

Non-destructive optical detection of pigment changes during leaf senescence and fruit ripening

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Reflectance spectra in the visible and near infra-red range of the spectrum, acquired for maple (*Acer platanoides* L.), chestnut (*Aesculus hippocastanum* L.), potato (*Solanum tuberosum* L.), coleus (*Coleus blumei* Benth.), leaves and lemon (*Citrus limon* L.) and apple (*Malus domestica* Borkh.) fruits were studied. An increase of reflectance between 550 and 740 nm accompanied senescence-induced degradation of chlorophyll (Chl), whereas in the range 400–500 nm it remained low, due to retention of carotenoids (Car). It was found that both leaf senescence and fruit ripening affect the difference between

reflectance (R) near 670 and 500 nm ($R_{678} - R_{500}$), depending on pigment composition. The plant senescing reflectance index in the form $(R_{678} - R_{500})/R_{750}$ was found to be sensitive to the Car/Chl ratio, and was used as a quantitative measure of leaf senescence and fruit ripening. The changes in the index were followed during leaf senescence, and natural and ethylene-induced fruit ripening. This novel index can be used for estimating the onset, the stage, relative rates and kinetics of senescence/ripening processes.

Introduction

During senescence, plant tissues undergo remarkable changes in colour as a result of changes in the content of, and proportions between, individual pigments (Chichester and Nakayama 1965, Knee 1972, Hendry et al. 1987, Knee 1988, Matile Ph Flash and Eller 1991, Biswall 1995, Buchanan-Wollastin 1998). This makes the application of non-destructive reflectance spectroscopy for assessment of the physiological state of plants and monitoring of senescence-induced events in vegetation very attractive (Guyot 1993, Merzlyak et al. 1997). There have been attempts to develop optical methods for evaluating fruit ripeness and quality (Chuma et al. 1981, Blanke and Notton 1992, Morita et al. 1992). In many cases (but not always), the changes in colour of senescing plant tissues are related to preferential degradation of chlorophyll (Chl) over carotenoids (Car), which result in yellowing and eventually the development of their bright yellow colour (Chichester and Nakayama 1965, Knee 1972, Hendry et al. 1987, Knee 1988, Matile Ph Flash

and Eller 1991, Biswall 1995). Therefore, an estimation of the proportion between pigments could serve as a marker of senescence.

Although plant tissues exhibit complicated optical properties (Osborne and Raven 1968, Vogelmann 1993, Richter and Fukshansky 1996), non-destructive Chl estimation was shown to be possible using reflectance in the green (550 nm) and red (700 nm) regions of the visible spectrum (Gitelson and Merzlyak 1996, 1997). In the blue region, Car and Chl exhibit strong and overlapping absorption that makes it difficult to separate their contribution to reflectance, even at late stages of leaf senescence or fruit ripening (see spectra in Chuma et al. 1981, Adams et al. 1990, Matile Ph Flash and Eller 1991, Morita et al. 1992, Gitelson and Merzlyak 1994a, 1996, Merzlyak and Gitelson 1995, Merzlyak et al. 1997). Recently, it has been found that for a number of diverse plant species, reflectance within certain spectral bands is highly correlated. In particular, such a correlation

Abbreviations – Car: carotenoids; Chl: chlorophyll; NIR: near infra-red; PSRI: plant senescence reflectance index; R: reflectance; T: transmittance.

has been observed between the bands near 500 and 670–680 nm at low reflectance values (Gitelson et al. 1996, Lichtenthaler et al. 1996, Merzlyak et al. 1997). However, in leaves containing low concentrations of pigments and, as a result, with higher reflectance, this close correlation failed, especially in yellowing autumn leaves of deciduous plants (Merzlyak et al. 1997).

In this work, we examined senescence-induced reflectance changes in great detail, using leaves and fruit of different species. We attempted to elucidate the mechanisms responsible for the relationships between reflectances at 500 and 670–680 nm and find basic criteria suitable for quantitative assessment of the senescence process in plants.

Materials and methods

Plants

Healthy mature to senescing leaves and ripening fruits homogeneous in colour, and without anthocyanin pigmentation, were used in the experiments. Summer-autumn leaves of deciduous trees, i.e. Norway maple (*Acer platanoides* L.) and horse chestnut (*Aesculus hippocastanum* L.), were collected in a park at Moscow State University (1992–1997). Potato (*Solanum tuberosum* L. cv. Lugovskoi) plants were grown under field conditions and examined in late June–early July 1996. Both green (exposed to sunlight) and yellowing (darkened) leaves were used. The leaves of the house plant, coleus (cockspurflower, *Coleus blumei* Benth. ~ *Plectranthus fruticosus* L'Herit.), which, during both natural senescence and ageing in vitro, turn a whitish colour, were also studied.

