

Use of near infrared reflectance spectroscopy to measure leaf water potential in grape vines

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Introduction

The Australian wine industry is facing an unprecedented challenge to maintain its international competitiveness. This is a severe, immediate challenge, but climate change models predict that such events will become more frequent.^{1,2} 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.²⁻⁴

Irrigation scheduling in vineyards is conventionally based on either direct measures of soil moisture status or on soil water balance calculations.^{3,4} 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 leaf water potential (Ψ_{leaf}) and stomatal conductance (g_s). The measure of stem water potential (Ψ_{stem}) was proposed as an alternative method since it is a more useful and integrative indicator of the water status of the plant.⁵ Nevertheless, this technique requires destructive sampling, pre-treatment for up to one hour. The method is also prone to operator and sampling errors if certain precautions are not taken, and when data are extrapolated to the whole plant or to vineyard scale. In this study the possibility of using a simpler and rapid method was investigated by applying multivariate data analysis and NIR spectroscopy to measure water status in grape-vines.

Materials and methods

The experiment was carried out during January and February 2009 in a commercial Chardonnay vineyard (Yalumba Nurseries). Five irrigation strategies were used in 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 to the vines in the vineyard (5 Mega L/ha/year; irrigations were scheduled to apply 6 mm in 4 hr). Determinations of water potential were performed with a pressure chamber on three plants per treatment and two leaves per vine. The measurements were made at midday (11:30 to 14:30 hr, solar time) on bagged (Ψ_{stem}) and uncovered (Ψ_{leaf}) leaves.^{6,7} On the same leaves, before Ψ_{leaf} was determined, NIR spectra were acquired from both leaf surfaces (adaxial = upper surface and abaxial = lower surface).

The diffuse reflectance spectra of the leaf surfaces were recorded using a contact probe attached by fibre optic cable to an ASD FieldSpec 3 (Analytical Spectral Devices, USA) which records spectra with 1 nm resolution (350–1850 nm). A Spectralon reference tile was used as a

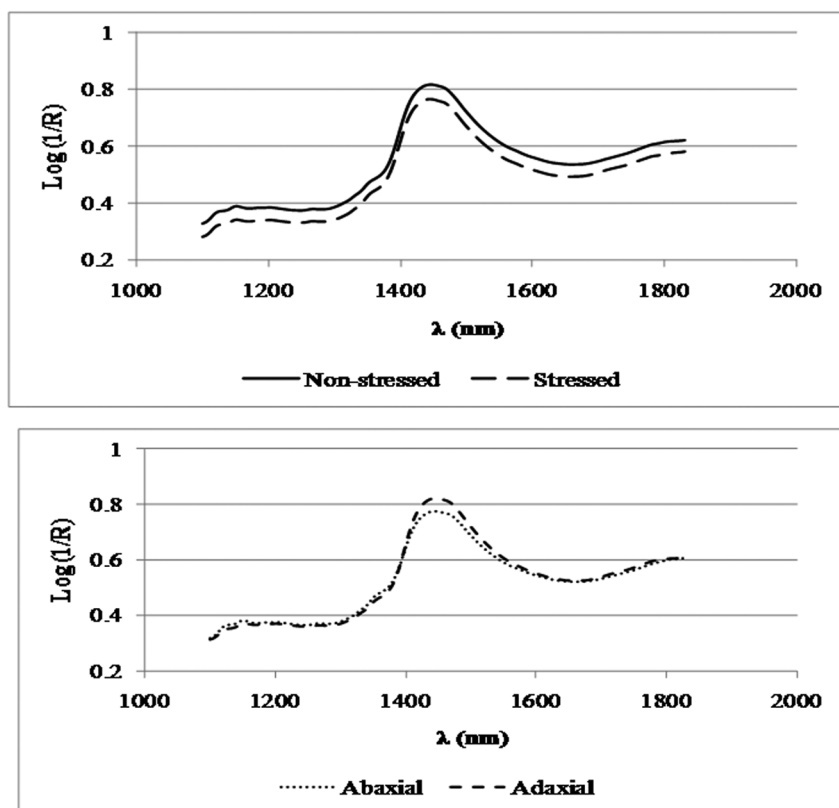


Figure 1. Near infrared spectra of leaves taken from stressed and non-stressed plants (a) and from the adaxial and abaxial leaf surface (b) of Chardonnay vines during 2008–2009 vintage.

white reference. The RS₃ software (RS₃, Analytical Spectral Devices, USA) was used to control the instrument and to acquire the NIR spectra.

The Unscrambler software (version 9.2 CAMO, Norway) was used for chemometric analysis and calibration development. Principal component analysis (PCA) was used to examine the patterns in the data.⁸ Calibration models were developed using partial least squares regression (PLS) with full cross validation. The coefficients of correlation for validation (*R*) and the standard error of cross validation (*SECV*) were calculated.⁸

Results and discussion

The NIR spectra obtained from the adaxial (upper) and abaxial (lower) surfaces of stressed ($\Psi_{\text{stem}} > -1.2$ MPa) and non-stressed ($\Psi_{\text{stem}} < -1.2$ MPa) Chardonnay leaves [Figures 1(a) and (b), showed absorption bands in the region around 1200 nm and between 1400–1450 nm. Water has a minor overtone in the 1150–1200 nm region. Bands in the 1400–1450 nm are related to the first overtone of the OH stretch of water.^{9,10} Overall, leaves of non-stressed plants gave a higher absorbance than stressed plants.^{11,12} 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 to the abaxial (0.75 a.u.) surfaces. The first three principal components explain more than 95% of the variation in the NIR spectra related to leaf surface. A visual analysis of the score plot of the second and third principal components (PC) of the leaf samples analysed using NIR spectroscopy (Figure 2)

