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Effects of saline reclaimed waters and deficit irrigation on *Citrus* physiology assessed by UAV remote sensing

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ABSTRACT

The aim was to assess the usefulness of spectral data to detect structural and physiological changes in *Citrus* crops under water and saline stress. Multispectral images were acquired from a fixed-wing Unmanned Aerial Vehicle (UAV) while concomitant measurements of gas exchange, plant water status, leaf structural traits and chlorophyll were taken in a commercial farm located in southeast Spain with two *Citrus* species, grapefruit and mandarin irrigated for eight years with saline reclaimed water (RW) combined with regulated deficit irrigation (RDI). Measurements at leaf scale and airborne flights were carried out twice a day, at 7 and 10 GMT. Irrigation with RW decreased gas exchange and leaf dry mass per unit area (LMA) on grapefruit. However, salinity from RW resulted in an increase in pressure potential (Ψ_P) on mandarin and allowed maintaining net photosynthesis (A) and stomatal conductance (g_s) when vapour pressure deficit increased. On both crops, leaf total chlorophyll (ChlT) concentrations were significantly reduced by RW. Moreover, RDI decreased A, g_s and stem water potential (Ψ_s) on grapefruit, independently of water quality. Regarding spectral data, red wavelength (R) was significantly correlated with Chl T ($p < 0.001$), except when mandarin was subjected to stressful climatic conditions (at 10 GMT); since R was influenced, in addition to Chl T, by the plant water and gas exchange status. Near infrared (NIR) was a useful indicator of Ψ_s, A and g_s on both crops. The normalized difference vegetation index (NDVI) was clearly related to gas exchange in both species and to Ψ_s only on mandarin. Finally, we combined data from both *Citrus* species and the best indicators were NIR and R. The novelty of this study was to show that diurnal changes in physiological and structural traits of *Citrus* irrigated with RW combined with RDI can be determined by multispectral images from UAVs.

1. Introduction

Irrigation water is not always available (mainly in summer) in the semi-arid Mediterranean areas due to water scarcity (Pedrero et al., 2015). Therefore, irrigation scheduling needs to be precise, and this requires strategies to optimize irrigation water productivity (Tapsuwan et al., 2014). One technique currently in use is the regulated deficit irrigation (RDI) strategy, where water deficits are imposed only during the crop developmental stages that are least sensitive to water stress (Chalmers et al., 1981). Furthermore, current climate change predictions indicate increases in the frequency and intensity of drought periods (Garcia-Galiano et al., 2015; Stocker et al., 2013). In order to overcome this issue, the use of non-conventional water sources such as reclaimed water (RW) (RD 1620/2007) would be an alternative for farmers. On the one hand, RW can be beneficial to crops due to its concentration of macronutrients (N,P,K) (Pedrero et al., 2013); bearing in mind that an excess of them could be lost through leaching and other processes (Romero-Trigueros et al., 2014a). On the other hand, RW may have risks for agriculture because of its high concentration of salts. Therefore, inappropriate management of irrigation with RW can exacerbate problems of secondary salinization and soil degradation

Abbreviations: A, net photosynthesis (μmol m^{-2} s^{-1}); AF, airborne flight; C, control treatment; Chl T, total chlorophyll (mg $g_{FM}$$^{-1}$); Chl *a*, chlorophyll *a* (mg $g_{FM}$$^{-1}$); Chl *b*, chlorophyll *b* (mg gFM^{-1}); EC, electrical conductivity (dS m^{-1}); ET_c, crop evapotranspiration (mm month^{-1}); ET_o, reference evapotranspiration (mm month^{-1}); GMT, Greenwich Mean Time; gs, stomatal conductance (mmol m^{-2} s^{-1}); LMA, leaf dry mass per unit area (g m^{-2}); NDVI, normalized difference vegetation index; NIR, near infrared wavelength; ns, not significant; R, red wavelength; RDI, regulated deficit irrigation; RS, remote sensing; RW, reclaimed water; SE, standard error; TW, transfer water; t_1, time 1; t_2, time 2; UAV, unmanned aerial vehicle; VPD, vapour pressure deficit (KPa); WWTP, tertiary wastewater treatment plant; Ψ_s, steam water potential (MPa); Ψ_π, osmotic potential (MPa); Ψ_P, pressure potential (MPa).

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at the medium-long term, and finally result in negative impacts on crop physiology, growth, crop quality, etc. (Romero-Trigueros et al., 2014b).

In order to be successful, RDI strategies and improved agricultural management need a reliable characterization of the plant water status. This is achieved by measurements at leaf scale, and up-scaling this information to the canopy/field level. Measuring the spectral response of canopies is a non-destructive and rapid method to signal stress early in orchards (Jones and Vaughan, 2010). The acquisition of this information with remote sensing (RS) techniques has proven useful and cost-effective compared to more time-consuming and laborious field techniques based on leaf sampling (González-Dugo et al., 2012).

Traditional RS approaches have also a number of drawbacks: satellite imagery often suffers from issues with cloud cover, and remote sensors that are fixed on towers within crop fields are relatively expensive when data from several plots needs to be collected (Anderson and Gaston, 2013). However, in recent years, the use of unmanned airborne vehicles (UAVs) increased thanks to technological advances, cost reductions and the size of sensors. These UAVs could be operated by the farmers themselves to diagnose crop features such as water stress and then adjust their water management practices as needed. Hence, UAV technology can fill the gap of knowledge between the leaf and the canopy by improving both the spatial and the temporal resolution of data on vegetative status (Gago et al., 2015). Nevertheless, the reliability of aerial RS approaches must be assessed with plant-truth data carried out in the field, i.e. with measurements related to plant water status (leaf water potential), gas exchange (net photosynthesis and stomatal conductance), chlorophyll content and leaf structure (Berni et al., 2009b; Contreras et al., 2014; Gago et al., 2013; González-Dugo et al., 2012, 2013; Lelong et al., 2008; Zarco-Tejada et al., 2012).

