The potential of the spectral 'water balance index' (WABI) for crop irrigation scheduling

Tal Rapaport[1,2], Uri Hochberg[3], Amnon Cochavi[2], Arnon Karnieli[1] and Shimon Rachmilevitch[2]

[1]The Swiss Institute for Dryland Environmental and Energy Research, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Midreshet Ben-Gurion 84990, Israel; [2]The French Associates Institute for Agriculture and Biotechnology of Drylands, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Midreshet Ben-Gurion 84990, Israel; [3]The Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA

Summary

- Hyperspectral sensing can detect slight changes in plant physiology, and may offer a faster and nondestructive alternative for water status monitoring. This premise was tested in the current study using a narrow-band 'water balance index' (WABI), which is based on independent changes in leaf water content (1500 nm) and the efficiency of the nonphotochemical quenching (NPQ) photo-protective mechanism (531 nm).
- The hydraulic, photo-protective and spectral behaviors of five important crops – grapevine, corn, tomato, pea and sunflower – were evaluated under water deficit conditions in order to associate the differences in stress physiology with WABI suitability.
- Rapid alterations in both leaf water content and NPQ were observed in grapevine, pea and sunflower, and were effectively captured by WABI. Apart from water status monitoring, the index was also successful in scheduling the irrigation of a vineyard, despite phenological and environmental variability. Conversely, corn and tomato displayed a relatively strict stomatal regime and/or mild NPQ responses and were, thus, unsuitable for WABI-based monitoring.
- WABI shows great potential for irrigation scheduling of various crops, and has a clear advantage over spectral models that focus on either of the abovementioned physiological mechanisms.

Introduction

Water scarcity is a major restriction for worldwide agriculture, especially in arid and semi-arid regions, where it is the most common limiting factor for plant growth and development (Boyer, 1982; Morison *et al.*, 2008). With global climate models predicting an intensification in aridity on the one hand, and the rise in human population on the other (IPCC, 2007), the increasing pressure on water resources makes urgent the need to minimize the footprint of irrigated crops whilst maintaining high yield and quality (Cominelli *et al.*, 2009). To this end, an understanding of the underlying plant physiology mechanisms is essential, as they are the most preliminary and reliable indicators of crop water status changes and efficient water use (Chaves & Oliveira, 2004; Morison *et al.*, 2008). Nonetheless, physiological measurements might require intrusive equipment and proficient manpower, and can also be time- and cost-inefficient due to substantial variability in the field. A plausible solution to these issues is the implementation of suitable remote and proximal sensing instruments, which can offer a faster, inexpensive, broader and nondestructive alternative for assessing the physiology status of crops (Mulla, 2013); specifically, visible (VIS)-to-shortwave infrared (SWIR) hyperspectral spectrometers and cameras could serve as a replacement, as their sensors are sensitive enough to detect even slight

variations in numerous biochemical properties of leaves (Blackburn, 2007; Goetz, 2009; Jetz *et al.*, 2016). Over the years, such narrow-band VIS-to-SWIR tools have, indeed, been successful in tracking minor alterations in various physiological variables of plant vitality and productivity – such as water status, nutrient and pigment contents, and chlorophyll fluorescence – and significantly promoted the field of precision agriculture (Mulla, 2013).

Despite the vast progress, the full potential of hyperspectral sensing still remains to be fulfilled, as narrow-band datasets can substantially benefit from dimensionality reduction techniques (Thenkabail *et al.*, 2011; Mariotto *et al.*, 2013); in other words, as only a few of hundreds of wavelengths are, in fact, sensitive to biochemistry whereas the rest are redundant – negatively affecting the reliability of derived indices – optimal spectrum selection processes are essential. By now, a variety of imaging spectroscopy studies have managed to tackle this issue by employing advanced statistical methods, which have highlighted the most physiologically relevant wavebands and demonstrated the superiority of targeted indices over traditional and nonspecific spectral models (e.g. Hansen & Schjoerring, 2003; Renzullo *et al.*, 2006; Cho *et al.*, 2007; Blackburn & Ferwerda, 2008). In this regard, the recently published 'water balance index' (WABI; Rapaport *et al.*, 2015), a narrow-band, physiology-based product of 'partial least squares-regression' modeling (PLS-R; Geladi & Kowalski, 1986;

Wold *et al.*, 2001), shows promise for accurate tracking of water status changes in Cabernet Sauvignon grapevines (*Vitis vinifera* L.). In practice, this spectral model simultaneously monitors modifications in the water status of the leaf and related alterations in the efficiency of the nonphotochemical quenching (NPQ) photo-protective mechanism, which rapidly regulates the heat dissipation of excess, harmful light energy to maintain the functionality of the photosynthetic apparatus (Ort, 2001). These physiological traits are spectrally manifested by SWIR reflectance changes *c.* 1500 nm, which are directly (Sims & Gamon, 2003; Eitel *et al.*, 2006) and indirectly (Curran, 1989; Herrmann *et al.*, 2010) sensitive to leaf water content fluctuations and are found within an atmospheric window, and by VIS reflectance changes *c.* 531 nm, which are indicative of xanthophyll cycle epoxidation state transitions between the Violaxanthin (limiting light conditions) and Zeaxanthin (excessive light conditions) pigments (Gamon *et al.*, 1990, 1992).

Other than in Cabernet Sauvignon, the high importance of quick, reversible water status-associated NPQ responses was also demonstrated in numerous grapevine cultivars (Bota *et al.*, 2001; Flexas *et al.*, 2002) and various crop species, including sorghum (Cousins *et al.*, 2002), wheat (Lu & Zhang, 1999), barley (Robredo *et al.*, 2010), cotton (Zhang *et al.*, 2010), olive (Sofo *et al.*, 2009), peanut (Shahenshah & Isoda, 2010), soybean (Inamullah & Isoda, 2005) and sunflower (Tezara *et al.*, 1999). As both NPQ and water status changes are also spectrally detectable in the leaves of various plants via reflectance modifications *c.* 531 nm (Sims & Gamon, 2002) and 1500 nm (Sims & Gamon, 2003), the combination of these wavelengths as WABI may also prove to be a suitable irrigation tool for a wide variety of agricultural crops. Nevertheless, the function of the index cannot be blindly generalized due to the inherent variability in leaf physiology, anatomy and – consequently – spectral features (Blackburn, 2007), especially when considering species that respond to drought by maintaining a strict stomatal activity and stable water contents (Tardieu & Simonneau, 1998), or C_4 crops that are more resilient to photochemical perturbations (Brugnoli *et al.*, 1998). Furthermore, the actual capability of WABI to autonomously schedule irrigation under variable field conditions and throughout a prolonged water stress is as yet unknown, as previously it has been tested only on specific phenological stages and not throughout a continuous growing season (Rapaport *et al.*, 2015). Therefore, the main aims of the current study were (1) to test the potential of WABI in monitoring preliminary, water status-related physiological changes in the leaves of several important, widespread crops; and (2) to examine the consistency of WABI during an entire growing season and to assess its capability for spectral regulation of irrigation under changeable field conditions.

In order to accomplish the first objective, a short-term dehydration experiment was conducted under net house conditions with four agriculturally and economically important species – corn (*Zea mays* L.), pea (*Pisum sativum* L.), tomato (*Solanum lycopersicum* L.) and sunflower (*Helianthus annuus* L.) – that vary markedly in their hydraulic and photo-protective drought responses (Tardieu & Simonneau, 1998). At one end of the scale, the monocotyledonous, C_4 corn applies a strict stomatal regime

when facing relatively slight reductions in soil and leaf water contents (Tardieu *et al.*, 1993), maintains very high water-use efficiency and photochemical efficiency even under well-developed drought conditions, and may substantially increase heat dissipation through NPQ only under severe water deficit conditions (Xu *et al.*, 2008; Chen *et al.*, 2016). At the other end of the scale, the dicotyledonous, C_3 sunflower permits much looser stomatal control until it reaches much lower soil and leaf water content values (Tardieu *et al.*, 1996), experiences consequent rapid decreases in CO_2 assimilation and photosynthetic capacity, and requires the use of the NPQ mechanism in order to avoid photo-damage during moderate water stress (Maury *et al.*, 1996; Tezara *et al.*, 1999, 2002). It was hypothesized that WABI will prove more effective at monitoring preliminary water status changes in species with sharp hydraulic and photo-protective responses (such as sunflower) than in those that are more hydraulically and photo-protectively stable (such as corn). To accomplish the second objective, a long-term, seasonal-scale water stress experiment was conducted in a vineyard of Cabernet Sauvignon, one of the most widespread grapevine cultivars in the world. The idea was to maintain the grapevines at a moderate stress level, in which their NPQ responses are readily reversible and their photochemistry does not experience any irreversible adverse effects (Flexas *et al.*, 2000; Guan *et al.*, 2004; Dobrowski *et al.*, 2005), and wherein their grape quality characteristics are optimized in relation to yield reduction (Cifre *et al.*, 2005; Lovisolo *et al.*, 2010). It was hypothesized that WABI will be able to track slight water status variations throughout the continuous grape growing season and, therefore, consistently maintain the grapevines at their designated moderate water stress level.

Materials and Methods

Plant materials and growth conditions

The net house experiment was conducted during September 2014, at the Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Israel (30°51′N/34°46′E). Seedlings of corn (*Z. mays* L. cv Sweety), pea (*P. sativum* L. cv Karina), tomato (*S. lycopersicum* L. cv Charlotte) and sunflower (*H. annuus* L. cv D.Y.3) were grown in 10-l pots that contained a mixture of fine-clayey soil (55% clay, 25% silt and 20% sand) and potting media at a 2 : 1 (v/v) ratio. The pots were randomly sorted inside the net house, put at a sufficient distance from one another in order to minimize the shading effect of one plant over another, and irrigated daily up to their full capacity until the beginning of the trial. Throughout every midday (11:00–13:00 h) section of the experimentation period, the photosynthetic active radiation (PAR) flux range was 600–700 μmol photons m^{-2} s^{-1}, the relative humidity range was 35–45%, and the air temperature range was 28–31°C.

