Impacts of water stress, environment and rootstock on the diurnal behaviour of stem water potential and leaf conductance in pistachio (Pistacia vera L.)

Houssem Memmi 1,2, Jose F. Couceiro 1, Carmen Gijón 1 and David Pérez-López 2,3

1 Centro Agrario “El Chaparrillo”, Junta de Comunidades de Castilla-La Mancha. 13071 Ciudad Real, Spain. 2 Universidad Politécnica de Madrid, Ciudad Universitaria s/n. 28040 Madrid, Spain. 3 CEIGRAM, Centro de Estudios e Investigación para la Gestión de Riesgos Agrarios & Medioambientales. 28040 Madrid, Spain

Abstract

Little information is available on the diurnal behaviour of water potential and leaf conductance on pistachio trees despite their relevance to fine tune irrigation strategies. Mature pistachio trees were subject to simultaneous measurements of stem water potential (Ψ x) and leaf conductance (g l) during the day, at three important periods of the irrigation season. Trees were grown on three different rootstocks and water regimes. An initial baseline relating Ψ x to air vapor pressure deficit (VPD) is presented for irrigation scheduling in pistachio. Ψ x was closely correlated with VPD but with a different fit according to the degree of water stress. No evidence of the variation of Ψ x in relation to the phenology of the tree was observed. Furthermore, midday Ψ x showed more accuracy to indicate a situation of water stress than predawn water potential. Under well irrigated conditions, g l was positively correlated with VPD during stage II of growth reaching its peak when VPD reached its maximum value (around 4 kPa). This behaviour changed during stage III of fruit growth suggesting a reliance of stomatal behaviour to the phenological stage independently to the tree water status. The levels of water stress reached were translated in a slow recovery of tree water status and leaf conductance (more than 40 days). Regarding rootstocks, P. integerrima showed little adaptation to water shortage compared to the two other rootstocks under the studied conditions.

Additional key words: diurnal pattern; Pistacia atlantica; Pistacia integerrima; Pistacia terebinthus; water stress recovery.

Abbreviations used: ETo (potential crop evapotranspiration, in mm); ETa (actual crop evapotranspiration, in mm); ETc (potential crop evapotranspiration, in mm); ETo (reference evapotranspiration, in mm); Kc (crop coefficient); Kr (reduction coefficient); PA (Pistacia atlantica Desf.); PI (Pistacia integerrima L.); PT (Pistacia terebinthus L.); RDI (regulated deficit irrigation); VPD (air vapor pressure deficit, in kPa); Ψ x (stem water potential, in MPa); g l (leaf conductance, in mmol/m 2·s).

Authors’ contributions: Conceived and designed the experiments, performed the experiments and analysed the data: HM, JFC, CG and DPL. Contributed materials-analysis tools and wrote the paper: HM and DPL. Critical revision of the manuscript: JFC and CG.

Citation: Memmi, H.; Couceiro, J. F.; Gijón, C.; Pérez-López, D. (2016). Impacts of water stress, environment and rootstock on the diurnal behaviour of stem water potential and leaf conductance in pistachio (Pistacia vera L.). Spanish Journal of Agricultural Research, Volume 14, Issue 2, 0804. http://dx.doi.org/10.5424/sjar/2016142-8207.

Received: 24 Jun 2015. Accepted: 17 May 2016

Copyright © 2016 INIA. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial (by-nc) Spain 3.0 Licence, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Funding: Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Ministerio de Economía y Competitividad, Spain (Project AGL2013-45922-C2-1-R); grant to Houssem Memmi.

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Houssem Memmi: houssem.memmi@gmail.com.

Introduction

Cultivated for a long time in Mediterranean semi-arid areas, pistachio (Pistacia vera L.) has been considered as a drought-tolerant species (Spiegel-Roy et al., 1977; Behboudian et al., 1986; Rieger, 1995), mainly for its ability to survive under extreme conditions of water stress (Kanber et al., 1990; Goldhammer, 1995). Nonetheless, the expansion of pistachio cultivation in countries like the USA has been linked to a change in the type of orchard management, with the aim of increasing yield mainly by transforming pistachio from a traditionally rainfed cultivated crop to an irrigated crop. However, the negative balance between water needs and its availability has provoked the claim of deficit irrigation strategies to maximize the rate of returns in front of water availability. Consequently, precision in irrigation has taken another level and the recourse to the study of the different mechanisms connecting the tree water relations to water deficit strate-
gies and the search of effectiveness in detecting stress is henceforth indispensable for a precise application of irrigation.

The water status of a tree or a tree organ embodies a dynamic property controlled by the relative rate of water loss and water uptake. Thus, it would be expected to change with variation in soil moisture, environment and endogenous tree factors, making it a variable component along the day and season (Sánchez-Blanco, 1983). Plant water potential is certainly the most commonly used parameter as indicator of water status and irrigation timing in fruit trees (Klepper, 1975), mainly for its physiological relevance in the control of the water pathway in the tree (Ritchie & Hinkley, 1975). Stem water potential (water potential of a covered leaf) has been reported to be the most representative index of tree water status compared to the water potential of an exposed leaf. Nevertheless, the time of taking this measure has made the subject of several studies and disagreement between researchers, where the effectiveness of detecting water stress was compared between measures at predawn and midday in many species.

In any case, theoretical predictions are that water potential will show a marked diurnal pattern where the degree of depression is closely related to variation in water availability and evaporative demand of the air along the day. These factors of variation limit the use and interpretation of stem water potential (Ψ,) to the role of checking (“How well are we doing?”?) and make it an unsatisfactory tool for an independent use in irrigation scheduling. To overcome this concern, the development of references by the correction of this parameter for diurnal and environmental variations could develop predictions of the behaviour of plants under specific water conditions and climate. Such references allow the establishment of specific protocols for irrigation by the only means of water potential. To reach this objective, sampling at different water availabilities under the same environment during different times of the day and season make possible to segregate the effect of each factor from the other and to determine the connection between fact and cause.

Goldhamer et al. (2005) suggested an initial approach based on shaded leaf water potential measurements for a fully irrigated mature pistachio orchard, recommending a range from −0.7 MPa to −1.2 MPa in which the shaded leaf water potential decreases gradually along the season.

