Photochemistry and Photobiology, 2002, 75(3): 272–281

Assessing Carotenoid Content in Plant Leaves with Reflectance Spectroscopy¶

Anatoly A. Gitelson*[1,2], Yoav Zur[2], Olga B. Chivkunova[3] and Mark N. Merzlyak[3]

[1]School of Natural Resource Sciences, University of Nebraska—Lincoln, Lincoln, NE; [2]Department of Geological and Environmental Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel and [3]Department of Cell Physiology and Immunology, Faculty of Biology, Moscow State University, Moscow, Russia

Received 8 August 2001; accepted 20 December 2001

ABSTRACT

Spectral reflectance of maple, chestnut and beech leaves in a wide range of pigment content and composition was investigated to devise a nondestructive technique for total carotenoid (Car) content estimation in higher plant leaves. Reciprocal reflectance in the range 510 to 550 nm was found to be closely related to the total pigment content in leaves. The sensitivity of reciprocal reflectance to Car content was maximal in a spectral range around 510 nm; however, chlorophylls (Chl) also affect reflectance in this spectral range. To remove the Chl effect on the reciprocal reflectance at 510 nm, a reciprocal reflectance at either 550 or 700 nm was used, which was linearly proportional to the Chl content. Indices for nondestructive estimation of Car content in leaves were devised and validated. Reflectances in three spectral bands, 510 ± 5 nm, either 550 ± 15 nm or 700 ± 7.5 nm and the near infrared range above 750 nm are sufficient to estimate total Car content in plant leaves nondestructively with a root mean square error of less than 1.75 nmol/cm^2.

INTRODUCTION

Carotenoids (Car) and chlorophylls (Chl) are the main pigments of green leaves. Car are usually represented by two (α- and β-) carotenes and five xanthophylls (lutein, zeaxanthin, violaxanthin, antheraxanthin and neoxanthin), which exhibit strong light absorption in the blue region of the spectrum and are nonuniformly distributed in photosystems and individual pigment–protein complexes of chloroplasts (1–4). Several specific physiological functions have been attributed to Car because of their unique physicochemical and photophysical functions: structural role in the organization of photosynthetic membranes, participation in light harvesting, energy transfer, quenching of Chl excited states and singlet oxygen, and interception of deleterious free oxygen and organic radicals (2,4–9). The reversible conversion of violaxanthin to zeaxanthin *via* antheraxanthin (violaxanthin cycle) is considered to be an important mechanism of excess energy dissipation in chloroplasts (2,4–7,10). The retention of Car in the progress of Chl breakdown (3) has been suggested to be a mechanism of photoprotection during leaf senescence (11). The changes of leaf Car content and their proportion to Chl are widely used for diagnosing the physiological state of plants during development, senescence, acclimation and adaptation to different environments and stresses (1,3,4,12–18).

During the last decade, several attempts have been undertaken to develop nondestructive techniques for Car content assessment at both the leaf and canopy level (15,19–23). For senescing leaves, reflectance indices sensitive to the [Car]/[Chl] ratio have been reported (14,17,21,22,24). It was shown that in different plant species, high photon flux induces small reversible changes of reflectance near 530 nm, attributable to the transformation of violaxanthin cycle xanthophylls. The corresponding reflectance indices applicable for nondestructive estimation of the cycle and photosynthetic activities have been suggested (14–16,25).

Chappelle *et al.* (20) used ratio analysis of reflectance spectra to find a spectral band sensitive to pigment content. A ratio spectrum obtained by dividing the mean reflectance spectrum of soybeans grown at a high nitrogen level by the mean reflectance spectrum of soybeans grown at a medium nitrogen level had a small peak around 500 nm that was attributed to Car absorption. They recommended using a ratio R_{760}/R_{500}, where R_{760} and R_{500} are the reflectances at 760 and 500 nm, respectively, as a quantitative measure of Car. Blackburn (21) suggested that the optimal individual waveband for Car estimation is located at 470 nm and used the pigment-specific ratio R_{800}/R_{470} and a pigment-specific normalized difference $(R_{800} - R_{470})/(R_{800} + R_{470})$ for Car content assessment.

To retrieve the [Car]/[Chl] ratio for a range of individual leaves and conditions, Penuelas *et al.* (24) proposed a structure-insensitive pigment index in the form $(R_{800} - R_{445})/(R_{800} - R_{680})$. Merzlyak *et al.* (17) found that the difference $R_{680} - R_{500}$ depends on the pigment composition; the index $(R_{680} - R_{500})/R_{750}$ was found to be sensitive to [Car]/[Chl] and was used as a quantitative measure of leaf senescence and fruit ripening.

¶Posted on the web site on December 31, 2001.

*To whom correspondence should be addressed at: School of Natural Resource Sciences, University of Nebraska—Lincoln, 113 Nebraska Hall, Lincoln, NE 68588-0517, USA. Fax: 402-472-4608; e-mail: gitelson@calmit.unl.edu

Abbreviations: Car, carotenoids; Chl, chlorophyll; CRI, Carotenoid Reflectance Index; NIR, near infrared; RMSE, root mean square error.

Analyzing the coefficient of variation of reflectance spectra of yellow to dark-green leaves representing a wide-ranging process of senescence, Gitelson *et al.* (see fig. 2 in [26]) found a prominent narrow peak in the range 500 to 520 nm. The magnitude of the peak decreased when yellow leaves with a high [Car]/[Chl] ratio were excluded from analysis. When only green to dark-green leaves were analyzed, the peak disappeared. This suggested that the peak might be attributed solely to Car absorption and that reflectance in the range 500 to 520 nm is maximally sensitive to the Car content.

In an attempt to find spectral bands that are maximally sensitive to Car content in green leaves, Zur *et al.* (27) suggested normalization of the leaf absorbance spectra (A_λ) to the red Chl absorbance at 678 nm (A_{678}). As a result, the contribution of Chl was significantly reduced, and in the spectra of the standard deviation of A_λ/A_{678} for leaves with a wide range of Chl and Car content, they found a prominent peak at 510 to 520 nm. This peak was pronounced even for the group of green to dark-green leaves. In the same range, around 510 nm, the coefficient of determination of the relationship reflectance *vs* [Car] had a peak; so reflectance in this range was sensitive to Car content (27). Importantly, it was shown that Chl also contributed considerably to absorbance and reflectance in the range 510 to 520 nm. The greater the Chl content in the leaves, the greater the contribution of Chl to absorbance and reflectance in this range was (27).

The preceding results (11,20,24,26,27) indicate that reflectance between 470 and 520 nm is sensitive to Car content and that Chl also plays a significant role in this spectral range. Chl absorption in the range 470 to 500 nm was found to be so strong that the relationship reflectance *vs* [Chl] leveled off and became almost invariant at moderate-to-high Chl content (26,28–33). Therefore, the problem of leaf Car assessment is closely related to the removal of the Chl contribution from reflectance in the range that is most sensitive to Car content.

Relationships between reflectance in the visible range and leaf Chl content are essentially nonlinear (34). First-difference transformation of the apparent absorbance, log of reciprocal reflectance $(R)^{-1}$, was found to be the best predictor for nitrogen and Chl in fresh bigleaf maple leaves (35). For a variety of plant species and in a wide range of pigment content and composition, reciprocal reflectance in spectral bands located quite far from the main absorption bands of pigments, near 550 and 700 nm, was linearly related to Chl (26,28–32). This feature of leaf reflectance has been used to estimate Chl content in leaves accurately (30,31). To estimate anthocyanin content nondestructively, reciprocal reflectance at 700 nm, $(R_{700})^{-1}$, was used as a measure of Chl content to remove the Chl contribution from reflectance around 550 nm (33). However, it should be examined whether this approach can be used for Car retrieval from the leaf reflectance spectra.

Thus, the main obstacle in devising a nondestructive technique for Car content estimation is the fact that the Car content is much lower than the Chl content in green plants and that Car exhibit absorption wavebands overlapping with Chl (17,20–22). Therefore, to develop a technique for nondestructive estimation of leaf Car, one needs:

- to find spectral bands where reflectance is maximally sensitive to Car content and minimally sensitive to other pigments, leaf structure and thickness;
- to remove the contribution of Chl to the reflectance in the spectral bands chosen; and
- to devise a model that relates reflectance and Car content in a wide range of Car and Chl variation.