As WABI was shown to be sensitive to slight water status changes in the net house-grown sunflowers (see the Results section 'Net house experiment'), the suitability of the spectral model for this crop was also tested under field conditions. The experiment was conducted during the 2016 growing season, from

anthesis (May) to physiological maturity (mid-June), at a commercial plot in Patish, Israel (31°19′N/34°33′E). This study site is located within the semi-arid Northern Negev region, which is annually characterized by an average evapotranspiration of *c.* 1600 mm and an average precipitation (December–April) of *c.* 400 mm (Portnov & Safriel, 2004). Sunflower seeds (cv Sopremo) were sown in a sandy-loam soil (15% clay, 30% silt and 55% sand; east–west orientation; 1 m space between two adjacent plant rows) and the young plantlets were later thinned to an average of 3.5 plants m^{-1} at the fourth-true-leaf stage. Before the onset of the trial, irrigation was applied twice a week in accordance with the agricultural practice, using 2 l h^{-1} drippers. Throughout each midday section of the experimentation period, the ambient conditions were typical for the season, including a PAR flux range of 1900–2200 µmol photons m^{-2} s^{-1}, a relative humidity range of 20–50%, and an air temperature range of 26–41°C.

The vineyard experiment was conducted during the 2015 growing season, from anthesis (May) to harvest (late July), at the Ramat Negev Agro-Research and Development Center, Israel (30°98′N/34°70′E). This study site is located within the arid Negev Desert Highlands region, which is annually characterized by an average evapotranspiration of *c.* 1800 mm and an average precipitation of < 200 mm (Portnov & Safriel, 2004). The trial included 6-yr-old Cabernet Sauvignon grapevines at their third year of production, which were originally grafted onto Ruggeri-140 rootstocks (*Vitis berlandieri* × *Vitis rupestris*), planted in a silty-loam soil (15% clay, 50% silt and 35% sand; north–south orientation; 1.5 m apart within a 10-plant row; 3.5 m apart between two adjacent plant rows) and trained onto a vertical trellis (bilateral cordon 'T'). Throughout the growing season, irrigation was applied twice a week using 2 l h^{-1} drippers, with the watering amounts being determined by the evapotranspiration (ET$_C$) model of Netzer *et al.* (2009) and a dynamic irrigation factor (see the Materials and Methods section 'Experimental design'). Throughout each midday section of the experimentation period, the ambient conditions were typical for the season, including a PAR flux range of 1800–2150 µmol photons m^{-2} s^{-1}, a relative humidity range of 20–39%, and an air temperature range of 26–40°C.

Experimental design

The net house experiment began 3 wk after planting and consisted of eight plants per species. While four plants per species were continuously irrigated and regarded as the control (C) group, a terminal water deficit (WD) was imposed on the other four up to the point of wilting, which was assessed visually. Throughout the trial, the stress level of both treatment groups was quantified daily by taking midday measurements of leaf relative water content (RWC), stomatal conductance (g$_s$), net CO$_2$ assimilation (A$_N$) and NPQ, together with complementary hyperspectral readings. All measurements were performed on a single sun-exposed, youngest matured leaf per plant, under clear skies.

The sunflower field experiment began after the opening of the flower buds and included 15 inner plants from a single row in the center of the plot, which was utterly disconnected from the irrigation system in order to impose a terminal water deficit. During the trial, the progressing stress level of the sunflowers was quantified on a weekly basis through midday RWC assessments and complementary hyperspectral readings. All measurements were performed on a single sun-exposed, youngest matured leaf per plant, under clear skies.

The vineyard experiment was conducted on 18 plants from three rows in the center of the field (i.e. six inner plants from each grapevine row) and consisted of two stages. In the first stage, which lasted for *c.* 3 wk, the goal was to establish a robust correlation between midday values of WABI and of stem water potential (Ψ$_S$) – a common parameter for water status detection in grapevine (Chone *et al.*, 2001; Acevedo-Opazo *et al.*, 2012) – under conditions of a moderate water deficit (i.e. *c.* −1.0 MPa; De Bei *et al.*, 2011). Technically, this was done by applying an irrigation factor of 0.25 × ET$_C$ whenever Ψ$_S$ > −0.9 MPa, in order to decrease the water potential towards −1.0 MPa; an irrigation factor of 0.50 × ET$_C$ whenever −1.1 MPa < Ψ$_S$ < −0.9 MPa, in order to sustain that stress degree; and an irrigation factor of 0.75 × ET$_C$ whenever Ψ$_S$ < −1.1 MPa, in order to increase the water potential towards −1.0 MPa. Overall, during that period, the WABI–Ψ$_S$ correlation was established for stem water potential values ranging between −0.8 MPa and −1.2 MPa. Thereafter, during the second stage of the trial (until harvest), irrigation was applied in a similar manner, except that the Ψ$_S$ values were spectrally predicted based on the spectral–physiological regression. Due to the regularly high evaporative demands in the vineyard, the consequent quick changes in leaf water status, and the relatively narrow range of Ψ$_S$ that was required to be maintained throughout the trial, physiological and hyperspectral measurements were taken twice a week and performed on a single sun-exposed, youngest matured leaf per plant, under clear skies.

Physiological and spectral measurements

Leaf RWC measurements were based on a slightly altered version of the protocol of Chen *et al.* (2016); namely, the fresh weight (FW) of the leaf was recorded immediately after its excision, the turgid weight (TW) was recorded after a 4-h immersion in distilled water, and the dry weight (DW) was recorded after 48-h drying in an oven at 65°C:

$$RWC(\%) = \frac{FW - DW}{TW - DW} \times 100 \qquad \text{Eqn 1}$$

The g$_s$ (presented in units of mol H$_2$O m^{-2} s^{-1}) and A$_N$ (presented in units of µmol CO$_2$ m^{-2} s^{-1}) gas exchange parameters were obtained using a portable LI-6400 Infrared Gas Analyzer (Li-Cor Inc., Lincoln, NB, USA), using a constant CO$_2$ concentration of 400 µmol mol^{-1}, PAR flux density of 650 µmol photons m^{-2} s^{-1}, relative humidity of 35% and temperature of 29°C.

NPQ calculations (Bilger & Bjorkman, 1990) were derived from measurements of maximum fluorescence of dark-adapted-

(F_m; 30-min darkening) and light-adapted (F'_m) samples, which were acquired by applying saturating pulses with a Mini-PAM Photosynthesis Yield Analyzer (Heinz Walz GmbH, Effeltrich, Bavaria, Germany):

$$NPQ = \frac{F_m - F'_m}{F'_m} \qquad \text{Eqn 2}$$

Measurements of Ψ_S (presented in units of MPa) were conducted with an Arimad-3000 Pressure Chamber (MRC Inc., Holon, Israel), using the protocol of Turner (1988); in short, a leaf was first covered for 1 h with aluminum foil over a plastic bag, before it was excised and immediately placed in the chamber with its petiole protruding out. The pressure on the lamina was then gradually increased until the emergence of sap from the tip of the petiole.

Hyperspectral signatures of the adaxial side of leaves were obtained with a FieldSpec Pro-FR Portable Spectrometer (Analytical Spectral Devices Inc., Boulder, CO, USA) equipped with an 1800-12S Integrating Sphere (Li-Cor Inc., Lincoln, NE, USA), as described by Herrmann *et al.* (2017). The system was set to measure the reflected radiant flux within the 400–1600 nm spectrum at a 1-nm wavelength resolution, and the energy readings were then normalized into a reflectance (ρ) base by taking supplementary measurements of $BaSO_4$ 'white reference' standards. WABI calculations were performed according to the formulation of Rapaport *et al.* (2015), which implies an increase in value as leaf RWC decreases (causing reduced absorption and increased reflectance *c.* 1500 nm) and/or as light energy is in excess (the de-epoxidation of Violaxanthin into Zeaxanthin causes a reduction in reflectance *c.* 531 nm; Gamon *et al.*, 1990), and vice versa:

$$WABI = \frac{\rho_{1500} - \rho_{531}}{\rho_{1500} + \rho_{531}} \qquad \text{Eqn 3}$$

In this context, it is important to note that the normalization of the reflectance at 531 nm to that of potential reference wavelengths *c.* 540–600 nm – which was suggested by Gamon *et al.* (1992) in order to minimize the effects of overlapping spectral features on the xanthophyll signal – was not beneficial in the current study; this is because the stressing agent produced a change in ρ for most of the green band (see Figs 5, 8 and 10) and, thus, no wavelength within the 540–600 nm spectral region was idle and could be used as a reference. This general 'green shift' suggests that other chloroplast-related physiological processes – unrelated to xanthophyll activity – are at work and, consequently, the 531 nm signals of the current study might not be exclusively associated with NPQ changes (Gamon *et al.*, 1990). Additionally, it is noteworthy that the substitution of the 1500 nm component with a wavelength closer to 1600 nm is potentially useful for remote sensing purposes, as the latter may further reduce atmospheric interferences. Nonetheless, as the proximal sensing measurements of the current study were performed at a minimal distance from the leaf, and because the physiological suitability of

the 1500 nm region was found to be greater – the shorter wavelength was ultimately chosen for the model.

Statistical analyses

Physiological and spectral differences were examined with one-way analysis of variation (ANOVA) models, followed by Tukey's honest significant difference (HSD) post-hoc tests (Tukey, 1949). Before each comparison, the necessary assumptions of residuals' normality (Shapiro & Wilk, 1965) and homogeneity of variances (homoscedasticity; Bartlett, 1937) among the groups were checked and met. Linear and nonlinear regression models were also preceded by tests for the assumptions of residuals' normality, independence and homoscedasticity. Analyses of the ANOVA models and of the correlations were performed with STATISTICA 12 (Statsoft Inc., Tulsa, OK, USA) and were considered significant at $P < 0.05$.

Results

Net house experiment

Withholding irrigation from the WD groups of all species ultimately led to their wilting, after variable drought periods (Table 1). Specifically, the pea and tomato plants wilted after 4 d and presented final RWC values (Fig. 1) of 86.93% and 71.01%, respectively (C = 94.12% and 93.87%, respectively), the sunflower plants wilted after 5 d and displayed a final RWC of 77.61% (C = 92.90%), and the corn plants wilted after 10 d and exhibited a final RWC of 85.93% (C = 94.30%). Throughout the trial, the decrease in leaf water content was accompanied by a reduction in g_s (Fig. 2a), with final values of 0.05 mol H_2O m^{-2} s^{-1} (C = 0.26 mol H_2O m^{-2} s^{-1}) for pea, 0.03 mol H_2O m^{-2} s^{-1} (C = 0.25 mol H_2O m^{-2} s^{-1}) for tomato, 0.06 mol H_2O m^{-2} s^{-1} (C = 0.70 mol H_2O m^{-2} s^{-1}) for sunflower, and 0.04 mol H_2O m^{-2} s^{-1} (C = 0.12 mol H_2O m^{-2} s^{-1}) for corn. Following the decrease in stomatal conductance, a consequent reduction in A_N was also observed during the experiment (Fig. 2b), with final values of 3.26 µmol CO_2 m^{-2} s^{-1} (C = 11.12 µmol CO_2 m^{-2} s^{-1}) for pea, 1.96 µmol CO_2 m^{-2} s^{-1} (C = 13.93 µmol CO_2 m^{-2} s^{-1}) for tomato, 8.90 µmol CO_2 m^{-2} s^{-1} (C = 18.42 µmol CO_2 m^{-2} s^{-1}) for sunflower, and 4.96 µmol CO_2 m^{-2} s^{-1} (C = 23.81 µmol CO_2 m^{-2} s^{-1}) for corn. It should be noted that the dehydration patterns of the species cannot be compared on a temporal scale, as besides being influenced by their stomatal conductance, the water status of the plants was also affected by their capacitance and total canopy area – which were not assessed in this experiment.

