

# Responses of several soil and plant indicators to post-harvest regulated deficit irrigation in olive trees and their potential for irrigation scheduling



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## ABSTRACT

The response of olive trees to deficit irrigation during post-harvest has been little evaluated because low rainfall often precludes the need to irrigate at this phenological stage in the Mediterranean Basin where olive is mostly cultivated. In many growing areas of Argentina, the lower latitude and continental climate leads to harvesting table olives in mid-summer when evapotranspiration is still high and rainfall is low. We assessed the responses of soil moisture and several plant-based indicators to post-harvest regulated deficit irrigation (RDI) in two growing seasons in order to: 1) determine the responses of the indicators to a range of irrigation levels; 2) elucidate the relationships between the different soil and plant variables; and 3) evaluate the appropriateness of the indicators for scheduling irrigation. Three RDI treatments (66, 33, 0% crop evapotranspiration; ETC) and a control (100% ETC) were applied for 75 days from mid-summer to mid-fall in a cv. 'Manzanilla fina' orchard during 2009 and 2010. The treatments received irrigation equivalent to the control during the rest of the season. Soil relative extractable water (REW%), midday stem water potential ( $\Psi_s$ ), leaf conductance (gl), sap flow, and trunk diameter variations were the variables evaluated. The RDI treatments generated a wide range of REW values (0–125%) with all of the plant indicators being affected to some degree. Midday stem water potential increased linearly with REW until it reached a break point at 48% REW, above which  $\Psi_s$  maintained a plateau at  $-1.75$  MPa. The increase in maximum trunk diameter (MXTD) showed strong relationships with REW,  $\Psi_s$ , and gl. Trunk growth rate (TGR) showed a very early response to water-withholding in both seasons, and trunk growth decreased along with  $\Psi_s$  until it reached a constant negative growth rate of  $-12 \mu\text{m d}^{-1}$  at a  $\Psi_s$  of  $-2.7$  MPa. Trunk maximum daily shrinkage was much less responsive to irrigation than either MXTD or TGR. Our results during post-harvest RDI in an arid region suggest that automated soil moisture sensors can be used to schedule irrigation at different water stress levels if reliable soil moisture values can be measured, and indicate that a continuous recording of trunk diameter has sufficient enough potential for irrigation scheduling that further investigation of MXTD and TGR is warranted.

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## 1. Introduction

Irrigation has been a key tool in obtaining high yields in the new olive orchards of Argentina, especially in areas where rainfall is often minimal (Gómez-del-Campo et al., 2010; Searles et al., 2011). The large, commercial orchards that have been

established there over the last two decades typically estimate water requirements using the FAO method (Allen et al., 1998), and drip irrigation is then applied to satisfy 100% of crop evapotranspiration (ETc). A controlled field study conducted over two years in Northwest Argentina with a broad range of irrigation levels found that yield was maximized with irrigation amounts corresponding to a seasonal crop coefficient ( $K_c$ ) of approximately 0.7–0.8 (Correa-Tedesco et al., 2010). Whole-tree transpiration and soil evaporation from several intervals during the season also provided similar  $K_c$  values using sap flow and microlysimeter measurements, respectively (Rousseaux et al., 2009; Figuerola et al., 2013). Additionally, the observed transpiration values were consistent with calculated values from the model approach of Orgaz et al. (2007).

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As external pressures such as increasing population and industry result in direct and indirect competition for water resources, the proper scheduling of irrigation amounts and its timing will become more critical (Fernández and Torrecillas, 2012). In this regard, plant-based indicators have been suggested to be a potentially viable alternative to the FAO method in olive and other fruit trees in a number of recent reviews (Fernández and Cuevas, 2010; Ortuño et al., 2010; Fernández, 2014a). Traditional, discontinuously measured variables such as midday stem water potential and leaf conductance (e.g., Moriana et al., 2010, 2012) and continuously measured variables including sap flow and stem or trunk diameter variations (e.g., Moriana and Fereres, 2002; Fernández et al., 2008, 2011a; Tognetti et al., 2009; Cuevas et al., 2013) have all shown potential for scheduling irrigation in olive orchards to various degrees depending on tree age, crop load, planting density, and other factors. Less emphasis has been placed recently on soil indicators possibly because the soil moisture of drip-irrigated orchards includes both wet and dry areas that can be difficult to integrate for scheduling irrigation. Nevertheless, transpiration has been shown to decrease sharply below a critical soil moisture threshold in drip-irrigated orchards (Rallo and Provenzano, 2013).

Continuous, plant-based indicators can offer an advantage in comparison to discontinuous indicators because they are automated, which may be particularly important in detecting the onset of water stress and scheduling either sustained or regulated deficit irrigation (RDI). Deficit irrigation is becoming more common in many Mediterranean Basin regions due to the competition for water mentioned earlier and also due to the need to limit tree size by reducing vegetative growth in high and super high density orchards (Gómez-del-Campo, 2013a; Connor et al., 2014). Trunk diameter variations (TDV), which include a number of derived indexes based on minimum and maximum daily trunk diameter, can be combined with sap flow to detect water deficit when applying RDI in older trees with large root systems in moderately dense orchards (Fernández et al., 2011a), but sap flow may be preferable in denser orchards with restricted root systems (Cuevas et al., 2013). Moriana et al. (2013) and Girón et al. (2015) have recently reported that different TDV indexes could be used for scheduling RDI at different phenological stages, although the actual irrigation applied due to the established TDV thresholds was sometimes very low, and more evaluation is likely warranted.

Although several studies have evaluated the relative strengths and weaknesses of different plant indicators in detecting water stress, much less emphasis has been placed on the physiological relationships between them. Ultimately, an understanding of these relationships provides an important tool for managing orchards and increasing crop performance (Fernández, 2014b). The available evidence indicates that many of the relationships are complex. For example, the relationship between midday stem water potential ( $\Psi_s$ ) and the TDV index maximum daily shrinkage (MDS) appears to be concave, which means that the same value of MDS can occur both at low and high  $\Psi_s$  depending on the degree of water stress (Moriana et al., 2000; Ortuño et al., 2010; Pérez-López et al., 2013). In contrast, negative linear relationships were reported under RDI treatments between  $\Psi_s$  and the daily difference of maximum trunk diameter ( $D_{MXTD}$ ), another common TDV index used in olive (Fernández et al., 2011b; Cuevas et al., 2013). For stomatal conductance and soil relative extractable water (%), either a weak linear or no relationship was apparent between these variables and  $D_{MXTD}$ .

To date, the vast majority of the studies assessing the response of continuous plant indicators to RDI in olive trees have been conducted in a handful of orchards under Mediterranean climate conditions in central and southern Spain. Under Mediterranean conditions, RDI is most often employed during the summer months in olive oil cultivars when evapotranspiration is greatest (Alegre

et al., 1999; Gómez-del-Campo, 2013a,b), although alternative RDI strategies have also been suggested for both oil and table olive cultivars (see review by Fernández, 2014b). In the growing areas of Argentina, the lower latitude and continental climate result in warm spring temperatures that accelerate fruit maturation and green table olives are harvested in mid-summer with little or no rainfall occurring after harvest. Currently, little or no information on olive tree response to RDI in the phenological period immediately following harvest is available (Girón et al., 2015). This post-harvest period may represent up to 20% of the annual irrigation in our region, and insufficient irrigation could lead to reductions in crop production the next growing season.