Apple fruits (*Malus domestica* Borkh. cv. Antonovka obyknovennaya) were grown in the garden of Moscow State University (1997) or obtained from Michurinsk, in the Tambov region, Russia (1995). After-ripening (maturation) of apples was followed during incubation of detached fruits at 20°C. Lemon (*Citrus lemon* Burm. cv. Novogruzinsky) fruits were collected from trees grown in a greenhouse under natural illumination at 25°C. Both attached fruits, undergoing natural ripening on a tree, and detached fruits treated with ethylene, were examined. In the latter case, fruits with similar reflectance spectra were selected. Then each fruit was placed in a 3-l glass vessel. The vessels were sealed and known quantities of ethylene were injected through rubber septa. Every 1–2 days, the vessels were ventilated to prevent hypoxia and CO₂ accumulation and new portions of ethylene were added. Ethylene was prepared and its concentrations in the vessels, as well as those of oxygen and carbon dioxide, were measured with gas chromatography, as described previously (Rakitin and Rakitin 1986).

Chloroplast isolation

Green coleus leaves were ground in 4.0 ml of extraction medium containing 0.066 M K-phosphate buffer, pH 6.8, 0.3 M NaCl and 0.1% (w/v) bovine serum albumin (Sigma, St. Louis, MO). The homogenate was filtered through four layers of nylon and centrifuged at 800 g for 1 min. The

pellet was discarded and the supernatant centrifuged at 1000 g for 4 min. Then the chloroplast fraction was resuspended in the same medium and washed by centrifugation at 1000 g for 4 min. The chloroplasts were suspended in 0.066 M K-phosphate buffer, pH 6.8, containing 0.3 M NaCl. Then the pellet was resuspended in the same medium and washed by centrifugation at 1000 g for 4 min. The chloroplasts (broken chloroplasts of class II) were suspended in 0.066 M K-phosphate buffer, pH 6.8, containing 0.3 M NaCl.

Pigment analysis

Leaves and fruit peels were rapidly ground with a porcelain mortar and pestle in methanol and with calcium carbonate added to prevent Chl pheophytinisation. Homogenates were centrifuged for 3–4 min in glass tubes at 3000 g. Extracts were immediately assayed spectrophotometrically. Specific absorption coefficients of Chl *a* and Chl *b* and total Car reported by Lichtenthaler (1987) were used. It was assumed that the molecular mass for Car was 570. Pigment content was expressed on a leaf area basis.

Spectral measurements

Reflectance and transmittance spectra were measured with a spectrophotometer (150-20 Hitachi, Noka Works Hitachi Ltd., Tokyo, Japan) equipped with a 150-mm integrating sphere attachment (part 150-0901) and interfaced to a personal computer. Reflectance spectra (*R*) from the adaxial surface of leaves, as well as those of whole fruits, were recorded against barium sulphate as a standard at a spectral resolution of 2 nm. Black velvet with reflectance of less than 0.5% over the whole spectral range studied was used as a background in leaf reflectance measurements. Remission spectra $f(R_\infty)$ for maple and coleus leaves were calculated from reflectance measurements as $f(R_\infty) = [(1 - R)^2 / 2R]$ (Wendlandt and Hecht 1966).

Results

Spectral features of senescing leaves and ripening fruits

Reflectance spectra of coleus and maple leaves (with quite close Chl content) which, during senescence, turn whitish and yellow, respectively, exhibited similar reflectance spectra at wavelengths longer than 550 nm. A lowering in Chl concentration brought about a remarkable difference in reflectance between 400 and 530 nm. In coleus, a progressive increase of reflectance occurred and the absorption bands of Chl *a* (near 440 nm), Chl *b* and Car (460–480 nm) became apparent as gaps in reflectance spectra. At the stage where Chl breakdown was nearly complete (upper curve in Fig. 1, upper panel), small minima near 480, 450 and 430 nm attributable to Car were observed. In contrast, in senescing maple leaves reflectance between 400 and 520 nm remained low. Spectral changes similar to maple were recorded in chestnut and potato leaves (data not shown).

Lemon and apple fruits exhibited much higher reflectance in the NIR region of the spectrum (75–85%) compared with

leaves (45–50%) (Fig. 2). Between 550 and 750 nm, fruit ripening was accompanied by spectral changes similar to those in senescing leaves (cf. Figs. 1 and 2). As Chl breakdown progressed in lemon (Fig. 2, upper panel, curves 1–6) and in apple fruits (Fig. 2, lower panel, curves 1–6), reflectance over 400–500 nm increased monotonously. In lemon fruit, at the late stage of Chl degradation, a significant decrease of reflectance in the blue range was observed (Fig. 2, upper panel, insert); reflectance decreased monotonously from 550 nm to shorter wavelengths without any spectral details. Apples, with low Chl content, had reflectance minima near 460, 455 and 425 nm (Fig. 2, low panel, insert).

