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Coping with chemical complexity in mammal-plant interactions: near-infrared spectroscopy as a predictor of *Eucalyptus* foliar nutrients and of the feeding rates of folivorous marsupials

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Abstract We investigated the utility of near-infrared reflectance spectroscopy (NIRS) as a means of rapidly assaying chemical constituents of *Eucalyptus* leaves and of directly predicting the intake of foliage from individual trees by greater gliders (*Petauroides volans*) and common ringtail possums (*Pseudocheirus peregrinus*). The concentrations of total nitrogen, neutral detergent fiber, condensed tannins and total phenolics could be predicted accurately by partial least squares regression models relating the near-infrared reflectance spectra of foliage samples to analyses performed using standard laboratory procedures. Coefficients of determination (r^2) for all four constituents ranged between 0.88 and 0.98, and standard errors of prediction between 0.80 mg g^{-1} dry matter (DM) for total nitrogen and 5.14 quebracho equivalents g^{-1} DM for condensed tannins. Near-infrared spectral-based models of food intake had r^2 values of 0.90 and 0.95 with a standard error of prediction of 3.4 and 8.3 g DM $kg^{-0.75}$ day^{-1} for greater gliders and common ringtail possums respectively. We used the predictive model of food intake for greater gliders to examine the relationship between leaf palatability and documented food preferences of animals in the wild. Ranked differences in leaf palatability across four *Eucalyptus* species were consistent with documented food preferences of greater gliders in the wild. We conclude that NIRS provides a powerful tool to predict foraging behaviour of herbivores where forage choices are determined by compositional attributes of food.

Keywords Leaf chemistry · Herbivory · Plant secondary metabolite · Nutrition

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Introduction

The diets of mammalian browsers are chemically complex, containing a range of nutrients, indigestible tissues and plant secondary metabolites (Cork and Foley 1991; Hanley 1997). Understanding how these components interact to determine nutritional quality of the diet has been the subject of much research. Nutritional quality of browse is usually inferred indirectly from measures of nutrients such as total nitrogen and soluble carbohydrates, refractory components such as fiber and lignin and plant secondary metabolites such as tannins (Oates et al. 1980; Waterman et al. 1980; Cork and Pahl 1984). Combinations of these measures have been used to develop indirect, predictive indices of feeding (e.g. Waterman et al. 1980; Cork 1992) but cannot address the two fundamental components of nutritional quality, food intake and the digestibility or metabolizability of that food (Robbins 1983).

Many studies using indirect techniques were performed in the 1980s but interest in this approach has declined for two reasons. First, it is apparent that variation in chemical composition both within and amongst individual trees is an important determinant of mammalian browsing (Ganzhorn 1988; Hjalten et al. 1996; Lawler et al. 1998; Pass et al. 1998). This means that sampling and subsequent nutritional analysis needs to be much more intensive than has been attempted in the past. However, to do so, often requires an extensive time commitment and laboratory resources. Consequently, analytical constraints may limit the sampling intensity (Foley et al. 1998).

A second problem is the links between compositional analyses and nutritional quality are not well defined (Foley 1992; Hagerman et al. 1992; Waterman and Kool 1994; Hjalten et al. 1996). Consider for example, the interpretation of the concentration of total nitrogen (N) in plant tissue. If this variable is to be linked to animal foraging, we need to know the proportion of N that occurs as amino acids rather than nitrate or cyanide, whether any amino acids are limiting, the protein requirements of the animal, some knowledge of the animal's digestive physiology and the presence of other factors that might either enhance or diminish the availability of N to the tissues. Synergisms with poorly defined components such as tannins make defining what is high- and low-quality food virtually impossible from compositional analyses alone.

Foley et al. (1998) have argued that near-infrared reflectance spectroscopy (NIRS) offers ecologists a powerful tool to address the problem of sampling intensity. Correctly calibrated, NIRS allows multiple components of plant samples to be measured quickly, non-destructively and with minimal sample preparation. Near-infrared spectroscopy has been used previously in agriculture to estimate chemical components of pastures and other fodder species with a high degree of accuracy and precision (e.g. reviewed in Givens et al. 1997) but has been applied only rarely in nutritional ecology (e.g. Brooks et al. 1984). Near-infrared spectral characteristics of reflect the underlying chemical bonds that make up different nutrients and plant secondary metabolites. Therefore, NIR spectra can "capture" chemical information about a plant sample accurately. However, correlations must be sought between spectra and samples of known composition to quantify this information, because peaks in an NIR spectrum cannot necessarily be assigned to single functional groups (Foley et al. 1998).

This paper presents evidence that NIRS is suitable for measuring a range of primary constituents and plant secondary metabolites found in *Eucalyptus* foliage. We then argue that, if NIRS can predict foliar components known to influence foliage intake by marsupials, it may be possible to predict intake directly by modelling the relationship between spectral characters of foliage and a standard measure of the amount of food an animal is prepared to eat in the laboratory. We believe that this approach could enable ecologists to sidestep some of the difficulties of interpreting the effects of poorly defined foliar attributes on an animal's willingness to eat a particular foliage. We explored this approach by testing whether NIRS could predict accurately the intake of *Eucalyptus* foliage by two marsupial browsers, the common ringtail possum (*Pseudocheirus peregrinus*) and the greater glider (*Petauroides volans*). We then investigated whether NIRS could be used in field-based studies of feeding in folivorous marsupials by comparing our predictions of diet selection with those observed in free-living greater gliders.

