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Hyperspectral remote sensing of plant pigments

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Abstract

The dynamics of pigment concentrations are diagnostic of a range of plant physiological properties and processes. This paper appraises the developing technologies and analytical methods for quantifying pigments non-destructively and repeatedly across a range of spatial scales using hyperspectral remote sensing. Progress in deriving predictive relationships between various characteristics and transforms of hyperspectral reflectance data are evaluated and the roles of leaf and canopy radiative transfer models are reviewed. Requirements are identified for more extensive intercomparisons of different approaches and for further work on the strategies for interpreting canopy scale data. The paper examines the prospects for extending research to the wider range of pigments in addition to chlorophyll, testing emerging methods of hyperspectral analysis and exploring the fusion of hyperspectral and LIDAR remote sensing. In spite of these opportunities for further development and the refinement of techniques, current evidence of an expanding range of applications in the ecophysiological, environmental, agricultural, and forestry sciences highlights the growing value of hyperspectral remote sensing of plant pigments.

Key words: Anthocyanins, carotenoids, chlorophyll, hyperspectral, optical properties, pigments, radiative transfer, reflectance, remote sensing.

Introduction

The importance of plant pigments

Plant pigments are of tremendous significance in the biosphere. Indeed, it is argued that the chlorophylls are Earth's most important organic molecules as they are necessary for photosynthesis, the carotenoids are essential

for plant and mammal survival through their photosynthetic and nutritional functions, while other pigment groups are key to the physiology of plants and the organisms with which they interact (Davies, 2004).

Within leaf chloroplasts the antenna pigments absorb solar radiation and transfer the energy to the reaction centre pigments which initiates photosynthesis (Richardson *et al.*, 2002). The most important of these pigments are the chlorophylls (*Chls*; chlorophyll *a* and *b*) which are of interest in their own right, but, from a physiological perspective, *Chls* concentration is important for several reasons. *Chls* have a dominant control upon the amount of solar radiation that a leaf absorbs, therefore, foliar concentrations of *Chls* controls photosynthetic potential and, consequently, primary production. The molecular structure of the *Chls* incorporates a large proportion of total leaf nitrogen and several studies have found that foliar *Chls* concentration provides an accurate, indirect estimate of plant nutrient status (Filella *et al.*, 1995; Moran *et al.*, 2000). *Chls* generally decrease under stress and during senescence and the ratio of *Chl a* to *Chl b* changes with abiotic factors such as light (Fang *et al.*, 1998), therefore, measurements of total *Chl* and *Chl a* and *Chl b* individually, can provide useful insights into plant–environment interactions (Richardson *et al.*, 2002).

Carotenoids (*Cars*) are the second major group of plant pigments, composed of carotenes and xanthophylls. *Cars* can absorb incident radiation and contribute energy to photosynthesis as they are essential structural components of the photosynthetic antenna and reaction centre complexes (Bartley and Scolnik, 1995). The fraction of photosynthetically active radiation absorbed by a plant canopy (APAR) has been related to net primary productivity as a function of a light use efficiency (LUE) coefficient defining the carbon fixed per unit radiation intercepted (Landsberg *et al.*, 1996). Such studies assume that the contribution of each pigment to the energetics of photosynthesis is equal, but this is an insufficient interpretation, as

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the concentration of *Chl a* is the limiting factor in the utilization of light for photosynthesis, because it receives energy absorbed by *Chl b* and *Cars* (Chappelle *et al.*, 1992; Kim *et al.*, 1994). Thus, the photosynthetic potential of two plants may differ even though their APAR is equal, depending upon the concentrations of individual pigments. Furthermore, when incident radiation exceeds that needed for photosynthesis, *Cars* that compose the xanthophyll cycle dissipate excess energy and protect the reaction centres. This is achieved by the conversion of the xanthophyll pigments from the epoxidized (violaxanthin) state to the de-epoxidized (antheraxanthin and zeaxanthin) state (Demming-Adams and Adams, 1996). Xanthophyll pigment composition is therefore closely related to the photosynthetic LUE. The dual photosynthetic and photoprotective roles of the carotenoids have also been evident in studies demonstrating two levels of regulation of carotenoid synthesis, the first dependent on chlorophyll synthesis and the second on LUE (Härtel and Grimm, 1998). Thus, while changes in *Chls* are indicative of stress and phenological stage, *Cars* concentration provides much complementary information on vegetation physiological status (Young and Britton, 1990).

Anthocyanins (*Anths*) are water-soluble flavonoids which are the third major group of pigments in leaves, but there is no unified explanation for their presence and function. For certain species or particular phenological stages *Anths* can become the dominant pigment group and they are a common component of all leaves. *Anths* can modify the light environment within a leaf and have the potential to regulate photosynthesis and limit photoinhibition and photobleaching (Barker *et al.*, 1997) thereby having a photoprotective function (Steyn *et al.*, 2002; Close and Beadle, 2003). *Anths* may act as osmotic adjusters offering plants resistance to freezing and drought stress (Chalker-Scott, 1999) and there is evidence that *Anths* have antioxidant properties that aid recovery after foliar injury (Gould *et al.*, 2002). While the roles of *Anths* are currently the focus of some debate, it is clear that information on the dynamics of their concentrations is key to understanding the physiological reaction and resistance of plants to different environmental stress factors brought about by episodic events or seasonal fluctuations.

