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Monitoring water stress and fruit quality in an orange orchard under regulated deficit irrigation using narrow-band structural and physiological remote sensing indices

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ABSTRACT

This paper deals with the monitoring of water status and the assessment of the effect of stress on citrus fruit quality using structural and physiological remote sensing indices. Four flights were conducted over a citrus orchard in 2009 using an unmanned aerial vehicle (UAV) carrying a multispectral camera with six narrow spectral bands in the visible and near infrared. Physiological indices such as the *Photochemical Reflectance Index* (PRI_{570}), a new structurally robust PRI formulation that uses the 515 nm as the reference band (PRI_{515}), and a chlorophyll ratio (R_{700}/R_{670}) were compared against the *Normalized Difference Vegetation Index* (NDVI), *Renormalized Difference Vegetation Index* (RDVI) and *Modified Triangular Vegetation Index* (MTVI) canopy structural indices for their performance in tracking water status and the effects of sustained water stress on fruit quality at harvest. The irrigation setup in the commercial orchard was compared against a treatment scheduled to satisfy full requirements (based on estimated crop evapotranspiration) using two regulated deficit irrigation (RDI) strategies. The water status of the trees throughout the experiment was monitored with frequent field measurements of stem water potential (Ψ_x), while titratable acidity (TA) and total soluble solids (TSS) were measured at harvest on selected trees from each irrigation treatment. The high spatial resolution of the multispectral imagery (30 cm pixel size) enabled identification of pure tree crown components, extracting the tree reflectance from shaded, sunlit and aggregated pixels. The physiological and structural indices were then calculated from each tree at the following levels: (i) pure sunlit tree crown, (ii) entire crown, aggregating the within-crown shadows, and (iii) simulating a lower resolution pixel, including tree crown, sunlit and shaded soil pixels. The resulting analysis demonstrated that both PRI formulations were able to track water status, except when water stress altered canopy structure. In such cases, PRI_{570} was more affected than PRI_{515} by the structural changes caused by sustained water stress throughout the season. Both PRI formulations were proven to serve as pre-visual water stress indicators linked to fruit quality TSS and TA parameters ($r^2 = 0.69$ for PRI_{515} vs TSS; $r^2 = 0.58$ vs TA). In contrast, the chlorophyll (R_{700}/R_{670}) and structural indices (NDVI, RDVI, MTVI) showed poor relationships with fruit quality and water status levels ($r^2 = 0.04$ for NDVI vs TSS; $r^2 = 0.19$ vs TA). The two PRI formulations showed strong relationships with the field-measured fruit quality parameters in September, the beginning of stage III, which appeared to be the period most sensitive to water stress and the most critical for assessing fruit quality in citrus. Both PRI_{515} and PRI_{570} showed similar performance for the two scales assessed (sunlit crown and entire crown), demonstrating that within-crown component separation is not needed in citrus tree crowns where the shaded vegetation component is small. However, the simulation conducted through spatial resampling on tree + soil aggregated pixels revealed that the physiological indices were highly affected by soil reflectance and between-tree shadows, showing that for TSS vs PRI_{515} the relationship dropped from $r^2 = 0.69$ to $r^2 = 0.38$ when aggregating soil + crown components. This work confirms a previous study that demonstrated the link between PRI_{570}, water stress, and fruit quality, while also making progress in assessing the new PRI formulation (PRI_{515}), the within-crown shadow effects on the physiological indices, and the need for high resolution imagery to target individual tree crowns for the purpose of evaluating the effects of water stress on fruit quality in citrus.

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1. Introduction

Water scarcity is a major constraint to irrigated agriculture in many areas of the world. In the Mediterranean region, rainfall is scarce and irregularly distributed throughout the year, and climate change models predict even more arid conditions in the coming decades (Bates et al., 2008). Although citrus are able to withstand considerable drought (Salter and Goode, 1967), they are cultivated widely in semi-arid climates where water resources are becoming the major limiting factor for irrigated agriculture. Therefore, irrigation scheduling needs to be more precise, and this requires the development of methodologies to optimize irrigation water productivity. 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). This practice was originally proposed to control vegetative vigor in high-density orchards, to reduce production costs and to improve fruit quality. However, it also saves irrigation water, with the concomitant benefits of reduced drainage losses (Fereres and Soriano, 2007).

The fruit quality of citrus is affected by water stress due to changes in juice properties, such as increases in sugar concentrations and acidity (Chartzoulakis et al., 1999). In some cases, the water stress reduces fruit quality (Mougheith et al., 1977). When water deficits are imposed, as in RDI, yield and fruit size are not generally affected (Girona, 2002), whereas some quality parameters such as total soluble solids and titratable acidity increase (Crisosto et al., 1994; Girona et al., 2003; Mills et al., 1994). Responses to RDI are variable, depending on the timing and severity of the water deficits (Girona et al., 2003; Marsal and Girona, 1997), which in turn depends on orchard management. Although there is increasing knowledge in the application of deficit irrigation strategies and in the relationships between water stress and fruit quality (Baeza et al., 2007; Ginestar and Castel, 1996; Gonzalez-Altozano and Castel, 1999; Myers, 1988), the introduction of these strategies into commercial orchards is being hindered by a lack of reliable indicators of water status that account for the heterogeneity naturally found in orchards. As fruit quality is of paramount importance for harvest strategy and growers' revenues, indicators to depict fruit quality are also desirable. Remote sensing of fruit quality has been attempted by different means, such as determining the vigor or total leaf area in vineyards (Johnson et al., 2001, 2003; Lamb et al., 2004) or relating quality parameters in water-stressed mandarin trees to spectral changes in the red and green channels (Kriston-Vizi et al., 2008). High spatial resolution airborne imagery was also implemented to detect relationships of olive fruit size, weight, and oil content against crown temperature (Sepulcre-Cantó et al., 2007), while the visible part of the spectrum was also used in fruit quality detection of orange, peach and nectarine (Suárez et al., 2010).

Canopy thermal infrared radiation has long been implemented in remote sensing as a method of pre-visual detection of water stress (Cohen et al., 2005; Idso et al., 1981, 1978; Jackson et al., 1977, 1981; Jackson and Pinter Jr., 1981; Leinonen and Jones, 2004; Möller et al., 2007; Sepulcre-Cantó et al., 2006, 2007; Wanjura et al., 2004). Thermal imagery acquired over vegetation is sensitive to canopy instantaneous transpiration rate at the time of image acquisition because temperature rises due to the reduction in evaporative cooling under stress conditions. On the other hand, hyperspectral imagery in the visible and near-infrared (NIR) has been proven capable of accurate estimation of crop physiological status through optical indices related to leaf biochemistry and canopy structure (Haboudane et al., 2004; Zarco-Tejada et al., 2005). However, most of the optical indices track the effects of long-term water stress on plants and thus are not useful in early detection and monitoring of water stress strategies such as RDI. Nevertheless, the visible spectral region has been suggested for pre-visual water stress detection based on indices that use bands located at the specific wavelengths where photosynthetic pigments are affected by stress conditions. The most promising index to track photosynthetic condition is the Photochemical Reflectance Index (PRI) (Gamon et al., 1992), which has been proposed to assess vegetation water stress based on xanthophyll pigment composition changes (Peguero-Pina et al., 2008; Suárez et al., 2008, 2009, 2010; Thenot et al., 2002).

The PRI index is related to the de-epoxidation state of the xanthophyll cycle (Gamon et al., 1990, 1992; Peñuelas et al., 1995) and was initially developed as a method to remotely assess photosynthetic efficiency using narrow-band reflectance (Gamon et al., 1992; Peñuelas et al., 1995). However, the attributes of PRI are more complex since it can also serve as an index of relative chlorophyll:carotenoid levels. Over longer time scales (weeks–months), changes in pigment content and ratios due to leaf development, aging or chronic stress have been reported to play a significant role, along with xanthophyll pigment epoxidation, in the PRI signal (Gamon et al., 2001; Peñuelas et al., 1997; Sims and Gamon, 2002; Stylinski et al., 2002). Together, these responses to the de-epoxidation state of the xanthophyll cycle and to chlorophyll/carotenoid ratios ensure that PRI provides an effective measure of relative photosynthetic rates across a wide range of conditions, species and functional types (Filella et al., 1996; Gamon et al., 1992; Gamon and Qiu, 1999; Peñuelas et al., 1995; Stylinski et al., 2002).

Consequently, PRI can be more descriptive of plant physiology and photosynthetic functioning, and thus the effects of water deficits on yield and vigor, than thermal indicators. In line with this hypothesis, recent work by Suárez et al. (2010) demonstrated that high-resolution PRI time series calculated from pure sunlit vegetation pixels exhibited a good relationship with fruit quality, whereas crown airborne temperature acquired over the same trees did not yield comparable results and did not correlate well with fruit quality. Thus, PRI from pure sunlit tree crowns may be a better estimator of fruit quality than other established water stress indicators that are related directly to transpiration, such as crown temperature, since fruit quality is more closely tied to photosynthesis and carbon metabolism.