Materials and methods

Chemical composition of eucalypt foliage

Sampling of foliage

Ninety samples of eucalypt foliage were collected from trees in both the north-eastern tropical zone (north-eastern Queensland) and south-eastern temperate zone (south-eastern and north-eastern New South Wales) of Australia, within the distribution ranges of both greater gliders and common ringtail possums. These collections spanned young and mature foliage of 85 species of eucalypt, which were growing on a wide range of soil types and under a range of different climates (Cork 1992; A.M. McIlwee, unpublished work). Each sample consisted of 30–50 g (wet mass) of foliage that was cut close to the mid-crown (north-eastern quadrant) of a single eucalypt tree. Each sample was frozen in the field either on a bed of solid CO_2 ("dry ice") or else in a field-portable freezer and on return to the laboratory was stored at –20°C. All samples were freeze-dried and ground to pass a 1-mm screen in a Udy Cyclone grinder.

Analytical procedures

We measured four chemical attributes of the foliage in duplicate and expressed all our results as a percentage of dry matter after correcting for residual moisture by drying a duplicate sample in a porcelain crucible at 80°C for 24 h. Not all samples were analysed for all constituents. Foliar nitrogen was determined by a semi-micro Kjeldahl procedure with selenium as a catalyst using a Gerhardt Vapodest nitrogen analyser. Recoveries of $(NH_4)_2SO_4$ standards were always between 99.5 and 100.7%. Neutral detergent fiber (NDF), a measure of the cell wall constituents of the leaves, was determined as described by Van Soest et al. (1991) but using the filter bag apparatus described by Komarek (1994). Polar phenolic constituents were extracted from samples of ground foliage with 50% aqueous acetone, for 30 min, at 4°C, and in the dark (Cork and Krockenberger 1991). Total phenolics ("Hagerman phenolics") were assayed on portions of these extracts as described by Hagerman and Butler (1989) and condensed tannins were assayed by the butanol-HCl method (Porter et al. 1986). Quebracho, purified as recommended by Robbins et al. (1991) was used as the standard for both assays and the results are expressed in quebracho equivalents. The standard error of the laboratory procedures was calculated following the method of Smith and Flinn (1991) to give an estimate of the precision of laboratory procedures.

Measurements of the dry matter intake of greater gliders and common ringtail possums

This part of the research was approved by the Animal Experimentation Ethics Committees of Monash University and James Cook University and conforms to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Dry matter intake (DMI) of natural foliage diets was measured for greater gliders (*Petauroides volans*) and common ringtail possums (*Pseudocheirus peregrinus*) in a series of experiments with captive animals. Six greater gliders were caught by hand in eucalypt woodland near Taravale, in north-eastern Australia and eight common ringtail possums were caught at Kilcunda, in south-eastern Australia. Animals were housed in individual metabolism cages as described by Foley and Hume (1987) and Foley (1992) and maintained inside a room maintained at 20–25°C under a 12 h:12 h light:dark cycle. All animals were provided with fresh water ad libitum and maintained on foliage from several eucalypt species. Once branches of foliage were cut from trees, they were stored for up to 7 days in large polythene bags in the dark at 4°C. Ends of the branches were left standing in water.

Greater gliders were fed foliage from six individual trees of five different eucalypt species (*Eucalyptus tereticornis*, *E. acmenoides*. *E. crebra*, *E.* (=*Corymbia*) *intermedia* and *E. grandis*) in a series of Latin square designs. Each experiment was restricted to the individuals of one species of eucalypt and so consisted of six individual trees fed over six days. Greater gliders are strictly nocturnal, have few energy reserves and must feed each night to survive (Foley 1987). Therefore, we fed each foliage overnight from 1730 hours to 0500 hours, after which it was replaced with high-quality young tips of *E. tereticornis* foliage ad libitum. This approach was necessary to ensure that each animal ate sufficient food each night during the experiment to minimise carry-over effects that might result from different degrees of hunger. All food was removed before midday, to prevent animals from feeding at least 6 h before the next set of experimental leaves were offered. Animals were weighed at 3-day intervals and their mass did not change by more than 5% throughout the course of the experiments. We measured the amount of dry matter consumed between 1730 and 0500 hours as described by Foley (1992).

Common ringtail possums were fed on six to eight individual trees of three eucalypt species. These were *E. ovata*, *E. viminalis* and *E. polyanthemos*. Four separate experiments were conducted in total with eight samples of tree foliage from *E. ovata* individuals, six individuals of *E. polyanthemos*, six individuals of *E. ovata* again and six individuals of *E. viminalis*. All experiments were designed as Latin squares (Lawler et al. 1998) and, as with greater gliders, test foliage was removed at 0500 hours and a palatable form of *E. ovata* foliage was offered. Under this regime, all animals maintained constant mass. Again, we measured the amount of dry matter consumed between 1730 and 0500 hours as described by Foley (1992).

During experiments, additional bunches of foliage were placed in an empty cage and used to correct for any increase or decrease in the mass of the leaves due to changes in water content. These changes were insignificant. Bunches of foliage were sampled in a way consistent with the feeding of the animals. These samples were stored at –20°C and later freeze-dried and ground as described and used for chemical analyses and NIR spectroscopy.