Thus, we conducted a study to assess the responses of soil moisture and several plant-based indicators to post-harvest RDI. The specific objectives of our study over two growing seasons were to: 1) determine the responses of the various indicators to a range of irrigation levels (100, 66, 33, and 0% ETC); 2) elucidate the relationships between the different soil and plant variables; and 3) evaluate the appropriateness of the indicators for scheduling irrigation.

## 2. Materials and methods

### 2.1. Experimental site

The post-harvest RDI experiment was conducted during 2009 and 2010 in a commercial olive orchard located about 15 km to the east of Aimogasta, La Rioja, in northwest Argentina (lat. 28° 35' S, long. 66° 42' W; 790 m above mean sea level). The trees (*Olea europaea* L. cv. 'Manzanilla Fina') were 10-years-old at the start of the experiment. Row orientation was north-south with a tree spacing of 4 m within the row and 8 m between rows (i.e., 312 trees ha<sup>-1</sup>). The climate is arid with an annual reference evapotranspiration near 1600 mm and an annual rainfall of about 100 mm, which mostly occurs between late spring and early fall (Searles et al., 2011). The growing season starts in September for our Southern Hemisphere location and a 12-month annual cycle from September until the following August (i.e., the end of winter) was considered in this study. The soil texture was between loamy sand and sandy loam with a particle size distribution of 78% sand, 17% silt, and 5% clay. Organic matter was about 1% and some gravel (<10%) was present below a soil depth of about 1.25 m. The volumetric soil water content (cm<sup>3</sup> cm<sup>-3</sup>) at field capacity and wilting point were estimated to be 0.16 and 0.06, respectively, above a soil depth of 1.25 m based on field measurements. Saxton and Rawls (2006) have reported typical values of soil available water (cm<sup>3</sup> cm<sup>-3</sup>) of 0.07 for loamy sand-textured soils and 0.10 for sandy loam-textured soils according to particle size distribution.

Tree canopy volume was trained as a free-vase with an average volume of 38 m<sup>3</sup>, and there was no pruning during the course of the study. The trees were fertilized by the farmer/grower in the spring and summer of the first growing season before the start of the post-harvest RDI treatments in mid-summer. During the second season, 500 g N, 90 g P (as P<sub>2</sub>O<sub>5</sub>), and 400 g K (as K<sub>2</sub>O) were applied per tree before the RDI treatments based on foliar nutrient analysis. There was no fertilization during the treatment period in either season.

### 2.2. Irrigation treatments and experimental design

After all fruit were removed at mid-summer (February 15) during the green table olive harvest, three post-harvest RDI treatments along with a control (100% of crop evapotranspiration, ETC) were applied for 75 days (22 February–May 5) in 2009 and 2010. We evaluated RDI in this period because table olive harvest is early in our region, and irrigation is normally applied after harvest to maintain soil moisture through the fall and winter months when rainfall is

low. The RDI treatments were 66% ETc (T66), 33% ETc (T33), and no irrigation (T0). In order to implement the RDI treatments and the control, two drip irrigation lines per row with 8 emitters per tree were used. The drip lines were spaced approximately 1 m apart (i.e., 0.5 m on each side of the tree trunk), and the drip emitters were installed continuously at 1-m distances along the drip lines. The emitter discharge rate was 6, 4, and 2 l h<sup>-1</sup> for the control, T66, and T33, respectively. For T0, the drip lines had no emitters and no irrigation was applied during the experimental period. The irrigation frequency was 3–4 days per week and there were no effective precipitation events (>7 mm) during the RDI period in either 2009 or 2010. Before and after the treatments, all the trees were irrigated with 100% ETc as is common in our arid region. Tree crop load at harvest prior to the RDI treatments was medium to high in both 2009 and 2010.

The irrigation requirements were calculated every two weeks using the standard FAO56 formula for crop evapotranspiration (ETc = ET<sub>o</sub> × K<sub>c</sub> × K<sub>r</sub>) where ET<sub>o</sub> is the reference evapotranspiration estimated using the Penman-Monteith equation over grass (Allen et al., 1998), K<sub>c</sub> is the crop coefficient, and K<sub>r</sub> is the coefficient of reduction associated with percentage crop cover (Fereres and Castel, 1981). Climate data for determining ET<sub>o</sub> were taken from an automatic weather station (Davis Instruments, CA USA) located in a large, cleared area with bare soil 1 km from the experiment. The weather station included temperature and humidity sensors, a shortwave pyranometer to measure solar radiation, and an anemometer for wind speed. Values of ET over bare soil were adjusted to reference conditions over grass using Annex 6 of Allen et al. (1998). A crop coefficient (K<sub>c</sub>) of 0.7 was used during the growing season (spring through fall) to estimate 100% ETc based on a previous study in the same orchard (Correa-Tedesco et al., 2010), and a K<sub>c</sub> of 0.4 was applied during the winter months (Rousseaux et al., 2008, 2009). The K<sub>r</sub> was 0.76 in 2009 and 0.82 in 2010.

The experimental design was a randomized complete block design with five blocks. Each block contained one plot of each irrigation level for a total of 20 plots (i.e., 5 blocks × 4 irrigation levels). A plot consisted of seven consecutive trees in the same row, and the measurements were performed on the central three trees. Multiple tree rows were not used for a given plot because a previous study at this orchard determined that moderate irrigation levels did not wet the soil at the midpoint between the rows due to the 8-m distance between rows and the somewhat sandy soil texture (Rousseaux et al., 2009). In the current study, non-experimental trees in the orchard were irrigated with only 33% ETc during the RDI period to reduce the risk of lateral water movement between rows.

### 2.3. Soil moisture

The soil moisture was determined once prior to initiating the RDI treatments, every 15 days during the experimental period, and once after full irrigation (100% ETc) was restored. One soil core per plot was taken to a depth of 1.25 m at a distance of 0.2 m from a drip emitter using an Edelman-type auger (Eijkelkamp Agrisearch Equipment, EM Giesbeek, The Netherlands). The samples were taken two days after an irrigation event to allow for the proper drainage of excessive water near the emitter. Each 1.25-m core was separated at 0.25-m depth intervals in the field, these subsamples were weighed in the laboratory, and then dried in an oven at 105 °C until a constant weight was reached. Gravimetric water content (%) was converted to volumetric soil water content (%) by multiplying by the soil bulk density (1.2 g cm<sup>-3</sup>). Lastly, the relative extractable water (REW, %) was calculated using the formula, REW = (R - R<sub>min</sub>) / (R<sub>max</sub> - R<sub>min</sub>), where R is the current volumetric soil water content, R<sub>min</sub> is the lowest measured soil water content during the experiment, and R<sub>max</sub> is the soil water content at field

capacity (Fernández et al., 1997; Gómez-del-Campo and Fernández, 2007).

### 2.4. Stem water potential, leaf conductance, and sap flow

Similar to the soil water content measurements, stem water potential (Ψ<sub>s</sub>) and leaf conductance (g<sub>l</sub>) were performed once every 15 days during the RDI treatments with an additional measurement before and after applying the treatments. The Ψ<sub>s</sub> was measured at midday under clear sky conditions on one stem per plot with a pressure chamber (Bio-control, model 0–8 MPa, Buenos Aires, Arg). The stems employed were short, ungnified stems with at least two fully expanded leaf pairs found inside the tree canopy near the main trunk. The stems were covered with polyethylene bags and aluminum foil for an hour before the measurements to reduce leaf transpiration as recommended by Fulton et al. (2001). The g<sub>l</sub> was measured in three mature, sun-lit leaves per plot at midday using a porometer (Delta-T Devices Ltd, model AP4, Cambridge, UK). Midday was chosen in order to facilitate the comparison of g<sub>l</sub> with Ψ<sub>s</sub> at the same time of day, although maximum g<sub>l</sub> values likely occurred earlier in the day as observed by Moriana et al. (2002).

