Non-destructive measurement of grapevine water potential using near infrared spectroscopy

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Abstract
Background and Aims: Near infrared (NIR) spectroscopy techniques are not only used for a variety of physical and chemical analyses in the food industry, but also in remote sensing studies as tools to predict plant water status. In this study, NIR spectroscopy was evaluated as a method to estimate water potential of grapevines.

Methods and Results: Cabernet Sauvignon, Chardonnay and Shiraz leaves were scanned using an Integrated Spectronic (300–1100 nm) or an ASD FieldSpec®, 3 (Analytical Spectral Devices, Boulder, Colorado, USA) (350–1850 nm) spectrophotometer and then measured to obtain midday leaf water potential using a pressure chamber. On the same shoot, the leaf adjacent the one used for midday leaf water potential measurement was used to measure midday stem water potential. Calibrations were built and NIR showed good prediction ability (standard error in cross validation (SECV) <0.24 MPa) for stem water potential for each of the three grapevine varieties. The best calibration was obtained for the prediction of stem water potential in Shiraz ($R = 0.92$ and a SECV = 0.09 MPa).

Conclusion: Differences in the NIR spectra were related to the leaf surface from which the spectra were collected, and this had an effect on the accuracy of the calibration results for water potential. We demonstrated that NIR can be used as a simple and rapid method to detect grapevine water status.

Significance of the Study: Grapevine water potential can be measured using NIR spectroscopy. The advantages of this new approach are speed and low cost of analysis. It may be possible for NIR to be used as a non-destructive, in-field tool for irrigation scheduling.

Abbreviations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Psi_{\text{leaf}}$</td>
<td>Midday leaf water potential; $\Psi_{\text{stem}}$</td>
</tr>
</tbody>
</table>

Keywords: abaxial, adaxial, NIR, optical property

Introduction
The amount of water available for irrigation is declining worldwide because of a quantitative and qualitative deterioration of water resources (Eastham and Gray 1998). The Australian wine industry, in particular, is facing an unprecedented challenge to maintain international competitiveness in the current drought. The increasing shortages of water and costs of irrigation are leading to an emphasis on the development of new methods of irrigation and irrigation scheduling that minimise water use and maximise water use efficiency (WUE) (Jones 2004). According to Al-Kaisi and Yin (2003) WUE maximisation should be considered as a key subject for research because of water scarcity issues. Grapevines are an intensive crop in semi-arid regions, so irrigation would be more effective if scheduled appropriately and if dosages and timing are applied to maximise WUE. In order to achieve this, the crop water status must be monitored accurately and reliably (Jones 2007).

Irrigation scheduling in vineyards is conventionally based on direct measures of soil moisture status and/or on soil water balance calculations (Jones 2004, 2007). Soil water measurements rely on the availability of many commercial systems and are relatively easy to apply; however, these approaches are prone to cumulative errors, require many sensors and may not be representative because of soil heterogeneity (Jones 2004). Alternative approaches are based on the physiological knowledge of grapevine response to water stress, thus sensing the plant response to water deficits rather than sensing the soil moisture status directly.
The most common direct sensing methods are the measures of midday leaf water potential (Ψ_stem), and leaf conductance to water vapor (g) largely dependent on stomatal conductance (Flexas et al. 2010). The pressure chamber technique of measuring Ψ_stem, which is a destructive method, has been assessed in several cases for grapevine as a relatively simple and rapid measurement (Naor et al. 1997, Trégoat et al. 2002, Williams and Araujo 2002, Sibille et al. 2007). A good correlation exists between Ψ_stem and g has been found for many grapevine varieties, but not for those showing near-isolythic behaviour (Schulz 2003, Cifre et al. 2005). This may reduce the utility of Ψ_stem as an indicator of water stress for the latter varieties (Cifre et al. 2005).

Leaf water status within a canopy is variable because it depends on the transpiration rate that a particular leaf has at the moment of measurement (Choné et al. 2001). As a partial solution to this variability, the measurement of midday stem water potential (Ψ_stem) was proposed because it is a more integrative indicator of whole-vine water status (Choné et al. 2001). Nevertheless, traditional methods for measuring Ψ_stem require destructive sampling and pretreatment for up to 1 h.

