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THE SPECTRAL CONTRIBUTION OF CAROTENOIDS TO LIGHT ABSORPTION AND REFLECTANCE IN GREEN LEAVES*

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1.0 ABSTRACT

Absorbance and reflectance spectra in the visible and near infrared range of the spectrum, acquired for maple (*Acer platanoides* L.) leaves were studied. Standard deviation of absorbance spectra showed that in yellow to green leaves, with chlorophyll content at least up to 30 nmol/cm^2, there is a spectral feature at 520 nm attributable to carotenoids. Reflectance around 520 nm also correlates closely with carotenoids content in yellow to green leaves. Thus, this spectral feature at 520 nm could be used as a measure of carotenoids content in green leaves and plants.

2.0 INTRODUCTION

Remote sensing techniques that estimate pigment content in higher plants are a prominent tool for determining the plants physiological state. The methods for remote estimation of chlorophyll content in leaves are quite well established (Thomas and Gausman. 1977; Chappelle et al., 1992; Gitelson and Merzlyak, 1996a, 1996b, 1997; Datt, 1998; Gamon and Surfus, 1999). However, for carotenoids, remote sensing techniques are still not well developed.

In higher plants, carotenoids fulfill many functions; among them the most important are their involvement in light harvesting and photo-protection (Demmig-Adams et al., 1996; Edge et al., 1997). Recent studies show that carotenoids play a key role in adaptation of plants to light stress and other unfavorable ecological conditions. Some reflectance indexes for measuring carotenoids content from reflectance spectra have been reported (Chappelle et al., 1992, Datt 1998). There are two main obstacles in devising a non-destructive methods for estimating carotenoids content in green plants: the first is that carotenoids are present at lower levels than chlorophyll; the second is that carotenoids exhibit overlapping absorption of chlorophyll wavebands. Moreover, the absorption of visible light by carotenoids in green leaves is not well understood at present (Demmig-Adams et al., 1996). Nevertheless, it was shown that in different plant species high photon flux induces small reversible changes of in absorbance (Bilger et al., 1989) and reflectance (Gamon et al., 1990, 1992) near 535 nm due to the transformation of carotenoids of violaxanthin cycle. For senescing leaves with low chlorophyll content, reflectance indexes sensitive to the carotenoids / chlorophyll ratio have been developed (Penuelas et al., 1995 and Merzlyak et al., 1999).

To develop a technique for non-destructive estimation of pigment content, it's essential to find spectral bands where reflectance is maximally sensitive to a pigment of interest and minimally

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sensitive to other pigments. In this study, we employed the approach proposed by Gitelson and Merzlyak, (1994, 1996b, 1997) and Gitelson et al., (1996) to use statistical parameters (standard deviation (STD) and/or coefficient of variation of absorbance and reflectance) to search for the spectral signature of carotenoids in green leaves.

3.0 MATERIALS AND METHODS

Healthy young mature and senescing leaves without visible anthocyanin pigmentation were used in the experiments. Norway maple leaves (*Acer platanoides* L.) were collected in a park at Moscow State University (1992-1998).

To extract pigments, leaves were rapidly grounded with a porcelain mortar and pestle in methanol or acetone and with calcium carbonate added to prevent chlorophyll pheophytinization. Homogenates were centrifuged for 3-4 min in glass tubes at 3000 G. Resulting extracts were immediately assayed spectrophotometrically. Specific absorption coefficients of chlorophyll *a* and chlorophyll *b* and total carotenoids reported by Lichtenthaler (1987) were used. A molecular weight of 570 for carotenoids was assumed.

Adaxial reflectance (R) and transmittance (T) spectra of the leaves were measured with a 150-20 Hitachi spectrophotometer equipped with an integrating sphere attachment with a spectral resolution of 2 nm interfaced to a computer. Reflectance spectra for barium sulfate were recorded to use as a standard. Absorbance (A) was calculated as log [(100-R)/T].

