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SECONDARY TREATED WASTEWATER AS A SUPPORT STRATEGY FOR TREE CROPS IRRIGATION: NUTRITIONAL AND PHYSIOLOGICAL RESPONSES ON APPLE TREES

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Abstract

Wastewater for irrigating tree crops may act both as water and mineral nutrients source, offering potential agronomical and environmental advantages. This work investigated the effect of an entire season supply of secondary treated wastewater (STW) on the nutritional and physiological responses of 3 year old apple trees. Trees (Gala /M9) were individually grown on 40-L pots filled with a sandy-loamy soil and drip irrigated with: 1)Tap water (TW) (without any mineral fertilizer inputs); 2) Tap water plus mineral fertilized inputs (TW+MF) and 3) STW (without any mineral fertilizer inputs). Each treatment was applied to five individual trees. Daily leaf carbon assimilation rates were promoted by STW, compared to TW trees, although TW+MF trees showed the highest values. Although STW provided a "fertigation-like" effect, the tree nutrient demand was only partially fulfilled. Leaf mineral concentration resulted mostly in the optimal range for STW and TW+MF, except TW, which showed nutritional deficiencies, especially on leaf rather than on fruit tissues. No heavy metal contamination was recorded in STW leaves nor in fruit tissues. A decrease in STW-irrigated tree stem water potentials suggested a moderate salinity stress that indirectly improved fruit quality parameters. Irrigating with STW did not enhance shoot growth compared to TW+MF, promoting instead fruit yield. Results indicate how STW may be suitably reused as a precious resource for supporting the traditional fresh-water supplies in irrigating fruit tree crops. Moreover, the application of STW could allow to partially save tree mineral fertilization needs, thanks to its nutritional inputs.

Key words: fertilization, nitrogen, plant water relations, water reuse, water scarcity

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1. Introduction

Recycling treated municipal wastewater for agricultural irrigation purposes may reduce the water volumes extracted from natural water sources especially in areas facing water shortages. This practice could contribute to recycle nutrients and reduce the amount of pollutants discharged into the waterways (Hanjra et al., 2012). For instance, through wastewater irrigation practices, most of the eutrophication-related elements (i.e. N and P) could be conveniently reused as fertilizers rather than lost in fresh-water bodies.

Nowadays, the reuse of treated wastewater in agriculture is highly encouraged as the amount of collected and treated wastewater is likely to increase considerably with population growth and urbanization. However, treated wastewater must be carefully managed to protect the environment and public health. Scientific knowledge of such practice on both annual and perennial crops intended for human consumption are highly required (Pedrero et

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al., 2010), especially when its use in agriculture is increasing in the Mediterranean Countries.

Compared to freshwater, treated wastewater has a higher mineral and organic matter (OM) concentration, representing a precious source of nutrients to "fertigate" crops which in turn can provide benefits on plant physiological and nutritional status (Khurana and Singh, 2012). Literature mostly confirms that tertiary treated wastewater (TTW) can be suitably reused as water resource to irrigate tree crops in water-scarce Mediterranean areas (Mendoza-Espinoza et al., 2008; Pedrero and Alarcon, 2009; Pedrero et al., 2013; Petousi et al., 2015; Vivaldi et al., 2013). In Europe a univocal legislation regulating the reuse of treated wastewater in agriculture is currently missing and each Country adopts its own regulation. In Italy, for instance, the reuse of secondary treated wastewater (STW) for irrigation purpose is still not admitted.

The fertilization effect of STW on cultivated crops remains underestimated. Indeed, STW supplies significant amount of OM as well as plant-available nutrients (Chen et al., 2008). Thus, a large-scale utilization of STW to irrigate crops would reduce the need of chemical inputs in agriculture. The use of wastewater in agriculture has been demonstrated to positively affect soil fertility and productivity (WCED Report, 1987). However, most of these studies were addressed using TTW, in which the amount of nutrients, especially nitrogen (N) and phosphorous (P), were significantly depleted as a consequence of the cleaning treatments (Pescod, 1992), while the effect of the STW is only beginning to be explored. On the other hand, although the agronomic validity would need to be demonstrated, the use of STW in agriculture implies environmental (i.e. soil pollution, phytotoxicity), food safety risks and social acceptance obstacles as well (Bernstein, 2011; Fatta-Kassinos et al., 2009; Muchuweti et al., 2006).

