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Remote Multi-Colour Fluorescence Imaging of Selected Broad-Leaf Plants

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ABSTRACT

An imaging fluorescence lidar system was used for remote multi-spectral measurements on selected broad-leaf plants cultivated in the Botanical Garden of the University of Karlsruhe. The technique allows to characterise vegetation in terms of chlorophyll content via sensing the chlorophyll fluorescence ratio F690/F735 and also by the blue to red fluorescence ratio F450/F690 in images of one or several leaves. Remotely evaluated data were compared with *in vivo* chlorophyll fluorescence emission spectra, measured in the near distance up to 20 cm, for the same leaf samples. In addition, the leaves were physiologically characterized via determination of the variable chlorophyll fluorescence ratio, the Rfd690-values. Leaves of different plants with different chlorophyll content were tested. We also studied the effect of a block of photosynthesis by the herbicide diuron on the fluorescence ratios F690/F735 and F450/F690. The results obtained indicate that fluorescence imaging may prove to be a valuable tool in the remote assessment of the vegetation status in relation to environmental stress and herbicide treatment.

INTRODUCTION

Laser-induced fluorescence (LIF) signatures of plant leaves exhibit maxima in the blue region near 450 nm (F450), green region near 530 nm (F530) and in the red and far-red region near 690 and 735 nm (F690 and F735) (Chappelle et al., 1984; Lang and Lichtenthaler, 1991; Stober and Lichtenthaler, 1992). Both of the two red fluorescences originate from protein-bound chlorophyll molecules in the chloroplasts of the mesophyll of the leaf. The blue-green fluorescence, already detected 60 years ago (Kautsky and Hirsch, 1934), and rediscovered more recently (Chappelle, 1984a and b), can be regarded as a mixed signal of various secondary plant compounds such as plant phenolics (Goulas et al., 1990; Lang et al., 1991). Recent fluorescence microscopic studies showed that the blue-green fluorescence emission of green leaves primarily emanates from the cell walls of the epidermis layer of leaves (Stober and Lichtenthaler, 1993b). The chlorophyll fluorescence ratio F690/F735 is inversely related to the *in vivo* chlorophyll content (curvilinear relationship) and represents a non-destructive method for the determination of the in vivo chlorophyll content of leaves (Lichtenthaler and Rinderle, 1988; Hák et al., 1990; Szábo et al. 1992). Since all long-term stress events reduce the chlorophyll content of leaves and increase the fluorescence ratio F690/F735, the ratio F690/F735 can be applied as a stress indicator of terrestrial vegetation (Hák et al., 1990; D'Ambrosio et al., 1992; Lichtenthaler and Rinderle, 1988; Lichtenthaler et al., 1990).

LIF signatures, when remotely sensed, could provide information on the state of health of plants of terrestrial vegetation. Besides the ratio F690/F735 one can apply other fluorescence ratios which possess complementary information. Such an additional ratio is that of the blue to green fluorescence F450/F530 which exhibits a linear relationship to the pigment ratio chlorophylls/carotenoids and therefore reflects both chlorophyll and carotenoid content (Stober and Lichtenthaler 1992). Another one is the ratio of blue to red fluorescence, F450/F690, which increases with increasing

age of conifers and which was demonstrated to change under water stress (Lichtenthaler et al., 1991). In addition, the fluorescence ratio F450/F690 can increase considerably with increasing stress due to the fact that the exciting UV-light can no longer penetrate the epidermis layer of stressed plants (Stober and Lichtenthaler, 1993b). Excitation spectra of the blue, green and chlorophyll fluorescence gave the information that the optional wavelength for the simultaneous excitation of the blue-green and the red fluorescence for a future European LIDAR fluorosensor lies in the range >370 to 395 nm (Stober and Lichtenthaler, 1992, 1993a; Lichtenthaler et al., 1993).

Here we describe the fluorescence signatures of leaves with different chlorophyll content as measured from a near distance up to 20 cm to the leaf and compare these with the LIF signatures which were remotely sensed by fluorescence imaging spectroscopy in a distance of 50 m using the Swedish mobile fluorescence LIDAR system. The physiology of leaves was measured via the potential photosynthetic activity of the intact leaves as determined by means of the variable chlorophyll fluorescence ratio, Rfd690 value, which is a vitality index of leaves (Lichtenthaler and Rinderle, 1988). The results come from a joint measuring campaign in June 1992 in the Botanical Garden of the University of Karlsruhe performed within the European EUREKA research program LASFLEUR (Eu 380).

MATERIAL AND METHODS

PLANTS

The plants applied in this investigation were cultivated in the Botanical Garden of the University of Karlsruhe. These were *Bergenia cordifolia* L., *Clivia nobilis* L., *Iris germanica* L. and *Nicotiana tabacum* L. aurea mutant Su/su. The cherry-laurel (*Prunus laurocerasus* L.) was grown in a private garden in Karlsruhe-Durlach.

FLUORESCENCE EMISSION SPECTRA

Fluorescence emission spectra of leaves from 430 to 800 nm (including the blue-green fluorescence and the red chlorophyll fluorescence emission) were recorded with the LS-50 Spectrofluorometer (Perkin-Elmer, Überlingen, Germany) operating with a pulsed Xenon-lamp. Due to the suppliers' solid sample holder the fluorescence was excited at an angle of 30° and sensed in an angle of 60° from the upper leaf side. The spectral slit widths were adjusted to 10 nm for the excitation and the emission monochromators. A 430 nm cut-off filter was placed in the emission beam in order to avoid scattering light. The fluorescence spectra were measured of fully illuminated leaves which were at the steady-state level of the chlorophyll fluorescence induction kinetics in order to simulate the outdoor situation at sunlight. The fluorescence of the leaf samples was excited at 397 nm, a wavelength region which was found very suitable to excite blue-green as well as red chlorophyll fluorescence (Stober and Lichtenthaler, 1993a, Edner et al., 1993b). Shorter wavelengths between 300 and 370 nm gave a strong blue-green fluorescence signal but only a very weak chlorophyll fluorescence signature (Stober and Lichtenthaler, 1993a). This is why the excitation range of > 370 to 400 nm is recommended as an excitation wavelength for the future airborne LASFLEUR LIDAR fluorosensor, which is termed to sense the blue-green and the red fluorescence emission bands of terrestrial vegetation (Günther et al., 1991, Stober and Lichtenthaler, 1992; Lichtenthaler et al., 1992, 1993). The spectrofluorometer was spectrally calibrated with a standard tungsten lamp.

