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RESEARCH ARTICLE

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Deficit irrigation in commercial mandarin trees: water relations, yield and quality responses at harvest and after cold storage

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Abstract

Two experiments were conducted on a commercial farm of late ‘Fortune’ mandarin trees in order to know the response of regulated deficit irrigation (RDI), mainly applied during the most harmful ripening stages, on plant water relations, yield and fruit quality at harvest and after cold storage at different temperatures. Control trees were irrigated to satisfy maximum crop evapotranspiration, while RDI-1 and RDI- 2 represented a 20% and 40% reduction, respectively, in the water applied. Total yield and fruit quality at harvest were not significantly affected by either treatment. Late stage II of fruit growth was the most sensitive period to water stress, while deficit irrigation applied during flowering and stage I of fruit growth resulted in a significantly higher number of fruits per tree and an improvement in irrigation water productivity compared with the Control treatment. In both experiments, skin chroma decreased during cold storage, at the same time as titratable acidity fell. Fruit quality (titratable acidity, skin C* and ascorbic and glutamic acids) were more affected by cold storage than by differences between the RDI treatments. The use of trunk diameter fluctuation was useful for restoring the RDI irrigation to levels of the Control at the end of early stage II. From a quality point of view, any difference between treatments found at harvest tended to diminish during the subsequent shelf-life after cold storage. Quality traits (titratable acidity, ascorbic and glutamic acid) could be used as chilling biomarkers.

Additional keywords: *Citrus*; regulated deficit irrigation; fruit growth; storage performance; organic acids content.

Abbreviations used: A_{C02} (maximum net CO_2 assimilation rate); E_m (transpiration rate); g_s (maximum stomatal conductance); MDS (maximum daily trunk shrinkage); MI (maturity index); MNTD (minimum daily trunk diameter); MXDT (maximum daily trunk diameter); RDI (Regulated deficit irrigation); RGR (fruit relative growth rate); TA (titratable acidity); TCSA (trunk cross-section area); TDF (trunk diameter fluctuations); TGR (trunk daily growth rate); TSS (total soluble solid content); WUE (Water use efficiency); WUE_i (Instantaneous water use efficiency); Ψ_s (midday stem water potential); S_Ψ (water stress integral).

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Introduction

Citrus is one of the most important crops cultivated in Mediterranean climates worldwide, where water is the most limiting factor for fruit production. Furthermore, in such areas, the crop water status needs to be constantly monitored (Puerto *et al.*, 2013) in order to maintain the fruit quality and irrigation water productivity, especially when deficit irrigation strategies are being applied (García-Tejero *et al.*, 2012). In this respect, regulated deficit irrigation (RDI) strategies have been proposed to improve water use efficiency (WUE) and ameliorate water scarcity (Chalmers *et al.*, 1981; Ruiz-Sanchez *et al.*, 2010; García-Tejero *et al.*, 2011; Pérez-Pastor *et al.*, 2016). Such strategies are based on reducing water intake during certain times of the growing season, while fully covering the needs of the crop during the most sensitive phenological stages to water stress

(González-Altozano & Castel, 2003a). Phenological stages in citrus can be represented as a sigmoid curve divided into three fruit growth stages: stage I, which corresponds to the period that runs from bud-break-flowering until fruit set; stage II, known as the rapid fruit growth stage, and stage III, which coincides with the ripening period and is regarded as a non-critical period (García-Tejero *et al.*, 2010). The benefits of RDI techniques have been reported in several citrus crops, including lemon (Domingo *et al.*, 1996; Pérez-Perez *et al.*, 2016), sweet orange (Pérez-Pérez *et al.*, 2009; Aguado *et al.*, 2012; Gasque *et al.*, 2016), grapefruit (Pérez-Pérez *et al.*, 2014; Romero-Trigueros *et al.*, 2017) and mandarin (González-Altozano & Castell, 2003a,b; Conesa *et al.*, 2014).

RDI requires careful selection of the moment, intensity and duration of the water deficit application, all of which depend on the stage of plant development (Pérez-Pastor *et al.*, 2016). RDI saves water to a greater extent than conventional irrigation strategies while maintaining productivity and fruit quality at harvest and during postharvest, which is also essential (reviewed by Ruiz-Sánchez *et al.*, 2010). Indeed, drought stress applied during flowering and fruit set might increase the fall of flowers and young fruits (Doorenbos *et al.*, 1980).

Apart from saving water, RDI in citrus trees might improve the fruit quality by increasing the total soluble solids (TSS) content (González-Altozano & Castel, 2003a; Romero-Trigueros *et al.*, 2017). Pérez-Pérez *et al.* (2009) and García Tejero *et al.* (2012) suggested a moderate water-stress applied during stage III of fruit growth in citrus to improve TSS. Aguado *et al.* (2012) applied a water-stress ratio of 0.75 during the ripening period resulting in an increase in the maturity index (TSS divided by titratable acidity). Moreover, there are few references to the effect of RDI on the post-harvest behaviour of citrus (Conesa *et al.*, 2014; Frías, 2017).

When a deficit-irrigation strategy is being applied, it is crucial to monitor the crop water status to assess the physiological response and take suitable irrigation decisions. Some reports consider the trunk diameter fluctuations (TDF) index for irrigation in citrus trees (reviewed by Fernandez & Cuevas, 2010 and Ortuño *et al.*, 2010). However, irrigation scheduling based on trunk growth diameter or maximum daily trunk shrinkage (MDXT), which is derived from TDF, has received no attention in *Citrus* for determining the duration of each phenological stage. Pagán (2012) observed the benefits of applying RDI conditions in 'Fortune' mandarin trees grown in saline conditions. Moreover, the same study suggested that the slow-down in MXDT at the end of early stage II of fruit growth as a criterion for restoring irrigation to control levels in order to avoid reductions in yield and fruit diameter. However, there are no references in the relevant literature to using this criterion under real conditions.

In this study, two experiments were performed in a commercial citrus orchard. The first aimed to obtain preliminary results about how water restrictions affect plant water relations, yield and fruit quality when RDI is applied during the most harmful period for water stress. With the results obtained, the second experiment included a new RDI treatment applied from flowering to stage II. Both experiments also investigated the influence of these RDI treatments on some fruit quality traits during cold storage and after a subsequent simulated retail sale period. The use of TDF-indices as a criterion for restoring irrigation to Control levels was also assessed in order to define a practical recommendation for irrigation management when water stress is being imposed.

Material and methods

Plant material, experimental design and site description

Two experiments were carried out in a commercial farm of 20-year-old, drip-irrigated mandarin trees (*Dancy tangerine* × *Clementine* (*Citrus clementine Hort. Ex Tanaka* × *Citrus reticulata Blanco*)), grafted onto Cleopatra mandarin (*Citrus reshni Hort. Ex. Tanaka*) trees. The orchard was located in La Palma (Cartagena, SE Spain). The soil with a bulk density of 1.31 g/cm^3 had a clay loam texture (11.45%, 30% and 58.55% sand, silt and clay particle size, respectively) with a medium level of organic matter (1.8%). Trees, spaced at 6 m × 4 m were irrigated by a drip irrigation system with two lines per row of trees. Each tree was irrigated with a total of 6 emitters (4 emitters with 4 L/h and 2 emitters with 2 L/h). The application of each irrigation treatment is explained in detail below. Irrigation water was a mixture of desalinized water with water from the Tajo-Segura Water Transfer System, with an electrical conductivity of 1.42 dS/m and pH of around 8.

