Relationships between fruit growth and oil accumulation with simulated seasonal dynamics of leaf gas exchange in the olive tree

Hernandez-Santana, V.¹, Fernandes, R.D.M.¹, Perez-Arcoiza A.¹, Fernández, J.E.¹, Garcia, J.M.², Diaz-Espejo, A.^{1,*}

¹ Irrigation and Crop Ecophysiology Group, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC). Avda Reina Mercedes 10, 41012 Sevilla, Spain.

² Instituto de la Grasa (IG-CSIC), Edificio 46, Campus Universitario Pablo de Olavide, Ctra. de Utrera, Km. 1, 41013 Sevilla, Spain

*adiaz@irnase.csic.es

ABSTRACT

Carbohydrates availability, which are directly related to photosynthesis (A_N), and turgor are the main determinants of fruit growth. Since stomatal conductance (g_s) is the main limiting factor of A_N in fruit trees in most environments, and is strongly regulated by turgor, its measurement is pivotal to understanding fruit growth dynamics. Despite its relevance, the use of g_s to estimate A_N faces the major limitation of being difficult to measure in an automated and continuous manner. Based on these observations, and considering the control that the stomata exert on transpiration, and thus on sap flux density (J_s) under conditions of high coupling to the atmosphere, we conducted a multifaceted experiment in olive trees. The main objective was to assess the use of continuously modelled A_N , derived using a simulated g_s , as a tool to study fruit growth and oil accumulation and other components of vegetative above-ground growth (leaf area and number of shoot internodes) in a super-high-density olive orchard under different irrigation levels. Sixteen olive trees under four different irrigation treatments (two control and two deficit irrigated, with one and two drip lines each) were continuously monitored with J_s sensors from May to November 2016. Gas exchange, fruit growth, number of shoot internodes and leaf area were measured periodically. Stomatal conductance was empirically simulated through J_s , and A_N was modelled using previously simulated g_s and a biochemical model of photosynthesis. Results showed that A_N can be accurately modelled from simulated g_s , obtained in turn from J_s measurements divided by pressure deficit. Moreover, the approach was shown to be sensitive enough to infer the response of g_s and A_N to soil water content and vapour pressure deficit. Interestingly, accumulated A_N was significantly related to fruit growth and oil content for all the irrigation treatments which determine the slope of these relations. In contrast, the relationship with leaf area was only significant for the control irrigation treatments, where the number of shoot internodes increased significantly more than in the water-stressed trees. Our results show that under water stress conditions trees prioritize fruit growth and oil content accumulation over vegetative growth, suggesting a higher sink strength for reproductive growth than for vegetative growth. We conclude that the use of sap flow and the proposed approach provides reliable g_s and A_N data, and allows the modelling of the relations between carbon assimilation and allocation, which is helpful to estimate fruit growth.

38 *Keywords:* stomatal conductance, photosynthesis, irrigation scheduling, water stress, sap flux density

INTRODUCTION

Water is an increasingly scarce resource, but central to achieve a more productive agricultural output, to feed the growing world population (Foley et al., 2011; Mueller et al., 2012). Plants require water for growth and tissue expansion, with water absorbed by plants through their roots being lost through the stomata while CO_2 is taken up for photosynthesis. Stomatal opening is the main factor determining CO_2 uptake, and therefore, the synthesis of photoassimilates in the plant (Flexas and Medrano 2002). Furthermore, stomatal closure is one of the earliest responses to water deficit (Martin-StPaul et al. 2017), markedly affecting water uptake and water movement through the plant. Stomatal conductance (g_s) is, therefore, a sensitive water stress indicator, directly related to plant function, shoot and fruit growth and, eventually, to yield (Jones and Tardieu, 1998). In addition to affecting the amount of carbohydrate available for the fruit, stomatal regulation ultimately determines the fruit water status through its effect on both leaf and fruit water potential (Ho et al. 1987; Fishman and Génard, 1998; Leuchadel et al. 2007).

Despite it being important to know g_s for ecophysiological studies and agricultural applications such as irrigation scheduling, its continuous and automated recording is not yet possible. However, new approaches have been recently proposed to conduct g_s estimations continuously, either by modifying the inverted equation of Penman-Monteith (Kucera et al. 2017), or by using its simplified version (McNaughton and Jarvis, 1983; Jarvis and McNaughton, 1986) as proposed by Hernandez-Santana et al. (2016a). Specifically, Hernandez-Santana et al. (2016a) proposed simulating g_s from sap flux density (J_s) values measured in the trunks of trees, divided by the air vapour pressure deficit (D). This avoids the uncertainty derived from upscaling from single point measurements of J_s in the trunk to the whole tree transpiration (Swanson, 1994; López-Bernal et al. 2010; Hernandez-Santana et al. 2015; Berdanier et al. 2016). The automated estimation of g_s opens the possibility of using it both as a reliable water stress indicator and as an input in photosynthesis models which, when applied to fruit tree orchards, could help to predict yield. The mechanistic model of photosynthesis by Farquhar et al. (1980) has proven to be robust and easily applicable to many species and environments (Farquhar et al., 2001; von Caemmerer, 2013). This model has been parameterised for many species of high agronomic interest (Diaz-Espejo et al., 2006; Egea et al., 2011; Greer et al., 2012; Kattge and Knorr, 2007). After a correct parametrization, the main difficulty to its application rests in the estimation of g_s . However, once we have a value for g_s , A_N can be estimated from environmental variables, such that we can predict which region of the A_N - g_s relationship our crop is in at any given moment. Considering the close analogy between the A_N - g_s relationship and yield-transpiration (Flexas et al., 2010; Medrano et al., 2002; Morison et al., 2008; Parry et al., 2005), i.e., the two relations can be considered equivalent, the combination of sap flux density measurements and Farquhar et al.'s photosynthesis model may overcome the difficulty of applying a target level of

water stress in deficit irrigated trees. For example, the known hyperbolic curve usually found between A_N and g_s justifies the maintenance of g_s values below the plateau region in which A_N hardly increases, but g_s , and thus tree water use, still grows significantly, thereby decreasing water use efficiency (Parry et al. 2005).

