Spectrometric prediction of secondary metabolites and nitrogen in fresh *Eucalyptus* foliage: towards remote sensing of the nutritional quality of foliage for leaf-eating marsupials

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Abstract. Near-infrared reflectance spectroscopy provides an excellent means of assessing the chemical composition of *Eucalyptus* foliage but the standard methods of drying and grinding the samples limit the speed at which spectra can be collected and thus are unsuitable for measurements in the field. We investigated whether reliable spectra could be collected from whole fresh and dry leaves of *E. melliodora* and *E. globulus* and whether we could predict the concentration of total nitrogen, the volatile terpene, 1,8 cineole and the phenolic antifeedant compound, sideroxylonal A, from these spectra. Water absorbance peaks did not obscure the absorption spectrum of 1,8 cineole and so cineole concentration was readily predicted from spectra of whole, fresh *E. melliodora* leaves. Similarly, both total nitrogen and sideroxylonal A could be predicted from spectra of fresh leaf in *E. melliodora* even though water absorption obscured some spectral features. The predictions of cineole and total nitrogen concentration in *E. globulus* were not as good as those in *E. melliodora*, possibly due to interference from waxes on the leaf surface of *E. globulus* juvenile foliage. Overall, these results suggest that certain important ecological attributes of *Eucalyptus* foliage can be predicted from spectra of whole fresh leaves. Thus, it is feasible to investigate the collection of spectra by portable or airborne spectrophotometry.

Introduction

Trees of the genus *Eucalyptus* dominate more than 90% of Australian forests and woodlands (Williams and Woinarski 1997). Associated with these forests is a unique and diverse fauna including species such as the koala (*Phascolarctos cinereus*) that depend on *Eucalyptus* leaves for food (Moore and Foley 2000). Conservation of this fauna depends largely on resolving conflicts between agricultural urbanisation and forestry activities and the reservation of areas of high habitat quality for the animals (Cork and Catling 1996). However, it has proven difficult to identify such sites reliably because, until recently, we had little idea of what made some *Eucalyptus* trees suitable as food for koalas and related marsupials.

Recent studies have shown that the nutritional quality of *Eucalyptus* for marsupial herbivores is driven largely by a group of lipophilic phenolic plant secondary metabolites called formylated phloroglucinol compounds (FPC) (Lawler *et al.* 1998). For example, Lawler *et al.* (2000) showed that 86% of the variance in feeding rates of common ringtail possums (*Pseudocheirus peregrinus*) on *E. polyanthemos* foliage in the laboratory was due to the variable

concentrations of a single compound called sideroxylonal A. Thus, koalas (B. D. Moore, unpubl. data), brushtail possums (*Trichosurus vulpecula*) (Wallis *et al.* 2002) and Christmas beetles (*Anoplognathus* spp.) (M. Matsuki, R. Floyd and W.J. Foley, unpubl. data) behave similarly.

In contrast to previous suggestions (e.g. Southwell 1978; Hume and Esson 1993), Lawler *et al.* (1999) showed that the volatile terpenes, that are so characteristic of *Eucalyptus*, do not themselves deter feeding at the concentrations found in foliage. However, marsupials can use the smell and taste of terpenes (in particular 1,8 cineole) as a cue to the concentration of FPCs in a food (Lawler *et al.* 1999) because there is a significant correlation between the foliar concentrations of cineole and sideroxylonal. *Eucalyptus* generally contains relatively low concentrations of nutrients, particularly protein. Not surprisingly, foliar nitrogen (N) also appears to play a role in diet selection and in determining the long-term suitability of habitats (Cork and Catling 1996).

The link between plant secondary compounds and animal feeding has been partly applied to field studies by using near-infrared spectrophotometry as a means of rapidly analysing foliar samples for sideroxylonal and total N. Although this

has been generally successful (Lawler *et al.* 2000; McIlwee *et al.* 2001), the scale at which these analyses can be made is not large enough to provide useful information for management of animal populations or to answer landscape-level questions. Analyses are still limited by the time required to cut foliage from trees and to dry, grind and collect spectra manually (Lawler *et al.* 2000; McIlwee *et al.* 2001).

Drying *Eucalyptus* foliage reduces the concentration of the volatile terpenes that are a critical cue for diet selection (Lawler *et al.* 1999). Grinding the foliage produces a homogeneous material that reduces light scattering but is expensive in time and labour. If we are to increase the scale of ecological measurements by spectroscopy, we have to consider remote sensing where attributes such as chemical composition (Foley *et al.* 1998) or foliage palatability (McIlwee *et al.* 2001) can be measured directly in a large number of tree crowns.

The success of studies in other systems to remotely predict chemistry of tree crowns (e.g. Gastelluetchegorry *et al.* 1995; Zagolski *et al.* 1996; Martin and Aber 1997) encouraged us to consider measuring specific plant secondary metabolites and nutrients in *Eucalyptus* forests. Although the complexities introduced by atmospheric interference and tree architecture must be addressed in the future, we believe there are many intermediate steps. The first step is to ensure that spectrometric predictions of specific aspects of foliar chemistry, which describe the nutritional quality of the foliage for herbivores, are not compromised by either loss of data in the water-absorbing parts of the spectrum or by the collection of spectra from whole leaves. Water is a particularly strong absorber of near-infrared light and can mask other spectral features that are critical to quantifying some foliar constituents.

