PHOTOSYNTHETICA 38 (4): 521-529, 2000

Measurement of differences in red chlorophyll fluorescence and photosynthetic activity between sun and shade leaves by fluorescence imaging

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Abstract

With a flash-lamp chlorophyll (Chl) fluorescence imaging system (FL-FIS) the photosynthetic activity of several thousand image points of intact shade and sun leaves of beech were screened in a non-destructive way within a few seconds. The photosynthetic activity was determined *via* imaging the Chl fluorescence at maximum F_p and steady state fluorescence F_s of the induction kinetics (Kautsky effect) and by a subsequent determination of the images of the fluorescence decrease ratio R_{Fd} and the ratio F_p/F_s. Both fluorescence ratios are linearly correlated to the photosynthetic CO_2 fixation rates. This imaging method permitted to detect the gradients in photosynthetic capacity and the patchiness of photosynthetic quantum conversion across the leaf. Sun leaves of beech showed a higher photosynthetic capacity and differential pigment ratios (Chl *a/b* and Chls/carotenoids) than shade leaves. Profile analysis and histogram of the Chl fluorescence yield and the Chl fluorescence ratios allow to quantify the differences in photosynthetic activity between different leaf parts and between sun and shade leaves with a high statistical significance.

Additional key words: carotenoid and chlorophyll contents; *Fagus sylvatica*; flash radiation pulses; fluorescence decrease ratio; pigment ratios; vitality index.

Introduction

Plants, when exposed to high or low irradiance during growth, react with a variety of adaptations, *i.e.*, the formation of sun and shade leaves as well as sun and shade chloroplasts (Lichtenthaler 1981, Meier and Lichtenthaler 1981). Sun leaves with sun chloroplasts possess a much higher photosynthetic capacity on a leaf area and Chl basis, higher Chl *a/b* ratio, higher saturation irradiance of photosynthetic CO_2 fixation, a much lower concentration of light-harvesting Chl proteins (LHC2), as well as smaller grana stacks and more exposed thylakoid membranes than shade leaves with their low-irradiance chloroplasts (Lichtenthaler *et al.* 1981, Lichtenthaler and Burkart 1999). Plants are also exposed to a variety of biotic and abiotic stressors that affect growth, physiological functions, and yield (Lichtenthaler 1996, 1998). Stressors affect either directly or indirectly the function of the photosynthetic apparatus and the performance of leaves and often modify their optical and fluorescence properties. Early stress detection in plants, before visual damage symptoms are detectable, is required in order to reactivate the plant's vitality by suitable countermeasures.

In the last 20 years, Chl fluorescence signatures of plants have been applied as an efficient tool to describe and investigate the photosynthetic quantum conversion at physiological conditions as well as to detect stress and senescence in the photosynthetic apparatus (for reviews see Lichtenthaler and Rinderle 1988, Krause and Weis 1991, Govindjee 1995). Details on various Chl fluorescence parameters and fluorescence ratios, which

Received 28 August 2000, *accepted 23 November* 2000
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This paper is dedicated to Prof. Dr. Stanislav Procházka, Mendel University Brno, on the occasion of his 60th anniversary.
Abbreviations: Chl, chlorophyll; FL-FIS, flash-lamp fluorescence imaging system; F_d, fluorescence decrease from F_p to F_s; F_p, maximum Chl fluorescence at the given high irradiance pulse; F_s, steady state Chl fluorescence; F_p/F_s, ratio of maximum to steady state Chl fluorescence; PPFD, photosynthetic photon flux density; R_{Fd}, variable chlorophyll fluorescence decrease ratio.
Acknowledgements: This research was sponsored in part by EU grants to H.K. Lichtenthaler within the INTERREG II research project and the Inco-Copernicus project QAAFFI which are gratefully acknowledged. We wish to thank Mrs Gabrielle Johnson, Rastatt, for English language assistance, Mrs Inge Jansche for word-processing, and Ms Julia Reichert for technical assistance.

were collected by point-data measurements of leaves, were given by Schreiber *et al.* (1986), Lichtenthaler and Rinderle (1988), Genty *et al.* (1989), D'Ambrosio *et al.* (1992), Schindler and Lichtenthaler (1996), Lichtenthaler and Miehé (1997), and Lichtenthaler and Burkart (1999). The red Chl fluorescence provides ample information on the photosynthetic apparatus as first discovered by Kautsky (Kautsky and Hirsch 1931, Lichtenthaler 1992, Govindjee 1995). The Chl fluorescence induction kinetics of pre-darkened leaves (known as Kautsky effect) exhibit a fast fluorescence rise to a maximum (F_m or F_p) within *ca.* 200 ms and then a slow decline, parallel to the onset of photosynthesis, within 5 min to the much lower steady state fluorescence value F_s. The ratio of this fluorescence decrease, F_d (= $F_p - F_s$) to the steady state fluorescence F_s (ratio F_d/F_s), also known as variable Chl fluorescence ratio R_{Fd}, and the ratio F_P/F_S were established as indicators of the potential photosynthetic capacity of leaves (Lichtenthaler and Rinderle 1988, Tuba *et al.* 1994, Babani and Lichtenthaler 1996, Lichtenthaler and Miehé 1997).

