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Spectral reflectance characteristics of eucalypt foliage damaged by insects

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Abstract. Variables related to foliar damage, leaf morphology, spectral reflectance, chlorophyll fluorescence and chlorophyll content were measured from leaves sampled from mature eucalypts exhibiting symptoms of crown dieback associated with bell miner colonisation located in Olney State Forest, near Wyong, New South Wales. Insect-damaged mature leaves and healthy young expanding leaves of some species exhibited a conspicuous red coloration caused by the presence of anthocyanin pigmentation. For the mature leaves, the level of red coloration was significantly correlated with insect herbivory and leaf necrosis. Significant correlations were also found between the level of red pigmentation and the following four spectral features: maximum reflectance at the green peak (550 nm); the wavelength position and maximum slope of the red edge (690–740 nm) and the maximum reflectance at 750 nm in the near-infrared portion of the electromagnetic spectrum. While it has been shown that anthocyanin pigments are synthesised in some eucalypt species in response to certain abiotic stresses causing photoinhibition and activation of photoprotective mechanisms, this work proposes that biotic agents such as leaf damaging insects and fungal pathogens may induce a similar response in eucalypt foliage resulting in increased levels of anthocyanins. The potential of anthocyanin levels to be related to leaf ontogeny for some eucalypt species was also illustrated in the reflectance spectra. Thus, it is essential that leaf age be considered. This work demonstrates that the identification of a number of key features of leaf spectra can provide a basis for the development of a robust forest health indicator that may be obtained from airborne or spaceborne hyperspectral sensors.

Introduction

Leaf reflectance properties and resultant spectral curves are the product of the integrative effects of genetic, developmental and environmental factors through their influence on physical and biochemical properties of leaves (Mohammed *et al.* 2000). Previous studies, based principally on Northern Hemisphere tree species, have identified diagnostic features from the spectral responses in the visible and near-infrared regions of the electromagnetic spectrum of leaves exhibiting symptoms of injury from a range of stress-inducing agents including insect damage (e.g. Rock *et al.* 1988; Leckie *et al.* 1989; Hoque *et al.* 1992; Vogelmann *et al.* 1993; Carter 1994). While several studies have examined the spectral features of eucalypt leaves (Thomas and Barber 1974; O'Neill *et al.* 1990; Greaves and Spencer 1993; Datt 1998, 1999*a*, 1999*b*) they used relatively healthy leaves.

The success of scaling up and applying spectral features identified at the leaf scale to tree canopies has been mixed (Leckie *et al.* 1989; Greaves and Spencer 1993; Yoder and Pettigrew-Crosby 1995; Curran *et al.* 1997; Sampson *et al.* 2000) largely due to significant variability in canopy reflectance compared with leaf samples. The reflectance of canopies is not only influenced by leaf properties but also tree-crown density, leaf shape and orientation, background canopy reflectance and measurement geometry (Asner 1998). Nevertheless, diagnostic features identified in the leaf spectra have the potential to form the basis of future spectral indices or algorithms of canopy health (Mohammed *et al.* 2000; Sampson *et al.* 2000; Zarco-Tejada *et al.* 2000).

Leaf optical properties are governed by surface and internal structure properties as well as by concentration and distribution of biochemical components. Thus, remote analysis of reflected light can be used to assess both the

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structure and the physiological status of a plant (e.g. Peñuelas and Filella 1998; Zarco-Tejada *et al.* 2000; Carter and Knapp 2001). Leaves are subjected to a large array of stressful abiotic and biotic agents including insect herbivores and fungal pathogens. In Australia, outbreak populations of herbivorous insects are a common cause of unhealthy eucalypt canopies. Types of insect damage to eucalypt leaves include: damage or removal of the waxy cuticle; destruction of cell walls and intercellular integrity; reduction in moisture and chlorophyll content and an increase in non-green pigments. The extent and severity of damage can vary from localised small spots to large proportions of leaf tissue. Therefore, it would be expected that herbivorous insects could significantly influence leaf reflectance depending on the species composition and density of insects present.

Anthocyanins and photoinhibition

Leaf colour is a function of the composition and concentrations of the leaf pigments including chlorophylls, carotenoids and anthocyanins. Anthocyanins are responsible for the red to purple coloration of leaves (Sharma and Crowden 1974; Chalker-Scott 1999; Gould *et al.* 2000). Changes in leaf pigmentation are a common trait of 'evergreen' eucalypts (Brooker and Kleinig 1999; Datt 2000). As with the other leaf pigments, anthocyanin content changes with ontogeny and senescence (Datt 2000; Hoch *et al.* 2001). While there is no general consensus for the presence of anthocyanins (Gould *et al.* 2000), several authors have presented evidence of their possible photoprotective function (Gould *et al.*1995; Chalker-Scott 1999; Gamon and Surfus 1999; Neill and Gould 1999; Close *et al.* 2000; Hoch *et al.* 2001). If the absorption of light energy exceeds the photosynthetic capacity of the photosynthetic apparatus then plants are able to induce photoprotective mechanisms in order to minimise permanent damage from excess energy. This photoinhibition of photosynthesis results in a decline in quantum yield and hence in photosynthetic efficiency (Björkman and Demmig-Adams 1994). Mechanisms associated with photoinhibition include increased emission of energy either as heat or red and far-red chlorophyll fluorescence (Lichtenthaler and Miehé 1997), increase of xanthophyll-cycle pigment activity (e.g. Demmig-Adams and Adams 1996) or possibly the production of anthocyanins. Many stress-inducing environmental factors can affect the light intensity required for photoinhibition by altering either the rate of damage or the rate of repair of the photosynthetic apparatus (Critchley 1988). Generally, any stress that reduces the capacity of photosynthetic carbon metabolism, e.g. low temperatures, drought or nutrient deficiencies increases the probability of photoinhibition (Hoch *et al.* 2001). Because of the relationship between the increased emission of chlorophyll fluorescence and photochemical efficiency, the chlorophyll fluorescence parameter, F_v/F_m (the ratio of variable to maximal chlorophyll fluorescence) is often used as a measure of stress-induced photoinhibition (e.g. Ögren and Evans 1992; Holly *et al.* 1994; Close *et al.* 2000).