4.0 RESULTS

4.1 ABSORBANCE SPECTRA

Absorbance spectra of maple leaves in a wide range of pigment contents and compositions are shown in Fig. 1. The absorbance spectra of green to dark green leaves indicate that, in the blue and the red regions, over 95 per cent of the light is absorbed by the leaf. In the blue range, there are two small peaks at 440 and 470 nm that can be attributed to the absorption of chlorophyll *a* and chlorophyll *b* together with carotenoids, respectively (French, 1960; Merzlyak et al., 1999).

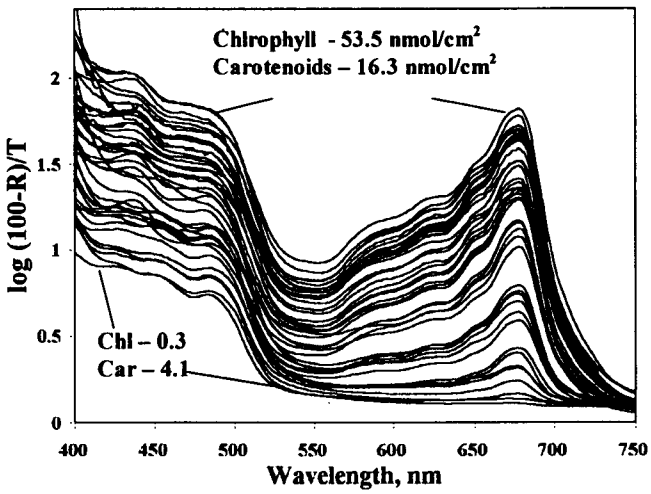

Figure 1: Absorbance Spectra of Dark Green (upper curve) to Yellow (lower curve) Maple Leaves With Wide Variety of Pigment Content and Composition.

The absorption in the green range is also very high, reaching 90 per cent. In the red range, the strong absorption of chlorophyll *a* near 680 nm and a shoulder at 650 nm due to absorption by chlorophyll *b* can be seen. In yellowing leaves with a decline in chlorophyll content, the absorbance in the green and the red ranges decreases significantly. In contrast, the absorption in the blue range remains high (80-90 per cent) due to the strong absorption of retained carotenoids (Merzlyak and Gitelson, 1995; Merzylak et al. 1999).

Yellow leaves do not absorb at 678 nm (lower curve in Fig. 1); therefore we can infer that the absorbance in the blue range for yellow leaves is mainly related to carotenoids. The resemblance between the spectral features near 420, 460 and 475 nm and absorption bands of carotenoids in extracts (Lichtenthaler, 1987) suggests that carotenoids are the major absorbers in yellow leaves. As the chlorophyll content in the leaves rises, the spectral features of the carotenoids diminish due to the strong and overlapping absorption by chlorophyll. Thus, in order to find specific spectral features of carotenoids, it is essential to reduce the influence of chlorophyll absorption. This is accomplished by normalization of the absorbance spectra to the absorbance at 678 nm (the main red absorption peak of chlorophyll *a*), which decreases significantly the contribution of chlorophyll absorbance to the total absorbance in all the visible range.

Standard deviation (STD) of absorbance, normalized to absorbance at 678 nm (A_{678}), of three leaf groups with different chlorophyll content are presented in Fig. 2. The first group contained yellowish-green to dark green leaves with chlorophyll content higher than 10 nmol/cm^2; the second group contained slightly green to dark green leaves with chlorophyll content higher than 20 nmol/cm^2; the last group contained green to dark green leaves with chlorophyll content higher than 30 nmol/cm^2. All three STD spectra were generally similar. At 440 and 460 nm, where chlorophylls are strong absorbers, the STD values were low as a result of the normalization. In the spectral region between 550-620 and near 700 nm, where Chl absorption is weaker than in the blue, the STD values were almost equal. This equivalence is consistent with a very strong covariance between the optical properties of leaves at 550 nm and near 700 nm (Gitelson and Merzylak, 1994, 1996, 1997; Gitelson et al., 1996).