Although irrigation of fruit tree crops normally does not wet the plant canopy (preventing external contamination), investigations on the potential consequences of STW irrigation on the tree-root absorption pathway are required before its diffusion on a large scale.

Finally, these studies are of extreme importance to support the legislator in promulgating new regulations about treated wastewater reuse in agriculture. Among the irrigated fruit tree crops, apple is within the most important cultivated species in Italy with a total area over 57.000 ha and an annual production of about 2.4 Mt (Istat, 2018), the most important European producer after Poland.

The aim of this work was to investigate the effect of STW (treated according to the Italian Decree (DME, 2006) as irrigation water on the nutritional and physiological responses of bearing apple trees.

2. Material and methods

2.1. Experimental set up

We carried out a 1-year experiment at the experimental farm of the University of Bologna located in Cadriano (BO), on 15 bearing 3-year old apple trees (Malus×domestica Borkh) cv. Gala grafted on M9. Trees were grown in 40-L pots each, filled with an alkaline, poorly fertile sandy-loamy soil (USDA classification) and maintained under a shading hail net. Trees were trained as slender spindle, irrigated by four drippers per tree of 2 L h^{-1} and managed according to the local Integrated Standard Crop Management practices (ICM, 2010) for pruning, thinning, pest and disease management. Climate of the region is temperate sub-continental with warm and humid summers and cold winters. The average annual temperature was 14.1 °C, while annual precipitation was equal to 750 mm.

Starting 48 days (May 15th) after full bloom (DAFB) till 174 DAFB (September 5th), three irrigation treatments were set up, with 5 replicates (single tree) each: 1) irrigation with tap water (TW) 2) irrigation with tap water and fertilization with mineral inputs (TW+MF) and 3) irrigation with secondary treated wastewater (STW). Trees irrigated with STW did not receive additional fertilizer sources. STW (DME, 2006) was provided by the local urban wastewater treatment plant, managed by HERA S.p.a (Italian multi-utility). Along the season, TW+MF trees received 7.83, 1.56, 5.97, and 0.49 g $tree^{-1}$ of N, P, K and Mg as commercial mineral fertilizers, respectively, split in 3 interventions starting from 48 DAFB. Trees were irrigated twice a day to balance crop evapotranspiration (ET_c) rate.

2.2. Irrigation water chemical and microbiological characterization

Samples of STW and TW were collected at two weeks intervals throughout the irrigation season for chemical analyses, then stored at 5°C. Mineral concentration was determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Ametek Spectro Arcos EOP, Kleve, Germany) on liquid samples as such. pH was measured with a pH-meter XS PH510 (Eutech Instruments, Singapore) whereas electrical conductivity (EC) was determined by a conductimeter (METERLAB, CDM 210, Radiometer Analytical, France). Finally, total organic carbon (TOC) and total dissolved N were measured in the water samples by an elemental analyzer TOCVcpn- TNM1 (Shimadzu Corp., Kyoto, Japan).

The abundance of *E. coli* and *Salmonella* spp., was determined on STW samples by the membrane filtration method. Briefly, for *E. coli* enumeration, membranes were placed onto a Chromocult ES (VWR) agar and incubated at 37 °C for 24 h. *Salmonella* spp. Relative abundance was performed according to procedure UNI EN ISO 19250:2013.

The annual nutrient input was calculated multiplying the concentration of the dissolved elements in TW and STW water by the amount of water provided throughout the season. The TW+MF annual nutrient input is the contribution of the TW annual nutrient input plus the mineral fertilizer supply.

2.3. Tree nutritional status

Leaf mineral concentration was assessed on ten fully expanded leaves per replicate, randomly selected from annual shoots on the second half of July. Petioles were removed, then leaf limbs were washed, oven-dried, weighed, milled and analysed. N was determined by the Kjeldahl method (Schuman et al., 1973) while P, K, Ca, Mg, S, Fe, Cu, B, Na, Zn, Mn were determined by ICP-OES after digestion with nitric acid (HNO_3) by a microwave lab station (Ethos TC-Milestone, Bergamo, Italy). The same procedure was adopted to asses mineral concentration of fruit peel and fruit pulp on fruit sampled at commercial harvest.

