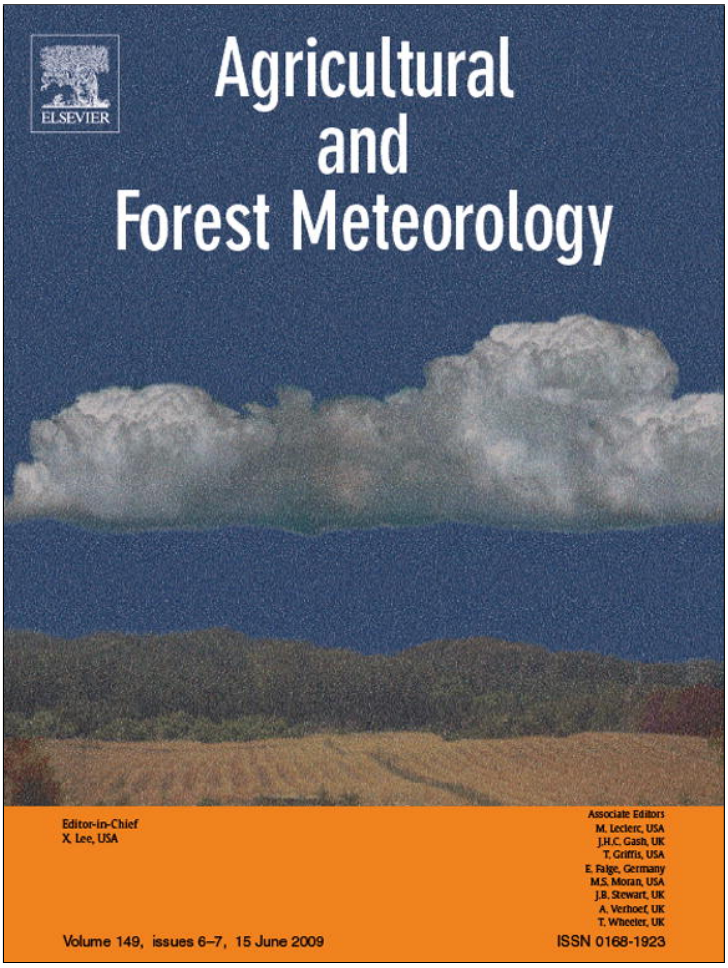
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Short communication

Estimation of chlorophyll content in *Eucalyptus globulus* foliage with the leaf reflectance model PROSPECT

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ABSTRACT

This communication presents the performance of the PROSPECT leaf optical model to derive chlorophyll content (*Cab*) estimates from reflectance and transmittance spectra of *Eucalyptus globulus* foliage. Estimates were compared to measured chlorophyll of 100% acetone extractions. The analysis showed that recent modifications to the absorption coefficients used in the PROSPECT model resulted in improved estimates of chlorophyll for both adult and juvenile leaves. Results were better for adult leaves, with estimates within 5 $\mu g\,cm^{-2}$ for the juvenile data set and 3 $\mu g\,cm^{-2}$ for adult leaves. Accurate estimates of canopy chlorophyll content in eucalypt plantations through the numerical inversion of leaf-level radiative transfer (RT) models such as PROSPECT coupled to canopy RT models would improve our ability to assess and monitor eucalypt plantation growth, health and condition.

1. Introduction

Chlorophyll content is strongly related to photosynthetic functioning of vegetation and this capacity varies in a range of environmental and phenological conditions. Periods of medium- to long-term stress can often be detected by decreases in chlorophyll content (Lichtenthaler, 1996; Zarco-Tejada et al., 2002), while short-term changes can be more easily detected via carotenoid metabolism (Demmig-Adams and Adams, 1996). Methods to accurately detect changes in chlorophyll content in agricultural and forest landscapes via remote sensing have been developed using spectral indices (Coops et al., 2003; Datt, 1998; Haboudane et al., 2002; Wu et al., 2008), radiative transfer (RT) models (Jacquemoud and Baret, 1990) and fluorescent approaches (Zarco-Tejada et al., 2002; Zarco-Tejada et al., 2006). Coupled leaf and canopy RT models refine the development of spectral indices, which are insensitive to canopy structure, illumination geometry, and background reflectance for estimating foliar chlorophyll concentrations from canopy reflectance (Zhang et al., 2008).

