

Use of high spectral resolution remote sensing to determine leaf palatability of eucalypt trees for folivorous marsupials

Steve Dury¹, Brian Turner¹, Bill Foley² and Ian Wallis²

¹ Department of Forestry, School of Resource Management and Environmental Science, Australian National University, Canberra, ACT 0200 (e-mail: Stephen.Dury@anu.edu.au)

² Division of Botany and Zoology, Australian National University, Canberra, ACT 0200 (e-mail: William.Foley@anu.edu.au)

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ABSTRACT

In this study we assess the feasibility of remotely measuring canopy biochemistry, and thus the potential for conducting large-scale mapping of habitat quality. A number of studies have found nutrient composition of eucalypt foliage to be a major determinant of the distribution of folivorous marsupials. More recently it has been demonstrated that a specific group of secondary plant chemicals, the diformylphloroglucinols (DFPs), are the most important feeding deterrents, and are thus vital determinants of habitat quality. We report on the use of laboratory spectroscopy to attempt to identify one such DFP, sideroxylonal-A, in the foliage of *Eucalyptus melliodora*, one of the few eucalypt species browsed by folivorous marsupials. Reflectance spectra were obtained for freeze-dried, ground leaves using near infrared spectroscopy (NIRS) and for both oven-dried and fresh whole leaves using a laboratory-based (FieldSpec) spectroradiometer. Modified partial least squares (MPLS) regression was used to develop calibration equations for sideroxylonal-A concentration based on the reflectance spectra transformed as both the first and second difference of absorbance (Log 1/R). The predictive ability of the calibration equations was assessed using the standard error of calibration statistic (SECV). Coefficients of determination (r^2) were highest for the ground leaf spectra (0.98), followed by the fresh leaf and dry leaf spectra (0.94 and 0.87, respectively). When applied to independent validation sub-sets, sideroxylonal-A was most accurately predicted from the ground leaf spectra ($r^2 = 0.94$), followed by the dry leaf and fresh leaf spectra (0.72 and 0.53, respectively). Two spectral regions, centred on 674 nm and 1394 nm, were found to be highly correlated with sideroxylonal-A concentration for each of the three spectral data sets studied. Results from this study suggest that calibration equations derived from modified partial least squares regression may be used to predict sideroxylonal-A concentration, and hence leaf palatability, of *Eucalyptus melliodora* trees, thereby indicating that the remote estimation of habitat quality of eucalypt forests for marsupial folivores is feasible.

INTRODUCTION

The Australian environment is characterised by poor quality soils and high levels of solar radiation. This dispropor-

tionate balance of soil nutrients to carbon availability (via photosynthesis) allows plants a high investment in carbon-based secondary metabolites, as exemplified by *Eucalyptus*, the tree genus which dominates most Australian forests [Landsberg & Cork, 1997]. As a result, the foliage of these trees is often a poor quality food resource to the extent that large portions of Australian forests do not support viable populations of leaf-eating mammals [Braithwaite *et al*, 1983; Cork & Catling, 1996]. Only four mammalian species, all marsupials, have digestive and metabolic adaptations that permit them to eat eucalypt foliage. These are the common ringtail possum (*Pseudocheirus peregrinus*), the greater glider (*Petauroides volans*), the koala (*Phascolarctos cinereus*), and, to a lesser extent, the common brushtail possum (*Trichosurus vulpecula*). Clearing has removed much of the forest from the most productive soils, and contemporary forest management activities remain a source of conflict between conservation organisations and timber/farming industries [National Forest Inventory, 1998]. Identification of the habitat requirements for these arboreal, folivorous marsupials has therefore become an important priority among forest ecologists in Australia.

Pausas *et al* [1995] modelled a number of factors (eg, foliage and soil nutrients, site characteristics, forest structure) thought to explain the distribution of folivorous marsupials in eucalypt forests, and concluded that the main factor predicting their occurrence is leaf nutrient quality. These results are consistent with other studies that have shown leaf nutrient quality to be a major determinant of the distribution of folivorous marsupials in eucalypt forests [Braithwaite *et al*, 1983, 1984; Norton, 1987; Kavanagh & Lambert, 1990; Cork, 1992].

Eucalypt foliage presents particular nutritional challenges through having low concentrations of nutrients, high concentrations of components that interfere with digestion (eg, lignin and tannins) and a range of potentially toxic allelochemicals, mainly carbon-based chemical defenses such as phenolics and terpenoids (essential

oils), that deter herbivores because of their taste or post-ingestive consequences [Fox & Macauley, 1977; Cork & Foley, 1991, 1997; Cork, 1996]. Phenolics may comprise up to 40 percent of foliar dry matter in eucalypts, much higher than in other forests in the world [Fox & Macauley, 1977; Cork, 1996].

Early studies of Plant Secondary Metabolite (PSM)–herbivore interactions focused on non-absorbable phenolics, such as tannins, because they are ubiquitous and because it was believed that they interfered with digestive processes by binding protein in the gastrointestinal tract [McArthur & Sanson, 1993]. However, it is becoming increasingly evident that the role of tannins as reducers of protein digestibility has been over-emphasised [Foley *et al*, 1999; Pass & Foley, 2000].

Recent advances in the chemical identification of *Eucalyptus* PSMs have resulted in better correlative relationships between feeding and individual absorbable phenolic constituents of eucalypt foliage [Lawler *et al*, 2000]. In particular, a newly discovered group of PSMs, the diformylphloroglucinol compounds (DFPs), have been found to play an important role in deterring feeding by marsupial folivores [Lawler *et al*, 1998a,b, 1999, 2000; Pass *et al*, 1998]. Lawler *et al* [1998a] developed a method to quantify sideroxylonals, a subset of the DFPS, and have shown that in *Eucalyptus* species where sideroxylonal is the predominant DFP, it explains the majority of variation in feeding by marsupial folivores among individual trees [Lawler *et al*, 2000].

