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Crown-scale evaluation of spectral indices for defoliated and discoloured eucalypts

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Remote sensing for evaluation of canopy health in plantation eucalypts is a realistic option for forest managers in the near future if reliable and robust methods of spectral analysis can be developed. Pot-grown eucalypts of three species important to the Australian plantation industry were used for crown-scale spectral (400–1000 nm) evaluations of vegetation indices as indicators of common symptoms of stress. When defoliation treatments (in *E. globulus*) or exposure to cold and nutrient deprivation (in *E. pilularis*) resulted in large differences in leaf cover, the red edge position and slope indices, two normalized difference vegetation indices (NDVIs), modified chlorophyll absorption ratio index 2 (MCARI2) or modified triangular vegetation index 2 (MTVI2) were most strongly correlated to leaf cover. However the NDVIs were significantly affected by soil background in a study with *E. globulus*. The percentage of red leaves resulting from stress treatment was most strongly correlated with the anthocyanin reflectance index (ARI) and red-green index (RGI) in both *E. grandis* and *E. pilularis*, however the RGI was affected by background type in the *E. globulus* study while the ARI was not. Exposure to cold and nutrient deprivation led to marked changes in leaf cover for *E. pilularis* but not in *E. grandis* and a much more reduced level of chlorophyll in *E. pilularis* than is suspected in *E. grandis*. In *E. globulus*, defoliation from the upper crown was easier to detect with spectral data than from the lower crown. Results were generally comparable to studies of eucalypt crown condition from native forests.

1. Introduction

1.1 *Detection of stress in eucalypts*

The possibility of routine assessments of forest canopy health utilizing remote sensing technologies has increased in recent years through advances in our understanding of the spectral reflectance properties of vegetation, including eucalyptus-dominated forests (Coops *et al.* 1997, 2003b, 2004, Datt 1998, 1999b, Stone *et al.* 2001, 2005, Pietrzykowski *et al.* 2006). During the growth of forest trees, a multitude of stressors (biotic or abiotic) can result in the development of stress symptoms (or strains) (Lichtenthaler 1996). The strain may be expressed as a

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reduction in chlorophyll content, accumulation of secondary metabolites such as anthocyanins or loss of photosynthetic tissue (by defoliation or necrosis). All of these symptoms result in a consequential decrease in growth. Quantification of the location, extent and severity of these strains in plantation forests will enable this information to be integrated into forest management systems for timber growth and supply analysis.

Chlorophyll content is widely regarded as a generic indicator of plant stress (Carter 1993, Lichtenthaler 1996, Zarco-Tejada *et al.* 2002, Gitelson *et al.* 2003, Sampson *et al.* 2003) and methods to quantify chlorophyll content in eucalypts with remote sensing have been developed (Datt 1998, 1999b, Coops *et al.* 2003b). However, chlorophyll content can vary widely with leaf age in eucalypts (Choinski Jr *et al.* 2003, Close *et al.* 2004, 2005, Stone *et al.* 2005) and other genera (Mohammed *et al.* 2000, Sims and Gamon 2002). In eucalypts a common response to stress is production of anthocyanins, for example during photoinhibitory conditions (Close and Beadle 2003) or from biotic leaf damage (Stone *et al.* 2000, Smith *et al.* in press). Detection of anthocyanins is possible with remote sensing (Curran 1990, Gamon and Surfus 1999, Gitelson *et al.* 2001) and has been investigated in eucalypt forests to a limited extent (Coops *et al.* 2004, Stone *et al.* 2005).

In addition to alterations in leaf pigments, premature abscission of foliage, production of fewer and smaller leaves and contracted crowns can arise in eucalypts from abiotic (Pook 1985, Stone and Bacon 1994, Snowdon 2000, Thomson *et al.* 2001) or biotic damage (Shearer and Smith 2000, Stone *et al.* 2000, 2005, Stone and Coops 2004). These types of symptoms, which influence crown size and density, have a direct impact on biomass production of young eucalypt plantations (Jordan *et al.* 2002, Pinkard *et al.* 2006). Crown discolouration (chlorosis and/or redness) and defoliation (along with a reduction in leaf area index, LAI) are therefore two important indicators of stress in eucalypts. It is relevant to separate the effects of red discolouration from chlorosis and defoliation because seasonal and phenological changes in eucalypt leaves are often involved in a "red flush" of new growth which should not be considered part of response to poor health. Timely detection and spatial quantification of symptoms associated with stress will empower forest managers to make cost-effective decisions related to the management of underlying damaging processes.

1.2 *Vegetation indices for defoliation, leaf area index and red discolouration*

Total green biomass and related features of vegetation such as crown density, LAI and defoliation can be related to spectral information and most rely on the detection of chlorophyll content. While leaf pigments such as chlorophyll and anthocyanin absorb light in the visible wavelengths (Curran 1990, Curran *et al.* 1991, Gamon and Surfus 1999), the influences of leaf and crown structure are generally found in the near infrared (NIR) regions (Asner 1998). Vegetation indices (VIs) use information from both these wavelength regions to enable structurally normalized analysis of pigment content. We chose a number of published VIs to test relationships with symptoms exhibited by defoliated and stressed eucalypts (equations provided in table 1).

A recent test of 61 spectral indices for chlorophyll content against large databases of simulated and experimental spectra from various plant genera (le Maire *et al.* 2004) found that one of the best indices (DMI, table 1) was that developed with eucalypt leaves (Datt 1999b, Maccioni *et al.* 2001) and therefore this was included in

Table 1. Hyperspectral indices used in this study.

Index	Equation	Comments	Reference
Datt/Maccioni index (DMI)	$DMI=(R_{780}–R_{710})/(R_{780}–R_{680})$	Developed for chlorophyll content of eucalypt leaves	(Maccioni *et al.* 2001) after (Datt 1999b)
Far red to red index (FRRI)	$FFRI=R_{750}/R_{700}$	Chlorophyll content	(Gitelson *et al.* 1996)
Lower red edge slope (RE_{ls})[1]	$RE_{ls}=(R_{710}–R_{690})/(710–690)$	Chlorophyll content	(Curran 1990, Coops *et al.* 2004)
Total red edge slope (RE_T)[1]	$RE_T=(R_{740}–R_{690})/(740–690)$	Chlorophyll content	(Curran 1990, Coops *et al.* 2004)
Normalised Difference Vegetation Index (NDVI)	$NDVI=(R_{NIR}–R_{RED})/(R_{NIR}+R_{RED})$ $NDVI_{LANDSAT}$ R_{RED}=*630 to 690 nm,* R_{NIR}=*760 to 900 nm;* $NDVI_{narrow}$ R_{RED}=*645 to 655 nm,* R_{NIR}=*755 to 765 nm*	Vegetation index for chlorophyll and energy absorption	(Rouse 1974)
Red edge position (REP)	Determined using first and second derivatives with the Lagrangian interpolation method	Chlorophyll content	(Demetriades-Shah *et al.* 1990, Dawson and Curran 1998)
Soil Adjusted Vegetation Index (SAVI)	$SAVI=(1+L)(R_{NIR}–R_{RED})/(R_{NIR}+R_{RED}+L)$, *where L=0.5* for intermediate vegetation densities and the NIR and RED bands are used as for $NDVI_{LANDSAT}$	Improved NDVI for influence of soil.	(Huete 1988)
Optimized SAVI (OSAVI)	$OSAVI=(1+0.16)(R_{800}–R_{670})/(R_{800}+R_{670}+0.16)$	Improved SAVI, with single wavelengths	(Rondeaux *et al.* 1996)
Combined index of TCARI and OSAVI	$TCARI/OSAVI=\{3[(R700–R670)–0.2(R700–R550)(R700/R670)]\}/OSAVI$	Reduces the effects of non-photosynthetic materials and soil for chlorophyll estimation	(Haboudane *et al.* 2002)
Modified chlorophyll absorption ratio index 2 (MCARI2)	$MCARI2=\frac{1.5[2.5(R_{800}-R_{670})-1.3(R_{800}-R_{550})]}{\sqrt{\left[(2R_{800}+1)^2-\left(6R_{800}-5\sqrt{R_{670}}\right)-0.5\right]}}$	To calculate LAI with low sensitivity to chlorophyll content, atmospheric and soil effects	(Haboudane *et al.* 2004)
Modified triangular vegetation index 2 (MTVI2)	$MTVI2=\frac{1.5[1.2(R_{800}-R_{550})-2.5(R_{670}-R_{550})]}{\sqrt{\left[(2R_{800}+1)^2-\left(6R_{800}-5\sqrt{R_{670}}\right)-0.5\right]}}$	To calculate LAI with low sensitivity to chlorophyll content, atmospheric and soil effects	(Haboudane *et al.* 2004)
Red-green index (RGI)[1]	$RGI=(R_{600:700})/(R_{500:600})$.	Anthocyanin content	(Gamon and Surfus 1999)
Anthocyanin reflectance index (ARI)	$ARI=(R_{550}^{-1})–(R_{700}^{-1})$	Anthocyanin content	(Gitelson *et al.* 2001)
Carter stress index (CSI)[1]	$CSI=R_{695}/R_{760}$	Generic detection of stress	(Carter 1994)