So far mostly Chl fluorescence signatures of single leaf spots have been measured (*e.g.*, Lichtenthaler and Rinderle 1988, Krause and Weis 1991, D'Ambrosio *et al.* 1992, Babani and Lichtenthaler 1996, Schindler and Lichenthaler 1996). The disadvantage of such point-data measurements is the fact that they provide only limited information on the state of health of leaves and their photosynthetic apparatus, as a single leaf spot is often not representative of the whole leaf. Over the last 6 years high-resolution multi-colour fluorescence imaging techniques for whole leaves have been developed (Lang *et al.* 1994, Edner *et al.* 1995, Lichtenthaler *et al.* 1996, 1997, Buschmann and Lichtenthaler 1998). These techniques offer the possibility to screen gradients and irregularities of Chl fluorescence signatures over the whole leaf area. Imaging of the red Chl fluorescence (F_{690}) during the induction kinetics with determination of the R_{Fd} and F_p/F_s ratio images should thus provide quick information on the photosynthetic performance of the leaf, should allow to detect gradients and to study a loss of photosynthetic activity under stress.

The fluorescence imaging results were obtained using an expensive laser-equipped fluorescence imaging system (Laser-FIS) (Lang *et al.* 1994, Lichtenthaler *et al.* 1996). Here we describe a new, compact, and much cheaper flash lamp induced fluorescence imaging system (FL-FIS) for routine analysis of leaves. Using this Karlsruhe FL-FIS we tested if the differential photosynthetic activity of green shade and sun leaves can be screened *via* Chl fluorescence imaging and if the photosynthetic activity is evenly distributed across the leaf area or shows gradients.

Materials and methods

Plants: Fully developed sun and shade leaves of beech (*Fagus sylvatica* L.) were taken from a 50-year-old, solitary standing beech tree on the University Campus. On sunny days the shade leaves in the inner tree part received *ca.* 80 μmol m^{-2} s^{-1}, whereas the sun leaves were exposed to a maximum PPFD between 1 700 to 2 000 μmol m^{-2} s^{-1}.

Determination of pigments: The contents of chlorophylls (*a*+*b*) and total carotenoids (x+c) of beech leaves was determined in the same 100 % acetone extract solution using the re-evaluated extinction coefficients and equations of Lichtenthaler (1987).

The Karlsruhe fluorescence imaging system (FL-FIS): A new compact flash-lamp fluorescence imaging system (FL-FIS) (Fig. 1) was established which is based on the Karlsruhe/Strasbourg laser-induced fluorescence imaging system (Laser-FIS) for plants (Lichtenthaler *et al.* 1996). The expensive Nd-YAG laser was replaced by a Xenon flash lamp as an excitation source (300 W, *Cermax*, UV-enhanced, *Perkin Elmer Optoelectronics*, Cambridge, UK) with a pulse energy of 0.7 J and a flash duration of 20 μs operated at 16.6 Hz. Blue and green fluorescence as well as red and far-red Chl fluorescence can be excited simultaneously using a UV-A transmission filter (*DUG 11*, golden filter, *Schott*, Germany, range 280-400 nm, λ_{max} = 340 nm). A blue filter was applied (*Corning* No. *9782*, range 370–600 nm, λ_{max} = 465 nm) (Fig. 2) to solely excite the red Chl fluorescence F_{690}. By blue radiation excitation a higher Chl fluorescence yield is obtained than by UV-A excitation. This is due to the fact that a higher proportion of blue radiation penetrates into the green leaf mesophyll and excites more Chl molecules than UV-A radiation which is partially absorbed by the flavonols and cinnamic acids in the leaf epidermis (Buschmann and Lichtenthaler 1998, Lichtenthaler and Schweiger 1998). Thus, when screening the photosynthetic activity *via* Chl fluorescence induction kinetics it is advisable to apply blue radiation excitation. In contrast, when the screening aims at the detection of early stress and strain events and damage, UV-A excitation is recommended since the fluorescence ratio blue to red (F_{440}/F_{690}) and blue to far-red (F_{440}/F_{740}) are very early stress indicators (Lichtenthaler and Miehé 1997, Buschmann and Lichtenthaler 1998).

The fluorescence signals were detected using a gated intensified video camera with an CCD array of 565×754 elements (CCIR video output, 8 bit resolution on Frame grapper, objective: *Nikon* 35 mm; *Optronis*, Kehl,

Germany). The image intensifier tube (2nd gen, *S25* photocathode, *P43* screen, gatable to 50 ns, coupling by fiber reducing taper; *Optronis*, Kehl, Germany) was gated synchronously with the flash lamp (gating time 100 µs). The fluorescence images of leaves can be sensed in the four fluorescence bands (440, 520, 690, and 740 nm) by applying appropriate interference filters (*Oriel*, France; 10 nm half-band width). The latter are installed into a filter wheel in front of the CCD-camera. The red Chl fluorescence band F_{690} of green leaves shows a much higher amplitude during the fluorescence induction kinetics than the far-red Chl fluorescence band F_{740}. For this reason we have measured the F_{690} band.

