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Signature Analysis of Leaf Reflectance Spectra: Algorithm Development for Remote Sensing of Chlorophyll

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Summary

The goal of the study is to investigate the basic spectral properties of plant leaves to develop spectral indices more sensitive to chlorophyll concentration than the presently widely used Normalized Difference Vegetation Index. These indices can serve as indicators of stress, senescence, and disease in higher plants. The spectral reflectance of senescing leaves of two deciduous species (maple and chestnut) as well as their pigment content were measured. Spectral indices were developed using reflectances corresponding to wavelengths with maximum and minimum sensitivity to variation in pigment concentration. The signature analysis of reflectance spectra indicated that, for a wide range of leaf greenness (completely yellow to dark green leaves), the maximum sensitivity of reflectance coincides with the maximum absorption of chlorophyll *a* at 670 nm. However, for yellow-green to green leaves (minimum chlorophyll *a* as low as 3–5 nmol/cm^2), the reflectance near 670 nm is not sensitive to chlorophyll concentration due to saturation effects. Therefore, it seems inappropriate to use this spectral band for pigment estimation in yellow-green to green vegetation. The spectral bands ranging from 400 to 480 nm and above 730 nm are not sensitive to chlorophyll concentration as found for 670 nm. The reflectances at these wavelengths could be used as references in the vegetation indices. Maximum sensitivity to chlorophyll *a* concentration was found at 550–560 nm and 700–710 nm. Reflectances at 700 nm correlated very well with that at 550 nm for a wide range of chlorophyll concentrations for both plant species studied. The inverse reflectance, $(R_{550})^{-1}$ and $(R_{700})^{-1}$ are proportional to chlorophyll *a* concentration; therefore indices R_{750}/R_{550} and R_{750}/R_{700} are directly proportional (correlation $r^2 > 0.95$) to chlorophyll concentration. These indices were tested for a wide range of chlorophyll *a* concentration, using several independent data sets. The estimation error in the derivation of chlorophyll concentration from the indices is assessed to be less than 1.2 nmol/cm^2.

Key words: Reflectance spectra of leaves, remote sensing, vegetation indices.

Abbreviations: Chl *a* and *b* = chlorophyll *a* and *b;* Car = total carotenoids; STD = standard deviation.

Introduction

Plant senescence, diseases and many long-term stresses result in a loss of chlorophyll content, therefore it is extremely important to develop technique for non-destructive estimation of chlorophyll content of a vegetation. The application of remote sensing technology in the visible part of the electromagnetic spectrum to yield information about the state of vegetation is limited by a complexity of interacting factors involved in the reflectance response (Andrieu and Baret, 1993; Baret and Guyot, 1991; Curran et al., 1991; Horler et al., 1983; Huete et al., 1994). Not enough is yet understood about the peculiarities, specific features and optical properties of a leaf (Horler et al., 1983; Fukshansky, 1981; Vogelmann and Björn, 1986). Nevertheless, several specific bands in the reflectance spectrum were successfully used

to monitor chlorophyll and carotenoid contents in intact leaves (Baret et al., 1987; Baret et al., 1992; Buschmann and Nagel, 1993; Chappelle et al., 1992; Curran et al., 1991; Gitelson and Merzlyak, 1994 a, b; Kim et al., 1994; McMurtrey III et al., 1994).

We found in previous experiments with yellowing autumn leaves of two deciduous species that the bands near 550 and 700 nm were the regions with the maximal sensitivity of reflectance to a variation in chlorophyll (Chl) concentration. Several indices utilizing spectral features located outside the main bands of photosynthetic pigment absorption, were developed for estimation of Chl concentration (Gitelson and Merzlyak, 1994 a, b).

The first objective of this research was to understand in more detail the specific spectral features of the plant photosynthetic tissues through examination of the reflectance spectra of both mature and senescing green leaves covering a wide range of pigment concentration. Specific wavelengths sensitive to pigment variation were discovered and the algorithms for Chl assessment at leaf level using reflectances at 550, 700, and longer 750 nm were developed. They were tested by independent data sets for a range of Chl *a* from 0.3 to 44.8 nmol/cm^2 for maple and chestnut leaves.

The second objective was to determine which bands in the reflectance spectrum could be employed for estimation of pigment quantities and other characteristics of vegetation canopies in the presence of non-photosynthetic materials. In addition to the reflectance at 550 and 700 nm, the reflectances at 500 and 670 nm were also found to be highly correlated in yellow-green to dark green leaves. It was suggested to use the indices $[(R_{700}/R_{550})-1]$ and $[(R_{670}/R_{500})-1]$ as indicators of the contribution of non-photosynthetic materials and apply them to remote estimation of pigments in canopies.

