD) orchards (Connor et al., 2014), as orchards with densities over 1500 trees ha⁻¹ are usually named (Vossen et al., 2004), and in areas where local conditions can induce uncontrolled tree vigor (Correa-Tedesco et al., 2010). An excessive growth of the canopy produces difficult mechanical harvesting (Leon et al., 2007) and more importantly, a reduction of the long-term orchard productive life from mutual shading problems which conduct to an irregular distribution of the incident solar radiation into the canopy (Connor et al., 2006; Gómez-del-Campo and García, 2012; Connor et al., 2014). In dry areas where water is scarce, a deficit irrigation strategy (DI) is needed (Morison et al., 2008), specially under future climatic predictions (IPCC, 2014). Besides the substantial water saving that can be achieved using deficit irrigation (DI) strategies (Moriana et al., 2003; Caruso et al., 2013; Fernández et al., 2013; Padilla-Díaz et al., 2016), they could help to control excessive vegetative growth. This is the case of regulated deficit irrigation (RDI), one of the most effective DI strategies for SHD orchards (Fernández

et al., 2013). RDI can help to reduce the problem of excessive growth because it consists of replacing the crop evapotranspiration (ET_c) in the phases of the growing cycle when the crop is most sensitive to water stress, specially vegetative growth, and reducing irrigation for the rest of the cycle (Chalmers et al., 1981). The irrigation periods coincide in olive partially with the periods of maximum rate of both vegetative growth and fruit growth and ripening, reducing the competition for resources at critical stages (Connor and Fereres, 2005).

Stressed plants frequently display altered morphology which promotes plant survival by changing development including, for example, reduced growth, altered resource allocation between above-grown and below-grown tissues and a shift from vegetative to reproductive growth (Gifford and Evans, 1981; Allahverdiyeva et al., 2015). Actually, fruit competition for carbohydrates can subsequently lead to a reduced vegetative growth of shoots and roots (Génard et al., 2008) because fruit and seed growth dominate the growth of vegetative tissues (Wardlaw, 1990). In agricultural cultivars, the allocation partitioning patterns by which limited carbon is distributed from photosynthesizing leaves to heterotrophic plant organs and tissues have been largely modified through plant breeding and agricultural practices to increase productivity (Gifford and Evans, 1981; Genard et al., 2008). The effects of water deficit on photosynthesis is well described for olive (Angelopoulos et al., 1996; Giorio et al., 1999; Moriana et al., 2002), as it is also on fruit and olive oil yield (Moriana et al., 2003; Tognetti et al., 2006; Gucci et al., 2007; Fernández et al., 2013) and vegetative growth (Iniesta et al., 2009; Gomez-del-Campo, 2010). However, relationships between limited photosynthesis and plant growth patterns are not well established. The use of different irrigation levels to modify the growth patterns of aboveground organs such as leaves, trunks and fruits through the control of photosynthesis limitation may constitute a tool to avoid excessive vegetative biomass production and optimize reproductive growth (Connor et al., 2014) as well as saving a considerable amount of water, but more information is needed to unravel these relationships.

Thus, the main hypothesis we want to assess in this work is that there would be an optimum RDI level that would help to control excessive tree growth without severely penalizing crop production. Specifically, we hypothesize that the photosynthesis reduction caused by a RDI strategy would limit the increase of leaf area and trunk diameter (vegetative organs) to a greater extent than that of fruit weight (reproductive organs). We further hypothesize that for saturating light conditions, the photosynthesis limitation driven by stomatal closure would be mainly determined by soil water deficit but also by the effect of its interaction with high levels of vapor pressure deficit. Thus, the main objective of this work was to evaluate different RDI strategies as tools to control excessive vegetative growth of olive trees mediated by photosynthesis decline without penalizing yield. Our specific objectives were: (i) to assess the photosynthesis limitations produced by the interaction of different levels of soil water deficit and vapor pressure deficit and (ii) to determine the effect of limited photosynthesis on final leaf area, trunk diameter, fruit and virgin olive oil (VOO) yield and the effect of reduced photosynthesis on the increase rates of the former variables.

