Future opportunities of proximal near infrared spectroscopy approaches to determine the variability of vineyard water status

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Abstract

Background and Aims: Non-destructive, reliable, fast and automated plant-based methods for the assessment of the water status of a grapevine are necessary to design irrigation strategies. The goal of this work was to test the capability of near infrared (NIR) spectroscopy using a vehicle-mounted and remote NIR sensor without plant contact (contactless) to assess the water status of grapevines in the vineyard.

Methods and Results: An NIR spectrometer (1100–2100 nm) mounted on an all-terrain vehicle was used to acquire spectra (contactless, in stop-and-go mode) from leaves of water-stressed and non-stressed vines of Riesling at two timings during the season. Calibration and cross-validation models yielded $R_c^2 = 0.95$ and $R_{cv}^2 = 0.88$ for the estimation of the stomatal conductance measured in the same grapevines.

Conclusions: The study demonstrates that NIR spectroscopy may become a potential tool for on-the-go assessment of proximal plant water status, although further research will be required for full confirmation.

Significance of the Study: The NIR technology tested, capable of being installed on vineyard machinery, paves the way to collect data on plant water status at high spatial and temporal resolution to assist in irrigation scheduling.

Keywords: *contactless NIR sensing, near infrared spectrometer, plant-based method, stomatal conductance, water stress*

Introduction

A wide range of plant-based sensing methods exists, and their advantages and pitfalls have been extensively reviewed (Jones 2004, Fernández 2014, Jones and Grant 2016). Among these methods, some directly assess plant water status, such as the relative water content (RWC) (Barrs and Weatherley 1962), the plant water potential, either leaf (Ψ_{leaf}) or stem water potential (Ψ_{stem}) (Choné et al. 2001) and the cavitation and embolism phenomena caused by water conductance (Lovisolo and Tramontini 2010). Others are based on the plant's physiological reactions to water scarcity. In this group, the measurement of the stomatal conductance (g_s), thermography (Jones et al. 2002), sap-flow meters and sensors measuring leaf turgor and fluctuations of trunk or stem diameter (Fernández 2014) can be mentioned. Many of these plant-based sensors, however, are used mainly for research purposes as some are slow, too labour demanding (as they are not automated), difficult to operate with and to interpret, destructive in many cases and most of the time only capable of measuring a small number of vines per hectare in a reasonable time period. Such constraints limit any real-time mapping of the spatial heterogeneity or even more of utmost viticultural interest providing temporal dynamics of the vine water status or progress in fruit development. Jones (2004) and Fernández (2014) summarised some of the main features of the ideal plant-based sensing method, which should be non-destructive, sensitive to water variation, capable of providing a reliable and early response, as well as inexpensive, easy to operate and suitable for automation.

Near infrared (NIR) spectroscopy is a non-destructive and fast technique that has been extensively used for evaluating food composition (Cen and He 2007), with many potential applications also for plant phenotyping (Cozzolino 2014). The NIR region (750–2500 nm) contains information referring to the relative proportion of CH, NH and OH bonds of organic molecules. Because water is the predominant constituent of leaves, their NIR reflectance spectra are dominated by the water spectrum, which shows overtone bands of the OH bonds at 760, 970 and 1450 nm and a combination band at 1940 nm (Nicolaï et al. 2007). One of the advantages of NIR spectroscopy is its ease of use in combination with chemometrics for quality and quantitative analysis. Multivariate statistical approaches, mainly regressive tools, together with spectra filtering and pre-processing, are largely used in modelling procedures. Unlike destructive data derived from wet chemistry analysis, NIR spectra are usually aimed at building calibration and prediction models of specific parameters or attributes (Geladi 2003, Cozzolino et al. 2011, Dambergs et al. 2015). Validating methods are also applied, as well as indexes of statistical significance and robustness (Williams and Sobering 1996, Fearn 2002). This approach has been followed in the few studies that have investigated the application of portable NIR spectrophotometers for the assessment of grapevine water status (Santos and Kaye 2009, De Bei et al. 2011, Vila et al. 2011, Gutiérrez et al. 2016, Tardaguila et al. 2017). These authors have shown the potential and promising capabilities of in-field portable NIR spectroscopy for non-destructively assessing plant water status, with values of correlation