PROSPECT (Jacquemoud and Baret, 1990) is a leaf optical model based on the principles of radiative transfer. One of the benefits of the model is that it can readily be coupled with canopy reflectance models (Baret et al., 1992; Zhang et al., 2008) to facilitate direct modelling of the impact of chlorophyll, water and leaf dry matter constituents on the reflectance of a complete plant canopy. This coupled leaf and canopy modelling approach also opens up the possibility of inverting the model, using non-linear methods, in order to estimate foliar properties such as chlorophyll content, directly from canopy scale measurements of reflectance.

Eucalyptus globulus is an internationally important plantation species and forest health assessment is an expensive component of management (Carnegie et al., 2008). Hyperspectral imagery has the potential to offer spatially explicit information on forest health and condition (Coops et al., 2004). While foliar nitrogen is used in many crops as a basis for productivity assessments and stress detection (Baret et al., 2007; Smith et al., 2002) the relationship between foliar nitrogen and growth is not clear in eucalypts except when nitrogen is very low. Determination of chlorophyll content in production forests is also important for detection of stress to enable remedial action to be planned and the potential impact of stress events on stand growth quantified.

Vegetation indices for chlorophyll have been developed and tested on some eucalypt species (Coops et al., 2003; Datt, 1998, 1999) including *E. globulus* (Barry et al., 2008; Pietrzykowski et al., 2006). Previous work has been performed to model the reflectance of eucalypt forests as a whole (Jupp et al., 1986) but this has involved assumptions about the mean reflectance of foliage and the evaluation of reflectance modelling of eucalypt leaves has not yet been specifically addressed. Radiative transfer models of leaf

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reflectance offer the capacity to account for extraneous influences on chlorophyll estimates by explicitly dealing with factors including foliage internal and surface structure and the geometry of illumination and viewing angles. This has the potential to increase the transferability of chlorophyll assessment methods to deal with variations in both leaf form and measurement methodologies and overcome the limitations of regressive empirical models. In this paper we test the performance of two versions of PROSPECT to predict the chlorophyll content of *E. globulus* leaves.

2. PROSPECT versions 3 and 4

We used a version of the PROSPECT model (version 3, P3) adapted for spectral absorption coefficients at 1 nm intervals (Jacquemoud, 2004; Renzullo et al., 2006) and with the maximum angle of beam interception (α) set to 0° instead of 60°. We also tested the PROSPECT model with an updated set of absorption coefficients (version 4, P4) as described by Féret et al. (2008). The main difference between the two versions of the model is that the chlorophyll absorption coefficients are increased in magnitude in P4 across the 400–700 nm range, particularly between 400 and 500 nm. P4 also differs from P3 by setting α to 40° rather than 60°. The value α defines the maximum angle between the normal to the leaf surface and the beam direction, effectively defining a cone of possible interception angles about the leaf surface normal. A smooth leaf surface illuminated by a collimated beam will have an α close to zero while a rough leaf surface, even if illuminated by collimated light will have larger values of α, since small facets of the leaf surface, oriented at different angles, will be illuminated at a range of angles.

Using measured reflectance (R_{meas}) and transmittance (T_{meas}) spectra from 400 to 2500 nm, PROSPECT model estimates of total chlorophyll (*Cab*, μm cm^{-2}), leaf water content (*Cw*), dry matter content (*Cm*) and mesophyll structure parameter (*N*) were derived using inversion. The merit function for the inversion is shown in Eq. (1), where the subscript mod refers to modelled estimates of reflectance and transmittance. The derived estimates were then compared to measured values of *Cab*, *Cw* and *Cm*. Estimates of *N* could not be validated since this parameter described the scattering power of the leaf internals material and is only indirectly associated with physical structure:

$$\chi^2 = \sum[(R_{meas} - R_{mod})^2 + (T_{meas} - T_{mod})^2] \qquad (1)$$

Using the measured values for *Cab*, *Cw* and *Cm*, and a value of *N* derived from the inversion, modelled reflectance (R_{mod}) and transmittance (T_{mod}) spectra from 400 to 2500 nm were generated. The accuracy of these modelled spectra was tested against the measured data and the effect of altering α on the spectra was determined. We found little measurable difference in the reproduced spectra for α set to 0°, 40° or 60° (Fig. 1). We therefore maintained 0° for all further analyses. This supports our belief that eucalyptus leaves can be treated as optically flat. This is due to the layer of leaf wax that covers the surface of juvenile leaves, and the smooth glossy surface of adult leaves.