2. MATERIALS AND METHODS

2.1. Orchard and climate characteristics

The study was conducted from 2010 to 2015. From 2010 to 2012 we used a slightly different strategy in the timing and level of water stress than in the period 2013-2015 (explained in the following section). The data from 2010 and 2013 were not used in this work because the effects of the irrigation treatments of previous years could not be totally disregarded. The study plots were located in a commercial SHD olive orchard near Seville, southwest Spain (37° 15' N, -5° 48' W). Trees were 4-year-old in 2010. They were 'Arbequina' trees planted at 4 m × 1.5 m (1667 trees ha⁻¹), in rows oriented N-NE to S-SW. The trees, with a single trunk and branches from 0.6 to 0.7 m above ground, were manually pruned in December-January each year. The orchard soil (Arenic Albaqualf, USDA 2010) had a sandy loam top layer and a sandy clay layer downwards. The trees were planted at the top of 0.4 m high ridges. The amount of fertilizer was changed every month to match the crop needs

(Troncoso et al., 2001). Further details on the orchard characteristics can be found in Fernández et al. (2013).

Climate in the area is Mediterranean, with mild rainy winters and hot, dry summers. Most of the annual rainfall occurs between late September and May. Average values in the area of potential evapotranspiration (ET_0) and precipitation (P) were 1528 mm and 540 mm, respectively, for the 2002–2014 period. For that period, average maximum and minimum air temperature were 24.9 °C and 10.7 °C, respectively. The hottest months are July and August.

2.2. The RDI strategy

In 2011 and 2012 we followed the RDI strategy described in Fernández et al. (2013), and in 2014 and 2015 we used a slightly different one described in Padilla-Díaz et al. (2016) (Fig. 1). Briefly, we considered three periods along the olive growing cycle on which the crop is highly sensitive to water stress and irrigation supplies must replace or be close to the crop water needs. Period 1 goes from the last stages of floral development to full bloom (DOY 118, 116, 111 and 115 for 2011, 2012, 2014 and 2015, respectively), period 2 occurs at the end of the first phase of fruit development (June) and period 3 refers to a period of ca. 3 weeks prior to ripening, after the midsummer period of high atmospheric demand (from late August to mid-September). Between periods 2 and 3 (late June-late August), the olive tree is highly resistant to drought and irrigation supplies can be reduced (Alegre et al., 2002; Moriana et al., 2003; Iniesta et al., 2009; Fernández et al., 2013; Padilla-Díaz et al., 2016). Indeed, if irrigation is enough on period 3, the olive tree shows an outstanding capacity for recovering from water stress (Lavee et al., 1990; Moriana et al., 2007; Fernández et al., 2013; Padilla-Díaz et al., 2016). In 2011 and 2012 we did not irrigate between periods 1 and 2. In 2014 and 2015 we decided to irrigate between periods 1 and 2, if rainfall supplies were far from replacing the crop water needs. According to Hammami et al. (2011), severe water stress between period 1 and 2 could limit fruit size.

In 2011 and 2012 we imposed three irrigation treatments, a control (100C) and two RDI treatments (30RDI and 60RDI). In the 100C treatment the trees were irrigated daily to replace 100% of the irrigation needs (IN). IN on a daily basis were calculated as $IN = ET_c - P_e$, being ET_c the maximum potential crop evapotranspiration calculated with the single crop coefficient approach (Allen et al., 1998) and P_e the effective precipitation which according to Orgaz and Fereres (2008), was calculated as 75% of the precipitation recorded in the orchard. Details on the ET_c calculations are given by Fernández et al. (2011). The two RDI treatments were aimed to replace 30% and 60%, respectively, of IN. Details of both RDI strategies are given in Fig. 1a. In 2014 and 2015 we had a 100C treatment irrigated as explained before and a single RDI treatment, aimed to replace 45% of IN (45RDI) (Fig. 1b). The actual irrigation amounts (IA) supplied to each treatment are given in Table 1.

We used a randomized block design with four 12 m × 16 m plots per treatment (four replicates), having each plot four rows. The plots used were the same for the four years studied but monitored trees were different along the years. Each plot contained eight central trees surrounded by 24 border trees. All measurements were made on the central trees. In all plots the irrigation system consisted of one drip line per tree row with a 2 L h⁻¹ dripper every 0.5 m, corresponding three drippers to each tree. The irrigation scheduling (time, frequency and duration of irrigation) was controlled by an irrigation controller (Agronic 2000, Sistemes Electrònics PROGRÉS, S.A., Lleida, Spain).