Materials and Methods

The experiments were performed in October 1991, 1993 on horse chestnut (*Aesculus hippocastanum* L.), and in October 1992–1993 on Norway maple (*Acer platanoides* L.) leaves. Leaves of both trees were collected in the Botanical Garden of the Moscow State University, as described previously (Gitelson and Merzlyak, 1994 a; Merzlyak and Gitelson, 1995). In addition to the senescing samples, mature green leaves of both species collected in July of 1994 were also examined. This sampling scheme intended to cover a variation of pigment concentrations as high as possible in each experiment. Only leaves having homogeneous dark green, green, green-yellow, yellow-green and yellow color without anthocyanin pigmentation were selected.

Hemispherical reflectance spectra were recorded from 400 to 750 nm for the upper surface of the leaves with a Hitachi 150-20 spectrophotometer equipped with an integrating sphere. The spectral resolution was 2 nm. The reflectance spectra were measured against barium sulfate as a reference standard. A light trap was designed to eliminate the specular reflected component of the radiance, and black velvet was used as a background to absorb the light passing through the leaf (Gitelson and Merzlyak, 1994 a; Merzlyak and Gitelson, 1995). The reflectance was expressed as a ratio of the radiance of the leaf to the radiance of the reference. The spectra were recorded for the sections of the leaves between main veins (maple) or with a removed main vein (chestnut).

Chl *a*, *b* and total carotenoid (Car) concentrations in the leaves were determined in acetone extracts and calculated using equations and specific extinction coefficients reported by Lichtenthaler (1987). The average mol.wt for Car of 570 was used.

Results

Pigment content in the leaves

Chl *a*, *b* and Car concentrations in the leaves are presented in Fig. 1 where data are sorted by Chl *a*+*b* concentration. The dominant pigment, Chl *a*, ranged from 0.3 to 44.8 nmol/cm^2 in maple leaves and from 0.5 to 42.4 nmol/cm^2 in chestnut leaves. The green leaves collected both in summer and autumn (Chl *a*+*b* > 20 nmol/cm^2) contained approximately equal proportions of pigments. Although the leaves of both species lost Chl *a* and *b* during the progression of autumn senescence, relatively high concentrations of carotenoids were present (Gitelson and Merzlyak, 1994 a; Merzlyak and Gitelson, 1995; Merzlyak and Hendry, 1994). Only trace amounts of chlorophylls were detected in completely yellow leaves.

Reflectance spectral changes in the leaves

The representative reflectance spectra of the maple leaves which changed color from dark green to completely yellow contained decreasing amounts of Chl are shown in Fig. 2. The reflectance spectral features were found to be similar for both species studied. Although the spectra obtained with *A. platanoides* leaves will be considered later, the results being very close to those obtained with *A. hippocastanum* leaves.

The maximum reflectance was found at 750 nm and was independent of pigment concentration and the stage of leaf development. The lowest reflectance was observed in the blue range of the spectrum from 400 to 500 nm. For completely yellow leaves (Chl *a* from 0.3 to 1 nmol/cm^2), the reflectance in the above range was much higher than that of the other

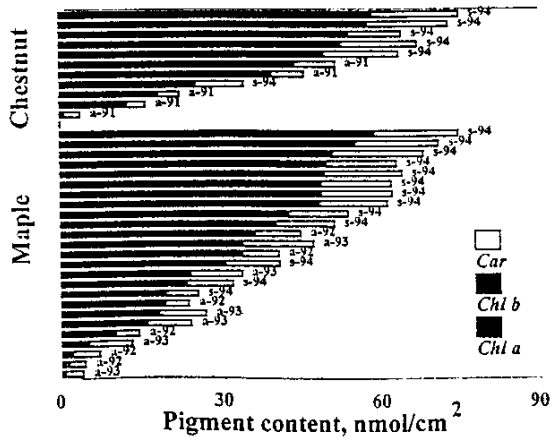

Fig. 1: Pigment concentrations in mature and senescent maple and chestnut leaves. Car is total carotenoids, Chl *a* and Chl *b* are chlorophyll *a* and *b*, respectively. An example of abbreviations: a-91 are autumn leaves, collected in 1991, s-94 are mature leaves collected in summer 1994.

