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Estimation of leaf total chlorophyll and nitrogen concentrations using hyperspectral satellite imagery

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SUMMARY

Remotely sensed estimates of biochemical parameters of agricultural crops are central to the precision management of agricultural crops (precision farming). Past research using *in situ* and airborne spectral reflectance measurements of various vegetation species has proved the usefulness of hyperspectral data for the estimation of various biochemical parameters of vegetation. In order to exploit the vast spectral and radiometric resources offered by space-borne hyperspectral remote sensing for the improved estimation of plant biochemical parameters, the relationships observed between spectral reflectance and various biochemical parameters at *in situ* and airborne levels needed to be evaluated in order to establish the existence of a reliable and stable relationship between spectral reflectance and plant biochemical parameters at the pixel scale. The potential of the EO-1 Hyperion hyperspectral sensor was investigated for the estimation of total chlorophyll and nitrogen concentrations of cotton crops in India by developing regression models between hyperspectral reflectance and laboratory measurements of leaf total chlorophyll and nitrogen concentrations. A comprehensive and rigorous analysis was carried out to identify the spectral bands and spectral indices for accurate retrieval of leaf total chlorophyll and nitrogen concentrations of cotton crop. The performance of these critical spectral reflectance indices was validated using independent samples. A new vegetation index, named the plant biochemical index (PBI), is proposed for improved estimation of the plant biochemicals from space-borne hyperspectral data; it is simply the ratio of reflectance at 810 and 560 nm. Further, the applicability of PBI to a different crop and at a different geographical location was also assessed. The present results suggest the use of space-borne hyperspectral data for accurate retrieval of leaf total chlorophyll and nitrogen concentrations and the proposed PBI has the potential to retrieve leaf total chlorophyll and nitrogen concentrations of various crops and at different geographical locations.

INTRODUCTION

Measurement of various crop canopy variables during the growing season provides an opportunity for site-specific application of fertilizers to improve grain yields and quality. Important variables in this context are leaf area and total above ground biomass (Jamieson *et al*. 1998; Asseng *et al*. 2000). Further, leaf chlorophyll and nitrogen concentration in the leaf dry matter (LDM) are indicators for crop nitrogen requirements. Nitrogen is a key component of chlorophyll and, as such, different levels of nitrogen in any given plant will generally be reflected in the concentration of chlorophyll in plant leaves (Donahue *et al*. 1983). Nitrogen deficiency results in chlorosis, i.e. yellowing of leaves due to a drop in chlorophyll content. When nitrogen supply surpasses the nutritional needs of the vegetation, the excess is eliminated by runoff and water infiltration leading to pollution of aquatic ecosystems (i.e. eutrophication; Wood *et al*. 1993). This loss of nitrogen to the environment represents an economic loss for farmers. Nevertheless,

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inappropriate reduction of nitrogen supply could result in reduced yields and, subsequently, substantial economic losses. With this dilemma, the optimal and rational solution is an adequate assessment of nitrogen status and its variability in agricultural landscapes. Since yield is determined by crop condition at the earlier stages of growth, it is necessary to provide farmers with nitrogen status at these stages in order to supply appropriate rates based upon an accurate assessment of plant growth requirements and deficiencies.

The spatial and temporal variations of nitrogen status must be determined in order to match the crop requirements as closely as possible. Traditional methods of pigment analysis, through extraction and spectrophotometric or high performance liquid chromatography (HPLC) measurement, require destruction of the measured leaves and thus do not permit measurement of changes in pigments over time for a single leaf. In addition, the techniques are time consuming and expensive, thus making assessments of overall vegetation health of landscapes and ecosystems impractical. Remotely sensed estimates of the spatial and temporal variations in pigment concentrations can be important for providing improved assessments of vegetation physiological dynamics, estimating productivity and discriminating species. In the face of such potential, many workers have become involved in developing, evaluating and refining hyperspectral transformations for quantifying plant pigment concentrations through a series of empirical based studies.

Evidence from both leaf- and canopy-scale experiments suggest that relationships exist between pigment concentrations and narrowband reflectance, though there is little agreement over the optimum wavelengths.

Most studies have focused on the chlorophylls, and a fewer number of studies identify two spectral regions that are most strongly correlated with pigment concentrations: at around 680 nm, the absorption peak for Chlorophyll (Chl) *a*, and 550 nm, where absorption is at a minimum in the visible region. For example, Mariotti *et al.* (1996) found 550 nm (R_{555}) to be the narrow waveband most highly correlated with Chl *a* concentration in corn (*Zea mays* L.) and sunflower (*Helianthus annuus* L.) leaves grown under different iron treatments. Filella *et al.* (1995) studied wheat (*Triticum aestivum* L.) canopies under different fertilization treatments and found narrowband reflectance at both 550 (R_{550}) and 680 nm (R_{680}) to have curvilinear relationships with Chl *a* concentration. R_{550} was found to be sensitive over a wider range of Chl *a* concentrations up to 700 mg/m^2, while R_{680} became insensitive above 500 mg/m^2.

Yoder & Daley (1989) found that nitrogen concentration can be determined spectroscopically with visible bands (Thomas & Oerther 1972; Tsay *et al.* 1982; St.-Jacques & Bellefleur 1991), but the results rely on close correlation between nitrogen and chlorophyll, because pigments determine most spectral features between 400 and 700 nm (Gates *et al.* 1965; Woolley 1971; Thomas & Gausman 1977). Peterson *et al.* (1988) reported good relationships between near and mid-infrared reflectance features of fresh conifer foliage and lignin and nitrogen concentrations, although the predictive power was low compared to that found with dried, ground material. However, Johnson & Billow (1996) were able to predict the nitrogen concentration of fresh Douglas-fir needles precisely (coefficient of determination (R^2) = 0·93) with three shortwave infrared (SWIR) bands and the standard error of prediction with the fresh foliage was lower than that with ground, dried foliage. Martin & Aber (1993) used first-difference Airborne Visible/Infrared Imaging Spectrometer (AVIRIS) data to predict nitrogen and lignin concentrations at Harvard Forest, Massachusetts, and Blackhawk Island, Wisconsin, and reported an R^2 of 0·85 between laboratory measured and spectral data derived nitrogen and lignin.