Image correction was carried out taking into account non-uniform excitation of the beech leaves. The software *Camille 1.05* (*Photonetics*, Kehl, Germany) with modifications allows a centralised control of all FL-FIS components *via* the PC. Applying computer-aided data processing, false colour images of the measured fluorescence intensity and the Chl fluorescence ratios (R_{Fd}-values and F_p/F_s) were obtained by a pixel to pixel division procedure. The images are expressed in false colours, whereby red is the highest fluorescence intensity and blue is zero fluorescence as indicated in the image scale. Also the distribution of the lowest and highest values of the two fluorescence ratios R_{Fd} and F_p/F_s are indicated in false colours from red (highest) to blue (zero value).

Imaging procedures: Beech sun and shade leaves, which had been pre-darkened for 20 min, were irradiated for 1 s with "white light" (PPFD 1 500 µmol $m^{-2} s^{-1}$) in order to determine maximum Chl fluorescence F_p. The red Chl fluorescence was excited and the images were sensed at the upper adaxial leaf side. After sensing the F_p value the leaves were irradiated continuously. Further F_{690} images were taken at 2, 5, and 8 min after onset of irradiation. One hundred image accumulations were chosen as a suitable number of successive readout images to obtain a good signal to noise ratio. After their measurement, the same amount of background images was acquired and subtracted automatically. The whole procedure of accumulation and subtraction of the background images took 12 s. The Chl fluorescence decline F_d from F_p to the steady state fluorescence F_s, reached after 2 or 5 min, was taken to determine the fluorescence ratio $R_{Fd} = F_d/F_s$ and the ratio F_p/F_s. The F_p and F_s values given in Table 2 are the mean fluorescence counts of the pixels of either shade or sun leaves; they were obtained by dividing the sum of the fluorescence counts of all pixels by the total number of the leaf pixels.

The histograms of the frequency distribution of Chl fluorescence intensity and the heights of the fluorescence ratios (R_{Fd} and F_p/F_s) over the whole leaf area are based on all leaf pixels (>100 000 pixels). The leaf profiles, in turn, were measured from a selected area line across the leaf (black and yellow lines in Fig. 3) and are based on *ca.* 5000 pixels.

Results and discussion

Pigment contents and ratios: Fully developed green beech leaves sampled in July 2000 were used for this investigation. Sun and shade leaves of beech differed in their amounts of Chls and carotenoids as well as in their pigment ratios. Sun leaves possessed a higher pigment content per leaf area unit and also exhibited higher values for the ratio Chl *a/b* (Table 1) compared to shade leaves. The mass ratio Chls/carotenoids, in turn, was significantly lower in sun than shade leaves. The Chl and carotenoid amounts were relatively evenly distributed over the whole leaf area, as was proved by multiple pigment determination of different leaf parts indicating only a low pigment variation of <3 %.

Table 1. Chlorophyll, Chl (*a+b*), and total carotenoid content (x+c) [mg m^{-2}(leaf area)] as well as pigment ratios Chl *a/b* and Chl/carotenoids, (*a+b*)/(x+c) in fully functional green shade and sun leaves of a solitary standing beech tree. Mean of 10 determinations with a standard deviation of maximal ± 3 % (pigment concentrations) and less than ± 1 % (pigment ratios). The difference between shade and sun leaves is highly significant ($p<0.001$).

Leaf-type	Shade leaf	Sun leaf
Chl (*a+b*)	445.2	521.5
Carotenoids (x+c)	83.5	127.1
Chl *a/b*	2.67	3.45
(*a+b*)/(x+c)	5.33	4.10

These differences in pigment ratios between sun and shade leaves were described before. They are due to the high-irradiance adaptation response of the photosynthetic pigment apparatus of sun leaves with much less light-harvesting Chl *a/b* proteins (LHC2) and more reaction centres on a total Chl basis compared to shade leaves which exhibit higher and broader grana thylakoid stacks and primarily invest into the pigment antenna (Lichtenthaler *et al.* 1981, 1982a,b). As a consequence, sun leaves of beech with their sun-type chloroplasts possess considerably higher photosynthetic CO_2 fixation rates on a Chl and leaf area basis than shade leaves (Lichtenthaler 1981, Lichtenthaler *et al.* 1981).

Chl fluorescence images of the shade leaf: The photosynthetic activity of fully developed green and 20-min pre-darkened shade leaves was studied by imaging the red Chl fluorescence in the 690 nm range of the upper adaxial leaf side during the Chl fluorescence induction kinetics (Kautsky effect). By applying the Karlsruhe

flash-lamp fluorescence imaging system (FL-FIS, cf. Fig. 1), the red Chl fluorescence F_{690}, as excited here by blue radiation, was imaged at maximum fluorescence F_p (1 s after onset of irradiation) and after 2, 5, and 8 min of irradiation. The F_s was reached after 5 min of "white light" exposure. The Chl fluorescence intensity at F_p was not evenly distributed over the whole leaf area: gradients were clearly visible (Fig. 3). F_p was particularly low at the leaf veins and at the top and basis of the leaf. With the onset of photosynthetic quantum conversion, F_p continuously declined within 5 min to F_s as seen by a change of the red false colour (highest Chl fluorescence intensity) to a light-blue false colour (low Chl fluorescence intensity) (Fig. 3*A,B,C*). In most leaf parts the decline proceeded fast and was almost finished after 2 min (cf. Fig. 3*B* and *C* taken 2 and 5 min after onset of irradiation). The F_{690} intensity was, however, not evenly distributed over the leaf area, showing higher F_s particularly at the left leaf rim.

