

Leaf water relations in *Diospyros kaki* during a mild water deficit exposure

I. Griñán^{a,1}, P. Rodríguez^{b,1}, Z.N. Cruz^c, H. Nouri^{d,e}, E. Borsato^f, A.J. Molina^g, A. Moriana^h, A. Centenoⁱ, M.J. Martín-Palomo^h, D. Pérez-Lópezⁱ, A. Torrecillas^j, A. Galindo^{d,*,1}

^a Dpto. Producción Vegetal y Microbiología, Grupo de Investigación de Producción Vegetal y Tecnología, Universidad Miguel Hernández de Elche, Ctra. de Beniel, km 3, 2., E-03312 Orihuela, Alicante, Spain

^b Centro de Investigación Obonuco. Corporación Colombiana de Investigación Agropecuaria (CORPOICA), Vía Pasto-Obonuco km 5, Pasto, Nariño, Colombia

^c Department of Physiology and Biochemistry, Instituto Nacional de Ciencias Agrícolas (INCA). Ctra. de Tapaste, km 3.5, San José de Las Lajas, Mayabeque, Cuba

^d Dept. of Water Engineering & Management. Faculty of Engineerin Technology, University of Twente. P.O. Box 217, 7500 AE, Enschede, the Netherlands

^e Division of Agronomy, University of Göttingen, Von-Siebold-Strasse 8, 37075, Göttingen, Germany

^f Dept. of Land, Environment, Agriculture and Forestry, University of Padova, 35020 Agripolis, Italy

^g Surface Hydrology and Erosion Group, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), E-08034, Barcelona, Spain

^h Dpto. Ciencias Agroforestales, ETSIA, Universidad de Sevilla. Crta de Utrera km 1, E-41013 Sevilla, Spain

ⁱ Dpto. Producción Vegetal, Fitotecnia, ETSIAAB, Universidad Politécnica de Madrid. Ciudad Universitaria s/n, E-28040 Madrid, Spain

^j Dpto. Riego. Centro de Edafología y Biología Aplicada del Seguro (CSIC). P.O. Box 164, E-30100 Espinardo, Murcia, Spain

ARTICLE INFO

Keywords:

Diospyros kaki
Gas exchange
Sap flow
Trunk diameter fluctuations
Water relations
Water stress

ABSTRACT

The resistance mechanisms (stress avoidance and stress tolerance) developed by persimmon plants (*Diospyros kaki* L. f. grafted on *Diospyros lotus* L.) in response to mild water stress and the sensitivity of continuously (on a whole-day basis) and discretely (at predawn and midday) measured indicators of the plant water status were investigated in 3-year old 'Rojo Brillante' persimmon plants. Control (T0) plants were drip irrigated in order to maintain soil water content at levels slightly above soil field capacity (102.3% of soil field capacity) and T1 plants were drip irrigated for 33 days in order to maintain the soil water content at around 80% of soil field capacity. The results indicated persimmon plants confront a mild water stress situation by gradually developing stomata control (stress avoidance mechanism) and exhibiting some xeromorphic characteristic such as high leaf relative apoplastic water content, which could contribute to the retention of water at low leaf water potentials. In addition, sap flow measurements made by the heat-pulse technique were seen to be the most suitable method for estimating persimmon water status, because it provided the highest signal intensity (actual value/reference value):noise (coefficient of variation) ratio in almost all intervals of time considered and provides continuous and automated registers of the persimmon water status in real time.

1. Introduction

The decrease in the profitability of some Mediterranean fruit tree industries in recent years has led to the search for other fruit trees as alternatives. This situation has provided very important collateral advantages, including such as the enrichment of biodiversity, which is fundamental for ecosystem functioning, more sustainable agricultural production and increased food and nutritional security (Thrupp, 2000; Toledo and Burlingame, 2006; Chappell and LaValle, 2011). In this sense, persimmon (*Diospyros kaki* L. f.) tree culture in the Spanish Mediterranean basin is steadily increasing, aided by its excellent adaptation to temperate warm climates, high yields, high commercial value of the fruit, and excellent post-harvest storage life. Persimmon is

native to the mountains of central China and Japan (Mowat and George, 1994; Llácer and Badenes, 2002; George et al., 1997) and is included in the list of so-called underutilized or minor fruit crop species.

Badal et al. (2010) suggested that the irrigation water requirements of persimmon are quite large. So, besides genetic factors, water deficit is considered as the main environmental factor affecting unstable persimmon fruit production (physiological fruit drop and biennial bearing) (Suzuki et al., 1988; Yamamura et al., 1989; Yakushiji et al., 2013). As a consequence, irrigation may be the main limiting factor for persimmon culture in Mediterranean agrosystems due to the persistent shortage of water resources. For this reason, persimmon irrigation will need to be based on the use of very precise deficit irrigation management strategies that are able to significantly reduce the amount of irrigation water

* Corresponding author at: Current address: P.O. Box, 217, 7500 AE Enschede, the Netherlands.

E-mail addresses: hamideh.nouri@uni-goettingen.de (H. Nouri), a.galindo@utwente.nl (A. Galindo).

¹ These authors contributed equally to this work.

necessary with minimum effects on yield and fruit quality.

Under deficit irrigation conditions, the continuous and precise control of tree water status is crucial in order to prevent a potentially beneficial water stress from becoming too severe and ending in a reduction in the yield or fruit quality (Johnson and Handley, 2000). In this sense, the use of plant-based water status indicators may be considered as an ideal tool for precise deficit irrigation scheduling in fruit trees, as has been reported by Naor (2000); Lampinen et al. (2001); García-Orellana et al. (2007); Ortuño et al. (2009a, b), Ortuño et al. (2010) and Conejero et al. (2011). A suitable plant-based water stress indicator for use in irrigation scheduling practices has to be sufficiently sensitive, consistent and reliable for detecting minimum changes in the plant water status. Moreover, it is important to consider that the magnitude of any plant-based water status indicator, even in a well-watered tree, is not constant over a period of days with different environmental conditions. Therefore, the absolute values of these indicators, registered without considering the evaporative demand, might be meaningless. For this reason, for irrigation scheduling it is better to use the concept of signal intensity (SI), normalizing the absolute values with respect to values in non-limiting soil water conditions (Fernandez and Cuevas, 2010; Ortuño et al., 2010).

The irrigation protocol for trees using plant-based water status indicators consists of maintaining the plant-based water status indicator SI at around a threshold value, decreasing the irrigation rate when the SI does not exceed the threshold value, and increasing the irrigation rate when the SI exceeds the threshold value. When fruit trees are grown with high frequency irrigation the irrigation water amounts to be applied are usually estimated daily (Conejero et al., 2011), every three days (Conejero et al., 2007) or weekly (Velez et al., 2007).

The discrete measurement of predawn or midday leaf water potential (Ψ_{pd} or Ψ_{md}) and midday (12 h solar time) stem water potential (Ψ_{stem}) are the most widely used approaches for evaluating plant water status (McCutchan and Shackel, 1992; Naor, 2000). However, in recent years the possibility of obtaining real time, continuous and automated registers of the plant water status, avoiding frequent trips to the field and a significant input of manpower, has led to the increased use of alternative indices using plant sensors such as sap flow (SF) and maximum daily trunk shrinkage (MDS), a single parameter obtained from trunk diameter monitoring, which can be used for full and deficit irrigation scheduling in fruit trees (García-Orellana et al., 2007; Ortuño et al., 2009a, b; Conejero et al., 2011; Moriana et al., 2013).