The overlap of absorption spectra of the different photosynthetic pigments within leaves has been seen as an important limitation in the use of reflectance in individual narrowbands for estimating pigment concentrations. Furthermore, the influence of leaf and canopy structure upon the reflectance spectrum has often been identified as a major factor confounding the relationship between pigment concentration and reflectance. Therefore, a number of studies have proposed pigment indices which are ratios of reflectance in narrow visible and near-infrared (NIR) bands. Penuelas *et al.* (1995) suggested that the use of a NIR band minimizing the effects of radiation interactions with the leaf surface and internal structures. Their structure insensitive pigment index (SIPI) used ratios of reflectance at 800, 445 and 680 nm and was found to have a strong curvilinear relationship with the ratio between carotenoids (Cars) and Chl *a* ratio for leaves of a range of species. Gitelson *et al.* (1996*a*) found that a green normalized difference vegetation index (NDVI), employing a green band centred on 500 nm rather than a red band as in the traditional NDVI, was highly correlated with Chl *a* concentrations in maple and chestnut leaves and sensitive over the range 3–450 mg/m^2. Blackburn (1998) found that ratios of narrow NIR and visible wavebands, termed the pigment-specific simple ratio (PSSR) and pigment-specific normalized difference (PSND), have strong correlations with the concentrations of Chl *a*, Chl *b* and Cars at both leaf and canopy scales. Yoder & Pettigrew-Crosby (1995) reported that SWIR bands were the best predictors for nitrogen, visible bands best for chlorophyll. However, in the SWIR region, the absolute differences in the reflectance at critical bands were extremely small, and the bands of high correlations are narrow. In addition, the best SWIR wavebands from leaf scale are not good predictors of chemical content or concentration at the canopy scale. As a consequence of the different measurement conditions, there exists some disagreement in the selection of wavebands.

The present brief literature review illustrates that there is currently little consensus over which spectral transformations have the strongest relationships with the concentrations of plant photosynthetic pigments. While some appear to be applicable across a range of species, there is limited evidence of the effectiveness of different transformations over a range of scales. A number of previous studies have tackled this problem at the leaf scale, and some at the canopy scale, but few have systematically explored the relationships between spectral reflectance and photosynthetic pigment concentrations at landscape scale.

To derive the benefits of advances being made in the space-borne hyperspectral imaging technology for agricultural crop studies at the operational level, these relationships should be validated using space-borne hyperspectral reflectance data and we must be able to generalize across species and phenological stages.

The objective of the present study was to develop spectral indices for prediction of leaf chlorophyll and nitrogen concentration in semiarid agricultural crops (cotton and rice) using space-borne hyperspectral reflectance data. In particular, the aims were to (i) identify critical spectral reflectance indices for predicting total cotton crop chlorophyll (Chl *t*) and leaf nitrogen concentration (LNC) and (ii) develop and evaluate a critical reflectance index for monitoring rice crop Chl *t* and LNC using space-based hyperspectral data (Hyperion). Further, an attempt was made to evaluate whether leaf nitrogen accumulation (LNA), which is also an indicator of leaf nitrogen content in vegetation, could be used an as alternative to LNC. The intended outcome is to enable the application of the necessary amounts of fertilizer nitrogen when and where it is needed to optimize crop nitrogen management and yield.

MATERIALS AND METHODS

Study area

The study site for the present work lies in Guntur district, Andhra Pradesh State of India, situated between 16°7′31″ – 16°50′55″ N and 79°40′37″ – 79°44′49″ E. The annual rainfall of the district is 889 mm, while the climate is semi-arid and generally warm in summer. The soils in general are very fertile and are broadly classified as Black cotton (0·70 of the total area of the district), Red loamy (0·24 of the area) and Sandy loamy (0·06). The predominant cereals grown in the district are *Oryza sativa* (rice), *Sorghum vulgare* (jowar or sorghum) and *Pennisetum glaucum* (bajra or pearl millet), with the main pulses being *Vigna mungo* (black gram), *Vigna radiata* (green gram or mung bean) and *Cajanus cajan* (red gram or pigeon pea), with cotton, chillies, sugarcane, turmeric and tobacco predominant among non-food and commercial crops. Most of the cultivation in the study area is based on canal irrigation. During the summer, 0·10–0·15 of the farms in the study area are irrigated from deep wells.

Data used

The data in the present study were collected from various sources, including reflectance data from EO-1 Hyperion hyperspectral imagery, laboratory measured Chl *t* and LNC values of field samples of cotton and rice, and ancillary data regarding crop management practices at the sample collection locations.

Hyperion image pre-processing

A single Hyperion image taken on 28 September 2003 (Path/Row No. 143/48), representing the kharif (wet) season of 2003 (June–November) was used. The Hyperion image was georeferenced with the help of Survey of India (SOI) toposheets after selecting 30 Ground Control Points (GCPs), ensuring proper distribution throughout the image with root mean squared error (RMSE) of 0·31 pixel. The image was resampled with a 30 m pixel size using the nearest neighbourhood method. The image processing was carried out using ERDAS IMAGINE 8.6 software (Leica Geosystems, Norcross, GA, USA).

Since Hyperion operates from space, with consequently modest surface signal levels and full-column atmospheric effects, its data demand careful processing. The striping was minimized using the Fast Fourier Transform technique (Peleg 1998). This 'destriped' Hyperion image was converted into a radiance image with the help of gain and offset coefficients, supplied along with the satellite data. Further, this radiance image was used to compute the equivalent surface reflectance by performing atmospheric corrections using Fast Line-of-sight Atmospheric Analysis of Hypercubes (FLAASH) atmospheric correction software module (Matthew *et al.* 2003).

Collection of leaf samples and laboratory measurement of Chl t*, LNC and LNA*

A 2-day field campaign was conducted on 29–30 September 2003, immediately after the date that the satellite passed over the area, to collect leaf samples of cotton and rice crops. At that time, most of the rice crops were at the late vegetative growth stage and

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some fields at visual panicle initiation. Cotton fields were at the mature stage. In order to collect samples of cotton and rice leaves simultaneously, only those sites where large patches of both cotton and rice crops were to be found within few hundred metres were selected for sample collection. At each of 109 collection sites, the top five fully expanded leaves were harvested from each crop, placed in a cooler over ice and transported to a privately owned contract research company operating in the study area (M/s SVS Agro-Genetics Research Services Pvt. Ltd, Guntur, Andhra Pradesh State, India), where facilities were available for the extraction of Chl *t* and LNC in the laboratory. A total of 545 leaves were collected for each crop.