2.3. Soil water content and meteorological measurements

In every plot, we installed two access tubes for a Profile probe (Delta-T Devices Ltd, Cambridge, UK) at 0.5 m from the tree trunk, as described in greater detail in Fernández et al. (2013). One of the access tubes was at 0.1 m from a dripper, i.e., in the soil volume wetted by irrigation and the other one at 0.4 m from the dripper, i.e. in drying soil during the irrigation season. The Profile probe was calibrated *in situ* (Fernández et al., 2011). Measurements of volumetric soil water content (θ_v) in each access tube were taken at 0.1, 0.2, 0.3, 0.4, 0.6 and 1.0 m depths, 1–2 times per week, 2h after irrigation. From the estimated θ_v values we calculated the relative extractable water (REW) in the

root zone (0-45 cm, Diaz-Espejo et al. 2012) following Granier (1987) as $REW = (R - R_{min}) / (R_{max} - R_{min})$, where R (mm) is the soil water content, R_{min} (mm) the minimum soil water content measured during the experiments, and R_{max} (mm) is the soil water content at field capacity ($0.18 \text{ m}^3 \text{ m}^{-3}$, Fernández et al. 2013). We calculated a weighted average for each plot, considering that the weighting factors were 0.97 and 0.03, for the ϑ_v measured at 0.1 m and 0.4 m from the dripper, respectively.

Thirty-minute average values of main meteorological variables were recorded by a Campbell weather station (Campbell Scientific Ltd., Shepshed, UK) located in the center of the area covered by the experimental plots. All meteorological sensors were located just above the canopies. The station recorded 30 min average values of wind speed (u), air temperature (T_a), air humidity (RH_a), global solar radiation (R_s), net radiation (R_n), photosynthetically active radiation (PAR), and rainfall (P). We calculated vapor pressure deficit (D_a) as a function of T_a and RH_a .

2.4. Gas exchange measurements

Measurements of maximum stomatal conductance ($g_{s,max}$) and net photosynthesis ($A_{n,max}$) were conducted once every 2 weeks during the irrigation season of every experimental year, from June to October, having a total of 10, 11, 12 and 13 measurements days in 2011, 2012, 2014 and 2015, respectively. On each measurement day we sampled one leaf per tree from two representative central trees per plot of each irrigation treatment, at 08.00–09.00 GMT, the time for maximum daily stomatal conductance in olive (Fernández et al., 1997). In 2011-2012 we had three irrigation treatments, four plots, two leaves and thus we had a total of $n = 24$ measurements every day we measured gas exchange; in 2014-2015 we had just 2 irrigation treatments, four plots, two leaves and thus $n = 16$ measurements every day we measured gas exchange. We used a Li-cor LI-6400 portable photosynthesis system (Li-cor, Lincoln NE, USA) with a $2 \text{ cm} \times 3 \text{ cm}$ standard chamber, to measure gas exchange in the 4th–5th leaf from the apex of current year shoots from the outer part of the canopy facing SE, at ca. 1.5 m above ground. Measurements were made in sunny days to make sure

the radiation was above the limiting threshold for $g_{s,max}$, reported for olive by Fernández et al. (1997) as around $500 \mu\text{mol m}^{-2} \text{s}^{-1}$. The gas exchange measurements were conducted at ambient light (1100 to $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$) and CO_2 ($370 - 400 \mu\text{mol mol}^{-1}$) natural conditions.

To disentangle the effect of REW and D_a on $A_{n,max}$ reduction, we divided all the $g_{s,max}$ and $A_{n,max}$ values by the maximums found in each irrigation treatment and classified these fractions in three D_a classes (low, medium and high) for 30RDI, 45RDI, 60RDI and 100C. We established these three D_a groups based on the D_a - $g_{s,max}$ relationships established for olive by Fernández et al. (1997). These authors found that maximum stomatal conductance values were obtained in a D_a range between 0.8 kPa and 1.6 kPa. They also observed a proportional $g_{s,max}$ decrease with increasing D_a up to values of 3.5 kPa. Consequently, we used the maximum $g_{s,max}$ and $A_{n,max}$ values measured in the D_a range of 0.8-1.6 kPa to normalize all the categories. We established a low D_a group with $g_{s,max}$ and $A_{n,max}$ values measured between 1.7-2.6 kPa, a medium D_a group with $g_{s,max}$ and $A_{n,max}$ found from 2.7 to 3.5 kPa and a high D_a group for all the values of $g_{s,max}$ and $A_{n,max}$ measured at higher D_a of 3.5 kPa. In our case, the maximum D_a found on any sampling day was 4.7 kPa. For this analysis we only used the measurements between periods 2 and 3, i.e., from late June to late August, when the greatest differences on REW between treatments occurred.

