



Combining leaf physiology, hyperspectral imaging and partial least squares-regression (PLS-R) for grapevine water status assessment



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ABSTRACT

Physiological measurements are considered to be the most accurate way of assessing plant water status, but they might also be time-consuming, costly and intrusive. Since visible (VIS)-to-shortwave infrared (SWIR) imaging spectrometers are able to monitor various bio-chemical alterations in the leaf, such narrow-band instruments may offer a faster, less expensive and non-destructive alternative. This requires an intelligent downsizing of broad and noisy hyperspectra into the few most physiologically-sensitive wavelengths. In the current study, hyperspectral signatures of water-stressed grapevine leaves (*Vitis vinifera* L. cv. Cabernet Sauvignon) were correlated to values of midday leaf water potential (Ψ_1), stomatal conductance (g_s) and non-photochemical quenching (NPQ) under controlled conditions, using the partial least squares-regression (PLS-R) technique. It was found that opposite reflectance trends at 530–550 nm and around 1500 nm – associated with independent changes in photoprotective pigment contents and water availability, respectively – were indicative of stress-induced alterations in Ψ_1 , g_s and NPQ. Furthermore, combining the spectral responses at these VIS and SWIR regions yielded three normalized water balance indices (WABIs), which were superior to various widely-used reflectance models in predicting physiological values at both the leaf and canopy levels. The potential of the novel WABI formulations also under field conditions demonstrates their applicability for water status monitoring and irrigation scheduling.

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1. Introduction

Grapevine (*Vitis vinifera* L.), one of the most planted fruit crops in the world, is commonly grown in Mediterranean and other semi-arid climate regions that are prone to water stress (Flexas et al., 2010). In such environments, under harsh conditions of strong incoming radiation, low water availability and large evaporative demands, yield quality and wine production are highly dependent on irrigation (Chaves et al., 2010). Since excess water inputs are known to impair both grape content and color (Bravdo et al., 1985; Esteban et al., 1999), yet smaller, regulated supplements were shown to improve berry composition (Chapman et al., 2005; Dry et al., 2001), deficit irrigation (DI) techniques have been widely adopted as the leading watering strategy for wine

vines (Chaves et al., 2010). Although the most accurate way of monitoring DI is by measuring relevant physiological variables (Cifre et al., 2005), acquiring these preliminary indicators of stress might also entail intrusive equipment and proficient manpower. Furthermore, as spatial variability (Bramley, 2005; Trought and Bramley, 2011) and genotype-based hydraulic differences (Hochberg et al., 2015) within a vineyard may also require numerous samplings, physiology tools become also time- and cost-inefficient (Hall et al., 2002). Therefore, a faster, simpler, cheaper and non-destructive alternative is necessary to advance DI regulation in grapevine (Cifre et al., 2005).

Visible (VIS)-to-shortwave infrared (SWIR) imaging spectrometers are able to detect slight modifications in leaf bio-chemistry, allowing a quick and non-intrusive assessment of plants' physiological status (Blackburn, 2007; Mariotto et al., 2013). Over the last decade, such hyperspectral instruments have been shown to have great potential for precision agriculture, through proximal and

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remote estimations of various water-related variables (e.g., Rodríguez-Perez et al., 2007; Serrano et al., 2010, 2012; Zarco-Tejada et al., 2005, 2013). Nonetheless, there seems to be room for improvement in the predictive power that is presented in many studies, which has been derived from the application of widely-used, so-called “universal” indices (e.g., Yu et al., 2000); namely, as the same stressing agent may produce distinctly different spectral responses across species and growing environments (Carter and Knapp, 2001), the effectiveness of a general spectral model – tested on a given plant and exported to others – is highly questionable. Specifically, the spectral signature of a water-stressed leaf (directly or indirectly expressed as changes in water content, chlorophyll content or fluorescence, etc.) is unique and should be better monitored via specific spectral regions. This claim is firmly supported by numerous studies, which demonstrate the superiority of indices that are targeted for specific variables or species over non-specific formulations (e.g., Cho et al., 2007; Fu et al., 2014; Hansen and Schjoerring, 2003; Huang et al., 2004; Li et al., 2014., 2008; Ramoelo et al., 2013; Yi et al., 2014). Furthermore, in the absence of grapevine-designated models in the literature, the search for the most suitable spectral index may involve a tiresome trial-and-error selection process, featuring dozens of “universal” models (e.g., Martin et al., 2007; Rodríguez-Perez et al., 2007; Zarco-Tejada et al., 2005). Hence, studying the unique spectral–physiological connection of wine vines under water deficit is necessary for constructing more reliable indices and, ultimately, for improving DI regulation.

While hyperspectra often consist of hundreds of correlated, redundant bands (predictors) that invariably lead to large volumes of noise (Mariotto et al., 2013; Thenkabail et al., 2011), the scope of complementary physiological data (predictees) is immeasurably smaller. Consequently, the creation of robust and meaningful indices necessitates the intelligent isolation of the most physiologically-sensitive and least collinear wavelengths (Yi et al., 2014). Such dimensionality-reduction of a spectrum can be efficiently performed with the partial least squares-regression (PLS-R) technique (Wold, 1982), an advanced extension of multiple linear regression (MLR) modeling. Unlike the latter, PLS-R is able to correlate collinear, noisy and distribution-free datasets, even when the number of predictors greatly exceeds the number of predictees (Geladi and Kowalski, 1986; Wold et al., 2001). To date, despite successful implementations in studies of various species and stress factors (e.g., Feret et al., 2011; Hansen and Schjoerring, 2003; Herrmann et al., 2011; Li et al., 2014; Nguyen and Lee, 2006; Ramoelo et al., 2013; Yi et al., 2014), only a couple of water deficit-related grapevine works (de Bei et al., 2011; Santos and Kaye, 2009) have harnessed the PLS-R tool for spectral–physiological analyses. Whereas seemingly important SWIR bands were highlighted in these two studies, no actual index application was developed or tested under either controlled- or field conditions. Moreover, although narrow-band leaf signatures were collected from a minimal distance – restricting the source of spectral variation to anatomical and biochemical properties alone (Eitel et al., 2006; Zarco-Tejada et al., 2005) – they were not validated at the canopy-scale or tested against the effects of background reflectance and atmospheric noise.