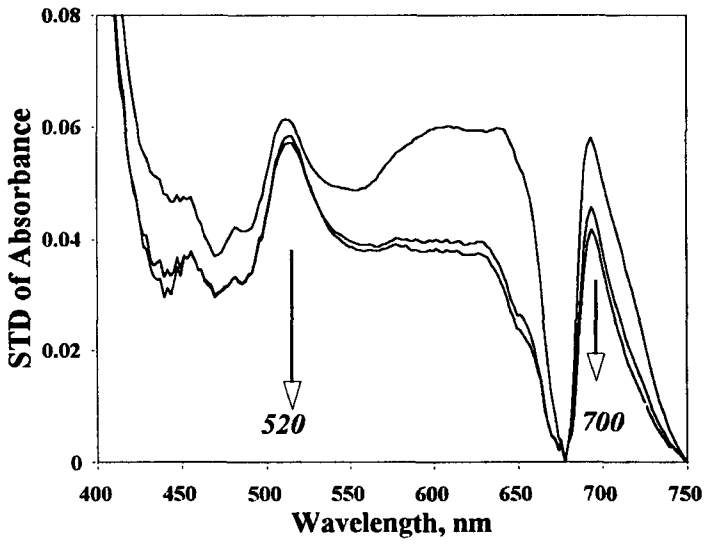

Figure 2: STD of Normalized Absorbance Spectra to the Absorbance at 678 nm.

For a wide range of chlorophyll content, including green leaves, a peak at 520 nm appears in the STD curves. The troughs in the main absorbance bands of chlorophylls at 440 nm and 460 nm (Fig. 2) show that the normalization to the absorbance at 678 nm significantly reduced the contribution of chlorophyll to the total

absorbance. This indicates that the range near 520 nm shows maximal sensitivity to variation of carotenoids content.

Reflectance at 550 nm is closely correlated to chlorophyll content for a wide range of leaves greenness (Gitelson and Merzylak, 1994, 1996). Thus, we suggest that absorbance at 550 nm (A_{550}) might be useful as a measure of chlorophyll content as well. Absorbance at 520 nm is influenced by the absorbance of both chlorophyll and carotenoids; therefore, the difference A_{520}-A_{550} would probably be more dependent on carotenoids absorbance than on chlorophyll absorbance.

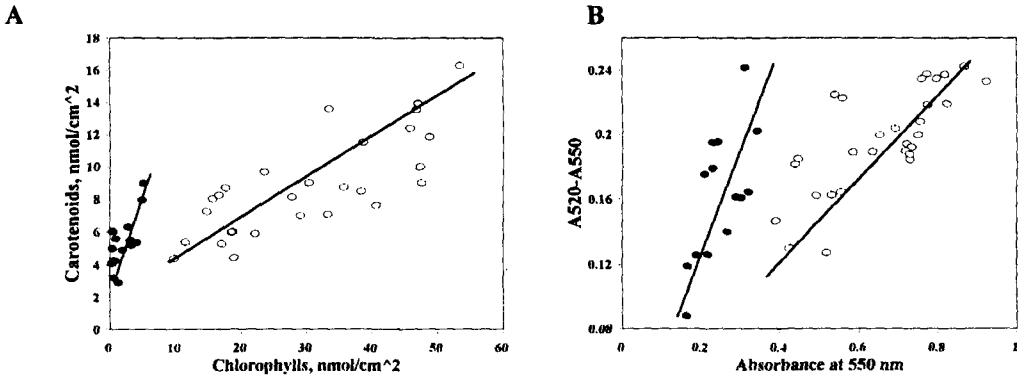

Figure 3: (a) Carotenoids Content vs. Chlorophylls Content in Maple Leaves. (b) The Difference in the Absorbance at 520 and at 550 nm vs. Absorbance at 550 nm.

The partial contribution of carotenoids and chlorophylls to the absorbance at 520 nm can be demonstrated by comparing the relations "chlorophylls vs. carotenoids" and "A_{550} vs. A_{520}-A_{550}". Fig. 3a shows the relation between chlorophylls and carotenoids contents. The relations between two kinds of pigments are very different at the last stage of senescence (when chlorophyll < 10 nmol/cm^2) and before this stage in slightly green to dark green leaves (chlorophyll > 10 nmol/cm^2). Fig. 3b shows that a similar relation exists between the absorbance at 550 nm and the difference A_{520}-A_{550}. The resemblance between Fig. 3a and Fig. 3b shows that carotenoids have a significant role in the absorption at 520 nm.