Daily meteorological information (temperature, relative humidity and precipitation (T, RH, Prec) was obtained from an automatic weather station (CA52-www.siam.es) close to the experimental site. The air vapour pressure deficit (VPD) was calculated every day using air temperature and RH data. Crop evapotranspiration (ETc) was weekly determined from the product of reference evapotranspiration (ET_0,), the crop coefficient (kc, between 0.2-0.6) and the correction factor *vs* the shaded area (kr).

Irrigation treatments and storage experiments

The phenological stages were determined as a function of fruit development (stages I, II and III, representing the typical sigmoid model of growth in citrus crops) (González-Altozano & Castel, 2003b). The slow-down of MXDT was used as criterion for dividing stage II of fruit growth into two periods (early and late). Details of the application of both RDI treatments are summarized in Fig 1.

Experiment 1 (2009-2010)

Three irrigation treatments were established: (i) Control treatment (Control) irrigated at 100% ETc throughout the season, (ii) RDI-1 irrigated as the Control, except for the early stage II and from late stage III to harvest (50% of Control) and late stage II (80% of Control) (Fig. 1), and (iii) RDI-2 irrigated as the Control at 100% ETc throughout the season. RDI-2 trees were actually irrigated as Control trees in Experiment 1, but they were termed as RDI-2 trees to maintain consistency with their name in the second experiment. Fruits were stored at 3 ºC and 90 ± 5% RH for 50 d followed by a shelf-life period of 3 d at 25 ºC and 90 ± 5 % RH.

Experiment 2 (2010-2011)

Three irrigation treatments were established: (i) Control treatment (Control) irrigated at 115% ETc to ensure non-limiting soil water conditions; (ii) RDI-1, irrigated as the Control except for early stage II (50% Control), and (iii) RDI-2, irrigated as the Control except from flowering-fruit set (April) to late stage II (November) at 70% of the Control (Flowering-Fruit set), 50% of Control (stage I), 70% of Control (early stage II), 80% of Control (late stage II) (Fig. 1). Fruits were stored at 5 ºC and 90 ± 5% RH for 34 d with or without a subsequent shelf-life period of 3 d at 25 ºC and 90 ± 5% RH. Both cold storage experiments ended when the percentages of fruit suffering chilling injury exceeded 10%.

The experimental design of both experiments consisted of completely randomized blocks with three replicates for each treatment. Each replicate consisted of three adjacent tree rows with 6 trees per row. Measurements of plant water status and fruit production were taken in 4 trees of the central row, the other trees serving as borders. Agricultural practices such as pruning, weed control, fertilization and banding were the same for all the trees of the experiment and were carried out by the technical department of the commercial orchard following usual criteria for the area.

The experimental design of both cold storage experiments consisted of 30 fruits per treatment (ten fruits for each replicate). Fruit quality traits – skin color (skin C*), hardness, total solid soluble content, titratable acidity, maturity index and the percentages of peel and juice – were measured at harvest and during storage, as suggested by Conesa *et al.* (2014).

Measurements of plant water stress indicators

Midday stem water potential (Ψ_s, MPa) was measured in 6 mature leaves per treatment (two leaves per replicate) located on the south side, selected from the middle-third of the tree, using a pressure chamber (Soil Moisture Equipment Co., Model 3000) following the recommendations outlined by Hsiao (1990). In order to estimate the intensity of stress endured by deficit treatments, the water stress integral was calculated from the values of Ψ_s, according to the equation defined by Myers (1988):

$$S_\Psi = \left| \sum_{i=0}^{i=t} (\overline{\Psi}_{i,i+1} - \Psi_c) n \right| \qquad [1]$$

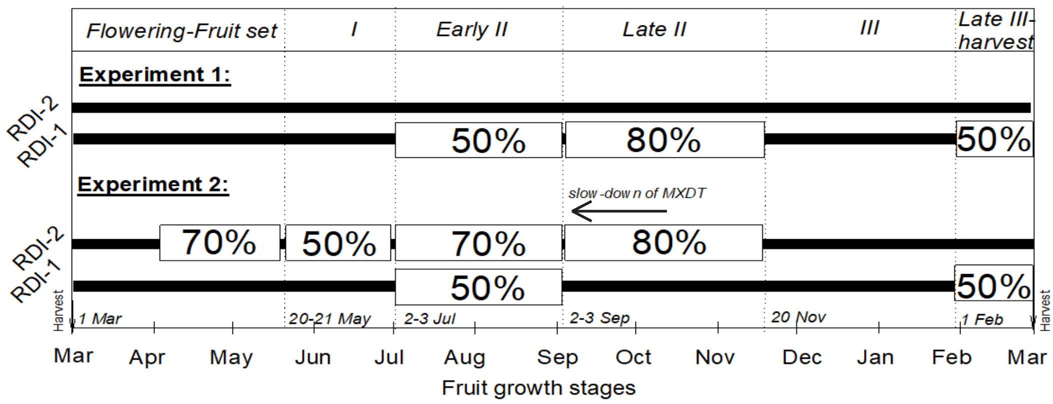

Figure 1. Irrigation strategies used in both RDI treatments (RDI-1 and RDI-2) and control treatment during the study period. Horizontal black bars indicate the irrigation levels of the Control. Dotted lines delimit the phenological stages. The arrow indicates the slow-down of MXDT.

where t is the number of measurements of Ψ_s, $\Psi_{i,i+1}$ is the average Ψ_s for any interval (MPa), Ψ_c is the value of the maximum Ψ_s measured during the season, and n is the number of days in each interval. All values were referred to those of the Control treatment.

Gas exchange measurements were taken every two weeks between 09.00 and 11.30 h in daylight hours from 6 mature leaves per treatment (2 leaves per replicate) exposed to the sun, as described by Pérez-Pérez *et al.* (2008a). Maximum net CO_2 assimilation rate (A_{CO2}, µmol/m^2·s), maximum stomatal conductance (g_{sm}, mmol/m^2·s), and transpiration rate (E_m, mmol/m^2·s) were measured at a photosynthetic photon flux density (PPFD) ≈ 1200 µmol/m^2·s above the photosynthesis light saturation intensity for citrus leaves (Sinclair & Allen, 1982). Near constant ambient CO_2 concentration (Ca ≈ 350 µmol/mol) and leaf temperature (Tleaf ≈ 30 °C) were measured with a portable gas exchange system CIRAS-2 (PP Systems, Hitchin, Hertfordshire, UK). Instantaneous water use efficiency (WUEi) was calculated as the ratio between A_{CO2} and E_m (µmol/mmol).