The primary aim of the current study was, thus, to combine leaf physiology variables, VIS-SWIR hyperspectral proximal measurements and PLS-R in order to construct wine vine-specific indices for water status monitoring. Under controlled conditions and at the leaf level, it was hypothesized that these PLS-based models will prove more efficient at predicting relevant physiological values than various “universal” water stress-related models. Nevertheless, in order to evaluate the potential of the former indices for commercial vineyards, they should also be tested alongside the widely-used indices at the canopy level, under field conditions. Accordingly, the

specific objectives of the study were (1) to identify the most sensitive wavelengths to water stress at the leaf level, under greenhouse conditions; (2) to utilize these indicative bands for deriving specific indices, and to compare the latter's predictive ability with those of selected “universal” models; (3) to validate index performance at the canopy-scale, from a few meters height, under controlled conditions; and (4) to test index potential at the same canopy-scale, under field conditions. These four continuous goals were divided into three independent water deficit experiments, which were conducted throughout the three growing seasons of 2012–2014; specifically, the first greenhouse experiment (2012) was aimed at highlighting diagnostic wavelengths through PLS-R modeling and utilizing them for index construction; the second greenhouse experiment (2013) was aimed at validating the PLS-based indices at the canopy-scale; and the third experiment (2014) was aimed at canopy-scale validation under vineyard conditions.

2. Methodology

2.1. Plant materials and growth conditions

The first and second greenhouse experiments included two- and three-year-old Cabernet Sauvignon vines (*V. vinifera* L. cv.), respectively, which were grafted onto Richter-110 rootstocks (*Vitis berlandieri* × *Vitis rupestris*). The plants were grown in 10 L pots that contained 9 L of RAM8 media (Tuff Merom Golan Inc., Merom Golan, Israel), and were annually-enriched with a controlled-release ‘Multigan’ fertilizer (Elgo Irrigation Inc., Caesarea, Israel). Drip irrigation to field capacity was applied on a daily basis, and day/night temperatures within the greenhouse were maintained at 28/18 °C, respectively. Throughout each of the experiments, all vines were pruned to allow the growth of a single shoot and flower buds were removed upon emergence to sustain the plants in a vegetative state.

The third experiment took place in a five-year-old vineyard of the Ramat Negev Agro-Research and Development Center, Israel (30°98'N/34°70'E), which was in its second year of production. The Negev Desert Highlands region is characterized by an average annual precipitation of less than 100 mm and pan evaporation of over 1700 mm (Portnov and Safriel, 2004). The vines were grafted onto Ruggeri-140 rootstocks (*V. berlandieri* × *V. rupestris*), planted in a sandy-loam soil (1.5 m apart within a row, 3.5 m distance between rows) and trained onto a vertical trellis (cordon T) in a north–south orientation. To physically prevent water- and root-crossing between the vines, each experimental block was partitioned with a 1.5 m deep plastic buffer.

2.2. Experimental design

The first greenhouse experiment lasted for 30 days and began once 16 plants had reached a size of 12–16 leaves. While eight vines were maintained at a field capacity irrigation regime and regarded as controls (C), the other eight were subjected to a progressive degree of water deficit (WD), as described by Hochberg et al. (2015). All pots were randomly arranged in the greenhouse and placed at a sufficient distance from one another and from the structure's edges in order to minimize shading effects. Midday physiological and spectral measurements were conducted on a weekly basis between 11:00 and 13:00, and were accompanied by supporting morphological observations. The second, validation greenhouse experiment was identical in design to the first, but lasted 18 days and included measuring points on the first and last days of experiment (DOE).

Irrigation in the third, vineyard experiment was applied according to the crop evapotranspiration (ET_c) model of Netzer et al.

(2009), which is based on frequent measurements of potential evapotranspiration (ET_0) and leaf area index (LAI) throughout the growing season. Three watering treatments of $0.5 * ET_C$, $0.35 * ET_C$ and $0.2 * ET_C$ – reflecting common, low and very low irrigation amounts, respectively – were applied in the field between anthesis (May) and harvest (August). Before anthesis and after the harvest, all treatments were uniformly irrigated according to the $0.5 * ET_C$ level. At a single time point during veraison (July), physiological measurements were conducted on five vines per treatment, while spectral images were acquired from three plants per treatment. As in the greenhouse experiments, all midday measurements were taken between 11:00 and 13:00.

2.3. Physiological and morphological measurements

During all three experiments, physiological values of midday leaf water potential (Ψ_l ; reflecting leaf water availability), stomatal conductance (g_s ; reflecting the plant's main response to water limitation, via stomata aperture modification) and non-photochemical quenching of chlorophyll fluorescence (NPQ; reflecting dissipation of excessive energy as heat, when stomata closure leads to low CO_2 availability) were measured. All three variables are known for their relevance to grapevine irrigation scheduling, due to their preliminary and indicative response to water status changes (Acevedo-Opazo et al., 2010; Cifre et al., 2005; Flexas et al., 2002; Girona et al., 2006).

Gas exchange and fluorescence measurements were performed with a portable LI-6400 Infrared Gas Analyzer (Li-Cor Biosciences Inc., Nebraska, USA). The system maintained the leaf chamber at the ambient temperature, at a steady CO_2 level of $400 \mu mol mol^{-1}$ and at a quantum yield (PAR) degree of $1000 \mu mol photons m^{-2} s^{-1}$. Values of NPQ were derived from the maximal fluorescence of dark-adapted (F_m ; 30-min adaptation) and light-adapted (F_m') samples. All measurements were conducted on a single sun-exposed, youngest fully-matured leaf per plant.

Midday leaf water potential values were obtained with an Arimad-3000 Pressure Chamber (MRC Inc., Holon, Israel), according to the protocol of Turner (1988). In short, a plastic bag was placed over the lamina, just before the petiole's excision. The detached leaf was then immediately placed inside the measuring chamber, while still covered by the bag. Thereafter, the pressure on the lamina was progressively increased until an initial amount of sap emerged from the petiole. All Ψ_l values were obtained from a single sun-exposed, youngest fully-matured leaf per plant.

In order to strengthen the physiological findings throughout the first and second greenhouse experiments, supporting morphological measurements were also taken; these included calculations of total foliage areas according to the model of Rapaport et al. (2014), and counting of the number of nodal positions along each vine's branch.

2.4. Spectral measurements

During the first greenhouse experiment, adaxial signatures were collected with a FieldSpec Pro-FR Portable Spectrometer (Analytical Spectral Devices Inc., Colorado, USA) equipped with an 1800-12S Integrating Sphere (Li-Cor Biosciences Inc., Nebraska, USA). The system was set to measure radiance flux units within the 400–2000 nm spectrum, at a 1 nm spectral resolution. Normalized values of reflectance (ρ) were derived from supplementary readings of white reference standards ($BaSO_4$ tablets) and dark current (noise). In order to spectrally-represent an entire vine with a single leaf – considering the effect of water stress on the age variability within a plant's canopy – the reflectance profile method of Rapaport et al. (2014) was applied at every measuring point. Twelve commonly used “universal” narrow-band indices were

calculated for each plant (Table 1), including two versions of the photochemical reflectance index (PRI; Gamon et al., 1992), the red-edge inflection point (REIP; Horler et al., 1983), the sum of red-edge first derivatives ($\sum d\rho$; Filella and Penuelas, 1994), the modified normalized difference vegetation index (mNDVI; Gitelson et al., 1996), the structure insensitive pigment index (SIPI; Penuelas et al., 1995), the normalized difference vegetation index (NDVI; Jordan, 1969), the normalized difference infrared index (NDII; Hardisky et al., 1983), the normalized difference water index (NDWI; Gao, 1996), the water index (WI; Penuelas et al., 1993), the maximum difference water index (MDWI; Eitel et al., 2006) and the moisture stress index (MSI; Rock et al., 1986).