Near-infrared spectroscopy

Near-infrared reflectance spectroscopy is commonly used in agriculture and standard methods have been recommended by the American Society for Testing and Materials (Anon 1995). In our work, we followed these standard procedures carefully and so give only brief details of our procedures.

Sub-samples of dried, ground foliage were allowed to equilibrate for one week in a room held at 15% relative humidity (RH) and 22°C to ensure that the residual moisture content of each sample was similar and so reduce background signals or noise in the O-H band of the spectra. The spectrum of each sample was recorded by a NIR Systems 6500 scanning spectrophotometer with a spinning cup attachment. The instrument was housed in a room maintained at 22–24°C and 55–60% RH and all samples were scanned in a custom-built, small-ring cup with an optical-grade quartz cover. At the beginning of each day, we checked the accuracy of the wavelengths, the repeatability of measurements and at regular intervals throughout the day, a standard sample of sugar cane leaf of known chemical composition was scanned to serve as a control. Near-infrared spectra were measured as the log 1/*R* for each sample, where *R*=the reflectance of monochromatic light between 400 and 2500 nm at 2-nm intervals. We collected duplicate spectra of each sample and accepted duplicates only if the root mean square of the difference between them was less than 50.

We modelled the relationship between spectral characters and our assays of the chemical composition and intake of the foliage using two different multivariate modelling procedures. Modified partial least squares regression (Shenk and Westerhaus 1991) was our preferred method. It is recognized to be one of the more robust methods of modelling multidimensional data since it uses all available spectral information and far fewer samples are needed for calibration (Shenk and Westerhaus 1991). In contrast, multiple linear regression models include only those wavelengths that have been selected and usually require more samples to derive useable models (Shenk and Westerhaus 1991). Raw spectral data were transformed to remove the possible influence of particle size differences between samples. Particle size can lead to spectral variation that is unrelated to the chemical composition of samples. To overcome this problem, we used standard normal variate and detrend transformations as outlined by Barnes et al. (1989). These procedures separate physical and chemical variance between samples and leave transformed spectra free from effects of multi-collinearity and differences in particle size.

Two independent validation tests were used to assess the accuracy of all our NIRS-based models as recommended by the standard procedures (Anon 1995). These were:

1. Cross-validation of the whole sample set. In this procedure, the population is divided into arbitrary groups and a prediction is made of the values for one group based on calibrations developed from the remaining groups. It is a particularly useful method where sample sizes are limited, as all available measurements are used to derive the model without the need to maintain separate validation and calibration sets. It is also recommended for use with modified partial least squares regression to prevent overfitting the model (Shenk and Westerhaus 1991).
2. Comparisons were made between the standard error of prediction for samples excluded from the calibration set and the standard error of prediction for each calibration set.

Predicted intake and food-tree use in free-living greater gliders

Models relating foliar spectral characters to the intake of foliage from single eucalypt trees by greater gliders were used to assess the relationship between leaf palatability and the foraging preferences of animals in the wild. One hundred young and mature leaf samples were collected among four eucalypt species *Eucalyptus acmenoides*, *E.(=Corymbia) intermedia*, *E. tereticornis* and *E. crebra* over 25 sites, all within the distribution range of greater gliders in north-eastern Australia (A.M. McIlwee, unpublished work). A detailed study of foraging behaviour of greater gliders had been made at one of these sites by Comport et al. (1996) and these data were used to characterise the feeding preferences of greater gliders in the wild. A total of 440 independent foraging observations were available from the study of Comport et al. (1996) and we used these to determine food-tree use by greater gliders by comparing the total proportion of feeding observations for each eucalypt species (P_f) with its frequency of occurrence in the study area (P_t). Preferences are expressed as an index of feeding selectivity (SI) that are directly comparable between different eucalypt species, by means of the formula $SI=(P_f-P_t)/P_t$. This index produces a value of 0 when tree species are used in the same proportion as their occurrence, –1 when tree species are not used at all, and positive values indicate that animals forage on a species in a greater proportion than its occurrence.

The spectra of all samples were collected using a NIR Systems 6500 scanning spectrophotometer (as outlined above). The spectral variation of samples was assessed by calculating the Mahalanobis distance of each sample from the spectral mean of the greater glider food intake calibration set. All spectra were less than three *H* distances from the calibration mean, which are within the standard limits over which predictions can be made (Shenk and Westerhaus 1993).

Results

Chemical composition of *Eucalyptus* foliage

The precision of the analytical technique for each attribute is measured by the standard error of duplicate labora-

Table 1 Performance of laboratory methods and near-infrared spectroscopy (NIRS) predictive equations used to measure attributes of leaf chemistry in eucalypts. Laboratory accuracy assessment: N^1 represents the total number of samples. Standard error of the laboratory (SEL) is: $SEL=\sqrt{[\Sigma(y_1-y_2)^2/N]}$ where y_1 and y_2 are duplicates of analyses. NIRS equation performance: summary of modified partial least squares linear regression statistics for NIRS predicted values for chemical attributes and actual measurements made by laboratory analysis. N^2 represents the number of samples in the calibration set (N^1 less laboratory outliers). The slope is the deviation of the regression line from the equation $y=x$ (where y is the NIRS predicted value and x is the laboratory measured value). The performance of NIRS equations is measured by coefficient of determination (r^2), standard error of the cross-validation (SECV) and standard error of prediction (SEP) for 30 samples independent of calibration set