However, there is no straightforward relationship between A_N and yield. Indeed, the fraction of photoassimilates going to each organ of the plant depends on a series of complex rules. Carbon allocation partitioning links source and sink plant organs through various regulations and interactions among different plant processes related to carbon metabolism (Génard et al. 2008). When A_N is limited by low soil water availability, the allocation partitioning patterns can change based on priority rules. These allocation patterns have been widely studied (Le Roux et al. 2001), although they are not easy to implement in agriculture because the mechanisms by which dry matter is distributed among organs are not fully understood (Marcelis, 1996). Nevertheless, biomass allocation patterns have been manipulated in agricultural species for years to achieve a gain in productivity by increasing the plant harvest index (the ratio of grain/fruit weight to total plant weight), which reflects the partitioning of photoassimilates between the grain/fruit and the rest of the plant (Sinclair, 1998; Morison et al. 2008). Indeed, the plant harvest index can be increased by using deficit irrigation strategies (Morison et al. 2008). In addition to saving water, deficit irrigation strategies can reduce vegetative growth without decreasing yield, i.e., they are effective in decreasing the carbon spent on vegetative growth without decreasing fruit growth as shown previously in olive (Iniesta et al. 2009; Dag et al. 2010; Hernandez-Santana et al. 2017). Thus, under limited available water, fruits augment their already strong carbohydrate sink capacity and have higher priority for assimilates than vegetative organs, leading to a reduced vegetative growth of roots, stem and leaves (Génard et al. 2008; Hacket-Pain et al. 2017). Therefore, knowing the irrigation strategy that can modify the growth patterns of a plant to enhance the biomass allocation to fruits rather than to vegetative organs, would allow us to control excessive vegetative growth with a reduced impact on fruit growth. In addition, this would lead to a net water saving in agriculture. Recent results indicate that 30-45% of the irrigation needs of a super-high-density olive orchard could be useful for this purpose (Hernandez-Santana et al. 2017). However, these results were based on values obtained at the end of the irrigation season of different years, and did not provide information about the dynamics during the fruit growth period.

In this work, we simulated the dynamics of g_s and A_N continuously over an irrigation season (late May to early November) in a hedgerow olive orchard with high plant density. Fruit size, oil content, leaf area and number of shoot internodes were frequently measured, to derive the correlation between accumulated A_N and fruit development. We followed the approach described in Hernandez-Santana

et al. (2016a) to simulate g_s from continuous and automated J_s/D measurements, and then used the photosynthesis model by Farquhar et al. (Farquhar et al. 1980) to estimate A_N . Our hypothesis was that this approach would allow the estimation of the temporal dynamics of fruit size and oil content since g_s is highly sensitive to water stress, the main limiting factor of A_N and a major regulator of the incoming and outgoing water fluxes of the fruit. We further hypothesized that knowing the dynamics of accumulated A_N would allow the dynamics of fruit growth, oil accumulation, leaf area and the number of shoot internodes to be simulated over the course of the season. Finally, we hypothesized that vegetative growth would be more affected by deficit irrigation than fruit growth. Thus, our specific aims were (1) to calibrate and validate an approach to simulate g_s and A_N based on automated J_s measurements, and (2) to assess the use of continuously simulated A_N as a tool to study fruit growth, oil accumulation and other components of vegetative above-ground growth (leaf area and shoot length) in a super-high density olive orchard under different levels of irrigation.

2.- MATERIALS AND METHODS

2.1.- Orchard and climate characteristics

The experiment was conducted along the irrigation season (late May to early November) of 2016, in a commercial super-high-density orchard near Seville (Spain) (37° 15' N, -5° 48' W). At that time, the 'Arbequina' olive trees used were 10 years old. They were planted in a 4 m × 1.5 m formation (1667 trees ha⁻¹), in rows oriented N-NE to S-SW. The trees, with a single trunk and shoots from 0.6 to 0.7 m above the ground, were pruned in December-January each year. The orchard soil (Arenic Albaqualf, USDA 2010) had a sandy loam top layer and a sandy clay layer underneath. 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).

The area has a Mediterranean climate with hot and dry weather from May to September, being mild and wet for the rest of the year. Most of the annual rainfall occurs between late September and May. Average values in the area of potential evapotranspiration (ET_o) and precipitation (P) were 1531 mm and 509 mm, respectively, for the 2002–2016 period. For that period, average maximum and minimum air temperature values were 25.3 °C and 10.9 °C, respectively. The hottest months are July and August.

We applied four irrigation treatments: two control treatments, in which irrigation fulfilled tree water demand, with one (100C-1L) or two (100C-2L) dripper lines, and two sustained deficit irrigation treatments (SDI) in which 45% of the water added to control was applied along the whole irrigation

season, with one (45SDI-1L) or two (45SDI-2L) dripper lines. The objective of these sub-treatments is to alter the equilibrium between root and leaf area with localised irrigation by drippers which have an important effect on transpiration as shown preliminarily in Diaz-Espejo et al. (2012). However, this aim is beyond the scope of this work. Moreover, the dripper lines were doubled in 2016 and thus, no effect was observed between the treatments with one and two dripper lines in this work. All lines were close to the trunk and each line had a 2 L h^{-1} dripper placed every 0.5 m. We used a complete randomized design, with four plots (replicates) per treatment. Each plot consisted of 8 central trees surrounded by 16 border trees. For the 100C treatment, the trees were irrigated daily to replace 100% of the irrigation needs (IN). These were calculated on a daily basis as $IN = ET_c - P_e$, with ET_c being 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. Estimation of ET_c in this orchard has proven satisfactory in several studies (Diaz-Espejo et al., 2012; Fernández et al, 2013; Egea et al., 2016). The 45SDI treatments were aimed to replace 45% of IN. In the 2L plots, the irrigation time was scheduled to be half that of the 1L plots, so that the trees received the same volume of water despite having double the number of drippers. An irrigation controller (Agronic 2000, Sistemes Electrònics PROGRÉS,S.A., Lleida, Spain) was used to apply the calculated irrigation amounts (IA).

2.2. Meteorological measurements

Thirty-minute average values of main meteorological variables were recorded by a weather station (Campbell Scientific Ltd., Shepshed, UK) located in the centre of the area covered by the experimental plots. All meteorological sensors were located just above the tree canopies. The station recorded 30 min average values of air temperature (T_a), air humidity (RH_a) and photosynthetically active radiation (PAR), among other variables. Vapour pressure deficit (D) was calculated as a function of T_a and RH_a .

