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Short-Term Response of Young Mandarin Trees to Desalinated Seawater Irrigation

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Abstract: Water deficit and increasing pressure on water resources in semi-arid regions has led to the spread of irrigation with non-conventional water resources, such as desalinated seawater (DSW). The few existent studies to date, mainly carried out in Israel and Spain, have shown that suitable management of irrigation with DSW must be performed to avoid agronomic problems and reductions in crop productivity and fruit quality in the mid-long term. To the best of our knowledge, in the case of citrus, fruit production, and quality, information on the effects of DSW irrigation is not available. In this study, we evaluated the short-term agronomic and economic effects of irrigating a mandarin orchard during two crop cycles (2017–2019) with (i) fresh water (FW), (ii) desalinated seawater (DSW), and (iii) a mix of water composed of 50% FW and 50% DSW. Stem water potential ($\Psi_s < -1$ MPa) and gas exchange parameters (net photosynthesis; $A > 6.5$ μmol/m^2/s and stomatal conductance; $g_s >$ 65 mmol/m^2/s) indicated that trees were well irrigated throughout the experiment. The concentration of Na^+ and B^{3+} in the DSW always exceeded the maximum thresholds for irrigation water proposed in the literature for citrus, and the concentration of Na^+ in the leaves exceeded the maximum threshold in summer 2018. Nonetheless, symptoms of toxicity were not observed. Significant differences among treatments were not observed for Ψ_{stem}, A, g_s, Na^+, Cl^-, and B^{3+} in leaves (except in the summer months), yield components, fruit quality, or the economic assessment. The lack of such differences was explained by the large standard deviations caused by the youth of the trees, with figures that on occasion could represent more than 100% of the mean value. These results may justify the agronomic and economic viability of the irrigation of young trees with DSW in the short-term, but further research, considering the effects on adult trees in the long term is still needed.

Keywords: phytotoxicity; food security; non-conventional water resources; water productivity; economic assessment

1. Introduction

Feeding nine billion people in 2050 will require increasing world food production by 70–100%, which will only be possible under intensive irrigated agriculture [1]. However, in arid and semiarid regions, freshwater availability is being reduced by climate change, which creates imbalances between renewable resources and total demands and jeopardizes the sustainability of irrigated agriculture, its resilience, and hence food production [2]. A clear example is the Segura River Basin (SRB), an irrigation area of 262,400 ha located in southeastern Spain, which supports an annual structural water deficit above 400 hm^3; i.e., 1524 m^3/ha [3]. In this hydrologically stressed region, the compulsory allocation of water creates significant conflicts among aggravated users, with farmers being especially affected during drought periods. In order to redress this limitation of conventional water resource availability, and to continue providing society with food, farmers complement their share of conventional water resources with other non-conventional resources, such as desalinated seawater (DSW) [4].

The DSW represents an abundant and steady coastal water source, which effectively removes the climatological and hydrological constraints. In the SRB, 184.3 hm^3 of DSW is annually used for irrigation representing 74% of the total production of DSW in the basin and 11.9% of its agricultural demand [5].

Limited fundamental scientific researches evaluated the agronomic, economic, environmental, social, and policy implications of DSW irrigation in agriculture. As positive aspects, studies state that (i) it may provide drought risk-buffering value [4]; (ii) its low salinity can produce significant increases in the quality and quantity of crop yields, especially when replacing low quality water supplies in water stressed regions [6]; (iii) it may reverse previous problematic trends of soil salinization attributed to irrigation with poor-quality water, and provide a path to sustainable irrigated agriculture in arid and semiarid environments [5]; and (iv) the replacement of traditional water sources with DSW represents new water policies and water management options [7,8]. On the contrary, DSW produced through reverse osmosis (RO) does not seem to be problem free, especially when compared with other conventional water resources. The main significant limitations are: (i) the lack of plant nutrients [9]; (ii) the compliance of stringent B^{3+}, Na^+, and Cl^- standards for agricultural irrigation, especially for sensitive crops; i.e., most woody crops [10]; (iii) the effects of Na^+ accumulation in soil structure and productivity [11]; (iv) the energy requirements and resulting costs [12]; (v) the high emission of greenhouse gases that further exacerbates climate change [13]; and (vi) the impacts of massive brine disposal on oceanic life [14]. Despite these references, the agronomic issues associated to irrigation with DSW have been poorly evaluated. Some of the existent results, mainly obtained in Israel and Spain, can be consulted in Maestre-Valero et al. and Martinez-Alvarez et al. [15,16]. Consequently, studies dealing with the analysis of the effects of irrigation with DSW on crops and soils are necessary to bring down barriers and improve the farmers' perceptions and acceptance of DSW, which might, in turn, condition its sustainable use [8].

In this sense, a young mandarin tree orchard was irrigated with DSW for two consecutive seasons and the main agronomic implications, providing special reference to the effects on tree water relations, leaf mineral concentrations, vegetative growth, gas exchange parameters, soil salinity and sodicity, and fruit yield and quality were evaluated. A simple economic assessment of the irrigation with DSW was also performed.

2. Material and Methods

2.1. Experimental Plot and Irrigation Treatments

The experiment began in October 2017 and was carried out for 21 months at a commercial mandarin orchard located in Torre Pacheco, Murcia, Spain (37°47′30″ N; 1°03′85″ W; 30 m above sea level). The experiment finished by mid-June 2019 and covered two crop cycles.

This area is characterized by a Mediterranean semi-arid climate with warm, dry summers and mild winter conditions. The average annual reference evapotranspiration (ET_0) and rainfall are 1326 mm and 300 mm, respectively.

The experimental plot consisted of 0.5 ha cultivated with 3-year old (in 2017) mandarin trees (cv. Safor) grafted on Macrophylla rootstock (*Citrus Macrophylla*) with a tree spacing of 5 m × 3 m.

The soil had a loamy sandy texture (20% clay, 20% loam, and 60% sand) within the first 50 cm depth, with an average bulk density of 1.3 g/cm^3. The soil electrical conductivity (EC_e) before the experiment showed large variability, ranging between 4.4 and 8.4 dS/m and the sodium adsortium ratio (SAR_e) between 2.9 and 3.9 [mmol/L]$^{0.5}$.

The experiment was laid out as a randomized complete design with four blocks and three experimental plots per block. The standard plot, which covered about 180 m^2 was made up of twelve trees, organized in three adjacent rows with four trees per row. The two central trees of the middle row were used for measurements, with the other ten trees being guard trees.