Comparison of spectral reflectance changes in senescing coleus and maple leaves

To study the spectral features of senescence-related reflectance changes, remission spectra of coleus and maple leaves

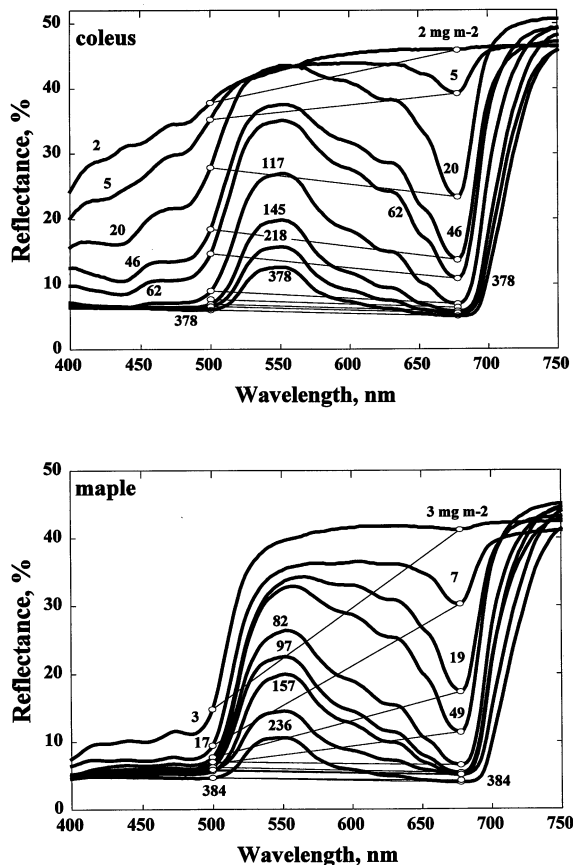


Fig. 1. Reflectance spectra of mature to senescent maple and coleus leaves. The numbers in the figure indicate the total Chl content in mg m^{-2} . Reflectances at 500 and 678 nm are shown as symbols and connected by thin lines to show the difference between them. During senescence, a decrease in Chl content in coleus leaves was followed by almost synchronous increase of reflectance at both 500 and 678 nm. Only at the late stages of senescence, when Chl content dropped to values below 20 mg m^{-2} , did R_{678} become higher than R_{500} . For maple leaves, a drop in Chl content caused a significant increase in reflectance at 678 nm, whereas reflectance at 500 nm remained almost invariable; thus, the difference between them ($R_{678} - R_{500}$) increases substantially, quantitatively indicating senescence.

