

# Evaluating the performance of xanthophyll, chlorophyll and structure-sensitive spectral indices to detect water stress in five fruit tree species

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Abstract This study assessed the capability of several xanthophyll, chlorophyll and structure-sensitive spectral indices to detect water stress in a commercial farm consisting of five fruit tree crop species with contrasting phenology and canopy architecture. Plots irrigated and non-irrigated for eight days of each species were used to promote a range of plant water status. Multi-spectral and thermal images were acquired from an unmanned aerial system while concomitant measurements of stomatal conductance  $(g_s)$ , stem water potential  $(\Psi_s)$  and photosynthesis were taken. The Normalized Difference Vegetation Index (NDVI), red-edge ratio ( $R_{700}/R_{670}$ ), Transformed Chlorophyll Absorption in Reflectance Index normalized by the Optimized Soil Adjusted Vegetation Index (TCARI/ OSAVI), the Photochemical Reflectance Index using reflectance at 530 (PRI) and 515 nm [PRI(570-515)] and the normalized PRI (PRInorm) were obtained from the narrow-band multi-spectral images and the relationship with the in-field measurements explored. Results showed that within the *Prunus* species,  $\Psi_s$  yielded the best correlations with PRI and  $PRI_{(570-515)}$  (r<sup>2</sup> = 0.53) in almond trees, with TCARI/OSAVI (r<sup>2</sup> = 0.88) in apricot trees and with PRI<sub>norm</sub>, R<sub>700</sub>/R<sub>670</sub> and NDVI (r<sup>2</sup> from 0.72 to 0.88) in peach trees. Weak or no correlations were found for the Citrus species due to the low level of water stress reached by the trees. Results from the sensitivity analysis pointed out the canopy temperature  $(T_c)$ and  $PRI_{(570-515)}$  as the first and second most sensitive indicators to the imposed water conditions in all the crops with the exception of apricot trees, in which  $\Psi_s$  was the most

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sensitive indicator at midday.  $PRI_{norm}$  was the least sensitive index among all the water stress indicators studied. When all the crops were analyzed together,  $PRI_{(570-515)}$  and NDVI were the indices that better correlations yielded with Crop Water Stress Index,  $g_s$ and, particularly,  $\Psi_s$  ( $r^2 = 0.61$  and 0.65, respectively). This work demonstrated the feasibility of using narrow-band multispectral-derived indices to retrieve water status for a variety of crop species with contrasting phenology and canopy architecture.

Keywords Fruit crop · Multispectral imagery · Remote sensing · Water stress detection

## Abbreviations

gs	Stomatal Conductance
$\Psi_{\rm s}$	Stem Water Potential
CWSI	Crop Water Stress Index
T <sub>c</sub>	Canopy Temperature
UAS	Unmanned Aerial System
NDVI	Normalized Difference Vegetation Index
$R_{700}/R_{670}$	Red Edge Ratio (reflectance at 700 nm divided by the reflectance at 670 nm)
TCARI	Transformed Chlorophyll Absorption in Reflectance Index
OSAVI	Optimized Soil Adjusted Vegetation Index
PRI	Photochemical Reflectance Index (using reflectance at 530 and 570 nm)
PRInorm	Normalized Photochemical Reflectance Index
PRI(570-515)	Photochemical Reflectance Index (using reflectance at 515 and 570 nm)

# Introduction

Continuous advances in technology have promoted the use of unmanned aerial systems (UAS) for a large range of applications (Anderson and Gaston 2013). Precision agriculture is one of the most promising applications (Mulla 2013; Gago et al. 2015) since low-cost UAS can be equipped with robust sensors providing very high resolution, such as miniaturized narrow-band and hyperspectral and thermal cameras used to remotely monitor vegetation (Bendig et al. 2012). Farm assessment by remote sensing using UAS enables crop monitoring from a closer range and a higher frequency than is currently possible with satellites. Inter- and intra-field variability of crops can then be assessed in detail providing farmers with crucial information to better optimize farm management and increase farmers' profitability (Mulla 2013).

Water availability is becoming the most limiting factor for crop production in much of the world (Field 2014). This fact has increased the emphasis that policy-makers are placing on both demand and supply options to water management. Remote sensing takes on special significance within this context since it enables a better monitoring of large cultivated areas making it easier to assess the proper functioning of irrigation systems (for example, by identifying water-stressed areas or irrigation leaks with thermal images) and the precise management of plants water stress, which has been extensively pointed out in the literature (Taghvaeian and Neale 2011) as a key factor to ensure the success of water-saving irrigation strategies based on irrigating plants below their full water requirements.

High resolution thermal imagery has been successfully used in a variety of crops to assess the variability in plant water status at the field and farm scales (Bellvert et al. 2013; Berni et al. 2009; Gonzalez-Dugo et al. 2012). Bellvert et al. (2013) mapped the spatial variability in leaf water potential of different vineyards based on high resolution thermal

imagery and then used that information for scheduling irrigation. Similarly, Gonzalez-Dugo et al. (2013) identified water-stressed areas from thermal images in a farm composed of five fruit tree crops and based on the relationship between the Crop Water Stress Index (CWSI) and the stem water potential ( $\Psi_s$ ) established a CWSI threshold for scheduling irrigation.

Notwithstanding the suitability of thermal sensing for plant water stress assessment, alternative indices less sensitive to variations in the air vapor pressure deficit and more related to biophysical parameters such as the chlorophyll or xanthophylls pigment content are currently of interest (Zarco-Tejada et al. 2013). That is because in the field, in order to cope with drought, plants usually exhibit adaptive mechanisms at leaf level such as the dissipation of excitation energy (associated with an increase in the concentration of de-epoxidized xanthophyll cycle components), decrease in leaf chlorophyll concentration and down-regulation of photosynthesis (Chaves et al. 2002).

