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Original article

Deficit irrigation strategies and their impact on yield and nutritional quality of pomegranate fruit

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Summary

Introduction – **The cultivation of drought tolerant crops and the application of deficit irrigation (DI) strategies are necessary agronomic measures for sustainable agriculture in arid regions of the world and in areas that are experiencing recurrent water shortages (*i.e.*, Central California). Pomegranate is a drought tolerant fruit tree and is extensively cultivated in arid and semi-arid regions of the world. The objective of this study was to evaluate the physiological response of pomegranate trees subject to DI for two years relative to fruit yield and quality.** *Materials and methods* – **In this 2-year study, pomegranate trees (*Punica granatum* L. 'Wonderful') grown in Central California were treated with four different DI treatments [35, 50, 75 and 100% evapotranspiration (ET_{lys})] and tree physiological responses were evaluated relative to fruit yield and nutritional quality (including pH, soluble solids, total phenolic compounds, anthocyanin and non-anthocyanin compounds, and mineral elements).** *Results and discussion* – **The DI strategies, as low as 35% Et_c, did not significantly affect the yield, fruit color, pH, concentration of soluble solids, total phenolic compounds, anthocyanin and non-anthocyanin compounds, and mineral elements.** *Conclusion* – **Longer-term studies are needed to better predict physiological responses to water deficit management at orchard and individual tree level relative to productivity and nutritional quality of the pomegranate fruit.**

Keywords
USA, pomegranate, *Punica granatum*, water stress, sustainable agriculture, phenolics

Significance of this study

What is already known on this subject?

- Deficit irrigation strategies and cultivation of drought-tolerant crops are agronomic measures used in arid regions and in areas with recurrent water shortages.

What are the new findings?

- The application of deficit irrigation strategies, as low as 35% of ET_c, did not affect fruit yield and nutritional quality of pomegranate within a given year.

What is the expected impact on horticulture?

- Deficit irrigation may be a strategy to reduce water usage without impacting fruit nutritional quality, and to increase agricultural sustainability in arid regions.

Résumé

Stratégies d'irrigation déficitaire et leur impact sur le rendement et la qualité nutritionnelle des fruits du grenadier.

Introduction – **La culture d'espèces végétales tolérantes à la sécheresse et l'application de stratégies d'irrigation déficitaire (DI) sont des méthodes agronomiques permettant d'assurer une agriculture durable dans les zones arides ou qui connaissent des pénuries d'eau récurrentes (*i.e.*, le centre de la Californie). Le grenadier est une espèce arboricole fruitière tolérante à la sécheresse, largement cultivée dans les régions arides et semi-arides du monde. L'objectif de cette étude était d'évaluer la réponse physiologique des grenadiers soumis à une DI pendant deux ans, à partir du rendement et de la qualité des fruits.** *Matériel et méthodes* – **Les grenadiers (*Punica granatum* L. 'Wonderful') cultivés en Californie centrale ont été soumis durant deux ans à quatre traitements de DI différents [35, 50, 75 et 100% évapotranspiration (ET_{lys})]. Les réponses physiologiques des arbres ont été évaluées par des mesures de la production fruitière et de qualité des fruits (y-compris le pH, les solides solubles, composés phénoliques totaux, anthocyanes et composés non-anthocyanes, et éléments minéraux).** *Résultats et discussion* – **Une stratégie de DI aussi basse que 35% Et_c n'a pas affecté de façon significative le rendement fruitier, la couleur des fruits, le pH, les concentrations en matières solubles, en composés phénoliques totaux, anthocyanes et composés non-anthocyanes, ni en éléments minéraux des fruits.** *Conclusion* – **Des études à plus long terme sont nécessaires pour mieux prédire les réponses physiologiques à la gestion de déficit en eau de vergers ou d'arbres individuels en matière de productivité fruitière et de qualité nutritionnelle des grenades.**

Mots-clés
États-Unis, grenade, *Punica granatum*, stress hydrique, agriculture durable, composés phénoliques

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Introduction

In arid regions of the world, farmers use deficit irrigation strategies to supply water at levels below full crop evapotranspiration throughout the growing season or at specific phenological stages. Deficit irrigation (DI) is often conducted as a response to water shortage, inconsistent water supply, and recurrent droughts. The strategy putatively has potentially various advantages such as maximization of water productivity (the ratio of marketable yield to the amount of water consumed by the crop), reduction of disease and pest risk, reduction of nutrient losses through leaching, and increased fruit nutritional quality (Geerts and Raes, 2009). In arid regions, growing crops that are water stress-resistant and tolerant of arid environments is essential for sustaining agriculture.