CHLOROPHYLL FLUORESCENCE INDUCTION KINETICS

Fluorescence induction kinetics of tobacco leaves were performed using the two wavelength fluorometer LITWaF which is equipped with a He/Ne laser (632.8 nm) as previously described (Lichtenthaler and Rinderle, 1988). Before the measurement of the induction kinetics all samples were predarkened for 20 min. The variable fluorescence ratio Rfd, a vitality index, was determined from the maximum fluorescence fm, the fluorescence decrease fd to the steady state fluorescence fs according to the definition of Rfd = (fm - fs)/fs = fd/fs. The Rfd690-value as determined at 690 nm is a vitality index and is an indicator of the photosynthetic functionality of the leaf chlorophyll (Lichtenthaler and Rinderle, 1988). The light intensity of the exciting red laser-light (He-Ne-laser emission 632.8 nm) of the LITWaF instrument used was relatively high (500 mol photons m^{-2} s^{-1}) and gave fairly well reproducible Rfd-values. The latter only then become low when the exciting red laser-light is much below 300 mol m^{-2} s^{-1}.

PIGMENT DETERMINATION

The spectrophotometric determination of the photosynthetic pigments, total chlorophylls a+b and total carotenoids x+c (xanthophylls and carotenes) after extraction of leaf segments in 100 % acetone, was carried out applying the UV-2001-PC spectrophotometer (Shimadzu, Duisburg, Germany) using the redetermined extinction coefficients and equations of Lichtenthaler (1987), that allow a simulta-

neous determination of chlorophylls a and b as well as total carotenoids (x+c) in the same extract solution.

TREATMENT WITH THE HERBICIDE DIURON

The photosynthetic electron transport chain in tobacco leaves was fully blocked by treating the lower leaf side with the herbicide diuron (=DCMU, Merck, Darmstadt, Germany). The herbicide was applied from a concentrated solution with a very fine paint brush to the lower leaf side. After 30 min the photosynthetic electron flow was fully blocked, as seen from a very a high chlorophyll fluorescence level and the absence of the variable fluorescence (see Fig. 8), which was sensed from the upper leaf side.

REMOTE MULTI-COLOUR FLUORESCENCE IMAGING

The remote measurements were performed using the Swedish mobile fluorescence LIDAR laboratory (Edner et al., 1993a, 1993b). The target plants were placed at a distance of about 50 m from the LIDAR system, where they were illuminated from above with the laser-light via a nearby flat mirror. The excitation wavelength near 397 nm was generated by Raman-shifting the frequency-tripled output from a Nd:YAG laser in a high-pressure deuterium cell. An output pulse energy of about 30 mJ was achieved at a repetition rate of 20 Hz. The outgoing beam was transmitted in a divergent beam to illuminate an area of 30-cm diameter at the target position. The laser-induced fluorescence light was collected by a 40-cm diameter telescope and directed into a second Cassegrainian telescope, which had its first mirror cut into four segments. By tilting the mirror segments, each segment produced an image on different parts of an image intensified CCD camera connected to the Cassegrainian telescope. Furthermore, different interference filters or Schott coloured-glass filters were placed before each mirror segment matching selected wavelength intervals of the fluorescence spectra. Two of the images were used to detect the two chlorophyll fluorescence peaks, using interference filters centred at 685 nm and 740 nm, respectively, and with a bandwidth of 10 nm (FWHM). The other two images recorded the blue fluorescence with a 470 nm (10 nm bandwidth) interference filter and a BG7 colour glass filter transmitting in the blue-green spectral region. The gate-width of the CCD image intensifier was set at 20 ns to efficiently suppress the influence of daylight illumination of the target. Typically, the images were recorded during 100 laser shots. The fluorescence images were read out to a PC 486 computer. Computer processing made it possible to generate a new image, pixel by pixel, from the four sub-images using a suitable designed spectral contrast function, which enhances features of interest. The resulting image could be shown on the screen in false colours.

Alternatively, remote fluorescence spectra could be collected with the same LIDAR system in a specific point within the target area. In this mode of operation, a flip-in mirror was used to guide the fluorescence into a 600 nm optical quartz fibre, which was connected to an optical multichannel analyser (OMA) system. The OMA system consisted of a 27-cm spectrometer and an image intensified 1024 channel diode array. Both remote systems were spectrally calibrated using a 200 W standard tungsten filament lamp that was placed at a distance of 50 m from the lidar van.
Due to the differential pixel size of the illuminated samples, leaf spots (in the case of the spectrofluorometer) and large leaf areas (in the case of the remote sensing system), the absolute values of the fluorescence ratios obtained with both fluorescence systems were somewhat different, yet the characteristic differences and changes in fluorescence ratios, due to differences in chlorophyll content, could be sensed by the spectrofluorometer and the fluorescence imaging system.

RESULTS

The leaves of all investigated plants were characterized by their content of photosynthetic pigments (chlorophylls and carotenoids) and by the pigment ratios chlorophyll a/b as well as total chlorophylls to total carotenoids (a+b)/(x+c). A characterization of the investigated leaves by pigment content and pigment ratios is necessary, since a part of the emitted blue-green and red fluorescence is reabsorbed by the photosynthetic pigments (Stober and Lichtenthaler, 1992). The shape of the fluorescence spectra and the fluorescence ratios therefore changes in dependence on the pigment content of leaves. This fact can be used to differentiate by fluorescence between fully green photosynthetically active leaves and stressed or senescent leaves which have lost part of their photosynthetic pigments.The two pigment ratios, mentioned above, contain information whether the leaves are in good physiological conditions or are partly or extremely stressed. High values for the ratio (a+b)/(x+c) of 5 to 6 indicate a photosynthetically intact photosynthetic apparatus. When the leaves and the photosynthetic apparatus are under stress, chlorophylls are faster broken down than the carotenoids and the pigment ratio declines and shows in senescent leaves very low values of 2 to 3 for (a+b)/(x+c). The ratio a/b, in turn, amounts to values of 2.6 to 3.1 under physiological conditions of leaves, except for aurea mutant where the a/b ratio can reach values of 3.5 to 4.5. In senescent or stressed leaves the a/b ratio, however, eventually declines to values of 2 or even lower ones.

Therefore the knowledge of the pigment content and pigment ratios of the investigated leaves is a prerequisite in order to correctly interpret the fluorescence signals of different leaves, which may be in a different physiological state. For this reason the pigment status of all investigated leaves was determined. The inverse correlation between the chlorophyll fluorescence ratio F690/F735, as described before (Hák et al., 1990; Lichtenthaler and Rinderle, 1988), was confirmed for the leaves applied in this investigation (Table 1). Those leaves, which had a lower chlorophyll content were characterized by increased values of the fluorescence ratio F690/F735.

The dark-green large *Bergenia* leaves were photosynthetically fully functional as seen from high values of the variable chlorophyll fluorescence ratio Rfd690 of 3.8 (± 0.3) as shown in Table 1. The upper leaf-side showed a lower chlorophyll fluorescence emission and a lower fluorescence ratio F690/F735 than the lower leaf side (Fig. 1). The higher value of the ratio F690/F735 of the lower leaf-side is due to its lower chlorophyll content as compared to the upper adaxial leaf half with its densely packed palisade cells. In yellowish-green senescent *Bergenia* leaves, which had a much lower chlorophyll content the ratio F690/F735 showed very high values of 2.47 (± 0.3) (Table 1). The high Rfd-value of 2.7 indicated that the chlorophyll still present in this senescent leaf was organized in a functional way and was photochemically active. The blue-green fluorescence emission of the dark green *Bergenia* leaf was fairly low as compared to other plants, which resulted in a low value for the blue to red fluorescence ratio F450/F690 of 0.19 (Table 1).