The sap flow was measured for a period of five days before applying the RDI treatments and for a similar period six weeks after the start of RDI. Flow was measured in a total of six trees using the heat balance method (Dynamax Inc., Flow Model 32, TX, USA). The six trees included two trees each from the control, T33, and T0. A sensor was placed on the main trunk of each tree after the trunk was cleaned and the sensor was covered with a very thin layer of silicone. The trunks had a diameter of about 17 cm, and the data were stored in a data logger (Campbell Scientific, model CR10X, Logan, UT, USA) that recorded values every 60 s and averaged these values every 15 min. Long periods of measurements were not used because a previous study found that the trunk of olive trees is sensitive to overheating (i.e., cracks) with extended heat applications (Rousseaux et al., 2009). Thus, it was not possible to use these sap flow sensors as a continuous indicator of water stress in this study.

### 2.5. Trunk diameter variations

Trunk diameter was measured continuously during 2009 and 2010 from one month before the start of the RDI treatments until one month after the treatments finished using microdendrometers (Phytech, model DE-1 M, Rehovot, Israel). Each dendrometer consisted of a linear variable displacement transducer with a spring-loaded rod to detect the variations (resolution = 5 μm) in trunk diameter. The dendrometers were installed in the main trunk at a height of 40 cm above ground level in 3 trees of each irrigation level (i.e., 3 trees × 4 irrigation levels = 12 trees in total). The tree canopy shaded the dendrometers and aluminum foil covered each dendrometer to further reduce heat load and to avoid soil particulate accumulation on the sensors. The diameter variations were recorded every hour using individualized data loggers for each dendrometer and downloaded wirelessly to a notebook computer. Daily values of maximum (MXTD) and minimum (MNTD) trunk diameter were obtained from the data records. Three indicators were then calculated: 1) the absolute increase in MXTD of each tree since the beginning of the measurements prior to the start of the RDI period; 2) the trunk growth rate (TGR) of each tree as the average of the differences between MXTD<sub>day + 1</sub> and MXTD<sub>day</sub> for 5 consecutive days; and 3) maximum daily shrinkage (MDS) as the difference between MXTD and MNTD on a given day. Alternative indices were considered including the daily difference of MXTD (D<sub>MXTD</sub>; Cuevas et al., 2013) and of TGR (D<sub>TGR</sub>; Moriana et al., 2010) between the RDI and the control trees, but a preliminary analysis indicated that

the use of these indices did not change the interpretation of the data in our study.

### 2.6. Signal intensity, coefficient of variation, and sensitivity

The potential of the volumetric soil water content and physiological variables for scheduling irrigation was determined using the signal intensity (SI), the coefficient of variation (CV), and sensitivity. In order to obtain a comparable SI for all variables, the SI was calculated as the value of the control divided by the value of the treatment, or the reverse. The CV, a measure of the variability or noise between measurements, was obtained as the ratio of the average to the standard deviation. Sensitivity was calculated as SI divided by CV (Fernández and Cuevas, 2010), and is essentially a signal-to-noise ratio.

### 2.7. Statistical analysis

Analysis of variance (ANOVA) was performed to determine the effect of post-harvest RDI on the soil and physiological variables for each growing season and date using a statistical software package (INFostat; University of Córdoba, Córdoba, Arg). Tukey's test was used to detect differences between means with a significance level of  $P < 0.05$ . Linear and nonlinear relationships between variables were calculated with GraphPad Prism 5 (GraphPad Software, San Diego, USA).

## 3. Results

### 3.1. Meteorological data and irrigations applied

The daily maximum temperature decreased from about 32 to 26°C and the minimum from 20 to 9°C during the post-harvest RDI treatment period in both seasons (Fig. 1A and B). Additionally, the daily ETo decreased from approximately 5.3 to 2.5 mm (Fig. 1C and D). Precipitation was minimal during the RDI period with a total of 10 and 16 mm in 2009 and 2010, respectively, and was not included in the crop water calculations. Irrigation during the RDI period was 0–178 mm in 2009 and 0–193 mm in 2010 depending on the irrigation level applied (Table 1). The reduction in annual irrigation was 7, 13, and 20% for T66, T33 and T0, respectively.

### 3.2. Relative extractable water, stem water potential, and leaf conductance

The REW (%) was similar in all plots when they were irrigated with 100% ETC before the RDI period in both growing seasons (Fig. 2A and B). The 100% ETC control was well-irrigated with REW ranging from 70 to 125% over the course of the 2009 and 2010 RDI periods. The REW values of T66 showed no significant differences with the control, while the REW decreased rapidly in the T0 in both seasons with marked differences compared to the control in REW after just the second week of RDI. The T33 had moderate decreases in REW compared to the control. When measured five weeks after re-watering, the REW in all of the treatments was similar to that of the control.

The  $\Psi_s$  of all the trees was low (–2.4 MPa) just before the beginning of the experiment in 2009 possibly due to several days of maximum temperatures greater than 35°C (Figs. 1A, 2C). However, the  $\Psi_s$  of the control remained above –1.9 MPa during the RDI period in both seasons. The  $\Psi_s$  of T66 did not differ significantly from the control (Fig. 2C and D) as was the case for REW. The  $\Psi_s$  of T0 fell sharply in the first 2–4 weeks after the start of RDI and then maintained fairly constant values between –2.7 to –3.8 MPa. No statistically significant differences in the  $\Psi_s$  of T33 and the control were detected in 2009, but the  $\Psi_s$  of T33 decreased

gradually to –3.0 MPa towards the end of the RDI period in 2010. After re-watering,  $\Psi_s$  of the treatments showed no differences with the control.

The  $g_l$  of the control was typically around 200 mmol m<sup>–2</sup> s<sup>–1</sup> in both growing seasons (Fig. 2E and F). The  $g_l$  of T66 was very similar to that of the control in 2009, while some slight reductions compared to the control were apparent in 2010. As occurred for  $\Psi_s$ , the  $g_l$  of T0 fell rapidly at the start of the RDI period and remained around 100 mmol m<sup>–2</sup> s<sup>–1</sup> until re-watering. The  $g_l$  of T33 had lower values than the control in 2010, but not in 2009. The control and all treatments showed similar values after re-watering.

### 3.3. Trunk diameter variations and sap flow

Trunk growth in the control increased in an approximately linear manner over the course of the RDI period in both seasons based on the daily MXTD values, while little trunk growth or even trunk shrinkage occurred in T0 (Fig. 3A and B). In contrast, trunk growth of T33 and T66 was intermediate between T0 and the control. Significant differences in MXTD between irrigation levels were apparent from the fifth week of treatment until the end of the RDI period in 2009, and from the third week onwards in 2010. The TGR of the control averaged 27 and 21  $\mu\text{m d}^{-1}$  over the RDI period in 2009 and 2010, respectively, and the TGR of T0 was significantly less than that of the control even as early as the first week of the RDI period (Fig. 3C and D). As would be expected, the TGR values of T33 and T66 were intermediate. Shortly after re-watering, there were no differences in TGR between the control and the RDI treatments. Lastly; although few precipitation events occurred in either year, precipitation led to large variations in TGR values two weeks after the start of RDI in 2009 and some other dates.