This study forms part of a larger project initiated to develop a reliable, robust indicator of health for Australian eucalypt forests derived from digital imagery to be obtained from airborne or spaceborne hyper-spectral sensors (Stone *et al.* 2000). The objective of this preliminary paper was to relate distinguishing features obtained from reflectance spectra to a range of morphological and physiological leaf parametres obtained from eucalypt leaves damaged by insects. An additional aim was to examine the correlation between insect damage, level of red discoloration (anthocyanins) and chlorophyll fluorescence (F_v/F_m) of three species of eucalypts because of the significant influence non-green pigments have on leaf reflectance spectra (Curran *et al.*1991; Gamon and Surfus 1999; Neil and Gould 1999). We also visually compared the reflectance spectra of leaves differing in leaf ontogeny and insect damage.

Materials and methods

Study site, tree species and sample collection

Trees sampled were from two plots separated by 1.2 km and located in a mixed-aged moist regrowth eucalypt forest within Olney State Forest (33°9′00″S, 151°20′30″E), approximately 12 km east of Morisset, New South Wales. Both plots had similar floral structural and species compositions. The overstorey is dominated by *Eucalyptus pilularis* Smith, *E. saligna* Smith, *E. paniculata* Smith, *Angophora floribunda* (Smith) Sweet and several rainforest species including *Acmena smithii* (Poiret) Merr. & Perry, *Guioa semiglauca* (F.Muell.) Radlk. and *Glochidion ferdinandii* (Muell. Arg.) Bailey. At one site, most trees were healthy with crowns in good condition. At the other site, tree crowns exhibited a range of decline symptoms associated with colonisation by bell miners (*Manorina melanophrys*) (Stone 1996, 1999). Bell miners are insectivorous social birds that aggressively defend their territories against other insectivorous bird species and predators. Eucalypt trees with this form of crown dieback tended to have high levels of foliar damage caused by a range of different herbivorous insects. Presence of the bell miners appears to interfere with the effectiveness of both vertebrate and invertebrate natural enemies of some herbivorous insects (e.g. other insectivorous birds, insect parasitoids and spiders). This results in elevated populations of a diverse range of herbivorous insects on the foliage of susceptible eucalypts, including sap-sucking insects (in particular psyllids), leaf chewing beetles and leaf-mining moth larvae (Stone 1996, 1999).

Over their life span, eucalypt leaves are exposed to a multitude of biotic and abiotic stress-inducing agents and their cumulative effects become confounded with the natural maturation and aging processes. Most leaves sampled in this study were approximately 1 year old and were considered fully mature but not phenologically old. The reflectance spectra of younger expanding foliage were also obtained from selected trees in order to identify discriminating features between young foliage with a red coloration, typical of many healthy eucalypt species (Brooker and Kleinig 1990) and mature foliage with a reddish coloration, often associated with stressed or damaged eucalypt foliage (e.g. Moore 1961; Ladiges and Kelso 1977; Wall and Keane 1984; Close *et al.* 2000; C. Stone, pers. obs.).

Leaves were obtained by shooting down small branches with a .22-calibre rifle. Two sampling schemes were carried out, the first during October 1998 to examine sources of variability and the second during September 1999 to examine the relationship between leaf damage and leaf reflectance spectra. Sampling was done in spring as the mature foliage would have approximately 1 year's cumulative damage. In the first sampling scheme four mature trees of both *E. saligna* and *E. paniculata* were selected: two healthy trees of each species and two visually assessed as having unhealthy crowns. Crown condition was visually assessed by the method used in Stone (1996). For each tree, two small branches were sampled from each of four crown aspects (north, south, east and west), providing eight small branches per crown. For the second sampling, twenty mature trees were selected from across both sites so as to represent a range of crown decline symptoms from very healthy vigorous canopies to crowns exhibiting severe canopy dieback. From these trees a single small branch located approximately in the upper mid-crown was shot down. For both samplings, the branches were immediately transported to a nearby State Forest New South Wales field-workers depot for assessment.

Leaf assessments

Table 1 lists the array of damage and morphological leaf traits assessed for both sets of foliage. Approximately 20 mature leaves were used per sample. The three variables, percentage of leaf area missing, actual leaf area and potential leaf area were measured by the methodologies described in Stone and Bacon (1995). Areas of necrotic tissue and reddish or purple discoloration per leaf were visually estimated. The ratio of maximum potential length along the midrib to maximum potential breadth was also obtained and used as a surrogate for leaf shape and juvenility (Brooker and Kleinig 1990). Measurements of F_v/F_m, the ratio of variable to maximum chlorophyll fluorescence (a fluorescence-based index of photosystem II photochemical efficiency) were made with a portable chlorophyll fluorometer (Fluorescence Induction Monitor 1500: ADC BioScientific Ltd, UK). A single leaf clip was placed on 1 of 20 mature leaves per sample for 15-min dark-adaptation before recording the F_v/F_m values.

A SPAD chlorophyll meter from Minolta Co., Ltd (model 502, Japan) was used to obtain a relative measure of chlorophyll concentration from the same leaves used for the F_v/F_m measurements (Monje and Bugbee 1992). The SPAD instrument utilises 650 and 940 nm wavelengths to estimate relative chlorophyll levels. The determination of actual chlorophyll content requires calibration with acetone-extractable leaf chlorophyll (chlorophyll $a + b$). Highly significant linear relationships ($r^2 > 0.95$) have been found between acetone-extractable leaf chlorophyll (chlorophyll $a + b$) and values obtained from the SPAD meter for several species of *Eucalyptus* including *E. occidentalis*, *E. camaldulensis* and *E. grandis* (K. Montagu, SFNSW, pers. comm.). These relationships appear to hold true even for leaves with low levels of chlorophyll (i.e. <0.3 mmol m^{-2}) but which were noticeably red in coloration. We did not determine whether the anthocyanins in our study might have interfered with the SPAD absorptance values at 650 nm (e.g. Curran *et al.* 1991). However, it is noted that Smillie and Hetherington (1999) determined that *in vivo* anthocyanin absorption is primary below 600 nm. In addition, Neill and Gould (1999) reported that higher concentrations of anthocyanins were associated with enhanced absorptance and reduced reflectance of the green–yellow wavelengths while red reflectance (between 600–700 nm) was better correlated to declining chlorophyll concentrations than to anthocyanin levels.