2.4. Vegetative growth and daily photosynthetic assimilation rates assessment

Three shoots per tree were selected and their length was recorded at 34, 41, 48, 54, 60, 68, 76, 83, 93, 104, 128 and 157 DAFB. Furthermore, for each tree leaf net assimilation rate (A) was measured at about 9:00, 13:00 and 16:00 hours at 174 DAFB using a portable gas analyser (Li-COR 6400, LI-COR, Lincoln, Nebraska, USA). Measurements were carried out on one fully-expanded leaf per plant. Light intensity inside the cuvette was maintained constant as recorded by the photosynthetic photon flux density (PPFD) sensor immediately before the measurements. Cumulative daily photosynthesis (∑A) (from 9:00 to 17:00) was then calculated as described by Tozzi et al. (2018) using the following equation (Eq. 1):

$$\sum_y = \sum_{i=t0}^{t1} \left(\frac{yt_0 + yt_1}{2}\right) i \sum_{yt1}^{t2} \left(\frac{yt_1 + yt_2}{2}\right) i \qquad (1)$$

where: y is the variable A whereas t_0, t_1 and t_2 correspond to the A values recorded at 9:30, 13:30 and 16:30, respectively. Cumulative daily photosynthesis (∑A) was then multiplied by the total leaf area per tree. Leaf area was estimated by multiplying the total leaf number by the average leaf area, measured by a leaf area meter (LI-3000 A, LI-COR, Lincoln, Nebraska, USA) on three replicates per tree, each of 30 grams of leaves.

2.5. Tree leaf and stem water potential

The daily patterns of leaf and stem water potentials (WP) were assessed at 115 (pre-harvest) and at 150 DAFB (post-harvest). Measurements were performed at 6:00, 9:00, 13:00 and 16:00 hour using a Scholander pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Leaf water potential was measured on well exposed leaf per tree following the recommendations of Turner and Long (1980). Similarly, stem water potential was measured on leaves previously covered with aluminium foils and placed in plastic bags for at least 90 minutes prior measurements, to allow equilibration with the stem (Naor et al., 1995).

2.6. Fruit growth rate, tree yield and fruit quality

The diameter of 8 fruit per treatment, randomly chosen, was recorded at 60, 68, 76, 83, 94, 104, 117 and 128 DAFB, using a digital caliper provided with an external memory (http://www.hkconsulting.it/). At commercial harvest, yield was assessed for each tree. Fresh weight, dry matter content, flesh firmness, soluble solids content (SSC), skin lightness (L*) and a* b*colour components were assessed on the harvested fruits. Flesh firmness was assessed by a 53220 FTA Fruit Texture Analyser (T.R. Turoni srl, Italy) equipped with a 11 mm plunger. Soluble solids content was determined on the fruit juice by a digital refractometer (ATAGO CO., LTD, Japan) and peel colour was measured using a Minolta CR-400 (Konica Minolta Sensing Americas, Inc, USA).

2.7. Statistical analysis

Shoot length and fruit growth were analysed using a linear mixed model function. A one-way ANOVA followed by a Tukey HSD test using R software (www.r-project.org) was used to establish differences among treatments for daily leaf and stem water potential, daily cumulative photosynthesis and fruit quality parameters. Data of the tissue mineral concentration were analysed according to a complete randomized block design. When the analysis of variance showed a statistical effect, means were separated by the SNK Test (SAS 9.0, SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Water quality

As expected, mineral concentration was lower in TW than STW (Table 1). This latter showed a moderately alkaline pH and a relatively low EC and SAR indexes, indicating low risks of soil salinization. Values were even within the Italian legal thresholds for a direct utilization of treated wastewater sources, as TTW, in the agricultural sector (DME, 2003). TOC was almost 10-fold higher in STW compared to TW, with potential benefits on soil microbial activity, CEC and nutrient availability (Beutler et al., 2014). Dissolved mineral nutrients supplied through STW irrigation allowed to save 50.3 % and 75.1 % of N and P, respectively, compared to the reference mineral fertilized treatment (TW+MF). Similar results were achieved in open field by Vivaldi et al. (2017) and Pedrero et al. (2012) on nectarine trees. *E. coli* mean concentration in STW was 4 CFU 100 mL^{-1}, below the Italian *E. coli* threshold for irrigation water (DME, 2003). No *Salmonella* spp. were detected in STW water samples. Ag, Al, As, Be, Cd, Co, Cr, Hg, Mo, Sb, Sn, Ti, Tl, and V concentration either in STW or TW was below the instrumental detection limit (dl).