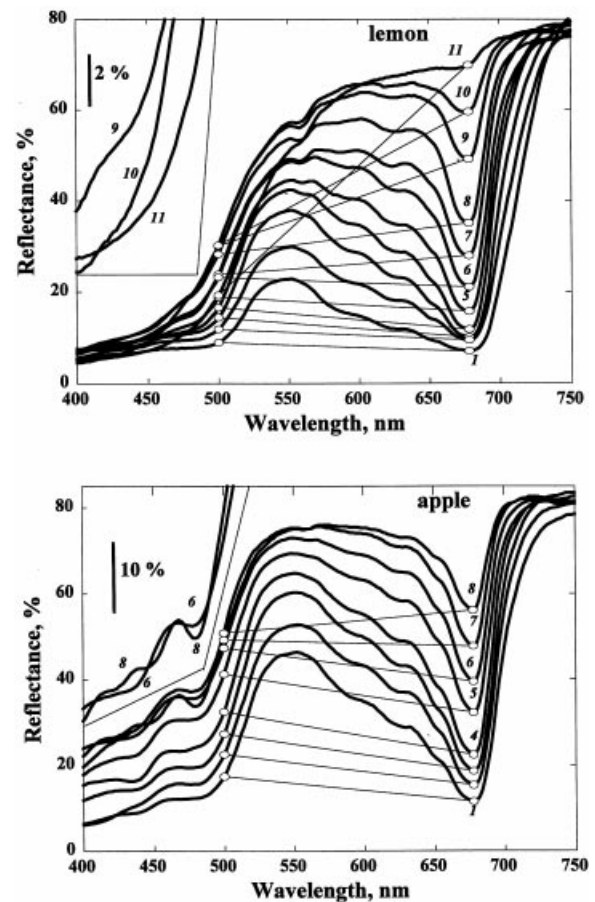


Fig. 2. Reflectance spectra of lemon and apple fruits during ripening. In both panels, the bottom spectra correspond to early stages; the top ones correspond to the late stages. To show the difference between reflectances at 500 and 678 nm, they are presented as symbols and connected by thin lines. Insert: reflectance in the blue at the late stage of ripening for lemon and apple fruits. For lemon, reflectance in the blue decreased during ripening, while for apple, it was almost invariable. During ripening for apple and lemon, reflectance increased at 500 and 678 nm: the difference between them ($R_{678} - R_{500}$) remains roughly the same. Later in lemon (spectra 8–11), R_{678} significantly increased, whereas R_{500} remained the same or even decreased (see insert). Thus, as senescence progressed, the difference ($R_{670} - R_{500}$) increased significantly.

were analysed. A remission function was calculated as $f(R_{\infty}) = k/s$ where k is the absorption coefficient and s is the scattering coefficient. Thus, an absorption spectrum may be identified by reflectance technique (Wendlandt and Hecht 1966), and it allows comparison of absorption properties of various plant leaves (Merzlyak and Gitelson 1995). The average remission spectra of leaves with a Chl content of $< 100 \text{ mg m}^{-2}$ had similar values between 550 and 750 nm (Fig. 3). However, at shorter wavelengths between 400 and 500 nm, the difference was noticeable: the remission function for coleus leaves was much smaller than that for maple leaves. The shape of the remission function of coleus leaves was very similar to the absorption spectrum of isolated chloroplasts. The difference $f(R_{\infty})$ 'maple-coleus' spectrum showed bands near 480, 460 and 420–430 nm (the latter as a shoulder). In addition, a tail of UV-absorbing substances can be seen in the range 400–410 nm in the difference spectrum.

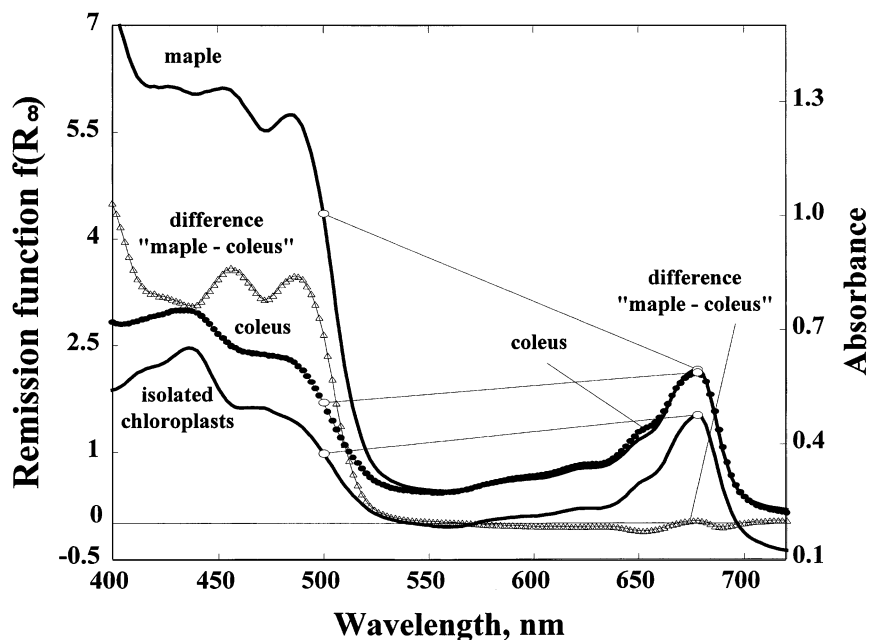


Fig. 3. Average Kubelka-Munk remission spectra of coleus ($n = 15$) and maple ($n = 14$) leaves with Chl content $< 100 \text{ mg m}^{-2}$, their difference and absorption spectrum of isolated coleus chloroplasts. Reflectance spectra – left scale, absorption spectrum – right scale. The wavelengths 500 and 678 nm are shown as symbols and connected by thin lines. Below 550 nm, the $f(R_\infty)$ for maple leaves is considerably higher than that for coleus leaves. Chl content was virtually the same in both species, but Car content was different. In maple leaves it was higher than that in coleus; as a consequence, there was much higher absorption in maple leaves in blue. The difference 'maple-coleus' remission spectrum exhibits characteristic Car absorption bands near 460 and 480 nm. The shape of coleus leaf remission and absorption spectra of isolated coleus chloroplasts was nearly the same.