Greenhouse experiments have shown that water stress periods of even eight days may have a significant effect on the content and organization of chlorophyll in the mesophyll (Albert and Thornber 1977). A variety of narrow-band optical indices obtained from remote sensing data have been related to leaf chlorophyll concentration (Haboudane et al. 2002; Zarco-Tejada et al. 2004). Combination of indices such as the Transformed Chlorophyll Absorption in Reflectance Index (TCARI) and the Optimized Soil Adjusted Vegetation Index (OSAVI) to give the TCARI/OSAVI index (Table 1), have been shown as more robust indices to estimate chlorophyll concentration than simple indices because of a lower sensitivity to soil background and crop leaf area index variations (Haboudane et al. 2002; Zarco-Tejada et al. 2004).

The narrow-band Photochemical Reflectance Index (PRI, Table 1) proposed by Gamon et al. (1992), which is based on the xanthophylls cycle activation as a mechanism to dissipate the excess of energy when photosynthesis declines under conditions of stress, has been also successfully tested as a water stress indicator in several studies (Peguero-Pina et al. 2008; Suárez et al. 2010; Hernández-Clemente et al. 2011). These authors, however, also pointed out that PRI is highly affected by factors such as canopy structure, viewing and illumination geometry effects, and background, which challenge its widespread use as a water stress indicator. Different formulations of PRI (based on different wavelength

**Table 1** Formulations used to obtain the vegetation indices: Photochemical Reflectance Index (PRI),normalized PRI (PRI<sub>norm</sub>), PRI using the reflectance band at 515 nm [PRI<sub>(570–515)</sub>], red edge ratio (R700/R670), Normalized Difference Vegetation Index (NDVI) and Transformed Chlorophyll Absorption inReflectance Index normalized by the Optimized Soil Adjusted Vegetation Index (TCARI/OSAVI)

Index	Formulation	References
PRI	$(R_{570} - R_{530})/(R_{570} + R_{530})$	Gamon et al. (1992)
PRI <sub>(570–515)</sub>	$(R_{570} - R_{515})/(R_{570} + R_{515})$	Hernández-Clemente et al. (2011)
PRI <sub>norm</sub>	PRI <sub>570</sub> /[RDVI (R <sub>700</sub> /R <sub>670</sub> )]	Zarco-Tejada et al. (2013)
R700/R670	R <sub>700</sub> /R <sub>670</sub>	Part of TCARI index
NDVI	$(R_{800} - R_{670})/(R_{800} + R_{670})$	Rouse et al. (1974)
TCARI/ OSAVI	$\begin{array}{l} [3[(R_{700}-R_{670})-0.2\;(R_{700}/R_{550})\;(R_{700}/R_{670})]]/[(1+0.16)\\ (R_{800}-R_{670})/(R_{800}+R_{670}+0.16)]\;R_{700}/R_{670} \end{array}$	Haboudane et al. (2002)

Within the formulation column,  $R_{\lambda}$  means the reflectance signal at " $\lambda$ " nm wavelength

references) have been studied in this sense searching to overcome these limitations (Hernández-Clemente et al. 2011). Recently, Zarco-Tejada et al. (2013) proposed a modified PRI-based index (PRI<sub>norm</sub>) to track the diurnal trends of water stress using a combination of a structural index (Renormalized Difference Vegetation Index, RDVI) and an index sensitive to the leaf chlorophyll content (the red edge ratio,  $R_{700}/R_{670}$ ) to normalize PRI. The PRI<sub>norm</sub> index was tested in an experimental vineyard site in a diurnal setting, yielding higher correlations than PRI with the CWSI and the commonly used water stress indicators, leaf water potential and stomatal conductance ( $g_s$ ).

Spectral indices have been usually evaluated in orchards consisting of a single crop. It is not uncommon for farms to be made up of several crop species with different canopy architectures, nutrient status and even phenological stages. Under these circumstances, the possibility of using a single multi-spectral index that could provide reliable information regarding the plant water status of all the co-existing crops in the farm would considerably simplify the farm assessment.

With that objective in mind, a study was performed in a commercial farm consisting of five fruit tree crop species in which the capability of xanthophyll, chlorophyll and structure-sensitive spectral indices to track the effects of water stress on trees was assessed. The sensitivity of these indices to the imposed drought conditions were assessed by means of a sensitivity analysis as other authors (Goldhamer et al. 1999; Moriana and Fereres 2002; Intrigliolo and Castel 2006) have reported for the evaluation of alternative indirect physiological indicators. Thus, the specific objectives of the present work were: (i) to assess the sensitivity of xanthophyll, chlorophyll and structure-sensitive spectral indices to water stress conditions; (ii) to explore the relationship between the spectral indices and  $\Psi_s$ ,  $g_s$  and CWSI for each of the fruit tree crop species studied, and; (iii) to identify which of the spectral indices could more accurately track the water stress effects on trees when the five fruit tree crop species were assessed together.

# Materials and methods

#### Site characteristics and irrigation treatments

The study was performed in July 2010 in the same 42-ha commercial farm described in Pérez-Sarmiento et al. (2010) and Gonzalez-Dugo et al. (2013), located in the Mula Valley ( $37^{\circ}55'N$ ,  $1^{\circ}26'W$ ), Murcia (Spain), where the climate is considered as semi-arid Mediterranean. The annual reference evapotranspiration (ET<sub>0</sub>) and rainfall for the experimental season were 1182 and 445 mm, respectively.