However, there are some serious drawbacks that prevent the wide use of PRI at crown or field scale. PRI is affected by a series of parameters such as variability of leaf pigment concentration (due to reflectance overlapping of chlorophyll a + b and carotenoid with xanthophyll pigment de-epoxidation), amount of leaf biomass, canopy structure, viewing-illumination geometry, background reflectance and sensor spectral responses (Barton and North, 2001; Grace et al., 2007; Suárez et al., 2008). The reference band in the formulation of each index is supposed to compensate for a number of unwanted effects. However, in PRI formulation the mechanistic basis for wavelength selection has been fully explored only at the leaf scale (Gamon et al., 1993), and is poorly supported at canopy and larger scales, where a variety of alternate wavebands have been used, often based on statistical correlations (Gamon et al., 1992; Inoue et al., 2008). It is not entirely clear if the best wavelengths for assessing sensitivity to xanthophyll pigments at the leaf scale (531 and 570 nm) are necessarily the best wavelengths at progressively larger scales (canopy level), where multiple scattering due to the canopy structure and background alter the sensitivity of the bands related to the xanthophyll cycle feature. In particular, previous work conducted in forestry used airborne imagery and radiative transfer modeling methods to examine five formulations of PRI (based on 531 nm as a xanthophyll-sensitive spectral band, using five different reference wavelengths) for detecting stress levels, while minimizing the effects of crown architecture and structure. The study proposed a more robust version of PRI, less sensitive to canopy structural and biochemical parameters, that uses the 515 nm band as the reference band instead of the 570 nm band (Hernández-Clemente et al., 2011). In such work, the methods conducted by Hernández-Clemente were adapted to the bandset of the AHS airborne imager, using the 512 nm band in the modified PRI formulation.

The present study examines the potential of high resolution optical imagery in detecting water status and fruit quality of a citrus orchard. The traditional (PRI_{570}) and the proposed modified formulation (PRI_{515}) are calculated along with structural and chlorophyll indices to compare and evaluate their performance. Moreover, high spatial resolution airborne imagery was used to assess the effects of canopy structure and within-crown and between crown shadowing on the spectral indices used to monitor water stress and fruit quality.

2. Materials and methods

2.1. Study site

The study was carried out in 2009 in a 0.6-ha plot of orange trees in a commercial orchard located near La Campana, Seville (37.8°N, 5.4°W). The orange trees (*Citrus sinensis* L. cv. Powell) were planted in 1997 at a spacing of 7 m × 4 m (358 trees/ha) in deep, alluvial soil with a loam to sandy-loam texture. The climate in the area is Mediterranean, characterized by warm, dry summers and cool, wet winters, with an average annual rainfall over 550 mm and ET_o (calculated with the Penman–Monteith equation) (Allen et al., 1998) over 1300 mm.

Four treatments were carried out in a randomized block design, and each treatment was replicated four times. Individual plots comprised 12 trees (three rows with four trees each), thus 16 plots were monitored (Fig. 1). Trees were irrigated with an automated drip system, with 10 pressure compensation emitters (1.6 l/h) per tree. Irrigation management in the orchard (scheduled according to commercial practices, referred to as *Field* treatment) was compared with three other treatments: one fully irrigated and two deficit irrigation treatments. The deficit treatment was applied during period II (initial fruit enlargement) as this is the period during which trees are least sensitive to water stress. Two levels of water deficit were applied, a moderate and severe stress. After the stress period was completed, the water status was recovered followed by full irrigation. In summary, the irrigation schedule conducted in the experiment comprised the following treatments and dates: (i) ET, where irrigation was scheduled to satisfy full requirements (based on estimated crop evapotranspiration), (ii) RDI-1, with an application dose similar to ET, except for the deficit irrigation period (between 11-June and 22-September), where only 37% ET was applied, and (iii) RDI-2, similar to RDI-1, but with water application during the deficit irrigation period equal to 50% ET. Crop evapotranspiration was estimated using the Food and Agriculture Organization (FAO) method (Allen et al., 1998) from a crop coefficient (adapted to local conditions) and the reference evapotranspiration obtained from an automatic weather station located 10 km from the study site.

2.2. Field measurements

Xylem water potential measurements (Ψ_x) is the standard method to assess the water status (Shackel et al., 1997). In such protocol, leaves located near the trunk are enclosed in bags or covered by aluminum foil for 30 min to stop transpiration and to match the leaf water potential to the xylem. Nevertheless, previous results (Goldhamer and Fereres, 2001) in almonds indicated that, in dense orchards, shaded leaves located near the trunk do not need to be covered, as transpiration is already very low, and display the same water potential as the xylem. Tests made in this citrus orchard supported these findings (data not shown). Therefore, two shaded leaves near the trunk were sampled on one tree per plot at local midday using a pressure chamber to measure the water potential (PWSC Model 3000, Soil Moisture Equipment Corp., California, USA). Measurements were conducted once every 10 days, with more intense monitoring during the high water stress period (June–September 2009). A water stress integral was calculated as the integral of stem water potential, following Myers (1988). This calculation enabled the analysis of the effect of the cumulative water stress over the whole period.

To avoid border effects, the two central trees per plot were harvested individually on 8-April-2010. All fruits from each tree were weighted, and a subsample of 70 fruits was weighted and their diameter measured. Eight fruits per tree were selected randomly for a physiochemical and organoleptic characterization of TSS and TA, used to calculate the ratio (TSS/TA). Fruit juice from the eight fruits was extracted and filtered together to get a single sample per tree. Titratable acidity in the fruit juice was measured with a pH meter (pH-Burette 24, Crison, Spain) using the procedure described by Garner et al. (2008). Soluble solids content (°Brix) was determined in a small sample of fruit juice using a hand-held refractometer (Atago, ATC-1E, Japan).

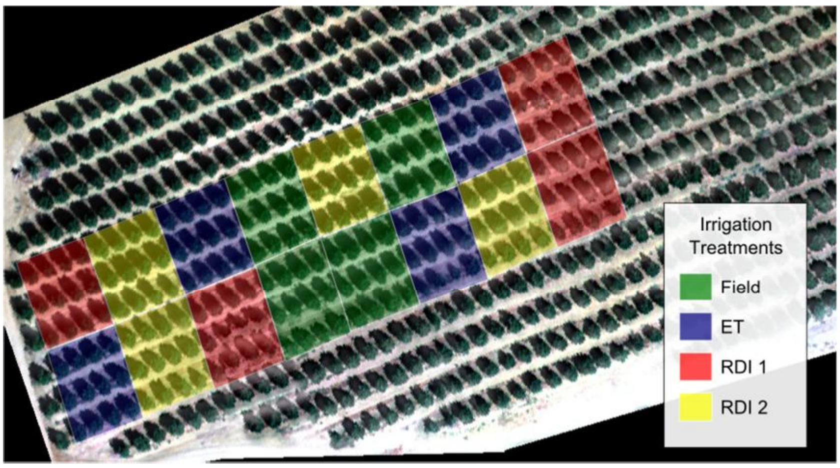

Fig. 1. Overview of the orange orchard used for the experiment in the present study. The plots with the four different irrigation treatments are represented by the indicated colors. Field refers to irrigation management based on commercial practices, ET is the treatment designed to satisfy full plant requirements, and RDI 1 and 2 are the two different deficit irrigation treatments.

2.3. Airborne imagery

Four flights were conducted above the study site during the growing period of 2009, using an unmanned aerial vehicle (UAV) (Berni et al., 2009) at 250 m above ground level with a payload consisting of a 6-band multispectral camera (MCA-6, Tetracam, Inc., California, USA). The first image was acquired on 19-June, before any signs of water stress. The second one was acquired on 5-August, when water stress was already obvious in the RDI treatments. The last two flights were conducted in September, one at the beginning of the month (4-September), the time of the highest levels of stress, and the last flight at the end of the month (25-September), 3 days after full irrigation was resumed for RDI treatments.

The multispectral camera installed on board the UAV platform consisted of six image sensors with a 25 mm diameter and bandpass filters of 10 nm FWHM (Andover Corporation, NH, USA). The image resolution was 1280 × 1024 pixels with a 10-bit radiometric resolution and optical focal length of 8.5 mm, yielding a ground-based spatial resolution of 30 cm at 250 m altitude. The bands were centered at 515, 530, 570, 670, 700 and 800 nm except for the 19-June flight, when the 515 nm band was replaced by 550 nm. Geometric calibration of airborne data was conducted as explained in Berni et al. (2009). The camera was radiometrically calibrated using coefficients derived from measurements made with a uniform calibration body (integrating sphere, CSTM-USS-2000C Uniform Source System, LabSphere, NH, USA) at four different levels of illumination and six different integration times. Radiance values were later converted to reflectance values using total incoming irradiance simulated with sunphotometer (Microtops, Solar Light Inc.) data taken in the field at the time of imagery acquisition.