Plant senescence reflectance index

Green leaves with a Chl content of $> 100\text{--}120 \text{ mg m}^{-2}$, as well as green lemon fruit, possessed approximately equal reflectance values at 500 and 678 nm (the thin lines in Figs. 1 and 2 are almost horizontal). The ranges with synchronous changes of reflectance at 500 and 678 nm were different for the plant species studied (Fig. 4): up to 7–8% for maple leaves, up to 15–18% for potato, coleus leaves and lemon fruits, and up to 20–22% for apple fruits. During the course of Chl breakdown, R_{678} increased while R_{500} remained almost the same or increased slightly. In lemon fruit, R_{500} changed about 8% while R_{678} varied from 20 to 60% (Figs. 2 and 4).

We propose the following index which is sensitive to senescence-induced reflectance changes (plant senescence reflectance index, PSRI):

$$\text{PSRI} = (R_{678} - R_{500}) / R_{750}$$

In dark to light-green coleus and maple leaves (Chl content $100\text{--}700 \text{ mg m}^{-2}$), the PSRI was similar for both species and remained virtually unchanged (Fig. 5A). In leaves with low Chl content (less than 100 mg m^{-2}), the index increased sharply in maple, whereas in coleus it decreased. The index in coleus increased when Chl content reached ca $10\text{--}20 \text{ mg m}^{-2}$ and occurred at a much slower rate than that in maple leaves (Fig. 5A). The index was also plotted for all samples studied (including fruits) versus the R_{750}/R_{700} ratio (Fig. 5B), which was found to be a precise measure of Chl content (Gitelson and Merzlyak 1994a,b, 1996, 1997, Lichtenthaler et al. 1996). The behaviour of this relationship was similar to that of PSRI versus Chl (Fig. 5A): when R_{750}/R_{700} was high, the index was close to zero, then it decreased slightly when the ratio ranged between 1.2 and 1.7, and, finally, it increased sharply.

Fig. 6 shows the relationship of PSRI with the Car/Chl ratio in leaves. In coleus, when the Car/Chl ratio did not vary, neither did the index. At late stages of senescence, when the Car/Chl ratio increased, so did the index. In contrast, during senescence of maple leaves both the Car/Chl ratio and the PSRI underwent a sharp increase. The PSRI versus Car/Chl for chestnut leaves was found to be similar to maple (data not shown). Regression (r^2) between PSRI values and the Car/Chl molar ratio for maple and chestnut leaves were 0.92 and 0.90, respectively.

To compare relative senescence-induced reflectance changes in different plant tissues, the PSRI was plotted versus reflectance ratio R_{678}/R_{750} (Fig. 7 and Table 1). At a low R_{678}/R_{750} ratio (ca < 0.15), all leaves and lemon fruits exhibited an almost invariable PSRI (R_{500} was slightly

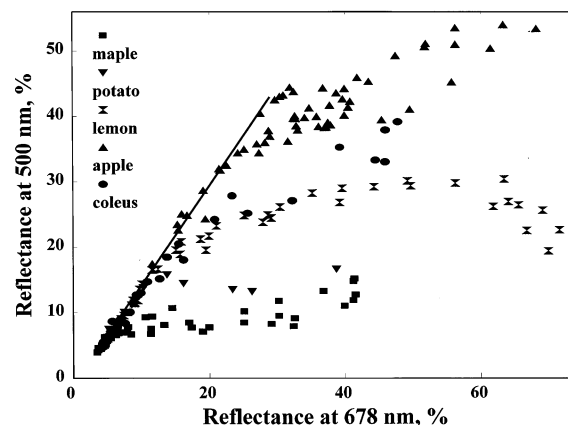


Fig. 4. Relationships between reflectance at 500 and 678 nm for leaves and fruits. For mature green plant tissues, a linear relation R_{500} versus R_{678} existed (solid line represents linear best fit function). During senescence, R_{500} remained virtually unchanged whereas R_{678} increased significantly; as a result, there is deviation of points from a linear line.