Imagery RS technologies are mainly based on canopies' wavelength reflectances in the visible, such as red, green and blue, and non-visible range of the spectrum, such as near-infrared (NIR). The remote monitoring of these specific reflectances is commonly performed using visible, multispectral and hyper-spectral cameras (Baluja et al., 2012; Zarco-Tejada et al., 2012, 2013a,b). This reflectance can be used as an indicator of plant status because of its relationship with, among others, leaf pigment composition, plant biophysical or structural parameters and physiological status (Jones and Vaughan, 2010). Red wavelengths (R) (660 to 680 nm) specifically are absorbed by leaf chlorophyll (Ollinger, 2011). Because salty environments harm or reduce the functionality and content of chlorophyll in the leaves, reflectance may be proportionally reduced. In the NIR (750–1400 nm) domain, the spectral response depends on the multiple scattering of light inside the leaf that is mainly controlled by its internal structure, such as mesophyll thickness and water content (Bonilla et al., 2015).

Composite indices integrating data from both domains, such as the Normalized Difference Vegetation Index (NDVI), have shown positive correlations with water stress indicators (water potential and stomatal conductance) in a number of crops (Gago et al., 2015; Glenn et al., 2008). In most cases, the indicators used for this purpose are related to canopy structural changes in different days of the year or growth season, but approaches related with diurnal physiology changes along a single day are rare (González-Dugo et al., 2015).

In the last years, research focused on checking the different vegetation indices acquired from the UAVs equipped with multi-spectral cameras and then comparing them to field-collected measurements of plant-physiological and structural increased (Berni et al., 2009a; Contreras et al., 2014; Lelong et al., 2008; Zarco-Tejada et al., 2013a,b). Drought is one of the most studied stress impulses (Baluja et al., 2012; Gago et al., 2015; Pôcas et al., 2015; Rodriguez-Pérez et al., 2007; Stagakis et al., 2012; Zarco-Tejada et al., 2012); however, research on saline stress from RW using UAV technology is limited (Contreras et al., 2014). Besides, studies that evaluate saline and/or water stress tolerances over extended periods are scarce because of the cost and time required for extended periods of time (i.e. multiple years).

Salinity stress harms *Citrus* mainly in two ways: (1) by specific-ion toxicity and (2) by osmotic effects caused by the accumulation of salts. If the stress factor remains, changes in the leaf pigments can arise. In this sense, negative effects of salinity on the chlorophyll content have been reported in *Citrus* species (Papadakis et al., 2004; Romero-Trigueros et al., 2014b), which constitute one of the most important commercial fruit crops worldwide. The experiment reported on here is the first one to evaluate the diurnal effects of prolonged exposure (eight years) to RW and deficit irrigation on grapefruit and mandarin trees under field conditions by i) measurements of plant water status, gas exchange and chlorophyll in order to obtain the plant-truth data and ii) spectral data, acquired with an UAV, both carried out twice over the course of the day. In addition, the current work sought to assess the usefulness of multispectral imagery to determine the structural and physiological diurnal changes in *Citrus* crops under water and saline stress.

2. Materials and methods

2.1. Site description and irrigation treatments

The experiment was conducted in 2015 in a commercial *Citrus* orchard, located at the northeast of the Region of Murcia in Campotéjar (38°07′18″N, 1°13′15″W, 132 m above sea level) with a BSk climate by Köppen-Geiger classification (Peel et al., 2007).The 1-ha experimental plot was cultivated with i) 11 year-old 'Star Ruby' grapefruit trees (*Citrus paradisi* Macf) grafted on Macrophylla rootstock [*Citrus Macrophylla*] planted at 6 × 4 m and ii) 14 year-old mandarin trees (*Citrus clementina* cv Orogrande) grafted on Carrizo citrange *(Citrus sinensis* L. Obs. x *Poncirus trifoliate* L.) planted at 5 × 3.5 m. Irrigation was scheduled on the basis of crop evapotranspiration (ET_c) accumulated during the previous week. ET_c values were estimated by multiplying reference evapotranspiration (ET_o), calculated with the Penman-Monteith methodology (Allen et al., 1998), by a monthly local crop coefficient according to Pedrero et al. (2015) for grapefruit and Nicolás et al. (2016) for mandarin. All trees received the same amount of N, P_2O_5 and K_2O through a drip irrigation system: 215–110–150 kg ha^{-1} year^{-1} for grapefruit and 215–100–90 kg ha^{-1} year^{-1} for mandarin, respectively. Weeds were eradicated in the orchard by applying the farmers' commonly used pest control methods.

The experimental plot has been irrigated with two different water sources since 2007. In one case water was pumped from the Tajo-Segura canal (transfer water, TW) and in the other case water was pumped from the North of "Molina de Segura" tertiary wastewater treatment plant (WWTP) (reclaimed water, RW). The latter had high salt and nutrient levels (Table 1) with high electrical conductivity (EC) close to 4 dS m^{-1}, while for the transfer irrigation water the EC values were close to 1 dS m^{-1}. Saline water was automatically mixed with water from TW at the irrigation control-head to lower its EC to ≈3 dS m^{-1} in order to establish a constant EC during the experiment. This high level of salinity observed in the RW was mainly due to the high concentration of Cl^- and Na (Table 1). The boron concentration in RW was considerably higher than that in TW. Moreover, higher concentrations of N, P and K were observed in RW than in TW. The pH was more basic in TW than RW (Table 1). No differences in the concentration of heavy metals were found between the irrigation water sources (data not shown).

Two irrigation treatments were established for each water source. The first treatment was a control (C) irrigated throughout