Attributes[a]	Laboratory accuracy			NIRS equation preformance							
				Regression equation statistics							
	N^1	Mean	SEL	N^2	Mean	Range	Slope	r^2	SECV	SEP	Significance
N	85	12.37	0.52	84	12.79	7.17–20.1	0.964	0.961	0.759	0.79	***
NDF	85	39.78	1.46	81	39.35	14.6–59.8	0.980	0.978	3.166	3.62	***
CT	90	22.04	3.54	86	22.16	0.04–67.3	0.903	0.949	4.830	5.14	***
HP	90	40.90	4.47	85	40.45	14.2–60.2	0.871	0.877	4.511	4.45	***

Coefficient of determination represented by r^2, with significance levels for regression at $P<0.001$ (***)

[a] Attributes include nitrogen concentration (*N*), neutral detergent fibre (*NDF*), condensed tannins (*CT*) and total phenolics by Hagerman procedure (*HP*). Concentrations expressed as mg g^{-1} dry matter (DM) (N), % DM (NDF), % quebracho equivalents g^{-1} DM (CT and HP)

tory assays for each sample analysed (Table 1). The semi-micro Kjeldahl technique proved the most accurate laboratory assay and the greatest source of error in the laboratory analysis was associated with the measurement of total phenolics.

There were highly significant ($P<0.0001$) relationships between the values derived by chemical analyses and the values predicted by NIRS for all four foliar constituents (Fig. 1, Table 1). Slopes of the regression lines between values measured by chemical analysis and those predicted by NIRS did not differ significantly from 1.0, indicating that no significant bias existed in the prediction of any constituent by NIRS. Given that NIRS-derived predictions include both calibration and laboratory errors, a comparison of the standard error of the laboratory procedures and standard errors of prediction for each constituent (0.50–4.47% vs. 0.80–4.45% respectively) suggests that calibration errors are similar to those of the laboratory. This result demonstrates that NIRS is a reliable technique for quantifying various nutritional fractions of *Eucalyptus* foliage.

Dry matter of intake by greater gliders and common ringtail possums

Table 2 summarises the different predictive models that were tested in order to find the best-fit model that describes the relationship between food intake measured in standard feeding experiments and values predicted by NIRS. The relationship between food intake and NIRS predicted values were highly significant for all modelling approaches tested. The modified partial least squares model with standard normal variate scatter correction provided the most accurate models of food intake for both greater gliders and common ringtail possums. Mathematical transformations further improved the per-

Table 2 The effects of regression procedure, scatter correction, spectral transformations and inclusion of visible wavelengths on the performance of NIRS intake equations. Effects of treatments on the performance of NIRS predictions are measured by the standard error of the calibration (SEC), standard error of the cross-validation (SECV) and the coefficient of determination (r^2)

Treatment	Greater gliders			Ringtail possums		
	SEC	SECV	r^2	SEC	SECV	r^2
Regression method						
PCR	6.786	6.822	0.791	8.460	9.420	0.726
PLS	3.198	5.343	0.820	7.025	9.005	0.811
MPLS	2.569	4.398	0.884	3.538	7.382	0.921
Scatter correction						
No correction	2.974	5.052	0.845	4.934	8.253	0.909
Detrend	2.899	5.341	0.852	4.538	7.382	0.921
SNV	2.519	4.398	0.884	3.785	6.732	0.945
Math treatments						
First derivative	2.569	4.398	0.884	3.785	6.732	0.945
Second derivative	2.118	4.296	0.921	3.469	7.299	0.954
Wavelengths						
408–2492 nm	2.382	3.685	0.900	3.772	6.583	0.946
1108–2492 nm	2.289	4.398	0.884	5.405	8.321	0.888

Regression procedures include principal components regression (*PCR*), partial least squares regression (*PLS*), modified partial least squares regression (*MPLS*). Scatter correction is used to remove the non-linearity in spectra due to effects such as differences in particle size and the scattering of light. Detrend is a baseline correlative method which removes the linear and quadratic curvature of each spectrum. Standard normal variate (*SNV*) correction scales each spectrum to a standard deviation of 1.0. First and second derivative algorithms perform optimal N-point smoothing of data before derivatives are calculated. Derivatives are taken on log $1/R$ values (where R is reflectance of composite spectrum)

