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Influence of deficit irrigation timing on the fruit quality of grapefruit (*Citrus paradisi* Mac.)

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Abstract

The irrigation necessities for grapefruit production are very high. Due to the scarcity of water resources, growers use deficit irrigation (DI) - which could affect the fruit quality. Different DI strategies were studied: Control (irrigated at 100% ETc) and T1, T2 and T3 (50% ETc at phases I, II and III of fruit growth, respectively). Strategy T1 only delayed external maturation depending on the duration of the water stress. High water stress in T2 delayed fruit maturation, increased acidity and reduced the sugar concentration. Under T2, trees suffering moderate water stress showed increased flavonoid and phenolic contents but decreased lycopene levels. External maturation was delayed in T3 when severe stress occurred during the first part of phase III. Strategy T3 advanced internal ripening when moderate water stress occurred during the first 40 days of phase III, increasing sugar accumulation, promoted by the high acidity of the fruits. Moderate water stress also increased β-carotene, flavonoids and phenolics levels.

Keywords

Total soluble solids, acidity, maturity index, sugar accumulation, total phenolics, carotenoids, ascorbic acid, antioxidant activity

1. Introduction

Citrus is the most-economically-important fruit crop in the world and there is increasing demand for "high quality fresh citrus", driven by World Health Organization recommendations. In a broad sense, fruit quality in citrus is related with many physical properties and with the chemical components of the fruit, among them sugars, acids, flavour compounds, volatiles and other nutraceutical substances. It is well known that citrus fruits are rich sources of dietary fibre and bioactive compounds, including vitamin C, phenolics and flavonoids, with potential health-promoting properties (Ross & Kasum, 2002). Grapefruits in particular are a rich source of vitamin C, flavonoids,

carotenoids (provitamin A) and other nutraceutical compounds. These bioactive compounds are known to act as free-radical scavengers, to modulate enzymatic activity and to protect against a variety of diseases, particularly cardiovascular diseases and some forms of cancer (Pellegrini, del Rio, Colombi, Bianchi & Brighenti, 2003). Hence, the intake of these substances in the habitual diet is highly recommended because of their antioxidant properties. In this respect, the improvement of fruit quality has been a major breeding objective in the citrus industry. In recent years, research activities in agriculture have been aimed at finding new production strategies for improving the nutraceutical properties of products.

The limited water supply worldwide makes it necessary to reduce the quantity of water used for irrigation; for example, by applying management strategies focusing on the maximisation of the water-use efficiency. Deficit irrigation (DI) is based on the application of amounts of irrigation water lower than those needed by the crop (Romero et al., 2006). Under water deficiency, fruit quality is affected (Yakushiji, Morinaga & Nonami, 1998) and the main effects are reflected in organoleptic fruit parameters due to changes in juice properties, such as increases in sugar concentrations and acidity (Chartzoulakis, Michelakis, & Stefanoudaki, 1999). Several studies have shown the advantages of DI strategies with regard to improving the fruit quality in citrus trees (Aguado, Frías, García-Tejero, Romero, Muriel, & Capote, 2012; Pérez-Pérez, Robles & Botía, 2009). The sugar accumulation in mandarin fruits due to water stress is not caused by dehydration, but rather by active osmoregulation - glucose and fructose being largely responsible for this (Yakushiji et al., 1998). Not only classical parameters such as sugar content or juice acidity are affected by water deficit, Navarro, Pérez-Pérez, Romero & Botía (2010) found significant increments in the ascorbic acid content,

although other authors - such as Buendía, Allende, Nicolás, Alarcón & Gil (2008) - did not find a direct relationship between DI and the content of vitamin C.

In other cases, the fruit quality has been found to decrease due to DI (Mougheith, El-Ashram, Amerhom, & Madbouly, 1977). For example, Clementine citrus trees exhibited decreased fruit quality, depending on the phenological stage at which the DI was applied (Romero et al., 2006; Navarro et al., 2010). Several authors have pointed out the great importance, with respect to fruit quality, of the growth periods during which DI is imposed (García-Tejero, Romero, Jiménez, Martínez, Durán & Muriel, 2010; Navarro et al., 2010). During the final fruit-growth period and the beginning of the ripening period, DI improves the fruit quality by increasing the values of some important quality parameters regarding taste, flavour and nutrition, particularly: the acidity and sugar content, through active osmotic adjustment (Sanchez-Blanco, Torrecillas, León & del Amor, 1989; Ginestar & Castel, 1996), and the non-reducing/reducing sugars ratio, total phenolic acids and lycopene (Navarro et al., 2010). Although DI during the active fruit-growth phase also could increase acidity, sugars and some bioactive compounds, it produces an important delay in the maturation process (Navarro et al., 2010). As well as the phenological phase when the DI is introduced, the effects of water deficits also depend on the intensity and duration of the water-stress period. Noteworthy results suggest that a severe DI during fruit maturity improves juice quality without lowering the maturity index (García-Tejero et al., 2010). Depending on the water-stress level reached during the fruit development, DI promotes osmotic adjustment in fruits by passive accumulation of soluble sugars and/or by active carbon import into fruits (Navarro et al., 2010), the fruit acidity playing an important role in these processes (Hockema & Etxeberría, 2001).