2.2. Irrigation System, Management and Treatments

The irrigation system consisted of a single drip line laid on the soil surface next to each tree row. Two self-pressure compensating on-line emitters per tree provided a discharge of 4 L/h each. Emitters were placed at 0.50 m from the trunk and spaced 0.75 m apart. The irrigation doses were scheduled on the basis of the daily crop evapotranspiration (ET_c) accumulated during the previous week. The daily ET_c values were estimated by multiplying the daily reference evapotranspiration (ET_0), calculated with the Penman–Monteith methodology [17], by the month-specific crop coefficients for citrus provided by Castel et al. [18]. In addition, a reduction coefficient (K_r = 0.22), which accounted for an eventual decrease in evapotranspiration because of the partial soil covering by the crop canopy (young mandarin trees), was used [19]. The meteorological data to calculate ET_0 were collected from the nearest automatic weather station (TP-91) belonging to the *Sistema de Información Agraria de Murcia*. Additionally, two soil water content probes (HydraProbe II—Stevenswater, Portland, OR, USA) per plot were installed at 25 cm and 50 cm depths and connected to an automatic datalogger CR1000 (Campbell Scientific, Logan, UT, USA) to continuously monitor soil moisture and check that the trees did not suffer water stress during the experiment. No leaching fraction was added to the irrigation doses regardless of water salinity.

The irrigation control head of the entire experimental area was equipped with pumps, a fertigation system, electrovalves, an automatic irrigation programmer, and filters. All the treatments received the same amounts of fertilizer ($N–P_2O_5–K_2O$), applied through the drip irrigation system (174–98–220 kg/ha/year from Oct-2017 to Sept-2018 and 110–35–98 kg/ha/year from Oct-2018 to Jun-2019), irrespective of the water quality. Pest control practices and pruning were those commonly used by growers in the area, and no weeds were allowed to develop within the orchard.

The treatments were established by supplying three water sources to the irrigation system; the first source (FW), with an average electrical conductivity (EC_w) of 1.40 ± 0.30 dS/m, was provided by the *Campo de Cartagena* irrigator's community. The second source (DSW) was obtained from the desalination plant of *Escombreras* (EC_w = 0.91 ± 0.10 dS/m). The third treatment (MW) was a mix of water composed of 50% FW and 50% DSW (EC_w = 1.19 ± 0.30 dS/m).

2.3. Irrigation Water Quality

One water sample from each water source was collected in glass bottles, transported in an ice chest to the laboratory and stored at 5 °C before being processed for physical and chemical analyses. Water samplings were performed on a monthly basis throughout the experiment. An inductively coupled plasma (ICP-MS Agilent Technologies, Model 7900, Santa Calara, CA, USA) was used to determine the concentration of Na^+, K+, Ca^{2+}, Mg^{2+}, and B^{3+}. Anions (Cl^-, NO_3^-, PO_4^{3-}, and SO_4^{2-}) were quantified by ion chromatography with a liquid chromatograph (Thermo Scientific Dionex, Model ICS-2100, Thermo Scientific, Basel, Switzerland). The EC_w was determined using a conductivity instrument GLP-31 (Crison Instruments S.A., Barcelona, Spain); pH was measured with a pH-meter GLP-21 (Crison Instruments S.A., Barcelona, Spain). The sodium adsorption ratio (SAR_w) was also calculated from the concentration of Na^+, Ca^{2+}, and Mg^{2+} measured in the irrigation water.

2.4. Soil Characterization

The area surrounding one central tree in each replicate was selected to collect data for the analyses. Three soil samples were collected per replicate four times per year at 0–25 cm and 25–50 cm depths and 0.30 m away from the emitter. A total of 144 samples were collected during the experiment. Soluble salt contents were determined in the saturated paste extract as described by Rhoades [20]. The electrical conductivity of the saturated paste extract (EC_e) was measured with a conductivity GLP-31 meter (Crison Instruments, Barcelona, Spain). An inductively coupled plasma (ICP-MS Agilent Technologies, Model 7900, Santa Calara, CA, USA) was used to determine the concentration of water soluble Na^+, Ca^{2+}, and Mg^{2+}. The sodium adsorption ratio (SAR_e) was also calculated from the concentration of Na^+, Ca^{2+}, and Mg^{2+} measured in the saturated paste extract. The sodicity risk due to the irrigation water quality was analyzed based on the relation of the SAR_w and the EC_w. Extractable B^{3+} was determined in soil samples by refluxing 20 g soil with 40 mL hot water (boiling) for a period of 5 min. One aliquot from the filtered extract was then used for measuring B^{3+} using an inductively coupled plasma (ICP-MS Agilent Technologies, Model 7900, Santa Calara, CA, USA).

2.5. Determinations in Plants

2.5.1. Vegetative Growth

Before the beginning of the experiment, all trees' canopy height and perimeter and trunk perimeter were measured. Vegetative growth (canopy perimeter and diameter) was also measured at the end of each season after the harvest. The canopy equatorial area was calculated from the perimeter of the tree's foliage, measured with ranging rods.

2.5.2. Leaf Water Relations

Mid-day stem water potential" is known as the best leaf-based physiological response indicator for recording moisture status of the citrus tree. From May 2018, monthly measurements of stem water potential (Ψ_{stem}) were performed at solar noon (12:00 h Greenwich Mean Time), using a Scholander-type pressure chamber (model 3000; Soil Moisture Equipment Corp., Santa Barbara, CA, USA) and following Turner [21] recommendations. Two mature, fully expanded leaves and close to the trunk were taken from the two central trees of each replicate. Before the collection and measurement, the leaves were enclosed within polyethylene bags covered with aluminum foil, at least 2 h prior to the measurement [22].

On the same dates, a leaf from the same shoot was used to measure the leaf water potential (Ψ_{leaf}). The Ψ_{leaf} was also measured at midday using the pressure chamber. Following Ψ_{leaf} measurement, the leaves were immediately frozen and stored at −80 °C for determination of leaf osmotic potential (Ψ_{osm}) by a Wescor 5520 vapor pressure osmometer. Leaf turgor potential was calculated as the difference between Ψ_{leaf} and Ψ_{osm}.