To overlap with the VIS-SWIR spectral range of the first greenhouse experiment, an AISA-HS Camera (Specim Inc., Oulu, Finland; 400–1000 nm range, 1 nm spectral resolution) and an IK-1523 Camera (ABS Inc., Jena, Germany; switchable narrow-band filters from 900 to 1700 nm) were simultaneously used in both the second and third canopy-scale experiments. The instruments were fixed side-by-side on a 4.5 m-tall construction, which was stationed directly above the plants to comply with the non-Lambertian nature of vine foliage (Hall et al., 2003). Since the cameras produced two unaligned radiance products for each vine, due to a slight difference in viewing angles, the images were geometrically-rectified with the ArcGIS 10.1 software (ESRI Inc., California, USA). The images were then normalized to a reflectance base with the help of complementary dark current readings and a 'white reference' Spectralon Panel (Labsphere Inc., New Hampshire, USA), which was included in every picture frame. All radiometric corrections and image processing procedures were done with the ENVI 4.6 software (Exelis Visual Information Solutions Inc., Virginia, USA).

In order to validate the first experiment's leaf-scale regressions for the canopy level, the reflective signature of four spectrally-representative leaves – selected according to the protocol of Rapaport et al. (2014) – was obtained from each vine of the second experiment. Evaluation of index potential under the third experiment's field conditions included a selection of a small, representative foliage portion from each vine.

2.5. Statistical analyses

Physiological and morphological differences were examined with one-way analysis of variation (ANOVA) models, followed by

Table 1
Summary of all reflectance models in this study. ρ = reflectance value at a given wavelength.

Index acronym	Formulation	Reference
PRI-1	$\frac{\rho_{531}-\rho_{550}}{\rho_{531}+\rho_{550}}$	Gamon et al. (1992)
PRI-2	$\frac{\rho_{531}-\rho_{570}}{\rho_{531}+\rho_{570}}$	
REIP	$\max d\rho (680-780)$	Horler et al. (1983)
$\sum dRE$	$\sum d\rho (680-780)$	Filella and Penuelas (1994)
mNDVI	$\frac{\rho_{750}-\rho_{705}}{\rho_{750}+\rho_{705}}$	Gitelson et al. (1996)
SIPI	$\frac{\rho_{800}-\rho_{445}}{\rho_{800}+\rho_{680}}$	Penuelas et al. (1995)
NDVI	$\frac{\rho_{800}-\rho_{675}}{\rho_{800}+\rho_{675}}$	Jordan (1969)
NDII	$\frac{\rho_{820}-\rho_{1650}}{\rho_{820}+\rho_{1650}}$	Hardisky et al. (1983)
NDWI	$\frac{\rho_{860}-\rho_{1240}}{\rho_{860}+\rho_{1240}}$	Gao (1996)
WI	$\frac{\rho_{900}}{\rho_{970}}$	Penuelas et al. (1993)
MDWI	$\frac{\max \rho[1500-1750]-\min \rho[1500-1750]}{\max \rho[1500-1750]+\min \rho[1500-1750]}$	Eitel et al. (2006)
MSI	$\frac{\rho_{1600}}{\rho_{820}}$	Rock et al. (1986)
WABI-1	$\frac{\rho_{1490}-\rho_{531}}{\rho_{1490}+\rho_{531}}$	The current study
WABI-2	$\frac{\rho_{1500}-\rho_{538}}{\rho_{1500}+\rho_{538}}$	
WABI-3	$\frac{\rho_{1485}-\rho_{550}}{\rho_{1485}+\rho_{550}}$	

honest significance difference (HSD) post-hoc tests (Tukey, 1949). Prior to each comparison between the treatment levels, the necessary assumptions of residuals' normality (Shapiro and Wilk, 1965) and homoscedasticity (Bartlett, 1937) were checked and met. All ANOVA models and correlation analyses (simple index-physiology regressions) were created with the Microsoft Excel 2010 (Microsoft Corporation Inc., Washington, USA) software and were considered significant at $p < 0.05$.

PLS-R analyses were performed with the PLS_Toolbox 7.03 software (Eigenvector Research Inc., Washington, USA) running under a MATLAB 7.12 environment (The Mathworks Inc., Massachusetts, USA). In order to ease the process of model construction, all independent (wavelength) and dependent (physiology) variables were mean-centered beforehand (Geladi and Kowalski, 1986). Once a regression was built, the variable importance in projection (VIP) index (Wold et al., 1993) – reflecting the weighted sum of squares of the PLS weights – was calculated for all predictors in order to determine their significance to the model (Chong and Jun, 2005; Westad et al., 2013); practically, the VIP-statistic score assesses the contribution (covariance) of each wavelength to both the physiological and spectral variations, and recommends whether it should be excluded from the regression. Since the mean of squared-VIP scores over the variables is 1 (Wold et al., 2001), this threshold was set for removing unimportant narrow-bands, and the process was repeated on the reduced spectrum until no more improvements were found in the model (Andersen and Bro, 2010). Finally, to estimate their applicability to other datasets, the best PLS-R models were cross-validated (CV) with the random subsets method, according to the suggestions of the PLS_Toolbox manual.

The quality of each PLS-R model was evaluated in light of its latent variable (LV) amount, ability to explain calibration (reference) physiological variance (R_c^2), ability to explain CV physiological variance (R_{cv}^2), root mean squared error of calibration (RMSEC), root mean squared error of cross-validation (RMSECV), and its RMS ratio (RMSECV/RMSEC). In general, a good model should have the lowest possible LVs – determined according to the optimal RMSs to avoid over-fitting (de Bei et al., 2011; Feret et al., 2011), the highest possible coefficients of determination, and a close-to-one RMS ratio (Bjorsvik and Martens, 2008; Williams, 2001). To better assess the applicability of the PLS-regressions for external datasets, the ratio of performance to inter-quartile distance (RPIQ) index (Bellon-Maurel et al., 2010) was calculated for each model; this measure is derived by dividing the inter-quartile range ($Q_3 - Q_1$) of a reference dataset by its RMSECV, and facilitates the comparison to results from other validation populations. Nonetheless, as the RPIQ index is relatively new and, thus, no scale has been published for it to date, the value of 2 was considered the threshold for a model's robustness (e.g., Camps et al., 2014; McDowell et al., 2012).

