Narrow band spectral indexes for chlorophyll determination in soybean canopies [*Glycine max* (L.) Merril]

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Photosynthetic pigments are essential for plant development. Quantifying these pigments in great extensions of agricultural crops is an important objective in remote sensing for agricultural purposes. This information can be used to produce a more accurate estimation of the physiological state of the vegetation, for species discrimination and productivity estimation. The aim of the present study was to (a) evaluate the potential for estimating chlorophyll content of crop canopies, using narrow band spectral indexes, and (b) in this respect compare the performances of NDVI (a multispectral wide band index) and two narrow band vegetation indexes ($R_{750/700}$ and $R_{750/550}$). Experiments were carried out under greenhouse conditions whereby soybean [*Glycine max* (L.), Merril] was monitored with a high-resolution spectroradiometer (10 nm at 365-1,126 nm range) during the phenological cycle of the crop. Chlorophyll (*a*, *b* and total) contents were determined weekly in the laboratory. A statistical correlation analysis was performed between narrow band spectral indexes against chlorophyll content and r^2 coefficients near 0.84 were obtained. For NDVI r^2 was around 0.51. These analyses showed that $R_{750/700}$ and $R_{750/550}$ ratios are very useful indexes for chlorophyll determination and very effective compared with NDVI (one of the wide band indexes widely used). Thus, it can be stated that hyperspectral remote sensing has great potential for providing a reliable estimate of photosynthetic pigment content at the canopy level through evaluated indexes and other such indexes that might arise. Thus, further studies should be carried out for evaluating other indexes at the canopy level, both in the laboratory and under field conditions, using spectroradiometers and hyperspectral images, aimed at providing information for agricultural purposes.
Key words: canopy chemistry, hyperspectral remote sensing, remote sensing in agriculture, spectroradiometry.

Índices hiperespectrais de vegetação para a determinação do conteúdo de clorofila em dosséis de soja [*Glycine max* (L.) Merril]: Os pigmentos fotossintéticos são essenciais ao desenvolvimento das plantas e sua quantificação em grandes extensões de culturas agrícolas é uma das metas mais importantes do sensoriamento remoto aplicado à agricultura. Essa informação pode ser utilizada para determinar, mais precisamente, o estado fisiológico das plantas, discriminar as espécies plantadas e estimar a produtividade. O presente trabalho teve como objetivos: (a) avaliar o acerto da estimativa do conteúdo de clorofila em dosséis de culturas, usando índices espectrais de bandas estreitas, e (b) comparar os desempenhos do Índice de Vegetação Diferença Normalizada (NDVI) em relação a dois índices de bandas estreitas ($R_{750/700}$ e $R_{750/550}$). Desenvolveu-se um experimento em condições de casa de vegetação cuja cultura da soja [*Glycine max* (L.) Merril] foi monitorada, durante o seu ciclo fenológico, com um espectrorradiômetro de alta resolução espectral (10 nm no intervalo de 365 a 1.126 nm). Determinaram-se os conteúdos de clorofila (*a*, *b* e total), semanalmente, em laboratório, e calcularam-se correlações estatísticas entre os índices de bandas estreitas e o conteúdo de clorofilas, obtendo-se coeficiente r^2 de 0,84, enquanto para o índice NDVI, obteve-se 0,51. Segundo as análises realizadas, as razões $R_{750/700}$ and $R_{750/550}$ constituem importantes índices para a determinação das clorofilas em dosséis, sendo mais efetivas em comparação ao NDVI, um dos índices de banda larga mais utilizados. Dessa forma, pode-se dizer que os índices avaliados (e outros que possam ser gerados) possuem grande potencial para estimar o conteúdo de pigmentos fotossintéticos em dosséis agrícolas.
Palavras-chave: espectrorradiometria, química de dossel, sensoriamento remoto hiperespectral, sensoriamento remoto em agricultura.