The farm consisted of five orchards planted in 1999 with almond (*Prunus dulcis* cv. Garrigues and cv. Ramillete), apricot (*Prunus armeniaca* cv. Bulida), peach (*Prunus persica* cv. Catherine), orange (*Citrus sinensis* cv. Lane Late) and lemon (*Citrus x limon* cv. Fino 49) trees. Each orchard was divided into 2–4 irrigation units, which enabled different irrigation managements and the development of contrasting plant water status. The number of irrigation units and other characteristics such as tree spacing, canopy ground cover and number of emitters per tree used within each orchard are shown in Table 2. At the time of the study (July), almond, peach, lemon and orange trees were being irrigated daily. In order to generate different levels of tree water status in these orchards, irrigation was withheld for eight days prior to the measurements in one single irrigation unit in the almond, lemon and orange orchards, and in two irrigation units in the peach

Species	Tree spacing (m)	CGC (%)	# emitters tree <sup><math>-1</math></sup> (4 l h <sup><math>-1</math></sup> )	# irrigation units
Almond	$6 \times 8$	40	5	3
Apricot	$6 \times 8$	65	5	2
Peach	$4 \times 6$	48	3	4
Lemon	$6 \times 8$	41	5	2
Orange	$4 \times 6$	51	3	3

 Table 2
 Tree spacing, canopy ground cover (CGC) and number of emitters used per tree for each of the orchards in the study. The number of irrigation units established per orchard is also shown

orchard. Apricot trees, on the other hand, had already been harvested and water had not been applied since 24 days before the measurements date. Thus, to generate different water status in the apricot orchard, irrigation was resumed in one single unit eight days prior to the measurements.

### Airborne imagery and image processing

Multispectral images were acquired on July 7th at 13.00 h (local time; UTC + 1 h) with a 6-band multispectral camera (MCA-6, Tetracam Inc., California, USA) installed on a twometer wingspan fixed-wing UAS platform. The image resolution was  $1280 \times 1024$  pixels with 10-bit radiometric resolution and optical focal length of 8.5 mm. The UAS (mX-SIGHT, UAV Services and Systems, Germany) was controlled by an autopilot for autonomous flying (AP04, UAV Navigation, Madrid, Spain) following a flight plan of around 1 h at 350 m above the ground and 5.8 kg take-off weight using waypoints to acquire imagery from the entire orchards under study. At this flight altitude, the camera delivered a ground resolution of 200 mm pixel-size. The autopilot had a dual CPU controlling an integrated attitude heading reference system (AHRS) based on a L1 GPS board, 3-axis accelerometers, gyros and a 3-axis magnetometer (Berni et al. 2009). The ground control station and the UAS were radio-linked, transmitting position, altitude and status data at 20 Hz frequency.

The bandsets chosen for this study were centered at 515, 530, 570, 670, 700 and 800 nm (10 nm bandwidths). The high-resolution of the multispectral imagery enabled the identification of every single crown within the orchard. A region of interest was established in the center of each crown to extract pure vegetation reflectance and to avoid soil background effect. Then, the different indices were calculated at the object level (crown). The average crown reflectance derived from the imagery of well-watered and deficit-irrigated trees for each of the five tree crop species studied is shown in Fig. 1. Reflectance values obtained for the six spectral bands enabled the calculation of vegetation indices sensitive to variations in canopy structure, chlorophyll and xanthophyll pigment content (Table 1).

The Normalized Difference Vegetation Index (NDVI) was used to track changes in canopy structure. Effect of treatments on the leaf chlorophyll concentration was assessed with the red edge index ( $R_{670}/R_{700}$ ), which uses the reflectance at 670 and 700 nm wavelengths, and the Transformed Chlorophyll Absorption in Reflectance Index normalized by the Optimized Soil Adjusted Vegetation Index (TCARI/OSAVI). Finally, PRI, PRI using reflectance at 515 nm [PRI<sub>(570-515)</sub>] instead of 530 and PRI<sub>norm</sub> were also determined.



Fig. 1 Average crown reflectance derived from the imagery of well-watered (WW) and deficit irrigated (DI) trees for each of the five fruit tree crops studied

The spectral indices were compared with  $T_c$  and CWSI, which were determined in a parallel study by Gonzalez-Dugo et al. (2013) in the same trees. Thermal images were obtained from a thermal camera (MIRICLE 307, Thermoteknix Systems Ldt., Cambridge, UK) also installed on the UAS during the study.

#### Field data collection

Concomitant measurements of  $\Psi_s$  and  $g_s$  were taken during the flight in selected irrigated and non-irrigated trees from each species. Measurements were carried out by five teams composed of 3–5 people each with experience taking in-field determinations.

The  $\Psi_s$  determinations were carried out with five Scholander pressure chambers (Model 600 Pressure Chamber, PMS Instrument Company, Albany, USA) in two mature leaves per tree covered with aluminum foil for at least 90 min before measurements. The number of trees used for the  $\Psi_s$  and other in-field determinations within each irrigation unit are shown

in Table 3. The g<sub>s</sub> was measured in 2-4 sunny leaves per tree using a diffusion porometer (SC-1 porometer, Decagon, WA, USA) in the lemon, orange and peach crops and a portable photosynthesis system (LI-6400 Li-Cor, Lincoln, NE, USA) equipped with a LI-6400/40 Leaf Chamber Fluorometer and a LICOR 6400-01 CO<sub>2</sub> injector in the almond and apricot crops. Leaf gas exchange was measured on fully expanded leaves placed in a  $200 \text{ mm}^2$  leaf cuvette. The CO<sub>2</sub> concentration in the cuvette was maintained at 400  $\mu$ mol·mol<sup>-1</sup> ( $\approx$  ambient CO<sub>2</sub> concentration). Measurements were performed at saturating light intensity of 1800  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup> and at ambient temperature and relative humidity. The airflow was set to 300  $\mu$ mol s<sup>-1</sup>, which enabled also recording the leaf net  $CO_2$  assimilation. Additionally, three leaf samples were taken from selected irrigated and non-irrigated trees of all the species with the exception of peach to determine the leaf chlorophyll content (Table 3). For the leaf chlorophyll determination, approximately 30 mg leaves were sampled from the same, avoiding major veins. Chlorophyll was eluted from the leaf by submerging them in 3 ml of N, N-dymethylformamide in the dark for at least 72 h. The amount of absorbance was read at 647 nm and 664.5 nm with a Thermo Spectronic (model Helios alpha, UVA No. 092009, England) and used to calculate fresh mass-based chlorophyll content according to the equations of Inskeep and Bloom (1985).

Weather conditions at the time of flights were recorded with a portable weather station (Model WXT510, Vaisala, Finland) placed just outside the orchard. Mean values for the air temperature, vapor pressure deficit and wind speed at the time of flight were 31.9 °C, 3.76 kPa and 1.9 m s<sup>-1</sup>, respectively.

