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ABSTRACT

The goal of this work is to develop non-destructive techniques that can conveniently, rapidly and accurately assess crop physiological status and objectively evaluate plant responses to environmental factors, both natural and anthropogenic. High spectral resolution reflectance and absorption spectra of different and unrelated plant species were analyzed to determine spectral variability and information content in the visible and near-infrared spectrum at leaf and canopy levels. Techniques were developed to quantitatively retrieve chlorophyll, carotenoid and anthocyanin content from reflectance in a wide range of pigment content and composition.

Techniques for vegetation fraction retrieval those based on channels in visible range of the spectrum were developed and validated. Despite the fact that the reflectance contrast among the visible channels is much smaller than between the visible and near infrared, the sensitivity to moderate to high values of vegetation fraction is much higher than for NDVI and the error in vegetation fraction prediction did not exceed 10 per cent.

Leaf pigment content estimation

Optical properties of the leaves of several plant species in a wide range of chlorophyll (Chl), carotenoid (Car) and anthocyanin (Anth) contents have been investigated (Gitelson and Merzlyak, 1997; Zur et al., 2000; Gitelson et al., 2001a) in order to develop techniques for non-destructive estimation of pigment content and composition. It was found that the reflectance in spectral band near 700 nm was sensitive to Chl content only, whereas reflectance around 550 nm was sensitive to both Chl and Anth content, and near 500-520 nm was sensitive to both Car and Chl content. The technique was developed to remove Chl effect from reflectance near 550 nm and 500-520 nm. Algorithms for Chl, Car and Anth contents estimating were devised and, finally, validated by few independent data sets.
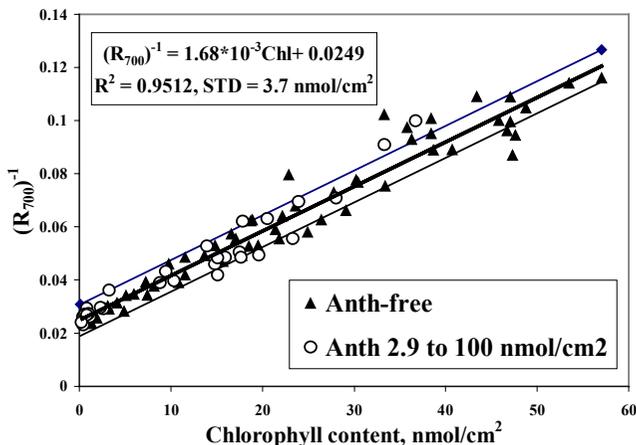
FIGURE 1. Inverse reflectance at 700 nm vs. Chl content for Anth-containing and Anth-free leaves.

Chlorophylls. Reflectance in spectral bands near 550 nm and 700 nm was found to be sensitive to Chl content (Gitelson and Merzlyak, 1994; 1996; Blackmer et al., 1994; Lichtenthaler et al., 1996; Scheppers et al., 1996; Gamon and Surfus, 1999). For many unrelated plant species, inverse reflectance $(R_{550})^{-1}$ and $(R_{700})^{-1}$ was a precise measure of Chl, and the reflectance ratios in near infrared range (R_{NIR}) to those in the green (R_{550}) and red edge (R_{700}) regions, R_{NIR}/R_{550} and R_{NIR}/R_{700}, were linearly proportional to Chl content (determination coefficient $r^2 = 0.95$; root-mean square variation < 4 nmol/cm^2) for Chl ranged from 3 to 75 nmol/cm^2 (Gitelson and Merzlyak, 1997). In this study, we investigated the sensitivity of these indices to Anth content. Absorption by Anth *in vivo* at 550 nm (Gitelson et al., 2001a) prevents to use the index R_{NIR}/R_{550} for Chl estimation in Anth-containing leaves. Nevertheless, it has been found that reciprocal reflectance at 700 nm $(R_{700})^{-1}$ remained linearly proportional to Chl content in leaves with Anth content ranged from 0.3 to 100 nmol/cm^2 and Car content from 3 to 18 nmol/cm^2 (Fig. 1). Thus, for Chl assessment in Anth-free leaves, we suggest using reflectance ratios R_{NIR}/R_{550} and R_{NIR}/R_{700}. Inverse reflectance $(R_{700})^{-1}$ or ratio R_{NIR}/R_{700} can be employed for Chl estimation in leaves with a wide range of Chl, Car, and Anth variation.