4.2 REFLECTANCE

In order to study spectral behavior of leaves reflectance, the data was divided into few groups based on their chlorophyll content. The first group included yellow leaves with very low chlorophyll content, and the last one included all leaves studied with chlorophyll ranging from 0 to 60 nmol/cm^2. The STD of reflectance of these groups are shown in Figure 4. In the blue range, STD was very low and three peaks near 425, 452 and 485 nm appear. For yellow to greenish leaves with chlorophyll content ranging up to 20 nmol/cm^2 (curves 1-4), there are several pronounced spectral features. The peak around 680 nm is due to absorption by chlorophyll *a* in the red range (Gitelson and Merzlyak, 1996a,b). As the chlorophyll content rises, the peak becomes wider. For green to dark green leaves with chlorophyll > 20 nmol/cm^2, there is a broad range from 550 to 620 nm and a narrow zone around 700 nm that show high sensitivity to pigment variation. As chlorophyll increases, the STD shows a trough near 680 nm, indicating that the reflectance around 680 nm is more sensitive to low chlorophyll content (i.e., yellow to greenish leaves) and less sensitive to moderate to high chlorophyll content (Gitelson and Merzylak, 1997).

For four groups of leaves (1-4) with chlorophyll content of less than 15 nmol/cm^2, a pronounced peak in the STD near 520 nm appears. This peak shows the sensitivity reflectance to variation of carotenoids content in leaves. The sensitivity becomes lower with increase in chlorophyll content; for groups 4 and 5 with chlorophyll ranging from 0 to 20 nmol/cm^2 and 0 to 30 nmol/cm^2; only a shoulder near 520 nm can be seen.

This is due to an increase in the chlorophyll absorption at 520 nm in those groups of leaves. As the chlorophyll content in the leaves increases, this spectral feature disappears.

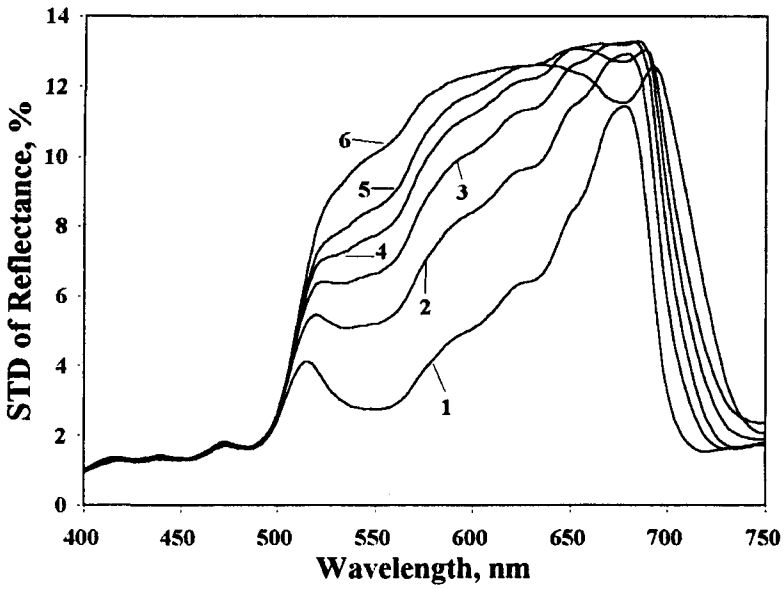

Figure 4: STD of Reflectance for Maple Leaves with Different Chlorophyll Content (in nmol/cm^2): *1*: 0 < Chl <5; *2*: 0 < Chl <10; *3:* 0 < Chl <15; *4:* 0 < Chl <20; *5:* 0 < Chl <30; *6:* 0 < Chl <60.