Micrometric TDF were monitored in 6 selected trees (two per replicate) in Control and RDI-1 treatments, using a set of linear variable displacement transducers (LVDT; Solartron Metrology, Bognor Regis, UK, model DF ±2.5 mm, precision ±10 µm) installed on the northern side of trunks and 40 cm above the ground. The transducers were mounted on holders built of aluminum and invar-an alloy comprising 64% Fe and 35% Ni that has minimal thermal expansion. Measurements were recorded by a CR1000X data-logger (Campbell Scientific, Inc., Logan, USA) every 30 s and averaged every 15 min. Several indices were derived from TDF according to Goldhamer & Fereres (2001): maximum (MXTD) and minimum (MNTD) daily trunk diameter (µm), maximum daily trunk shrinkage (MDS = MXTD – MNTD) and trunk daily growth rate (TGR, calculated as the difference between MXTD measurements obtained during two consecutive days).

Water use efficiency, fruit growth and yield components

WUE was calculated as the rate of yield and the total irrigation applied (kg/m^3). Fruits were fully harvested in one pick. The commercial picking dates depended on the needs of the commercial farm. In this case, the dates were 22nd February and 3rd March in the first and second experiment, respectively. Trunk perimeter was measured with a tape-measure in 4 trees per replicate to determine trunk cross-section area (TCSA, cm^2). The effects of RDI treatments on the distribution of photosynthetic resources were also calculated as the ratio between yield/TCSA and yield/ΔTCSA (González-Altozano & Castel, 2003b).

Yields components (total yield, number of fruit per tree, fruit weight and fruit diameter) were measured according to Conesa *et al.* (2014). An evaluation during flowering was also made weekly in order to obtain the percentage of fruit set (%), fruit drop (%), and the ratios (fruit/branch and fruit/flower) from early March to fruit drop (end of June). The measurements were taken in four fruit-bearing branches, 15-30 cm long per tree and six trees per treatment (2 trees per replicate). Phenological status was determined during the second experiment according to the classification defined by the BBCH scale (Agustí *et al.*, 2003).

The dynamics of fruit growth was determined weekly from fruit set to harvest with a digital caliper (Mitutoyo, CD-15D) using 45 fruits chosen randomly per treatment (15 fruits per replicate). The relative growth rate (RGR) was determined based on the values of the equatorial diameters at the beginning of the experiment (Conesa *et al.*, 2014).

Organic acids content

Organic acids in the first experiment were extracted according to Obando-Ulloa *et al.* (2009). After squeezing, the juice was filtered through four layers of cheesecloth and then centrifuged for 15 min at 12,000 rpm and 4 °C. The supernatant was immediately treated with physical (Oasis HLB 6 cc 500 mg LP Waters Co., Milford, MA, USA) and chemical (Millex-HV PVDF Durapore 0.45 µm, 13 mm Millipore, Billerica, MA, USA) filters. All the samples were thermostated at 5 °C in an auto-sampler prior to analysis using a photodiode array (PDA) detector (model 2996; Waters) connected to an HPLC (model Alliance 2695; Waters). The juice was diluted 1:10 for quantification of citric, ascorbic and succinic acids using a Rezex Roa- Organic acid H + (8%) 300 × 7.80 mm column (Phenomenex Inc., Torrance, CA, USA) working at room temperature, with a guard column KD-0138-03B. The mobile phase was 0.005 N H_2SO_4 with a constant flow of 0.5 mL/min. Malic acid was diluted 1:10 and quantified at room temperature using another column (LUNA C18 5 µm, 250 × 4.6 mm; Phenomenex Inc.) and a water/methanol/trifluroacetic solution (97.7/2.2/0.1 v/v/v) at a constant flow of 0.7 mL/min. The glutamic acid was quantified at 1:100 dilution according to the method described by Obando-Ulloa *et al.* (2009). All acids were quantified using a 10 µL volume for injections, UV absorbance readings at 210 nm and calibration curves were prepared with external standards of organic acids (Sigma-Aldrich, Spain) in the linear range of concentrations (usually 0 to 1000 mg/L).

Statistical analysis

A fully randomized two-way analysis of variance (ANOVA) using treatment (Control, RDI-1 and RDI-2) and cold storage time as factors was performed; or a two-way ANOVA using treatment and shelf-life as factors. Data analysis was carried out using Statgraphics Plus for Windows vers. 5.1 (Manugistics, Inc., Rockville MD, USA). *Post hoc* pairwise comparison between all means was performed by Duncan´s multiple range test at $p<0.05$. For elucidating the possible effect of RDI treatments on variability of the production measurements at harvest, an additional one-way ANOVA of the variable residuals[2] previously obtained from each ANOVA model reported above was evaluated (Romero & Zúñica, 2005). Linear and nonlinear regressions between plant water stress indicators were fitted using Sigma Plot 2000 (Systat, Richmond, CA, USA).

Results

Water applied and climatology

The average water applied in the Control treatment was around 630 mm during the experimental period and the reductions in water applied in RDI-1 compared with the Control were 17% and 21% during the first and second experiment, respectively (Table 1). Of note was the reduction of 39% in water consumption in RDI-2 compared with the Control in the second season (Table 1). As can be observed, the first season was very wet (annual rainfall 678 mm) and the annual ET_0 was 1183 mm (Table 1). In the second season, the climatology was characterized by low temperatures, occasionally below 0 ºC during the winter, and the annual ET_0 and total rainfall registered were 1202 mm and 273 mm, respectively (Table 1). The highest VPD values were registered in early stage II of fruit growth - about 3.56 kPa and 3.29 kPa in the first and second experiment, respectively (Fig. 2A). ETc was 580.3 mm and 576.5 mm during the first and second experiment, respectively.

Table 1. Reference evapotranspiration (ET_0), crop evapotranspiration (ETc) precipitation (mm), irrigation water applied and percentage reduction of water applied (%Red) to respect control in: regulated deficit irrigation (RDI) treatments during both experiments.

	Experiment 1		Experiment 2		Total	
	(mm)	%Red	(mm)	%Red	(mm)	%Red
ETo	1202		1183		2385	
ETc	580.3		576.5		1156.8	
Rainfall	678		273		951	
Control	563		697		1260	
RDI-1	480	-17	576	-21	1039	-21
RDI-2[z]	639	12	502	-39	1153	-9

[z]RDI-2 performed as a Control treatment in the first experiment.

Water relations and fruit growth

During the studied seasons, a similar pattern of Ψ_s was observed in both RDI strategies (Fig. 2E), reflecting the irrigation volume applied and the evapotranspirative demand. The Control treatment showed Ψ_s values close to -1.0 MPa during both experiments (ranging between -1.3 MPa in early stage II and -0.5 MPa during stage I). As regards RDI-1 during the first season, this treatment registered Ψ_s values below -2.0 MPa at the end of early stage II, while maximum differences of Ψ_s with respect to the Control (around 1.1 MPa) were registered in September (late stage II). In the second experiment, Ψ_s values in RDI-2 were lower than the corresponding Control values until the second-half of late stage II of fruit growth, and the greatest differences in Ψ_s compared with the Control ($\Delta_\Psi \approx 0.8$ MPa) were reached in August (early stage II). Coinciding with higher VPD values (Fig. 2A), RDI-1 also showed maximum differences of about 0.5 MPa with respect to the Control in early stage II. Related to this, the maximum values of the water stress integral (S_Ψ) were registered in early stage II in both RDI treatments (Fig. 3). RDI-1 presented an accumulated S_Ψ of 106.4 MPa*day during the first experiment, whereas the accumulated S_Ψ in RDI-2 was 27% higher than in RDI-1 during the second experiment (Fig. 3B).