Near infrared (NIR) spectroscopy has been used as a non-destructive technique to analyse components of several agricultural products (Osborne et al. 1993, Batten 1998, Cozzolino et al. 2006). The NIR region of the electromagnetic spectrum (730–2300 nm) contains several wavelengths that are strongly influenced by the presence of water, and the state of water in the measured sample. The NIR spectral region is dominated by weak overtones and combinations of vibration bands from molecular bonds of hydrogen attached to atoms such as nitrogen, oxygen and carbon (Murray 1993, Batten 1998). Strong NIR absorption bands of water are found around 1400–1440 nm and between 1900 to 1950 nm and have often been applied to quantitative analysis of water content in food (Murray 1993, Batten 1998, Williams 2001, Büning-Pfaue 2003, Cozzolino et al. 2006).

Wavelength bands related to water have also been utilised in NIR reflectance with remote sensing applications to determine water content and water status of plants (Hunt et al. 1987, Bowman 1989, Hunt and Rock 1989, Peñuelas et al. 1993, 1997a,b, Ceccato et al. 2001, Maki et al. 2004). Recently, Santos and Kaye (2009) attempted to use NIR spectroscopy to assess Ψ_stem in grapevines. However, their measurements of Ψ_stem using the pressure chamber (Boyer 1967) were not made in the field at the time of the NIR measurements in some experiments, potentially compromising the accuracy of Ψ_stem determinations. Nevertheless, in a laboratory experiment, they obtained good calibrations (R = 0.84) for the prediction of Ψ_stem in Cabernet Sauvignon and Thompson Seedless. Rodríguez-Pérez et al. (2007) obtained significant correlations for Ψ_stem and Ψ_stem-Ψ_sporadically for grapevines using the ratio of leaf reflectance at specific wavelengths when measured at the canopy level, but correlation coefficients were generally low. They asserted that measurements of leaf reflectance may provide a better approach to standardise water status measurement for specific grapevine varieties and they identified several vegetation indices that may be useful for remote sensing of grapevine water stress.

In NIR spectroscopy, calibration is a key mathematical process, which uses multivariate regression techniques relating NIR optical measurements (absorbance values) at selected wavelengths to reference values measured by conventional chemical or physical methods (Murray 1993, Batten 1998, Williams 2001). Once calibrated, the advantages of NIR spectroscopy are the speed of the analysis, simplicity in sample preparation, multiplicity of analysis, and the nonrequirement for the use of chemical reagents (Murray 1993, Batten 1998, Williams 2001). In contrast, water potential measurements can be restrictive, because they are slow, labour intensive, and are therefore expensive (Jones 2004).

This study reports the results of a multivariate analysis of the NIR absorption spectrum and physiological measures of water potential in pot and field grown Shiraz, Cabernet Sauvignon and Chardonnay grapevines.

Materials and methods

Field experiment 1 – Cabernet Sauvignon and Shiraz

The experiment was carried out in the Coombe vineyard at the Waite Campus of the University of Adelaide, South Australia (34°58′3.47″S; 138°38′0.43″E), during the season 2006–07. Measurements were made on own-rooted Cabernet Sauvignon and Shiraz vines, planted in 1991 with a vine spacing of 1.8 m in the row and 3 m between rows. Rows were oriented North-South. The training system was a bilateral spur-pruned cordon with the shoots vertically positioned. Vines were drip irrigated by in-line drippers discharging 1.5 L/h. Drippers spacing within the row was 0.8 m. Six plants for each variety were selected and Ψ_stem, Ψ_sporadically and g were measured on six occasions for Cabernet Sauvignon and eight occasions for Shiraz, at approximately 7-day intervals, from February to March 2007. A Scholander pressure chamber (Scholander et al. 1965) was used to measure Ψ_stem as described by Meron et al. (1987). Measurements were made at midday (1200 to 1400 hours, solar time) on two fully expanded and undamaged leaves for each plant chosen from the mid-upper part of the canopy. One leaf was collected from the midday sunlit side of the canopy and one from the shadowed side. Measurements on both the sunlit and shaded sides of the canopy were used to build the calibration. No leaves from secondary shoots were used. Ψ_stem was measured immediately after Ψ_stem on the same shoot using the leaf below the one used to measure Ψ_stem. For Ψ_sporadically measurements, leaves were covered for 60 min with a ziplock aluminium foil-coated plastic bag before the measure, in order to allow Ψ_stem to equilibrate with Ψ_sporadically (Begg and Turner 1970). After the equilibration period, the leaves were cut and Ψ_sporadically was measured following the same procedure already described for Ψ_stem. A maximum of 30 s elapsed between cutting the leaves and the measurements. The same pressure chamber operator did all measurements with the objective of normalising interpretation of the moment that sap emerges from the petiole. Leaf conductance (g) was measured using a diffusion porometer (AP4, Delta-T Devices, Cambridge, UK) before the leaf was cut for Ψ_stem measurement.