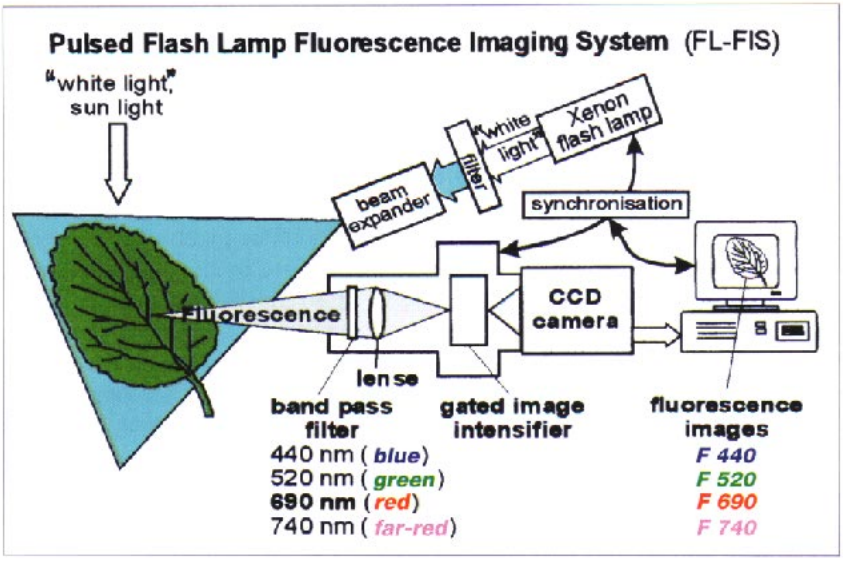

Fig. 1. The Karlsruhe flash-lamp fluorescence imaging system (FL-FIS) for sensing blue, green, red, and far-red fluorescence images of whole leaves. With a pulsed Xenon lamp and a UV-transmission filter, all four fluorescence bands (F_{440}, F_{520}, F_{690}, and F_{740}) are excited. A blue transmission filter is applied when only the red chlorophyll fluorescence (F_{690}) is excited. F_{690}, shown here for a single leaf pixel, is simultaneously collected from more than hundred thousand pixels of the leaf using a CCD video camera, and is stored and processed by the image processing system of a PC.

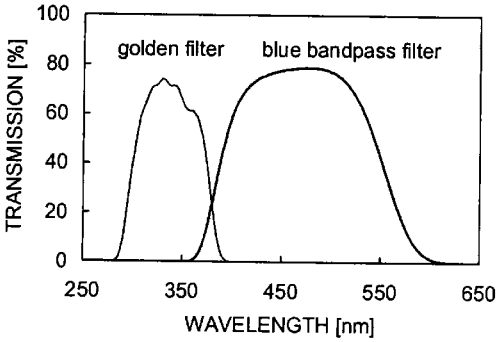

Fig. 2. Transmission range of the UV-A transmission filter (golden filter) applied for simultaneous excitation of the blue, green, red, and far-red leaf fluorescence and of the blue band pass filter used for the excitation of the red Chl fluorescence F_{690}.

The images of R_{Fd} after 2 and 5 min of irradiation (Fig. 3*D,E*) indicate that also the photosynthetic activity is not evenly distributed over the whole leaf area. Low R_{Fd}-values (and thus low photosynthetic rates), as seen in light blue instead of yellow and red spots, were found near the leaf basis, the leaf top, and near the left leaf rim. Similar lower values were found for F_p/F_s (Fig. 3*G*).

During the Chl fluorescence induction kinetics, the values of both fluorescence ratios increased during irradiation. Final values were reached after 5 min as seen by comparing Fig. 3*D* with 3*E* and Fig. 3*F* with 3*G*. The highest photosynthetic activity seen as red spots of R_{Fd}-values (Fig. 3*E*) and as red spots at F_p/F_s in Fig. 3*G* was not found in a larger area of the leaf, but only as individual spots distributed over various parts of the leaf area. Thus the photosynthetic quantum conversion exhibits a patchiness across the leaf. This had also been reported before for the stomata opening (Cheesemann 1991, Terashima 1992). A longer irradiation did not minimise this patchiness.

Fluorescence histogram of the shade leaf: On the basis of more than 100 000 fluorescence pixels of the shade leaf of beech, a frequency distribution of the Chl fluorescence intensity of all leaf pixels was performed.

This histogram for F_p and F_s (at 2 and 5 min of "white light" exposure) shown in Fig. 4 indicates that the fluorescence intensity after 2 min of irradiation, $F_{s(2')}$, was very close to the final minimum $F_{s(5')}$ reached after 5 min of irradiation. The histogram also documents that the variation of F_p is broader than that of F_s.