Variations in the relative concentrations of *Chls*, *Cars*, and *Anths* have recently been the subject of an emerging theory that proposes that the 'bright' foliar colours (i.e. low *Chl*, higher *Cars* and/or *Anths*) displayed by some plant species in autumn could be a result of coevolution between insects and trees (Archetti, 2000; Hamilton and Brown, 2001). It is proposed that the 'bright' colours are a signal of the defensive commitment of the plant which it uses to reduce parasite load. The coevolution theory may even be a general explanation for 'bright' colours of leaves, i.e. an explanation for inter- and intraspecific variations in the proportions of the major groups of pigments in leaves

(Archetti and Brown, 2004). Whether or not this theory holds up to ongoing experimental scrutiny, variations in the absolute and relative concentrations of the different pigment groups have undoubted significance for plant physiological functioning and the interactions between flora and fauna (Bartley and Scolnik, 1995).

Given the demonstrable importance of plant pigments, information concerning the temporal dynamics and spatial variations of pigments can, from an applied perspective, provide key contributions to a wide range of scientific investigations and environmental/agricultural management endeavours. However, current capabilities for providing this information are limited. Traditional techniques for measuring foliar pigment concentrations involve extraction with a solvent and spectrophotometric analysis using standard procedures. This is possible because pigments have differing spectral absorption properties and simple combinations of absorbance values at a number of wavelengths can be used accurately to determine individual pigment concentrations from mixed extracts (Lichtenthaler, 1987). Pigment assay, particularly for individual carotenoids, can also be performed using high performance liquid chromatography (Dunn *et al.*, 2004). However, these wet laboratory techniques are time- and labour-intensive and therefore expensive. Thus, for whole canopies, pigments must be quantified by extrapolation from a limited number of samples, which introduces inaccuracies. Moreover, the destructive nature of traditional methods can restrict the ability to monitor the temporal dynamics of pigments, particularly at the individual leaf scale. Faster, non-destructive measurements of total leaf *Chl* concentration can be obtained using hand-held field instruments such as the Minolta SPAD (Markwell *et al.*, 1995), but there is still a requirement to extrapolate spatially from a restricted number of samples and measurements of *Chl a* and *Chl b*, *Cars*, or *Anths* cannot be made.

The potential for remote quantification of pigments

The spectral absorbance properties of pigments are manifest in the reflectance spectra of leaves and this offers the opportunity of using measurements of reflected radiation as a non-destructive method for quantifying pigments. A useful overview of the physical basis and applications of radiation measurements to enable remote sensing capabilities in plant ecophysiology is provided by Jones *et al.* (2003). Multispectral remote sensing systems such as the Landsat Thematic Mapper have been operational for several decades and spectral indices based on their broad wavebands have been developed for quantifying vegetation biophysical properties such as leaf area index (*LAI*). However, a number of studies have demonstrated that broad band multispectral data are inadequate for the remote sensing of vegetation biochemical properties and that narrow band (high spectral resolution; usually a bandwidth

of 10 nm or less) hyperspectral data are required (Broge and Mortensen, 2002).

With the advent of airborne [e.g. Airborne Visible/Infrared Imaging Spectrometer (AVIRIS) and HyMap] and, more recently, experimental spaceborne imaging spectrometers [e.g. Compact High Resolution Imaging Spectrometer (CHRIS) and Hyperion], with high spectral and radiometric resolutions and signal:noise ratios, there have been opportunities to acquire vegetation reflectance spectra and test methods for imaging plant pigment concentrations. Ustin *et al.* (2004) outline recent advances in the technology and applications of airborne and spaceborne imaging spectrometry for remote sensing of biophysical processes and properties. The forthcoming generation of operational spaceborne imaging spectrometer missions [e.g. Hyperspectral Environment and Resource Observer (HERO) and Environmental Mapping and Analysis Program (EnMAP)] will facilitate the development of a greater range of practical applications. At the same time as airborne and spaceborne technologies are developing, there is growing interest, particularly from the precision agriculture sector, in the development of field-based instruments that can be used to obtain plant spectra (Inoue and Peñuelas, 2001; Noh *et al.*, 2005; Zillman *et al.*, 2006). Such instruments can be mounted on tractors or mobile irrigation equipment and are able to obtain spectral data at a higher spatial resolution and temporal frequency and lower cost than airborne or spaceborne instruments. The maturation of sensor technology and growing operational deployment of ground-based, airborne and spaceborne instruments will greatly enhance the capabilities for routinely acquiring hyperspectral data and potentially, for quantifying plant pigments over a wide range of spatial scales, repeatedly.