After collecting leaf samples at each measurement site, its exact location (i.e. a plot) was marked on the Hyperion colour composite map with the help of GPS readings, taken simultaneously. These were used as references for subsequent spectral data extraction from the Hyperion image. A cork borer was used to remove five discs from each leaf, avoiding areas with large veins. Leaf discs were placed into vials containing 4·0 ml dimethyl sulphoxide (DMSO) and kept at room temperature overnight in the dark to extract pigments. Absorbances of the extract at 470, 648 and 664 nm were recorded and concentrations of Chl *a*, *b* and total chlorophyll (Chl $t = \text{Chl } a + \text{Chl } b$) were computed following the formulae given by Chappelle *et al.* (1992).

Chlorophyll concentration of the extract and the total disk surface area of 98 mm^2 were used to compute Chl *t* per unit projected leaf area. The top fully expanded leaves were sampled for Chl *t*, as these leaves express early symptoms of nitrogen stress, such as chlorosis. The leaves used for pigment analysis were pooled and dried at 70 °C for 72 h, and LNC was determined subsequently on dried, ground samples, according to standard micro-Kjeldahl method (Nelson & Sommers 1972).

Measurements of plant biomass components were obtained by randomly harvesting four to five hills (clumps of plants transplanted together) for the rice crop. From each sample, a sub sample of 10–20 tillers was taken from each plot, placed in separate bags, oven dried at 70 °C to constant weight and then weighed for measurement of leaf dry weight per unit ground area. Similarly for the cotton crop, all leaves of a single cotton plant canopy from each plot were cut and put in separate bags, oven dried at 70 °C to constant weight and then weighed for measurement of leaf dry weight per unit ground area. The LNA was calculated as the product of LNC per unit dry weight and leaf area index (LAI). A total of 109 observations each for cotton and rice crops for total chlorophyll and nitrogen concentration were computed from samples collected during field visits. The total set of leaf data was divided into subsets for calibration (57 for nitrogen, 57 for chlorophyll) and validation (52 for nitrogen, 52 for chlorophyll) by random assignment.

Correlation analysis of Chl t *LNC and LNA with spectral reflectance from Hyperion*

Pixel-based retrieved reflectance spectra from the calibrated Hyperion image at the leaf sample measurement plots were extracted from the image. Four to five homogenous pixels were extracted and averaged for each measurement plot. Linear regression analysis was carried out amongst Chl *t*, LNC, LNA and spectral reflectance and various band ratios. The value of the coefficient of determination was used as the criterion for the selection of spectral reflectances and band ratios sensitive to the variability presented in Chl *t*, LNC and LNA. Finally, all the sensitive band ratios were ranked, based on the precision and accuracy of retrieval of Chl *t*, LNC and LNA to optimize the estimation of Chl *t*, LNC and LNA from the Hyperion data.

Data analysis

Coefficients of determination (R^2) for individual wavebands at 10-nm resolution were plotted to determine spectral regions of greatest sensitivity to Chl *t*, LNC and LNA. The R^2 value indicates the fraction (0–1) of variability in Chl *t*, LNC and LNA that can be accounted for by plant reflectance features. The maximum value of R^2 was used to identify the wavebands, from which a denominator reflectance band could be selected for computing simple ratios with reflectance values of Hyperion at 10 nm intervals between 400 and 2500 nm as the numerator. Values for R^2 from the linear relationship between all possible reflectance ratios and leaf constituents were computed. Linear regression analysis was used to develop relationships between the waveband ratios with maximum R^2 and Chl *t*, LNC and LNA for cotton crops. Apart from all the possible waveband ratios, the classical NDVI, which is a widely used vegetation index for mapping and monitoring of vegetation, was also tested for the prediction of Chl *t*, LNC and LNA.

The regression model developed was validated using 52 independent samples of Chl *t*, LNC and LNA from cotton crops. Further, in order to evaluate the applicability of potential waveband ratios to various crops, the best waveband ratio obtained from the present study was used to estimate Chl *t*, LNC and LNA of rice crop using linear regression method. For validation of the regression model, proportional standard error of estimation was calculated to test the goodness of fit between the estimated and observed values.