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coefficients of validation (r_v) above 0.84 for the prediction of Ψ_{leaf} (Santos and Kaye 2009) or Ψ_{stem} (De Bei et al. 2011, Gutiérrez et al. 2016, Tardaguila et al. 2017). Although successful, the step forward towards the true implementation of NIR spectroscopy for in-field appraisal of grapevine water status would be its automation, and this can only occur if NIR spectra could be acquired contactless, at a given distance from the plant.

The aim of this study was to test the capability of NIR spectroscopy, operating contactless from a vehicle, to assess the water status of grapevines in the vineyard, in order to implement such technology for the non-destructive determination and future assessment of the spatial variability and temporal dynamics of a vineyard water status.

Materials and methods

Experimental site

Field experiments were conducted in an established vineyard of *Vitis vinifera* L. cv. Riesling (clone Gm 198–25; grafted to rootstock SO4 Gm47) located close to Geisenheim, Germany (49°59′20″ N; 7°55′56″E) during season 2014. Vines were trained to a vertical shoot positioning (vertical shoot positioning-type) trellis system in a north–south row orientation. Row and vine spacing were 2.10 and 1.05 m, respectively. Two levels of vine water status were imposed in the vineyard: water stress and Control (non-stressed vines). The experimental layout consisted on two replication blocks, each of them comprising three adjacent rows. Of these, only the central row was used for the measurements, while the other two were considered edge rows. Water-stressed and Control treatments were established in alternating rows, and half of the row was used (16 vines) for the treatment.

Water stress was induced by covering the inter-row on both sides of the vine (to withhold natural precipitation) using a removable plastic sheet from flowering onwards. No additional water nor plastic shielding were applied to non-stressed Control vines; soil was managed with natural cover crop consisting mainly of various grass species (*Festuca* spec.).

Acquisition of NIR spectra

An NIR spectrometer (Polytec, Waldbronn, Germany) covering the spectral range between 1100 and 2100 nm, connected through optic fibre (PSS-H-A03 sensor head; Polytec) was used for contactless spectral detection of vine water status. The spectrometer was an NIR optical device, based on a polychromator as the reflection light source selector, and indium gallium arsenide diode array detectors, which operated at a rate of 28 Hz. A distance sensor head, which is a reflection device designed for measurements over larger distances, that is 150 to 600 mm to the target, was used. This was based on an integrated 20 W Tungsten-halogen Lamp (Polytec, Waldbronn, Germany) lamp for sample illumination. The instrumentation was mounted to and carried on an all-terrain vehicle (ATV) (Kawasaki Mule 610 4 × 4, Akashi, Japan), which moved at a groundspeed of approximately 2 km/h (Figure 1). Measurements with the NIR sensor were conducted statically (stop-and-go) on 16 vines subjected to stressed and non-stressed water treatments, including the two sampling dates (July and September) at two distances to the canopy: 0.25 and 0.50 m, approximately. In both cases, the sensor head was mounted at a height of 1.40 m above ground. The spot size changed from 1.6-cm diameter at a distance of 0.25 m to 2 cm when the distance to the canopy was 0.50 m.

At each sampling date, eight random vines within the two water treatments were measured. Data were recorded from both sides of the canopy. For each vine, three fully sun-exposed leaves located just above the bunch zone were randomly selected and tagged, and 100 spectra (2 nm wavelength increments) per leaf within the range of 1100–2100 nm were collected from a single spot per leaf and stored in an outdoor computer. In total, 96 measurements were conducted (two sampling dates × eight vines × three leaves × two target distances). For each leaf, the acquired spectra were then averaged to a mean representative spectrum per leaf. Different leaves were measured at each sampling date. To improve discrimination between other materials than leaves, spectra of shoots, wire, posts and canopy gaps were recorded separately at a distance to the canopy of 0.50 m (Figure 2).