INTRODUCTION

Chlorophylls are essential for plant development, as they are responsible for the capture of incident solar energy necessary for photosynthesis. With the development of hyperspectral remote sensing in the 1980s, the possibility has arisen to quantify each of these pigments individually in extensive agricultural crop areas (Dawson et al., 1998a,b; Fourty and Baret, 1998; Green et al., 1998; Martin et al., 1998; Dawson et al., 1999; Jago et al., 1999, Kokaly and Clark, 1999; Gitelson et al., 2002). Such information can be used for the determination of the physiological state of the vegetation (detecting stresses), for species discrimination (monitoring phenological characteristics) and for productivity estimation (measurement and interpretation of absorbed photosynthetically active radiation) (Blackburn, 1998a,b). Furthemore, in the last decade, attention has been focused on the utilisation of non-destructive techniques to quantify elements from leaves (Blackburn and Steele, 1999). One possible way to make use of the hyperspectral sensor data for the determination of pigment concentration is to use spectral reflectance indexes. These indexes use narrow spectral bands in the form of addition, ratio or multiplication, and their objective is to minimise interfering effects in the spectral data (for example the soil background and structural factors), thus allowing more specific data for the determination of the photosynthetic components of the vegetation.

The present work was carried out considering the prospect of new sensors of very fine spectral resolution (named hyperspectral sensors), which are already in use or forthcoming (currently used in aircraft and satellites). It is assumed that significant relationships exist between the spectral reflectance of an agricultural crop and its agronomic parameters. The main goal of the present investigation is the evaluation of photosynthetic pigments of the soybean crop through spectral indexes, using both the narrow and wide band spectral data. Thus, experiments were carried out under greenhouse conditions whereby soybean [*Glycine max* (L.), Merril] plants were monitored at weekly intervals with a high-resolution spectroradiometer, throughout the phenological cycle of the crop.

MATERIAL AND METHODS

The experiments were performed under greenhouse conditions, in the Department of Plant Physiology at the State University of Campinas (UNICAMP), at Campinas, São Paulo, Brazil), in August, 2000, using a totally randomized statistical design. A precocious soybean cultivar (IAC 17) was planted in trays containing washed vermiculite, and maintained in the greenhouse. When the plants produced fully developed cotyledons (V_C stage, according to the phenological scale proposed by Alvares Filho, 1988), the seedlings were transferred to 16 black plastic trays (60 cm x 40 cm x 20 cm), each containing about 40 L of soil. The soil of each tray was prepared from a mixture of 200 L of Black Soil, 400 L of a Ferric Oxisol ("Latossolo Vermelho Eutroférrico" according to the Brazilian Soil Taxonomy, as defined in Embrapa, 1999), 120 L of an Entisol ("Neosolo Quartzarenico" Embrapa, 1999), 80 L of vermiculite and 2 kg of chemical fertilizer NPK (4-14-8). The spacing used in each box between lines and between plants was 8 cm resulting in 40 plants per tray.

Hyperspectral data for soybean were collected at close-range using a Spectron Engineering SE-590 portable spectroradiometer. The system detects and records spectral data in 252 usable bands in the range 365 to 1126 nm. Mean wavelength spacing between midpoints of adjacent bands was about 3 nm. The sensor was configured to acquire eight individual radiance measurements, which were internally averaged and stored as a single data file. The controller was connected to a portable computer, which initiates the scanning procedure, graphically displays the reflectance values of the target, and stores the data. The sensor was positioned at a height of 100 cm above the canopy. The sensor head was then positioned over three different sample locations within each plot and radiant flux was measured. A white Spectralon (Labsphere, 2004) reflectance standard was used to calibrate the spectroradiometer to the total incoming radiant flux. All canopy radiance data were imported into Microsoft's Excel Spreadsheet software and the bidirectional reflectance factor (%) was calculated as:

$$R_{\lambda\ soybean}(\%) = (L_{\lambda\ soybean}\ [\mu W.cm^{-2}.sr^{-1}.nm^{-1}]) \ /\ (L_{\lambda\ panel}\ [\mu W.cm^{-2}\ sr^{-1}\ cm^{-1}])*100,$$

where $L_{\lambda\ soybean}$ is the measured radiance for soybean at wavelength λ; $L_{\lambda\ panel}$ is the measured radiance for the calibration panel at wavelength λ; $R_{\lambda\ soybean}$ is the canopy reflectance (%) for soybean at wavelength λ.