[1]These four indices were tested in a eucalypt crown health study with CASI data (Coops *et al.* 2004).

our study. The spectral region known as the "red edge" (Curran 1990), which interfaces the visible and NIR, is particularly useful to characterize stressed vegetation and we used a number of indices with wavelengths from this region, including the slope and red edge position (REP, table 1) (Demetriades-Shah *et al.* 1990, Dawson and Curran 1998). Carter (1993) developed a "generic stress" index using wavelengths from the red edge which was consistently greater in plants stressed by a range of causes, compared to healthy plants. Assessments of remotely sensed indices for a mixed eucalypt species forest found that the total and lower slope of the red-edge were well correlated with crown density (Coops *et al.* 2004). These were used in our study (see table 1). Another simple algorithm using wavelengths from the red edge region was also tested (Gitelson *et al.* 1996).

The normalized difference vegetation index (NDVI), which was developed with the red and NIR wavebands available from Landsat data (Rouse 1974), can be correlated to green biomass and has been used to estimate LAI for a variety of vegetation types, including broadleaved forests (Fassnacht *et al.* 1997). A strong positive relationship between NDVI and LAI of a *Eucalyptus maculata* forest was shown (Coops *et al.* 1997) but effects of changing understorey were noted as a possible source of variation. As LAI in young eucalypt plantations is low, for example 2.3–3.8 in 2–3-year-old *E. globulus* (Tomé and Pereira 1991), the influence of changing soil or understorey composition may limit the use of NDVI. We tested NDVI with our data because it is a commonly used algorithm for many applications and suitable for broad wavelength band, multispectral instruments. We used wavelengths equivalent to Landsat bands as well as a narrow band alternative (table 1) similar to that used with the Digital Multi-Spectral Video (DMSV) in Australia, operated by Specterra Services Pty Ltd.

Several indices which adjust NDVI for atmospheric (Kaufman and Tanre 1992) and soil (Huete 1988) influences have been developed. These indices may be more reliable and less noisy than the NDVI (Rondeaux *et al.* 1996). Indices which have been designed to improve NDVI for background effects (SAVI, OSAVI, TCARI/OSAVI; table 1) were tested in our studies. Haboudane *et al.* (2004) developed vegetation indices to minimize the effect of leaf chlorophyll content on prediction of green LAI and the two best indices are further discussed here (MTVI2 and MCARI2, table 1). This may be particularly relevant for cases where trees are defoliated (e.g. by leaf chewing insects) but chlorophyll content within individual leaves does not change or increases in a compensatory manner (Pinkard *et al.* 2007) rather than decreasing which is more typical of most stress responses.

Green regions of leaf and canopy reflectance spectra are influenced by pigments such as anthocyanin and carotenoids in addition to chlorophyll content. A red-green index (RGI, table 1) has been well correlated with percentage leaf damage in eucalypts (Coops *et al.* 2004) and also to anthocyanin content (Gamon and Surfus 1999). The anthocyanin reflectance index (ARI, table 1) which was developed to detect anthocyanin content (Gitelson *et al.* 2001) uses similar wavelengths and both indices are tested here.

1.3 *A crown-scale approach*

The most common remote sensing approach for monitoring vegetation health has been to firstly develop robust spectral indices that are related to the symptom of interest and minimize extraneous influences. This approach is usually conducted

through leaf-scale studies, using leaves with varying symptom levels or with varying concentrations of pigments such as chlorophyll (Carter 1993, 1994, Datt 1999a, Gamon and Surfus 1999, Mohammed *et al.* 2000, Maccioni *et al.* 2001, Stone *et al.* 2001, 2005, Coops *et al.* 2003b, Coops and Stone 2005, Pontius *et al.* 2005, Pietrzykowski *et al.* 2006). While diagnostic features of spectra from individual leaves contribute to assessment of tree crown health, reflectance of canopies is also influenced by whole-tree features such as foliar density, leaf shape and leaf orientation (Asner 1998) as well as non-vegetation components and background reflectance (Rondeaux *et al.* 1996, Zarco-Tejada *et al.* 2005). For example, a simple ratio index using reflectance at 850 and 710 nm was developed to predict chlorophyll content from a range of *Eucalyptus* species (Datt 1999b) and while predictions of chlorophyll content developed from remotely sensed data were moderately correlated with laboratory analysis of foliar chlorophyll content, the index was strongly affected by soil and water in mixed pixels (Coops *et al.* 2003b). Therefore vegetation indices developed with leaf-level relationships must be "scaled-up" to test their utility at the crown and canopy level.

Vegetation indices can be incorporated into the "scaling-up" process for remote sensing by a number of methods (Zarco-Tejada *et al.* 2001). These include direct application of leaf-level relationships between optical indices and leaf properties to the canopy-measured reflectance, often with single-crown delineation (Coops *et al.* 2003a, 2004). Alternatively, canopy reflectance can be investigated using canopy radiative transfer models such as SAILH (Verhoef 1984) which are coupled to leaf radiative transfer models, such as PROSPECT (Jacquemoud and Baret 1990, Jacquemond *et al.* 1996). Robust vegetation indices can then be developed on the basis of this synthetic data. Validation of the modelling methods show that they function well and have the ability to assess the performance of vegetation indices against a number of vegetation, environmental and atmospheric variables (Haboudane *et al.* 2004, Zarco-Tejada *et al.* 2004, 2005). However leaf models such as PROSPECT may benefit from further improvement (le Maire *et al.* 2004) and have not been tested to date for eucalypts.