2.3. Measurement of predawn leaf water potential

Measurements of predawn leaf water potential (Ψ_{pd} , MPa) were conducted before dawn every other week from July to the beginning of November, with a Scholander-type pressure chamber (PMS Instrument Company, Albany, Oregon, USA). On each measurement day we sampled two leaves per tree of four trees per irrigation treatment. These values were used as a proxy of soil water potential.

2.4. Sap flux density measurements

The Compensation Heat Pulse (CHP) method (Green et al., 2003) was used to derive sap flux density (J_s , mm h^{-1}) values in the sapwood of sampled trees (Tranzflo NZ Ltd., Palmerston North, New

Zealand). CHP consists of measuring the time for the temperature difference registered by two temperature probes located 10 mm downstream and 5 mm upstream of a heater probe to become 0 after the release of a heat pulse. Details on the calibration and testing of the technique for the olive tree, as well as on data analysis, are given in Fernández et al. (2001, 2006). One or two central trees per plot, in three plots per treatment, were monitored with one sap flow probe set on the east-facing side of trunks, 0.3–0.4 m above-ground; four trees were used per treatment to give a total of 16. Each temperature probe measured J_s at a depth 5 mm below the cambium, given that we observed in a previous study (Hernandez-Santana et al. 2016a) that J_s measured at this depth was strongly related to g_s measured in new, sun-exposed leaves. Measurements were made every 30 min by releasing heat pulses (60 J; 60 W over 1 s) at that frequency for the entire experimental period (from May 29 to November 8, 2016). A CR1000 datalogger connected to a AM25T multiplexer (Campbell, Campbell Scientific Ltd., Shepshed, UK) was used to release the heat pulses and to collect probe outputs.

2.5. Gas exchange measurement and modelling

Stomatal conductance was simulated after Hernandez-Santana et al. (2016a), i.e. from the collected J_s/D values and measured g_s . The simulated g_s values were the input of the model by Farquhar et al. (Farquhar et al. 1980) to estimate A_N . Briefly, we established regressions between g_s measured in new, sun-exposed leaves, and J_s measured in the trunk of the same tree, divided by D values calculated from the weather station at the orchard. The J_s/D vs. g_s calibration equations were established using 10-23 data points from each instrumented tree (Table 1).

The stomatal conductance values used for the equations were obtained from measurements of g_s and A_N , which were conducted on four clear days from May to August, every 30-60 min from dawn to noon, in three sun-exposed current-year leaves per instrumented tree. The sampled leaves were on the SE-facing side of the canopy, i.e. on the sun-exposed side. In addition, we used a dataset obtained from measuring maximum g_s and A_N ($g_{s,max}$ and $A_{N,max}$) in two leaves per tree, conducted every other week in every tree instrumented with sap flow probes, from mid-July to the beginning of October, at 8:00-9:00 GMT, this being the time for maximum stomatal opening in olive leaves (Fernández et al., 1997). Data from five randomly selected days from this last dataset were not included in the calibration but, instead, were used to validate the calibration g_s equations. We used two portable photosynthesis systems (Li-cor 6400-XT, LI-COR, Lincoln NE, USA), with a 2 cm × 3 cm standard chamber, at ambient light and CO₂ conditions for these measurements.

A_N was modelled from the simulated g_s values following the procedure described in the Appendix. The specific temperature responses for olive were taken from Diaz-Espejo et al. (2006). The maximum rate of ribulose biphosphate (RuBP) carboxylation (V_{cmax}), maximum rate of electron

transport ($J_{\max}$) and mesophyll conductance to CO₂ (g_m) were determined from five A_N-C_i response curves measured in the instrumented trees of the 100C and 45SDI plots, regardless of the number of dripper lines. The curves were measured between 09:00 and 13:00 GMT during the experimental period (June 14th, 15th, 21st and 23rd) using two LI-6400 portable photosynthesis systems (LI-COR, Lincoln, NE, USA) at ambient temperature, saturating photosynthetic photon flux density ($1600 \mu\text{mol m}^{-2} \text{s}^{-1}$) and an ambient CO₂ concentration (C_a) of between 50 and $1500 \mu\text{mol mol}^{-1}$ as described in Hernandez-Santana et al. (2016b). After steady-state photosynthesis was achieved, the response of A_N to varying C_i was measured by lowering C_a stepwise from 390 to $50 \mu\text{mol mol}^{-1}$, returning to $390 \mu\text{mol mol}^{-1}$ and then increasing C_a stepwise from 390 to $1500 \mu\text{mol mol}^{-1}$. Each $A-C_i$ curve comprised 16 measurements, each made after at least 3 min at each C_a . Maximum carboxylation rate ($V_{\max}$) was estimated by the curve-fitting method proposed by Ethier and Livingston (2004). Diffusion leaks were corrected following the procedure by Flexas et al. (2007). Rubisco kinetic parameters were taken from Bernacchi et al. (2002). The validation of the Farquhar et al. (Farquhar et al. 1980) equation to simulate A_N was conducted with the measured dataset as described above. Accumulated A_N is calculated summing up the quantity of simulated A_N every 30 mins until the moment the value is showed.

2.6. Leaf area measurements

Measurements of leaf area were made at dawn every other week from June to October, with a LAI-2200 Plant Canopy Analyzer (Li-cor, Lincoln, NE, USA). We followed the approach proposed by Villalobos et al. (1995) for olive orchards. In this way, measurements were taken at eight locations per plot with the LAI-2200 Plant Canopy Analyzer, in each of the four plots per irrigation treatment (100C-1L, 100C-2L, 45SDI-1L, 45SDI-2L). Four of those locations per plot were just underneath the two central tree rows of the plot, providing the maximum leaf area index ($\text{LAI}_{\max}$). The other four locations were in-between the two central rows of each plot, where LAI is minimum ($\text{LAI}_{\min}$). The fraction of groundcover (GC) was used as a weighting factor to calculate the average leaf area index (LAI_{avg}) as $\text{LAI}_{\text{avg}} = \text{LAI}_{\max} \text{GC} + \text{LAI}_{\min} (1 - \text{GC})$. Finally, the average tree leaf area per plot was calculated by multiplying LAI_{avg} by the ground area per plot, and dividing this by the number of trees in the plot.

2.7. Shoot growth

Shoot growth was assessed by measuring the number of internodes of four current-year shoots per tree, each from a cardinal point of the canopy and randomly selected in two trees per plot in each treatment ($n = 32$). Measurements were made every 15 days from June 13 to October 31.

