

DUAL EFFECT OF THE PRESENCE OF FRUITS ON LEAF GAS EXCHANGE AND WATER RELATIONS
OF OLIVE TREES

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ABSTRACT

The presence of fruits provokes significant modifications in the plant water relations and leaf gas exchange. The underlying processes driving these modifications are still uncertain and likely depend on the water deficit level. Our objective was to explain and track the modification of leaf water relations by the presence of fruits and water deficit. With this aim, net photosynthesis rate (A_N), stomatal conductance (g_s), leaf osmotic potential (Ψ_π), leaf soluble sugars, and daily changes in a variable related to leaf turgor (leaf patch pressure) were measured in olive trees with and without fruits at the same time, under well-watered (WW) and water stress (WS) conditions. Leaf gas exchange was increased by the presence of fruits, this effect being observed mainly in WW trees, likely because under severe water stress, the dominant process is the response of the plant to the water stress and the presence of fruits has less impact on the leaf gas exchange. Ψ_π was also higher for WW trees with fruits than for WW trees without fruits. Moreover, leaves from trees without fruits presented higher concentrations of soluble sugars and starch than leaves from trees with fruits for both WW and WS, these differences matching those found in Ψ_π . Thus, the sugar accumulation would have had a dual effect because on the one hand, it decreased Ψ_π , and on the other hand, it would have downregulated A_N , and finally g_s in WW trees. Interestingly, the modification of Ψ_π by the presence of fruits affected turgor in WW trees, whose change can be identified with leaf turgor sensors. We conclude that the plant water relationships and the leaf gas exchange are modified by the presence of fruits through their effect on the export of sugars from leaves to fruits. The possibility of automatically identifying the onset of sugar demand by the fruit through the use of sensors, in addition to the water stress produced by soil water deficit and atmosphere drought, could be of great help for the fruit orchard management in the future

1. INTRODUCTION

The olive tree is traditionally grown in the Mediterranean basin, and although it has been historically cultivated under rain-fed conditions, its positive productivity response to irrigation has increased the surface of irrigated trees (Orgaz and Fereres 2008). Water scarcity in the regions where olive grows demands to develop a specific strategy to apply deficit irrigation (Moriana et al. 2003, Dell'Amico et al. 2012, Fernández et al. 2013) and to study the response of the olive tree to water stress (Connor and Fereres 2005, Fernández 2014). Although the response of olive trees to soil water deficit has been extensively studied (Lavee 1996, Connor and Fereres 2005, Fernández 2014), the effect that the fruit, specifically the fruit load, may have on this response has been neither as widely examined nor included in deficit irrigation strategies. However, studying the effect of fruit load on the response of olive to water stress is relevant because previous studies have found that fruit load plays a relevant role in modifying tree water consumption (Bustan et al. 2016), plant water relations (Martín-Vertedor et al. 2011, Naor et al. 2013, Bustan et al. 2016), fruit size (Trentacoste et al. 2010) and yield and oil accumulation (Naor et al. 2013). However, the importance of the effect of crop load on tree-water relationships has been reported to be variable, likely dependent on the level of tree water stress (Martín-Vertedor et al. 2011, Naor et al. 2013). Moreover, other studies have confirmed that fruit growth has a preference over vegetative growth (stem or leaves) especially under water stress (Iniesta et al. 2009, Dag et al. 2011, Hernandez-Santana et al. 2018, Rosati et al. 2018), fruits being major water (Girón et al. 2015, Fernandes et al. 2018) and carbon (C) sinks (Hernandez-Santana et al. 2018).

Despite these findings on the importance of fruit load for modifying plant carbon and water relations, the mechanisms explaining the modification of leaf gas exchange by fruit load have not been explored thoughtfully. Indeed the higher demand in fruit-bearing plants

compared to plants with a low crop load or without fruits have been reported to increase leaf photosynthesis and stomatal conductance (Naor et al. 2013, Bustan et al. 2016). The modification of stomatal conductance produced by different fruit loads and water availability could be mediated by a photosynthesis reduction generated by the accumulation of soluble sugars in the leaf provoked by a decrease of C sinks (i.e., fruits) in trees with low fruit load compared to trees with great fruit loads. The inhibition of photosynthesis by end-product is a well-tested effect (Kelly et al. 2013) which has been already shown to occur in a large number of crops species (Goldschmidt and Huber 1992). The sugar accumulation in the leaves and consequent reduction of photosynthesis can be produced by the decrease of sink strength of the plant (Herold 1980, Paul and Foyer 2001) produced, for example, by a low fruit load. Indeed, in Bustan et al. (2011) stored non-structural carbohydrates in olives decreased in summer, under maximum carbohydrate demand for fruit growth and oil production. However, despite the known function of some soluble sugars on osmotic potential (Martínez-Vilalta et al. 2016), they have been rarely used to explain the observed effects of different levels of fruit loads on plant water relations (Dell'Amico et al. 2012, Girón et al. 2015, Fernandes et al. 2018). Changes on leaf osmotic potential, as a consequence of soluble sugar dynamics, together with concomitant fluctuations of leaf water potential (Naor et al. 2013, Bustan et al. 2016) due to the presence of fruit, could have an impact on leaf turgor pressure, which can be monitored by a sensor in field-grown olives trees (Hernandez-Santana et al. 2021). Thus, as the fruit load could have an effect on leaf turgor pressure through its effect on the leaf osmotic potential, this variable could be used to identify the sink effect of fruits on tree water relations. Moreover, because stomatal behaviour and leaf turgor pressure are closely interrelated (Buckley 2019), the measurement of this variable allows the estimation of stomatal conductance (Rodríguez-Dominguez et al. 2019).

Hence, this work aims to explain and track the modification of leaf water relations and leaf gas exchange by the presence of fruits and water deficit in olive trees. Specifically, we aim to (i) study the leaf sugar effects and water deficit on leaf-water relations and (ii) explore a

method to identify the effect of fruit-sink effect on leaf-water relations. We hypothesize that photosynthetic regulation would be affected by the sink demand of fruits, and thus, the leaf gas exchange would be lower in trees without fruits than in trees with fruits, with water deficit modulating this response. Sugar accumulation in the leaf under low sink demand would also decrease the osmotic potential of leaves, and hence, decrease the turgor loss point. We hypothesize further that we would be able to detect these changes with a leaf turgor sensor. Thus, we would be able to have a method to identify the effect of fruit sink demand on tree water relations. If successful, the monitoring of turgor could be used to identify the onset of fruit sugar demand in commercial orchards.