To the best of our knowledge, research on the response, at plant water relations level, of persimmon plants to drought is very scarce. Nevertheless, Yakushiji et al. (2013) showed that predawn leaf turgor potential (Ψ_{ppd}) began to decrease when Ψ_{pd} fell below ca. -0.7 to -0.8 MPa, and that the response of fruit water status to drought clearly depends on the fruit growth stage. Yamamura et al. (1989) indicated that even a moderate water deficit (leaf water potential (Ψ_{leaf}) values around -1.8 MPa) increased fruit drop. Also, Badal et al. (2010) assessed the usefulness of the MDS, as a persimmon water deficit indicator.

Bearing the above in mind, the purpose of the present study was (i) to evaluate the sensitivity of continuous and discretely measured indicators of the plant water status to use in irrigation scheduling in persimmon trees, and (ii) to analyze leaf water relations in order to clarify the response mechanisms (stress avoidance and stress tolerance) developed by persimmon plants in response to mild water stress.

2. Materials and methods

2.1. Experimental conditions, plant material and treatments

The experiment was carried out during the summer of 2016 at a farm located near the city of Murcia (Spain) (38°1'N, -1°3'W). The soil is a Calcaric fluvisol with clay texture. Soil volumetric water contents (θ_v)

at saturation, field capacity and permanent wilting point were 0.48, 0.42 and 0.28 $m^3 m^{-3}$, respectively. The irrigation water had an electrical conductivity of between 1.2 and 1.4 dS/m and a Cl^- concentration ranging from 20 to 35 $mg l^{-1}$.

The climate of the area is typically Mediterranean, with mild winters, low annual rainfall, and hot dry summers. During the experimental period, average daily maximum and minimum air temperatures were 32 and 19 °C, respectively, the mean daily air vapour pressure deficit (VPD_m) (Allen et al., 1998) ranged from 0.89 to 2.64 kPa, and reference crop evapotranspiration (ET_o, Allen et al., 1998) was 171 mm. No rainfall was recorded during the experimental period.

The plant material consisted of 3-year old persimmon trees (*Diospyros kaki* L. f. cv. 'Rojo Brillante' grafted on *Diospyros lotus* L.). Tree spacing followed a 3 m x 5 m pattern. Pest control and fertilization practices were those normally used by the growers, and no weeds were allowed to develop within the orchard.

Two irrigation treatments were considered, in which irrigation was carried out daily and during night time using a drip irrigation system with one lateral pipe per tree row. From day of the year (DOY) 218–251, in order to guarantee non-limiting soil water conditions, control plants (treatment T0) were irrigated using six emitters (each delivering 4 $l h^{-1}$) per plant in order to maintain soil water content in the 0–60 cm soil depth at levels near constant and slightly above soil field capacity. In the T1 treatment water was applied at 70% of control trees.

2.1.1. Measurements

θ_v was measured with a portable FDR sensor (HH2, ΔT , U.K.) previously calibrated. The measurements were made in four plots per treatment. The access tubes for the FDR sensor were placed in the irrigation line at about 30 cm from an emitter. The data were obtained at 0.10, 0.20, 0.30, 0.40 and 0.60 m depth. Ψ_{leaf} was measured on the south facing side and the middle third of the trees, in two fully developed leaves per tree of each replicate, using a pressure chamber (PMS 600-EXP, PMS Instruments Company, Albany, USA), as recommended by Turner (1988). After measuring Ψ_{leaf} , the leaves were frozen in liquid nitrogen and the osmotic potential was measured after thawing the samples and expressing sap, using a vapour pressure osmometer (Wescor 5600, Logan, USA). Leaf turgor potential (Ψ_p) values were derived as the difference between osmotic and water potentials. The Ψ_{stem} was measured in a similar number and type of leaves as used for Ψ_{leaf} , enclosing leaves in a small black plastic bag covered with aluminium foil for at least 2 h before measurements in the pressure chamber. Leaf conductance (g_{leaf}) in attached leaves was measured with a porometer (Delta T AP4, Delta-T Devices, Cambridge, UK) on the abaxial surface of the leaves and in a similar number and type of leaves as used for the Ψ_{leaf} measurements.

At the end of the experimental period, two pressure-volume (PV) curves were performed per replicate in order to determine values of leaf osmotic potential at full turgor (Ψ_{os}), leaf water potential at the turgor loss point (Ψ_{tlp}), leaf bulk modulus of elasticity (ϵ), relative water content at the turgor loss point (RWC_{tlp}) and relative apoplastic water content (RWC_a) (Tyree and Hammel, 1972; Tyree and Richter, 1981, 1982; Savé et al., 1993). For this, leaves were excised at predawn and resaturated by dipping the petioles in distilled water for 24 h in darkness at 4 °C. The resaturated leaves were weighed using an analytical balance (± 0.1 mg precision), placed in the pressure chamber (lined with damp filter paper) and slowly pressurized (0.025 MPa s^{-1}) until the balance pressure was reached (when the leaf sap appeared through the cut petiole protruding from the chamber). Once depressurized, the leaf was allowed to transpire outside the pressure chamber on the laboratory bench at room temperature (22 ± 2 °C). Leaves were repeatedly weighed and their balance pressures were determined over the full range of the pressure gauge (Kikuta and Richter, 1986). Data for initial saturated weight, intermediate fresh weight (corresponding to

values for Ψ_{leaf} , and final dry weight (at 80 °C for 48 h) were used to calculate the relative water content (RWC) (Barrs and Weatherley, 1962).

The curves were drawn using a type II transformation (Tyree and Richter, 1982). The reciprocal of Ψ_{leaf} was plotted against RWC, and the resultant relationships displayed both linear and non-linear regions. Extrapolation on the straight portion of the curve obtained for a value of RWC = 1 gave the reciprocal of the Ψ_{os} and extrapolation to the abscissa gave RWC_a. The Ψ_{tlp} and RWC_{tlp} were estimated as the intersection between the linear and curvilinear portions of the PV curve. The ϵ of leaf tissue at 100% RWC (RWC_o) was estimated according to Patakas and Noitsakis (1999) as ϵ (MPa) = $(\Psi_{\text{os}} - \Psi_{\text{stlp}}) (100 - \text{RWC}_a) / (100 - \text{RWC}_{\text{tlp}})$, where Ψ_{stlp} is the osmotic potential at the turgor loss point and Ψ_{os} values correspond to those obtained from the analysis of the PV curves.

The micrometric trunk diameter fluctuations (TDF) were measured throughout the experimental period on four trees per treatment, using a set of linear variable displacement transducers (LVDT) (model DF \pm 2.5 mm, accuracy \pm 10 μ m, Solartron Metrology, Bognor Regis, UK) attached to the trunk, with a special bracket made of Invar, an alloy of Ni and Fe with a thermal expansion coefficient close to zero (Katerji et al., 1994), and aluminium. Sensors were placed on the north side, 10 cm above the graft point of each tree, and were covered with silver thermoprotected foil to prevent heating and wetting of the device. Measurements were taken every 10 s and the datalogger (model CR10X, Campbell Scientific, Logan, UT, USA) was programmed to report 15 min means. MDS was calculated as the difference between maximum and minimum daily trunk diameter.

SF was measured using the compensation heat-pulse technique (Swanson and Whitfield, 1981) in the same trees used for TDF measurements throughout the experimental period. One set of heat pulse probes was located above the LVDT sensors on each tree. Each set consisted of a heater needle of 1.8 mm diameter and two temperature probes also of 1.8 mm diameter installed in parallel holes drilled radially in the trunks at 10 mm downstream and 5 mm upstream. Each heat-pulse probe had three thermocouple sensors to monitor the sap velocity at a radial depth of 5, 12 and 21 mm below the cambium. Sap velocity was measured following the procedure of Green et al. (2003), using the theoretical calibrations of Swanson and Whitfield (1981) to account for the probe-induced effects of wounding. The volume fractions of wood and water determined by López-Bernal et al. (2014) were used. The temperature signals and the corresponding heat-pulse velocities were recorded at 30 min intervals using heat-pulse instrumentation controlled by a datalogger (CR10X, Campbell Scientific Ltd., Logan, Utah).