Grapefruit (*Citrus paradisi* Macf.), considered a profitable alternative to other, more-widely-cultivated citrus crops, needs a very-high amount of irrigation for fresh fruit production, forcing citrus growers to reduce the water applied. Thus, we have evaluated the sensitivity of the most-important fruit quality parameters of grapefruit to the application of DI, in order to increase the value-added properties of these fruits as a source of juice with nutritionally-important qualities. The objective of this study was to identify the alterations in the fruit quality of 'Star Ruby' grapefruit trees and evaluate the sensitivity of different fruit-growth phases to DI strategies during two consecutive years. This work is part of a study to evaluate the agronomical and physiological responses of 'Star Ruby' grapefruit to the same DI strategies during this period of time (Pérez-Pérez, Robles & Botía, 2014).

2. Material and methods

2.1. Plant Material

The study was carried out during 2007 and 2008 at the experimental station of the IMIDA in Torre Pacheco, Murcia (South-eastern Spain). The experiment was performed on 14-year-old 'Star Ruby' grapefruit trees (*Citrus paradisi* Mac.) grafted on Cleopatra mandarin (*Citrus reshni* Hort. Ex Tanaka) rootstock. More details of orchard characteristics and climatic data during the experimental period are given in Pérez-Pérez et al. (2014). The lay-out of the experiment took the form of three completely-randomised, selected plots. Each treatment consisted of nine trees (three trees per treatment and plot). There were four irrigation treatments, a control and three deficit-irrigation treatments, which were applied in two consecutive years. The control treatment involved irrigation during the whole season at 100% ETc. The DI treatments consisted of irrigation at 100% ETc, except during different phenological stages of fruit growth (T1: phase-I, cell division, just after bloom and fruit set; T2: phase-II, cell

elongation, rapid fruit-growth period; T3: phase-III, final fruit-growth period, ripening and harvest) when irrigation was applied at 50% ETc. To determine the levels of water stress, the midday stem water potential (Ψ_{md}) was measured as previously described (Pérez-Pérez et al., 2014). The water-stress integral was calculated using the Ψ_{md} data, according to the equation defined by Myers (1988):

$$S_{\psi} = \left| \sum_{i=0}^{i=i} (\overline{\Psi}_{i,i+1} - c)n \right|$$

where S_{Ψ} is the water stress integral (MPa·day), $\Psi_{i,j+1}$ is the average Ψ_{md} for any interval i,i+1 (MPa), *c* is the maximum Ψ_{md} measured during the study and *n* is the number of days in the interval.

2.2. Samples

When fruits had reached commercial size (1st December 2007 and 2nd December 2008), nine fruits per tree were collected randomly from six trees per treatment, to analyse fruit quality. The fruits were squeezed with an electric fruit juicer and the juice strained with a 2-mm-mesh sieve. The nine fruit extracts from the same tree were combined and considered as a single sample for chemical analysis. Thus, six samples of nine fruits were analysed separately for each treatment. The juices were placed in separate, sealed amber vials and kept frozen at -80 ºC before analysis, taking one vial of each sample for each chemical determination.

2.3. Chemicals

The 2,2´-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) was obtained from the Fluka Chemical Co., and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), sucrose, fructose, glucose, catechin and Folin-Ciocalteu reagent from the Sigma-Aldrich Chemical Co. Other reagents were of analytical grade.

2.4. Fruit colour

The external fruit colour was measured in nine fruits per treatment, using a tri-stimulus colour difference meter (Minolta CR-300 colorimeter), on three locations

around the equatorial plane of the fruit. The Hunter parameters *a* and *b* were used, and colour was expressed as *a/b* since this ratio has a high correlation with the visual appreciation of the fruit colour.

2.5. Juice yield, TSS, TA and TSS/TA ratio

The titratable acidity (TA), total soluble solids (TSS) and juice yield were determined according to conventional methods (Kimbal, 1999). The maturity index (MI) was expressed as the TSS×10/TA ratio.

2.6. Carotenoids determination

Carotenoids were determined according to the method of Nagata & Yamashita (1992), with the modifications made by Navarro et al. (2010). Briefly, 5 mL of fruit juice were mixed with 20 mL of acetone-hexane (4:6). Automatically, two phases separated and an aliquot was taken from the upper phase for measurement of the optical density at 663, 645, 505 and 453 nm in a spectrophotometer. Lycopene and β-carotene were calculated according to the equations of Nagata & Yamashita (1992):

$$\text{Lycopene (mg/100 mL)} = -0.0458\ A_{663} + 0.204\ A_{645} + 0.372\ A_{505} - 0.0806\ A_{453}$$

$$\beta\text{-Carotene (mg/100 mL)} = 0.216\ A_{663} - 1.22\ A_{645} - 0.304\ A_{505} + 0.452\ A_{453}$$

Lycopene and β-carotene were expressed as mg L^{-1}, by multiplying the concentrations obtained from the Nagata & Yamashita equations by a factor of 40.

2.7. Sugar determination

The concentrations of soluble sugars (glucose, fructose and sucrose) in the fruit juice were determined by HPLC (Merck Hitachi), as described by Navarro, Flores, Garrido & Martinez (2006). Samples were passed through a Sep-Pak C_{18} cartridge, preconditioned with methanol (4 mL) and water (10 mL), to remove interfering compounds. Before use, the residual water in the cartridge was expelled with air. The first 2 mL of each

sample were discarded and the next 1 mL was used for analysis, after filtration through a 0.45-µm Millipore filter. The HPLC analysis was performed using a LiCrospher 100 NH_2 5 µm column, coupled with a refraction index detector. The program used an isocratic mobile phase of acetonitrile:water (85:15), with a flow rate of of 1 mL min^{-1}. The calibration curves ranges were 100–5000 mg L^{-1} for glucose (r^2 = 0.996), fructose (r^2 = 0.996) and sucrose (r^2 = 0.995).