Table 1. Chemical and microbiological parameters of tap water (TW) and secondary treated wastewater (STW) (n=7 ± SE) and estimated annual nutrient inputs supplied trough the water source (TWni, STWni) and from the mineral fertilizers (TW+MFni)

Chemical parameters	*Irrigation water*		*Nutrition elements*	*Annual nutrient inputs*		
	[1]TW	*[2]STW*		*[3]TWni*	*[4]STWni*	*[5]TW+MFni*
pH	7.43 ± 0.04	8.31 ± 0.92				
EC (dS m^{-1})	0.47 ± 0.01	1.21 ± 0.04				
SAR	0.63 ± 0.03	1.85 ± 0.04				
N (urea 46%)			**N (urea 46%) (g $tree^{-1}$)**			5.52
NH_4-N (mg L^{-1})	0.02 ± 0.01	1.02 ± 0.09	**NH_4-N (g $tree^{-1}$)**	0.02	0.37	1.88
NO_3-N (mg L^{-1})	3.28 ± 0.60	11.6 ± 0.74	**NO_3-N (g $tree^{-1}$)**	1.18	4.17	1.63
P (mg L^{-1})	0.03 ± 0.01	3.28 ± 0.33	**P (g $tree^{-1}$)**	0.01	1.18	1.57
K (mg L^{-1})	4.81 ± 1.10	23.2 ± 0.68	**K (g $tree^{-1}$)**	1.73	8.32	7.70
Ca (mg L^{-1})	57.3 ± 5.91	72.8 ± 3.7	**Ca (g $tree^{-1}$)**	20.6	26.21	20.6
Mg (mg L^{-1})	17.2 ± 1.92	26.2 ± 2.12	**Mg (g $tree^{-1}$)**	6.11	9.43	6.60
S (mg L^{-1})	17.1 ± 1.24	28.7 ± 1.25	**S (g $tree^{-1}$)**	6.11	10.3	6.11
Na (mg L^{-1})	20.7 ± 0.73	82.9 ± 1.04	**Na (g $tree^{-1}$)**	7.43	29.8	7.43
Cu (µg L^{-1})	6.08 ± 1.10	15.9 ± 1.39	**Cu (mg $tree^{-1}$)**	2.18	5.72	2.18
Fe (µg L^{-1})	6.00 ± 0.50	22.9 ± 2.31	**Fe (mg $tree^{-1}$)**	2.16	8.24	2.16
B (µg L^{-1})	83.7 ± 4.71	180.7 ±6.47	**B (mg $tree^{-1}$)**	30.1	64.8	30.1
Zn (µg L^{-1})	10.3 ± 1.70	42.9 ± 7.20	**Zn (mg $tree^{-1}$)**	3.70	15.4	3.70
TOC (mg L^{-1})	1.13 ± 0.21	10.4 ± 1.71	**TOC (g $tree^{-1}$)**	0.40	3.74	0.40
***E. coli* (CFU 100 mL^{-1})**	0	4 ± 2				
Salmonella spp.	0	0				

[1]Tap Water. [2]Secondary Treated Wastewater. [3]TWni Tap water annual nutrient input. [4]STWni Secondary treated wastewater annual nutrient input. [5]TW+MFni Tap water plus mineral fertilized annual nutrient input

3.2. Tree nutritional status

Trees irrigated with TW (without fertilization) exhibited a leaf N concentration far below the optimal threshold (Table 2), while the overall values of leaf mineral concentration found in TW+MF and STW irrigated trees were close to the optimal range for the same variety (Cheng and Raba, 2009). Leaf and fruit N concentrations were statistically enhanced by the TW+MF, despite a larger canopy development. Intermediate values were recorded in trees irrigated with STW (Table 2). This indicates that N exclusively provided by STW (< 6.0 g $tree^{-1}$) was not enough to satisfy tree nutrient requirements and in line with what reported by Pereira et al., 2011 on citrus tree nutrition. Indeed, leaf N concentration of mineral-fertilized trees were significantly higher as a consequence of higher N inputs (9.0 g $tree^{-1}$).

On the contrary, leaf P and Ca concentration in TW+MF trees were significantly decreased, likely due to the dilution and partitioning effect induced by a larger vegetative biomass. An opposite trend was exhibited in the TW trees, with higher concentration for P and Ca (Table 2). Concerning micronutrients, TW+MF increased leaf Fe and Mn concentrations while no effects was detected on leaf Cu, B and Na concentrations, regardless of the irrigation treatment (Table 2). An increased concentration of Fe, Cu and Mn was induced by TW+MF in fruit peel and pulp (Table 2). Instead, a decreasing trend was detected in P, Ca, B and Na concentrations from TW+MF to TW (Table 2). The reiterate supplied of STW as irrigation water likely promoted an increased in the soil microbial biomass, due to the naturally high microbial abundance and biodiversity of this water source.