Nonetheless, when the stomatal behavior of the net house species was normalized to their leaf water content (Fig. 3) – cancelling the leaf area factor – remarkable differences in water management were revealed. On the one hand, the drying corn leaves presented a low and constant stomatal conductance (an insignificant decrease down to 0.10 mol H_2O m^{-2} s^{-1} on Day 6), which allowed them to maintain the highest RWC values of all species throughout the trial (an insignificant decrease down to 89.85%

Table 1 Statistical data for the physiological variables of the water deficit groups throughout the net house experiment

Species	Physiological variable	Control	Moderate stress	Severe stress (wilting)	F-test
Corn	RWC (%)	93.26 ± 2.47	89.90 ± 2.02 (Day 4) $P > 0.05$	85.93 ± 3.84 (Day 10) $P < 0.05$	$F_{(2,9)} = 6.48$ $P < 0.05$
	g_s (mol H_2O m^{-2} s^{-1})	0.13 ± 0.02	0.10 ± 0.03 (Day 6) $P > 0.05$	0.04 ± 0.01 (Day 10) $P < 0.01$	$F_{(3,12)} = 13.58$ $P < 0.01$
	A_N (µmol CO_2 m^{-2} s^{-1})	24.22 ± 2.32	20.47 ± 2.17 (Day 6) $P > 0.05$	4.96 ± 1.89 (Day 10) $P < 0.01$	$F_{(3,12)} = 74.70$ $P < 0.01$
	NPQ	2.68 ± 0.18	2.93 ± 0.32 (Day 6) $P > 0.05$	3.28 ± 0.27 (Day 10) $P < 0.05$	$F_{(3,12)} = 2.96$ $P > 0.05$
Pea	RWC (%)	95.40 ± 3.57	90.31 ± 3.27 (Day 3) $p > 0.05$	86.93 ± 4.34 (Day 4) $P < 0.05$	$F_{(2,9)} = 5.15$ $P < 0.05$
	g_s (mol H_2O m^{-2} s^{-1})	0.29 ± 0.07	0.08 ± 0.03 (Day 3) $P < 0.01$	0.05 ± 0.02 (Day 4) $P < 0.01$	$F_{(2,9)} = 31.13$ $P < 0.01$
	A_N (µmol CO_2 m^{-2} s^{-1})	10.92 ± 1.79	6.59 ± 1.72 (Day 3) $P < 0.01$	3.26 ± 0.86 (Day 4) $P < 0.01$	$F_{(2,9)} = 25.64$ $P < 0.01$
	NPQ	2.99 ± 0.29	–	3.98 ± 0.28 (Day 4) $P < 0.01$	$F_{(1,6)} = 22.24$ $P < 0.01$
Tomato	RWC (%)	92.43 ± 2.76	78.23 ± 5.35 (Day 3) $P < 0.01$	71.01 ± 6.25 (Day 4) $P < 0.01$	$F_{(2,9)} = 18.95$ $P < 0.01$
	g_s (mol H_2O m^{-2} s^{-1})	0.24 ± 0.08	0.09 ± 0.04 (Day 3) $P < 0.01$	0.03 ± 0.01 (Day 4) $P < 0.01$	$F_{(2,9)} = 19.09$ $P < 0.01$
	A_N (µmol CO_2 m^{-2} s^{-1})	14.95 ± 2.99	3.37 ± 1.24 (Day 3) $P < 0.01$	1.96 ± 1.79 (Day 4) $P < 0.01$	$F_{(2,9)} = 44.55$ $P < 0.01$
	NPQ	2.46 ± 0.25	–	2.76 ± 0.18 (Day 4) $P > 0.05$	$F_{(1,6)} = 3.80$ $P > 0.05$
Sunflower	RWC (%)	94.18 ± 4.26	83.87 ± 2.55 (Day 4) $P < 0.05$	77.61 ± 6.22 (Day 5) $P < 0.01$	$F_{(2,9)} = 13.27$ $P < 0.01$
	g_s (mol H_2O m^{-2} s^{-1})	0.75 ± 0.11	0.12 ± 0.02 (Day 4) $P < 0.01$	0.06 ± 0.01 (Day 5) $P < 0.01$	$F_{(3,12)} = 53.92$ $P < 0.01$
	A_N (µmol CO_2 m^{-2} s^{-1})	18.75 ± 2.43	10.71 ± 2.93 (Day 4) $P < 0.05$	8.90 ± 3.32 (Day 5) $P < 0.01$	$F_{(3,12)} = 8.11$ $P < 0.01$
	NPQ	2.87 ± 0.29	–	3.87 ± 0.27 (day 5) $P < 0.01$	$F_{(1,6)} = 25.29$ $P < 0.01$

Values of leaf relative water content (RWC), stomatal conductance (g_s), net CO_2 assimilation (A_N) and nonphotochemical quenching (NPQ) represent averages of four samples $\pm$ SD. The significance values (P) in the moderate and severe stress columns are a product of Tukey's honest significant difference test (Tukey, 1949), and relate to the difference between each of these deficit levels and the control stage.
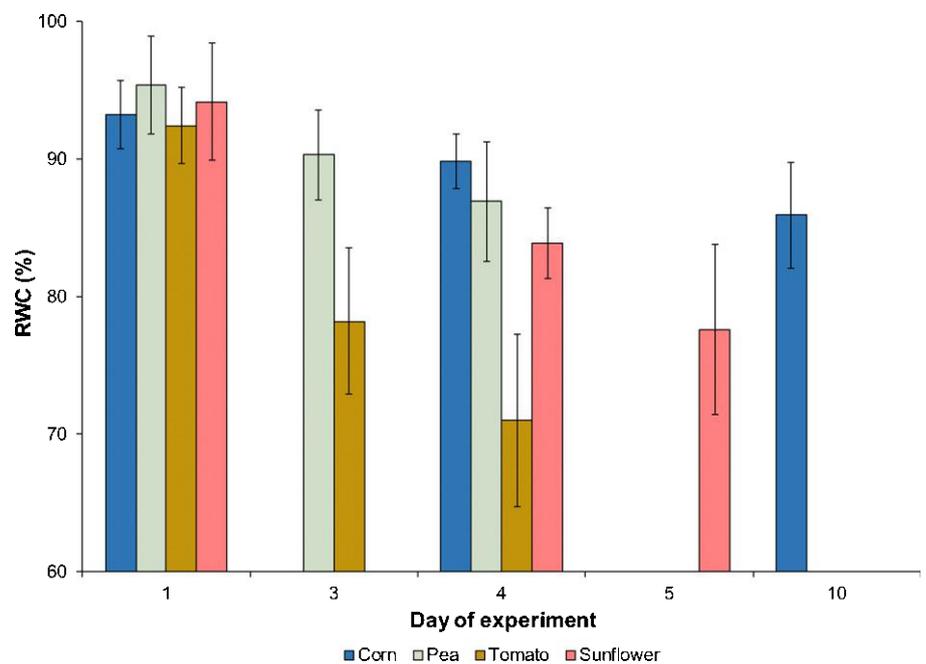

Fig. 1 Leaf relative water content (RWC) changes in the water deficit treatment groups throughout the net house experiment. Each column represents an average of four replicates $\pm$ SD.

on Day 4). On the other hand, the WD sunflower leaves presented the highest g_s from the beginning of the experiment (0.75 mol H_2O m^{-2} s^{-1}) until just before the point of wilting (0.12 mol H_2O m^{-2} s^{-1} on Day 4), leading to a significant RWC decline (down to 83.87% on Day 4). In between these ends, the stomatal behavior of the stressed pea and tomato groups were fairly similar throughout the trial, presenting a significant g_s reduction (down to 0.08 and 0.09 mol H_2O m^{-2} s^{-1} on Day 3, respectively) that was stronger than that of corn yet weaker than that of sunflower. Nevertheless, pea demonstrated a relatively conservative water status (with an insignificant decrease down to

90.31% on Day 3), making its hydraulic behavior more similar to that of corn, whereas tomato showed a much higher desiccation rate (with a significant decrease down to 78.23% on Day 3) and a hydraulic behavior that was closer to that of sunflower.