3. Data acquisition

Leaves of *E. globulus* of two ages growing at a site in SE Tasmania already described (O'Grady et al., 2006) were sampled. Young trees had been planted as seedlings in the field for 4 months, were an average of 1.7 m height and were composed of leaves of juvenile morphology only. Mature trees planted in an adjacent stand were approximately 4.5 years old, an average of 8.0 m height and dominated by foliage of adult morphology. All young and mature

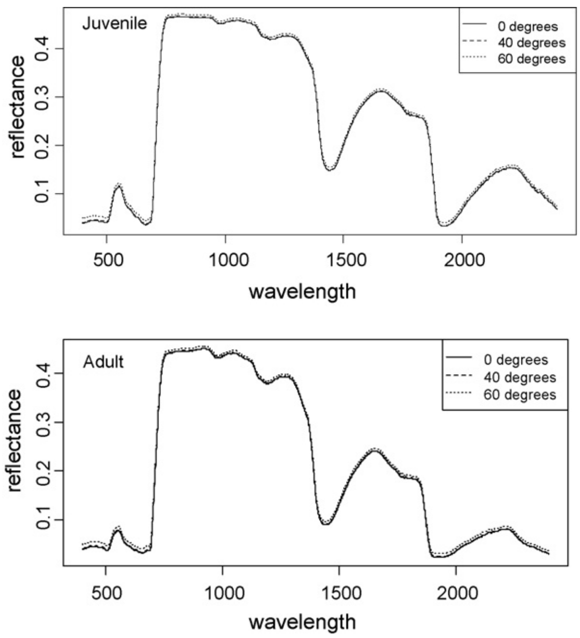

Fig. 1. *R* and *T* spectra generated by forward modelling P4 with averaged measured data (and derived *N* values from this data set) for juvenile and adult *Eucalyptus globulus* leaves, using angle of beam interception (α) set at 0°, 40° and 60°.

trees were in good condition, having been provided with fertiliser, adequate water and weed and pest control when required. A total of 64 juvenile leaves were sampled from 8 young trees; 4 branches were selected in cardinal directions and the youngest partly-expanded leaf and an older fully-expanded leaf were sampled. A total of 48 adult leaves were sampled from 4 mature trees; 3 young recently formed leaves and 3 older leaves were selected from two selected branches on each tree. We sampled foliage of different ages to provide a range of chlorophyll contents, as content increases as leaves mature (Barry et al., 2009; Stone et al., 2005). All leaves were fully sun-lit when sampled.

Reflectance and transmittance were recorded within 5 min after leaves were removed from the trees, during which time they were kept in the shade. Adult *E. globulus* leaves hang vertically and are essentially isobilateral in morphology, however the presence of reddening on one side of the leaf indicated which side had experienced most sun exposure. This side of the leaf was therefore used to measure reflectance and transmittance. In contrast, juvenile leaves are presented horizontally and there is a distinct difference between the abaxial and adaxial side. Therefore the adaxial side was used for measurements of reflectance and transmittance. An Analytical Spectral Devices FieldSpec® Pro FR spectroradiometer (ASD, Boulder, USA) was used with a LiCor integrating sphere attached to collect stable bi-hemispherical reflectance measurements. Due to low-spectral intensity of the halogen lamp used for the integrating sphere above 1600 nm and the resulting noise in the measured spectra, reflectance and transmittance was only considered below this wavelength. Reflectance and transmittance were recorded from the same leaf position on one side of the mid-vein. Spectra were corrected for stray light by subtraction from both the transmittance and reflectance data, and a conversion factor between the barium sulphate surface of the integrating sphere and a Spectralon® reference panel.

Immediately following spectral data collection, leaves were sampled for destructive analysis. The side of the leaf opposite to that used for spectral assessment was retained for pigment analysis and immediately stored in liquid nitrogen. Three leaf discs (0.50 cm^2 each) were punched from the other side of the leaf, a fresh weight obtained and then they were stored for oven drying to obtain *Cw* and *Cm*. Pigment extraction was later completed using a method which involved grinding in liquid nitrogen and a triple extraction with 100% acetone, followed by centrifugation and UV–vis spectrophotometry (Martin et al., 2007).