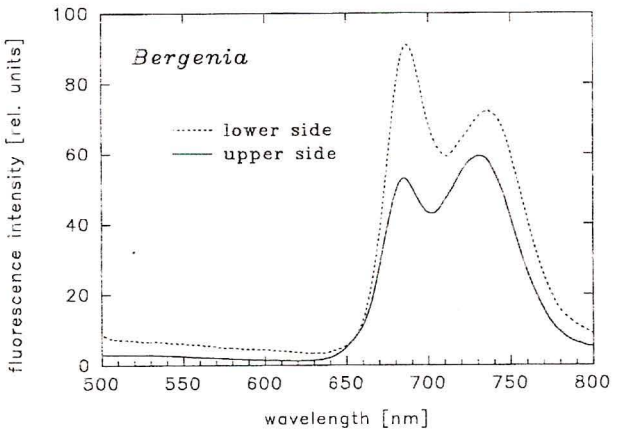


Fig. 1 - Chlorophyll fluorescence emission spectra from the upper and lower side of a green leaf of Bergenia with emission maxima near 690 and 735 nm. The fluorescence emission ratio F690/F735 is higher for the abaxial lower leaf half (1.27) than the chlorophyll-rich, adaxial upper leaf half (0.90) with its densely packed palisade parenchyma cells. (For pigment content and fluorescence ratios see Table 1).

Remote fluorescence images were recorded for the upper and the lower side of the same fully green *Bergenia* leaves. In Fig. 2 simultaneously recorded fluorescence images in four wavelength bands (upper left) are shown. The left leaf had its upper side exposed to the detection system and the right leaf the lower side. The two fluorescence images at 685 and 740 nm were used to form an image of the 685/740 ratio (upper right). The yellow colour of the right leaf indicates a higher fluorescence ratio as compared to the left leaf. The fluorescence ratios for all the pixels of the F685/740 image are presented in the diagram in the lower right part of Fig. 2, where the number of pixels with a certain ratio is shown as a function of the chlorophyll fluorescence ratio. As can be seen, the diagram shows two peaks, which are resolved as the two leaves in the lower diagram. From this diagram the mean value of the F685/F740 ratio for the left leaf (with the upper side exposed) is 0.9, the ratio for the right leaf (with the lower side exposed) is about 1 .4. These values are in good agreement with the values obtained with the near distance spectrofluorometer (s. Table 1). A photograph of the scene with a circle inserted to indicate the aperture of the telescope, is shown in the lower left part of the figure.

Due to the broad spectral band-width of the filters applied, the chlorophyll fluorescence ratios F690/F735 as measured with the spectrofluorometer can directly be compared with the F685/F740 ratio as determined using the remote fluorescence imaging system. Small differences in the values of this ratio between direct measurement and remote sensing are due to the fact that the excitation sources and the detectors of the instruments as well as the pixel sizes of the leaves were different in both modes of measurement.

In the case of *Clivia* leaves the blue-green fluorescence of dark-green leaves was higher than in *Bergenia* and considerably increased with decreasing chlorophyll content (Fig. 3). The red chlorophyll fluorescence emission near 690 nm, in turn, increased at the beginning of senescence to a higher value (leaf 2) and then decreased, whereas the fluorescence band near 735 nm decreased with decreasing chlorophyll content (Fig. 3). Consequently the values of the ratio F690/F735 increased with decreasing chlorophyll content (Table 1) whereas the fluorescence ratio blue/red (F450/F690) decreased from 2.1 (green) via 0.91 to 0.64 (yellowish green leaf).

Fig. 4 shows remotely recorded fluorescence images for three leaves of *Clivia* of increasing degree of senescence from left to right. In this case a ratio of the broad-band blue emission and the F685 image is displayed (right). The most senescent leaf (the right one) showed the highest value of the ratio F*blue*/F685, as indicated by the yellow colour in Fig. 4 (right part). This was also observed with the spectro-

Table 1: Pigment content (chlorophylls a+b, carotenoids x+c; in g cm^{-2}), pigment ratios (Chl a/b, chlorophyll/carotenoids (a+b)/(x+c)) and chlorophyll fluorescence ratios (F690/F735 and Rfd690) in leaves of different plants. Mean of 6 determinations, standard deviation 6% or less. The fluorescence was excited and sensed on the upper leaf side, except for *Bergenia* where also the lower leaf side was (n.d. = not determined).

Plant	a+b	x+c	a/b	(a+b)/(x+c)	Rfd690	F690/F735	F450/F690
Bergenia							
dark green	51.8	10.2	2.8	5.1			
upper leaf side					3.8	0.90	0.19
lower leaf side					3.4	1.45	n.d.
yellowish-green	8.4	4.0	2.6	2.1	2.7	2.47	n.d.
Clivia							
dark-green	86.5	16.8	3.0	5.1	2.7	0.86	2.1
light-green	19.5	6.5	2.6	3.0	2.1	1.44	0.91
yellowish-green	3.4	1.6	1.9	2.1	1.6	4.25	0.64
Prunus							
green	41,8	8.1	3.2	5.2	3.4	0.73	0.17
light-green	26.4	6.8	2.4	3.9	2.7	1.04	0.13
Nicotiana tabacum							
aurea form Su/su	18.6	5.1	3.8	3.7	2.9	1.47	0.14
aurea + DCMU	18.6	5.1	3.8	3.7	0.0	1.75	0.06
Iris germanica							
green	65.9	13.9	3.1	5.1	2.5	1.07	0.75
light-green	27.5	6.1	2.9	4.5	2.7	1.29	0.41
pale-green	13.6	3.1	2.8	4.4	2.4	1.89	0.21

fluorometer as mentioned above. The large changes in the ratio of blue to red fluorescence, with decreasing chlorophyll content and senscence emphasizes the applicability of the ratio F450/F690 in the stress detection of plants.

In the case of cherry-laurel (*Prunus laurocerasus*) we also measured several leaves with different chlorophyll content which could also be seen and judged from the different value of the fluorescence ratio F690/F735 (Table 1). All leaves were photosynthetically active as seen from Rfd-values of 2.7 to 3.4. These *Prunus* leaves from plants grown in the garden had a very low blue-green fluorescence emission (Fig. 5) and consequently the fluorescence ratio blue/red (F450/F690) gave low values (Table 1).

Remote fluorescence images are in this case shown for 685 nm and 740 nm (Fig. 6). An image of the F685/F740 ratio was formed (not shown here) and the corresponding ratio is given in a table in Fig. 6 for several locations on the leaves. The mean value over all the leaves was 0.82, which is close to the value 0.73 obtained with the spectrofluorometer.