Of note was the sharp fall in the Ψ_s trend of all treatments during both experiments in stage III (coinciding with the winter months), reaching less than –1.8 MPa. A strong dependence was established between the Ψ_s values of the Control (correctly irrigated trees) and the minimum temperature during the previous night [$\Psi_{s,md}$=0.465 ln (Tmin) - 2.347; r^2= 0.72, ($p<0.01$)] (Fig. 4).

In the first experiment, the g_{sm} and the A_{CO2}/g_{sm} ratios were sensitive to the deficit applied in RDI-1 during late stage II and late stage III to harvest, respectively. In the second experiment, the lowest values of A_{CO2} (≈ 4.0 µmol/m^2·s) and g_{sm} (≈ 50 mmol/m^2·s) obtained in RDI-2 during early stage II (summer) also underlined the effects of water restrictions on the physiological response of this cultivar (Fig. 5). However, the deficit imposed during stage I did not significantly influence the leaf gas exchange parameters (Fig. 5). The year effect was only significant at $p<0.05$ in the g_{sm} of the second experiment, which is consistent with the longer deficit applied in RDI-2 (Fig. 5D).

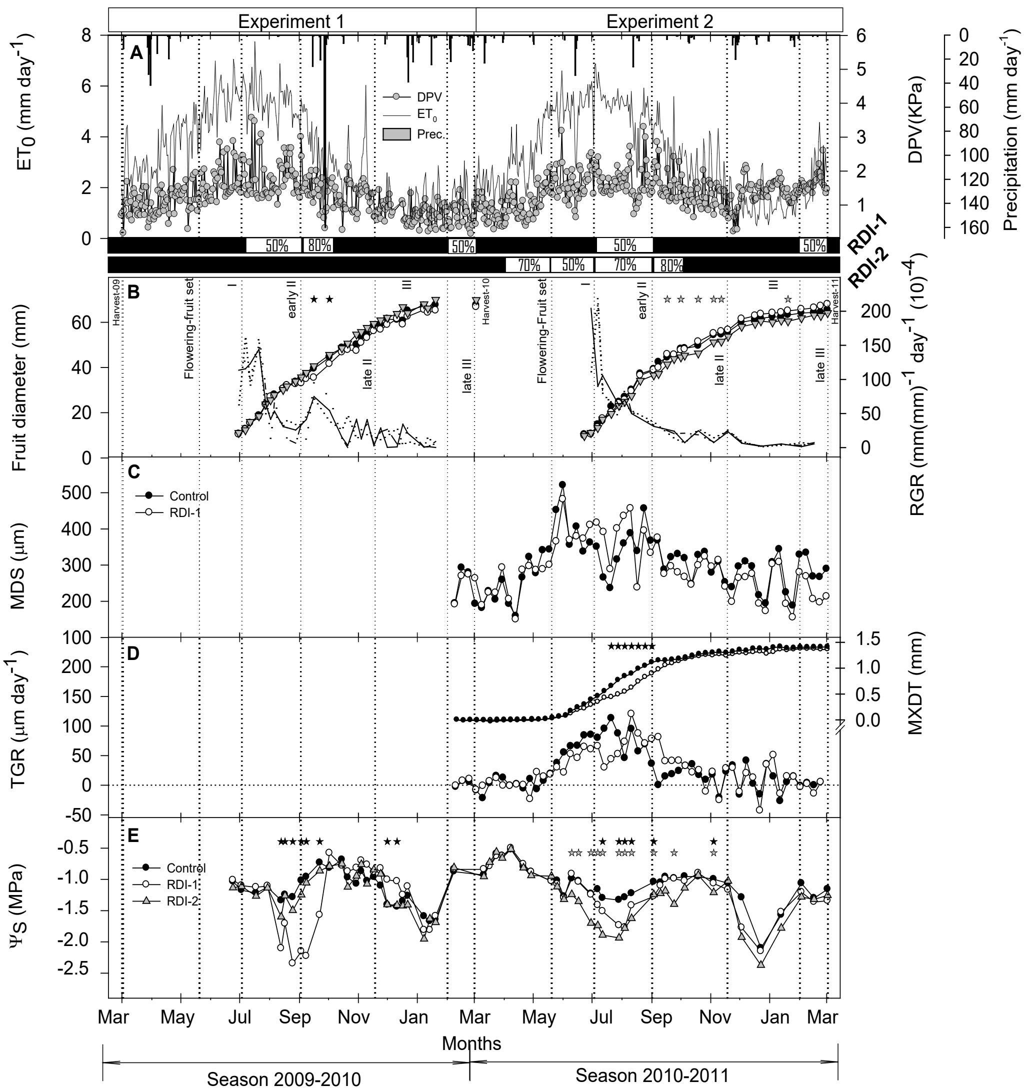

Figure 2. Seasonal evolution of (A) climatic parameters (VPD kPa, ET_0 mm/day and precipitation mm), (B) equatorial diameter (mm) and fruit relative growth rate (RGR mm/mm·day (10^{-4})), (C) maximum daily shrinkage (MDS µm), (D) maximum daily trunk growth (MXDT mm), trunk daily growth rate (TGR µm/day) and (E) midday stem potential (Ψ_s MPa) during the observation period. Each point is the average of 30 fruits, 6 leaves and 6 sensors per treatment. Vertical bars indicate the percentages of the irrigation applied compared with the Control in RDI-1 and RDI-2. The asterisks indicate significant differences with respect to the Control.

The most pronounced differences in TGR between Control and RDI-1 (around 30 µm/d) occurred when the MDS of the trunks in RDI-1 was 70 µm greater than in the Control (Fig. 2C). At that moment, the difference in Δ_Ψ was around 0.5 MPa (Fig. 2E). Despite the deficit applied in early stage II, TGR values in RDI-1 were significantly higher than in the Control in August as a result of summer rainfall events (Fig. 2A). Consistent with this, a compensatory increase in the MDXT of RDI-1 was observed, reaching similar values to the Control (Fig. 2D). Moreover, a good linear relationship was found between MDS and $\Psi_{s,md}$ [MDS= 1.39 $\Psi_{s,md}$ + 3.02; r^2= 0.88 (p<0.001)] during the study (Fig. 4). Reflecting the maximum differences in Ψ_s observed in the late stage II of the first experiment (Fig. 2E), fruit RGR in RDI-1 slowed down and the mean equatorial diameter fell by 10% compared with the Control value (Fig. 2B). However, the abundant rainfall registered in September (335 mm) promoted a higher fruit RGR in RDI-1 and, as a result, there was a compensatory effect on the equatorial diameter (Fig. 2B). In the second experiment, the maximum differences in Ψ_s compared with the Control were reached in early stage II (around 0.5MP and 0.8MPa in RDI-1 and RDI-2, respectively),