With their decline in leaf water content and gas exchange rates, all species but tomato ultimately presented a significant increase in NPQ (Fig. 4). In particular, the average values at the wilting point of corn, pea and sunflower were 3.28 (C = 2.53), 3.98 (C = 2.84) and 3.81 (C = 2.91), respectively, whereas that of tomato was merely 2.76 (C = 2.49). From a spectral perspective, these NPQ increases were not necessarily accompanied by a
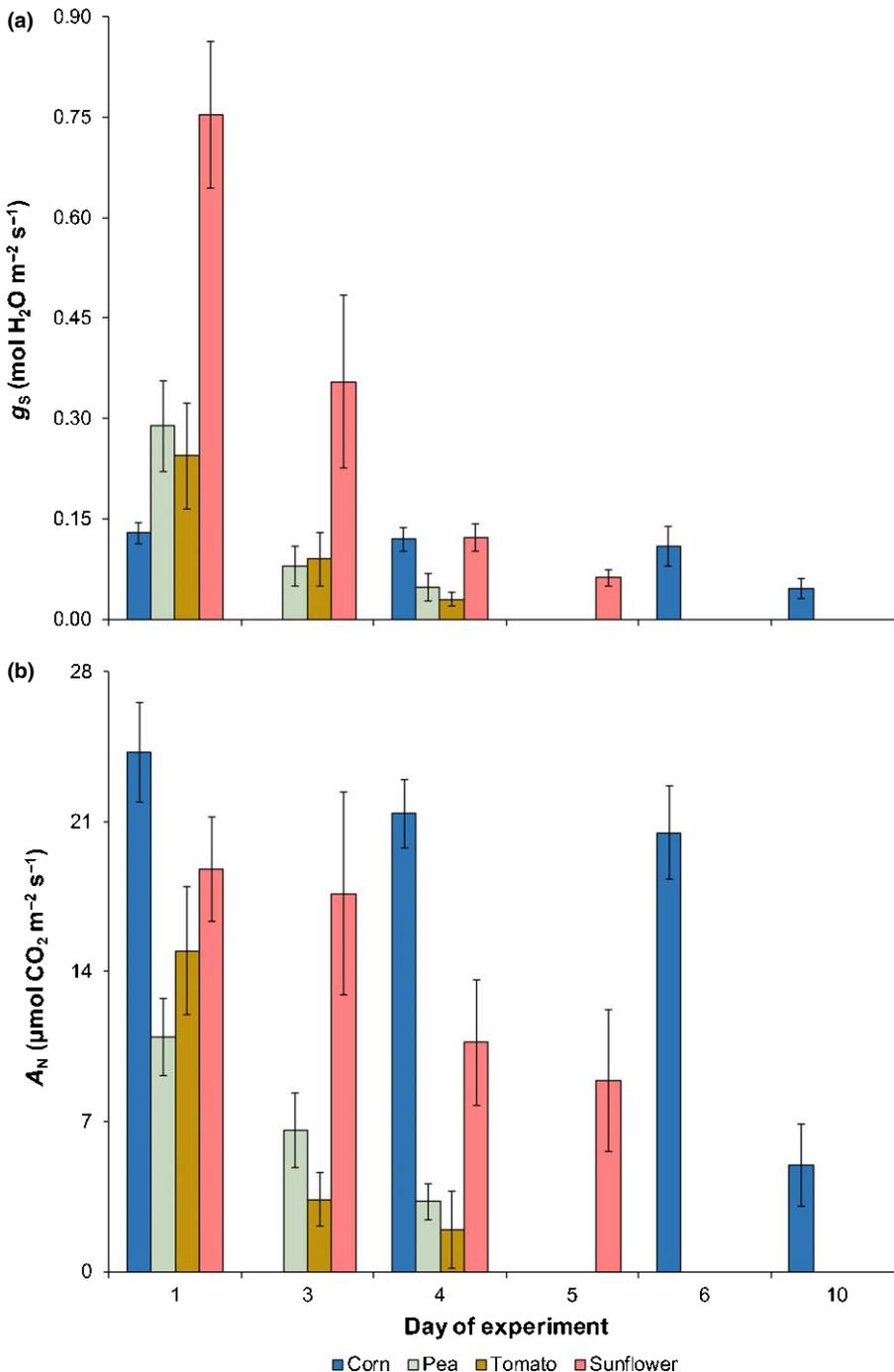

Fig. 2 (a) Leaf stomatal conductance (g_s) and (b) net CO_2 assimilation (A_N) changes in the water deficit treatment groups throughout the net house experiment. Each column represents an average of four replicates ± SD.
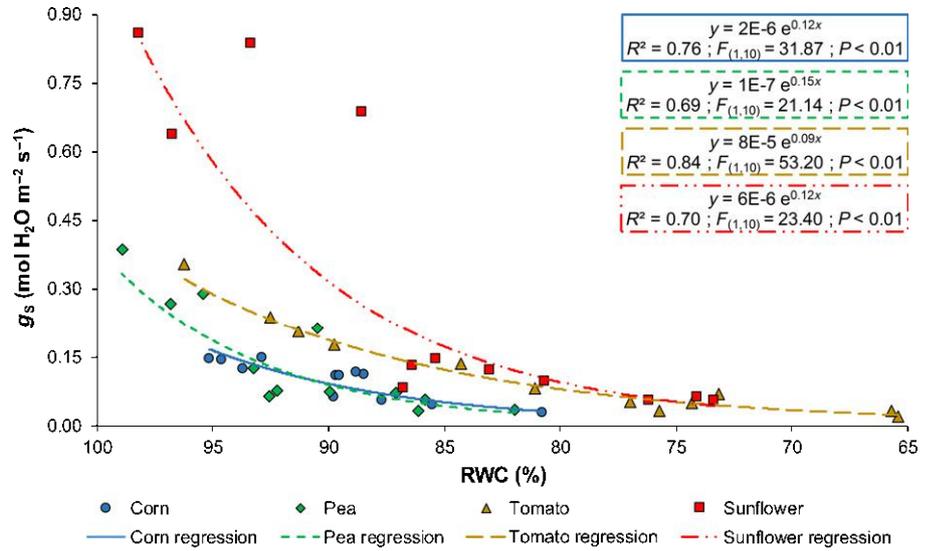
Fig. 3 Stomatal behavior of the water deficit treatment groups throughout the net house experiment. Each regression between the stomatal conductance (g_s) and relative water content (RWC) variables stretches from the fully turgid stage up to the wilting point.
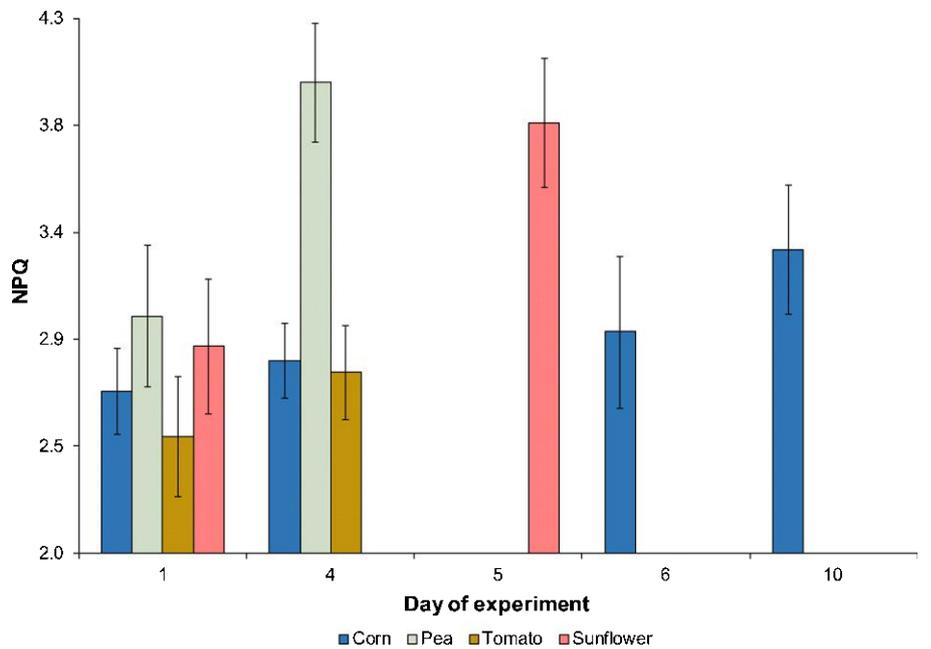

Fig. 4 Nonphotochemical quenching (NPQ) changes in the water deficit treatment groups throughout the net house experiment. Each column represents an average of four replicates $\pm$ SD.

reflectance decrease trend $c.$ 531 nm (Table 2); namely, whereas sunflower (Fig. 5a) and pea (Fig. 5b) showed a 1.56% significant decrease and a 1.22% insignificant decrease until their respective wilting points, corn (Fig. 6a) and tomato (Fig. 6b) presented a constant increase along the entire VIS spectrum. The RWC decreases were also not necessarily accompanied by significant reflectance increase patterns $c.$ 1500 nm; namely, whereas sunflower demonstrated a significant ρ rise of 2.21% even at the mild–moderate WD stage, and pea exhibited a significant increase of up to 3.62% at its wilting point, tomato and corn exhibited 2.49% and 0.49% insignificant increases until their respective wilting points.

Following these spectral outlines, WABI was successful in differentiating between the sunflower treatment groups even at the moderate stress stage (0.10 index unit change) and between the pea treatment groups at the severe stress stage (0.13 index

unit change; Table 2). Although the reflective patterns of tomato and corn were generally unfavorable, the index was still capable of differing between the control and severe stress groups of the latter species (0.10 index unit change) due to its relatively large changes at 531 nm. All three physiological variables were also found to be best correlated with the sunflower and pea WABI models. Specifically, in the case of the RWC measure (Fig. 7a), sunflower and pea presented coefficients of determination (R^2) of 0.83 and 0.73, respectively, whereas tomato and corn displayed respective values of 0.30 and 0.37; in the case of the g_s measure (Fig. 7b), the equivalent R^2 values were sunflower 0.69, pea 0.71, tomato 0.22 and corn 0.28; in the case of the NPQ measure (Fig. 7c), the equivalent R^2 values were sunflower 0.63, pea 0.53, tomato 0.17 and corn 0.24. The spectral-physiological regressions of sunflower and pea were also spanned across wider WABI value ranges of 0.22

Table 2 Statistical data for the spectral variables of the water deficit groups throughout the net house experiment

Species	Spectral variable	Control	Moderate stress	Severe stress (wilting)	F-test
Corn	ρ 531 nm (%)	10.55 ± 0.69	11.57 ± 0.83 (Day 6) $P > 0.05$	13.35 ± 0.93 (Day 10) $P < 0.01$	$F_{(3,12)} = 9.33$ $P < 0.01$
	ρ 1500 nm (%)	14.78 ± 0.64	15.02 ± 0.76 (Day 6) $P > 0.05$	15.27 ± 0.41 (Day 10) $P > 0.05$	$F_{(3,12)} = 0.54$ $P > 0.05$
	WABI	0.17 ± 0.04	0.13 ± 0.03 (Day 6) $P > 0.05$	0.07 ± 0.03 (Day 10) $P < 0.01$	$F_{(3,12)} = 6.30$ $P < 0.01$
Pea	ρ 531 nm (%)	14.07 ± 0.16	13.50 ± 0.88 (Day 3) $P > 0.05$	12.85 ± 0.99 (Day 4) $P > 0.05$	$F_{(2,9)} = 2.49$ $P > 0.05$
	ρ 1500 nm (%)	19.24 ± 0.37	20.75 ± 1.30 (Day 3) $P > 0.05$	22.86 ± 0.82 (Day 4) $P < 0.01$	$F_{(2,9)} = 15.85$ $P < 0.01$
	WABI	0.15 ± 0.02	0.21 ± 0.02 (Day 3) $P > 0.05$	0.28 ± 0.05 (Day 4) $P < 0.01$	$F_{(2,9)} = 15.72$ $P < 0.01$
Tomato	ρ 531 nm (%)	10.33 ± 0.60	10.63 ± 0.32 (Day 3) $P > 0.05$	10.82 ± 0.55 (Day 4) $P > 0.05$	$F_{(2,9)} = 0.94$ $P > 0.05$
	ρ 1500 nm (%)	15.55 ± 0.39	16.50 ± 1.54 (Day 3) $P > 0.05$	18.04 ± 0.04 (Day 4) $P > 0.05$	$F_{(2,9)} = 3.40$ $P > 0.05$
	WABI	0.21 ± 0.04	0.21 ± 0.05 (Day 3) $P > 0.05$	0.25 ± 0.04 (Day 4) $P > 0.05$	$F_{(2,9)} = 1.29$ $P > 0.05$
Sunflower	ρ 531 nm (%)	12.54 ± 0.63	11.70 ± 0.63 (Day 4) $P > 0.05$	10.98 ± 0.65 (Day 5) $P < 0.05$	$F_{(3,12)} = 4.37$ $P < 0.05$
	ρ 1500 nm (%)	16.01 ± 0.99	18.22 ± 0.61 (Day 4) $P < 0.05$	19.42 ± 1.49 (Day 5) $P < 0.01$	$F_{(3,12)} = 8.97$ $P < 0.01$
	WABI	0.12 ± 0.02	0.22 ± 0.04 (Day 4) $P < 0.01$	0.28 ± 0.04 (Day 5) $P < 0.01$	$F_{(3,12)} = 16.35$ $P < 0.01$