Also the leaves of the chlorophyll-deficient mutant tobacco showed a low blue-green fluorescence emission and a typical red chlorophyll fluorescence (Fig. 7). After treatment with the herbicide diuron (DCMU), which blocked the photosynthetic electron transport, the total chlorophyll fluorescence emission considerably increased and also augmented the fluorescence ratio F690/F735 by 17% from a value of 1.47 to 1.75 (Table 1). This incerase happened at a constant pigment content, and therefore was only due to the loss of the photosynthetic function. Since the block of photosynthetic electron transport by DCMU had only little effect on the blue fluorescence emission, the blue/red fluorescence ratio F450/F690 decreased significantly from 0.14 to 0.06. The variable chlorophyll fluorescence, as seen in the induction kinetics at 690 and 735 nm region applying the LITWaF instrument (Fig. 8) became fully lost by DCMU treatment and the leaves showed, as expected, only the maximum fluorescence levels. Consequently the Rfd-values were zero in the diuron-treated leaves (Table 1). The results indicate that the fluorescence ratios F450/F690 and F690/F735 change when photosynthesis declines and thus can also provide information on short-term stresses which block photosynthesis but do not yet lead to a lower pigment content.

An example of fluorescence imaging of a tobacco leaf treated with DCMU is shown in Fig. 9. In the lower part of the figure the ratio of the broad band blue fluorescence and the 685 nm fluorescence is shown. For comparative reasons only the right half of the leaf was treated with DCMU. Consequently, the right half of the leaf shows a lower value of the F*blue*/F685 ratio than the left half. Additionally, two

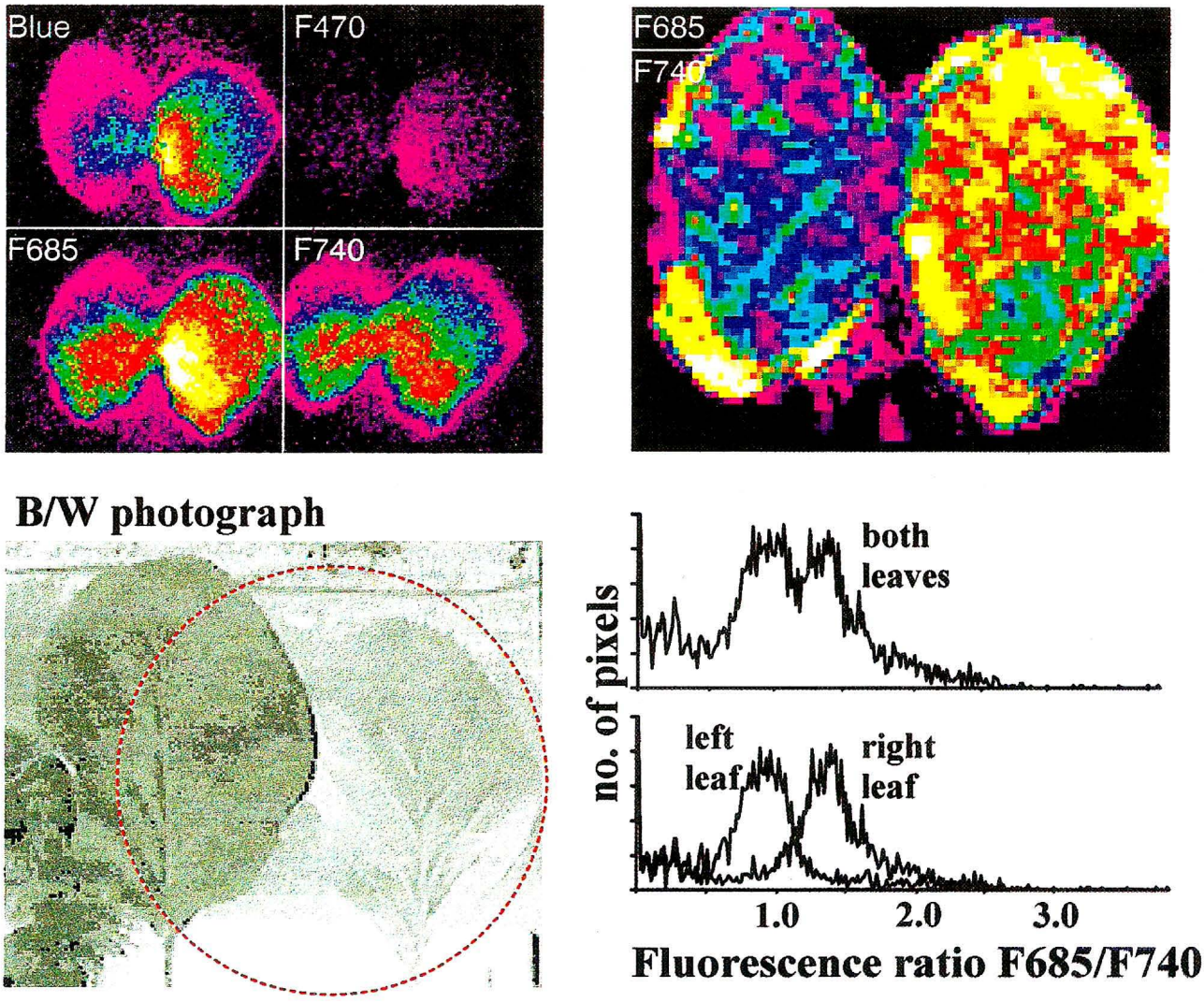


Fig. 2 - Four simultaneously recorded remote fluorescence images (upper left) of two leaves of Bergenia. The optical filters used were BG7 and interference filters at 470 nm, 685 nm and 740 nm, respectively. A false colour image was produced by division of images at 685 nm and 740 nm (upper right). The leaf to the right has its lower side facing the detector and shows a higher ratio F685/F740 as compared with the left leaf, which has its upper side exposed to the detector. A black/white photograph with the imaged area indicated, is also shown (lower left). Two diagrams of the distribution of pixel fluorescence ratio within the processed image are included (lower right).

remotely recorded fluorescence spectra from the two leaf halves are included in the figure. As can be seen, the DCMU treated areas showed a higher chlorophyll fluorescence in relation to the non-treated areas. It should be noted, that the remote spectra were recorded from an area that was about as large as the leaf. Thus, the spectral information may be diluted between the two leaf halves, which might explain the small disagreement with the near-field spectra.

Also in the case of *Iris* leaves we could demonstrate that the chlorophyll fluorescence emission of photosynthetically intact leaves (Rfd-values of 2.4 to 2.7) was higher in leaves with a lower chlorophyll content (Fig. 10 and Table 1). The chlorophyll fluorescence ratio F690/F735 rose with decreasing chlorophyll content (Table 1). The fluorescence emission in the blue region was also higher at a lower chlorophyll level, yet the rise was not as large as that of the chlorophyll fluorescence (Fig. 10). The fluorescence ratio blue/red, F450/F690, decreased from values of 0.75 (green leaf 1) via 0.41 (leaf 2) to 0.21 of the very pale *Iris* leaf. Whereas the fluorescence ratio F690/F735 increased, that of F450/F690 decreased, thus both fluorescence ratios can be used to describe the differences in pigment composition of the *Iris* leaves.