2.1.2. Statistical design and analysis

The design of the experiment was completely randomized with four replications, each replication consisting of three adjacent tree rows, each with seven trees. Measurements were taken on the inner tree of the central row of each replicate, which were very similar in appearance (leaf area, trunk cross sectional area, height, ground shaded area, etc.), whereas the other trees served as border trees. Statistical analysis was performed by an analysis of variance using the general linear model (GLM) of SPSS (SPSS, 2002). Values for each replicate were averaged before the mean and the standard error of each treatment were calculated.

3. Results

In the T0 treatment, θ_v between 0 and 0.60 m in depth was nearly constant and slightly above field capacity (102.3% of θ_v values at field capacity) (Fig. 1). In the T1 treatment, θ_v values decreased achieving constant values of around 81% of soil field capacity from DOY 222–251.

Ψ_{pd} values in T0 plants were very high and fairly constant throughout the experimental period, while in T1 plants Ψ_{pd} values were

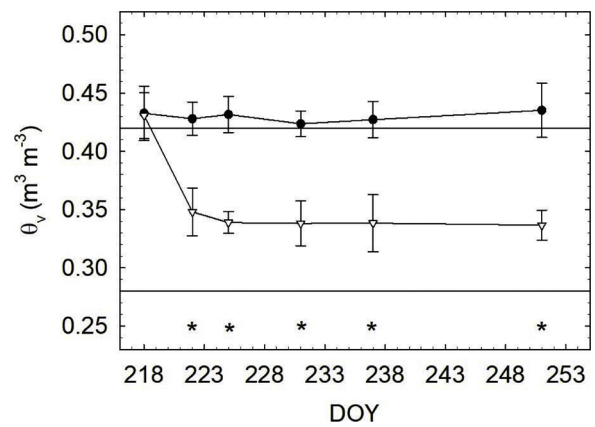


Fig. 1. Soil volumetric water content (θ_v) to a depth of 0.60 m (mean \pm SE) in the T0 (closed circles) and T1 (open triangles) irrigation treatments during the experimental period. The lower horizontal line represents θ_v at permanent wilting point and the upper horizontal line represents θ_v at field capacity. Asterisks indicate significant differences at $P \leq 0.05$ ($n = 4$).

significantly lower than in T0 plants, being characterized by a slight decrease from the beginning of the experiment to DOY 222, when minimum values were reached (Fig. 2A). Ψ_{md} values in T0 plants during the experimental period were fairly constant and higher than those in T1 plants, whereas in T1 plants the Ψ_{md} values gradually decreased, achieving minimum values on DOY 231 and increasing slightly thereafter (Fig. 2B). Ψ_{ppd} and leaf turgor potential at midday (Ψ_{pmd}) values in both irrigation treatments were always above zero, indicating that turgor was maintained during the experimental period. However, both parameters showed differences in the response to irrigation treatments (Figs. 2C and 2D). The Ψ_{ppd} values in both treatments were high and showed some tendency to fluctuate (Fig. 2C). Furthermore, Ψ_{ppd} values in T1 plants were always lower than in T0 plants. Ψ_{pmd} values in T1 plants were also lower than those in T0 plants, even though Ψ_{pmd} values in T1 plants showed a gradual but clear tendency to decrease during the experimental period, reaching minimum values of 0.36 MPa at the end of the experiment on DOY 251 (Fig. 2D).

Ψ_{stem} values in both irrigation treatments were higher than the corresponding Ψ_{md} values throughout the experimental period and behaved somewhat similarly to Ψ_{pd} values (Figs. 3 A, 2 B and 2 A, respectively). So, Ψ_{stem} values of T0 plants were almost constant during the experimental period while in T1 plants they showed a tendency to decrease, almost all the time with lower values than those observed in T0. The g_{lmd} values in T0 plants were nearly constant during the experimental period, whereas g_{lmd} values in T1 plants were clearly lower than in T0 plants, gradually decreasing during the experimental period (Fig. 3B).

During the experimental period, regardless of the treatment, Ψ_{leaf} values exhibited a similar circadian rhythm on the five measuring dates, reaching maximum values at predawn, decreasing rapidly in the morning and reaching minimum values at around 14.00–17.00 h, after which they gradually recovered (Fig. 4). Differences between the circadian Ψ_{leaf} values of the T0 and T1 varied from day-to-day. At the end of the experimental period (DOY 251), the daily Ψ_{leaf} pattern in T1 plants was characterized by a gradual decrease, reaching minimum values at 14.00 h and showing only a very slight recovery during the afternoon.

At sunrise, the increase in radiation induced stomatal opening while g_{leaf} increased to reach maximum values between 10.00 and 14.00 h, after which it progressively decreased (Fig. 4). T0 plants showed higher g_{leaf} values than those in plants under water deficit (T1) during most of the day and but specially when daily maximum g_{leaf} values were achieved. Differences in g_{leaf} values between T0 and T1 plants gradually increased due to the response of T1 plants to the deficit irrigation,

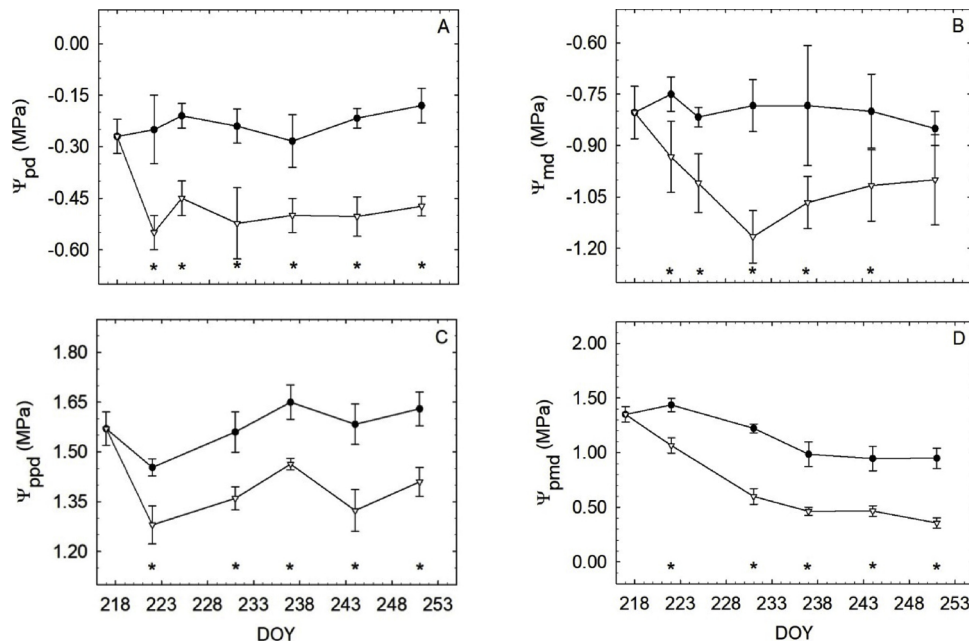


Fig. 2. Predawn leaf water potential (Ψ_{pd} , A), midday leaf water potential (Ψ_{md} , B), predawn leaf turgor potential (Ψ_{ppd} , C) and midday leaf turgor potential (Ψ_{pmd} , D) values for persimmon plants in T0 and T1 treatments during the experimental period. Symbols as in Fig. 1.

which gradually decreased the duration of maximum stomatal opening. Low and near constant g_{leaf} values were registered during most of the day from DOY 231–251 (Fig. 4). Ψ_p values showed a similar circadian rhythm on the five studied dates, characterized by maximum values at predawn and minimum values at 12.00–17.00 h (Fig. 4). Ψ_p values in T1 plants tended to be lower than in T0 plants, especially in the central hours of the day (12.00–17.00 h).