Carotenoids. Car and Chl have overlapping absorption bands in the blue range of the spectrum, making it difficult to distinguish between them by remote sensing techniques (Merzlyak et al., 1999). To find spectral range that is sensitive to Car content, absorption and reflectance spectra of leaves in a wide range of Car (1 to 18 nmol/cm^2) and Chl (0.1 to 75 nmol/cm^2) content were studied. The standard deviation (STD) of absorbance spectra, normalized to Chl absorption maximum at 670 nm, peaks at 520 nm; this peak is attributed to Car absorption (Zur et al., 2000). The determination coefficient of the relationship 'Reflectance vs. Car' also shows prominent peak in the range 500-520 nm. The technique was devise to remove Chl effect on reflectance in this spectral region and the index for non-destructive Car estimating was developed. The Carotenoids Reflectance Indices in the forms

$$CRI_{550} = (R_{510})^{-1} - (R_{550})^{-1} \text{ and } CRI_{700} = (R_{510})^{-1} - (R_{700})^{-1}$$

were found to be proportional to Car (r^2 = 0.93, an estimation error of Car < 2 nmol/cm^2) and almost insensitive to Chl content (Fig. 2).
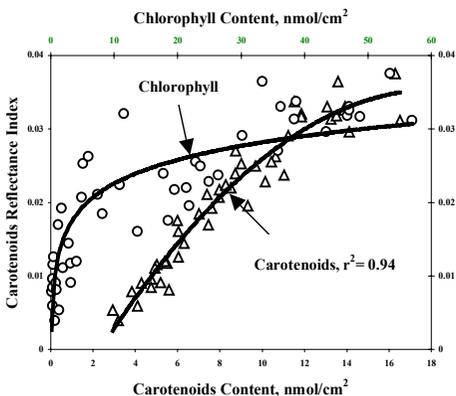

FIGURE 2. Index for carotenoids estimation plotted versus Car and Chl content

Anthocyanins. Comparing absorption spectra of anthocyanin-containing and anthocyanin-free leaves with the same chlorophyll content, absorption spectra of anthocyanin in intact leaves were derived. The main spectral feature of anthocyanin absorption *in vivo* was a peak around 550 nm which magnitude was closely related to anthocyanin content (Fig. 3). A technique was developed to subtract Chl contribution to reflectance in this spectral region and quantitatively non- destructively retrieve Anth content from reflectance in a wide range of pigment content and composition (Gitelson et al., 2001a). Anthocyanin Reflectance Index in the form

$$ARI = (R_{550})^{-1} - (R_{700})^{-1}$$

allowed an accurate estimation of anthocyanin accumulation (STD within 3.9 nmol/cm^2), even in minute amounts, in intact stressed and senescing leaves (Fig. 4).

Vegetation fraction and density estimation

The decreased reflectance in the NIR, occurring at or near the midseason of a growing crop, can be a limiting factor in the use of that spectral region for estimating of vegetation fraction (VF). In order to evaluate the information content inherent in reflectance spectra and devise a technique for remote estimation of vegetation fraction, spectral behavior of reflectance and absorption of a wheat canopy with vegetation fraction ranged from 0 to 100% were studied in visible and near-infrared ranges of the spectrum. The MODIS blue (459-479 nm), green (545-565 nm), and red (620-670 nm) channels as well as MERIS red edge channel (695-705 nm) were used in developed Visible Atmospherically Resistant Indices (VARI)