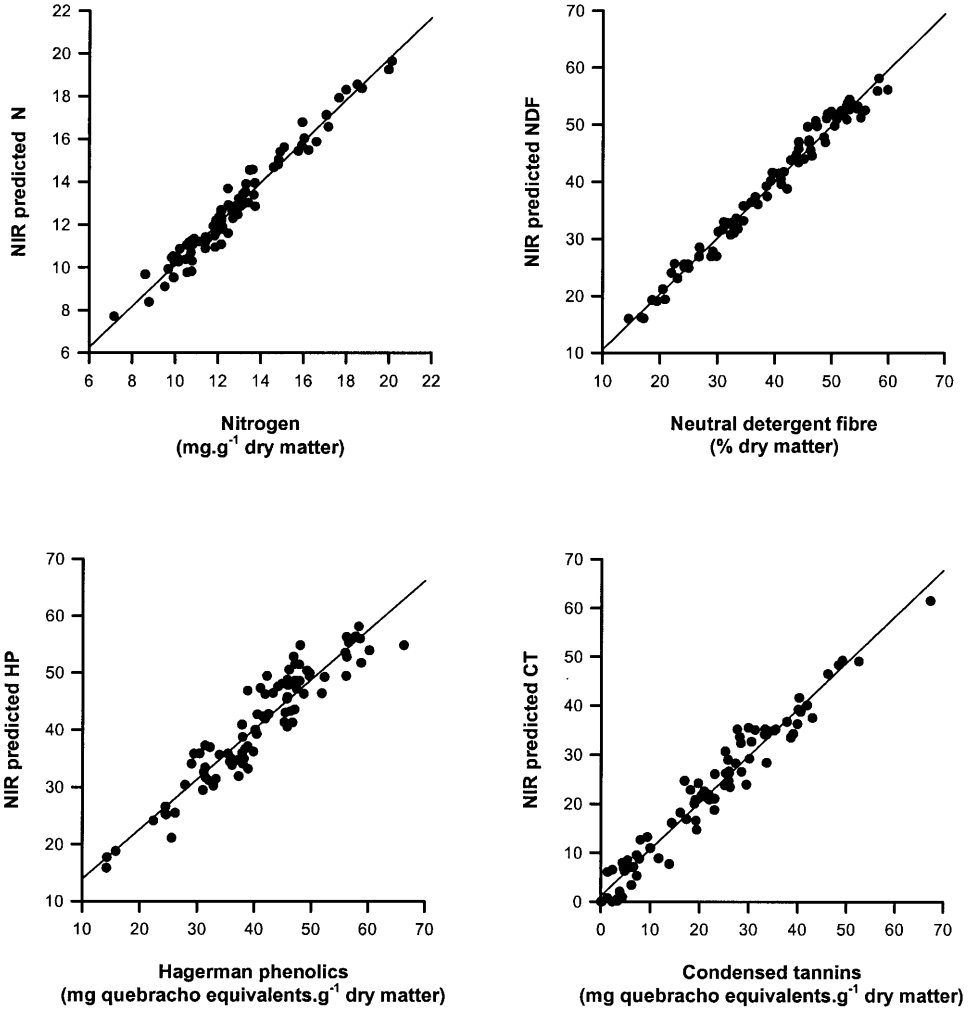

Fig. 1 Relationships between values predicted by standard laboratory assays, and those predicted by a model based on partial least squares regression, relating near-infrared reflectance (NIR) spectra of *Eucalyptus* foliage to these standard assays for four components of *Eucalyptus* foliage. Significant relationships were found between actual and predicted constituent values for nitrogen (N) (r^2=0.96, SE=0.490, n=84; $F_{1,83}$=2161, P<0.0001), neutral detergent fiber (NDF) (r^2=0.98, SE=1.64, n=81; $F_{1,80}$=3968, P<0.0001), condensed tannins (CT) (r^2=0.95, SE=3.08, n=86; $F_{1,85}$=1764, P<0.0001) and total phenolics by the Hagerman procedure (HP) (r^2=0.87, SE=3.46, n=85; $F_{1,84}$=618, P<0.0001). Concentrations are expressed as: mg g^{-1} dry matter (DM) (N), % DM (NDF), mg quebracho equivalents g^{-1} DM (condensed tannins and total phenolics)

formance of models, with the second derivative of log 1/*R* being most successful. Including data points from both visible and NIR parts of the spectrum also enhanced the accuracy of the models. However, we omitted visible wavelengths from the final models because our objective was to characterise food intake based strictly on chemical composition and not on aspects of colour.

The results for the highest ranked models for food intake for greater gliders and ringtail possums are illustrated in Fig. 2 and summarised in Table 3. These data show that NIRS-based models of food intake are at least as good as the models derived for the chemical constituents of the leaves described in Table 1. Assessment of calibration performance using an independent validation set gave standard error of prediction of 3.4 and 8.3 g DM $kg^{-0.75}$ for dry matter intake of greater gliders and ringtail possums respectively. This demonstrates that the models have a high predictive power outside the calibration set from which they are derived. However, for ringtail possums, despite a close match between actual and predicted intakes using the validation equation (as indicated by the high r^2 and standard error of calibration), the performance of the equation was low, as the standard error of the prediction was approximately 2.5 times greater than the standard error of calibration (8.3 vs. 3.5 DM $kg^{-0.75}$). It is likely that the performance of the ringtail possum validation equation was limited by the low number of samples and the loss of important spectral variability from the calibration.

Comparison of food intake predicted by spectral modelling with dietary preferences of free-living greater gliders

There were significant differences in our NIRS-based predictions of food intake of greater gliders amongst tree species and leaf ages (Fig. 3a). Our models predicted that animals would consistently eat more young foliage than old for all four eucalypt species. *E. acmenoides* had the highest potential intake for both young and mature foliage. *E.* (=*Corymbia) intermedia* and *E. tereticornis* were predicted to be eaten in significantly lower amounts than *E. acmenoides.* However, the predicted intake of these species was significantly higher than that of *E. crebra.* This pattern reflected the feeding preferences of greater gliders reported by Comport et al. (1996), as shown in Fig. 3b.