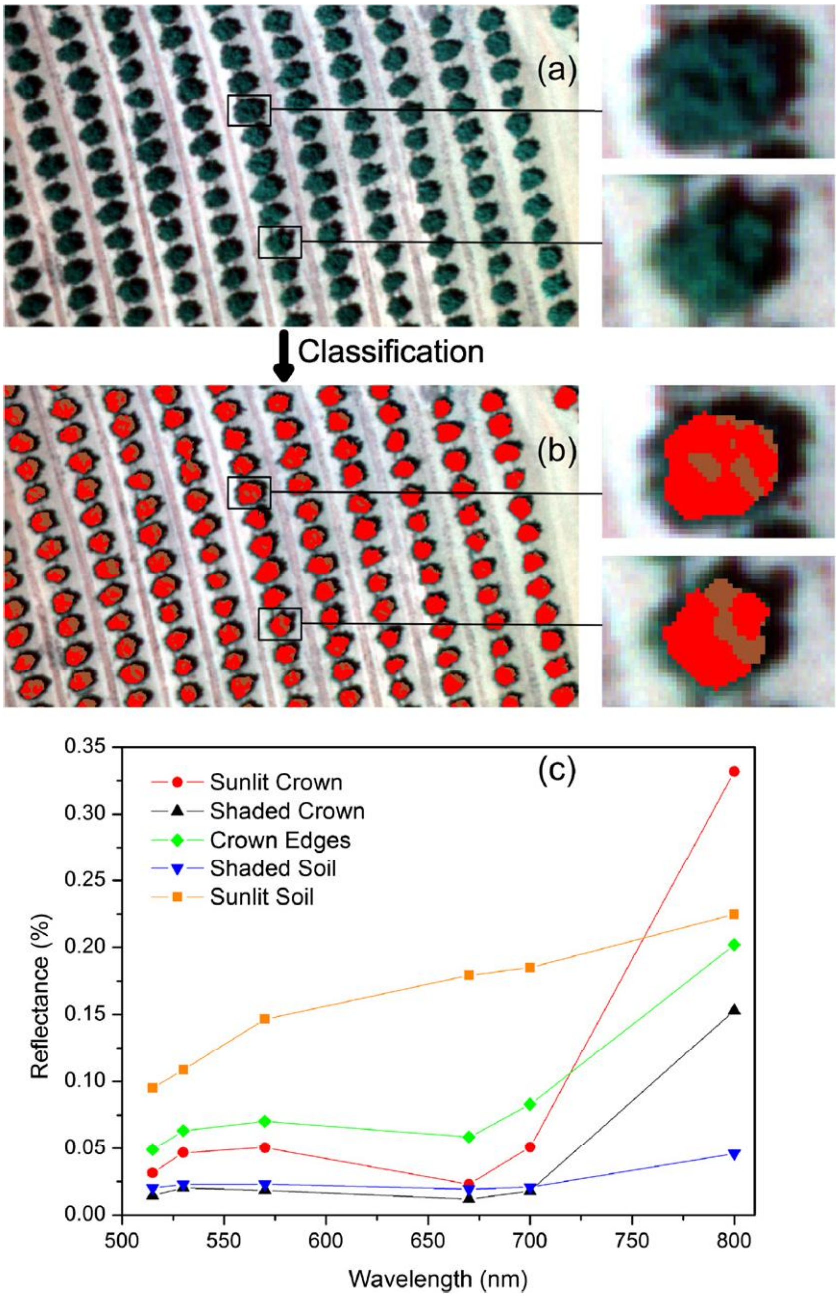

Fig. 2. Example of the classification of the 6-band airborne imagery. A part of an image over the study area is presented in true color before classification (a) and after classification (b). The sunlit crown class is displayed in red, while the shaded crown class is displayed in brown. The spectral signatures of the scene components used for the classification are also included (c).

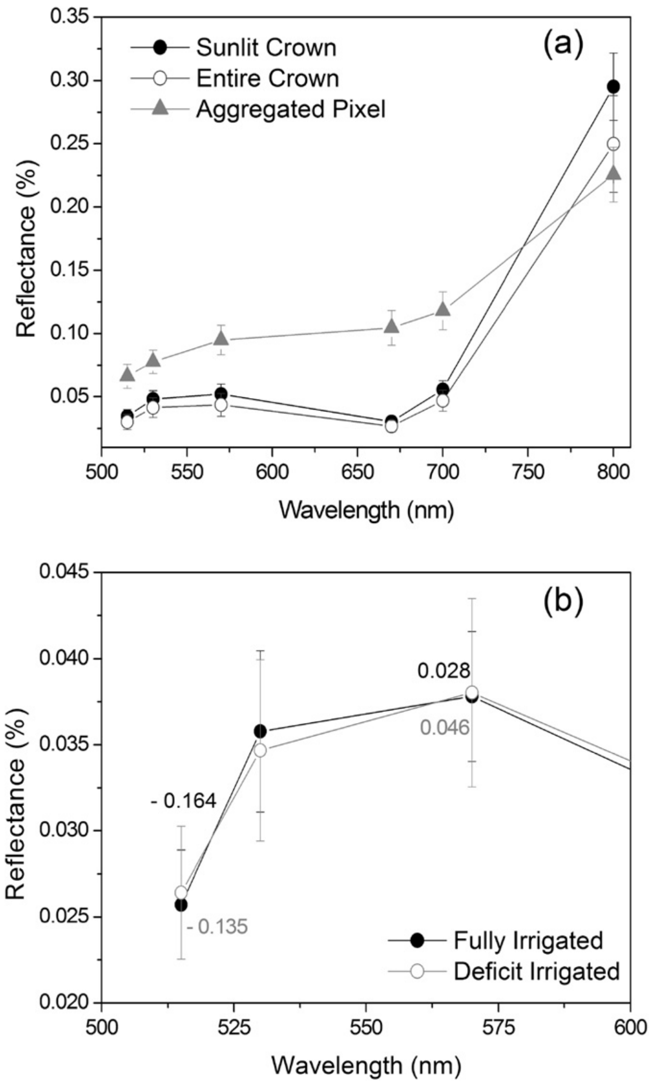

Fig. 3. Reflectance spectra of the 6-band camera in the three different spatial scales (sunlit crown, entire crown and aggregated pixel) extracted from the fully irrigated trees of 4-September (a). Comparison of the reflectance of the three bands used in the two PRI formulations for the fully irrigated (ET) and deficit irrigated trees (RDI 1) at crown level (b). The PRI_{515} and PRI_{570} values are displayed next to the corresponding reference band (515 nm and 570 nm) for each spectrum.

To avoid any subjective effects when processing the imagery for feature extraction, a fully automated processing method for extracting crown reflectance was used. Every object in the images was classified in five groups using their spectral signatures (Fig. 2), enabling identification of the following classes: (i) pure sunlit crown, (ii) shaded crown, (iii) sunlit soil, (iv) shaded soil, and (v) crown edges. The *crown edge* class was used

Table 2
Stem water potential (Ψ_x) measurements (mean values and standard deviation in parenthesis, MPa) for each irrigation treatment in each flight conducted during the growing period. The measurements of 22-September are also presented for comparison purposes.

	Field	ET	RDI-1	RDI-2
19-June	−0.98 (±0.28)	−0.99 (±0.20)	−0.92 (±0.25)	−0.90 (±0.26)
5-August	−0.92 (±0.19)	−0.92 (±0.16)	−2.19 (±0.31)	−1.06 (±0.15)
4-September	−1.15 (±0.09)	−1.08 (±0.11)	−2.49 (±0.25)	−1.18 (±0.05)
22-September	−0.96 (±0.09)	−0.94 (±0.03)	−2.90 (±0.65)	−1.12 (±0.34)
25-September	−0.88 (±0.04)	−1.00 (±0.19)	−2.80 (±1.15)	−1.20 (±0.12)

to avoid the pixels of the crown that are mixed with soil. The spectral information was then extracted from each individual tree (32 trees per image) in three spatial scales: (i) sunlit crown pixels (*Sunlit*); (ii) entire crown scale (*Crown*), and (iii) aggregated reflectance, including the full tree crown reflectance and the soil and shadows around each crown (*Aggr*). For this purpose, regions of interest (9 m × 4.5 m) comprising each individual crown and the adjacent soil pixels were created, and the spectral information was extracted (aggregated pixel scale). The reflectance of each band in the three different spatial scales used in this study is presented in Fig. 3a, showing the spectral differences for the 515 nm, 530 nm and 570 nm bands as a function of water stress level (Fig. 3b).

The methodology for the calculation of vegetation indices for all scales used in this study was *object based,* not *pixel based*, meaning that each object (e.g., one tree crown) was treated as an independent reflectance signature used to calculate all indices. For this purpose, the reflectance for each object (*sunlit crown; entire crown; aggregated pixels*) was calculated, and the spectral indices were computed. The indices used in this study are presented in Table 1. The first three indices (NDVI, RDVI and MTVI) are primarily related to leaf biomass and canopy structure (Broge and Leblanc, 2001; Haboudane et al., 2004; Roujean and Breon, 1995; Zarco-Tejada et al., 2005). The R_{700}/R_{670} red-edge index (Kim et al., 1994) is used in the present study as sensitive to chlorophyll content. The PRI_{570} index was proposed as a pre-visual water stress indicator (Peguero-Pina et al., 2008; Suárez et al., 2008, 2009; Thenot et al., 2002) and demonstrated to be sensitive to fruit quality changes caused by water stress (Suárez et al., 2010). The PRI_{515} index was recently proposed by Hernández-Clemente et al. (2011) as more robust to canopy structural changes.

The spectral indices and field measurements were initially calculated at the tree level, with all airborne and field data further processed at the plot level. The two central trees of each plot were used to compute the plot-level indices, avoiding plot edge effects, and to ensure agreement with the tree-level field measurements. As for Ψ_x, the time series for each index was integrated according to Myers (1988).

Table 1
Overview of the structural and physiological indices used in this study, their formulation and reference.