Values of reflectance (ρ) at 531 and 1500 nm and of the 'water balance index' (WABI) represent averages of four samples $\pm$ SD. The significance values (P) in the moderate and severe stress columns are a product of Tukey's honest significant difference test (Tukey, 1949), and relate to the difference between each of these deficit levels and the control stage.

and 0.19, respectively, in comparison to the respective 0.12 and 0.17 ranges of tomato and corn.

Sunflower field experiment

Following the net house results, the applicability of WABI was also tested in a commercial sunflower field. Throughout the trial, the continuous depletion of soil moisture significantly decreased the leaf RWC average from 93.15% on Day 1 to 84.93% on Day 14, and ultimately caused wilting at 76.91% on Day 21 (Table 3). Spectrally, the drying of the plants was accompanied by VIS and SWIR reflectance patterns that were similar to those of the net house experiment (Fig. 8); namely, a significant reduction of 0.91% was observed at 531 nm by the point of wilting, whereas a significant increase of 1.12% was observed at 1500 nm even at the moderate stress stage, on Day 14. Consequently, the WABI model also showed a significant increase of 0.40 index values even after 2 wk, and displayed a strong correlation to leaf RWC up to the wilting of the plants (Fig. 9). It is important to note that this spectral–physiological regression was, however, substantially different from that of the net house trial, which was spanned across a much wider WABI value range.

Vineyard experiment

During the first 3 wk of the trial, changes in the stem water potential values of the Cabernet Sauvignon grapevines were accompanied by the expected alterations in both VIS and SWIR reflectance; an example from this training period of WABI (Fig. 10) demonstrates that a transition from -0.88 MPa ($\Psi_S > -0.9$ MPa; a degree that is above the optimal stress range and requires a low irrigation amount) to -1.11 MPa ($\Psi_S < -1.1$ MPa; a degree that is below the optimal stress range and requires a high irrigation amount) was followed by significant ρ changes of 1.67% and 1.90% at 531 and 1500 nm, respectively (Table 4). Consequently, a significant difference of 0.10 was found between the WABI values of these two deficit degrees – enabling the spectral model to effectively distinguish between them and, thus, to potentially determine the irrigation amounts that are required to maintain the plants at their designated stress range (-1.1 MPa $< \Psi_S < -0.9$ MPa). Furthermore, the readily reversible nature of the 531 and 1500 nm wavelengths resulted in a strong, robust, negative linear correlation ($R^2 = 0.92$) between the values of Ψ_S (ranging from -0.8 to -1.2 MPa) and those of WABI (ranging from 0.34 to 0.43) during this first part of the experiment (Fig. 11).

Using this statistical relation, it was decided to set the 0.36 and 0.42 index values (representing the -0.9 and -1.1 MPa limits, respectively) as the spectral irrigation thresholds for the rest of the growing season, throughout the second stage of the trial; namely, from the end of WABI's training period and until the harvest, the vineyard was irrigated with the low, $0.25 \times ET_C$ level in case an average index value of 0.36 or lower (i.e., $\Psi_S > -0.9$ MPa) was measured; irrigated with the common, $0.50 \times ET_C$ level in case an average index value of $0.36 < WABI < 0.42$ (i.e., -1.1 MPa $< \Psi_S < -0.9$ MPa) was measured; and
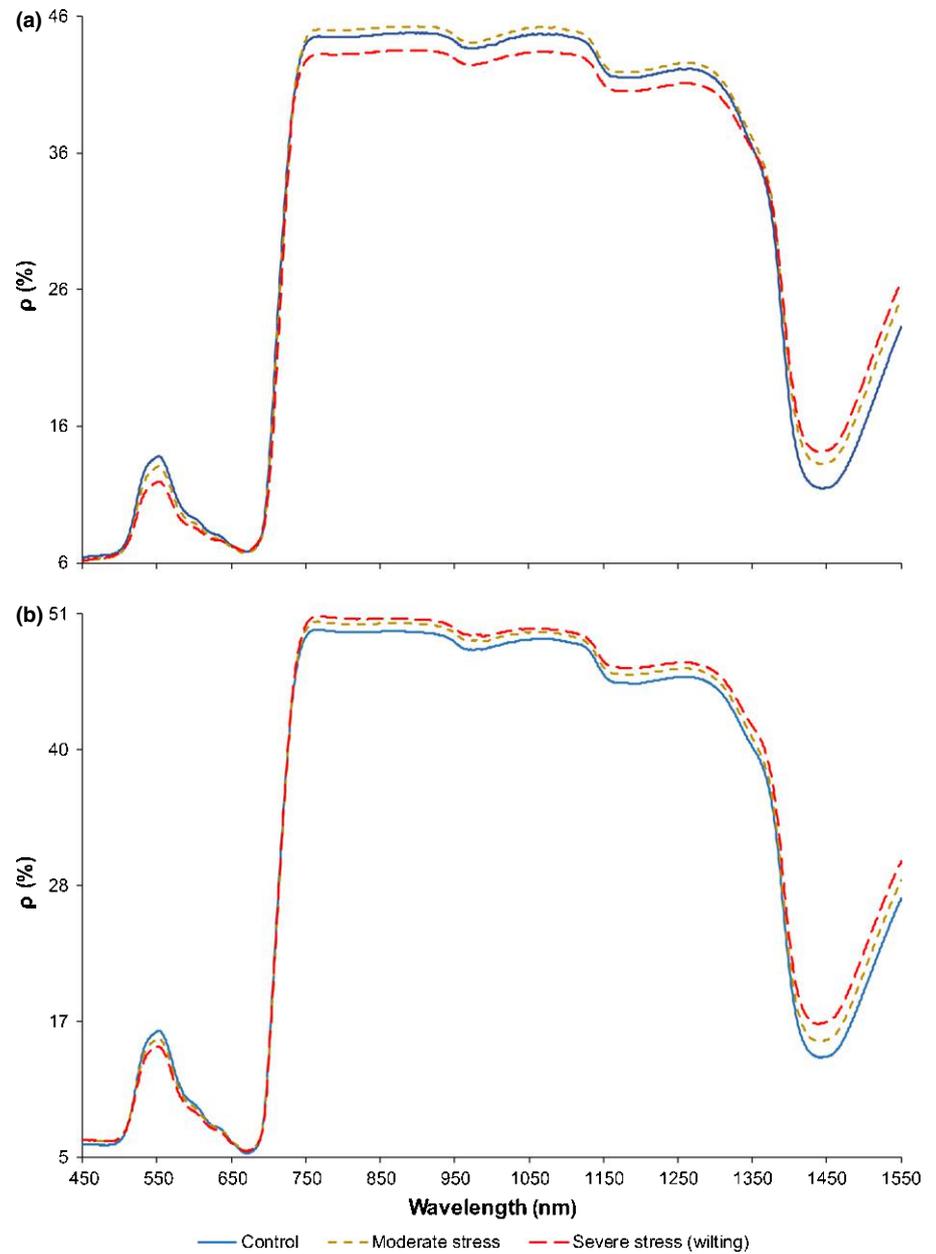
Fig. 5 Reflectance (ρ) changes of the (a) sunflower and (b) pea water deficit treatment groups relative to wavelength throughout the net house experiment. Each graph represents an average signature of four replicates.

irrigated with the high, $0.75 \times ET_C$ level in case an average index value of 0.42 or higher (i.e., $\Psi_S < -1.1$ MPa) was measured. Interestingly, the regression between the WABI values and the actual Ψ_S values in the second stage of the experiment was very similar to that of the first stage (Fig. 11), implying that the reflectance responses to NPQ and leaf RWC were consistent during the entire growing season. The vast majority of the predictions made by the index thresholds (Fig. 12a) was, thus, successful in identifying the correct physiological stress degree (Fig. 12b). Indeed, WABI mistakenly predicted a Ψ_S value that required a common ($0.5 \times ET_C$) irrigation amount, instead of a low (-0.88 MPa) and high (-1.12 MPa) degree, respectively, on only two occasions out of seventeen, on Julian days 174 and 192. It should also be noted that – as in the abovementioned case of the net house- and field-grown sunflowers – the WABI–Ψ_S

vineyard correlations of the current study were different from the glasshouse-based Cabernet Sauvignon regression of Rapaport *et al.* (2015) (which was originally based on leaf water potential values and adapted through the linear model of Williams & Araujo (2002); Fig. 11).