Accordingly, we tested whether we could predict the concentration of total N, sideroxylonal A and a major volatile terpenoid, 1,8 cineole, in fresh and dried whole leaves of *E. melliodora*, a widely distributed woodland species that has been a focus of much of our previous work (e.g. Wallis *et al.* 2002).

However, *Eucalyptus* is highly variable. One major feature of this variation is the presence of juvenile and adult leaves on the same tree (heteroblasty) and oftentimes the juvenile leaves are glaucous (covered with a waxy layer) particularly on the abaxial (facing away from the stem) surface. This wax could potentially interfere with the spectra of desired compounds and so we studied whether we could predict foliar N and cineole in fresh juvenile and adult foliage in a second eucalypt species, *E. globulus*.

Materials and methods

Samples and chemical analysis

We collected foliage from 50 individual *E. melliodora* trees growing in open woodland within 20 km of Canberra. All samples were of fully expanded adult foliage from the mid- to upper crown. The samples were

kept in polythene bags and held at 4°C for a maximum of 2 h before transport to the laboratory. The visible and near-infrared spectra (400–2500 nm) of each sample were collected using a NIR Systems 6500 spectrometer (details below).

We measured the concentration of 1,8 cineole in hexane extracts of two leaves from each sample by gas chromatography using tridecane as an internal standard (Edwards *et al.* 1993). Within 4 h of collection, we placed a second subsample in an oven for dry matter determination (3 days at 40°C) so that we could express the cineole concentrations in terms of dry leaf weight.

We then freeze-dried all the samples that had been scanned when fresh and then collected near-infrared spectra of the whole dried leaves. Those samples were then ground to pass a 1-mm screen in a Cyclotec 1093 Sample Mill (Tecator, Sweden) and we again collected near-infrared spectra of the powdered samples. Thus, for each of the 50 samples collected we had near-infrared spectra of whole leaves when both fresh and dried and also the ground leaf.

We measured foliar cineole concentration on the dried leaf samples as described above and sideroxylonal A concentration by HPLC as previously described (Lawler *et al.* 2000). We also measured total N of all dried, ground samples by a Kjeldahl procedure with selenium as a catalyst and standardising the assay against ammonium sulfate. All samples were analysed in duplicate and the maximum variation accepted between duplicates was <2% for all constituents.

Eucalyptus globulus *foliage samples*

We conducted a second series of experiments with foliage from *E. globulus* because this species of eucalypt carries morphologically different adult and juvenile foliage. In addition, juvenile foliage is often highly glaucous and we wanted to know whether this would affect the quality of the spectra that we collected. We collected adult foliage from 30 *E. globulus* trees growing in a 15-year-old experimental plantation in Canberra and juvenile leaves from a further 15 trees. All samples were of fully expanded foliage from the mid-crown and were transported to the laboratory within 90 min of collection. *E. globulus* foliage does not contain sideroxylonals (Eschler *et al.* 2000) and so we restricted these experiments to the estimation of foliar cineole and total N. We collected spectra in the same way as described for *E. melliodora* except that we explored whether presenting the glaucous surface to the spectrometer had any impact on the predictions derived from the spectra.

Spectra of pure compounds

We collected reflectance spectra of purified 1,8 cineole and sideroxylonal A so that we could relate specific features of the spectra from foliage samples to authentic spectra. Pure cineole (99.9% by GLC), which is an oil at room temperature, was re-distilled from a commercial supply (Sigma, Castle Hill, NSW). We scanned cineole in a liquid cuvette in the transport module. Sideroxylonal A was extracted and purified as previously described (Eschler and Foley 1999) and about 1 g of the off-white crystals was placed directly into a small spinning cup sample holder for presentation to the spectrometer.

Spectral measurement and data analysis

All spectra were collected with a NIR Systems Model 6500 scanning monochromator housed in a room maintained at 22°C and at 55% RH. This instrument measures spectral reflectance at wavelengths between 400 and 2498 nm with a bandwidth of 10 nm but with an interpolated data gap of 2 nm. Whole (fresh and dried) leaf samples were scanned using the NIR Systems Transport Module with a large rectangular sample cell, whereas dried, ground samples were scanned using the Spinning Cup Module and a small sample cup. We collected at least two replicate spectra of reflected monochromatic light between 400 and 2498 nm at 2-nm intervals and averaged these replicates only if the

standard error of the difference between the two spectra [expressed as log $(1/R)/10^6$] was less than 200. If the standard error was greater than 200, we re-collected the spectra in duplicate. This procedure ensured the quality of the spectral data collected (Shenk and Westerhaus 1993*a*). The reflectance (R) readings were converted to absorbance (A) values by the equation $A = \log (1/R)$ and each spectrum was converted to a first-derivative spectrum to emphasise subtle changes in slope as well as reducing variation due to particle size and intercorrelations between spectral data at different wavelengths (Osborne *et al.* 1993). The instrument was maintained and operated, spectral data manipulated and calibrations developed with the NIRS3 software package (InfraSoft International, Port Matilda, PA; Shenk and Westerhaus 1993*b*).