Traditional methods for assessing foliar chemistry involve the collection of field samples, their drying and grinding, followed by laboratory analyses. These measurements are costly and time consuming, and impractical to apply over large areas. By sensing variations in solar energy absorbance caused by the presence of foliar biochemical compounds, near infrared spectroscopy (NIRS) can significantly reduce the time spent on laboratory analyses. Examination of spectral characteristics obtained with NIRS has revealed strong relationships between functional groups of PSMs and specific wavelengths [Windham *et al*, 1988; McClure & Williamson, 1988; Clark *et al*, 1987; Roberts *et al*, 1993; Mika *et al*, 1997]. It has been also found to be a useful tool for modelling aspects of the intake and digestion of forages containing PSMs. By applying an NIRS-based model to feeding by common ringtail possums fed three species of *Eucalyptus* foliage, Lawler *et al* [2000] explained more than 80 percent of the variation in foliage intake. This matched the explanatory power of a detailed and time-consuming investigation of the secondary chemistry of the plants.

In this study we report on the use of laboratory spectroscopy to attempt to measure leaf palatability through

the estimation of sideroxylonal-A concentration in the foliage of individual *Eucalyptus melliodora*. This will give an indication of the potential for conducting regional-scale mapping of tree palatability/habitat quality through the use of airborne/spaceborne imaging spectroscopy. Reflectance spectra obtained in the laboratory using whole, fresh leaf samples are analysed to establish whether calibration equations derived from the radiometric properties of *E. melliodora* foliage can be used to predict sideroxylonal-A concentration, and the results are compared with those obtained using whole, dry leaf samples and freeze-dried, ground leaf material.

METHODS

Leaf samples were collected in June 1998 and April 1999 from 85 *E. melliodora* growing in open woodland approximately 20 km east of Canberra. All samples were of fully-expanded adult foliage from the mid to upper canopy. An Analytical Spectral Devices (ASD) FieldSpec FR spectroradiometer, which senses in the spectral range 350 to 2500 nm with a spectral resolution of between 3 and 10 nm, was used in the laboratory to measure spectral reflectance of both the intact fresh leaf samples and oven-dried leaves (70°C for 48 hours) from each tree.

Reflectance measurements of the fresh leaf samples were made within 4 hours of the leaves being detached. Experiments on signal degradation with time showed that there was no change in the spectral reflectance of the leaves within the first 4 hours after detachment. A constant source of illumination was provided by a modified Makita high intensity reflectance probe. Multiple leaf layers were used to ensure reflectance saturation. Average reflectance (R) was calculated from ten spectral recordings per tree, and converted to Log (1/R), a form analogous to absorbance and, assuming Beer's Law, approximately linear with concentration. This procedure was repeated for sub-samples of oven-dried leaves from each tree. Near infrared spectroscopy (NIRS) was used to collect spectra from a sub-sample of freeze-dried, ground leaf material from each tree. Leaf sideroxylonal-A concentrations (mg per g of leaf dry matter) were determined on freeze-dried leaf material using a procedure described by Lawler *et al* [2000].

Both first and second derivatives (multiple order differentiation of the Log (1/R) data) were calculated. A modified partial least squares (MPLS) regression technique was used to regress spectral data against the corresponding sideroxylonal-A concentrations. This method is superior to stepwise multiple linear regression for determination of leaf chemistry by NIRS, and is less likely to result in overfitting [Shenk & Westerhaus, 1991; Bolster *et al*, 1996]. PLS reduces the spectral data to a few combinations of the absorbance values that not only explain

much of the spectral information but also relate to the sample reference (*ie*, sideroxylonal-A) values. We used cross validation to estimate the optimal number of terms for the calibration, in which the population is arbitrarily divided into a small number of groups, and a prediction is made of the values for one group based on calibrations developed from the remaining groups. In turn, predictions were made for all groups with the average of predictions for all groups [Shenk & Westerhaus, 1993].

Although this means the requirement to maintain separate validation and calibration sets is no longer strictly necessary, we have nonetheless done so as an additional means of verification. Of the 85 samples, 60 were randomly selected for calibration (for both dry and fresh leaf data-sets) and 25 retained as validation samples. Fifty samples are recommended as the minimum for calibration, although a higher percentage should yield more accurate predicted values [Shenk *et al*, 1978]. The calibration data-set was used to establish the coefficients in the regression equations. The resulting calibration equations were then used to predict the sideroxylonal-A concentrations of the validation data sets. Validation samples were combined with the calibration set to derive the final regression equations. The predictive ability of the calibration equations was assessed using the coefficients of multiple determination (r^2) and, foremost, the standard error of calibration statistic (SECV), which is based on an iterative calibration-validation algorithm. The equation with the lowest SECV and highest r^2 is selected as the best calibration [Mark & Workman, 1991].

Scatter is a nonlinear function that can distort the relationship between the spectrum and the reference, although a number of scatter correction techniques are available that minimize the variations of scattering due to variable particle-size, and account for variation in base-

line shift and curvilinearity [Barnes *et al*, 1989]. Four methods of scatter correction were compared: using a Standard Normal Variate (SNV) and de-trending transformation (evaluated together and separately), a multiplicative scatter correction (MSC), and using raw spectra only (*ie*, no scatter correction).

RESULTS

The 'best' predictive equations obtained for the first and second derivative transformations of the Log (1/R) data for each of the three sets of leaf spectral measurements are reported in Table 1. Spectra below 500 nm were eliminated from the FieldSpec spectrometer measurements prior to analysis, because of inconsistent readings encountered in this spectral region. This prevented subsequent comparisons across data sets for these wavelengths.

FREEZE-DRIED, GROUND LEAF MATERIAL

The regression models using the first and second derivative transformations of the Log (1/R) data sets predicted the validation data sets with coefficients of determination (r^2) of 0.94 and 0.89, respectively (Figure 1a,b).

The 'best' calibration regressions for the first and second derivative transformations gave similar results in terms of r^2 and SECV (see Table 1). The regression equations of the combined calibration and validation data sets show that while both have r^2 of 0.98, the first derivative transformation was superior to the second through having a lower SECV.