2.8. Fruit growth and yield determination

Fruit growth was determined from fruit dry weight and fruit diameter measurements. For fruit weight we randomly sampled six fruits per plot for each treatment; these were oven-dried for 48 hours and then weighed on a digital balance (XS105 Dual Range, Mettler Toledo, Greifensee, Switzerland). Fruit weight was measured from the beginning (May 30) to the end of the irrigation season (November 9) every other week.

In addition, in one tree from each of the 100C-2L and 45SDI-2L treatments we installed two fruit dendrometers (FI-XSSF, Solfranc, Tarragona, Spain) to monitor the seasonal courses of fruit equatorial diameter, logging these every 5 minutes. The fruit dendrometers were connected directly to CR1000 dataloggers (Campbell Scientific Ltd., Shepshed, UK). When the diameters of the fruits were close to the maximum range of measurement of the fruit dendrometers, an offset was added to the fruit dendrometer, by changing the position of one of its ends. The fruit dendrometers were installed on August 17 (day of year (DOY) 230) and were removed from the experimental plots a few days before harvesting (November 9, DOY 313). Calculations of accumulated daily fruit equatorial diameter increment and A_N were started the day the fruit dendrometers were installed. To better understand the seasonal dynamics of fruit growth in relation to A_N , we also calculated the accumulation of the two variables over a period of three days; this value was divided by the number of days to obtain the rate per day such that fruit growth reflects the effect of a previously fixed carbon. Three days was assumed to be a reasonable period of time to integrate/translate the accumulated A_N into fruit growth.

Four central trees were also manually harvested at the end of the irrigation season (November 9) to determine total fruit yield in each experimental plot. Samples were oven-dried and weighed separately to determine the dry fruit weight. The number of fruits was calculated from the total fruit yield and dry fruit weight.

2.9. Oil accumulation

Oil accumulated in the fruits was determined from June to November, both in 2014 and 2015. We manually collected around 100 fruits from a height of ca. 1.5 m for five out of the eight central trees of each plot (the three remaining trees were used to determine yield, as described above). After grinding the fruits ca. 20 g of fresh paste was obtained, which was dehydrated at 105°C. The oil of the dehydrated paste was chemically extracted by using hexane as a solvent during 4 hours (Soxhlet's method, García et al., 2013). Results were expressed as percentage of oil per fruit dry weight.

2.10. Statistical analyses

Relationships, coefficient of determination (R^2) and probability values (P) were determined using SigmaPlot (version 12.0, Systat Software Inc., San Jose, California, USA). Estimation accuracy was

determined by the calculation of root mean square error (RMSE) and mean absolute error (MAE). We also calculated the mean bias error (MBE) to estimate the deviation of the predicted g_s . The closer that R^2 is to 1 and that RMSE and MAE are to 0, the better is the agreement between measured and calculated g_s values. The sign of MBE indicates whether simulated g_s is being underestimated (negative) or overestimated (positive). More details on the formulas used here and their meaning can be found in Abbas et al. (2011) and Walther & Moore (2005).

We used linear mixed models (LMM) with Tukey's post-hoc comparisons to analyse the effects of irrigation treatment (fixed factor) on mean fruit number and yield as dependent variables at $P < 0.05$. No random factor was necessary as only one measurement was made per plot. When no normal or heterocedastic residuals were obtained, appropriate transformation of the variable was used. We also used analysis of covariance (ANCOVA) including the interaction term (accumulated A_N * irrigation treatment) to test whether the slopes of the regression lines for fruit weight, leaf area, oil content and number of shoot internodes were significantly different. For these analyses, we considered that the variables were linearly related even though for dry fruit weight and oil content a linear relation did not provide the best fit. This same analysis was conducted when comparing measured and modelled g_s and A_N to detect any effect of irrigation treatment. These analyses were conducted with R software (R Core Team, version 3.2.0, 2015) using R packages "nlme R" (Pinheiro et al., 2011) and "multcompR" (Hothorn et al., 2008).

3. RESULTS

3.1. Simulation of stomatal conductance and net photosynthesis rate

The J_s/D vs. g_s relationships derived from each instrumented tree all had high R^2 values (between 0.48 and 0.86, with the majority of trees being over 0.6) and were highly significant (all P values were < 0.01 except for one, which was 0.012) (Table 1). The validation results confirmed the good agreement between measured and simulated g_s values calculated with the calibration equations. Moreover, the observed RMSE and MAE values were small and close to 0 for every instrumented tree, while MBE did not show any under- or overestimating bias for g_s . The validation of the simulated g_s and A_N , calculated by treatment, also showed good agreement with measured g_s and A_N (Fig. 1). The high R^2 (0.7), the slopes close to 1, and the y-intercepts close to zero in both cases, indicated a good level of accuracy between observed and simulated g_s and A_N . No bias produced by the irrigation treatments was detected.

3.2. Automated and continuous simulation of stomatal conductance and net photosynthesis rate

Once the strong correlation between the dynamics of J_s measured in the outer xylem of the trunk and that of g_s in new, sun-exposed leaves in the canopy was confirmed, and after validating the Farquhar et al. model to estimate A_N in the monitored olive trees, we inferred the time dynamics of g_s and A_N for 170 days at 30 min intervals. The simulated daily values of maximum stomatal conductance ($g_{s,max}$) and net photosynthesis rate ($A_{N,max}$) (Fig. 2a) showed that the seasonal dynamics of these variables were mainly driven by D and the irrigation treatment, i.e. $g_{s,max}$ and $A_{N,max}$ decreased as D increased (Fig. 2c), but they decreased with soil water potential (predawn leaf water potential, Ψ_{pd} , was used as a proxy). Thus, lower $g_{s,max}$ and $A_{N,max}$ values were simulated in the 45SDI trees than in the 100C trees (Fig. 2d). The number of drip lines had no effect on these results.

3.3. Relationship between accumulated net photosynthesis and growth

Accumulated A_N was linearly related to the measured variables associated with vegetative growth, i.e. with leaf area (Fig. 3a) and number of shoot internodes (Fig. 3b). As mentioned in the Material and Methods section, accumulated A_N was calculated for the condition where the first day of measurement of fruit growth was taken as the baseline ($A_N=0$). The relationships between accumulated A_N and leaf area for the control irrigation treatments were strong and significant (100C-1L: $R^2=0.95$, $p=0.002$; 100C-2L: $R^2=0.84$, $p=0.0002$), but they were not for water-stressed trees (45SDI). The number of shoot internodes correlated well with A_N (100C-1L: $R^2=0.95$, $p<0.0001$; 100C-2L: $R^2=0.98$, $p<0.0001$; 45SDI-1L: $R^2=0.76$, $p=0.001$; 45SDI-2L: $R^2=0.91$, $p<0.0001$), although in the case of 45SDI trees the slopes of the equations were very small despite the significant relationship (0.01-0.02 in 45SDI trees versus 0.04 in 100C).