Daily SF values in T0 plants were characterized by a more pronounced fluctuation than was seen in T1 plants, where they decreased gradually, showing differences between treatments from DOY 221 onwards and remaining almost constant from DOY 223 onwards (Fig. 5A). MDS values in T1 plants were higher than in T0 plants. In addition, differences in MDS values between treatments were significant the day immediately after the beginning of the experiment, 2 days earlier than the differences in SF became evident (Fig. 5A and B). In contrast with the behaviour observed in daily SF values, no differences between treatments were observed in MDS values from DOY 226–230, on DOY 235 and on DOY 238. The regression analysis between SF and Ψ_{pd} , Ψ_{md} , Ψ_{stem} and g_{lmd} , obtained by pooling data for the whole observation period, demonstrated that decreases in SF values were associated with decreases in Ψ_{pd} , Ψ_{md} , Ψ_{stem} and g_{lmd} values (Fig. 6).

In order to study the sensitivity of the measured plant-based water status indicators we considered both continuously and discretely recorded plant-based indicators (SF, MDS, Ψ_{pd} , Ψ_{md} , Ψ_{stem} and, g_{lmd})

during increasing intervals of time from the beginning to the end of the experimental period (Table 1). The SI values increased in all plant-based water stress indicators considered in response to water deficit. However, during the experimental period different behaviours were observed. So, at the beginning of the deficit irrigation period the MDS SI (T1/T0) and Ψ_{pd} SI (T1/T0) increased more sharply than SI of the other indicators. After DOY 222, SF SI (T0/T1) values tended to be higher than the SI values of other indicators. Nevertheless, from DOY 218–226 and 218–231, Ψ_{pd} SI (T1/T0) values were similar to those observed in the SF SI. When the mean SI values were considered in relation to their noise for all the plant-based water stress indicators (Table 1), the described behaviours changed. The data indicated that Ψ_{md} mean noise was very low, leading it to show the highest Ψ_{md} signal:noise ratio at the beginning of the experimental period (DOY 218–222 and 218–226). However, from DOY 226 to the end of the experiment, the substantial increase in the SF SI led to a higher signal:noise ratio for all the following intervals of time considered, even though Ψ_{md} signal:noise ratios were close to those of the SF signal:noise ratio.

At the end of the experimental period (DOY 251), no significant differences in Ψ_{os} , Ψ_{tlp} , C, RWC_{tlp} or RWC_a values were found between T0 and T1 plants (Table 2). Nevertheless, it is important to point out that RWC_a values were very high in both treatments.

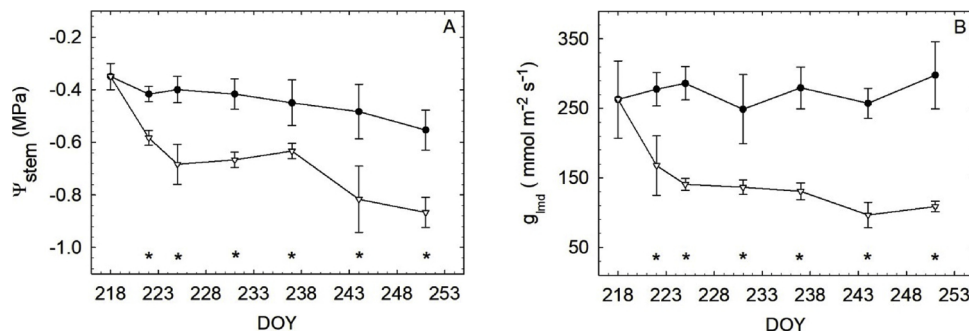


Fig. 3. Midday stem water potential (Ψ_{stem} , A) and midday leaf conductance (g_{lmd} , B) values for persimmon plants in T0 and T1 treatments during the experimental period. Symbols as in Fig. 1.

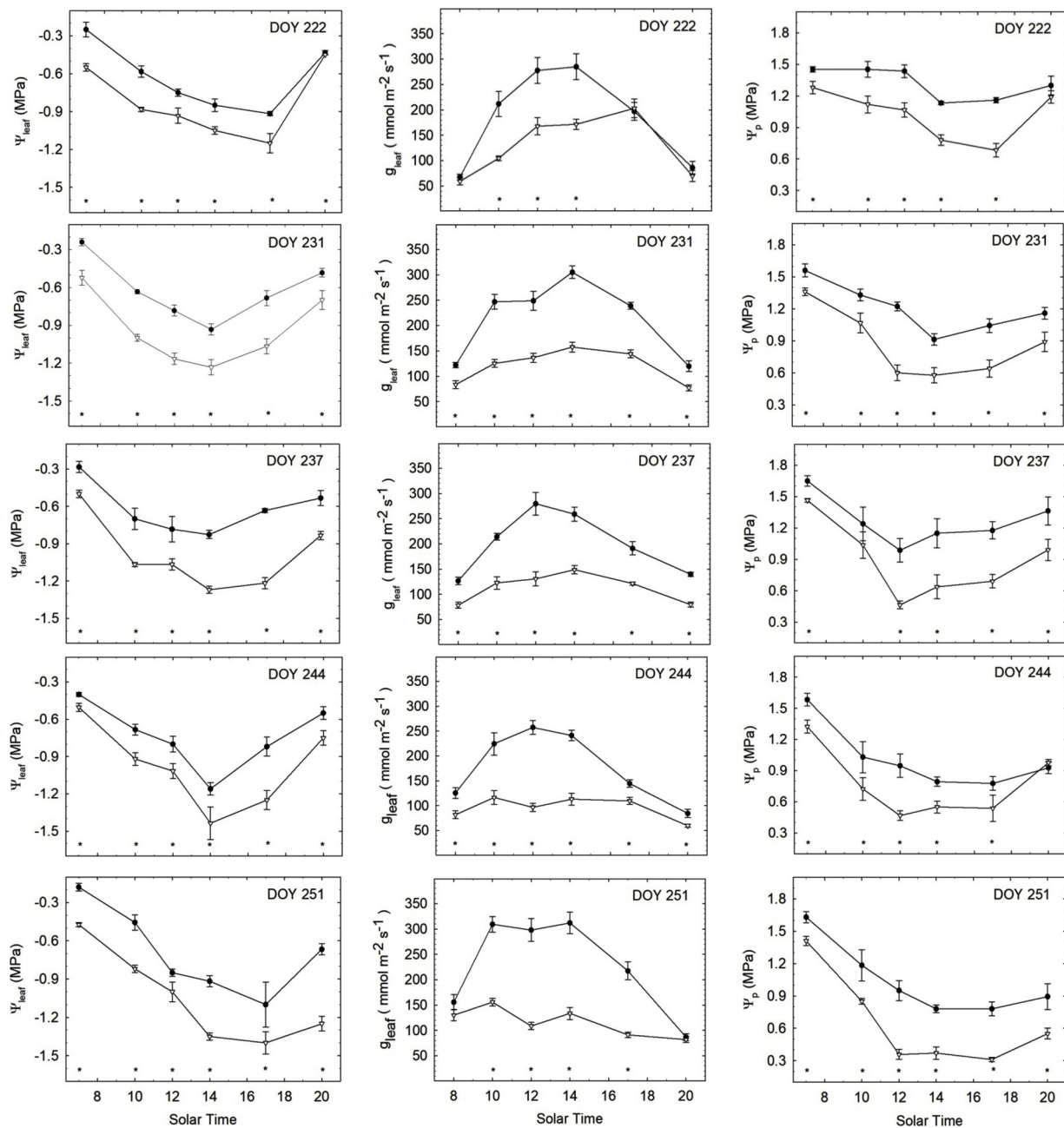


Fig. 4. Diurnal course of leaf water potential (Ψ_{leaf}), leaf conductance (g_{leaf}) and leaf turgor potential (Ψ_p) values for persimmon plants in T0 and T1 treatments at five different times during the stress period (DOY 222, 231, 237, 244 and 251). Symbols as in Fig. 1.