The green *Iris* leaves were also investigated remotely with the imaging system and images at 685 and 740 nm are shown in Fig. 11. The processed image (not shown) was evaluated and the corresponding values of the chlorophyll fluorescence ratio F685/F740 are summarised in the table of Fig. 11. The mean value of the ratio F685/F740 was 0.90, which is in fairly good agreement with that determined with the spectrofluorometer of 1 .07 (Table 1).

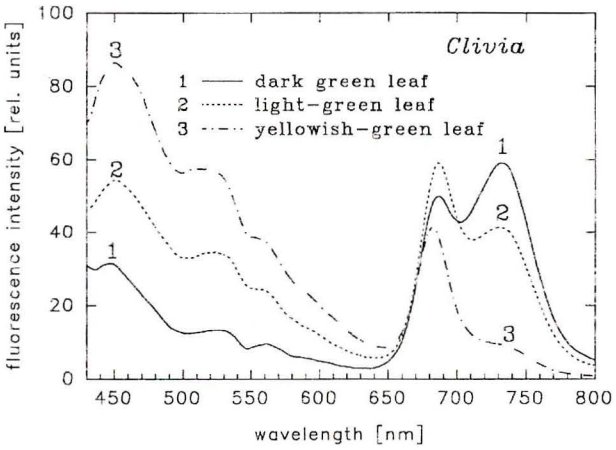


Fig. 3 - Blue-green and red fluorescence emission spectra of Clivia leaves with different chlorophyll content. The blue and green fluorescence emission shows maxima near 450 nm and 530 nm respectively and the red chlorophyll fluorescence maxima near 690 and 735 nm. (For pigment content etc. see Table 1).

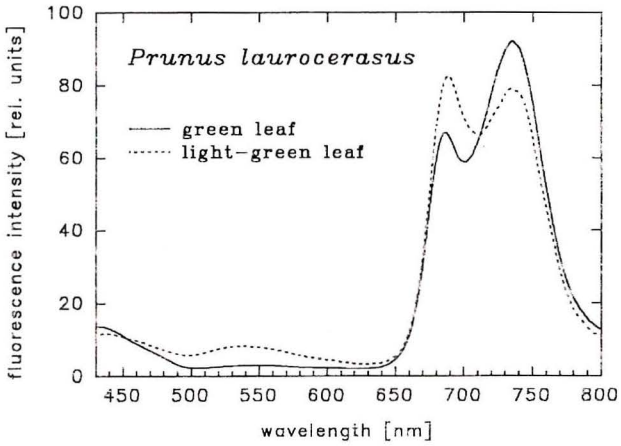
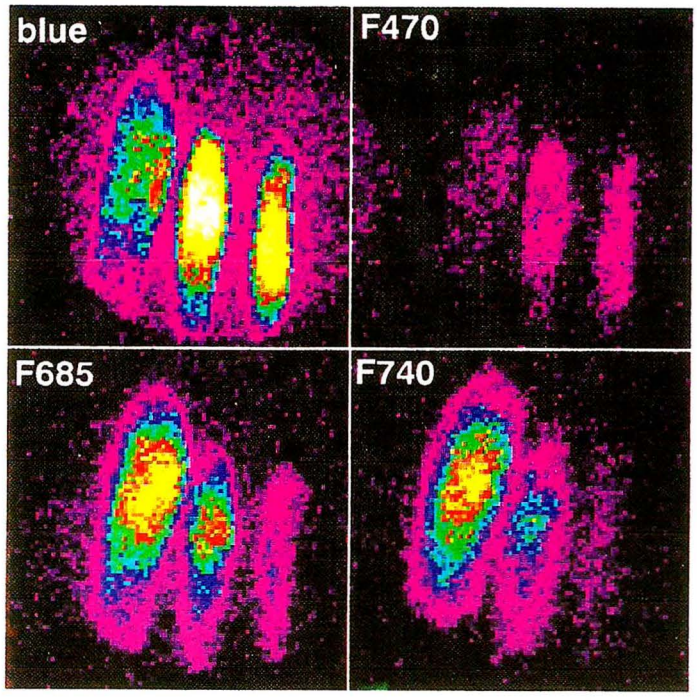


Fig. 5 - Fluorescence emission spectra of a green and a light-green leaf of cherry-laurel (Prunus laurocerasus) with different pigment content (s. Table 1).



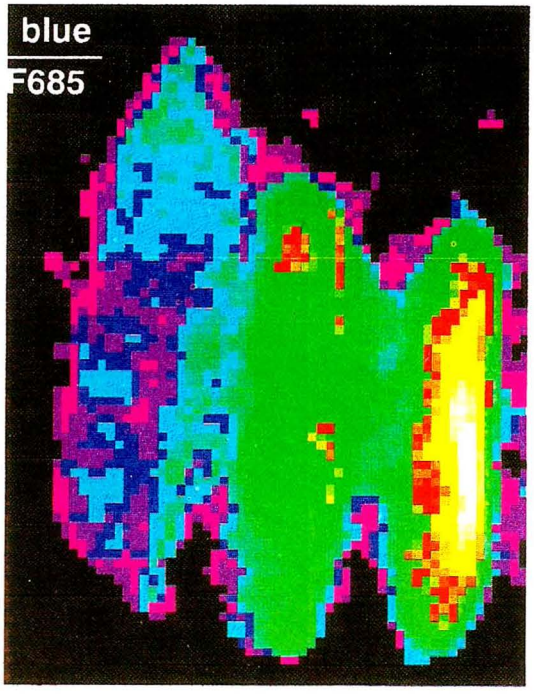
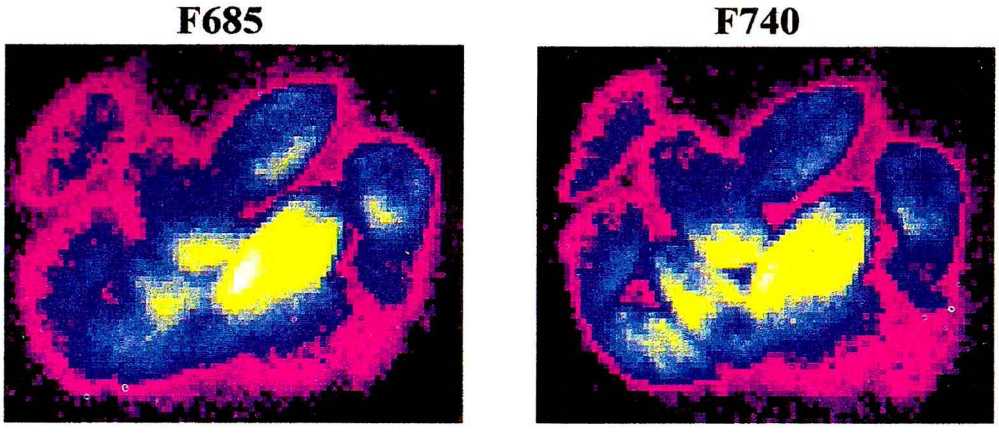
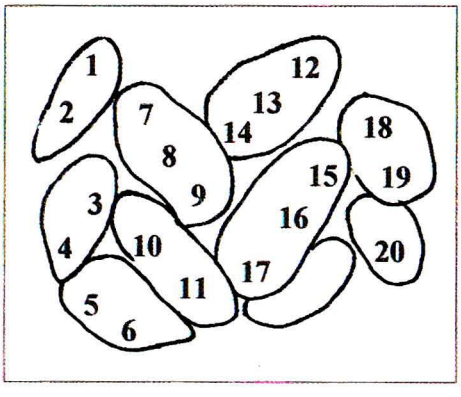