A reflectance spectrum was acquired on the adaxial surface using the same leaf where g and Ψ_stem were measured. The time elapsed between the spectra acquisition and the water potential measurements was about 30 s for Ψ_stem, and 120 s for Ψ_sporadically. No spectra were acquired from the leaf used for Ψ_stem measurement. The spectrophotometer (custom made, Integrated Spectronics, Sydney, Australia) was equipped with a 10 W halogen lamp as the light source and a silicon diode array detector able to collect spectra from the visible and NIR regions of the spectrum to give a total range of 300 to 1100 nm at 3.2 nm wavelength resolution, producing a total of 220 data points. Leaf samples were placed in front of the lens (diameter approximately 40 mm) for scanning. A spectrally black surface was put on the underside of the leaf to minimise differences in background reflectance.

Lab View software (Version 5.1, National Instruments, Austin, Texas, USA) was used to control the spectrophotometer and to acquire the NIR spectra.

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Field experiment 2 – Chardonnay

The experiment was carried out during January (7th–8th) and February (12th) 2009 in a commercial Chardonnay vineyard at Qualco, South Australia (34° 60.76'S; 139° 50.55.76'E). The vines were 5 years old and grafted on Ramsey rootstock. The vines were trained on a two-wire vertical trellis system with a row spacing of 1.8 m between vines and 3.05 m between rows. Five irrigation strategies were used for this study: full irrigation (Control), and reductions to 50, 30, 20 and 10% of the control. The control treatment represented the amount of water that is normally applied in the vineyard (5 ML/ha/year). The trial was a randomised block design with four blocks and was established in 60 rows made of 90 vines each, covering a total area of 3.69 ha. In the block, each treatment was made of three rows and the rows were divided in three sections of 30 plants each. In the middle row of each treatment, the middle plant in each section was selected for measurements.


The experiment was carried out in a glasshouse at the Waite Campus of the University of Adelaide, South Australia (34°58’3.47”S; 138°38’0.43”E), during May and September 2009, on pot-grown Cabernet Sauvignon and Shiraz vines. The water potential measurements were conducted on ten plants per variety. The same 20 plants were measured in four occasions in May and five occasions in September at about 7-day intervals. One shoot for each plant was chosen and \( \Psi_{\text{stem}} \) was measured on a fully expanded leaf using a pressure chamber as described before. The adjacent leaf was bagged for 1 h prior to measurement and \( \Psi_{\text{stem}} \) was measured immediately after \( \Psi_{\text{leaf}} \) as previously described. Six NIR spectra were acquired for each leaf on six different positions evenly distributed over the abaxial leaf surface; two distal along the mid vein and four proximal (two on each half of the leaf, halfway between the mid vein and leaf margin) using the ASD spectrophotometer. No spectra were acquired from the leaf used for \( \Psi_{\text{stem}} \) measurement. The time elapsed between the spectra acquisition and the water potential measurements was about 30 s for \( \Psi_{\text{leaf}} \) and 120 s for \( \Psi_{\text{stem}} \).

Data analysis and interpretation

Spectral and associated laboratory reference data were exported to The Unscrambler® software (version 9.2 CAMO, Oslo, Norway) for chemometric analysis and calibration development. Principal component analysis (PCA) was used to examine any relevant and interpretable pattern in the data (Otto 1999, Naes et al. 2002). PCA was also used to explore the spectral data set for outliers. Calibration models were developed using partial least squares regression (PLS) with full cross validation. The NIR region 750–1050 nm was used to develop the calibration with the spectra obtained from the Integrated Spectronics spectrophotometer while for the ASD FieldSpec® 3 the range 1100–1830 nm was selected. The cross validation was performed using six segments, with 19 samples for each segment. The coefficients of correlation in validation (R) and the standard error in cross validation (SECV) were calculated. The optimum number of terms in the PLS calibration models was determined as indicated by the lowest number of factors that gave the closest to minimum value of the PRESS (prediction residual error sum of squares) function in cross validation, in order to avoid over fitting of the models (Naes et al. 2002).

Water potential and g data collected from the Chardonnay field experiment were analysed using analysis of variance (ANOVA) with Cohort Costat software (Version 6.2, Cohort Software, Monterey, CA, USA). Mean separations were determined using the Student-Newman-Keuls test.