In this paper, leaf reflectance spectra of three plant species in a wide range of pigment content and composition have been investigated. The first step in our research was to find specific Car features inherent in reflectance spectra. Second, having found the spectral band around 510 nm that was sensitive to the Car content, a technique was developed to remove the Chl effect from the reflectance in this spectral range. Finally, several reflectance indices for total leaf Car content estimation were devised and validated by independent data sets.

MATERIALS AND METHODS

Juvenile, mature and senescent leaves of Norway maple (*Acer platanoides* L.) and horse chestnut (*Aesculus hippocastanum* L.) were collected in a park at Moscow State University in spring, summer and fall in 1992–2000. Second-flush beech leaves (*Fagus sylvatica* L.) grown on the University of Karlsruhe campus were taken in August 1996 and August 2000. Leaves were visually selected according to their difference in color. Leaves healthy and homogeneous in color without anthocyanin pigmentation or visible symptoms of damage were used in the experiments.

The leaf pigments were quantitatively determined from the same leaf samples used for reflectance measurement. Circular pieces were cut from the leaves and extracted with 100% acetone or methanol using a mortar. The pigment extracts were centrifuged for 3 to 5 min in glass tubes to make the extract transparent. The resulting extracts were immediately assayed spectrophotometrically. The specific absorption coefficients of Chl *a* and Chl *b* and total Car reported by Lichtenthaler (1) and an average molecular weight for Car of 570 were used.

Adaxial reflectance (R) spectra of the leaves were taken in a spectral range between 400 and 800 nm against barium sulfate as a standard with a spectral resolution of 2 nm. A Hitachi 150-20 spectrophotometer (measurements of maple and chestnut leaves) and a Shimatzu 2101 PC spectrophotometer (measurements of beech leaves) equipped with integrating spheres were used in the study.

RESULTS AND DISCUSSION

Pigment content and composition

Pigment content and composition in the leaves varied widely. In maple leaves, Chl ranged from 0.1 to 53.5 nmol/cm^2, and Car changed from 1.6 to 16 nmol/cm^2 (Table 1, Fig. 1a). In beech leaves, the Chl range was even wider, from 1.2 to 67.5 nmol/cm^2, and that of Car from 3.0 to 13.7 nmol/cm^2. Car were dominant pigments in leaves with a total pigment content of less than 5 nmol/cm^2; they constituted more than 97% of the total pigment content (Fig. 1a,b). With an increase in total pigment content, the Car fraction decreased sharply; Chl became the major portion of pigments in leaves with a total pigment content >20 nmol/cm^2 (Fig. 1b). With a further increase in leaf greenness, the ratio of Car to total pigment content, [Car]/([Chl] + [Car]), decreased only a little: from 30% in slightly green to 20% in dark-green leaves. The relationship between Car and Chl was linear, with a coefficient of determination, r^2, higher than 0.82. Even higher linear correlation (r^2 exceeded 0.87) occurred between Car and the total pigment content (Fig. 1b); however, a slope of

Table 1. Pigment content and composition in leaves studied

Species	Year	Device	No. of leaves	Chl min–max	Chl mean	Car min–max	Car mean	Chl/Car min–max	Chl/Car mean
Maple	1992–1998	Hitachi	46	0.1–53.5	17.1	2.9–16.3	8.4	0.0–4.1	1.6
Maple	1999–2000	Hitachi	22	0.1–38.4	16.6	1.6–10.9	6.8	0.0–4.5	2.2
Chestnut	1992–1998	Hitachi	26	1–47	18.4	5.2–16.9	10.7	0.2–2.8	1.5
Beech	1996	Shimatzu	38	9.5–75	41.7	5.5–25.1	16.4	0.8–4	2.4
Beech	2000	Shimatzu	28	1.4–36.7	21.5	963.7–14.8	19.2	0.4–2.9	2.2

this relationship for low pigment content ([Car + Chl] < 10 nmol/cm^2) was much higher than the slope for greener leaves ([Car + Chl] > 15 nmol/cm^2), underscoring the dominant role of Car in yellow to yellowish-green leaves. For all leaves studied, [Chl] and the total pigment content, [Car + Chl], correlated closely with [Car + Chl] = 1.18·[Chl] + 5.41 with r^2 = 0.99 and a coefficient of variation of less than 7.3%.

Reflectance spectral changes in the leaves

To study the reflectance spectral features of yellow to dark-green leaves, the average reflectance was calculated for different groups of leaves (Fig. 2a). The first group contained yellow leaves with a total pigment content of <5 nmol/cm^2 (average Chl content was 0.4 nmol/cm^2 and that of Car was 3.6 nmol/cm^2). The second group included leaves with 5 nmol/cm^2 < [Car + Chl] < 10 nmol/cm^2; the average Car content was still higher than that of Chl. In the third (10 nmol/cm^2 < [Car + Chl] < 15 nmol/cm^2) and the fourth (15 nmol/cm^2 < [Car + Chl] < 20 nmol/cm^2) groups' averages, the Car content was quite close to the Chl content; the leaves in the fourth group were greener than in the third. In the last three groups, the average Chl content increased from 17 to 34 nmol/cm^2, whereas the average Car content increased only slightly from 7.3 to 10.5 nmol/cm^2. In other words, the leaf groups had different average total pigment contents, increasing with the group number. The first and second groups can be considered to represent an advanced process of stress or senescence, when leaf color turns from completely yellow to yellowish green. Leaf groups 4 and 5 corresponded to different stages of stress or senescence or both when they were still green, but the suppression of biosynthesis or the increased degradation of the green pigments or both had already begun. Groups 6 and 7 represented green (both juvenile and mature) leaves at different stages of their development; this range ended with dark-green leaves represented by group 7.

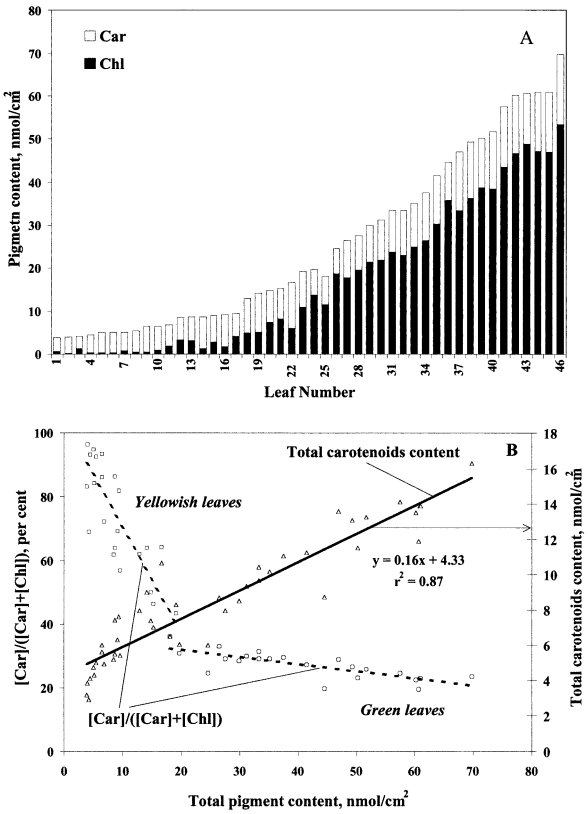

Figure 1. Relationships between total Chl and total Car contents in maple leaves. (a) Total Chl and total Car contents in maple leaves (1992–1998 data set). (b) Ratio of Car content [Car] to total pigment content [Chl] + [Car] (left scale) and total Car content (right scale) plotted *vs* total pigment content. Dotted lines represent linear best-fit functions [Car]/[Chl] + [Car] *vs* total pigment content for yellowish leaves (total pigment content <20 nmol/cm^2) and for green to dark-green leaves (total pigment content >20 nmol/cm^2). Solid line represents the best-fit function of total Car content *vs* total pigment content with r^2 = 0.87.

