Effects of Reclaimed Waters on Spectral Properties and Leaf Traits of Citrus Orchards

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ABSTRACT: Effects resulting from the use of reclaimed waters on mandarins and grapefruits are evaluated by measuring the spectral responses of their canopies and the anatomy and the chlorophyll content of their leaves against control trees irrigated with waters provided by an interbasin transfer. Spectral responses from the red (R) and near-infrared (NIR) wavelength bands, and its normalized ratio (NDVI), were acquired from a hyperspatial flight conducted after a low-moderate exposition to reclaimed waters. Chlorophyll and leaf and palisade/spongy ratio thicknesses were analyzed after a moderate-high exposition. Significant differences between controls and treatments were detected in mandarins in R and leaf chlorophyll, but not in grapefruits, likely because of their higher tolerance to saline waters. Reused waters did not affect either NIR–NDVI or anatomy traits. Hyperspatial sensing techniques are suitable for detecting chlorophyll dynamics, but NIR information and related vegetation indices may mask the detection of periods of saline stress in citrus orchards. *Water Environ. Res.*, **86,** 2242 (2014).

KEYWORDS: reclaimed waters, citrus, irrigation, salinity, hyperspatial imagery, leaf anatomy, chlorophyll content.

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Introduction

Climates of dry lands are characterized by mean annual precipitation values that are lower than potential evaporation. Under these conditions, vegetation develops water use strategies that aim to maximize transpiration up to annual rates similar to the water inputs supplied by rainfall after accounting for evaporation (Eagleson, 1982; Santoni et al., 2010). The quasi-equilibrium between precipitation and actual evapotranspiration makes deep drainage almost negligible in the long term and promotes dry and salty vadose zones. As a consequence of the soil dryness and the high salt concentrations in the soil profile, the agronomic potential of semiarid regions is limited (Safriel et al., 2005). Because irrigation is strongly constrained in these regions resulting from water scarcity and shortages, farmers typically search for alternative sources of water (e.g., reclaimed urban effluents) and apply "deficit irrigation" strategies that aim to maximize the yield of crops and orchards accepting a certain level of water stress (Fereres and Soriano, 2007; Lawhon and Schwartz, 2006; Paranychianakis and Chartzoulakis, 2005; Pedrero et al., 2010; Pereira et al., 2002). The use of reclaimed waters for irrigation is considered an environment-friendly option to lower the demand for water of high-quality standards and to control the pollution of groundwater resulting from their direct discharge to open channels. Another advantage is that the use of reclaimed waters in irrigation agriculture can improve the nutritional requirements of fruit cultivars (Levine and Asano, 2004; Pedrero et al., 2012; Pedrero et al., 2013). Despite their potential environmental and nutritional benefits, the high concentration of salts and phytotoxic elements typically observed in these waters could exacerbate the problems of secondary salinization and soil degradation and cause negative effects on the growth, yield, and quality of cultivars if the water is not appropriately applied (Beltran, 1999; Kalavrouziotis, 2011; Kitamura et al., 2006; Paranychianakis and Chartzoulakis, 2005; Pedrero et al., 2012; Pedrero et al., 2013; Scanlon et al., 2010; Schoups et al., 2005). These negative effects on soils and crops may even be increased when irrigation is supplied by dripping against other less-efficient techniques (Mounzer et al., 2013). For these reasons, preliminary assessments on the tolerance and response of orchards and crops to the interactive effects of salinity and phytotoxic elements in high-efficiency irrigated agrosystems are critical to minimize the environmental risks related to the adoption of these strategies in dry lands.

Salinity stress harms citrus orchards in two principal ways: (1) by specific-ion toxicity and (2) by osmotic effects caused by the accumulation of salts in the soil profile (Ferguson and Grattan, 2005; Parida and Das, 2005). In general specific-ion toxicities and osmotic effects generate a reduction of the net gas exchange in leaves, closure of stomata, and a decrease in the water use efficiency. If the stress factor remains, changes in the content of leaf pigments (e.g., chlorophyll, anthocyanin, carotenoids) can arise, giving rise to leaf bronzing or yellowing (e.g., García-Sanchez et al., 2002; Zekri, 1991). This may lead finally to the appearance of symptoms of chlorosis and necrosis. The extended exposure to these stress conditions may result in yield and growth suppression of orchards (Romero-Aranda et al., 1998).

Negative effects of salinity on the chlorophyll content and anatomic morphology of leaves have been reported in trees of native ecosystems as *Arbutus unedo* (Navarro et al., 2007), *Bruguiera parviflora* (Parida et al., 2004), and *Prosopis tamarugo* (Valenti et al., 1991); in such crops as cowpea, bean, and cotton (Garzón and García, 2011; Longstreth and Nobel, 1979); and in such orchards as wine grapes (Qin et al., 2012) and fruit trees, including species of *Citrus* (Romero-Aranda et al., 1998; Zekri, 1991), *Tamarindus* (Gebauer et al., 2004), *Morus* (Vijayan et al.,