Single-crown studies as a basis for developing spectral indices are rarely used, but represent an intermediate between leaf-scale and canopy-scale reflectance studies (Leckie *et al.* 1988, Yoder and Pettigrew-Crosby 1995). Gaining reflectance spectra from plant material at this level provides a simple way to test the influence of non-vegetation features and/or whole-tree alterations such as defoliation. One study of defoliation has assessed individual balsam fir crowns, in which single tree crowns with defoliation ranging from nil to complete were used. Ground vegetation was removed to simplify the data and a boom-arm held a spectroradiometer probe above each crown (Leckie *et al.* 1988). In the absence of hyperspectral imagery of stressed eucalypt plantations, this approach allows an investigation of whole-plant factors on reflectance at high spectral resolution, with the benefit that subject material can be manipulated and its condition accurately quantified. Constraints on using hyperspectral imagery at a suitable resolution for research in eucalypt plantations are currently based on cost. If there was no cost constraint, an alternative or additional method of developing indices for canopy health would be with airborne hyperspectral imagery and delineating trees for which ground-based information (e.g. LAI, extent of discolouration or defoliation) was collected (Coops *et al.* 2004) or with the canopy and leaf radiative transfer models discussed previously.

1.4 *Objectives of this study*

In this study we investigated two important attributes presented as symptoms of crown damage; defoliation and discolouration. This was conducted with pot-grown *Eucalyptus* of three species important to the Australian plantation industry, using a spectroradiometer operating from 400 to 1000 nm. In addition, use of an artificial substrate allowed for the effect of background surface to be tested. Digital photographs captured "crown-scenes" with a matching field of view to the spectroradiometer probe which were assessed using image analysis software. This enabled a measure of the projected leaf "cover" to be made and also proportions of discoloured leaves to be calculated. *E. globulus*, *E. grandis* and *E. pilularis* with varying crown conditions were used to test the effectiveness of a selected number of spectral indices.

2. Methods

2.1 *Plant material*

Six *E. globulus* saplings in 20 cm diameter pots were used. Plants were raised in an outdoor growing area, applied with slow-release fertilizer, irrigated daily and were all healthy in appearance. The plants were ca. 12 months old when used in January 2005. Average tree height was 112 cm (range 90–125 cm) at this time. Plants were assessed with full foliage and were then subjected to a defoliation treatment. The top defoliation involved removing all leaves above a point on the stem at 50% of its length, while the bottom treatment removed leaves from below this point. As the lower stem (approx. 30 cm) had no branches or leaves, the bottom treatment usually involved removal of less leaves in total than the top treatment. Therefore the two treatments will be referred to as "top/severe" and "bottom/moderate" defoliation.

Ten *E. grandis* plants of one clone were produced from cuttings prepared by Forests New South Wales from mother plants of Orara East provenance. Ten *E. pilularis* of one clone were similarly produced from mother plants of Queens Lake provenance. For both species, at ca. nine months of age, plants were transferred into 8 cm diameter pots, given slow-release fertilizer and kept in a glasshouse between 15 and 25°C. Plants were later re-potted to 20 cm diameter pots. Half of the plants of each species were subjected to a "stress" treatment which involved placing them in an outdoor growing area for an eight-week period beginning on 12 April 2005 (mean minimum and maximum daily temperatures for June were 4.7 and 12.8°C respectively) and giving no additional fertilizer. As a consequence, the new leaves were red due to photoinhibitory conditions. Reddening of leaves due to anthocyanin production in eucalypts is a common response to low nutrition, low temperatures and high light (Close *et al.* 2001a, Close and Beadle 2003). In contrast, the "healthy" treatment consisted of keeping plants in a glasshouse at between 15 and 25°C and applying liquid fertilizer when needed. The new leaves of these plants were green and relatively soft.

Due to the different growing conditions of the treatments, height and form of the plants differed. The "stressed" *E. pilularis* were an average height of 85.6 cm, while the "healthy" were 141.4 cm and a number of leaves had a tendency for curling due to the humidity of the glasshouse. The "stressed" *E. grandis* had an average height of 115.0 cm, while the "healthy" plants averaged 111.3 cm in height. For all *E. grandis*, lower leaves exhibited a mild oedema condition and for the "healthy" plants the upper leaves also showed this symptom, possibly due to glasshouse

humidity. At the time the experiment was conducted (early June 2005), plants were ca. 14 months old, based on the time cuttings were first made (March 2004). The plants had undergone approximately eight weeks of stress treatment by this time.

2.2 *Background surfaces*

A black canvas was used for all experiments as a background surface. Two different soils were also used as backgrounds with the *E. globulus* plants. A yellow mudstone soil (''bright'') and a brown sandy soil (''dull'') were both collected from an *E. globulus* plantation in the Barnback region of southern Tasmania. Soils were dried and sieved through a 9 mm mesh before use.

2.3 *Spectral analysis*

Reflectance data were obtained with a dual-channel spectroradiometer (UniSpec-DC, PP Systems, Hammerhill, MA, USA) recording across the range of 300–1100 nm with a 3.1–3.4 nm sampling interval (dependent on wavelength), 3.7 nm resolution and 0.1 nm repeatability. The useful sampling range is between approx. 400 nm and 1100 nm due to the transmittance properties of the foreoptics. The dual channel system uses 2.1 mm diameter glass foreoptics; channel 1 (CH1) had a cosine receptor (UNI435) attached and channel 2 (CH2) had a 100 mm stainless steel ferrule covering a polished fibre tip (UNI684, 25° field of view). Integration time was set to 200 ms and 20 scans were averaged for each recorded spectrum. A Spectralon™ panel (PP Systems Hammerhill, MA, USA) was used as a white reference for spectrometer calibration. Data was collected on anintegral PC using UniWin-DC V1.5 software (PP Systems, Hammerhill, MA, USA).

To obtain reflectance measurements of individual crowns, trees were placed indoors in a small room sealed from external light and illuminated by four 150 W halogen globes which were equidistant from the crown centre. Halogen light sources provide a low and uniform irradiance over the spectral range of interest. A purpose-built aluminium boom was attached to the ceiling and this included a mount for a digital camera and a fitting to hold the spectroradiometer fibre optic probe directly vertical. The camera lens was placed a close as possible to the fibre optic tip; approximately 3 cm horizontally and 2 cm vertically offset. The tree base was surrounded by a piece of plywood to provide a false background platform and cover the black plastic pot. The plywood was then covered by black canvas, or the two different soil types described previously for *E. globulus*. Soil was spread out evenly across the plywood at a depth of between 5 and 10 mm. The probe was positioned 2.0 m above the background platform and therefore an area of the surface of radius 0.44 m was captured. A ring of black tubing was placed on the background which was equivalent to the field of view captured by the probe. This allowed the digital photograph image to be interpreted only for the approximate area sensed by the probe.

As the height of each plant varied, care was taken to ensure that approximately the same length of stem was exposed above the platform to provide uniformity. For the experiment with *E. grandis* and *E. pilularis*, the height of plants was altered so that a maximum of approx. 1 m of stem length was visible above the background platform. For example, the lip of the pots of ''red'' *E. pilularis* plants was positioned 5 cm below the platform, while the ''green' *E. pilularis* were positioned between 60 and 66 cm below in order to expose only 1 m of stem length. For these taller plants,

branches level with the platform were removed and those beneath it were not included in the experiment. As there was little difference between heights of the *E. globulus* plants, all plant pots were positioned at the same height, with the lip of the pot level to the background platform.