2. MATERIALS AND METHODS

2.1. Orchard and climate conditions

The experiment was conducted in 2018 (from July to November) in a super-high-density olive orchard (*Olea europaea* L. cv. Arbequina) near Utrera (Seville, southwest Spain) (37° 15' N, -5° 48' W). The olive trees used were 12 years old and planted in rows N-NE to S-SW oriented, in a 4 m × 1.5 m spacings (1667 trees ha⁻¹). The soil of the orchard had a sandy top layer and a bottom clay layer (Arenic Albaquaf, USDA 2010, https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_050915.pdf). Further details on the orchard characteristics can be found in Fernández et al. (2013).

In the area, the climate is Mediterranean with mild, rainy winters and hot, dry summers. During the months of the experiment, there were rarely rain events because annual rainfall occurs mainly between late September to May. The only rainfall events (5 days with >10 mm) occurred after DOY 283 (10th of October), but they did not affect the differential irrigation treatments in our experiment. Average values of potential evapotranspiration (ET₀) and precipitation (P) in the region are 1482 mm and 500 mm, respectively, for the 2002–2018 period

(data recorded at the nearby of the study area, Los Molares station, 37° 10' 34" N -5° 40' 22" W, 77 m above sea level; averages provided by the Regional Government of Andalusia). For the same period, average maximum ($T_{a,max}$) and minimum ($T_{a,min}$) air temperatures were 24.8 °C and 10.6 °C, respectively. The hottest months are July and August, whose $T_{a,max}$ values are over 40 °C. Additionally, at least once per year between July and August, the vapour pressure deficit (VPD) values reach over 7 kPa.

2.2. Irrigation and treatments

Two irrigation treatments were applied in twelve trees: six well-watered (WW) trees with a full irrigated (FI) regime, which were irrigated daily to replace 100% of the irrigation needs (IN), and another six water stressed (WS) trees subjected to a sustained deficit irrigation (SDI) regime, whose trees received 50% of IN (50 SDI). IN were calculated daily based on the maximum potential crop evapotranspiration (ET_c) described in Allen et al. (1998) as $IN = ET_c - P_e$, being P_e the effective precipitation calculated as 75% of the precipitation recorded in the orchard. Further details can be found in Fernández et al. (2013). The SDI treatment was carried out from day of the year (DOY) 196, however, we applied an irrigation recovery on DOY 285 to see its effect on the trees. In addition, due to technical problems, an irrigation event occurred on DOY 261. Each tree row was irrigated with one dripper line located close to the trunk with a 2 L h⁻¹ dripper every 0.5 m.

To study the effect of fruit presence or absence, we applied two different treatments in each irrigation treatment: three trees with a presence of fruits (+) which are considered a control where the tree fruit load had not been modified, and three trees with an absence of fruits (-) in which all fruits have been detached from the tree. Fruit removal was performed on DOY 182 (July 1) before all the measurements and then the experiment started. The number of fruits per tree was estimated in eight adjacent trees. No significant differences were found between treatments: WW trees had 8786±790 fruits and WS 10124±518 fruits. Moreover, in our study

plot we have never seen any evidence of alternate bearing (see for example Fernández et al. (2013) and Hernandez-Santana et al. (2017) for a summary of the yield of the period 2010-2012 and 2011-2015, respectively)

2.3. Olive growing cycle

The olive growing cycle is widely known and a graphical representation of the most important processes and the times at which they occur in our study area can be found in Hernandez-Santana et al. (2017). Olive growing cycle starts in our experimental area in mid-February with the shoot growth. Bloom usually occurs in April, thus fruit growth starts in May and continues until mid-September, when fruit growth rate becomes slower than in the previous months. From the beginning of the fruit growth period, cell division in the fruit occurs, being maximum until July when starts to slow down until the end of August, when it is completely stopped. Maximum rate of pit hardening is normally detected in June and could be extended along July and August. Finally, ripening begins in September and lasts until harvest, which normally happens at the end of October-beginning of November. Oil accumulation starts sometime after fruit growth in June and continues until harvest.

2.4. Gas exchange and leaf water potential measurements

Net photosynthesis rate (A_N) and stomatal conductance (g_s) values were measured weekly with a LI-6400 portable photosynthesis system (Li-Cor, Lincoln NE, USA) with a 2 cm × 3 cm standard chamber and ambient light and CO₂ conditions (400 ppm). Measurements were taken at 10:00–11:00 GMT during summer (DOY 191-261 inclusive) and 12:00–13:00 GMT during the autumn (DOY 268-302 inclusive) period to reach the minimum and maximum daily A_N and g_s according to Fernández et al. (1997). Leaf gas exchange measurements were performed on three young, fully mature, and developed leaves per tree from the southeast part of the canopy approximately 1.5 m above the ground. Leaf water potential at midday ($\Psi_{\text{leaf, md}}$) was measured with a Scholander-type pressure chamber (PMS Instrument Company, Albany OR, USA) in two

leaves per tree. Briefly, after collecting the leaves, they were enclosed in an open regular plastic bag and stored in an icebox with ice packs not touching the bagged leaves to avoid abrupt changes in leaf temperature that might result in condensation. This plastic bag was introduced in a closed, zip plastic bag where a moistened paper towel was introduced. We also exhaled into this bag, so the high humidity and CO₂ conditions prevents transpiration from happening. Measurements were conducted late in the afternoon. Full details of the procedure followed can be found in Rodriguez-Dominguez et al. (2022).

2.5. Fresh fruit weight (FW), dry weight (DW), and oil content

Six fruits per studied tree were collected every two weeks. In the laboratory, the fruit FW values were taken using an accurate electronic balance (Balance XS105, Mettler Toledo, Columbus OH, USA). Then, fruits were immediately placed in an oven at 75 °C for at least 72 h until constant weight and their DW was recorded.

The oil content was measured from six fruits per studied tree every two weeks. The fruits were frozen, and at the end of the experiment, the oil extraction analyses were performed from fruit mesocarp tissue by the method used in Hara and Radin (1978). Oil content (%) was determined by gravimetric quantification of total lipid weight after solvent evaporation in an *Eppendorf*[®] centrifugal vacuum concentrator Basic Model 5301 (Eppendorf, Hamburg, Germany).

2.6. Leaf osmotic potential measurements

Ten green and mature leaves per each tree were collected on DOY 219, 226, 233, 240, 254, 261, 268, 283, and 295 at 13:30 h. Specifically, in the WW+ and WS+ treatments, efforts were made to collect them near the fruits. Leaves were cut, covered with aluminium foil paper, and immediately introduced in liquid nitrogen. Back in the laboratory, the samples were stored in a freezer at -80 °C until further analysis. To calculate the leaf osmotic potential (Ψ_{π}), we used two foliar 7 mm diameter disks per sample, between the midrib and margin obtained with a cork

borer. Then, we punctured the fruit 15–20 times with forceps to equilibrate the sample. We used a PSYPRO Thermocouple Psychrometer Water Potential System (Wescor Inc., South Logan, UT, USA) and let the sample be in equilibrium *ca.* 2 h before it was measured. Measurements were calculated by using the regression model proposed by Bartlett et al. (2012) based on temperature dependence for constant equilibrium of chemical processes.