On the other hand, stem water potential has proven to be a useful index of stress in a number of fruit tree species and fluctuations of this parameter under full irrigated conditions was found to be closely linked to vapour pressure deficit (VPD) variations (McCutchan & Shackel, 1992; Shackel, 2011; Williams & Baeza, 2007). The determination of a preliminary relation between Ψ, and VPD for well irrigated and stressed pistachio trees represents one of the objectives of this work.

As for water potential, stomatal conductance responds to a multitude of factors. However, while diurnal water potential profile can be mostly generalized for the majority of fruit species, stomatal behaviour differs. In the absence of water stress, in every diurnal cycle, stomatal conductance profile can oscillate, according to the species, between two behaviours; some species “prioritizes” saving water loss through stomatal closure, however others are characterized by countering the negative effect of excess heat by the opposite mechanism (stomatal opening) (Fereres et al., 2012). Pistachio is a deciduous species; the majority of deciduous trees are characterized by a morning peak of stomatal opening with a substantial midday depression to maximize water use efficiency. By this way, the rate of carbon uptake is maximized per unit of water loss. However, few other deciduous species maintain a continuous stomatal opening to soften the heat effect at the expense of water loss. Pistachio has been reported to follow the first group of species (Goldhamer, 2012), however, such comportment does not explain at all, the dual behaviour of this crop as drought-tolerant as well as a species with an elevated water consumption and keep its behaviour somewhat ambiguous. One reason for this discrepancy can be the change of stomatal behaviour according to the requirement of each pheno- logical stage. Such theory requires the study of stomatal behaviour at different key stages of pistachio growth.

Turner (1991) stated the fundamental role of roots in directing the stomatal pattern. Such founding would be traduced in a consistent difference of stomatal behaviour when different rootstocks are used. *Pistacia integerrima* L. (PL), *Pistacia atlantica* Desf. (PA), *Pistacia terebinthus* L. (PT) and UCB-I (a cross between PI and PA) are the most widely used rootstocks today for pistachio cultivation. PI is recognized as the most vigorous rootstock, PA as being medium vigour, and PT being the smallest according to studies in the USA (Ferguson et al., 2005). There are few studies comparing the water relation responses between pistachio rootstocks. Germana (1997) mentioned that potted PA induced a more intense transpiration and net photosynthetic activity when it was compared to PT, which may limit its use in water-deficient areas. Ferguson et al. (2005) considered UCB-I as the most preferable commercial rootstock under irrigated conditions, and PT under rainfed conditions.

This work was undertaken to investigate the mechanisms relating pistachio water status to stomatal behaviour by the means of their diurnal cycles. The re-
Diurnal behaviour of stem water potential and leaf conductance in pistachio

The three rootstocks used were PT, PA and PI. These rootstocks were irrigated with three different treatments: a Control and two RDI strategies, T1 and T2.

The phenological stages taken into account in the implementation of RDI treatments were those suggested by Goldhamer (1995): stage I, from sprouting until the end of rapid nut growth (when pericarp reaches its maximum volume); stage II, from maximum nut size until the beginning of kernel growth (commonly known as the period of shell hardening); and stage III, from the beginning of kernel growth until harvest (this period starts after a fresh weight lag phase behaviour).

Contrary to stone fruits, the growth of the pericarp and the growth of the seed (kernel) in pistachio fruits are separated in time. The period separating these two processes (stage II of fruit growth) has been reported to be the most tolerant phase to water stress and make the subject of water deficit phase for water stress treatments.

Each experimental plot was formed by two trees used for measurements, surrounded by trees under the same conditions (rootstock and irrigation). In total, eighteen plots were established in the field. Six plots for each irrigation treatment and six for each rootstock. The design was factorial and completely randomized.

Material and methods

Site description and plant material

The study was conducted during 2012 (“on” year), on a 14-year-old pistachio trees cv. Kerman orchard, situated in “La Entresierra” research station, Ciudad Real (Central Spain) (L 3°56’W; L 39°0’N; altitude 640 m). Pistachio trees were budded on three different rootstocks: P. terebinthus, P. atlantica and P. integerrima. Tree spacing was set at 7 m x 6 m (238 trees/ha). Pistachio trees, cv. Peter, were used as male trees and were distributed uniformly throughout the field, in a proportion of 10%. Irrigation was performed daily using a drip irrigation system with twelve self-compensating emitters (each delivering 4 L/h tree). Irrigation water has an electrical conductivity of 2.6-2.9 dS/cm. Irrigation lines were separated 1 meter from the tree line and the distance between drippers was 1 m as well (Fig. 1).

The soil at the experimental site is an alkaline (pH 8.1) shallow soil with a discontinuous petrocalcic horizon located at 0.50 m (Petrocalcic Palexeralfs), with a clay loam texture, low electrical conductivity (0.2 dS/m), 1.05% of organic matter, 0.12% of nitrogen, 17 · 10⁻⁴ mol/kg of potassium levels and high cationic exchange capacity (0.186 mol/kg). The soil volumetric water content for the first 0.3 m depth is 22.8% at field capacity (soil matric potential -0.03 MPa) and 12.1% at permanent wilting point (soil matric potential -1.5 MPa), and from 0.3 to 0.5 m it was 43.0 and 21.1%, respectively.

The climate is Mediterranean with an average annual rainfall of 397 mm, mostly distributed outside a four-month summer drought period. Rainfall, air temperature, solar radiation and humidity data were collected from an automatic weather station situated near the field.

Experimental design

The experimental design was factorial and completely randomized. Two factors were involved: rootstocks and irrigation; with three levels for each factor.

Figure 1. Distribution of the capacitance probes in the plot. Large circles represent the trees, the medium ones the access tubes, and the small ones the drippers. Distances are expressed in meters (m).
Irrigation regimes

The three water regimes were:

Control trees: During the irrigation season, control plants received water to cover estimated crop evapotranspiration (ETc) losses by fully replenishing all soil water extracted. ETc was estimated according to daily reference evapotranspiration (ETo), calculated using the Penman–Monteith equation (Allen et al., 1998), a crop factor for pistachio (Kc) based on the time of the year (Goldhamer, 1995) and a corrector coefficient for the canopy size (Kr) (Fereres & Goldhamer, 1990). The mean Kc values used were 0.65, 1.19 and 1.10 for stages I, II and III, respectively (Goldhamer, 1995). Kr was set according to the canopy volume of each irrigation treatment, as no significant difference was found previously in the canopy volume of the different rootstocks (Memmi et al., 2015). In order to ensure the maintenance of no-water-stress conditions and to establish a reference, midday Ψx and soil water balance were considered to check and modify the ETc in case of misestimating. A Ψx value of -1.0 MPa, considered for several woody crops as a situation of an absence of water stress (Shackel et al., 1997) was taken as threshold. When Ψx values of that week were below -1.0 MPa, irrigation amounts were increased a 10%, as suggested by Goldhamer & Fereres (2001).