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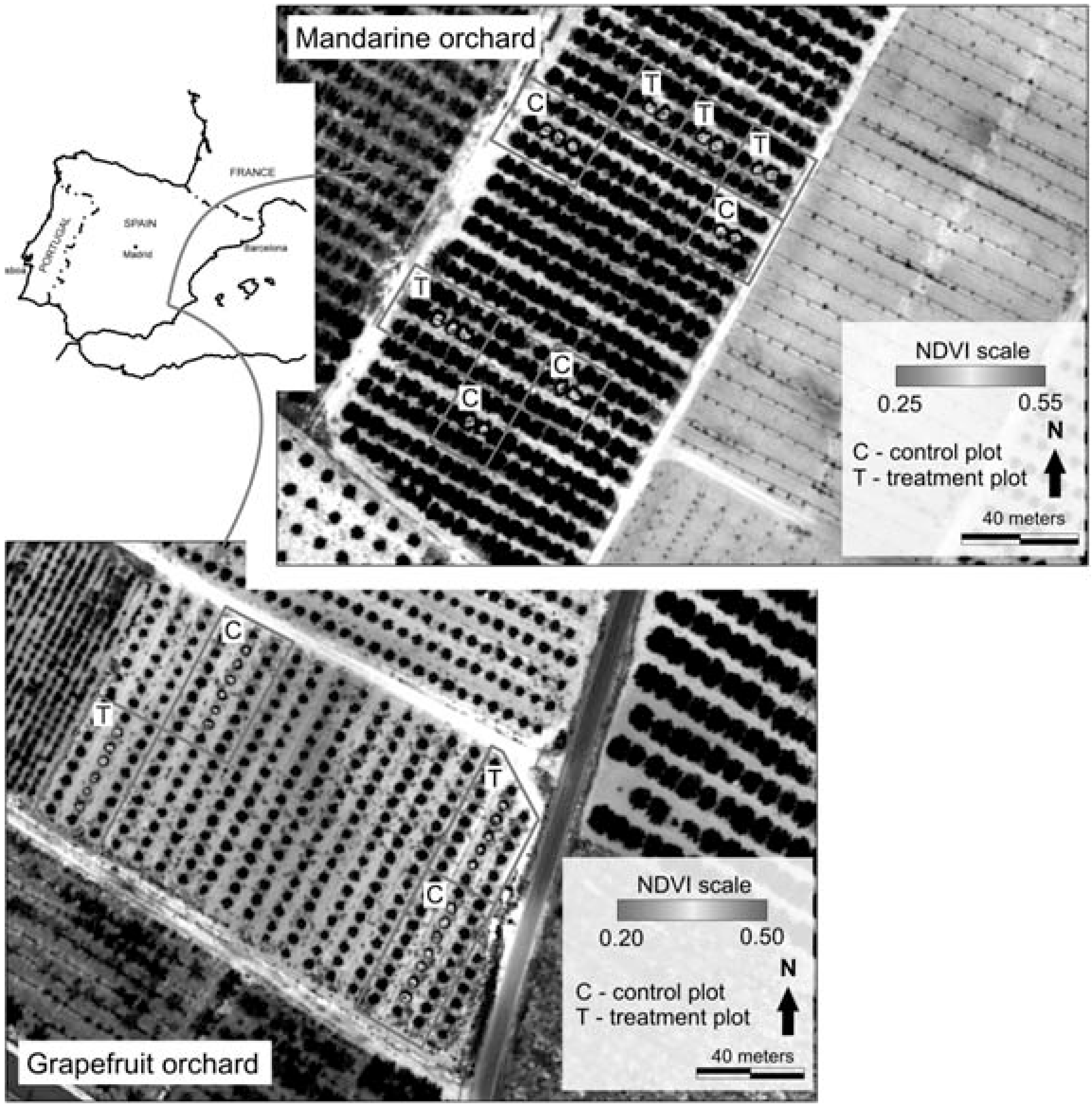

Figure 1—Location map showing the mandarin and grapefruit orchards studied. Control and treatment plots were irrigated with waters of good agronomic quality provided by an interbasin aqueduct, and with reclaimed water supplied by a local water resource recovery facility.

2008), and *Olea* (Bongi and Loreto, 1989; Karimi et al., 2009; Vigo et al., 2005). In general, there is a broad agreement that salinity reduces leaf chlorophyll contents of crops and trees (Parida and Das, 2005). However, the degree of salinity effect on leaf morphology, anatomy, and physiology is still poorly understood as each specie has a different tolerance to the accumulation of salts in the soil. Several studies have shown that spongy and total leaf mesophyll layer thicknesses increase with salinity in *Citrus* (Romero-Aranda et al., 1998) and *Olive* spp. (Bongi and Loreto, 1989; Kchaou et al., 2010) because of the accumulation of water in the mesophyll cells and the increase of the succulence (Nastou et al., 1999; Romero-Aranda et al., 1998; Zekri, 1991).