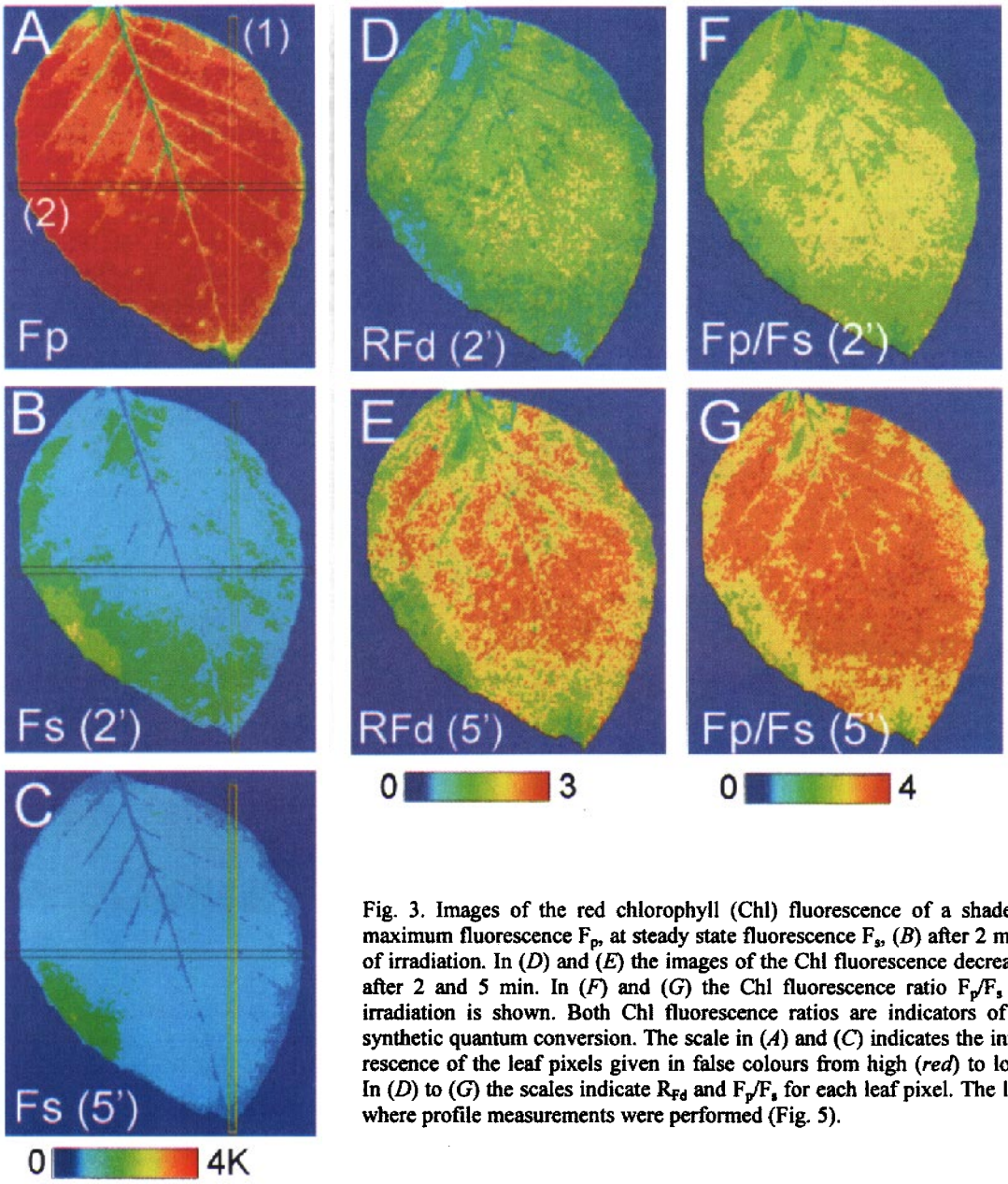

Fig. 3. Images of the red chlorophyll (Chl) fluorescence of a shade leaf of beech (*A*) at maximum fluorescence F_p, at steady state fluorescence F_s, (*B*) after 2 min, and (*C*) after 5 min of irradiation. In (*D*) and (*E*) the images of the Chl fluorescence decrease ratio R_{Fd} are shown after 2 and 5 min. In (*F*) and (*G*) the Chl fluorescence ratio F_p/F_s after 2 and 5 min of irradiation is shown. Both Chl fluorescence ratios are indicators of the degree of photosynthetic quantum conversion. The scale in (*A*) and (*C*) indicates the intensity of the Chl fluorescence of the leaf pixels given in false colours from high (*red*) to low fluorescence (*blue*). In (*D*) to (*G*) the scales indicate R_{Fd} and F_p/F_s for each leaf pixel. The lines show the leaf part where profile measurements were performed (Fig. 5).