T1 trees: This treatment was irrigated as control along the season except during stage II. During this stage, T1 trees were not maintained to the same midday Ψx reference indicated for control (theoretical value of -1 MPa) but have been stressed to drop their midday Ψx value to a threshold of -1.5 MPa.

T2 trees: This treatment was stressed during the same period than T1 but led to a higher level of stress in which midday Ψx threshold was maintained to a value of around -2.0 MPa during stage II.

In order to be more accurate, the implementation of RDI in this work is not governed by the traditional method of applying a percentage of the ETc applied to control treatment but tries to combine the benefits of RDI on water saving with the benefit of midday Ψx to detect stress. To achieve this goal, the irrigation protocol for stressed treatments was derived from the methodology proposed by Moriana et al. (2012) for olive trees and was adapted to pistachio by considering a midday Ψx threshold of -1.00 MPa as an index for no water stress conditions;

Irrigation started when the measured Ψx values were lower than the threshold values considered. The first irrigation event was always 1 mm. Then, each increase or decrease of irrigation water supply was scheduled according to the percentage of difference derived from the relation between measured Ψx, and the set threshold for that stage: if deviation rate was <10%, the variation in the irrigation was 0.25 mm/day; if deviation rate was between 10 and 20%, the variation in the irrigation was 0.5 mm/day; if deviation rate was between 20 and 30%, the variation in the irrigation was 1 mm/day; and if deviation rate was >30%, the variation in the irrigation was 2 mm/day. Irrigation was stopped if this approach indicated a negative application.

Soil moisture monitoring and evapotranspiration measurements

The monitoring of soil moisture was done using a portable capacitance probe model (Diviner 2000 Sentek Pty. Ltd., Stepney South, Australia) employing the default calibration supplied by the manufacturer. Four access tubes measuring 50 cm in length were installed for each experimental plot. Two access tubes were located in the middle between tree axis and drip line, at 50 cm from each one. One access tube was installed in front of a dripper at 50 cm from the drip line, and 50 cm from tree axis. The other access tube between drip line and tree axis was located in front of the middle of two drippers, at 50 cm from the other access tube.

The other two access tubes were located in the same perpendicular line from the tree axis as the previous access tube, in the middle, between drip line and the middle of the row, in fact, at 1.75 m from the other two access tubes, and 2.25 m from the tree axis (Fig. 1).

A weighted average based on the area of each moisture zone was calculated to determine the soil water content. Further details are presented in Memmi et al. (2015).

Real crop water consumption for each treatment and period was derived from the soil water balance calculated according to FAO methodology (Allen et al., 1998). Irrigation and effective precipitation considered as 70% of total precipitation according to the recommendations of Orgaz & Fereres (2008) for this zone and type of soils, were taken as soil water inputs while actual crop evapotranspiration (ETa) was established as soil water outputs. Soil water content balance was taken into account as input or output depending on its signs. Deep percolation of irrigation water, runoff and capillary rise were considered as minimum due to the irrigation system type and soil characteristics and were supervised by an established moisture pattern for this field.
Tree water status and gas exchange measurements

Water relations were characterized along the season, with three diurnal cycles of midday $\Psi_x$ and abaxial leaf conductance. $\Psi_x$ was measured using a pressure chamber (Soil Moisture Equip., Santa Barbara, CA, USA) (Hsiao, 1990). One leaf was taken from each tree in each experimental plot for measurements. The leaf subject to measurement was selected from the middle and shaded side of the canopy to avoid a sharp change in its state. Then it was covered with aluminium foil 1 hour before measurement, stopping transpiration, and enabling the leaf to come into balance with the water potential of the stem (Begg & Turner, 1970). Owing to the difficulty to determine $\Psi_x$ in pistachio trees due to turpentine exudation, a piece of blotting paper was placed above the petiole cutoff to facilitate measuring as paper does not become moist with turpentine, but it does with xylem water. Measurements of abaxial leaf conductance were taken from two sun-exposed and fully expanded leaves per tree from predawn to sunset using a steady state porometer (Model LICOR-1600, UK). Both parameters were measured throughout the day, approximately every two hours from predawn until dusk.

The daily cycles were performed on: 2 July 2012, corresponding to phenological stage II and a water stress period; 24 July 2012, corresponding to the beginning of change from stage II to stage III and the initiation of rehydration (one week after the start of rewatering); during this day, cloudiness was the cause of stopping measurements earlier than expected; and 7 August 2012, corresponding to stage III and a period of no water stress.

Statistical analysis

The results were subjected to ANOVA. Means were compared using the Tukey test. Significance was set at $p < 0.05$. Data were also subjected to regression analysis. To check the regression model hypothesis (linearity, homoscedasticity, normality and independency), Kolmogorov-Smirnov was used with the Lilliefors correction and the Shapiro-Wilk tests for normality and Bartlett’s test for homoscedasticity. Linearity was observed in the graphics and independency was assumed due to the way that data were obtained.

Slopes and F-interception were analysed comparing equality of variances first by Bartlett’s test. When homoscedasticity was checked, slopes and f-interception were compared using an F-test, where significance was set at $p < 0.05$.

Results

Climatic parameters and amounts of water applied

The meteorological conditions during the daily cycles are presented in Fig. 2. The minimum temperature was respectively, 11.82 °C, 16.91 ºC and 13.51 °C and the maximum was 31.41 °C, 34.27 °C and 33.67 °C. From the first to the last day of the study, the maximum values of VPD were 4.04 kPa, 4.24 kPa and 4.42 kPa respectively and it was registered approximately in the early afternoon.