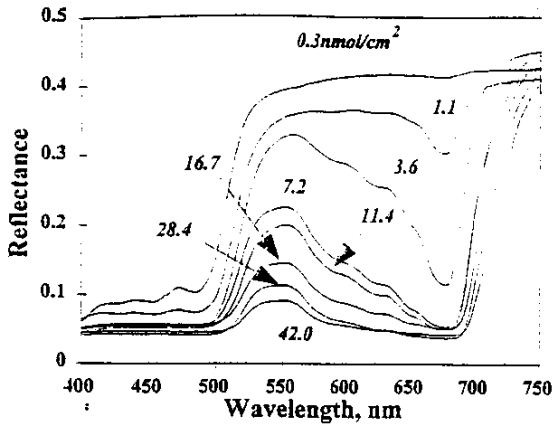

Fig. 2: The representative reflectance spectra of maple leaves containing different concentration of pigments. Chl *a* concentrations in nmol/cm² are indicated at reflectance curves.

leaves, and the typical three carotenoid absorption bands were clearly seen (Fig. 2). A very small increase in Chl *a* and *b* concentrations from 0.3 to 1 nmol/cm² induced a significant decrease in reflectance and shifted the «green edge» (rise of the reflectance in the range from 500 to 550 nm) at the reflectance spectra toward longer wavelengths. This alteration in the shape of the spectrum occurred at virtually the same carotenoid concentrations. An increase in pigment concentration from 3 to 44.8 nmol/cm² did not lead to any variation in the reflectance in this spectral range.

An increase in reflectance occurred near 500 nm for all leaves studied. For a completely yellow leaf (Chl *a* = 0.3 nmol/cm²), a wide plateau up to 750 nm came after this increase. In the leaves with Chl *a* > 3 nmol/cm², the prominent maximum of reflectance occurred in the green range of the spectrum. In yellow-green leaves, this peak was wide and reached 30–40 %, while for green-yellow to green leaves (Chl *a* > 3 nmol/cm²), it became narrow and was of decreased magnitude. The reflectance near 550 nm did not exceed 10 % in green leaves (Chl *a* > 20 nmol/cm²).

A decrease in reflectance followed the «green» peak. The reflectance in the range 600–650 nm varied from about 5 % for dark-green leaves up to 40 % for yellow ones. In dark-green to yellow-green leaves (Chl *a* > 3 nmol/cm²), the reflectance near 670 nm (i.e., the red maximum of Chl *a* absorption) was low and remained virtually the same in the range of Chl *a* variation from 5 to more than 44 nmol/cm². Only when the Chl *a* dropped to a level of less than 3 nmol/cm², was a considerable increase in reflectance observed.

A minimum near 670 nm was followed by a sharp increase in reflectance towards longer wavelengths. The slope of the reflectance increase (the «red edge») varied widely, decreasing with increase in Chl concentration.

To better understand the nature of the observed reflectance changes and to find which spectral bands are maximally sensitive to variation in pigment concentrations, the standard deviation (STD) of the reflectance was studied. STD was calculated for different groups of leaves. The first group contained leaves with a Chl *a* concentration from 0.3 (minimum concentration found in the experiments) to 44.8 nmol/cm² (maximum concentration). The second group included leaves with Chl *a* from 0.6 to 44.8 nmol/cm². In the third group, the minimum Chl *a* concentration was 1.1 nmol/cm² and maximum Chl remained the same (44.8 nmol/cm²). In other words, the groups had different minimum Chl *a* concentrations, while the maximum concentration remained the same. The first group of leaves can be considered to represent of a wide-ranging process of senescence, when the color of the leaves turn from dark green to completely yellow. The following groups, in order, corresponded to different stages of senescence (or/and stress and disease). This range ended with leaves in the very early stages of stress and senescence, when they were still green, but the suppression of biosynthesis and/or increased degradation of green pigments already had been initiated. In the last group, the summer leaves with natural variation in Chl *a* concentration due to light adaptation (between leaves in the sun and those in shade) were presented. Since the range of variation in Chl concentration was different for each group of the leaves, for their comparison STD of reflectance, STD(R), was normalized to STD of chlorophyll *a*, STD(Chl *a*), and the data are presented in Fig. 3.