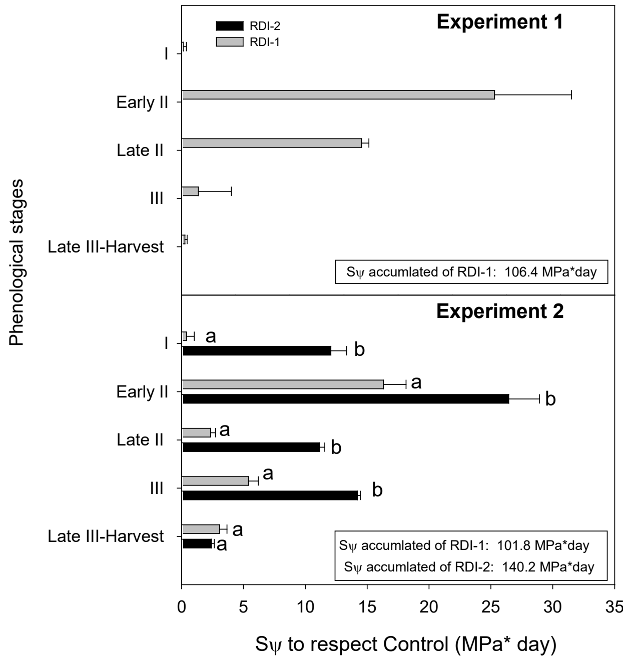

Figure 3. Accumulated water stress integral (S_Ψ, MPa*d) with respect to Control treatment of RDI-1 (▭) and RDI-2 (▬) treatments during each phenological stage of experiment 1 (A) and experiment 2 (B). Maximum values of $\Psi_{s,md}$ used were 0.58 and 0.49 MPa during the first and second experiments, respectively.

but had no negative effect on fruit growth (Fig. 2B). However, the moderate deficit applied ($\Delta_\Psi \approx 0.5$MPa) in RDI-2 during late stage II, promoted a significant decrease in the rate of fruit growth in this treatment (Fig. 2B). Consequently, the fruit diameter of RDI-2 at harvest was significantly lower (4.5%) than in the Control (Table 2).

Yield, resources distribution and irrigation water use efficiency

Non-significant differences in the means or variability of yield, fruit weight and fruit diameter were found among the three treatments assayed during both experiments (Tables 2 and 3). By contrast, the interaction treatment × season for fruit weight was significant (Table 3), and related to the significant increase in the number of fruits of RDI-2 trees in the second experiment compared with the rest of the combinations. The percentage of fruit set in the RDI-1 treatment was lower than in the Control (11 ± 5 and 40 ± 8%, respectively), but RDI-2 produced similar results to the Control (38 ± 5%) (Table S1 [suppl.]). A logarithmic relationship was found between total yield (Y) and fruit set percentage (FS): [$Y=66.98\cdot\ln(FS) -242.7$; $r^2=0.93$; $p<0.001$].

On the other hand, neither the irrigation treatment nor the experiment affected the distribution of resources (yield: TCSA ratio and the increment of TCSA) during this study (Table S2 [suppl.]). Nevertheless, during the second experiment the more severe RDI (RDI-2) resulted in significantly higher WUE values than were observed in the Control or RDI-1 treatments (6.44 *vs* 3.86 or 3.53).

Fruit quality at harvest and after storage conditions

In the first experiment, the fruit composition and skin color at harvest were affected by RDI but differences were not very pronounced for RDI-1, and any changes in TSS were significant (Fig. 6; Table 2 and 4). At harvest, RDI-2 showed the more vivid fruit (higher C* values) and RDI-1 the dullest (slightly lower C* values) (Fig. 6).

In the second experiment, RDI-2 TSS levels at harvest were above the corresponding Control levels (Table 2). Also, the statistics for the second experiment indicated a significant effect of temperature (T) as regards TA and hardness, together with a decrease in TA and a peak of hardness at the end of storage (Fig. 6). The interpretation of the former effect is that RDI-2 fruit were more acidic and softer than Control fruit irrespective of the storage condition (Fig. 6 and Table 4). In contrast, RDI-1 showed the highest peel percentage but only in the first experiment (Table 2).

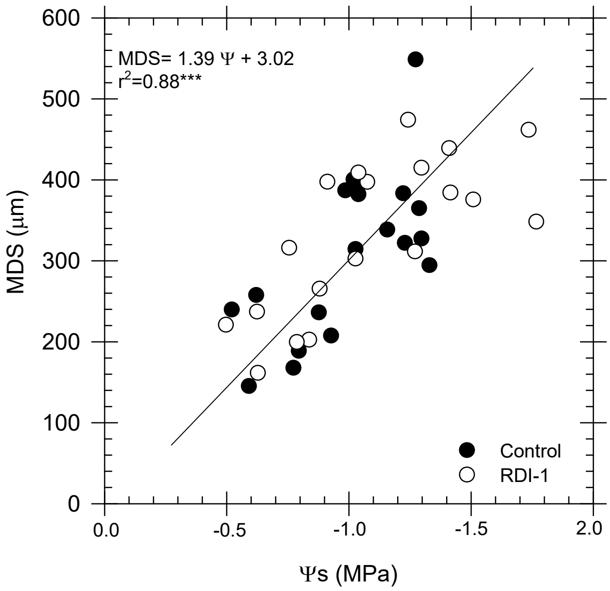

Figure 4. Linear relationship between maximum daily shrinkage (MDS) and midday stem water potential ($\Psi_{s,md}$). The black circles (•) are values from the Control treatment and the white circles (○) are values from RDI treatments.