Incipient signals of vegetation stress in orchards can be detected in a rapid and nondestructive way by measuring the spectral response of their leaves and canopies. The acquisition of this information with remote sensing techniques have been proved useful against more costly and time-consuming field techniques based on the soil core or vegetation sampling, or other electrical and electromagnetic device-based approaches strongly limited to the early stages of the crops (Wang et al., 2002). Absorption patterns in the visible region of the electromagnetic spectrum, which includes the red (R) (660 to 680 nm) domain, is positively related with the concentration of photosynthetic (chlorophyll a+b) and accessory (carotenoids, anthocyanins) leaf pigments (Chappelle et al., 1992; Datt, 1998; Ollinger, 2011; Sims and Gamon, 2002). Because salty environments harm or reduce the functionality and content of chlorophyll in the leaves, reflectance may be proportionally reduced. In the near-infrared (NIR) (750 to 1400 nm) domain, the spectral response of leaves depends on the multiple scattering of light inside the leaf that is mainly controlled by

Table 1—Water quality measurements for irrigation waters provided from the Tajo-Segura interbasin aqueduct (control) and from a water resource recovery facility (treatment). Values represent Avg ± 1*Std. Error from 36 measurements for water electrical conductivity (EC, dS m^{-1}), sodicity adsorption ratio (SAR), and chemical composition (Na and Cl in meq L^{-1}; B, in mg L^{-1}). Significant differences (Tukey test, *p*-level < 0.05) between types of water are marked with different letters.

Variable	Control	Treatment
EC	1.4 ± 0.1 a	3.4 ± 0.3 b
SAR	1.9 ± 0.8 a	4.0 ± 1.7 b
Na	6.9 ± 3.6 a	17.8 ± 8.8 b
Cl	1.2 ± 0.8 a	31.9 ± 16.2 b
B	0.2 ± 0.1 a	0.9 ± 0.2 b

its internal structure (e.g., leaf and mesophyll thickness, palisade/spongy mesophyll thickness ratio, percentage of intercellular air space in the spongy layer) (Grant, 1987; Hoque and Remus, 1996; Pinter et al., 2003; Slaton et al., 2001). Among these indicators, the palisade/spongy thickness ratio has been suggested as a suitable indicator of stress conditions (Slaton et al., 2001). Because differential absorption of light in the red and NIR spectral domains is strongly related with most of the biophysical parameters of vegetation, other composite indices integrating data from both domains (e.g., normalized difference vegetation index [NDVI]) have been also proposed as useful indicators to (1) discriminate the photosynthetical tissues of the plant from the non-photosynthetical and the soil background signals, and (2) detect signals of water and saline stress (Bannari et al., 1995; Glenn et al., 2008).

Among the remote sensing techniques available at the present, hyperspatial or high-resolution imagery improves the capability to isolate tree crowns and retrieve their pure spectra without the "spectral noise" resulting from the soil background (Greenberg et al., 2005; Leckie et al., 2003). Nowadays, the use of airborne platforms and unmanned aerial vehicles with hyperspatial sensors are receiving an increasing interest in agriculture precision for the monitoring and surveillance of rangelands, crops, and orchards (e.g., Herwitz et al., 2004; Zarco-Tejada et al., 2012).

This study provides results on the effects of prolonged exposure (more than 9 years of experimental irrigation) to reclaimed water on two species of *Citrus* spp. (mandarin and grapefruit).

Material and Methods

Study Area and Experimental Design. The study was conducted in two commercial citrus orchards located at Campotejar (Figure 1), in the municipal district of Molina de Segura (Murcia, SE Spain). The region is characterized by a Mediterranean semiarid climate, with a mean annual precipitation of 350 mm. Most of the rainfall is concentrated during the November through May period. Mean annual temperature is 17 °C, with the highest values (up to 40 °C) reached during the dry summer period. Potential evapotranspiration has been estimated at ~900 mm y^{-1} according the Penman–Monteith equation.

The first farm was planted in 2002 with mandarin (*Citrus clementina* cv Orogrande) grafted on Carrizo citrange (*Citrus sinensis* L. Obs. x poncirus trifoliate L.). The second farm was planted in 2005 with "Star Ruby" grapefruit (*Citrus paradise* Macf.) grafted on Macrophylla rootstock (*Citrus macrophylla*). Soils in both farms are classified as an association of haplic and petrocalcic calcisols according the FAO-ISRIC-ISSS (1998) soil classification code, showing an aridic soil moisture regime, a very weak ocric A horizon, and, frequently, a petrocalcic horizon.