Index	Formulation	Reference
NDVI (Normalized Difference Vegetation Index)	$\frac{R_{800}-R_{670}}{R_{800}+R_{670}}$	Rouse et al. (1974)
RDVI (Renormalized Difference Vegetation Index)	$\frac{R_{800}-R_{670}}{\sqrt{R_{800}+R_{670}}}$	Roujean and Breon (1995)
MTVI (Modified Triangular Vegetation Index)	$0.5[120(R_{800}-R_{570})-200(R_{670}-R_{570})]$	Based on Broge and Leblanc (2001) and Haboudane et al. (2004)
R_{700}/R_{670} (Chlorophyll-related Index)	$\frac{R_{700}}{R_{670}}$	Kim et al. (1994)
PRI_{570} (Photochemical Reflectance Index)	$\frac{R_{570}-R_{531}}{R_{570}+R_{531}}$	Gamon et al. (1992)
PRI_{515} (Modified Photochemical Reflectance Index)	$\frac{R_{515}-R_{531}}{R_{515}+R_{531}}$	Hernández-Clemente et al. (2011)

3. Results

3.1. Water status detection

Of the different irrigation levels applied, only the *RDI-1* treatment exhibited extreme water stress levels, while the deficit treatment (*RDI-2*) and commercial management (*Field*) closely followed the water potential levels of the fully irrigated plots (*ET*) (Table 2). The water potential measurements conducted on *RDI-1* trees showed a decline from the very beginning of the deficit irrigation period, reaching a minimum on 22-September (Ψ_x = −2.90 MPa). The first flight was conducted at the start of the deficit irrigation period, when all treatments showed similar behavior in terms of water potential (Ψ_x ranging between −0.9 and −0.99 MPa for all irrigation treatments) (Table 2). The second and the third flights were conducted during the deficit irrigation period. On the third flight, RDI-1 plots showed water potential levels doubling the values measured for ET trees (Ψ_x = −2.49 MPa for RDI-1; Ψ_x = −1.08 MPa for ET). The last flight was conducted on 25-September, 3 days after the end of the deficit irrigation period, and thus with all plots fully irrigated for those 3 days (Ψ_x = −2.80 MPa for RDI-1; Ψ_x = −1.00 MPa for ET). The higher Ψ_x standard deviation for the RDI-1 plots found in the last flight (25-September) was due not only to the transitional phase of water stress recovery, but also to a technical error that occurred during this 3-day period and prevented two of the four RDI-1 plots from receiving full irrigation. The mean value of Ψ_x for the plots that were re-watered was over −1.8 MPa, in line with expected recovery, while the two plots that remained under dry conditions showed values below −3.5 MPa. This technical error did not have any effects on the results of this work other than finding a higher standard deviation for Ψ_x on the RDI-1 plots on 25-September.

The multispectral imagery was used to extract the proposed vegetation indices and was assessed against field-measured Ψ_x data for each date (Fig. 4). It can be observed that the NDVI, RDVI and MTVI structural indices showed significant relationships with Ψ_x measurements only at the end of September, when the sustained water deficit levels affected tree crown structure (Fig. 4a–c). On the other hand, the two PRI formulations exhibited significant relationships with water status at the beginning of September (PRI_{515} vs Ψ_x, r^2 = 0.49) (Fig. 4e and f), also showing sensitivity to the *re-watering* phase. Of the two PRI formulations, PRI_{515} showed better relationships against Ψ_x than PRI_{570} at the end of September (Fig. 4e and f). None of the indices exhibited relationships with Ψ_x in the June or August flights, when stress levels were still lower than at the end of the deficit irrigation period.

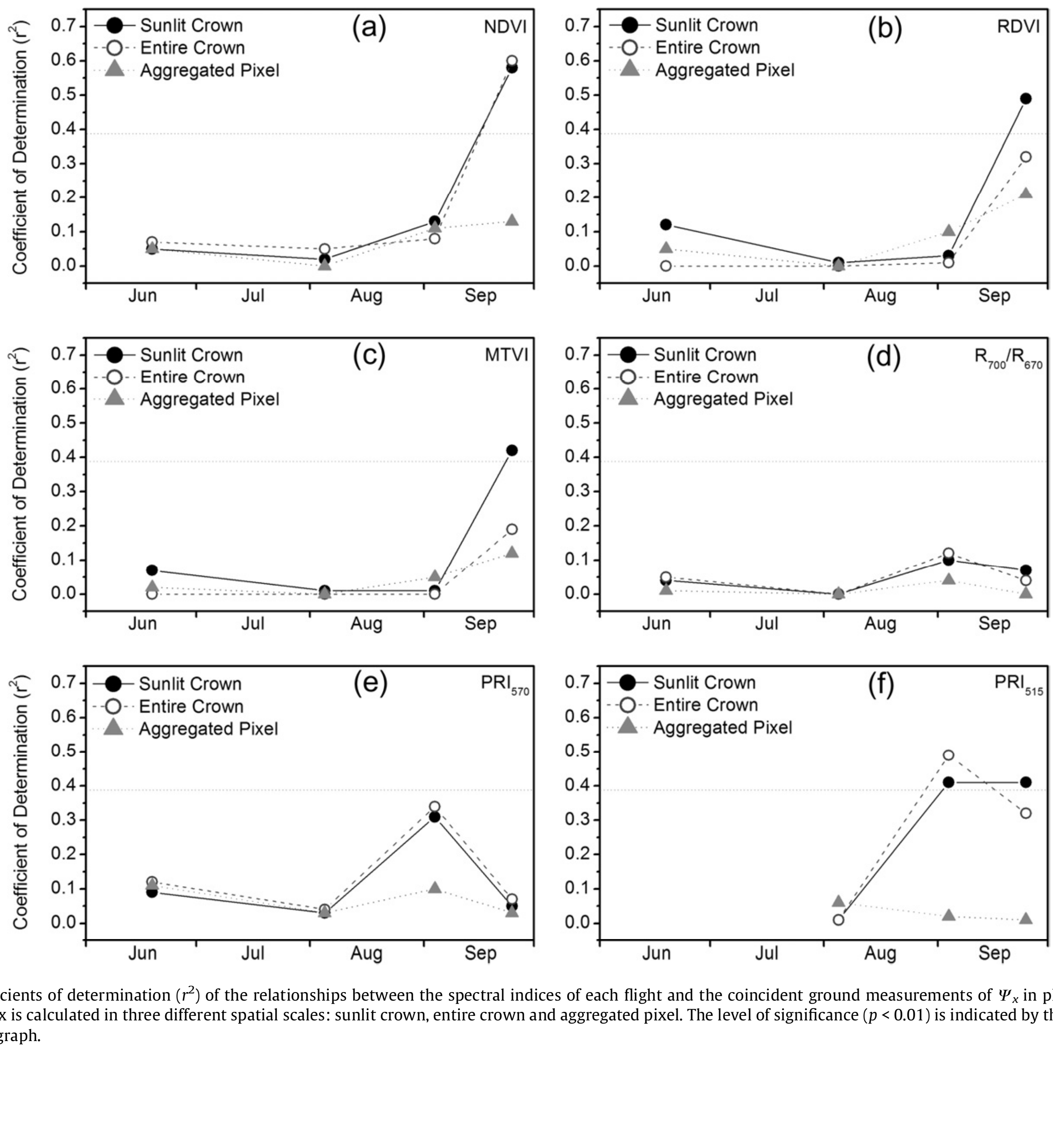

Fig. 4. Coefficients of determination (r^2) of the relationships between the spectral indices of each flight and the coincident ground measurements of Ψ_x in plot level. Each spectral index is calculated in three different spatial scales: sunlit crown, entire crown and aggregated pixel. The level of significance (p < 0.01) is indicated by the gray dotted line in each graph.

A detailed assessment of the relationships obtained between the structural indices (NDVI, RDVI and MTVI) and Ψ_x for the last flight conducted (Fig. 5) reveals that the RDI-1 trees presented extremely low values for all three indices. The two RDI-1 outlayers shown in Fig. 5 did not receive irrigation between 4-September and 25-September (date of flight), reaching the most severe water stress as measured in the field ($\Psi_x = -4.0$ MPa). Under such extreme conditions, the canopy structure and very likely the pigment content of the trees in that RDI-1 plot were affected by the long-term water stress intensified in the last days of the deficit irrigation trial. Such extreme conditions were detected by the structural indices shown in the Fig. 5.

On 25-September, RDI treatments were in a transitional phase to full irrigation that started on 22-September. PRI values were better correlated with Ψ_x measured on 22-September (Fig. 6b and d) rather than with the actual Ψ_x (Fig. 6a and c). Comparing both PRI formulations at this stage, when water stress had already altered canopy structure, PRI_{515} yielded a better correlation than PRI_{570}. The tendency of the relationship with Ψ_x for the trees that showed extremely low values of NDVI, RDVI and MTVI was maintained for PRI_{515} (Fig. 6d), but not for PRI_{570} (Fig. 6b).

3.2. Fruit quality detection

Fruit quality was strongly related to Ψ_x over the course of the experiment except for the June data (Table 3), yielding $r^2 = 0.54$ (Ψ_x vs TSS), $r^2 = 0.81$ (Ψ_x vs TA), and $r^2 = 0.71$ (Ψ_x vs TSS/TA) for the integral of the time series throughout June–October. A similar level of relationships was found for single dates, obtaining the best results for the August and September flight dates (Table 3).

The spectral indices extracted from each flight and assessed for their relationship to the fruit quality parameters TSS, TA and TSS/TA yielded different results for structural indices (Table 4) as compared to physiological indices (Table 5). Each flight date was examined separately, along with the integral of the two September flights and all four time-series flights. It was observed that NDVI, the most commonly used structure-related greenness index, did not show a significant relationship with fruit quality in any of the flights, even when the September or all time-series data were integrated (Table 4). Only RDVI and MTVI indices showed some degree of relationship for TA and TSS/TA at the beginning of the experiment (June flight date) (Table 4 and Fig. 7), although these relationships are absent when the stress differences among treatments started.