Each week, the following soybean agronomic parameters were determined: (a) plant height (from the stem base to the highest visible node); b) shoot fresh mass and shoot dry mass (obtained in a forced-draught oven at 80ºC, for at least 48 h); c) leaf area index, LAI (obtained using a 3100C LiCor Area

Meter); d) percentage of green vegetation cover over the soil (obtained by digital photography processed using the SPRING software (INPE, 2000); e) phenological stage, identified during the whole cycle of the crop, according to Alvares Filho (1988); f) colorimetric determination of pigment concentration (chlorophylls *a* and *b* and total chlorophylls), using three 25 mg samples per box (to better represent the top of the canopy, ten leaves were harvested from the superior part of ten plants from each box; these leaves were then cut into small disks and weighed to 25 mg). Pigments in the discs were extracted with dimethyl sulfoxide (Hiscox and Israelstam, 1979) and the data obtained from spectrophotometer readings at 470, 646 and 663 nm were used to calculate the concentration (Lichtenthaler, 1987).

Spectral indexes (narrow bands) for the chlorophyll determination at different stages of crop development were calculated according to the spectroradiometric data. The following indexes were analysed: (a) the ratios $R_{750/700}$ and $R_{750/550}$ (Lichtenthaler et al.,1996); and (b) *NDVI*, proposed by Rouse et al. (1973). The linear regression analysis (Neter e Wasserman, 1974) was used to analyse the results and establish any existing relationships between the indexes and the data of pigment concentration obtained in the laboratory.

RESULTS AND DISCUSSION

Soybean development in greenhouse conditions: During the phenological development of soybean, data were collected that allowed the analysis of the following parameters: height, shoot fresh mass, shoot dry mass, total leaf area, ratio of leaf area, net assimilation rate, relative growing rate, percent covering and leaf area index. These parameters were used for a growth analysis procedure, carried out according to Beadle (1993), in order to determine crop development under greenhouse conditions. The soybean plants presented normal development under greenhouse conditions, revealing an assimilatory system sufficient and efficient for the production and accumulation of dry material. The work of Ferri (2002) discusses the results of this growth analysis in detail.

Table 1 presents values for chlorophyll obtained during the phenological development of soybean plants and figure 1 shows the spectral behaviour for three different phenological stages of soybean development. These data show that the normal development indicated by the growth analysis is also shown by the spectral graphs obtained during three distinct stages of the crop's biological cycle. The figures represent three stages of the crop: V_C - soybean plant with developed cotyledons; V_5 - soybean plant with five nodes on the main stem bearing fully expanded leaves; and R_8 - 95% of the pods having reached ripe pod colour.

Table 1. Mean and standard deviation (SD) of chlorophyll *a* and *b* and total chlorophyll during the phenological development of soybean.

Stages	Chlorophyll *a*		Chlorophyll *b*		Total chlorophyll	
	Mean	SD	Mean	SD	Mean	SD
VC	5.67	0.22	1.07	0.06	6.74	0.26
V1	7.19	0.72	1.30	0.15	8.49	0.86
V2	8.95	1.33	1.72	0.34	10.67	1.66
V3	9.67	1.71	1.53	0.37	11.20	1.93
V5	18.11	3.13	4.39	0.95	22.50	4.07
R2	11.48	1.42	2.31	0.39	13.79	1.79
R4	12.49	1.05	2.71	0.27	15.20	1.29
R5	11.18	1.42	2.92	0.53	14.10	1.85
R6	7.82	1.56	1.22	0.45	9.04	1.99
R7	4.97	2.03	1.30	0.51	6.27	2.53
R8	3.11	1.74	0.80	0.40	3.93	2.11