The obtained STD(R)/STD(Chl *a*) spectra showed several notable features. They clearly indicated different spectral behavior for groups of leaves containing «completely yellow to green» leaves (samples with minimum Chl *a* <1.1 nmol/cm²) as compared to «yellow-green to green» (samples with mini-

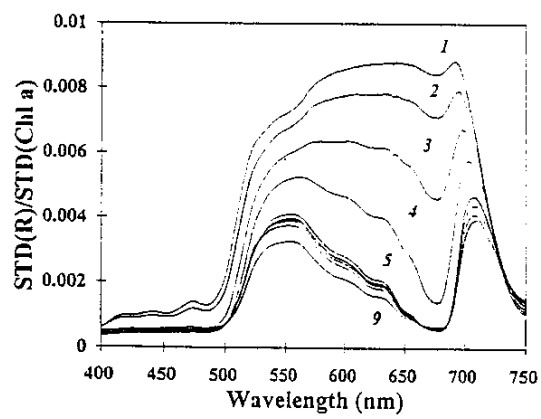

Fig. 3: The ratio of the standard deviation of reflectance, STD(R), normalized to the standard deviation of chlorophyll *a*, STD(Chl *a*), for groups of maple leaves selected from 25 samples, listed in Fig. 1. The groups had different minimal Chl *a* concentrations: 1–0.3 nmol/cm²; 2–0.6; 3–1.1; 4–3.6; 5–7.1; 6–11.4; 7–12.6; 8–14.0; 9–17.0. Maximum Chl *a* concentration was 44.8 nmol/cm² for all leaf groups. The first group of leaves can be considered to represent of a wide-ranging process of senescence, when the color of the leaves turn from dark-green to completely yellow. The following groups, in order, corresponded to different stages of senescence (or/and stress and disease). This range ended with leaves in the very early stages of stress and senescence, when they were still green, but the suppression of biosynthesis and/or increased degradation of green pigments already had been initiated. In the last group with minimal Chl *a* concentration of 17 nmol/cm², the summer leaves were presented with natural variation in Chl *a* concentration due to light adaptation (i.e., between leaves in the sun and those in shade).

mum Chl *a* > 3 nmol/cm^2) leaves. For leaves with 44.8 > Chl *a* > 0.3 nmol/cm^2, three distinct maxima due to carotenoid absorption near 425, 450 and 490 nm could be seen (Fig. 3). Between 550 and 660 nm, a broad peak of STD existed. Near 670 nm, a small minimum occurred. It was followed by insignificant peak near 690 nm. Then a sharp decrease of STD towards longer wavelengths was recorded. STD was low and almost the same in the blue and the near infra-red ranges of the spectrum. In leaves with 44.8 > Chl *a* > 1 nmol/cm^2, the spectral behavior was very different from the group of completely yellow to green leaves. In the blue range, the STD decreased two-three fold and spectral features were not detected. The minimum near 670 nm became prominent. The small peak at 690 nm was transformed to a noticeable maximum which shifted towards the longer wavelengths when the minimal Chl *a* concentration increased.

When the minimal Chl *a* in the leaf group was more than 3 nmol/cm^2, the broad maximum between 550 and 650 nm was transformed to a quite small narrow peak centered at 550 nm. The magnitude of the ratio STD(R)/STD(Chl *a*) at 670 was very low and remained virtually the same for the leaf groups with a minimal Chl *a* of more than 3 nmol/cm^2. The existence of two spectral bands (one, wide near 550 nm and the other, narrow near 700 nm), where variation of reflectance was found to be much higher than at 670 nm for leaf groups with minimal Chl *a* > 1 nmol/cm^2, was an important finding. It is also interesting to note that the variation of reflectance near 550 and 700 nm remained almost the same for leaf groups with a minimum Chl *a* concentration of more than 3 nmol/cm^2.

Reflectances in the range of 520 to 650 nm were strongly correlated with reflectance near 700 nm and were much more weakly correlated with reflectances in the blue (400 to 480 nm), in the near infra-red ranges and near 670 nm (data not shown). For all leaves studied an extremely high correlation ($r^2 > 0.99$) was found between the reflectance at 700 nm, R_{700}, and at 550 nm, R_{550} (Fig. 4).

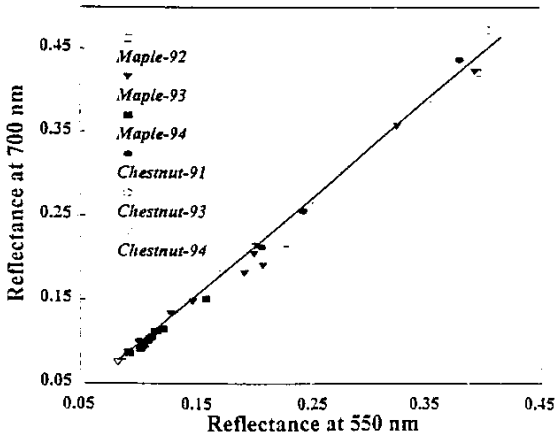

Fig. 4: The reflectance at wavelength 700 nm versus that at 550 nm. For all leaves and both species studied in different years in the range of Chl *a* concentration from 0.3 to 44.8 nmol/cm^2 the correlation between the reflectances at 550 and 700 nm was extremely high ($r^2 > 0.99$).