Figure 2 depicts the correlation between first derivative transformed NIRS absorbance and the sideroxylonal-A concentration of *E. melliodora* samples. The three major correlation peaks for the first derivative transformation

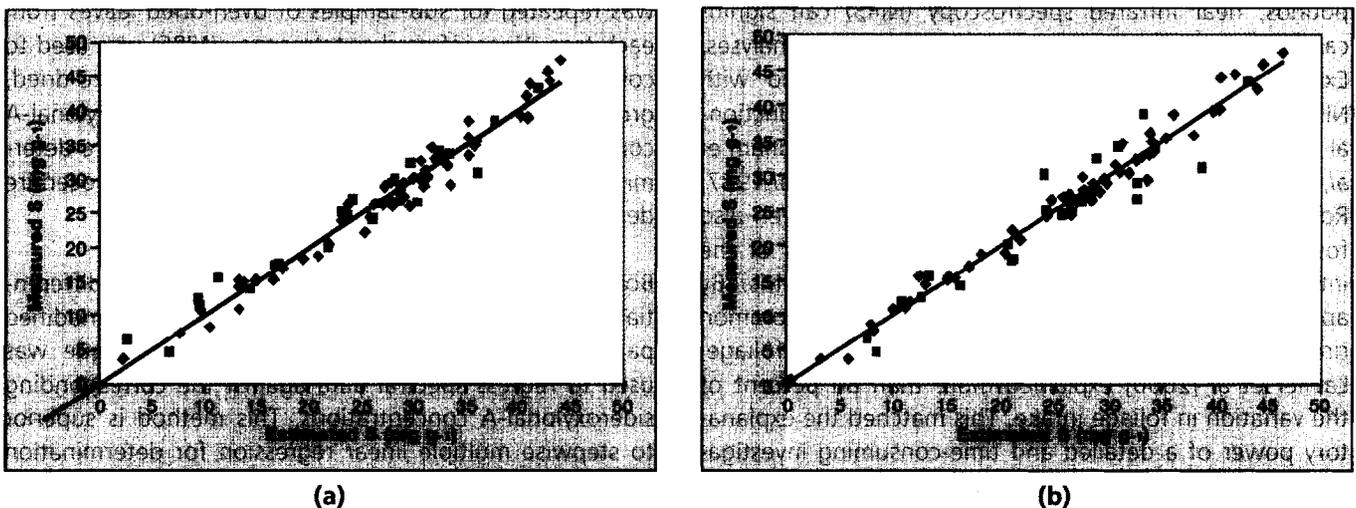


FIGURE 1: Plot of measured versus predicted sideroxylonal-A concentration (S), using calibration regression equations generated for freeze-dried, ground leaf data set, for a) first derivative and b) second derivative Log (1/R) data. The calibration data is represented by diamonds and the validation data by squares.

of the Log (1/R) data occur at a broad band of wavelengths centred on 856 nm, a narrower band centred on 1394 nm, and a third peaking at 1902 nm. Other correlation peaks occur at wavelength bands centred on 450, 568, 618, 674, 1222, 2320, 2406 and 2478 nm.

The three major correlation peaks for the second derivative transformation of the Log 1/R data occur at 1156 nm, 1334 nm and 1910 nm. Other notable correlation peaks ($r > 0.57$) occur at 486, 574, 626, 682, 886, 916, 1156, 1240, 1334, 1404, 1458, 1910, 2136, and 2244 nm.

WHOLE DRY LEAVES

The regression model using the first derivative transformation of the validation data set resulted in a more reliable prediction ($r^2 = 0.72$; Figure 3a) than that obtained using the second derivative transformation ($r^2 = 0.58$; Figure 3b).

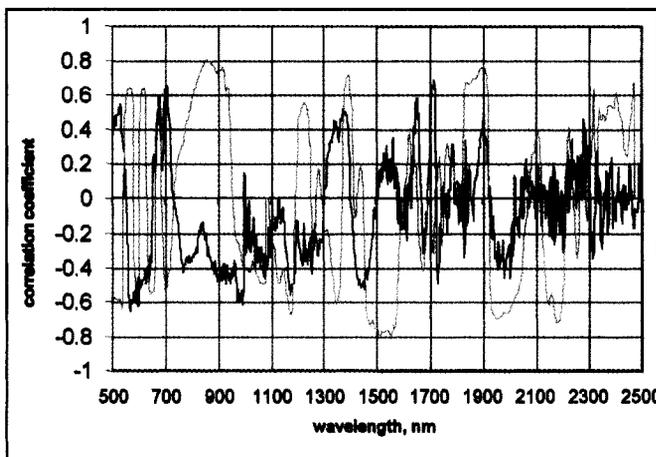


FIGURE 2: Correlogram of sideroxydonal-A concentration by wavelength for freeze-dried ground leaf material (lighter line), compared with correlations for fresh leaf (darker line).

The first derivative transformation of both the calibration and combined calibration and validation data had the lowest SECVs (6.64 and 5.98 respectively), with corresponding coefficients of determination of 0.82 and 0.85 (Table 1).

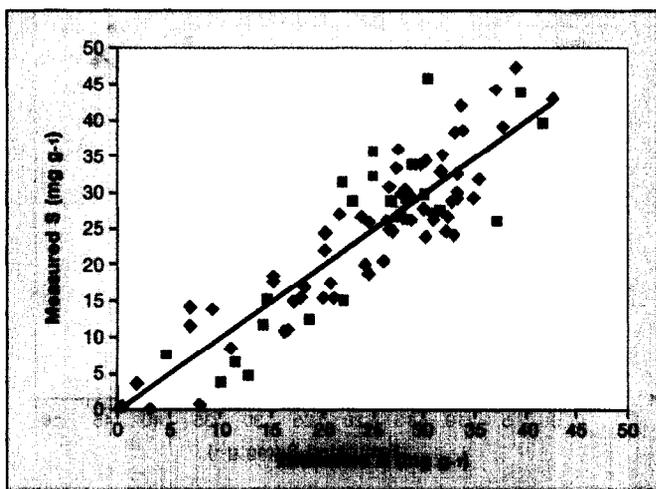
The correlation between first derivative transformed spectrometer absorbance and the sideroxydonal-A concentration of *E. melliodora* samples is depicted in Figure 4. The major correlation peaks for the first derivative transformation of the Log (1/R) data occur at wavelength bands centred on 526, 561, 671, 1417, 1647 and 1800 nm. Other minor correlation peaks occur at wavelength bands centred on 615, 715, 787, 1711, 1985 and 2013 nm.