The water balance along the study is presented in Table 1. During stage I, there was no water stress in any of treatments. From the commencement of stage II until 02/07/2012, Control treatment was irrigated with 114.41 mm, which means 81% of ETc, although there was a soil water accumulation positive balance.
Table 1. Reference evapotranspiration (ETo), precipitation, irrigation amount, soil water content balance and real consumed water (ETa) for each treatment: Control (C), T1 and T2.

| Water regimes              | Stage II | Rewatering initiation | Stage III |
|----------------------------|----------|-------------------------|-----------|
| Period                     | 05/06/2012 | 02/07/2012 | 16/07/2012 | 23/07/2012 | 06/08/2012 |
| ETo (mm)                   | 189.4    | 100.3       | 51.4       | 101.1      |             |
| ETo (mm/day)               | 6.7      | 7.2         | 7.3        | 6.7        |             |
| Precipitation (mm)         | 0.2      | 0.1         | 0          | 16         |             |
| Irrigation (mm)            | C  114   | 61.7        | 37.3       | 66         |             |
|                            | T1  47.1 | 40.1        | 42.2       | 69.8       |             |
|                            | T2  9.1  | 24          | 39.9       | 70.2       |             |
| Soil water content balance (mm) | C  9.7   | 4.7         | 2.5        | 4.5        |             |
|                            | T1  -6.1 | -0.7        | 5.1        | 11         |             |
|                            | T2  -12  | -1.5        | 4.1        | 8.9        |             |
| ETa (mm)                   | C  105   | 57.1        | 34.8       | 77.5       |             |
|                            | T1  53.4 | 40.9        | 37.1       | 74.7       |             |
|                            | T2  21.4 | 25.7        | 35.7       | 77.3       |             |
| ETa (mm/day)               | C  3.7   | 4.1         | 5          | 5.2        |             |
|                            | T1  1.9  | 2.9         | 5.3        | 5          |             |
|                            | T2  0.7  | 1.8         | 5.1        | 5.2        |             |

of 9 mm. T1 was irrigated only with 33.46% and T2 a 6.45% of ETc. The reduction in irrigation produced a consumption of the soil water reserve, which was reflected by a negative value of soil water balance (Table 1). In the following two weeks, irrigation in Control was 62 mm, 40 mm in T1 and 24 mm in T2, but with a soil water balance near zero, although negative in both deficit treatments, minor reductions of water consumption (based on ETa) was produced compared to Control.

When initiating the rewatering, irrigation was the same for all treatments, around 40 mm; this quantity was sufficient to cover crop water needs for all treatments without any additional depletion in soil water reserve. Water consumption was around 5 mm/day. Similar results were obtained in the period from 23/07/2012 to 06/08/2012 (Table 1).

Stem water potential and abaxial leaf conductance diurnal course throughout rehydration

Stem water potential diurnal pattern

On 2 July 2012 (in the middle of the water stress period), Ψx decreased in all treatments from predawn until a minimum was reached at around 14:00 h when a gradual recovery began (Fig. 3A). Control reached a maximum of -0.64 MPa and a minimum of -1.33 MPa. T1 treatment reached a maximum of -0.90 MPa and a minimum of -1.65 MPa while T2 reached a maximum of -1.18 MPa and a minimum of -2.11 MPa. Statistical differences between all treatments were found during the whole day.

On 24 July 2012 (one week after initiating rewatering), diurnal pattern of Ψx followed a similar curve shape than the first diurnal cycle, and significant differences in Ψx values were measured (Fig. 3B). The minimum of Ψx was observed at around 16:00 h for all treatments. Control treatment reached a maximum of -0.72 MPa at predawn and a minimum of -1.37 MPa. T1 treatment reached a maximum of -0.88 MPa at about 06:30 h and a minimum of -1.59 MPa. T2 treatment reached a maximum of -1.00 MPa between predawn and 06:30 h and a minimum of -1.64 MPa. Statistical differences were found between Control and the other two treatments throughout the day and between all treatments only at predawn, at 06:30 h and at 13:38 h. The extent of differences of Ψx between treatments was clearly smaller than the previous day of measurement (Fig. 3B). On 7 August 2012, statistical differences between all treatments were found at approximately 08:30 h and 13:30 h, with T2 being the treatment with the lowest values, and between Control and the other treatments before noon (at 10:40 h) and late afternoon (17:50 h) (Fig. 3C). Control reached a maximum of -0.71 MPa and a minimum of -1.33 MPa. T1 reached a maximum of -0.85 MPa and a minimum of -1.41 MPa.
Diurnal behaviour of stem water potential and leaf conductance in pistachio

was strong and significant (Fig. 4A). In the T1 treatment, the first two diurnal cycles have been included in the same equation regression, which was significantly different from the Control ones (Fig. 4B). Data measured during the drought cycle presented a significantly different fit in which the slope of the equation was significantly lower (-0.23 vs -0.16), while no significant differences were found between y-interception. Data in T2 trees were not grouped and each date had a significantly different fit (Fig. 4C). The regression equation of the last date was near to Control though significantly different
from it. The slopes of the daily cycles were lower with the water stress.

Regarding rootstocks, during stage II, PT, PA and PI reached respectively a maximum of -0.88 MPa, -0.97 MPa and -0.85 MPa, and a minimum of -1.68 MPa, -1.72 MPa and -1.69 MPa during the first diurnal cycle. A significant difference was found between rootstocks only at 06:30 h although PA maintained lower values than the other rootstocks during the whole day (Fig. 5A). At the beginning of the rehydration process, significant differences were found between rootstocks at predawn (04:00 h), early morning (06:30 h) and during the afternoon, approximately at 13:30 h and 16:00 h (Fig. 5B). Trees budded on PT manifested a better general water status than PA and PI during almost all the day and PA a better water status than PI specially at the second half of the day which corresponds to the time of VPD maximum values. The values ranged from -0.80 to -1.46 MPa for PT, -0.92 to -1.51 MPa for PA and -0.87 to -1.62 MPa for PI between predawn and mid-afternoon. Two weeks after rewatering, significant differences between PI and the other rootstocks were found from late morning until the last measurement. Statistical differences between all rootstocks were perceived at 10:40 h and 17:50 h where PT reached a value of -1.20 MPa, PA a value of -1.23 MPa and PI a value of -1.32 MPa at this time (Fig. 5C). In this interval of time, VPD had a maximum of 4.42 kPa.