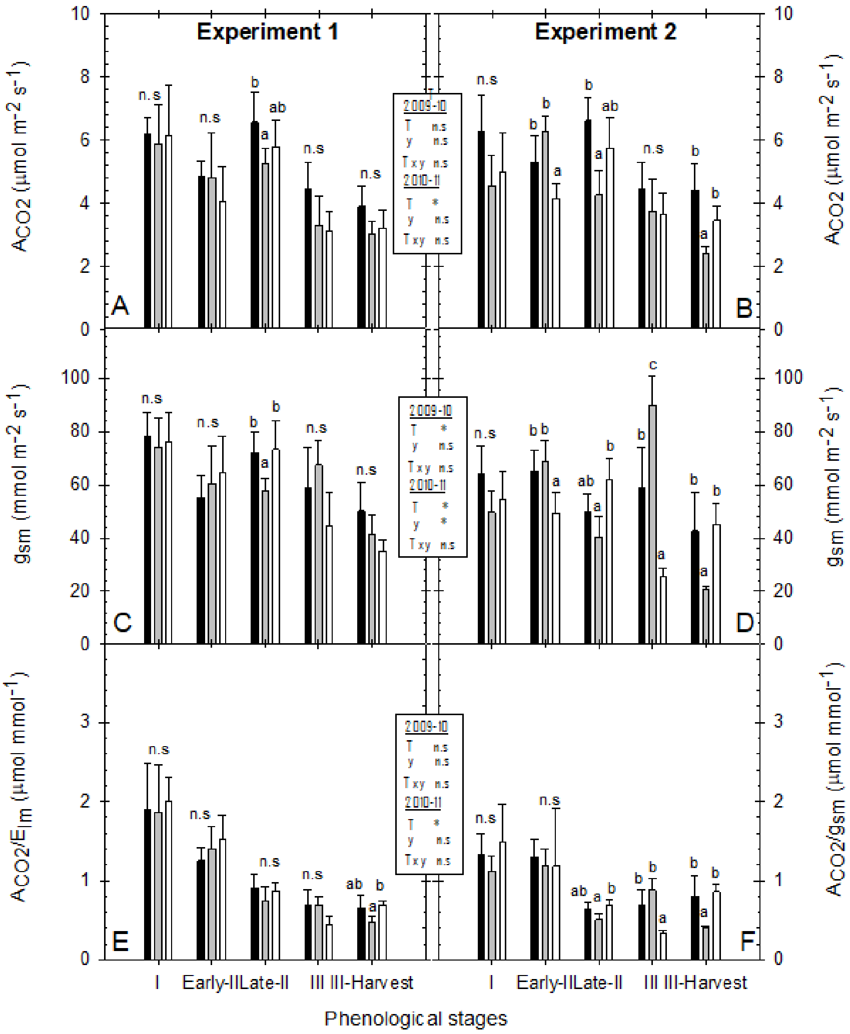

Figure 5. Mean values of gas exchange parameters for each phenological stage during both experiments: (A, B) maximum net CO_2 assimilation rate (A_{CO2}, μmol/m²·s), (C,D) maximum stomatal conductance (g_{sm} mmol/m²·s), and (E,F) instantaneous water use efficiency (A_{CO2}/E_m, μmol/mmol). The treatments were: Control (▬), RDI (▭) and RDI-2 (▭). The square indicates the results of a two-way analysis of variance using Treatment (T), year (y) as factors and T × y interaction.

During cold storage in the first experiment, the decrease in TA followed the same pattern as ascorbic acid, and the opposite to glutamic acid (Figs. 6A and 7), but did not follow the trend of citric acid (mean values of 26.6 ± 4 g/L throughout the experiment). Malic and succinic acids were not significantly affected at any time during the study period (means of 332.3 ± 21 and 550.1 ± 66, mg/L, respectively).

Discussion

The most undesirable period to reduce the irrigation dose was late stage II in both experiments, as also suggested by Domingo *et al.* (1996), because the differences in stem water potential (Δ_Ψ) of around 1.1 MPa and 0.5 MPa during the first and second experiment, respectively, were maximal, decreasing the fruit relative growth rate (Fig. 2). Application of a continuous water stress based on the signal intensity derived from equatorial diameter gave similar results to those obtained in the present experiments (Conesa *et al.*, 2014). Moreover, a moderate water stress applied in certain periods between April (flowering-fruit set) and July (stage I) could decrease the number of fruits per tree since could cause higher flower drop and, consequently, a poorer fruit set and yield (Tables 1 and S1 [suppl.]). Nevertheless, in early mandarin, González-Altozano & Castel (2003b) reported that RDI applied between flowering and fruit set significantly reduced the final

Table 2. Mean values of yield and quality parameters at harvest during both experiments.

Parameters	Experiment 1 (2009-2010)				Experiment 2 (2010-2011)			
	Control	RDI-1	RDI-2	p^z	Control	RDI-1	RDI-2	p
Yield (t/ha)	29.5 ± 1.41	18.6 ± 2.52	25.5 ± 1.45	NS	27.4 ± 8.71	19.1 ± 21.40	36.1 ± 9.42	NS
Number of fruits (fruits/tree)	572 ± 89	366 ± 129	455 ± 109	NS	492 ± 101ab	315 ± 125b	685 ± 102a	*
Fruit weight (g)	125.3 ± 15.71	128.6 ± 12.51	146.6 ± 8.41	NS	145.6 ± 12.11	151 ± 13.15	132.3 ± 7.89	NS
Fruit diameter (mm)	66.7 ± 2.51	67.5 ± 1.70	69.2 ± 2.45	NS	67.9 ± 1.15ab	68.4 ± 0.57b	65.3 ± 0.89a	*
Peel percentage (% w/w)	25.6 ± 0.51a	27.5 ± 0.78b	25.1 ± 0.49a	*	26.6 ± 0.72	24.0 ± 0.63	25.1 ± 0.83	NS
Juice percentage (% w/w)	46.9 ± 0.89	43.1 ± 3.51	46.5 ± 1.25	NS	42.6 ± 2.8a	51.3 ± 1.08b	46.4 ± 0.64ab	*
Total soluble solids (TSS, ºBrix)	12.2 ± 0.09	12.1 ± 0.07	11.5 ± 0.12	NS	12.9 ± 0.06a	13.6 ± 0.06b	14.2 ± 0.040c	*

Means within rows followed by different letters in each experiment are significantly different according to a Duncan multiple range test ($p \leq 0.05$). * and NS indicate significant or non-significant effects at $p \leq 0.05$, respectively. [z] ANOVA of the residuals^2 was not significant, indicating the absence of variability in these results.

Table 3. Analysis of variance of the yield parameters at harvest during both experiments using a treatment and season of study (of the experiment 1 and 2) as factors.

Source	d.f.	Sum of squares													
		Total yield (kg/tree)		Crop load (no. of fruits/tree)		Fruit weight (g)		Fruit diameter (mm)		WUE (kg/m^3)[x]		Yield/TCSA (kg/cm^2)[y]		Yield/ΔTCSA (kg/cm^2)[y]	
Treatment (T)	2	2228.62	NS	248482.0	NS	68.42	NS	8.41	NS	7.49	NS	0.0316	NS	101.35	NS
Season (S)	1	295.48	NS	566.16	NS	173.47	NS	2.43	NS	2.33	NS	0.0013	NS	8.58	NS
T × S	2	563.54	NS	144853.0	NS	2174.03	*	35.64	NS	13.63	NS	0.0078	NS	15.02	NS
Residual	12	4974.22		425993.0		3073.78		66.54		32.28		0.0762		1861.62	
Total variance explained (%)	17	61.8		65.8		64.1		62.9		63.3		60.6		51.6	

d.f.: Degrees of freedom. [x] Mean of irrigation water productivity efficiency as the ratio [yield/irrigation volume applied]. [y] Mean of production efficiency as the ratio [yield/trunk cross-section area] and the ratio of [yield/Δ trunk cross-section area].

yield, probably because the reserves are essential for the initial development of the small fruits (Pérez-Pérez *et al.*, 2008b).

The torrential rainfall event registered in September (335 mm) (Fig.2A) during the first experiment, very typical of Mediterranean conditions (Pérez-Pastor *et al.*, 2009), together with the restoration of irrigation in late stage II after this event, promoted a compensatory effect in the equatorial diameter of RDI-1 and RGR (Pagán, 2012). Romero *et al.* (2006) in 'Clementina de Nules' also reported an overgrowth during stage II after a severe deficit applied during stage I of fruit growth, due to a more negative potential in the fruits of the RDI treatments, which led to osmotic adjustment (Pérez-Pérez *et al.*, 2008b). In 'Búlida' apricot fruit, Pérez-Pastor *et al.* (2014) found a similar compensatory effect after restoring irrigation to Control levels during stage III due to the greater dry fruit growth rates with respect to fresh fruit growth rates that occurred during stages I and II.