The highest correlations for the second derivative transformation of the Log (1/R) data occur at 531, 622, 645, 685, 761, 1438 and 1800 nm.

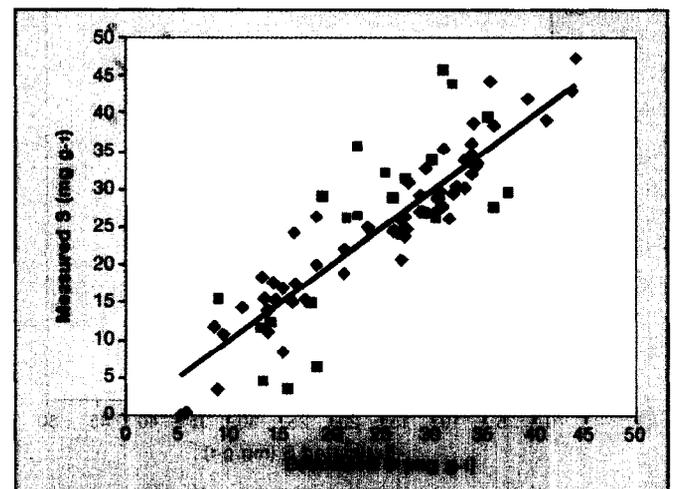
WHOLE FRESH LEAVES

The validation data sets were predicted with coefficients of determination of 0.49 and 0.53 for the regression models using the first and second derivative transformations of the Log (1/R) data sets, respectively (Figure 5a,b).

The 'best' calibration data set regressions for the first and second derivative data transformations were similarly ranked in terms of r^2 and SECV, although the SECV was lower for the former (see Table 1). The regression equations of the combined calibration and validation data sets show that the predictive value of the first derivative data transformation was superior to the second derivative data transformation through having a lower SECV (5.22 against 6.06) despite the higher r^2 (0.95 against 0.91) of the latter.



(a)



(b)

FIGURE 3: Plot of measured versus predicted sideroxydonal-A (S), using calibration regression equations generated for whole, oven-dried leaf data set, for a) first derivative and b) second derivative Log (1/R) data. The calibration data is represented by diamonds and the validation data by squares.

TABLE 1: Summary of results from modelling the relationship between spectral characteristics of *E. melliodora* foliage and the foliar concentration of sideroxylonal-A

	<i>r</i> ²	SECV	Mathematical Treatment*	Scatter correction	N
<i>Freeze-dried ground leaf</i>					
All samples	0.98	2.53	1441	SNV	81
	0.98	3.04	2441	SNV/Detrend	83
Calibration set	0.98	3.12	2441	SNV/Detrend	58
	0.97	3.22	1441	SNV	59
<i>Whole oven-dried leaf</i>					
All samples	0.85	5.98	1881	Detrend	82
	0.87	6.86	2881	SNV/Detrend	81
Calibration set	0.82	6.64	1881	SNV/Detrend	59
	0.89	6.90	2881	SNV/Detrend	59
<i>Whole fresh leaf</i>					
All samples	0.91	5.22	1881	SNV	81
	0.94	6.06	2881	SNV/Detrend	85
Calibration set	0.91	5.71	1881	SNV/Detrend	59
	0.94	6.13	2881	SNV/Detrend	60

*Note: "Mathematical treatment" refers to the derivatives and range of data points over which the derivative and smoothing functions were calculated. Thus 1,4,4,1 refers to first derivative, a gap of 4 nm (ASD spectrometer) or 8 nm (near infrared spectrometer, which records reflectance every 2 nm), and a smoothing over 4 data points.

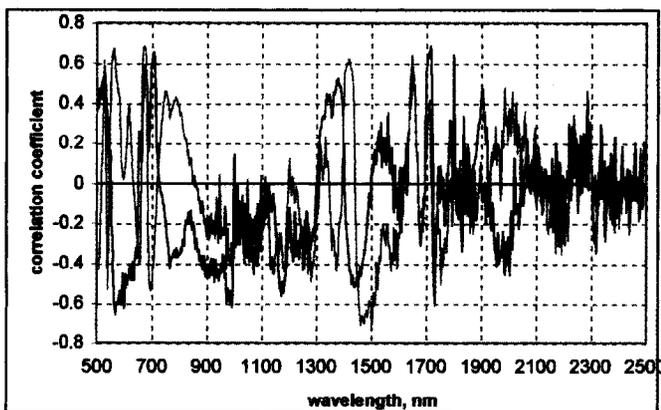
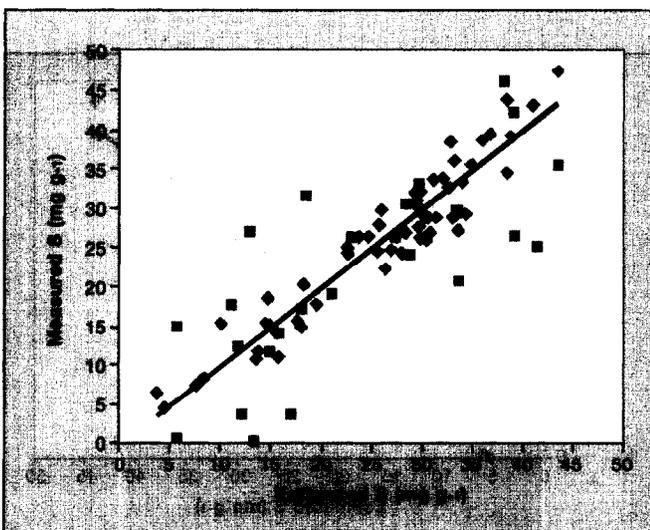


FIGURE 4: Correlogram of sideroxylonal-A concentration for oven-dried ground leaf material (lighter line), compared with correlations for fresh leaf (darker line).

