Respuesta espectral y fisiológica de Cítricos regados con agua regenerada salina y estrategias de riego deficitario.

Spectral and physiological responses of Citrus irrigated with saline reclaimed water combined with deficit irrigation strategies

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Resumen

El ensayo consistió en evaluar la fisiología de árboles de mandarino (M) y de pomelo (P) regados durante 8 años con agua regenerada salina (AR) y agua del trasvase Tajo-Segura (AT) y diferentes estrategias de riego: control (C) y riego deficitario controlado (RDC), mediante la medida del contenido en clorofila, el intercambio gaseoso, el estado hídrico de la planta y de datos espectrales en los dominios rojo (R) e infrarojo cercano (NIR). El 7 de julio se llevaron a cabo dos vuelos aéreos, uno a las 09.00 h (AF1) y otro a las 11.00 h (AF2), en una finca localizada en el noreste de la Región de Murcia al mismo que tiempo que se registraban el resto de parámetros citados. Los resultados mostraron que el riego con AR afectó negativamente a la síntesis de clorofila tanto en M como en P. Respecto a los datos espectrales, en M el valor de R se incrementó significativamente en los tratamientos de RDC, pero no en el control de AR dado que la salinidad dio lugar a un amento del turgor de hoja que permitió mantener los valores de intercambio gaseoso y de potencial hídrico similares al control de AT. Sin embargo, en P, el valor de R disminuyó en todos los tratamientos, especialmente en AR-RDC. Por otra parte, en AF2 los valores de R y NIR fueron inferiores que en AF1. Además, ambos cultivos mostraron cambios significativos entre los cuatro tratamientos en el índice diferencial de vegetación normalizado (NDVI) para ambos vuelos. Por consiguiente, se demuestra que las propiedades espectrales son herramientas más adecuadas que las relacionadas exclusivamente con la síntesis de clorofila para evaluar a corto plazo y de forma global los cambios fisiológicos diarios, provocados por las condiciones atmosfércias y el estrés hídrico, de cítricos regados con AR y estrategias de riego deficitario.

Abstract

We reported the results of an experiment using the spectral data from the Red (R) and Near-Infrared (NIR) domains, chlorophyll content, gas exchange and plant water status to evaluate the physiology of mandarin (M) and grapefruit (G) trees irrigated with saline reclaim water (RW) and transfer water (TW) and different irrigation strategies: control (C) and regulated deficit irrigation (RDI). Two airborne flights at 09.00 (AF1) and 11.00 am (AF2) on July 7, 2015 using a fixed-wing Unmanned Aerial Vehicle (UAV) (eBee from SenseFly) were conducted at a commercial orchard located in the northeast of the Region of Murcia. Results suggested a negative effect on the chlorophyll system of M and G after eight years of exposure to RW. In M trees, R values only were significantly increased in RDI treatments but not in saline RW-C treatment due to the increasing of turgor which allowed to maintain gas exchange and water potential similar to TW-C. However, in G trees, all treatments were affected, mainly RW-RDI. Moreover, all reflectance values in R and NIR regions decreased from AF1 to AF2. In addition, normalized difference vegetation index (NDVI) values showed in both crops significant changes within treatments from AF1 to AF2 too. Thus, R, NIR and NDVI were sensitive to diurnal physiological changes induced by atmospheric conditions and water stress. The results of this study highlight the key role that spectral properties can play in detecting physiological processes and responses on the short-term in plant irrigated with RW and with different strategies of irrigation, addition to the role in the chlorophyll synthesis.

Palabras claves: agua depurada salina; clorofila; mandarino; pomelo; propiedades espectrales.

Keywords: chlorophyll content; grapefruit; mandarin; saline reclaimed water; spectral properties.

Introduction

Citrus is one of the most important commercial fruit crops in the world, including southeastern Spain where climate is semi-arid Mediterranean with warm and dry summers and mild winter conditions. In these kinds of regions irrigation water is not always available due to water scarcity and shortage. Therefore, many citrus orchards suffer severe drought periods, mainly in the summer season. In order to overcome this issue, the use of non-conventional water sources such as reclaimed water (RW) would be an alternative for farmers since its volume is progressively augmenting. RW can be beneficial to crops when used as fertilizer (Levine and Asano, 2004), bearing in mind that excess of these nutrients, for instance nitrates, could be lost in ecosystem through leaching, etc. (Romero-Trigueros et al., 2014a). Conversely, use of RW may have risks for agriculture since it often has a higher concentration of salts than that found in natural water resources. Therefore, an inappropriate management of irrigation with RW can exacerbate problems of secondary salinization and soil degradation at the medium-long term, finally resulting in negative impacts on the plant physiologic, growth, quality of cultivars, etc. (Romero-Trigueros et al., 2014b). 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. If the stress factor remains, changes in the content of leaf pigments (e.g., chlorophyll) can arise, giving rise to leaf bronzing or yellowing (García-Sanchez et al., 2002). Specifically, negative effects of salinity on the chlorophyll content and anatomic morphology of leaves have been reported in Citrus (Romero-Aranda et al., 1998). 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 leaf pigments (Ollinger, 2011). 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 its internal structure (Pinter et al., 2003). Because differential absorption of light in the R 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 detect signals of water and saline stress (Gago et al., 205; Glenn et al., 2008). In most cases, the indicators used for this purpose are related with canopy structure, but using approaches related with diurnal physiology changes are rare (Gonzalez-Dugo et al., 2015). This study provides results on the diurnal effects of prolonged exposure of RW and deficit irrigation on two species of Citrus. A first study to evaluate the impact of the irrigation water quality on the heatlh Citrus orchards were realized by Contreras et al. (2014), see references herein for other recent experiences in other crop systems.