4. Discussion

Throughout the experimental period and based on the fact that (i) θ_v values in T0 treatment were slightly above field capacity (Fig. 1), (ii) Ψ_{pd} , Ψ_{md} and Ψ_{stem} values (Figs. 2A, 2B and 3A) were nearly constant and very high in relation to the values already reported for other authors for full irrigated persimmon plants (Badal et al., 2010; Buesa et al., 2013), and (iii) Ψ_{pd} values depend mainly on soil moisture levels (Elfving et al., 1972; Torrecillas et al., 1988; Sellin, 1996), we conclude that T0 plants were under non-limiting soil water conditions. Moreover, considering that the tree water relations under flooding conditions are characterized by a substantial decrease in leaf conductance and leaf water potential as a consequence of the effects of chemical signals from roots and an increase in the resistance to water flowing through the plant (Ruiz-Sánchez et al., 1997; Dell'Amico et al., 2001), the water relations of T0 plants indicated the absence of any waterlogging

because leaf turgor was maintained (Ψ_{ppd} and $\Psi_{\text{pmd}} > 0$), and high and near constant values of Ψ_{pd} , Ψ_{md} , Ψ_{stem} , SF and g_{lmd} were observed (Figs. 2A, B, 3A, B and 5A).

Regarding the T1 treatment, the fact that minimum θ_v values were around 81% of field capacity and minimum Ψ_{pd} , Ψ_{md} , Ψ_{stem} and g_{lmd} values were around -0.50 MPa, -1.17 MPa, -0.87 MPa and 99.66 $\text{mmol m}^{-2} \text{s}^{-1}$, respectively, indicated that T1 plants were under a mild degree of water deficit during the experimental period (Figs. 2A, 2B, 3A and 3B) (Cruz et al., 2012; Rodríguez et al., 2012; Torrecillas et al., 2018). In addition, the rate of development of water stress in T1 plants was very low because the Ψ_{pd} , Ψ_{stem} and Ψ_{md} values decreased by only around 0.01, 0.02 and 0.01 MPa per day basis (2A, 3A and 2B, respectively) (Hale and Orcutt, 1987).

The progressive decrease in g_{lmd} in T1 plants, and the tendency to shorten the duration of maximum stomatal opening in its circadian rhythm as stress progressed (Figs. 3B and 4) indicated that stomata

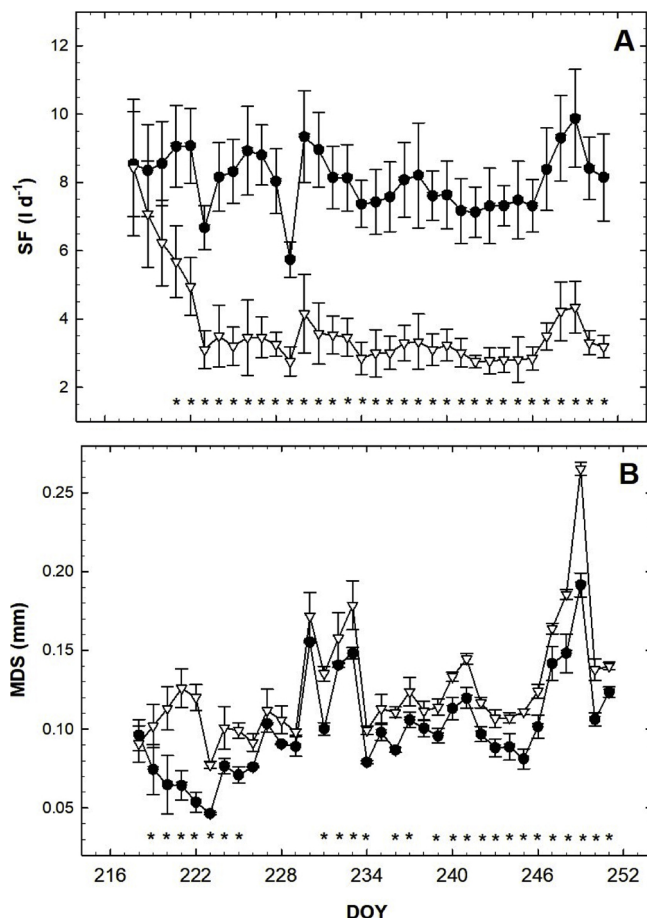


Fig. 5. Daily sap flow (SF) (A) and maximum daily trunk diameter shrinkage (MDS) (B) in T0 and T1 plants during the experimental period. Symbols as in Fig. 1.

regulation is a key mechanism in controlling leaf water status because leaf turgor was maintained in T1 plants (Fig. 2C and D) and persimmon plants did not develop any other stress tolerance mechanism such as elastic adjustment (ϵ decrease) or active osmotic adjustment (Ψ_{os} decrease) in our experimental conditions (Table 2). The decrease in g_{lmd} values of woody crop leaves in response to water deficit has been reported as a stress avoidance mechanism in response to water deficit, which improves water use efficiency (Rieger and Duemmel, 1992; Girona et al., 1993).

The behaviour of ϵ , Ψ_{os} , Ψ_{tlp} and RWC_{tlp} values, which did not change as a result of water deficit in T1 plants (Table 2), was similar to the results obtained by other authors (Sánchez-Blanco et al., 1991; Savé et al., 1995; Torrecillas et al., 1996) suggesting that the ϵ and Ψ_{os} affect the RWC_{tlp} and Ψ_{tlp} values, respectively. RWC_a values in persimmon plants (around 58%) (Table 2) were similar to those found for grapes

Table 1

Mean signal intensity (actual value/reference value or reference value/actual value), mean noise (coefficient of variation), and signal:noise ratio of maximum daily trunk shrinkage (MDS), sap flow (SF), predawn (Ψ_{pd}), midday stem (Ψ_{stem}) and midday (Ψ_{md}) water potentials and midday leaf conductance (g_{lmd}) at different intervals of the experimental period. For each interval, mean signal or mean noise values that do not have a common letter are significantly different according to Duncan's multiple range test ($P \leq 0.05$).