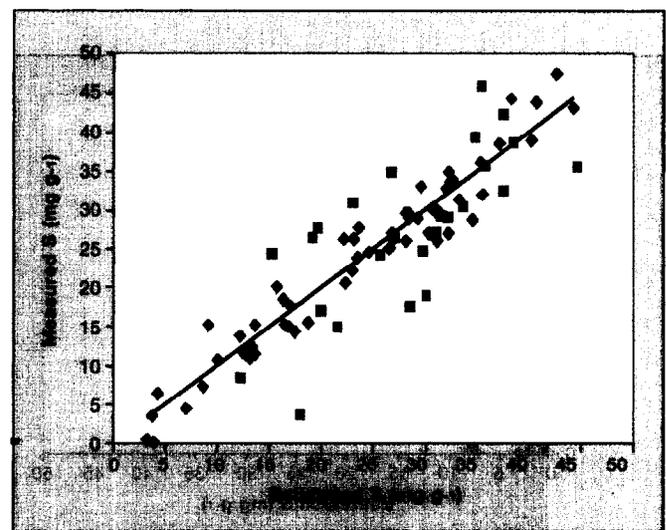
The major correlation peaks for the first derivative transformation of the Log (1/R) data (plotted in Figures 2 and 4) occur at wavelength bands centred on 528, 674, 707, 1378, 1651, 1716, 1904 and 2286 nm. The highest correlations for the second derivative transformation of the Log (1/R) data occur at 528, 556, 701, 1199, 1391, 1664 and 1713 nm.

DISCUSSION

Comparison of the results for the ground, dry and fresh leaf data sets reveals that both the first and second derivative transformations of the Log (1/R) data are equally capable of predicting sideroxylonal-A concentrations. No single scatter correction consistently featured in the



(a)



(b)

FIGURE 5: Plot of measured versus predicted sideroxylonal-A, using calibration regression equations generated for the whole, fresh leaf data set, for a) first derivative and b) second derivative Log (1/R) data. The calibration data is represented by diamonds and the validation data by squares.

selected models. The optimal mathematical treatment for each of the three leaf treatment data sets occurred when the derivative was calculated over a gap of 8 nm.

Using first derivative transformations of the Log (1/R) data, two spectral regions, centred on 674 nm and 1394 nm (although the latter varied between 1378 and 1417 nm) appeared highly correlated with sideroxylonal-A concentration across the three leaf treatments. The former coincides with the only spectral region picked out from each treatment using second derivative transformation data, namely at 682, 685 and 701 nm, for the ground, dry and fresh leaf data sets, respectively. Two wavelength regions (568/561 nm and 618/615 nm) exhibited high correlation in the ground and dry leaf data sets, but not in the fresh leaf data set. Three wavelength regions (526/528 nm, 1647/1651 nm and 1711/1716 nm) showed high correlation in the dry and fresh leaf data sets, but not in the ground leaf data set.

The second derivative transformations gave fewer matching regions of high correlation. Apart from the 685 nm region, highlighted in all three datasets, the only other matching correlations were at 626/622 nm (ground and dry leaf data sets), 531/528 nm (dry and fresh leaf data sets), and 1404/1391 nm (ground and fresh leaf data sets).

The results from the fresh leaf measurements are of particular interest for assessing the capability to remotely estimate an important measure of habitat quality (concentrations of plant secondary chemicals) of eucalypt forests for folivorous marsupials. By comparing calibration equations and wavelength correlations obtained from reflectance measurements of whole, fresh leaves with those from dry/ground leaf material, it is possible to judge whether wavelengths identified from the MPLS calibration equations of the fresh leaf spectra are important, or perhaps merely artefacts of the data. Since the higher correlations in the fresh leaf first derivative transformation spectral regions centred on 674 and 1378 nm were identified in the other data sets, one may presume that these regions are significant. 1378 nm is near an area of strong water absorption, and in the dry and ground leaf spectra the strongest correlations were actually centred at slightly longer wavelengths, around 1405 nm, where it is likely that the dominant absorption by water obscured that of sideroxylonal-A in the corresponding fresh leaf spectra. Other likely important spectral regions are those centred on 528 nm, 1651 nm, and 1716 nm, identified in both the fresh and dry whole-leaf data sets. Flinn *et al* [1996] found that phenolics have a major absorbance around 1650 nm, and this wavelength has been used to separate forage samples containing high and low concentrations of phenolic constituents. The three correlation peaks identified in the freeze-dried,

ground leaf spectra between 2300-2500 nm were not identified in either the fresh or dry leaf spectra. This may be due to the 'noisier' spectra obtained in this region when measured by the FieldSpec spectroradiometer. In future analyses we will investigate the predictive ability of regression models using only the wavelengths highlighted above, to further minimise the risk of overfitting.

A recent study has highlighted a role for terpenes in marsupial folivore-*Eucalyptus* interactions, whereby they appear to act as a cue to concentrations of toxic DFPCs in the leaves (*ie*, a conditioned flavour aversion) [Lawler *et al*, 1999]. Indeed, the same authors note that correlations between terpenes (*eg*, cineole) and DFPCs have been found in several eucalypt species. It is possible, therefore, that wavelength correlations with sideroxylonal-A concentration may in fact relate to absorbance features of terpenes. As long as the correlation between the two substances is consistent this does not pose a problem and may even serve to enhance our predictions of DFPC's. To gain a better understanding of whether the wavelengths highlighted in this study are a direct consequence of absorption by sideroxylonal-A we plan to collect spectra of purified sideroxylonal-A, extracted as described in Lawler *et al* [2000]. Although Wessman *et al* [1988] pointed out that the exact wavelengths of pure compound absorption will not necessarily appear in near infrared spectroscopy equations, due to the presence of other compounds in the mixture tending to broaden and confound their effect, if the regression equations include terms that appear close to the constituent's absorbing wavelengths we can have greater confidence in the robustness of the equations, particularly for the estimation of sideroxylonal-A concentration at sites distant from the training sites.