Fluorescence profiles of the shade leaves: With the software of the Karlsruhe fluorescence imaging system one can also perform individual Chl fluorescence profiles and profiles of the Chl fluorescence ratios of the pixels at any horizontal or vertical leaf image line. The horizontal leaf profile (black line in Fig. 3*A*) of F_p and F_s showed spikes of a decrease at the main leaf vein and at the green point indicating that the Chl content and consequently the Chl fluorescence yield was lower in these leaf regions (Fig. 5*A*). The lower Chl content at the main leaf vein and at that single leaf point was also observed visually. The profiles of photosynthetic activity and quantum conversion, the ratios R_{Fd} and F_p/F_s, however, revealed that in those leaf areas the photosynthetic quantum conversion was the same as in the other leaf parts exhibiting higher F_p and F_s. This indicates that in leaf veins or other leaf points with a lower Chl content the process of photosynthetic quantum conversion is as functional as in the other leaf parts. The R_{Fd}-values and their related ratio F_p/F_s are independent of the Chl amount

present in a leaf pixel and indicate if the differential amounts of Chl present in a leaf point are functionally organised. Similar results were obtained by checking a leaf vertical fluorescence profile (yellow line in Fig. 3*A*).

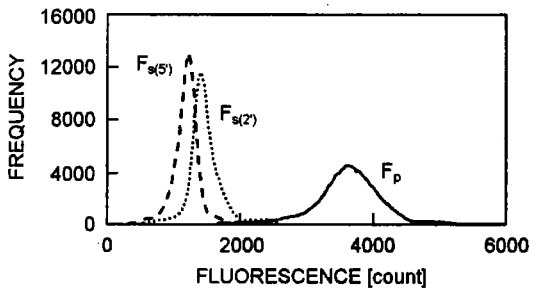

Fig. 4. Histogram of the frequency distribution of the Chl fluorescence intensity at maximum (F_p) and steady state fluorescence (F_s) after 2 and 5 min (2' and 5') of irradiation measured here for all pixels of the whole shade beech leaf. The frequency distribution is based on more than 100 000 individual leaf pixels.

The F_p profile showed the same variability with decrease spikes in the side leaf veins, which were, however, not seen in R_{Fd} and F_p/F_s (Fig. 5).

Chl fluorescence images of the sun leaf: In the pre-darkened sun leaf of beech, F_p was fairly evenly distributed over the whole leaf area (Fig. 6*A*), the Chl fluorescence yield was, however, considerably lower than in the shade leaf. This is because F_{690} is partially reabsorbed by the *in vivo* Chl (overlapping of absorption and fluorescence emission bands of Chl *a*-forms) as documented for leaves of various plant species (Lichtenthaler and Rinderle 1988, Hák *et al.* 1990, D'Ambrosio *et al.* 1992, Gitelson *et al.* 1998). For this reason the Chl fluorescence yield decreases with increasing Chl content of leaves (Stober *et al.* 1994, Babani and Lichtenthaler 1996) and is lower in sun leaves than shade leaves, since sun leaves possess a higher Chl content than shade leaves (cf. also Table 1).

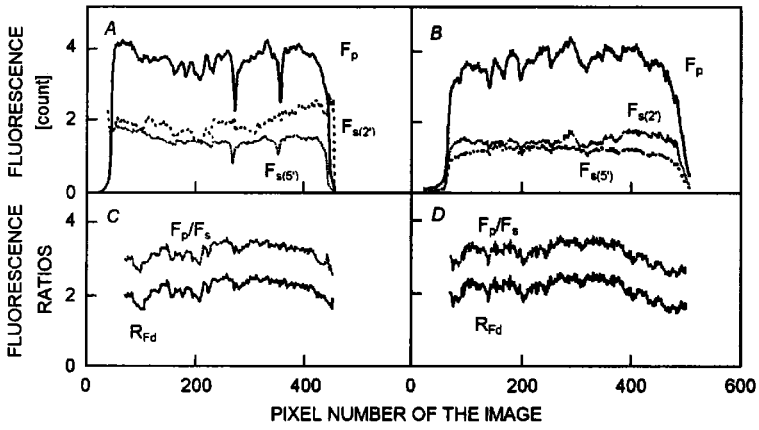

Fig. 5. Profiles of (*A*, B) Chl fluorescence intensity at F_p and F_s and (*C, D*) of the two Chl fluorescence ratios, R_{Fd} and F_p/F_s across the leaf area along the horizontal (*A, C*) and vertical (*B, D*) lines shown in Fig. 3. The profile of the shade leaf is based on the individual fluorescence information of more than 5 000 leaf pixels.

Upon irradiation with "white light" the actual Chl fluorescence F decreased continuously during the induction kinetics as seen by the F_s values taken here at 1 and 5 min of irradiation (Fig. 6*B,C*). Further irradiation (*e.g.*, for 8 min) did not result in lower F_s (Fig. 6*D*). In contrast to F_p, F_s exhibited a large difference in the individual leaf parts. F_s was particularly high near the leaf rim of the left lower part of the sun leaf and in the lower part of the median leaf vein. R_{Fd} and F_p/F_s demonstrated that the photosynthetic quantum conversion was not evenly distributed over this sun leaf, and both ratios exhibited low values especially in those leaf parts (Fig. 6*E,D*) which showed relatively high F_s-values.