Table 1. Damage, morphological and physiological leaf traits assessed on sampled leaves

Damage variable
  Percentage leaf area missing due to insect herbivory
  Estimation of percentage necrotic tissue on each leaf
  Estimation of percentage area of reddish or purple discoloration on each leaf
Morphological variables
  Estimated potential leaf area (cm^2)
  Actual leaf area (cm^2)
  Ratio of maximum potential leaf length to maximum leaf breadth
  Leaf thickness (mm)
  Specific leaf mass (mg cm^{-2})
Physiological variables
  Ratio of variable to maximum chlorophyll fluorescence (F_v/F_m)
  Relative measure of chlorophyll content (SPAD values)

Leaf-reflectance measurements

The leaf-reflectance measurements were undertaken on leaves sampled from the same small branches and at the same time as the other leaf parameters were being assessed. A Personal Spectrometer II (Analytical Spectral Devices Inc., Boulder, Colorado, USA), which measures radiation in the visible and near-infrared (NIR) wavelengths from 400 to 1000 at a spectral bandwidth of approximately 3 nm, was used to obtain the leaf-reflectance spectra. A 150-W halogen bulb through a fibre optic annual ring was used as the light source to illuminate the leaves. Leaves were arranged into a 10 × 10-cm stack, six layers thick. Leaf stacks rather than single leaf layers were used so as to simulate the reflectance from leaves in a canopy; for this study, six leaf layers gave the maximum NIR reflectance. For each sample, five reflectance measurements were taken and later averaged to obtain a mean reflectance spectrum.

Data analysis

On visually examining the reflectance spectra of each sample of leaves, four spectral features were chosen for inclusion in the correlation analysis with the other leaf traits. The four spectral features were: the maximum reflectance at the green peak and having a wavelength at approximately 550 nm (GPmax); the maximum slope of the electromagnetic spectrum in the region between 690 and 740 nm, known as the chlorophyll red edge and calculated from the first derivative maxima at the red edge (REdr); the wavelength position of the red-edge maximum slope (REP); and the reflectance at 750 nm which occurs on the upper shoulder region of the NIR reflectance plateau (Ref750).

Reflectance spectra were taken of small clusters of leaves from individual shoots requiring the other leaf traits to be converted and used on a per shoot basis. Thus, the sampling unit was a small terminal shoot from the upper central crown of each tree as opposed to individual leaves. This approach is also a more valid measure of insect herbivory (Landsberg 1989). Analysis of the data was done by procedures in either the SAS System for Windows v6.12 or S-Plus v4.5 PC statistical software packages. The distribution of all the parameters was examined by normal probability plots and with very few exceptions, distributions proved unimodal, symmetric and coherent. Correlations between the leaf traits and the four chosen features of the reflectance spectra were examined by two methods, through the production of a reduced-rank regression biplot (ter Braack 1994) and also a Pearson's correlation matrix. Relations between the leaf traits and the reflectance variables on the biplot are disclosed by projecting the leaf trait points orthogonally onto the arrows representing the reflectance variables. The relative position of each arrow enables the leaf traits to be ranked in order of association with each reflectance variable (ter Braak 1994).

Results

Sources of variation

Sources of variation for the three leaf-damage parameters (percentage leaf area missing, estimated percentage necrotic

Table 2. Variance component estimates for damage and shape parameters of leaves sampled from mature *Eucalyptus saligna* and *E. paniculata* in a forest stand affected by symptoms of bell miner (*Manorina melanophrys*) canopy decline

GI = grey ironbark (*E. paniculata*); SbG = Sydney blue gum (*E. saligna*)

Tree component (effects)	Leaf area missing (%)	Estimated necrotic area (%)	Estimated reddish discoloration (%)	Estimated potential leaf area (cm^2)	Max. leaf length : max. leaf breadth	Leaf thickness (mm)
Leaf	103.6	1.5	0.00039	36.0	3.8	25.2
Shoot (10 leaves)	160.9	2.8	0.00049	86.8	4.6	46.7
Aspect (2 shoots)	253.1	4.5	0.00094	176.5	9.3	183.1
Tree (4 aspects)	1040.0	38.4	0.00567	318.6	56.6	977.0
Crown condition (healthy *v.* unhealthy)	9279.2	575.8	0.05368	2281.2	546.5	5444.2
Species (GI & SbG)	29.0	34.6	0.00519	16353.2	61.9	5536.5

area and estimated percentage reddish discoloration), as well as the three leaf-shape parameters (total potential leaf area, maximum leaf length to breadth ratio and leaf thickness) were examined initially with respect to sampling scale (Table 2). Not unexpectedly, the magnitudes of the variance components for all the dependent leaf variables increased as the sampling unit increased (leaf, shoot, aspect, tree). All three leaf-damage parameters have a large variance component due to tree-crown condition (Table 2). Tree-crown condition was also the most important effect on leaf length to breadth ratio and to a much lesser extent, leaf thickness. However, leaf size was more dependent on tree species than crown condition (Table 2).

A similar pattern is reflected in the results presented in Table 3. There were significant differences ($P < 0.05$) for all three measures of leaf damage and for leaf shape (length : breadth ratio) between trees with canopies visually categorised as healthy and trees with unhealthy crowns. However, this distinction was less apparent for leaf size, thickness and specific mass. The chlorophyll fluorescence parameter F_v/F_m was significantly lower for leaves from the unhealthy crowns. However, the measure of chlorophyll content, the SPAD values, were not significantly different. This could be due, in part, to the fact that both the chlorophyll fluorometer and the SPAD chlorophyll-meter measurements were obtained from small point readings, randomly selected on leaves, excluding any necrotic patches. Obviously, there is no functioning chlorophyll in the necrotic patches. One consistent trend was the greater variability of the damaged foliage for all leaf traits measured compared with foliage from healthy crowns.