Thereby, other than the direct nutritional contribution provided by the STW (nutrients under mineral forms dissolved in the water), the effect of the STW-derived microorganisms on the native soil OM on tree uptake, cannot be disjointed (Smith, 1991). The availability of these elements could then allow a significant reduction in fertilizer application while still partially meeting tree nutrient requirements (Pereira et al., 2011), as it has been reported from other studies on fruit trees (Pedrero et al., 2012; Petousi et al., 2015; Segal et al., 2011; Vivaldi et al., 2017).

It is worth to mention that heavy metals accumulation in STW vegetal tissues (i.e. leaves and fruits) was not observed, excluding potential contamination risk for human health. Similar results were observed in olive trees irrigated with reclaimed wastewater by Petousi et al. (2015).

3.3. Vegetative growth and daily photosynthetic assimilation rate

TW+MF shoot length was characterized by a fast increase until 60 DAFB, while afterwards shoot growth rate was much slower and proceeded until 157 DAFB (Fig. 1). Shoots on TW+MF trees were statistically longer from 60 DAFB on, compared to the other treatments, reaching an average length of 32.0 cm $shoot^{-1}$ at the end of season. Shoot growth on STW and TW irrigated trees showed comparable growth patterns, with limited and slow growth rates, reaching a maximum length of 15.2 cm $shoot^{-1}$ and 9.9 cm $shoot^{-1}$, respectively (Fig. 1). It has to be taken into account the different yield of the treatments, that was the highest in the STW treatment, penalizing the STW vegetative growth (Grappadelli et al., 1994).

Table 2. Leaf, fruit peel and pulp macro and micronutrient concentration in TW+MF, STW and TW irrigated trees

Tissue	***N***	***P***	***K***	***Ca***	***Mg***	***S***	***Fe***	***Cu***	***B***	***Na***	***Zn***	***Mn***
Treatment	***g kg^{-1}***					***mg kg^{-1}***						
Leaf												
TW	11.9 c	2.41 a	14.5 a	13.5 a	2.56 a	0.70 b	45.8 b	8.80 a	27.0 a	63.4 a	15.4 a	23.0 b
TW+MF	19.9 a	1.15 b	14.0 a	10.9 b	2.27 a	0.98 a	90.0 a	9.80 a	25.2 a	52.8 a	10.8 b	34.4 a
STW	16.6 b	2.65 a	13.5 a	13.3 a	2.32 a	0.93 a	54.4 b	9.40 a	24.6 a	62.2 a	14.6 a	31.2 a
Significance	***	***	ns	*	ns	***	***	ns	ns	ns	*	**
Fruit Peel												
TW	2.00 b	0.30 b	3.50 c	1.34 a	0.89 a	0.14 c	37.5 a	1.90 a	24.6 a	27.1 a	2.44 a	5.80 c
TW+MF	2.72 a	0.31ab	3.85 a	0.82 c	0.81 b	0.28 a	44.1 a	2.10 a	11.9 c	19.6 a	2.44 a	7.46 a
STW	2.31 b	0.33 a	3.70 b	0.99 b	0.84 ab	0.22 b	50.3 a	1.40 a	14.8 b	23.1 a	2.73 a	6.55 b
Significance	*	*	**	***	*	***	ns	ns	***	ns	ns	***
Fruit Pulp												
TW	1.01 b	0.61 a	6.05 a	0.36 a	0.22 a	0.08 c	6.20 c	1.76 b	31.8 a	39.0 a	1.75 a	1.13 c
TW+MF	2.72 a	0.43 c	5.12 b	0.23 c	0.21 a	0.14 a	11.0 a	2.34 a	10.0 c	22.6 b	0.70 a	1.70 a
STW	1.34 b	0.56 b	5.40 b	0.29 b	0.22 a	0.11 b	8.40 b	1.75 b	14.5 b	25.5 b	2.15 a	1.44 b
Significance	**	***	**	**	ns	***	***	**	***	**	ns	***