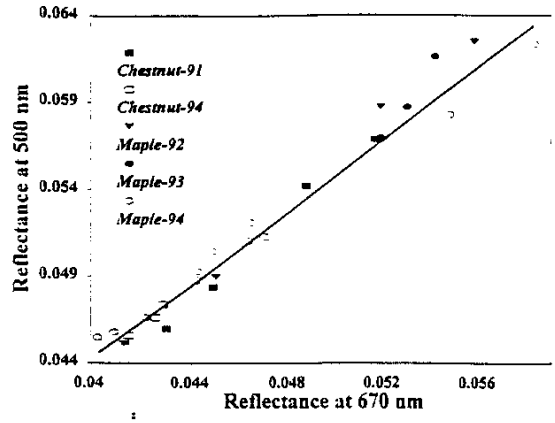

Fig. 5: The reflectance at wavelength 670 nm versus that at 500 nm for a yellow-green to dark-green maple and chestnut leaves with minimum Chl *a* concentration > 3 nmol/cm^2. The determination coefficient, r^2, for this relationship was more than 0.97.

Another feature of the reflectance spectra was a strong correlation of R_{670} with the reflectances in the range 500–520 nm (Fig. 5). For all leaves studied (Chl *a* > 0.3 nmol/cm^2), the maximum correlation coefficient was at 520 nm (data not shown). For green-yellow to green leaves (Chl *a* > 3 nmol/cm^2), the maximum correlation was R_{670} with R_{500} ($r > 0.98$). This spectral feature of «yellow-green» to «green» vegetation was found also for chestnut leaves.

Algorithms for chlorophyll detection

The index for Chl estimation should be maximum sensitive to Chl and invariant with respect to other factors. Therefore it would be very useful to find the wavelength where only one dominant factor influences the reflectance variation. The ratio STD(R)/STD(Chl *a*) (Fig. 3) clearly indicates such specific spectral bands with a high sensitivity of reflectance to Chl *a* variation – near 550 and 700 nm. Fig. 6 demonstrates the relationships between Chl *a* concentration and reflectances at 550, 700 and 670 nm. There is a hyperbolic relationship between Chl *a* and R_{550} and R_{700} (Fig. 6). For the linear function Chl *a* versus $(R_{700})^{-1}$ and $(R_{550})^{-1}$, the correlation was very strong ($r^2 > 0.95$) with an error of Chl *a* estimation of less than 2.8 nmol/cm^2. Reflectance at 670 nm decreased sharply when Chl *a* increased up to 3–5 nmol/cm^2; thereafter R_{670} was almost pigment-concentration independent. Therefore, it would be inappropriate to use the reflectance near 670 nm as a sensitive term in the index for Chl estimation. The lowest variation of reflectance took place in the near infra-red (above 750 nm) and in the blue (shorter than 500 nm) ranges of the spectrum.

Thus, the reflectances near 700 nm and in the range from 540 to 600 nm were the only features found to be sensitive to Chl *a* concentration which could be used in constructing the index. R_{750} can be taken as a term insensitive to Chl *a* concentration. Taking into consideration that $(R_{700})^{-1}$ and $(R_{550})^{-1}$ are directly correlated to Chl *a* and that R_{750} virtually did not depend on Chl *a*, the indices R_{750}/R_{550} and R_{750}/R_{700}