Fig. 4 - Four simultaneously recorded remote fluorescence images (left) of three leaves of Clivia. The optical filters used were BG7 and interference filters at 470 nm, 685 nm and 740 nm, respectively. A false colour image (right) was produced by division of images captured through the BG7 and 685 nm filters. The leaves represent three different stages of senescence with a fully green leaf to the left and the most senescent leaf to the right.

Concerning the values of the fluorescence ratios blue/red and red/far-red we could not detect a dependence on the intensity of the excitation light, though the absolute fluorescence values were different. This particularly applied when the red chlorophyll fluorescence was measured, as was done in this paper, at the steady state of the chlorophyll fluorescence induction kinetics (Kautsky effect). Correspondingly under these measuring conditions the fluorescence ratios of the illuminated leaves (white light or day light) were stable and reproducible.





Data point	F685/F740	Data point	F685/F740
1	0.82	11	0.74
2	0.77	12	0.85
3	0.66	13	0.97
4	0.73	14	0.94
5	0.68	15	0.89
6	0.70	16	0.91
7	0.77	17	0.99
8	0.63	18	0.94
9	0.77	19	0.99
10	0.80	20	0.92
mean: 0.82		std: 0.11	

Fig. 6 - Two simultaneously recorded images of a set of ten leaves of Prunus at 685 nm (upper left) and 740 nm (upper right), respectively. A sketch of the scene is included (lower left). The table (lower right) shows calculations of the ratio F685/F740 based on the two fluorescence images above.

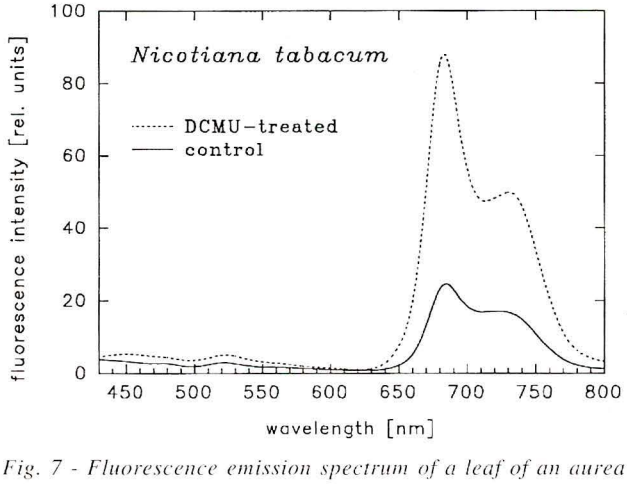


Fig. 7 - Fluorescence emission spectrum of a leaf of an aurea mutant (Su/su) of Nicotiana tabacum before and after addition of the herbicide DCMU (diuron) which blocked the photosynthetic electron transport.

DISCUSSION

A multi-colour imaging system was used with good results for remote fluorescence recording of plants and the data obtained were compared with laboratory recordings of the fluorescence emission spectra and chlorophyll fluorescence induction kinetics. In parallel, all plants were analysed in terms of content of photosynthetic pigments: chlorophylls (a+b) and carotenoids (x+c) in order to correlate differences in the fluorescence emission and in the fluorescence ratios blue/red (F450/F690) and red/far-red (F690/F740). The ratio of green to yellow pigments, (a+b)/(x+c), declines with senescence and increasing stress to plants and is, together with the Rfd-values as vitality index, a very suitable stress indicator (Lichtenthaler and Rinderle, 1988), which can efficiently be applied in ground-truth measurements, which always have to parallel the remote sensing screening. The