In yellow leaves, represented by the first group, the [Car]/[Chl] ratio was high; thus, reflectance was governed solely by Car (Fig. 2b). In the blue range, where Car absorb, reflectance was around 10%. A sharp increase in reflectance from 10% at 480 nm to almost 40% at 550 nm was observed; at longer wavelengths, reflectance remained practically unchanging in a wide spectral range including near infrared (NIR). Chl content in these leaves was so low that only a small trough could be seen in the red absorption band of Chl near 680 nm.

An increase in the total pigment content (the second to the fourth groups) led to a slight decrease of reflectance in the blue range and a significant decrease in the green and especially in the red, where reflectance dropped to less than 10% in the fourth group. A further increase in leaf greenness (groups 5 to 7) did not change the reflectance in the blue and the red. The only spectral ranges where reflectance was sensitive to pigment variation were between 510 nm to 650 nm and in the red edge around 700 nm. The variation of reflectance in the NIR range (750 to 800 nm) was small and did not exceed 5% against a background reflectance of 40%.

In the blue range, the spectra of reciprocal reflectance were fairly sensitive to the variation in pigment content between the leaf groups studied (Fig. 2c). An increase in total pigment content in groups 1 to 4 manifested itself in a con-

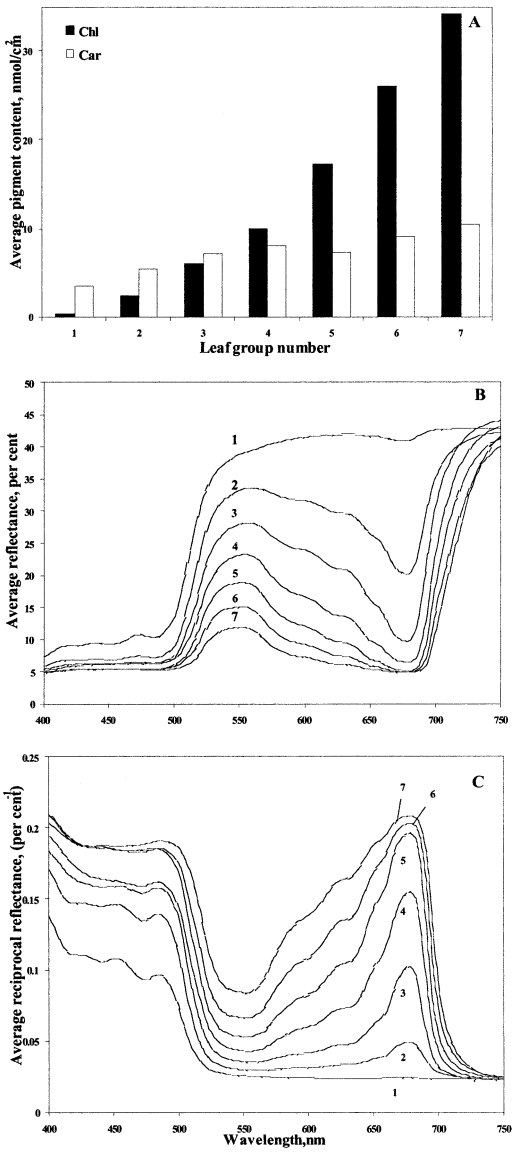

Figure 2. Pigment content, reflectance and reciprocal reflectance spectra of different maple leaf groups. (a) Average Chl and Car content in groups of yellow (group 1) to dark green (group 7) leaves. (b) Average reflectance spectra for groups of yellow (group 1) to dark green (group 7) leaves. (c) Average reciprocal reflectance spectra for groups of yellow (group 1) to dark green (group 7) leaves.

siderable increase of $(R_\lambda)^{-1}$, indicating a significant increase in Car and Chl absorption. Considerable changes also occurred in the spectral ranges of the green edge around 500 nm and the red edge around 700 nm. An increase in pigment content led to an increase in the reciprocal reflectance and to a shift of both edges toward longer wavelengths. Reciprocal reflectance in the NIR was small and almost constant.

The first leaf group is best suited to evaluate general optical properties of Car and to find their specific spectral features inherent in the (R_λ) and $(R_\lambda)^{-1}$ leaf spectra. Chl content in these leaves was negligible and the [Car]/[Chl] ratio >9. Thus, the peaks at 430, 460 and 480 nm in the $(R_\lambda)^{-1}$ spectrum (Fig. 2c) corresponded to Car absorption. A sharp decrease in absorption near 500 nm formed the green edge, a

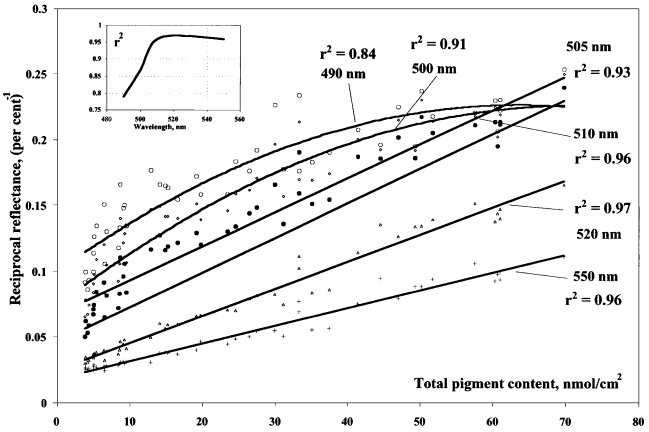

Figure 3. Reciprocal reflectance in the range 490 to 550 nm plotted *vs* total pigment content in maple leaves. At wavelengths 490 and 500 nm, the relationship $(R_\lambda)^{-1}$ *vs* [Chl + Car] was essentially nonlinear. At 505 nm, the relationship became linear with a high sensitivity of $(R_\lambda)^{-1}$ to the pigment content. It remained linear in the range 505 to 550 nm with a decrease in the slope at longer wavelengths. Insert: the coefficient of determination for the linear relationship $(R_\lambda)^{-1}$ *vs* [Chl + Car]. In the range 510 to 550 nm, r^2 was more than 0.96.

narrow transition region where absorption by Car changes from a high at 480 nm to almost negligible near 530 nm. In an attempt to find a spectral band that is maximally sensitive to Car content, this spectral region between 480 and 530 nm is of primary interest. Note that at 550 nm and beyond, there is no evidence of Car absorption; $(R_\lambda)^{-1}$ remained almost the same in the range from 540 nm to 750 nm.

The second leaf group allowed us to understand how Chl affected absorption in this range. In this group, Car increased slightly compared with the first group (from 3.6 to 5.4 nmol/cm^2), whereas Chl increased six-fold. The increase in Chl manifested itself as a sharp decrease of R_λ and an increase of $(R_\lambda)^{-1}$ around 680 nm. A significant increase in $(R_\lambda)^{-1}$ also occurred in the range 480 to 510 nm; Chl and Car are factors governing spectral characteristics of reflectance and $(R_\lambda)^{-1}$ in this spectral range. At 550 nm and longer wavelengths, R_λ and $(R_\lambda)^{-1}$ are governed by Chl absorption only.

The green edge range between 490 and 530 nm is a transition zone between the range with strong absorption by Chl and Car (in the range 400 to 480 nm) and the green range (around 550 nm) where pigment absorption is weaker and relates only to the Chl content. Having found that the reciprocal reflectance in the green range is an accurate measure of Chl content (28 to 32), we hypothesized that $(R_\lambda)^{-1}$ in the green edge range related to both pigment (Car and Chl) contents. To find evidence, we studied the relationships between $(R_\lambda)^{-1}$ and the total pigment content [Chl + Car] (Fig. 3). In the range 400 to 500 nm, the relationship of $(R_\lambda)^{-1}$ *vs* [Chl + Car] was essentially nonlinear; it leveled off for total pigment content around 30 nmol/cm^2. At 505 nm, the relationship became linear with a high sensitivity of $(R_\lambda)^{-1}$ to the total pigment content in the whole range of its variability. In the range 510 to 550 nm, $r^2\{(R_\lambda)^{-1}$ *vs* [Chl + Car]$\}$ was higher (0.96) (Fig. 3, insert). Thus, the reciprocal reflectance in the range of the green edge between 505 and 550 nm is indeed a measure of the total pigment content.