We modelled the relationship between cineole, total N or sideroxylonal concentrations and spectral characteristics of whole fresh leaves, whole dried leaves and ground, dried leaves by modified partial least squares regression (MPLS: Shenk and Westerhaus 1991), following the standard principles described by the American Society for Testing and Materials (Anon 1995). We tested a variety of combinations of derivative and smoothing functions as well as the application of the standard normal variate and/or detrend scatter correction described by Barnes *et al.* (1989). We ranked the resulting models on the criteria of lowest SECV and only report the highest-ranked model in each case.

The MPLS approach requires cross-validation to prevent overfitting (i.e. using too many terms in the equation) and to select the optimum number of terms for each calibration equation (Osborne *et al.* 1993; Davies 1998). In all cases, the number of MPLS was never greater than $[(n/10) + 1]$. Cross-validation involves dividing the sample set into 'n' groups and performing the calibration on '$n-1$' groups, with the remaining group being used as an independent validation set. This exercise is repeated until all samples have been cross-validated. The residuals of each prediction are then pooled to provide a standard error of cross-validation (SECV). A final regression model is then fitted to the data, using the number of factors determined by the cross-validation procedure. Cross-validation is an efficient procedure because all samples are used for both calibration and validation and avoids the need to set aside samples for a validation set (Osborne *et al.* 1993; Shenk and Westerhaus 1993*b*). Another advantage of cross-validation is that outliers from the prediction residuals are identified readily (Shenk and Westerhaus 1991, 1993*b*).

Cross-validation may be criticised because it lacks independent validation. Thus, we made an additional validation of all models for *E. melliodora* leaf samples with a separate set of 12 samples. We calculated the co-efficient of determination (r^2) and the standard error of prediction (SEP) for each model based on this separate sample set.

Results

Spectra of pure compounds

The first derivative of the absorbance spectrum of pure cineole (Fig. 1*a*) showed major peaks at 1700–1800 nm and 2250–2450 nm. The first-derivative absorbance spectrum of pure sideroxylonal A (Fig. 1*b*) was more complex than that observed for 1,8 cineole. In particular, there were significant peaks between 1700 and 1800 nm and between 2100 and 2300 nm.

Eucalyptus melliodora *foliage*

Cineole content ranged from 1.3 to 20.0 mg g^{-1} (dry matter) in *E. melliodora* leaf samples. The freeze-dried specimens of *E. melliodora* foliage lost between 26 and 84% (mean 65%) of the cineole measured in the fresh leaves. The concentration
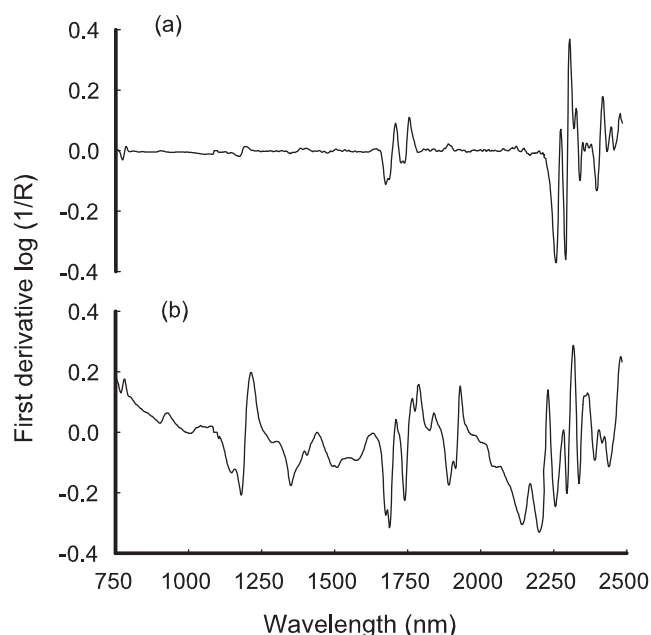

Fig. 1. First-derivative near-infrared absorbance spectrum of (*a*) 1,8 cineole and (*b*) sideroxylonal A.

of sideroxylonal A was between 2.4 and 23.9 mg g^{-1} (dry matter).

Figure 2 shows the (average) first-derivative absorbance spectra of *E. melliodora* foliage for fresh leaves and for freeze-dried whole leaves. The greatest difference between the two spectra is, as expected, near the absorption peaks for water (particularly between 1400 and 1450 nm and between 1900 and 1950 nm). However, the derivatised absorbance spectrum of pure cineole (Fig. 1*a*) does not show any peaks near these wavelengths, although it does show absorbance peaks that border the water peak at about 2250 nm. This suggests that the absorbance spectrum of cineole is unlikely to be significantly masked by absorbance peaks from water
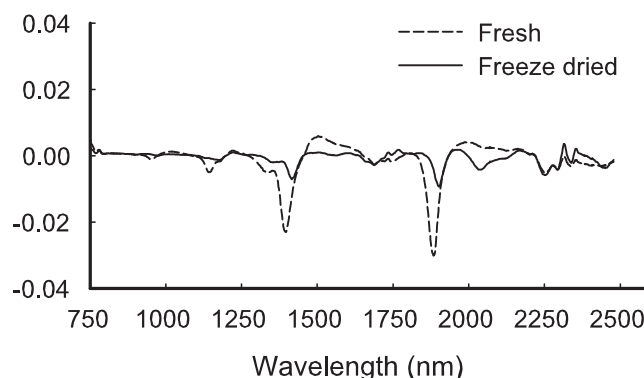

Fig. 2. Average first-derivative near-infrared absorbance spectra of adult *Eucalyptus melliodora* foliage samples, scanned fresh (*n* = 50) (dotted line) and freeze-dried whole leaves (*n* = 49) (solid line).