Alongside developments in hyperspectral data acquisition there has been an increasing intensity of research focused on developing techniques for analysing plant spectra in order to quantify pigment concentrations. To extract pigment information the range of other factors which also influence vegetation reflectance spectra must first be taken into account. The internal structure of leaves, with large numbers of refractive discontinuities between cell walls and intercellular air spaces, scatters incident radiation and allows a large proportion to pass back through the upper epidermis to be observed as reflected radiation. Pigments, water, and other biochemicals absorb certain wavelengths of radiation which reduces reflectance in these regions. However, because of the overlapping absorption features of the pigments (Fig. 1) and other constituents, it may be difficult to relate reflectance at a single wavelength to the concentration of an individual pigment. Furthermore, leaf reflectance can vary independently of pigment concentrations due to differences in internal structure, surface characteristics (e.g. hairs/waxes) and moisture content. The reflectance spectrum of a whole canopy is subject to even more controlling factors, notably,

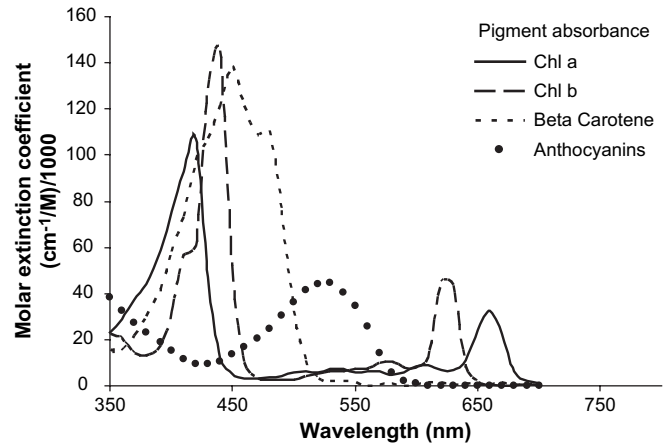


Fig. 1. Absorption spectra of the major plant pigments.

effects of variations in number of leaf layers (*LAI*), orientation of leaves (leaf angle distribution; *LAD*), % canopy ground coverage, presence of non-leaf elements, areas of shadow and soil/litter surface reflectance. This range of factors, at leaf and canopy scales, obscures the relationships between spectral reflectance and concentrations of individual pigments.

Further issues arise because of the nature of hyperspectral data. While high spectral resolution is required in order to resolve the fine spectral differences between individual pigments, sensing in a large number of adjacent narrow wavebands can produce reflectance spectra containing considerable spectral autocorrelation. The autocorrelation causes redundancy within hyperspectral data sets, therefore it is necessary to employ appropriate techniques with which to characterize the main sources of spectral variability and to identify optimal wavebands that offer maximum informational content.

The following section reviews the progress in tackling this series of issues in order to quantify pigment concentrations from hyperspectral remotely-sensed data. A combination of research approaches have been used, incorporating experimental studies, usually where pigment concentrations are manipulated through stress treatments, and observational studies, often exploiting pigment variations due to natural phenological changes.

The development of hyperspectral analytical approaches

Figure 2 displays the reflectance spectrum over the visible (400–700 nm) and near infrared (here 700–1000 nm) wavebands of a typical green leaf. This figure demonstrates the low reflectance across the visible, with a slight peak in the green (about 550 nm), with reflectance rising rapidly at the so-called ‘red edge’ leading to a plateau of high reflectance in the near-infrared, where pigments no longer absorb radiation. Several groups of spectral variables have been identified as being of value in characterizing

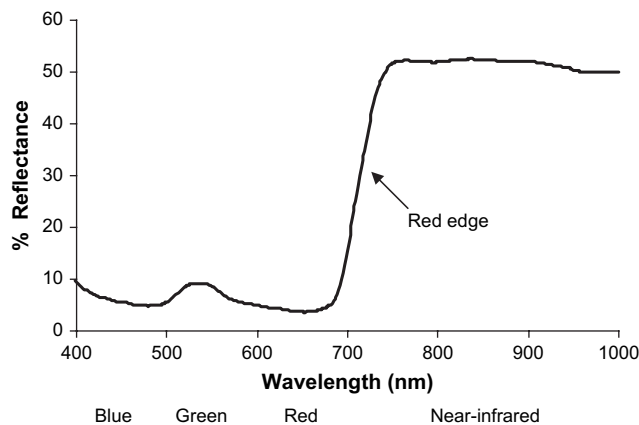


Fig. 2. Reflectance spectrum of a typical green leaf over the visible (400–700 nm) and near infrared (here 700–1000 nm) wavebands.

the way in which reflectance spectra change as pigment concentrations vary and empirical relationships between these spectral variables and pigment concentrations have been defined. An alternative group of approaches for quantifying pigments from hyperspectral data are based on the numerical inversion of physically-based leaf and canopy radiative transfer models.

Reflectance in individual narrow wavebands

While there is little agreement on the optimal wavelengths, there is good evidence from modelling studies that, at wavelengths where absorption coefficients of pigments are low, reflectance is more sensitive to high concentrations, while spectral regions with high absorption are more sensitive to lower pigment concentrations (Jacquemoud and Baret, 1990; Yamada and Fujimara, 1991). This is confirmed by empirical evidence which has demonstrated that reflectance at wavelengths corresponding to the lower and upper flanks of the major chlorophyll absorption feature in the red region (i.e. approximately 530–630 nm and a narrower band around 700 nm, respectively) is most sensitive to pigment concentrations over the normal range found in most leaves (Lichtenthaler *et al.*, 1996; Mariotti *et al.*, 1996; Carter and Knapp, 2001; Buscaglia and Varco, 2002; Carter and Spiering, 2002; Zhao *et al.*, 2003) and canopies (Filella *et al.*, 1995; O'Neill *et al.*, 2002). In studies incorporating low concentrations of pigments, as found in early immature and later senescent leaves and canopies with low leaf area and canopy cover, reflectance at wavelengths corresponding to the centre of the major absorption features are most sensitive to pigment concentrations (Blackburn 1998a, b; Sari *et al.*, 2005).