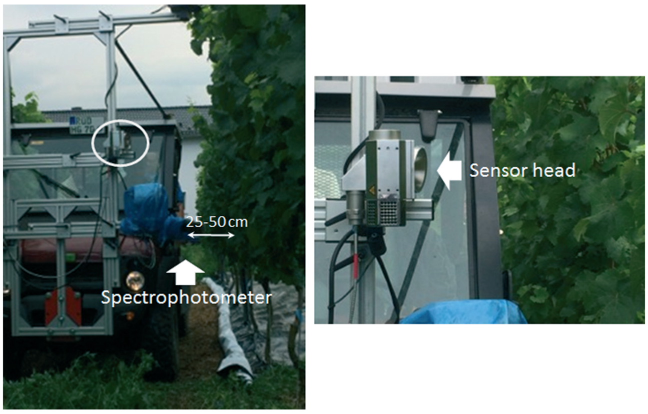

Figure 1. Illustration of the set-up of the near infrared system operating from the moving all-terrain vehicle.

Ground-truthing: measurement of stomatal conductance (g_s)

With the aim of ground-truthing (at the leaf level) the spectral measurement of grapevine leaf water status, stomatal conductance was chosen as the reference method and measured using a portable porometer (AP4, Delta-T, Cambridge, England).

On two cloudless days during the two sampling periods, July and September 2014, the same three fully sun-exposed leaves tagged in each of the 16 measured vines, of either water stressed or non-stressed vines (Control), were measured (two spots per leaf were measured and then averaged)

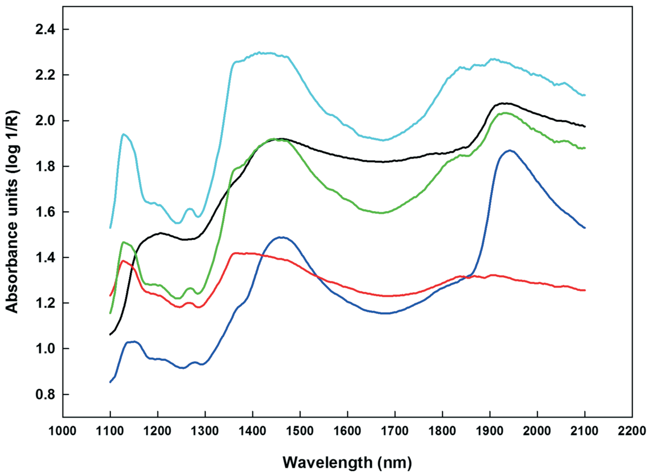

Figure 2. Spectral absorbance of leaves (—), bunches (—) and other constituents of the canopy, wooden post (—), grapevine shoot (—) and gap (—), acquired contactless from a 50 cm distance.

approximately 1 h prior to the spectral data acquisition around midday. Ninety-six measurements of g_s were made.

Chemometric analysis

Raw spectra were recorded with PSS software (Polytec), while subsequent statistical pretreatment for absorbance (log 1/R) transformation, together with chemometric calculation, was obtained after data exportation in UNSCRAMBLER v9.7 software (CAMO ASA, Oslo, Norway). The absorbance spectra were used as the *X*-variable, and g_s was used as the *Y*-variable to build the models for water status evaluation.

In addition to simple absorbance, mean normalised spectra, as well as statistical filtering operations [(e.g. standard normal variate (SNV) transformation, multiplicative scattering correction (MSC)], first derivative of Savitzky–Golay filter or second derivative of Savitzky–Golay were tested for regression model calculation. Principal component analysis (PCA) allowed the differentiation of spectral variations of the data sets and performed a pattern recognition analysis consisting of the discrimination between stressed and unstressed leaf samples.

Table 1. Stomatal conductance of stressed and non-stressed *Vitis vinifera* cv. Riesling vines determined with a portable porometer.

Water status	Data set (*n*)	Stomatal conductance [mmol $H_2O/(m^2·s)$]			
		Mean	SD	Min	Max
Stressed	24	86.0	22.5	38.5	126.0
Non-stressed	24	233.8	56.7	104.0	365.0

Data are values per leaf including the two measuring dates. Data set (*n*), number of leaves measured of each water status in the two timings; Max, maximum; Mean, average values in each water treatment; Min, minimum; SD, standard deviation.