Table 3 Performance of NIRS equation predicting food intake (g DM $kg^{-0.75}$) for greater gliders (*GG*) and common ringtail possums (*RTP*): summary of observed voluntary intake statistics and summary of modified partial least squares regression statistics for NIRS predicted voluntary intake. N^C and N^V represent the number of samples used in calibration and validation sets respectively. The slope is the deviation of the regression line from the equation $y=x$ (where y is the NIRS predicted value and x is the observed value). Equation performance is measured by the coefficient of determination (r^2) (observed intake), the standard error of the calibration (*SEC*) and standard error of prediction (*SEP*) for N^V (number of samples) (NIR equation performance)

Equation	N^C	N^C	Observed intake (g DM $kg^{-0.75}$)			NIR equation performance			
			Mean	Range	SD	Slope	r^2	SEC	SEP
Calibration									
GG	30	–	26.9	12.8–46.9	7.8	0.96	0.943	1.80	n.a
RTP	25	–	22.6	1.3–50.12	16.2	0.95	0.945	3.79	n.a
Validation									
GG	20	10	26.9	12.8–46.9	8.0	0.99	0.901	2.51	3.42
RTP	16	9	24.1	2.5–50.1	16.0	0.85	0.953	3.47	8.29

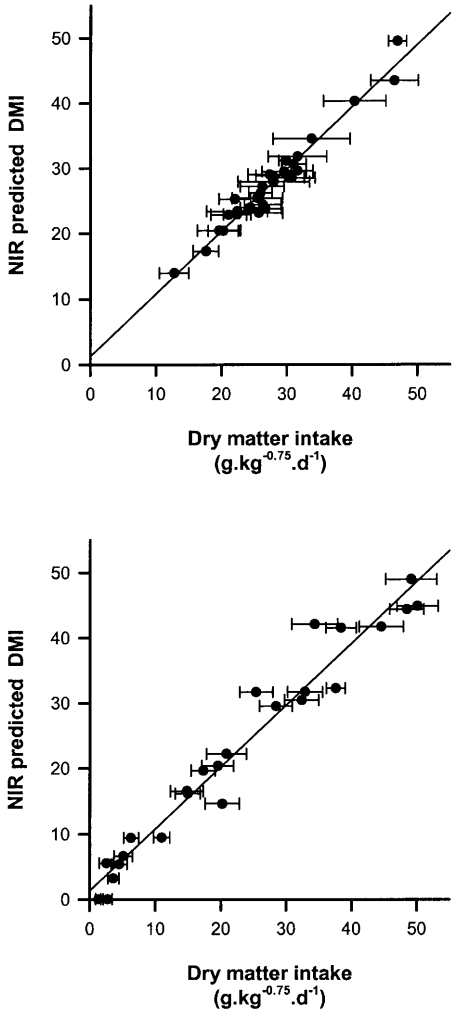

Fig. 2 Relationship between mean voluntary dry matter intake (DMI, ±SE) of *Eucalyptus* foliage for **a** greater gliders and **b** common ringtail possums, and the intake predicted by a model based on partial least squares regression relating the near-infrared spectra of *Eucalyptus* foliage to these measures of intake of *Eucalyptus* foliage. Significant relationships between actual and predicted intake were found for both greater gliders (r^2=0.94, SE=1.80, n=30; $F_{1,29}$=272, P<0.0001) and common ringtail possums (r^2=0.95, SE=3.79, n=25; $F_{1,24}$=505, P<0.0001)

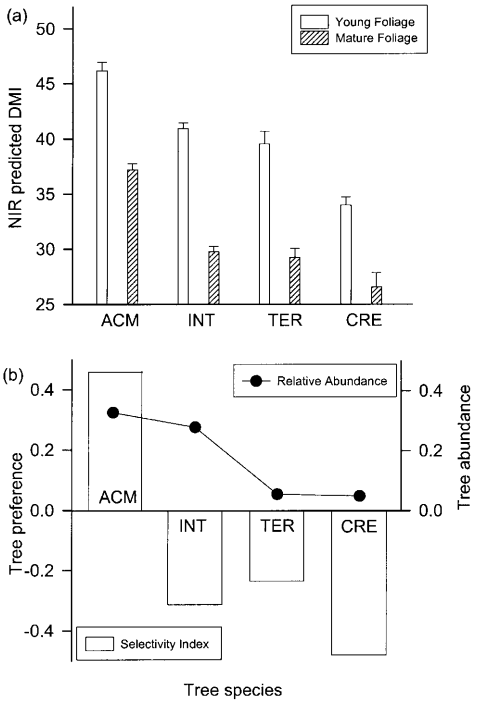

Fig. 3 **a** Mean predicted dry matter intakes (g DM $kg^{-0.75}$ day^{-1}) of greater gliders with 95% confidence limits, for 100 young and mature foliage samples of each of four *Eucalyptus* species based on a model relating spectral characteristic of *Eucalyptus* foliage to its measured intake by captive greater gliders. Predicted intakes differed significantly between tree species ($F_{3,792}$=277; P<0.0001) and leaf age ($F_{1,792}$=1203; P<0.0001). **b** Observed feeding preferences greater gliders in the wild, expressed as a selectivity index, and proportions of each tree species in the study area. Data of Comport et al. (1997) (*ACM Eucalyptus acmenoides*, *INT E.* (*=Corymbia) intermedia*, *TER E. tereticornis*, *CRE E. crebra*)