On the other hand, the relationships between both dry fruit weight and oil content with A_N were strong and highly significant for all treatments, with power relationships providing the best fit ($R^2=0.92-0.99$, $p<0.0001$). However, the rate of increase in dry fruit weight was steeper at the beginning of the measurement period (Fig. 3c) than at the end, in contrast to the opposite holding true for oil accumulation (Fig. 3d).

An analysis of the regression equations suggests that the rate of increase of number of shoot internodes (Figure 3b) and olive oil accumulation were statistically different between 100C trees and 45SDI trees (different slopes), with either one or two dripper lines. In contrast, the slopes of the regression curves between dry fruit weight and accumulated A_N (Figure 3c) were similar for all treatments, indicating a similar growth rate for fruits, regardless of the irrigation treatment.

Overall, and for all treatments, we observed a good agreement between accumulated A_N and both the number of shoot internodes and leaf area. However, the final fruit weight and the oil content were very similar despite the final accumulated A_N being different for the different treatments (Figure 3). No statistical differences were found when comparing the number of fruits or the yield

among treatments (Table 2), although the average yield reduction for the 45SDI treatments was 14% compared to the 100C yield.

The accumulated increase in fruit diameter measured with fruit dendrometers followed the seasonal dynamics of A_N (Fig. 4), with the increase in fruit diameter being slightly higher in plot 9 tree (45SDI-2L) than in plot 15 tree (100C-2L), despite A_N being higher in the latter tree than in the former. The average fruit diameter was higher in the 100C-2L tree (7.966 mm) than in the 45SDI tree (7.598 mm) on DOY 231, at the beginning of the measurement period. However, the temporal dynamics of the fruit diameter increment were closely related to the rate of A_N accumulation in the two monitored trees for each irrigation treatment (Figure 5).

4. DISCUSSION

Automated simulation of leaf gas exchange

This study builds on the g_s simulation method reported in Hernandez-Santana et al. (2016a) and presents a new, simplified approach for tracking gas exchange in a continuous manner and for studying its relationship with leaf area, number of shoot internodes, fruit growth and oil accumulation in olive trees. These results open the gate to develop a novel approach to manage the level of stress of the plant by irrigation, although still some uncertainties must be explored like the determination of photoassimilates partitioning to fruits vs other plant organs under a range of water stress levels, the effect of xylem-fruit water potential gradients on fruit growth or how to scale-up from fruit growth to yield considering different crop loads. Stomatal conductance was simulated from sap flow-related measurements and easy-to-record meteorological values. The proposed approach is sensitive enough to infer stomatal response to soil water availability and to D, and to quantify their impact on A_N (Figure 2). The approach can be fully automated and provides values representative of the whole canopy (thereby avoiding errors derived from the natural variability among leaves). Importantly, it does not require any upscaling, or for any assumptions to be made about any parameter. Moreover, it does not require the measurement of total leaf area or environmental variables other than D in contrast to other approaches using sap flow data to simulate gas exchange at the canopy level (Granier et al. 2000; David et al. 2007; López-Bernal et al. 2015; Kucera et al. 2017). Using direct measurements of J_s allows the use of sap flow probes with a single temperature sensor, which makes the method cheaper, as demonstrated by López-Bernal et al. (2017). In addition, the number of sensors required to assess the level of water stress is also reduced as it is not necessary to apply the signal intensity concept, in which measurements should be made both in treatment and in reference trees (Goldhamer and Fereres, 2004).

If used for scheduling irrigation, the described approach has two potential advantages: it is based on absolute values of $g_{s,max}$, which have a solid physiological meaning in relation to plant water stress (Medrano et al., 2002), and allows quantification of the A_N limitation imposed by deficit irrigation, which is an indicator of yield (Hernandez-Santana et al., 2017). Moreover, g_s and A_N tracking overcomes one of the shortcomings of other water stress indicators widely used for scheduling irrigation, which do not consider key intrinsic processes related to fruit development (Bustan et al. 2011). Although the relationships between gas exchange measurements and fruit growth or oil accumulation are not straightforward, g_s and A_N provide more information on fruit growth than other water stress indicators. Stomata regulate the carbohydrate source and, indirectly, the incoming-outgoing fluxes in the fruit, through their effect on leaf, stem and fruit water potentials, driven in turn, by osmotic pressure and hydrodynamics (Zonia and Munnik, 2007).

Growth partitioning under water stress

The proposed approach is also able to predict A_N , which thus increases the value of the approach for the management of irrigation. This is due to A_N being closely related to plant growth, function, productivity and yield, in response to water deficit (Flexas et al. 2004; López-Bernal et al. 2015; Ramírez et al. 2016). As mentioned, A_N is, together with the accumulation of water, i.e. turgor, one of the determinants of fruit growth (Fishman and Génard, 1998). Our data show that reduced soil water availability, induced by SDI, modified the supply of photoassimilates through the reduction of A_N , and altered the relationships between these photoassimilates and the growth of different plant organs (Figure 3). We found that fruit growth was less affected in water-stressed trees than was leaf area and the number of shoot internodes. The reduced slopes of the A_N -shoot growth relationship in 45SDI trees and the non-relationship between accumulated A_N and leaf area for that treatment, confirmed that vegetative growth in our water-stressed trees was reduced. The steeper slopes of the same relationships in well-irrigated trees indicated that growth occurred to a point suggesting that well-irrigated trees were photosynthesising enough to promote both fruit and vegetative growth. However, under conditions of water stress, 45SDI trees dedicated most of their A_N to fruit growth and oil accumulation (Fig. 3). Other authors working with forest and agronomical species reported similar results (e.g., Dag et al. 2010; Müller-Haubold et al. 2013; Selas et al. 2002; Intrigliolo and Castel, 2007; Hacket-Pain et al. 2017; Hernandez-Santana et al. 2017), demonstrating that the competing sink relationships between vegetative growth (stem or leaves) and fruit production for newly assimilated carbon were dependent on drought. This indicates that the biological costs of reproduction increase strongly under conditions of environmental stress, penalizing vegetative growth but having no detrimental effect on vegetative growth under favourable climatic conditions (Hacket-Pain et al. 2017). Accordingly, studies on leaf-fruit water relations in olive suggest that vegetative growth is more sensitive to drought than fruit growth, and that this sensitivity can be

modulated by crop load (Dell'Amico et al. 2012; Girón et al. 2015). However, this is the first time that the hypothesis of a higher response of vegetative growth versus reproductive growth to water stress has being tested in olive. We showed that the relationship between A_N and fruit dry matter depends on the level of irrigation. Thus, in future implementations of the model, the prediction of fruit dry matter accumulation should consider the increment towards reproductive sinks under water stress conditions. In agricultural species, carbon allocation to reproductive organs is particularly important in economic terms because of its impact on fruit yield.