DOY		Mean signal	Mean noise	Signal:noise
218-222	MDS	1.65a	0.31bc	5.33
	SF	1.40bc	0.24cd	5.95
	Ψ_{pd}	1.60ab	0.53a	3.01
	Ψ_{stem}	1.20cd	0.24cd	5.03
	Ψ_{md}	1.12d	0.15d	7.37
	g_{lmd}	1.33cd	0.35b	3.76
218-226	MDS	1.54c	0.26b	5.82
	SF	1.85a	0.32ab	5.71
	Ψ_{pd}	1.78ab	0.40a	4.50
	Ψ_{stem}	1.37cd	0.26b	5.30
	Ψ_{md}	1.16d	0.12c	9.88
	g_{lmd}	1.56bc	0.34ab	4.57
218-231	MDS	1.40bc	0.27abc	5.24
	SF	2.04a	0.27abc	7.56
	Ψ_{pd}	1.88a	0.33a	5.62
	Ψ_{stem}	1.43bc	0.22bc	6.46
	Ψ_{md}	1.24c	0.18c	7.00
	g_{lmd}	1.63b	0.29ab	5.62
218-237	MDS	1.34c	0.24abc	5.49
	SF	2.16a	0.23abc	9.40
	Ψ_{pd}	1.86b	0.30a	6.28
	Ψ_{stem}	1.42c	0.19abc	7.40
	Ψ_{md}	1.27c	0.16c	8.05
	g_{lmd}	1.73b	0.28ab	6.26
218-244	MDS	1.30c	0.22ab	5.85
	SF	2.25a	0.20ab	11.13
	Ψ_{pd}	1.94b	0.28a	6.92
	Ψ_{stem}	1.47c	0.20ab	7.24
	Ψ_{md}	1.27c	0.15b	8.65
	g_{lmd}	1.89b	0.30a	6.18
218-251	MDS	1.29c	0.20bc	6.37
	SF	2.29a	0.18bc	12.55
	Ψ_{pd}	2.03b	0.28ab	7.36
	Ψ_{stem}	1.48c	0.19bc	7.96
	Ψ_{md}	1.25c	0.14c	8.84
	g_{lmd}	2.01b	0.32a	6.35

(51–63%) (Rodrigues et al., 1993), to the lower limit of the range found for *Pinus ponderosa* (57–81 %) (Hardegree, 1989), and to the higher limit found for pomegranate (42–58%) (Rodríguez et al., 2012) and almond (42–59%) (Torrecillas et al., 1996). On the other hand, persimmon RWC_a values were high compared with other tree species such as apricot (27–42%) (Torrecillas et al., 1999), peach (29–44%) (Mellisho et al., 2011), *Eucalyptus globulus* (14–27%) (Correia et al., 1989) and *Quercus alba* (26–31%) (Parker and Pallardi, 1987). High RWC_a values represent a xeromorphic characteristic (Cutler et al., 1977), and are a consequence of thicker cell walls or differences in cell wall structure (Hellkvist et al., 1974), which could contribute to the

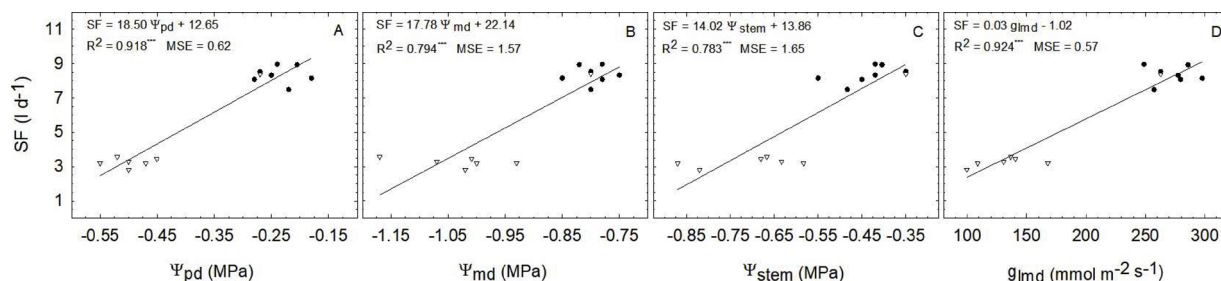


Fig. 6. Relationships between sap flow (SF) and predawn leaf water potential (Ψ_{pd} , A), midday leaf water potential (Ψ_{md} , B), midday stem water potential (Ψ_{stem} , C) and midday leaf conductance (g_{lmd} , D) values for persimmon plants in T0 and T1 treatments during the measurement period. Symbols as in Fig. 1.

Table 2

Effect of water stress on leaf osmotic potential at full turgor (Ψ_{os}), leaf osmotic potential at turgor loss point (Ψ_{tlp}), leaf bulk modulus of elasticity (C), relative water content at turgor loss point (RWC_{tlp}) and relative apoplastic water content (RWC_a) of persimmon plants in T0 and T1 treatments at the end of the experimental period. Means with the same letter across each row do not differ significantly at $P \leq 0.05$ ($n = 4$).

Parameters	T0	T1
Ψ_{os} (MPa)	-1.41a	-1.50a
Ψ_{tlp} (MPa)	-2.76a	-3.32a
C (MPa)	2.50a	3.38a
RWC_{tlp} (%)	77.50a	76.95a
RWC_a (%)	58.09a	56.94a

retention of water when water potential decreases (Torrecillas et al., 1996).

SF, Ψ_{pd} , Ψ_{md} , Ψ_{stem} and g_{lmd} encompass different time scales, because the last four are point measurements, taken at predawn or at midday, and are considered as indicators of the minimum (Ψ_{pd}) and maximum (Ψ_{md} , Ψ_{stem} and g_{lmd}) daily plant water deficit, whereas SF is an integrative indicator, which reflects the continuous sap flow records on a diurnal basis. Despite these facts, the relationships between SF and Ψ_{pd} , Ψ_{md} , Ψ_{stem} and g_{lmd} (Fig. 6) were high and constant, indicating that SF can be used to indicate the water status of young persimmon trees.

Assuming that any comparison of the sensitivities of different plant-based water status indicators for diagnosing water deficit must consider the strength of each indicator in the context of its variability (Goldhamer and Fereres, 2001; Naor and Cohen, 2003), it can be observed that Ψ_{md} was the most suitable indicator for persimmon irrigation scheduling when short periods of time are considered, because it showed the highest signal:noise ratio during the first 4 or 8 days of the experimental period (DOY 218–222 and 218–226) (Table 1). However, as the interval of time considered grew (DOY 218–231, 218–237, 218–244 and 218–251) SF SI sharply increased and SF noise was maintained, leading it to show the highest signal:noise ratio for these intervals of time (Table 1). Moreover, taking into consideration that during the two first periods of time considered (DOY 218–222 and 218–226) the SF signal:noise ratio, despite being lower than that showed by Ψ_{md} was relatively high, it could be concluded that SF is a more suitable indicator than Ψ_{md} for irrigation scheduling because it can provide continuous and automated registers of the plant water status in real time, avoiding frequent trips to the field and a significant input of manpower since frequent Ψ_{md} readings are needed.

In this respect, Ortuño et al. (2004) indicated that in young trees continuously measured plant water status indicators were more immediate and sensitive than discretely measured indicators for detecting water stress. Also, other authors indicated that MDS and SF revealed significant differences between irrigation treatments even in the absence of differences in Ψ_{stem} (Goldhamer et al., 1999; Remorini and Massai, 2003). By contrast, in persimmon plants, Badal et al. (2010) assessed the feasibility of using MDS, Ψ_{stem} , g_{lmd} and fruit diameter variations and concluded that although MDS can be successfully used as continuous plant water stress indicator, Ψ_{stem} was the most sensitive plant water stress indicator.

The above results indicated that persimmon plants exposed to mild water stress are able to gradually develop stomata control (a stress avoidance mechanism). Also, under water stress the high relative apoplastic water content could contribute to the retention of water. So, both drought resistance characteristics could have contributed to the leaf turgor maintenance observed during the experimental period. In addition, the discrete and continuously recorded plant-based indicators showed different degrees of sensitivity for diagnosing persimmon tree water status. Overall, SF measurements made by the heat-pulse technique are the most suitable method for estimating persimmon water

status, because it showed the highest signal:noise ratio in almost all intervals of time considered, while providing continuous and automated registers of the persimmon water status in real time.