Two water sources of different agronomic quality were employed in each farm (Table 1). The first source of water, considered hereafter as the "control", was provided from the Tajo-Segura interbasin aqueduct. Control waters had low levels of salinity (electrical conductivity less than 1.5 dS m^{-1}) and their agronomic quality for irrigation were considered as good. The second source of water, the "treatment", consisted of reclaimed waters pumped from a water resource recovery facility (WRRF) located in the surrounding area. The treatment in the WRRF consists of a conventional activated sludge process on local urban effluents followed by a tertiary treatment with coagulation–flocculation, lamellar sedimentation, sand filtration, and UV disinfection. Treated waters had higher levels of salinity (electrical conductivity 3.0 to 4.0 dS m^{-1}), and chloride, boron, potassium, and sodium concentrations 26, 4.5, 3.3, and 2.5 times higher than in the control waters, respectively. According to the electrical conductivity and water quality guidelines published by the Food and Agriculture Organization of the United Nations (Ayers and Westcot, 1985), the reclaimed water used in this study may generate severe risks of salinization and strong reductions in the water extraction capacity of the crops. Boron content in reclaimed waters during the experiment was higher than the phytotoxic range for sensitive crops (>0.7 mg L^{-1}, Table 1). In the treated plots, Pedrero et al. (2013) have measured boron contents in mandarin leaves higher than the phytotoxic limits although at the time of those measurements, no visual symptoms of toxicity were observed. In this study, the authors analyzed plots in which irrigation inputs were supplied to meet 100% of the crop water requirements during the second phase of fruit growth (from late June to mid-August). Irrigation was supplied by dripping with a single irrigation line for each row of trees and three emitters per plant supplying a rate of 4 L h^{-1}. The adoption of drip irrigation against other less-water-efficient techniques (e.g., sprinkling or furrow irrigation) ensures sufficient stressful conditions for the assessment of the saline tolerance of the orchards. Trees rows are sufficiently separated (plant spacing of 5 × 3.5 m and 6 × 4 m in mandarin and grapefruit, respectively) to minimize the competition of trees for the water and nutrient resources.

Airborne Imagery and Data Processing. An airborne flight campaign was conducted in the region on August 14, 2009. A high-resolution (25 cm), 16-bit, multispectral camera carried in a Partenavia P68C-type aircraft was used for the acquisition of the airborne imagery. Raw imagery was rectified, and raw data in digital numbers from the red (R, 610 to 660 nm) and near infrared (NIR, 835 to 885 nm) regions were used for this study. The NDVI was computed as

$$NDVI = (NIR - R)/(NIR + R) \quad (1)$$

in which NIR and R are the radiance at the top of the sensor (in this study, coded as digital numbers). At the time of acquisition, that is, in the middle of the summer, physiological activity of mandarins and grapefruits in the region is at its maximum rate,

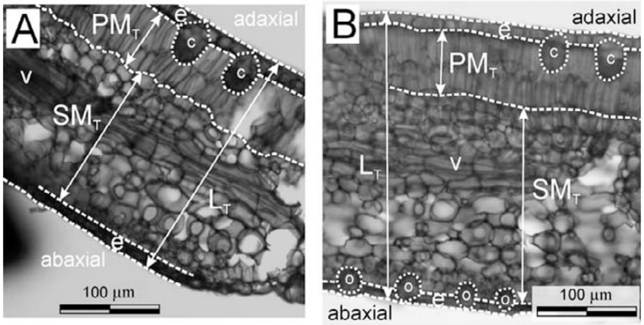

Figure 2—Transverse section of a representative leaf of mandarin (A) and grapefruit (B) trees irrigated with waters pumped from the Tajo-Segura interbasin aqueduct. PM_T = palisade-meshophyll layer thickness; SM_T = spongy-meshophyll layer thickness; L_T = total leaf thickness; e = epidermis; c = cystolite; o = oil channel.

which makes these dates the best suitable period to detect the negative effects of salinity on the spectral response of canopy trees. All pixel values from the red and NIR spectral domains, and the resulting NDVI were extracted from all the canopy trees located in the center of each experimental plot to minimize edge effects. Tree crowns were manually masked from the NDVI map layer to exclude the green canopy surface from the canopy edge and the soil background. Once masked, outlier values found in each tree crown were additionally removed before computing the average value per tree. The average number of usable pixels per tree was 90. Canopy variability observed was in average less than 10% for all the variables considered. Statistical differences between treatments were assessed using the nonparametric Mann–Whitney *U* test.

Chlorophyll Content and Leaf Anatomical Properties. The interactive effects of salinity and phytotoxic elements on the citrus performance were additionally evaluated 4 years after the airborne flight using laboratory analyses. These analyses were performed in January 2013 over a representative sample of fresh leaves collected from the top of the canopy in four trees located in the central rows of each experimental plot (grapefruit vs mandarin, control vs treatment).

For the chlorophyll determination, small disks of fresh leaves were cut from the mid-lamina area and put inside scintillation vials to reach a total weight of 0.03 ± 0.01 g. Major veins were avoided during the sampling. Three milliliters of N, N-dimethylformamide were added to the fresh material and maintained 72 hours in darkness. After 24 hours of shaking, chlorophyll a, b, and total (see equations below) were quantified with the equations of Inskeep and Bloom (1985) after recoding absorbance at 647 and 664 nm with a spectrophotometer:

$$Chla = 12.70 * A_{664.5} - 2.79 * A_{647} \quad (2)$$

$$Chlb = 20.70 * A_{647} - 4.62 * A_{664.5} \quad (3)$$

$$ChlT = 17.90 * A_{647} - 8.08 * A_{664.5} \quad (4)$$

where A is absorbance in 1.00-mm cuvettes.

Measurements of the leaf total thickness (T), palisade- (PM), and spongy- (SM) layer thickness, and the PM/SM ratio were determined (Figure 2). To count this, fresh leaf material was fixed in FAA (formaldehyde, ethanol, acetic acid, water). After 48 hours in the solution, transverse sections of the leaves were obtained by freehand cutting, colored with safranin, and finally mounted in DPX (a synthetic resin consisting of a mixture of distyrene, a plasticizer, and xylene) (D'Ambrogio de Argüeso, 1986). Measurements were taken and analyzed with an Olympus BX40 light microscope and the ProgRes C12 plus Capture Pro 1.1.0 software. Statistical differences for chlorophyll content and anatomical traits between treatments were assessed using the nonparametric Mann–Whitney *U* test.