# Statistical analysis

Sensitivity of the spectral indices related to the water stress conditions was assessed by means of a sensitivity analysis (sensitivity defined as signal to noise ratio) based on that proposed by Goldhamer and Fereres (2001) and comparison with traditional indicators of plant water status. When there were significant differences between treatments, the value "signal" for  $\Psi_s$ ,  $T_c$  and the vegetation indices was calculated as the ratio between the average value for the water-stressed and control treatments while, for  $g_s$ , it was obtained from the ratio between the average value for the average coefficient of variation (CV) among trees from the same treatments as the signal value.

U	e e e e e e e e e e e e e e e e e e e								
Species	$\Psi_{\rm s}$		gs		Photosynthesis		Chl		
	WW	DI	WW	DI	WW	DI	WW	DI	
Almond	10	12	10	12	10	12	6	6	
Apricot	6	4	6	4	6	4	6	4	
Peach	5	5	5	5	-	-	-	_	
Lemon	5	5	5	5	-	-	4	4	
Orange	12	6	5	3	-	-	6	6	

**Table 3** Number of trees in which measurements of stem water potential  $(\Psi_s)$ , stomatal conductance  $(g_s)$ , leaf net  $CO_2$  assimilation (photosynthesis) and leaf chlorophyll content (Chl) were taken within each irrigation treatment

"WW" and "DI" mean well-watered and deficit irrigated trees, respectively

The relationship between the spectral indices and  $\Psi_s$ ,  $g_s$  or CWSI for each of the crop species studied, as well as when data from all the crops were pooled together, was explored by correlation analyses.

# **Results and discussion**

#### Sensitivity of the indicators to detect water-stressed areas

Results obtained from the sensitivity analysis showed that  $T_c$  was the indicator with the lowest variability and highest sensitivity in three out of the four crop species included in the analysis (Table 4). Data from the orange orchard were not included in the analysis due to the non-significant differences in plant water status observed between treatments. Apricot was the only crop in which  $\Psi_s$  resulted in the most sensitive indicator to the imposed irrigation treatments, followed by PRI<sub>(570–515)</sub> and T<sub>c</sub>. These results were mainly

**Table 4** Sensitivity analysis of the stem water potential ( $\Psi_s$ ), stomatal conductance ( $g_s$ ), Crop Water Stress Index (CWSI), Photochemical Reflectance Index (PRI), normalized PRI (PRI<sub>norm</sub>), PRI using the reflectance band at 515 nm [PRI<sub>(570-515)</sub>], red edge ratio ( $R_{700}/R_{670}$ ), Normalized Difference Vegetation Index (NDVI) and Transformed Chlorophyll Absorption in Reflectance Index normalized by the Optimized Soil Adjusted Vegetation Index (TCARI/OSAVI) for each of the species assessed in the experiment

	$\Psi_{s}$	gs	T <sub>c</sub>	NDVI	R <sub>700</sub> / R <sub>670</sub>	TCARI/ OSAVI	PRI	PRI <sub>(570-515)</sub>	PRInorm
Almond									
Signal	2.04	4.38	1.06	1.12	1.01	1.31	1.66	1.11	1.58
Noise	0.16	0.27	0.02	0.12	0.12	0.11	0.20	0.03	0.45
Sensitivity (signal/noise)	12.66	16.47	56.06	9.37	8.29	11.39	8.41	41.97	3.52
Lemon									
Signal	1.18	1.11	1.05	1.02	1.20	1.09	0.98	1.01	0.85
Noise	0.09	0.26	0.01	0.03	0.08	0.04	0.18	0.02	0.36
Sensitivity	13.44	4.36	124.10	35.40	14.81	25.43	5.48	65.12	2.38
Apricot									
Signal	1.76	3.90	1.13	1.13	0.94	1.51	0.91	1.07	0.79
Noise	0.02	0.19	0.05	0.07	0.07	0.07	0.07	0.04	0.28
Sensitivity	77.18	20.32	24.91	16.21	13.86	21.60	13.59	25.74	2.82
Apricot (11:00)									
Signal	2.11	2.21	1.08						
Noise	0.07	0.40	0.02						
Sensitivity	31.24	5.51	52.74						
Peach									
Signal	2.84	2.95	1.17	1.14	0.75	0.92	0.91	1.13	0.51
Noise	0.22	0.59	0.03	0.13	0.19	0.08	0.17	0.08	0.39
Sensitivity	12.76	5.02	40.47	9.08	3.96	9.08	5.22	14.30	1.30

Data obtained from measurements taken at 13:00 h. Bold numbers highlight the highest sensitivity value obtained for each species

due to the low CV observed in  $T_c$  within trees from the same treatment in the almond, lemon and peach orchards, and the low tree to tree variability observed in  $\Psi_s$  in apricot trees at 13:00 h compared to the other indicators (Table 4). Gonzalez-Dugo et al. (2013) determined  $T_c$  in trees of the same orchards used in this experiment at different times (09:00, 11:00 and 13:00 h). Their results (Gonzalez-Dugo et al. 2013) showed, in fact, that apricot was the species with the highest variability in  $T_c$  at any of the three measurement times and that CV within this crop was lowest at 11:00 h. Taking this information into account, the analysis was repeated for this species with  $\Psi_s$ ,  $g_s$  and  $T_c$  data obtained at 11:00 h (Table 4). For this time frame, results revealed that sensitivity of  $T_c$  was higher than that of  $\Psi_s$  and  $g_s$  (no multispectral data were available at 11:00 h).

Interestingly,  $PRI_{(570-515)}$  was found to be the second most sensitive indicator to the imposed water-stressed conditions in all the cases. The results obtained for  $PRI_{(570-515)}$  contrast with those obtained for  $PRI_{norm}$  which, along with  $g_s$ , was the indicator with the highest noise and least sensitivity. The multispectral indices NDVI, TCARI/OSAVI,  $R_{700}/R_{670}$  and  $PRI_{570}$  had, in general, intermediate values of sensitivity (Table 4).