2.5.3. Leaf Analysis

To perform the analyses, leaves from non-fruiting twigs located in the central part of the tree were sampled monthly for each of the seasons. Ten leaves per tree were sampled in the two central trees of each replicate. Adhered soil and dust particles were removed from leaf samples by washing with deionized water and freeze-drying. Dried leaves were ground and digested in a microwave digestion system (Cem Corporation, Matthews, NC, USA) with a mix of hydrochloric acid (0.5 mL) (Suprapur, Merk), nitric acid (9 mL) (Suprapur, Merk), and hydrogen peroxide (1 mL) (Sigma Aldrich, San Luis, MO, USA) (USEPA-3052). Leaf macronutrients (P, K^+, Ca^{2+} and Mg^{2+}), micronutrients (Fe, Mn and Zn) and phytotoxic elements (Na^+ and B^{3+}) were determined by inductively coupled plasma mass spectrometry (ICP-MS Agilent Technologies, Model 7900, Santa Calara, CA, USA). Chlorides were extracted from 0.5 g of ground leaves with 25 mL deionized water and measured by ion chromatography with a liquid chromatograph (Thermo Scientific Dionex, Model ICS-2100, Switzerland). Nitrogen was determined by the Kjeldahl method [23]. Additionally, proline was extracted from 50 mg of freeze-dried leaves with sulphosalicylic acid (3%) and quantified according to the protocol described by Bates et al. [24] and modified by Torrecillas et al. [25].

2.5.4. Gas Exchange Parameters

Gas exchange was measured between 09:00 and 11:00 h on a weekly basis from May 2018 to May 2019 on selected clear days. Measurements were made on healthy, fully expanded mature leaves (one leaf on each of the six trees per treatment), exposed to the sun, from young branches in exterior mid-canopy positions. The net CO_2 assimilation rate (A), stomatal conductance to water vapor (g_s) and transpiration rate (E) were measured with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) equipped with a broadleaf chamber (6.0 cm^2). The leaf chamber temperature was maintained between 28 and 32 °C, leaf to air vapor pressure deficit at 2.5 ± 0.5 kPa and the relative humidity of the chamber at 30–40% during measurements. The molar air flow rate inside the leaf chamber was 500 $\mu mol\ mol^{-1}$. Portable 12-g cartridges of high-pressure, liquefied, pure CO_2 were attached to the console by an external CO_2 source assembly and were controlled automatically by a CO_2 injector system (6400-01 Li-Cor, Lincoln, NE, USA). All measurements were taken at a reference CO_2 concentration similar to ambient (400 $\mu mol\ mol^{-1}$) and a saturating photosynthetic photon flux of 1200 $\mu mol\ m^{-2}\ s^{-1}$ [26,27], using a red/blue light source (6400-02B LED, LI-COR, Lincoln, NE, USA) attached to the leaf chamber. Gas exchange parameters were calculated automatically by the internal program of the LI-6400, based on the equations of von Caemmerer and Farquhar [28].

2.5.5. Yield and Fruit Quality

In February 2018 and 2019, six inner trees per treatment; i.e., two per replicate, were evaluated to determine (i) number of fruits per tree, (ii) yield in total kilograms per tree, and (iii) fruit weight. As the trees were young, only one pick was necessary at each harvest period to collect all the mandarins. Each season, 54 fruits per treatment (18 fruits per block) were randomly selected to study the fruit quality. In 2018, the fruits were cut in the equatorial area and the juice content (JC) and soluble solid content (SSC) were determined. To do so, the fruits were squeezed and the juice filtered and weighted. The SSC of the juice was measured at 25 °C with a digital refractometer (Atago, Palette PR100). In 2019, in addition to these measurements, the equatorial and longitudinal diameters were determined and the fruit fractions, juice, peel, and pulp, were separated, weighed, and expressed as percentages; the peel thickness was measured at three points with a digital caliper; the tritratable acidity (TA) (expressed as percentage of citric acid in the juice) was determined by titration with 0.1 N NaOH to pH 8.1, using an automatic titrator (CRISON Titro-Matic 2S, Crison Instruments S.A., Barcelona, Spain); and the maturity index (MI) was expressed as the SSC × 10/TA ratio.

2.5.6. Economic Assessment

A simple economic analysis was performed. It should be noted that this only considered the economic return calculated each season for the yield and the prices received by growers for selling the fruit (0.4 €/kg) as income, and the use of water and its cost under each scenario; i.e., FW = 0.35 €/m^3, DSW = 0.60 €/m^3, and MW = 0.47 €/m^3 as outlay.

2.6. Statistical Analysis

Statistical analysis was performed as a weighted analysis of variance (ANOVA; statistical software IBM SPSS Statistics v. 21 for Windows). The Shapiro–Wilk test ($P < 0.05$) was used to evaluate the normality of the data. Tukey's Honestly Significant Difference (HSD) test ($P \leq 0.05$) was used for mean separation. Unless otherwise stated, the significance level was $P \leq 0.05$.

3. Results and Discussion

3.1. Irrigation Water Quality and Volume Applied

The water quality parameters for the FW, DSW, and MW are shown in Table 1. Notable differences between the FW and DSW were observed throughout the experiment. Reverse osmosis membranes do not only separate the undesirable salts from the water, but also remove minerals that are essential nutrients for plant growth [29]; especially divalent ions such as Ca^{2+}, Mg^{2+}, and $SO_4{}^{2-}$ with higher molecular mass. In this sense, Ca^{2+}, Mg^{2+}, and $SO_4{}^{2-}$ in the DSW were only 37.3%, 20.4%, and 8.0% of those measured in the FW from Oct-2017 to Sept-2018 and 50.9%, 33.7%, and 21.6% of those measured in the FW from Oct-2018 to June-2019. However, the concentrations of Na^+ and Cl^-, in the DSW were still high and similar to those found in the FW (Table 1). In the case of Na^+ and Cl^-, the selectivity of the one-stage RO process is high; 99.81% and 98.93%, respectively. However, both ions represent the largest part of the salts in marine water; 10.8 g/L and 19.5 g/L (30.2% and 54.3%, respectively), which explains their high concentrations in the DSW. The boron concentration in the DSW was similar to that found in the FW, a circumstance that was attributed to the low selectivity for B^{3+} of the one-stage RO process (71.11%) due to its low molecular mass (10.811 g/mol), which hinders its retention by the RO membranes. Additionally, in Spain, no legal regulation exists to condition the quality of the DSW devoted for irrigation, but its production is regulated by the Royal Decree 140/2003, which establishes the sanitary criteria for the quality of DSW for human consumption and the maximum B^{3+} concentration at 1 mg/L. It is of note that Na^+ and B^{3+} concentrations surpassed the maximum published threshold for citrus irrigation [4,30,31] and hence, detrimental effects on crops might occur (Table 1).