With the continuing clear-cutting of Australia's forests it is imperative to accurately assess the quality of forest blocks as habitat for arboreal marsupials. Management of forests for wildlife will require accurate maps of the diversity and extent of habitats over large areas and often difficult terrain. Even with NIRS it will be impractical to assess all but the smallest forest blocks, or subsections of larger blocks. High spectral resolution airborne imaging spectrometry offers scope for a faster, large scale assessment of habitat quality. This is the first stage in part of a wider study aimed at tracking minor absorption features in reflectance measurements from the laboratory to the field. High spectral resolution airborne or satellite data offers scope for rapid, large-scale assessments of habitat quality. However extraction of the spectral signature of leaf biochemical composition at the canopy level faces additional problems, such as sensor limitations (*eg*, signal to noise ratio), atmosphere (selective absorption and scattering), physical characteristics of the canopy (*eg*, geometry/ structure, Leaf Area Index,

Leaf Angle Distribution) and background (eg, soil and understorey reflectance) [Baret & Fourty, 1997; Dawson *et al*, 1999]. Methods to reduce the effect of unwanted background reflectance in airborne imaging spectrometry [Dury & Jia, 2001] are also currently being evaluated, to determine whether leaf palatability and hence habitat quality can be measured at the landscape level. If successful, this will provide the first opportunity to extend laboratory-based data to a scale sufficient to answer fundamental questions at the landscape level pertinent to the management and conservation of arboreal folivores.

CONCLUSION

In this study we have demonstrated that laboratory spectroscopy can be used to measure the concentration of a eucalypt PSM (sideroxylonal-A, known to play an important role in deterring feeding by marsupial folivores), using intact, fresh leaf samples, with reasonable predictive value. This is despite the dominant effect of absorption by water in fresh leaves, which obscures some but not all of the useful absorption features. A number of wavelength regions highly correlated with sideroxylonal-A concentration in the fresh leaf spectra were also found to be highly correlated in the dry and/or ground leaf spectra, suggesting these are likely to have some physical significance rather than being attributable to noise. In particular the first derivative transformation spectral regions centred on 674 nm and 1378 nm are likely to be useful for predictive purposes since these were identified in all three data sets. These results indicate that the remote estimation of habitat quality of eucalypt forests for folivorous marsupials is feasible, dependent on the successful extraction of the foliar spectra from airborne/spaceborne imaging spectrometry data.

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REFERENCES

- Barnes, R.J., M.S. Dhanoa & S.J. Lister, 1989. Standard normal variate transformation and detrending of NIR spectra. *Applied Spectroscopy* 43: 772-777.
- Baret, F. & T. Fourty, 1997. The limits of a robust estimation of canopy chemistry. *Proceedings, 7th Symposium on Physical Measurements and Signatures in Remote Sensing, Courcheval, April 7-11, pp. 413-420.*
- Bolster, K.L., M.E. Martin & J.D. Aber, 1996. Determination of carbon fraction and nitrogen concentration in tree foliage by near infrared reflectance: a comparison of statistical methods. *Canadian Journal of Forest Research* 26: 590-600.
- Braithwaite, L.W., M.L. Dudzinski & J. Turner, 1983. Studies on the arboreal marsupial fauna of eucalypt forests being harvested for woodpulp at Eden, NSW. II. Relationship between the faunal density, richness and diversity, and measured variables of the habitat. *Australian Wildlife Research* 10: 231-247.
- Braithwaite, L.W., J. Turner & J. Kelly, 1984. Studies on the arboreal marsupial fauna of eucalypt forests being harvested for woodpulp at Eden, NSW. III. Relationships between faunal densities, eucalypt occurrence and foliage nutrients, and soil parent materials. *Australian Wildlife Research* 11: 41-8.
- Clark, D.H., M.H. Ralphs & R.C. Lamb, 1987. Total alkaloid determinations in larkspur and lupine with near infrared reflectance spectroscopy. *Agronomy Journal* 79: 485-490.
- Cork, S.J., 1992. Polyphenols and the distribution of arboreal, folivorous marsupials in Eucalyptus forests of Australia. In: R.W. Hemingway (Ed.), *Plant Polyphenols: Synthesis, Properties, Significance*. Plenum Press, New York, NY, pp. 653-663.
- Cork, S.J., 1996. Optimal digestive strategies for arboreal herbivorous mammals in contrasting forest types: why koalas and colobines are different. *Australian Journal of Ecology* 21: 10-20.
- Cork, S.J. & P.C. Catling, 1996. Modelling distributions of arboreal and ground-dwelling mammals in relation to climate, nutrients, plant chemical defences and vegetation structure in the eucalypt forests of southeastern Australia. *Forest Ecology and Management* 85: 163-175.
- Cork, S.J. & W.J. Foley, 1991. Digestive and metabolic strategies of arboreal and mammalian folivores in relation to chemical defences in temperate and tropical forests. In: R.T. Palo & C.T. Robbins (Eds), *Plant Defences Against Mammalian Herbivory*. CRC Press, Boca Raton, FL, pp. 133-166.
- Cork, S.J. & W.J. Foley, 1997. Digestive and metabolic adaptations of arboreal marsupials for dealing with plant antinutrients and toxins. In: N.R. Saunders & L.A. Hinds (Eds), *Marsupial Biology, Recent Research, New Perspectives*. UNSW Press, Sydney, pp. 215-226.
- Dawson, T.P., P.J. Curran, P.R.J. North & S.E. Plummer, 1999. The propagation of foliar biochemical absorption features in forest canopy reflectance: a theoretical analysis. *Remote Sensing of Environment* 67: 147-159.
- Dury, S.J. & X. Jia, 2001. Use of a hybrid supervised and unsupervised classification model to determine nitrogen concentration of eucalypt tree foliage using HyMap data. *Proceedings, IEEE International Geoscience and Remote Sensing Symposium (IGARSS 2001), Sydney, 9-13 July 2001. CD ROM.*
- Flinn, P.C., Edwards, N.J., Oldham, C.M., McNeil, D.M., 1996. Near infrared analysis of the fodder shrub tagaste (*Chamaecytisus proliferus*) for nutritive value and anti-nutritive factors. In: A M C Davies, P Williams (editors), *Near infrared spectroscopy: the future waves*. NIR, Chichester, pp. 576-580.
- Foley, W.J., G. Iason, C. McArthur, 1999. Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores – how far have we come in 25 years? In: H-J.G. Jung & G.C. Fahey (Eds), *Fifth International Symposium on the Nutrition of Herbivores, San Antonio, TX, April 1999*. American Society of Animal Science, Savoy, IL, pp. 203-274.
- Fox, L.R. & B.J. Macauley, 1977. Insect grazing on Eucalyptus in response to variation in leaf tannins and nitrogen. *Oecologia* 29: 145-162.
- Kavanagh, R.P. & M.J. Lambert, 1990. Food selection by the Greater Glider, *Petauroides volans*: Is foliar nitrogen a determinant of habitat quality? *Australian Wildlife Research* 17: 285-299.
- Landsberg, J. & S.J. Cork, 1997. Herbivory: interactions between eucalypts and the vertebrates and invertebrates that feed on them. In: J.E. Williams & J.C.Z. Woinarski (Eds), *Eucalypt Ecology: Individuals to Ecosystems*. Cambridge University Press, Cambridge, pp. 342-372.
- Lawler, I.R., W.J. Foley, B.M. Eschler, D.M. Pass & K. Handasyde, 1998a. Intraspecific variation in Eucalyptus secondary metabolites determines food intake by folivorous marsupials. *Oecologia* 116: 160-169.