Pomegranate (*Punica granatum* L.) is a fruit cultivated since ancient times throughout the Mediterranean region and is considered to have originated in Persia. Pomegranate is grown extensively in arid and semi-arid regions of the world for its requirement of hot and dry climate and high alkaline soils. A pomegranate fruit tree already possesses drought tolerance characteristics typical of xeromorphic plants, such as high leaf relative apoplastic water content and the ability to confront water stress by developing complementary stress avoidance and stress tolerance mechanisms, including active osmotic adjustment and leaf conductance decreases that control water loss (Rodriguez *et al*., 2012).

There is an increasing demand for pomegranate fruit and juice due to its reported large quantities of healthy bioactive compounds, mineral nutrients and antioxidants (Viuda-Martos *et al*., 2010). Very little is known, however about effects of drought and reduced irrigation on pomegranate fruit nutritional characteristics and especially on levels of antioxidants, which are compounds that may provide health benefits in addition to basic nutritional value (Gil *et al*., 2000; Mellisho *et al*., 2012; Mena *et al*., 2013; Pena *et al*., 2013). Over one hundred different types of potential antioxidants called phenolics have been identified in pomegranate fruits and seeds (Sentandreu *et al*., 2013). Phenolics are secondary plant metabolites that are often produced by plants in response to various stresses and some of them may have human health benefits for the prevention of chronic diseases (Teixeira da Silva *et al*., 2013).

The goal of our work is to evaluate the physiological response of pomegranate trees subject to DI relative to fruit yield and quality. In this study, pomegranate fruit juice was obtained by mechanical pressing the whole fruit; a method based on the industrial practice of juicing the fruit for commercial purposes. Four DI treatments were applied: 35, 50, 75 and 100% evapotranspiration (ET_{lys}) based on pomegranates grown in a lysimeter located in the field. Fruit were harvested for two consecutive years and analyzed for yield, pH, soluble solids, total phenolic compounds, anthocyanin and non-anthocyanin compounds, and mineral elements. The objective of this study was to gain information about DI strategy effects on pomegranate water stress responses and associated consequences on fruit nutritional quality.

Materials and methods

Field site

One-year-old pomegranate trees (*Punica granatum* L. 'Wonderful', donated by Wonderful Orchard Inc. at Del Ray, California, USA), were field planted on February 25, 2010, at USDA Parlier (CA) on a sandy loam soil. The soil is classified as a Hanford sandy loam (coarse-loamy, mixed, superactive, non-acid, thermic Typic Xerothents) field soil with a sand/silt/clay distribution of 55%, 40%, and 5%, respectively. Physical soil properties include: pH 7.7, bulk density of 1.4 g cm^{-3}, organic matter of 7.4 g kg^{-1}, and cation exchange capacity of 6.8 $cmol_c$ kg^{-1} (equivalent to 6.8 meq 100 g^{-1}).

Deficit irrigation treatment

Pomegranate trees were fully irrigated for the first 2 years after planting to insure stand establishment and reasonably uniform tree size prior to beginning the DI treatments. We used sustained deficit irrigation that applied a fixed amount of water throughout the growing season. Deficit irrigation (DI) treatments began in 2012. Four treatments were applied: 35, 50, 75 and 100% evapotranspiration (ET_{lys}) based on pomegranates grown in a lysimeter located in the field [see Schneider *et al.* (1996)] for a complete description of lysimeter). Actual crop evapotranspiration or ET_c was measured by the weighing lysimeter and potential evapotranspiration or ET_0 was obtained from a nearby (1 km) California Irrigation Management Information Systems (CIMIS) weather station located on the University of California Kearney, Agricultural Research and Extension Center. We accumulated water use up to 4 mm based on the lysimeter and then initiated irrigation. The rate of accumulation was dependent on the irrigation treatment with the percentage of the daily use being accumulated until the 4 mm threshold was reached, *e.g.*, it takes twice as long to reach 50% as the 100% treatment. As a result, we irrigated with a different frequency for each treatment but with the same depth of application. The applied water was measured with electronic water meters in each treatment and data, including values of ET_0, are shown in Figure 1. Trees were drip irrigated with single surface drip tubing containing 0.002 m^3 h^{-1} snap on turbulent flow emitters spaced 0.45 m apart. A total of four replications per treatment were used in this study. The trees were not fully matured in 2012 and additional pruning was performed during the growing season to shape the trees. Individual plots consisted of three rows with the center row being used for data collection (described below).