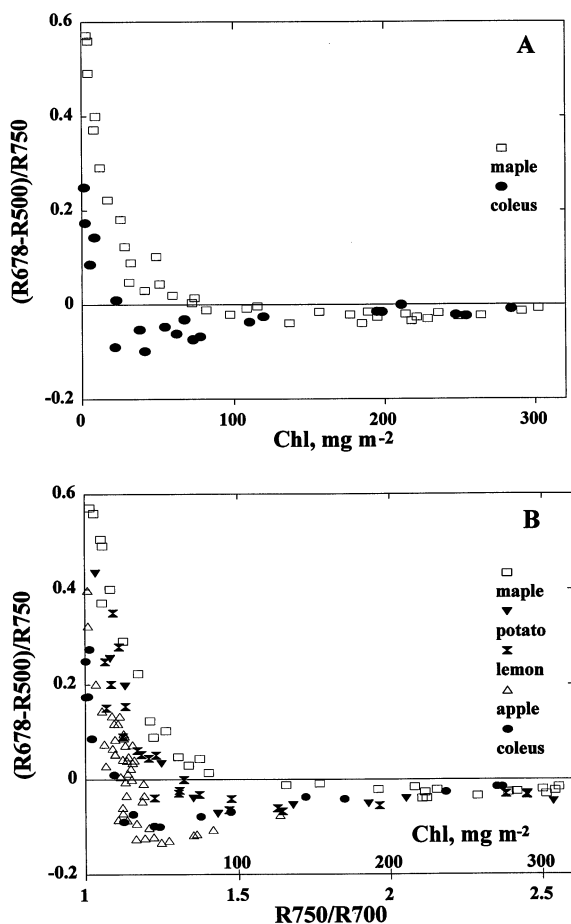


Fig. 5. The relationships of $(R_{678} - R_{500})/R_{750}$ and Chl content for coleus and maple leaves (A), and $(R_{678} - R_{500})/R_{750}$ versus Chl content and ratio R_{750}/R_{700} for all species (B). The ratio R_{750}/R_{700} is a precise measure of Chl content. For moderate to high Chl content ($\text{Chl} > 100 \text{ mg m}^{-2}$), the index $(R_{678} - R_{500})/R_{750}$ was negative and almost invariable. For senescing maple leaves with $\text{Chl} < 80\text{--}100 \text{ mg m}^{-2}$, $(R_{678} - R_{500})/R_{750}$ increased drastically and for coleus it increased slightly.

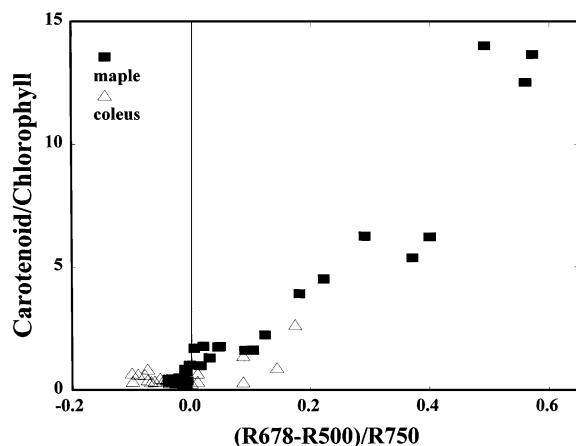


Fig. 6. Total Car to Chl molar ratio versus the index $(R_{678} - R_{500})/R_{750}$. During senescence of coleus leaves, the Car/Chl ratio remains within narrow limits and the PSRI slightly increases. For maple leaves, the Car/Chl ratio varies widely. The PSRI increases with Car/Chl.

higher than R_{678}). A subsequent decrease of the PSRI to lower values was observed in ripening fruits, coleus and potato leaves (but not in the leaves of deciduous plants). Intersection of the PSRI with the abscissa was observed at different ranges of R_{678}/R_{750} . In all samples, when $R_{678} > R_{500}$, the PSRI correlated closely with the reflectance ratio R_{678}/R_{750} . In lemon fruit, two lines fit this increase. Lower slopes (a coefficient k in Table 1) were observed in coleus leaves and at early stages of lemon yellowing. A high slope of the PSRI versus R_{678}/R_{750} was found during maturation of apple fruits.

The PSRI was used to characterise the effect of ethylene on lemon fruit ripening (Fig. 8). In untreated fruits, the PSRI was almost constant during the whole period of the experiment. In ethylene-treated fruits, negative PSRI values were observed 18–19, 9–10 and 6 days at ethylene concentrations of 0.1, 1.0 and 10 ppm, respectively, and about 4–5 days at ethylene concentrations of 100–200 ppm. Then, the index increased significantly. S-shape kinetics of the PSRI changes were dependent on ethylene concentrations and increased with concentration of the plant hormone.

Discussion

The data presented in this paper demonstrate similar senescence-induced changes in reflectance spectra of leaves and fruits (see also Adams et al. 1990, Morita et al. 1992, Merzlyak and Gitelson 1995, Merzlyak et al. 1997). The characteristic changes of reflectance spectra occurred when leaf Chl content reached approximately 100 mg m^{-2} and when the R_{750}/R_{700} became less than ca 1.5 (Figs. 1, 2, 5, and Table 1). A remarkable difference between reflectance spectra of coleus and maple related to the difference in pigment transformation during senescence was observed: in coleus, degradation of Car and Chl occurred simultaneously and only trace amounts of Car were present in senescent leaves, whereas in maple, Car pigments were retained (Fig. 6; see also Merzlyak and Gitelson 1995, Merzlyak et al. 1997). The pigments responsible for lower reflectance of senescent maple leaves in the range 400–500 nm exhibit absorption bands characteristic of carotenes and xanthophylls (Fig. 3). The same bands were presented as gaps in reflectance spectra of autumn leaves of deciduous plants (Fig. 1; see also Adams et al. 1990, Gitelson and Merzlyak 1994a,b, Merzlyak and Gitelson 1995, Merzlyak et al. 1997), apples (Fig. 2) and other fruits (Morita et al. 1992) when Chl degradation was largely complete.