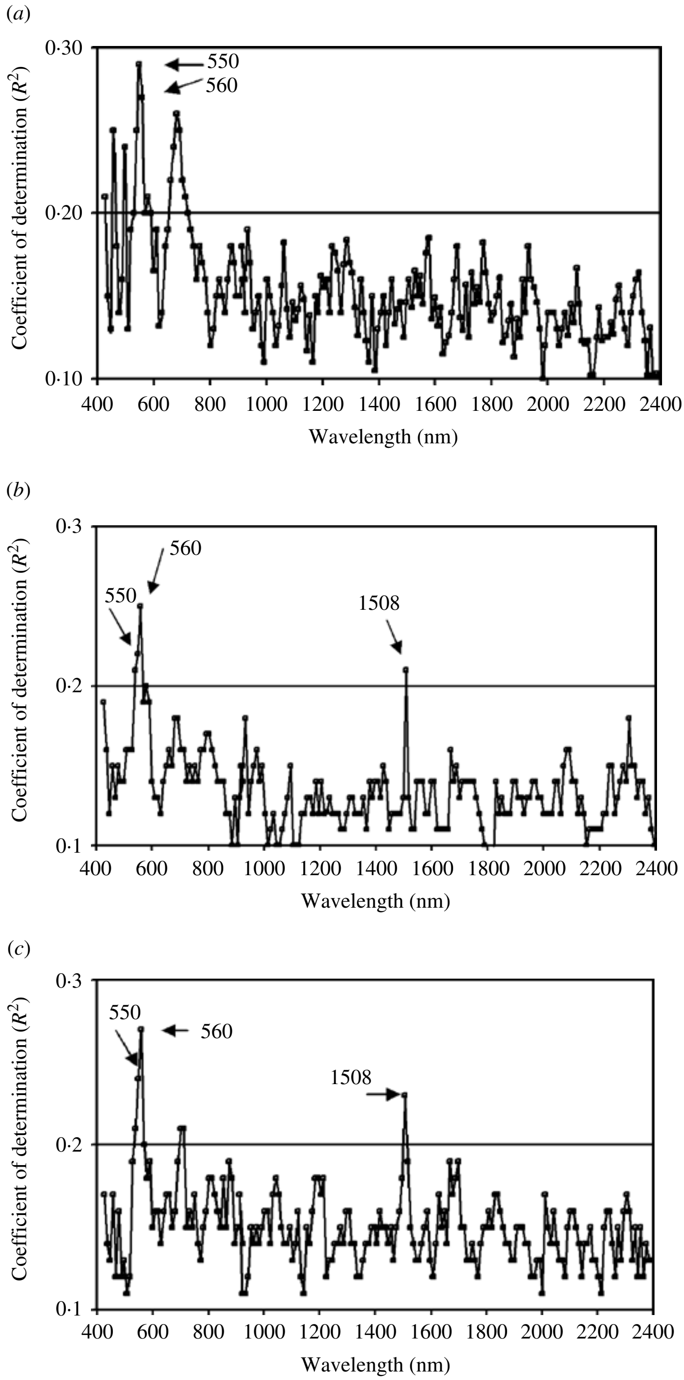

Fig. 1. Variation of R^2 between spectral reflectance across all Hyperion bands and (*a*) Chl *t*, (*b*) LNC and (*c*) LNA.

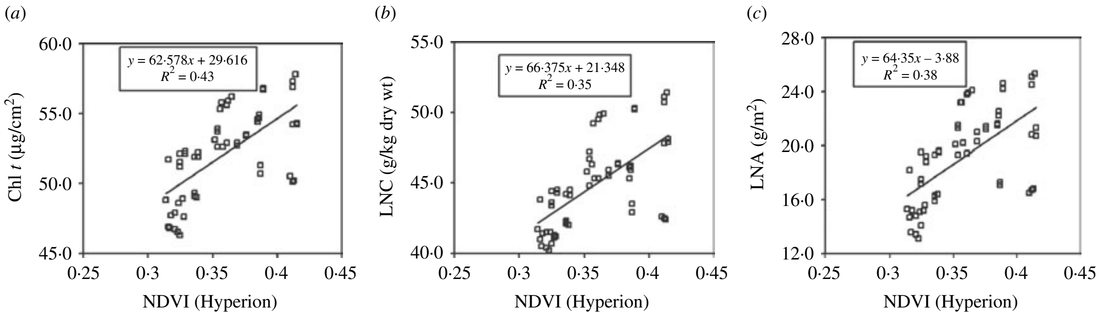

Fig. 2. Relationship between NDVI and (*a*) Chl t, (*b*) LNC and (*c*) LNA.

RESULTS

Correlation of Chl t*, LNC and LNA to Hyperion waveband reflectance*

It is clear that Chl *t* shows a better correlation coefficient in the visible region in the entire wavelength spectrum of 400–2500 nm (Fig. 1 *a*). In particular, the spectral reflectance at 457, 540, 550, 560, 671, 681 and 691 nm had correlation coefficients of 0·25, 0·25, 0·29, 0·27, 0·24, 0·26 and 0·25, respectively, which were higher than that of the entire range of the spectrum between 400 and 2500 nm. The correlations observed at 457, 671, 681 and 691 nm are indicative of the strong absorbance of chlorophylls in the blue and red regions of the spectrum. Because the blue wavelength region peak overlaps with the absorbance of the Cars, the 457 nm wavelength is not generally used in estimation of chlorophyll content. Similarly, correlations observed at 671, 681 and 691 nm wavelengths have proved to be less useful for prediction of chlorophyll content in comparison to reflectance at slightly longer (towards red-edge) or shorter (green-peak) wavelengths, because relatively low chlorophyll contents are sufficient to saturate the red absorption, thus reducing those spectral indices based on red wavelengths. A moderate and consistent correlation was observed between the Chl *t* and the red-edge region (680–730 nm), supporting the importance of red-edge region for the study of various vegetation processes.

Similar trends have been observed between spectral reflectance of individual wavebands and LNC (Fig. 1 *b*). Here again, the narrow wavebands in the green wavelengths have shown high correlation amongst all the bands, however, with a fine spectral shift of 10 nm for the best correlation; thus 560 nm is the best sensitive band. Besides the green reflectance plateau, a sharp increase in the correlation is observed in the SWIR wavelength region at 1508 nm (R^2 = 0·21). This may be due to the presence of a weak characteristic absorption feature of LNC at around 1510 nm.

It is evident that LNA has reflectance characteristics similar to LNC, with an exception in the blue wavelengths (Fig. 1 *c*). However, LNA shows slightly higher correlation to reflectance at 560 nm than LNC, indicating improved relationship between spectral reflectance and leaf nitrogen while taking ground-sampling area into consideration.

Clearly, the wavebands 550 and 560 nm offer the highest correlations of single band reflectances to Chl *t*, LNC and LNA, respectively. However, the highest correlation obtained is less than 0·3 for either of above two wavebands thus indicating the limitations of using a single waveband for the estimation of Chl *t*, LNC and LNA.

Correlation of NDVI with Chl t*, LNC and LNA*

The broadband version of NDVI has been used in numerous studies involving a range of applications involving identification, quantification, mapping and monitoring of vegetation and vegetation bio-physio-chemical parameters. However, its application to the estimation of leaf constituents, like Chl *t*, LNC and LNA of agricultural crops using space-borne narrowbands is yet to be explored. In the present case, a moderate positive correlation (R^2=0·43) has been observed between NDVI and Chl *t* (Fig. 2). However, NDVI showed a lower correlation with LNC (R^2= 0·35) and LNA (R^2=0·38), indicating the inability of the red and NIR based NDVI to estimate leaf pigments and biochemicals.