Discussion

In the current study, the narrow-band 'water balance index' (WABI) demonstrated its abilities to: (1) monitor preliminary water status changes in net house-grown sunflower and pea plants; (2) sensitively track water status alterations in field-grown sunflowers; and (3) autonomously schedule the irrigation of a vineyard throughout an entire growing season. Those achievements were a product of the combination between the 531 and
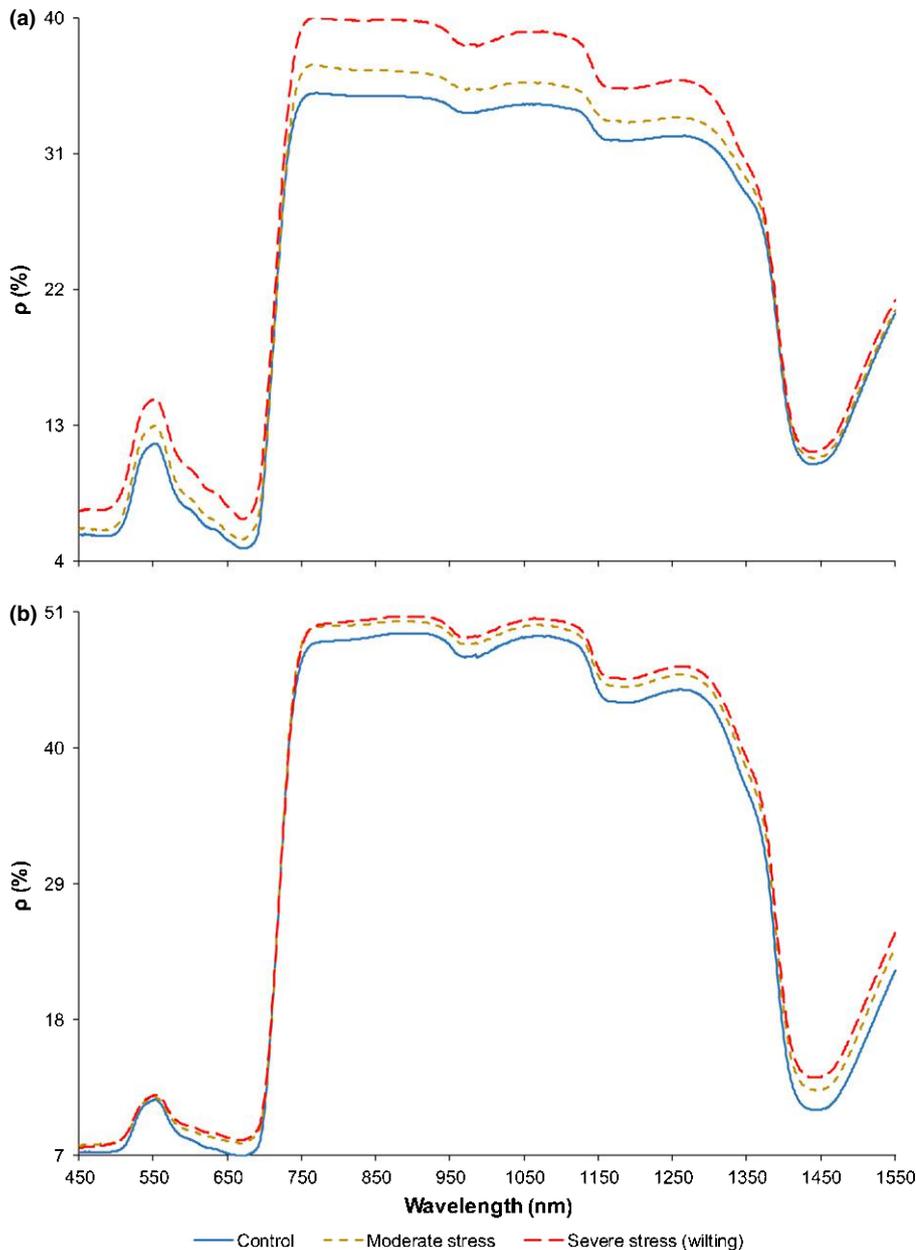
Fig. 6 Reflectance (ρ) changes of the (a) corn and (b) tomato water deficit treatment groups relative to wavelength throughout the net house experiment. Each graph represents an average signature of four replicates.

1500 nm wavelengths, representing two physiological mechanisms that are rapidly reactive within the operating range of the abovementioned crops, independent of one another, and stable through environmental and phenological changes. As previously shown by Rapaport *et al.* (2015), the dual-response structure of WABI has a clear advantage over many other spectral models, including those that focus only on either nonphotochemical quenching (NPQ) or leaf water status variations. For instance, although WABI was able to detect the development of moderate water stress in both the net house- and field-grown sunflowers, the 531 nm-exclusive 'physiological reflectance index' (PRI; Gamon *et al.*, 1992) failed to do so (Supporting Information Table S1); this is because the NPQ-related reflectance decrease of this plant (Gamon *et al.*, 1990; Gamon & Surfus, 1999; Magney *et al.*, 2014) was significant

enough only during severe deficit, close to the points of wilting and irreversible damage, whereas the loose stomatal regime and, thus, the rapid leaf dehydration of the crop (Tardieu *et al.*, 1996; Tardieu & Simonneau, 1998) caused a significant shortwave infrared (SWIR) reflectance increase much earlier. Similarly, WABI could outperform a 1500 nm-exclusive index throughout the vineyard experiment, by consistently showing a higher sensitivity to modifications in the water status of the plant (Table 4); this can be explained by mild relative water content (RWC) alterations, which generally characterize grapevine leaves within the narrow deficit range of the trial ($-1.2 \, MPa < \Psi_S < -0.8 \, MPa$; Cifre *et al.*, 2005) and lead to minimal SWIR changes, and by substantial NPQ and 531 nm reflectance variations that occur even during small water status alterations (Evain *et al.*, 2004; Dobrowski *et al.*, 2005).
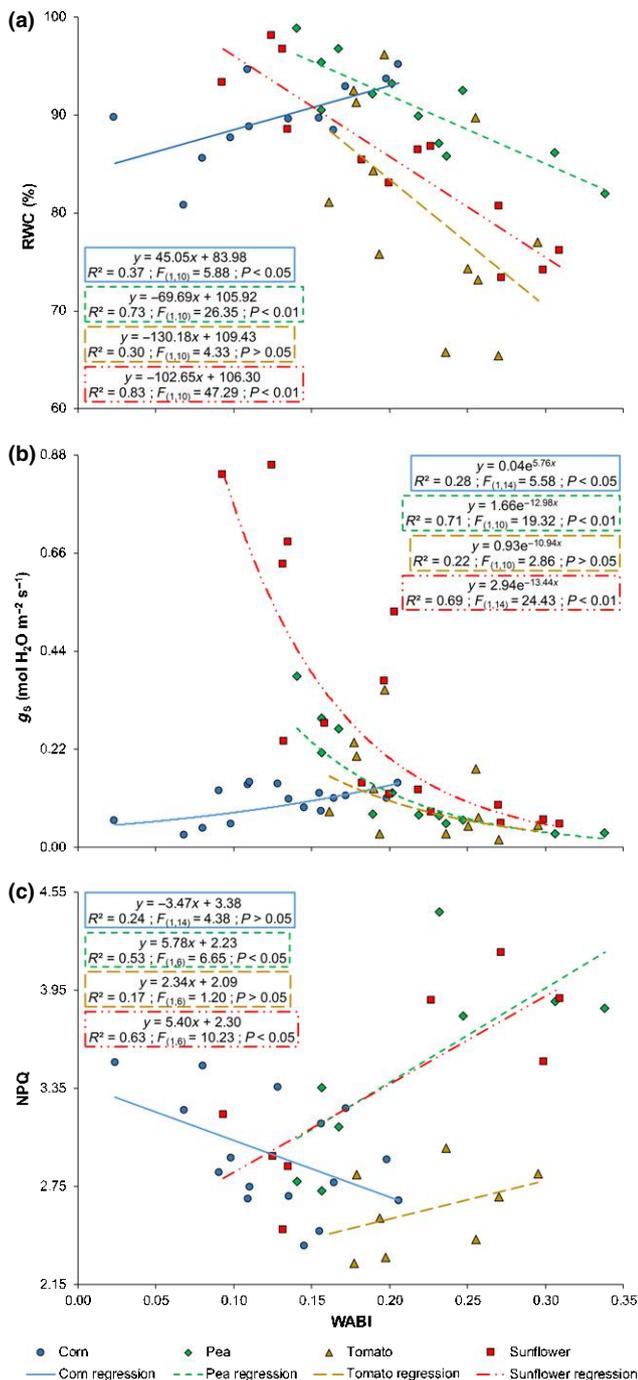
Fig. 7 Spectral–physiological correlations of the water deficit treatment groups throughout the net house experiment. Each regression between the (a) relative water content (RWC), (b) stomatal conductance (g_s) and (c) nonphotochemical quenching (NPQ) variables and the 'water balance index' (WABI) variable stretches from the fully turgid stage up to the wilting point.

supported in the current study by the stress-induced physiological (Figs 1–4; Table 1) and spectral features (Figs 6, 7; Table 2) of both corn and tomato. Regarding corn, the tight stomatal regime of the plant (Tardieu et al., 1993; Tardieu & Simonneau, 1998) led to an insignificant SWIR reflectance increase, and the gradual NPQ increase (Saccardy et al., 1998; Xu et al., 2008; Chen et al., 2016) was accompanied by a visible (VIS) reflectance rise, implying that the dominant photo-protective mechanism of this species may be chloroplast movement (Zygielbaum et al., 2012). In the case of tomato, the reduction in leaf RWC resulted in an insignificant SWIR increase, and a negligible NPQ rise (Biehler et al., 1997; Haupt-Herting & Fock, 2000; Mishra et al., 2012; Shao et al., 2015) did not cause a significant VIS reflectance change (Sarlikioti et al., 2010; Marino et al., 2014), suggesting that this species may avoid photo-inhibitory damage by other means, such as utilizing the photo-respiratory pathway or the Mehler reaction (Haupt-Herting & Fock, 2000). Although the 531 and 1500 nm responses of the abovementioned plants were unsuitable for WABI-based monitoring of water status changes, it should be noted that substantial near infrared (NIR) reflectance variations did, however, follow the stress development in both species (Fig. 6). As this observation is in line with other drought-related studies of corn (Woolley, 1971; Schepers et al., 1996; Zygielbaum et al., 2009) and tomato (Okamura et al., 2001; Marino et al., 2014), it is likely that a cheaper VIS–NIR broadband sensor may be sufficient for water stress detection in those cases.

Apart from genotypic variability, the diversity in the physiological features and responses of plants is also known to be greatly affected by differences in their growing environments (Chaves et al., 2010; Lovisolo et al., 2010). This is especially noticeable when comparing between the phenotypic traits of clones that were acclimated to distinct environmental conditions and stressing agents. In the context of WABI, it was demonstrated previously that the hydraulic and photo-protective characteristics of a given genotype are prone to considerable alterations upon a change in various factors, including soil texture (Tramontini et al., 2013), salinity (Stepien & Johnson, 2009), light intensity (Magney et al., 2014), light quality (i.e. spectral composition; Sellin et al., 2011), light duration (i.e. photo-period; Frechette et al., 2016), air temperatures (Flexas et al., 1999), atmospheric vapor pressure deficit (Soar et al., 2006) and wind (Huang et al., 2016). As the variations in leaf water status and NPQ do not necessarily coincide with one another, it is reasonable to assume that the correlativity of the spectral model and the physiological variables (i.e. the efficiency of the index) can vary greatly from one place to another. This assumption was supported in the current study with the difference between the WABI-stem water potential (Ψ_S) regressions of the glasshouse- and field-grown Cabernet Sauvignon clone (Fig. 11), and, to some extent, with the difference between the WABI–RWC sunflower regressions of the net house-grown 'D.Y.3' cultivar and the field-grown 'Sopremo' cultivar (Fig. 9). Therefore, the index cannot be automatically or blindly generalized across growing environments and there must always be site-specific calibration to well-known stress indicators (such as RWC, Ψ_S, stomatal conductance (g_s) or NPQ) before its use.