Estimation of fruit growth and oil content

Our results support the use of the described approach to simulate the impact of water management on fruit and oil yields, which are the major production targets for farmers. More work is needed, however, to achieve the objectives of predicting fruit growth and thus, crop yield, which is beyond the scope of the present study. First, predicting fruit growth in terms of fresh weight might even be more complicated because it is determined by water besides biomass accumulation, which in turn, is a consequence of changes in water flows into and out of the fruit with the parent plant and also of transpiration losses from the fruit (Greenspan et al. 1994; 1996; Mathews and Shackel, 2005). This water fruit balance has been described to change toward the end of fruit development (Mathews and Shackel, 2005). Moreover, these results may apply particularly well in olive where the water component is less important than in other species where water flows are major determinants of fruit growth such as grape (Greenspan et al. 1996), peach (Morandi et al. 2007), pear (Morandi et al. 2014), etc. Secondly, the relationship between A_N and yield is not so straightforward, with yield determined not only by fruit size but also by fruit number. Fruit number is highly associated with the number of leafy shoots from the previous year, and therefore, leaf area plays a key role in determining yield, as was shown for the studied orchard by Hernandez-Santana et al. (2017). Naor et al. (2013) reported that in trees with a higher crop load, fruit size decreased, suggesting a stronger competition for assimilates among fruits. This was not the case in our study, in which the number of fruits and their size was statistically similar for all treatments. However, it is likely that under more stressful conditions, or under other conditions in which the ratio between leaf area and crop load is further reduced, we could have observed a limitation in yield produced by the number of fruits, as we did in other years in the same orchard (Fernández et al. 2013; Hernandez-Santana et al. 2017). The effect of the irrigation strategy on yield, whether regulated or sustained, is not the objective of this work and one irrigation season is not enough to evaluate the effects of an irrigation strategy on yield. Still, our study shows a remarkably tight correlation between fruit growth and accumulated A_N (Fig. 5). In our case, the main driving variable for A_N was D , which has a direct effect on stomatal regulation and confirms that diffusional limitations of photosynthesis are the main constraints for plant production in our environment (Flexas et al., 2014). Mechanistic models dealing with fruit

growth (Léchaudel et al. 2005; Lescourret and Génard 2005) could facilitate the calculation of yield based on gas exchange modelling.

As oil production is a key feature of olive cultivation, we also evaluated oil accumulation and observed that the final oil content in the fruit was slightly higher in water-stressed trees than in well-irrigated trees (Figure 3d). Although accumulated A_N did not start from 0 (Figure 3d) – because the baseline used to calculate accumulated A_N was the first day that fruit growth was measured – the dynamics of oil content accumulation in relation to A_N would have been similar if the baseline selected was different. In agreement with our results, other authors reported for olive that oil content in the fruit was less affected by water stress than shoot growth (Gomez-del-Campo, 2013), fruit size (Gomez-del-Campo et al. 2014) or yield (Dbara et al. 2016). It has been demonstrated that the amount of water in the fruit is most sensitive to decreases in irrigation supplies than is the amount of oil, which is less impacted by water deficit (Gomez-del-Campo et al. 2014). In our study, both fruit weight and oil content were expressed in dry weight terms to avoid misinterpretations due to the impact of the irrigation treatment on the fruit water content. The fruit dendrometers, however, measured the fruit diameter “*in vivo*”, thus including the effect of changes on fruit water content. The use of fruit dendrometers is promising as these instruments work automatically and provide data with a high temporal resolution (Figure 4, 5). Similarly to trunk dendrometers, however, the interpretation of the signal to separate the effect of water relations from biomass accumulation is challenging (Fernández and Cuevas, 2010; Mencuccini et al. 2013; De Swaef et al. 2015). In our case, the slightly higher fruit growth in the 45SDI-2L trees, as compared to the 100C-2L trees, suggests that growth is less affected by the relatively mild atmospheric demand typical of the end of the summer (Figure 2) in the smaller fruits of the 45SDI-2L. However, these results should be considered with caution because we used just two fruit dendrometers per treatment.

Consequences for scheduling deficit irrigation

Our findings increase the understanding of the trade-off between vegetative growth and fruit growth, and show that oil accumulation in the olive is dependent on soil water availability. A priority in dry environments, which will become even drier in the future, will be to produce more with less water. To face this challenge, irrigation management must be adapted to maximize fruit production while avoiding excessive tree growth. Excessive growth is, in fact, a major problem in super-high-density orchards (Connor et al. 2014, León et al. 2007). Applying deficit irrigation strategies designed to optimize the carbon allocation patterns could lead to trees of reduced size, i.e., requiring less water, without severely penalizing production. Indeed, the large gain in productivity among fruit tree species is often the result of the manipulation of the carbon allocation pattern within the plant, the

objective being to increase the number and size of fruits produced as a result of higher fruit competition for carbohydrates, as has been conducted in agricultural species for years (Génard et al. 2008). Deficit irrigation can extend the modification of these patterns further by enhancing fruit load and quality while controlling vegetative growth. Combined with pruning and a properly designed fertilization strategy, this approach will help to achieve the right balance between fruit and vegetative growth.

While a sustained irrigation strategy was used in the present study, regulated deficit irrigation (Chalmers et al. 1981) is often recommended for super-high-density olive orchards (Fernández et al., 2013). However, information is lacking concerning the main processes underlying crop performance to irrigation, which makes it a challenging task to design regulated deficit irrigation strategies that are pertinent to specific orchard conditions and production targets. Scheduling irrigation with a mechanistic approach as was used here could help to establish a regulated deficit irrigation strategy for each case. Although the relationships established between g_s , A_N , plant and fruit dry weight, as well as oil content, could help to establish an irrigation deficit approach based on physiological measurements, they still have to be assessed further in different olive orchards and under various environmental conditions.