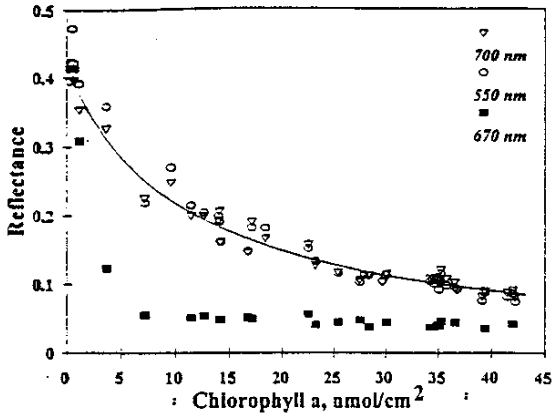

Fig. 6: The reflectance at the wavelengths 550, 670, and 700 nm versus Chl *a* concentrations for maple leaves. Reflectance at 670 nm, R_{670}, decreased sharply when Chl *a* increased up to 3–5 nmol/cm^2; thereafter R_{670} was almost pigment-concentration independent. There is a hyperbolic relationship between Chl *a* and R_{550} and R_{700}. The solid line represents the equation $Chl = -7.2 + 4.01 \cdot (R_{700})^{-1}$ with determination coefficient $r^2 > 0.95$.

were chosen for Chl *a* assessment. These indices were compared with analytically-measured Chl *a* and Chl *a*+ *b* concentrations. For leaves measured in 1991 and 1992 linear regressions were obtained (Gitelson and Merzlyak, 1994 a, b):

– for maple leaves

$$Chl\,a = -(8.16 \pm 1.96) + 8.59 \cdot R_{750}/R_{550} \quad (1)$$

with $r^2 > 0.97$,

– for chestnut leaves

$$Chl\,a = -(11.52 \pm 1.69) + 12.50 \cdot R_{750}/R_{700} \quad (2)$$

with $r^2 > 0.98$. For the relationship Chl *a*+ *b* versus the above reflectance ratios, r^2 was higher than 0.96.

The models (1) and (2) were validated by independent data sets obtained in 1993 and 1994. We performed the validation employing Eq. 1 and 2 to calculate the Chl *a* concentration using reflectance data measured in 1993 and 1994. The resulting predicted Chl *a* and Chl *a*+ *b* values were compared to the analytically-measured chlorophyll concentrations in 1993 and 1994 (Fig. 7). The correlation between predicted and measured Chl *a* concentrations was $r^2 = 0.99$ for both R_{750}/R_{550} and R_{750}/R_{700} ratios, with an error in the Chl *a* estimation of less than 1.26 nmol/cm^2 and 1.14 nmol/cm^2, respectively. A minimal estimation error of Chl *a*+ *b* (less than 1.3 nmol/cm^2) was obtained for R_{750}/R_{700} ratio.

Discussion

Mechanisms responsible for these revealed spectral signatures have to be understood in order to ascertain whether the parameters of above algorithms will be stable for a wide range of pigment concentrations in the leaves and can be applied to a number of plant species. The leaves studied (Fig. 1) covered a very wide range of pigment concentrations (corresponding to leaf color change from completely yellow to dark green). They could be considered as a model of different physiological states of a plant, which revealed the following important relationships.

(1) Near 670 nm, as well as in the blue region of the spectrum (400 to 500 nm), the relationship reflectance versus Chl *a* was saturated for a concentration of the pigment more than 3 nmol/cm^2, whereas near 550 and 700 nm the relation was monotonous and reflectance remained sensitive to pigment concentration for Chl *a*>40 nmol/cm^2 (Figs. 2–3, 6).

(2) For all leaves and both species studied in the range of Chl *a* concentration from 0.3 to 44.8 nmol/cm^2 the correlation between the reflectances at 550 and 700 nm was extremely high ($r^2 > 0.99$) (Fig. 4).

(3) For all leaves, a strong correlation between R_{670} and reflectance in the range 500–520 nm was found. For green-yellow to green leaves (Chl *a*> 3–5 nmol/cm^2) the correlation coefficient peaked at 500 nm and reached r>0.98.

The range near 700 nm belongs to «the red edge» which is a unique feature of green vegetation (e.g., Baret et al., 1992; Curran et al., 1991; Horler et al., 1983; Fukshansky, 1981). It results from two optical properties of plant leaves: high Chl *a* absorption resulting in low reflectance near 670 nm and high internal leaf scattering causing large near infra-red reflectance. Thus, in the wavelength range lower than 700 nm the reflectance is primarily determined by strong Chl *a* absorption, while beyond 700 nm, it is governed less by absorption and much more by scattering (e.g., Horler et al., 1983; Fukshansky, 1981). The reasons for the high sensitivity of this spectral band to Chl *a* concentration is its location which is rather far from the main absorption bands of chloroplast pigments. This prevents the saturation of relationship reflectance versus chlorophyll (Gitelson and Merzlyak, 1994 a). On the