Differences between sun and shade leaves in Chl fluorescence yield and photosynthetic quantum conversion can be quantified by comparing the mean values of the more than 100 000 leaf pixels and also by contrasting the results of fluorescence histograms. The higher Chl fluorescence yield at F_p and F_s of shade leaves as compared to sun leaves is shown in Table 2 on the basis of fluorescence images of four sun and shade leaves of beech. Also R_{Fd} and F_p/F_s, which indicate the photosynthetic quantum conversion and photosynthetic activity of leaves (Lichtenthaler and Rinderle 1988, Tuba *et al.* 1994, Babani and Lichtenthaler 1996), showed the expected higher values in sun than shade leaves of beech (Table 2). This agrees with older observations of sun and shade leaves of the beech (Lichtenthaler 1981, Lichtenthaler *et al.* 1981, Rinderle 1990). These differences in R_{Fd} and F_p/F_s of sun and shade leaves were also seen in the corresponding histogram (Fig. 7) which is based on the Chl fluorescence images of the shade leaf (Fig. 3) and the sun leaf (Fig. 6).

From the Chl fluorescence images taken at different times during the Chl fluorescence induction kinetics (Kautsky effect) upon irradiation of pre-darkened leaves, one can also determine the development of R_{Fd}-values of all leaf pixels. These values were higher in sun than shade leaves during the whole induction kinetics (Fig. 8*A*). The final values were reached in both cases after 5-min irradiation and did not further increase after 8 min of irradiation.

One can also measure and compare the relative decline in Chl fluorescence from the fluorescence images taken during the Chl fluorescence induction kinetics. Since the absolute Chl fluorescence of shade leaves is higher than that of sun leaves, the relative fluorescence decline was expressed as ratio of the actual Chl fluorescence F (measured after 1, 5, and 8 min of irradiation, respectively) over F_p. This relative fluorescence decline (F/F_p) was lower in shade than sun leaves (Fig. 8*B*).

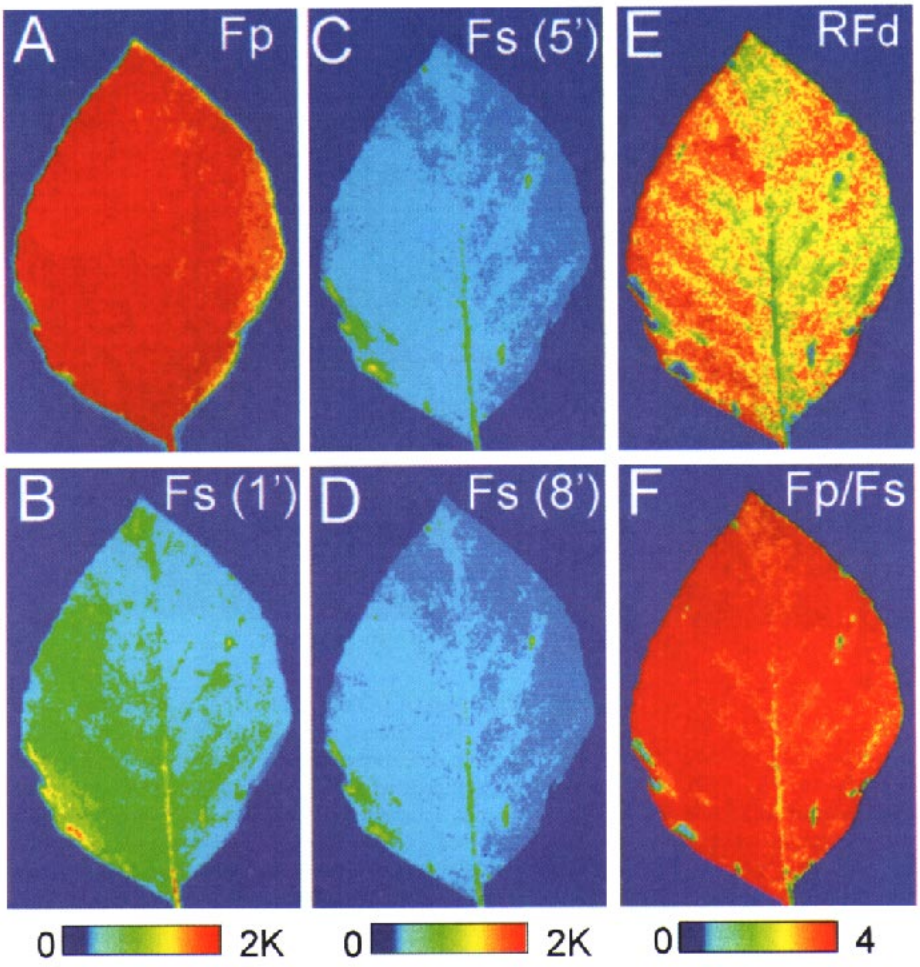

Fig. 6. Images of the red Chl fluorescence of a sun leaf of beech (*A*) at maximum fluorescence F_p, at steady state fluorescence F_s after 1 min (*B*), 5 min (*C*), and 8 min (*D*) of irradiation during the Chl fluorescence induction kinetics. In (*E*) and (*F*) the images of the Chl fluorescence decrease ratio ($R_{Fd} = F_d/F_s$) and the Chl fluorescence ratio F_p/F_s after 5 min are shown. The scale in (*A*) to (*D*) indicates the intensity of the Chl fluorescence of the leaf pixels given in false colours from high (*red*) to zero (*blue*) fluorescence. In (*E*) and (F) the scale indicates the values of the two fluorescence ratios.