2.7. Soluble sugars and starch analyses

Additional four green and mature leaves were collected on DOY 219, 254, 283, and 295 at 13:30 h. They were frozen immediately in liquid nitrogen and then stored at -80 °C. Then, they were lyophilized (VirTis BenchTop 2 K Freeze Dryer, SP Industries Inc.) for 48 h and their DW was recorded. For the extraction of soluble sugars from the polar fraction, 50 mg of DW were used and 20 volumes (1000 µl) of 80% EtOH + 0.1% formic acid were added. The samples were incubated in a heating block at 80 °C with gentle shaking for 1 h, they were then centrifuged and the supernatant was reduced to the aqueous phase in an *Eppendorf*[®] centrifugal vacuum concentrator Basic Model 5301 (Eppendorf, Hamburg, Germany) at 50 °C. This process was repeated twice and the tubes were immediately frozen in liquid nitrogen and stored in a freezer overnight. The following day, they were resuspended in 20 volumes of sterile dH₂O.

The concentration of soluble sugars (glucose, *myo*-inositol, mannitol, galactose, fructose, and sucrose) were determined by anion exchange chromatography using a sample dilution (sample:sterile dH₂O 1:100) but only glucose, *myo*-inositol, and mannitol showed significant concentrations. Thus, glucose, *myo*-inositol, and mannitol were the soluble sugars shown in this work. Chromatographic analyses of soluble sugars were conducted in a Metrohm (Herisau, Switzerland) 930 compact ICFlex ion chromatograph equipped with a pulsed amperometric detector (PAD). The chromatographic separation was performed on a Metrosep Carb 2 column (4 mm × 150 mm, Metrohm) equipped with a guard column (Metrosep Carb 2 Guard, 5 mm × 30 mm, Metrohm). Isocratic elution mode was applied using a mobile phase

composed by a mixture of NaOH 0.3 M and CH₃COONa 0.01 M at a flow rate of 0.5 ml min⁻¹ in a 17 min run at a column temperature of 30 °C, and an injection volume of 20 µl. The electrochemical detector was equipped with a gold working electrode and a palladium reference electrode. The cell of the pulsed amperometric detector was kept at 35 °C. Sugar identification and quantification were carried out using retention times and the related sugar calibration curve, respectively.

For starch quantification, glucose extraction from starch was performed. The pellet obtained in the previous process was washed twice with sterile dH₂O, resuspended in 9 volumes (450 µl) of sterile dH₂O and stored at 4 °C overnight. The starch in the pellet was gelatinized by introducing the samples at 100 °C for 2 h. Once at room temperature, 1 volume (50 µl) of 1 M CH₃COONa, pH 4.5, containing 100 U ml⁻¹ of α-amylglucosidase was added to transform starch into glucose. It was checked using a Lugol solution (sterile dH₂O: Lugol 10: 1). Then, the extracted glucose was determined as described before.

The contribution of soluble sugars to the osmotic potential was calculated from the concentration of each osmolite by using the Boyle-van't Hoff relation as Aranda et al. (2021) and Ranney et al. (1991). Briefly, osmotic potential = RDW x c x R x T, where RDW represents the ratio of leaf dry weight to leaf water content (estimated as the difference between leaf fresh weight and dry weight), c is the concentration of each osmolyte, R is the gas constant and T is the temperature at 25 °C.

2.8. Turgor-related sensors

To study maximum daily changes in leaf turgor pressure, leaf turgor pressure sensors ("ZIM probe") were installed on one leaf in three WW+ and WW- trees. No leaf turgor pressure sensors were installed in WS trees because under water stress conditions leaves are in a turgorless state and out of the minimum range that they can measure (Ehrenberger et al. 2012). This is not a limitation in our study since if the turgor is too low, the conditions for fruit growing

are not achieved, and the measurement of turgor is irrelevant (Hernandez-Santana et al. 2021, for more information on this topic). The basis of these sensors consists of clamping the leaf between two metal pads with two magnets. The principle of the turgor-related sensors was described in detail by Westhoff et al. (2009) and Zimmermann et al. (2008). The relative leaf turgor pressure (P) is measured as a function of the pressure that the turgor sensor exerts on the leaf and is referred to as leaf patch pressure (P_p). The magnets exert a pressure on the leaf that it is counteracted by its turgor pressure. The higher the turgor pressure of the leaf, the lower the P_p (more details on the method in Zimmermann et al. 2008, Westhoff et al. 2009, Ehrenberger et al. 2012). Therefore, the minimum daily value of the sensor ($P_{p, \min}$) corresponds to the maximum daily turgor pressure of the leaf. Seasonal changes in $P_{p, \min}$ are interpreted as changes in the maximum turgor of the leaf. To monitor more clearly the trend of these seasonal changes, the difference in $P_{p, \min}$ between two consecutive days was calculated ($\Delta P_{p, \min}$). Positive values of $\Delta P_{p, \min}$ indicate that the maximum turgor reached by the leaf every day is decreasing, and in the context of our study it would suggest that the osmotic potential is increasing due to a lower concentration of osmolytes in the leaf. The use of these sensors is not valid in the WS treatment when leaves are in a nearly turgorless state as defined by Ehrenberger et al. (2012). Under water stress conditions, like those imposed in the WS treatment, the conditions for fruit growth are not fulfilled, as demonstrated by Hernandez-Santana et al. (2021). Therefore, turgor sensors were only used to study the presence or absence of fruits in the WW treatment.

2.9. Statistical analyses

Data for A_N , g_s , $\Psi_{\text{leaf, md}}$, and Ψ_{π} of each measurement day were analysed by a two-way ANOVA, being water stress treatment (WW and WS) and the presence/absence of fruits (+ and -) the factors considered. Data for fruit DW and oil content were analysed by a one-way ANOVA. The assumptions of normality and homoscedasticity in the data were verified before performing an ANOVA. Statistical analyses were carried out using SigmaPlot[®] software (Systat Software, San Jose, CA).

3. RESULTS

3.1. Fruit dry weight and oil content

Although differences between WW+ and WS+ trees were found in both fruit dry weight and oil content during some days in the studied period (Fig. 1), the synthesis of oil was less affected by water stress than the fruit dry weight. The increment rate of fruit dry weight occurred at a slower pace in the WS than in the WW treatment from DOY 220 to DOY 263, generating the differences between both irrigation treatments during the period of higher water stress. However, during that period, the rate of increment of oil content was similar in both treatments, suggesting that the plant promoted the oil synthesis process over fruit dry weight increment, and that the level of stress was not enough to impair it. After irrigation recovery, no differences were found between treatments in either variable.