Similar to the decrease in ETo over the RDI period (Fig. 1A and B), the MDS decreased from approximately 300  $\mu\text{m}$  at the beginning of RDI to between 60 and 230  $\mu\text{m}$  at the end of RDI period (Fig. 3E and F). In 2009, no significant differences in MDS between irrigation levels were observed. Towards the end of the 2010 RDI period, the MDS values of T0 and T33 were consistently greater than those of T66 and the control.

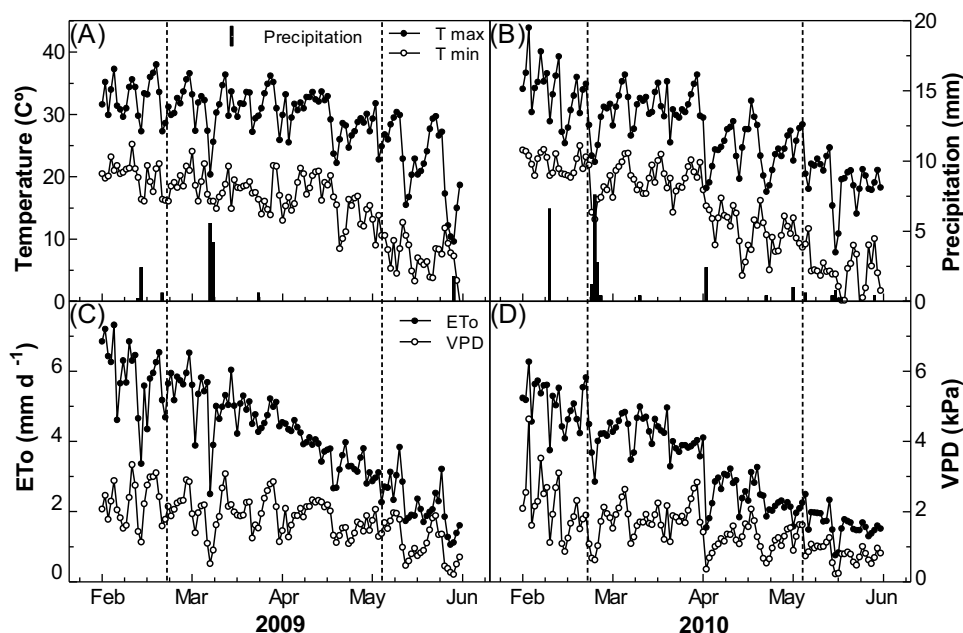
Sap flow in the control (T100) and the measured RDI treatments (T33 and T0) was similar in the summer of 2009 and 2010 before the RDI period (Fig. 4A and B). After 6–7 weeks of no irrigation, a significant reduction in sap flow (–57%) was found in T0 compared to the control in 2009 ( $P < 0.05$ ), and a similar tendency (–63%) for T0 was observed in 2010 ( $P = 0.06$ ). No significant differences were observed between the control and T33 possibly due to the low number of replicates per irrigation level ( $n = 2$  trees). Sap flow values of the fall measurements were lower in all the irrigation levels than those of the summer in accordance with the decrease in ETo.

### 3.4. Relationships between soil and physiological variables

A bilinear relationship was obtained between soil REW (%) and  $\Psi_s$  when combining data from the different irrigation levels and seasons (Fig. 5A;  $r^2 = 0.62$ ).  $\Psi_s$  increased linearly with REW until it reached a break point at 48% REW, above which  $\Psi_s$  maintained a plateau at –1.75 MPa. A weak exponential relationship between REW and  $g_l$  was observed with  $g_l$  reaching a plateau at 196 mmol m<sup>–2</sup> s<sup>–1</sup> (Fig. 5B;  $r^2 = 0.24$ ).

The increase in MXTD during the RDI period was better related to the discontinuously measured variables (REW,  $\Psi_s$ ,  $g_l$ ) with  $r^2$  values between 0.61 and 0.78 than were either TGR or MDS (Fig. 6). The relationships between the increase in MXTD and REW or  $g_l$  were linear, while an exponential relationship with  $\Psi_s$  was determined (Fig. 6A–C). This exponential relationship indicates that trunk growth was near zero during the RDI period when the  $\Psi_s$  was lower than about –2.5 MPa. TGR showed a weak linear





**Fig. 1.** Daily maximum and minimum temperature and precipitation in 2009 (A) and 2010 (B) along with daily reference evapotranspiration (ETo) and vapor pressure deficit (VPD) in 2009 (C) and 2010 (D). The dashed vertical lines indicate the beginning and end of the post-harvest regulated deficit irrigation period.

**Table 1**

Reference evapotranspiration (ETo) and irrigation during the post-harvest regulated deficit irrigation period (RDI) in 2009 and 2010. Annual irrigation is also shown for the 12 months from early spring (September) to the end of the following winter (August).

Year	ETo (mm)	Treatment (ETc%)	Irrigation during RDI (mm)	Annual irrigation (mm)
2009	323	100	178	897
		66	119	838
		33	59	778
		0	0	719
2010	313	100	192	954
		66	128	890
		33	64	826
		0	0	762

relationship with REW and a bilinear relationship with  $\Psi_s$  (Fig. 6D and E). Fairly similar to the exponential relationship for MXTD, the bilinear relationship shows that trunk growth reached a constant negative value below  $-2.7$  MPa. No relationship between TGR and  $g_1$  was apparent (Fig. 6F). The MDS had a positive linear relationship with REW and a negative linear relationship with  $g_1$ , while MDS showed a quadratic, polynomial relationship with  $\Psi_s$  (Fig. 6G–I). In all cases, the  $r^2$  values between MDS and the soil or plant variables were low (0.10–0.19).

### 3.5. Signal intensity, coefficient of variation, and sensitivity

When irrigation was withheld (i.e., T0), the SI calculated showed significant differences between the measured variables for some dates in both seasons (Fig. 7A and B). In 2009, the SI of volumetric soil water content was sometimes greater than the SI of  $\Psi_s$  and  $g_1$  under T0 conditions, while the SI values of MDS were near 1.0 because the treatment and control values of MDS were similar. In 2010, volumetric soil water content also had fairly high SI values under T0. Soil REW(%) was not used in the SI analysis because it is already relativized and unrealistic SI values were obtained.