Discussion

This study has shown that the chemical composition of eucalypt foliage can be predicted rapidly, and accurately using NIR spectroscopy. For example, up to 200 samples can be processed in a single day, and all four constituents measured here can be predicted simultaneously. This re-

Table 4 Summary of published NIRS empirical models predicting voluntary food intake (for mammalian herbivores. Errors between observed intake and NIRS predicted intake are measured by the coefficient of determination (r^2) and the standard error of prediction (SEP)

Forage	*n*	Intake (g DM $kg^{-0.75}$)		NIR prediction		Reference
		Species	Range	r^2	SEP	
Mixed samples	76	Sheep	40–114	0.62	7.8	Norris et al. (1976)
Grazed pasture	21	Cattle	53–112	0.72	9.6	Ward et al. (1982)
Faecal samples	36	Elk	40–113	0.80	8.9	Brooks (1984)
Grazed pasture	53	Cattle	75–129	0.66	7.8	Redshaw et al. (1986)
Grazed pasture	44	Sheep	66–116	0.55	8.4	Redshaw et al. (1986)
Grazed pasture	136	Cattle	n.a	0.86	5.5	Steen et al. (1995)
Foliage	30	GG	13–47	0.90	3.3	This study
Foliage	25	RTP	3–50	0.95	7.8	This study

sults in savings of at least 80% on normal laboratory costs and this effectively removes a major problem with intensive sampling of natural plant populations. Furthermore, NIRS provides a powerful method for dealing with chemical complexity of foliage diets as NIR spectra capture compositional data sufficiently well to allow robust models of food intake to be produced.

Several studies have shown that NIRS is able to predict the composition of various plant materials with a high level of accuracy and precision (e.g. reviews in Givens et al. 1997; Foley et al. 1998). Consequently, we were not surprised that NIRS could predict these attributes in *Eucalyptus* foliage. Predictions of total polar phenolics were less accurate than other chemical components. However, given the enormous structural variation in compounds that are detected by the phenolic assay, as a group total phenolics are difficult to define chemically. Therefore, we were not surprised that the accuracy of NIRS was reduced for this constituent compared with analyses of total nitrogen. A comparison between the standard error of the laboratory analyses of phenolics and the standard error of cross-validation supports this conclusion.

Previous and ongoing studies of the nutritional ecology of folivorous marsupials have argued strongly for the roles of leaf nitrogen, fiber, phenolic constituents and tannins in determining levels of food intake and diet selection (Cork and Pahl 1984; Foley and Hume 1986; Cork and Sanson 1990; Kavanagh and Lambert 1990; Cork 1992; Lawler et al. 1998, 2000; Pass et al. 1998). Food intake is closely linked to chemical composition of the foliage in common ringtail possums irrespective of differences in digestibility of the foliage (Lawler et al. 1998, 2000). This means that if NIR spectra can capture information about chemical makeup of a foliage, then it is likely that we can predict intake directly without necessarily measuring particular chemical components of the leaf. Our results show NIRS spectra alone are able to explain more than 90% of the variation in mean dry matter intake for both greater gliders and common ringtail possums.

The models that we derived to predict our standard measure of food intake for both greater gliders and ringtail possums show a similar or better accuracy than similar NIRS equations used to predict the intake of large grazing mammals, as illustrated in Table 4. This difference may be because compositional differences have a greater impact on the food intake of browsers than grazers. In grazers, animal-related factors such as mastication can have a major effect on the level of digestibility of the diet and hence the subsequent intake (Cork and Foley 1991). Also, the small size and high mass-specific energy requirements of arboreal folivores (Cork and Foley 1991) should make them more sensitive to variations in diet quality than large grazing herbivores. In this study, a substantially higher variation in food intake was observed in comparison to other feeding studies for these two marsupials (Foley 1987, 1992; McArthur and Sanson 1991) but this was because the earlier studies focused more on differences in digestibility, and not differences in intake, as a cause of variation in nutritional quality of different foliages.

The ability of NIRS to capture the compositional features of foliage in a more holistic way than selected chemical analyses is, to our view, a major advantage of the method. This is because food intake for most herbivores is determined by a range of factors, both nutritional and anti-nutritional (many unknown), which are mediated by the specific digestive and toxicological constraints imposed by an individual animal's physiology (Bozinovic and del Rio 1996). Consequently, studies that seek correlations between selected compositional measures and animal feeding will rarely succeed. However, it is important to note that the intake values used to calibrate our models were derived with captive animals in a laboratory setting. Many non-forage factors influence food intake in the wild. These may include the effect of mixed-species diets (and hence the ability to choose between alternatives) and reproductive state. Based on this inherent limitation, our models might best be described as “potential intake” and an assessment of their utility in the field is required, as described above using the data of Comport et al. (1996).