Results

Spectra interpretation

Figure 1 shows averaged NIR spectra obtained during the 2006–07 season with the Integrated Spectronic spectrophotometer from the adaxial surface of leaves of field grown Cabernet

![Figure 1. Near infrared spectra collected from Shiraz (S) and Cabernet Sauvignon (CS) leaves of stressed (\( \Psi_{\text{stem}} < -1.2 \text{ MPa} \)) and non-stressed (\( \Psi_{\text{stem}} > -1 \text{ MPa} \)) plants. Spectra were taken from the adaxial leaf surface using the Integrated Spectronic spectrophotometer during the season 2006–07.](image-url)
Sauvignon and Shiraz vines under non water stressed (\(\Psi_{\text{stem}} > -1 \text{ MPa}\)) and water stressed (\(\Psi_{\text{stem}} < -1.2 \text{ MPa}\)) conditions (Lampinen et al. 2001, Trégoat et al. 2002, Williams and Araujo 2002, Ferreyra et al. 2003, Cifre et al. 2005, Sibille et al. 2007). The main features of the spectra are absorption bands in the 970 nm region. Non-stressed vine leaves showed a higher absorbance in the whole spectrum compared with the stressed vines for both varieties.

The spectra collected with the ASD spectrophotometer from the adaxial and abaxial surfaces of stressed (\(\Psi_{\text{stem}} < -1.2 \text{ MPa}\)) and non-stressed (\(\Psi_{\text{stem}} > -1 \text{ MPa}\)) Chardonnay leaves (Figure 2a,b), show absorption bands in the region between 1400–1450 nm. Overall, leaves of non-stressed plants gave a higher absorbance in the whole spectrum (1100–1830 nm) compared with stressed plants (Figure 2a). Interpretation of the average spectra of adaxial and abaxial surfaces of the leaves at 1445 nm showed a higher absorbance in the adaxial (0.83 a.u.) compared with the abaxial (0.75 a.u.) (Figure 2b).

### Table 1. Calibration statistics for midday stem water potential (\(\Psi_{\text{stem}}\)), midday leaf water potential (\(\Psi_{\text{leaf}}\)), and leaf conductance (\(g\)) calibrations prepared with NIR spectra collected on the adaxial surfaces of fully expanded attached leaves of field grown Cabernet Sauvignon and Shiraz vines.

<table>
<thead>
<tr>
<th></th>
<th>Cabernet Sauvignon</th>
<th>Shiraz</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>84</td>
<td>71</td>
</tr>
<tr>
<td>SD (MPa)</td>
<td>0.29</td>
<td>0.14</td>
</tr>
<tr>
<td>Range (MPa)</td>
<td>-0.72, -1.65</td>
<td>-0.93, -1.52</td>
</tr>
<tr>
<td>SECV (MPa)</td>
<td>0.23</td>
<td>0.11</td>
</tr>
<tr>
<td>R</td>
<td>0.87</td>
<td>0.67</td>
</tr>
<tr>
<td>(\Psi_{\text{stem}}) calibration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\Psi_{\text{leaf}}) calibration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g. calibration</td>
<td>Cabernet Sauvignon</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>71</td>
</tr>
<tr>
<td>SD (MPa)</td>
<td>49.3</td>
<td>81.2</td>
</tr>
<tr>
<td>Range (MPa)</td>
<td>18–286</td>
<td>27–375</td>
</tr>
<tr>
<td>SECV (MPa)</td>
<td>31.7</td>
<td>78.7</td>
</tr>
<tr>
<td>R</td>
<td>0.58</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Data were obtained during the season 2006–07. \(n\), number of samples used in calibration; R, coefficient of correlation; SD, standard deviation; SECV, standard error of cross validation.

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differences between treatments were small. Only the 10% treatment was significantly different from the rest, and did not represent a water stress level for the plants.

The g was measured in January and results show only significant differences between the control and the rest of treatments, which is in contrast with the significant differences observed for $\Psi_{stem}$ among treatments (Table 2).

Table 3 summarises the NIR calibration statistics obtained for the prediction of water potentials in Chardonnay. Similar correlation coefficients were obtained for both $\Psi_{leaf}$ ($R = 0.67$; SECV = 0.26 MPa) and $\Psi_{stem}$ ($R = 0.67$; SECV = 0.24 MPa) when spectra were collected on the adaxial leaf surface.