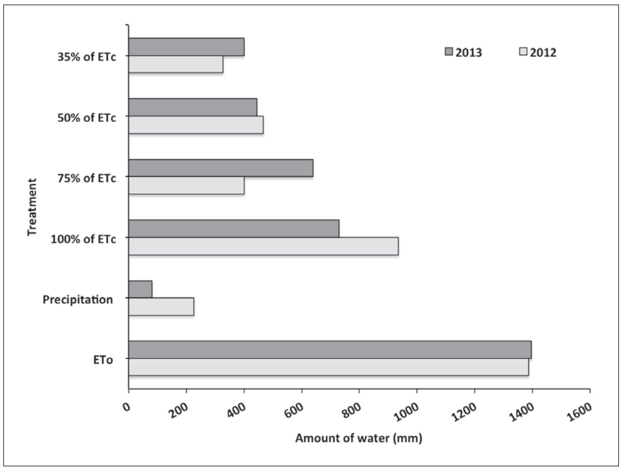

FIGURE 1. Amount of water applied to each irrigation treatment during 2012 and 2013, precipitation and reference evapotranspiration (ET_0) obtained from the California Irrigation Management Information Systems (CIMIS) weather station located 1 km away from the experimental field site. Precipitation in both years occurred outside the primary growing season and would have only contributed to supply the soil water for future use (ET_c: actual crop evapotranspiration).

Fruit harvest and sample preparation

Fruits were manually harvested in 2012 and 2013. Before harvest of the whole tree, 10 fruits per tree from 5 trees in the central row of each plot were manually picked. These fruit were weighed, washed, cut in quarters and juiced manually using a French press (Alpine Cuisine, Los Angeles, CA). Total yield comprised the sum of the two harvests (weight of the 10 fruits per tree that were juiced plus weight of all other fruits per tree). A method for juicing the pomegranate fruit that was developed based on the industrial practice of juicing the fruit for commercial purposes. Higher concentrations of hydrolysable tannins that seem to play an important role in human health (Gil *et al.*, 2000) are contained in the peel and the industry utilizes the whole fruit in the juicing process to include most of the antioxidants contained in the pomegranate fruit. Fruit juice was analyzed for pH, soluble solids, concentration of total phenolics, anthocyanin and non-anthocyanin compounds, and mineral elements as described below.

Soluble solids, color, pH

Soluble solid (SS) content was determined in juice using a digital refractometer (3810 PAL-1, Atago, Tokyo, Japan) and expressed as °Brix. The color of fruit and juice samples was determined using a Chroma Meter CR-400 optical sensor (Konica Minolta Sensing, Inc., Osaka, Japan) according to the CIE Lab scale (CIE Colorimeter Committee, 1974). The system provides the values of three color components; *L**, and the chromaticity coordinates, *a** and *b** (Hunter, 1942). *L** defines lightness where lower values indicate darker color (0 = black) and higher values indicate lighter color (100 = white). Negative *a** values indicate green and positive values red color, while negative *b** values imply blue and positive values yellow color. The objective color was calculated as chromaticity or chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and hue angle ($h° = \arctan(b^*\ a^{*-1})$). The instrument was calibrated using a standard white and a standard black reflective plate. Each color value reported is the mean of three determinations at 22–24°C.

Fruit juice pH was determined at room temperature using an Orion 420A pH-meter (Thermo Scientific, Waltham, USA).

Total phenolics (TP)

Total phenolic concentrations were measured according to Singleton *et al.* (1998), using the Folin-Ciocalteu reagent assay. Absorbance was measured at 756 nm using a Spectra Max plus 384 spectrophotometer (Molecular Devices, Sunnydale, CA). Total phenol concentrations were standardized against gallic acid (GA) and expressed as mg gallic acid equivalents (GAE) mL^{-1} fruit juice. The linearity range for this assay was determined as 50–250 mg mL^{-1} GA, giving absorbance range of 0.50–2.55 AU.

Composition of polyphenols by liquid chromatography

The analysis of single phenolics was carried out to identify variation in specific compounds (anthocyanin and non-anthocyanin) because analysis of total phenolic comprises the bulk of all antioxidants and does not discriminate for single compounds.

In the juice samples, phenolic compounds were analyzed using a Shimadzu (Columbia, MD) high-performance liquid chromatography (HPLC) system equipped with a Shimadzu ZR-ODS C18 column and a photodiode array detector set at 280 nm (for non-anthocyanins) and 520 nm (for anthocyanins). Compounds were identified as described previously by Sentandreu *et al.* (2013), Rashed *et al.* (2013) and Wallis *et al.* (2012).

All compounds after identification were quantified at 280 nm, with a standard curve of cyanidin chloride (Sigma-Aldrich, St. Louis, MO, USA) used to convert derivatives of cyanidin and delphinidin to g amounts, a standard curve of pelargonidin chloride (Sigma) to convert derivatives of pelargonidin to g amounts, a standard curve of ferulic acid (Sigma) to convert quinic acid and citric acid to g amounts, and a standard curve of ellagic acid (Sigma) to convert all remaining compounds to g amounts.

Mineral elements

A standard procedure was used to determine the mineral element concentrations in fruit samples (Bañuelos and Lin, 2010). Fruit juice samples were wet digested with HNO_3-H_2O_2-HCl, as described by Bañuelos and Akohoue (1994). The NIST wheat flour (SRM 1567) was used as an external quality control standard for elemental analysis in plant material. Mineral elements were analyzed by an inductively-coupled plasma optical emission spectrometer (Agilent 7500cx, Santa Clara, USA) according to Agilent manufacture protocol.