Reflectance in multiple narrow bands

In response to difficulties in relating reflectance in individual narrow bands to pigment concentration due to variations in the multiple controlling factors on reflectance

at leaf and canopy scales (see section entitled 'The potential for remote quantification of pigments') and the observed non-linearity of relationships between pigments and reflectance, many analytical approaches have used reflectance in multiple narrow bands. Most so-called 'spectral pigment indices' have employed ratios of narrow bands within areas of the spectrum that are sensitive to pigments and those areas not sensitive and/or related to some other control on reflectance. Thus, pigment indices have been proposed as a means of solving the problems of the overlapping absorption spectra of different pigments (Chappelle *et al.*, 1992) and the effects of leaf structure, leaf surface interactions, and canopy structure (Peñuelas *et al.*, 1995). Most pigment indices have been derived for *Chl* quantification and are based on ratios of narrow bands in the visible and near-infrared (Schepers *et al.*, 1996; Blackburn 1998a, b), while some use ratios of narrow bands only in the visible (Filella *et al.*, 1995), in the red edge region (Vogelman *et al.*, 1993), or in the near-infrared shoulder and red edge regions (Gitelson and Merzlyak, 1997). Most of these indices employ reflectance in two narrow wavebands, although some use three (Chappelle *et al.*, 1992); most relate to total *Chl*, while some differentiate *Chl a* and *Chl b* (Datt, 1998); the majority have been developed at the leaf scale. le Maire *et al.* (2004) provide a comprehensive listing of the *Chl* spectral indices published until 2002.

Recent work has tended to focus on improving the robustness and generality of *Chl* indices by testing and refinement over a range of species and physiological conditions. Interestingly, much of this work has resulted in the incorporation of more narrow wavebands, usually chosen from the near-infrared, red edge, and visible regions. Indices using three bands have been found to be generally applicable at the leaf scale (Sims and Gamon, 2002; Gitelson *et al.*, 2003; le Maire *et al.*, 2004), while at the canopy scale both three (Dash and Curran, 2004; Gitelson *et al.*, 2005) and four band indices (Thenkabail *et al.*, 2002) have been developed. The merits of exploiting reflectance in several narrow bands are further demonstrated in studies that have attempted to quantify leaf and canopy *Chl* concentrations by subjecting multiple bands and, in some cases, the whole spectrum to principal components (Yao and Tian, 2003) and factor analysis (Coops *et al.*, 2002), artificial neural networks (Tumbo *et al.*, 2002; Chen *et al.*, 2006) and stepwise multiple regression (O'Neill *et al.*, 2002; Osborne *et al.*, 2002). However, it has been found, when working on a single crop type, that a normalized difference ratio employing just two narrow wavebands was superior to a multiple regression model using five wavebands (Goel *et al.*, 2003) or a partial least squares regression model based on the whole spectrum (Hansen and Schjoerring, 2003). **Indeed, other work using single species has confirmed the efficiency of ratios of reflectance in two narrow wavebands selected**

from the near-infrared, red edge, and visible regions for *Chl* estimation for leaves (Zhao *et al.*, 2005) and even at the canopy scale (Coops *et al.*, 2003; Ferri *et al.*, 2004). Overall, this indicates that, in an attempt to identify universal *Chl* indices, it may be important to incorporate more of the sources of spectral variability in plant reflectance data by using several narrow wavebands, while just two wavebands can result in a more accurate predictive model when calibrated for a specific plant type.

Most research has focused on *Chls* and less attention has been paid to quantifying *Cars* from reflectance spectra. Significant overlap in the absorption features of *Chl* and *Cars* (Fig. 1) and the low concentrations of *Cars* with respect to *Chl* in most leaves can present difficulties in defining suitable spectral indices for *Cars*. Spectral indices based on three narrow bands in the near-infrared, red edge, and visible regions of the spectrum, have shown promise for estimating the ratio of *Cars:Chl* (Peñuelas *et al.*, 1995; Merzlyak *et al.*, 1999). Robust measures of leaf *Cars:Chl* ratios over a range of species have been obtained using the photochemical reflectance index (PRI) (Sims and Gamon, 2002). PRI is a normalized difference ratio of reflectance in narrow bands either side of the peak in green reflectance (550 nm) and was originally developed to estimate short-term fluctuations in different xanthophyll pigments (Gamon *et al.*, 1992). A number of spectral indices have been developed to estimate total *Cars* from reflectance (Chappelle *et al.*, 1992; Blackburn, 1998a; Datt, 1998), but these have tended to have somewhat limited transferability across species or scales (Blackburn, 1998b; Sims and Gamon, 2002). Nevertheless, an index employing three narrow bands in the visible and red edge (i.e. 510 nm, 550 or 700 nm) and near-infrared regions appears to offer a robust solution at the leaf scale (Gitelson *et al.*, 2002; Gitelson and Merzlyak, 2004).