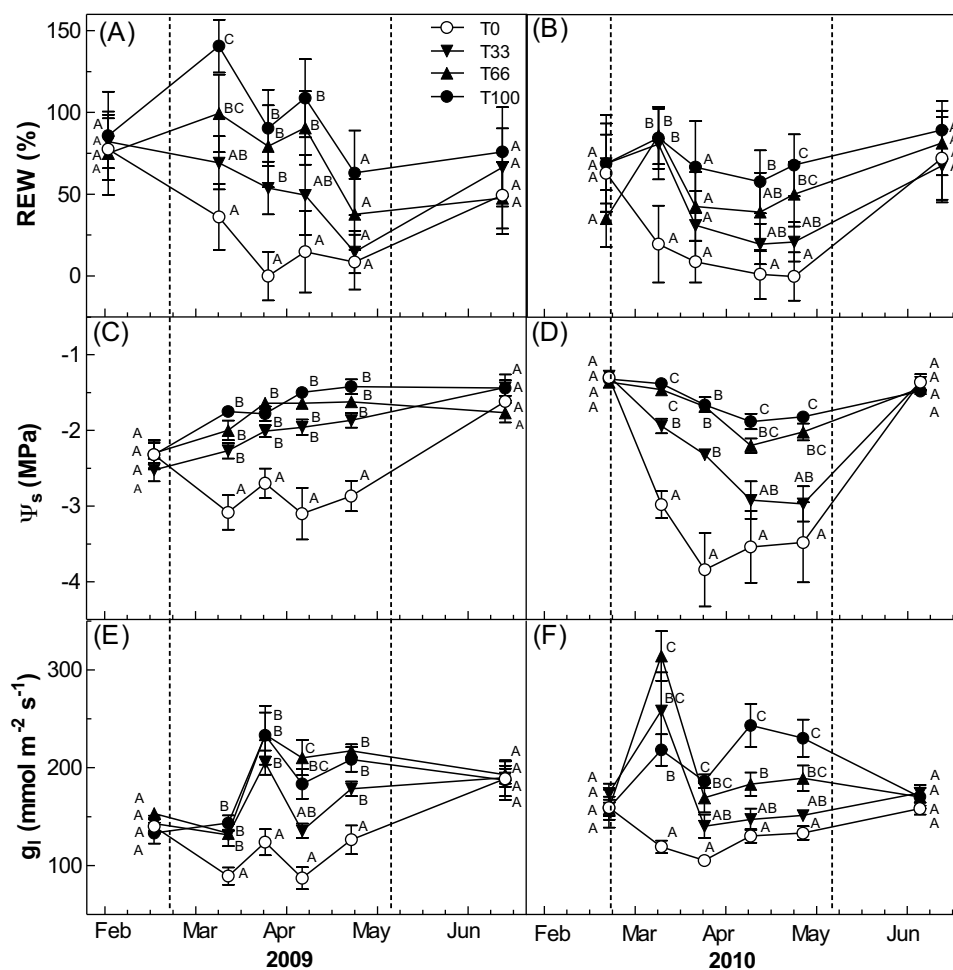
As also found by Moriana et al. (2010), the increase in MXTD and TGR could not be included in the T0 SI calculations (Fig. 7A and B) because of negative values on many dates (Fig. 3), but MXTD was included in the T33 calculations (Fig. 7C and D). The SI of MXTD tended to be greater than those of the other variables under T33

(Fig. 7C and D). Although both volumetric soil water content and MXTD had high SI values, significant differences in SI with the other measured variables were only detected on some dates due to their high CV (Table 2). The high CV values for volumetric soil water content and MXTD led a low sensitivity (SI/CV), or low signal-to-noise ratio. In contrast,  $\Psi_s$  tended to show a high sensitivity.

## 4. Discussion

Evapotranspiration is high and rainfall is very infrequent during the post-harvest period that starts in mid-summer for table olive cultivars such as 'Manzanilla' in northwestern Argentina (Searles et al., 2011). The climatic conditions and early harvest provided us the opportunity to evaluate the effects of RDI on several plant and soil indicators during post-harvest. Irrigation during this phenological phase has received little attention in olive because rainfall during post-harvest in the Mediterranean Basin where most olive is cultivated often precludes the need to irrigate (Girón et al., 2015).

The well-irrigated control and the RDI treatments successfully resulted in a wide range of soil REW (%) from 0 to 125%. High levels of soil moisture maintained  $\Psi_s$  in the control trees between  $-1.3$  and  $-1.8$  MPa. Dell'Amico et al. (2012) has reported similar  $\Psi_s$  values in low fruit load trees with no decrease in yield being likely if  $\Psi_s$  is above a threshold of  $-1.8$  MPa. No decrease in  $\Psi_s$  was apparent in the 66% RDI treatment, and REW measured to a soil depth of 1.25 m was almost never lower than 50% in this



**Fig. 2.** The soil relative extractable water (REW; A, B), stem water potential ( $\Psi_s$ ; C, D), and leaf conductance ( $g_l$ ; E, F) for the control (T100) and the post-harvest regulated deficit irrigation treatments (RDI; T66, T33 and T0) in 2009 and 2010. Each data point represents a mean  $\pm$  standard error ( $n = 5$  plots per irrigation level). The dashed vertical lines indicate the beginning and the end of the RDI period.

**Table 2**

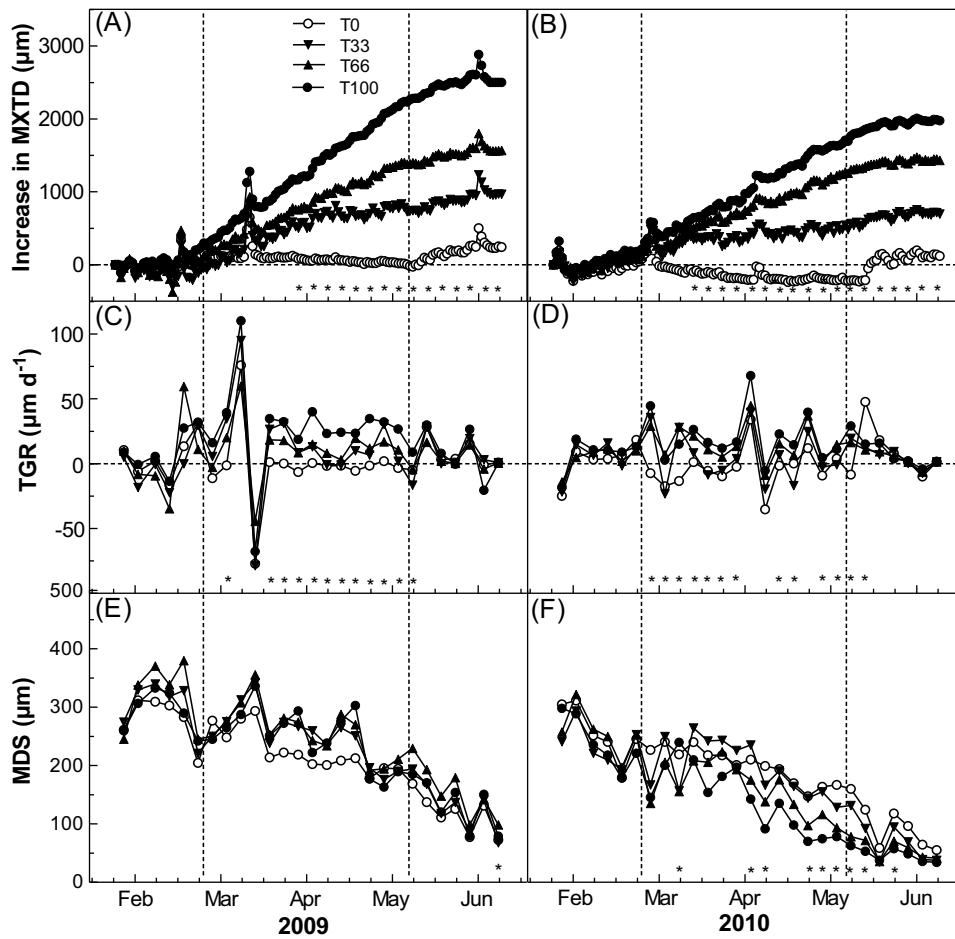
Coefficient of variation (CV) and sensitivity (signal intensity/CV) of volumetric soil water content (SWC), stem water potential ( $\Psi_s$ ), leaf conductance ( $g_l$ ), increase in maximum trunk diameter (MXTD), and maximum daily shrinkage (MDS) during the post-harvest regulated deficit irrigation period (RDI) in 2009 and 2010.