Statistical analysis

Results were examined by factorial analysis of variance (ANOVA) with year and treatment as main factors influencing the biochemical and quality parameters evaluated. Statistically significant differences were assumed for $P \le 0.05$ and statistical data analysis were performed using Gretl (*G*nu *Re*gression, *E*conometrics and *T*ime-series *L*ibrary) (Baiocchi and Distaso, 2003).

Results and discussion

Yield and fruit quality parameters (color, pH, soluble solids)

We did not observe any significant differences in fruit yield among DI treatments, both in 2012 and in 2013 (Figure 2). However, yield in 2013 was significantly ($P \ge 0.004$) higher than in 2012 independent of treatment (Figure 2). This different yield is attributed to the age of the tree and pruning that was performed during 2012 to shape the tree (during dormancy). The total fruit yield (kg $tree^{-1}$) reported in this study was about 5 times lower than that reported by Galindo *et al.* (2014), probably because the trees were grown under different irrigation and weather conditions and because of genetic distinction between the two varieties of pomegranate ('Mollar de Eche' *vs.* 'Wonderful'). These authors (Galindo *et al.*, 2014) measured the total fruit yield in pomegranate trees that were irrigated above the crop water requirements for most of the growing season, except for a couple of months before harvest when irrigation was withheld. In addition, the trees received varying amounts of rain (from 0.9 to 84.0 mm) during the experiment (Galindo *et al.*, 2014). In our experiment, the trees were grown under arid conditions and were treated with deficit irrigation during the growing season. In 2012 and 2013 the rainfall was below 1.0 mm during the pomegranate growing season in Central California from April through November) (Figure 3), and the average air temperature was above 25°C (Figure 4). The total fruit yields reported in this study are, however, higher than those measured by Singh *et al.* (2011). In their study, the pomegranate trees were grown under arid conditions that affected fruit size and weight (smaller arils).

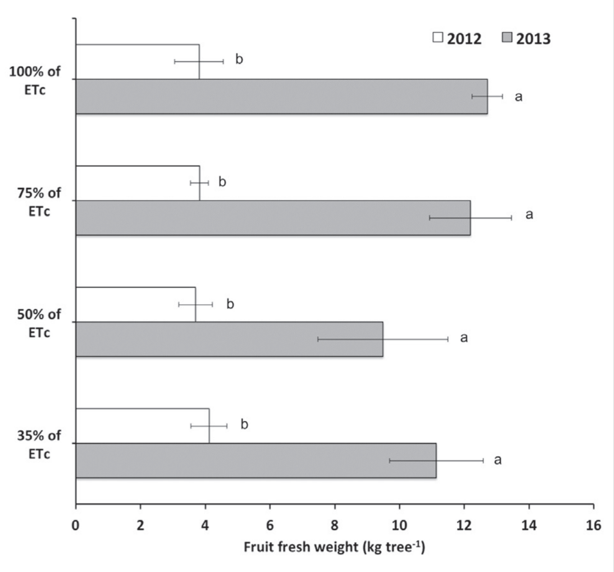

Figure 2. Fruit fresh weight (kg tree^{-1}) of pomegranate (*Punica granatum* L. 'Wonderful') grown over two years under various deficit irrigation treatments (calculated as % ET_c). Bars and error bars represent means and standard errors ($n=4$), respectively. Similar letters indicate no significant ($P \leq 0.05$) difference between treatments (ET_c: actual crop evapotranspiration).

Fruit peel color was similar among all treatments and years, except for the DI treatment of 35% ET_c in 2013, which showed a significantly lighter color than the other treatments (Table 1). Values of a^* (red-greenness) were comparable to those reported by Mellisho *et al*. (2012), Mena *et al*. (2013), and Pena *et al*. (2013), who studied the effect of deficit irrigation treatments on pomegranate fruit physical and chemical characteristics. The measurements of color intensity (L^*), chroma (C^*), and hue angle (h°) were lower in this study than values previously reported (Mellisho *et al*., 2012; Mena *et al*., 2013; Pena *et al*., 2013), indicating that the fruits in this study were of darker red color. This different juice color can be due to different fruit characteristics between 'Wonderful' analyzed in this study and 'Mollar de Elche' previously studied (Mellisho *et al*., 2012; Mena *et al*., 2013; Pena *et al*., 2013).