# Relationships between the spectral indices and $\Psi_s$ , $g_s$ or CWSI within each orchard

Scaling up observations of plant water status from the leaf to the field, or even the farm level, requires validation of remote sensing data with ground-truth data. Here, data obtained from the multispectral imagery of selected trees within each orchard were compared with those of in-field measurements of  $\Psi_s$  and  $g_s$  as well as CWSI, which had been already validated for this particular farm by Gonzalez-Dugo et al. (2013).

Within the *Citrus* species, only indices sensitive to leaf chlorophyll content were significantly correlated with  $\Psi_s$ ,  $g_s$  or CWSI (Table 5). When  $R_{700}/R_{670}$  was plotted against  $g_{s_1}$  orange trees yielded a coefficient of determination (r<sup>2</sup>) of 0.62 (p < 0.05). In lemon trees, however,  $R_{700}/R_{670}$  and TCARI/OSAVI were significantly correlated with  $\Psi_s$  $(r^2 = 0.41; p < 0.05)$  and CWSI  $(r^2 = 0.64; p < 0.001)$ , respectively. The structural and photochemical indices were not correlated with  $\Psi_s$ ,  $g_s$  or CWSI in any of the Citrus species, which could be related to the small range of plant water status reached in these two orchards compared to the others. The evaluation of structure, xanthophyll and chlorophyll sensitive indices in orange trees to detect plant water stress has been also studied by Zarco-Tejada et al. (2012). These authors found that in spite of the wide range of  $\Psi_s$  reached in that study (from -0.5 to -2.0 MPa) compared to the work presented here, PRI, TCARI/ OSAVI and NDVI, although sensitive, were poorly correlated with  $\Psi_s$ . In that case, the PRI using the band 515 as a reference, proposed by Hernández-Clemente et al. (2011), and the structure-sensitive indicators RDVI, MTVI1 or TVI were shown as more robust water stress indicators for orange trees. Other recent studies (Romero-Trigueros et al. 2016), however, have reported significant changes in NDVI in grapefruit and mandarin trees as a consequence of short-term changes in  $\Psi_s$ .

Better correlations than in *Citrus* were obtained for the crops from the genus *Prunus* (Table 5). In almond trees,  $PRI_{(570-515)}$ , PRI, NDVI and TCARI/OSAVI indices were statistically correlated with  $\Psi_s$ ,  $g_s$  and CWSI with  $r^2$  ranging from 0.32 to 0.79. The  $\Psi_s$  was better correlated with  $PRI_{(570-515)}$  and PRI ( $r^2 = 0.53$  and 0.52, respectively; p < 0.001). The  $g_s$  yielded the highest correlation with TCARI/OSAVI ( $r^2 = 0.65$ ; p < 0.001) while CWSI yielded the highest correlation with PRI<sub>(570-515)</sub> and NDVI ( $r^2 = 0.79$  and 0.70, respectively; p < 0.001). Measurements of photosynthesis taken in almond trees were highly correlated with TCARI/OSAVI (Fig. 2). No correlations, however, were obtained

Table 5         Coefficients of determination obtained for the relationships between the Photochemical Reflec-
tance Index (PRI), normalized PRI (PRI <sub>norm</sub> ), PRI using the reflectance band at 515 nm [PRI <sub>(570-515)</sub> ], red
edge ratio (R700/R670), Normalized Difference Vegetation Index (NDVI) and Transformed Chlorophyll
Absorption in Reflectance Index normalized by the Optimized Soil Adjusted Vegetation Index (TCARI/
OSAVI) and the more classical water stress indicators: stem water potential ( $\Psi_s$ ), stomatal conductance ( $g_s$ )
and Crop Water Stress Index (CWSI) for each of the crops and cultivars studied

	PRI	PRI(570-515)	PRInorm	R700/R670	NDVI	TCARI/OSAVI
Almond						
$\Psi_{s}$	0.52***	0.53***	0.13	0.01	0.33**	0.39**
gs	0.52***	0.45**	0.14	0.01	0.32*	0.65***
CWSI	0.32**	0.79***	0.01	0.07	0.70***	0.45**
Almond 'Garrigues'						
$\Psi_{s}$	0.46*	0.66**	0.21	0.00	0.74***	0.37*
gs	0.75***	0.78***	0.49*	0.10	0.75***	0.72***
CWSI	0.55**	0.77***	0.31	0.03	0.89***	0.57**
Almond 'Ramillete'						
$\Psi_{\rm s}$	0.68**	0.72**	0.24	0.00	0.13	0.54*
gs	0.36	0.59**	0.07	0.01	0.51*	0.61**
CWSI	0.37	0.79***	0.07	0.05	0.65**	0.79***
Orange						
$\Psi_{\rm s}$	0.16	0.10	0.11	0.02	0.02	0.04
gs	0.25	0.20	0.32	0.62*	0.13	0.00
CWSI	0.08	0.07	0.10	0.06	0.00	0.07
Lemon						
$\Psi_{\rm s}$	0.02	0.02	0.10	0.22	0.02	0.41*
gs	0.15	0.38	0.14	0.23	0.00	0.08
CWSI	0.03	0.03	0.19	0.64***	0.14	0.18
Apricot						
$\Psi_{\rm s}$	0.00	0.16	0.03	0.04	0.32	0.88***
gs	0.10	0.06	0.23	0.21	0.42*	0.77***
CWSI	0.06	0.18	0.38	0.34	0.69**	0.82***
Peach						
$\Psi_{\rm s}$	0.21	0.58*	0.81***	0.88***	0.72**	0.29
gs	0.27	0.56*	0.84***	0.93***	0.71**	0.31
CWSI	0.17	0.61***	0.67***	0.62***	0.68***	0.20

\* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001

with the other chlorophyll sensitive indicator,  $R_{700}/R_{670}$ . When comparing results from the two almond cultivars used in the study (*cv*. 'Garrigues' and *cv*. 'Ramillete'), the relationships obtained between PRI<sub>(570–515)</sub> or TCARI/OSAVI and  $\Psi_s$ ,  $g_s$  and CWSI were similar. Nevertheless, PRI and NDVI relationships with  $\Psi_s$ ,  $g_s$  and CWSI were weaker for *cv*. 'Ramillete' (r<sup>2</sup> ranging from 0.13 to 0.68) than for *cv*. 'Garrigues' (r<sup>2</sup> ranging from 0.46 to 0.89).