Table 1. **Results from modelling (by partial least squares with cross-validation) the relationship between spectral characteristics of *Eucalyptus melliodora* foliage and the foliar concentration of 1,8 cineole, sideroxylonal A and total nitrogen**

The r^2-value indicates the degree of correlation between the predicted values and the actual measured values; SECV is the standard error of the cross-validated predictions; 'Math. treatment' contains the derivatives and range of data points over which the derivative and smoothing functions were calculated; wavelength range indicates the range over which the model was derived; the standard normal variate (SNV)/detrend scatter-correction procedure was that described by Barnes *et al.* (1989); SEP is the standard error of prediction derived from a separate validation set ($n = 12$)

Sample	r^2	SECV	Math. treatment	Wavelength range (nm)	Scatter correction	n	Validation (SEP)
			1,8 Cineole				
Fresh leaf	0.97	1.60	2441	750–2492	None	49	1.13
Freeze-dried whole leaf	0.94	1.52	2441	1108–2492	None	46	0.92
Freeze-dried ground leaf	0.88	1.81	2441	750–2492	None	48	1.41
			Sideroxylonal				
Fresh leaf	0.90	2.69	1441	1108–2492	SNV/detrend	48	3.33
Freeze-dried whole leaf	0.95	2.10	1441	1108–2492	None	45	3.65
Freeze-dried ground leaf	0.94	2.05	1441	1108–2492	None	48	4.04
			Total N				
Fresh leaf	0.92	0.07	2441	1108–2492	SNV/detrend	50	0.11
Freeze-dried whole leaf	0.97	0.04	2441	1108–2492	SNV/detrend	47	0.05
Freeze-dried ground leaf	0.98	0.04	2441	1108–2492	SNV/detrend	48	0.05

and that it is possible to estimate the concentration of cineole from the spectra of whole fresh leaves. In contrast, the prominent peak of sideroxylonal A, at about 2250–2300 nm, suggests some potential for water-derived features to overlap when predicting sideroxylonal concentrations in whole fresh leaves. However, no major difference was found between the spectra for fresh leaves and freeze-dried leaves in this wavelength region.

We were able to successfully model the relationship between absorbance spectra and foliar concentration of cineole, sideroxylonal and N in both fresh and dried whole leaf (Table 1). Although we explored a range of alternative procedures including changing the derivative, the number of data points and the smoothing functions for generating these models, we report here only those with the lowest standard error of cross-validation. In all instances, the modified partial least squares regression approach proved the most useful. In all cases the r^2-value refers to the correlation between the measured values for each constituent and the value predicted by the model.

Our predictions of both cineole and sideroxylonal concentration depended strongly on wavelengths that were important features of the derivatised absorbance spectra for purified compounds, as shown by the correlograms in Figs 3 and 4. Correlograms allow assessment of the wavelengths that have the greatest correlation with the constituent values. If the correlogram shows a high correlation coefficient at a certain wavelength it means that there is a high correlation between the first derivative of the absorbance by the samples at that wavelength and the concentration of the compound in those samples. We have shown first-derivative correlograms to facilitate comparison with the first-derivative spectra of pure compounds (Fig. 1). Note that the best model for cineole concentration is derived using the second-derivative spectra but the results for this model were similar to the best model using first-derivative spectra. The vertical dashed lines in Figs 3 and 4 highlight the important absorbance features of the purified compounds. For both cineole and sideroxylonal, there is close agreement between the wavelengths at which these occur and the importance of those wavelengths to the models predicting the concentrations of these compounds in both fresh and dried whole leaves. This suggests that the models are detecting unique chemical attributes of the foliage.