Fruit juice pH was significantly higher in 2012 for all treatments, except for 75% Et_c, which showed a significantly higher pH in 2013 than in 2012 (Table 1). Hence fruit became slightly more acid with time. However, no effects of treatments were observed in both years. Similarly, no effect of treatments was observed for soluble solids (Table 1), but a higher soluble solid concentration was measured in 2013 for all treatments, except for the fully irrigated treatment (100% ET_c) (Table 1). Concentrations of soluble solids and pH values in this study were comparable to those previously reported (Mellisho *et al*., 2012; Mena *et al*., 2013; Pena *et al*., 2013). Navarro *et al.* (2015) and Intrigliolo and Castel (2010) reported high acidity and increased soluble solid contents in the fruits (of grapefruit and plum, respectively) of trees suffering moderate water stress. In our study, the trees were grown in Central California in years of recurrent drought and high temperatures. All the trees showed a uniform response independently of treatment due to the extreme environmental conditions.

Total phenolic and anthocyanin and non-anthocyanin compounds

Wahid and Ghazanfar (2006) reported that the increased synthesis of total phenolics is a response of plants to protect themselves from ion-induced oxidative stress in case of pathogen attack, high salinity, high temperature, or water stress. In our study, no significant differences were observed ($P \geq 0.1$) among DI treatments in total phenolics for both 2012 and 2013 (Figure 5). Total phenolics were significantly higher ($P \geq 0.009$) in 2013 than in 2012, possibly due to high temperatures (>40°C) recorded in 2013 (Figure 4). As described above, other authors have reported an increase in pH, soluble solids, and phenolic compounds in fruit trees that suffered moderate water stress (Navarro *et al*., 2015). Values of total phenolics are comparable to the values reported by Orak *et al.* (2012) and Tzulker *et al.* (2007), who studied antioxidant activities in pomegranate juice and peel from various pomegranate varieties grown in Turkey and Israel, respectively. Total phenolics concentrations in this study were one order of magnitude lower than reported by Mellisho *et al*. (2012) and Mena *et al*. (2013). In contrast, Di Nunzio *et al.* (2013) reported 10 times lower total phenolic concentrations in pomegranate ('Wonderful') juice than

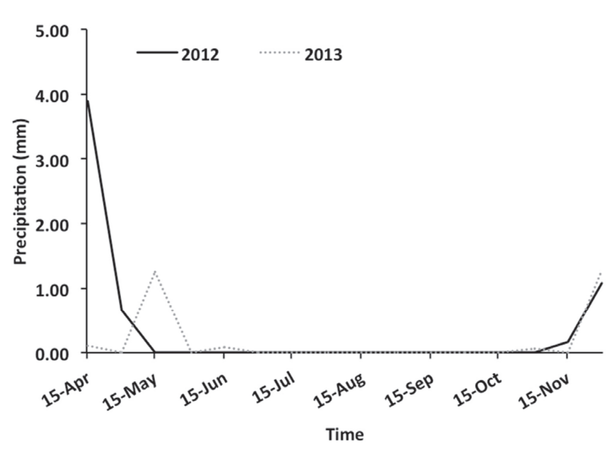

Figure 3. Water precipitation recorded in 2012 and 2013 by CIMIS (California Irrigation Management Information System) weather station located 1 km away at the University of California Kearney Research Station in Parlier, CA, USA.

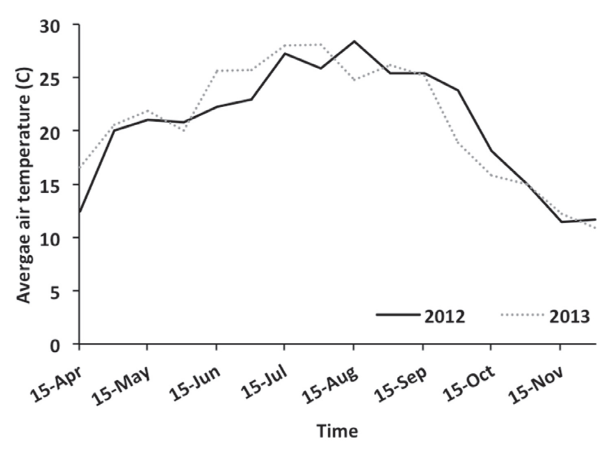

Figure 4. Average air temperatures recorded in 2012 and 2013 by CIMIS (California Irrigation Management Information System) weather station located 2 km away at the University of California Kearney Research Station in Parlier, CA, USA.

TABLE 1. Measurement of color parameters [lightness (L^*), red-greenness (a^*), blue-yellowness (b^*), chroma (C^*) and hue angle (h°)] of fruit peel, and soluble solid (°Brix) contents and pH of fruit juice of pomegranate (*Punica granatum* L. 'Wonderful'). Fruits were harvested in 2012 and 2013 and the trees were grown under various deficit irrigation treatments (calculated as % Et_c) (ET_c: actual crop evapotranspiration). Values represent means ± standard errors (n=4).