Leaf reflectance spectra

There is greater variation within each mean spectral response of insect-damaged leaves compared with healthy leaves (Fig. 1). Correlations amongst the leaf traits (Table 4) reveal that estimated percentage reddish discoloration of the mature leaves is significantly correlated with both percentage leaf area missing and estimated percentage necrotic area. While leaf size, thickness and specific mass were poorly correlated with the leaf damage traits, leaf shape (length : breadth ratio) was well correlated to leaf damage as well as leaf thickness. A salient relationship appears to be the high correlation between the level of reddish discoloration and chlorophyll fluorescence (F_v/F_m) (Figs 2 and 3*a*). The SPAD values (relative chlorophyll content) were also significantly correlated to reddish discoloration but not to the same degree as the F_v/F_m parameter (Fig. 2 and Table 4). The correlation biplot (Fig. 2) visually summarises the relationships between the leaf traits (points) and the spectral reflectance parameters (arrows). There appears to be two groupings amongst the leaf traits, those occurring above the arrows (e.g. SPAD, F_v/F_m and RED) and a second group (e.g. leaf ratio, percentage area missing, thickness, total area and actual area) occurring below the arrows. The relationship of GPmax and REP with the first group is stronger than with the second group while for the other two reflectance parameters, REdr and Ref750, the situation is reversed.

Both approaches (Fig. 2 and Table 4) demonstrate that the slope of the red edge (REdr) is highly correlated to the other three spectral features but most-strongly associated with the reflectance at 750 nm (Ref750). The magnitude of the green peak is more highly correlated with the red edge features than reflectance of the NIR plateau (Ref750). All four spectral features are significantly correlated to the reddish discoloration and, to a lesser degree, leaf area missing but not well correlated to the amount of necrotic area (Table 4). The slope of the red edge (REdr) appears to be a better predictor of leaf area missing (percentage herbivory) than the red-edge position (REP) (Fig. 2).

The significant positive correlation between the SPAD values and red-edge position (REP) suggests that with decreasing chlorophyll content the red edge moves to shorter wavelengths. This shift of the red edge to shorter wavelengths during senescence or stress-induced chlorosis has been demonstrated by many others (e.g. Rock *et al.* 1988; Datt 2000; Carter and Knapp 2001) and is due to a reduction in the depth and breadth of the chlorophyll absorption feature. While relative chlorophyll content (SPAD values) is significantly correlated with all four spectral features, especially the red-edge features, the relationship with the reddish discoloration of leaves is

Table 3. Comparison of mean leaf traits per shoot between eucalypt crowns visually assessed as unhealthy or healthy

Means in each column (mean ± standard deviation) followed by different letters are significantly different ($P < 0.05$; Student's *t*-test)

Tree species	Mean leaf traits per shoot									
	Leaf area missing (%)	Estimated necrotic area (%)	Estimated reddish discoloration (%)	Estimated potential leaf area (cm^2)	Actual leaf area (cm^2)	Max. leaf length : max. leaf breadth ratio	Leaf thickness (mm)	Specific leaf mass (mg cm^{-2})	Chlorophyll fluorescence (mean F_v/F_m)	SPAD value (chlorophyll content)
	Tree crowns visually assessed as unhealthy									
E. paniculata (*n* = 6)	13.6a ± 5.2	7.6a ± 2.8	41.3a ± 18.3	12.3a ± 3.2	10.6a ± 2.6	4.45a ± 0.79	0.39a ± 0.04	20.0ab ± 1.7	0.77a ± 0.03	39.6a ± 2.1
E. saligna (*n* = 7)	16.1a ± 4.7	19.6a ± 12.9	27.6a ± 20.3	23.2b ± 4.6	19.5b ± 4.3	3.91a ± 0.42	0.38a ± 0.03	20.1ab ± 1.7	0.78a ± 0.02	46.0bc ± 1.3
	Tree crowns visually assessed as healthy									
E. paniculata (*n* = 2)	4.2b ± 0.53	0.25b ± 0.35	0.60b ± 0.21	20.7b ± 2.9	19.8b ± 4.3	6.33b ± 0.36	0.42a ± 0.06	19.2b ± 0.5	0.82b ± 0.003	43.1b ± 1.3
E. saligna (*n* = 2)	4.6b ± 1.62	1.9b ± 0.6	2.77b ± 0.95	18.6ab ± 4.8	17.7b ± 4.3	6.68b ± 1.04	0.40a ± 0.02	18.6b ± 0.8	0.82b ± 0.002	47.5cd ± 0.5
E. pilularis (*n* = 3)	4.7b ± 2.5	3.5ab ± 1.8	0.9b ± 0.6	20.7b ± 6.6	19.6b ± 5.8	6.33b ± 1.03	0.59a ± 0.24	22.5a ± 1.9	0.82b ± 0.006	50.4d ± 2.1

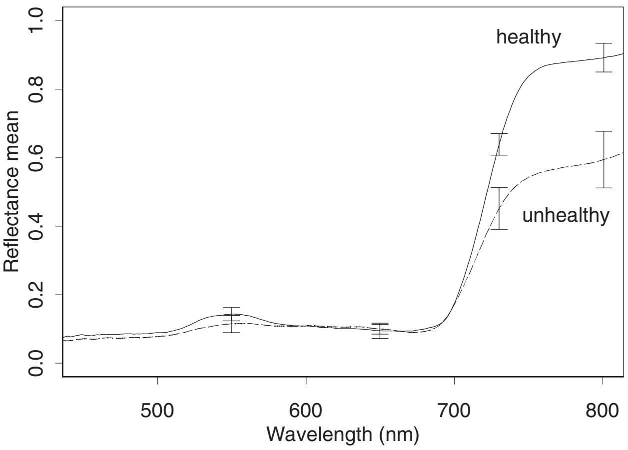

Fig. 1. The mean reflectance curves obtained for foliage from an *Eucalyptus paniculata* with a healthy crown compared with insect-damaged foliage from another *E. paniculata*. Vertical bars illustrate standard deviation errors of the mean of five replicates at key wavebands.