Potential intake and greater glider feeding preferences

It is difficult to transfer predictions of leaf palatability to animal foraging preferences in the wild, as animal pref-

erences are no doubt influenced by a variety of circumstances. Given that there is a complex set of ecological circumstances that can influence the foraging choices of herbivores, one could probably not expect a precise match between foraging behaviour and estimates of nutritional quality. This may be particularly relevant to greater gliders if they are dependent on young leaf growth to meet their nutritional requirements (Kavanagh and Lambert 1990; Comport et al. 1996) as variations in leaf production may have a large impact on foraging choices. Nonetheless, both the marked selection of greater gliders for *E. acmenoides* foliage over other tree species, and the strong avoidance of *E. crebra* were predicted by our model, suggesting that nutritional quality influences foraging patterns strongly in this species.

Our model of food intake in greater gliders predicted consistent differences between the intake of young and mature foliage, as would be expected from the field studies outlined above. The spectral variation between samples also suggests that intraspecific variation in leaf palatability is low compared to interspecific variation for these eucalypt species. However, the leaves used in the calibration were from sites in the same region, but not the same trees, as in the study of Comport et al. (1996). Their data also do not consider foraging preferences within eucalypt species. Field studies of koalas (e.g. Hindell and Lee 1987) and field and laboratory data of ringtail possum food preferences (Cork and Pahl 1984; Lawler et al. 1998; this study) suggest that with appropriate calibration and the increased sampling power made possible by NIR spectroscopy, a far greater understanding of the foraging decisions made by individual animals will be developed by examining spectral variation of all individual trees within a study site.

Empirical versus mechanistic models in nutritional ecology

This study has been a component of larger studies that have examined the chemical basis of food selection by folivorous marsupials (Foley 1992; Pass et al. 1998; Lawler et al. 1998). This knowledge has given us a sound appreciation of the role of different components in *Eucalyptus* foliage that constrain animal feeding. However, in cases where the underlying mechanisms behind feeding have not been clearly identified, we ask how valid is it to use simple empirical models to predict complex physiological and behavioural outcomes? It could be argued that food intake is the complex outcome of many factors that will always remain poorly understood but should a lack of complete understanding prevent us from attempting to quantify such attributes? We suggest that NIRS may confer valuable benefits in two areas.

Firstly, examination of NIRS calibrations may provide insights into the aspects of plant chemistry that determine the characteristic of interest, even though peaks in NIR spectra cannot be unequivocally be attributed to a single functional group. For example, Clark and Lamb (1991) surveyed the use of different NIR wavelengths as predictors of dry matter digestibility in ruminants. They found that regardless of forage type, wavelengths associated with C-H groups typical of plant fiber were important. Similarly, in a study of resistance of sugar cane to stem borer attack, Rutherford and Van Staden (1996) used important wavelengths in calibration equations to implicate alcohols and carbonyl components in resistance. Therefore, an initial empirical approach became an analytical tool that substantially increased progress towards identifying resistance factors through many bioassays.

Applying this approach to our data, we used stepwise regression to identify the most important wavelengths used to predict both food intake and leaf constituents. Using just three wavelengths, stepwise regression was able to explain 76% of the variation in food intake in greater gliders and 82% in ringtail possums. Wavelengths 1132 nm and 1572 nm identified in models of food intake by greater gliders were also highlighted by stepwise regression as being important for determining condensed tannin and total phenolic concentrations respectively (A.M. McIlwee and W.J. Foley, unpublished work). Although these compounds may be important determinants of foliage quality it is worth noting that these connections do not demonstrate any causative relationships. However, they do provide some indication of where further research efforts could best be directed. No correlation was found between important wavelengths used in models describing food intake in common ringtail possums and models of any of the four foliar constituents measured. However, this result is not surprising since food intake in common ringtail possums is largely controlled by a single group of non-polar phenolics that were not measured in this study (Pass et al. 1998; Lawler et al. 1998).

A second reason for adopting an empirical approach to predicting animal performance is the great rapidity with which a useable predictive model can be developed relative to a mechanistic understanding. The stark difference in the rate of development of predictive equations using NIRS, as opposed to attempts using mechanistic approaches is clearly evident. For example, the effect of secondary chemistry of *Eucalyptus* on the foraging behaviour of marsupial folivores has been the subject of much study over the past two decades (e.g. Southwell 1978; Foley and Hume 1987; Hume and Esson 1993). However, the complexity of the secondary chemistry and the unknown effects of this on different marsupial folivores meant that little progress was made. Recently, however, detailed chemical studies finally identified compounds that explained 80% of the variation in feeding between individual trees by common ringtail possums, and koalas (Pass et al. 1998; Lawler et al. 1998; I.R. Lawler, W.J. Foley, and B.M. Eschler, unpublished work; B.D. Moore and W.J. Foley unpublished work). This required the combined efforts of five chemists and zoologists and 4 years of full-time work using bioassay-guided fractionation. In contrast, it took a single after-

noon to develop the initial predictive NIR models, which estimate the feeding rates of folivorous marsupials with greater accuracy than our detailed chemical investigations have so far produced. Furthermore these models incorporate all leaf attributes thought to affect rates of food intake.

If caution is used, models can potentially be developed for management purposes without any knowledge of the underlying chemistry (although we emphasise that it will also begin to identify aspects of the chemistry as outlined above). If samples of all food species within a defined habitat are represented in the spectral variation of a calibration set then application of that calibration over the entire area will be valid. Extension of that calibration to other areas or species will require expansion of the calibration set, depending on the purpose for which the calibration is to be used.

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