Only a small number of studies have examined the opportunities for quantifying *Anths* concentration from reflectance spectra. Gamon and Surfus (1999) observed increased reflectance in the red region associated with increased *Anths* in immature leaves and proposed a broadband red:green ratio as an index of *Anths*. However, a subsequent test of this index using a number of different species found no relationship with *Anths* (Sims and Gamon, 2002). This concurs with the findings of Neill and Gould (1999) who found broadband red reflectance to be independent of leaf *Anths* concentration. The presence of *Chl* is the main factor which obscures relationships between reflectance and *Anths*. In an attempt to overcome this problem, an index has been proposed that uses narrow band green (550 nm) and early red edge (700 nm) reflectance (Gitelson *et al.*, 2001). This approach was refined to account for the effects of varying leaf cellular structure by also incorporating a near-infrared band and the index was found to be an accurate predictor of *Anths* over a range of species (Gitelson and Merzlyak, 2004).

Derivative spectra and continuum removal

A first derivative spectrum (Fig. 3) displays the variation with wavelength in the slope of the original reflectance spectrum (Fig. 2). Likewise, a second derivative calculates the slope of the first derivative, and so on. It has been suggested that spectral derivatives have important advantages over spectral reflectance, such as their ability to reduce variability due to changes in illumination or soil/litter reflectance (Curran *et al.*, 1991; Elvidge and Chen, 1995). The majority of workers have used derivatives to define the wavelength position of the red edge (λ_{RE}) and have illustrated relationships between λ_{RE} and total chlorophyll (*Chl tot*) concentration for both leaves and canopies (Lichtenthaler *et al.*, 1996; Mariotti *et al.*, 1996; Jago *et al.*, 1999). A common method for defining λ_{RE} has been to identify the wavelength position of the peak (maximum amplitude) of the first derivative in the region of the red edge. This peak can be clearly seen in Fig. 2 at approximately 710 nm. A number of alternative methods for calculating λ_{RE} based on first and second derivatives have also been proposed (Miller *et al.*, 1990; Dawson and Curran, 1998).

However, several studies have identified the existence of two or more peaks in first derivative spectra in the region of the red edge (Boochs *et al.*, 1990; Clevers *et al.*, 2004; Smith *et al.*, 2004) and this can lead to a bimodal distribution of λ_{RE} values corresponding to low and high *Chl* concentrations. Thus, λ_{RE} tends to jump at a certain threshold of *Chl* and this prevents predictive relationships being established between λ_{RE} and *Chl*. The existence of double or multiple peaks in first derivative spectra has been attributed to the characteristic absorption spectra of *Chl* (le Maire *et al.*, 2004) with a possible contribution from *Chl* fluorescence (Zarco-Tejada *et al.*, 2003). In an attempt to overcome the problems of the λ_{RE} jump, le Maire *et al.* (2004) introduced a new index that is based on the difference between the amplitude of two bands (approximately 30 nm wide) within the lower and upper wavelength sides of the red edge feature in the first

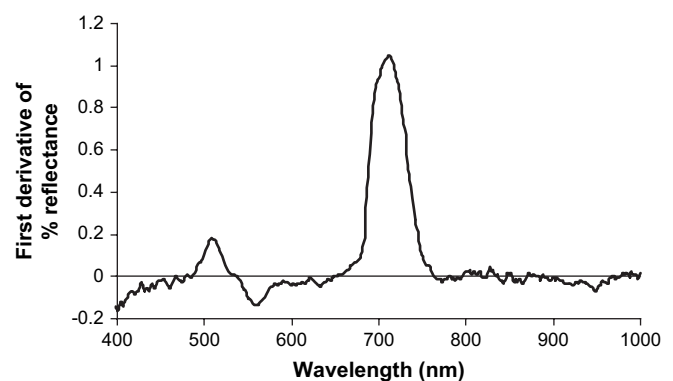


Fig. 3. The first derivative of the reflectance spectrum of a typical leaf (i.e. the spectrum shown in Fig. 2).

derivative. This 'Double Difference Index' showed good predictive accuracy for *Chl* at the leaf scale. An alternative solution is provided by Cho and Skidmore (2006) who developed a method for calculating λ_{RE} that does not depend on characterizing a peak in the first derivative. The technique uses linear extrapolation of straight lines fitted to the flanks of the red edge feature in the first derivative. This avoids the λ_{RE} jump and ensures that λ_{RE} varies continuously and is closely correlated with *Chl* concentration (measured as total nitrogen in their study). Furthermore, the authors demonstrate how this approach can be applied to imaging spectrometer data that may have a lower spectral resolution than field or laboratory-based spectroradiometers.

The amplitude of first and second derivatives of reflectance at individual and multiple combinations of wavelengths has been correlated with *Chl* concentrations at leaf and canopy scales (Boochs *et al.*, 1990; Adams *et al.*, 1999; Endo *et al.*, 2000) as has the amplitude of the first derivative of pseudo absorbance (the logarithm of the reciprocal of reflectance) (Yoder and Pettigrew-Crosby, 1995; Blackburn, 1999). A small number of studies have also demonstrated relationships between the amplitude of spectral derivatives and *Cars* at leaf and canopy scales (Blackburn, 1998b; Blackburn and Steele, 1999; Guan *et al.*, 2005). A 'Brown Pigment Index' has been developed using second derivative spectra to measure the distance between the position of the reflectance red edge and the wavelength with the minimum reflectance slope in the near-infrared (Peñuelas *et al.*, 2004). This index was correlated with the concentrations of oxidized compounds in stressed leaves.