3. Results

3.1. Physiological and morphological analyses

Throughout the first greenhouse experiment, the WD treatment was presenting progressive signs of water stress – from a mild level on the 7th DOE to a severe degree on day 28 (Fig. 1). In terms of Ψ_1 (Fig. 1A), a significant decline to -0.95 MPa ($C = -0.69$ MPa) was already observable by the end of the first week, and the potential was further decreased to -1.63 MPa by the end of the experiment. Consequently, a significant g_s reduction to 0.10 mol H_2O m^{-2} s^{-1} ($C = 0.18$ mol H_2O m^{-2} s^{-1}) was also noticeable by day 7, and this rate was further declined to a minimum of 0.02 mol H_2O m^{-2} s^{-1} by the 28th DOE (Fig. 1B). In accordance with the decrease in leaf water potential and stomatal conduc-

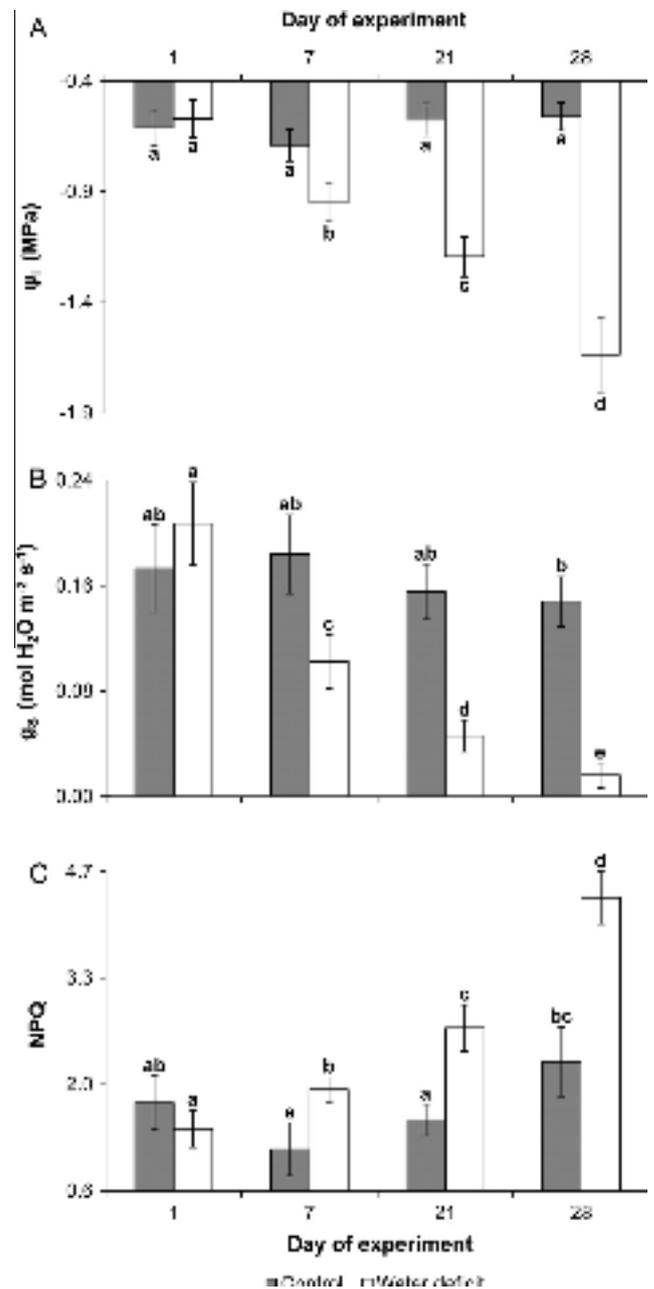


Fig. 1. Physiological changes throughout the first greenhouse experiment. (A) Water potential (Ψ_1); (B) stomatal conductance (g_s); (C) non-photochemical quenching (NPQ). Error bars represent means \pm standard deviations (8 plants). Lower-case letters indicate significance of differences.

tance, significant NPQ increases to 1.89 ($C = 1.13$) and up to 4.32 were apparent by day 7 and 28, respectively (Fig. 1C). These physiological changes of the WD group were accompanied by significant morphological alterations, which were already noticeable by the 14th DOE (Fig. 2); specifically, a smaller number of 19 nodes ($C = 24$; Fig. 2A) and a smaller foliage area of 3450 cm^2 ($C = 4240$ cm^2 ; Fig. 2B) were evident by the end of the second week, and were insignificantly different from their counterparts of the 28th DOE – indicating a growth inhibition.

As in the first experiment, the distinct irrigation regimes of the second greenhouse experiment created considerable physiological differences between the treatments, which peaked as the WD group was reaching a moderate-severe stress level by the 18th DOE; specifically, the last day of the experiment was characterized

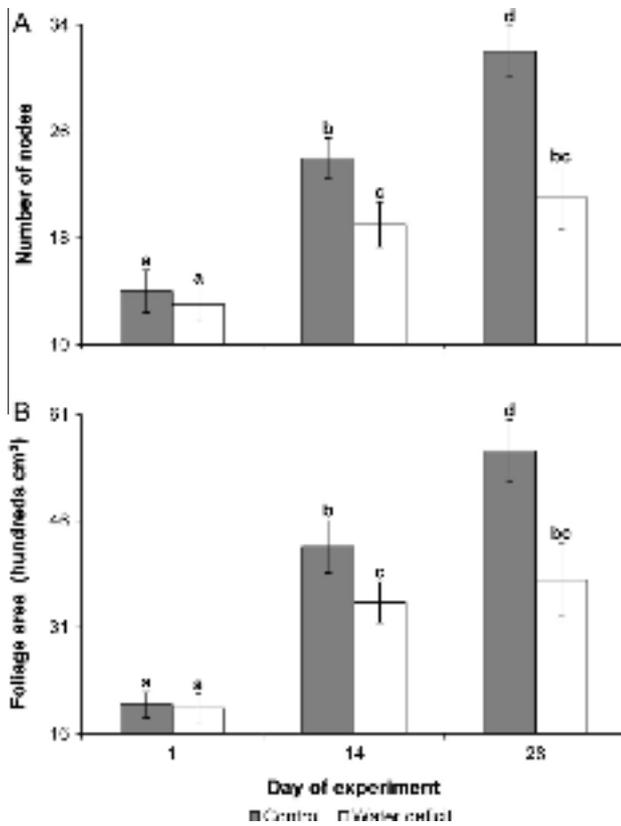


Fig. 2. Morphological changes throughout the first greenhouse experiment. (A) Number of nodes; (B) foliage area. Error bars represent means \pm standard deviations (8 plants). Lower-case letters indicate significance of differences.

by significantly lower Ψ_1 and g_s averages of -1.24 MPa ($C = -0.53$ MPa) and 0.04 mol $\text{H}_2\text{O m}^{-2} \text{s}^{-1}$ ($C = 0.22$ mol $\text{H}_2\text{O m}^{-2} \text{s}^{-1}$), respectively, and a consequent, significantly higher NPQ average of 2.67 ($C = 1.36$). The most significant morphological differences were also noticeable by the end of the experiment, with the reduced WD foliage of 3070 cm^2 ($C = 4470$ cm^2) spreading over merely 8 nodes ($C = 18$).