*ns, *, ** and ***: effect not significant or significant at p ≤0.05, p ≤0.01 and p ≤0.001, respectively. Within the same tissue, means followed in column by the same letter are not statistically different (p ≤0.05, SNK Test)*

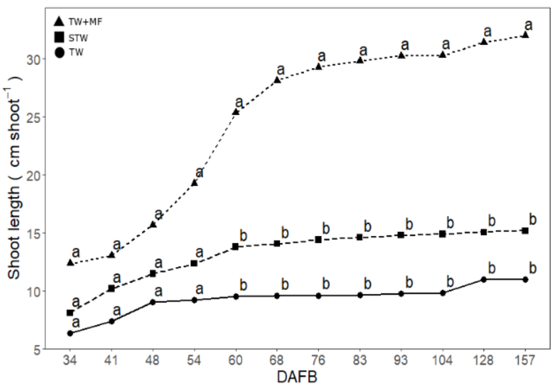

Fig. 1. Seasonal pattern of shoot growth (n=15) for TW+MF, STW and TW. Different letters indicate significant differences with P value <0.05

The different shoot growth rate is a direct consequence of the total N that trees received in the different treatments. TW+MF trees received a higher amount of N, which likely sustained tree growth. This indicates that irrigation with STW may partially contribute to partially fulfil plant nutrient requirements. Therefore, nutrients supplied by STW should be taken into account in the fertilization schedule. In our conditions, results suggest that the use of STW cannot replace traditional fertilization for young apple trees and mineral nutrients must be integrated by alternative sources. On the other hand, irrigation with STW was not detrimental to plant growth (Petousi et al., 2015; Segal et al., 2011) and nutritional status, indicating that STW is a potential water source to irrigate apple trees. Treatments significantly affected tree photosynthetic daily assimilation rate (Fig. 2). Compared to the TW-irrigated trees, irrigation with STW more than doubled the cumulative amount of assimilated C estimated at the end of the season (Fig. 2) with values of 13.4 and 5.04 g CO_2 d^{-1} in STW and TW irrigated trees, respectively. However, the C assimilated in TW+MF trees was the highest, with a value of 19.8 g CO_2 d^{-1} (Fig. 2). These differences are likely the consequence of the different nutrient supplies and canopy areas among the irrigation treatments. Tree canopy area was on average 0.68 ± 0.07 m^2 tree^{-1}, 0.29 ± 0.04 m^2 tree^{-1} and 1.42 ± 0.09 m^2 tree^{-1} for the STW, TW, and TW+MF irrigated trees, respectively.

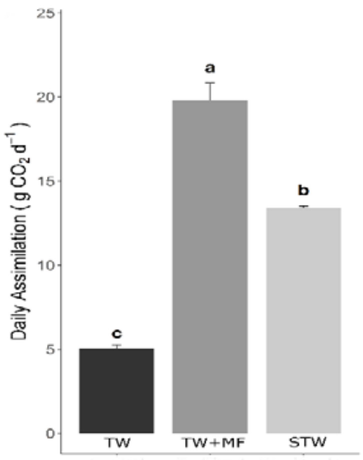

Fig. 2. Effect of the irrigation treatment on the cumulative daily canopy CO_2 assimilation (n=5; Avg. ± SE) measured at the end of the season. Columns with different letters indicate significant differences at P <0.05

3.4. Water relations

Leaf and stem water potentials (WP) showed a decreasing pattern from early morning to afternoon on both the day of measurements (Fig. 3). In pre-harvest (115 DAFB) leaf WP on STW and TW+MF trees were statistically more negative in comparison to TW trees (Fig. 3, b). This difference seems related to the higher water demand of STW and TW+MF trees, which can be mainly attributed to their larger leaf area and fruit yield (Chapter 4.5) compared to TW. No difference was found among treatments on the post-harvest leaf WP (150 DAFB), except at 9:00 A.M. In this case, STW trees showed slightly more negative water potentials.