Material and Method

Study area and experimental design. The experiment was conducted at a commercial *Citrus* orchard, located in the northeast of the Region of Murcia, 7 km north of Molina de Segura (38º07'18”N, 1°13’15”'W) in 2015. The experimental plot of 1 ha was cultivated with i) 10 year-old 'Star Ruby´grapefruit trees (*Citrus paradisi* Macf) grafted on Macrophylla rootstock [*Citrus Macrophylla*] planted at 6 x 4 meters and ii) 13 year-old mandarin trees (*Citrus clementina* cv Orogrande) grafted on Carrizo citrange *(Citrus sinensis* L. Obs. x poncirus trifoliate L.) planted at 5 x 3.5 meters. The experimental plot of Grapefruit (G) and Mandarin (M) trees was irrigated with two different water sources during approximately eight years of cultivation. The first irrigation water was pumped from the Tajo-Segura canal (Transfer Water, TW) and the second water source came from the North of “Molina de Segura” tertiary wastewater treatment plant (Reclaimed Water, RW) characterized by having high salt and nutrients levels. RW showed EC values close to 3 $dS \cdot m^{-1}$, while for TW the EC values were lower, close to 1 $dS \cdot m^{-1}$. Two irrigation treatments was established for each water quality: a control irrigation treatment (C) in which the crop will be irrigated according to water requirements (100% ETc), and a regulated deficit irrigation (RDI) treatment, applying only 50% of ETc during the second phase of rapid fruit growth. The irrigation scheduling will be weekly and based on the calculations of reference evapotranspiration (ETo) by the Penman-Monteith equation (Allen et al., 1998). The experimental design of each irrigation treatment was 4 standard experimental plots and distributed following a completely randomized design. Each replica was made up of 12 trees, organized in 3 adjacent rows. The middle rows were used for measurements and the rest were guard trees.

Spectral and physiological response. A flight campaign was conducted in study area on July 7, 2015 using a fixed-wing Unmanned Aerial Vehicle (UAV) (eBee from SenseFly). Two flights were conducted over the tree plots: the first was at 09.00 a.m. (AF1), while the second at 11.00 a.m (AF2). The eBee UAV is capable of approximately 40 minute flights at cruise speeds of around 50 km/h and can be flown either manually or using an autopilot. When using the autopilot the eBee follows the waypoints of a flight plan created using flight planner software (eMotion). A constant radio link between the flight planner software and the UAV allows for inflight monitoring and control. The UAV is mounted with a GPS receiver, altimeter, wind meter and a digital camera that is electronically triggered by the autopilot system to acquire images at the correct positions. The digital camera has a 16 megapixel sensor, i.e. 4608 by 3456 pixels, and captures JPEG format images in the Green, Red and Near Infrared light range. Following previous experiences in the area (Contreras et al., 2014), the spectral data from the Red (R) and Near-Infrared (NIR) domains were used to evaluate the status of vegetation. Vegetation gives a strong reflection in the range of 0.7-0.9 microns (near infrared), while a weak reflection in the range of 0.6-0.7 microns (red) due to the absorption by chlorophyll. Additionally, and from both spectral signatures, the Normalized Difference Vegetation Index (NDVI), as an indicator of greenness, was also computed as:

$$NDVI = (NIR - R)/(NIR + R)$$

where NIR and R are the radiance at the top of the sensor (in this study, coded as digital numbers). The center of each tree crowns was manually detected and a buffer zone of 1 m radius was adopted to exclude the impact of soil background on the overall spectral response of the trees. Finally average values for each spectral domain was computed and statistically analyzed. Total leaf chlorophyll was measured according to equations of Inskeep & Bloom (1985).

Statistical analysis was performed as a weighted analysis of variance (ANOVA; statistical software IBM SPSS Statistics v. 21 for Windows. Chicago, USA).