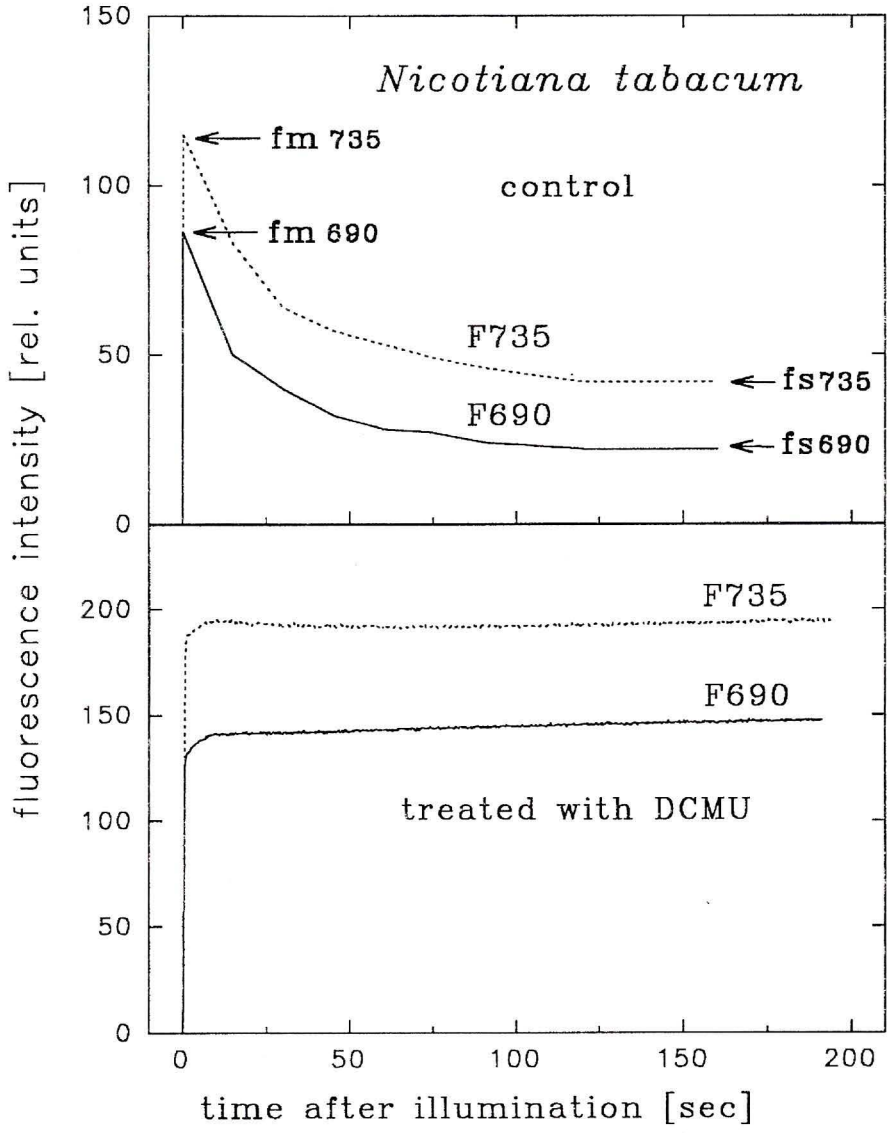


Fig. 8 - Chlorophyll fluorescence induction kinetics (slow component of the Kautsky effect) in 20 min predarkened leaf of aurea tobacco measured in the 690 and 735 nm region before and after addition of the herbicide DCMU. The portions of maximum and steady state fluorescence fm and fs are indicated for both wavelength regions. The variable fluorescence ratio Rfd = (fm - fs)/fs is higher in the 690 than 735 nm region (Rfd690 = 2.9; Rfd735 = 1.7). The DCMU treated leaf did not show any variable chlorophyll fluorescence.

correlation between potential photosynthetic activity of leaves and the variable chlorophyll fluorescence ratio (Rfd690-values), as determined from the chlorophyll fluorescence induction kinetics (Kautsky effect), has been well established before (Lichtenthaler and Rinderle, 1988).

It was also shown in this investigation for *Bergenia* and *Clivia* leaves that the ratio (a+b)/(x+c) declined in senescent yellowish-green leaves from normal values of 5.1 to those of 2.1, indicating a faster breakdown of chlorophylls than carotenoids. In both cases the red/far-red chlorophyll fluorescence increased ratio F690/F735 increased from values below 0.9 to high values of 2.47 (*Bergenia*) and 4.25 (*Clivia*).

The fluorescence ratio blue/red (F450/F690) was different for green leaves of all investigated plants and decreased with decreasing chlorophyll content in all examined plants, no matter how high the original value had been (Table 1). From these results we conclude that this fluorescence ratio offers interesting additional possibilities to characterize the pigment content and physiological state of the leaves and plants. At long-term stress conditions and early senescence the level of leaf chlorophylls declines, which can be sensed in a non-destructive way via the fluorescence ratios red/far-red (F690/F735) and blue/red (F450/F690) as has been shown here by the near-field spectrofluorometer measurements. Even at the same pigment content both fluorescence

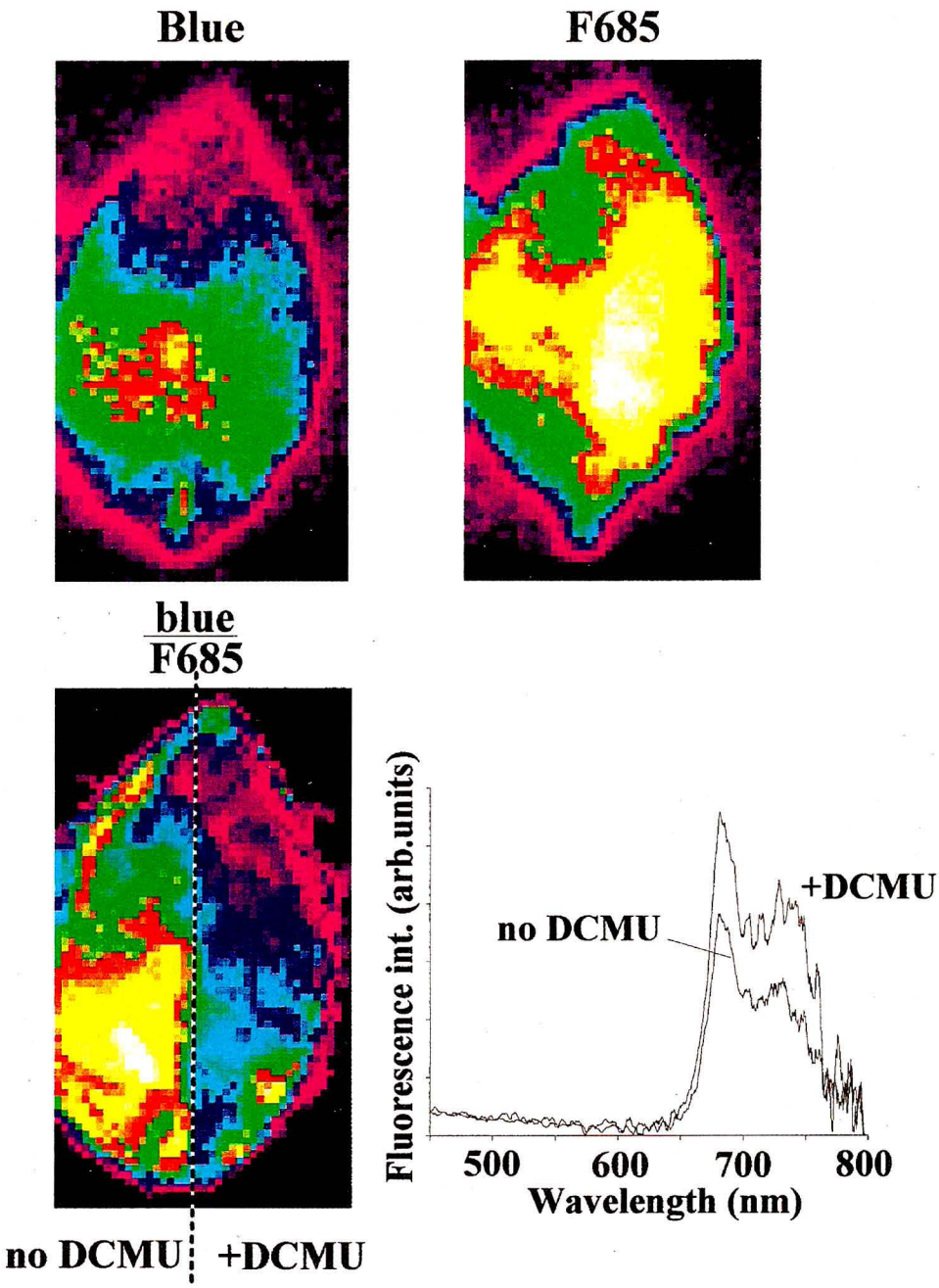


Fig. 9 - Two simultaneously recorded images of a leaf of tobacco (upper leaf side) in the broadband blue region (BG7) (upper left) and at 685 nm (upper right), respectively. To the lower left a processed image is shown obatined from division by the two images above. The right side of the leaf was treated with the photosynthesis herbicide DCMU 20 min. prior to the investigation. Remote fluorescence spectra of the two leaf halves are included.

ratios blue/red and red/far-red also changed as shown here after DCMU-treatment, when the photosynthetic quantum conversion declined, due to the block of the photosynthetic electron transport. This demonstrates that both fluorescence ratios can be applied to check the physiological state of the photosynthetic apparatus e. g. at short-term stress conditions when the photosynthetic activity declines but has not or not yet resulted in a loss of pigments. It is of particular interest that the fluorescence ratio F690/F735 increased and the blue/red fluorescence ratio (F450/F690) decreased in both cases a) with decreasing chlorophyll content and b) with a loss of photosynthetic function as caused here by DCMU-treatment.