Results and Discussion

Effects of Water Quality in Grapefruit and Mandarin: Imagery Results. At the time of the airborne flight, mandarin trees irrigated with saline-reclaimed waters showed 5% lower reflectance values in the red wavelength domain than trees irrigated with waters pumped from the interbasin aqueduct (p-value < 0.05) (Table 2, Figure 3A). By contrast, no differences were found between treatments for reflectance values in the NIR regions (Figure 3A). Because of the differences in the red reflectance values, NDVI computed in mandarins was 1.06 times significant higher (p-value < 0.05) in plots irrigated with high-quality waters than in those ones with saline-reclaimed waters (Figure 4B).

In grapefruits, no reflectance differences in red and NIR and in the NDVI were nevertheless found between controls and treatments (Figures 3 and 4). Reflectance in the red region was 1.10 to 1.17 times higher (p-value < 0.05) in grapefruits than in mandarins (Figure 3), whereas in control plots, NIR reflectance was on average 10% lower in grapefruits than in mandarins for the date of the airborne flight (Figure 4A).

Despite of the high electrical conductivity and boron contents of the reclaimed waters used during the experiment and the use of the drip irrigation technique, the fact that no differences were

Table 2—Comparisons between control (C) and treatment (T) water qualities for mandarin (Man) and grapefruit (Gra) trees in measurements of red, near infrared, and NDVI. The number of trees analyzed for C and T appears in the rows n1 and n2. Significant statistical differences are represented by asterisk symbols: * (p-level < 0.01), ** (p-level < 0.05), * (p-level < 0.1) (n.s. = not significant).**

			Red			Near infrared			NDVI		
T1 vs T2	n1	n2	T1	T2	*p*	T1	T2	*p*	T1	T2	*p*
Man-C vs Gra-C	9	13	1930 ± 28	2254 ± 32	***	5248 ± 69	5731 ± 77	***	0.46 ± 0.01	0.43 ± 0.00	***
Man-C vs Man-T	9	9	1930 ± 28	2037 ± 40	**	5248 ± 69	5208 ± 55	n.s.	0.46 ± 0.01	0.44 ± 0.01	**
Gra-C vs Gra-T	13	11	2254 ± 32	2233 ± 23	n.s.	5731 ± 77	5730 ± 88	n.s.	0.43 ± 0.00	0.44 ± 0.01	n.s.
Man-T vs Gra-T	9	11	2037 ± 40	2233 ± 23	***	5208 ± 55	5730 ± 88	***	0.44 ± 0.01	0.44 ± 0.01	n.s.

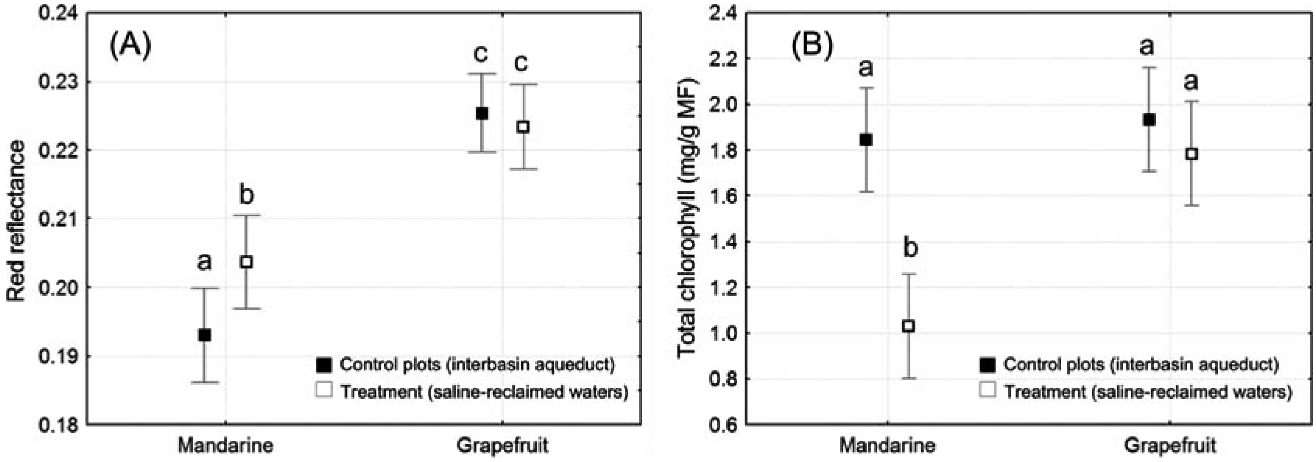

Figure 3—Measurements of (A) canopy red reflectance, and (B) total leaf chlorophyll in mg g^{-1} MF, for mandarin and grapefruit trees. Vertical bars denote 0.95 confidence intervals, and different letters mark significant differences among treatments according to the non-parametric Mann–Whitney *U* test (*p*-value $<$ 0.05).