Measurements of absorption feature depths have been obtained by fitting a continuum to vegetation reflectance spectra (Kokaly and Clark, 1999), which is a similar approach to the use of spectral derivatives, in the sense that it can enhance spectral features of interest while suppressing unwanted effects. This approach has been extended by normalizing to the band depth at the centre and the area of the absorption feature (Curran *et al.*, 2001). Stepwise regression was then used to identify optimal combinations of band depths for providing accurate estimates of *Chl tot*, *a* and *b* in dried and ground pine needles. Furthermore, continuum removal analysis was found to be superior to the use of standard derivative analysis for quantifying tree canopy biochemical properties from airborne imaging spectrometry data, though total N was examined rather than pigment concentrations specifically (Huang *et al.*, 2004).

Radiative transfer (RT) models

A number of physically based models have been developed which account for the interactions of incident radiation with leaves and canopies and are able to simulate reflectance spectra. In the context of the remote sensing of pigments,

such models have been used in both forward and inverse modes. In forward mode, RT models have often been used to generate large databases of reflectance spectra using wide ranges and combinations of the input variables that describe the biochemical and biophysical properties. Such databases have then been used to identify spectral variables or indices that are sensitive and robust predictors of pigment concentrations. At the leaf scale, such studies have been undertaken by using the PROSPECT (le Maire *et al.*, 2004) and LIBERTY models (Coops and Stone, 2005). Other studies have been carried out using coupled leaf and canopy RT models to understand the way in which leaf reflectance properties are influenced by the larger number of controlling factors at canopy scale (Demarez and Gastellu-Etchegorry, 2000). Coupled models have enabled the development and refinement of spectral indices, for estimating foliar *Chl* concentrations from canopy reflectance data, that are insensitive to factors such as canopy structure, illumination geometry, and soil/litter reflectance (Broge and Leblanc, 2000; Daughtry *et al.*, 2000). Such approaches have also been used in defining predictive relationships that have been applied to hyperspectral imagery to generate maps of *Chl* (Haboudane *et al.*, 2002; Zarco-Tejada *et al.*, 2004b, 2005a).

The numerical inversion of RT models based on measured reflectance spectra has also been used to quantify pigments. At the leaf scale, *Chl* has been predicted accurately using the PROSPECT (Jacquemoud *et al.*, 1996; Demarez *et al.*, 1999; Renzullo *et al.*, 2006) and LEAFMOD (Ganapol *et al.*, 1998) models in inverse mode. The inversion of coupled leaf and canopy RT models has been demonstrated for quantifying *Chl* from canopy hyperspectral data (Jacquemoud *et al.*, 1995, 2000). A useful hybrid approach has been proposed which uses spectral indices that are correlated with foliar pigment concentrations as the merit (error minimization) function in the numerical inversion of RT models (Zarco-Tejada *et al.*, 2001). It has been demonstrated that an index based on the ratio of reflectance in narrow bands in the red edge (e.g. at 750 and 710 nm), provides a suitable merit function for the inversion of coupled leaf and canopy models, with which to predict *Chl* from airborne hyperspectral data (Zarco-Tejada *et al.*, 2001, 2004a; Sampson *et al.*, 2003).

RT model inversion techniques offer the potential of a more generic approach to quantifying pigments from hyperspectral data than spectral pigment indices and other approaches that are based on empirical calibrations. However, intercomparisons have demonstrated that the predictive accuracy of different canopy models varies according to the canopy variable being estimated (Jacquemoud *et al.*, 2000) and the method used for the inversion (Weiss *et al.*, 2000). It has also been recognized that measurement and model uncertainties and model equifinality make inversion an ill-posed problem (Combal *et al.*, 2002). Solutions can be found by using *a priori* information

concerning the canopy under investigation, but this requirement could restrict the operational use of the inverse modelling approach.

Prospects and future directions

Intercomparison of hyperspectral approaches

When attempting to identify a robust, generic solution, there is currently only limited evidence with which to rank the performance of the range of existing hyperspectral approaches for quantifying plant pigments. Many studies have tested a number of approaches using data from only one or a small number of different species and there has been a paucity of comprehensive testing of the whole range of approaches outlined above across many species. Useful intercomparisons have been performed across a large number of species using a limited number of different hyperspectral approaches (Sims and Gamon, 2002) and across a limited number of species using a large number of different approaches (le Maire *et al.*, 2004). Interestingly, there is some agreement between these studies in terms of the optimal approaches that are identified. Both studies found that the best predictors of leaf *Chl* concentrations were simple ratio and normalized difference ratios that were modified to incorporate a waveband in the blue region as a correction for leaf surface (specular) reflectance. However, the wavelengths of the optimum three narrow bands for use in these indices differed between the two studies. Intercomparison studies such as these could usefully be extended by incorporating hyperspectral approaches to pigment quantification that are based on the numerical inversion of RT models, by testing methods at the canopy scale as well as the leaf scale and evaluating approaches for estimating independently *Chl a* and *b*, *Cars*, and *Anths*.