Relationships of $(R_\lambda)^{-1}$ *vs* [Chl + Car] (Fig. 3) together

with quantitative relationships between pigments (Fig. 1) showed that in green leaves Chl plays a principal role in light absorption, and Car absorption takes place against a high background of Chl absorption. Car is a fraction of total pigment content; it accounts for no more than 30% of the total pigment content for [Chl + Car] > 20 nmol/cm^2.

The main problem then was to remove the Chl contribution from the reflectance and to find a spectral band where the removal could be done with maximal efficiency. It is especially important for estimation of the early stages of plant stress or senescence when a process of green pigment degradation begins in still-green leaves and the Chl absorption is much higher than that of Car.

As mentioned previously, the Chl and Car content in leaves are strongly dependent (Fig. 1b). For example, in the yellow to dark-green maple leaves studied, r^2\{[Car] *vs* [Chl]\} was higher than 0.81 with a *P*-value <0.01 and a coefficient of variation <8.7%. Thus, to find a spectral band that is sensitive to the Car content, one should analyze leaf groups where Car and Chl are slightly dependent. We calculated the coefficient of determination for the relationships $(R_\lambda)^{-1}$ *vs* [Chl] and $(R_\lambda)^{-1}$ *vs* [Car] for maple leaves with a slight dependence of Car and Chl content. Figure 4a shows the relationships $r^2\{(R_\lambda)^{-1}$ *vs* [Chl]\} and $r^2\{(R_\lambda)^{-1}$ *vs* [Car]\} for leaves with a total Chl content lower than 10 nmol/cm^2. Yellow to yellowish-green leaves (groups 1 to 3 in Fig. 2a) were included in the analysis. The coefficient of determination of the relationship [Car] *vs* [Chl] for these leaves was lower than 0.45 with a covariance of <3.0. Spectral characteristics of these leaves were governed mainly by Car and to a lesser extent by Chl. In the range 480 to 495 nm, which is quite close to the main absorption bands of both Car and Chl *b*, $r^2\{(R_\lambda)^{-1}$ *vs* [Car]\} was about 0.8; a prominent peak of r^2 with a magnitude of >0.9 occurred around 505 nm. At longer wavelengths, the correlation became weaker, dropping to less than 0.6 at 550 nm. $r^2\{(R_\lambda)^{-1}$ *vs* [Chl]\} had very different spectral behavior. A low correlation ($r^2 < 0.5$) took place in the range 480 to 500 nm, increasing toward a longer wavelength and reaching 0.8 at 550 nm.

Figure 4b shows r^2 for the relationships $(R_\lambda)^{-1}$ with Chl and Car for yellow to green leaves (groups 1 to 5 in Fig. 2a) with the total Chl <20 nmol/cm^2. The coefficient of determination of the relationship r^2\{[Car] *vs* [Chl]\} for these leaves was lower than 0.26. Spectral characteristics of the leaves were governed by both Car and Chl. The behavior of r^2 was generally the same as for yellow to yellowish-green leaves, whereas r^2 was lower for Car and much higher for Chl. In the range 530 to 550 nm, $r^2\{(R_\lambda)^{-1}$ *vs* [Chl]\} was higher than 0.92.

In Fig. 4c, r^2 for the relationships of $(R_\lambda)^{-1}$ with Car and Chl for yellow to dark-green maple leaves (0.14 < total Chl < 54 nmol/cm^2) are shown. High $r^2\{(R_\lambda)^{-1}$ *vs* [Chl]\} values (>0.9) in the range 505 to 550 nm indicated a strong contribution of Chl to $(R_\lambda)^{-1}$ in this spectral region; it was even higher than that of Car. Note once again that we should keep in mind the close correlation between pigments when analyzing relationships between the pigment content and the reciprocal reflectance for the whole data set with a wide range of total Chl (Fig. 4c).

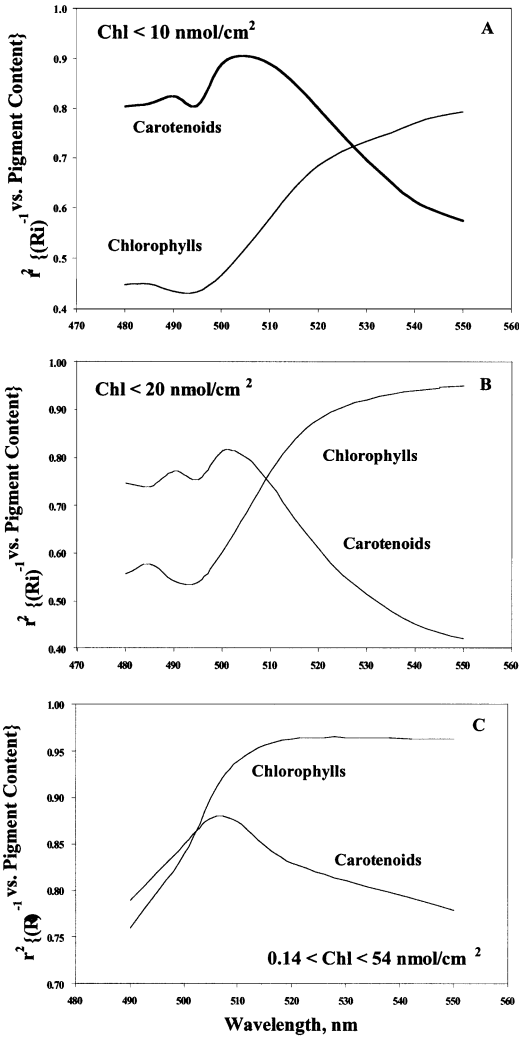

Figure 4. The coefficient of determination for linear relationships $(R_\lambda)^{-1}$ *vs* pigment content. Solid lines represent best-fit functions. (a) Leaves with total Chl content of less than 10 nmol/cm^2. (b) Leaves with total Chl content of less than 20 nmol/cm^2. (c) Leaves with total Chl content ranging from 0.14 to 54 nmol/cm^2.

Algorithm development and validation

Both Chl and Car contribute to reflectance in the range 500 to 520 nm, where $(R_\lambda)^{-1}$ was found to be maximally sensitive to Car content (Fig. 4, see also references [1,17,27]). Therefore, the challenge was to find a way to accurately subtract the Chl contribution from the reflectance in the range around 510 nm and apportion the remainder to Car.

In the range near 550 nm, both Chl, Chl *a* and Chl *b*, participate in light absorption (1). In the presence of trace amounts of Chl (<0.3 nmol/cm^2) and considerable quantities of Car (>6 nmol/cm^2), there is no evidence of a Car contribution to leaf absorption and reflectance at 550 nm (Fig. 2b,c, the first leaf group; see also references [17] and [30]). Thus, reflectance at 550 nm and longer wavelengths is governed by Chl absorption. It was found that the relationship between R_{550} *vs* [Chl] and R_{700} *vs* [Chl] is hyperbolic; thus, a close linear correlation exists between $(R_{550})^{-1}$ and [Chl] as well as between $(R_{700})^{-1}$ and [Chl] (28–33). For all leaves studied in this work, the relationships $(R_{550})^{-1}$ *vs* [Chl] and

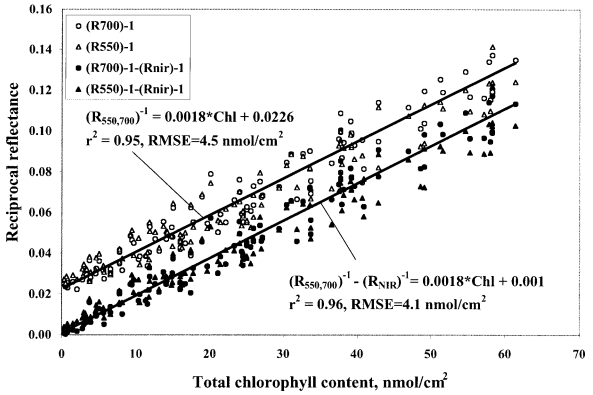

Figure 5. Reciprocal reflectance at 550 and 700 nm, $(R_{550,700})^{-1}$, and the difference $(R_{550,700})^{-1} - (R_{NIR})^{-1}$ *vs* total Chl content in maple, beech, and chestnut leaves. R_{NIR} is the reflectance in the range 750 to 800 nm. Solid lines are best-fit functions. Subtraction of $(R_{NIR})^{-1}$ from $(R_{550})^{-1}$ and $(R_{700})^{-1}$ drastically (more than 20-fold) decreased the intercept; function $(R_{550})^{-1} - (R_{NIR})^{-1}$ and $(R_{700})^{-1} - (R_{NIR})^{-1}$ became linearly proportional to the Chl content with r^2 higher than 0.95 and RMSE < 4.1 nmol/cm^2.