Apricot trees were in the stage of postharvest when the experiment was performed. In contrast to that obtained for almond trees, no correlations were found between  $\Psi_s$ ,  $g_s$  or CWSI and any of the PRI formulations studied. NDVI was significantly correlated with  $g_s$  ( $r^2 = 0.42$ ; p < 0.05) and CWSI ( $r^2 = 0.69$ ; p < 0.01) but not with  $\Psi_s$ . Among the spectral indices, TCARI/OSAVI was the indicator that yielded the best correlations in apricot trees with  $\Psi_s$ ,  $g_s$  and CWSI ( $r^2$  ranging from 0.77 to 0.88; p < 0.001). TCARI/OSAVI is an index related to the chlorophyll content and not an index directly linked with the water status. Nevertheless, all the trees in this study were managed similarly before the beginning of the experiment. As aforementioned, apricot had already been harvested and water had not been applied for 24 days before the measurements date. In this case, and in order to increase the range of water status for this crop, irrigation was resumed in one single unit eight days prior to the measurements. Thus, the variation that is observed in the index might be related to the contrasted water status and the natural variability observed in the field.

As also observed in almond trees, TCARI/OSAVI was highly correlated with photosynthesis (Fig. 2). Differences observed in the relationship between TCARI/OSAVI and photosynthesis between apricot and almond trees may be related to differences in the phenological stage between these two crops.

Results obtained in the peach orchard were somewhat different from those reported for almond and apricot trees (Table 5). High correlations were obtained for all the spectral indices with the exception of PRI and TCARI/OSAVI. PRI<sub>(570–515)</sub>, PRI<sub>norm</sub>, R<sub>700</sub>/R<sub>670</sub> and NDVI yielded similar r<sup>2</sup> compared with CWSI (0.61 to 0.68; p < 0.001). PRI<sub>norm</sub> and R<sub>700</sub>/R<sub>670</sub>, however, were the spectral indices with the highest correlations with  $\Psi_s$  and  $g_s$  (r<sup>2</sup> from 0.81 to 0.93; p < 0.001).

The  $PRI_{norm}$  has been reported as a more reliable water stress indicator ( $r^2 = 0.82$  when compared against  $\Psi_s$ ) than PRI ( $r^2 = 0.53$ ) in grapevines (Zarco-Tejada et al. 2013). Here, that was the case for peach trees but the opposite was observed for almond trees (Table 5).

Most of the studies found in the literature assessing the use of spectral information to detect plant water stress have been conducted in one particular crop, generally crops of high economic interest such as olive (Berni et al. 2009; Suárez et al. 2008; Zarco-Tejada et al. 2004) and grapevines (Gago et al. 2015 and references therein). In the present study, multi-spectral indices were assessed in a farm composed of crops with different canopy architecture, nutrient status and phenology, which meant that a specific indicator performed better in some crops than in others. The use of a single multi-spectral indicator that

would be sensitive enough to variations in the plant water status of several crops would facilitate the assessment of irrigation needs at the farm scale.

## Relationships between the spectral indices and $\Psi_s$ , g<sub>s</sub> or CWSI at farm scale

Pooling data from all the fruit tree crops species together,  $PRI_{(570-515)}$ ,  $PRI_{norm}$ ,  $R_{700}/R_{670}$ and NDVI showed a statistically significant correlation with  $\Psi_s$  with  $r^2$  ranging between 0.15 and 0.65 (Table 6). Linear relationships were found for  $PRI_{(570-515)}$ ,  $PRI_{norm}$  and NDVI while  $R_{700}/R_{670}$  was best-fitted by a polynomial curve (Fig. 3). Among these indicators,  $PRI_{(570-515)}$  and NDVI were those which yielded the highest  $r^2$  (0.61 and 0.65, respectively; p < 0.001).

Although weak, statistically significant correlations were also found between  $PRI_{(570-515)}$ , PRI and NDVI with  $g_s$  (r<sup>2</sup> of 0.16–0.18; p < 0.01), and between  $PRI_{(570-515)}$ ,  $PRI_{norm}$ ,  $R_{700}/R_{670}$  and NDVI with CWSI (r<sup>2</sup> ranging from 0.19 to 0.33; p < 0.01) (Table 6).

Mean chlorophyll content within the farm ranged from 0.10  $\mu$ g mm<sup>-2</sup> in lemon trees to 0.41  $\mu$ g mm<sup>-2</sup> in almond trees (Table 7). Both, the TCARI/OSAVI and the R<sub>700</sub>/R<sub>670</sub> indices were statistically correlated with leaf chlorophyll concentration when data from all the species were pooled together (Fig. 4). The red edge ratio yielded higher r<sup>2</sup> (0.67; p < 0.001) than TCARI/OSAVI (0.40; p < 0.001).

The PRI<sub>norm</sub>, which was originally formulated to deal with contrasting canopy architecture and pigment content, did not perform as was expected. The non-linear relationship observed between water potential and the red edge ratio ( $R_{700}/R_{670}$ ) shown in Fig. 3 might be responsible for the low performance observed for this index. Results obtained for the NDVI were also surprising considering that water restrictions were just applied during eight days and considerable canopy structure effects were not expected. Instead of that, NDVI yielded the best correlations with  $\Psi_s$ . Recent studies, however, have also shown a response of NDVI to short-term changes in  $\Psi_s$  (Romero-Trigueros et al. 2016).