Relationships between measured and modelled data were explored using linear regression. A small number of data points with large residuals and high leverage were selectively removed from the data set. The standard error of the estimate (SEE) was determined, which is equivalent to the root mean squared error (RMSE) commonly use to evaluate accuracy of modelled to measured data.

4. Results

Measured reflectance and transmittance spectra were generally typical of healthy green foliage (Fig. 2). Transmittance was higher for juvenile leaves compared to adult leaves throughout most of the spectrum, apart from between 400 and 500 nm where no transmittance was recorded for the juvenile leaves, possibly due to foliar wax. Chlorophyll content in juvenile leaves of young trees ranged from 21 to 54 μg cm^{-2} while in adult leaves of mature trees the range was 25–103 μg cm^{-2} and averages are shown in Table 1.

Both juvenile and adult leaf data sets were tested with PROSPECT independently and also as a combined set. The *N* values determined for juvenile leaves ranged from 1.40 to 2.04 with PROSPECT version 3 and 1.49–2.15 with PROSPECT version 4, while for the adult leaves the range was 1.21–2.33 and 1.26–2.47, respectively. Based on inverse modelling using both reflectance and transmittance spectra within the 400–1600 nm wavelength range, estimated values of *Cab* were a good fit to measured *Cab* for adult leaves (R^2 = 0.96, 0.95) using either version of the model (Table 1). However, the SEE was almost halved (from 5.5 to 3.5 g cm^{-2}) when the PROSPECT version 4 model was used (Table 1). The fit between modelled and measured *Cab* for juvenile leaves was not as close (R^2 = 0.87, 0.88) however the SEE was again almost halved (from 9.2 to 5.1 g cm^{-2}) when version 4 of the model was used. These relationships are also plotted (Fig. 3) and

Table 1
Average measured *Cab* content and statistics derived from linear regression of measured and modelled values for *Cab* for both data sets and when combined, using P3 and P4. SEE is the standard error estimate (equivalent to root mean square error, RMSE).

	Measured mean (±)	Model	R^2	SEE	F. Pr.
Adult leaves					
Cab (μg cm^{-2})	67.02 ± 3.78	P3	0.96	5.51	<0.001
		P4	0.95	3.52	<0.001
Juvenile leaves					
Cab (μg cm^{-2})	42.30 ± 1.73	P3	0.87	9.20	<0.001
		P4	0.88	5.09	<0.001
Combined data					
Cab (μg cm^{-2})	52.51 ± 2.19	P3	0.84	11.57	<0.001
		P4	0.86	6.57	<0.001