5. CONCLUSIONS

Our results demonstrate that fruit growth can be simulated using accumulated photosynthesis, being this relation dependant on the level of irrigation. . Photosynthesis is not the only determinant of growth, being the other key variable involved in fruit growth, turgor pressure, set by stem/fruit water potential gradients,

also related to fruit growth via g_s , at least in a daily basis. Future efforts must be focused on studying the interrelations among leaf, stem and fruit water relations, as well as the physiological traits determining photoassimilate partitioning between both organs. In this sense, the use of sap flow measurements for monitoring tree performance emerges as a powerful tool to understand the mechanisms involved in the dynamics of fruit growth in tree crops and their relationship with leaf gas exchange. Although sap flow monitoring can be used directly to evaluate the seasonal response to irrigation management, we used it in our approach to simulate g_s empirically. Knowing the diurnal and seasonal dynamics of g_s allows us not only to estimate transpiration with tree leaf area, but also to estimate photosynthesis. Moreover, our approach could also be used to develop process-based models of g_s , which could help us understand why crops respond differently under a given environmental condition. Mechanistic models can provide information about plant responses to increased atmospheric CO_2 , providing an approach to test mechanisms by which plants can acclimate

to changes in the environment. Although the use of the methodology proposed in this study should be further tested in different species and under a range of management and environmental conditions, the estimation of g_s and its use in mechanistic models emerge as the core component of the new generation of methods and tools to schedule irrigation and predict yield in the future.

ACKNOWLEDGMENTS

This work was funded by the Spanish Ministry of Science and Innovation (research project AGL2015-71585-R). Antonio Montero, Alfonso Perez-Martin, Saray Gutierrez-Gordillo, M. Dolores Raya-Sereno and Rafael Romero helped us with the field measurements. Our thanks to the owner of Internacional Olivarera, S.A.U. (Interoliva), for allowing us to perform the experiments in the Sanabria orchard.

APPENDIX

Biochemical model of photosynthesis

This appendix presents equations necessary to reproduce the model used in this study to estimate net photosynthesis rate (A_N). We estimated stomatal conductance to H_2O (g_s) from sap flow as described in the Material and Methods section, following the procedure described in Hernandez-Santana et al. (2016). Then A_N was estimated following the widely used biochemical model by Farquhar et al. (1980).

The supply function for net photosynthesis rate (A_s) is described by Fick's law as

$$A_s = g_{CO_2, total} \cdot (C_a - C_c)$$

where C_a is ambient CO_2 , C_c is chloroplastic CO_2 and $g_{CO_2, total}$ is the total conductance to CO_2 ($1/g_{CO_2, total} = 1/g_{s, CO_2} + 1/g_m$). In the former equation $g_{s, CO_2} = g_s / 1.6$.

We used the biochemical model of Farquhar *et al.* (1980) and the gas exchange equations of von Caemmerer & Farquhar (1981) to simulate the demand function for net photosynthesis rate (A_d) based on the RuBP-carboxylation-limited and RuBP-regeneration-limited rates (A_c and A_q) as follows:

$$A_d = \min \{A_c, A_q, A_p\} - R_d,$$

$$A_c = V_{cmax} \frac{C_c - \Gamma^*}{C_c + K_c \left[1 + \left(\frac{\Gamma^*}{K_o} \right) \right]},$$

$$A_q = J \frac{(C_c - \Gamma^*)}{4(C_c + 2\Gamma^*)},$$

where V_{cmax} is the carboxylation capacity, J is the potential electron transport rate, C_c is the chloroplastic CO_2 concentration, Γ^* is the photorespiratory CO_2 compensation point, K_c and K_o are the Michaelis constants for RuBP carboxylation and oxygenation, respectively, O is the oxygen concentration and R_d is the rate of non-photorespiratory CO_2 release.

A nonrectangular hyperbolic function (Farquhar and Wong 1984) was used to calculate J as a function of irradiance (PPFD),

$$\theta J^2 - (\alpha \text{ PPFD} + J_{\text{max}}) J + \alpha \text{ PPFD} J_{\text{max}} = 0,$$

where J_{max} is the maximum rate of electron transport under saturating irradiance, α is the quantum efficiency of electron transport and θ describes the degree of curvature of the function.

Table A1. Values of the carboxylation capacity (V_{cmax}), the maximum potential electron transport rate (J_{max}) and mesophyll conductance to CO_2 (g_m) used in this study. These parameters were estimated from the analysis of A_N-C_i measured under field conditions as explained in Material and Methods.

	WW	WS
V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	199.4±19.4	162.2± 14.7
J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	154.2± 8.4	170.5± 10.6
g_m ($\text{mol m}^{-2} \text{s}^{-1}$)	0.341± 0.064	0.226± 0.040

Several parameters in this photosynthesis model have a high dependence on temperature. We used the following equations to describe these temperature dependencies:

$$y(T_k) = y_{25} \exp(c_1 - c_3 T_k^{-1}) \left(\frac{1 + \exp(c_2 - c_4 T_r^{-1})}{1 + \exp(c_2 - c_4 T_k^{-1})} \right),$$

where $y(T_k)$ is the value of a parameter at a temperature T_k (kelvins); y_{25} is the parameter's value at $T_k = 298.15 \text{ K}$ (25°C) (for V_{cmax} , J_{max} and g_m those shown in Table 1); $T_r = 298.15 \text{ K}$; and c_1 , c_2 , c_3 and c_4 are empirical parameters whose values used in this study for olive are suitable in Table A2.

At steady state the demand and supply functions are equal ($A_d = A_s$), and A_N is given by the intersection of A_s and A_d :

$$A_N = A_s \cap A_d.$$

This intersection leads to a quadratic equation whose positive root is equal to A_N .