A number of indices were chosen for investigation with this data (table 1), as discussed in the introduction. Application of the MCARI2 and MTVI2 indices gave identical results, therefore they are presented together.

2.4 *Analysis of leaf material from digital photographic images*

Colour analysis was used to quantify different components (i.e. leaves as opposed to background) of the crown scene in the digital photographs, using WinFolia software (Regent, Canada). This allowed the area of different leaf colours (i.e. all shades of red, compared to all shades of green) to be separately quantified, or alternatively these could be added together to give total leaf cover visible in the crown scenes. This data relates to a two-dimensional plane of the field of view of the spectroradiometer, similar to measures such as the green vegetation fraction (horizontal density) used at a larger scale (Montandon and Small 2005). While leaf area index was not estimated for each plant, the data derived from the photographs ensures that only parts of the crown scanned by the spectroradiometer are included, because in some cases less than 100% of the crown width was enclosed by the field of view.

2.5 *Leaf pigments of* E. pilularis

Three weeks after the stress treatment of the healthy and stressed clonal *E. pilularis*, leaves were destructively sampled for pigment analysis. Five leaves from leaf pairs 2 or 3 were selected from each of five plants of the stressed and healthy treatments.

Leaf discs were taken at three positions on the leaf to determine specific leaf area. The remainder of the leaf was immediately frozen at -20°C until processing.

Leaves were allowed to thaw in the dark at 2°C for 30 minutes before processing. The leaf tissue was cut into thin slivers with scissors and a random subsample (150–200 mg) was used. For chlorophyll extraction, leaf tissue was homogenized for approximately 30 seconds with a Polytron PT2100 (Kinematica AG, Switzerland) in 10 ml 80% aqueous acetone (pH adjusted to approximately 7.8 with 1 N NH_4OH solution), then a 5 ml rinse of the homogenizer was added to the sample. Samples were stored in the dark at 2°C for two hours to extract, followed by centrifugation at 13 000 rpm for 10 minutes. Samples were diluted with acetone and absorbance was measured at 645 and 663 nm using a spectrophotometer (Cary 1E UV-Vis, Varian). Chlorophyll content was calculated using the following formulae (Arnon 1949): Chlorophyll a (mg/ml)$=(0.0127 \times A_{663})$–$(0.00269 \times A_{645})$; Chlorophyll b (mg/ml)$=(0.02269A_{645})$–$(0.00468 \times A_{663})$.

Anthocyanin extraction was conducted with the remaining sliced leaf material. The thawed material (100–150 mg) was homogenized as above but with 6 ml of acidified methanol (40 ml of concentrated H_2SO_4:1760 ml of methanol) and a 3 ml rinse was added to the sample. Samples were heated to the point of gentle boiling in a hot water bath for 1.5 minutes (Close *et al.* 2001b). Samples were then left at 2°C in the dark for approximately 10 hours to extract and then centrifuged for 10 minutes at 13 000 rpm. Any necessary dilutions were made and absorbance at wavelengths 530 and 657 nm was measured as above. Anthocyanin content was

calculated using the following formula (Mancinelli *et al.* 1975) and results were expressed as $\mu g\,cm^{-2}$. Total anthocyanin content $= A_{530} - (0.25 \times A_{657})$.

2.6 *Statistics*

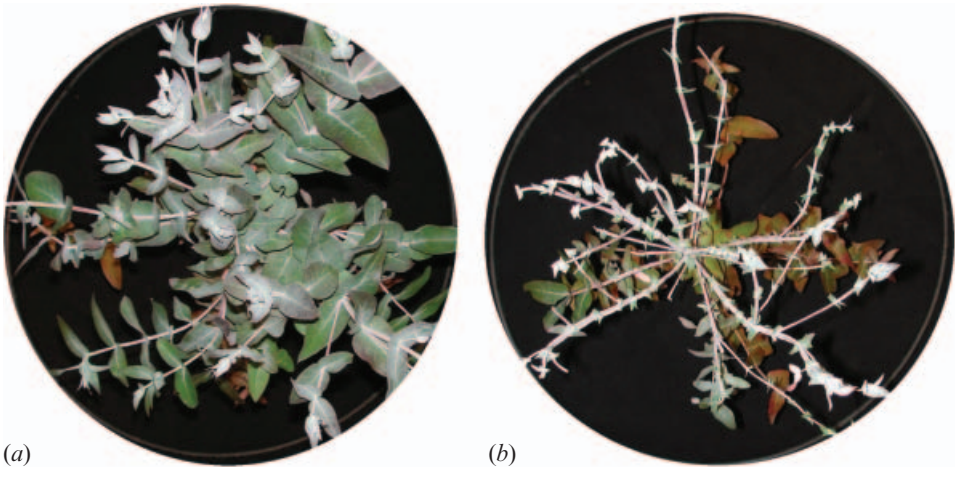
All statistics were completed with Genstat for Windows (Genstat 2003). One-way ANOVA were used to compare data from plants assessed with different background surfaces and for the *E. grandis* and *E. pilularis* stress experiments. Analysis of the defoliation study was complicated by the fact that the controls (before defoliation) were not independent from the defoliation treatment, so an ANOVA could not be performed. That is, spectra were first collected from the plants with full foliage, then they were defoliated and spectra were obtained again. For this reason the difference between results before and after defoliation was determined for each type of defoliation (top or bottom) then a *t*-test was conducted which compared these differences. For all datasets, linear regressions were performed to correlate spectral indices with leaf area data. Significant differences were assessed at the 5% level.

3. Results and discussion

3.1 *Effect of defoliation pattern for* E. globulus

While the same length of stem (i.e. 50%) was defoliated in the bottom and top defoliation treatments, the crown scene photographs revealed that the treatments had a very different effect on how much foliage was visible (horizontal density) from above the crown (figure 1). Leaf analysis from the photographs showed that there was little difference in visible leaf cover after defoliation when leaves were removed from the bottom of the plants, whilst a large difference when plants were defoliated from the top (*t*-test $P = 0.008$, table 2). The top defoliation treatment reduced the horizontal foliage density to less than two-thirds of that of a healthy plant (table 2).

Overall reflectance brightness was more substantially reduced by defoliating the tops of the crowns than for the removal of lower crown foliage (figure 2) and this was particularly pronounced in the NIR plateau. This is unlike balsalm fir in which



(*a*) (*b*)

Figure 1. Examples of crown scenes obtained from photographs taken 2 m above the substrate, related to the spectroradiometer field of view; (*a*) *E. globulus* with black canvas background. (*b*) The same *E. globulus* after 50% "top/severe" defoliation.

Table 2. Leaf cover percentage and averaged values (±SE) of spectral indices (n=3) for healthy *E. globulus* when subjected to two different defoliation treatments. OSAVI, SAVI and TCARI/OSAVI were not tested for this study. The *t*-test compared the differences between plants before and after defoliation for the top and bottom treatments.