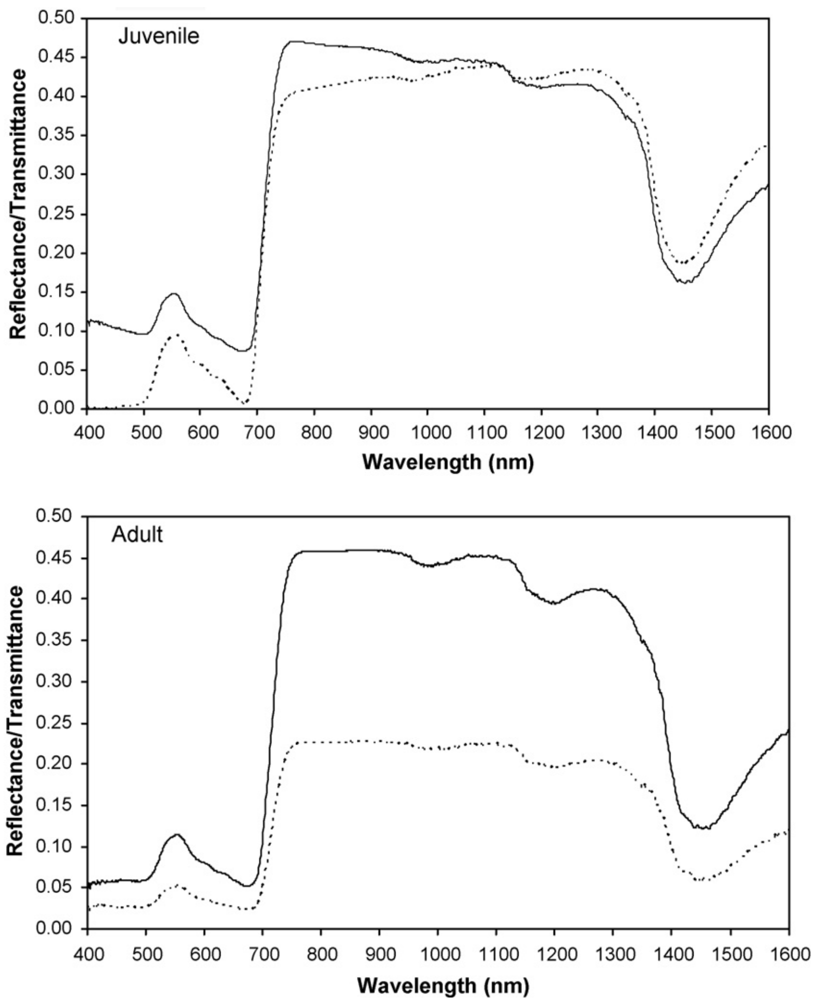

Fig. 2. Average *R* (solid line) and *T* (dashed line) spectra for juvenile *E. globulus* leaves from young trees and adult *E. globulus* leaves from mature trees.