2.5. Leaf area measurements

Measurements of leaf area were made at dawn on the same sampling days where gas exchange was measured with a LAI-2000 Plant Canopy Analyzer (Li-cor, Lincoln, NE, USA) as described in Cuevas et al. (2013), following the measurement strategy proposed by Villalobos et al. (1995) for olive orchards. This measurement strategy consists on measuring at eight points per plot with the LAI-2000 Plant Canopy Analyzer in each of the four plots (replicates) per irrigation treatment (30RDI, 45RDI, 60RDI, 100C). In each plot, four points at different locations were measured just underneath the tree row of each plot, where the leaf area index (LAI) is maximum (LAI_{max}), and other four points were measured in the midpoint between two rows of each plot where LAI is minimum (LAI_{min}). The

average LAI (LAI_{avg}) was calculated using the fraction of ground cover (GC) as a weighting factor [$LAI_{avg} = LAI_{max} GC + LAI_{min} (1-GC)$]. The average tree leaf area per plot was calculated as LAI_{avg} multiplied by the ground area per plot and divided by the number of trees in that plot.

2.6. Trunk diameter measurements

We instrumented one central tree per plot with dendrometers, having three plots per treatment. We used two standard Verdtech stations as described by Cuevas et al. (2010). They included Plantsens radial dendrometers (Verdtech un Nuevo Campo S.A., Spain), connected to one addWAVE A733GSM remote telemetry unit (Adcon Telemetry, Austria), for data storage and transmission and one solar panel that supplies power to the system. The dendrometers were placed in the north side of the trunks, at about 0.3–0.4 m above ground, in locations free of scars. The contact point of each dendrometer was glued to the surface of the living tissues of the bark with a standard mastic for pruning wounds. The stations stored 15 min averages throughout the whole irrigation season every experimental year.

2.7. Fruit and virgin olive oil yield

Four central trees were manually sampled every three or four weeks from July to each year's harvesting day (DOY 299 in 2011, DOY 318 in 2012, DOY 325 in 2014 and DOY 310 in 2015), with at least five measurements per year. The samples were then oven-dried and weighed separately to determine the dry fruit weight. In addition, in each year's harvesting day, the fruit of four trees unsampled previously was collected, as it has been described, to determine total fruit yield. From the different fruit samples we took a subsample of 2 kg per plot before being oven dried, for oil extraction with an Abencor system (Comercial Abengoa S.A., Seville, Spain) (Martínez et al., 1975) to determine VOO yield.

2.8. Normalized increase rate

For the trunk diameter, leaf area and dry fruit weight we calculated the normalized increase rate (%) as $(V_f - V_i) / (V_i)$, where V_i and V_f are the values measured at the beginning and at the end of the irrigation season, respectively.

2.9. Statistical analyses

We used linear mixed models (LMM) with Tukey's post-hoc comparisons to analyze the effects of the irrigation treatment (fixed factor) on mean $A_{n,max}$, leaf area, trunk diameter and fruit weight, their normalized increase rates, and fruit and VOO yield, as dependent variables at $\alpha < 0.05$. The same analysis was used to determine the $A_{n,max}$ and $g_{s,max}$ differences under different D_a conditions. We used leaf identity within plot as the random factor structure in the $A_{n,max}$ and $g_{s,max}$ analyses to describe appropriately our experimental design and deal with the non-independent nature of the spatial experimental design. In the rest of comparisons the random factor was not necessary as we only have one measurement per plot. When no normal and heterocedastic residuals were obtained, appropriate transformation of the variable was used. We also conducted a partial correlation analysis between $A_{n,max}$ and total fruit yield, keeping the leaf area as the control variable. These analyses were conducted with R software (R Core Team, version 3.2.0, 2015) using R packages "nlme R" (Pinheiro et al., 2011), "multcomp R" (Hothorn et al., 2008) and "ppcor" (Kim, 2015). The relationship between $g_{s,max}$ and $A_{n,max}$, $A_{n,max}$ and leaf area, trunk diameter and fruit and VOO yields and leaf area and fruit yield were determined with SigmaPlot (version 12.0, Systat Software Inc., San Jose, California, USA).