3.2. Leaf gas exchange

Higher values of A_N and g_s were measured in WW than in WS trees, independently of the fruit presence (Fig. 2). Differences were as high as 3-fold between water treatments. When fruit treatments were compared within a single water treatment, no differences were found for the WS trees. However, significant differences emerged in the WW trees from DOY 260 to DOY 270, WW+ showing higher A_N and g_s than WW- trees. When the irrigation recovery was applied on DOY 285, we observed that, although there were still differences between both irrigation treatments, these differences became smaller than in the previous period.

3.3. Leaf water and osmotic potential

We observed significant differences in $\Psi_{\text{leaf, md}}$ between WW and WS trees from DOY 226 to 285 (Fig. 3A). In most of the dates during the deficit irrigation period, $\Psi_{\text{leaf, md}}$ in WS+ was significantly more negative than in WS- trees. Differences disappeared when irrigation was recovered on DOY 285. The sudden increase of $\Psi_{\text{leaf, md}}$ on 260 was due to technical problems in

the irrigation system, but the effects of the irrigation treatments were maintained as WW was still significantly less negative than WS.

Regarding Ψ_{π} , we found significant differences between the WW and WS trees from DOY 226 to DOY 283 (Fig. 3B). The WW trees presented higher leaf Ψ_{π} , than the WS trees. However, in contrast to $\Psi_{\text{leaf, md}}$, we found differences between WW+ and WW- from DOY 261 onward, WW+ presenting higher Ψ_{π} values than trees without fruits. On the contrary, no differences between WS+ and WS- trees for most dates were observed. After irrigation recovery, differences in Ψ_{π} were maintained between WW+ and WW-, but Ψ_{π} from WS+ trees became significantly higher than in WS- trees.

3.4. Changes in soluble sugars and starch and relationship with leaf osmotic potential

The highest concentrations of soluble sugars and starch were measured in WS- trees (Fig. 4). In addition, leaves from trees without fruits presented higher concentrations of soluble sugars and starch than leaves from trees with fruits for both irrigation treatments. These differences were found in the period of deficit irrigation, and trends matched those found in leaf Ψ_{π} . This pattern was noticed mainly in *myo*-inositol and mannitol. Starch accumulated 3- to 4-fold in leaves of trees without fruits than in trees with fruits in both WW and WS treatments (Fig. 4D).

Our results show that mannitol was related to leaf Ψ_{π} (Fig. 5), especially at moderate values of Ψ_{π} (above -4 MPa). Lower Ψ_{π} values than -4 MPa did not correspond with higher concentrations of mannitol, suggesting that this sugar was not responsible for the lowest Ψ_{π} values measured. The contribution of mannitol to the osmotic potential estimated by Boyle-van't Hoff equation showed that mannitol represented between 17-20% of the osmotic potential measured in the range of concentrations observed. A mannitol concentration of 20 mg g⁻¹ DW represents -0.5 MPa, meanwhile a concentration of 35 mg g⁻¹ DW represents -0.87 MPa.

3.5. Dynamics of turgor estimated with turgor-related sensors

$P_{p,min}$ represents the maximum turgor that a leaf achieves in a day. Thus, in terms of turgor-related probes functioning, it means that the lower the value is, the higher the leaf turgor is (Fig. S1). In the studied period, fruitless trees, WW- (Fig. 6), showed a constant value of $P_{p,min}$, suggesting that no changes in maximum turgor occurred. However, a remarkable increase was monitored in the WW+ trees, starting on DOY 254, indicating a reduction in the maximum turgor of the leaf. The same conclusion can be inferred independently from Figure 3, where differences were found in Ψ_{π} with no changes in water potential. $P_{p,min}$ in WW+ was much higher than WW- during the period DOY 254–275. During those days, g_s (Fig. 2) and leaf Ψ_{π} (Fig. 3) were also significantly higher in WW+ than WW-.

4. DISCUSSION

According to our hypothesis, the decrease of g_s and A_n observed in WW- compared to WW+ (Fig. 2) can be explained by sugar accumulation in the leaves (Figs. 4 and 5) as the dynamics of the studied variables were similar. When the export of sucrose is impaired due to the absence of fruits, sugars accumulate in the leaves of WW- trees because the photosynthesis produces more carbohydrates than demanded. This carbohydrates accumulation in WW- trees would have been produced by the accumulation of photosynthesis due to the lower demand of sugars in the tree generated by the absence of fruits. The absence of these major sinks (Hernandez-Santana et al. 2018, Ryan et al. 2018) impairs the sugars exportation by the phloem. The accumulated soluble sugars perform different functions such as the already mentioned inhibition of photosynthesis (Goldschmidt and Huber 1992), the well-defined role in tree survival (Bustan et al. 2011, Tixier et al. 2018) and also a myriad of functions (metabolic, osmotic balance among different organs, etc) that requires maintaining relatively high concentrations of soluble sugars at all times (Martínez-Vilalta et al. 2016). Some of these functions of great relevance to cope with water stress are osmoregulation (key to maintaining turgor) and maintenance of vascular integrity (xylem and phloem) (Sala et al. 2012). However, there are crucial aspects of

these functions that are not yet fully understood (Adams et al. 2013, Hartmann and Trumbore 2016, Martínez-Vilalta et al. 2016), and thus, this work contributes to advance in the knowledge of the effect of temporal strong carbon sinks on the regulation of stomatal conductance and leaf turgor.

The acceptance of the hypothesis that sugars are the key regulating factor of the leaf gas exchange under these conditions of sink demand (i.e., fruit absence) means that non-stomatal limitations are the main constraints of photosynthesis under these circumstances (Dewar et al. 2022). In general, it is difficult to distinguish whether leaf-water content or sugar concentration is triggering the regulation of photosynthesis since these two variables are intimately linked to each other (Hölttä et al. 2017). Sugar concentration can increase not only because sugars cannot be transported via phloem to other plant organs, but because lower water content increases sugar concentration in the liquid phase. However, a lower water content does not seem to be the cause in this study. Leaf osmotic potential was lower in WW- than in WW+, as there were differences in leaf gas exchange during that period. However, despite fruit removal decreasing g_s , and consequently transpiration, in WW- with respect to WW+, both treatments showed similar leaf water potential. At the moderate water stress level measured in WW treatments in this study, it is known that olive leaves can maintain the relative water content constant along the season, even if there is osmotic adjustment (Diaz-Espejo et al. 2018). Thus, the water status of the plant does not seem to be playing a role in the downregulation of the leaf gas exchange and thus, the cause of this downregulation should be the lack of phloem transport of sugars.