Overall, NDVI and the  $PRI_{(570-515)}$  were the indices able to retrieve water status when the five fruit tree crops species were assessed together. These results suggest that these indicators could be used to detect water-stressed areas in farms composed of a variety of crop species with contrasting phenology and canopy architecture.

**Table 6** Coefficients of determination obtained for the relationships between the Photochemical Reflectance Index (PRI), normalized PRI (PRI<sub>norm</sub>), PRI using the reflectance band at 515 nm [PRI<sub>(570-515)</sub>], red edge ratio ( $R_{700}/R_{670}$ ), Normalized Difference Vegetation Index (NDVI) and Transformed Chlorophyll Absorption in Reflectance Index normalized by the Optimized Soil Adjusted Vegetation Index (TCARI/ OSAVI) and the stem water potential ( $\Psi_s$ ), stomatal conductance ( $g_s$ ) or Crop Water Stress Index (CWSI) when data for all the species were pooled together

	PRI(570-515)	PRI	PRInorm	R700/R670	NDVI	TCARI/OSAVI
Ψs	0.61***	0.01	0.15**	0.42***	0.65***	0.06
gs	0.18**	0.18**	0.02	0.00	0.16**	0.05
CWSI	0.19***	0.06	0.23***	0.27***	0.33***	0.10

\* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001



**Fig. 3** Relationships between the stem water potential ( $\Psi_s$ ) and the Photochemical Reflectance Index (PRI), normalized PRI (PRI<sub>norm</sub>), PRI using the reflectance band at 515 nm [PRI<sub>(570–515)</sub>], red edge ratio (R<sub>700</sub>/R<sub>670</sub>), Normalized Difference Vegetation Index (NDVI) and Transformed Chlorophyll Absorption in Reflectance Index normalized by the Optimized Soil Adjusted Vegetation Index (TCARI/OSAVI) for all the fruit species together

## Conclusions

The work presented here evaluated the capability of a series of narrow-band indices sensitive to biophysical parameters of different nature to track water stress effects on a farm composed of five fruit tree crop species. Structure, chlorophyll and xanthophyll sensitive indices were all able to detect differences between irrigation treatments. Nevertheless, not all the indices were correlated with  $\Psi_s$  in all the fruit tree crop species studied. Weak or no correlations were found for the *Citrus* species. While best correlations in almond trees were obtained with the PRI and PRI<sub>(570–515)</sub> indices, TCARI/OSAVI was

	Chl ( $\mu g mm^{-2}$ )			
	Well watered	Deficit irrigated	C.V.	n
Almond	$0.35\pm0.04$	$0.28 \pm 0.02$	25.71	12
Almond cv. Ramillete	$0.28\pm0.05$	$0.27\pm0.03$	23.74	6
Almond cv. Garrigues	$0.41\pm0.02$	$0.29\pm0.04$	21.80	6
Orange	$0.12\pm0.04$	$0.17\pm0.04$	28.07	12
Lemon	$0.14\pm0.03$	$0.10\pm0.00$	35.61	8
Apricot	$0.35\pm0.04$	$0.29\pm0.03$	26.61	10

 Table 7
 Mean leaf chlorophyll content (Chl) in well-watered and deficit-irrigated trees of each tree crop.

 The coefficient of variation (C.V.) within each fruit tree crop studied and the number of measurements (n) taken in each case are also shown



**Fig. 4** Relationships between leaf chlorophyll content and: **a** the Transformed Chlorophyll Absorption in Reflectance Index normalized by the Optimized Soil Adjusted Vegetation Index (TCARI/OSAVI) and; **b** the red edge ratio ( $R_{700}/R_{670}$ ) for all the crop species together

the index that best correlated with  $\Psi_s$  in apricot trees. Both chlorophyll and xanthophyll sensitive indices, on the other hand, correlated well with  $\Psi_s$  in peach trees.

Comparison of the sensitivity analysis performed for direct and indirect physiological water stress indicators measured in the study, pointed out  $T_c$  and  $PRI_{(570-515)}$  as the first and second most sensitive indicators to the imposed water conditions in all the crops with the exception of apricot trees. In fact,  $PRI_{(570-515)}$ , along with NDVI, were the spectral indices that better tracked differences in plant water status ( $r^2 = 0.61$  and 0.65, with  $\Psi_s$ , respectively) between treatments when all the fruit tree crop species were assessed together. These results demonstrate the feasibility of using multi-spectral narrow-band indices (i.e. 10 nm bandwidths) acquired from miniature cameras on board a UAS to retrieve water status for a variety of crop species with contrasting phenology and canopy architecture.