Parameters	Fruit parts	Years	Deficit irrigation (DI) in % Et_c			
			35%	50%	75%	100%
L^*	Peel	2012	18.50 ± 0.17 a[y]	18.10 ± 0.01 a	18.30 ± 0.34 a	17.90 ± 0.04 a
		2013	19.90 ± 0.22 a	18.80 ± 0.18 a	18.30 ± 0.16 a	18.30 ± 0.18 a
a^*	Peel	2012	11.50 ± 0.31 a	12.30 ± 0.01 a	12.10 ± 0.03 a	11.90 ± 0.06 a
		2013	10.60 ± 0.35 b	12.40 ± 0.38 a	12.90 ± 0.33 a	13.10 ± 0.38 a
b^*	Peel	2012	3.32 ± 0.25 a	3.32 ± 0.02 a	3.31 ± 0.02 a	3.32 ± 0.01 a
		2013	4.06 ± 0.24 a	3.67 ± 0.29 a	4.14 ± 0.24 a	3.90 ± 0.25 a
C^*	Peel	2012	11.90 ± 0.36 a	12.70 ± 0.02 a	11.90 ± 0.03 a	12.30 ± 0.05 a
		2013	11.40 ± 0.35 a	13.00 ± 0.40 a	13.60 ± 0.38 a	13.70 ± 0.43 a
h°	Peel	2012	16.00 ± 0.76 a	15.00 ± 0.07 a	15.30 ± 0.08 a	15.50 ± 0.08 a
		2013	20.80 ± 1.25 a	16.30 ± 1.16 a	17.10 ± 0.55 a	15.90 ± 0.53 a
pH	Juice	2012	3.95 ± 0.04 a	3.90 ± 0.06 a	3.60 ± 0.07 ab	3.80 ± 0.06 a
		2013	3.80 ± 0.03 b	3.72 ± 0.03 b	3.74 ± 0.02 b	3.74 ± 0.02 b
Soluble solids (°Brix)	Juice	2012	14.20 ± 0.42 a	14.30 ± 0.39 a	14.60 ± 0.58 a	15.50 ± 0.37 b
		2013	13.70 ± 0.53 b	15.40 ± 0.57 b	16.10 ± 0.19 b	15.50 ± 0.25 b

[y] Similar letters indicate no significant ($P \leq 0.05$) difference between treatments.

those recorded in this study. This may be due to a different mechanical process for extracting the fruit juice. In this study the whole fruit (including peel) was juiced, whereas only arils were juiced in the other studies mentioned. However, the concentration of total phenolics reported by various authors may also depend on the pomegranate variety and the agro-environmental conditions.

Similarly to the total phenolics, no significant differences were observed among DI treatments and years for all anthocyanin and non-anthocyanin compounds analyzed in 2012 and 2013 (Table 2). Data collected in 2012 are not shown because of their similarity with 2013. The concentrations of anthocyanin and non-anthocyanin compounds were comparable to those reported in pomegranates under deficit irrigation (Mellisho *et al*., 2012; Mena *et al*., 2013; Pena *et al*., 2013), but they were more than 10 times higher in this study compared to those reported for pomegranate 'Wonderful' (Wahid and Ghazanfar, 2006). Di Nunzio *et al.* (2013) obtained the juice from only the arils, whereas we used the whole fruit. It is known that pomegranate fruit peel contains high levels of tannins and phenolics (Gil *et al*., 2000).

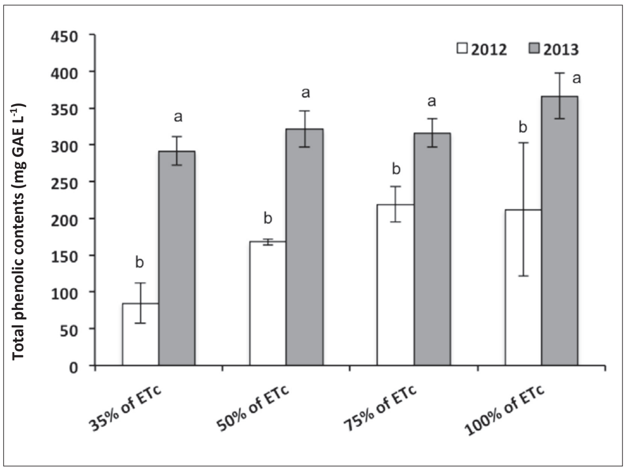

FIGURE 5. Total phenolic contents in pomegranate juice, expressed as mg gallic acid equivalent (GAE) per liter. Bars and error bars represent means and standard errors (n=4), respectively. Similar letters indicate no significant ($P \leq 0.05$) difference between treatments.

Mineral elements in fruit juice

There were no differences among treatments for all elements analyzed, except Na. Sodium concentration in fruit juice was similar among the three DI treatments used (Table 3) but it was significantly higher in all the DI treatments relative to control (100% Et_c) (Table 3). In addition, Na concentration increased with time, and was significantly higher in 2013 than 2012 (Table 3). Reduced irrigation water applied due to application of DI strategies can cause an increase in soil Na concentration in the root zone and hence an increased uptake and translocation of Na from the roots to the fruit.