	E. globulus (A) "bottom/moderate" defoliation			*E. globulus* (B) "top/severe" defoliation			Statistical analysis	
	Before defoliation	Defoliated	*Difference*	Before defoliation	Defoliated	*Difference*	*P* (*t*-test)	r^2 (% leaf cover)
Image analysis								
Leaf cover (%)[1]	61.8 (±2.11)	59.3 (±1.35)	*2.6*	62.1 (±5.07)	38.1 (±4.58)	*24.0*	**	–
Reflectance indices								
$NDVI_{LANDSAT}$	0.78 (±0.01)	0.77 (±0.01)	*0.01*	0.78 (±0.00)	0.68 (±0.00)	*0.10*	***	0.81***
$NDVI_{narrow}$	0.59 (±0.02)	0.57 (±0.02)	*0.02*	0.59 (±0.01)	0.44 (±0.01)	*0.15*	***	0.78***
MCARI2 or MTVI2	0.29 (±0.01)	0.28 (±0.01)	*0.01*	0.30 (±0.03)	0.15 (±0.02)	*0.15*	**	0.98***
DMI	0.47 (±0.03)	0.47 (±0.03)	*0.00*	0.50 (±0.02)	0.33 (±0.01)	*0.17*	*	0.68***
FRRI	2.13 (±0.11)	2.08 (±0.11)	*0.05*	2.19 (±0.06)	1.52 (±0.03)	*0.68*	*	0.75***
$RE_{ls} \times 100$	0.48 (±0.02)	0.45 (±0.02)	*0.03*	0.45 (±0.05)	0.28 (±0.03)	*0.17*	*	0.93***
$RE_T \times 100$	0.38 (±0.02)	0.36 (±0.02)	*0.02*	0.38 (±0.03)	0.18 (±0.02)	*0.19*	*	0.97***
RGI	0.81 (±0.01)	0.82 (±0.01)	*−0.01*	0.81 (±0.01)	0.88 (±0.01)	*−0.07*	**	0.77***
REP	703.89 (±0.86)	704.12 (±0.97)	*−0.22*	705.08 (±0.46)	697.07 (±1.09)	*8.01*	*	0.98***
ARI	1.73 (±0.21)	1.77 (±0.21)	*0.04*	1.69 (±0.22)	2.44 (±0.44)	*−0.75*	*NS*	0.55**
CSI	0.41(±0.02)	0.42 (±0.02)	*−0.01*	0.40 (±0.01)	0.61 (±0.01)	*−0.21*	*	0.82***

[1]Percentage of field of view.
***P<0.001. **P<0.01. *P<0.05. [NS]Not significant.

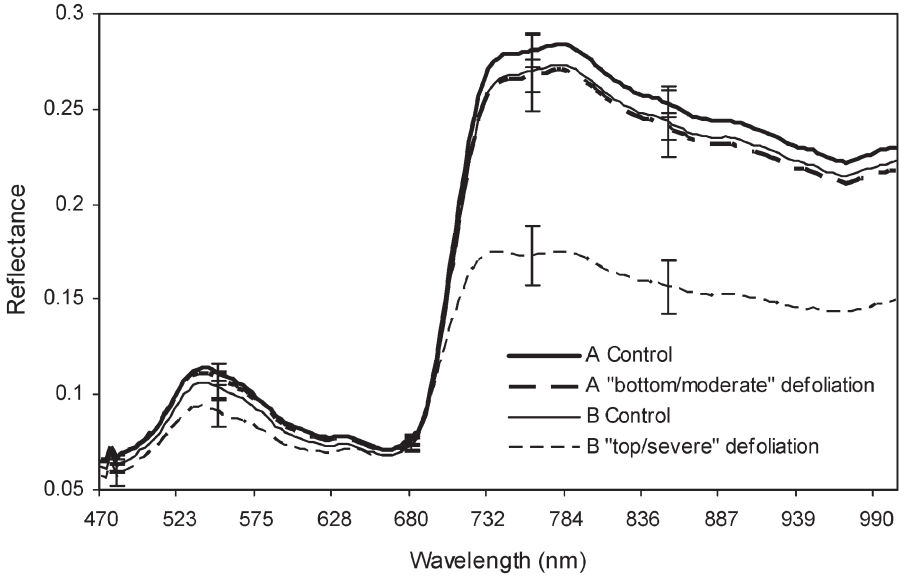


Figure 2. Averaged spectra (±SE) for two sets of *E. globulus* plants (*A, B*) which were defoliated with either of two different treatments.

reflectance was increased in the UV/VIS wavelengths but decreased in the NIR when defoliation became progressively more pronounced (Leckie *et al.* 1988), a trend which was also apparent for moderate to severely defoliated hemlock (Pontius *et al.* 2005). Decreased reflectance across the spectrum in our study may be due to the black background used and if a typical soil was used the lighter background would result in a different result. Decreased reflectance in the NIR region often indicates a change in the leaf or crown structure (Gitelson *et al.* 2002) which is commensurate with a loss of leaf area, however the visible wavelengths relate more specifically to pigment content. The reports of hemlock and balsam fir were naturally-induced declines involving foliage undergoing varying stages of chlorosis and defoliation and therefore spectral alterations in the UV/VIS region of the spectrum would be expected. As our study used artificial defoliation, there was no other stress effect on the remaining leaves and therefore less change in the UV/VIS regions of the spectra may be expected than if the remaining leaves were discoloured or damaged.

The *t*-test showed that for all indices the difference between the plants before and after defoliation with the top defoliation treatment was significantly greater than that for the bottom defoliation treatment (table 2). This would be expected as a result of both greater extent of leaf removal but also the pattern of removal. The broad and narrow band NDVI had the greatest statistical difference ($P<0.001$) while other indices were between $0.003<P<0.027$ or not significant in the case of the ARI. Correlations between leaf cover with each index showed that all were significantly correlated but the red edge slope and position indices and MCARI2 (or MTVI2) were the most strongly correlated. Using PROSPECT and SAILH models to simulate their data, Haboudane *et al.* (2004) also found that MCARI2 and MTVI2 generated similar but robust results in terms of estimating green LAI.

This study demonstrates that the VIS/NIR spectrum is sensitive to foliage loss from the top of the crown. Detection of loss from the upper crown is important for monitoring the effects of defoliation on growth, as studies of artificially defoliated young *E. globulus* in Tasmania showed that loss of all leaves in the upper 50% of the crown resulted in a dramatically greater effect on growth compared to loss of leaves in the lower 50% of the crown (Pinkard *et al.* 2006). Defoliation of tree crowns may occur in a number of patterns depending on the causal agent. For example in *E. globulus*, damage by the *Mycosphaerella* spp. fungi results primarily in loss of the older leaves in the lower crown (Carnegie and Ades 2000), while insect pests such as Crysomelids or *Gonipteris* spp. feed on new leaves, with loss most conspicuous in the upper crown (Loch and Floyd 2001). Further studies of this nature may be useful to discern to what extent damage to various parts of the crown can be detected using reflectance data.

Curran (1990) found no relationship between the red edge and chlorophyll content of whole canopies, due to the influence of understorey vegetation. However, Coops *et al.* (2004) found that the RE_T and RE_{ls} were well correlated with crown density when individual tree crowns were delineated in high resolution imagery and the reflectance data extracted for analysis. This agrees with results found here for *E. globulus* as the RE_{ls} and RE_T were very strongly correlated with leaf cover, as was the REP.

3.2 *Comparison of healthy and stressed (discoloured)* E. grandis *and* E. pilularis

Exposure to cold conditions and deprivation of nutrients (the ''stress'' treatment) resulted in red discolouration of the youngest leaves for both *E. grandis* and *E. pilularis*. Plants which remained indoors and were provided with nutrients (the ''healthy'' condition) did not develop any red discolouration. The stress treatment resulted in reduced growth of *E. pilularis*, such that the plants had significantly less (approximately two-thirds) of the leaf cover compared to the healthy plants (table 3). This is in contrast to *E. grandis* which maintained a similar (not significantly different) leaf cover to the healthy plants (table 4). Other studies of young *E. grandis* have shown that nutrient stress has little effect on growth (Rolando and Little 2003) which may explain the difference between the species here. These different responses to the stress treatment allow comparison of the effects of red discolouration (the percentage of which was essentially identical in both species, table 3) in reflectance spectra from single crowns with or without leaf cover reduction.