Reflectance Ratios $R_{750/700}$ and $R_{750/550}$: Hyperspectral remote sensing using spectral reflectance indexes that associate two or more narrow spectral bands in the form of a ratio, addition or multiplication may have potential for the determination of chlorophyll in agricultural crop canopies. According to Lichtenthaler et al. (1996), a non-destructive way to determine the chlorophyll content of vegetation canopies is the use of remote sensing techniques, however these determinations will only be accurate when high-resolution spectral radiometry is used. The authors suggested new ratios of two reflectances ($R_{750/700}$ and $R_{750/550}$) to determine the chlorophyll concentration in tobacco leaves, having found a high correlation between the spectral variables and the referred pigment (chlorophyll *a* and total). In the present work, the reflectances at 550 nm, 700 nm and 750 nm (represented by R_{550}, R_{700} and R_{750} respectively) presented variations with the increase in chlorophyll concentration during the growth cycle (figure 1) and, when the ratios suggested by Lichtenthaler et al. (1996) ($R_{750/700}$ and $R_{750/550}$) were applied, high values for correlation coefficients were obtained between the ratios and chlorophyll (*a* and total) (figures 2 and 3).

The results shown above for the ratios $R_{750/700}$ and $R_{750/550}$ are consistent with the results obtained by Lichtenthaler et al. (1996) and Gitelson and Merzlyak (1997). These authors found linear relationships with chlorophyll *a* content, with determination coefficients higher than 0,90. However, it is important to point out that they used an integrating sphere and individual leaves, while in the present work the remote sensing data were obtained with a spectroradiometer over the soybean canopy.

The fact that in the present work the focus was on canopies and nevertheless high and significant determination figures were obtained shows that, overall, $R_{750/550}$ and $R_{750/700}$ ratios can be considered good indicators of chlorophyll in plant canopies, when narrow band remote sensing techniques are used. This has an obvious application in the study of large areas of agricultural crops that can be monitored by airplanes, satellites or for use in precision farming.

$R_{750/550}$ and $R_{750/700}$ compared to NDVI: The NDVI index proposed by Rouse et al. (1973) is a broad-band vegetation index and has largely been employed to determine quantitative parameters of green phytomass through remote sensing, using wide spectral bands in the red and near infrared generally acquired by multispectral sensors in satellites. On the other hand, with the development of hyperspectral remote sensing, Lichtenthaler et al. (1996) have proposed the use of $R_{750/R550}$ and $R_{750/R700}$ ratios, where the reflectance values are those of very narrow bands (1 to 6 nm, depending on the equipment used) of green, red and infrared. Table 2 presents the mean values obtained for the ratios, comparing the reflectance of the experiment performed.

Figure 4 shows the profile of $R_{750/550}$ and $R_{750/700}$ together with NDVI indexes at different stages of soybean crop development. As the differences in amplitude for NDVI and for ratios $R_{750/550}$ and $R_{750/700}$ are high, the graphical comparison of these parameters required normalisation such that maximum values are transformed to 1.0.

Similar index profiles were found at all stages of crop development. Ratios $R_{750/550}$ and $R_{750/700}$ presented a peak at stage R4 which did not occur with the NDVI index. Gitelson et al. (1996) and Gitelson and Merzliak (1997) also found high relationships of these indexes for chestnut leaves. It should be pointed out that the afore-mentioned authors only used leaves from plants of different ages and an integrating sphere to determine the reflectance of the leaves. However, in our work, we used data obtained over canopies,

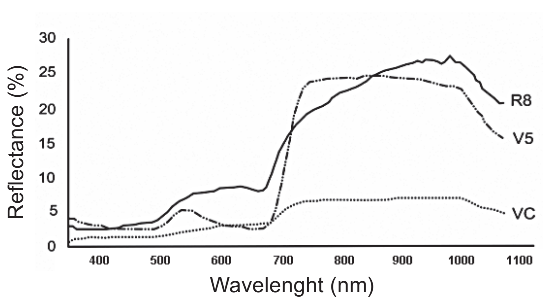

Figure 1. Soybean mean reflectance spectra at three different phenological stages of crop development.