**Abaxial leaf conductance diurnal pattern**

On 02/07/2012 (corresponding to stage II, drought period), abaxial leaf conductance (g_l) in fully irrigated Control trees increased throughout the day, reaching a maximum g_l value of 315 mmol/m^2·s at around 16:00 h, followed by a gradual decline during the rest of the day (Fig. 6A). T1 and T2 reached a g_l peak earlier than Control. T1 and T2 peaked at 08:30 h, reaching a value of 242 and 220 mmol/m^2·s respectively and then declined gradually throughout the day. Statistical differences have been observed between Control and the other treatments from 11:00 h until sunset. Statistical differences between T1 and T2 were only observed at 14:00 h when VPD was near its maximum (3.91 kPa).

On 24/07/2012, the general behaviour of g_l diurnal pattern was similar for all treatments, characterized by an increase of g_l early in the morning reaching a maximum peak at 10:00 h when VPD reached 3.27 kPa and a gradual decrease throughout the day. Control treatment has been characterized by a different pattern compared to the previous diurnal cycle. There were statistical differences between all treatments at approximately 11:00 h and between Control and the other treatments at 13:30 h (Fig. 6B).

On 07/08/2012, the diurnal progress of g_l showed a similar behaviour for all treatments. It was characterized by an increase of g_l values early in the morning reaching a maximum peak at about 08:30 h and a con-
Diurnal behaviour of stem water potential and leaf conductance in pistachio

Continuous decrease during the rest of the day. Statistical differences were found between Control and the other treatments at 08:30 h, 13:15 h, and 15:30 h, and a difference only with T2 at approximately 11:00 h.

The relationship between \( g_l \) and VPD is presented in Fig. 7. Relationships were weaker than the ones obtained for \( \Psi_c \). In all treatments, there were significant differences between data obtained during the two first cycle (stage II) and the last date (stage III). In fact, there was no significant relationship between \( g_l \) and VPD during stage III in any of the treatments. The regression in all treatments suggests lower values for the same VPD during this last diurnal cycle than in the other two. The relationships during stage II were significantly different between treatments with a greater slope in Control (Control>T1>T2).

During the water stress period, significant difference in \( g_l \) was found between rootstocks at mid-afternoon, PI being the rootstock which showed the highest level of leaf conductance (247 mmol/m²·s) at about 16:00 h, and PA the

Figure 6. Diurnal pattern of abaxial stomatal conductance (\( g_l \)) of pistachio leaves under different water regimes on three dates. Each point is the mean of 12 measurements. Vertical bars indicate the standard error of the mean. Asterisks denote significant differences were found among treatments (\( p < 0.05 \)).

Figure 7. Relationship between air vapour pressure deficit (VPD) and stomatal conductance (\( g_l \)) for Control, T1 and T2 treatments. All data of the three dates of the experimental period are included.
Ѱx as water stress indicator for pistachio

The measurement of water potential at predawn has usually been taken as a reference to detect water stress in plants in several studies because it is assumed that the potential before dawn depicts the moment of equilibrium between tree and soil water potential (Shultz, 1996; Williams & Araujo, 2002). In this study, measurement of Ψx before dawn had showed its limitation even when statistical water stress differences were detected between treatments from the fact that it was not representative of a steady state; the maximum of Ψx did not take place only at predawn but a few hours later, when trees were in a rehydration process from stage II to stage III (Figs. 3B and 3C). In fact, the soil-plant water potential unbalance just before sunrise is quite common and has been mentioned in several studies (Sellin, 1996; Schmidhalter, 1997; Schmidhalter et al., 1998).

The fact that predawn water potential is biased by the spatial distribution of soil moisture makes it a weak recommended indicator for tree water status determination (Ameglio et al., 1999). Even when the soil humidity is uniform, Donovan et al. (2001) found disequilibrium between predawn water potential and soil water potential for well irrigated plants and attributed a great part of the unbalance to a putative mechanism of high concentration of leaf apoplastic solutes and/or to night time transpiration. Therefore, the use of a more specific indicator related to the current status of plant could be more useful. Unlike predawn Ψx measurements, significant differences have been found at midday or mid-afternoon with considerably smaller coefficients of variation (data not shown). During this time interval, the detection of water stress in pistachio trees could be the most adequate. In fact, measurement at midday showed its efficiency in several deciduous trees such as peach, almond and prune (Shackel et al., 1997) due to their high sensitivity to water deficit (McCutchan & Shackel, 1992). In pistachio, at a practical level, measurement of Ψx has the advantage of not being limited to midday time as with many other fruit trees for a better sensing of water stress, and a measurement at mid-afternoon might be more representative of water status than midday. In fact, differences between treatments were more evident at mid-afternoon than at midday. This practical specificity in pistachio is mainly due to its particular stomatal behaviour since the lower values of Ψx were obtained approximately at the moment of maximum evaporative demand producing the temporary imbalance, which occurs be-

![Figure 8](image-url)

**Figure 8.** Diurnal pattern of abaxial stomatal conductance (gℓ) of pistachio leaves related to different rootstocks (PT, *Pistacia terebinthus*; PA, *Pistacia atlantica* and PI, *Pistacia integerrima*) on three dates. Each point is the mean of twelve measurements. Vertical bars indicate the standard error of the mean. Asterisks denote significant differences between rootstocks (p < 0.05).
between the variant rate of roots water absorption, the xylem water transport and the stomata water loss (Medrano & Flexas, 2003).

The use of plant water status measurements has been suggested previously as a tool for irrigation scheduling (Shackel et al., 1997; Goldhamer et al., 1999; Moriana & Fereres, 2002), given the fact that the $\Psi_{s}$ measurement results in an accurate state of the dynamic relation integrated by water stress and environmental conditions (Moriana et al., 2012). The reference to a baseline under well-watered conditions and varying atmospheric situations is required for a correct use of midday/middle-afternoon $\Psi_{s}$ measurements as indicators in pistachio irrigation scheduling. The fact that this baseline is not affected by the phenological stage of the tree is very useful. As a primarily approximation, the relation represented in Fig. 4A, obtained between $\Psi_{s}$ and VPD could be a useful tool for this purpose. Further works are needed in order to evaluate the extrapolation of this equation to other conditions.