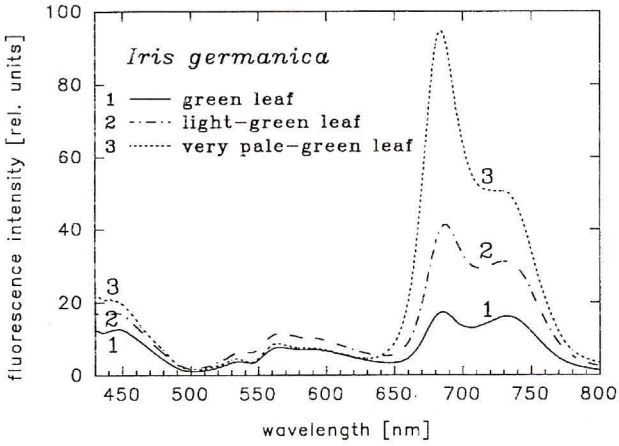
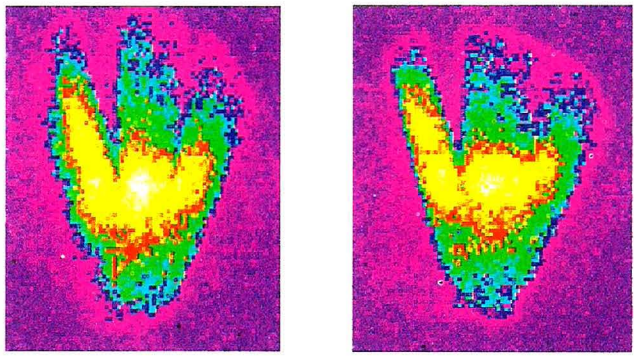
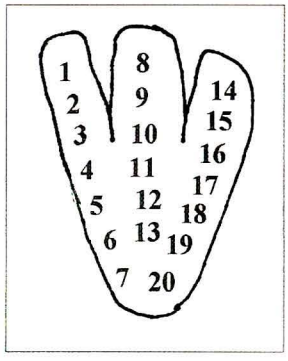


Fig. 10 - Fluorescence emission spectra in photosynthetically functional green leaf parts of Iris germanica. The upper leaf parts of these much elongated leaves were fully green (curve 1), whereas the lower leaf parts towards the leaf base showed a lower chlorophyll content (curve 2 and 3; see also Table 1).





Data point	F685/F740	Data point	F685/F740
1	0.85	11	0.86
2	0.72	12	0.99
3	0.87	13	1.07
4	0.90	14	0.83
5	0.93	15	0.79
6	0.81	16	0.91
7	0.98	17	0.86
8	1.00	18	0.85
9	0.92	19	0.94
10	0.94	20	0.93
mean: 0.90		std: 0.08	

Fig. 11 - Two simultaneously recorded images of three leaves of Iris at 685 nm (upper left) and 740 nm (upper right), respectively. A sketch of the scene is included (lower left). The table (lower right) shows calculations of the ratio F685/F740 based on the two fluorescence images above.

If these fluorescence ratios blue/red and red/far-red could also be remotely sensed, this would open new possibilities to judge the physiological state and pigment content of plants and terrestrial vegetation. Our LIDAR measurements demonstrated that blue fluorescence (F450 or F470) as well as the red (F685) and far-red fluorescence (F740) of plants can remotely be sensed by imaging spectroscopy (Figs. 2, 4, 6, 9 and 11). The advantage of the fluorescence imaging system (imaging mode of the LIDAR system) is that one obtaines very detailed fluorescence information on the fluorescence distribution within the target pixel of a smaller or larger leaf area and even of several leaves which were sensed together. A further advantage is the fact that with the computer-aided system one can calculate and present images of the fluorescence ratios and their variation within a leaf or the differences between different leaves. In contrast, the remote sensing of fluorescence emission spectra (spectral mode of the LIDAR system) only delivers one fluorescence emission spectrum of the target but does not allow to sense the gradient within a leaf or between different leaves of the same target pixel. In other words, imaging mode measurements provide information on the distribution of the fluorescences at particular wavelength regions over the target, whereas spectral mode measurements deliver the complete fluorescence emission spectrum of the target pixel. Though both fluorescence informations are complementary, it is only the imaging system that allows to sense the gradient and differential degree of damage within a plant target. A good correlation was found between near-field chlorophyll fluorescence recordings (spectrofluorometer) and remote fluorescence imaging (Lidar imaging system). This is essential for the possibilities of performing airborne fluorescence mapping of terrestrial vegetation for detection of early damages.

The imaging capability of our LIDAR system is a useful extension of available point monitoring systems. Besides the obvious advantage of displaying the fluorescence mapping in terms of false-coloured images, an imaging system overcomes the problem of mixing fluorescence from the leaves and from the stem of a plant or the grass below the plant. A further advantage of the multi-colour imaging system is the possibility of statistical analysis such as is presented in Fig. 2. Here the full statistical distribution of the fluorescence ratio within the leaf is displayed instead of just the mean value and a standard deviation (for which one has to assume a certain distribution). Thus this imaging technique is of value also for ground-based studies of individual leaves or plants. An important aspect of the imaging system is the split-mirror telescope, which enables a simultaneous recording of the fluorescence images at different wavelengths. This is advantageous compared to sequential

acquisition at the different wavelengths, where movements of the measuring platform or the target and pulse-to pulse fluctuations of the laser are a problem. The simultaneous recording in principle seems to allow single-shot image detection of the physiological status of plants.

CONCLUSIONS

The good agreements in the results of remote fluorescence imaging and the direct spectrofluorometer measurements indicate that future airborne multi-colour fluorescence imaging may become a powerful tool in the remote sensing of terrestrial vegetation. With images of the chlorophyll fluorescence ratio F685/F740, which rises under stress, one obtaines a quick overview on the chlorophyll distribution in a given area and its decline due to short-term or long-term stress events. With the blue/red fluorescence ratio, which decreases with increasing stress and chlorophyll degradation, there is a second ratio, F450/F690, which changes in the opposite direction. Also regeneration of pigment content and physiological function can be monitored by fluorescence imaging on the basis of both fluorescence ratios. Furthermore, a short-term loss of photosynthetic function, which is not associated with a loss in chlorophyll, as shown here in the experiment with the photosynthetic herbicide diuron, can also be detected with the multi-colour fluorescence imaging system. For early stress detection in green vegetation, ground-truth measurements of fluorescence kinetics will still be necessary to complement fluorescence imaging information and to determine the type of stress to which the plants were exposed.

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