Identification of sensitive waveband ratios

The analysis of all possible waveband ratios with Chl *t*, LNC and LNA revealed that waveband ratios 810/550, 810/560, 860/550 and 860/560 had correlation coefficients greater than 0·80 (Fig. 3). These waveband ratios were strongly correlated with Chl *t*, LNC and LNA (Fig. 4). However, the spectral ratios 810/550 and 860/550 are considered unreliable for estimation of Chl *t* because of the characteristic absorption of anthocyanin at 550 nm. The spectral ratios 810/560 (Fig. 4 *b*) and 860/560 (Fig. 4 *c*) appear to have stable responses and high correlations

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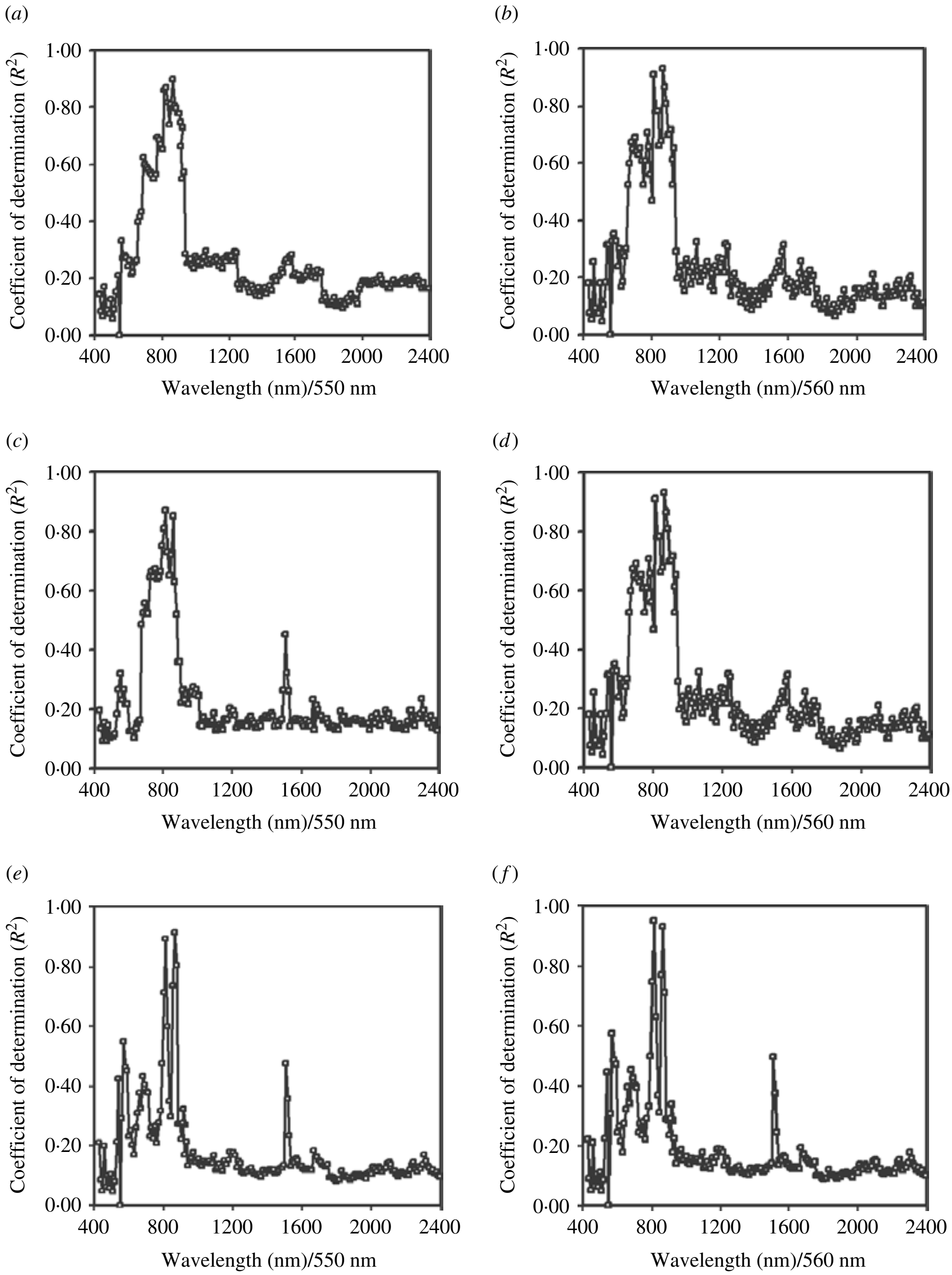

Fig. 3. Variation of R^2 between Chl t (*a*, *b*), LNC (*c*, *d*), LNA (*e*, *f*) and all possible band ratios of Hyperion with 550 and 560 nm as denominator respectively.

(R^2=0·91, 0·93) among all the possible waveband ratios of Hyperion data. Similarly, high and consistent correlations between LNC, LNA and waveband ratios 810/550, 860/550 and 860/560 were observed (Fig. 4). The correlation coefficient obtained from all the four waveband ratios are similar to that obtained for Chl *t*, thus indicating a possibility of adapting a single waveband ratio for simultaneous prediction of Chl *t*, LNC and LNA from reflectance measurements. The highest correlation coefficient is obtained for