Effect of adaxial and abaxial leaf surface on the NIR spectra. A visual analysis of the score plot of the second and third principal components (PCs) of the leaf samples analysed using NIR spectroscopy (Figure 4) reveals a clear separation between the two leaf surfaces. The cluster or separation indicates differences in the NIR spectra between the two sides of a leaf. The first three PCs explain more than 95% of the variation in the NIR spectra related to surface. Overall, similar loading weights were observed for the calibrations built using the adaxial surface, with a dominant peak at 1415 nm corresponding to that expected for water. Similarly, the calibration for the prediction of $\Psi_{leaf}$ using the abaxial leaf surface showed a dominant peak at 1415 nm. In contrast, the calibration for $\Psi_{stem}$ using the abaxial surface had a less dominant negative peak at 1418 nm and a more even distribution of the loading weights with wavelength (Figure 5).

Table 3 shows the NIR calibration statistics for the water potential of Chardonnay grapevine leaves obtained from the abaxial leaf surface. The $R$ for $\Psi_{stem}$ was 0.84 (SECV 0.18 MPa), a poorer calibration was obtained for $\Psi_{leaf}$ ($R = 0.80$, SECV 0.21 MPa). The best calibration was between NIR spectra collected from the abaxial surface and $\Psi_{stem}$.

Glasshouse experiment. The calibrations built for $\Psi_{stem}$ and $\Psi_{leaf}$ when more than one spectrum was collected for each Shiraz leaf, yielded the same and high correlation coefficient ($R = 0.92$) and low SECV (0.09 MPa for $\Psi_{stem}$ and 0.11 MPa for $\Psi_{leaf}$) (Table 4). Similarly, for Cabernet Sauvignon, no differences were observed between the $R$ in the two calibrations but

**Table 2.** Midday leaf water potential ($\Psi_{leaf}$), midday stem water potential ($\Psi_{stem}$) and leaf conductance ($g$) measured in a commercial Chardonnay vineyard with five irrigation treatments: control (fully irrigated), and 50, 30, 20 and 10% of the control.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>7–8 January</th>
<th>12 February</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Psi_{stem}$ (MPa)</td>
<td>$\Psi_{leaf}$ (MPa)</td>
</tr>
<tr>
<td>Control</td>
<td>-0.59$^a$</td>
<td>-0.90$^a$</td>
</tr>
<tr>
<td>50%</td>
<td>-0.96$^{ab}$</td>
<td>-1.24$^b$</td>
</tr>
<tr>
<td>30%</td>
<td>-1.07$^b$</td>
<td>-1.32$^b$</td>
</tr>
<tr>
<td>20%</td>
<td>-1.14$^b$</td>
<td>-1.44$^b$</td>
</tr>
<tr>
<td>10%</td>
<td>-1.27$^b$</td>
<td>-1.49$^b$</td>
</tr>
</tbody>
</table>

Measurements were made on the 7–8 January and 12 February 2009. Means followed by different letters are different at $P \leq 0.05$ (Newman-Keuls test).
this variety yielded lower $R$ and lower SECV compared with Shiraz. Results in Table 4 show that all the calibrations built with only one random spectrum per leaf had a lower $R$ and a higher SECV compared with those obtained including multiple spectra in the analysis. Moreover, better calibrations were obtained for $\psi_{\text{stem}}$ compared with $\psi_{\text{leaf}}$ for one spectrum per leaf.

### Figure 5

The first partial least square regression loading weights for the four calibrations performed in Chardonnay using leaf and stem water potential ($\psi_{\text{leaf}}$ and $\psi_{\text{stem}}$) and the adaxial (Ad) and abaxial (Ab) leaf surfaces, during the season 2008–09.

### Table 3

<table>
<thead>
<tr>
<th>$\psi_{\text{stem}}$ calibration</th>
<th>$n$</th>
<th>SD (MPa)</th>
<th>Range (MPa)</th>
<th>SECV (MPa)</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adaxial leaf surface</td>
<td>102</td>
<td>0.32</td>
<td>−0.25, −1.56</td>
<td>0.24</td>
<td>0.67</td>
</tr>
<tr>
<td>Abaxial leaf surface</td>
<td>102</td>
<td>0.32</td>
<td>−0.25, −1.56</td>
<td>0.18</td>
<td>0.84</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$\psi_{\text{leaf}}$ calibration</th>
<th>$n$</th>
<th>SD (MPa)</th>
<th>Range (MPa)</th>
<th>SECV (MPa)</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adaxial leaf surface</td>
<td>101</td>
<td>0.35</td>
<td>−0.35, −1.8</td>
<td>0.26</td>
<td>0.67</td>
</tr>
<tr>
<td>Abaxial leaf surface</td>
<td>102</td>
<td>0.35</td>
<td>−0.35, −1.8</td>
<td>0.21</td>
<td>0.80</td>
</tr>
</tbody>
</table>

$n$, number of samples used in calibration; $R$, coefficient of correlation; SD, standard deviation; SECV, standard error of cross validation.