Despite the dual-response advantage of WABI and its potential applicability for many other crops, the genotypic variability among plants – which diversifies their hydraulic (Tardieu & Simonneau, 1998) and photo-protective reactions (Takahashi & Badger, 2011) to water deficit – suggests that the spectral model might not be compatible to all of them. This suggestion was

Table 3 Statistical data for the physiological and spectral variables throughout the sunflower field experiment

Variable	Control	Moderate stress	Severe stress (wilting)	F-test
RWC (%)	93.15 ± 3.83	84.93 ± 3.16 (Day 14) $P < 0.01$	76.91 ± 4.79 (Day 21) $P < 0.01$	$F_{(3,56)} = 56.46$ $P < 0.01$
ρ 531 nm (%)	11.15 ± 0.88	10.68 ± 0.45 (Day 14) $P > 0.05$	10.24 ± 0.89 (Day 21) $P < 0.01$	$F_{(3,56)} = 4.58$ $P < 0.01$
ρ 1500 nm (%)	17.99 ± 0.72	19.11 ± 0.79 (Day 14) $P < 0.01$	19.85 ± 0.72 (Day 21) $P < 0.01$	$F_{(3,56)} = 15.15$ $P < 0.01$
WABI	0.24 ± 0.03	0.28 ± 0.03 (Day 14) $P < 0.01$	0.32 ± 0.05 (Day 21) $P < 0.01$	$F_{(3,56)} = 13.32$ $P < 0.01$

Values of leaf relative water content (RWC), of reflectance (ρ) at 531 and 1500 nm, and of the 'water balance index' (WABI) represent averages of 15 samples $\pm$ SD. The significance values (P) in the moderate and severe stress columns are a product of Tukey's honest significant difference test (Tukey, 1949), and relate to the difference between each of these deficit levels and the control stage.
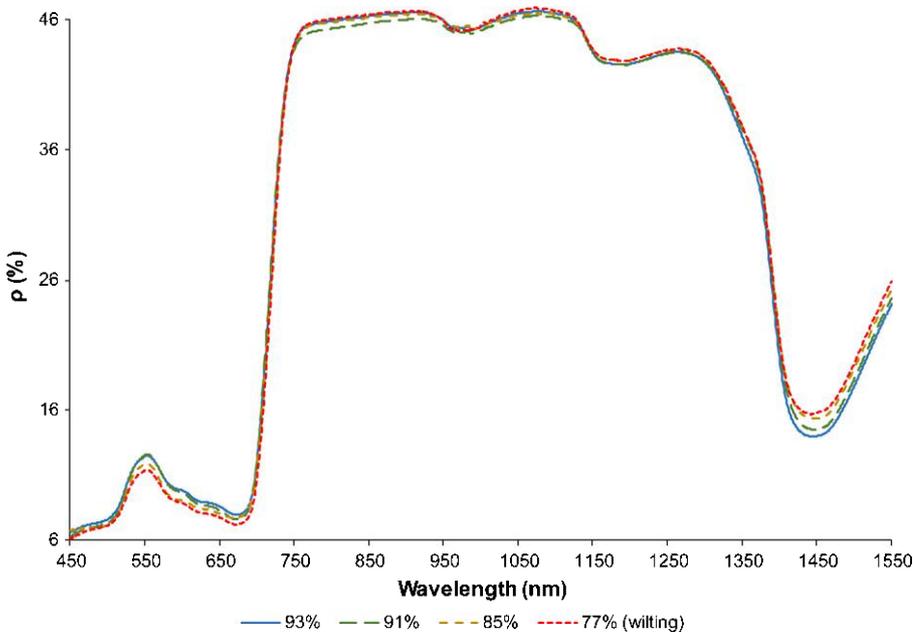
Fig. 8 Reflectance (ρ) changes relative to wavelength throughout the sunflower field experiment. Each graph represents an average signature of 15 replicates at a given leaf relative water content (%) value.
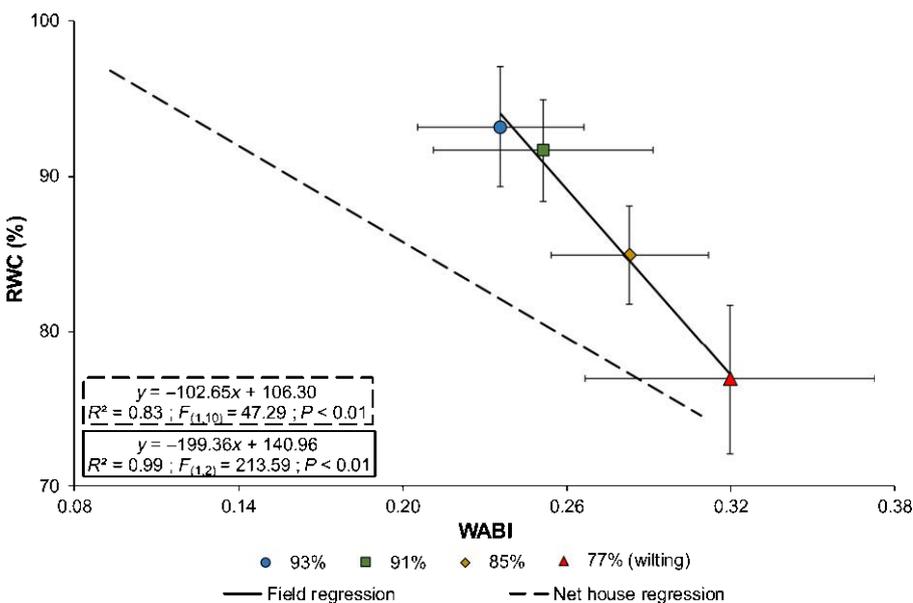
Fig. 9 The spectral–physiological correlation throughout the sunflower field experiment. Each datum of the leaf relative water content (RWC or %)-'water balance index' (WABI) regression represents a weekly average of 15 replicates $\pm$ SD, from the fully turgid stage up to the wilting point. The net house sunflower regression was adopted from Fig. 7(a).
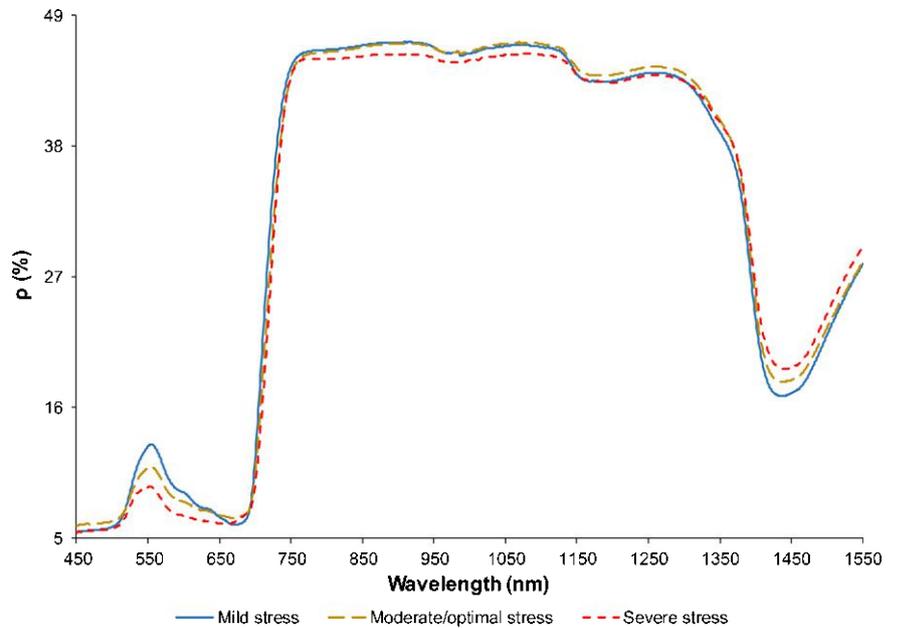
Fig. 10 Reflectance (ρ) changes throughout the training period of the 'water balance index' (WABI) relative to wavelength in the vineyard experiment. Presented are examples of the mild, moderate/optimal and severe stress degrees that required a low- (−0.88 MPa; $\Psi_S > -0.9$ MPa), common- (−1.03 MPa; -1.1 MPa $< \Psi_S < -0.9$ MPa) and high (−1.11 MPa; $\Psi_S < -1.1$ MPa) irrigation amounts, respectively. Each graph represents an average signature of 18 replicates.