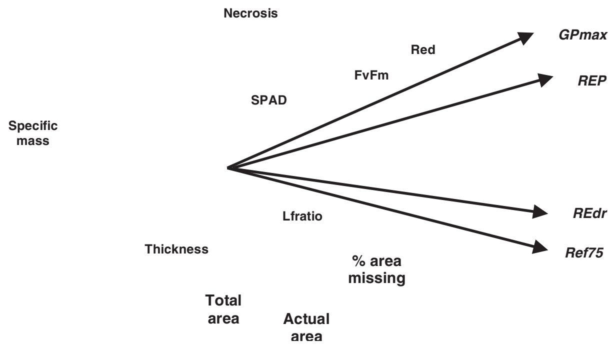

Fig. 2. A reduced-rank regression biplot illustrating the relationships between leaf traits and four spectral reflectance parameters of mature foliage sampled from *Eucalyptus saligna*, *E. paniculata* and *E. pilularis* exhibiting a range of canopy-decline symptoms associated with colonisation by bell miners. The goodness-of-fit of the biplot is 92% and n = 20. Relationships between the leaf traits and reflectance parameters on the biplot are disclosed by projecting a perpendicular line from each leaf-trait point onto the arrows representing the reflectance variables. The relative position of where each line strikes the arrow provides a visual ranking of the correlations between the leaf traits with the particular reflectance parameter. The closer the perpendicular line is to the arrowhead, the stronger the relationship. The biplot variable arrows can be extended through the origin in order for all projected points to be intercepted.

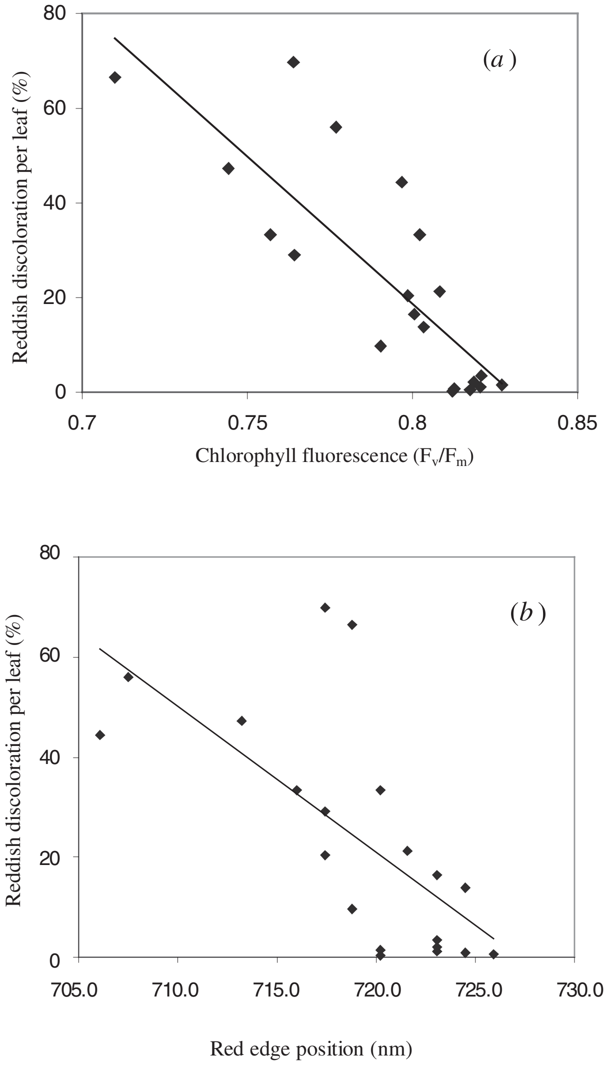

Fig. 3. (*a*) The relationship between sample means of estimated reddish discoloration per leaf and chlorophyll fluorescence (F_v/F_m) from 20 mature eucalypt trees (n = 9 for *Eucalyptus saligna*, n = 8 for *E. paniculata*, n = 3 for *E. pilularis*). There were about 20 leaves per sample from one shoot per tree. Percentage red discoloration = $-622 \times F_v/F_m + 516$, $r^2 = 0.68$, $P = 0.0001$. (*b*) The relationship between sample means of estimated reddish discoloration per leaf (%) and the spectral red-edge position (nm) from 20 mature eucalypt trees (n = 9 for *E. saligna*, n = 8 for *E. paniculata*, n = 3 for *E. pilularis*). There were about 20 leaves per sample from one shoot per tree. Percentage red discoloration = $-2.9 \times \mathrm{REP} + 2133$, $r^2 = 0.46$, $P = 0.001$).

stronger (Table 4). The significant negative relationship between reddish discoloration of leaves and the red-edge position (Fig. 3*b*) also demonstrate a ‘blue shift’ through the apparent movement of the red edge to shorter wavelengths with increasing levels of red pigmentation.

On some of the sampled branches were small amounts of immature foliage. While very little insect damage was observed on this young expanding foliage the degree of reddish coloration varied consistently between the eucalypt species. The expanding immature foliage from *E. paniculata* was pale green, that of *E. saligna* mostly pale yellow-green with some orange coloration while that of *E. pilularis* had a distinctive red coloration. A preliminary comparison of reflectance was made of this immature foliage compared

Table 4. Matrix presenting Pearson's correlation values for shoot means of leaf traits sampled from 20 trees (*Eucalyptus saligna*, *E. paniculata*, *E. pilularis*) exhibiting a range of canopy-decline symptoms associated with colonisation by bell miners

Ten to twenty mature leaves per shoot, depending on the parameter, $^*P \leq 0.05$; $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, all other values not significant