Table A2. Parameter values used in this study for responses of photosynthetic parameters to temperature. Values at 25°C (y_{25}) varied across leaves, treatments and species, except that we assumed $y_{25} = 272.38$ Pa for K_c , 165.82 kPa for K_o and 37.43 Pa for Γ^* , based on Bernacchi *et al.* (2002). $T_r = 298.15$ K.

parameter	c_1	c_2	c_3	c_4	source
y	(unitless)	(unitless)	(K)	(K)	
K_c	32.67	0	$c_1 \cdot T_r$	0	1
K_o	9.57	0	$c_1 \cdot T_r$	0	1
Γ^*	9.87	0	$c_1 \cdot T_r$	0	1
V_{cmax}	18.77	0	$c_1 \cdot T_r$	0	2
J_{max}	19.53	43.05	$c_1 \cdot T_r$	13608.7	2
g_m	32.27	45.87	$c_1 \cdot T_r$	13608.7	3
R_d	18.07	0	$c_1 \cdot T_r$	0	2

Sources: (1) Bernacchi *et al.* (2002); (2) Diaz-Espejo *et al.* (2006); (3) Diaz-Espejo *et al.* (2007).

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FIGURE CAPTIONS

Figure 1. Comparison of simulated (a) stomatal conductance (g_s) and measured g_s and (b) net photosynthesis rate (A_N). Measurements used to calculate the g_s calibration equations were not used in the validation results. Each point represents the average of four trees per treatment. Circles are control trees (100C), irrigated with one drip line (1L, black) and two drip lines (2L, white). Triangles represent trees under deficit irrigation treatments (45SDI) with one drip line (black) and two drip lines (white). Dashed line represents 1:1 relationship and the solid line the linear relationship between modelled and measured g_s . Bars are ± 1 SE.

Figure 2. Temporal variation of (a) simulated daily maximum stomatal conductance ($g_{s,max}$), (b) simulated daily maximum photosynthesis rate ($A_{N,max}$), (c) vapor pressure deficit (D) measured at $g_{s,max}$ and $A_{N,max}$, (d) predawn leaf water potential (Ψ_{pd}) in the four irrigation treatments at the Sanabria experimental orchard. Black and white circles represent well-irrigated trees (100C) with one (1L) and two drip lines (2L), respectively, and black and white triangles represent deficit irrigated trees (45SDI) with one and two drip lines, respectively. Each point is the average of four trees; error bars are not shown for clarity purposes.

Figure 3. Relationship between accumulated net photosynthesis (A_N) and (a) leaf area, (b) number of shoot internodes, (c) dry fruit weight and (d) oil content. Black and white circles represent well-irrigated trees (100C) with one (1L) and two drip lines (2L), respectively, and black and white triangles represent deficit irrigated trees (45SDI) with one and two drip lines, respectively. Solid black and grey line are well-irrigated trees (100C) with one and two drip lines, respectively and dashed black and grey represent deficit irrigated trees (45SDI) with one and two drip lines, respectively. Each point for leaf area and internode shoots is the average of four plots per treatment. Fruit weight symbols represent the average of 3-5 fruits. The calculation of accumulated A_N started on the first day that dry fruit weight was measured. Error bars are ± 1 SE.

Figure 4. Temporal dynamics of accumulated net photosynthesis (A_N) and fruit diameter increment measured in two trees, one well-irrigated (plot 15 tree, 100C-2L) and one deficit-irrigated (plot 9 tree, 45SDI-2L). The lines of fruit diameter correspond to the average of two fruits; standard errors not shown for clarity purposes. Initial diameter was 7.966 and 7.598 mm on average for the two fruits measured in 100-2CL and 45SDI-2CL trees, respectively

Figure 5. Increase in rate per day of net photosynthesis (A_N) and fruit diameter calculated as the sum of each variable for three days, divided by that number of days. The measurements were conducted in two fruits of two trees exposed to different irrigation treatments: the plot 9 tree is under deficit irrigation with two drip lines (45SDI-2L), while the plot 15 tree is well-irrigated with two drip lines (100C-2L).

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Table 1. Calibration equations descriptors to estimate stomatal conductance from the ratio between sap flux density and air vapour pressure deficit (slope, intercept, coefficient of determination (R^2), probability value (P) and calibration points (N)) and validation statistical parameters (root mean squared error (RMSE), mean absolute error (MAE) and mean bias error (MBE)).

Treatment	Tree	Slope	Intercept	R^2	P	N	RMSE	MAE mol m ⁻² s ⁻¹	MBE mol m ⁻² s ⁻¹
100C-1L	1	0.017	-0.138	0.834	<0.001	12	0.039	<0.001	-0.005
100C-1L	2	0.020	0.014	0.594	0.006	10	0.051	0.038	0.038
100C-1L	3	0.009	0.080	0.573	0.002	12	0.035	0.009	0.009
100C-1L	4	0.028	-0.055	0.514	0.009	11	0.038	0.032	0.032
100C-2L	1	0.018	0.035	0.534	0.007	11	0.041	0.012	0.012
100C-2L	2	0.007	0.047	0.499	0.008	12	0.042	0.029	0.029
100C-2L	3	0.013	0.037	0.758	<0.001	19	0.051	0.034	0.034
100C-2L	4	0.008	0.086	0.690	<0.001	12	0.062	<0.001	-0.025
45SDI-1L	1	0.010	0.028	0.669	<0.001	12	0.017	0.009	0.009
45SDI-1L	2	0.010	0.037	0.479	0.012	12	0.036	<0.001	-0.001
45SDI-1L	3	0.006	0.015	0.858	<0.001	19	0.031	<0.001	-0.004
45SDI-1L	4	0.007	-0.012	0.688	<0.001	15	0.035	<0.001	-0.014
45SDI-2L	1	0.009	0.030	0.502	0.010	11	0.015	0.007	0.007
45SDI-2L	2	0.004	0.015	0.844	<0.001	23	0.037	0.010	0.010
45SDI-2L	3	0.007	0.051	0.737	<0.001	18	0.025	<0.001	-0.005
45SDI-2L	4	0.013	0.059	0.634	<0.001	18	0.041	<0.001	-0.036

Table 2. Final fruit numbers per plot, and yield averaged for each treatment. No significant differences were found among the irrigation treatments.

	100C-1L	100C-2L	45RDI-1L	45RDI-2L
N Frutos ($\times 10^6$)	22.3 $\pm$ 2.32	25.5 $\pm$ 2.97	25 $\pm$ 1.73	20.9 $\pm$ 8.6
Yield (kg ha ⁻¹)	16817.7 $\pm$ 915.4	17835.5 $\pm$ 1082.5	16062.9 $\pm$ 931.7	14042.4 $\pm$ 832.7