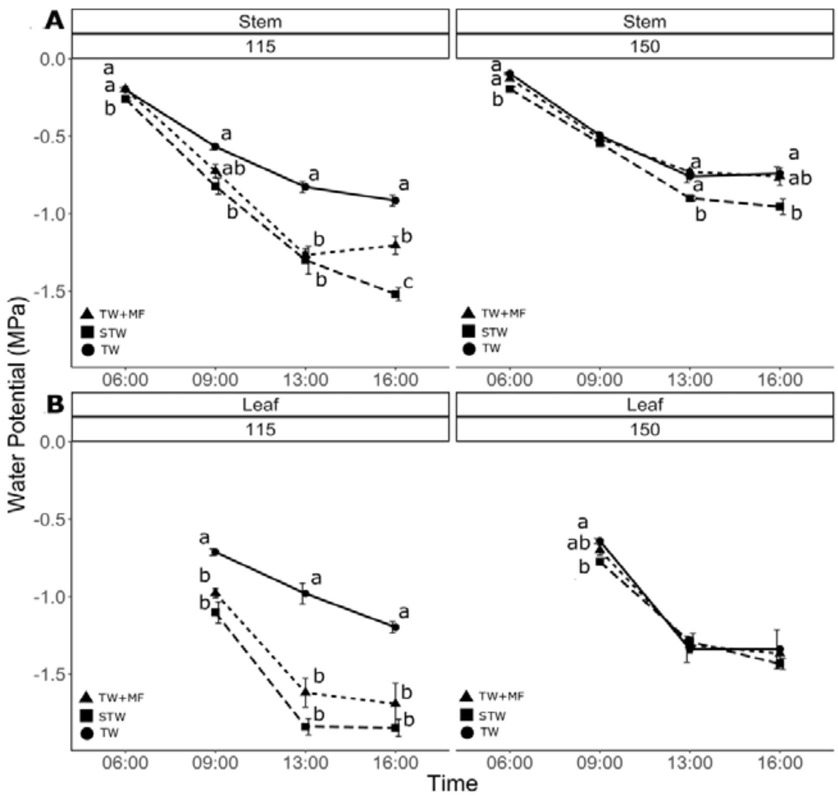

Fig. 3. Daily patterns of stem (A) and leaf water potentials (B) in TW, STW and TW+MF irrigated trees, measured at 115 and 150 DAFB. Each point represents the mean of 5 measurements. Within the same time, values with different letters indicate significant differences at P <0.05

Stem WP at 115 DAFB revealed more negative values on STW trees compared to the other treatments (Fig. 3a) during the whole day, except at midday. This result may indicate a slight salinity stress (Acosta-Motos et al., 2017; Segal et al., 2011) induced by the irrigation with STW that is strengthened by the stem pre-down (i.e. 6:00 AM) data, as a direct indicator of the water soil availability (Van Zyl, 1987). Apple tree is considered among the most sensitive tree crops to soil salinity (FAO, 2002). Such effect was confirmed on the post-harvest measurement, at 150 DAFB, when trees are characterized by a physiological recovering process (Fig. 3), due to the fruit unload (Naor et al., 1997). Indeed, STW trees showed lower stem WPs if compared to TW+MF and TW treatments, except at 9.00 A.M. This is line with the salinity stress hypothesis observed during pre-harvest conditions.

3.5. Fruit growth, yield and quality

The seasonal pattern of fruit growth was not statistically different among treatments (Fig. 4). STW trees showed slightly higher values in fruit dimeter for almost all the season compared to TW and TW+MF with a double yield if compared to the TW+MF. The fruit crop load was 1.2 ± 0.4 kg tree^{-1}, 0.6 ± 0.2 kg tree^{-1} and 0.2 ± 0.1 kg tree^{-1} for the STW, TW+MF, and TW treatments, respectively. Nicolás et al. (2016) and Pedrero et al. (2013; 2014) found that the use of STW increased yield in mandarin and grapefruit trees, respectively. Data indicate that STW did not negatively affect seasonal fruit development, despite the higher crop load.

Treatments affected most of the fruit quality parameters (Fig. 5). Fruit from TW+MF treated trees showed statistically higher b* and lightness (L*) values compared the other two treatments. Concerning dry matter and soluble solid content, TW+MF and STW trees showed statistically higher values if compared to TW trees. Skin lightness, as well as the b* colour component, was significantly increased in mineral-fertilized fruits, while similar values were measured for the other strategies (Fig. 5). Conversely a* component was statistically higher in the fruit skin of STW trees, followed by TW and TW+MF, respectively (Fig. 5). Fruit firmness was higher in TW trees compared to the other two treatments. No difference was detected in the fruit weight (data not shown).

The higher dry matter and soluble solid contents in STW-irrigated fruit, which were characterized also by a higher crop load, could be related to the chemical element concentrations and EC of the STW water (Table 1). In fact, many plants adapt to salt stress by enhancing the concentration of sugars, organic acids, proteins and amino acids which act as osmolytes to maintain plant turgor under salt stress. The presence of these metabolites often increases the nutritive quality and marketability of fruit and vegetables (Ahlem et al., 2011; Ahmed et al., 2009).