### Table 4

<table>
<thead>
<tr>
<th>$\psi_{\text{stem}}$ calibration</th>
<th>SD (MPa)</th>
<th>Range (MPa)</th>
<th>Six spectra/leaf</th>
<th>One spectrum/leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>SECV (MPa)</td>
<td>$R$</td>
<td>$n$</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>0.15</td>
<td>−0.54, −1.05</td>
<td>419</td>
<td>0.08</td>
</tr>
<tr>
<td>Shiraz</td>
<td>0.22</td>
<td>−0.48, −1.15</td>
<td>385</td>
<td>0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$\psi_{\text{leaf}}$ calibration</th>
<th>SD (MPa)</th>
<th>Range (MPa)</th>
<th>Six spectra/leaf</th>
<th>One spectrum/leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>SECV (MPa)</td>
<td>$R$</td>
<td>$n$</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>0.14</td>
<td>−0.69, −1.20</td>
<td>415</td>
<td>0.07</td>
</tr>
<tr>
<td>Shiraz</td>
<td>0.29</td>
<td>−0.45, −1.55</td>
<td>386</td>
<td>0.11</td>
</tr>
</tbody>
</table>

$n$, number of samples used in calibration; $R$, coefficient of correlation; SD, standard deviation; SECV, standard error of cross validation.
When all glasshouse samples of Cabernet Sauvignon and Shiraz were used to develop a global calibration for the prediction of $\Psi_{ecw}$ a high correlation coefficient ($R = 0.87$) and a low SECV (0.1 MPa) were observed (Figure 6).

Discussion

NIR reflectance spectroscopy may be used to predict $\Psi_{ecw}$ and $\Psi_{leaf}$ in grapevine because we have demonstrated that regions of the NIR spectrum are highly correlated with the water potential in three varieties of *Vitis vinifera* L. using different NIR spectrophotometers and over different ranges of wavelengths. A higher absorbance in the whole NIR spectrum was consistently associated with non-stressed vine leaves for the three varieties, with both types of spectrophotometers used. Peñuelas and Inoue (1999) observed an increase in the reflectance at all wavelengths with decreasing leaf water content in peanut and wheat leaves. These observations are in general agreement with previous studies showing that the NIR reflectance of a dried leaf is greater than that of a fresh leaf at all wavelengths (Thomas et al. 1966, Knipping 1970, Woolley 1971, Gausman 1974, Peñuelas et al. 1994, Jones et al. 2004). It is well known that water is a strong absorber in the NIR region of the electromagnetic spectrum, therefore non-stressed plants will have higher absorbance values in the NIR spectrum compared with stressed plants.

According to Carter (1991), when scanning single leaves, the water absorption bands that show the highest sensitivity to leaf water concentration are those in the 1300–2500 nm range. Peñuelas et al. (1993, 1997b) and Peñuelas and Filliéla (1998) showed that the weaker water absorption band between 950 and 970 nm is also effective and they defined a water index, initially as the ratio between the reflectance at 970 nm and that at 900 nm ($R_{970}/R_{900}$) (Peñuelas et al. 1993) and later as the inverse ($R_{900}/R_{970}$) (Peñuelas et al. 1997b). The latter ratio was highly correlated with the plant relative water content (RWC) in several trees, shrubs, crops and grasses. Combinations of wavelength bands sensitive to water (760, 970, 1450 and 1940 nm) have been used to generate other indices related to plant water status and soil water availability (Thomas et al. 1971, Hunt and Rock 1989, Danson et al. 1992, Mogensen et al. 1996, Bahrun et al. 2003, Rodríguez-Pérez et al. 2007). Some of these indices have been used to estimate crop water status (Peñuelas et al. 1993, 1997b, Jones et al. 2004, Rodríguez-Pérez et al. 2007) and in remote sensing studies aimed to asses vineyard conditions (Tuckor 1980, Broge and Leblanc 2001, Zarco-Tejada et al. 2005a,b). Furthermore, Eitel et al. (2006) found a correlation ($r^2 = 0.34$) between $\Psi_{leaf}$ and their proposed maximum difference water index (MDWI) when spectra were collected at leaf level for poplar trees. They defined MDWI as the spectral response at the leaf level to water status calculated from the maximum and minimum reflectance located between 1500 and 1750 nm.