Significant physiological differences were also found between all three irrigation treatments of the third, field experiment. In terms of leaf water potential, while the $0.5 * \text{ET}_C$ vines averaged at -1.16 MPa, the means of the $0.35 * \text{ET}_C$ and $0.2 * \text{ET}_C$ groups were -1.35 and -1.56 MPa, respectively. Accordingly, the commonly-irrigated treatment also had the highest stomatal conductance average of 0.33 mol $\text{H}_2\text{O m}^{-2} \text{s}^{-1}$, which was followed by the 0.14 and 0.05 mol $\text{H}_2\text{O m}^{-2} \text{s}^{-1}$ means of the low and very low watering groups, respectively. The reductions in Ψ_1 and g_s created an opposite increasing trend for NPQ, manifested by a gradient of 1.64 ($0.5 * \text{ET}_C$), 3.40 ($0.35 * \text{ET}_C$) and 5.63 ($0.2 * \text{ET}_C$).

3.2. PLS-R and spectral analyses

In general, all PLS models were akin and performed considerably well, having a relatively small number of LVs (5), strong R^2_C (≥ 0.91) and R^2_{CV} (≥ 0.81) coefficients, small errors of calibration ($\leq 11\%$) and CV ($\leq 15\%$) that were also close ($1.36 \leq \text{RMS ratio} \leq 1.58$), and sufficient RPIQ (≥ 2.69) values (Table 2). Specifically, the leaf water potential regression was the most robust of the three models, as it had the highest R^2_{CV} (0.88), the lowest RMSECV (0.15 MPa, or 9% error) and RMS ratio (1.36), and the best RPIQ (3.80) value. The g_s regression was only slightly weaker than the Ψ_1 model, with very close values of R^2_{CV} (0.85), RMSECV (0.03 mol $\text{H}_2\text{O m}^{-2} \text{s}^{-1}$,

Table 2

Quality evaluation of the partial least squares-regression (PLS-R) models. Each model was built from 32 observations (8 stressed vines over 4 time-repetitions) and included 5 latent variables. R^2_C = explained calibration physiological variance; R^2_{CV} = explained cross-validation physiological variance; RMSEC = root mean squared error of calibration; RMSECV = root mean squared error of cross-validation; RMS ratio = RMSECV/RMSEC; $Q_3 - Q_1$ = inter-quartile range; RPIQ = $(Q_3 - Q_1)/\text{RMSECV}$; * = value in (MPa); # = value in (mol $\text{H}_2\text{O m}^{-2} \text{s}^{-1}$).

Physiological variable	R^2_C	R^2_{CV}	RMSEC	RMSECV	RMS ratio	$Q_3 - Q_1$	RPIQ
Ψ_1	0.94	0.88	0.11* (7%)	0.15* (9%)	1.36	0.57*	3.80
g_s	0.95	0.85	0.02# (8%)	0.03# (12%)	1.52	0.11#	3.67
NPQ	0.91	0.81	0.35 (11%)	0.55 (15%)	1.58	1.48	2.69

or 12% error), RMS ratio (1.52) and RPIQ (3.67). The least accountable NPQ regression had the lowest R^2_{CV} (0.81), highest RMSECV (0.55, or 15% error) and RMS ratio (1.58), and a considerably lower RPIQ value (2.69) – indicating that the model's error in prediction was less than three times smaller than the inter-quartile range of the reference data.

Calculations of the VIP-statistics revealed the shared importance of the 530–550, 700–750, 1380–1420 and 1450–1590 nm regions for all three physiological variables (Fig. 3). Particularly, the local extrema above the score threshold – indicative of the most physiologically-sensitive narrow-bands – were found at 538, 710, 1400 and 1480–1510 nm for Ψ_1 ; 550, 712 and 1485 nm for g_s ; and 531, 710, 747 and 1490 nm for NPQ. From a spectral perspective (Fig. 4), the water stress was causing a progressive reflectance decrease at the green (530–550 nm; Fig. 4A) and red-edge regions (700–750 nm; Fig. 4B) regions, whereas a reflectance increase was observed around the SWIR regions (1380–1420 and 1450–1550 nm, Fig. 4C). Based on these opposing trends, various arithmetic combinations between the most diagnostic wavelengths were tested in predicting physiological values at the leaf level. Of all configurations, the difference between the reflectance trends at 530–550 nm and around the 1500 nm region was found to be the most relevant, leading to the creation of three water balance indices (WABIs; Table 1). Each of these simple and normalized models – WABI-1, WABI-2 and WABI-3 – was highly correlated to at least one physiological variable at the leaf-scale (Table 3), and presented a convincing separability between the irrigation levels of the first greenhouse experiment (Fig. 5). Conversely, the most successful “universal” model, the MDWI, explained only a low-to-medium proportion of the variance in the Ψ_1 (34%), g_s (45%) and NPQ (64%) variables (Table 2) and displayed a poor differentiation ability between the watering treatments.

When transitioned to the canopy-scale of the second greenhouse experiment, all three WABIs showed very analogous patterns of change with Ψ_1 (WABI-2; Fig. 5A), g_s (WABI-3; Fig. 5B) and NPQ (WABI-1; Fig. 5C), as compared with the first experiment's correlations. However, as in the case of the leaf level, the “universal” indices failed to present sufficient correlations or differentiation abilities between the irrigation levels of the second, canopy-scale experiment (data not shown). The success of the PLS-based models was also replicated in the third, vineyard experiment, as they presented even better separabilities than in the greenhouse experiments (Figs. 6 and 7); this is because extreme reflectance values were observed at the 530–550 and 1500 nm regions, likely due to lower relative humidity and water availability, higher light intensity and more developed canopies. Under such conditions, the most successful WABI-1 model could easily differentiate between all three irrigation levels (Fig. 7A), while