	Estimated necrotic area (%)	Estimated reddish discolor-ation (%)	Estimated potential leaf area (cm^2)	Actual leaf area (cm^2)	Leaf length : breadth ratio	Leaf thickness (mm)	Specific leaf mass (mg cm^{-2})	Chlorophyll fluorescence (mean F_v/F_m)	SPAD value (chlorophyll content)	Max. reflectance at Green Peak	1st derivative maxima at red edge	Reflectance at 750 nm	Red edge position (nm)
Leaf area missing (%)	0.3778	0.5840**	0.0339	-0.1943	–0.8562***	–0.3787	–0.0439	–0.5183*	–0.3472	–0.4906*	–0.5318*	–0.4146	–0.3532
Estimated necrotic area (%)	1	0.55635*	0.3329	0.2306	–0.4982*	–0.1987	–0.1217	–0.3428	–0.03311	–0.2322	–0.00439	0.0768	–0.1978
Estimated reddish discoloration (%)		1	-0.3182	–0.4388	–0.6103**	–0.3506	–0.1365	–0.8533***	–0.6557**	–0.59877**	–0.5803**	–0.5594*	–0.5783**
Estimated potential leaf area (cm^2)			1	0.9721***	–0.0773	–0.0459	–0.0208	0.3123	0.5030*	0.2204	0.4465*	0.5255*	0.4186
Actual leaf area (cm^2)				1	0.1190	0.0433	0.0056	0.4186	0.5506*	0.3233	0.5559*	0.6048**	0.4841*
Leaf length : breadth ratio					1	0.5648**	0.1158	0.5586*	0.4079	0.5049*	0.4911*	0.3572	0.3304
Leaf thickness (mm)						1	0.5241*	0.2325	0.4181	0.2252	–0.0115	–0.03681	0.0331
Specific leaf mass (mg cm^{-2})							1	–0.0659	0.1114	–0.24475	–0.2796	–0.3072	–0.2252
Chlorophyll fluorescence (mean F_v/F_m)								1	0.5986**	0.7529***	0.5798**	0.5017*	0.4064
SPAD values									1	0.53227*	0.4930*	0.4988*	0.6325 **
Max. reflectance at green peak										1	0.5621**	0.4663*	0.6441**
1st derivative maxima at red edge											1	0.9659***	0.0715***
Reflectance at 750 nm												1	0.7189***

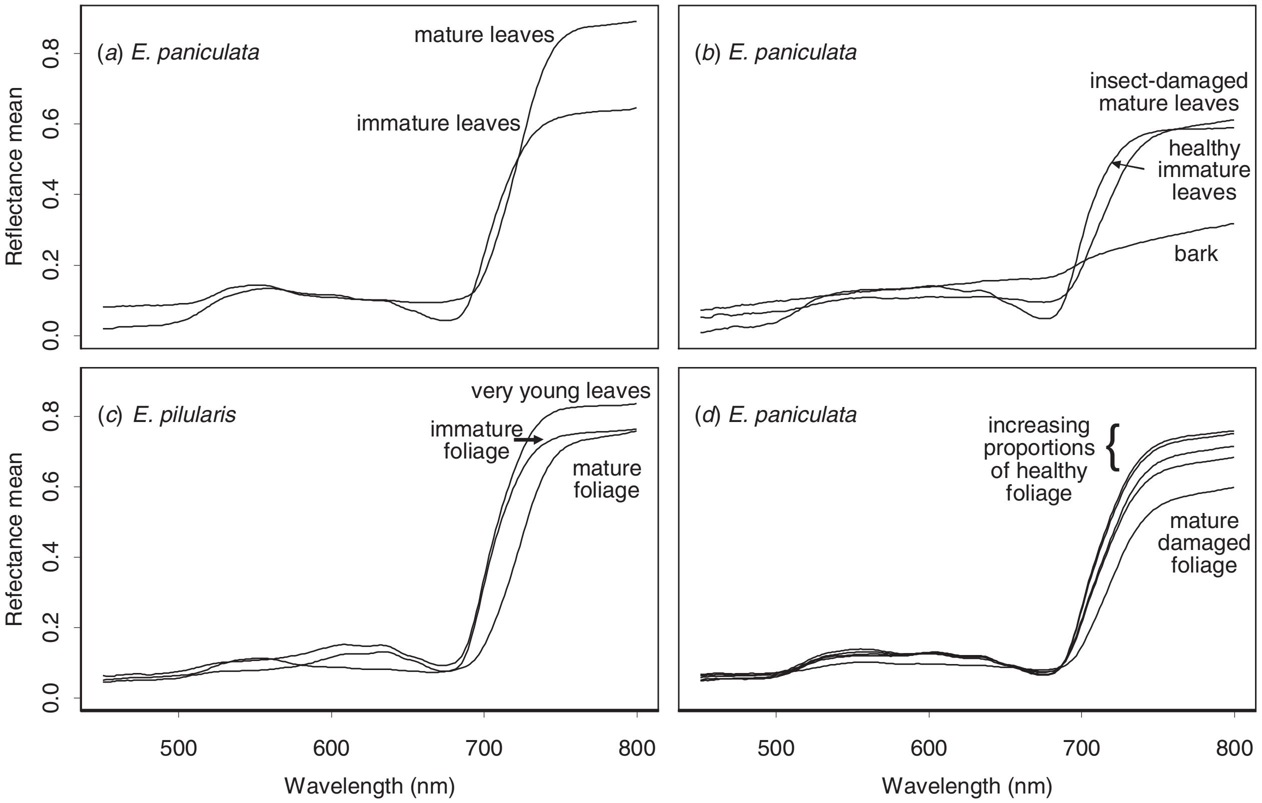

Fig. 4. Mean reflectance curves for (*a*) mature and immature foliage from an *Eucalyptus paniculata* with a healthy crown, (*b*) foliage from an *E. paniculata* with insect-damaged mature leaves and healthy immature leaves, (*c*) foliage from an *E. pilularis* with a healthy crown and (*d*) foliage from another *E. paniculata* with insect-damaged mature leaves and healthy immature leaves. There were five reflectance measurements per mean reflectance spectrum.

with mature foliage sampled from the same branch (Fig. 4*a*–*d*).