Eucalyptus globulus *foliage*

In *E. globulus*, the cineole content ranged from 7.5 mg g^{-1} to 24.6 mg g^{-1} (dry matter) in adult leaf samples and from 15.1 to 48.2 mg g^{-1} (dry matter) in juvenile leaf samples. The pattern in derivatised absorbance spectra found for adult fresh and freeze-dried whole *E. globulus* leaves (data not shown) resembles those for *E. melliodora* samples, with the greatest differences about the water absorbance wavelength range.
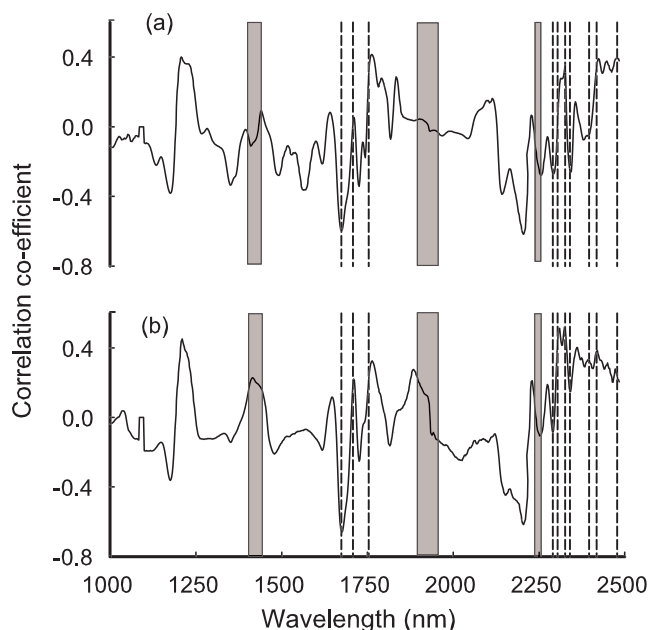
Fig. 3. Wavelength dependence of correlation between first-derivative absorbance spectra and 1,8 cineole concentration (mg g^{-1} dry matter) for (*a*) freeze-dried whole and (*b*) fresh *Eucalyptus melliodora* foliage. Dashed vertical lines indicate the most important absorbance features of pure 1,8 cineole. Grey bars indicate the regions of major water absorbance.
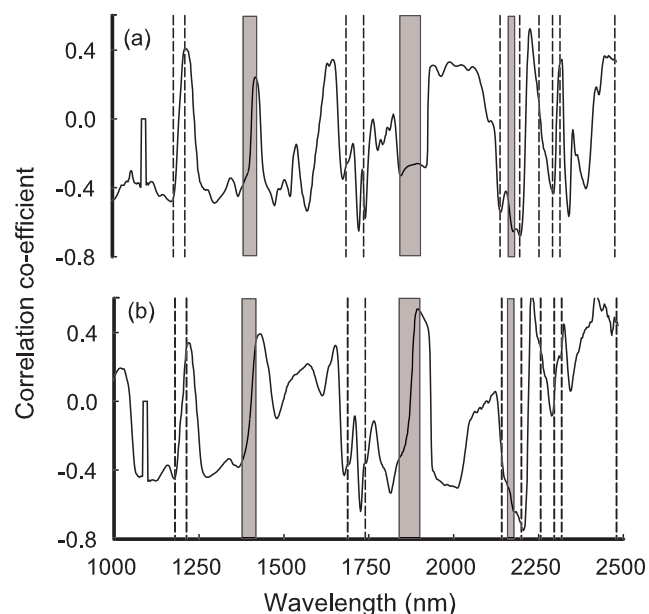
Fig. 4. Wavelength dependence of correlation between first-derivative absorbance spectra and sideroxylonal A concentration (mg g^{-1} dry matter) for (*a*) freeze-dried whole and (*b*) fresh *Eucalyptus melliodora* foliage. Dashed vertical lines indicate the most important absorbance features of pure sideroxylonal A. Grey bars indicate the regions of major water absorbance.

The models developed with the *E. globulus* adult and juvenile leaf samples do not predict cineole or N concentrations as well as those developed for *E. melliodora* (Table 2), even though there was close agreement between the cineole spectrum and the wavelengths that correlated most with cineole concentration (Fig. 5). This may be partly due to using fewer samples in the *E. globulus* studies. In addition, the agreement between the values predicted by the models and the actual measured values are better in the dried *E. globulus* material than in the fresh leaf samples. This suggests that there are other spectral features, apart from water content, which may affect the estimation of cineole in

Table 2. Results from modelling (by partial least squares with cross-validation) the relationship between spectral characteristics of different samples of *Eucalyptus globulus* foliage and the foliar concentration of 1,8 cineole and total nitrogen