Acknowledgments

We are grateful to the Arnau family from *Explotaciones Ecológicas Harisa S.L.* and Mr. J. Melgares from *Oficina Comarcal Agraria Huerta de Murcia* (Autonomous Community of the Region of Murcia) for all the help we have been given. AG and AJM acknowledge the postdoctoral financial support received from Ramón Areces Foundation and Juan de la Cierva program, respectively. IG is a predoctoral student at the Miguel Hernández University. Also, this work is a result of the PR internship (19925/IV/15) funded by the Seneca Foundation - Agency for Science and Technology in the Region of Murcia under the Jiménez de la Espada Program for Mobility, Cooperation and Internationalization.

References

- Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop Evapotranspiration-Guidelines for Computing Crop Water Requirements. Irrigation and Drainage, vol.56. FAO, Roma, pp. 56.
- Badal, E., Buesa, I., Guerra, D., Bonet, L., Ferrer, P., Intrigliolo, D.S., 2010. Maximum diurnal trunk shrinkage is a sensitive indicator of plant water, stress in *Diospyros kaki* (Persimmon) trees. Agric. Water Manage. 98, 143–147.
- Barrs, H.D., Weatherley, P.E., 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust. J. Biol. Sci. 15, 413–428.
- Buesa, I., Badal, E., Guerra, D., Ballester, C., Bonet, L., Intrigliolo, D.S., 2013. Regulated deficit irrigation in persimmon trees (*Diospyros kaki*) cv. 'Rojo Brillante'. Sci. Hortic. 159, 134–142.
- Chappell, M.J., LaValle, L.A., 2011. Food security and biodiversity: can we have both? An agroecological analysis. Agric. Hum. Values 28, 3–26.
- Conejero, W., Alarcón, J.J., García-Orellana, Y., Nicolás, E., Torrecillas, A., 2007. Evaluation of sap flow and trunk diameter sensors used for irrigation scheduling in early maturing peach trees. Tree Physiol. 27, 1753–1759.
- Conejero, W., Mellisho, C.D., Ortuño, M.F., Moriana, A., Moreno, F., Torrecillas, A., 2011. Using trunk diameter sensors for regulated deficit irrigation scheduling in early maturing peach trees. Environ. Exp. Bot. 71, 409–415.
- Correia, M.J., Torres, F., Pereira, J.S., 1989. Water and nutrient supply regimes and the water relations of juvenile leaves of *Eucalyptus globulus*. Tree Physiol. 5, 459–471.
- Cruz, Z.N., Rodríguez, P., Galindo, A., Torrecillas, E., Ondoño, S., Mellisho, C.D., Torrecillas, A., 2012. Leaf mechanisms for drought resistance in *Zizyphus jujuba* trees. Plant Sci. 197, 77–83.
- Cutler, J.M., Rains, D.W., Loomis, R.S., 1977. The importance of cell size in the water relations of plants. Physiol. Plant. 40, 255–260.
- Dell'Amico, J., Torrecillas, A., Rodríguez, P., Morales, D., Sánchez-Blanco, M.J., 2001. Differences in the effects of flooding the soil early and late in the photoperiod on water relations of pot-grown tomato plants. Plant Sci. 160, 481–487.
- Elfving, D.C., Hall, A.E., Kaufmann, M.R., 1972. Interpreting leaf water potential measurements with a model of soil-plant-atmosphere continuum. Physiol. Plant. 27, 161–168.
- Fernandez, J.E., Cuevas, M.V., 2010. Irrigation scheduling from stem diameter variations: a review. Agric. For. Meteorol. 150, 135–151.
- García-Orellana, Y., Ruiz-Sánchez, M.C., Alarcón, J.J., Conejero, W., Ortuño, M.F., Nicolás, E., Torrecillas, A., 2007. Preliminary assessment of the feasibility of using maximum daily trunk shrinkage for irrigation scheduling in lemon trees. Agric. Water Manage. 89, 167–171.
- George, A.P., Mowat, A.D., Collins, R.J., Morley-Bunker, M., 1997. The pattern and control of reproductive development in non-astringent persimmon (*Diospyros kaki* L.): a review. Sci. Hortic. 70, 93–122.
- Girona, J., Mata, M., Goldhamer, D.A., Johnson, R.S., DeJong, T.M., 1993. Patterns of soil and tree water status and leaf functioning during regulated deficit irrigation scheduling in peach. J. Am. Soc. Hortic. Sci. 118, 580–586.
- Goldhamer, D.A., Fereres, E., 2001. Irrigation scheduling protocols using continuously recorded trunk diameter measurements. Irrig. Sci. 20, 115–125.
- Goldhamer, D.A., Fereres, E., Mata, M., Girona, J., Cohen, M., 1999. Sensitivity of continuous and discrete plant and soil water status monitoring in peach trees subjected to deficit irrigation. J. Am. Soc. Hortic. Sci. 124, 437–444.
- Green, S., Clothier, B., Jardine, B., 2003. Theory and practical application of heat pulse to measure sap flow. Agron. J. 95 (6), 1371–1379.
- Hale, M.G., Orcutt, D.M., 1987. The Physiology of Plants Under Stress. 206 Pp. John Wiley & Sons, New York.
- Hardegee, S.P., 1989. Discrepancies between water potential isotherm measurements on *Pinus ponderosa* seedling shoots: xylem hysteresis and apoplasmic potentials. Plant Cell Environ. 12, 57–62.
- Hellkvist, J., Richards, G.P., Jarvis, P.G., 1974. Vertical gradients of water potential and tissue water relations in Sitka spruce trees measured with the pressure chamber. J. Appl. Ecol. 11, 637–667.
- Johnson, R.S., Handley, D.F., 2000. Using water stress to control vegetative growth and productivity of temperate fruit trees. HortScience 35, 1048–1050.