**Stomatal response to increasing atmospheric VPD, water availability and phenological stage**

According to several studies of stomatal behaviour in fruit trees, it was found that stomatal response throughout the day is characteristic of each species (Acherar & Rambal, 1992). During stage II, in fully irrigated trees, no closing response of $g_{s}$ was observed, reaching its peak approximately the same time VPD reached its maximum. A strong relationship was found between $g_{s}$ in fully irrigated trees and VPD ($R^{2}=0.96$), indicating that there is a low stomatal closure regulation for pistachio trees when water is not a limiting factor in this stage (Fig. 7A). This correlation presented a lower fit as water stress increased (Figs. 7B and 7C), which suggests that the association between $g_{s}$ and VPD is mainly governed by the water stress level in the tree. In this case, these results advocate that under water stress, tree gas exchange was more affected by factors related to water soil availability than by atmospheric components in pistachio.

During stage III, gas exchange was clearly affected in Control trees with a decrease by 20% in $g_{s}$ levels between stage II and stage III, and maximum $g_{s}$ peak was observed approximately 7 hours earlier (Figs. 6A and 6C). In addition, the correlation between VPD and $g_{s}$ on the third day was weaker compared to the first two days of the experiment, taking into account there were no significant changes in VPD levels throughout the experiment. Therefore, this change in

$g_{s}$ pattern can be regarded as physiological changes affecting tree water relations from a stage to another, rather than VPD or soil moisture conditions. Several authors reported that changes in water relation parameters in pistachio during stage II is characterized by an increase in $g_{s}$ values (Sajjadinia et al., 2010; Gijon et al., 2011). In almonds, several authors suggested that the seasonal decrease in gas exchange rates were related to changes in the hormonal balance or development of leaves (Sanchez-Blanco et al., 1991; Klein et al., 2001).

Depending on the phenological stage, the stomatal response of a full irrigated pistachio trees oscillates between two different behavioural “strategies”: (i) a strategy where the stomatal opening is substantial and prolonged during the day, approaching to an apple tree behaviour or (ii) a more conservative strategy which avoids high water loss traduced by a partial stomata closure at the middle of the morning which is more similar to almonds or olives trees behaviour. The first strategy is observed during shell hardening, a process of high energy consumption which could be the cause of little noticeable water stress effect at the final yield according to Goldhamer & Beede (2004). The second strategy, a more conservative approach, is recorded during the kernel filling stage which is considered imperative to the survival of the species.

On the other hand, water stress reduced the maximum $g_{s}$ in deficit treatments (T1 and T2). Under these conditions, the $g_{s}$ pattern followed by T1 and T2 treatments showed the ability of pistachio trees to regulate stomatal activity under water stress conditions by closing stomata early in the morning, anticipating an excessive decline in water potentials. This control from roots to shoots could be assigned to a hydraulic or/and a biochemical signal, which prevents excessive water loss as in other fruit trees: orange, Fereres et al. (1979); almond, Castel & Fereres (1982), Torrecillas et al. (1988); olive: Bongi & Loreto (1989), Giorio et al. (1999).

**Pistachio water recovery course**

Three weeks after the beginning of rehydration, a maintained positive soil water balance did not allow stressed trees to recover completely from stress levels of -1.5 MPa and -2.0 MPa maintained approximately for 40 days. Torrecillas et al. (1996) mentioned that a rapid recovery after a stress condition can be related to a greater physiological tolerance to drought, so the delay observed in the recovery of pistachio trees was not expected due to the great reputation of pistachio as a drought-tolerant species. Hence, the faster or slower
response to water renewal may be related to the extent of drought (level of stress and duration) and its possible progressive effects on different plant organs (short and long term effects).

Guerrero et al. (2005) imposed a similar extent of water stress as T1 and observed a rapid recovery for $g_l$ in pistachio trees; however, they concluded that more than three weeks are required for a complete recovery of water status in pistachio because of lagging trunk growth. Moreover, Rieger (1995) mentioned for potted pistachio plants the possibility that stomatal conductance could recover before hydraulic conductivity. In this study, a partial recovery was observed 3 weeks after the beginning of rewatering but a total recovery could not be confirmed when $\Psi$, drops to values around -2.2 MPa during stress period (T2 treatment).

Several studies involving acoustic sensing of woody tissues in other species showed that cavitation often occurred when the water potential falls below -1.5 MPa (Dixon et al., 1984; Peña & Grace, 1986; Tyree & Sperry, 1989). Then the recovery delay observed in this study may also be related to the complexity of the process for the refilling of xylem cavitation. The variant time necessary for each individual mechanism involved in the recovery to come to pre-stress conditions translates the complexity of the recovery process initiated by re-watering.

The unexpected delay for the recovery of pistachio from water stress should be taken into account and exceeding rewatering should start early for RDI major benefits specially under shallow soil conditions combined with drip irrigation system.

**Influence of rootstock on pistachio water relations**

The three different rootstocks studied led to different water relations in Kerman cultivar. During stage II, PA showed the lowest $\Psi$, and $g_l$ values during the day (Figs. 5 and 8). Spiegel-Roy et al. (1977) and Lin et al. (1984) described PA as an adapted species for very severe conditions and dry areas where higher stomatal control is exerted by PA compared to PI and PT. PT compared to other Mediterranean plants was considered by Sakcali & Ozturk (2004) as a water spender and described it as a partially water-tolerant species which cannot sustain long in harsh conditions. These results differ from those reported by Germana (1997) and Gijón et al. (2010) for potted pistachio plants, where PA is considered as a rootstock with little stomatal control compared to PT. In this study, PA maintained lower $g_l$ than PT and PI during stage II, but during stage III, PA displayed a greater $g_l$ than PT early morning and similar activity during the rest of the day. This variance between results could be explained by the wide genetic diversity that affects the spectrum of potential responses, and by the different responses given by the rootstock depending on whether it is combined or not to a scion.