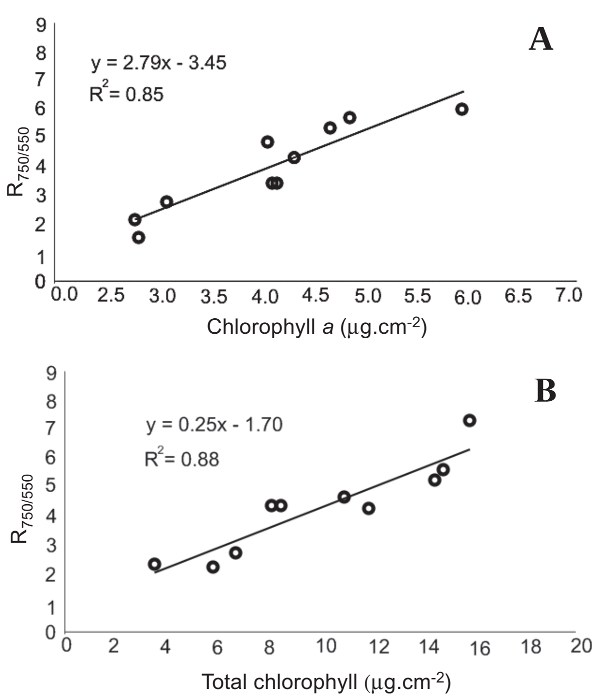

Figure 2. Mean ratio of linear relationships $R_{750/550}$ to chlorophyll *a* (A) and total chlorophyll (B) content (significant values at 5% probability level).

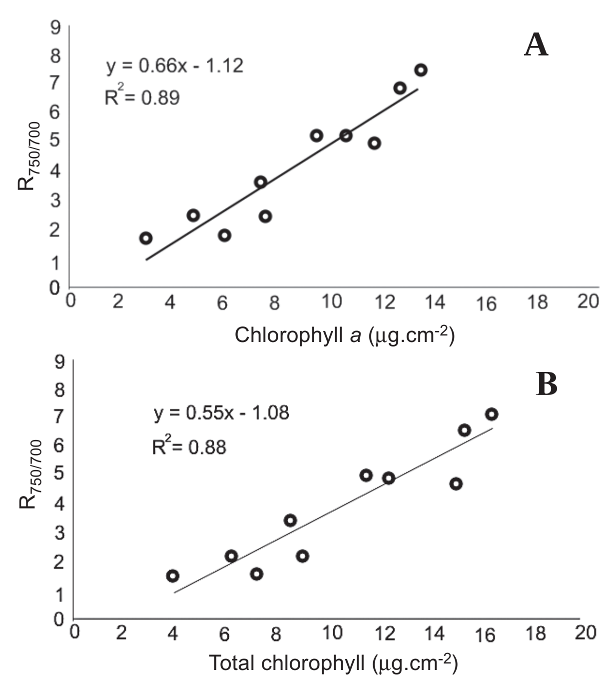

Figure 3. Mean ratio of linear relationships $R_{750/700}$ to chlorophyll *a* (A) and total chlorophyll (B) content (significant values at 5% probability level).

Table 2. Mean values for $R_{750/550}$, $R_{750/700}$ reflectance ratios and for NDVI, at different soybean phenological stages.

Stages	V_C	V_1	V_2	V_3	V_5	R_2	R_4	R_5	R_6	R_7	R_8
$R_{750/550}$	3.13	4.29	4.44	4.17	4.64	5.00	6.15	4.80	4.22	2.80	2.85
$R_{750/700}$	1.79	2.46	5.45	5.41	6.44	7.14	7.84	5.18	3.74	2.50	1.64
NDVI	0.41	0.42	0.84	0.83	0.84	0.85	0.86	0.85	0.79	0.70	0.37

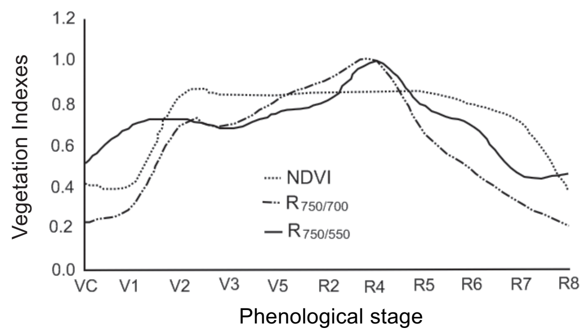

Figure 4. Mean $R_{750/550}$ and $R_{750/700}$ and NDVI index profiles at different phenological stages of soybean crop development (normalized curves).