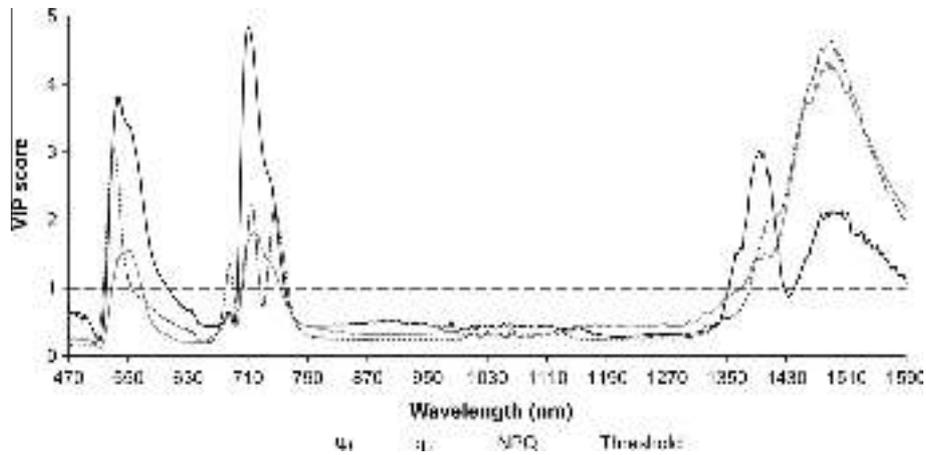


Fig. 3. Variable importance in projection (VIP) scores across the first greenhouse experiment's spectrum. The 530–550, 700–750, 1380–1420 and 1450–1590 nm regions were all shared by the leaf water potential (Ψ_1), stomatal conductance (g_s) and non-photochemical quenching (NPQ) variables.

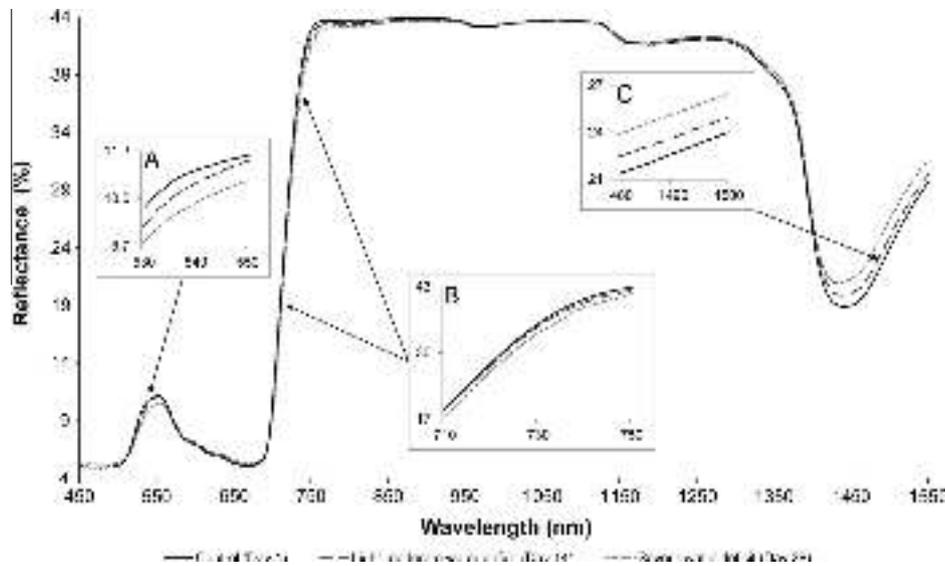


Fig. 4. Spectral changes throughout the first greenhouse experiment. Presented are the average control (day 1), light-moderate water deficit (day 14) and severe water deficit (day 28) reflectance patterns (8 plants). In reference to the variable importance in projection (VIP) recommendations (Fig. 3), a progressive reflectance decrease was observed in the 530–550 (A) and 710–750 (B) nm regions, while an increase was observed in the vicinity of 1500 nm (C).

Table 3

Goodness of fit of spectral–physiological regression models at the leaf level. ρ = reflectance value at a given wavelength. A bolded correlation coefficient (R^2) is the highest for a physiology variable. All g_s regression lines were exponential rather than linear.

Index acronym	Ψ_1	g_s	NPQ
PRI-1	0.19	0.17	0.45
PRI-2	0.32	0.26	0.56
REIP	0.34	0.18	0.20
$\sum dRE$	0.14	0.00	0.01
mNDVI	0.28	0.01	0.03
SIPI	0.17	0.23	0.22
NDVI	0.03	0.01	0.00
NDII	0.24	0.41	0.33
NDWI	0.04	0.25	0.22
WI	0.12	0.18	0.37
MDWI	0.34	0.45	0.64
MSI	0.31	0.29	0.43
WABI-1	0.72	0.37	0.86
WABI-2	0.89	0.39	0.64
WABI-3	0.61	0.80	0.14

the best of the “universal” models, PRI-2, could not easily separate between the treatments (Fig. 7B).

4. Discussion

Results from the first greenhouse experiment suggest that water stress-induced variation in Ψ_1 , g_s and NPQ is best explained by combining the reflectance decrease at 530–550 nm (e.g., Evain et al., 2004; Zarco-Tejada et al., 2013) with the opposed increase around 1500 nm (e.g., de Bei et al., 2011). The selection of these specific bands is an indication of the PLS-R success, as they are known to be directly- and indirectly-related to all of the examined physiological variables in many plant species, including grapevine (e.g., Eitel et al., 2006; Evain et al., 2004; Liakopoulos et al., 2006; Suarez et al., 2008); specifically, (1) the reflectance decrease around the sensitive 531/538 nm wavelengths is linked to the photoprotective (de-epoxidated) state of the xanthophyll cycle, through an increase in zeaxanthin content (Gamon et al., 1992); (2) the reflectance decrease at 550 nm is mainly associated with adjustments of photosynthetic and photoprotective pigments, such