Table 1. Chemical properties (electrical conductivity: EC_w, Sodium adsorptium ratio: SAR_w, pH, cations: Na^+, K^+, Ca^{2+}, Mg^{2+}, B^{3+} and anions: Cl^-, $NO_3{}^-$, $PO_4{}^{3-}$, and $SO_4{}^{2-}$) for fresh water (FW), desalinated seawater (DSW), and mixed water (MW). Data are divided into the two main periods of the study.

Parameters	Units	FW		DSW		MW	
		Oct-2017 to Sept-2018	Oct-2018 to Jun-2019	Oct-2017 to Sept-2018	Oct-2018 to Jun-2019	Oct-2017 to Sept-2018	Oct-2018 to Jun-2019
pH		7.7 ± 0.2	7.8 ± 0.1	7.9 ± 0.3	7.9 ± 0.8	7.7 ± 0.2	7.8 ± 0.1
EC_w	dS/m	1.7 ± 0.4	1.1 ± 0.0	0.9 ± 0.0	0.9 ± 0.2	1.4 ± 0.2	1.0 ± 0.1
Ca^{2+}	mg/L	65.1 ± 12.9	59.9 ± 9.4	24.3 ± 6.5	30.5 ± 18.0	45.2 ± 8.9	45.5 ± 9.2
Mg^{2+}	mg/L	44.4 ± 12.3	31.7 ± 5.2	9.1 ± 2.1	10.7 ± 10.7	27.9 ± 5.8	21.8 ± 6.4
Na^+	mg/L	195.1 ± 72.7	128.0 ± 25.3	152.6 ± 19.1	109.6 ± 38.2	175.9 ± 37.2	130.1 ± 7.2
K^+	mg/L	11.7 ± 2.8	7.2 ± 1.6	7.1 ± 1.0	5.9 ± 2.9	10.1 ± 2.2	6.4 ± 1.1
B^{3+}	mg/L	0.61 ± 0.28	0.49 ± 0.07	0.84 ± 0.11	0.85 ± 0.19	0.72 ± 0.11	0.71 ± 0.09
Cl^-	mg/L	298.2 ± 114.8	189.0 ± 24.2	244.5 ± 37.7	178.2 ± 63.2	266.6 ± 65.2	206.9 ± 15.3
$SO_4{}^{2-}$	mg/L	181.1 ± 48.6	159.5 ± 34.2	14.5 ± 4.1	34.6 ± 53.4	95.9 ± 25.6	98.9 ± 28.9
$NO_3{}^-$	mg/L	6.6 ± 1.7	8.7 ± 2.8	2.2 ± 2.2	4.3 ± 4.6	5.3 ± 1.9	4.9 ± 1.5
$PO_4{}^{3-}$	mg/L	LD * < 0.76	LD * < 0.76	LD * < 0.76	LD * < 0.76	LD * < 0.76	LD * < 0.76
SAR_w	$[mmol/L]^{0.5}$	4.5 ± 1.4	3.4 ± 0.7	6.8 ± 0.8	4.7 ± 1.6	5.1 ± 0.9	4.0 ± 1.0
Max threshold Na^+		115 mg/L Grattan et al. [30]					
Max threshold Cl^-		350 mg/L Hanson et al. [32]; 152–238 mg/L Grattan et al. [30]					
Max threshold B^{3+}		0.5 mg/L Martínez-Alvarez et al. [4]; Voutchkov and Semiat [31]					

*: LD: below the detection limit.

The EC_w of the DSW was 0.9 dS/m due to the large salt removal in the RO process. However, the high concentration of Na^+ and the low of Ca^{2+} and Mg^{2+} led to high SAR_w values (6.8 ± 0.8 $[mmol/L]^{0.5}$) from Oct-2017 to Sept-2018 and 4.7 ± 1.6 $[mmol/L]^{0.5}$ from Oct-2018 to Jun-2019), compared to the FW (Table 1).

All the experimental treatments received the same amount of irrigation water (1663 m^3/ha from Oct-2017 to Sept-2018 and 1025 m^3/ha from Oct-2018 to Jun-2019).

3.2. Soil Salinity and Sodicity

Figure 1 shows the evolution of EC_e and SAR_e for each treatment from October 2017 to June 2019, for 0–25 cm and 25–50 cm depths (FW, DSW, and MW).