Partial least squares (PLS) regression was used to build up the calibration model for g_s estimation. Internal cross validation [leave-one-out cross validation (LOOCV)] was applied in the calibration procedure; no outlier elimination was required. The performance of the models was evaluated by the standard error of calibration and the coefficient of determination in calibration(R_c^2). The number of latent variables, which yielded the minimum standard error in cross-validation value, was also specified in the model.

Results

The data set of g_s measurements used as the reference method for the NIR spectra to build the predictive models ranged from 38.5 to 365 mmol $H_2O/(m^2·s)$, involving measurements at the two sampling dates in grapevines subjected to the two water treatments (Table 1). Water stressed vs Control plants were significantly different ($P < 0.05$) in their g_s, and the mean value of this variable for the Control vines was almost fourfold that of the stressed vines.

In order to define the instrumental ability of the NIR system in the range of 1100–2100 nm, to measure thoroughly leaves of the vines, to verify and filter spectral responses of non-relevant information and hence to improve the accuracy of the application, required in future on-the-go operations, specific discrimination of material other than leaves in the spectral absorbance in the range of study was attempted. Figure 2 shows the distinct reflectance patterns of the individual organic matter (leaf, bunch and shoot), inorganic matter (post) or within the detection of possible gaps that occur regularly within vertical shoot positioning trellis systems. Spectra referred to leaf were clearly dominated by absorption peaks, which were assigned to the first overtone of the symmetric and asymmetric OH bond stretching and/or combination bands (1450 nm), and to the combination of the OH stretching band and to the OH bending band (1920–1950 nm), respectively. Stretching, combinations and bending are vibrational responses of the

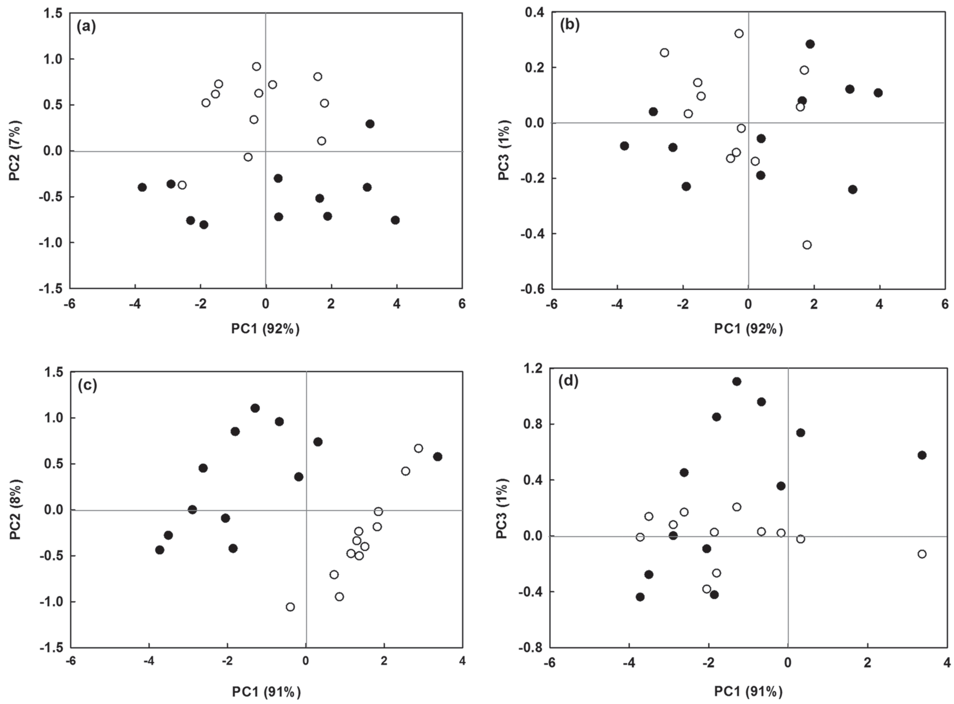

Figure 3. Principal component analysis from the spectra taken in July from Control (●) and water-stressed (○) vines. Bi-plot of (a) PC (principal component) 1 and PC2 and (b) PC1 and PC3, from spectra acquired at a distance of 0.25 m. Bi-plot of (c) PC1 and PC2 and (d) PC1 and PC3 from spectra acquired at a distance of 0.50 m.