2.8. Ascorbic acid

Ascorbic acid analysis in the fruit juice was performed according to the Official Methods of Analysis for Ascorbic Acid in Vitamin Preparations and Juices (method number 967.21, chapter 45, AOAC), with the modifications of Tounsi et al. (2010). Fruit juice samples were centrifuged at 1650 g for 5 min at 4 ºC. The supernatant was used for determination of the ascorbic acid content by titration with 2,6-dichlorophenolindophenol sodium salt hydrate (DIF). The results were expressed as mg ascorbic acid mL^{-1} juice.

2.9. Determination of total flavonoids

The content of total flavonoids was measured according to Dewanto, Wu, Adom and Liu (2002), with the modifications of Tounsi et al. (2010). Briefly, 250 µL of juice extract (diluted 1:1 with 80% methanol) were mixed with 75 µL of $NaNO_2$ (5%). After 6 min, 150 µL of 10% aluminium chloride were added and, 5 min later, 500 µL of 1 M NaOH. Finally, the mixture was adjusted to 2.5 mL with distilled water. The absorbance versus a prepared blank was read at 510 nm. The total flavonoid content of the samples (three replicates per treatment) was expressed as µg L^{-1} of catechin equivalents. The calibration curve range was 50–500 µg L^{-1} (r^2 = 0.996).

2.10. Extraction and determination of total phenolics

The total phenolics were determined by the Singleton, Orthofer & Lamuela-Raventos (1999) method. Briefly, 1 mL of fruit juice was extracted with 9 mL of 80%

methanol for 30 min at room temperature. After centrifugation at 5000 rpm for 10 min, the supernatant was taken for the determination of total phenolics by the Folin–Ciocalteu method. A reagent blank using H_2O was prepared. Folin–Ciocalteu phenol reagent (0.5 mL) was added to the mixture and it was shaken vigorously. After 5 min, 5 mL of 5% Na_2CO_3 solution were added, mixing at the same time. The solution was immediately diluted to 25 mL with distilled water, mixed thoroughly and then allowed to stand for 60 min before measurement: the absorbance was measured at 750 nm *versus* the prepared blank. The total phenolic content of the sample (three replicates per treatment) was expressed as mg L^{-1} of p-coumaric acid equivalent. The calibration curve range was 40–200 µg mL^{-1} ($r^2 = 0.994$).

2.11. Antioxidant activity determination

The test used to determine the antioxidant capacity of the fruit juice was the $ABTS^{\bullet+}$ radical cation assay, using Trolox to standardise the system (Miller, Rice-Evans, Davies, Gopinathan & Milner, 1993). The $ABTS^{\bullet+}$ radical cation was prepared by adding excess manganese dioxide (Sigma Chemical Co.) to a 5 mM aqueous stock solution of ABTS. This solution was diluted in 5 mM phosphate-buffered saline (PBS), pH 7.4, and pre-incubated at 30 ºC prior to use. The system was standardised by means of Trolox (Miller et al., 1993; Miller, Sampson, Candeias, Bramley, & Rice-Evans, 1996). After the addition of 1.0 mL of $ABTS^{\bullet+}$ radical solution to aliquots of Trolox or the juice extracts, the solutions were vortex-mixed for exactly 30 s and the absorbance at 734 nm was taken, exactly 30 min after initiation of mixing, in a spectrophotometer. A dose–response curve was derived for Trolox by plotting the absorbance at 734 nm as a percentage of the absorbance of the uninhibited radical cation solution (blank), according to the equation:

Inhibition of A_{734} (%) = $(1-A_f/A_0) \times 100$;

where A_0 is the absorbance of the uninhibited radical cation and A_f is the absorbance measured 30 min after the addition of the antioxidant sample.

Each sample was analysed in triplicate and referenced to a Trolox dose-response curve. The Trolox Equivalent Antioxidant Capacity (TEAC index) was calculated as the concentration (mM) of Trolox in phosphate buffer and shown as the antioxidative potential equivalent to the mL of juice used, at the 30th reaction minute.

2.12. Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) (SPSS 7.5.1 for Windows, standard version, 1996), with four treatments and six replicate trees per treatment. When there was a significant difference (P-value < 0.05), means were separated using Duncan's test.

3. Results and discussion

3.1. Fruit ripening

Although, in general, external and internal ripening coincide, the peel and pulp behave in many respects as separate organs and thus can be considered as distinct, physiologically. Under deficit irrigation, the delay or advance of the fruit maturation depends, among other things, on the stage of the fruit growth when it is applied, since it can affect the balance of titratable acidity and total soluble solids (Romero et al., 2006; Navarro et al., 2010; Aguado et al., 2012; Pérez-Pérez et al. 2009, 2014).