Scaling

Most research in this field has concentrated upon deriving relationships between the reflectance properties of leaves and their pigment concentrations. Indeed, such work has indicated that the best reflectance based indices performed better than the current commercial instrument (SPAD) for non-destructive *Chl* estimates (Richardson *et al.*, 2002; le Maire *et al.*, 2004). These leaf scale techniques may be valuable in some applications, but, in order to exploit the opportunities offered by imaging spectrometry for synoptic, simultaneous, and spatially continuous information, it is important that suitable methods are available for deriving estimates of foliar pigment concentrations from canopy scale reflectance spectra. Several strategies are available for the analysis of canopy spectra (Zarco-Tejada *et al.*, 2001), these involve: applying relationships between reflectance and pigments derived at the leaf scale directly to canopy reflectance spectra; deriving relationships directly

between canopy reflectance and ground-based measurements of pigment concentrations; using canopy RT models to scale up relationships between reflectance and pigments derived at the leaf scale; inverting canopy reflectance through coupled canopy and leaf RT models in order to estimate pigment concentrations; using hybrid approaches that employ a combination of the previous four strategies. While the advantages and disadvantages of these different strategies are reasonably well characterized in general terms and there are some useful studies that have explicitly tested one or two of the techniques, there is scope for much more extensive and systematic intercomparison. Research could usefully be directed towards producing guidelines to potential users of hyperspectral remote sensing of pigments. It may be beneficial to assess the relative merits of the five strategies according to: (i) different plant functional types or crop types; (ii) user requirements for absolute or relative accuracy of pigment estimates; (iii) the need to develop a local solution or a more general one that will operate across a range of vegetation types or environments; (iv) the requirement for data concerning pigments per unit leaf mass (often referred to as concentration) or per unit ground area (density); (v) the specific pigment or combination of pigments that are being quantified; (vi) the sensing and analytical capabilities of intended users; and (vii) the availability of prior information and ancillary data concerning the vegetation under investigation. There are also questions concerning the most effective strategies at particular scales of investigation and whether different approaches, within the limits of currently available relationships or models, become optimal as the investigations move from individual plants through canopies, stands, fields and communities up to landscapes, regions and biomes.

New methods of hyperspectral data analysis

A number of analytical approaches have emerged mainly from laboratory spectroscopy studies which hold promise for quantifying pigments from hyperspectral data. The capabilities of techniques such as modified Gaussian modelling (Sunshine and Pieters, 1993) and independent component analysis (Chen and Wang, 2001) for decomposing spectra and modelling component absorption features have been demonstrated and are worthy of further investigation in the present context. However, a technique that is already receiving some scrutiny and is showing considerable promise is that of wavelet decomposition (Graps, 1995). In laboratory spectroscopy, the ability of wavelet decomposition to remove the effects of background spectral variation when quantifying concentrations of components with fine absorption from mixtures has been demonstrated (Mittermayr *et al.*, 2001). In the present context, this offers the potential for both removing the effects of broader absorption features from the narrower features of specific pigments and for dealing with factors

which affect broader regions of vegetation reflectance spectra such as the leaf or canopy structure and soil/litter response. It has also been shown in the context of quantitative spectroscopy that wavelet decomposition has a capacity for noise suppression and it can deal with difficult situations where background varies between calibration and prediction data sets. These are potentially valuable characteristics for dealing with hyperspectral remotely-sensed imagery of vegetation. Wavelet decomposition has the potential to capture more of the information contained within high resolution spectra than narrow-band index approaches and offers the prospect of developing robust and extendible methods for pigment determinations.

The capabilities of a wavelet-based technique have been examined using reflectance spectra and pigment data collected for a range of plant species at leaf and canopy scales (Blackburn, 2006). For the combined data set and all of the individual vegetation types, methods based on wavelet decomposition appreciably outperformed the narrow-band spectral indices tested and stepwise selection of narrow-band reflectance. However, the relative performance of the three different techniques employed for selecting the wavelet coefficients for use in predictive models varied between vegetation types. There was considerable variability in the performance of predictive models according to the wavelet function used for spectral decomposition and the optimum wavelet functions differed between vegetation types and between individual pigments within the same vegetation type. The research indicates that wavelet analysis holds promise for the accurate determination of *Chl a* and *b* and *Cars*, but further work is needed to refine the approach. Such findings are supported by other preliminary work that has tested the abilities of wavelet decomposition for deriving predictive models for the remote sensing of total leaf nitrogen (Ferwerda and Jones, 2005) and *Chl* (Reum and Zhang, 2005).

Pigments under investigation

There is considerable scope for more extensive investigations of the hyperspectral remote sensing of pigments other than total *Chl*. The small number of studies that have investigated *Cars* and *Anths*, and even *Chl a* and *b* individually, belie the potential that information on their concentrations could provide. While a number of studies have demonstrated useful spectral indices for estimating these pigments at the leaf scale, the transferability across species and up to the canopy scale needs further investigation. Moreover, the range of strategies (as discussed in the section entitled 'Scaling') for achieving estimates of pigments other than *Chl* from canopy measurements have yet to be tested. Of particular importance in achieving this will be the extension of leaf and coupled leaf and canopy RT models to incorporate explicitly and individually the absorption coefficients of the range of pigments other than total *Chl*.