$\Psi$, of trees budded on PI was significantly lower than that of the two other rootstocks from the second diurnal cycle. Despite this, PI presented higher $g_l$ than the other two rootstocks. This result suggests that PI has less stomatal control than PT and PA and no discernible threshold for water potential maintenance, which could give it a little anhysohidric tendency. Duhme & Hinckley (1992) reported that the values of leaf osmotic potential at full turgor for PI were only 0.5 to 1.0 MPa higher than osmotic potentials at the turgor loss point for PT. This confirms that PI is less adapted than the other rootstocks to conditions of water scarcity. PI has been described as the most trunk and canopy size vigorous rootstock amongst the three (Ferguson et al., 2005), which could provide a higher capacity for energy interception and water transport, and can result in a major loss of water. According to the general trend of $g_l$, the influence of rootstocks on the degree of leaf conductance control when they are combined with cv. Kerman could be classified in this order, from the greatest to the lowest: PA > PT > PI.

In summary, midday stem water potential could be considered as a better indicator for water stress in pistachio than predawn stem water potential. Water stress conditions modified significantly the fit of the relationship between stem water potential and VPD. However, the phenological stage has no effect on this relationship. Relationship between leaf conductance and VPD has shown a positive linear correlation. Such behaviour was recorded during stage II of fruit growth where pistachio follows the tendency of a water spender species when all water requirements are fulfilled. Once water stress took place, pistachio showed an ability to reduce its water consumption by closing the stomata early in the morning. The recovery from water stress levels reaching -1.5 MPa and -2.0 MPa prolonged for 40 days could be considered a slow process since it takes around 4 weeks. Therefore, it should be taken into account for deficit irrigation scheduling. Concerning rootstocks, in contrast with previous works and to the general belief, *Pistacia atlantica* showed it could be a suitable rootstock for water deficit conditions. While, *Pistacia integerrima* showed less stomatal regulation, promoting water loss despite the major drop of water potential and showed less adaptation than *Pistacia atlantica* and *Pistacia terebinthus* to water stress conditions.
References

Acherar M, Rambal S, 1992. Comparative water relations of four Mediterranean oak species. Vegetation 99-100: 177-184. http://dx.doi.org/10.1007/BF01182224.

Allen RG, Pereira LS, 1979. Crop evapotranspiration. Guideline for computing crop water requirements. Irrig Drain Paper no. 56. FAO, Rome.

Ameglio T, Archer P, Cohen M, Valancogne C, Daudet FA, Dayau S, Cruijatz P, 1999. Significance and limits in the use of predawn leaf water potential for tree irrigation. Plant Soil 207: 155-167. http://dx.doi.org/10.1023/A:1026415302759.

Begg JE, Turner NC, 1970. Water potential gradients in field tobacco. Plant Physiol 46: 343-346. http://dx.doi.org/10.1104/pp.46.2.343.

Bongi G, Loreto F, 1989. Gas-exchange properties of salt-stressed olive (Olea europaea L.) leaves. Plant Physiol 90: 1408-1416. http://dx.doi.org/10.1104/pp.90.4.1408.

Donovan LA, Linton MJ, Richards JH, 2001. Predawn plant water potential does not necessarily equilibrate with soil water potential under well-watered conditions. Oecologia 129: 328-335. http://dx.doi.org/10.1007/s004420100738.

Duhme F, Hinckley TM, 1992. Daily and seasonal variation in water relations of macchia shrubs and trees in France (Montpellier) and Turkey (Antalya). Vegetation 99-100: 185-198. http://dx.doi.org/10.1007/BF0018225.

Fereres E, Goldhamer DA, 2001. Irrigation scheduling protocols using continuously recorded trunk diameter measurements. Irrig Sci 20 (3): 115-125. http://dx.doi.org/10.1007/s002710000034.

Goldhamer DA, Beebe RH, 2004. Regulated deficit irrigation effects on yield, nut quality and water-use efficiency of mature pistachio trees quality. J Hortic Sci Biotech 79: 538-545. http://dx.doi.org/10.1006/jhsb.2004.11511802.

Goldhamer DA, 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.

Goldhamer DA, Beebe RH, Michailides TJ, Salinas M, Doster M, 2005. Effects of regulated deficit irrigation on shell splitting and nut quality at harvest (2nd year report). In: Annual Report, crop year 2004-2005, pp: 104-106. California Pistachio Industry, Fresno, CA, USA.

Guerrero J, Moriana A, Pérez D, Couceiro JF, Olmedilla N, Gijón MC, 2005. Regulated deficit irrigation and the recovery of water relations in pistachio trees. J Physiol 26: 87-92. http://dx.doi.org/10.1093/treephys/26.1.87.

Hsiao TH, 1990. Measurements of plant water status. In: Irrigation of agricultural crops. Agron Monogr No. 30; Stewart BA, Nielsen DR (Eds.), pp: 243-280. Am Soc Agron, Madison, WI, USA.

Kanber R, Eylen M, Köksal H, Yüksek G, 1990. Güneydoğu Anadolu’da Antep fıstığı üretim ve su tüketiminin rölesi. Türkiye 1. Antep fıstığı Simpozyumu. Gaziantep, pp: 45-160. [In Turkish].

Klein I, Esperza G, Weinbaum SA, DeJong TM, 2001. Effects of irrigation deprivation during the harvest period on leaf persistence and function in mature almond trees. Tree Physiol 21: 1063-1072. http://dx.doi.org/10.1093/treephys/21.14.1063.

Klepper B, 1968. Diurnal pattern of water potential in woody plants. Plant Physiol 43: 1931-1934. http://dx.doi.org/10.1104/pp.43.12.1931.

Lin TS, Crane JC, Ryugo K, Polito VS, DeJong TM, 1984. Comparative study of leaf morphology, photosynthesis, and leaf conductance in selected Pistacia species. J Am Soc Hortic Sci 109: 325-330.

Germana C, 1997. The response of pistachio trees to water stress as affected by two different rootstocks. Acta Hortic 449: 513-519. http://dx.doi.org/10.17660/ActaHortic.1997.449.71.

Gijón MC, Giménez C, Pérez D, Guerrero J, Couceiro JF, Moriana A, 2010. Rootstock influences the response of pistachio (Pistacia vera L. cv kerman) to water stress and rehydration. Sci Hortic 125: 666-671. http://dx.doi.org/10.1016/j.scienta.2010.05.026.

Gijón MC, Giménez C, Pérez D, Guerrero J, Couceiro JF, Moriana A, 2011. Water relations of pistachio (Pistacia vera L.) as affected by phenological stages and water regimes. Sci Hortic 128: 415-422. http://dx.doi.org/10.1016/j.scienta.2011.02.004.