Column descriptions as for Table 1

| Sample	Preparation	r^2	SECV	Mathematical treatment	Wavelength range (nm)	Scatter correction	n
				1,8 cineole			
Adult leaf	Fresh, whole	0.74	3.57	2441	750–2492	None	30
Adult leaf	Freeze-dried, whole	0.91	2.71	1441	1108–2492	SNV	29
Adult leaf	Freeze-dried, ground	0.75	3.76	2441	750–2492	SNV/detrend	30
Juvenile leaf	Fresh whole	0.76	6.79	2441	1108–2492	SNV/detrend	15
Juvenile leaf	Freeze-dried, whole	0.83	6.85	1881	1108–2492	SNV	15
Juvenile leaf	Freeze-dried, ground	0.77	6.65	1441	1108–2492	SNV	15
				Total N			
Adult leaf	Fresh, whole	0.90	0.74	2641	1108–2492	SNV/detrend	29
Adult leaf	Freeze-dried, whole	0.93	0.6	1441	1108–2492	SNV/detrend	28
Adult leaf	Freeze-dried, ground	0.99	0.4	2441	1108–2492	SNV/detrend	30
Juvenile leaf	Fresh, whole	0.71	2.38	2441	1108–2492	SNV/detrend	15
Juvenile leaf	Freeze-dried, whole	0.93	1.36	2441	1108–2492	SNV/detrend	15
Juvenile leaf	Freeze-dried, ground	0.97	0.88	2441	1108–2492	SNV/detrend	15
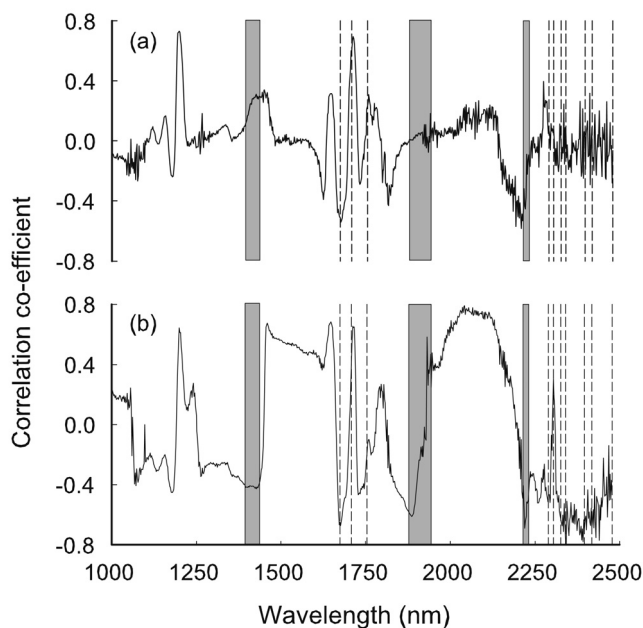
Fig. 5. Wavelength dependence of correlation between first-derivative absorbance spectra and 1,8 cineole concentration (mg g^{-1} dry matter) for (*a*) adult fresh and (*b*) juvenile fresh *Eucalyptus globulus* foliage. Dashed vertical lines indicate the most important absorbance features of pure 1,8 cineole. Grey bars indicate the regions of major water absorbance.

E. globulus leaves but not in leaves from *E. melliodora*. One possible candidate was surface wax (Li *et al.* 1997), and this was investigated further with juvenile *E. globulus* leaves.

Waxes occur on both sides of the juvenile leaf of *E. globulus* but are more pronounced on the abaxial side. The absorbance spectra for juvenile (fresh and freeze-dried) samples scanned with the abaxial surface presented to the light source of the spectrometer were slightly different from the spectra for samples scanned with the abaxial surface facing away from the light source (data not shown). This was especially the case for the fresh leaf samples. The wavelength ranges where the differences appear included some of the important absorbance ranges for pure cineole (data not shown). Models for cineole concentration had slightly lower standard errors and higher correlation coefficients when based on spectra collected with the abaxial surface facing towards the light source, suggesting that either cineole concentration is variable within the leaf or some components of the leaf waxes may well have interfered with the precision of the cineole estimates in *E. globulus*. As expected, a good model was obtained from freeze-dried, ground leaf samples.

Discussion

This work shows that certain ecologically important aspects of the chemical composition of *Eucalyptus* foliage are detectable in spectra of both whole dried leaf specimens and in whole fresh foliage. With whole dried leaf in place of ground leaf in broad scale studies of foliar composition, based on reflectance spectra, will result in significant savings because grinding is a major cost of collecting near-infrared spectra of plant material. Our success in obtaining spectra from whole fresh leaves containing features diagnostic of both foliar nitrogen and of specific secondary metabolites (cineole and sideroxylonal) suggests that it is worth exploring the possibility of remotely sensing the crown chemistry of *Eucalyptus*, with the aim of measuring important habitat parameters of folivorous marsupials.

Our results were most reliable for *E. melliodora* foliage which is much more consistent in appearance than *E. globulus* foliage. Clearly, more work needs to be conducted with *E. globulus* but one possible explanation is that the derivation of biochemical information may be compromised by the presence of wax on the surface of juvenile leaves. Most eucalypts have some degree of epicuticular wax (the principal component being β-diketones; Li *et al.* 1997), but in some species such as *E. globulus*, *E. cinerea* and *E. gunnii* it can impart a distinct glaucous sheen on the juvenile foliage.

One of the strengths of near-infrared spectroscopy is its ability to robustly model attributes without a unique chemical signal by applying the statistical approaches of chemometrics. However, when scaling up studies from dried, ground leaf to the level of whole crowns or canopies, reliance on absorption features that are clearly characteristic of the biochemical of interest are strongly preferred.

In general, there is no consensus on whether information on foliar biochemistry can be reliably extracted from near-infrared spectra of whole fresh material (Johnson 2001). Most focus has been on foliar N because of its importance in ecosystem productivity at the biome scale. However, as several studies (Jacquemoud *et al.* 1996; Johnson 2001) point out, researchers have often selected a diverse group of wavelengths during stepwise regression procedures without them necessarily being associated with known protein absorptions. Part of this difficulty may be due to different forms of N in foliage from different species. For example, some eucalypts contain a significant proportion of N as cyanogenic glycosides (Gleadow *et al.* 1998). In this study, we did not attempt detailed analysis of correlograms of N concentration, even though the models for predicting foliar N concentration were excellent. Instead we focused on characterised plant secondary metabolites and observed that the models derived using modified partial least squares regression depended on wavelengths characteristic of purified sideroxylonal and cineole. Furthermore, the wavelengths used to estimate cineole were similar in both tree species. Near-infrared spectroscopy has recently been used to measure the concentration of cineole in pharmaceutical preparations (Wilson *et al.* 2001). The

spectrum of pure cineole (presented as a standard normal variate second derivative), with major absorbances at 1700–1800 and at 2250–2400 nm, was similar to that recorded in this study.