Water stress applied during phase I (treatment T1) delayed grapefruit maturation in 2007 but not in 2008, probably due to the higher accumulated water stress at the end of phase I in 2007 relative to 2008 (15.5 and 13.8 MPa·day, respectively, Figure 1). Since the maturation process starts at the end of phase II, the high accumulated water stress at the end of this period in T2 trees (69 and 96 MPa·day in 2007 and 2008, respectively,

Figure 1) could be the cause of the low a/b ratio found in T2 fruits. In contrast, T3 delayed external fruit ripening during 2008 but not in 2007; but, in this case, the water stress in the first part of phase III was higher in 2008 than in 2007 (-2.0 and -1.7 MPa, respectively, Pérez-Pérez et al., 2014). According to these results, the most important delay in the external fruit maturation was produced when DI was imposed during fruit growth. However, it is during the maturation phase when the peel colour changes markedly (chloroplasts, containing carotenoids and chlorophyll, are transformed into chromoplasts, containing only carotenoids, Huff, 1984). The peel colouration of citrus fruits is governed by environmental and nutritional factors acting through the action of different hormonal signals, such as gibberellic acid, ethylene and abscisic acid (Rodrigo, Alquézar, Alós, Lado & Zacarías, 2013). Soil drying increases the ABA concentration in leaves (Zhang & Davies, 1990), so the high water stress reached during phase II of fruit growth could have modified the hormonal composition and affected the colour break in these fruits, resulting in a delay of external maturation.

The maturity index (MI, the balance between the TSS and the acidity) is a useful way to determine the internal fruit maturation, since, during ripening, the titratable acidity (TA) decreases (due to catabolism of citric acid and a dilution effect) and the sugars (usually expressed as TSS) increase. In this experiment, fruits were harvested on the same date, so the MI reflects the state of the internal ripening process of the fruit in each treatment.

Water stress applied during the early stages of fruit growth (T1) did not affect the internal fruit maturation and did not modify fruit quality parameters such as TSS, TA or juice percentage (Figure 3). Although external maturation was delayed by treatment T2 in both years, only in 2008 did this treatment significantly delay internal fruit maturation (Figure 3). The TA was increased by T2 in both years, but only in 2008 did

the TSS decrease - resulting in a general decrease in the MI in this year (Figure 3). High TA of the juice has also been found in trees subjected to low-moisture conditions (Mukai, Takagi, Teshima & Suzuki, 1996; Navarro et al., 2010). During the first half of phase II, developing fruits accumulate a considerable amount of organic acids in the vacuoles of the juice sac cells, which are gradually catabolised over the second half of phase II through phase III (Iglesias et al., 2007). So, the higher TA observed in T2 fruits, with respect to control fruits, was probably due to the higher accumulated water stress during phase II (Figure 1). This could have decreased the acid catabolism during the second half of phase II and phase III and led to a concentration effect due to the passive dehydration of the juice sacs. The T2 treatment produced a 15% reduction in fruit size at the end of phase II (data not shown) and passive dehydration of the juice sacs, leading to a significantly lower juice percentage (Figure 3).

The water stress applied in the T3 treatment was only effective during the first part of phase III, in both years. An average of -1.6 MPa was maintained during approximately 40 days in 2007, whereas in 2008, due to earlier rains, an average of -1.8 MPa was maintained only during the first 20 days (Pérez-Pérez et al., 2014). In spite of the rain, the accumulated water stress in treatment T3 during phase III (Figure 1) was high enough to alter the TSS and acid balance. During 2007, the TSS and TA were higher in T3 than in control fruits (Figure 3), but not due to a concentration effect since dehydration seems to have been absent. Passive dehydration of the juice sacs of citrus fruits is not the primary cause of differences in sugar accumulation among irrigation treatments (Barry, Castle & Davies, 2004; Yakushiji et al., 1998). The increase of TA found in T3 fruits could be related with a decrease in the acid catalysis that takes place throughout phase III. So, some mechanisms involved in acid degradation and in the metabolism of sugars during phase III were affected. The physiological roles of organic

acids could be related to sugar accumulation since vacuolar pH is related to sink strength (Hockema & Etxeberria, 2001).

3.2. Soluble sugars

Only the DI treatment applied during phase III (T3) in 2007 significantly increased the sugars in the juice of the fruits (Table 1). Drought stress imposed towards the end of fruit growth and expansion (phase III) increases fruit soluble solids without jeopardizing fruit size (Mills, Behboudian & Clothier, 1996); consequently, active osmotic adjustment may occur during this stage, as observed herein.

The accumulated water stress experienced by T3 trees in 2007 (63 MPa·day, Figure 1) produced an active accumulation of glucose (G), fructose (F) and sucrose (S), the three sugars contributing to the fruit osmotic adjustment, and, consequently, more total sugars than in control fruits. The increased partitioning of photosynthates to fruit is due to the higher sink strength (Hockema & Etxeberria, 2001; Yakushiji et al., 1998), determined by the ability of a sink to metabolise or store S. Sucrose synthase (SuSy) cleaves incoming S from the phloem, amplifying the gradient of sugar between the cytosol and the translocation stream. Drought stress significantly increased SuSy activity in these fruits, helping to achieve osmotic adjustment (Hockema & Etxeberria, 2001). On the other hand, lowering of the pH by drought stress triggers a cascade of events that leads to increases in the rate of total carbon import (Yakushiji et al., 1998; Hockema & Etxeberria 2001), due to two factors: higher rates of acid-mediated S hydrolysis in the vacuole (Sinclair, 1984) and a greater ability to sequester S into the vacuole due to a larger electrochemical potential gradient (Hockema & Etxeberria, 2001). Thus, the higher acidity values found in T3 fruits (Figure 3) might have accelerated both processes, increasing the sink strength of the fruit cells. So, both pH and SuSy could have been involved, both directly and indirectly, in the increase of the total carbon

import into T3-stressed fruits: as a result of these processes, the concentrations of F, G and S would increase, as was observed (Table 2). In these fruits, the S hydrolysis rate was higher than that of its synthesis, lowering the non-reducing/reducing sugars ratio (Table 1). In 2007, the T2 fruits also showed a lower ratio but, although the higher acids concentration was able to promote S hydrolysis (increasing the concentrations of F and G to maintain the osmotic gradient), in this case, these fruits did not accumulate S, leading to a decrease in the non-reducing/reducing sugars ratio (Table 1). In previous work with mandarin trees, high water stress increased the reducing sugars, whereas lower water stress increased the non-reducing sugars (Navarro et al., 2010).