- Lawler, I.R., W.J. Foley, B.M. Eschler & D.M. Pass, 1998b. Administration of a 5HT₃ receptor antagonist increases the intake of diets containing Eucalyptus secondary metabolites by marsupials. *Journal of Comparative Physiology B* 16: 611-618.
- Lawler, I.R., J. Stapley, W.J. Foley & B.M. Eschler, 1999. Ecological example of conditioned flavour aversion in plant-herbivore interactions: effect of terpenes of Eucalyptus on feeding by common ringtail and brushtail possums. *Journal of Chemical Ecology* 25: 401-415.
- Lawler, I.R., W.J. Foley & B.M. Eschler, 2000. Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. *Ecology* 81: 1327-1338.
- Mark, H.L. & D. Tunnell, 1985. Qualitative near infrared analysis using Mahalanobis distances. *Analytical Chemistry* 57: 1449-1456.
- Mark, H.L. & J. Workman, 1991. *Statistics in Spectroscopy*. Academic Press, San Diego, CA.
- McArthur, C. & G.D. Sanson, 1993. Nutritional effects and costs of a tannin in two marsupial arboreal folivores. *Functional Ecology* 7: 697-703.
- McClure, W.F. & R.E. Williamson, 1988. Near infrared analysis of tobacco. In: C.S. Creaser & A.M.C. Davies (Eds), *Analytical Applications of Spectroscopy*. Royal Society of Chemistry, London, pp. 109-132.
- Mika, V., A. Bartko, P. Nerušil & F. Smital, 1997. Use of NIR method for evaluation of rape quality. *Rost Vyroba* 43: 283-286.
- National Forest Inventory, 1998. *Australia's State of the Forests Report, 1998*. Bureau of Rural Sciences, Canberra.
- Norton, T.W., 1987. *Ecology of Greater Gliders, Petauroides volans Kerr 1792, in relation to variations in habitat quality in eucalypt forests in south-east NSW*. PhD thesis. ANU, Canberra.
- Pass, D.M., W.J. Foley & B. Bowden, 1998. Vertebrate herbivory on Eucalyptus – identification of specific feeding deterrents for common ringtail possums (*Pseudocheirus peregrinus*) by bioassay-guided fractionation of Eucalyptus ovata foliage. *Journal of Chemical Ecology* 24: 1513-1527.
- Pass, G.J. & W.J. Foley, 2000. Plant secondary metabolites as mammalian feeding deterrents: separating the effects of the taste of salicin from its post-ingestive consequences in the common brushtail possum (*Trichosurus vulpecula*). *Journal of Comparative Physiology B* 170: 185-192.
- Pausas, J.G., L.W. Braithwaite & M.P. Austin, 1995. Modelling habitat quality for arboreal marsupials in the south coastal forests of New South Wales. *Forest Ecology and Management* 78: 39-49.
- Roberts, C.A., P.R. Beuselinck, M.R. Ellersieck, D.K. Davis & R.L. McGraw, 1993. Quantification of tannins in birdsfoot trefoil germplasm. *Crop Science* 33: 675-679.
- Shenk, J.S., M.O. Westerhaus & M.R. Hoover, 1978. Infrared reflectance analysis of forages. *Proceedings, International Grain and Forage Harvesting Conference, Iowa State University, Ames, Iowa, Sept 25-29*. ASAE, St Joseph, MI, pp. 242-244.
- Shenk, J.S. & M.O. Westerhaus, 1991. Population definition, sample selection and calibration procedures for near infrared spectroscopy. *Crop Science* 31: 469-474.
- Shenk, J.S. & M.O. Westerhaus, 1993. Near infrared reflectance analysis with single and multiproduct calibrations. *Crop Science* 33: 582-584.
- Wessman, C.A., J.D. Aber, D.L. Peterson & J.M. Melillo, 1988. Foliar analysis using near infrared spectroscopy. *Canadian Journal of Forest Research* 18: 6-11.
- Wessman, C.A., 1990. Evaluation of canopy chemistry. In: R.J. Hobbs & H.A. Mooney (Eds), *Remote Sensing of Biosphere Functioning*. Springer-Verlag, New York, NY, pp. 135-156.
- Windham, W.R., S.L. Fales & C.S. Hoveland, 1988. Analysis for tannin concentration in *Sericea lespedeza* by near infrared reflectance spectroscopy. *Crop Science* 28: 705-708.