3. RESULTS

Values of $A_{n,max}$ followed a seasonal trend (Fig. 2) typical of the dry and hot conditions in the orchard (Fig. 3) and also showed the effects of the different irrigation treatments (Fig. 4). $A_{n,max}$ was similar for all the treatments, both at the beginning (19.95 ± 0.30 in 2011, 19.20 ± 1.67 in 2012, 20.33 ± 1.02 in 2014 and $13.17 \pm 0.92 \mu\text{mol m}^{-2} \text{s}^{-1}$ in 2015) and at the end of the experimental period (16.48 ± 2.13 in 2011, 20.12 ± 0.62 in 2012, 13.64 ± 2.79 in 2014 and $15.08 \pm 0.77 \mu\text{mol m}^{-2} \text{s}^{-1}$ in 2015) when

285 atmospheric demand was lower (Fig. 3) and the treatments were irrigated similarly (Fig. 4). However,
 286 $A_{n,max}$ decreased significantly ($p < 0.05$) in the RDI plots compared to 100C between late June and late
 287 August, when irrigation was reduced, to minimum values of 6, 7 and 3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in 30RDI, 60RDI
 288 and 45RDI, respectively. This trend was observed in the four studied years. This $A_{n,max}$ reduction was
 289 likely produced by the $g_{s,max}$ decline occurring under water deficit conditions, as suggested by the
 290 strong correlation between $A_{n,max}$ and $g_{s,max}$ ($y = 58.052 x^{0.7533}$, $R^2 = 0.89$, $p < 0.0001$, $n = 218$,
 291 relationship not shown). On each irrigation season $A_{n,max}$ in 100C trees remained fairly constant.
 292 To disentangle the effect of REW and D_a on $A_{n,max}$ reduction we divided the $g_{s,max}$ and $A_{n,max}$ by their
 293 maximums which were measured in the D_a range of 0.8-1.6 kPa. Those fractions were split in three
 294 classes based on D_a values, as explained before: low (1.7-2.6 kPa), medium (2.7-3.5 kPa) and high
 295 (3.6-4.7 kPa) (Fig. 5). This was made for each irrigation treatment. We compared the $g_{s,max}$ and $A_{n,max}$
 296 measured in 60RDI and 30RDI plots with the values of 100C plots obtained in 2011 and 2012 and the
 297 $g_{s,max}$ and $A_{n,max}$ of 45RDI with 100C values of 2014 and 2015 because we used a slightly different
 298 irrigation strategy in 2014 and 2015 compared to 2011 and 2012 (see Materials and Methods
 299 section). We found a marked effect of the RDI treatment (i.e., REW) and D_a on $g_{s,max}$ and $A_{n,max}$
 300 reduction. The differential irrigation effect on $g_{s,max}$ of 60RDI (Fig. 5a) and 45RDI (Fig. 5b) compared to
 301 100C was only observed for the highest D_a whereas $g_{s,max}$ in 30RDI was significantly lower than 100C
 302 for all the D_a classes. Accordingly, we observed very similar results for $A_{n,max}$ in response to irrigation
 303 treatments and D_a (Fig. 5c and Fig. 5d) in all the irrigation treatments except to 60RDI probably
 304 because the relationship between $g_{s,max}$ and $A_{n,max}$ is not linear.
 305 Accordingly with the $A_{n,max}$ decline in the RDI treatments as compared to 100C, the increase rate of
 306 leaf area decreased consistently and significantly with reduced irrigation, on the four years studied
 307 (Table 2). For example, in the year 2011 the leaf area increase rate was on average 0.1% in 30RDI
 308 plots, 25.4% in 60RDI plots and 48.2% in 100C plots. Contrary to these results, no significant
 309 differences were found in the increase rates of trunk diameter or fruit weight. However, despite the
 310 lack of significant differences produced by the irrigation treatments, the trunk diameter usually grew

311 more with increasing irrigation (Table 2). The fruit weight increased significantly more in 100C than in
 312 the 30RDI plots only in 2011, being the increase rates among all the irrigation treatments in the rest
 313 of the years statistically similar. The final weight of the fruit was also statistically indistinguishable
 314 among the treatments, for all the years studied (Table 2).