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# References

- Albert, R. S., & Thornber, J. P. (1977). Water stress effect on the content and organization of chlorophyll in mesophyll and bundle sheath chloroplast. *Plant Physiology*, 59, 351–353.
- Anderson, K., & Gaston, K. (2013). Lightweight unmanned aerial vehicles will revolutionize spatial ecology. Frontiers in Ecology and the Environment, 11(3), 138–146.
- Bellvert, J., Zarco-Tejada, T. J., Gonzalez-Dugo, V., Girona, J., & Fereres, E. (2013). Scheduling vineyard irrigation based on mapping leaf water potential from airborne thermal imagery. *Precision Agriculture*. doi:10.3920/978-90-8686-778-3\_8.
- Bendig, J., Bolten, A., & Bareth, G. (2012). Introducing a low-cost mini-UAV for thermal- and multispectral-imaging. *The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences*, 39(B1), 345–349.
- Berni, J. A. J., Zarco-Tejada, P. J., Suárez, L., & Fereres, E. (2009). Thermal and narrowband multispectral remote sensing for vegetation monitoring from an unmanned aerial vehicle. *IEEE Transactions on Geoscience and Remote Sensing*, 47(3), 722–738.
- Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P. P., Osorio, M. L., et al. (2002). How plants cope with water stress in the field. *Photosynthesis and growth. Annals of Botany*, 89, 907–916.
- Field, C. B., Barros, V. R., Dokken, D. J., Mach, K. J., Mastrandrea, M. D., Bilir, T. E., et al. (2014). IPCC, 2014: Climate change 2014: Impacts, adaptation and vulnerability. Part A: Global and sectoral aspects. Contribution of working group II to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Gago, J., Douthe, C., Coopman, R. E., Gallego, P. P., Ribas-Carbo, M., Flexas, J., et al. (2015). UAVs challenge to assess water stress for sustainable agriculture. *Agricultural Water Management*, 153, 9–19.
- Gamon, J. A., Peñuelas, J., & Field, C. B. (1992). A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency. *Remote Sensing of Environment*, 41, 35–44.
- Goldhamer, D. A., & Fereres, E. (2001). Irrigation scheduling protocols using continuously recorded trunk diameter measurements. *Irrigation Science*, 20, 115–125.
- Goldhamer, D. A., Fereres, E., Mata, M., Girona, J., & Cohen, M. (1999). Sensitivity of continuous and discrete plant and soil water status monitoring in peach trees subjected to deficit irrigation. *Journal of* the American Society for Horticultural Science, 124(4), 437–444.
- Gonzalez-Dugo, V., Zarco-Tejada, P. J., Berni, J. A. J., Suárez, L., Goldhamer, D. A., & Fereres, E. (2012). Almond tree canopy temperature reveals intra-crown variability that is water-stress dependent. Agricultural and Forest Meteorology, 154–155, 156–165.
- Gonzalez-Dugo, V., Zarco-Tejada, P. J., Nicolás, E., Nortes, P. A., Alarcón, J. J., Intrigliolo, D. S., et al. (2013). Using high resolution UAV thermal imagery to assess the variability in the water status of five fruit tree species within a commercial orchard. *Precision Agriculture*, 14(6), 660–678.
- Haboudane, D., Miller, J. R., Tremblay, N., Zarco-Tejada, P. J., & Dextraze, L. (2002). Integration of hyperspectral vegetation indices for prediction of crop chlorophyll content for application to precision agriculture. *Remote Sensing of Environment*, 81(2–3), 416–426.
- Hernández-Clemente, R., Navarro-Cerrillo, R., Suárez, L., Morales, F., & Zarco-Tejada, P. J. (2011). Assessing structural effects on PRI for stress detection in conifer forests. *Remote Sensing of Environment*, 115(9), 2360–2375.
- Inskeep, W. P., & Bloom, P. R. (1985). Extinction coefficients of chlorophyll a and b in N, N-dimethylformamide and 80% acetone. *Plant Physiology*, 60, 606–608.
- Intrigliolo, D. S., & Castel, J. R. (2006). Performance of various water stress indicators for prediction of fruit size response to deficit irrigation. Agricultural Water Management, 83, 173–180.
- Moriana, A., & Fereres, E. (2002). Plant indicators for scheduling irrigation of young olive trees. *Irrigation Science*, 21, 83–90.
- Mulla, D. (2013). Twenty five years of remote sensing in precision agriculture: Key advances and remaining knowledge gaps. *Biosystems Engineering*, 114, 358–371.
- Peguero-Pina, J. J., Morales, F., Flexas, J., Gil-Pelegrin, E., & Moya, I. (2008). Photochemistry, remotely sensed physiological reXectance index and de-epoxidation state of the xanthophyll cycle in *Quercus* coccifera under intense drought. *Oecologia*, 156, 1–11.
- Pérez-Sarmiento, F., Alcobendas, R., Mounzer, O., Alarcón, J., & Nicolás, E. (2010). Effects of regulated deficit irrigation on physiology and fruit quality in apricot trees. *Spanish Journal of Agricultural Research*, 8(S2), S86–S94.

- Romero-Trigueros, C., Nortes, P. A., Alarcón, J. J., Hunink, J. E., Parra, M., Contreras, S., et al. (2016). Effects of saline reclaimed waters and deficit irrigation on Citrus physiology assessed by UAV remote sensing. Agricultural Water Management. doi:10.1016/j.agwat.2016.09.014.
- Rouse, J. W., Haas, R. H., Schell, J. A., & Deering, D. W. (1974). Monitoring vegetation systems in the Great Plains with ERTS. In: S. C. Fraden, E. P. Marcanti & M. A. Becker (Eds.), *Third ERTS-1* symposium. NASA SP-351 (pp. 309–317). Washington D.C., USA: NASA.
- Suárez, L., Zarco-Tejada, P. J., González-Dugo, V., Berni, J. A. J., Sagardoy, R., & Morales, F. (2010). Detecting water stress effects on fruit quality in orchards with time-series PRI airborne imagery. *Remote Sensing of Environment*, 114, 286–298.
- Suárez, L., Zarco-Tejada, P. J., Sepulcre-Canto, G., Perez-Priego, O., Miller, J. R., Jimenez-Muñoz, J. E., et al. (2008). Assessing canopy PRI for water stress detection with diurnal airborne imagery. *Remote Sensing of Environment*, 112, 560–575.
- Taghvaeian, S., & Neale, C. M. U. (2011). Water balance of irrigated areas: A remote sensing approach. *Hydrological Processes*, 25, 4132–4141.
- Zarco-Tejada, P. J., Gonzalez-Dugo, V., & Berni, J. A. J. (2012). Fluorescence, temperature and narrowband indices acquired from a UAV platform for water stress detection using a micro-hyperspectral imager and a thermal camera. *Remote Sensing of Environment*, 117, 322–337.
- Zarco-Tejada, P. J., Gonzalez-Dugo, V., Williams, L. E., Suárez, L., Berni, J. A. J., Goldhamer, D., et al. (2013). A PRI-based water stress index combining structural and chlorophyll effects: Assessment using diurnal narrow-band airborne imagery and the CWSI thermal index. *Remote Sensing of Environment*, 138, 38–50.
- Zarco-Tejada, P. J., Miller, J. R., Morales, A., Berjón, A., & Agüera, J. (2004). Hyperspectral indices and model simulation for chlorophyll estimation in open-canopy tree crops. *Remote Sensing of Environment*, 90, 463–476.