Indicator	Treatment	Season	SWC	$\Psi_s$	$g_l$	MXTD	MDS
CV (%)	0	2009	39.7	12.4	20.5	na	19.3
		2010	28.0	14.9	11.7	na	17.5
	33	2009	26.6	10.4	14.3	21.1	14.9
		2010	36.6	10.2	21.6	58.1	17.3
Sensitivity (SI/CV)	0	2009	6.9	18.6	10.3	na	6.1
		2010	10.6	16.2	20.0	na	5.6
	33	2009	7.6	18.0	11.2	7.9	12.2
		2010	5.0	16.8	11.0	6.2	6.1

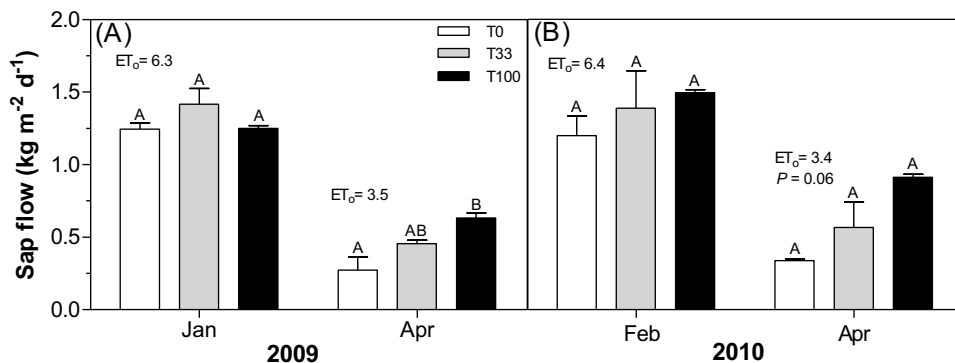
na = not assessed due to the negative values recorded for MXTD under T0.

treatment. The  $\Psi_s$  declined in both years during the first month of RDI when water was completely withheld (i.e., T0), and then remained fairly constant (albeit at very low values) the next 6 weeks until the end of the RDI period. Given that REW (%) was near zero in the upper 1.25 m of the soil after the first month in the T0 treatment, some root absorption of water likely occurred at deeper layers of the soil profile such that  $\Psi_s$  did not fall below  $-4$  MPa. Exploratory measurements with a soil corer during the second growing season found some soil moisture down to about 2 m-depth, and the soil volume explored by these mature trees was likely to be fairly large (18–24 m<sup>3</sup> per tree). An earlier study in this same orchard when the trees were only 5-years-old found 5% of the roots to be between a

soil depth of 1.0 and 1.5 m (Searles et al., 2009), but roots were not evaluated at a greater depth. Decreasing temperature the last 6 weeks of the study also potentially contributed to  $\Psi_s$  not further decreasing in the T0 treatment. In very mature trees with larger root systems (32 m<sup>3</sup> of soil explored per tree) and lower leaf areas than our trees, Fernández et al. (2008) determined that  $\Psi_s$  and transpiration did not start to decline until much later (i.e., 50 days after water withholding) than in our study. In contrast, a process-based model of water use in olive has shown that a low ratio of root area-to-leaf area such as may occur with high plant densities in shallow soils leads to a very rapid reduction in transpiration (Diaz-Espejo et al., 2012).



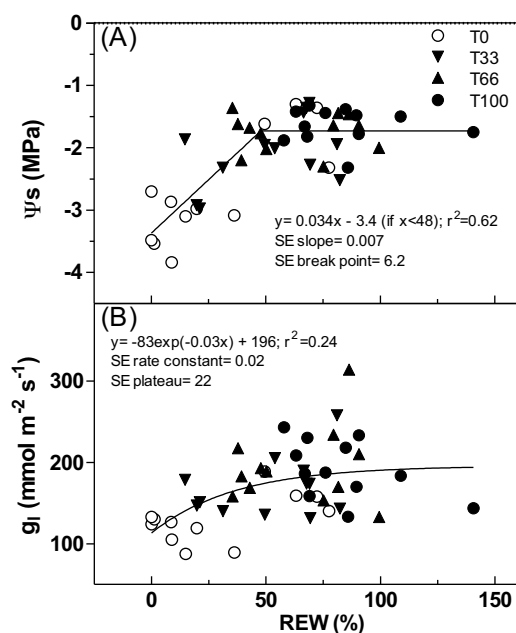
**Fig. 3.** The increase in maximum trunk diameter (MXTD; A, B), trunk growth rate (TGR; C, D) and maximum daily shrinkage (MDS; E, F) for the control (T100) and the post-harvest regulated deficit irrigation treatments (RDI; T66, T33 and T0) in 2009 and 2010. Each data point represents a mean  $\pm$  standard error ( $n=3$  trees per irrigation level). The dashed vertical lines indicate the beginning and the end of the RDI period.



**Fig. 4.** Sap flow per unit of leaf area for the control (T100) and two of the post-harvest regulated deficit irrigation treatments (RDI; T33 and T0) in 2009 (A) and 2010 (B). Each bar represents a mean  $\pm$  standard error ( $n=2$  trees per irrigation level).

Depending on how narrowly plants maintain  $\Psi_s$  under deficit irrigation, they may be classified as either isohydric (tight stomatal control) or anisohydric (little strict control) (Tardieu and Simonneau, 1998; Chaves et al., 2010). However, this classification is likely to be a matter of degree with most plant species being capable of a wide range of  $\Psi_s$  values (Maseda and Fernández, 2006). The  $\Psi_s$  was not significantly reduced on any of the measurement dates in the 33%  $ET_c$  treatment compared to the control during the first year of our study (Fig. 2C), but  $g_l$  was significantly reduced in early April after 6 weeks of RDI (Fig. 2E). This may indicate tight stomatal

control under mild water stress conditions in olive trees. Cuevas et al. (2010) has also found some evidence of isohydric behavior under mild water stress in large trees. In the T0 treatment with greater water stress even in the first few weeks following the start of RDI,  $\Psi_s$  decreased markedly along with  $g_l$ , which indicates anisohydric behavior as has been seen in other studies of olive with moderate to high levels of water stress (e.g., Moriana et al., 2012; Rosecrance et al., 2015). Such anisohydric behavior may be necessary to ensure adequate internal leaf  $CO_2$  concentrations and



**Fig. 5.** Relationships between soil relative extractable water (REW) and stem water potential ( $\Psi_s$ , A) or leaf conductance ( $g_l$ , B) when combining data from the control (T100), the post-harvest regulated deficit irrigation treatments (RDI; T66, T33 and T0), and the two seasons. Each data point represents the mean of 5 experimental plots for the dates shown in Fig. 2. The total number of data points is 48 (6 dates  $\times$  4 irrigation levels  $\times$  2 seasons). The standard errors (SE) of key parameters are shown to better interpret the nonlinear relationships.

consequently provide sufficient photoassimilates to the different plant organs.