organic groups to the electromagnetic excitement induced by using NIR spectroscopy. Considering the chemical constitution of the leaf, the hydroxyl (OH group) domination certainly means a significant correlation between NIR spectra and water presence (Curran et al. 2001).

Figure 3 represents the score plots of the PCA results calculated on the absorbance spectra, within the range 1100–2100 nm, respectively, detected at 0.25 m (Figure 3a,b) and 0.50 m (Figure 3c,d) of sensor head distance from the leaves. Although principal component (PC) 1 and PC 2 explained 99% of the residual variance for the two tested distances of detection, the score patterns demonstrated a better discriminative performance of the spectra acquired at a distance of 0.50 m (Figure 3c).

Scatter plots reporting calibration and cross validation (leave-one-out cross validation) results of the partial least squares regression for stomatal conductance estimation are reported in Figure 4a,b, respectively. Models were calculated after X-data manipulation with spectra mean normalisation, then a first derivative of Savitzky–Golay filtering (11 points of smoothing, second order). Positive and promising results were obtained and are represented by a coefficients of determination of calibration (R^2_c) equal to 0.96 and of cross validation (R^2_{cv}) equal to 0.86, together with a standard error of calibration and of cross validation of 18.1 and 45.6 mmol $H_2O/(m^2{\cdot}s)$, respectively.

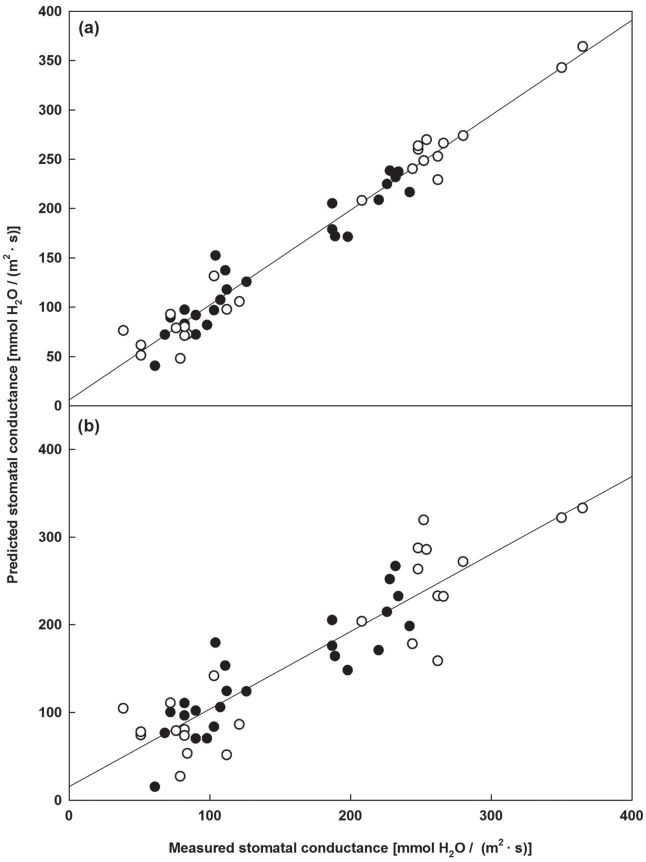

Figure 4. Relationship between predicted, using partial least squares regression, versus measured values of stomatal conductance from a distance of 0.50 m, in July and September, in leaves of *Vitis vinifera* L. cv. Riesling. Measurements were acquired in July (●) and in September (○). (a) Calibration [*n*, 48; R^2_c, 0.962; standard error of calibration, 18.10 mmol $H_2O/(m^2{\cdot}s)$]; (b) Cross validation [*n*, 48; R^2_c,0.862; standard error of calibration, 45.65 mmol $H_2O/(m^2{\cdot}s)$].