Besides inhibiting photosynthesis and stomatal conductance, sugars accumulation drives changes in the leaf-water relations, which is reflected in the osmotic potential decrease (Fig. 3). Indeed, part of the soluble sugars measured in the leaves of this study corresponded to sugars that have been described to play an important role in osmotic adjustment in olive (Lo

Bianco et al. 2013), such as mannitol (Flora and Madore 1993, Tattini et al. 1996, Lo Bianco and Avellone 2014, Lo Bianco and Scalisi 2017). However, not all the changes in osmotic potential were produced by the observed increase in the concentration of mannitol, especially in WS trees (Fig. 5). In olive trees, it has been reported that, apart from soluble sugars, other organic compounds, such as proline, betaines, or polyamines, are involved in the osmotic adjustment in response to abiotic stress (Gucci and Tattini 1997). In our case, the strongest correlation between leaf osmotic potential and mannitol occurred in the WW treatment, which corresponded to the highest values of osmotic potential. The concentrations of mannitol measured were calculated to contribute to around 20% to the osmotic potential, which aligns well with previous studies in olive trees (Dichio et al. 2009). The trend was similar for the high osmotic potential values of WS. However, at lower osmotic potential induced by more severe water stress, there was no correlation between leaf osmotic potential and mannitol. This lack of correlation could be explained by a greater contribution of other osmolytes as the ones already mentioned, present only under severe water stress conditions. Accordingly, for sugar accumulation trends (Fig. 4), we found greater starch concentrations in WW- than WW+. Different studies (Akinci and Losel 2010, Thalmann and Santelia 2017) suggest that starch is accumulated during the day, it is hydrolysed later on into organic acids and soluble sugars, which are transported during the night to guarantee carbon in the sink organs. Thus, starch can be a precursor of soluble sugars involved in osmotic and stomatal adjustment processes during abiotic stresses (Krasensky and Jonak 2012, Horrer et al. 2016). These processes would explain the lower amount of starch in trees with fruits compared to trees without fruits, regardless of the treatment.

Contrary to what happens in WW, the sugar differences between WS- and WS+ trees resulted in no differences in the leaf gas exchange variables (Fig. 2) or osmotic potential (Fig. 3B). Under the severe water stress imposed, stomatal conductance and photosynthesis were at minimum values in both treatments, and thus, the stomatal closure to prevent xylem disruption

(Scoffoni et al. 2017) may have prevented the effect of stress caused by the presence of fruit from being observed, since it is probably of lesser magnitude than that caused by soil water deficit.

However, the presence of fruits in this water-limited treatment imposed an extra negative impact on the plant water status, as suggests the lower leaf water potential. The lower $\Psi_{\text{leaf, md}}$ in WS+ than in WS- trees (Fig. 3) would have been likely produced by a preferential water movement towards the fruits due to their lower water potential than in the leaves, as observed in Fernandes et al. (2018). As a result, the leaf water potential, would have decreased more steeply in WS+ than in WS-.

Moreover, the leaves accumulated more soluble sugars and starch in WS- trees than WS+ trees (Fig. 4), similarly to WW. In the case of mannitol, the concentration was overall higher in WS than in WW. According to Gersony et al. (2020), a lower xylem than phloem water potential would have occurred in WS compared to WW trees, which could have prevented the sugars exportation to sink organs of the plant to a greater extent than in WW. This difficulty to export sugars to fruits or other sink organs could have contributed to the mannitol accumulation in the leaf. According to Gersony et al. (2020), this carbohydrate mobilization should happen during the night in water-stressed trees because large diurnal depressions in water potential may impede carbon export from leaves to other plant organs, such as fruits. As the xylem is the source of water for the phloem, when lower (more negative) potentials are recorded in the xylem, higher osmotic concentrations would be needed in the phloem to extract water from the xylem. Since xylem potentials partially recover at night, as seen in olive, movement of water from xylem to phloem would be more feasible at this time (Diaz-Espejo and Hernandez-Santana 2017). Testing this hypothesis of sugar transportation at night is beyond the objective of our work.

Finally, the effect of the presence of fruits on leaf turgor of WW trees was monitored with leaf turgor sensors. Our explanation for the significant difference between WW- and WW+ in $\Delta P_{p,\min}$ is that the higher osmotic potential in WW+ compared to WW- together with the lack of differences in leaf water potential between WW+ and WW- trees, lead to a lower turgor pressure in WW+ than in WW-. The major increase in $P_{p,\min}$, which is the maximum turgor of the leaf in a day, occurred in the same period when most of the changes in osmotic and leaf gas exchange happened. There is sufficient theoretical background to associate it as cause-effect because active growing fruits use sugars to increase their osmotic potential as a mean of attracting water (Kramer and Boyer 1995, Matthews and Shackel 2005). The independent measurement of turgor with the leaf turgor sensors confirms the expected consequences of sugar dynamics in the leaves due to the fruit presence. However, the highest increment of $P_{p,\min}$ in WW+ cannot be explained by an intense dry matter accumulation in the fruit in this period (Fig. 1). The higher demand for sugars from fruits to leaves corresponded to an increase of fruit oil synthesis (Fig. 1). This might have significant implications on the management of deficit irrigation strategies because with this method we could identify critical periods in terms of physiological processes related to the phenology of the crop in addition to the water stress produced by soil or atmosphere water deficit. Furthermore, our results reinforce the use of the leaf turgor sensors in physiological studies, providing more information than simply their use as indicator of water stress, and open new possibilities in ecophysiological studies. If according to the published works the leaf turgor sensors are a good proxy of actual turgor pressure (Zimmermann et al. 2008, 2010, Westhoff et al. 2009, R ger, Ehrenberger, et al. 2010, R ger, Netzer, et al. 2010, Ehrenberger et al. 2012), they can be used to derive the dynamics of parameters determining leaf water relations, like the osmotic potential at full turgor (which corresponds to an increase in $P_{p,\min}$). In our study, we hypothesized that the effect of sugar export from leaves to fruits would increase the leaf osmotic potential at full turgor, as we have demonstrated. The change of the osmotic potential at full turgor has profound ecophysiological

implications since it also involves the change in the turgor loss point (Bartlett et al. 2012), which deserves further verification in more species. In agronomy, regulated deficit irrigation strategies are designed considering the phenological stages when the crop is more sensitive to water stress or when a process needs to be promoted and facilitated (Fernández et al. 2013). Our results facilitate the identification of these sensitive periods, i.e when fruits demand more sugars, using sensors which work in a continuous and automatic manner. Thus, the changes in the leaf water relations are of such magnitude that they can be monitored and potentially used to manage a deficit irrigation strategy.