$(R_{700})^{-1}$ *vs* [Chl] were linear with $r^2 = 0.93$ and root mean square error (RMSE) < 4.8 nmol/cm^2 for the $(R_{550})^{-1}$ *vs* [Chl] relationship and $r^2 = 0.95$ and RMSE < 4.3 nmol/cm^2 for the $(R_{700})^{-1}$ *vs* [Chl] relationship (Fig. 5). The intercepts of both relationships represent reciprocal reflectance in the NIR range above 750 nm, $(R_{NIR})^{-1}$. In fact, subtraction of $(R_{NIR})^{-1}$ from $(R_{550})^{-1}$ and $(R_{700})^{-1}$ drastically (more than 20-fold) decreased the intercept; functions $(R_{550})^{-1} - (R_{NIR})^{-1}$ and $(R_{700})^{-1} - (R_{NIR})^{-1}$ were linearly proportional to the Chl content with r^2 higher than 0.95 and RMSE < 4.1 nmol/cm^2 (Fig. 5).

To remove the Chl contribution from the inverse reflectance in the green edge range, we used an inverse reflectance at either 550 or 700 nm. The differences $[(R_\lambda)^{-1} - (R_{550})^{-1}]$ and $[(R_\lambda)^{-1} - (R_{700})^{-1}]$ were calculated and plotted *vs* the Car content in the λ range from 505 to 525 nm (see solid lines representing the best-fit functions in Fig. 6). At 505 nm, the relationship had a high slope for Car <10 nmol/cm^2; with an increase in Car, the sensitivity of both differences declined and the curves leveled off. At 510 nm and longer wavelengths, the difference remained sensitive to Car content in the whole range of Car variations. In the range 510 to 525 nm, linear regressions between the differences and Car had $r^2 > 0.9$; the slopes of the relationships sharply decreased with wavelength, becoming three to four times smaller at 525 nm than at 510 nm (Table 2, Fig. 6). The closest linear correlation between the differences and Car with high sensitivity to the Car content was achieved in the range around 510 nm, where the product of the slope and the coefficient of determination reached the maximum for both differences (Table 2).

Thus, for Car content estimation, we devised and used the Carotenoid Reflectance Index (CRI), which represents the difference between the reciprocal reflectance at 510 nm, where both Car and Chl affect reflectance, and at either 550 or 700 nm, where only Chl affects reflectance:

$$^1\mathrm{CRI}_{550} = (R_{510})^{-1} - (R_{550})^{-1} \quad (1)$$

$$^1\mathrm{CRI}_{700} = (R_{510})^{-1} - (R_{700})^{-1} \quad (2)$$

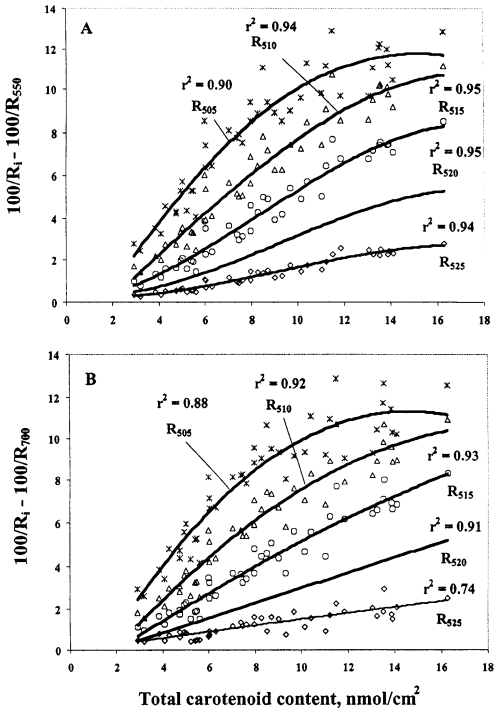

Figure 6. Indices in the form (a) $(100/R_i) - (100/R_{550})$ and (b) $(100/R_i) - (100/R_{700})$ *vs* total Car content in maple leaves. R_i is the reflectance in the range 505 to 525 nm. Solid lines represent best-fit functions.

In the indices, the first term is responsible for both Chl and Car absorption and the latter one for Chl absorption.

In Fig. 7, the relationships between $^1\mathrm{CRI}_{550} = [(R_{510})^{-1} - (R_{550})^{-1}]$ and both pigments are plotted (solid and thin lines represent polynomial best-fit functions for Car and Chl, respectively). The determination coefficient for the polynomial function CRI *vs* Car was $r^2 = 0.94$, and it was a little lower ($r^2 = 0.92$) for the linear function. To assess to what extent the Chl effect was removed from $(R_{510})^{-1}$ by subtraction of either $(R_{550})^{-1}$ or $(R_{700})^{-1}$, we analyzed the sensitivity of the suggested indices to the Car and Chl content as the slopes of the linear regressions between CRI *vs* [Car] and CRI *vs* [Chl] (Table 3). The maximal slope of CRI *vs* [Car] was in the range 505 to 510 nm and was at least five-fold higher than the slope of the relationship of CRI *vs* [Chl]. Note that the slopes of the relationships of CRI *vs* the pigment content in Table 3 are given for a whole range of pigment variation

Table 2. Coefficient of determination (r^2), slope and their product for the linear regression between Car content in maple leaves (1992–1998 data set) and indices in the form $[(R_\lambda)^{-1} - (R_{550})^{-1}]$ and $[(R_\lambda)^{-1} - (R_{700})^{-1}]$ for λ ranging from 505 to 525 nm

	$(R_{550})^{-1}$			$(R_{700})^{-1}$		
$(R_\lambda)^{-1}$	r^2	Slope	$r^2 \cdot$ Slope	r^2	Slope	$r^2 \cdot$ Slope
505	0.84	0.77	0.65	0.84	0.77	0.60
510	0.92	0.77	0.71	0.90	0.72	0.65
515	0.94	0.63	0.59	0.93	0.58	0.54
520	0.95	0.41	0.39	0.91	0.35	0.32
525	0.94	0.21	0.20	0.74	0.15	0.11

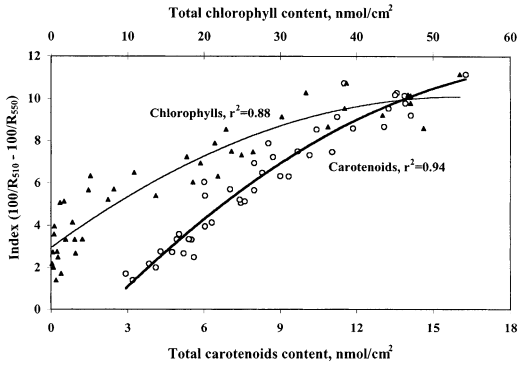

Figure 7. CRI = $(100/R_{510}) - (100/R_{550})$ plotted *vs* total Car and total Chl content in maple leaves. Solid line is the best-fit function of CRI *vs* Car; thin line is the best-fit function of CRI *vs* Chl. The sensitivity of CRI to the Car content was much higher than that to the Chl content.

from yellow to dark-green leaves. For green leaves with moderate to high Chl content, the slope of CRI *vs* [Chl] was even lower than the values presented in Table 3.