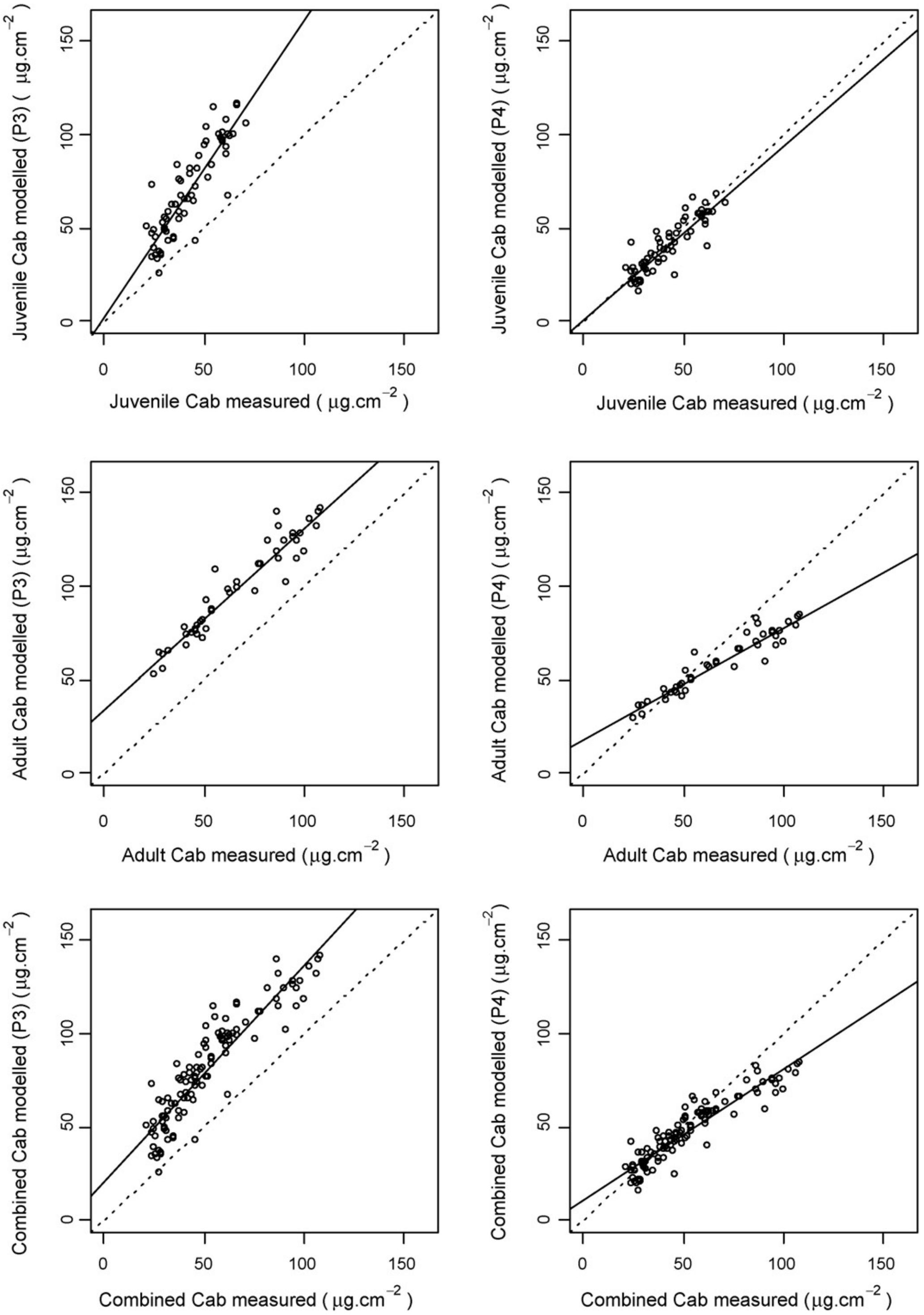

Fig. 3. Measured *Cab* plotted against *Cab* modelled with P3 and P4, for adult leaves and juvenile *E. globulus* leaves. The solid line is the regression of data points; dashed line is the 1:1 line.

compared to a 1:1 line of fit. When the two data sets were combined, the fit was still highly significant, but strength of the relationship was slightly decreased and the SEE was increased (Table 1, Fig. 3).

While the focus of our current study is on chlorophyll, estimations of *Cw* and *Cm* were derived for both data sets with PROSPECT versions 3 and 4 and compared to measured data. However, the lack of spectral information above 1600 nm for our data sets limits the retrieval of both *Cw* and *Cm* and results are not shown. The absorption coefficients for dry matter are highest above 2000 nm, while water has three major peaks of absorption including one below 1600 nm. To obtain a reliable indication of model performance for *Cw* and *Cm* data should be collected to 2500 nm if the signal quality is adequate.

5. Discussion and conclusion

This study shows that both versions of the PROSPECT model provide good predictions of *Cab* in *E. globulus* leaves of two different morphologies. Based on the SEE, version 4 of the model reduced the error associated with estimated *Cab* to around $5\,\mu g\,cm^{-2}$ for juvenile leaves and below that for adult leaves. These results are better than for multiple species studies (as may be expected due to uniformity in leaf structure) and other single species studies using PROSPECT version 3. For example, Renzullo et al. (2006) obtained $\pm 10\,\mu g\,cm^{-2}$ for grapevine of different varieties using the same PROSPECT version 3, although only reflectance was used. Féret et al. (2008) found that across data sets with a wide range of plant genera, an average of $\pm 9\,\mu g\,cm^{-2}$ was obtained with version 4.

The models performed better with adult *E. globulus* leaves than those of juvenile morphology. Juvenile *E. globulus* leaves have a high content of epicuticular wax compared to adult leaves, which may explain the relatively high reflectance and low transmittance in the visible wavelengths. The younger age class of juvenile leaves has a particularly high-wax content, and associated studies have shown that epicuticular wax of similar leaves from this site was an average of $89.5\,\mu g\,cm^{-2}$ for the younger age class and $75.5\,\mu g\,cm^{-2}$ for the older age class (S. Ugalde, N. Davies, K. Barry, unpublished data, University of Tasmania, 2007).

The promising results gained here with radiative transfer based modelling of leaf reflectance and transmittance suggest that integration of PROSPECT with a reflectance model may provide a valuable method of deriving canopy *Cab* for either young or mature *E. globulus* from measurements of reflectance at the canopy scale. For *E. globulus* stands in the transition from juvenile to adult foliage (where both foliage types are present) a layered canopy model may be the most suitable, such as the multi-layer SAIL model (le Maire et al., 2008) as this would allow chlorophyll content of the leaves of different morphology to be modelled independently within the crown.

As well as integrating PROSPECT version 4 with a canopy reflectance model to estimate canopy *Cab*, future goals of our research include estimating carotenoid content with PROSPECT version 5 (Féret et al., 2008) for *E. globulus* foliage. Preliminary testing of PROSPECT version 5 with our data showed poor results (J.-B. Féret, S. Jacquemoud, unpublished), possibly due to the effect of epicuticular wax. This will be investigated further because estimation of carotenoids as well as chlorophyll provides a better indicator of photosynthetic efficiency.

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