In this study, spectra were obtained at the leaf level and it was found that the most relevant water absorption peaks were in the regions of 970 and 1400–1450 nm for the Integrated Spectronics and the ASD FieldSpec® spectrophotometers, respectively. The absorption bands at 970 nm are related to the second overtone of the O-H stretch vibration of water (Murray 1986, Osborne et al. 1993, Williams 2001). Bands in the 1400–1450 nm are related to the first overtone of the OH stretch of water (Murray 1986, Osborne et al. 1993, Williams 2001). According to Eitel et al. (2006) the advantage of taking spectra at the leaf level is that the effect of background variables or atmospheric noise is eliminated so that variations in the spectra are only caused by leaf properties. However, we did not find correlations between $\Psi_{ecw}$ or $\Psi_{ecm}$ and some of the above mentioned indices (WI, MDWI) (data not shown). Nevertheless, water potential was used in this study rather than other measures related to plant water status, such as the RWC and equivalent water thickness (EWT), which is the hypothetical thickness of a single layer of water averaged over the whole leaf (Danson et al. 1992). Water potential provides information about the water status of the plant and the soil as an integrated soil–plant-atmosphere system (Kozlowski et al. 1991), compared with RWC and EWT, which may vary with cell elasticity and leaf development for equivalent water potentials. In this study, rather than using any specific wavelength, a multivariate analysis of the whole spectrum in the range 1100–1830 nm was used to build the calibrations. Previously, Santos and Kaye (2009) obtained good calibrations for the prediction of $\Psi_{ecw}$ in Cabernet Sauvignon and Thompson Seedless in a laboratory experiment, using the whole spectral range 1100–2300 nm. Therefore, the higher correlations obtained in this study and by Santos and Kaye (2009) compared with those obtained by Rodríguez-Pérez et al. (2007) using various vegetation indices ($0.55 < R < 0.67$), might be related to: (i) the use of a larger range of wavelengths rather than ratios of specific wavelengths; (ii) including regions known to be related to water content, and (iii) the collection of spectra at the leaf level instead of the canopy level.

As suggested by Eitel et al. (2006), variations in leaf properties are influenced by factors other than plant water status and these factors complicate the development of a direct relationship between plant water status and spectral indices. Varietal differences in leaf structure, such as the presence or absence of a thick cuticle or waxes, might negatively influence the amount of light transmitted or reflected from the adaxial leaf surface, hampering the penetration of the NIR light through the leaf. These variations might explain the differences in performance of the NIR calibration statistics obtained for the analysed samples. Additionally, factors such as the closely packed palisade tissue in the adaxial surface, compared with the air-filled spongy tissue in the abaxial surface, might play a role in the amount of light transmitted or reflected, as reported by Woolley (1971) and Gausman and Allen (1973). Sinclair et al. (1971) asserted that the spectral response of leaves depends on their surface and internal chemical and structural characteristics.

In this study, NIR calibration statistics from spectra collected using the abaxial surface of the Chardonnay leaves yielded better correlations with water potential compared with those collected on the adaxial surface. In particular, the collection of the NIR spectra in Shiraz samples from the abaxial surface might be one of the reasons why better calibrations were obtained for this variety in the glasshouse experiment compared with the results obtained in the field trial for the season 2006–07. However, this could be the result of factors other than the leaf surface, considering that different instruments and wavelength ranges were used in the two experiments. Differences have been observed previously between adaxial and abaxial surfaces of leaves (Woolley 1971, Walter-Shea et al. 1991, Slaton et al. 2001). The results from this study, in relation to the importance of leaf surface on the collection of the NIR spectra, have some practical implications in the way that canopy reflectance is used to predict plant water status in remote sensing studies. The PLS loading weights as a function of wavelength were examined to determine if particular NIR wavelengths tended to dominate the PLS calibrations obtained for $\Psi_{ecw}$ and $\Psi_{ecm}$ on the
two leaf surfaces (abaxial and adaxial). The similarities between the PLS loading weights for the calibrations obtained using the adaxial leaf surface suggested that wavelengths, in the region around 1400 nm, were important. In contrast, the differences between the loading weights for the two calibrations (Ψ^stem and Ψ^leaf) developed using the abaxial surface indicate a greater variability in reflectance when collecting spectra from this surface. Because these two calibrations yielded better R and SECV compared with those built using the adaxial surface, this may indicate that the spectra contain more information when collected from the abaxial surface, making them more suitable for the purpose of water potential calibrations. Interestingly, the calibration that gave highest R and SECV (Ψ^stem on the abaxial surface) also had the lowest absolute value of loading at 1400 nm relative to other wavelengths. This indicates that other wavelengths in the range 1100–1830 nm gave extra information that improved the calibrations relative to those that might be obtained from selection of only a few wavelengths known to be related to water.