315 As a result of these growth patterns, the final leaf areas of all RDI treatments were significantly lower
 316 (7.2 m^2 leaf per tree on average for all the RDI treatments and years) than the final leaf area in 100C,
 317 for the considered years (10.0 m^2 leaf per tree on average for the four years studied, Table 3). The
 318 trunk diameters were no significantly different, although greater trunk diameters were found for the
 319 100C trees than for the RDI trees (Table 3). Total fruit yield was always higher in 100C than in RDI but
 320 significant differences were only found in 2011 and 2012. However, these differences disappeared
 321 when total fruit yield was normalized by leaf area. VOO yield was consistently and significantly higher
 322 in 100C (1599 kg ha^{-1} on average for the four years studied) than in RDI (880 kg ha^{-1} , 1291 kg ha^{-1} and
 323 800 kg ha^{-1} in 30RDI, 45RDI and 60RDI, respectively). However, when VOO yield was expressed as
 324 percentage of the fruit weight, it was higher (2011), similar (2012) or lower (2014 and 2015) in 100C
 325 than in RDI plots (data not shown).

326 Concerning the relationships between mean annual $A_{n,\text{max}}$ per treatment and the final leaf area, trunk
 327 diameter and fruit and VOO yields per year and treatment, we found a very strong and significant
 328 relationship between mean $A_{n,\text{max}}$ and final leaf area ($y = 0.628x - 0.566$, $r^2 = 0.90$, $p < 0.0001$, $n = 10$)
 329 as well as with fruit yield ($y = 998.46x + 2832$, $r^2 = 0.66$, $p < 0.01$, $n = 10$) (Fig. 6). However, no
 330 significant relationship was found with trunk diameter or VOO yield (data not shown). The
 331 relationship of $A_{n,\text{max}}$ with fruit yield become not significant when fruit yield was divided by leaf area
 332 to eliminate its effect from the $A_{n,\text{max}}$ –fruit yield relationship (data not shown). These results were
 333 confirmed with the results of a partial correlation analysis between $A_{n,\text{max}}$ and total fruit yield,
 334 keeping the leaf area as the control variable (partial correlation coefficient= 0.51 and $p=0.16$). On the
 335 contrary, we found that the relationship between leaf area and fruit yield was strong and significant
 336 (Fig. 7, $y = 1384.1x + 5443.5$, $r^2 = 0.55$, $p < 0.05$, $n = 10$).

337

338 4. DISCUSSION

339 The database used in this research, with measurements every other week from May-June to
340 October-November during four years, allowed us to disentangle and describe new, consistent
341 relationships on the $A_{n,max}$ vs aboveground organs growth, specially fruit yield, and described the
342 interaction effect of soil water content and D_a on $A_{n,max}$. Stomatal conductance is the main limiting
343 factor of $A_{n,max}$, and although it had been already suggested that low soil water contents alter the
344 effect of D_a on olive stomata (Giorio et al., 1999; Moriana et al., 2002; Perez-Martin et al., 2009) our
345 results demonstrated that under the driest atmospheric conditions (3.6-4.7 kPa, Fig. 5) the effect of
346 soil water content on leaf gas exchange was also significantly affected. In all RDI treatments,
347 regardless the irrigation amount, $g_{s,max}$ and $A_{n,max}$ were similarly limited by high D_a . High atmospheric
348 demand has been identified as causing stomatal closure in olive (Fernández et al., 1997), which in
349 turn reduces $A_{n,max}$ (Giorio et al., 1999; Moriana et al., 2002; Naor et al., 2013), although this is the
350 first time that the interaction of D_a with soil water content was assessed to this detail in olive. Olive
351 characteristics such as the small leaf size together with the open canopies of olive orchards,
352 contribute to the highly responsive stomata to atmospheric demand in olive (Diaz-Espejo et al., 2012;
353 Fernández, 2014). The D_a -REW interaction contributes to explain why the differences caused by the
354 RDI treatments on trunk diameter or fruit yield are not so marked. Overall, our results suggest that
355 these findings on the D_a -REW interaction in the olive gas exchange must be taken into account for
356 the design of new practices to optimize irrigation management, like the reformulation of new
357 monthly fixed values of crop coefficients.