Conclusion: With the Karlsruhe fluorescence imaging system, FL-FIS, one can simultaneously measure the red Chl fluorescence of all leaf parts (more than 100 000 pixels per leaf) at various times during the light-induced Chl fluorescence induction kinetics. From the Chl fluorescence images at maximum fluorescence (F_m or F_p) and at steady state fluorescence F_s one can determine by data processing the images of the two related Chl fluorescence ratios R_{Fd} and F_p/F_s, which are indicators of the photosynthetic quantum conversion and photosynthetic activity of leaves. The linear relationship of these Chl fluorescence ratios to the photosynthetic CO_2 fixation rates had been shown for various leaves (Tuba *et al.* 1994, Babani and Lichtenthaler 1996).

Table 2. Mean yield of the red chlorophyll (Chl) fluorescence [counts per pixel] at maximum fluorescence F_p and at steady state fluorescence F_s after 5 min as well as mean values of Chl fluorescence ratios (R_{Fd} and F_d/F_s) of all leaf pixels of either shade or sun leaves of beech. Chl fluorescence and the ratios R_{Fd} and F_d/F_s as indicators of photosynthetic quantum conversion, photosynthetic activity and as a vitality index of the photosynthetic apparatus were determined with the fluorescence imaging system FL-FIS at the upper leaf side and separately for each leaf pixel. Mean from four separate leaves with a standard deviation of maximal ± 7 % (fluorescence intensities) and less than ± 3 % (fluorescence ratios) between the four leaves. These values are based on more than 100 000 pixels per leaf. The differences between shade and sun leaves in F_p, F_s, and the fluorescence ratios are highly significant ($p<0.001$).

Leaf-type	Shade leaf	Sun leaf
F_p	3300	1777
F_s	1134	470
R_{Fd}	2.1	2.8
F_p/F_s	3.1	3.8

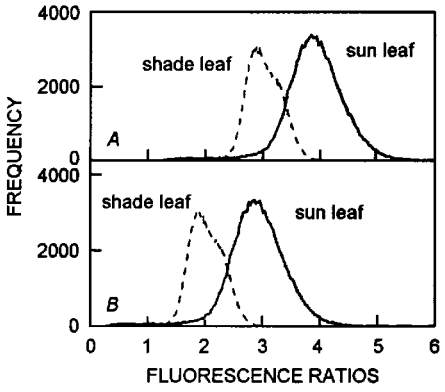

Fig. 7. Histogram of the frequency distribution of the two variable Chl fluorescence ratios, F_p/F_s (*A*) and R_{Fd} (*B*), measured for all pixels of shade and sun leaves of beech, 5 min after onset of irradiation. The frequency distribution is based on more than 100 000 individual leaf pixels.

Via R_{Fd} images of leaves one can simultaneously determine in a non-destructive way the photosynthetic capacity and rates of the various leaf parts including leaf veins and detect gradients in photosynthetic activity across the leaf area. *Via* the fluorescence histogram and leaf profile analysis, the differences in photosynthetic quantum conversion between leaves and leaf parts can be quantified. It allows the screening of the patchiness of the photosynthetic activity that is a general phenomenon of photosynthetically active green leaves (Cheeseman 1991, Terashima 1992). The Chl fluorescence imaging technique also permits to determine the differences in photosynthetic activity between sun and shade leaves and to detect early stress events which affect the rates of photosynthetic quantum conversion. The FL-FIS thus is a superior technique for ecophysiological plant research.

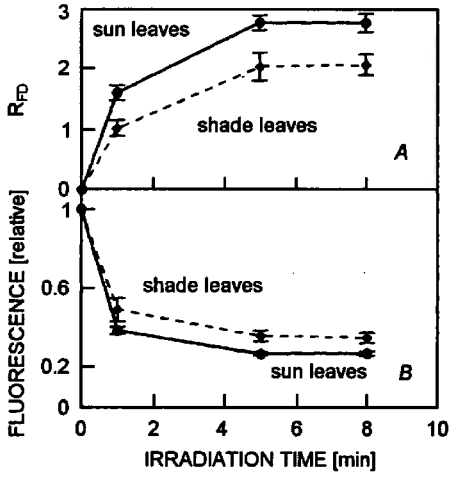

Fig. 8. Development of (*A*) the chlorophyll (Chl) fluorescence decrease ratios (R_{Fd}-values) and (*B*) the decline of the Chl fluorescence from maximum fluorescence F_p to steady state fluorescence F_s (expressed as F/F_p) during the Chl fluorescence induction kinetics in shade and sun leaves of beech from a second measurements series. Means of four images of the whole leaf area (more than 100 000 pixels per leaf) from four separate leaves (SD= ± 5 %) The differences between shade and sun leaves are highly significant ($p<0.001$).

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