A general decrease in the sugar content of the fruit juice was observed in T2 fruits in 2008 (Table 1). These fruits had significantly less F, G and S and, consequently, lower levels of total sugars than control fruits. Although treatment T2 produced an increase in fruit acidity, this did not affect the mechanisms of S cleavage in the vacuole in the same way or the S accumulation described above. During the last half of phase II, the trees had to cope with high water stress for longer and the accumulated water stress at the end of this period was higher in 2008 than in 2007 (96 and 69 MPa·day, respectively, Figure 1). These differences in S_Ψ at the end of phase II could have produced differences in sugar accumulation between the two years. In 2008, no import of sugars into the fruit was detected. Perhaps the longer phase II activated other pathways of sugar metabolism in the fruit cells, such as respiration, accelerating G and F degradation.

In general, water stress applied during phase I did not modify the F, G or S concentrations in fruit juice or the balance between non-reducing and reducing sugars (Table 1).

3.3. Lycopene and β-carotene.

Both lycopene and β-carotene are intermediates in the carotenoid biosynthesis pathway, β-carotene being a product of lycopene. The effects of reduced water availability on carotenoid accumulation have not been widely studied in citrus. In tomato fruits, drought stress consistently increases the lycopene and total carotenoid contents (Zushi & Matsuzoe, 1998) and also the β-carotene content (Favati, Loveli, Galgano, Miccolis, Di Tommaso, Candido, 2009). However, the carotenoids profile is different between tomato and citrus fruit. During tomato fruit ripening, there is massive accumulation of lycopene whereas xanthophylls accumulation is promoted in citrus fruits (Kato, Ikoma, Matsumoto, Sugiura, Hyodo & Yano, 2004). Previous studies involving greater water stress during phase II than in the present study pointed to lycopene and β-carotene accumulation in mandarin juice (Navarro et al., 2010). However, in our study, T2 fruits had lower lycopene concentrations in both years and a lower β-carotene concentration in 2008 than control fruits (Table 2). This could have been due to lower synthesis of these compounds, not diminished transport from other tissues, since the flesh can synthesise carotenoids rather than acquire them by transportation (Xu, Tao, Liu & Deng, 2006). No dilution effect was observed in these fruits; in fact, the juice percentage decreased (Figure 3). Probably, the concentrations of some lycopene precursors (such as phytoene) were decreased, or some enzymes involved in lycopene synthesis (phytoene desaturase or ζ-carotene desaturase) were deactivated, leading to low lycopene and β-carotene concentrations in these fruits.

In 2007, T3 fruits had higher β-carotene concentrations than control fruits (Table 2) - due to new synthesis, since the juice percentage of T3 fruits was not modified relative to the control fruits (Figure 3). The cyclisation of lycopene is a crucial branching point in the carotenoid biosynthesis pathway, yielding β,ε-carotenoids or β,β-xanthophylls (Kato et al., 2004). During the ripening of citrus fruits, the β,ε-carotenoid concentration

decreases while the β,β-xanthophylls are accumulated in the flavedo and juice sacs (Kato et al., 2004). One of the first products of this pathway is β-carotene. The high accumulated water stress in T3 trees during phase III in 2007 (Figure 1) probably affected the carotenoid biosynthesis pathway, promoting the β,β-xanthophyll pathway over the β,ε-carotenoid pathway.

3.4. Total flavonoids, total phenolics, ascorbic acid and antioxidant capacity of the grapefruit juice.

The total flavonoids concentration in the grapefruit juice was significantly increased in T2 fruits (Table 2), probably due to a concentration effect since these fruits had lower juice percentage than control fruits (Figure 3). Greater biosynthesis of flavonoids could have been the cause of their increase in T3 fruits, with respect to control fruits in 2007 (Table 3), as a response to the accumulated water stress suffered during the phase III of 2007 (Figure 1). Water stress stimulates the flavonoids biosynthesis pathway in grapevines and table grapes by upregulating some key regulatory enzymes implicated in flavonoids biosynthesis (Castellarin, Matthews, Di Gaspero, & Gambetta 2007).

The T2 fruits had 25% more total phenolics, compared with the control (Table 2). Similar to the total flavonoids, the increase in total phenolics in T2 fruits was probably due to both the lower juice content and new synthesis. Similar results have been found previously in Clemenules mandarin with DI applied during phase II (Navarro et al., 2010). Water stress activates phenolic compound biosynthesis in fruits suffering DI (Gómez-Rico et al., 2007; Stefanelli, Goodwin, & Jones, 2010).

In the present work, the ascorbic acid content of the fruit juice of 'Star Ruby' grapefruits was not modified by the water stress treatments (Table 2). Although some studies describe how low water availability increased the ascorbic acid content in mandarin (Navarro et al., 2010) or in tomato fruits (Favati et al., 2009), other studies

show that the vitamin C content of fruits was not influenced by DI (Buendía et al., 2008).