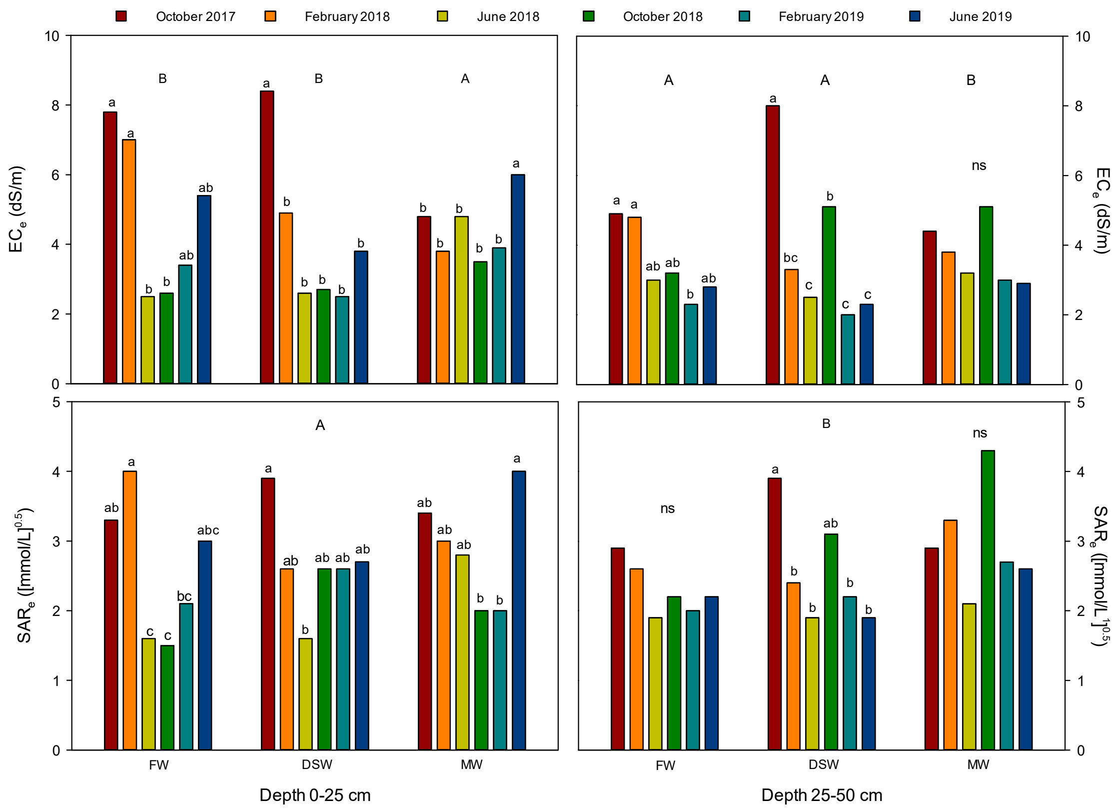

Figure 1. Electrical conductivity of the saturated paste extract (EC_e, dS/m) and sodium adsorption ratio (SAR_e) in the three irrigation treatments (fresh water, FW; desalinized sea water, DSW; and mix water, MW) from October 2017 to June 2019 for 0–25 cm and 25–50 cm depths. Values represent the mean for each sampling period (N = 3 for each sampling). Similar small letters in each bar indicate no significant differences among samplings; no small letters means no significant differences; and different capital letters in each treatment for surface and depth samplings indicate significant differences between depths, in both cases according to Tukey's HSD test ($P \leq 0.05$).

First, the large initial variability of the EC_e and SAR_e among samples collected at different points of the orchard should be highlighted. For example, while EC_e measured at 25 cm in the FW treatment in October 2017 was 7.8 dS/m, it was 4.8 dS/m in the MW treatment. A similar situation was found with SAR_e, especially at the 50 cm depth initial sampling. Such a huge initial non-homogeneity could be explained by the initial soil conditioning performed to establish the orchard, mainly due to the creation of ridges and the addition of organic matter. After that date, these high EC_e and SAR_e values began to be lower due to the rain which fell between January and June 2018 (103.8 mm). With salts having been leached from the soils, especially in FW and DSW treatments, new EC_e and SAR_e increases were again observed in June 2019.

Concerning the risk of soil sodicity, it is well known that high concentrations of Na^+ can damage the soil physical properties, causing clay dispersion which leads to: (i) the structural collapse of soil aggregates; (ii) decreased soil hydraulic conductivity; (iii) erosion problems; (iv) soil compaction; and (v) decreased soil aeration [11,33]. The results indicated that only irrigation with the DSW could induce a certain sodicity risk into the soil (Figure 2). As the DSW presented low EC_w, such sodicity risks could be corrected by adjusting the fertigation programs to increase the amount of Ca^{2+} and Mg^{2+} to displace Na^+ from the soil cation exchange complex, hence reducing the SAR_e or blending the DSW with FW or other non-conventional water resources such as reclaimed or underground waters. In any case, seasonal soil monitoring is strongly encouraged to detect any deterioration of the soil structure when DSW is used alone for crop irrigation, especially in soils with a high clay content, where the sodicity hazard may be more relevant [4].

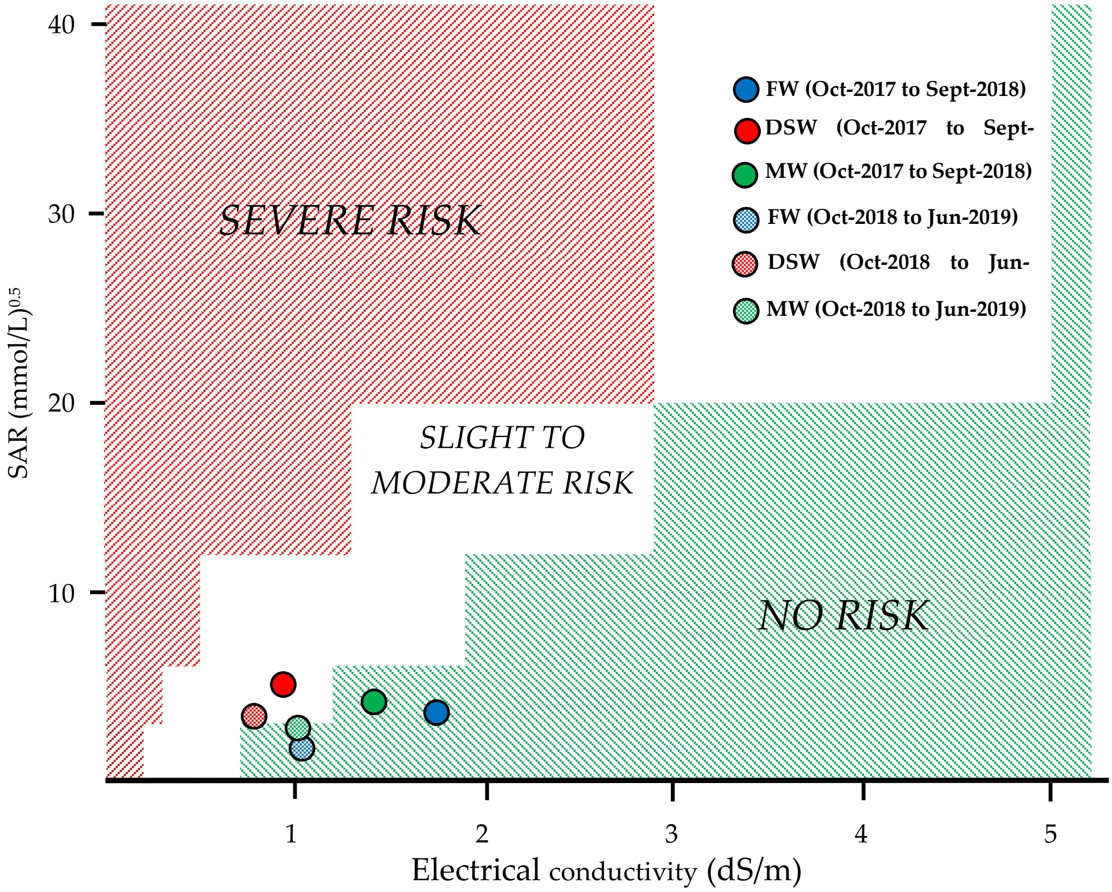

Figure 2. Soil sodicity potential risk evaluated using the sodium adsorption ratio (SAR_W) and the electrical conductivity (EC_W) of the irrigation water in the three irrigation treatments (fresh water; FW, desalinized seawater; DSW, and mix water; MW).

3.3. Leaf Mineral Analysis and Phytotoxic Elements

Figure 3 shows the mean leaf concentrations of Na^+, Cl^-, and B^{3+} for the periods from October 2017 to September 2018 and from October 2018 to June 2019 for the three irrigation treatments.