During the period of stress exposure, leaves of the *E. pilularis* plants were assessed for pigment content and specific leaf area. After three weeks of stress treatment the chlorophyll content of young leaves was approximately seven times less than corresponding leaves of the healthy plants, while anthocyanin content was over 3 times greater and specific leaf area was halved (table 5). While the crown spectra were acquired eight weeks after the stress treatment began, the pigments data from the three week analysis indicates that there were likely to be substantial differences in these factors also at eight weeks. This highlights that while leaf reddening was the ''visible symptom'' of the young leaves of stressed plants, anthocyanin accumulation is not the only change occurring which will affect the reflectance spectra (Karageorgou and Manetas 2006). Similar leaf assessments were not made for *E. grandis* therefore it is unknown if alterations in chlorophyll and anthocyanin content and specific leaf area were of a similar magnitude to *E. pilularis*. While

Table 3. *E. pilularis* leaf cover data and average values (±SE) for spectral indices (n=5) for healthy and stressed plants assessed eight weeks after the stress treatment with P values for one-way ANOVA and correlation coefficients (r^2) between indices and total leaf cover or the red leaf cover.

	Healthy	Stressed	P	r^2 (% leaf cover)	r^2 (% red)
Image analysis					
Total leaf cover[1]	39.8 (±1.6)	25.6 (±2.5)	**		
Green	39.8 (±1.6)	12.5 (±1.0)	***		
Red	0	13.2 (±1.6)	***		
Reflectance indices					
$NDVI_{LANDSAT}$	0.81 (±0.00)	0.73 (±0.01)	***	0.89^{NS}	0.72^{NS}
$NDVI_{narrow}$	0.57 (±0.03)	0.43 (±0.03)	**	0.94***	0.85^{NS}
MCARI2 or MTVI2	0.20 (±0.01)	0.14 (±0.01)	**	0.92***	0.40^{NS}
DMI	0.56 (±0.01)	0.29 (±0.01)	***	0.81***	0.59***
FRRI	2.43 (±0.06)	1.59 (±0.04)	***	0.86***	0.30**
$RE_{ls} \times 100$	0.25 (±0.01)	0.28 (±0.02)	NS	0.00^{NS}	0.53*
$RE_T \times 100$	0.24 (±0.01)	0.18 (±0.01)	**	0.93***	0.72^{NS}
RGI	1.29 (±0.01)	1.07 (±0.01)	***	0.70**	0.85***
REP	710.23 (±1.18)	694.98 (±1.16)	***	0.85***	0.66**
ARI	2.65 (±0.35)	6.17 (±0.49)	***	0.68**	0.72**
CSI	0.36 (±0.01)	0.56 (±0.02)	***	0.89***	0.65**

[1]Percentage of field of view.
***P<0.001. **P<0.01. *P<0.05. ^{NS}Not significant.

Table 4. *E. grandis* leaf cover data and average values (±SE) spectral indices (n=5) for healthy and stressed plants assessed eight weeks after the stress treatment with P values for one-way ANOVA and correlation coefficients (r^2) between indices and total leaf cover or the red leaf cover.

	Healthy[2]	Stressed	P (ANOVA)	r^2 (total leaf cover)	r^2 (% red)
Image analysis					
Total leaf cover[1]	23.6 (±2.1)	21.9 (±1.4)	NS		
Green	23.6 (±2.1)	8.9 (±1.2)	***		
Red	0	13.1 (±0.7)	***		
Reflectance indices					
$NDVI_{LANDSAT}$	0.74 (±0.01)	0.72 (±0.01)	*	0.64***	0.43^{NS}
$NDVI_{narrow}$	0.39 (±0.02)	0.34 (±0.01)	*	0.66**	0.46*
MCARI2 or MTVI2	0.14 (±0.01)	0.11 (±0.0)	**	0.50*	0.67**
DMI	0.38 (±0.02)	0.32 (±0.01)	*	0.13^{NS}	0.64*
FRRI	1.72 (±0.05)	1.57 (±0.02)	*	0.42^{NS}	0.52*
$RE_{ls} \times 100$	0.23 (±0.01)	0.20 (±0.01)	NS	0.85***	0.22^{NS}
$RE_T \times 100$	0.16 (±0.01)	0.13 (±0.01)	*	0.67**	0.43^{NS}
REP	699.07 (±1.37)	694.55 (±1.59)	NS	0.14^{NS}	0.32^{NS}
RGI	1.26 (±0.02)	1.06 (±0.01)	***	0.14^{NS}	0.95***
ARI	3.07 (±0.33)	7.56 (±0.16)	***	0.13^{NS}	0.97***
CSI	0.52 (±0.02)	0.58 (±0.01)	*	0.48*	0.52*

[1]Percentage of field of view.
[2]n=4.
***P<0.001. **P<0.01. *P<0.05. ^{NS}Not significant.

Table 5. Averaged values (±SE) for specific leaf area and pigments measured in *E. pilularis* plants three weeks after treatment began (n=25).

	Healthy	Stressed
Specific leaf area ($cm^2\,mg^{-1}$ DW)	0.26 (±0.01)	0.11 (±0.00)
Chlorophyll ($\mu g\,cm^{-2}$ FW)	39.2 (±1.6)	5.37 (±0.2)
Anthocyanin ($\mu g\,cm^{-2}$ FW)	13.8 (±0.8)	51.6 (±1.8)

responses to cold and nutrient deprivation stress are reasonably generic (Close *et al.* 2001a, 2001b) and both species favour sub-tropical to tropical climates, it is suspected that the chlorophyll content of *E. grandis* was not reduced as markedly by the stress treatment as *E. pilularis*.

The average reflectance spectra for each species and treatment shows differences throughout the visible and NIR range (Figure 3). The *E. pilularis* plants resulted in high reflectance across the NIR wavelengths for both the healthy and stressed plants compared to *E. grandis*. In both cases the healthy plants had higher reflectance in this spectral region than the plants subjected to the stress treatment which may be related to changes in specific leaf area (Stone *et al.* 2005) as well as whole-plant structural changes. The stressed *E. pilularis* plants had higher reflectance than the healthy plants in the red wavelengths (Figure 3), presumably due to reduced chlorophyll content, as detected in the leaf analysis earlier in the stress period (table 5). As reflectance in the stressed *E. grandis* spectra was not prominently increased in the chlorophyll well region compared to *E. pilularis*, this suggests that chlorophyll content was not as reduced by the stress treatment in *E. grandis* as in *E. pilularis*.