- Katerji, N., Tardieu, F., Bethenod, O., Quetin, P., 1994. Behavior of maize stem diameter during drying cycles: comparison of two methods for detecting water stress. *Crop Sci.* 34, 65–169.
- Kikuta, S.B., Richter, H., 1986. Graphical evaluation and partitioning of turgor responses to drought in leaves of durum wheat. *Planta* 168, 36–42.
- Lampinen, B.D., Shackel, K.A., Southwick, S.M., Olson, B., 2001. Deficit irrigation strategies using midday stem water potential in prune. *Irrig. Sci.* 20, 47–54.
- Llácer, G., Badenes, M.L., 2002. Situación actual de la producción de caquis en el mundo. *Agrícola Vergel* 242, 64–70.
- López-Bernal, A., Alcántara, E., Villalobos, F.J., 2014. Thermal properties of sapwood of fruit trees as affected by anatomy and water potential: errors in sap flux density measurements based on heat pulse methods. *Trees* 28, 1623–1634.
- McCutchan, H., Shackel, K.A., 1992. Stem water potential as a sensitive indicator of water stress in prune trees (*Prunus domestica* L. Cv. French). *J. Am. Soc. Hortic. Sci.* 117, 607–611.
- Mellisho, C.D., Cruz, Z.N., Conejero, W., Ortuño, M.F., Rodríguez, P., 2011. Mechanisms for drought resistance in early maturing cvar Flordastar peach trees. *J. Agric. Sci.* 149, 609–616.
- Moriana, A., Corell, M., Girón, I.F., Conejero, W., Morales, D., Torrecillas, A., Moreno, F., 2013. Regulated deficit irrigation based on threshold values of trunk diameter fluctuation indicators in table olive trees. *Sci. Hortic.* 164, 102–111.
- Mowat, A.D., George, A.P., 1994. Environmental physiology of persimmons. In: Schaffer, B., Anderson, P. (Eds.), *Handbook of Environmental Physiology of Fruit Crops Vol. 1*. CRC Press, Inc., Boca Raton, Florida, USA, pp. 209–232.
- Naor, A., 2000. Midday stem water potential as a plant water stress indicator for irrigation scheduling in fruit trees. *Acta Hortic.* 537, 447–454.
- Naor, A., Cohen, S., 2003. Sensitivity and variability of maximum trunk shrinkage, midday stem water potential, and transpiration rate in response to withholding irrigation from field grown apple trees. *HortScience* 38, 547–551.
- Ortuño, M.F., Alarcón, J.J., Nicolás, E., Torrecillas, A., 2004. Comparison of continuously recorded plant-based water stress indicators for young lemon trees. *Plant Soil* 267, 263–270.
- Ortuño, M.F., Brito, J.J., García-Orellana, Y., Conejero, W., Torrecillas, A., 2009a. Maximum daily trunk shrinkage and stem water potential reference equations for irrigation scheduling of lemon trees. *Irrig. Sci.* 27, 121–127.
- Ortuño, M.F., García-Orellana, Y., Conejero, W., Pérez-Sarmiento, F., Torrecillas, A., 2009b. Assessment of maximum daily trunk shrinkage signal intensity threshold values for deficit irrigation in lemon trees. *Agric. Water Manage.* 96, 80–86.
- Ortuño, M.F., Conejero, W., Moreno, F., Moriana, A., Intrigliolo, D.S., Biel, C., Mellisho, C.D., Pérez-Pastor, A., Domingo, R., Ruiz-Sánchez, M.C., Casadesu, J., Bonany, J., Torrecillas, A., 2010. Could trunk diameter sensors be used in woody crops for irrigation scheduling? A review of current knowledge and future perspectives. *Agric. Water Manage.* 97, 1–11.
- Parker, W.C., Pallardi, S.G., 1987. The influence of resaturation method and tissue type on pressure-volume analysis of *Quercus alba* L. Seedlings. *J. Exp. Bot.* 38, 535–549.
- Patakas, A., Noitsakis, B., 1999. Mechanisms involved in diurnal changes of osmotic potential in grapevines under drought conditions. *J. Plant Physiol.* 154, 767–774.
- Remorini, D., Massai, R., 2003. Comparison of water status indicators for young peach trees. *Irrig. Sci.* 22, 39–46.
- Rieger, M., Duemmel, M.J., 1992. Comparison of drought resistance among *Prunus* species from divergent habitats. *Tree Physiol.* 11, 369–380.
- Rodrigues, M.L., Chaves, M.M., Wendler, R., Davis, M.M., Quick, W.P., Leegood, R.C., Stitt, M., Pereira, J.S., 1993. Osmotic adjustment in water stressed grapevine leaves in relation to carbon assimilation. *Aust. J. Plant Physiol.* 20, 309–321.
- Rodríguez, P., Mellisho, C.D., Conejero, W., Ortuño, M.F., Cruz, Z.N., Galindo, A., Torrecillas, A., 2012. Plant water relations of leaves of pomegranate trees under different irrigation conditions. *Environ. Exp. Bot.* 77, 19–24.
- Ruiz-Sánchez, M.C., Domingo, R., Savé, R., Biel, C., Torrecillas, A., 1997. Effects of water stress and rewetting on leaf water relations of lemon plants. *Biol. Plant.* 39, 623–631.
- Sánchez-Blanco, M.J., Bolarín, M.C., Alarcón, J.J., Torrecillas, A., 1991. Salinity effects on water relations in *Lycopersicon esculentum* and its wild salt-tolerant relative species *L. Pennellii*. *Physiol. Plant.* 83, 269–274.
- Savé, R., Peñuelas, J., Marfá, O., Serrano, L., 1993. Changes in leaf osmotic and elastic properties and canopy architecture of strawberries under mild water stress. *HortScience* 28, 925–927.
- Savé, R., Biel, C., Domingo, R., Ruiz-Sánchez, M.C., Torrecillas, A., 1995. Some physiological and morphological characteristics of citrus plants for drought resistance. *Plant Sci.* 110, 167–172.
- Sellin, A., 1996. Base water potential of *Picea abies* as a characteristic of the soil water status. *Plant Soil* 184, 273–280.
- SPSS Inc, 2002. SPSS Professional Statistics v. 12 Business Intelligence Division, Chicago.
- Suzuki, A., Iwanaga, H., Murakami, Y., Maotani, T., 1988. Relationship between changes in ethylene evolution and physiological drop of persimmon fruit. *J. Japan. Soc. Hort. Sci.* 57, 167–172.
- Swanson, R.H., Whitfield, D.W.A., 1981. A numerical analysis of heat pulse velocity: theory and practice. *J. Exp. Bot.* 32, 221–239.
- Thrupp, L.A., 2000. Linking agricultural biodiversity and food security: the valuable role of agrobiodiversity for sustainable agriculture. *Int. Aff.* 76, 265–281.
- Toledo, Á., Burlingame, B., 2006. Biodiversity and nutrition: a common path toward global food security and sustainable development. *J. Food Anal.* 19, 477–483.
- Torrecillas, A., Ruiz-Sánchez, M.C., Del Amor, F., León, A., 1988. Seasonal variations on water relations of *Amygdalus communis* L. Under drip irrigated and non irrigated conditions. *Plant Soil* 106, 215–220.
- Torrecillas, A., Alarcón, J.J., Domingo, R., Planes, J., Sánchez-Blanco, M.J., 1996. Strategies for drought resistance in leaves of two almond cultivars. *Plant Sci.* 118, 135–143.
- Torrecillas, A., Galego, R., Pérez-Pastor, A., Ruiz-Sánchez, M.C., 1999. Gas exchange and water relations of young apricots plants under drought conditions. *J. Agric. Sci.* 132, 445–452.
- Torrecillas, A., Corell, M., Galindo, A., Pérez-López, D., Memmi, H., Rodríguez, P., Cruz, Z.N., Centeno, A., Intrigliolo, D.S., Moriana, A., 2018. Agronomical effects of deficit irrigation in apricot, peach and plum trees. In: García-Tejero, I.F., Durán-Zuazo, V.H. (Eds.), *Water Scarcity and Sustainable Agriculture in Semiarid Environment: Tools, Strategies and Challenges for Woody Crops*. Elsevier-Academic Press, pp. 87–109.
- Turner, N.C., 1988. Measurement of plant water status by the pressure chamber technique. *Irrig. Sci.* 9, 289–308.
- Tyree, M.T., Hammel, H.T., 1972. The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *J. Exp. Bot.* 23, 267–282.
- Tyree, M.T., Richter, H., 1981. Alternative methods of analysing water potential isotherms: some cautions and clarifications. I. The impact of non-ideality and of some experimental errors. *J. Exp. Bot.* 32, 643–653.
- Tyree, M.T., Richter, H., 1982. Alternative methods of analysing water potential isotherms: some cautions and clarifications. II. Curvilinearity in water potential isotherms. *Can. J. Bot.* 60, 911–916.
- Velez, J.E., Intrigliolo, D.S., Castel, J.R., 2007. Scheduling deficit irrigation of citrus trees with maximum daily trunk shrinkage. *Agric. Water Manage.* 90, 197–204.
- Yakushiji, H., Sugiura, H., Azuma, A., Yamasaki, A., 2013. Responses of water status and fruit quality of Japanese persimmon (*Diospyros kaki*) to drought stress. *Acta Hort.* 996, 265–270.
- Yamamura, H., Matsui, K., Matsumoto, T., 1989. Effects of gibberellins on fruit set and flower-bud formation in unpollinated persimmons (*Diospyros kaki*). *Sci. Hortic.* 38, 77–86.