Furthermore, González-Altozano & Castel (2003b) observed a compensatory growth in RDI fruits after water restrictions in stage I as long as the threshold values of Ψ_s did not exceed -1.2MPa. For their part, Ballester *et al.* (2011) indicated threshold Ψ_s values of around -1.3 and -1.5 MPa if fruit size reductions were to be avoided. In this sense, the values close to -1.8 MPa observed in RDI-2 during stage I and early II

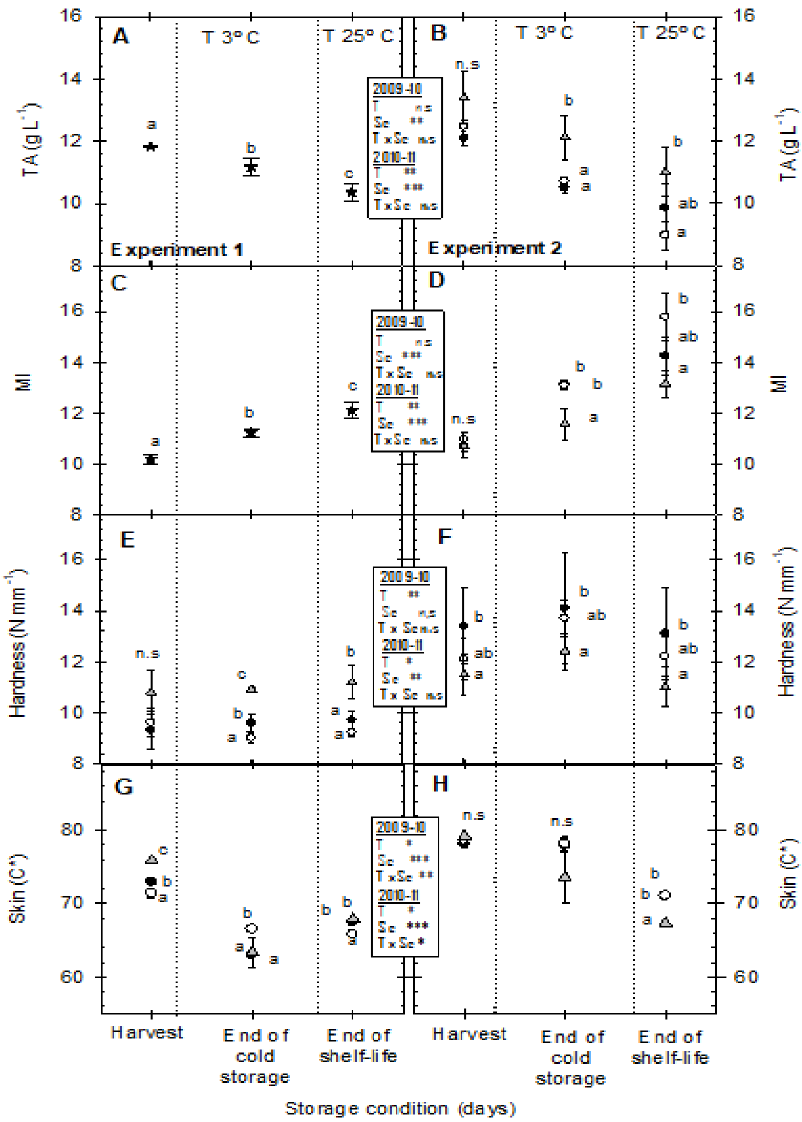

Figure 6. Evolution during both cold storage experiments of (A, B) titratable acidity (g/L); (C, D) maturity index (Total soluble solids*10/TA); (E, F) whole fruit hardness, (N/mm); (G, H) skin color (C* or chroma). Treatments were: Control, (•), RDI-1, (○), RDI-2 (△) or average per storage condition (✶) during both cold storage experiments. The average per storage condition was plotted when T or T × Sc was not significant. Each point is the mean of 3 replicates. Vertical black bars delimit the storage periods and indicate the temperatures assayed. The square indicates the results of a two-way analysis of variance using Treatment (T) or storage condition (Sc) as factors, and their interactions (T × Sc).

might have prevented higher RGR dynamics. Mandarin trees from the RDI treatments suffered a substantial decrease in Ψ_s during both growing seasons, although it was more pronounced during the periods that coincided with high evaporative demand (Ballester *et al.*, 2011). However, Gasque *et al.* (2016) found no negative effects on yield after RDI application when a threshold Ψ_s value of -2MPa was not exceeded in 'Navelina' citrus trees. Meanwhile, the lowest values of Ψ_s obtained in this study coincided with the winter period. Domingo *et al.* (1996) observed a similar decrease in leaf water potential in 'Fino' lemon during the same period, which might have been due to the lower temperatures reached by the soil in colder months (Terradas & Savé, 1992). In addition, the lower values of Ψ_s observed in RDI-2 would have been aggravated by the higher crop

Table 4. Analysis of variance of the quality parameters obtained in both experiments conducted in different seasons using treatments (Control, RDI-1, RDI-2) and storage conditions (at harvest, cold storage, cold storage plus additional shelf-life period) as factors.

Source	d.f.	Sum of squares													
		Skin (C*)		Peel percentage (% w/w)		Juice percentage (% w/w)		Hardness (N/mm)		TSS (ºBrix)		TA (g/L)		MI	
Experiment 1															
Treatment (T)	2	2.48	NS	22.4	NS	41.22	NS	12.59	**	0.59	NS	1.88	NS	0.67	NS
Storage conditions (Sc)	2	436.61	***	6.92	NS	89.02	NS	1.25	NS	1.45	NS	9.74	**	16.49	***
T × Sc	4	23.42	**	36.59	NS	319.95	*	3.12	NS	1.46	NS	0.87	NS	1.97	NS
Residual	18	20.27		239.06		411.21		11.68		3.90		12.15		6.96	
Total variance explained (%)	26	96.0		56.1		67.7		71.0		65.6		67.0		79.0	
Experiment 2															
Treatment (T)	2	37.11	**	14.17	NS	186.01	NS	16.85	*	1.87	NS	12.27	**	9.98	**
Storage conditions (Sc)	2	393.16	***	19.13	*	82.13	NS	9.03	**	1.18	NS	32.98	***	57.96	***
T × Sc	4	31.03	*	3.24	NS	267.45	NS	0.67	NS	1.20	NS	1.63	NS	5.62	NS
Residual	18	41.71		45.04		561.31		15.36		6.40		16.10		13.76	
Total variance explained (%)	26	92.3		64.4		66.2		73.2		62.5		79.7		86.4	

d.f.: degrees of freedom. TSS: total solid soluble content. TA: titratable acidity. MI: maturity index (MI= (TSS*10/TA). Means within columns followed by different letters were significantly different according to a Duncan multiple range test (p=0.05). *, **, ***: significant effect at p=0.05, 0.01, or 0.001, respectively. NS, non-significant.