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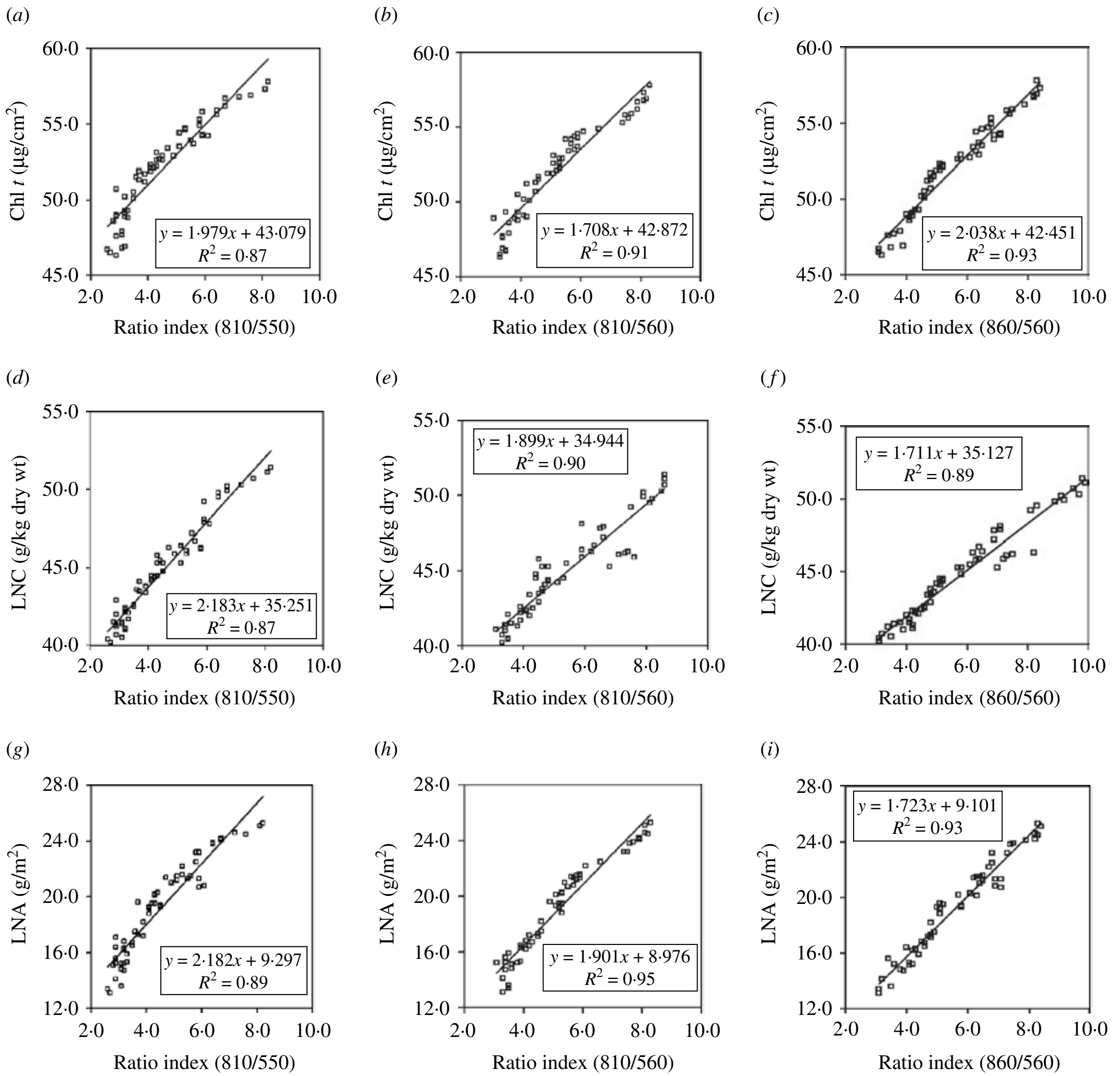

Fig. 4. Relationship between best waveband ratio indices and Chl *t* (*a*–*c*), LNC (*d*–*f*), LNA (*g*–*i*).

waveband ratios 810/560 (R^2=0·90) and 860/560 (R^2=0·89). It was observed that when the LNC exceeded 50 g N/kg dry wt, the regression on waveband ratios 810/550 and 860/550 exhibited a lower slope. The coefficient of determination of LNA was 5% higher than that of LNC.

To identify a single spectral index that is applicable to Chl *t*, LNC or LNA, all the regressions developed in the present study have been examined for precision (a correlation value close to 1·0 would indicate high precision) and accuracy (slope close to 1·0 when the intercept is 0 would indicate high accuracy), as described by Massart *et al.* (1988). Although the difference of coefficients of determination (R^2) of 810/550, 810/560, 860/550 and 860/560 to Chl *t* and LNC or LNA are less than 5%, the precision and accuracy of 810/550, 860/550 and 860/560 were less than that of 810/560. Hence, the waveband ratio 810/560 was selected as the best spectral index for the estimation of Chl *t* and LNC or LNA at landscape (pixel) scale and was named the PBI.

Validation of the proposed PBI

Evaluation of the performance of the proposed PBI for predicting plant biochemical concentrations requires application over a wide range of vegetation types and locations. In the present study, the

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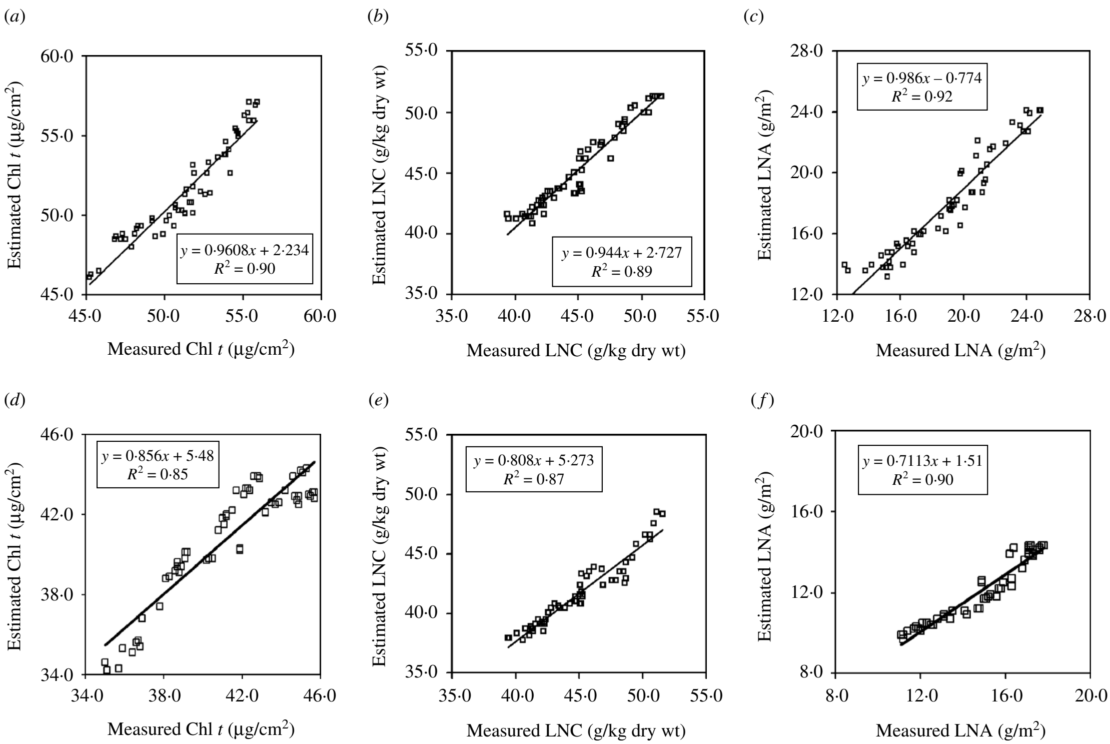

Fig. 5. Correlation between estimated and measured Chl *t*, LNC, and LNA of cotton (*a–c*) and rice (*d–f*).

proposed PBI was validated using independent samples of Chl *t*, LNC and LNA determined on cotton crops (Fig. 5). There was a strong and consistent correlation between laboratory measured and model estimated Chl *t* ($R^2 = 0{\cdot}90$), LNC ($R^2 = 0{\cdot}89$) and LNA ($R^2 = 0{\cdot}92$). The average proportional standard error of estimation between the predicted and observed values of Chl *t* was 0·015 while it was 0·012 and 0·020 for LNC and LNA respectively.