Results and Discussion

Spectral response: On the one hand, from AF1 to AF2 all M and G trees decreased reflectance values in the red wavelength domain and the NIR regions. On M, R and NIR reductions were about 12 and 9%, respectively, being in both cases more pronounced in TW-RDI treatment. On grapefruit, R and NIR reductions were about 12 and 13%, respectively, being in both cases more pronounced in RW-RDI treatment. On the other hand, among treatments within the same flight, the highest increase of R values respect to TW-C happened in AF1: 10% in mandarin of TW-RDI treatment and 9% in grapefruit of RW-RDI treatment. Regarding NDVI values, we also observed that there were significant changes within the treatment from AF1 to AF2. Thus, NDVI is sensitive to diurnal physiological changes induced by heat and water stress and not only tracks the effects in the long term, as other authors indicated (Haboudane et al., 2004, Dobrowski et al, 2005).

Physiology response: Regarding total leaf chlorophyll (Chl T), in both crops the treatments irrigated with RW showed the lowest values in both airborne flights, although more clearly in the second one. In the case of grapefruit, RW-RDI was the treatment more affected, supporting the spectral data. In addition, a significant correlation between average value of R and Chl T of all treatments was observed ($P<0.01$). In the case of mandarin, the Chl T found in both RW-C and TW-RDI was lower and higher, respectively, than expected as the spectral data and, therefore, no significant correlation between average value of R and Chl T of all treatments was found. Consequently, we observed that R values in mandarin were influenced, in addition to chlorophyll, for gas exchange data (since TW-RDI was the treatment with lower stomatal conductance) and for turgor potential (since RW-C was the treatment with higher turgor potential and gas exchange data similar to TW-C) (data not shown).

Moreover, the important decrease in R and NIR values from AF1 to AF2 was support with data of water and osmotic potential and photosynthetic activity (data not shown).

Conclusions

Results suggest that hyperspatial remote sensing techniques are more suitable for detecting global physiological processes and responses on the short and medium term than those related with the chlorophyll synthesis since spectral data including information regarding diurnal changes in the water status and gas exchange of the plant.

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Table 1. *Comparisons between treatments in measurements of red (R), near infrared (NIR) and normalized difference vegetation index (NDVI) for Mandarin (A) and Grapefruit (B). The values of each column followed by different letters are significantly different by Duncan's test (P <0.05).*

A		Airborne flight 1 (AF1)			Airborne flight 2 (AF2)		
	Treatment	**R**	**NIR**	**NDVI**	**R**	**NIR**	**NDVI**
Mandarin	**TW-C**	73.32±1.24b	160.77±1.75c	0.4136±0.0035a	65.82±0.75b	148.52±0.92b	0.4157±0.0033ba
	TW-RDI	80.78±1.22a	169.90±1.52a	0.3902±0.0041b	71.49±0.87a	154.36±0.96a	0.3951±0.0037c
	RW-C	74.13±1.14b	164.90±1.61cb	0.4102±0.0036a	66.01±0.82b	151.60±1.01a	0.4254±0.0035a
	RW-RDI	77.89±1.17a	167.06±1.54ba	0.3936±0.0039b	69.14±0.81a	152.13±1.03a	0.4069±0.0034b
B		**Airborne flight 1(AF1)**			**Airborne flight 2 (AF2)**		
	Treatment	**R**	**NIR**	**NDVI**	**R**	**NIR**	**NDVI**
Grapefruit	**TW-C**	71.62±1.03a	168.24±1.47b	0.4187±0.0033b	63.79±0.66a	148.10±1.06a	0.4082±0.0029b
	TW-RDI	74.97±1.58ab	162.20±2.44a	0.4062±0.0047a	66.50±0.82b	145.49±1.39a	0.3913±0.0025a
	RW-C	75.18±1.11ab	162.76±1.98ab	0.4067±0.0032a	65.76±0.77ab	145.80±1.18a	04014±0.0028b
	RW-RDI	78.30±1.34b	168.64±1.90b	0.3965±0.0039a	68.95±0.70c	148.90±0.95a	0.3981±0.0027a

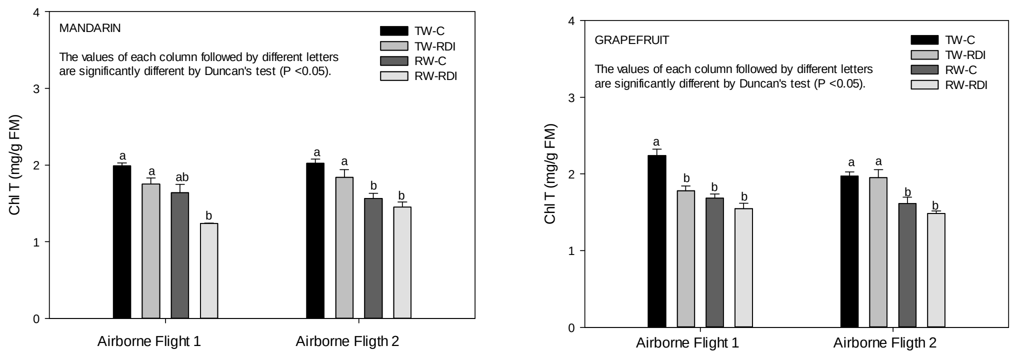

Figure 1. *Measurements of total leaf chlorophyll (Chl T) for mandarin (A) and grapefruit (B).*