Validation of the proposed technique has been carried out for maple, chestnut and beech leaves with a wide variation of pigment content (Table 1, Fig. 1a). In Table 4, the characteristics of the linear regression between $^1CRI_{700} = (R_{510})^{-1} - (R_{700})^{-1}$ and the Car content are summarized for these plant species and for the combined data set, which included all leaves studied. Coefficient *a* in the relationship $CRI_{700} = a\cdot[Car] + b$ ranged from 0.47 in beech to 0.72 in maple leaves measured during 1992–1998. They represented two extremes, whereas the relationships for maple 1999–2000 and chestnut were close to the relationship for the combined data set. The coefficient of determination, 0.61, was minimal for chestnut and maximal, 0.9, for maple 1992–1998. For all 122 leaves examined, the relationship was linear with $r^2 = 0.71$ and RMSE of Car estimation < 1.86 nmol/cm^2 (Fig. 8).

Both $(R_{550})^{-1}$ and $(R_{700})^{-1}$ relate closely to the Chl content (Fig. 5); nevertheless, it is not clear how precisely $(R_{550})^{-1}$ and $(R_{700})^{-1}$ represent the Chl effect on the reciprocal reflectance at 510 nm. The specific absorption of Chl *in vivo* is not known; in solution, it depends to a great degree on the type of solvent (see, for example, reference 1). Thus, the indices for Car estimation generally should have the forms

$$^2CRI_{550} = \{(R_{510})^{-1} - (R_{NIR})^{-1}\} - m\{(R_{550})^{-1} - (R_{NIR})^{-1}\} \quad (3)$$

$$^2CRI_{700} = \{(R_{510})^{-1} - (R_{NIR})^{-1}\} - k\{(R_{700})^{-1} - (R_{NIR})^{-1}\} \quad (4)$$

where *m* and *k* are coefficients accounting for the specific absorption by Chl at 510 nm. These coefficients might be different for $(R_{550})^{-1}$ and $(R_{700})^{-1}$, because at 550 nm, both Chl, Chl *a* and Chl *b*, affect reflectance, whereas at 700 nm, Chl *a* seems to be the main absorber. In solutions, the specific absorption of Chl *a* is higher at 550 than at 510 nm, whereas that of Chl *b* is lower at 550 than at 510 nm (1). Subtraction of $(R_{NIR})^{-1}$ makes both terms $\{(R_{550})^{-1} - (R_{NIR})^{-1}\}$ and $\{(R_{700})^{-1} - (R_{NIR})^{-1}\}$ directly proportional to the Chl content (Fig. 5) and term $\{(R_{510})^{-1} - (R_{NIR})^{-1}\}$ proportional to the total pigment content. For all leaves studied, the use of $k = m = 0.75$ enhanced the accuracy of the Car estimation; RMSE decreased from 1.92 for $k = m = 1$ to 1.75 nmol/cm^2, and r^2 increased from 0.71 to 0.77. For each plant species, RMSE does not exceed 1.6 nmol/cm^2.

Using our data sets, we tested the accuracy of the indices that had been developed previously for Car estimation (15,20,21,23) and compared it with the performance of the indices developed, CRI_{550} and CRI_{700}. As mentioned previously, RMSE of the Car estimation by $^2CRI_{550}$ and $^2CRI_{700}$

Table 3. Slope of the linear regression between indices $CRI_{550} = [(R_\lambda)^{-1} - (R_{550})^{-1}]$ and $CRI_{700} = [(R_\lambda)^{-1} - (R_{700})^{-1}]$ and Car and Chl content in the maple leaves 1992–1998 data set. λ ranged from 505 to 525 nm. Ratio Car/Chl is the ratio of the slope of the relationship {CRI *vs* [Car]} to {CRI *vs* [Chl]}

	CRI_{550}			CRI_{700}		
$(R_\lambda)^{-1}$	Slope CRI *vs* Car	Slope CRI *vs* Chl	Ratio Car/Chl	Slope CRI *vs* Car	Slope CRI *vs* Chl	Ratio Car/Chl
505	0.77	0.15	5.15	0.77	0.15	4.77
510	0.77	0.15	5.17	0.72	0.14	5.13
515	0.63	0.13	4.85	0.58	0.11	5.27
520	0.41	0.08	5.12	0.35	0.07	5
525	0.21	0.04	5.25	0.15	0.03	5

Table 4. Characteristics of linear regression between CRI in the form $(R_{510})^{-1} - (R_{700})^{-1}$ and Car content: CRI = $a \cdot$ Car + b. r^2 is coefficient of determination; RMSE is root-mean square error of Car estimation Coef. Var. is coefficient of variation. *P*-value for all data sets was less than 0.01

Species	Year	No. of leaves	*a*	*b*	r^2	RMSE nmol/cm^2	Coef. Var (%)
Maple	1992–1998	46	0.72	0.02	0.9	1.13	13.5
Maple	1999–2000	22	0.58	1.7	0.7	1.36	20.1
Chestnut	1992–1998	26	0.54	1.6	0.61	2.09	19.7
Beech	2000	28	0.47	0.93	0.87	1.12	11.2
All species together		122	0.56	1.16	0.71	1.86	20.7

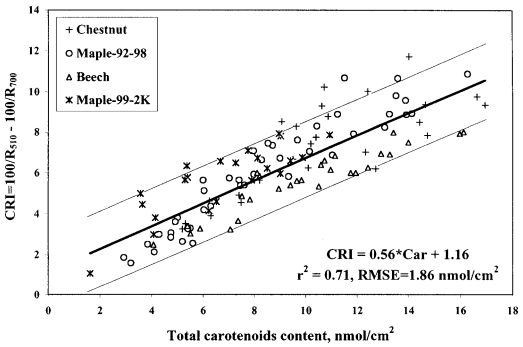

Figure 8. CRI = $(100/R_{510}) - (100/R_{700})$ plotted *vs* total Car content in maple, chestnut and beech leaves. Solid line is the best-fit function. Thin lines are RMSE of Car content estimation. Characteristics of the linear regressions for each plant species are given in Table 4.

Table 5. Comparison of reflectance indexes for Car estimation. Yellow to dark-green maple leaves (1992–1998) with pigment content 0.14 nmol/cm^2 < Chl < 53.5 nmol/cm^2 and 2.9 nmol/cm^2 < Car < 16.3 nmol/cm^2. r^2 is coefficient of determination of linear regression between the index and Car or Chl content. R_λ is reflectance at wavelength λ. (Sensitivity to Car)/(Sensitivity to Chl) was calculated as {Slope(Index *vs* [Car])}/Slope(Index *vs* [Chl]}·Slope([Chl] *vs* [Car]). Slope(Index *vs* [Car]) is the slope of linear regression between Index and Car, and Slope(Index *vs* [Chl]) is the slope of linear regression between Index and Chl. Slope(Chl *vs* [Car]) is the slope of the linear regression between Chl and Car; for this data set it was equal to 4.44. Thus, when Slope(Index *vs* [Car]) in cm^2/nmol was equal to 4.44, the index was equally sensitive to both Chl and Car contents, and the ratio (Sensitivity to Car)/(Sensitivity to Chl) was equal to 1. When Slope(Index *vs* [Car]) was more than 4.44, the index was more sensitive to Car content, and the ratio (Sensitivity to Car)/(Sensitivity to Chl) was more than 1. When Slope(Index *vs* [Car]) was less than 4.44, the index was more sensitive to Chl than to Car

Author	Chappelle *et al.* (1992)	Gamon *et al.* (1990)	Blackburn (1998)	Blackburn (1998)	Datt (1998)		This work	
Index	$\frac{R_{760}}{R_{500}}$	$\frac{R_{531}-R_{570}}{R_{531}+R_{570}}$	$\frac{R_{800}}{R_{470}}$	$\frac{R_{800}-R_{470}}{R_{800}+R_{470}}$	$0.0049\left[\frac{R_{672}}{R_{550}\cdot R_{708}}\right]^{0.748}$	$\frac{R_{NIR}}{R_{510}}$	$(R_{510})^{-1}-(R_{550})^{-1}$	$(R_{510})^{-1}-(R_{700})^{-1}$
r^2 {Index *vs* Car}	0.90	0.68	0.81	0.72	0.35	0.94	0.92	0.90
r^2 {Index *vs* Chl}	0.85	0.84	0.73	0.59	0.49	0.94	0.85	0.79
(Sensitivity to Car)/ (Sensitivity to Chl)	1.12	1.05	1.15	1.19	0.93	1.1	1.26	1.15

was about 10% lower than that of $^1CRI_{550}$ and $^1CRI_{700}$. In Tables 5 and 6, the coefficients of determination for the relationships Index *vs* [Car] and Index *vs* [Chl] as well as ratios of the sensitivities of the indices to Car and to Chl are shown. A ratio Sensitivity to [Car]/Sensitivity to [Chl] was calculated as {Slope(Index *vs* [Car])/([Car_{max}] − [Car_{min}])}/{Slope(Index *vs* [Chl])/([Chl_{max}] − [Chl_{min}]}. Slope(Index *vs* [Car]) is the slope of the linear regression Index *vs* [Car], normalized to the range of the Car variation; [Car_{max}] and [Car_{min}] are maximal and minimal Car values, respectively. Slope(Index *vs* [Chl])/([Chl_{max}] − [Chl_{min}]) is the slope of linear regression Index *vs* [Chl], normalized to the range of Chl variation; [Chl_{max}] and [Chl_{min}] are maximal and minimal Chl values, respectively. Thus, calculating the ratio of sensitivities to Car and to Chl (Tables 5 and 6), we took into account not only the slopes of the Index *vs* Car and Index *vs* Chl relationships but also the ranges of pigment variation.