found in the NIR response between control and experiment plots in mandarin and grapefruit suggests that no changes were induced in the leaf structure after 7 and 4 years of treatment, respectively. However, the differential response of mandarins against grapefruits in the red reflectance may indicate that (1) grapefruit presents a much higher salt tolerance than mandarins or (2) that the time of exposure to saline waters was not sufficiently extensive to promote the changes observed in the mandarins. Another source of potential uncertainty, which could explain the moderate (*p*-value $<$ 0.05) or negligible differences measured in the red reflectance in mandarins and grapefruits, respectively, may be related to the poor relationship between the reflectance at the red wavelength domain and the chlorophyll content when it exceeds values higher than 0.5 mmol m^{-2} (Sims and Gamon, 2002). Although not measured at the time of the airborne flight, the pool of total chlorophyll values acquired in the area (section 2) ranges from 0.6 to 1.0 mmol m^{-2} in the control plots, to 0.2–0.8 mmol m^{-2} in the treatment ones. To avoid this saturation effect, several authors have suggested regions around 550 nm (green) and 700 nm (in the R–NIR transition or "red edge") as more suitable spectral domains for evaluating changes in chlorophyll contents (e.g., Broge and Leblanc, 2000).

Effects of Water Quality in Grapefruit and Mandarin: Leaf Anatomy and Chlorophyll Content Results. In mandarin, total leaf chlorophyll was 1.8 times higher in trees irrigated with control water than in those irrigated with saline-reclaimed water (Figure 2B). Average leaf thickness, PM-, and SM-layer thickness measured in leaves of mandarins irrigated with control waters was 240, 60, and 160 μm respectively (Figure 5, Table 3). The PM/SM ratio was 0.38 ± 0.01 (Avg ± 1*Std. Error). No significant differences between irrigation treatments were observed for any leaf anatomical trait (Figure 5).

Leaf total chlorophyll measured in grapefruits at plots with both irrigation treatments was similar to the values observed in control plots of mandarins. Irrigation with reclaimed waters very weakly (*p*-value $<$ 0.1) reduced the leaf chlorophyll content

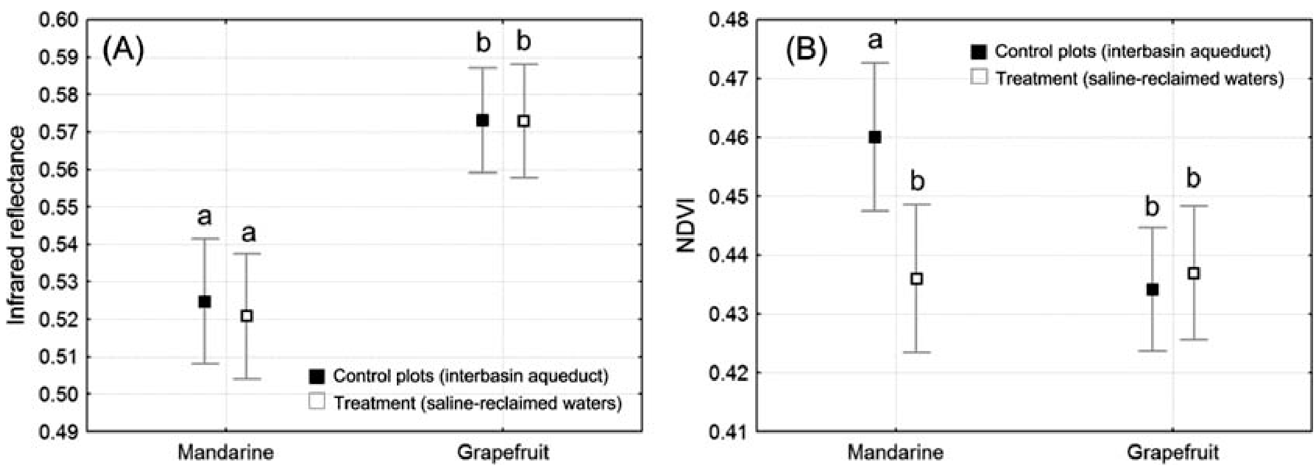

Figure 4—Canopy measurements of (A) near-infrared reflectance and (B) NDVI for mandarin and grapefruit trees. Vertical bars denote 0.95 confidence intervals, and different letters mark significant differences among treatments according to the nonparametric Mann–Whitney *U* test (*p*-value $<$ 0.05).

Table 3—Overview table with the basic statistics measured for different spectral properties, and anatomical and physiological traits in mandarin and grapefruit trees (SE = standard error).