The antioxidant activity of fruits and vegetables is important for assessing their nutritional value. The antioxidant activity of the fruit juice was decreased in T1 fruits in 2007, whereas no clear effect was found in any of the other treatments in 2007 and 2008 (Table 2). In spite of this, we found a high, positive correlation ($P < 0.001$) of the total phenolics and total flavonoids with the TEAC index ($r=0.71$ and $r=0.57$, respectively), confirming that, of the compounds determined in this study, they contributed the most to the antioxidant activity. Our data agree with those of other authors, who showed that a high total phenol content increases the antioxidant activity (Tounsi et al., 2011).

4. Conclusions

In general, when DI was imposed during the first stages of fruit development, fruit quality was not modified. Only when the water stress levels reached in treatment T1 were high enough was external fruit maturation delayed.

Application of DI during phase II was critical for fruit quality and its effects depended on the water stress intensity and duration. Severe water stress during phase II delayed external fruit maturation, but only delayed internal ripening in 2008 - due to the decline in sugars levels in T2 fruits. Also, acids, total flavonoids and total phenolics were increased in T2 fruits. Lycopene decreased in both years but β-carotene only did so in 2008, when the water stress lasted longer.

Phase III is also a critical period regarding the application of DI strategies. Water stress maintained during the first part of phase III promoted a greater accumulation of sugars than of acids in T3 fruits, bringing forward internal fruit ripening, due to the active accumulation of sugars - which contributed to fruit osmotic adjustment. Also, β-carotene, total flavonoids and total phenolics increased in the grapefruit juice.

The DI strategies affected the fruit quality parameters in different ways, depending on the stage of fruit growth and also on the water stress level reached in each phase. When DI was applied during phase I, the quality parameters were not affected but, depending on the stress duration, it affected external maturation. The application of DI during phase II improved some quality parameters but delayed maturation. Water stress during phase III improved the fruit quality by increasing the values of some important quality parameters, and advanced the maturation process when the stress was long enough.

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Legends of figures

Figure 1. Values of the water stress integral (S_Ψ) at the end of Phase I, Phase II and Phase III. Irrigation treatments: Control and three deficit irrigation (DI) treatments: T1 (phase-I), T2 (phase-II) and T3 (phase-III), during the experimental period (2007 and 2008).

Figure 2. External fruit colour (Hunter a/b) of 'Star Ruby' fruits in relation to the DI treatments: Control, T1 (phase-I), T2 (phase-II) and T3 (phase-III), during the experimental period (2007 and 2008).

Figure 3. Titratable acidity (TA), total soluble solids (TSS), maturity index and juice content (%) of 'Star Ruby' fruits in relation to the DI treatments: Control, T1 (phase-I), T2 (phase-II) and T3 (phase-III), during the experimental period (2007 and 2008).

Table 1. Effect of DI on the concentrations of total sugars, fructose, glucose and sucrose (g L^{-1}) and on the non-reducing/reducing sugars ratio in juice of 'Star Ruby' fruits in relation to the DI treatments: Control, T1 (phase-I), T2 (phase-II), T3 (phase-III), and during the experimental period (2007 and 2008).

	Total sugars	Fructose	Glucose	Sucrose	Non-reducing /reducing sugars
			2007		
Control	64.8b	14.0b	14.3b	36.5b	1.30a
T1	65.9b	13.9b	14.2b	37.4b	1.31a
T2	68.7b	15.3ab	16.4a	36.4b	1.13b
T3	75.7a	16.8a	17.3a	40.8a	1.17b
ANOVA	***	**	***	***	***
			2008		
Control	66.1ab	14.1a	14.1b	38.0a	1.35a
T1	67.1a	14.3a	14.8a	38.0a	1.30a
T2	61.9c	12.8b	13.3c	34.9b	1.30a
T3	64.7b	14.4a	15.2a	34.4b	1.13b
ANOVA	***	***	***	***	***

** and *** indicate significant differences between means at the 1 and 0.1% levels of probability, respectively. Values are the mean of 6 replicates. Values followed by the same letter within a column are not significantly different at the 0.05 level of probability, according to the Duncan test.

Table 2. Effect of DI on lycopene (mg L^{-1}), β-carotene (mg L^{-1}), ascorbic acid (mg L^{-1}), total phenolics (mg L^{-1}), total flavonoids (µg L^{-1}) and antioxidant activity (TEAC index), in juice of 'Star Ruby' fruits in relation to the DI treatments: Control, T1 (phase-I), T2 (phase-II), T3 (phase-III), and during the experimental period (2007 and 2008).

	Lycopene	β-carotene	Total flavonoids	Total phenolics	Ascorbic acid	Antioxidant activity
			2007			
Control	4.69a	1.91b	33.2c	717.4c	181.8	2.18a
T1	3.24b	1.85b	34.4c	660.0c	178.0	1.88b
T2	2.60b	1.82b	48.5a	899.2a	183.7	2.33a
T3	3.90ab	2.47a	41.9b	794.4b	188.8	2.15a
ANOVA	*	**	***	***	ns	***
			2008			
Control	4.77a	3.12a	48.5b	734.5b	176.1	2.23
T1	4.65a	2.89a	45.1b	753.6b	172.2	2.26
T2	2.80b	2.12b	55.9a	914.9a	175.5	2.30
T3	3.90a	2.91a	47.1b	772.9b	165.4	2.14
ANOVA	***	**	***	***	ns	ns

*, ** and *** indicate significant differences between means at the 5, 1 and 0.1% levels of probability, respectively. ns = not significant. Values are the mean of 6 replicates. Values followed by the same letter within a column are not significantly different at the 0.05 level of probability, according to the Duncan test.