Choné et al. (2001) suggested that Ψ^stem might be used, instead of Ψ^leaf, for vine irrigation management. Given that Ψ^stem is generally considered to be a more integrated and stable measure of plant water status compared with Ψ^leaf, this might explain better NIR calibration statistics obtained for the measurement of Ψ^stem. In most cases, there was greater variation in Ψ^leaf than Ψ^stem. Furthermore, there may be differences in tissue water potential for different positions of a leaf, perhaps because of patchiness in stomatal conductance (Downton et al. 1988). Water status measurements made on Chardonnay during this study showed that Ψ^stem reflects more the imposed water stress treatments than Ψ^leaf or g. These results are in agreement with those reported by other authors (Choné et al. 2001). These authors suggested that Ψ^stem might be used, instead of Ψ^leaf, for vine irrigation management. Given that Ψ^stem is generally considered to be a more integrated and stable measure of plant water status compared with Ψ^leaf, this might explain better NIR calibration statistics obtained for the measurement of Ψ^stem. It was shown that there was greater variation in Ψ^leaf than Ψ^stem and furthermore, there may be differences in tissue water potential and water content for different positions of a leaf, perhaps because of patchiness in stomatal conductance (Downton et al. 1988). This variation appears to be the reason why a single measured spectrum per leaf (spot size 20 mm^2) correlated less well with Ψ^leaf than with Ψ^stem. Taking the average of six spectra uniformly distributed over the lamina surface of a single leaf, resulted in equivalent correlations with Ψ^leaf and Ψ^stem (Table 4). However, Santos and Kaye (2009), found that repeated NIR scanning on the same leaf (15–20 spectra per leaf) may contribute to high levels of background noise in the spectra, requiring the use of the first derivative of the absorbance values to compute the best calibration.

Precision irrigation can be achieved in grapevines using Ψ^stem as a measure of vine water status, because it responds quickly and accurately to (i) vine water restriction; (ii) soil water availability; (iii) soil hydraulic conductivity; and (iv) the capacity of the vine to transport water from the soil to the atmosphere (Choné et al. 2000, 2001). Because NIR can be used as a surrogate and non-destructive measure of Ψ^stem, this technique can be used to accurately control water deficits imposed on vines with the objective of obtaining better WUE and high quality grapes for wine production (Ojeda et al. 2002, Coombe and Iland 2005, Pellegrino et al. 2005). NIR can be used as a physiological indicator for irrigation scheduling based on vine water demand, rather than relying on weather and/or soil moisture measurements, which do not consider the plant in the assessment.

For irrigation scheduling purposes, NIR has the potential to be used within the same general guidelines for vineyard water management as for Ψ^stem, which are: above –1.0 MPa (non-stress), between –1.0 to –1.2 MPa (moderate water restriction) and from –1.2 to –1.5 MPa (severe water restrictions) (Lampinen et al. 2001, Trégoat et al. 2002, Williams and Araujo 2002, Ferreyra et al. 2003, Cifre et al. 2005, Sibile et al. 2007). However, these thresholds may vary depending on the yield and quality aims and the climatic conditions. From our irrigation trial on Chardonnay, it was evident that even at 50% of the normally applied irrigation the vines would not be classified as stressed according to the values indicated above. The link between Ψ^stem and berry quality attributes was shown by Trégoat et al. (2002), who found a strong correlation between this parameter and anthocyanins, phenols and malic acid content in berries. These authors also found good correlations between midday Ψ^stem and grape berry water content and yield.

Conclusions

This study showed that grapevine Ψ^leaf and Ψ^stem can be measured non-destructively using NIR spectroscopy using appropriate calibrations. Observed differences in the NIR spectra were related to the leaf surface in which the spectra were collected, and this had an effect on the accuracy of the calibration statistics for water potential. The global calibrations built using data obtained from glasshouse and field studies on two varieties are indicative that, in the future, a universal calibration, able to predict water potential for all varieties in different environments can be built. Further studies will be carried out in order to address the physiological implications of different leaf surfaces and morphology on the accuracy of NIR calibrations for water potential and in order to build a universal calibration able to predict field water potential for all varieties in all environments.

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