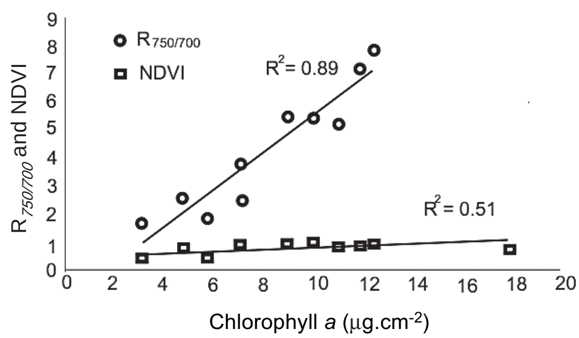

Figure 5. Mean relationships of the index $R_{750/700}$ and NDVI indexes to chlorophyll *a* content.

hence proving that such relationships have great potential for the determination of chlorophyll using crop canopies with data collected by air or orbital remote sensors.

Lichtenthaler et al. (1996) suggested the use of $R_{750/700}$, which is a simple spectral ratio, and figure 5 shows that a good relationship is obtained between this index and chlorophyll *a* content. The relationships of the indexes $R_{750/700}$ and NDVI in relation to chlorophyll *a* content are compared in figure 5.

A poor relationship between NDVI values and chlorophyll *a* content is shown, mainly when the index suggested by Lichtenthaler et al. (1996) is used. Table 2 shows that NDVI values varied from 0,37 to 0,86, a variation of 57 %, while the values of chlorophyll *a* varied from 3.11 to 18.11 $\mu g.cm^{-2}$ (82 %) (table 1). Higher variation for the values of ratios $R_{750/550}$ and $R_{750/700}$ (53.6 % and 75.8 %, respectively), may also be observed. Such variations were also reported by Lichtenthaler et al. (1996), who found much better relationships between the ratios when working with leaves of tobacco. Thus, it is possible to determine chlorophyll *a* and total chlorophyll concentrations in the vegetation canopy using the ratio Lichtenthaler et al. (1996) idealised for leaves. Indexes $R_{750/550}$ and $R_{750/700}$ presented, on average, a better relationship with chlorophyll *a* content than the NDVI index.

In conclusion, soybean plants developed in what might be considered a normal manner, even though cultivated under greenhouse conditions, since growth analysis suggested that the assimilatory system was both sufficient and efficient for the production and accumulation of dry material. From the V_3 stage, the spectral responses obtained by the spectroradiometer were already typically those of vegetation. At this point the display of relationships between spectral responses and pigment concentration began, which confirmed the validity of using hyperspectral images to determine phenological stages and other characteristics like crop vigour, health conditions and productivity. Ratios $R_{750/550}$ and $R_{750/700}$ proved to be very useful indexes, and more effective for the determination of chlorophyll *a* and total chlorophyll, when compared to the NDVI index, one of the most widely used broad band spectral indexes for remote sensing of vegetation. Our work has shown that, when one uses the evaluated narrow band vegetation indexes, it is possible to estimate photosynthetic pigments using spectral reflectance at the canopy level. Other narrow band indexes can be produced and further work must be conducted, both the laboratory and in the field, making use of spectroradiometers and hyperspectral images, in order to evaluate these and other indexes for remote sensing at the vegetation canopy level, evaluating their efficiency and applicability for future agricultural uses. Also, new experiments under similar conditions to those carried out here are also advisable, however with modifications of the conditions of data gathering, such as light and viewing angles, so that the effects of the environment are minimised. The possibility of using this approach for lignin and cellulose determination is also recommended, since these components are very important in many aspects, in view of the increasing number of reports in the literature which suggest they are likely candidates for hyperspectral remote sensing estimation.

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