For completely yellow to dark-green maple leaves (Table 5), $^1CRI_{550}$ and $^1CRI_{700}$ had the best correlation with Car and maximal sensitivity to the Car content. A fairly close correlation with Car also showed the ratio R_{760}/R_{500} (20), $^1CRI_{700}$ and the index in the form R_{NIR}/R_{510}. In the latter index, we used the approach of Chappelle *et al.* (20), changing R_{500} to R_{510}, which was found to be linearly proportional to the pigment content, whereas R_{500} leveled off for moderate to high Car content (Fig. 3). This index had a maximal r^2 value (0.93), but the sensitivity to Car was only slightly higher than that to Chl. When the reflectance at 470 nm was used as a term sensitive to the Car content in indices suggested by Blackburn (21), R_{800}/R_{470} and $(R_{800} - R_{470})/(R_{800} - R_{470})$, a good sensitivity to Car was achieved (1.15 to 1.19) with a fairly high r^2 (0.72 to 0.81). The index developed by Gamon *et al.* (15) to estimate the physiological status of plants $(R_{531} - R_{570})/(R_{531} + R_{570})$ was more sensitive to the Chl content than to Car.

To test the performance of the indices in detecting the Car content in slightly green to dark-green leaves, we excluded yellow leaves from the data set and present the comparison in Table 6. $^1CRI_{550}$ and $^1CRI_{700}$ displayed the best correlation and sensitivity to Car. Indices R_{NIR}/R_{510} as well as $0.0049\cdot[R_{672}/(R_{550}\cdot R_{708})]^{0.748}$ (23) had a smaller sensitivity to Car, whereas it was still higher than that to Chl. Indices R_{760}/R_{500} and $(R_{531} - R_{570})/(R_{531} + R_{570})$ presented in references (20) and (15), respectively, were more sensitive to the Chl content than to Car. Indices recommended in reference (21), R_{800}/R_{470} and $(R_{800} - R_{470})/(R_{800} - R_{470})$, showed a higher sensitivity to Car than to Chl, but the coefficient of determination was low ($r^2 < 0.56$). In green leaves with Chl >20

Table 6. Comparison of reflectance indexes for carotenoid estimation. Yellow-green to dark-green maple leaves (1992–1998) with pigment content 10 nmol/cm^2 < Chl < 53.5 and 6 nmol/cm^2 < Car < 16.3 nmol/cm^2. r^2 is coefficient of determination of linear regression between the index and Car or Chl content. R_λ is reflectance at wavelength λ. The slope of the relationship Chl *vs* Car for this data set was 4.16. It means that when the ratio of slopes (Index *vs* [Car])/(Index *vs* [Chl]) was equal to 4.16, the index was equally sensitive to both Chl and Car contents. When the ratio was more than 4.16, the index was more sensitive to Car content than to Chl content. When this ratio was less than 4.16, the index was more sensitive to Chl than to Car

Author	Chappelle *et al.* (1992)	Gamon *et al.* (1990/2/3/7)	Blackburn (1998)	Black-burn (1998)	Datt (1998)		This work	
Index	$\frac{R_{760}}{R_{500}}$	$\frac{R_{531}-R_{570}}{R_{531}+R_{570}}$	$\frac{R_{800}}{R_{470}}$	$\frac{R_{800}-R_{470}}{R_{800}+R_{470}}$	$0.0049\left[\frac{R_{672}}{(R_{550}\cdot R_{708})}\right]^{0.748}$	$\frac{R_{NIR}}{R_{510}}$	$(R_{510})^{-1}-(R_{550})^{-1}$	$(R_{510})^{-1}-(R_{700})^{-1}$
r^2 {Index *vs* Car}	0.75	0.53	0.53	0.56	0.79	0.82	0.81	0.75
r^2 {Index *vs* Chl}	0.75	0.69	0.54	0.57	0.89	0.90	0.60	0.64
(Sensitivity to Car)/ (Sensitivity to Chl)	0.97	0.96	1.19	1.44	1.06	1.06	1.27	1.09

nmol/cm^2, CRI was the only index showing a higher sensitivity to Car than to Chl.

Thus, the newly suggested indices showed the highest sensitivity to Car with a reasonably high (>0.75) coefficient of determination even in leaves with a high Chl content. It allowed detection of the early stages of stress or senescence or both when the leaves were still green, and only a slight decrease in the [Chl]/[Car] ratio was an indicator of the changes in the plant physiological state. The reason for the higher sensitivity and selectivity of the suggested CRI to Car is removal of the Chl effect from the reflectance in the green edge range, where reflectance is sensitive to both Car and Chl contents.

For nondestructive Car estimation, three spectral bands, 510 $\pm$ 5 nm, either 550 $\pm$ 15 nm or 700 $\pm$ 7.5 nm and the NIR band above 750 nm were found to be sufficient. The simulated reflectances in these spectral bands were used to calculate CRI; the RMSE of the Car estimation did not exceed those for the narrow channels mentioned previously.

The technique developed is the first step in quantifying Car content. The accuracy of a nondestructive Car estimation depends significantly on the Car composition. Thus, the next step is to investigate how the Car composition affects the accuracy of the total Car estimation and how to quantify nondestructively different Car types (carotenes, lutein, zeaxanthin, violaxanthin and neoxanthin). The problem is to determine the relationships between independent factors such as Chl *a* and Chl *b* and different types of Car, on the one hand, and reciprocal reflectances in the blue and green ranges and their combinations, on the other. From this, one will be able to choose the most appropriate range where reciprocal reflectance is most sensitive to the content of each type of Chl and Car and combinations of reciprocal reflectance that are most sensitive to each Car type. Among several advanced methods applicable in this situation, multidimensional scaling (36) appears most appropriate.

CONCLUSIONS

1. Reciprocal reflectance in the range 510 to 550 nm was found to be linearly related to the total pigment content in leaves. The finding allows for the accurate estimation of the total pigment content by nondestructive reflectance measurements.

2. The sensitivity of reciprocal reflectance to the Car content is maximal in a spectral range around 510 nm. Significantly, Chl also affects reflectance in this spectral range. To remove the Chl effect from the reciprocal reflectance at 510 nm, a reciprocal reflectance at either 550 or 700 nm was used.

3. On the basis of fundamental optical properties of Car in leaves revealed in this study and the optical properties of Chl, indices for nondestructive and selective estimation of the total Car content in leaves were devised. Reflectances in three spectral bands, 510 $\pm$ 5 nm, either 550 $\pm$ 15 nm or 700 $\pm$ 7.5 nm and the NIR range above 750 nm are sufficient to estimate nondestructively total Car content in leaves with a reasonable accuracy.

4. Once the optical properties of Car and Chl *in vivo* have been investigated and used, the same basic method would be applicable for other plant species. It should be stressed, however, that the accuracy of the proposed algorithms in other plant species must be verified. More studies are required to broaden the models offered in this work in order to devise comprehensive algorithms for remote sensing of Car pigments.