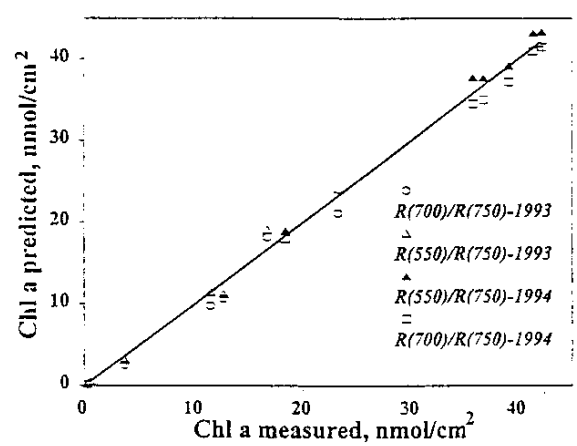

Fig. 7: The results of validation of the models (1) and (2) by independent data sets obtained in 1993 and 1994. Predicted chlorophyll *a* concentrations plotted versus measured in maple and chestnut leaves analytically. Predicted Chl *a* concentrations were calculated from Eq. 1 and 2, with the reflectances of the leaves measured in 1993 and 1994. The correlation between predicted and measured Chl *a* concentrations was $r^2 = 0.99$ for both R_{750}/R_{550} and R_{750}/R_{700} ratios, with maximum error in the Chl *a* estimation of less than 1.26 nmol/cm^2. Solid line presents equation $Chl_{pred} = Chl_{meas}$.

other hand, this band is too far from near infra-red range with high scattering, to provide enough sensitivity to Chl *a* variation. Apparently the reflectance near 700 nm is a fundamental spectral feature that is produced by an equilibrium between two these competing processes. Reflectance near 700 nm was also found to be the transition point at the red edge «between two more-or-less linear phases of the relationship ‹position of inflection point vs. Chl *a*›» (Horler et al., 1983). The high variation of R_{700} with Chl concentration is a result of the shift of the red edge and it is caused by the same physical processes (e.g., Baret et al., 1992; Curraet al., 1991; Gitelson et al., 1996; Horler et al., 1983; Vogelmann et al., 1993).

Reflectance at 550 nm, R_{550}, is located between two wide bands of strong pigment absorption. R_{550} is below the green edge in the reflectance spectrum, around 520 nm, on the long wavelength side of carotenoid as well as the blue Chl *a* and Chl *b* absorption band. The spectral behavior of this edge was found to be very similar to that of the red edge (Horler et al., 1983). The difference between them is that the green edge is primarily determined by Car, Chl *a*, and Chl *b* absorption, while the red edge is governed primarily by Chl *a*. (The contribution of Chl *b* to the absorption at near 700 nm is very low. The maximum for *in vivo* absorption of Chl *b* present in light-harvesting complex is assumed to be located around 650 nm.) At longer wavelengths R_{550} is located before the large range of absorption by chlorophylls. Thus, near 550 nm, two strong absorption processes reach their minimum, producing the monotonous relationship ‹Chl *a* versus R_{550}› with a high sensitivity to Chl *a* concentration.

Reflectances near 700 and 550 nm were found to be of equal sensitivity to variation in Chl *a*. Moreover, R_{550} and R_{700} correlated extremely well in both maple and chestnut leaves from senescent yellow to mature dark green (Fig. 4). The same phenomenon was discovered in soybean (Chappelle et al., 1992; Kim et al., 1994) and for corn leaves (McMurtrey III et al., 1994), when the plants were affected by nitrogen deficiency. This is consistent with the observations of Horler et al. (1983) where, again, a strong correlation between R_{540} and the position of the red edge was found. We also compared the values of R_{550} and R_{700} for young juvenile, mature and yellowing leaves of European beach (Fig. 3 in Tanner and Eller, 1986), and found that they were also very close to each other.

Such similarity between R_{550} and R_{700} could be understood if the absorption at these wavelengths was affected by pigments occurring exactly in the same proportion. Chl *a* is mainly responsible for absorbance near 700 nm. At 550 nm, as well as at longer wavelengths, both Chl *a* and *b* play a major, even dominant, role in light absorption. The contribution of carotenoids is probably much less, as indicated by the reflectance spectra of yellow leaves (Fig. 2). In the presence of trace amounts of both chlorophylls (<0.3 nmol/cm^2) and considerable quantities of carotenoids (>3 nmol/cm^2), no evidence for the contribution of carotenoids to reflectance at 550 nm exist (upper curve in Fig. 2). However, an increase in Chl *a* concentration up to 3 nmol/cm^2 on a background of approximately the same amounts of carotenoids led to a significant decrease in the reflectance near 550 nm.