Figure 1

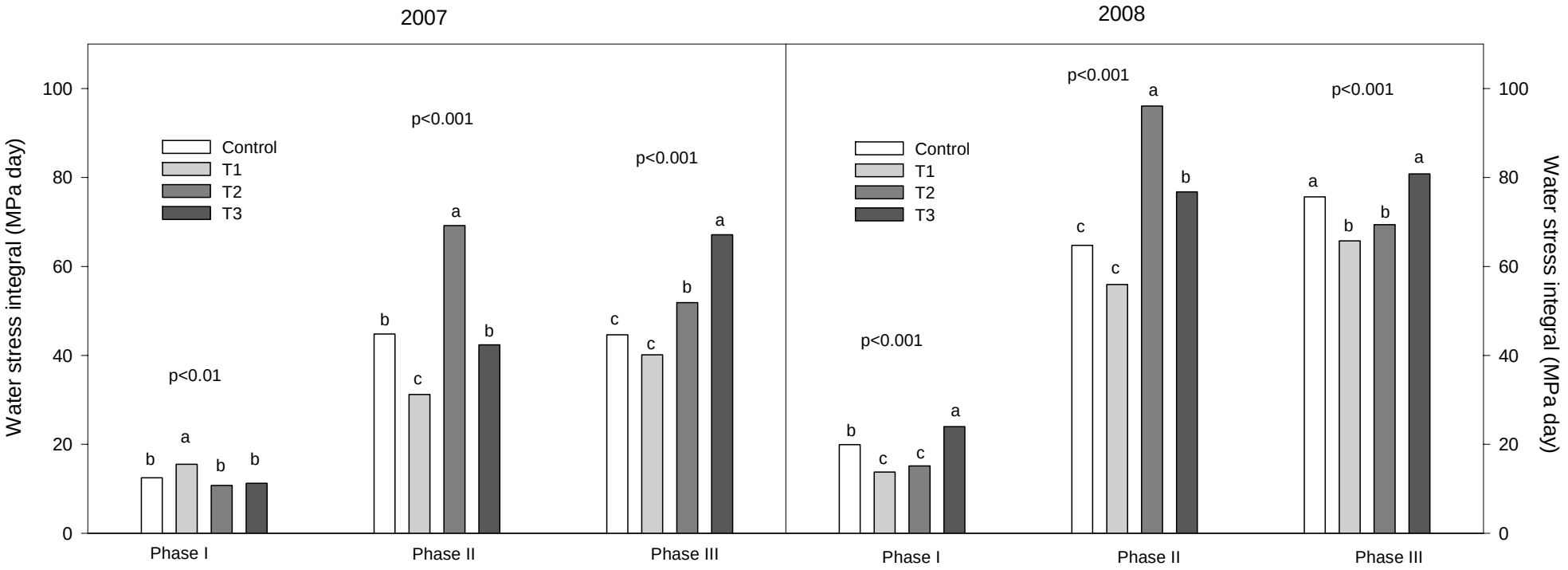

2007
2008
Water stress integral (MPa day)
Water stress integral (MPa day)
Control
T1
T2
T3
p<0.01
p<0.001
p<0.001
p<0.001
p<0.001
p<0.001
b
a
b
b
b
c
c
a
b
c
c
b
c
c
c
a
b
c
c
a
a
b
b
a
0
20
40
60
80
100
Phase I
Phase II
Phase III
Phase I
Phase II
Phase III