Analysis of the sensitivity of the physiological indices against TSS, TA and TSS/TA yielded more consistent and significant relationships (Table 5) than with structural indices. Statistically significant relationships between both PRI indices and fruit quality parameters were found in September, while none were detected for the June (beginning of the deficit irrigation period) and August flight dates. PRI indices calculated from the separate September flights along with the time-series integral yielded $r^2 = 0.69$ (PRI_{515} vs TSS), $r^2 = 0.58$ (PRI_{515} vs TA), and $r^2 = 0.38$ (PRI_{515} vs TSS/TA). These relationships are consistent for the two September flights and are in agreement with results obtained by Suárez et al. (2010) for PRI_{570}. The integral of the four flight dates did not yield such strong relationships with fruit quality parameters as the ones obtained for the September flights. The September PRI flight data for PRI_{570} (Fig. 8a and c) and PRI_{515} (Fig. 8b and d) showed similar sensitivity to TSS for both PRI formulations (Fig. 8a and b). A better relationship was found for the PRI_{515} when compare to TA (Fig. 8c and d).

The chlorophyll-related ratio R_{700}/R_{670} index yielded relationships with fruit quality parameters of $r^2 = 0.41$ (R_{700}/R_{670} vs TSS), $r^2 = 0.17$ (R_{700}/R_{670} vs TA), and $r^2 = 0.07$ (R_{700}/R_{670} vs TSS/TA) for the September flight dates, showing weaker relationships than those obtained when using PRI formulations.

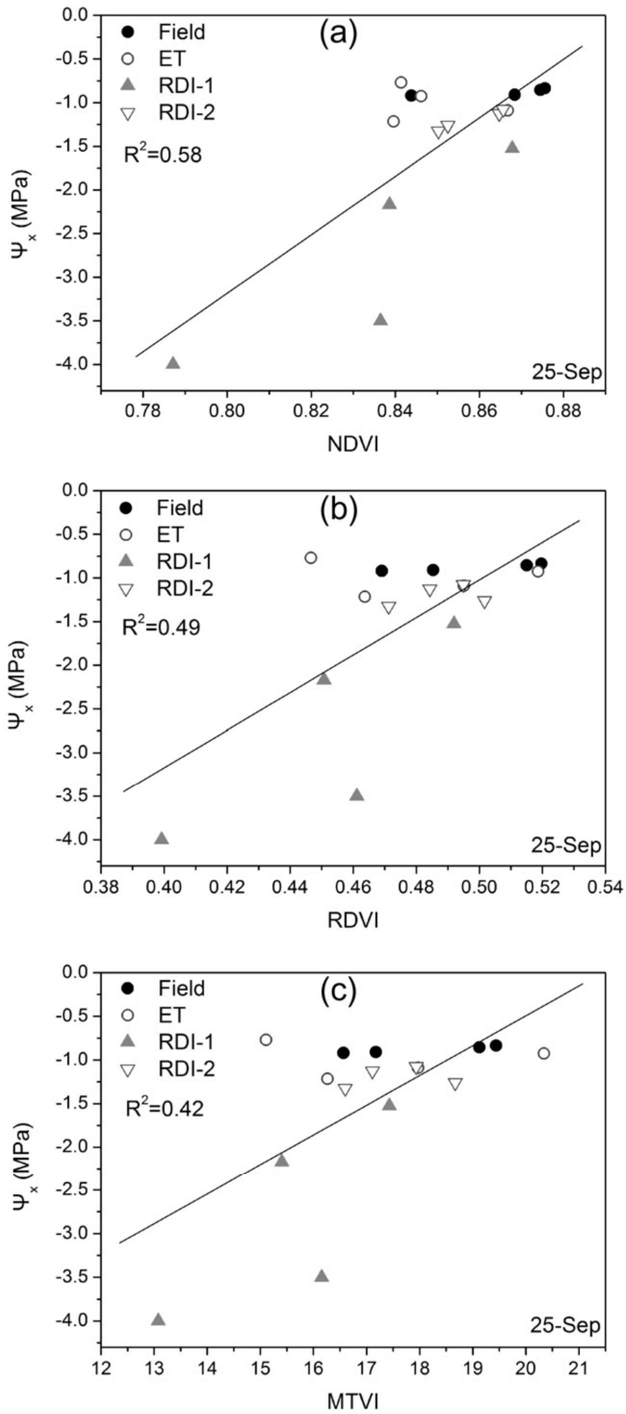

Fig. 5. Relationships obtained at the plot level between Ψ_x measurements conducted on 25-September and (a) NDVI, (b) RDVI, (c) MTVI calculated from sunlit crowns.

3.3. Spatial scale analysis

Comparing the performance of the structural and physiological indices for the two crown scales (sunlit crown vs entire crown), as opposed to Ψ_x, showed that NDVI was less affected by within-crown shadows (Fig. 4a) than RDVI (Fig. 4b) and MTVI (Fig. 4c), which seemed to be more affected by crown heterogeneity. Nevertheless,

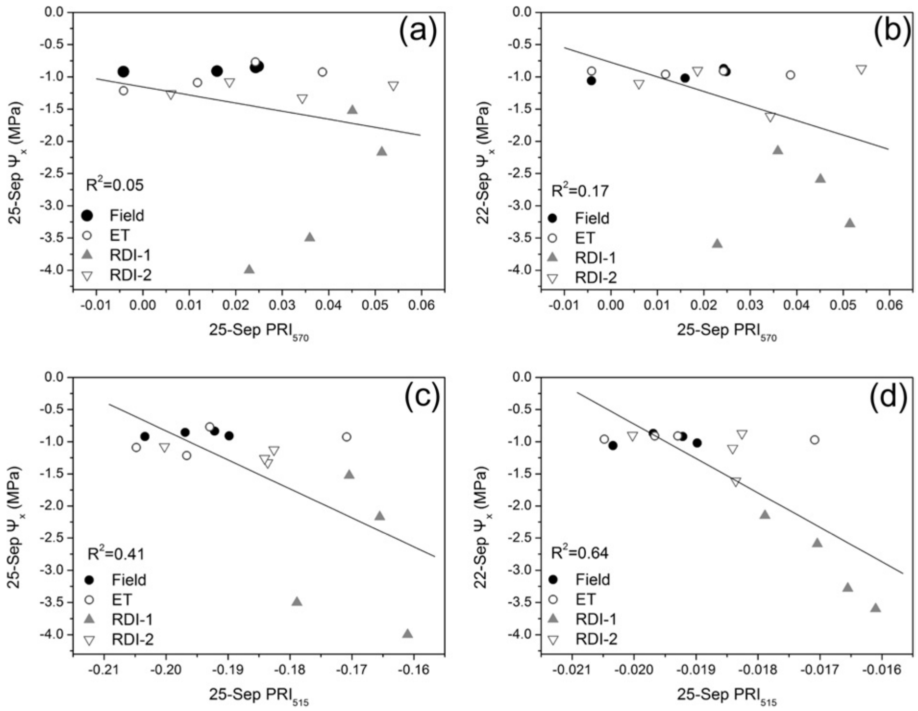

Fig. 6. Relationships between 25-September Ψ_x measurements and (a) PRI_{570}, (c) PRI_{515} of the same date calculated in sunlit crown scale. Relationships between 22-September Ψ_x measurements and (b) PRI_{570}, (d) PRI_{515} of the 25-September flight calculated in sunlit crown scale. All relationships are presented in plot level.

Table 3
Coefficients of determination (r^2) obtained for the relationships between fruit quality parameters (TSS, TA) and stem water potential (Ψ_x) measurements at each flight date and for the June–October integral. The significant relationships ($p < 0.01$) are displayed in bold.

	TSS (°Brix)	TA (%)	TSS/TA
19-June	0.02	0.01	0.00
5-August	0.38	**0.69**	**0.63**
4-September	**0.65**	**0.84**	**0.69**
25-September	0.31	**0.48**	**0.45**
September	**0.49**	**0.79**	**0.70**
June–October	**0.54**	**0.81**	**0.71**

the pixel purity level (pure crown vs entire crown, including crown shadows) did not improve the relationships between the structural indices and fruit quality parameters (Table 4). The R_{700}/R_{670}, PRI_{570} and PRI_{515} physiological indices showed nearly similar performance at the two crown scales, demonstrating that for citrus crowns the separation of within-crown sunlit and shaded pixels was not critical (Fig. 4d–f and Table 5).

However, the simulation conducted to assess the effects of the pixel size on the performance of the indices clearly showed the importance of the resolution to the indices' sensitivity to water potential and fruit quality parameters. When resolution exceeds crown size, performance is not preserved for any of the spectral indices (Fig. 4, Tables 4 and 5). For the aggregated pixel scale, all relationships decline to non-significant levels (i.e., $r^2 = 0.69$ for PRI_{515} vs TSS for pure crown spectra; $r^2 = 0.38$ for PRI_{515} vs TSS for aggregated crown + soil pixels; $r^2 = 0.58$ for PRI_{515} vs TA for pure crown spectra; $r^2 = 0.12$ for PRI_{515} vs TA for aggregated crown + soil pixels) (Fig. 9). Therefore, soil and tree shadows greatly affected all indices used, inhibiting the feasibility of detecting water status and fruit quality.

The maps created by using PRI_{515} calculated at the crown level (Fig. 10) and interpolated using single crown data (Fig. 11) demon-

Table 4
Coefficients of determination (r^2) of the relationships between fruit quality parameters and the three structure-related greenness indices in three different spatial scales (sunlit crown, entire crown and aggregated pixel). The relationships of every different flight in plot level are presented separately, along with the integrals of September and all measurements. The significant relationships ($p < 0.01$) are displayed in bold.