Discussion

Non-destructive, contactless NIR reflectance spectroscopy in the range of 1100–2100 nm, acquired proximally from a vehicle, under field conditions, has proved to assess successfully and reliably the stomatal conductance of grapevines, hence the plants' water status (from no stress to severe water stress conditions) in a commercial vineyard site. Likewise, reliable calibration and cross-validation models for a classical indicator of plant water status, such as the g_s, have been built from leaf spectra acquired with a new, fast, non-destructive NIR spectrophotometer in the vineyard.

Although g_s is a widely used plant-based indicator of a plant water status (Jones et al. 2002, 2009), mainly in physiological and agronomical research to evaluate different irrigation strategies (Speirs et al. 2013, Degaris et al. 2015, 2016), its measurement, that is manually performed in targeted leaves, is time and labour consuming, therefore expensive. In the present work, g_s was chosen as the reference method as it is a quick, non-invasive technique, closely linked to vine water use, because virtually all water transpired by the vine passes through the stomata (Loveys and Jones 2008). The tested NIR system mounted on an ATV and measuring in a stop-and-go way yielded a robust and remarkable estimation of the g_s values in seconds. Its performance and contactless operating mode paves the way for its implementation on-the-go in the near future. From the agronomical point of view, the determination and assessment of a vineyard's spatial variability and temporal dynamics of water status requires a vast number of plant water measurements (to be able to conduct a geostatistics analysis and mapping), spatially distributed, and these can be easily acquired with the tested NIR system.

The sensitivity yielded by the NIR spectroscopy in-field monitoring [45.65 mmol $H_2O/(m^2{\cdot}s)$] is similar to or even better than that of the new approaches of thermal imaging either from aerial or manual measurements (García-Tejero et al. 2016). Likewise, the correlations between the main thermal indices, such as the conductance index and the crop water stress index with g_s yielded differences larger than 100 mmol $H_2O/(m^2{\cdot}s)$ in the estimated g_s results for a given value of the corresponding thermal index (Fuentes et al. 2012, Grant et al. 2016, García-Tejero et al. 2016).

The present work builds on past studies using manual point of contact active NIR sensors for assessment of plant water status and represents the first step in developing a more practical on-the-go sensor system for this purpose. So far, most of the work using NIR spectroscopy to assess plant water status has operated with portable manual devices in contact with the leaf (Santos and Kaye 2009, De Bei et al. 2011, Gutiérrez et al. 2016) and has been validated against the measurement of the Ψ_{stem} or RWC (%) of leaves (Tardaguila et al. 2017). In these studies, the correlation coefficient of cross validation (r_{cv}) was around 0.84 in all cases, working with spectral data acquired on leaves, in different ranges, such as 1100–1830 (De Bei et al. 2011) or 1600–2500 nm (Gutiérrez et al. 2016, Tardaguila et al. 2017). In our study, the 1100–2100 nm spectral range comprised key absorption and overtone bands of the OH group, at 1450 nm and 1920–1950 nm. This may respond for the noteworthy values of R^2 obtained for both calibration and cross-validation models in the 1100–2100 nm spectral region. In terms of the prediction of g_s from NIR spectroscopy the number of studies is limited, and these have been conducted with contact, hand-held NIR devices. As an example, Warburton et al. (2014) obtained r_{cv} of 0.89 for g_s in *Eucalyptus grandis* seedlings, under a controlled environment, but no references for grapevines have been found in the literature. In this regard, the present study holds the predictive capability of NIR spectroscopy to estimate the stomatal conductance as an indicator of the plant's water status. In the present work, the NIR spectrum in the range 1200–2100 nm is dominated by water bands; therefore, the leaf water content could be successfully appraised with this technique. Although the g_s is a plant-based water status indicator, its relationship with the plant RWC, for example with the RWC on a leaf basis, is variable. This is clearly evidenced in the differential isohydric or anisohydric behaviour of diverse grapevine genotypes for a given plant RWC. This fact suggests that the NIR calibration and prediction models obtained in the present study for g_s may not be used to predict the plant RWC, but merely its g_s. This is confirmed by the results reported by Tardaguila et al. (2017) in a recent study. In this work, NIR-derived calibration and prediction models for RWC_{leaf} and Ψ_{stem}, measured on the same leaves of a various grapevine cultivars, were built. These models yielded an r_{cv} value that varied from 0.66 to 0.81 for RWC_{leaf} and from 0.77 to 0.93 for $_{stem}$.