5. CONCLUSIONS

In this work, we have shown that fruits behave as strong water and carbon sink organs and, therefore, their presence or absence are reflected at the leaf level in both leaf gas exchange regulation and water status. We conclude that under no water stress conditions, the impact of fruit absence on leaf water relations is mainly explained by leaf sugar accumulation, which downregulates A_N and g_s compared to trees with high fruit loads. Sugars accumulation decreases the leaf osmotic potential but not the water potential and, therefore, leaf turgor pressure is also affected. Interestingly, the water stress produced by the strong demand for carbon and water of a high fruit load can be tracked with the leaf turgor pressure sensors. In the future, this knowledge should be tested in trees with different fruit loads, and the use of turgor sensors could improve the application of irrigation strategies in some stages of the phenological cycle of the crop based on the sugar demand by fruits.

DATA AND MATERIALS AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPLEMENTARY DATA

Supplementary Data for this article are available at Tree Physiology Online.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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AUTHORS' CONTRIBUTIONS

AP-A, VH-S and AD-E were responsible for field experiments. AP-A performed gas exchange and leaf water potential measurements, harvesting of olive fruits and oil content determination. AP-A and LFP-R were responsible for leaf soluble sugars and starch extraction and RF-T was responsible of its determination by anion exchange chromatography. AP-A, VH-S and AD-E analysed the data. AD-E and VH-S designed the study. AP-A, VH-S and AD-E prepared the manuscript and collected contributions from all authors.

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FIGURE LEGENDS

Fig. 1. Evolution of **A)** fruit dry weight (DW) (g) and **B)** total oil content (%) in well-watered (WW) (blue) and water stressed (WS) (red) trees with presence of fruits (+). Irrigation recovery, that is represented by a grey area, started at DOY 285 (October 12). Data are mean \pm standard errors from six fruits per tree. Asterisks are shown when significant differences between irrigation treatments ($p \leq 0.05$) were found according to one-way ANOVA.

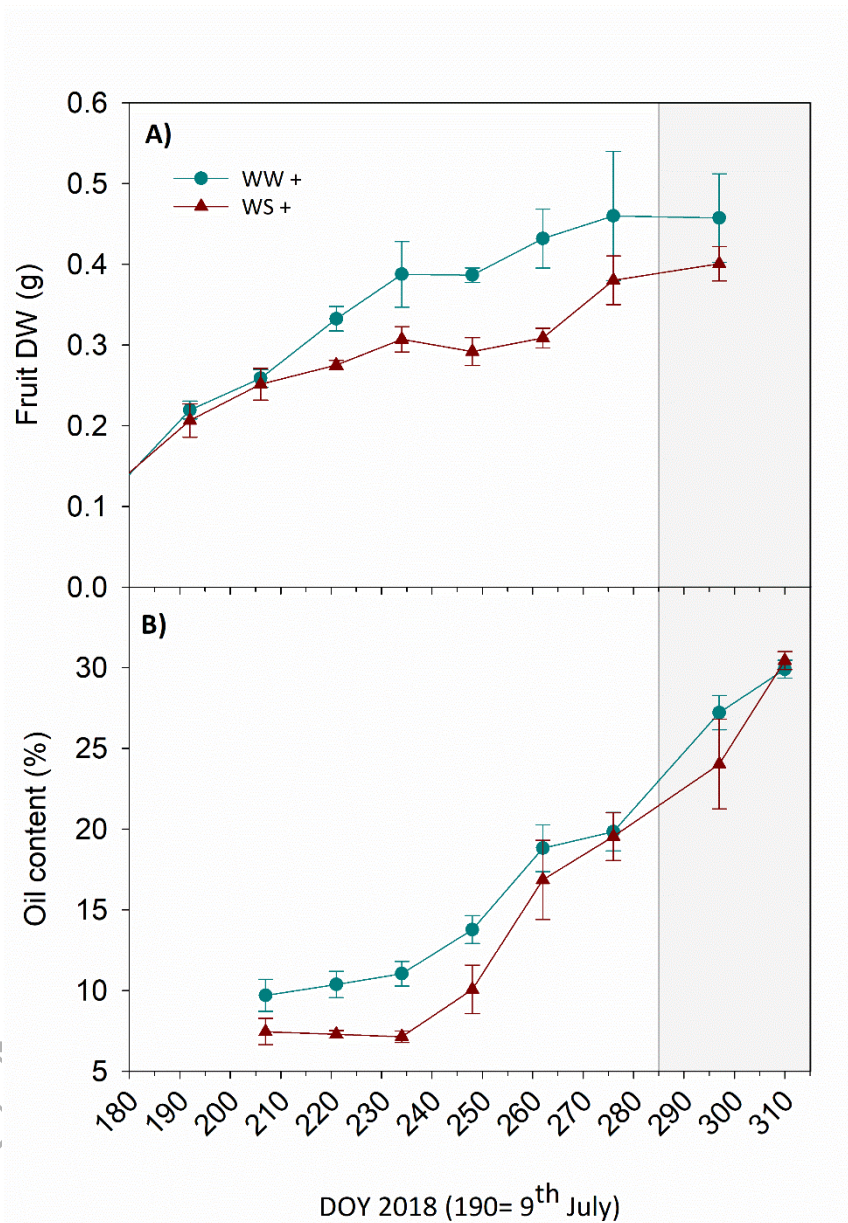


Fig. 2. Evolution from day of the year (DOY) 2018 190 to 310 (July 9 to November 6, 2018) of **A)** photosynthesis rate (A_N ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)) and **B)** stomatal conductance (g_s ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)); in well-watered (WW) (blue) and water stressed (WS) (red) trees, presence of fruits (+) and absence of fruits (-). Irrigation recovery started at DOY 285 (October 12), and it is represented by a grey area. Data are mean \pm SE from three different plots (1 tree = 1 plot) per treatment. Different letters indicate significant differences between irrigation treatments and fruit loads combined ($p \leq 0.05$) according to two-way ANOVA. The order of the letters follows the order of the treatments in the legend. Letters are not shown when no differences were found.