	NDVI			RDVI			MTVI		
	Sunlit	Crown	Aggr.	Sunlit	Crown	Aggr.	Sunlit	Crown	Aggr.
19-June									
TSS	0.29	0.32	0.04	0.38	0.00	0.00	0.15	0.03	0.01
TA	0.08	0.14	0.00	**0.55**	0.15	0.00	**0.44**	0.06	0.01
TSS/TA	0.02	0.07	0.01	**0.55**	0.28	0.01	**0.54**	0.17	0.00
5-August									
TSS	0.05	0.02	0.02	0.17	0.14	0.07	0.19	0.14	0.09
TA	0.02	0.03	0.00	0.07	0.03	0.01	0.08	0.03	0.02
TSS/TA	0.00	0.01	0.00	0.06	0.01	0.00	0.06	0.02	0.00
4-September									
TSS	0.15	0.02	0.28	0.01	0.04	0.17	0.03	0.06	0.07
TA	0.11	0.04	0.05	0.00	0.00	0.03	0.01	0.01	0.01
TSS/TA	0.09	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25-September									
TSS	0.04	0.04	0.05	0.03	0.05	0.16	0.03	0.04	0.18
TA	0.19	0.21	0.05	0.22	0.19	0.13	0.21	0.13	0.12
TSS/TA	0.24	0.27	0.03	0.29	0.20	0.06	0.27	0.13	0.06
September integral									
TSS	0.13	0.05	0.15	0.01	0.00	0.20	0.00	0.01	0.17
TA	0.26	0.20	0.06	0.12	0.05	0.09	0.07	0.02	0.07
TSS/TA	0.30	0.25	0.02	0.17	0.07	0.03	0.11	0.03	0.02
June–September integral									
TSS	0.03	0.00	0.01	0.15	0.11	0.00	0.16	0.10	0.00
TA	0.08	0.04	0.01	0.04	0.02	0.00	0.07	0.03	0.00
TSS/TA	0.09	0.06	0.00	0.03	0.02	0.00	0.05	0.02	0.00

Table 5
Coefficients of determination (r^2) of the relationships between fruit quality parameters and the chlorophyll index, along with the two PRI formulations in three different spatial scales (sunlit crown, entire crown and aggregated pixel). The relationships of every different flight in plot level are presented separately, along with the integrals of September and all measurements. The significant relationships ($p < 0.01$) are displayed in bold.

	R_{700}/R_{670}			PRI_{570}			PRI_{515}		
	Sunlit	Crown	Aggr.	Sunlit	Crown	Aggr.	Sunlit	Crown	Aggr.
19-June									
TSS	0.21	0.24	0.12	0.07	0.10	0.15			
TA	0.02	0.03	0.01	0.00	0.01	0.04			
TSS/TA	0.00	0.00	0.00	0.01	0.00	0.01			
5-August									
TSS	0.07	0.06	0.00	0.01	0.01	0.01	0.03	0.03	0.01
TA	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01
TSS/TA	0.00	0.00	0.00	0.01	0.01	0.02	0.01	0.01	0.04
4-September									
TSS	0.35	0.35	0.21	**0.57**	**0.58**	0.34	**0.63**	**0.64**	0.31
TA	0.06	0.07	0.01	0.33	0.36	0.10	**0.51**	**0.57**	0.07
TSS/TA	0.01	0.01	0.00	0.20	0.22	0.04	0.35	**0.39**	0.02
25-September									
TSS	0.36	0.33	0.31	**0.51**	**0.45**	0.31	**0.52**	**0.59**	0.36
TA	0.22	0.18	0.11	0.24	0.25	0.17	**0.45**	**0.45**	0.17
TSS/TA	0.13	0.10	0.04	0.12	0.15	0.09	0.31	0.28	0.08
September integral									
TSS	**0.44**	**0.41**	0.31	**0.63**	**0.62**	**0.41**	**0.66**	**0.69**	0.38
TA	0.18	0.17	0.06	0.34	0.36	0.18	**0.55**	**0.58**	0.12
TSS/TA	0.08	0.07	0.01	0.18	0.22	0.09	0.38	0.38	0.04
June–September integral									
TSS	0.34	0.32	0.21	0.26	0.27	0.24	**0.42**	**0.40**	0.11
TA	0.07	0.08	0.04	0.08	0.08	0.05	0.22	0.21	0.00
TSS/TA	0.01	0.01	0.00	0.01	0.01	0.00	0.10	0.09	0.01

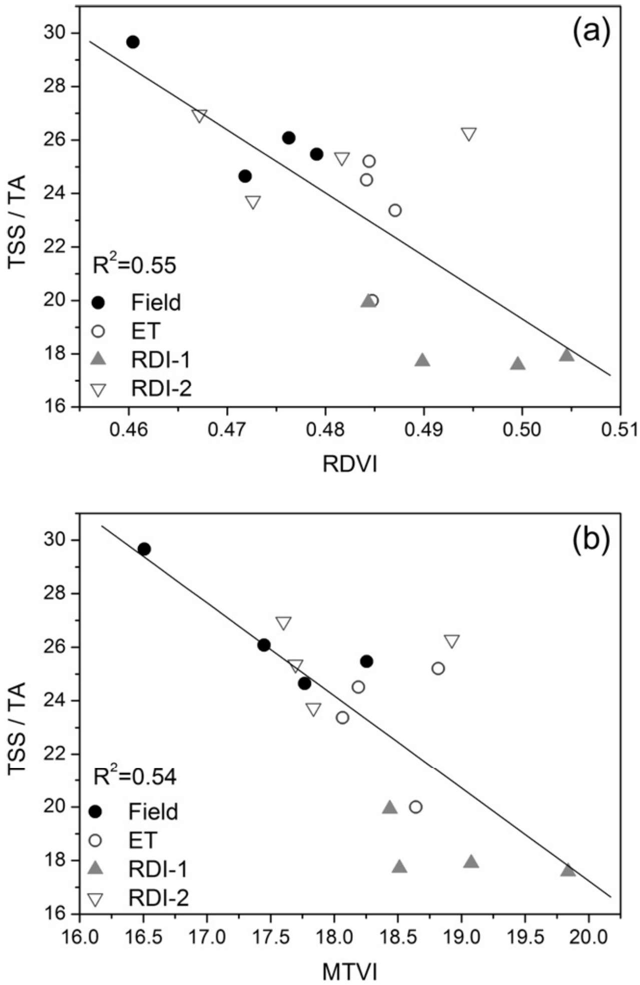

Fig. 7. Relationships in plot level between the fruit quality ratio TSS/TA and (a) RDVI, (b) MTVI in sunlit crown scale (19-June flight).

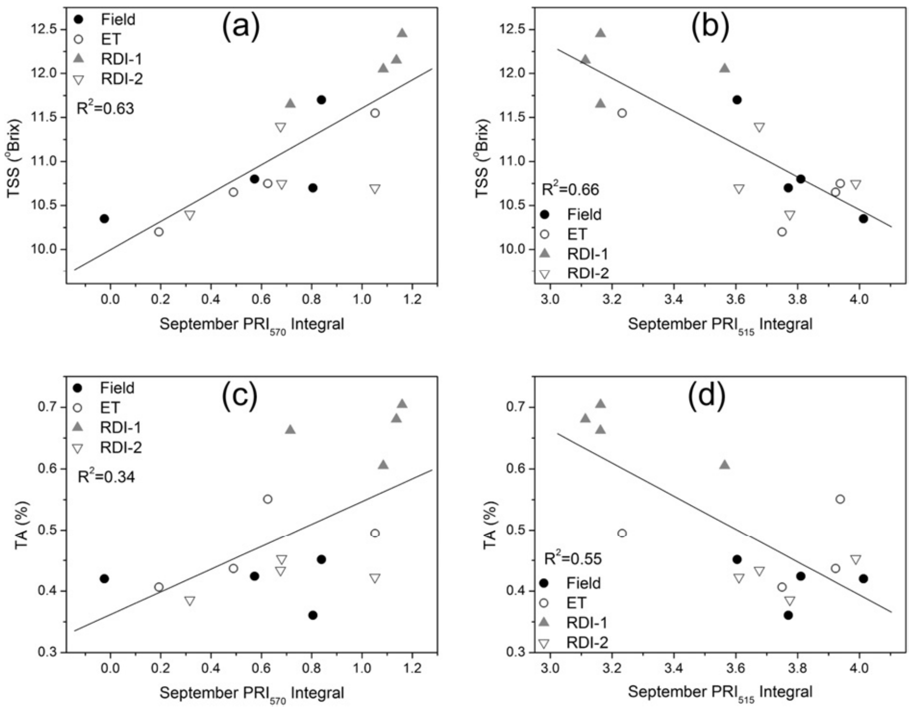

Fig. 8. Relationships between the fruit quality parameters TSS and TA and (i) the September PRI_{570} integral (a and c), (ii) the September PRI_{515} integral (b and d). The indices are calculated in sunlit crown scale, and the relationships are presented in plot level.