Sideroxylonal and cineole are well-defined compounds in contrast to entities such as lignin and total nitrogen that may exist in many forms or be of uncertain composition. In contrast to laboratory instruments, the airborne spectrometers such as AVIRIS (Vane *et al.* 1993) and HYMAP (Cocks *et al.* 1998), which could be used for remote sensing of foliar chemistry, can record only limited numbers of spectral bands in the region 400–2500 nm. The close association between absorption features of the purified compounds and the important features of the spectra-based models suggests that we may be able to assign particular diagnostic wavelengths to detecting these important biological molecules in at least two eucalypt species.

There has been significant recent interest in using reflectance spectrophotometry to detect or predict insect damage or stress in forest trees (Leckie *et al.* 1989; Luther and Carroll 1999; Stone *et al.* 2001). Both cineole and sideroxylonals have been implicated in differential insect damage of eucalypt crowns in Australia (Edwards *et al.* 1993; M. Matsuki, R. Floyd and W. J. Foley, unpubl. data) and there is a strong mechanistic understanding of how these effects might occur. What is notable from this study is that spectral regions >1000 nm are the most useful for predicting these constituents. Many imaging spectrometers, such as CASI (Compact Airborne Spectrographic Imager), do not detect this region. In contrast, hyperspectral instruments such as HYMAP and AVIRIS provide significantly more bands in the region 1000–2500 nm and consequently the opportunity to derive direct measures of foliar chemistry along with statistically based indices of vigour or insect damage. The value of spectral indices of forest processes can be enhanced only when there is a mechanistic basis underlying them (e.g. Stone *et al.* 2001).

The value of being able to remotely sense foliar attributes in *Eucalyptus* depends on identifying appropriate measurement scales for the particular question being asked. For example, questions about feeding by folivorous marsupials are posed at a much finer ecological scale than questions about stand productivity. There has been variable success in scaling up foliar spectral features to whole crowns or canopies in other systems. Although some modelling studies showed that leaf biochemical information was transmitted virtually unchanged from the leaf to the canopy in the near-infrared wavelengths (Kupiec and Curran 1995), others have been less positive. Previous work in conifer forests has shown that measuring the composition of the canopy is a realistic goal as shown by the strong correlations between near-infrared spectral data from an aerial platform and foliar N ($r^2 = 0.87$; Martin and Aber 1997), cellulose ($r^2 = 0.79$; Gastelluetchegorry *et al.* 1995) and lignin

($r^2 = 0.77$; Martin and Aber 1997). However, the crowns of individual *Eucalyptus* trees are usually more open than are those of conifers and this introduces significant difficulties in separating reflectance spectra of foliage from background spectra, such as bark and ground and in resolving problems of leaf shape and orientation that may vary significantly between and within trees. Resolving these difficulties is the next challenge to scaling up spectral measurements of *Eucalyptus* forests and making measurements of foliar secondary metabolites over entire landscapes that are critical to feeding choices of folivorous marsupials.

Acknowledgments

References

Anon (1995) 'Standard practices for infrared, multivariate, quantitative analysis.' Designation: E1655-94. (American Society for Testing and Materials: West Conshohocken, PA)

Barnes RJ, Dhanoa MS, Lister SJ (1989) Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Applied Spectroscopy* **43**, 772–777.

Cocks T, Jenssen R, Stewart A, Wilson I, Shields T (1998) The HyMap airborne hyperspectral sensor: the system, calibration and performance. 1st EARSeL Workshop on Imaging Spectroscopy, 6–8 October 1998, Zurich, Switzerland. pp. 37–42. (EARSeL: Paris)

Cork SJ, Catling PC (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.

Davies AMC (1998) Cross-validation: do we love it too much? *Spectroscopy Europe* **10**, 24–25.

Edwards PB, Wanjura WJ, Brown WV (1993) Selective herbivory by Christmas beetles in response to intraspecific variation in *Eucalyptus* terpenoids. *Oecologia* **95**, 551–557.

Eschler BM, Pass DM, Willis R, Foley WJ (2000) Distribution of foliar formylated phloroglucinol derivatives amongst *Eucalyptus* species. *Biochemical Systematics and Ecology* **28**, 813–824.

Eschler B, Foley WJ (1999) A new sideroxylonal from *Eucalyptus melliodora* foliage. *Australian Journal of Chemistry* **52**, 157–158.

Foley WJ, McIlwee A, Lawler IR, Aragones L, Woolnough A, Berding N (1998) Ecological applications of near-infrared spectroscopy—a tool for rapid, cost-effective prediction of the composition of plant tissues and aspects of animal performance. *Oecologia* **116**, 293–305.

Gastelluetchegorry JP, Zagolski F, Mougin E, Marty G, Giordano G (1995) An assessment of canopy chemistry with AVIRIS—a case study in the Landes forest, south-west France. *International Journal of Remote Sensing* **16**, 487–501.