Concentrations of B, Ca, K, Mg, and Mn in the fruit juice were significantly higher in 2012 relative to 2013 (Table 3). Those differences in nutrient concentrations in fruit juice between years may be attributed to reduced uptake and translocation of some mineral elements. High temperatures recorded in 2013 may have caused an even drier soil environment with reduced diffusion of water and nutrients to the plant's root system. Mineral elements were one order of magnitude lower in this study compared to data reported by Mellisho *et al.* (2012). The variation can be attributed to soil characteristics, *e.g.*, soil nutrient concentrations, as well as agronomic practices, *i.e.*, fertilization, or tree variety. Concentrations of Cu, Zn, and Mg were within the range reported by Tzulker *et al.* (2007) and Al-Maiman and Ahmad (2002), whereas concentration of Fe, P, Na, and Ca were 2 to 10 times lower in this study and K concentration was 10 times higher in this study than reported by Mirdehghan and Rahemi (2007) and Chaves *et al.* (2010). These variations may be due to soil characteristics, fertilization management, or pomegranate genetics.

Table 2. Concentrations of phenolic compounds and organic acids (µg mL^{-1}) detected in pomegranate juice collected in 2013 (ET_c: actual crop evapotranspiration). Values represent means ± standard error ($n = 4$). There were no significant ($P \le 0.05$) differences between treatments.

Compounds	Deficit irrigation (DI) in % Et_c			
	35%	50%	75%	100%
Anthocyanins				
Delphinidin-3-glucoside	613 ± 75	816 ± 80	771 ± 103	799 ± 109
Delphinidin-3,5-diglucoside	2,722 ± 283	3,469 ± 382	3,250 ± 318	3,561 ± 436
Cyanidin-3-glucoside	712 ± 131	847 ± 95	788 ± 136	755 ± 114
Cyanidin-3,5-diglucoside	472 ± 40	475 ± 56	459 ± 44	493 ± 74
Cyanidin-3-pentoside	423 ± 79	575 ± 109	580 ± 149	601 ± 158
Cyanidin-3-hexoside	758 ± 40	619 ± 31	734 ± 82	735 ± 31
Cyanidin rutinoside	342 ± 45	486 ± 98	398 ± 51	441 ± 73
Pelargonidin-3-glucoside	282 ± 17	203 ± 19	286 ± 16	284 ± 13
Pelargonidin-3-diglucoside	300 ± 50	381 ± 62	410 ± 54	448 ± 100
Non-anthocyanins				
Punicalin derivative 1	168 ± 14	142 ± 10	262 ± 66	194 ± 29
Punicalin derivative 2	596 ± 83	788 ± 124	710 ± 69	892 ± 168
Punicalagin derivative 1	141 ± 6	146 ± 12	153 ± 13	150 ± 14
Punicalagin derivative 2	532 ± 84	531 ± 126	613 ± 97	729 ± 138
Galloylhexahydroxydiphenoyl hexoside	170 ± 11	172 ± 22	196 ± 11	209 ± 11
Ellagic acid hexoside	334 ± 42	318 ± 34	450 ± 54	411 ± 29
Dihydrokaempferol hexoside	216 ± 27	195 ± 17	232 ± 18	177 ± 17
Quinic acid	20.9 ± 0.6	19.8 ± 0.6	20.7 ± 0.8	20.1 ± 0.9
Citric acid	68.3 ± 4.4	71.3 ± 4.6	70.6 ± 4.9	73.0 ± 3.8

Table 3. Concentration of mineral elements detected in pomegranate juice collected in 2012 and 2013 (ET_c: actual crop evapotranspiration). Values represent means ± standard errors ($n = 4$).