Table 4 Statistical data for the physiological and spectral variables throughout the first stage of the vineyard experiment

Variable	Mild stress	Moderate/optimal stress	Severe stress	F-test
Ψ_S (MPa)	−0.88 ± 0.07	−1.03 ± 0.04 $P < 0.01$	−1.11 ± 0.04 $P < 0.01$	$F_{(2,51)} = 106.27$ $P < 0.01$
ρ 531 nm (%)	10.44 ± 0.71	9.52 ± 0.61 $P < 0.01$	8.77 ± 0.54 $P < 0.01$	$F_{(2,51)} = 32.13$ $P < 0.01$
ρ 1500 nm (%)	22.18 ± 0.69	22.72 ± 0.96 $P > 0.05$	24.08 ± 0.84 $P < 0.01$	$F_{(2,51)} = 24.55$ $P < 0.01$
WABI	0.36 ± 0.03	0.41 ± 0.03 $P < 0.01$	0.46 ± 0.03 $P < 0.01$	$F_{(2,51)} = 53.08$ $P < 0.01$

Presented are examples of the three stress degrees during the training period of the 'water balance index' (WABI; see Fig. 10). Values of stem water potential (Ψ_S), of reflectance (ρ) at 531 and 1500 nm, and of WABI represent averages of 18 samples ± SD. The significance values (P) in the moderate/optimal and severe stress columns are a product of Tukey's honest significant difference test (Tukey, 1949), and relate to the difference between each of these deficit levels and the mild stress degree.
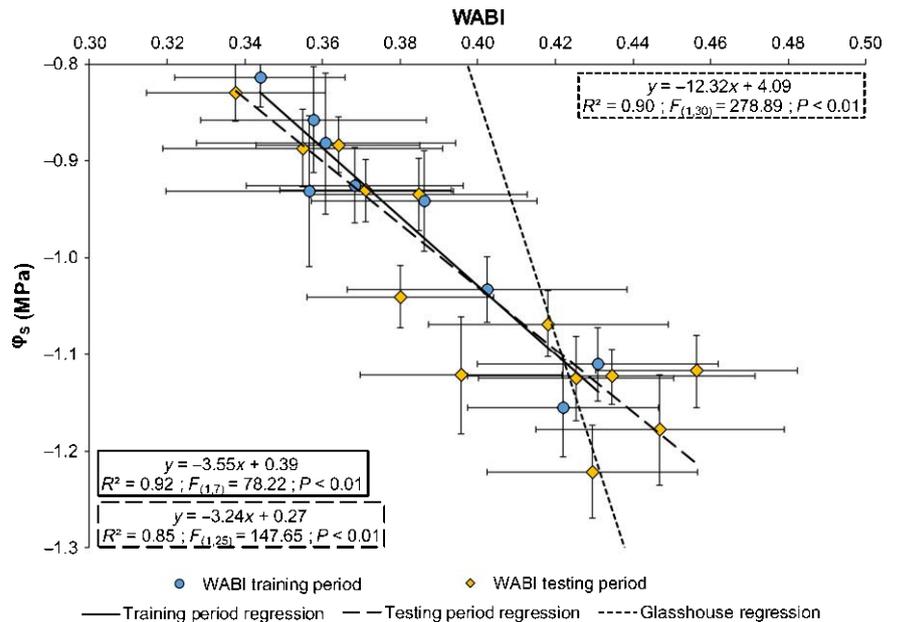

Fig. 11 The spectral–physiological correlations throughout the training and testing periods of the 'water balance index' (WABI) in the vineyard experiment. Each data point of the stem water potential (Ψ_S)–WABI regressions represents an average of 18 replicates ± SD. The glasshouse grapevine regression is based on the leaf water potential measurements of Rapaport *et al.* (2015), which were converted into Ψ_S values using the linear model of Williams & Araujo (2002).
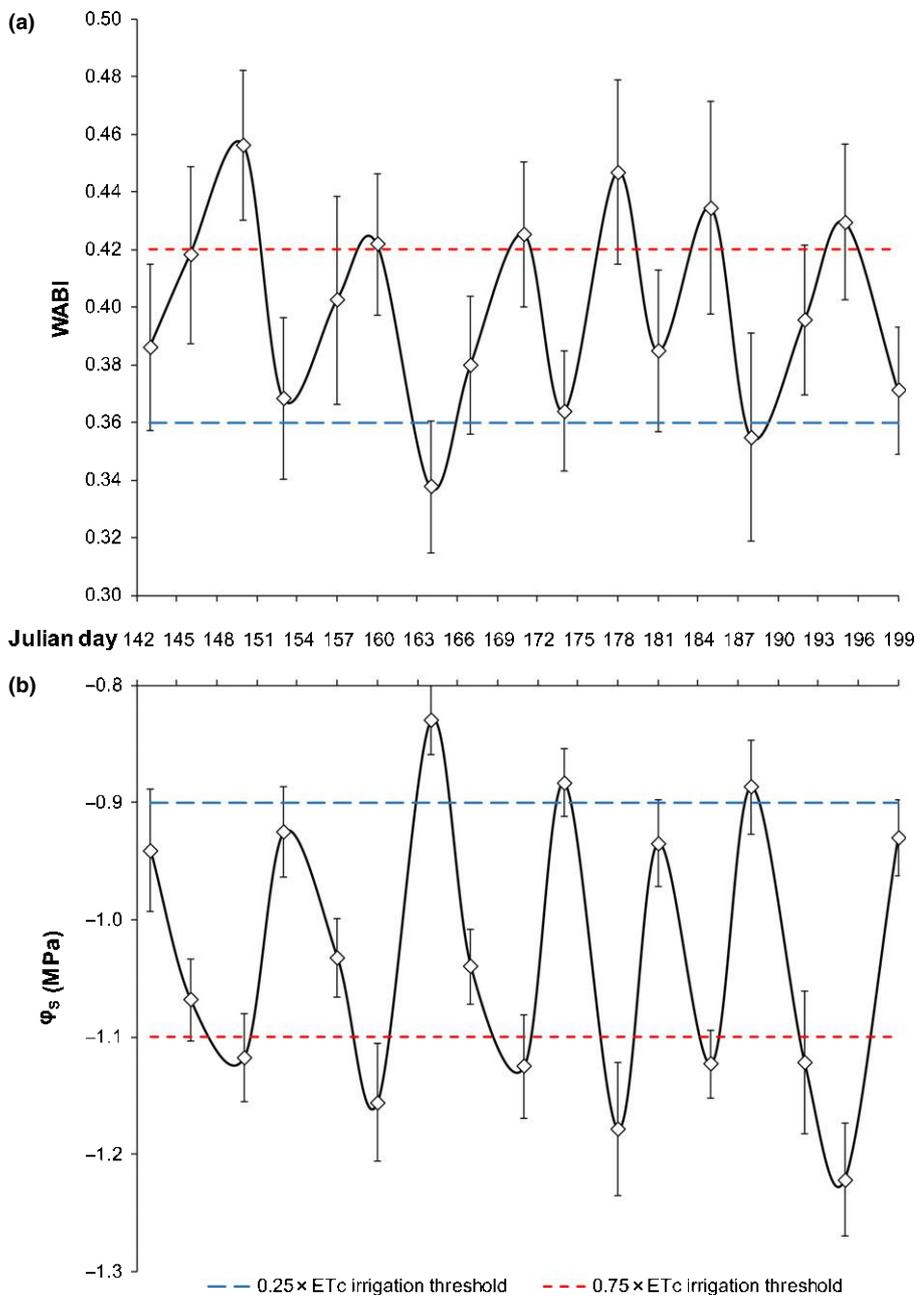
Fig. 12 Changes in (a) the 'water balance index' (WABI) and consequent changes in (b) in stem water potential (Ψ_S) throughout the testing period of the vineyard experiment. Each data point represents an average of 18 replicates $\pm$ SD. An average index value below 0.36 induced a low irrigation amount ($0.25 \times ET_C$); an average index value between 0.36 and 0.42 induced a common irrigation amount ($0.50 \times ET_C$); and an average index value above 0.42 induced a high irrigation amount ($0.75 \times ET_C$). ET_C, evapotranspiration.

Although site-specific calibrations should extend the use of WABI geographically for many crops, it is noteworthy that the success of the index is still drawn from leaf-level, proximal sensing measurements and, thus, it is currently confined to relatively small agricultural plots. Before the spectral model can be used for commercial irrigation irrigation purposes – i.e. monitor vast fields via remote sensing platforms – several important difficulties must be addressed. First, although the simple, two-wavelength structure of WABI is technically apt for simpler multispectral sensors, the requirement for simultaneous sensitivity to VIS and SWIR radiation would probably incur high production costs at the airborne level, especially for cameras. Secondly, whereas the NPQ-related spectral response is only a few nanometers wide, concentrated around the principal wavelength of 531 nm, remote

sensors are generally equipped with broader filters in order to increase the number of photons admitted to the detector, potentially losing focus on the physiological phenomenon to background noise (e.g., Suarez *et al.*, 2008). Thirdly, although the wavelength components of WABI do present a theoretical durability to atmospheric interferences (Sims & Gamon, 2003; Evain *et al.*, 2004; Rapaport *et al.*, 2015), a remote sensing approach would first require actual proof of their penetrability through dense layers of water vapor. Accordingly, future studies are encouraged to assess the contribution of wavelengths closer to 1600 nm, which may reduce the adverse effects of the atmosphere while still enabling sufficient sensitivity to leaf water status changes (e.g. Eitel *et al.*, 2006). Fourthly, because the physiological and, thus, spectral features of leaves of different ages or

developmental stages can be substantially different, remote predictions that are not based on youngest matured leaves can pose a significant source of bias and might not represent the true status of the canopy (Rapaport *et al.*, 2014). Therefore, before the actual WABI analysis, a signal or image must first be cleansed from the adverse effects of leaves that are either too young or old, using a designated algorithm. Lastly, remote estimations of various biochemical values might also be strongly biased by the biophysical properties of the leaf and canopy, such as their angle distribution and orientation (Magney *et al.*, 2014). For instance, while analyzing the spectral–physiological behavior of wilting corn canopies, Evain *et al.* (2004) noticed very large PRI variations that could not have been explained solely by NPQ alterations, due to changes in illumination conditions (i.e. canopy shadow fraction) and reflective angles (i.e. wilting; Gamon *et al.*, 1992). To address these issues, WABI can be applied from multiangular platforms (Hilker *et al.*, 2009) or extracted using 'terrestrial scanning laser' (TSL) technology (Gaulton *et al.*, 2013; Magney *et al.*, 2014), which can deal with illumination and observation geometry issues, separate the leaf signal from background radiation of nonphotosynthetic materials, and even operate in the dark to acquire baseline measurements for the reflectance at 531 nm.

To summarize, WABI was successful in scheduling the irrigation of a vineyard across an entire growing season and in monitoring early signs of water deficit in pea and sunflower plants. Those achievements are attributed to the rapid reactivity of leaf water content and the NPQ mechanism to alterations in water availability, which allowed the spectral detection of drought even in the face of phenological and environmental influences. Overall, the spectral model shows great potential for numerous crops, and might prove less suitable only for those plants that respond to drought by exhibiting both a strict stomatal regime and a mild NPQ increase. Nonetheless, before WABI can be commercialized and applied from the remote sensing level, various important challenges must be addressed, such as the production of affordable and suitable VIS–SWIR sensors and filters, the testing of atmospheric interference around the spectral components of the index, the isolation of youngest matured leaves through image processing, and the acquisition of a representative leaf signal in the face of biophysical-related influences.

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Author contributions

T.R., U.H., A.K. and S.R. designed the research; the study was performed by T.R.; data analysis and interpretation was carried out by T.R., U.H., A.C., A.K. and S.R.; and the manuscript was written by T.R., U.H., A.C., A.K. and S.R.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Table S1 The efficiency of the 'water balance index' (WABI) and of the 'physiological reflectance index' (PRI) throughout the sunflower net house- and field experiments

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