In *E. paniculata*, a distinguishing feature between the reflectance curves of healthy mature and immature leaves is the lower reflectance of the immature foliage in the NIR spectral region (Fig. 4*a*) while the red edge and green peak appear not too dissimilar. When the spectrum of healthy immature foliage is compared visually to insect-damaged mature *E. paniculata* foliage (Fig. 4*b*), the reflectance levels in the NIR are now much more similar. The spectral curve of *E. paniculata* twig bark possesses little structure with significantly lower levels of reflectance in the NIR region compared with both healthy and unhealthy foliage. Figure 4*c* compares healthy *E. pilularis* foliage from three leaf-age classes, very young red leaves, immature red leaves and mature green foliage. The spectra reflect the varying levels of green to red leaf pigments, with a higher level of reflectance in the red region of both young-leaf-age classes and an apparent 'red shift' of the red edge associated with leaf maturation (and increasing level of chlorophyll content). The shiny surfaces of the very young leaves compared with the other two age categories may contribute to the higher level of reflectance in the NIR region for the young red foliage (Fig. 4*c*). Figure 4*d* demonstrates changes in the spectra when increasing proportions of healthy foliage are added to insect-damaged mature *E. paniculata* foliage. With increasing contribution from healthy foliage, the magnitude of the green peak increases, the slope of the red edge increases and there is a reduction in NIR reflectance. One possible differing feature between Fig. 4*c* and *d* is the relative slopes of the red edge of healthy immature foliage and damaged mature foliage. Examination and analysis of many more foliage spectral curves are required to confirm the significance of this difference.

Discussion

Numerous studies have examined vegetation reflectance spectra in relation to the condition of tree foliage (e.g. Rock *et al.* 1988; Leckie *et al.* 1989; Hoque *et al.* 1992; Vogelmann *et al.* 1993; Wilson *et al.* 1998; Luther and Carroll 1999; Mohammed *et al.* 2000; Zarco-Tejada *et al.* 2000). This study, however, is the first to report on damaged eucalypt foliage. The most common source of foliar damage is from the large potential suite of insects and fungi associated with eucalypts. The ensuing changes in the biochemical, physiological and structural integrity of the leaves produces a range of physical and physiological symptoms of foliar condition. The leaf traits selected and assessed in this study were chosen as measures of these symptoms and hence foliar condition.

Both the maximum red-edge slope and green-peak features of the reflectance spectra were significantly correlated to leaf area missing (percentage herbivory); however, none of the four spectral features examined were good predictors of percentage necrosis. Peñuelas *et al.* (1995) suggest that reflectance features detecting changes in leaf pigments may be suitable for detecting pests and diseases that cause chlorosis but not necrosis. For stress-inducing agents causing predominantly structural changes such as necrosis, additional spectral features may be required. Roberts *et al.* (1998) attributed the reduced reflectance in the NIR of foliage in their study to an increase in necrosis and epiphytic growth on surfaces as the leaves aged.

While a significant relationship was found between the position of the maximum slope on the red edge and the SPAD values (relative chlorophyll content), it was not as strong as that demonstrated by others (e.g. Curran *et al.* 1995; Gitelson *et al.* 1996). One explanation is that in damaged eucalypt foliage the visible and red-edge regions are also affected by the absorption of other pigments including the anthocyanins (Curran *et al.* 1991; Neill and Gould 1999). Curren *et al.* (1991) presented results suggesting that the presence of a red leaf pigment interferes with the strong relationship between the red edge and chlorophyll concentration observed for healthy green leaves. Nevertheless, the movement of the maximum slope of the red edge towards shorter wavelengths with increasing levels of red pigmentation suggests a 'blue shift' of the red edge (Fig. 3*b*). This movement of the red edge to shorter wavelengths has been demonstrated for stress-induced chlorosis or during senescence in numerous other studies (e.g. Rock *et al.* 1988; Boochs *et al.* 1990; Curran *et al.* 1990; Carter and Miller 1994) and arises from the sensitivity of the red edge to chlorophyll content (Datt 2000). The movement of the red edge to shorter wavelengths with increasing reddish discoloration per leaf (Fig. 3*a*) suggests that a decrease in chlorophyll content corresponds to an increase in anthocyanin levels in the insect-damaged eucalypt leaves (Fig. 3*a*).

The chlorophyll fluorescence parameter F_v/F_m (a measure of photochemical efficiency and hence chlorophyll function) has frequently been used as a generic diagnostic tool for the detection of physiological stress (e.g. Close *et al.* 2000; Zarco-Tejada *et al.* 2000). Zarco-Tejada *et al.* 2000 claim that changes in chlorophyll function frequently precede changes in chlorophyll content and hence changes in chlorophyll fluorescence may be observed long before leaves become chlorotic. The results presented in Fig. 3*a* support other studies that have reported a reduction in photosynthetic efficiency (measured by fluorescence-based indices) associated with anthocyanin production (Close *et al.* 2000; Gould *et al.* 2000). Our results suggest a positive relationship between anthocyanin content and physiological stress expressed through both a decrease in chlorophyll function and concentration in eucalypt leaves damaged by insects.

Relationship between photoinhibition, anthocyanin levels and insect damage in eucalypt foliage

Reduced rates of photosynthesis have been shown to be caused by abiotic stresses such as cold temperatures and nutrient deficiency (e.g. Adams *et al.* 1994; Close *et al.* 2000). Our results indicate that biotic agents such as leaf-damaging insects and fungal pathogens may also induce response(s) in eucalypt leaves that affect the functioning of the photosynthetic apparatus. This is additional to other possible toxic effects and direct structural damage caused by these agents. The presence of elevated levels of reddish discoloration in eucalypt foliage from the effects of cold-induced photoinhibition (Close *et al.* 2000) and on insect-damaged eucalypt leaves suggests a possible generic stress response. Circumstantial evidence is also provided by Wall and Keane (1984) who reported that the purple margins developed around lesions of the eucalypt spot, caused by *Aulographina eucalypti*, were most noticeable during the cooler months and were due to an accumulation of anthocyanins. The purplish discoloration of *E. saligna* infested with the psyllid, *Glycaspis baileyi*, is also most obvious during late winter (Moore 1961; C. Stone, pers. obs.). These observations are due, in part, to the cumulation of damage on the dominant mature age class of leaves present in crowns over winter. However, the accumulation of anthocyanins may be a result of the leaves being more sensitive to cold-induced photoinhibition because of the damage caused by insects and fungal pathogens.