Some pigments, namely phytochromes, cryptochrome, and phototropin, present within leaves act as photoreceptors and allow plants to sense and respond to variations in the intensity, quality, and direction of solar irradiation through photomorphogenesis. While photoreceptor concentrations are very small compared to *Chls*, *Cars*, and *Anths*, they do have distinct absorption spectra and some impact upon the reflectance spectra of leaves and this offers the opportunity of characterizing photoreceptors *in situ* from reflectance spectra. While spectral indices designed for the major groups of pigments may ignore or discard residual information contained within the spectrum concerning photoreceptors, it may be possible that spectral decomposition techniques such as wavelet analysis could be employed, which have been shown to be sensitive to *Cars* in the presence of much higher concentrations of *Chl* (Blackburn, 2006). Indeed, spectroscopic methods have already been successfully developed to measure phytochromes in the presence of chlorophyll in leaf extracts (Lamparter *et al.*, 1994) and spectral indices for phytochromes have been applied to airborne hyperspectral imagery (Almeida and De Souza Filho, 2004), although their predictive accuracy was not tested. The composition, genesis, and functional roles of photoreceptors is under intense investigation and the development of non-invasive techniques for characterizing photoreceptors at leaf and canopy scales would facilitate substantial advances in this area of plant physiology.

Fusion of hyperspectral and LIDAR remote sensing

At the canopy scale, structural variables such as *LAI*, plant architecture, and the three-dimensional distribution of different plants can all have important influences on measured reflectance spectra and generally act to confound relationships between reflectance and leaf pigment concentrations. A possibility for measuring these structural properties is offered by LIDAR (Light Detection and Ranging) remote sensing. Large-footprint LIDARs that record the entire return pattern of each laser pulse enable detailed assessment of vegetation vertical structure, but with limited spatial sampling (a transect) and resolution. The more widely available small-footprint discrete return imaging LIDARs offer full spatial coverage and high spatial resolution. It has been demonstrated that forest canopy surface elevation models derived from such instruments can be used to create spatial filters that can be applied to (h-resolution) imaging spectrometer data from forests to extract spectral information from tree crowns, while removing extraneous spectral information from canopy gaps (Blackburn, 2002). This approach improved the accuracy of pigment estimates at the stand scale. Furthermore, recent investigations have demonstrated the possibility of measuring canopy *LAI* from imaging LIDAR (Riano *et al.*, 2004) and using this to scale-up estimates of foliar *Chl* concentration, derived from hyperspectral data, to quantify

total canopy *Chl* content (Solberg *et al.*, 2005). In addition, in the context of the hyperspectral remote sensing of pigments, LIDAR data could be used to derive site-specific model parameter ranges in generating look up tables for RT model inversion (Kotz *et al.*, 2004). Therefore, particularly for heterogenous canopies, there is considerable scope for further research to investigate the combined use of LIDAR and hyperspectral data.

Emerging applications

While the analytical approaches for hyperspectral remote sensing of plant pigments continue to be developed and refined, a wide range of applications of these techniques are emerging across various spatial scales. At the leaf scale, pigment indices applied to high resolution hyperspectral images of leaves has revealed the spatial patterns of damage to tissue and photosynthetic functioning in response to freezing and interacting light and temperature stresses (Nicotra *et al.*, 2003). Pigment indices applied to leaf spectra have provided efficient and sensitive measurements of large numbers of leaves that have been used to reveal variations in physiological stress along an elevation gradient and differences in tolerance between two coniferous species (Richardson *et al.*, 2001) and to assess the magnitude of physiological response to moderate changes in light and soil nutrient availability in temperate deciduous trees (Baltzer and Thomas, 2005). The non-destructive *in situ* monitoring of apple development has been achieved by using hyperspectral measurements of *Chl* and *Anth*s and this has provided a reliable method for determining optimum harvest date (Herold *et al.*, 2005). In the context of precision agriculture, pigment indices applied to data from field-based hyperspectral instruments have been used to determine crop nitrogen fertilizer requirements (Zillman *et al.*, 2006), to identify high grain-yielding genotypes (Gutierrez-Rodriguez *et al.*, 2004), to predict wheat grain protein content (Wang *et al.*, 2004), and to quantify gross primary production (Gitelson *et al.*, 2006). Pigment indices applied to airborne imaging spectrometry data have been used to produce accurate maps of within-field yield variability in cotton crops (Zarco-Tejada *et al.*, 2005b), the physiological condition of forests (Sampson *et al.*, 2000) and swamp vegetation (Gan *et al.*, 2002), and the distribution of different vegetation types (Fuentes *et al.*, 2001; Almeida and De Souza Filho, 2004). This array of examples reflects the increasing value of hyperspectral remote sensing of plant pigments across a range of pure and applied scientific disciplines.

Conclusion

The dynamics of plant pigments relate strongly to the physiological status of plants, therefore information concerning the temporal and spatial variations of pigments can

be a valuable indicator of a range of key properties and processes in vegetation and the wider ecosystem. The forthcoming generation of spaceborne hyperspectral sensors in combination with airborne and field instruments will open up the possibility of acquiring high resolution reflectance spectra from leaf to biome scales. Research over the last few decades has focused on developing analytical methods for extracting pigment concentrations from hyperspectral data and some convincing approaches have been developed, although no universal solutions have emerged. Progress can be made through extended intercomparison of techniques, the parallel refinement of empirically-derived approaches and RT modelling, and emphasis on defining the optimum strategies, given different circumstances and user requirements, for scaling methods up to the canopy level. A series of technological and analytical opportunities exist for enhancing the robustness, extendability and scope of hyperspectral methods for quantifying plant pigments. A range of studies have already benefited from the current capabilities and it can be anticipated that improvements in techniques will promote a wider diversity of applications.

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