358 The $A_{n,max}$ reduction caused by the limited available water in the soil of the RDI trees was one of the
359 main factors limiting the growth of the aboveground vegetative organs, as compared to the 100C
360 trees (Table 2). However, the reduction extent was different for each organ studied. As a result of the
361 $A_{n,max}$ reduction observed, the final leaf area of the RDI plots was significantly lower than in 100C
362 plots (Table 3) but no significant differences were found in trunk diameter. The fact that significant

differences were found on leaf area but not on trunk diameter can be explained by the annual tree pruning conducted in the study plots, which allowed an important renewal of part of the canopy. Leaf area was reduced with the pruning by $29\pm0.05\%$, $37\pm0.07\%$, $37\pm0.09\%$ and $22\pm0.01\%$ on average in 100C, 60RDI, 30RDI and 45RDI, respectively, for the period studied. Thus, leaf area was more affected by each year treatments than trunk diameter. Moreover, it is known that trees under water deficit conditions reduce their leaf area preferentially to trunk diameter, to additionally decrease the amount of water consumed (McDowell et al., 2008). Finally, the already described negative crop effect on trunk growth in different species regardless the tree water stress (Moriani et al., 2003; Intrigliolo and Castel, 2007; Tognetti et al., 2009) could have contributed to the lack of significant reduction in trunk diameter by the irrigation treatments. It is well known that fruits constitute strong carbohydrates sinks and have priority for assimilates with the consequence of a reduced availability for trunk growth (Intrigliolo and Castel, 2007).

In accordance with our hypothesis, the increase rate of fruit weight was not as affected by $A_{n,max}$ limitation produced by RDI, as it was the leaf area (Table 2). While leaf area increase was consistently and significantly lower in the RDI treatments compared to 100C, the increase rate of fruit weight was only significantly reduced in 2011 and only between 30RDI and 100C. The final weight of the fruits was not significantly different among treatments either, i.e., under limiting conditions for $A_{n,max}$, the plant reduced leaf area but not the average fruit weight. Interestingly, in all years the resulting annual fruit yield was higher in 100C plots than in RDI plots, although differences were only significant in 2011 and 2012 (Table 3). Thus, fruit yield was less affected than leaf area by the limitation of $A_{n,max}$ produced by RDI but showed more differences among treatments than single fruit weight. To explain these results we should consider the effect of total leaf area on fruit yield, besides the impact of $A_{n,max}$. Indeed, we found that fruit yield was significantly related to $A_{n,max}$ but also to leaf area. Moreover, we observed constant values of fruit yield when fruit yield was normalized by leaf area which removed the effect of leaf area from the fruit yield. These results suggest that fruit yield was controlled to a great extent by total leaf area. Larger trees can carry larger crop load,

simply because there are more potential flowering shoots. Therefore, to maximize the fruit yield under RDI it is not enough to enhance photosynthesis per leaf area but it is also necessary to consider the total leaf area. The implications of these results are remarkable since to control tree water stress and its effect on yield, stomatal conductance measured at leaf level is increasingly being suggested as indicator (Hernandez-Santana et al., 2016). Gas exchange measurements at leaf level cannot be determinant alone of the final yield because leaf area was demonstrated to have a marked effect on yield too. Our results highlight the importance of also considering the leaf area in the orchard management to optimize the yield and control irrigation. The fact that the differences produced by RDI treatments were significant in 2011 and 2012 but not in 2014 and 2015 may be explained by the different irrigation strategy in the timing in 2014 and 2015 compared to 2011 and 2012 as explained in Materials and Methods section. Different authors (Rapoport et al., 2004; Hammami et al., 2011) have highlighted the importance of avoiding severe tree water stress between period 1 and 2 due to the negative consequences on fruit development. Hammami et al. (2011) reported that the beginning of olive fruit development is characterized by active cell division, which produces the majority of the final cell number and it is strongly correlated with fruit size. Thus, excessive water stress in this period may reduce cell number and, in turn, fruit size. Finally, VOO yield usually increased with increasing irrigation. However, the same values expressed by fruit weight indicated that in some cases, these differences produced by different irrigation amounts were due to the greater fruit yield.

Overall, the RDI treatments yielded a significant reduction in leaf area compared to 100C but they did not curtail fruit weight or total yield to the same extent. The trunk diameter was the least influenced variable. Thus, considering that RDI could help to control vegetative growth, mainly leaf area, and reduce water supply, we suggest using either 30RDI or 45RDI as a tool to reduce excessive tree vigor in SHD olive orchards. Our results add new advantages to the RDI level (lower than 60RDI but slightly higher than 30RDI) proposed by Fernández et al. (2013) as the most successful for their objective of balancing water saving and oil production.