$$VARI_{green} = (R_{green} - R_{red})/(R_{green} + R_{red} - R_{blue})$$

and

$$VARI_{700} = (R_{700} - 1.7*R_{red} + 0.7*R_{blue})/(R_{700} + 2.3*R_{red} - 1.3*R_{blue})$$
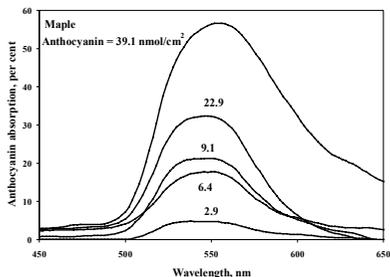
FIGURE 3. Anthocyanin absorption retrieved from absorption spectra of leaves with equal Chl content and different Anth content
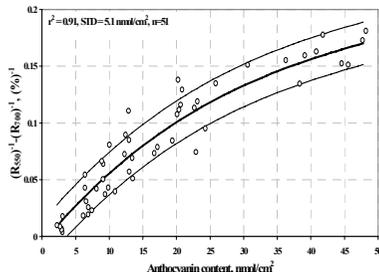
FIGURE 4. Index for anthocyanin estimation plotted versus anthocyanin content

The indices were found to be minimally sensitive to atmospheric effects allowing estimation of VF with an error of less than 10% in a wide range of atmospheric optical thickness (Gitelson et al., 1999; 2001b).

Another technique for vegetation fraction estimation was proposed (Gitelson et al., 2000; Stark et al., 2000). It was found that reflectance of the wheat with 100% vegetation fraction, despite various canopy structures and pigment contents, forms a vegetation line defined by close linear relationships reflectances R_{700} vs. R_{550} and R_{670} vs. R_{500}. The position and orientation of the lines in two-dimensional spectral space were repeatable for four types of wheat during two years of observations. Soils of very different types and wetness form soil lines in the R_{700} vs. R_{550} and R_{670} vs. R_{500} spectral spaces. The soil lines have also proven to be repeatable over the course of two years of data collection. Two-dimensional spectral space, as defined and constrained by soil and vegetation lines, includes all possible canopy reflectances with VF ranging from 0 to 100%. We suggest using the location of reflectance in these spectral spaces as a quantitative measure of VF. Predicted VF was proportional to measured VF with root-mean square variation of predicted values from measured of less than 10%. For the independent data set (cornfields in Nebraska), excellent correspondence between predicted and actually measured vegetation fraction values was found.

Despite the fact that the reflectance contrast among the visible channels is much smaller than between the visible and NIR, the sensitivity of both techniques to moderate to high values of VF was much higher than for NDVI. Suggested techniques will complement the widely used NDVI, ARVI, SAVI, and GARI (Gitelson et al., 1996), which is based on the green and the NIR bands. Therefore, we propose using only the visible range of the spectrum to quantitatively estimate vegetation fraction, and we are interested in applying the algorithms to new satellite technologies including the high spectral and radiometric resolution achieved in the recently launched SeaWiFS, MODIS, ASTER, MISR, and near future space systems such as HYPERION and MERIS.
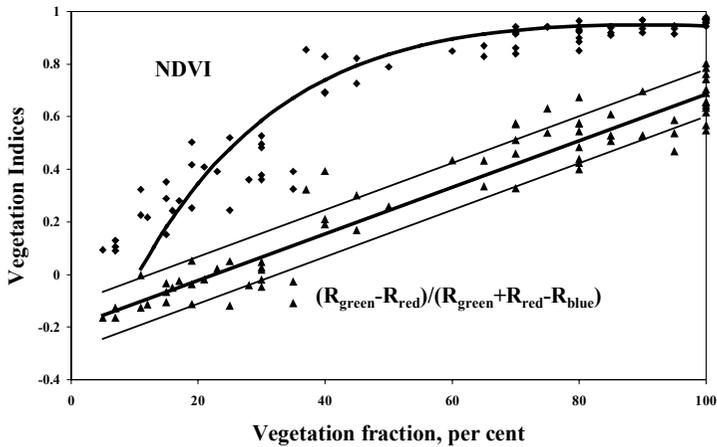
FIGURE 5. NDVI and newly developed vegetation index $(R_{green}-R_{red})/(R_{green}+R_{red}-R_{blue})$ versus vegetation fraction for wheat canopy

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