Acknowledgements—The authors are very thankful to Professor Harmut K. Lichtenthaller and Dr. Claus Buschmann, Karlsruhe University, for their help in collecting beech data and their helpful discussions. The study was supported partially by fellowships to A.A.G. (1996) and Y.Z. (2000) from the German Academic Exchange Service (DAAD), which is gratefully acknowledged.

REFERENCES

1. Lichtenthaler, H. K. (1987) Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol.* **148**, 331–382.
2. Palett, K. E. and A. J. Young (1993) Carotenoids. In *Antioxidants in Higher Plants* (Edited by R. G. Alscher and J. L. Hess), pp. 60–89. CRC Press Inc., Boca Raton, FL.
3. Biswall, B. (1995) Carotenoid catabolism during leaf senescence and its control by light. *J. Photochem. Photobiol. B* **30**, 3–14.
4. Demmig-Adams, B., A. M. Gilmore and W. W. Adams III (1996) *In vivo* functions of carotenoids in higher plants. *FASEB J.* **10**, 403–412.
5. Horton, P., A. V. Ruban and R. G. Walters (1996) Regulation of light harvesting in green plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 635–684.
6. Young, J. and H. A. Frank (1996) Energy transfer reactions involving carotenoids: quenching of chlorophyll fluorescence. *J. Photochem. Photobiol. B* **36**, 3–15.
7. Peterman, E. J. G., C. C. Gradinaru, F. Calkoen, J. C. Borst, R. van Grondelle and H. van Amerongen (1997) Xanthophylls in light-harvesting complex II of higher plants: light harvesting and triplet quenching. *Biochemistry* **36**, 12208–12215.
8. Edge, R. D., J. McGarvey and T. G. Truscott (1997) The carotenoids as anti-oxidants. *J. Photochem. Photobiol. B* **41**, 189–200.
9. Naqvi, K. R., B. B. Raji, T. Javorfi, I. Simidjev and G. Garab (1997) Quenching of chlorophyll *a* singlets and triplets by carotenoids in light-harvesting complex of photosystem II: comparison of aggregates with trimers. *Spectrochim. Acta Part A* **53**, 2659–2667.
10. Gruszecki, W., P. Kernen, Z. Krupa and R. J. Strasser (1994) Involvement of xanthophyll pigments in regulation of light-driven excitation quenching in light-harvesting complex of Photosystem II. *Biochim. Biophys. Acta* **1188**, 235–242.
11. Merzlyak, M. N. and A. A. Gitelson (1995) Why and what for the leaves are yellow in autumn? On the interpretation of optical spectra of senescing leaves (*Acer platanoides* L.). *J. Plant Physiol.* **145**, 315–320.
12. Lichtenthaler, H. K. (1996) Vegetation stress: an introduction to the stress concept in plants. *J. Plant Physiol.* **148**, 4–14.
13. Young, A. and G. Britton (1990) Carotenoids and stress. In *Stress Responses in Plants: Adaptation and Acclimation Mechanisms* (Edited by R. G. Alscher and J. R. Cumming), pp. 87–112. Wiley-Liss, New York, NY.
14. Penuelas, J. and I. Filella (1998) Visible and near-infrared reflectance techniques for diagnosing plant physiological status. *Trends Plant Sci.* **3**, 151–156.
15. Gamon, J. A., C. B. Field, W. Bilger, O. Bjorkman, A. L. Fredeen and J. Penuelas (1990) Remote sensing of the xanthophyll cycle and chlorophyll fluorescence in sunflower leaves and canopies. *Oecologia* **85**, 1–7.
16. Gamon, J. A. and J. S. Surfus (1999) Assessing leaf pigment content and activity with a reflectometer. *New Phytol.* **143**, 105–117.
17. Merzlyak, M. N., A. A. Gitelson, O. B. Chivkunova and V. Y. Rakitin (1999) Non-destructive optical detection of leaf senescence and fruit ripening. *Physiol. Plant.* **106**, 135–141.
18. Merzlyak, M. N. and O. B. Chivkunova (2000) Light stress

induced pigment changes and evidence for anthocyanin photoprotection in apple fruit. *J. Photochem. Photobiol. B* **55**, 154–162.
19. Thomas, J. R. and H. W. Gausman (1977) Leaf reflectance vs. leaf chlorophyll and carotenoid concentrations for eight crops. *Agron. J.* **69**, 799–802.
20. Chappelle, E. W., M. S. Kim and J. E. McMurtrey 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 Sens. Environ.* **39**, 239–247.
21. Blackburn, G. A. (1998) Quantifying chlorophylls and carotenoids at leaf and canopy scales: an evaluation of some hyperspectral approaches. *Remote Sens. Environ.* **66**, 273–285.
22. Blackburn, G. A. (1999) Relationships between spectral reflectance and pigment concentrations in stacks of deciduous broadleaves. *Remote Sens. Environ.* **70**, 224–237.
23. Datt, B. (1998) Remote sensing of chlorophyll *a*, chlorophyll *b*, chlorophyll *a*+*b*, and total carotenoid content in eucalyptus leaves. *Remote Sens. Environ.* **66**, 111–121.
24. Penuelas, J., F. Baret and I. Filella (1995) Semi-empirical indices to assess carotenoids/chlorophyll *a* ratio from leaf spectral reflectance. *Photosynthetica* **31**, 221–230.
25. Gamon, J. A., L. Serrano and J. S. Surfus (1997) The photochemical reflectance index: an optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. *Oecologia* **112**, 492–501.
26. Gitelson, A. A., Y. J. Kaufman and M. N. Merzlyak, (1966) Use of a green channel in remote sensing of global vegetation from EOS-MODIS. *Remote Sens. Environ.* **58**, 289–298.
27. Zur, Y., A. A. Gitelson, O. B. Chivkunova and M. N. Merzlyak (2000) The spectral contribution of carotenoids to light absorption and reflectance in green leaves. In *Proceedings of the Second International Conference on Geospatial Information in Agriculture and Forestry,* Vol. 2, pp. II-17–II-23. Lake Buena Vista, FL.
28. Gitelson, A. A. and M. N. Merzlyak (1994) Quantitative estimation of chlorophyll-*a* using reflectance spectra: experiments with autumn chestnut and maple leaves. *J. Photochem. Photobiol. B* **22**, 247–252.
29. Gitelson, A. A. and M. N. Merzlyak (1994) Spectral reflectance changes associated with autumn senescence of *Asculus hippocastanum* and *Acer platanoides* leaves. Spectral features and relation to chlorophyll estimation. *J. Plant Physiol.* **143**, 286–292.
30. Gitelson, A. and M. N. Merzlyak (1996) Signature analysis of leaf reflectance spectra: algorithm development for remote sensing of chlorophyll. *J. Plant Physiol.* **148**, 494–500.
31. Gitelson, A. and M. N. Merzlyak (1997) Remote estimation of chlorophyll content in higher plant leaves. *Int. J. Remote Sens.* **18**, 291–298.
32. Lichtenthaler, H. K., A. A. Gitelson and M. Lang (1996) Nondestructive determination of chlorophyll content of leaves of a green and an aurea mutant of tobacco by reflectance measurements. *J. Plant Physiol.* **148**, 483–493.
33. Gitelson, A. A., M. N. Merzlyak and O. B. Chivkunova (2001) Optical properties and non-destructive estimation of anthocyanin content in plant leaves. *Photochem. Photobiol.* **74**, 38–45.
34. Buschmann, C. and E. Nagel (1993) *In vivo* spectroscopy and internal optics of leaves as basis for remote sensing of vegetation. *Int. J. Remote Sens.* **14**, 711–722.
35. Yoder, B. J. and R. E. Pettigrew-Crosby (1995) Predicting nitrogen and chlorophyll concentrations from reflectance spectra (400–2500) at leaf and canopy scales. *Remote Sens. Environ.* **53**, 199–211.
36. Perekrest, V. T., T. V. Khachaturova, I. B. Beresneva, N. M. Mitrofanova, E. Kunstle, E. Wagner and L. Fukshansky (1995) Nonlinear regression-typological analysis of ecophysiological states of vegetation: a pilot study with small data sets. *Tree Physiol.* **15**, 765–774.