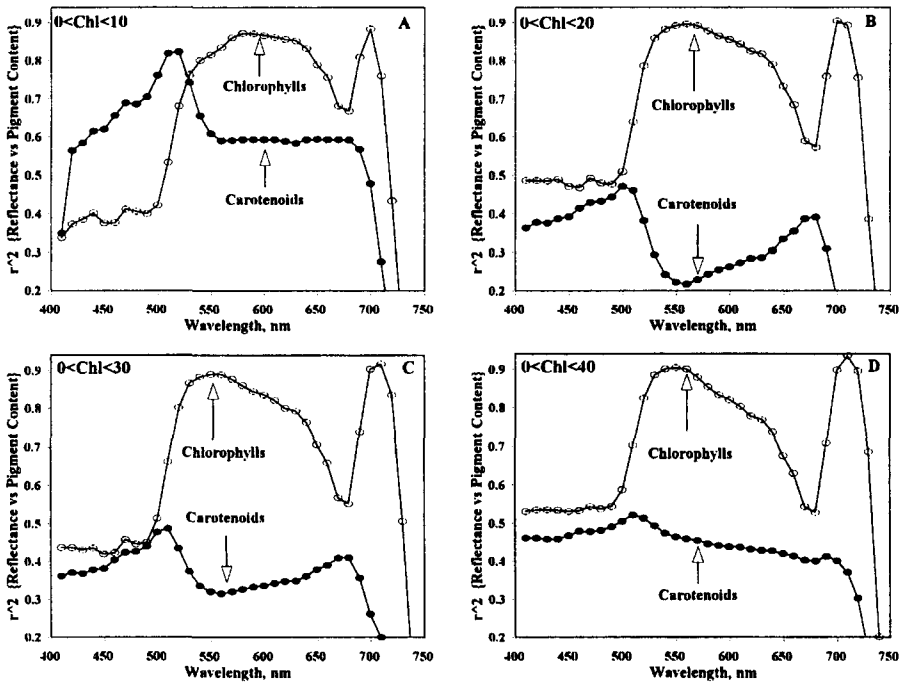

Figure 5: Correlation of Reflectance with Chlorophyll and Carotenoids for Groups of Leaves with Different Chlorophyll Content (shown in nmol/cm^2).

Correlation between reflectance and pigment content, chlorophyll or carotenoids, were also studied (Fig. 5). All leaves show very high correlation between chlorophyll content and reflectance in a wide range from 530 to 630 nm and around 700 nm. A minimum in the determination coefficient "chlorophyll vs. reflectance" in the bluemanifests insensitivity of reflectance to chlorophyll content in this spectral range.

In yellow to yellowish green leaves, the correlation coefficient (r) of the relationships between carotenoids and reflectance in the range 450 - 550 nm is higher than 0.8, reaching 0.95 near 520 nm (Figure 6A). With increase in chlorophyll content, the correlation becomes weaker. Nevertheless, in all relationships showed in Figure 6, a peak around 520 nm is prominent and the correlation is significant. Carotenoids do not absorb significantly beyond 550 nm (see Fig. 1 and Gitelson *et al*, 1996; Merzlyak *et al.*, 1999); thus, the correlation between reflectance and carotenoids at wavelengths longer than 550 nm is due to the correlation between carotenoids and chlorophyll content.

5.0 DISCUSSION AND CONCLUSIONS

Carotenoids and chlorophyll have overlapping absorption bands in the blue range (Fig. 1), making it difficult to distinguish between them by remote sensing techniques. Standard deviation of absorbance spectra normalized to absorbance of chlorophyll at 678 nm has a prominent peak at 520 nm that is probably related to carotenoids absorption (Fig. 2). This spectral feature is evident even in STD spectra of green leaves absorption. The STD of reflectance spectra (Fig. 4) also show high sensitivity of reflectance to carotenoids content. With increase in chlorophyll content in leaves, this feature becomes weaker. Gitelson *et al* (1996), analyzing coefficient of variation of reflectance spectra of senescing and mature leaves, also found a prominent peak near 520 nm that can be attributed to carotenoids absorption. Our findings are in agreement with the results of Bilger et al. (1989) and Gamon et al. (1990, 1992) that presented carotenoids-dependent light-induced spectral changes in leaves.

The significant correlation of reflectance near 520 nm with carotenoids content established in this study strongly suggests that this spectral range is sensitive to carotenoids content, particularly for yellow to green leaves with Chlorophyll < 30 nmol/cm^2.

6.0 ACKNOWLEDGEMENTS

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