The difference between reflectance around 680 and 500 nm ($R_{678} - R_{500}$) was found to be sensitive to pigment changes during leaf senescence and fruit ripening and, thus, can be used to quantitatively estimate the Car/Chl ratio (Figs. 3 and 6). At 500 nm, reflectance is controlled by combined absorption of Chl *a*, Chl *b* and Car, whereas around 680 nm by Chl *a* absorption solely. As a result, difference increases with a decrease in Chl content (Figs. 4 and 5), and with an increase in Car/Chl ratio. We have selected R_{500} for two main reasons: (1) it is distant from the absorption maximum of Chl *b* near 460 nm (Fig. 3), so the contribution of Car absorption at 500 nm is more than that

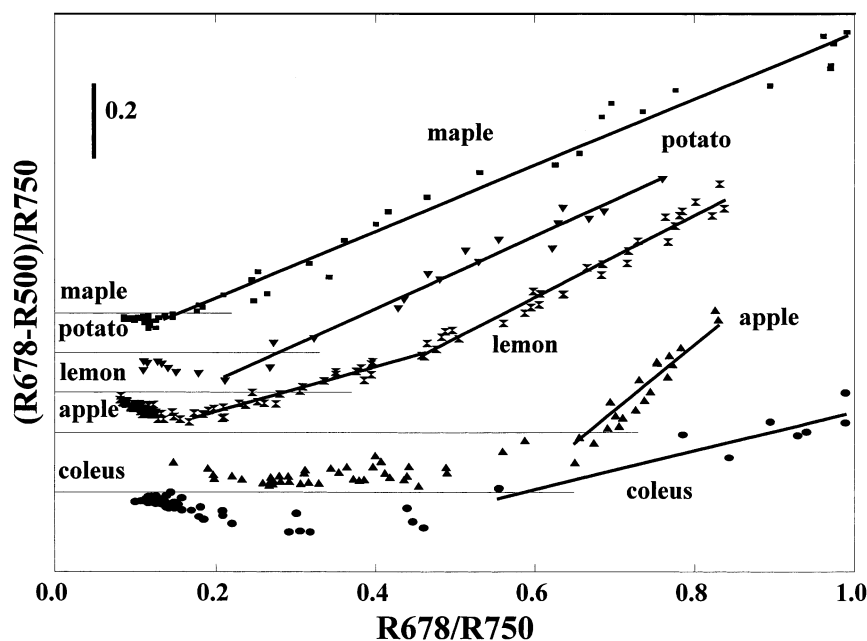


Fig. 7. The reflectance index $(R_{678} - R_{500})/R_{750}$ versus R_{678}/R_{750} for coleus, maple, potato leaves, apple and lemon fruits (see also Table 1). Horizontal thin lines correspond to zero index values for each species. Solid lines are the linear best fit functions calculated for positive index values.

of other pigments (e.g. Chappelle et al. 1992); and (2) at 500 nm, the uncertainties related to the contribution of UV-absorbing material present in senescing leaves and ripening fruits are smaller than at shorter wavelengths (see Figs. 1–3).

There are three relationships between the PSRI and R_{678}/R_{750} (Fig. 7 and Table 1). (1) All dark-green plant tissues with high Chl content possess small negative PSRI values (phase 1). In this phase, R_{678} and R_{500} changed synchronously and the difference $(R_{678} - R_{500})$ remained almost unchanged. (2) The second phase with low PSRI (ca. -0.1); PSRI increased slightly with an increase in R_{678} . This phase manifests early stage of senescence. (3) The third phase was characterised by a sharp increase of the PSRI in leaves and fruits not observed in coleus leaves. The relationships PSRI versus R_{678}/R_{750} were linear (r^2 of 0.94 – 0.98) up to the stage of complete Chl degradation (Fig. 7, Table 1). Apple fruit in the process of their maturation possessed the highest slope of the PSRI versus R_{678}/R_{750} across all species examined (Table 1). This can be explained by a significant increase in Car content in the peel of ripening apple fruit (Knee 1972, 1988). Thus, there is reason to believe that the close linear relationship between the PSRI and R_{678}/R_{750} is an indicator of Car retention or accumulation in senescing leaves and ripening fruits.