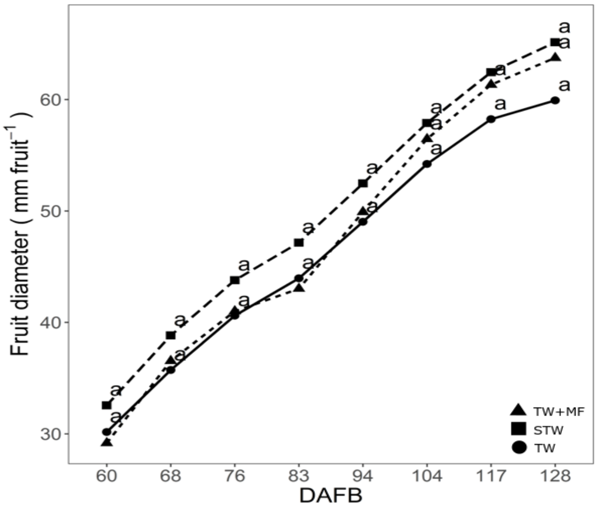

Fig. 4. Seasonal pattern of fruit growth (mm fruit^{-1}) of TW+MF, STW and TW irrigated trees (n=8)

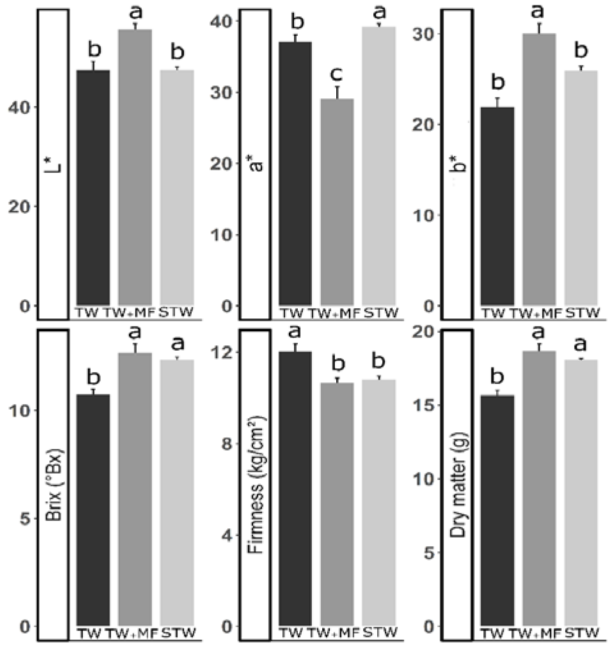

Fig. 5. Effect of the irrigation treatment on the fruit skin lightness (L*), a*; b*, soluble solid content, flesh firmness, and dry matter at commercial harvest (n=12; Avg. ± SE). Black, dark-grey and grey bars indicate TW, TW+MF and STW trees, respectively. Within the same parameter, columns with different letters indicate significant differences at P <0.05. (L* indicates skin lightness (black=0 while white=100) level; a* indicates redness-greenness component (red=100 while green=-100) and b* indicates yellowness-blueness (yellow=100 while blue=-100) component

It has been demonstrated on different crops (tomatoes, muskmelon, and cucumber), that fruit quality parameters such as soluble solid content, improved in fruits irrigated with reclaimed water (Basiouny, 1984; Biernbaum and Argo, 1995; Crisosto et al., 1994; Lurie et al., 1996; Pedrero et al., 2012). Our data indicate that even if the STW used was not highly saline, fruit quality parameters were positively affected by irrigation with this water.

4. Conclusions

Results suggest that STW can be adopted as a water source in the orchard irrigation management. In addition, this strategy conveniently contributes to fulfil tree nutrient requirements, with positive responses on the plant nutritional and physiological status. Recycling STW in agriculture allows to recover minerals (i.e. N and P) with positive ecological (e.g. limiting eutrophication problems) and agronomical (e.g. saving mineral inputs) implications.

In our conditions, irrigation with STW did not increase heavy metal concentration both in leaf and fruit tissues, indicating limited risks for human health. We observed a moderate plant water stress induced by the STW, likely induced by salinity. Nevertheless, this response may be associated with the improvement of the fruit quality parameters.

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