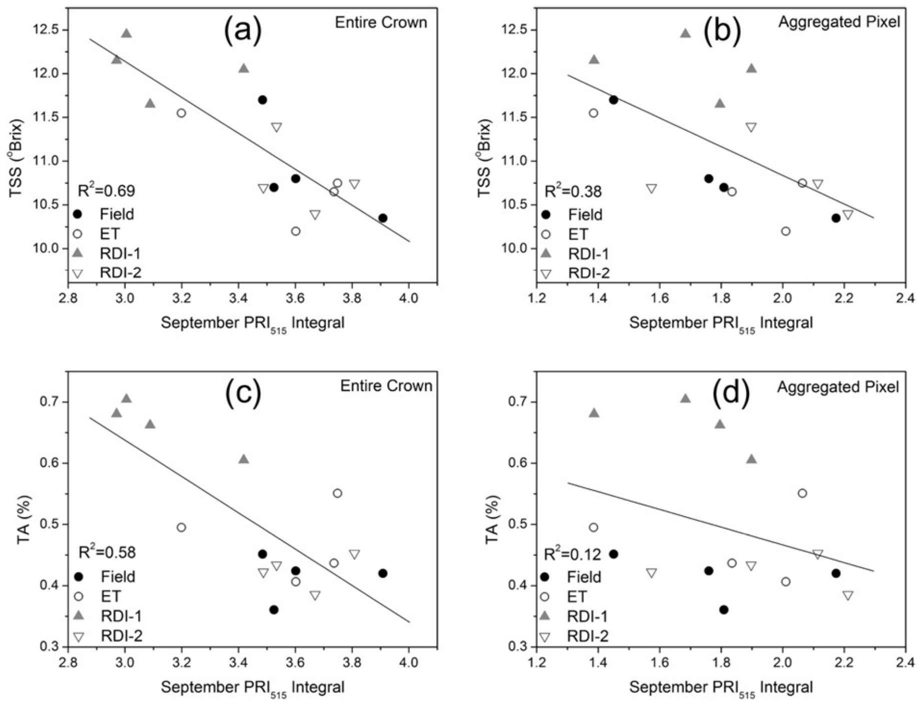

Fig. 9. Relationships in plot level between the September PRI_{515} integral and the fruit quality parameters TSS and TA for tree crowns (a and c) and aggregated pixels, including shaded and sunlit soil pixels (b and d).

strate that PRI_{515} spatial variability mimics the TSS fruit quality measurements conducted for each block under different irrigation levels. The figures depicting the spatial variation over the course of the experiment show that PRI_{515} data calculated from pure crown reflectance can be used to map spatial variability of fruit quality in citrus trees.

4. Discussion

High spatial and spectral resolution airborne imagery was used in this study to assess optical indices for monitoring water stress and fruit quality. Deficit irrigation strategies have demonstrated to be useful in conserving water, although crop water status must be accurately monitored to avoid yield or quality losses (Fereres and Soriano, 2007). The operational use of deficit irrigation strategies for correct field management requires the development of remote sensing indicators to track water stress levels and fruit quality in large areas. PRI has been proposed in recent literature as sensitive for detecting pre-visual stress (Peguero-Pina et al., 2008; Suárez et al., 2008, 2009; Thenot et al., 2002), but also sensitive to fruit quality (Suárez et al., 2010). In particular, PRI was demonstrated to be more appropriate in fruit quality detection than other established water stress indicators that are related directly to transpiration, such as crown temperature (Suárez et al., 2010), particularly when re-watering occurs after a long period under deficit levels. In such cases, transpiration levels may be recovered (i.e., temperature may not indicate water stress differences), but the photosynthetic apparatus may still be affected after sustained stress conditions (i.e., photosynthetic pigments may be damaged long after re-watering starts), hence supporting the need for photosynthetic-related indicators to account for the effect of water status on fruit quality.

In particular, the recovery of Ψ_x after watering can only last a few days, as long as hydraulic damage or embolism have not occurred (Brodribb et al., 2010; Galle et al., 2007). On the other hand, leaf photochemical activity does not display the same behavior after water deficits. The leaf photochemical mechanisms of plants subjected to severe water stress tend to recover more gradually during the re-watering phase. However, recovery rates are highly species-dependent (Galmes et al., 2007), while the severity and the duration of drought also seem to play a major role (Flexas et al., 2006). In general, after severe water stress, plants recover only 40–60% of the maximum photosynthesis rate during the day after re-watering, and recovery continues during the following few days. Nevertheless, maximum photosynthesis rates are not always recovered (Flexas et al., 2004; Galle et al., 2007; Kirschbaum, 1987; Souza et al., 2004). Moreover, while daily synthesis and removal of large amounts of zeaxanthin occurs rapidly in well-watered plants, the xanthophyll cycle becomes increasingly slower when photosynthesis rates have decreased due to long-term water stress (Demmig-Adams, 1990).

4.1. Assessing the robustness of a new PRI formulation, and its sensitivity to crown structure

Nevertheless, PRI cannot be readily applied at the canopy level due to several effects on the index. This index is known to be affected by a series of leaf- and canopy-level parameters (Barton and North, 2001; Grace et al., 2007; Suárez et al., 2008). A recent study conducted in forestry (Hernández-Clemente et al., 2011) introduced a more robust version of PRI, less sensitive to canopy structural and biochemical parameters. The proposed modified version of PRI (PRI_{515}), which uses the 515 nm spectral band as the reference band, is applied in the present study. Canopy structure variation within the orchard, as well as illumination and viewing angle geometry effects are minimized when using the new PRI formulation.

The results of the present study confirm that the photochemical reflectance indices based on the xanthophyll de-epoxidation signal on 531 nm can serve as pre-visual indicators of water stress. Previous work has shown that PRI_{570} can be used to assess pre-visual water stress at leaf level (Thenot et al., 2002; Winkel et al.,

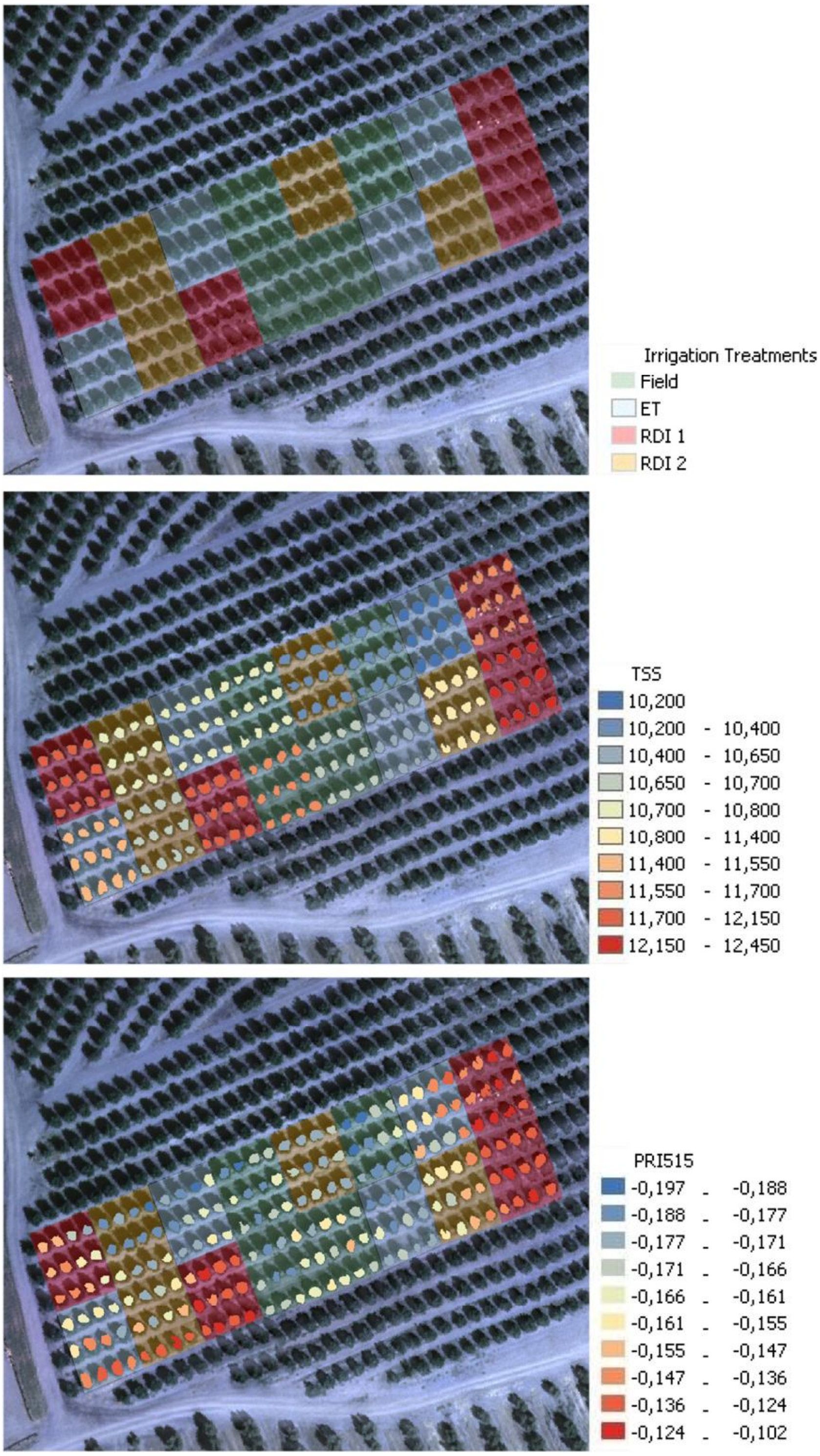

Fig. 10. Map of the spatial variability of tree crown PRI_{515} and TSS over the experiment.

2002), at canopy level (Dobrowski et al., 2005; Evain et al., 2004; Peguero-Pina et al., 2008; Sun et al., 2008) and using airborne imaging sensors (Suárez et al., 2008, 2009). However, a more thorough study assessing structural indices, the new PRI formulation and the effects of plot-level aggregation was lacking. In the present study, the relationships between PRI (both formulations) and Ψ_x are shown from the very beginning of September, a time when none of the structural or chlorophyll indices showed any sensitivity to water stress levels. Of the two PRI formulations used, PRI_{570} proved to be seriously affected by canopy structure alterations detected by the three structural indices at the end of the deficit irrigation experiment (Figs. 4e, 6a and b).