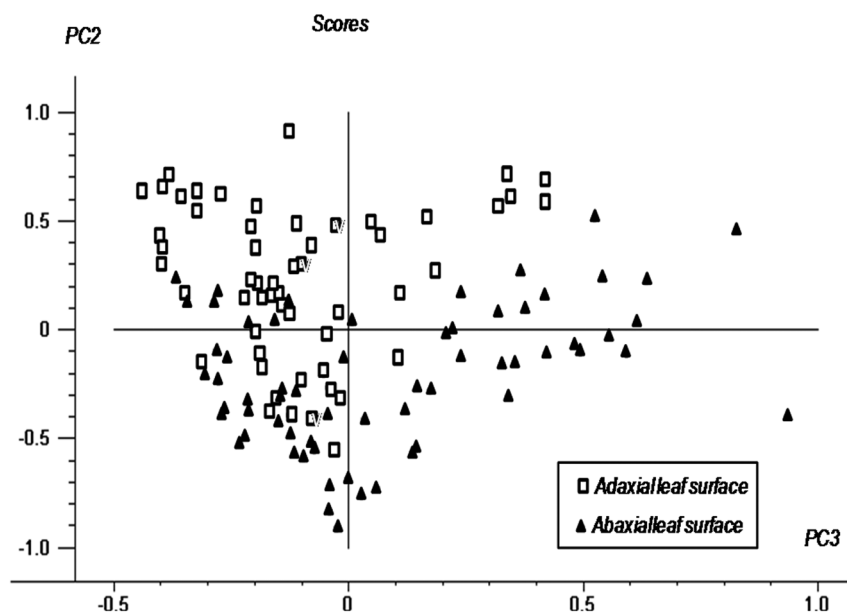


Figure 2. Score plot of the second and third principal components of the NIR spectra collected from the adaxial (□) and abaxial (▲) leaf surfaces of Chardonnay.

Table 1. Cross validation statistics for stem water potential (Ψ_{stem}) based on NIR spectra collected on the adaxial and abaxial surfaces of fully expanded attached leaves of field grown Chardonnay vines.

	<i>n</i>	<i>SD</i>	Range (MPa)	<i>SECV</i>	<i>R</i>
Ψ_{stem}					
Adaxial leaf surface	102	0.32	-0.25, -1.56	0.24	0.67
Abaxial leaf surface	102	0.32	-0.25, -1.56	0.18	0.84

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

reveals a clear separation between the two leaves surfaces used to collect the NIR spectra. The separation between samples indicates differences in the NIR spectra associated with different anatomical structures of the two sides of a leaf.

Water potential measurements conducted on the commercial Chardonnay vineyard show significant differences between irrigation treatments. The NIR calibration statistics for the water potential of grapevine leaves obtained from the abaxial leaf surface are shown in Table 1. The coefficient of correlation for Ψ_{stem} was 0.84 (*SECV* 0.18 MPa), where a poorer calibration was obtained for Ψ_{leaf} (*R* = 0.80, *SECV* 0.21 MPa). The best calibration was obtained using the abaxial surface of the leaf.

Conclusions

This study demonstrates that leaf and stem water potentials of the grapevine can be estimated non-destructively using NIR spectroscopy. Differences in the NIR spectra were observed 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.

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References

1. R. Ragab and C. Prudhomme, *Biosyst. Eng.* **81**, 3 (2002).
2. J. Cifre, J. Bota, J.M. Escalona, H. Medrano and J. Flexas, *Agric. Ecosys. Environ.* **106**, (2005).

3. H.G. Jones, *J. Exp. Bot.* **55**, 2427 (2004).
4. H.G. Jones, *J. Exp. Bot.* **58**, 119 (2007).
5. X. Chone, C. Van Leeuwen, D. Dubourdieu and J.P. Gaudillere, *Ann. Bot.* **87**, 477 (2001).
6. M. Meron, D.W. Grimes, C.J. Phene and K.R. Davis, *Irrig. Sci.* **8**, 215 (1987).
7. P.F. Scholander, E.D. Bradstreet, E.A. Hemmingsen and H.T. Hammel, *Science*. **148**, pp. 339–346 (1965).
8. T. Naes, T. Isaksson, T. Fearn, and A.M.C. Davies, *A User Friendly Guide to Multivariate Calibration and Classification*. NIR Publications Chichester, UK (2002).
9. I. Murray, “The NIR spectra of homologous series of organic compounds”, in *Proceedings of the International NIR/NIT Conference*, Ed by J. Hollo, K.J. Kaffka, and J.L. Gonczy. Akademiai Kiado, Budapest, Hungary, p.13 (1986).
10. P.C. Williams, “Implementation of near-infrared technology, in *Near Infrared Technology in the Agricultural and Food Industries*, Ed by P.C. Williams and K.H. Norris. American Association of Cereal Chemist, St Paul, Minnesota, USA, p. 145 (2001).
11. J. Penuelas, I. Filella, C. Biel, L. Serrano and R. Save, *Inter. J. Remote Sens.* **14**, 1887 (1993).
12. J. Peñuelas and Y. Inoue, *Photosynthetica* **36**, 355 (1999).