The responsiveness of trunk diameter variations (TDV) such as MXTD and TGR to deficit irrigation has long been recognized in olive trees (Michelakis, 1997; Moriana and Fereres, 2002; Moriana et al., 2003). In our post-harvest study, MXTD increased linearly throughout the RDI period in the well-watered, control trees in both seasons. Similar linear increases have been found in other studies throughout the growing season for trees with low crop loads (Moriana et al., 2003; López-Bernal et al., 2015). The significant differences in the increase in MXTD between the control trees and T0 were found after five weeks in 2009 and three weeks in 2010, while the differences with the 66% and 33% ETC treatments developed over longer time periods. Fernández et al. (2011a,b) also found in large trees that differences in MXTD developed over long time periods in RDI studies when employing low frequency irrigation treatments equivalent to 60 and 30% ETC. The increase in MXTD over a given period in any experiment is basically the sum of daily or weekly TGR values. We were able to detect differences in TGR between irrigation treatments as early as the first week of the experiment in both years using 5-day TGR averages in part because of the low humidity, low rainfall conditions of our region and lack of crop load in post-harvest. Rainfall and the consequent wetting and drying of the trunk often create large variations in trunk diameter such that TGR values are not representative of water stress (Girón et al., 2015). For example, a single rainfall event in early March 2009 precluded the possibility of detecting differences in TGR for several days. Lastly, few differences in MDS were observed between treatments as has been found by many authors (e.g., Moriana and Fereres, 2002; Cuevas et al., 2010, 2013; Moriana et al., 2010). The only exception was towards the end of the second RDI period when MDS was higher in the less irrigated treatments than the control and 66% ETC treatment.

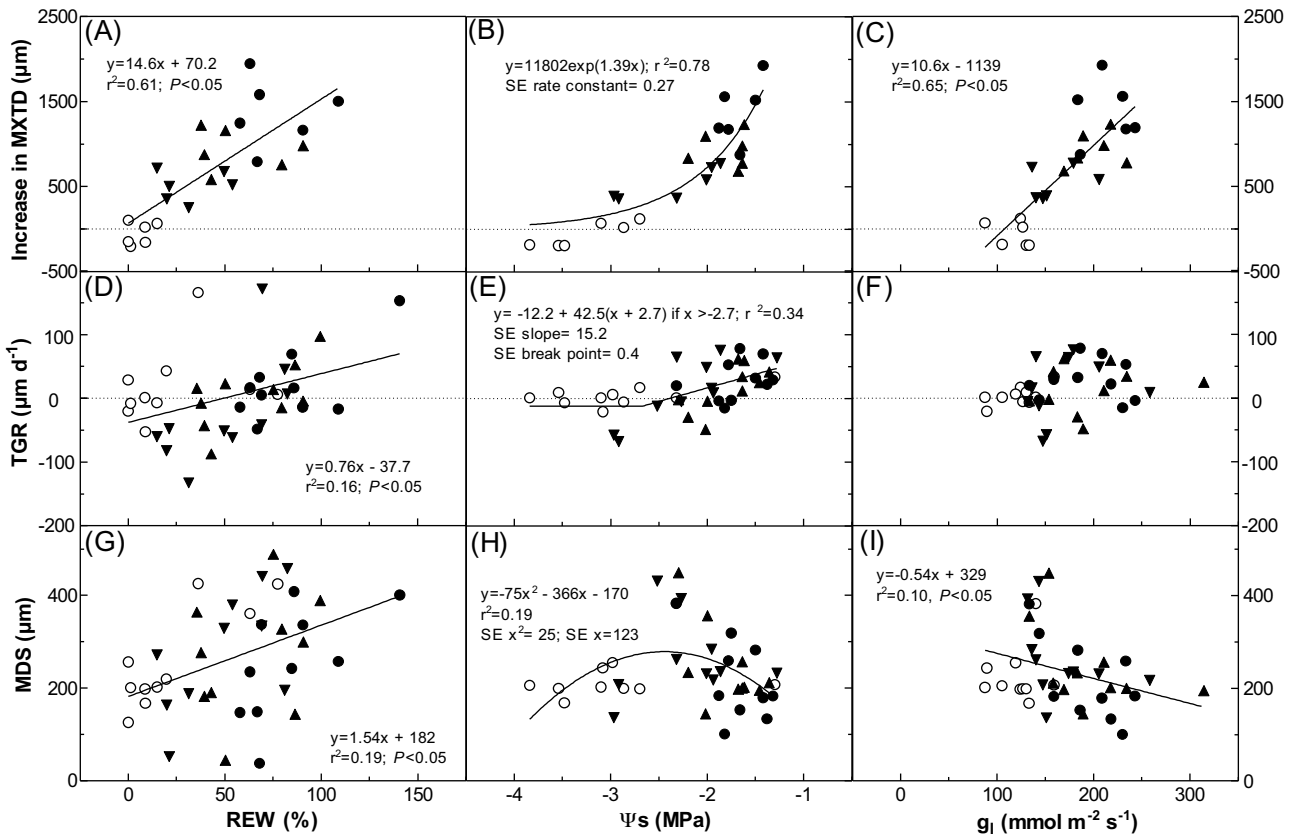
Although there was little replication, main trunk sap flow of these large trees decreased approximately 30 and 60% on a leaf

area basis for the 33% ETC and T0 treatments, respectively, when measured 6–7 weeks after the start of the post-harvest RDI period. In the second year of a sustained deficit irrigation study, Rousseaux et al. (2009) found a 30% decrease in sap flow when applying 66% ETC in an adjacent orchard with the same cultivar ('Manzanilla'), but this decrease was apparent only when it was expressed per tree due to adjustments in leaf area related to tree growth and leaf fall in response to prolonged, mild water stress. No response was apparent on a leaf area basis. In the current post-harvest RDI study, branch growth and leaf area accumulation were not affected (data not shown), and the decreases in sap flow on a leaf area basis appeared to largely follow the decreases found in  $g_l$ . The  $g_l$  decreases were approx. 30 and 45% for the 33% ETC and T0 treatments, respectively, during the same time period as the sap flow measurements. Lastly, decreases in sap flow of a similar magnitude to our results have been found by Cuevas et al. (2013) in southern Spain when assessing low frequency RDI treatments that partially mimic our T0 treatment.