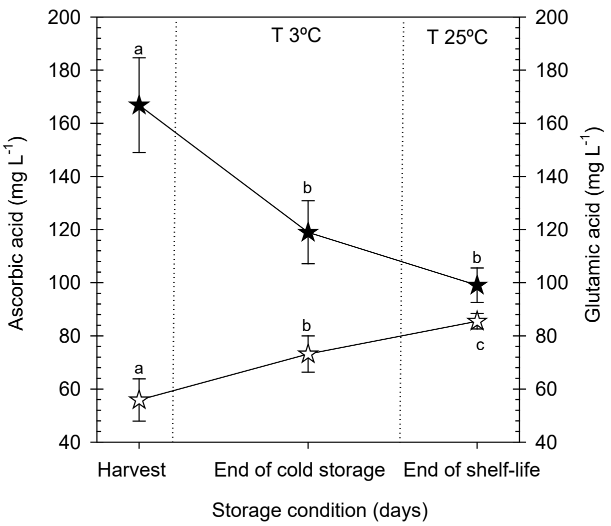

Figure 7. Juice ascorbic (★) and glutamic (☆) acid concentrations in the first experiment. Results are expressed as the mean ±SE (n=3 replicate × 3 treatments per storage condition; in mg/L) at harvest, after 50 d at 3 ºC, or after cold storage plus 3 d at 25 ºC). The effect of treatment or the interaction storage condition × treatment were not statistically significant at p<0.05 according to ANOVA. Vertical black bars delimit the storage periods and indicates the temperatures assayed.

load (Naor *et al.*, 2013). These findings suggest that the restoration of irrigation in RDI (applied until late stage II of fruit growth) can be considered as a good technique to recover the fruit size in adult mandarin orchards through compensatory effect.

In mature fruit trees under water deficit, an increase in MDS has been associated with a decrease in $\Psi_{s,md}$ (Ortuño *et al.*, 2010) which has also been found in this study. Both TGR and MXDT were slightly affected by the RDI strategies, as previously mentioned by other authors such as García-Tejero *et al.* (2012), probably due to the age of the trees and lower tissue elasticity, which is typical of adult trees (Egea *et al.*, 2009; de la Rosa *et al.*, 2013). In almond trees, MXDT was characterized by a sigmoid curve, as described by Nortes *et al.* (2005) (Fig. 2D). TGR was lower because of a reduction in the availability of carbohydrates for trunk growth as a result of the demand for photoassimilates of fruits (Pagán *et al.*, 2012). In line with this, MXDT was useful for restoring irrigation levels to Control levels at the end of early stage II, coinciding with a sharp decrease of the trunk growth. Consequently, the application of RDI strategies to adult mandarin trees grown in commercial orchards can be extended until late stage II when the competition between fruit and vegetative tissue for the resources decreases.

During RDI periods, gas exchange parameters (g_{sm} and A_{CO2}) showed lower values than the Control (Fig. 5), indicating that water losses were regulated via transpiration in response to water deficit (Pérez-Pastor *et al.*, 2009). Moreover, the A_{CO2}/E_m ratio increased in late stage II of RDI-2, meaning that carbon fixation was higher than losses of transpiration (Ehlenringer & Cook, 1984) (Fig.5E-F). This response has also been reported by other authors as a common feature in cultivated trees growing in Mediterranean climates in dry conditions (Pérez-Pastor *et al.*, 2009). In the case of g_{sm}, the interaction treatment × time of season was significantly different: it is known that in RDI treatments the stomatal closure is the gas exchange parameter most influenced by climatic conditions and by the changes in chemical signals such as abscisic acid or ethylene (Forner-Giner *et al.*, 2011).

The effects of RDI on fruit quality at harvest in citrus species are of great interest (González-Altozano & Castel, 2003a,b; Romero *et al.*, 2006; Pérez-Pérez *et al.*, 2009; Aguado *et al.*, 2012; Conesa *et al.*, 2014, Gasque *et al.*, 2016, Romero-Trigueros *et al.*, 2017), because some of them such as soluble solids or juice yield are minimal maturity indices and are strongly associated with postharvest flavour (Falagán *et al.*, 2015). The treatments assayed here apparently had more effect on external quality traits, such as hardness and skin chroma, than on internal compositional traits such as TA (Pérez-Pastor *et al.*, 2007) (Figs. 6 and 7). For example, RDI-2 in the second experiment resulted in slightly more acidic and softer fruit, both very important effects for mandarin fruit quality (Salvador *et al.*, 2006). More importantly, a shelf-life period of only 3 d at 25 °C was apparently sufficient to reduce the number of quality traits susceptible to present some differences among treatments compared with end of cold storage or at harvest (Figs. 6 and 7), in agreement with Conesa *et al.* (2014).

The trend of skin C* to decrease during cold storage in ´Fortune´ was associated with chilling damages during cold storage (Conesa *et al.*, 2014), probably as a result of degradation of the major peel mandarin pigments, such as the β-β-xanthophylls, the 9-Z-isomer of violaxanthinis or β-cryptoxanthin (Rodrigo *et al.*, 2013).

The loss of TA during cold storage in both experiments (Fig. 6), accompanied by a similar pattern in ascorbic acid (Fig. 7), also reported by Frías (2017) in ´Naveline´ orange, represent a very common trend in citrus due to their consumption as energetic substrates or their translocation to the skin (Murata, 1977b). Both trends could have been particularly exacerbated in this cold-sensitive cultivar (Conesa *et al.*, 2014) as a result of latent chilling damage during relatively long cold storage (Chalutz *et al.*, 1985). However, the main organic acid in citrus (citric acid) did not change in this experiment (see above), which could be associated with the variable behaviour of citric acid during postharvest cold storage (Murata, 1977a).

Finally, the apparent increase in glutamic acid during cold storage (Fig. 7) could also be explained by the link between proline (a typical stress metabolite that also increases during cold storage of mandarins according to Conesa *et al.* (2014) and the synthesis of other aminoacids such as glutamine during this period associated with proline levels (Malik *et al.*, 2013). Few studies have focused on organic acid metabolism in citrus fruit (Conesa *et al.*, 2014; Zhang & Xie, 2014). Apparently, severe water stress can affect citric acid metabolism and consequently producing changes in the citric acid content. However, our results provide evidence that RDI only produces transitory effects and we hypothesize that both fruit and plant can have mechanisms to avoid such dramatic changes in malic or citric acid composition, for example, mechanisms involving ascorbic and glutamic acid metabolism.

According to the obtained results in this work, the most undesirable period to apply RDI strategies would be the late stage II of fruit growth, this coinciding with the moment in which maximum differences in terms of stem water potential between treatments were reached. In the same vein, a moderate RDI strategy applied in adult mandarin trees between flowering and stage I of fruit growth resulted in a higher number of fruits per tree and higher irrigation water productivity with respect to the Control. Using MXDT to restore the irrigation to 100 % ETc at the end of early stage II resulted in water savings (20% of Control levels in the second experiment) without compromising yield or fruit quality at harvest. In this regard, most of the tested quality parameters were more affected by the length of cold storage than by RDI treatments. Moreover, the subsequent shelf-life period tended to minimize the differences among RDI and Control treatments found at harvest in some quality traits. Skin chroma, titratable acidity, ascorbic acid and glutamic acid were particularly affected by duration of storage conditions (chilling/length of cold storage biomarkers), while hardness was more affected by RDI, irrespective of the experiment considered.

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