During the two years of the experiment, concentrations of macro-and micro-nutrients (N, P, K^+, Ca^{2+}, Mg^{2+}, Fe, Mn, and Zn) measured in the leaves were within the optimum ranges proposed by Legaz et al. [34] (data not shown).

Regarding the Na^+ measured in the citrus leaves, no significant differences were observed among treatments and no trends of Na^+ increases were maintained over time throughout the experiment. Although the concentration of Na^+ in most of the tested irrigation waters exceeded the toxic threshold proposed for citrus by Grattan et al. by more than 50% [30] (115 mg/L; Table 1), almost all of the leaf samples were below the minimum threshold within the range for leaf phytotoxicity proposed by Grattan et al. [30] (Figure 3a). In any case, all values were found below the maximum Na^+ threshold proposed by Grattan et al. [30] for citrus (2.5 g/kg). Moreover, the trees were well supplied with K^+ (between 11 and 29 g/kg of dry matter) and with Ca^{2+} (between 17 and 54 g/kg of dry matter), which could have limited the Na^+ uptake by roots and its transport to the leaves [35,36].

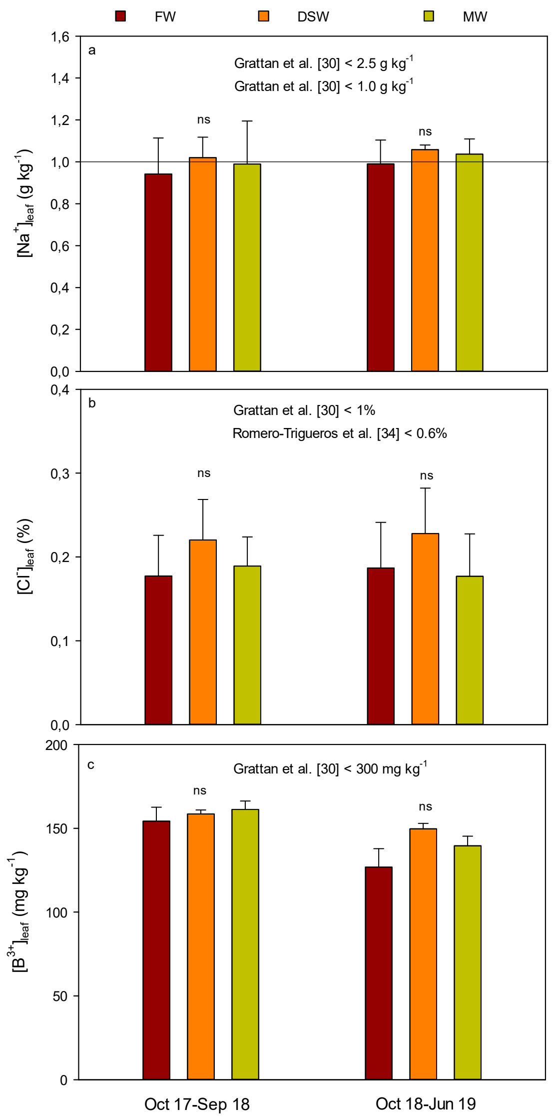

Figure 3. Mean leaf concentrations and standard deviations of (**a**) Na^+, (**b**) Cl^-, and (**c**) B^{3+} for the periods from October 2017 to September 2018 and from October 2018 to June 2019 for the three irrigation treatments (fresh water; FW, desalinated sea water; DSW, and mixed water; MW). ns means that significant differences were not detected among the treatments for each period ($P \leq 0.05$). References in the figures represent toxic thresholds found for citrus.

Regarding Cl^-, only sporadic significant differences among treatments occurred during the summer months in 2018 (from April 2018 to September 2018; data not shown). As observed for Na^+, no trends of Cl^- increase in leaves were observed throughout the experiment (Figure 3b). The concentration of Cl^- in the irrigation waters was well below the threshold proposed by Hanson et al. [31] (350 mg/L; Table 1) and never exceeded the leaf toxic threshold proposed by Romero-Trigueros et al. [37] and Grattan et al. [30]; i.e., 6 and 10 g/kg, respectively (Figure 3b).

The B^{3+} concentration in the irrigation waters exceed the maximum threshold proposed for citrus [4,31] (0.5 mg/L; Table 1). In this context, Abu-Daba'an and Al-Najar [38] irrigated a citrus orchard for ten consecutive years with a treated effluent with a B^{3+} concentration of 1.03 ± 0.45 mg/L and did not reach toxic levels for B^{3+} leaf concentration. However, they also concluded that, since B^{3+} is accumulative, if the same irrigation water is used for 30 years, leaf B^{3+} concentrations could reach that harmful level. In our short-term study, no trends of B^{3+} increase in leaves have been observed throughout the experiment. The critical leaf B^{3+} concentration in citrus when toxicity occurs falls in the

250–260 mg/kg dry weight range [39], although B^{3+} can become toxic at higher tissue concentrations of 100 mg/kg dry weight, ranges of 100–300 mg/kg dry weight tend to produce slight to moderate injury [30]. Leaf B^{3+} concentrations in our study ranged between 100–200 mg/kg with no differences between the DSW-irrigated trees and the control ones (Figure 3c). No visual leaf injury was observed in the first two years of study. On the other hand, diverse research works indicate that the foliar concentration might not be the only factor that determines the relative tolerance of different rootstocks to boron [40,41].

3.4. Plant Water Status and Leaf Gas Exchange

Figure 4 presents the average plant Ψ_{stem}, Ψ_{leaf}, Ψ_{osm}, and leaf turgor pressure measured from May 2018 to May 2019 for the three irrigation treatments.