4.2. Remote sensing of water stress and fruit quality using PRI

Results obtained for the detection of fruit quality changes as a function of water stress levels using PRI showed improved and consistent results (Table 5 and Fig. 8) when compared against

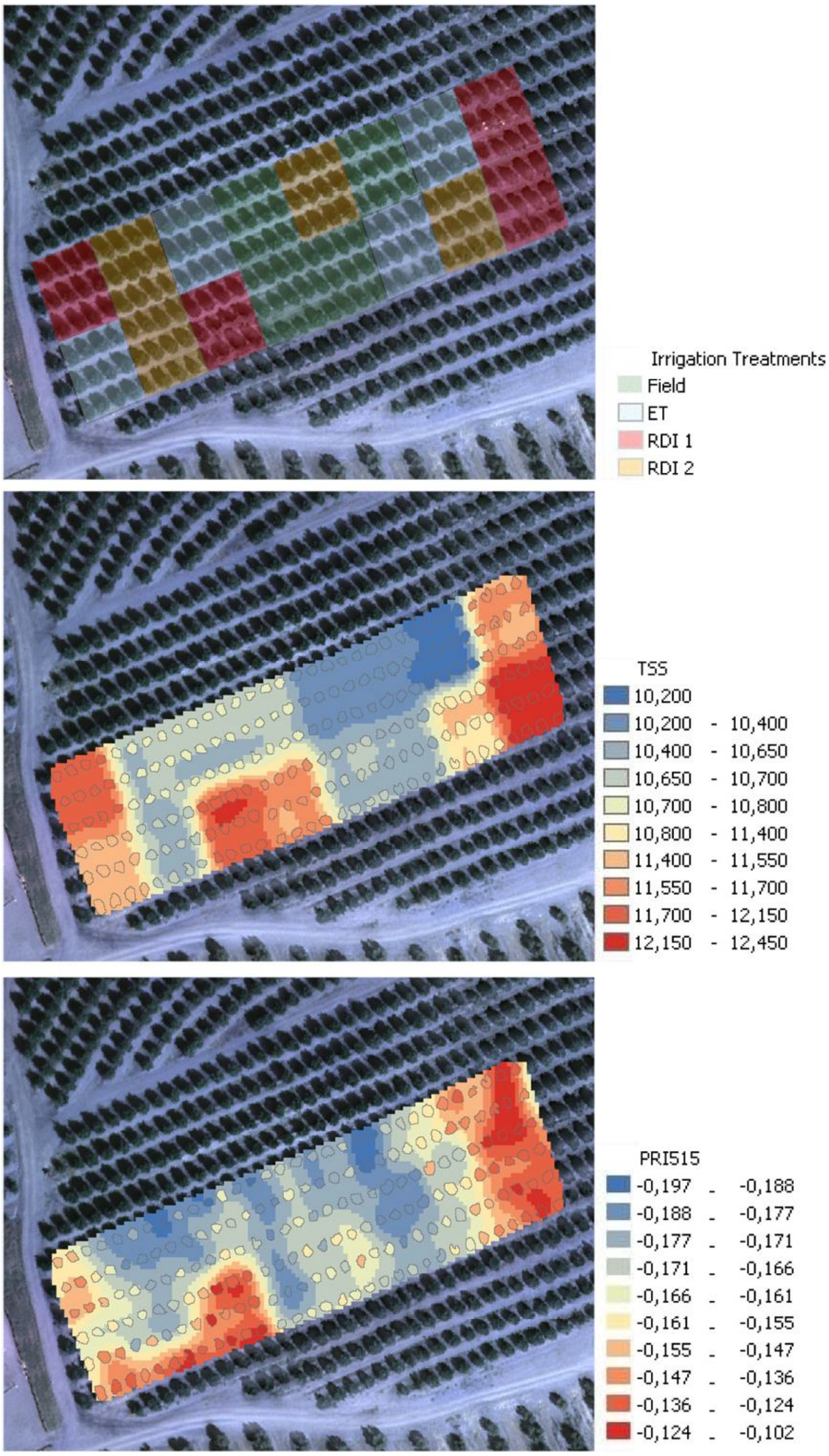

Fig. 11. Interpolated map of the spatial variability of PRI_{515} and TSS over the experiment.

water status detection. The relationship found between the photochemical reflectance indices and fruit quality in September can be explained by the fact that this period is not only the time of the most severe water stress levels, but also is a critical time in terms of the physiological stage of fruit growth in citrus. Previous experiments on '*Clementina de Nules*' and '*Navelina*' citrus trees (Gasque et al., 2010; Gonzalez-Altozano and Castel, 1999, 2000) have shown that the effects of RDI treatments depend on the phenological period in which the water restriction is applied. During period II, summer (initial fruit enlargement), the application of RDI did not affect yield or fruit quality, whereas during period III (end of summer–autumn) it resulted in a decrease in yield, fruit size and fruit quality parameters due to a slower growth rate in the final stages of crop development. September–October water stress levels and the timing chosen for recovery are critical for fruit growth and quality determination in citrus trees.

This work highlights the importance of spatial resolution to avoid non-vegetation or soil-mixed pixels. Previous studies investigated the effects of pixel size on the PRI_{570} index (Suárez et al., 2008, 2010), where aggregated pixels, including soil and shadows, were compared against pure sunlit tree crown pixels. Our results are in agreement with these findings, i.e., high resolution is needed to separate soil from vegetation pixels. Nevertheless, our work suggests that the separation of sunlit and shaded pixels does not improve the feasibility of stress detection in citrus. It can be hypothesized that crown architecture (mostly derived from training and pruning) plays a significant role in the importance of shaded crown within the total crown area. Further investigations in canopies other than the spherical crown structure of citrus, where shaded vegetation is small, are required.

4.3. Sensitivity of chlorophyll and structural indices to water stress levels and fruit quality

The chlorophyll ratio R_{700}/R_{670} presented weaker relationships for TSS than PRI in September (Table 5), which could lead to several hypotheses on the role of chlorophyll content in fruit quality under a sustained water deficit experiment. The results are consistent with the hypothesis that a sustained water stress deficit induces chlorophyll a + b degradation. In regard to the structural indices, both RDVI and MTVI showed significant relationships with fruit quality, but only in the first measurement of June, the beginning of the deficit irrigation period (Table 4). These two indices are designed to detect plant structural and physiological parameters such as leaf biomass (canopy size, density and vigor) and canopy chlorophyll content in a more robust and adequate manner than the traditional NDVI (Broge and Leblanc, 2001; Haboudane et al., 2004; Roujean and Breon, 1995; Zarco-Tejada et al., 2005). The parameters detected by the two indices are very important determinants of photosynthetic capacity influencing the synthesis and accumulation of sugars and acids in fruits (Pirie and Mullins, 1980; Smart and Robinson, 1991). In a relevant study by Suárez et al. (2010), some weak relationships between structural indices and fruit quality in orange species, as well as some stronger relationships between structural indices and fruit quality in peach species, were also found. An issue of interest in the results achieved is the negative relationship between the structural indices and fruit quality. This means that the trees with lower crown volume presented higher fruit quality (TSS/TA). This phenomenon could be related to the lower crown densities on the trees under stress due to the previous year's sustained water deficit levels.

5. Conclusions

The potential of using high spatial and spectral resolution airborne imagery to detect deficit irrigation strategies affecting fruit quality in a commercial citrus orchard is explored in the present study. A critical issue is to detect and monitor water stress accurately to apply regulated deficit irrigation strategies, assessing their effects on fruit quality parameters. For this purpose, structural, chlorophyll and photochemical indices were extracted and compared from four flights acquired over an orange orchard from June through September 2009. A new PRI formulation proposed for forest canopies, which implements 515 nm as the reference band (PRI_{515}), was applied for the first time in an orchard study to assess the structural effects of citrus tree crowns and shadows on the PRI indices. The results demonstrated that both PRI formulations were able to track water stress before any other canopy biochemical or structural changes were detectable by the NDVI, RDVI, MTVI or R_{700}/R_{670} indices. In the present study, long-term and severe water stress levels were imposed, driving some tree plots into apparent canopy structural alterations detected by the three structural indices used (NDVI, RDVI and MTVI). When canopy alterations occurred, the traditional PRI_{570} formulation was seriously affected, eliminating its relationship with water status. On the other hand, the modified PRI_{515} showed increased stability, and stronger and consistent relationships with water status, even when canopy structure was affected. Regarding the relationship between water status and photochemical reflectance indices, a time delay against water potential levels was observed, with leaf photochemical functioning presenting slower recovery rates during the re-watering phase than stem water potential.

Both PRI formulations were proven to be sensitive to fruit quality levels affected by water stress irrigation schemes. Strong and consistent relationships were found between both PRI formulations and the measured fruit quality parameters in the two September flights. September represents the beginning of stage III in the studied species, a time when the fruit enters the final stages of crop development. September water stress and the timing of recovery are critical for fruit quality determination in these species. Chlorophyll and structural indices in the same period presented poor relationships with fruit quality parameters. The modified formulation of PRI (PRI_{515}) also showed better performance for fruit quality levels than the traditional PRI_{570}.

Finally, both PRI formulations showed similar performance in the two spatial scales used for the assessment (sunlit crown and entire crown), demonstrating that they were not strongly affected by within-crown shadowing in citrus trees. However, the applicability of these physiological indices is limited when image resolution exceeds crown size in orchard fields: sunlit and shaded soil and between-tree shadows greatly affected PRI formulations, yielding weaker relationships against water status and fruit quality. Due to the shadow and soil effects on PRI formulations proposed in this study, high spatial resolution imagery to enable pure tree-crown identification is required to correctly assess water status and fruit quality in citrus orchards using remote sensing imagery.

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