Perhaps surprisingly, the use of the PBI derived from cotton for the prediction of Chl *t*, LNC and LNA of rice crops resulted in significant correlations ($R^2 = 0{\cdot}85$, 0·87 and 0·90, respectively). The average proportional standard error of estimation was 0·042, 0·049 and 0·031, respectively.

DISCUSSION

The present study has investigated the possibilities of scaling up the leaf and canopy level relationships observed between spectral reflectance and plant constituents. Linear regressions of numerous spectral indices formed with various band combinations of Hyperion imagery were analysed for predicting the foliar concentrations of Chl *t*, LNC and LNA. While the classical NDVI has been the most successful and widely used spectral vegetation index for the estimations of a range of vegetation state parameters, its application to the estimation of foliar biochemical composition has not yielded significant relationships at the landscape scale. The present results support previous observations that ratio of NIR to green wavelengths had a good relationship with chlorophyll per unit land area and nitrogen accumulation (Shibayama & Akiyama 1986; Takihashi *et al.* 2000). Similarly, in wheat, NDVI is found to be linearly related to LNA (Hinzman *et al.* 1986). However, the present study shows that the correlation coefficients between NDVI and Chl *t*, LNC and LNA are lower than that of any of the four Hyperion waveband ratios identified in the study.

This difference can be attributed to the fact that canopy reflectance is mainly influenced by LAI, background reflectance and Chl *t*. NDVI minimizes contributions of background reflectance but is relatively insensitive to chlorophyll concentration, whereas NIR/green ratio is responsive to both leaf chlorophyll concentration and background reflectance (Daughtry *et al.* 2000; Gitelson & Merzlyak 1997; Gitelson *et al.* 1996*b*). The green band (560 nm) is extremely sensitive to total chlorophyll concentration (Blackmer & Schepers 1994; Wang *et al.* 1998), while 810 nm, a NIR band related to leaf structure, tends to normalize the index with respect to canopy properties and provide a baseline for the leaf chlorophyll or nitrogen-sensitive band. This may increase the precision and accuracy of the NIR/green ratio compared with NDVI. Further, the ratio between reflectance of a stress-sensitive band and a stress-insensitive band could correct for the variation of canopy reflectance resulting from the variation in irradiance, leaf orientation, irradiance angles and shading (Tarpley *et al*. 2000).

Another interesting aspect of the present study is the increased precision of the estimation of leaf nitrogen using LNA instead of LNC. LNA provided larger differences between the reflectances at 560 and 550 nm wavelengths, making it more sensitive to nitrogen concentration. Moreover, LNA is the product of LNC and LDM per unit ground area, while LDM is the product of LAI and leaf mass per unit area (LMA), i.e. $LNA = LNC \times LDM = LNC \times LMA \times LAI$ (Shibayama & Akiyama 1986). Thus, LNA includes not only the impact of leaf area, but also the impact of specific leaf weight (SLW), which is associated with the leaf structure and components. The spectra obtained by remote sensing satellites reflect the complex information of whole vegetation, including the leaves, stems, spikes, soil and other backgrounds. Thus, estimation of canopy-based variables such as LNA may be more correct than leaf-based variables, such as LNC, using space-borne hyperspectral data.

The results obtained in the present study establish the use of space-borne hyperspectral remote sensing measurements for spatial estimation of plant leaf biochemicals at the landscape level, giving impetus to precision agriculture research by establishing actual working relationships between crop variables and reflectances at the landscape level. The proposed PBI avoids the existing practice of using various spectral indices for the estimation of Chl *t*, LNC and LNA, making use instead of a single spectral transformation for their estimation. Canopy reflectance in a single band often will be confounded by the variability in background reflectance and LAI. However, this can be circumvented by constructing spectral ratio indices which are sensitive to Chl *t*, LNC and LNA while minimizing the variations in canopy reflectance associated with background reflectance and LAI (Daughtry *et al*. 2000). This present study suggests the use of a limited set of wavebands for the construction of spectral indices, such as the PBI proposed, for optimal utilization of vast spectral resources.

The practical use of image-based estimates of Chl *t* and LNA for determining crop requirements needs simultaneous estimation of growth status such as leaf dry weight and leaf area. Given the spatio-temporal dynamics of crop-spectral reflectance interactions, the parameter-specific spectral bands identified in the present study require thorough validation by wider studies before being advanced for operational use. This is necessitated by the fact the empirical models are generally site-specific and data-specific. Although the relationships obtained with cotton crops in the present study appear to be transferable to rice crops, the robustness of the relationships need to be evaluated for a wider range of crops, preferably at various geographical locations and environments.

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REFERENCES

ASSENG, S., VAN KEULEN, H. & STOL, W. (2000). Performance and application of the APSIM Nwheat model in the Netherlands. *European Journal of Agronomy* **12**, 37–54.

BLACKBURN, G. A. (1998). Quantifying chlorophylls and carotenoids at leaf and canopy scales: an evaluation of some hyperspectral approaches. *Remote Sensing of Environment* **66**, 273–285.

BLACKMER, T. M. & SCHEPERS, J. S. (1994). Techniques for monitoring crop nitrogen status in corn. *Communications in Soil Science and Plant Analysis* **25**, 1791–1800.

CHAPPELLE, E. W., KIM, M. S. & MCMURTREY, J. III. (1992). Ratio analysis of reflectance spectra (RARS): an algorithm for the remote estimation of the concentrations of chlorophyll a, chlorophyll b, and carotenoids in soybean leaves. *Remote Sensing of Environment* **39**, 239–247.

DAUGHTRY, C. S. T., WALTHALL, C. L., KIM, M. S., DE COLSTOUN, E. B. & MCMURTREY, J. E. (2000). Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. *Remote Sensing of Environment* **74**, 229–239.

DONAHUE, R. A., BERG, V. S. & VOGELMANN, T. C. (1983). Assessment of the potential of the blue light gradient in soybean pulvini as a leaf orientation signal. *Physiologia Plantarum* **79**, 593–598.