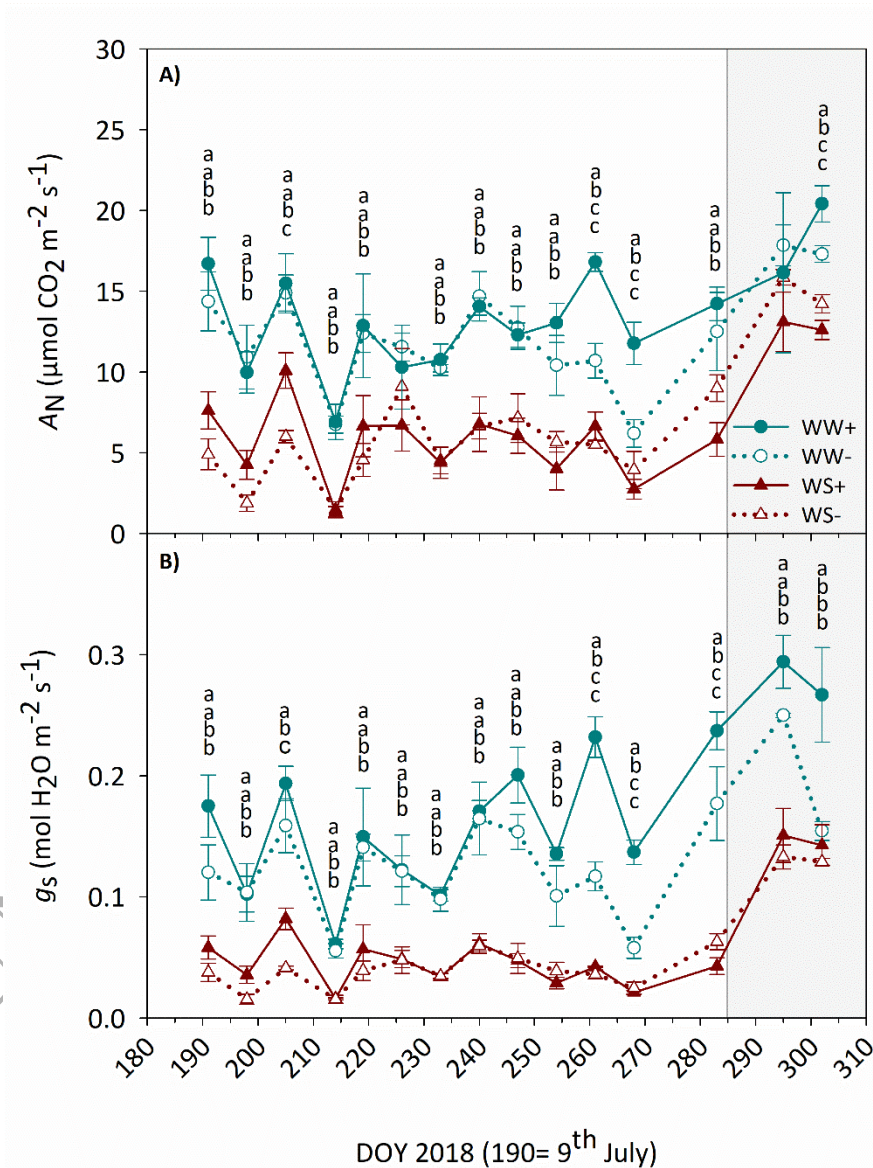
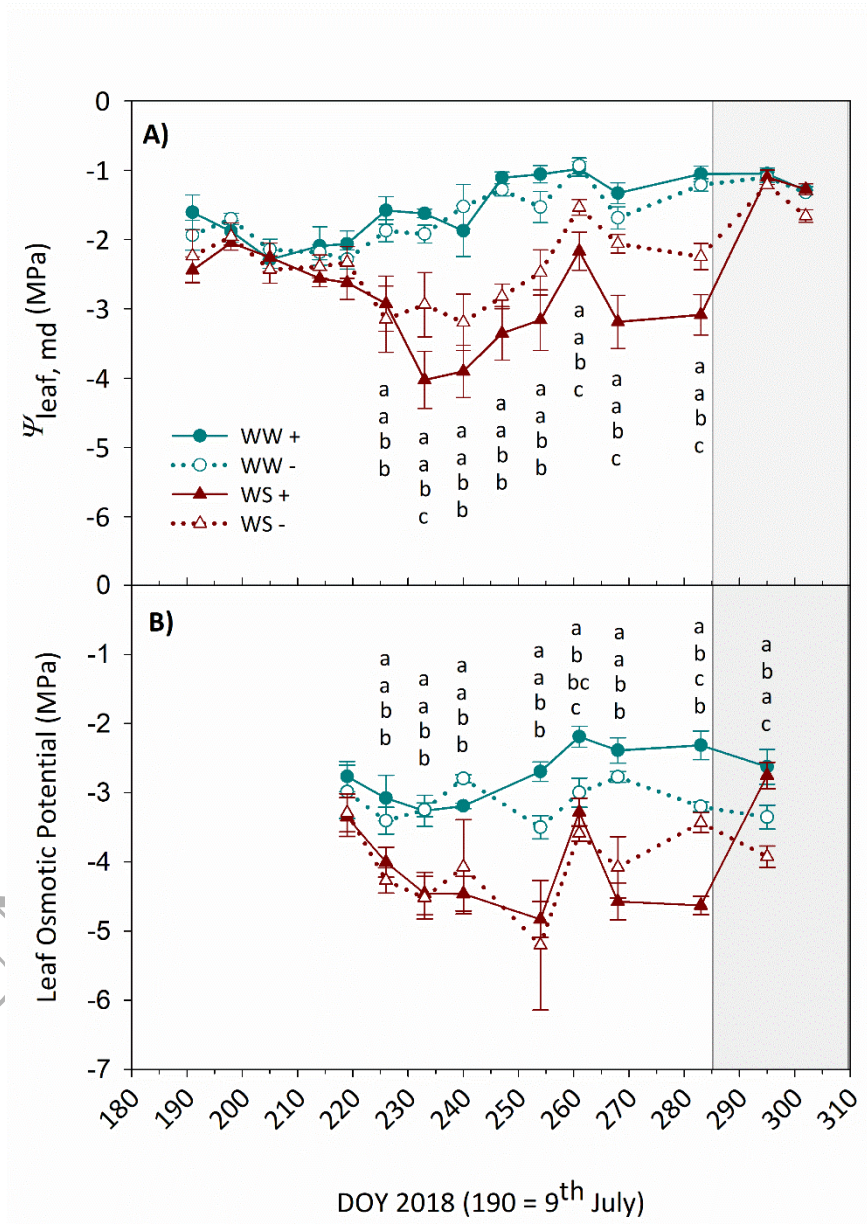


Fig. 3. Evolution from day of the year (DOY) 2018 190 to 310 (July 9 to November 6, 2018) of **A)** Leaf water potential at midday ($\Psi_{\text{leaf, md}}$ (MPa)) and **B)** leaf osmotic potential (Ψ_{π} (MPa)); in well-watered (WW) (blue) and water stressed (WS) (red) trees, with presence of fruits (+) and absence of fruits (-). Irrigation recovery started at DOY 285 (October 12) and it is represented by a grey area. Data are mean \pm standard errors from two leaves per tree. Different letters indicate significant differences between irrigation treatments and fruit loads combined ($p \leq 0.05$) according to two-way ANOVA. The order of the letters follows the order of the treatments in the legend. Letters are not shown when no differences were found.