	Mandarin—control				Mandarin—treatment			
Variables	**N**	**Avg ± SE**	**Min**	**Max**	**N**	**Avg ± SE**	**Min**	**Max**
Spectral properties								
Red reflectance	9	0.19 ± 0.00	0.18	0.20	9	0.20 ± 0.00	0.19	0.23
Infrared reflectance	9	0.52 ± 0.01	0.50	0.56	9	0.52 ± 0.01	0.50	0.55
NDVI	9	0.46 ± 0.01	0.43	0.49	9	0.44 ± 0.01	0.40	0.46
Anatomical properties								
Leaf thickness (μm)	4	243.09 ± 2.68	239.05	250.94	4	241.41 ± 15.88	209.69	284.56
PM thickness (μm)	4	60.52 ± 1.27	58.37	63.95	4	58.54 ± 0.96	56.28	60.63
SM thickness (μm)	4	161.44 ± 3.36	155.37	170.80	4	163.08 ± 14.73	136.04	205.05
PM/SM ratio	4	0.38 ± 0.01	0.34	0.40	4	0.37 ± 0.03	0.27	0.44
Chlorophyll content								
Chl A (mg/g MF)	4	1.37 ± 0.06	1.25	1.52	4	0.75 ± 0.16	0.46	1.07
Chl B (mg/g MF)	4	0.48 ± 0.02	0.43	0.55	4	0.28 ± 0.03	0.22	0.35
Chl T (mg/g MF)	4	1.84 ± 0.08	1.71	2.06	4	1.03 ± 0.17	0.71	1.41

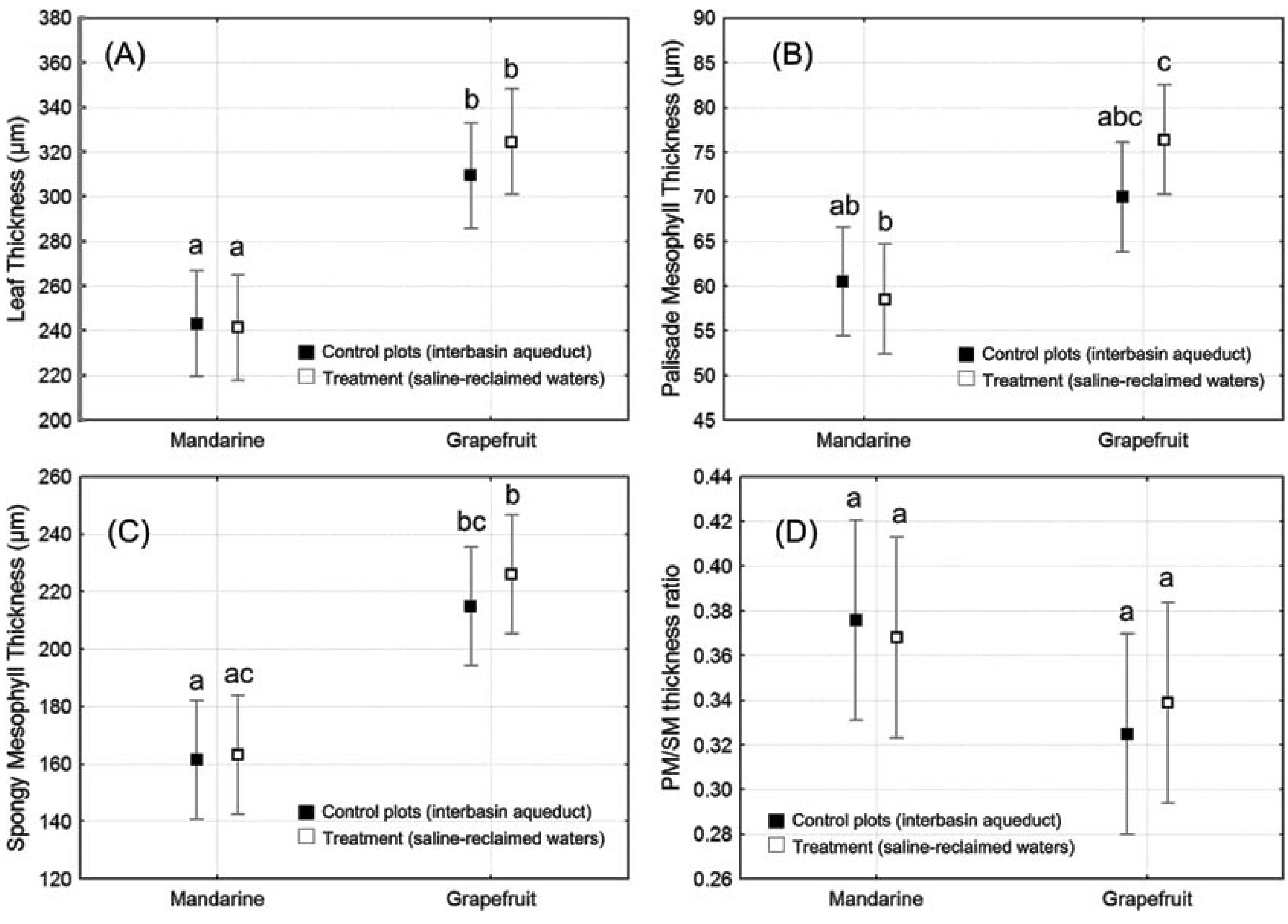

Figure 5—Anatomical traits of mandarin and grapefruit leaves: (A) Leaf thickness, (B) palisade mesophyll (PM) thickness, (C) spongy mesophyll (SM) thickness, and (D) PM/SM ratio. Vertical bars denote 0.95 confidence intervals, and different letters mark significant differences among treatments according to the nonparametric Mann–Whitney *U* test (*p*-value < 0.05).