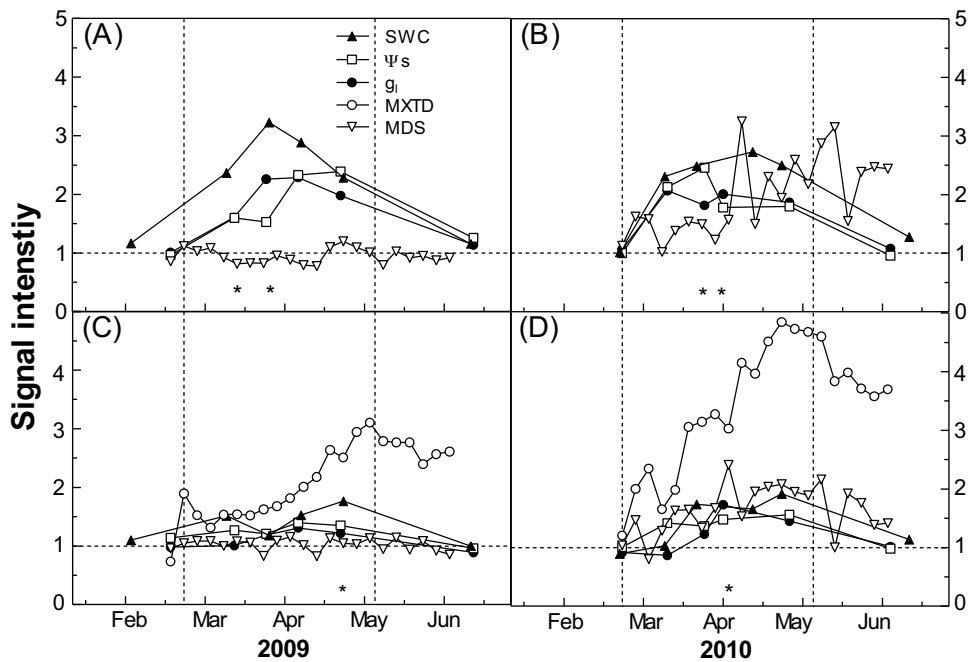
While the soil and physiological plant responses to specific irrigation treatments are important to document, obtaining relationships between soil and plant indicators and between individual plant indicators themselves is likely to more strongly further our understanding of plant functioning and how best we might schedule irrigation. Several studies have plotted the relationships between REW and water potential in olive over the last 20 years. Fernández et al. (1997) found that pre-dawn water potential ( $\Psi_{pd}$ ) remained relatively high in moist soil, but appeared to decrease sharply when REW values were less than 40%. Similar curvilinear relationships have been reported using either  $\Psi_{pd}$  (Tognetti et al., 2006, 2009) or  $\Psi_s$  (Moriana et al., 2002; Gómez-del-Campo, 2013a). A bilinear relationship was apparent in our study with  $\Psi_s$  decreasing sharply when REW fell below 48% (Fig. 5A). Under greater REW, a constant  $\Psi_s$  value of  $-1.75$  MPa occurred. Similarly, Rallo and Provenzano (2012) recently reported that  $\Psi_s$  fell sharply from a plateau somewhere between  $-1.5$  and  $-2.0$  MPa when a certain threshold for soil water content was reached. Taken together, the above results suggest that a threshold of 50% REW could conservatively be used to program irrigation if little or no reduction in  $\Psi_s$  is desired, and a lower REW could be used for regulating moderate water stress. While installing automated soil moisture measurement systems in large commercial farms ( $>100$  ha) may be costly and the measurements may not always be entirely accurate due to technological limitations (Evet et al., 2012), many commercial farms in our region monitor soil moisture continuously in order to have a real-time diagnostic tool for evaluating their irrigation needs.

Many fewer studies in olive have evaluated the relationships between more traditional approaches such as REW and  $\Psi_s$  with continuous TDV measurements. Under our post-harvest conditions, the highest correlation coefficients ( $r^2 = 0.61$ – $0.78$ ) were seen between the increase in MXTD with REW,  $\Psi_s$ , and  $g_l$  (Fig. 6A–C). Strong relationships have also been found between MXTD and water potential in both fairly large cv. 'Arbequina' trees (Fernández et al., 2011b) and in smaller 'Arbequina' trees grown under dense hedgerow conditions (Cuevas et al., 2013). In our study, the relationship was curvilinear with trunk growth falling sharply as  $\Psi_s$  decreased to about  $-2.5$  MPa. Below this value, trunk growth was minimal. A bilinear relationship between TGR and  $\Psi_s$  also showed a similar pattern (Fig. 6E) with TGR displaying a constant negative growth rate of  $-12 \mu\text{m d}^{-1}$  below  $-2.7$  MPa. Girón et al. (2015) have recently reported nonlinear relationships between several physiological parameters and fruit drop (%), with fruit drop increasing significantly below  $\Psi_s$  values of  $-2.2$  MPa and TGR values of  $-10 \mu\text{m d}^{-1}$ . A strict comparison of the results between these two studies is not feasible because of the differences in crop load and the phenological periods evaluated, but the similarity of some of the





**Fig. 6.** Relationships between soil relative extractable water (REW%), stem water potential ( $\Psi_s$ ), and leaf conductance ( $g_i$ ) with the trunk diameter indicators when combining data for the post-harvest regulated deficit irrigation treatments (RDI; T66, T33 and T0) and the two seasons. MXTD = increase in maximum trunk diameter; TGR = trunk growth rate; MDS = maximum daily shrinkage (MDS). Each data point represents the mean of the experimental plots using data from Fig. 2 and Fig. 3. The total number of data points is 40 (5 dates  $\times$  4 irrigation levels  $\times$  2 seasons) for the TGR and MDS relationships. The date after re-watering was not included because trunk growth was minimal late in the fall season regardless of irrigation level. For the MXTD relationships, only the last three dates of the RDI period were employed (3 dates  $\times$  4 irrigation levels  $\times$  2 seasons;  $n=24$ ) to correct for the cumulative nature of this variable. Please note that REW(%) and  $\Psi_s$  were not measured on exactly the same dates, which may affect the relationships obtained. The standard errors (SE) of key parameters are shown to better interpret the nonlinear relationships.



**Fig. 7.** Signal intensity (SI) of volumetric soil water content (SWC), stem water potential ( $\Psi_s$ ), leaf conductance ( $g_i$ ), increase in maximum trunk diameter (MXTD), and maximum daily shrinkage (MDS) for two post-harvest regulated deficit irrigation treatments (T0; A, B and T33; C, D) in 2009 and 2010. The dashed vertical lines indicate the beginning and the end of the RDI period. In order to obtain a comparable SI for all variables, the SI was calculated as the control divided by the treatment, or the reverse. The horizontal lines indicate that the values of the control and treatment are equal (SI = 1).

results provides sufficient promise that continuous trunk measurements have potential and warrant further investigation in olive.

Signal intensity (SI) can be a useful tool for assessing the relative response of different soil and plant parameters (Fernández and Cuevas, 2010), although calculations of SI for some parameters such as TGR are not always straightforward (Moriana et al., 2010). In our study, the increase in MXTD, soil moisture, and  $\Psi$ s tended to have a greater signal than gl and MDS. These results agree well with those found previously by Moriana and Fereres (2002). However, MXTD and soil moisture in our study showed a large degree of variability (CV%), which would require more measurements and greater equipment costs for commercial farms. In contrast, the measurements of  $\Psi$ s were much less variable between trees.

## 5. Conclusion

Our results show that irrigating with 100% of crop evapotranspiration during the post-harvest period from mid-summer when green table olives are harvested to mid-fall represents up to 20% of the annual irrigation for our warm, dry region in Northwest Argentina, and that deficit irrigation during this period led to reductions in soil REW%. These reductions; in turn, led to decreases in discontinuously measured plant indicators such as  $\Psi$ s and gl and continuously measured trunk diameter variations including MXTD and TGR. This information allowed relationships between soil and plant indicators to be determined. Given the strong non-linear relationship between soil REW(%) and  $\Psi$ s in our study and many others, defining thresholds for scheduling irrigation at different water stress levels based on soil REW(%) appears feasible if soil moisture can be measured accurately. Stem water potential was a reliable indicator of water stress in our study, although the measurements cannot be automated using traditional pressure chambers. However, alternative technologies such as leaf patch clamp pressure probes, which measure leaf turgor continuously, are also being developed (Padilla-Díaz et al., 2016). Over the last several years, the understanding of trunk measurements has greatly increased, and good relationships between soil REW(%) and  $\Psi$ s with trunk diameter variations were obtained in our study. Thus, trunk diameter variations show promise for scheduling irrigation during post-harvest in our arid region. Further studies should focus on testing these trunk diameter relationships under more conditions.

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