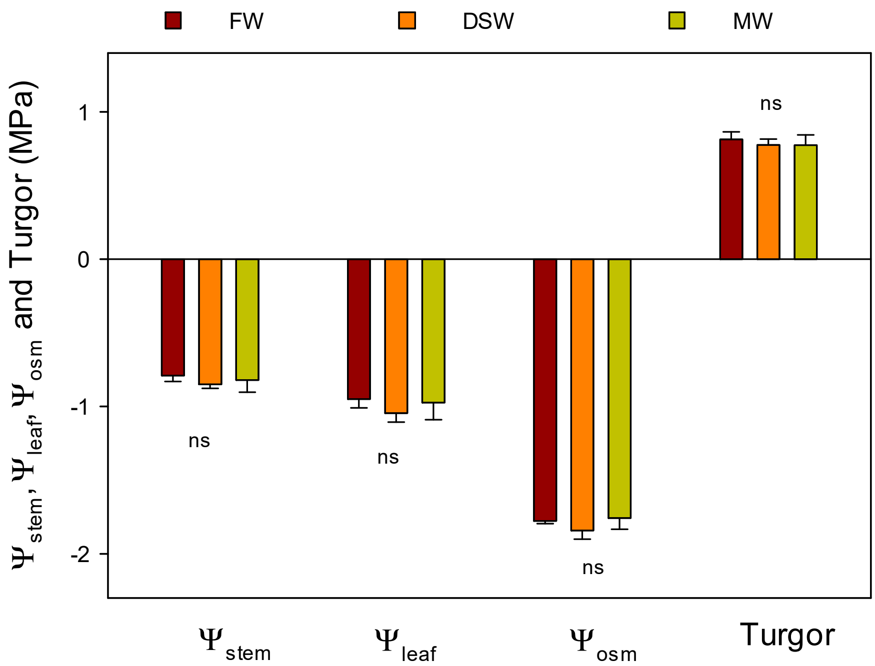

Figure 4. Mean plant water relations and standard deviations (Ψ_{stem}, stem water potential; Ψ_{leaf}, leaf water potential; Ψ_{osm}, osmotic potential and leaf turgor pressure; expressed in MPa) measured from May 2018 to May 2019 for the three irrigation treatments (fresh water; FW, desalinated sea water; DSW, and mixed water; MW). ns means that significant differences were not detected among treatments for each period ($P \leq 0.05$).

The plant water relations, analyzed as Ψ_{stem}, Ψ_{leaf}, Ψ_{osm}, and leaf turgor, irrespective of the treatment, remained quite constant during the experiment, and their values indicated the good water status of the trees (Figure 4) [42–44]. In fact, significant differences between treatments were only observed in August 2019 (data not shown), and were associated to a high-water demand period. At this date, Ψ_{leaf} and Ψ_{osm} were significantly lower in the DSW with regard to both the FW and the MW treatments (data not shown) but not enough to induce differences in leaf turgor. In this sense, it has been demonstrated that citrus can reduce Ψ_{osm}, which allows to reduce Ψ_{leaf} with no alteration of leaf turgor potential, thus avoiding leaf dehydration by leaf Cl^- and Na^+ accumulation under saline environmental stress [45]. In our study, Cl^- and Na^+ concentrations of DSW were similar to those found in the FW, so no Na^+ or Cl^- accumulation could be produced to reduce osmotic potential (Figure 4). Instead of that, in August 2018 a significant increase of proline was produced in citrus leaves of DSW-irrigated trees with regard to those irrigated with the FW or MW (data not shown). To maintain plant turgor when a lowering of leaf water status occurs many plants may also enhance biosynthesis of secondary metabolites, such as some amino acids, which may act as osmolytes under these situations [46].

Regarding the crop gas exchange, no differences in A, g_s, E, Ci, A/E, or A/g_s among treatments were observed throughout the experiment (Table 2). A and g_s ranged between 3.19 µmol/m^2/s and 0.027 mmol/m^2/s, observed in the DSW treatment in May 2019 and November 2018, respectively to 10.61 µmol/m^2/s and 0.111 mmol/m^2/s, observed in the FW treatment in September 2018. As expected, the highest values for A, E, and g_s were registered in summer due to the increase in the evapotranspirative demand ruled by a significant increase in the vapor pressure deficit and solar radiation (data not shown) [44]. All these increases in the gas interchange parameters during the summer time were mainly due to a higher stomatal opening; however, they did not change in the same way and a seasonal variation in leaf water use efficiency was observed, as was previously reported by Medrano et al. [47]. The ratio of the fluxed of net photosynthesis and conductance for water vapor (A/E), which indicates the cost of CO_2 assimilation per unit of water, decreased during the summer time, ranging from 6.9 to 2.8 µmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1} in November 2018 and May 2019 respectively in the DSW treatment (data not shown). Similarly, intrinsic water-use efficiency (A/g_s) values ranged from 160 to 74 µmol CO_2 m^{-2} s^{-1}/mol H_2O m^{-2} s^{-1} in November 2018 and May 2019 respectively in the DSW treatment, showing the lower plant efficiency to fix CO_2 per unit of H_2O lost during the summertime. Both instantaneous and intrinsic water-use efficiency did not show significant differences for the three water quality treatments in their mean values recorded from May 2018 to May 2019 (Table 2). However, values recorded in May 2019 in the DSW for A/E and A/g_s were significantly lower than those found in the FW and MW-irrigated trees (data not shown).

Table 2. Mean stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), net photosynthesis (A, µmol CO_2 m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), sub-stomatal CO_2 concentrations (Ci, µmol CO_2), instantaneous water-use efficiency (A/E, µmol CO_2 $m^{-2}s^{-1}$/mmol H_2O m^{-2} s^{-1}), intrinsic water-use efficiency (A/g_s, µmol CO_2 m^{-2} s^{-1}/mol H_2O m^{-2} s^{-1}) for the three water quality treatments (fresh water, FW; desalinated seawater, DSW; and mixed water, MW). Data represent the mean and the standard deviation of monthly values collected from May-2018 to May-2019. ns means non-significant differences between treatments according to Tukey's HSD test ($P \leq 0.05$).

Parameters	FW	DSW	MW	Significance
g_s	0.069 ± 0.006	0.064 ± 0.007	0.071 ± 0.012	ns
A	6.80 ± 0.32	6.56 ± 0.54	7.18 ± 0.82	ns
E	1.76 ± 0.09	1.69 ± 0.12	1.81 ± 0.22	ns
C_i	204 ± 12	199 ± 11	201 ± 13	ns
A/E	4.23 ± 0.07	4.20 ± 0.14	4.31 ± 0.13	ns
A/g_s	105 ± 7	108 ± 7	107 ± 9	ns

3.5. Yield and Fruit Quality

Concerning the soil salinity effects on the mandarin yield, several thresholds from which yield reductions could be expected have been proposed in literature. For instance, Ayers and Westcot [48] proposed a maximum EC_e value of 1.7 dS/m for citrus. More recently, Nicolás et al. [44] established a threshold of 2.21 dS/m for *Citrus Clementina* cv. Orogrande mandarins grafted on Carrizo citrange (*Citrus sinensis* [L.] Osb. × *Poncirustrifoliata* [L.]) rootstock. In our case, EC_e measured at second harvest (February 2019) ranged from 2.0 ± 0.3 dS/m observed in the DSW treatment at 50 cm depth to 3.9 ± 1.8 dS/m observed in the MW treatment at 25 cm depth. Therefore, these figures might have provoked notable yield reductions. However, during the two-year experimental period, no significant differences among treatments were observed in the yield components or in the fruit quality. This circumstance could be mainly explained by the large standard deviations caused by the youth of the trees, which for some values, represented more than 100% of the mean; a clear example of this is the crop fruit load for the FW or DSW in February 2018 (Table 3). Significant differences in tree canopy equatorial area, crop load, yield, yield/tree canopy equatorial area, fruit weight, and fruit quality parameters were not observed in the February 2019 harvest either (Table 4). As occurred in the February 2018 harvest, a large data variance was observed and attributed to the young age of the trees.