Figure 2

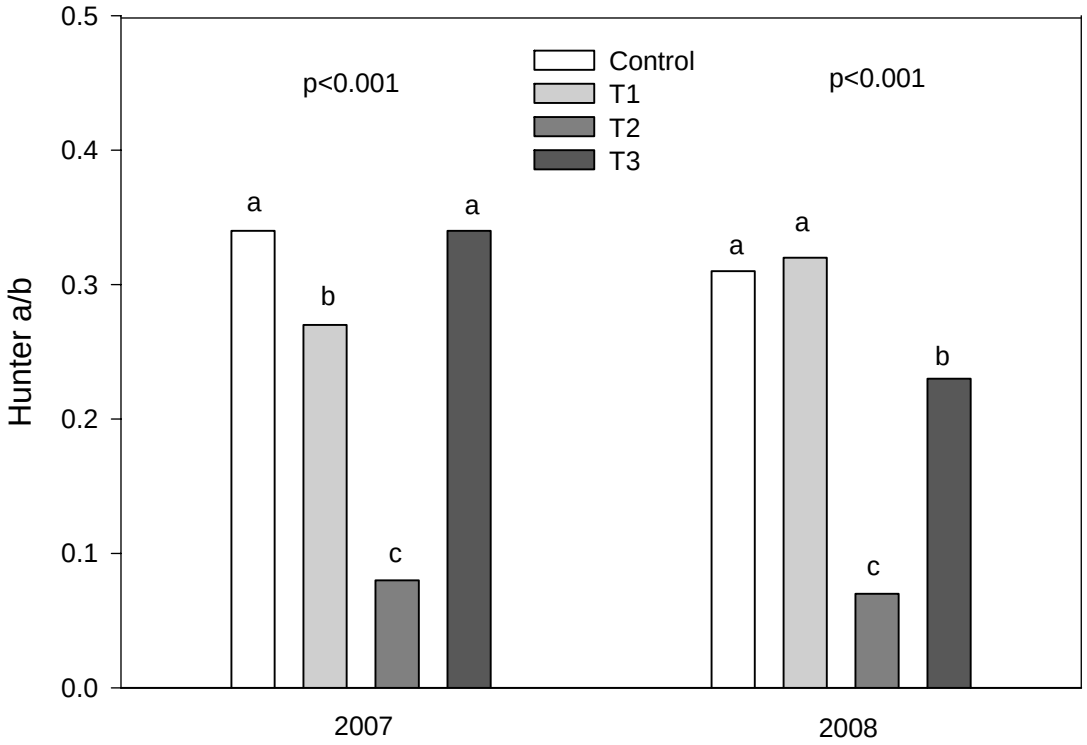
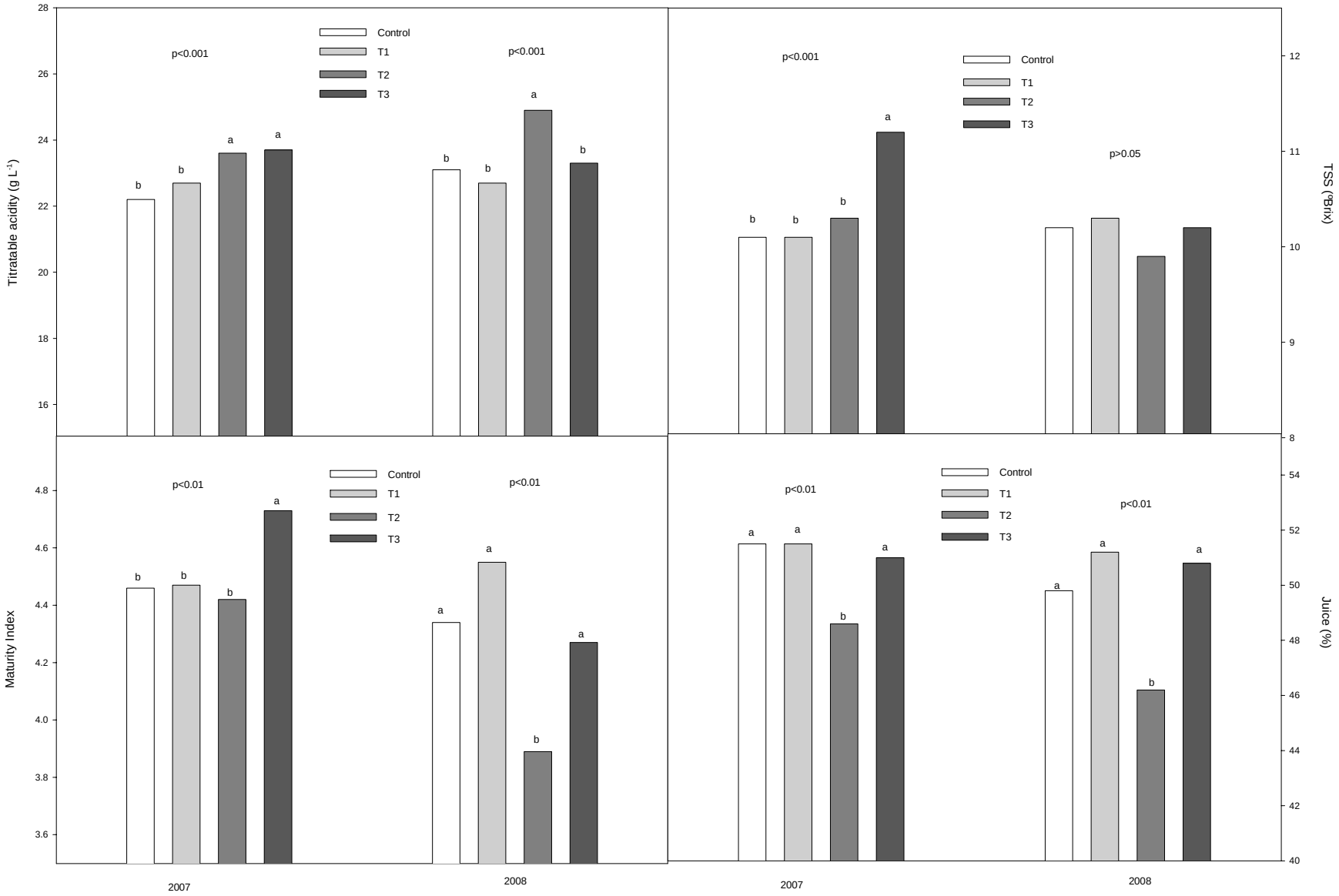

0.5
0.4
0.3
0.2
0.1
0.0
Hunter a/b
p<0.001
p<0.001
Control
T1
T2
T3
a
b
c
a
a
a
c
b
2007
2008

Figure 3


Titratable acidity (g L-1)
TSS (°Brix)
Maturity Index
Juice (%)
Control
T1
T2
T3
p<0.001
p<0.001
p<0.001
p>0.05
p<0.01
p<0.01
p<0.01
p<0.01
2007
2008
2007
2008

Highlights :

- The effects of deficit irrigation (DI) on fruit quality depended on the stage of fruit growth.
- DI during phase I only affected external fruit maturation.
- DI during phase II delayed fruit ripening but increased flavonoids and phenolics.
- DI during phase III increased the levels of bioactive compounds in juice.