In solution, pure chlorophylls possessed low, but measurable absorbance between 500 to 600 nm, with a molar extinction coefficient about 3–5 mM^{-1} cm^{-1}. The comparison of absorbance spectra of pure chlorophylls with those of maple leaf extracts in methanol indicated that the contribution of carotenoids at wavelengths near 550 nm and longer was at least 8–10-fold less than that of green pigments (Merzlyak, unpublished). Among the carotenoids present in the chloroplasts of higher plant leaves, only β-carotene exhibited an extremely small absorbance at 550 nm (Lichtenthaler, 1987). Therefore, the perfect covariation ($r^2 > 0.99$) between R_{550} and R_{700} (Fig. 4) may be explained by the fact that Chl *a* covaries reasonably well with Chl *b* ($r^2 > 0.96$) for the chestnut and maple leaves studied (data not shown), while the carotenoids did not contribute significantly to reflectance at 550 nm.

These results have confirmed and quantified our previous findings (Gitelson and Merzlyak, 1994 a, b). The developed indices work for leaves of both species studied, maple and chestnut in a very wide range of Chl concentrations. The indices are directly proportional to Chl *a* allowing precise estimation of Chl *a* and Chl *a*+*b* concentration at the leaf level. The reflectance near 700 nm is affected mainly by Chl *a*, whereas R_{550} is affected by both Chl *a* and *b*. Since these pigments covary reasonably well, total Chl concentrations can be determined from reflectance measurements at both wavelengths. The sensitivity of reflectance to Chl *a* concentration remained approximately the same in spectral band from 540 to 600 nm. Reflectance in this rather wide range can be used for estimating total Chl, whereas the reflectance near 700 nm is more suitable for Chl *a* assessment.

The index $\log(R_{800}/R_{550})$ was found to be a good indicator of chlorophyll content per leaf area for intact bean leaves (Buschmann and Nagel, 1993). They reported that the ratio R_{800}/R_{550} was also closely correlated with Chl *a* concentration ($r^2 > 0.88$). Carter found high sensitivity of the reflectances near 550 nm and 700 nm to stress of plants (Carter, 1993; 1994). The high sensitivity of R_{700} to Chl *a* concentration was also demonstrated by Chappelle et al. (1992), Kim et al. (1993), and McMurtrey III et al. (1994) for soybean and corn leaves. They constructed the index for Chl *a* estimation, using the ratio R_{700}/R_{670}. Taking into account that R_{670} virtually did not depend on Chl *a* concentration (see Fig. 2 and Table 9 in McMurtrey III et al., 1994), one can conclude that R_{700} was used as a term sensitive to Chl *a* concentration, whereas R_{670} was the insensitive one.

The parameters of the relationships between reflectance and pigment concentration depend on many factors; the primary ones are species, pigment composition, and developmental stage. It is indeed remarkable that the error of Chl *a* estimation in the range 0.3 to 44.8 nmol/cm^2 for two species studied was as low as 1.2 nmol/cm^2 considering the sources of «noise» in the algorithms.

A high correlation between reflectances at 500 and 670 nm was found for the leaves of both plant species with Chl *a* >3 nmol/cm^2 (Fig. 5). This means that absorbance by Chl *a*, *b* and Car at 500 nm and by both chlorophylls at 670 nm was almost similar over a wide range of pigment variation. The correlation of R_{500} and R_{670} was not so high if chlorophyll concentration dropped to less than 3–5 nmol/cm^2, while carotenoid concentration remained relatively high (i.e., for yellow-green and completely yellow leaves). Therefore, the closest

correlation between R_{500} and R_{670} took place when a certain proportion of green pigments and carotenoids existed. Apparently this phenomena is unique for yellow-green to green vegetation where a decrease in green pigment during senescence or disease is followed by a proportional decrease in carotenoid concentration.

These spectral features may be useful in construction the indices for the estimation of pigment concentration at the canopy level. The coincidence (for vegetation) of reflectances R_{500} with R_{670} and R_{550} with R_{700} over a wide range of pigment concentrations allows assessment of the effect of background reflectance (e.g. soil). The variation in background reflectance for the same Chl concentration can be recognized in differences between the ratio R_{500}/R_{670} (or/and the R_{700}/R_{550}). The indices $[(R_{700}/R_{550})-1]$ and $[(R_{670}/R_{500})-1]$ could be used to counteract the effects of background reflectance.

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