FILELLA, I., SERRANO, L., SERRA, J. & PENUELAS, J. (1995). Evaluating wheat nitrogen status with canopy reflectance indices and discriminant analysis. *Crop Science* **35**, 1400–1405.

GATES, D. M., KEEGAN, H. J., SCHLETER, J. C. & WEIDNER, V. R. (1965). Spectral properties of plants. *Applied Optics* **4**, 11–20.

GITELSON, A. A. & MERZLYAK, M. N. (1997). Remote estimation of chlorophyll content in higher plant leaves. *International Journal of Remote Sensing* **18**, 2691–2697.

GITELSON, A. A., KAUFMAN, Y. J. & MERZLYAK, M. N. (1996*a*). Use of a green channel in remote sensing of global vegetation from EOS-MODIS. *Remote Sensing of Environment* **58**, 289–298.

GITELSON, A. A., MERZLYAK, M. N. & LICHTENTHALER, H. K. (1996*b*). Detection of red edge position and chlorophyll content by reflectance measurements near 700 nm. *Journal of Plant Physiology* **148**, 501–508.

HINZMAN, L. D., BAUER, M. E. & DAUGHTRY, C. S. T. (1986). Effects of nitrogen fertilization on growth and reflectance characteristics of winter wheat. *Remote Sensing of Environment* **19**, 47–61.

JAMIESON, P. D., PORTER, J. R., GOUDRIAAN, J., RITCHIE, J. T., VAN KEULEN, H. & STOL, W. (1998). A comparison of the models AFRCWHEAT2, CERES-wheat, Sirius, SUCROS2 and SWHEAT with measurements from wheat grown under drought. *Field Crops Research* **55**, 23–44.

JOHNSON, L. F. & BILLOW, C. R. (1996). Spectrometric estimation of total nitrogen concentration in Douglas-fir foliage. *International Journal of Remote Sensing* **17**, 489–500.

MARIOTTI, M., ERCOLI, L. & MASONI, A. (1996). Spectral properties of iron-deficient corn and sunflower leaves. *Remote Sensing of Environment* **58**, 282–288.

MARTIN, M. E. & ABER, J. D. (1993). High spectral resolution remote sensing of forest canopy lignin, nitrogen and ecosystem process. *Ecological Applications* **7**, 431–443.

MASSART, D. L., VANDEGINSTE, B. G. M., DEMING, S. N., MICHOTTE, Y. & KAUFMAN, L. (1988). *Chemometrics: A Textbook*. Amsterdam: Elsevier.

MATTHEW, M. W., ADLER, S. M., BERK, A., FELDE, G. W., ANDERSON, G. P., GORODETZKY, D., PASWATERS, S. E. & SHIPPERT, M. (2003). Atmospheric correction of spectral imagery: evaluation of the FLAASH algorithm with AVIRIS data. *Proceedings of the SPIE* **5093**, 474–482.

NELSON, D. W. & SOMMERS, L. E. (1972). A simple digestion procedure for estimation of total nitrogen in soils and sediments. *Journal of Environmental Quality* **1**, 423–425.

PELEG, K. (1998). Fast Fourier-Transform based calibration in remote sensing. *International Journal of Remote Sensing* **19**, 2301–2315.

PENUELAS, J., FILELLA, I. & GAMON, J. A. (1995). Assessment of photosynthetic radiation-use efficiency with spectral reflectance. *New Phytologist* **131**, 291–296.

PETERSON, D. L., ABER, J. D., MATSON, P. A., CARD, D. H., SWANBERG, N., WESSMAN, C. A. & SPANNER, M. (1988). Remote sensing of forest canopy and leaf biochemical contents. *Remote Sensing of Environment* **24**, 85–108.

SHIBAYAMA, M. & AKIYAMA, T. A. (1986). A spectroradiometer for field use: VI. Radiometric estimation for chlorophyll index of rice canopy. *Japanese Journal of Crop Science* **55**, 433–438.

ST-JACQUES, C. & BELLEFLEUR, P. (1991). Determining leaf nitrogen concentration of broadleaf tree seedlings by reflectance measurements. *Tree Physiology* **8**, 391–398.

TAKIHASHI, W., NGUYEN-CONG, V., KAWAGUCHI, S., MINAMIYAMA, M. & NINOMIYA, S. (2000). Statistical models for prediction of dry weight and N accumulation based on visible and near-infrared hyper spectral reflectance. *Plant Production Science* **3**, 377–386.

TARPLEY, L., REDDY, K. R. & SASSENRATH-COLE, G. F. (2000). Reflectance indices with precision and accuracy in predicting cotton plant leaf nitrogen concentration. *Crop Science* **40**, 1814–1819.

THOMAS, J. R. & GAUSMAN, H. W. (1977). Leaf reflectance vs. leaf chlorophyll and carotenoid concentration for eight crops. *Agronomy Journal* **69**, 799–802.

THOMAS, J. R. & OERTHER, G. F. (1972). Estimating nitrogen content of sweet pepper leaves by reflectance measurements. *Agronomy Journal* **64**, 11–13.

TSAY, M. L., GJERSTAD, D. H. & GLOVER, G R. (1982). Tree leaf reflectance: a promising technique to rapidly determine nitrogen and chlorophyll content. *Canadian Journal of Forestry Research* **12**, 788–792.

WANG, K., SHEN, Z. Q. & WANG, R. C. (1998). Effects of N nutrition on the spectral reflectance characteristics of rice leaf and canopy. *Journal of Zhejiang Agricultural University* **24**, 93–97.

WOOD, C. W., REEVES, D. W. & HIMELRICK, D. G. (1993). Relationships between chlorophyll meter reading and leaf chlorophyll concentration, N status, and crop yield: a review. *Proceedings of the Agronomy Society of New Zealand* **23**, 1–9.

WOOLLEY, J. T. (1971). Reflectance and transmittance of light by leaves. *Plant Physiology* **47**, 656–662.

YODER, B. J. & DALEY, L. S. (1989). Development of a visible spectroscopic method for determining chlorophyll a and b *in vivo* in leaf samples. *Spectroscopy* **5**, 44–50.

YODER, B. J. & PETTIGREW-CROSBY, R. E. (1995). Predicting nitrogen and chlorophyll content and concentrations from reflectance spectra (400–2500 nm) at leaf and canopy scales. *Remote Sensing of Environment* **53**, 199–211.