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Fig. 4. Leaf soluble sugars (mg g^{-1} DW). **A)** Myo-inositol, **B)** glucose, **C)** mannitol and **D)** starch from day of the year (DOY) 2018, 219 to 295 (August 7 to October 22) in well-watered (WW) (blue) and water stressed (WS) (red) trees; and presence of fruits (+) and absence of fruits (-) trees. Irrigation recovery, that is represented by a grey area, started at DOY 285 (October 12). Data are mean \pm standard errors from four leaves per tree. Different letters indicate significant differences between irrigation treatments and fruit loads combined ($p \leq 0.05$) according to two-way ANOVA. The order of the letters follows the order of the treatments in the legend. Letters are not shown when no differences were found.

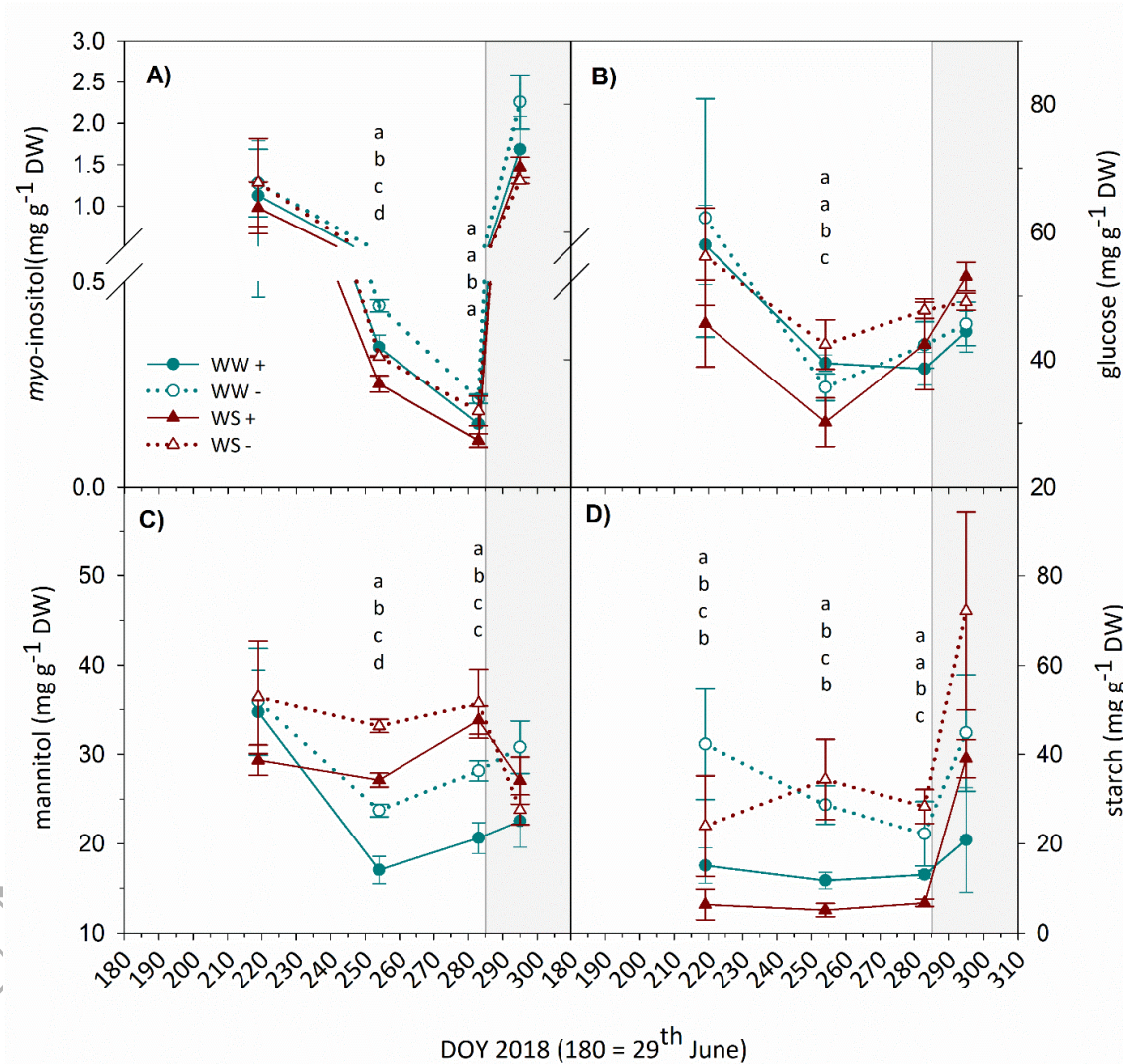
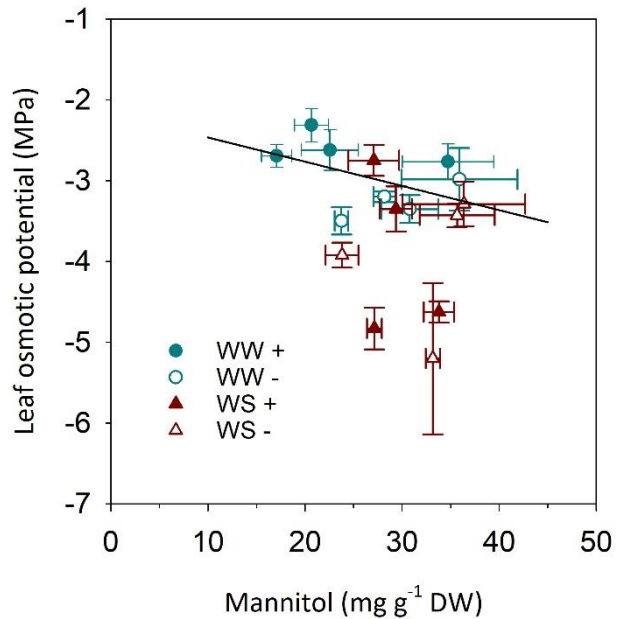


Fig. 5. Relationship between mannitol content (mg g^{-1} DW) and leaf osmotic potential (Ψ_{π} (MPa)) measured at midday in well-watered (WW) (blue) and water stressed (WS) (red) trees, presence of fruits (+) and absence of fruits (-). Data are mean \pm standard errors from three trees per treatment. Every dot is one date from each treatment. Regression line is plotted using all values higher than -4 MPa ($R^2 = 0.25$ $p \leq 0.05$).



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Fig. 6. Evolution of the maximum daily variation of leaf turgor ($\Delta P_{p,\min}$ (kPa)) from day of the year (DOY) 2018, 244 to 280 (September 1 to October 7) in well-watered (WW) (blue) trees with presence of fruits (+) and absence of fruits (-). Data are mean \pm standard errors from three trees per treatment. Asterisks are shown when significant differences between irrigation treatments ($p \leq 0.05$) were found according to one-way ANOVA.