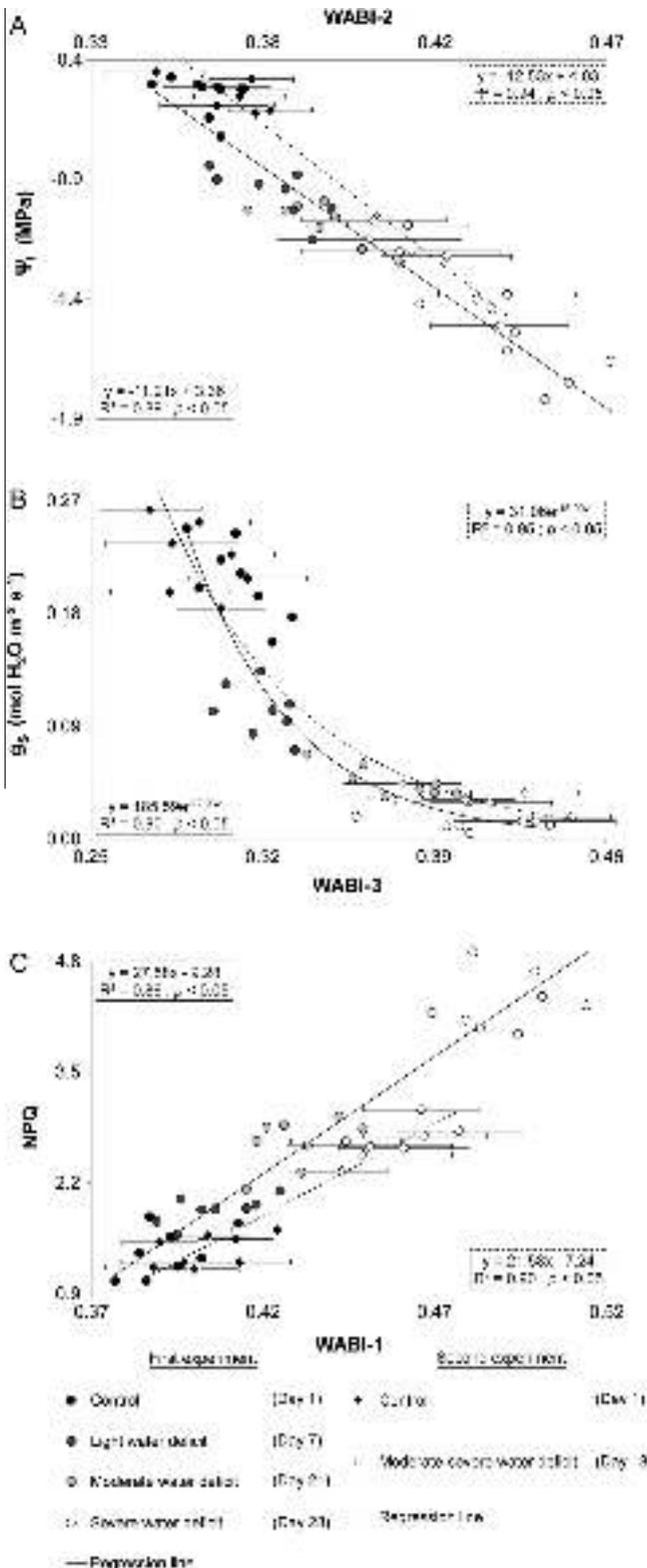


Fig. 5. Leaf- (first experiment) and canopy-scale (second experiment) spectral-physiological regressions. Each point in the second experiment’s dataset represents a four-leaf mean with its standard deviation (8 plants).

as anthocyanin (Gitelson et al., 2006; Steele et al., 2009); and (3) the reflectance increase around 1500 nm is strongly affiliated with a direct decrease in leaf water content (Carter, 1991; Eitel et al., 2006).

Thus, each WABI model monitors two independent mechanisms simultaneously – i.e., alterations in chlorophyll fluorescence and in leaf water content – which allows for a better assessment of vines’ physiological status. This advantage appears to be even more important and biologically-logical considering the ongoing debate about the most suitable parameter for irrigation scheduling, and in light of the frequent uncoupling of physiology variables in grapevine (Cifre et al., 2005); for example, while the coupling of Ψ_l and g_s may depend on grapevine cultivar (Hochberg et al., 2013) and leaf age (Patakas and Noitsakis, 1999), the coupling of NPQ and g_s may depend on heat and radiation conditions (Luo et al., 2011). These dependencies explain why a widely-used, “universal” model such as the PRI – based on a single response at 531 nm and on another spectrally-idle, reference band – would be inferior to the WABIs in predicting NPQ (or other variables), which may rely on other factors besides water availability (Gilmore, 1997).

In contrast to the rapid spectral changes in the abovementioned VIS and SWIR bands, relatively small reflectance alterations were observed in the red and near infrared (NIR) parts of the spectrum, as was exemplified by the NDVI and SIPI “universal” models. These observations are an indication of the late response of chlorophyll content and leaf structure to water stress, as was shown for grapevine and other plant species (e.g., Baluja et al., 2012; Dobrowski et al., 2005; Gamon et al., 1992; Serrano et al., 2010; Suarez et al., 2008). In the context of the red-edge region, although several diagnostic wavelengths were highlighted by the PLS-R analyses – generally supporting previous findings of sensitivity to chlorophyll content and fluorescence (e.g., Dobrowski et al., 2005; Gitelson et al., 1996; Thenkabail et al., 2014) – these spectral features presented insufficient differentiation abilities between the irrigation treatments and were less correlated to changes in Ψ_l , g_s and NPQ, as depicted by the mNDVI, REIP, and $\sum d\rho$ models. It should also be noted that while the 1400–1450 nm region was found to be of some importance in the current study, especially to changes in leaf water status (e.g., de Bei et al., 2011; Rodriguez-Perez et al., 2007), this band was not incorporated in the WABI models due to its susceptibility to water vapor absorption (Eitel et al., 2006; Sims and Gamon, 2003) and since the 1500 nm region was generally better correlated to changes in all physiological variables.

Under controlled conditions, all three WABI models also performed very well at the canopy level, presenting close similarities to the spectral-physiological correlations of the first experiment and plausible differentiation abilities. These results are supported by previous studies that emphasized the sensitivity of the 530–550 (e.g., Blackburn, 2007; Suarez et al., 2008) and 1500 nm (e.g., Eitel et al., 2006) general regions at the crown-scale. The effectiveness of the SWIR band under humid greenhouse terms is especially encouraging, as it may alleviate the concern regarding water vapor absorbance (Sims and Gamon, 2003) – at least for a proximal sensing system – and present an alternative for more susceptible wavelengths around 1400–1450 nm (e.g., de Bei et al., 2011; Rodriguez-Perez et al., 2007; Santos and Kaye, 2009). Such potential feasibility was demonstrated in the third, vineyard experiment of this study, where the WABI models presented excellent canopy-scale separabilities that were even better than those of the leaf level; this is probably because the higher leaf area density (=canopy water content) in the field enhances water absorption features, resulting in clearer differences between stress levels (Rodriguez-Perez et al., 2007).

Despite the auspicious proximal sensing results, several technical issues might still limit the incorporation of WABI-based monitoring systems into commercial vineyards. Firstly, a remote sensing approach would first require proof of the SWIR (1500 nm) band’s penetrability through thick atmospheric layers (Sims and Gamon, 2003). Secondly, at the aerial and satellite levels, VIS reflectance values would have to be corrected for numerous