Table 3—(Extended)

	Grapefruit—control				Grapefruit—treatment			
Variables	**N**	**Avg ± SE**	**Min**	**Max**	**N**	**Avg ± SE**	**Min**	**Max**
Spectral properties								
Red reflectance	13	0.23 ± 0.00	0.21	0.26	11	0.22 ± 0.00	0.21	0.24
Infrared reflectance	13	0.57 ± 0.01	0.52	0.62	11	0.57 ± 0.01	0.51	0.60
NDVI	13	0.43 ± 0.00	0.40	0.45	11	0.44 ± 0.01	0.39	0.46
Anatomical properties								
Leaf thickness (μm)	4	309.49 ± 12.08	286.00	340.11	4	324.55 ± 8.10	306.87	343.50
PM thickness (μm)	4	69.96 ± 4.96	58.16	82.42	4	76.40 ± 2.12	71.69	81.75
SM thickness (μm)	4	214.86 ± 8.32	198.53	235.26	4	226.06 ± 7.91	211.25	246.77
PM/SM ratio	4	0.32 ± 0.01	0.29	0.35	4	0.34 ± 0.01	0.30	0.36
Chlorophyll content								
Chl A (mg/g MF)	4	1.46 ± 0.05	1.39	1.61	4	1.36 ± 0.02	1.32	1.40
Chl B (mg/g MF)	4	0.48 ± 0.02	0.44	0.55	4	0.42 ± 0.01	0.41	0.45
Chl T (mg/g MF)	4	1.93 ± 0.07	1.84	2.15	4	1.78 ± 0.02	1.73	1.82

observed in grapefruits, that is, 8% lower in the treated than in the control plots (Figure 3B). However like in the mandarins, no significant differences between irrigation treatments were observed when anatomy leaf traits were evaluated (Figure 5).

Grapefruits irrigated with high-quality waters were 27% thicker than mandarins, and PM- and SM layers were in average 1.16 and 1.33 times thicker in grapefruits than in mandarins. The PM/SM in control plots of grapefruits was 0.32, that is, 14% lower than the average value measured in control plots of mandarins (Figures 2 and 5).

This study's irrigation experiment with reclaimed waters shows that more than 11 years in mandarins and 8 years in grapefruits were not sufficient to promote changes in their leaf anatomies. Despite the few studies that have described leaf anatomical changes resulting from a prolonged exposure to saline waters, most of them have reported an increase in total leaf thickness and a decrease of the intercellular spaces in the SM layer (Nastou et al. 1999; Romero-Aranda et al., 1998). Increases in leaf thickness after irrigation with saline waters have been also observed for other tree cultivars such as olive (Bongi and Loreto, 1989; Kchaou et al., 2010). Remote sensing and field laboratory measurements were taken in different years so it is not possible to find a quantitative correspondence for this study. However, the general pattern of red reflectance decrease observed in canopies of mandarins irrigated with reclaimed waters was in agreement with the leaf chlorophyll decrease measured after 4 years of the airborne flight. These differences were not observed in grapefruits, possibly because of their higher tolerance to salinity stress or because the time of exposure to salts was not sufficient to generate the expected changes (the experiment in grapefruits started 4 years after the mandarin's 1 year). The decreasing trend in total chlorophyll resulting from the prolonged exposure to saline waters has been also reported in *Citrus* species (Nastou et al., 1999; Romero-Aranda et al., 1998; Zekri, 1991), and in other cultivars as *Prunus salicina* (Ziska et al., 1990), *Vitis* spp. (Qin et al., 2012) and *Psidium guajava* L. (Ali-Dinar et al., 1999).

According the findings by Mounzer et al. (2013), this study's results regarding the saline effects of reclaimed waters on both canopy spectral and leaf anatomic properties could have been exacerbated even more if regulated deficit irrigation would have been applied during the growing period of orchards.

Conclusions

Negative effect of salinity on the chlorophyll system of mandarins after 7 years of exposure to saline reclaimed waters (electrical conductivity in the 3.0 to 4.0 dS m^{-1} range, boron contents >0.7 mg L^{-1}) was demonstrated by lower leaf chlorophyll contents and red reflectance values measured at the canopy level using a hyperspatial airborne flight. Grapefruit trees showed a higher tolerance to salinity and, accordingly, no significant changes in both variables were detected.

No differences between controls and treatments in both orchards were observed for the NIR spectral response of canopies, nor in the morphology and anatomy of leaves.

Results suggest that hyperspatial remote sensing techniques are more suitable for detecting physiological processes and responses on the short- and medium-term than those related with the chlorophyll synthesis. Because 10 years of drip irrigation with reclaimed water was not sufficient to promote changes in the leaf and mesophyll layer thicknesses and in the NIR spectral canopy response, vegetation indices related with leaf anatomic traits and NIR information (e.g., the NDVI) may be less suitable for detecting the gradual stress process in citrus orchards. Negative effects on *Citrus* spectral and anatomical traits may be exacerbated by using regulated deficit irrigation techniques or as a result of the accumulation of other phytotoxic elements (e.g., boron).

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