The good and even enhanced discriminative capability of the system at 0.50 m compared with 0.25 m is another positive result. Staying away from the canopy at a further distance will be safer for the on-the-go operations. Moreover, in future on-the-go measurements, automated removal of spectra corresponding to material other than leaves will be required. For a vehicle moving at a speed of 5 km/h, an acquisition rate of 28 Hz (as it is the one of the used NIR system) yields approximately 20 measurements per linear metre of vineyard row, which is a sufficient and representative number of data, even if some of them (corresponding to material other than leaf) have to be discarded. The leaf-class spectra per a given row distance may then be aggregated and averaged and maps could be finally prepared.

One of the main advancements of the tested NIR-based method is its ability to widen the spatial representation of the plant water status of a whole vineyard with respect to conventional methods. Moreover, one of the requirements of ideal water status indicators for optimised irrigation scheduling is their ability of automation (Jones 2004, Fernández 2014), and this new spectroscopic method can be fully automated. This way, the capability of the non-destructive, contactless NIR device to provide a large number of measurements of grapevine transpiration rapidly, to appraise the variation of the water status of a vineyard and help to define optimised irrigation strategies is a novel and remarkable outcome, highly useful for the wine industry. Compared with other existing techniques, such as thermography, NIR spectroscopy does not require references, such as T_{dry} and T_{wet} (Jones et al. 2002, Fuentes et al. 2012), and as mentioned before, its sensitivity is similar or better to that of thermography-based solutions. Commercially, thermography is currently mostly acquired from remote aerial platforms (mostly remotely piloted aircraft system or unmanned aerial vehicles). In comparison to aerial thermography, the NIR spectroscopy assesses the lateral side of the canopy instead of the zenital view (Baluja et al. 2012) and suffers no limitation in operational performance time as no batteries exhaust (i.e. In drones typical operational times reach a maximum of 20 min). Moreover, legal issues and lack of time flexibility derived from unmanned aerial vehicle piloting are not applicable to the proximal NIR approach. Finally, once the models are built, no additional expertise in data interpretation is needed. The choice of a site-specific versus a global model should be further investigated, as the scale of applicability changes in the two approaches, but the validity of the built models against the reference values (stomatal conductance or water potential) would remain. Finally, from the breeding perspective, this methodology can contribute to mitigate partly the bottleneck existing in this community for in-field high, throughput phenotyping.

Further investigation involving different cultivars, locations and seasons would contribute to refine better and to demonstrate the robustness of the ATV-mounted, contactless NIR method for the assessment of vine water status; however, the novelty and power of the system has been demonstrated. In the near future, this proximal technique, with powerful instrumentation, may become of high relevance with practical input of data immediately gained from the canopy or vine. Implementing mapping to such a single factor will make the variability within the vineyard visible and hence impact on management practices.

Conclusion

A suitable and promising tool for proximal, contactless NIR spectroscopy-based for assessing vine water status was developed. It was capable of providing a large number of measurements from a terrestrial vehicle, in order to appraise the within-field variability of grapevine water status. Because of the power in data acquisition, such technology could either be implemented in phenotyping activities involving either unmanned ground vehicles or through installation on vineyard machinery. This offers a great opportunity to collect data on water status with a high spatial and temporal resolution. Once implemented this will be of great interest for any type of management practice. Next steps to effectively put in place NIR spectroscopy to map water status variability and irrigation scheduling should include the testing of this technology on-the-go and the definition of reference or threshold values beyond which irrigation is necessary under various environmental conditions.

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