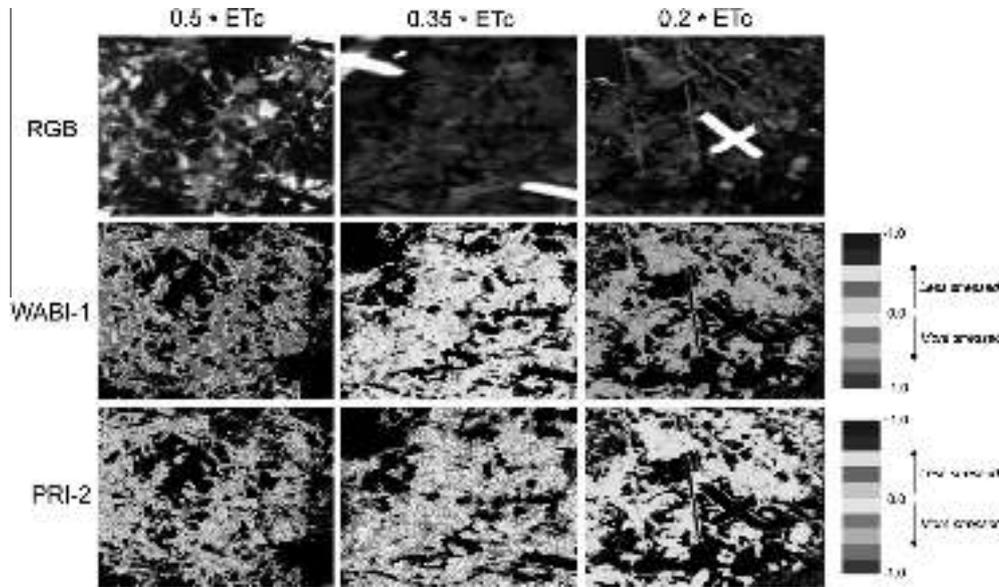


Fig. 6. Visual separability of selected canopy-scale indices in the vineyard experiment. Each red–green–blue (RGB) image displays a portion of a single, representative vine of either the common ($0.5 * ET_c$), low ($0.35 * ET_c$) or very low ($0.2 * ET_c$) irrigation regimes. The separability of the most successful model in the current study, WABI-1, and that of the best “universal” model, PRI-2, were based on a resolution of 0.2 index values (the color gradients in the legend are divided into ten 0.2 increments) and were tested on the uppermost layer of leaves, which was initially isolated by running an NDVI mask on each RGB image. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

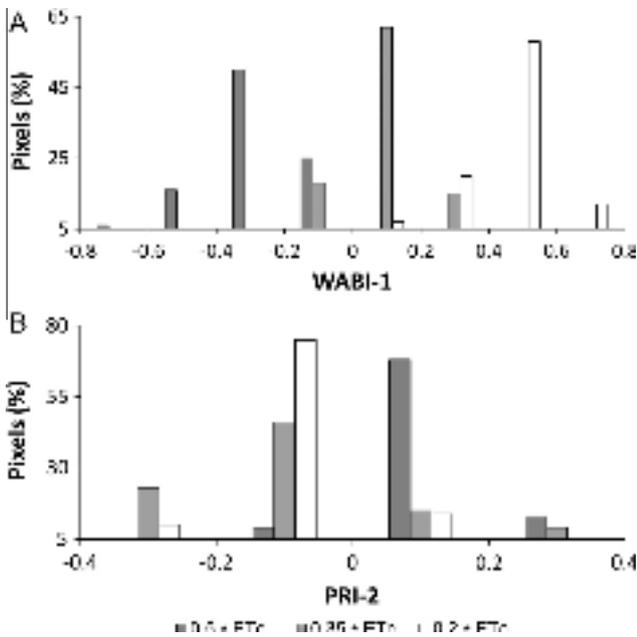


Fig. 7. Frequency histograms of selected canopy-scale indices in the vineyard experiment. In reference to the visual separabilities of Fig. 6, each column represents the overall percentage of pixels within a 0.2 index value range, for the common ($0.5 * ET_c$), low ($0.35 * ET_c$) or very low ($0.2 * ET_c$) irrigation regimes.

biases that originate from leaf orientation, shadow/sunlit fractions, canopy architecture, viewing and illumination geometry, and background effects (Gamon et al., 1990; Hall et al., 2008; Hilker et al., 2008). Thirdly, the physiological variability within a tree’s canopy – originating from the abovementioned factors and from biological and phenological differences (Rapaport et al., 2014) – would have to be taken into account. Fourthly, applying the WABIs through a single modern spectral instrument – designed to overcome the slowness and complexity of handling multiple sensors and analyzing their datasets – would probably incur high financial costs. Nev-

ertheless, it is important to note that the simple WABI structure allows using multi-spectral equipment, which is generally cheaper, faster and simpler than hyperspectral instruments. Moreover, as suggested by the VIP results, slight deviations from the most important narrow-bands are permitted – especially in the SWIR region – and should allow the purchase of broader, less expensive filters.

Disregarding the technical obstacles, the novel spectral indices of this study were shown to have great potential for DI monitoring. As was hypothesized, the combination between physiological variables, VIS-SWIR narrow-band reflectance signatures, and the bridging PLS-R technique yielded robust indices that were far more sensitive than various “universal” models. The WABI advantage can be attributed not only to the obvious application of proper wavelengths, but also to the use of a structure-independent SWIR band – in contrast to the biased NIR channels of some widely-used models (Carter, 1991; Ollinger, 2011; Sims and Gamon, 2003). This also explains the relative success of the SWIR-based MDWI, which was found to be more effective than NIR-dependent indices at the leaf level of this study and in other works (e.g., Eitel et al., 2006). Nonetheless, the WABIs were still superior to the best “universal” models, as the former combined the most relevant SWIR band with the most relevant VIS bands – leading to better correlativity and to the detection of even mild water deficit levels.

In general, water content and NPQ are both responsive to water availability in most species, with the exception of extreme isohydric cases and those plants that utilize either C_4 or CAM metabolism (e.g., corn; Tardieu and Simonneau, 1998; Xu et al., 2008). Therefore, it is likely that the suggested WABI models will be relevant for many C_3 species under various growing conditions and, accordingly, laborious PLS-R processes will not be compulsory in future applications. Nevertheless, since slight deviations from the specific wavelengths of the current study are probable, due to technical (i.e., measurement-related), biological or environmental factors (Gamon et al., 1990), spectral fine-tuning is recommended; namely, a relatively simple investigation for determining the maximal response around the highlighted bands (i.e., in the vicinity of 530–550 and 1500 nm) is important for optimizing model perfor-

mance prior to application. Furthermore, it is also important to note that a variable degree of modification is expected between genotypes and environmental conditions and, thus, WABI values should be site-specifically calibrated according to well-known physiological stress indicators (such as g_s or Ψ).

5. Conclusions and outlook

The significance of combining leaf physiology, hyperspectral imaging and the PLS-R technique was demonstrated in this water stress study, with the development and implementation of three robust WABI models. These novel spectral indices – which seem simultaneously responsive to independent changes in chlorophyll fluorescence (530–550 nm) and water content (1500 nm) – were found superior to a variety of widely-used indices in predicting physiological values, under both greenhouse and field conditions. Nonetheless, the transition into commercial usage still requires the examination of the WABI models under various environments and growing stages. Additionally, WABI applicability from air- and space-borne remote sensing platforms requires proof and should be examined in future studies. Overall, since many plant species present a similar spectral–physiological response to water deficit, the suggested WABI models could prove to be a potent irrigation tool for many crops.

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