In maple (Fig. 7, Table 1) and chestnut leaves (data not shown), a direct transition between phases 1 and 3 occurred at R_{678}/R_{750} as low as 0.15 – 0.16 , corresponding to a Chl content of ca 100 mg m^{-2} (Fig. 5). In potato leaves, a short phase 2 did exist and the PSRI increase occurred at higher R_{678}/R_{750} values than that of maple leaves (Fig. 7 and Table 1). These data indicate that the contribution of Car to light absorption, which may protect the plant's photosynthetic apparatus against harmful effects of light during senescence (Merzlyak and Gitelson 1995), appears in the leaves of deciduous plants at earlier stages of Chl degradation than in darkened (potato) leaves.

Table 1. Parameters of linear regressions $\text{PSRI} = (R_{678} - R_{500})/R_{750}$ (positive values) versus R_{678}/R_{750} for senescing leaves and ripening fruits: $(R_{678} - R_{500})/R_{750} = c + m R_{678}/R_{750}$ (see also Fig. 7). Two fits are given for lemon fruit.

Plant material	n	r^2	$c \cdot 10^2$ Mean \pm SE	$m \cdot 10^2$ Mean \pm SE
Leaves				
Maple	33	0.98	-1.29 ± 0.34	8.33 ± 0.19
Chestnut	14	0.94	-1.31 ± 0.19	8.77 ± 0.22
Potato	17	0.97	-2.53 ± 0.27	9.08 ± 0.42
Coleus	8	0.92	-2.84 ± 0.33	4.84 ± 0.44
Fruits				
Lemon	27	0.92	-1.61 ± 0.13	5.64 ± 0.33
Lemon	31	0.97	-5.78 ± 0.24	10.27 ± 0.35
Apple	22	0.85	-10.89 ± 0.41	16.47 ± 1.56

n, number of observations; r^2 , a determination coefficient.

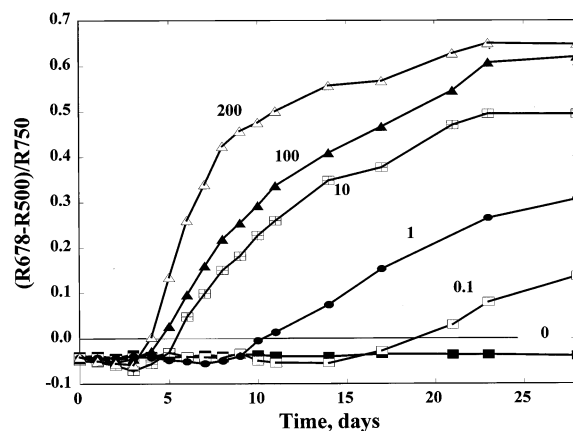


Fig. 8. The reflectance index $(R_{678} - R_{500})/R_{750}$ changes in lemon fruits under ethylene treatment. The numbers in the graph indicate the concentrations of added ethylene (ppm). The index remains invariable for non-treated fruits, whereas for treated fruits, the index increases with an increase in ethylene concentration.

In ripening lemon fruits, two stages of the PSRI increase were found (Fig. 7 and Table 1). In reflectance spectra of those fruits with low Chl content, a significant decrease of reflectance between 400 and 500 nm occurred during ripening, but no spectral features attributable to Car pigments were found (Fig. 2, curves 9–11). Therefore, it is likely that other pigments (see Merzlyak et al. 1997, 1998) reduce the reflectance at 500 nm and, together with Car, were responsible for the two-phase relationship between the PSRI and R_{678}/R_{750} (Figs. 2, 6 and 7), as well as for kinetics of PSRI changes (Fig. 8) observed in ripening lemon fruit.

The changes of the PSRI during pigment transformation in lemon fruits were dependent both on time of incubation and ethylene concentration (Fig. 8). Therefore, the PSRI can be used to quantitatively describe the kinetics of a ripening process.

This work presents a non-destructive technique for estimating the characteristic features and time-course of changes accompanying natural and, possibly stress-induced, leaf senescence and fruit ripening. The quantitative assessment of these events is based on the difference in transformation of Chl and Car. The analysis carried out in this paper indicates that relative changes of reflectance near 500 nm (and at shorter wavelengths, Figs. 1–3) and near 680 nm follow the retention or accumulation of Car during senescence. In addition, the relationships between the PSRI and R_{678}/R_{750} (Fig. 7 and Table 1) make it possible to determine the stage of leaf senescence and fruit ripening with a high degree of precision.

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