Elements	Units	Years	Deficit irrigation (DI) in % Et_c			
			35%	50%	75%	100%
B	mg kg^{-1}	2012	4.43 ± 0.69 a[y]	11.30 ± 1.71 ab	5.09 ± 0.89 a	5.84 ± 0.72 a
		2013	2.32 ± 0.29 c	2.50 ± 0.30 c	2.60 ± 0.10 c	2.44 ± 0.14 c
Ca	mg kg^{-1}	2012	54.1 ± 2.7 a	65.1 ± 5.1 a	51.0 ± 2.2 a	38.5 ± 3.6 b
		2013	39.8 ± 4.1 b	42.8 ± 4.3 c	45.7 ± 2.5 c	54.2 ± 2.7 c
Cu	mg kg^{-1}	2012	0.85 ± 0.12 a	1.05 ± 0.01 a	0.63 ± 0.08 a	0.53 ± 0.09 a
		2013	0.91 ± 0.12 a	0.75 ± 0.07 a	0.72 ± 0.05 a	0.85 ± 0.12 a
Fe	mg kg^{-1}	2012	0.29 ± 0.01 a	0.43 ± 0.03 a	0.29 ± 0.01 a	0.24 ± 0.02 a
		2013	0.34 ± 0.05 a	0.43 ± 0.12 a	0.27 ± 0.02 a	0.29 ± 0.01 a
K	g kg^{-1}	2012	1.68 ± 0.08 a	2.02 ± 0.09 a	1.71 ± 0.08 a	1.82 ± 0.07 a
		2013	1.54 ± 0.07 b	1.49 ± 0.08 b	1.63 ± 0.05 b	1.68 ± 0.08 b
Mg	mg kg^{-1}	2012	67.8 ± 4.1 a	80.6 ± 3.9 a	70.7 ± 4.0 a	61.1 ± 3.8 a
		2013	55.2 ± 2.9 b	55.5 ± 1.8 b	55.1 ± 1.4 b	67.8 ± 4.1 b
Mn	mg kg^{-1}	2012	0.52 ± 0.06 a	0.62 ± 0.08 a	0.58 ± 0.05 a	0.46 ± 0.07 a
		2013	0.31 ± 0.01 b	0.46 ± 0.17 b	0.31 ± 0.01 b	0.52 ± 0.06 b
Na	mg kg^{-1}	2012	2.81 ± 0.62 a	3.04 ± 0.43 a	3.52 ± 0.51 a	1.48 ± 0.13 b
		2013	4.55 ± 0.47 b	5.06 ± 0.86 b	4.24 ± 0.43 b	2.81 ± 0.62 c
P	mg kg^{-1}	2012	133 ± 21 a	138 ± 20 a	78 ± 17 a	105 ± 18 a
		2013	148 ± 15 a	118 ± 17 a	141 ± 6 a	133 ± 21 a
S	mg kg^{-1}	2012	49.1 ± 3.8 a	58.7 ± 2.8 a	47.0 ± 3.5 a	44.5 ± 3.7 a
		2013	52.2 ± 1.4 a	43.9 ± 4.0 a	45.6 ± 1.8 a	49.1 ± 3.8 a
Zn	mg kg^{-1}	2012	1.37 ± 0.08 a	1.92 ± 0.09 a	0.89 ± 0.04 a	1.21 ± 0.06 a
		2013	1.04 ± 0.08 a	0.99 ± 0.07 a	0.96 ± 0.04 a	1.31 ± 0.08 a

[y] Similar letters indicate no significant ($P \le 0.05$) difference between treatments for each element listed.

Conclusion

The effect of deficit irrigation (DI) on pomegranate fruit quality characteristics has been only recently studied and results are contradictory. For example, Mellisho *et al.* (2012) reported that pomegranate trees treated with a more pronounced water deficit (75%) during the second half of the fruit growth phase did not show changes in chemical characteristics of the fruit but only on fruit size. In a study on the effect of sustained deficit irrigation (SDI) on bioactive compounds in pomegranates, Pena *et al.* (2013) showed that after long term storage, the fruit grown under SDI had higher sensory and nutritional quality and a longer shelf-life than fruit irrigated at 100% ET_0. Similarly, Mena *et al.* (2013) studied the effect of deficit irrigation strategies on pomegranate nutrition quality and concluded that moderate (43%) and severe (12%) water stress treatments in pomegranates induced a more yellowish fruit color, lower antioxidant activity and lower total phenolic compound, punicalagin, and total anthocyanin contents than fruit from control trees.

Other authors have shown that deficit irrigation affected wine grape quality by increasing anthocyanin and phenolic concentrations (Chaves *et al.*, 2010; Santesteban *et al.*, 2011). In addition, the effect of salts, *i.e.*, sodium and chloride, has been shown to influence phenols and antioxidants compounds in pomegranate (Bhantana and Lazarovitch, 2010). This study is the first to report on the absence of increased phenolic and polyphenols content in fruit with application of deficit irrigation strategies, as well as absence of any effect of DI strategies on yield, fruit color, pH, soluble solids, and mineral element concentrations. The discrepancy between this study and previous studies mentioned above, can be attributed to the different pomegranate variety (*P. granatum* L. 'Mollar de Elche' versus 'Wonderful'), the agro-climatic conditions, and environmental factors. In addition, long-term (3 to 5 years) studies are necessary to obtain a steady-state physiological response of trees exposed to stress such as a reduction in amount of water applied with irrigation. This study reports results obtained after two years of treatment with DI strategies, whereas results reported by Mellisho *et al.* (2012), Mena *et al.* (2013), and Pena *et al.* (2013) refer to one-year treatment.

The application of deficit irrigation may be a strategy to reduce water usage and increase agricultural sustainability on arid and drought-stricken regions. Our two-year study has shown that application of deficit irrigation strategies, as low as 35% of ET_c, did not affect yield within a given year and fruit nutritional quality of pomegranate. Long-term studies are needed to better predict physiological responses to water deficit in crops and trees relative to nutritional quality and productivity.

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