Gleadow RM, Foley WJ, Woodrow IE (1998) Enhanced CO_2 alters the relationship between photosynthesis and defence in cyanogenic *Eucalyptus cladocalyx*. *Plant Cell and Environment* **21**, 12–22.

Hume ID, Esson C (1993) Nutrients, antinutrients and leaf selection by captive koalas (*Phascolarctos cinereus*). *Australian Journal of Zoology* **41**, 379–392.

Jacquemoud S, Ustin SL, Verdebout J, Schmuck G, Andreoli G, Hosgood B (1996) Estimating leaf biochemistry using the PROSPECT leaf optical properties model. *Remote Sensing of the Environment* **56**, 194–202.

Johnson LF (2001) Nitrogen influence on fresh-leaf NIR spectra. *Remote Sensing of the Environment* **78**, 314–320.

Kupiec JA, Curran PJ (1995) Decoupling effects of the canopy and foliar biochemicals in AVIRIS spectra. *International Journal of Remote Sensing* **16**, 1731–1739.

Lawler IR, Foley WJ, Eschler B, Pass DM, Handasyde K (1998) Intraspecific variation in secondary metabolites determines food intake by folivorous marsupials. *Oecologia* **116**, 160–169.

Lawler IR, Foley WJ, Eschler BM (2000) Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. *Ecology* **81**, 1327–1338.

Lawler IR, Stapley J, Foley WJ, Eschler BM (1999) Ecological example of a conditioned food aversion in plant-herbivore interactions: the effect of terpenes of *Eucalyptus* leaves on feeding by common ringtail and brushtail possums. *Journal of Chemical Ecology* **25**, 401–415.

Leckie DG, Ostaff DP, Teillet PM, Fedosjevs G (1989) Spectral characteristics of tree components of balsam fir and spruce damaged by spruce budworm. *Forest Science* **35**, 582–600.

Li H, Madden JL, Potts BM (1997) Variation in leaf waxes of the Tasmanian *Eucalyptus* species. 1. Subgenus *Symphyomyrtus*. *Biochemical Systematics & Ecology* **25**, 631–657.

Luther JE, Carroll AL (1999) Development of an index of balsam fir vigour by foliar spectral reflectance. *Remote Sensing of the Environment* **69**, 241–252.

Martin ME, Aber JD (1997) High spectral resolution remote sensing of forest canopy lignin, nitrogen and ecosystem processes. *Ecological Applications* **7**, 431–443.

McIlwee AM, Lawler IR, Cork SJ, Foley WJ (2001) Near infrared spectroscopy as a predictor of *Eucalyptus* foliar nutrients and of the feeding rates of folivorous marsupials. *Oecologia* **128**, 539–548.

Moore BD, Foley WJ (2000) Feeding and diet selection in koalas. *Australian Journal of Zoology* **48**, 317–333.

Osborne BG, Fearn T, Hindle PH (1993). 'Practical NIR spectroscopy with applications in food and beverage analysis.' (Longman Scientific and Technical: Essex, UK)

Shenk JS, Westerhaus MO (1991) New standardisation and calibration procedures for near infrared reflectance spectroscopy. *Crop Science* **31**, 469–474.

Shenk JS, Westerhaus MO (1993*a*) Near infrared reflectance analysis with single and multiproduct calibrations. *Crop Science* **33**, 582–584.

Shenk JS, Westerhaus MO (1993*b*) 'Monograph: analysis of agriculture and food products by near-infrared reflectance spectroscopy.' (InfraSoft International: Port Matilda, PA, USA)

Southwell IA (1978) Essential oil content of koala food trees. In 'The koala: proceedings of the Taronga Symposium on koala biology, management and medicine'. (Ed. TJ Bergin) pp. 62–74. (Zoological Parks Board of New South Wales: Sydney)

Stone CA, Chisholm L, Coops N (2001) Spectral reflectance characteristics of eucalypt foliage damaged by insects. *Australian Journal of Botany* **49**, 687–698.

Vane G, Green RO, Chrien TG, Enmark HT, Hansen EG, Porter WM (1993) The airborne visible infrared imaging spectrometer (AVIRIS). *Remote Sensing of the Environment* **44**, 127–143.

Wallis IR, Watson ML, Foley WJ (2002) Secondary metabolites in *Eucalyptus melliodora*: field distribution and laboratory feeding choices by a generalist herbivore, the common brushtail possum (*Trichosurus vulpecula*). *Australian Journal of Zoology* **50**, 507–519.

Williams JE, Woinarski JCZ (1997) 'Eucalypt ecology: individuals to ecosystems.' (Cambridge University Press: Cambridge)

Wilson ND, Watt RA, Moffat AC (2001) A near-infrared method for the assay of cineole in *Eucalyptus* oil as an alternative to the official BP method. *Journal of Pharmacy and Pharmacology* **53**, 95–102.

Zagolski F, Pinel V, Romier J, Alcayde D, Fontanari J, Gastelluetchegorry JP, Giordano G, Marty G, Mougin E, Joffre R (1996) Forest canopy chemistry with high spectral resolution remote sensing. *International Journal of Remote Sensing* **17**, 1107–1128.