Our results open the gate for a rational and objective control of leaf area, and therefore maximum yield, based on leaf gas exchange, photosynthesis or, its main determinant, stomatal conductance.

5. CONCLUSIONS

The assessment of the relationships between limited photosynthesis and the growth of aboveground tree organs allowed us to conclude that RDI can be used as an effective tool to control tree vigor, mainly through leaf area reduction, without decreasing fruit yield to the same extent. The plant water stress induced by RDI reduces stomatal conductance and thus, photosynthesis rate, which results on the decline of leaf area but not on the decrease of single fruit growth or the total fruit yield normalized by leaf area. While leaf area is determined by photosynthesis, fruit yield is conditioned also by leaf area. Thus, the most important variables that need to be controlled to limit tree growth without curtailing the yield are photosynthesis and leaf area. For a given leaf area we have a potential yield that must be optimized with the deficit irrigation strategy. The 30RDI or 45RDI strategies lead to greater water savings than 60RDI, with a similar effect on leaf area and fruit yield and thus, either a 30RDI or 45RDI strategy is preferred. More work is needed on the effect of plant leaf area since it determines both crop water requirements and yield. We also conclude that an optimal irrigation strategy in areas with very high vapor pressure deficit should consider that high atmospheric demand can exert a remarkable limitation on photosynthesis regardless the available water in the soil, i.e., the level of water reduction imposed by the applied RDI strategy.

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Figure captions

Figure 1. Regulated deficit irrigation strategies applied in the years 2011 and 2012 (a) and 2014 and 2015 (b). Both strategies are basically the same with the difference that in 2014 and 2015 we irrigated between Period 1 and Period 2. Also, different irrigation levels were applied in 2011 and 2012 as compared to 2014 and 2015, as detailed in the graphs. IN applies to the irrigation requirements, calculated as $IN = ET_c - P_e$, where ET_c is the crop evapotranspiration and P_e the effective

precipitation (see text for details). AW = available soil water; i.e. = irrigation event. After Fernández et al. (2013) and Padilla-Díaz et al. (2016).

Figure 2. Seasonal course of maximum net photosynthesis measured in well-watered trees (100C) and deficit irrigation treatments (60RDI, 45RDI, 30RDI) in the irrigation season for years 2011, 2012, 2014 and 2015. Each point represents the average of eight measurements in four plots per irrigation treatment and bars are ± 1 SE error bars. Different letters show significant differences ($p < 0.05$).

Figure 3. Maximum vapor pressure deficit determined for each day of the irrigation seasons in 2011, 2012, 2014 and 2015.

Figure 4. Seasonal course of relative extractable water calculated from measurements of soil water content in the root zone (0-45 cm) of three central trees per treatment during the irrigation season of 2011, 2012, 2014 and 2015. Part of the data of 2011 and 2012 were shown in Fernández et al. (2013) and 2014 in Padilla-Díaz et al. (2016).

Figure 5. Stomatal conductance (a and c) and net photosynthesis (b and d) measured from late June to late August in 2011 and 2012 and in 2014 and 2015, divided by their maximum and grouped according to the vapor pressure deficit measured. Different letters show significant differences ($p < 0.05$). Error bars are ± 1 SE.

Figure 6. Relationship between the annual mean of maximum net photosynthesis and final leaf area (black circles) and fruit yield (white circles) in years 2011, 2012, 2014 and 2015. Values were collected from all plots. Solid line is the regression curve for photosynthesis-leaf area ($y = -0.5660 + 0.6280 x$, $r^2 = 0.90$, $p < 0.0001$) and dotted line is the one for photosynthesis-fruit yield ($y = 2832.0040 + 998.4605 x$, $r^2 = 0.66$, $p < 0.001$). Error bars are ± 1 SE for each plot.

Figure 7. Relationship between final leaf area and fruit yield for all the plots in years 2011, 2012, 2014 and 2015. Solid line is the regression ($y = 1384.1 x + 5443.5$, $r^2 = 0.55$, $p < 0.05$, $n = 10$). Error bars are ± 1 SE for each plot.