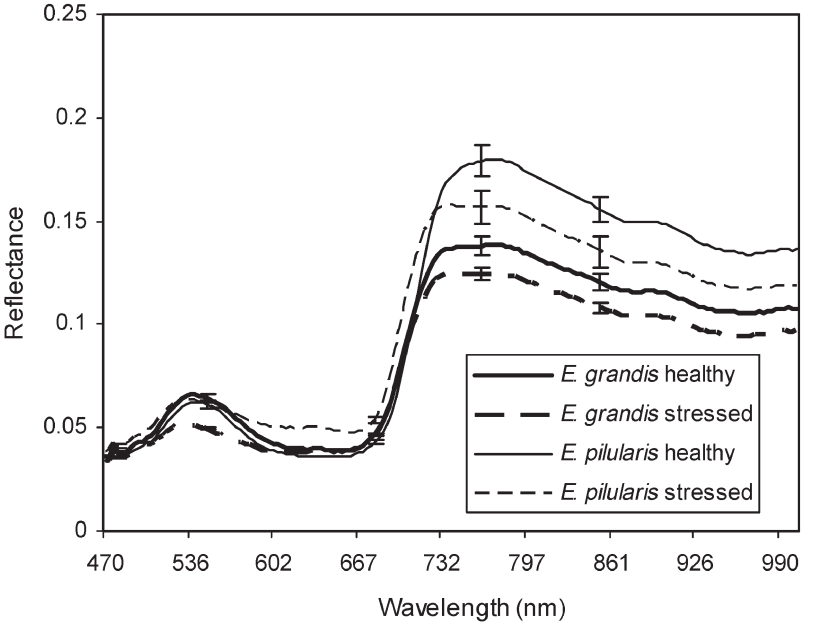


Figure 3. Averaged reflectance spectra (±SE) for healthy *E. grandis* and *E. pilularis* and "stressed" *E. grandis* (red discolouration) and *E. pilularis* (red discoluration and reduced leaf cover) plants.

Shifts of the red edge slope maxima to lower wavelengths have been well linked to decreased chlorophyll content (Curran 1990, Filella and Peñuelas 1994, Gitelson *et al.* 1996, Datt 2000). Comparison of red edge position shifts in this data showed that stressed *E. pilularis* plants had a REP shifted approximately 15 nm lower from the values of healthy *E. pilularis* (table 3) but this was only approximately 5 nm (table 4) for *E. grandis*. While the absolute difference in the shift was greater for *E. pilularis*, the position of the red edge maxima was essentially identical (695 nm) for the stressed plants of both species (tables 3 and 4).

Calculation of a range of spectral indices using the *E. pilularis* data revealed that all except the RE_{ls} could be used to detect significant differences above the 10% level between the crown scenes of the healthy and stressed plants, based on the one-way ANOVA (table 3). Regression between the results for each spectral index and the percentage total leaf cover showed that a number of indices had strong and highly significant correlations, with $NDVI_{narrow}$, RE_T and MCARI2 (or MTVI2) all explaining >90% of the variance in cover. These indices have been well correlated with leaf area index in other studies (Carlson and Ripley 1997, Coops *et al.* 2004, Haboudane *et al.* 2004). The REP, CSI, DMI and FFRI also had strong and significant relationship with leaf cover (table 3). As the latter two indices were designed specifically for chlorophyll content estimation, the data here supports their value in studies of vegetation stress. Regression of indices against the percentage of red leaves visible in the crown scene revealed that while most of those indices which performed best for estimation of leaf cover ($NDVI_{narrow}$, RE_T and MCARI2 or MTVI2) were not significantly correlated with percentage red leaves for *E. pilularis* (table 3) the REP, DMI and CSI were reasonably well correlated. The RGI had the strongest and most significant relationship with percentage of red leaves, followed by the ARI (table 3) which supports their intended purpose.

Analysis of the *E. grandis* spectral data revealed different results, in that only three indices detected significant differences above the 10% level between the healthy and stressed plants with the one-way ANOVA (table 4). Those indices which produced most significant results were the RGI and ARI, again, as expected. This highlights that the main difference between the healthy and stressed *E. grandis* plants was the proportion of red leaves, not a change in leaf cover (table 4) as opposed to *E. pilularis*. This is also highlighted by the fact that the ARI and RGI gave highly significant and very strong correlations with the percentage of red leaves for *E. grandis* (table 4).

One index that has provided contradictory results for both species is the RE_{ls}. This was not associated with a significant difference between the healthy and stress treatments for either species. It had a significant and strong relationship with percentage total leaf cover for *E. grandis* (table 4) but not for *E. pilularis* (table 3). Coops *et al.* (2004) found that the slope of the total red edge was more strongly correlated to crown density than the lower red edge slope for some native eucalypt species, to a degree which is similar to results found here with *E. grandis* for correlation with total leaf cover (table 4). In our study of *E. pilularis* the lower red edge slope was not significantly different for stressed and healthy plants nor was it correlated to the percentage leaf cover data (table 3). The presence of anthocyanins may weaken the relationship between the red edge indices and chlorophyll content (Curran *et al.* 1991) in the stressed plants and contribute to contradictory results for the RE_{ls}.

In general, this study highlights the way in which different eucalypt species respond to the same stress and how that influences spectral behaviour. That is, exposure to cold and nutrient deprivation in *E. pilularis* led to marked changes in leaf cover but not in *E. grandis*, and a much more reduced level of chlorophyll in *E. pilularis* than is suspected in *E. grandis*. Species differences were also examined in native eucalypt-dominated forests which were in poor or good health (Coops *et al.* 2004). While all four indices (RE_{ls}, RE_T, CSI and RGI) tested in that study produced strong and significant correlations with red leaves in *E. paniculata*, the correlations of all indices were poor for *E. saligna* and only the RGI was significant across all species tested. In addition to considerations of different responses to stress, phenological differences are also likely to contribute to variation within and between crowns (Stone *et al.* 2005).

3.3 *Use of different backgrounds for* E. globulus

Although the plants were moved slightly while the background surface was changed, analysis of the photographs showed there was no significant difference in the amount of leaf material in the spectroradiometer field of view between the treatments for this study (table 6). Averaged reflectance data reveal that across the whole spectrum, there were distinct and consistent differences between spectra of the same plants when background type changed (figure 4). The yellow mudstone soil (''bright'') had highest reflectance throughout the spectrum, followed by brown sandy soil (''dull'') and the black canvas. Comparison between the dull and bright soil in this study is similar in pattern to simulated data for canopy reflectance of grapevines with either bright or dark soil (Zarco-Tejada *et al.* 2005).

Calculation of the NDVI spectral indices and those designed to improve upon it (SAVI, OSAVI, TCARI/OSAVI, MCARI2 and MTVI2) showed that significant

Table 6. Leaf cover percentage and averaged values (±SE) of spectral indices ($n=6$) for healthy *E. globulus* with differing background surfaces (''bright''=yellow mudstone soil, ''dull''=brown sandy soil) with *P* values from a one-way ANOVA.