RESUME

Dans cette étude nous évaluons la faisabilité de mesure à distance de la biochimie de la canopée et par conséquent du potentiel pour entreprendre une cartographie à grande échelle de la qualité de l'habitat. Un certain nombre d'études ont montré que la composition de nutriment du feuillage d'eucalyptus est un déterminant majeur de la distribution de marsupiaux folivores. Plus récemment on a démontré qu'un groupe spécifique de produits chimiques de plante, les diformylphloroglucinolés (DFPs), sont les plus importantes menaces alimentaires et par conséquent les déterminants de la qualité de l'habitat. Nous rendons compte de l'utilisation de la spectroscopie de laboratoire pour tenter d'identifier un de ces DFPS, le sideroxydonal-A, dans le feuillage d'Eucalyptus melliodora, une des rares espèces d'eucalyptus dans lesquels viennent brouter les marsupiaux folivores. Des spectres de réflectance ont été obtenus pour des feuilles mortes séchées-congelées en utilisant la spectroscopie proche infrarouge (NIRS) et pour des feuilles entières, les unes séchées au four et les autres fraîches, en utilisant un spectro-radiomètre de laboratoire (FieldSpec). Une régression par moindres carrés partiellement modifiée (MPLS) a été utilisée pour développer des équations de calibration permettant de déterminer la concentration de sideroxydonal-A basée sur les spectres de réflectance transformés selon la différence première et seconde d'absorbance (Log 1/R). La capacité prédictive des équations de calibration a été évaluée en utilisant l'erreur type de la statistique de calibrage (SECV). Les coefficients de détermination (r^2) ont été plus élevés pour les spectres des feuilles mortes (0.98), suivi par les spectres des feuilles fraîches et des feuilles séchées (0.94 et 0.87 respectivement). Quand on l'applique à une validation indépendante de sous-séries, le sideroxydonal-A a été prédit de la manière la plus précise à partir des spectres de feuilles mortes ($r^2 = 0.94$), suivi par les spectres de feuilles sèches et de feuilles fraîches (0.72 et 0.53 respectivement). Deux régions spectrales, centrées sur 674 nm et 1394 nm, ont été trouvées très fortement corrélées avec une concentration de sideroxydonal-A pour chacune des trois données spectrales étudiées. Les résultats de cette étude suggèrent que les équations de calibrage dérivées de la régression par moindres carrés partiellement modifiée peuvent être utilisés pour prédire la concentration de sideroxydonal-A, et par conséquent la comestibilité des feuilles, d'arbres d'Eucalyptus melliodora, et indiquant de ce fait que l'estimation à distance de la qualité de l'habitat de forêts d'eucalyptus pour des marsupiaux folivores est faisable.

RESUMEN

En este estudio, evaluamos la factibilidad de realizar mediciones bioquímicas de dosel mediante teledetección, así como el potencial de cartografiar la calidad de hábitats a gran escala. Varios estudios encontraron que la composición nutricional del follaje de eucalipto era un factor determinante mayor en la distribución de los marsupiales folívoros. Más recientemente, se ha demostrado que un grupo específico de compuestos químicos secundarios en plantas, los diformilphloroglucinolés (DFP), constituye el más importante grupo de inhibidores de alimentación, y que estos son por lo tanto factores determinantes vitales en la calidad de los hábitats. Reportamos sobre el uso de espectroscopía de laboratorio en un intento para identificar uno de estos DFPS, el sideroxydonal-A, en el follaje de Eucalyptus melliodora, una de las pocas especies de eucalipto ramoneadas por marsupiales folívoros. Se obtuvieron espectros de reflectancia para hojas caídas al suelo, secadas por congelación, mediante espectroscopía en el infrarrojo cercano (NIRS), así como para hojas enteras secadas en estufa y para hojas enteras frescas mediante un espectroradiómetro de laboratorio (FieldSpec). Se usó una regresión parcial modificada de mínimos cuadrados (MPLS), para desarrollar ecuaciones de calibración para la concentración de sideroxydonal-A basada en los espectros de reflectancia transformados a la vez en la primera y segunda diferencia de absorbancia (Log 1/R). Se

evaluó la capacidad predictiva de las ecuaciones de calibración en base al error estándar de la estadística de calibración (SECV). Los coeficientes de determinación (r^2) resultaron ser los más altos para los espectros de las hojas encontradas en el suelo (0.98), seguidos por los espectros de hojas frescas y por los de hojas secas (0.94 y 0.87, respectivamente). Cuando se aplicó a sub-conjuntos de validación independientes, la predicción más precisa de sideroxylonal-A se obtuvo a partir de los espectros de hojas caídas al suelo ($r^2 = 0.94$), seguidos por los espectros de hojas secas y por los de hojas frescas (0.72 y 0.53, respectivamente). Dos regiones espectrales, centradas en 674 nm y 1394

nm, resultaron ser altamente correlacionadas con la concentración de sideroxylonal-A en cada uno de los tres conjuntos de datos espectrales estudiados. Los resultados de este estudio sugieren que se pueden usar ecuaciones de calibración derivadas de la regresión parcial modificada de mínimos cuadrados para predecir la concentración de sideroxylonal-A en árboles de *E. melliodora*, y por consiguiente para predecir la palatabilidad de sus hojas, lo que indica que es factible evaluar, por sensoramiento remoto, la calidad de hábitat en bosques de eucaliptos para marsupiales folívoros.