Giorio P, Sorrentino G, D’Andria R, 1999. Stomatal behaviour, leaf water status and photosynthetic response in field-grown olive trees under water. Environ Exp Bot 42: 95-104. http://dx.doi.org/10.1016/S0098-8472(99)00023-4.

Hsiao TH, 1990. Measurements of plant water status. In: Irrigation of agricultural crops. Agron Monogr No. 30; Stewart BA, Nielsen DR (Eds.), pp: 987-1017. Am Soc Agron, Madison, WI, USA.
McCutchan H, Shackel KA, 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.

Medrano H, Flexas J, 2003. Respuestas de las plantas al estrés hídrico. In: Ecofisiología: Una ciencia de síntesis; Reigosa MJ, Pedrol N, Sánchez–Moreiras A (Eds.), pp: 253-286. Paraninfo S.A., Madrid.

Memmi H, Gijón MC, Couceiro JF, Pérez-López D, 2015. Water stress thresholds for regulated deficit irrigation in pistachio trees: Rootstock influence and effects on yield quality. Agr Water Manage 164: 58-72. http://dx.doi.org/10.1016/j.agwat.2015.08.006.

Moriana A, Pérez D, Prieto MH, Ramírez M, Pérez JM, 2012. Midday stem water potential as a useful tool for estimating irrigation requirements in olive trees. Agr Water Manage 112: 43-53. http://dx.doi.org/10.1016/j.agwat.2012.06.003.

Orgaz F, Fereres E, 2008. Riego. In: El cultivo del olivo; Barranco D, Fernandez-Escobar R, Rallo L (eds), pp: 337-362. Mundi-Prensa, Madrid.

Peña J, Grace J, 1986. Water relations and ultrasound emissions before, during and after a period of water stress. New Phytol 103: 515-524. http://dx.doi.org/10.1111/j.1469-8137.1986.tb02889.x.

Rieger M, 1995. Offsetting effects of reduced root hydraulic conductivity and osmotic adjustment following drought. Tree Physiol 15: 379-385. http://dx.doi.org/10.1093/treephys/15.6.379.

Ritchie GA, Hinckley TM, 1975. The pressure chamber as an instrument for ecological research. Adv Ecol Res 9: 165-254. http://dx.doi.org/10.1016/S0027-0107(08)60290-1.

Sakcali MS, Ozturk M, 2004. Eco-physiological behavior of some Mediterranean plants as suitable candidates for reclamation of degraded areas. J Arid Environ 57: 1-13. http://dx.doi.org/10.1016/S0140-1963(03)00099-5.

Sajjadinia A, Ershadi A, Lokhabadi H, Khayyat M, Gholami M, 2010. Gas exchange activities and relative water content at different fruit growth and developmental stages of on and off cultivated pistachio trees. Aust J Agric Eng 1: 1-6.

Sánchez-Blanco MJ, 1983. Aportación al diagnóstico del estrés hídrico en citricos y frutales. Master’s thesis. Univ. Murcia, Spain. 80 pp.

Sánchez-Blanco MJ, Ruiz-Sánchez MC, Planes J, Torrecillas A, 1991. Water relations of two almond cultivars under anomalous rain-fall in non-irrigated culture. J Hortic Sci Biotech 66: 403-408.

Schmidhalter U, 1997. The gradient between pre-dawn rhizoplane and bulk soil matric potentials, and its relation to the pre-dawn root and leaf water potentials of four species. Plant Cell Environ 20: 953-960. http://dx.doi.org/10.1046/j.1365-3040.1997.d01-136.x.

Schmidhalter U, Burucs Z, Camp KH, 1998. Sensitivity of root and leaf water status in maize (Zea mays) subjected to mild soil dryness. Aust J Agric Eng 25: 307-316. http://dx.doi.org/10.1071/pp97047.

Sellin A, 1996. Base water potential of Picea abies as a characteristic of soil water status. Plant Soil 184: 273-280. http://dx.doi.org/10.1007/BF00010456.

Shackel KA, 2011. A plant-based approach to deficit irrigation in trees and vines. Hort Sci 46: 173-177.

Shackel KA, Ahmadi H, Biasi W, Buchner R, Goldhamer DA, Gurusuinghe S, Hasey J, Kester, D, Krueger B, Lampinen B, et al., 1997. Plant water status as an index of irrigation need in deciduous fruit trees. HortTechnol 7: 23-29.

Shultz HR, 1996. Water relations and photosynthetic responses of two grapevine cultivars of different geographical origin during water stress. Proc. Workshop Strategies to Optimize Wine Grape Quality. Acta Hortic 427: 251-266. http://dx.doi.org/10.17660/ActaHortic.1996.427.30.

Spiegel-Roy P, Mazigh D, Evenari M, 1977. Response of pistachio to low soil moisture conditions. J Am Soc Hort Sci 102: 470-473.

Torrecillas A, Ruiz-Sánchez MC, 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. http://dx.doi.org/10.1007/BF02371216.

Torrecillas A, Alarcón JJ, Domingo R, Planes J, Sánchez Blanco MJ, 1996. Strategies for drought resistance in leaves of two almond cultivars. Plant Sci 118: 135-143. http://dx.doi.org/10.1016/0168-9452(96)04434-2.

Turner NC, 1991. Measurement and influence of environmental and plants factors on stomatal conductance in the field. Agr Forest Meteorol 54: 137-154. http://dx.doi.org/10.1016/0168-9452(91)90003-9.

Tyree MT, Sperry JS, 1989. Vulnerability of xylem to cavitation and embolism. Annu Rev Plant Physiol Plant Mol Biol 40: 19-38. http://dx.doi.org/10.1146/annurev.pp.40.060189.000315.

Williams PH, Araujo MB, 2002. Apples, oranges, and probabilities: integrating multiple factors into biodiversity conservation with consistency. Environ Model Assess 7: 139-151. http://dx.doi.org/10.1023/A:1015657917928.

Williams LE, Baega P, 2007. Relationships among ambient temperature and vapor pressure deficit and leaf and stem water potentials of fully irrigated, field-grown grapevines. Am J Enol Vitic 58: 173-181.