	Bright soil	Dull soil	Black canvas	*P* (ANOVA)
Image analysis				
Leaf cover[1]	60.9±2.7	61.8±3.3	62.0±2.5	NS
Reflectance indices				
$NDVI_{LANDSAT}$	0.68 (±0.01)	0.73 (±0.01)	0.78 (±0.01)	***
$NDVI_{narrow}$	0.43 (±0.01)	0.49 (±0.01)	0.59 (±0.01)	***
SAVI	0.99 (±0.01)	1.06 (±0.01)	1.13 (±0.01)	***
OSAVI	0.38 (±0.01)	0.42 (±0.01)	0.48 (±0.01)	***
TCARI/OSAVI	0.47 (±0.02)	0.39 (±0.01)	0.33 (±0.02)	***
MCARI2	0.29 (±0.02)	0.29 (±0.01)	0.30 (±0.01)	NS
MTVI2	0.29 (±0.02)	0.29 (±0.01)	0.30 (±0.01)	NS
DMI	0.48 (±0.02)	0.50 (±0.01)	0.48 (±0.02)	NS
FRRI	1.68 (±0.04)	1.84 (±0.04)	2.15 (±0.06)	*
$RE_{ls} \times 100$	0.53 (±0.02)	0.49 (±0.02)	0.47 (±0.02)	NS
$RE_T \times 100$	0.43 (±0.02)	0.40 (±0.02)	0.38 (±0.02)	NS
REP	704.84 (±0.27)	705.52 (±0.36)	704.48 (±0.21)	NS
RGI	1.00 (±0.01)	0.94 (±0.01)	0.81 (±0.01)	***
ARI	1.29 (±0.09)	1.53 (±0.10)	1.71 (±0.13)	NS
CSI	0.55 (±0.02)	0.49 (±0.01)	0.41 (±0.01)	***

[1]Percentage of field of view.

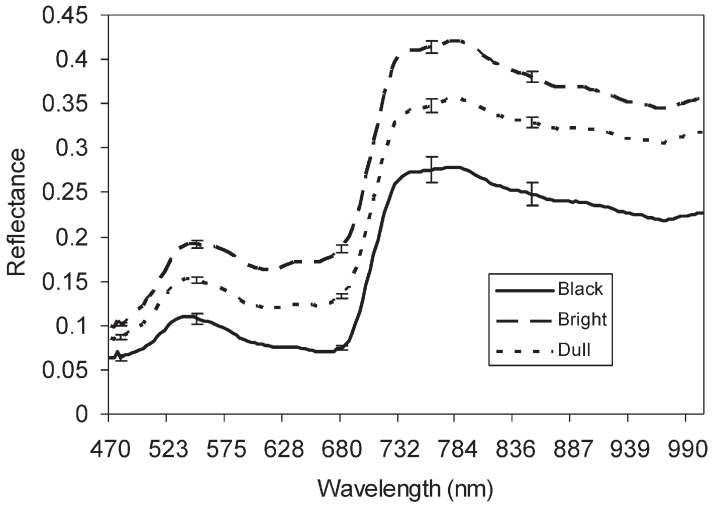


Figure 4. Averaged spectra (±SE) of healthy *E. globulus* (n=6) when different background surfaces were used (''black''=canvas, ''bright''=yellow mudstone soil, ''dull''=brown sandy soil).

differences between the crown scenes were detected for all but the latter two indices. Whilst SAVI (Huete 1988) and OSAVI (Rondeaux *et al.* 1996) were developed to minimize the influence of differences in soil background, they did not make any significant improvement from the broad or narrow band NDVI used in this study and nor did the combined index. However, the more recently developed combined indices of MCARI2 and MTVI2 (Haboudane *et al.* 2004) were not influenced significantly by the changing background surface.

The red edge slope and position indices, the DMI and ARI showed no significant effect of differing background between the crown scenes. In assessments of spectral indices suitable for grapevine condition, the red edge indices provided good correlations with chlorophyll whilst soil type changed, as opposed to NDVI and other traditional indices which were greatly influenced by soil type (Zarco-Tejada *et al.* 2005). Although absolute reflectance was altered with each background type, the slope of the red edge feature was not altered and therefore indices using the slope of the red edge may have the potential for remote sensing of plantations where soil types change substantially and there is little understorey. Curran (1990) used spectral mixture analysis to demonstrate that if background vegetation had a similar red edge to the canopy, then the overall reflectance would be an average of the two, but if the understorey vegetation was different then the canopy relationship with red edge would be discontinuous. Our vegetation simulation is simplistic, in that it only includes one stratum, the canopy. In a realistic field environment, however, understorey vegetation may impact on the apparent success of this index. The fact that the DMI was unaffected by background in our study but a similar index (i.e. Datt 1999a, 1999b) was poorly correlated with chlorophyll in a study of native eucalypt forest (Coops *et al.* 2003b) may be cause to question the general applicability of these results.

The CSI and RGI differed significantly between the background types and therefore while they have shown promise to detect leaf cover change and red discolouration respectively, the indices may not be robust for applications where

background changes in soil occur. The FRRI did not detect a difference between the two soils but the black canvas produced a significantly different result (table 6). While the RGI showed a significant difference between background types, the difference between averages values was actually smaller than for the ARI (the values for which had high standard error and therefore resulted in no significant difference being detected by the ANOVA). Therefore there is some uncertainty as to whether the ARI or RGI would perform better under realistic field conditions.

4. Conclusion

A number of conclusions can be drawn from these crown-level studies:

- When defoliation treatments (in *E. globulus*) or stress (in *E. pilularis*) resulted in large differences in leaf cover, the red edge indices (REP, RE_T and RE_{ls} for *E. globulus* but only REP and RE_T in the *E. pilularis* experiment), two NDVIs and MCARI2 (or MTVI2) were most strongly correlated to leaf cover. The red edge indices and MCARI2 or MTVI2 were unaffected by the background types used in this study while the NDVIs were affected.
- The percentage of red leaves was most strongly correlated with the ARI and RGI in both *E. grandis* and *E. pilularis*. Based on results from the *E. globulus* background study, the RGI may be more affected by background differences than the ARI, however the performance of these indices needs to be further compared in realistic field environments.
- Defoliation from the upper crown was much easier to detect with spectral data than defoliation from the lower crown, although more leaves were removed from the upper crown treatment.
- To develop robust indices it is important to understand how particular stresses affect plant condition, as it may vary with species. For example, exposure to cold and nutrient deprivation in *E. pilularis* lead to marked changes in leaf cover but not in *E. grandis* and a much more reduced level of chlorophyll in *E. pilularis* than is suspected in *E. grandis*.
- Performance of indices tested in native eucalypt forests with poor health (Coops *et al.* 2004) was in reasonable agreement with our results in terms of correlations with red edge indices being strong for leaf cover (cf. crown density) and the RGI being good for redness and damage. Our study suggests there are numerous other indices which may perform as well or better and warrant testing with remotely-sensed data of eucalypt plantations.
- No single VI was robust in all three trials and therefore detection of different symptoms of stress (i.e. defoliation, chlorosis or reddening) requires use of individual indices. These types of damage, as well as understorey, soil and atmospheric effects need to be characterized for specific damaging processes and plant structures in order to select the optimal VI (e.g. Broge and Leblanc 2001).

The value of the experimental system used in this study is that it is easy to manipulate and quantify plant and background factors and therefore assess their contribution to spectral data. However, it remains an artificial system and application of indices will differ to a field situation. The spectroradiometer is suitable for use in the field but would be limited to young plants due to technical difficulties in positioning the probe far enough above the crown. Further studies of

this nature may be useful as an intermediate between leaf- and canopy-scale research to improve the capability of remote sensing to assess forest health.

Studies such as this will assist in developing the best vegetation indices for detection and/or quantification of a variety of eucalypt plantation crown conditions, at a suitable resolution with sensors currently available in Australia. Some of the most promising indices in this study use narrow wavebands (i.e. RE_T, MCARI2 or MTVI2 and the ARI) and will require use of hyperspectral instruments or preselection of narrow filtered wavebands for multispectral instruments such as DMSV (Stone *et al.* 2004). However, the RGI can be used with broad-band multispectral data such as that from QUICKBIRD and IKONOS (Wulder *et al.* 2006) and may be valuable in some situations.

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