Table 3. Tree canopy equatorial area, crop load, yield, yield/tree canopy equatorial, and fruit weight for the two experimental seasons and for the three irrigation treatments (fresh water, FW; desalinated seawater, DSW; and mixed water, MW). Each value is the average ± standard deviation of the measurements performed in two inner trees per treatment (six central trees in each replicate per treatment). ns means non-significant differences between treatments according to Tukey's HSD test ($P \leq 0.05$).

Season	Treatment	Tree Canopy Equatorial Area (m^2)	Crop Load (Fruits/Tree)	Yield (kg/Tree)	Yield/Tree Canopy Equatorial Area (kg/m^2)	Fruit Weight (g)
February 2018	FW	1.2 ± 0.3	10.7 ± 14.9	1.3 ± 1.9	1.1 ± 1.6	120.3 ± 5.5
	DSW	1.4 ± 0.4	31.5 ± 33.7	4.9 ± 4.2	3.5 ± 2.9	138.3 ± 18.9
	MW	1.5 ± 0.7	23.0 ± 8.1	3.1 ± 1.4	2.1 ± 0.9	134.7 ± 30.1
	Significance	ns	ns	ns	ns	ns
February 2019	FW	3.1 ± 0.4	118.3 ± 78.5	13.9 ± 8.2	4.5 ± 2.6	122.7 ± 15.2
	DSW	3.3 ± 0.7	97.0 ± 54.1	11.4 ± 6.2	3.6 ± 1.9	120.3 ± 11.3
	MW	3.2 ± 1.0	161.2 ± 58.1	18.1 ± 6.2	5.5 ± 1.8	113.7 ± 9.6
	Significance	ns	ns	ns	ns	ns

Table 4. Fruit quality parameters: equatorial and longitudinal diameters, juice and soluble solid content, peel thickness, titratable acidity, and maturity index for the two experimental seasons and for the three irrigation treatments (fresh water, FW; desalinated seawater, DSW; and mixed water, MW). ns means non-significant differences between treatments according to Tukey's HSD test ($P \leq 0.05$).

Season	Treatment	Equatorial Diameter (mm)	Longitudinal Diameter (mm)	Juice Content (%)	Soluble Solid Content (°Brix)	Peel Thickness (mm)	Titratable Acid (%)	Maturity Index
February 2018	FW	-	-	50.7 ± 2.3	11.7 ± 1.6	-	-	-
	DSW	-	-	50.3 ± 1.6	12.4 ± 0.8	-	-	-
	MW	-	-	51.1 ± 2.2	12.7 ± 0.5	-	-	-
	Significance	-	-	ns	ns	-	-	-
February 2019	FW	66.1 ± 2.3	56.3 ± 2.3	46.2 ± 1.77	13.5 ± 1.1	2.9 ± 0.2	1.4 ± 0.2	9.5 ± 0.6
	DSW	66.0 ± 2.2	56.2 ± 1.2	45.3 ± 1.64	14.2 ± 0.8	3.0 ± 0.2	1.6 ± 0.1	9.0 ± 0.8
	Significance	65.3 ± 2.5	56.8 ± 2.4	43.7 ± 1.79	14.0 ± 0.9	2.8 ± 0.2	1.5 ± 0.3	9.5 ± 1.1
	ANOVA	ns	ns	ns	ns	ns	ns	ns

Concerning the quality parameters, the measured juice content and soluble solid content agreed with the results previously published by Nicolás et al., Asharaf and Harris, and Navarro et al. [44,46,49,50] on mandarin trees irrigated with fresh water in southeast Spain.

3.6. Economic Assessment

The irrigation with the DSW almost doubled the cost of the irrigation water (Table 5). In 2019, the income from selling the fruit was lower than the FW due to lower yields, although significant differences were not observed due to the large variability in yield, as commented in Section 3.5. income-outlay results shown in Table 5 for October 2018 to June 2019 indicated that the MW treatment was the most economically feasible due to the higher yield in proportion to the increase in the cost of water. Please note that significant differences among FW and DSW treatments were not detected. In addition, the large variability in yield must be taken into consideration.

Table 5. Economic assessment of the mandarin production under the three scenarios selected. For each year and row, means with the same letter are not significantly different according to Tukey's HSD test ($P \leq 0.05$).

Title	Oct-2017 to Sept-2018			Oct-2018 to Jun-2019		
€/ha	FW	DSW	MW	FW	DSW	MW
Water cost	582	998	790	543	931	737
Fruit selling income	270 ± 394a	1017 ±872a	643 ± 290a	2885 ± 1702a	2366 ± 1287a	3757 ± 1287a
Income−Outlay	−312 ± 187a	19 ±126a	−146 ± 499a	2342 ± 1159ab	1435 ± 356b	3020 ± 549a

FW: fresh water; DSW: desalinated seawater; MW: mixed water.

4. Conclusions

This study has evaluated the short-term response of young mandarin trees to irrigation with desalinated seawater (DSW). The young age of the trees has favored the increase in the variability of the results and therefore, no significant differences among treatments have been observed in (i) the plant water status; (ii) the net photosynthesis and the stomatal conductance; (iii) the leaf phytotoxicity, except for Na^+ and B^{+3} in summer months; (iv) the yield components; and (v) the fruit quality. Although Na^+ and B^{+3} concentrations in the irrigation waters, irrespective of the treatment, have exceeded the toxic threshold proposed for citrus in the scientific literature, the Na^+ concentration in the leaves has only exceeded this threshold in three months from April to June in 2018. To summarize, although the results presented herein may, to a certain extent, justify the agronomic and economic viability of the use of DSW to irrigate young trees in the short-term, studies which consider such effects in the mid-long term, when the variability of the yield data is notably reduced, are still needed.

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