A relationship between photosynthetic efficiency, photoinhibition and another group of non-green pigments, the pale yellow xanthophylls-cycle carotenoids, is well established (e.g. Demmig-Adams and Adams 1996). Gamon *et al.* (1997) developed a reflectance indicator called the photochemical reflectance index (PRI), on the basis of the relationship between a fluorescence parameter of photochemical efficiency, $\Delta F/F_m$ and the xanthophyll cycle pigment zeaxanthin. For stressed or damaged eucalypt leaves, however, an increase in anthocyanin pigments may be a more dominant visual characteristic and these symptoms become more noticeable during cooler conditions because of the proposed link to photoinhibition. Although the relationship between an increase in anthocyanin content and mature eucalypt foliage damaged by abiotic stresses has previously been reported, for example, cold temperatures and nutrient deficiency (e.g. Ladiges and Kelso 1977; Close *et al.* 2000) this is the first report linking biotic stress-inducing agents such as herbivorous insects or fungal pathogens to photosynthetic function and anthocyanin content. While abiotic and biotic stress-inducing agents can produce damage symptoms independently, together their effects may be synergistic.

This hypothesis is still speculative and requires testing over a wide range of herbivorous insects and fungal pathogens and in combination of various biotic and abiotic stress-inducing agents. For example, this induced response may more commonly occur with certain types of insect guilds, such as sap-suckers, leaf-mining insects or fungal leaf spots. Eucalypts do have other stress-coping mechanisms, for example eucalypt behaviour during moisture stress (van der Moezel *et al.* 1989). If, however, it can be demonstrated that biosynthesis of anthocyanins in mature eucalypt foliage is induced by a range of abiotic and biotic factors then spectral features that predict these changes in leaf pigmentation may form a basis of a generic indicator of eucalypt leaf condition. Our hypothesis differs from that developed for the PRI, in that, instead of utilising the close relationship between photochemical efficiency and increased levels of the photoprotective xanothophyll-cycle pigments, a link between photochemical efficiency and anthocyanins could be similarly investigated for eucalypts. For example, testing a red–green reflectance index similar to that proposed by Gamon and Surfus (1999).

Given the small sample size and single geographic location of this analysis, these results are provisional but do present a direction for further investigation. Additionally, they suggest that more sophisticated reflectance indices than those based on the broad-band reflectances (e.g. the Normalised Difference Vegetation Index) may need to be considered in order to detect this proposed link between photosynthetic function and anthocyanin content in chronically stressed or damaged mature eucalypt foliage. More work is required to extend this proposition from the leaf sample scale to the increasingly complex canopy scale.

Leaf ontogeny, pigmentation and spectral reflectance

The presence of anthocyanins in young expanding foliage of certain species of eucalypts (e.g. *E. pilularis* and 'ash-type' eucalypts) is not peculiar to eucalypts (Sharma and Crowden 1974; Chalker-Scott 2000). Ali *et al.* (1999) working with mango leaves, demonstrated that anthocyanin content increased before leaf enlargement but decreased rapidly as the lamina expanded. In these examples, the anthocyanins as well as the xanothophyll-cycle pigments may have photoprotective roles during early leaf development when chlorophyll synthesis is occurring (Gamon and Surfus 1999). This study indicates that several features appear common to both insect-damaged mature eucalypt leaves and young expanding leaves relative to healthy mature eucalypt foliage, especially when both the damaged and young leaf classes have high levels of red pigmentation (Figs 1 and 4). As previously mentioned, when comparing healthy mature foliage with insect-damaged leaves there is a shift of the red edge towards shorter wavelengths (Fig. 3*b*). Likewise, the spectral red edge associated with healthy but young red foliage occurs at shorter wavelengths relative to healthy mature foliage (Fig. 4*b*). Differences in NIR reflectance may also occur for both healthy young foliage and unhealthy mature foliage (Figs 1 and 3*c*) relative to healthy foliage. From these observations and by others (e.g. Rock *et al.* 1994; Roberts *et al.* 1998), levels of reflectance in the NIR region initially increases with leaf maturation and decreases as the leaf accumulates damage with aging. A spectral feature, which may distinguish between unhealthy mature foliage and healthy foliage, is the slope of the red edge (Figs 1 and 4*d*.) Confirmation of these observations requires examination of more spectral curves than utilised in this study. From the analysis of red-edge wavelengths and associated first derivative curves (from red-edge slopes) Datt (2000) was able to distinguish between young and mature healthy leaves sampled from *E. gummifera* (Sol. Ex Gaertner) Hochr and leaves sampled from drought stress from *E. gummifera*.

In addition to pigment changes arising through leaf maturation, Gitelson and Merzlyak (1994 and references therein) demonstrated that events occurring in senescencing leaves are very close to those taking place under stress, for example in response to herbicides, pollution, extreme temperatures, drought and diseases. Therefore, the natural senescence process in eucalypt leaves may also include a regulated reduction in chlorophyll content as well as an increase in non-green pigments, especially anthocyanins (Hoch *et al.* 2001). Because of the potential presence of high levels of anthocyanins in healthy eucalypt foliage depending on leaf ontogeny, it is essential that leaf age be taken into account when interpreting reflectance spectra. Because of the confounding effect of leaf ontogeny and damage, knowledge of the relative proportions of young and mature foliage is required. This has important implications for scaling up to the canopy scale and the interpretation of remotely sensed spectra (Mohammed *et al.* 2000). In south-eastern Australia, an ideal time would be during winter, before the commencement of new season's growth. This also has the advantage of maximising the presence of anthocyanins in stressed or damaged mature foliage through the additional effects of cold-induced photoinhibition.

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