Journal of Applied Botany and Food Quality 81, 77 - 85 (2007)

University of Bonn, Institute of Crop Science and Resource Conservation – Horticultural Science

UV-B induced damage and recovery processes in apple leaves as assessed by LIF and PAM fluorescence techniques

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(Received March 30, 2007)

Summary

The capability of laser-induced chlorophyll fluorescence (LIF) and pulse-amplitude-modulated (PAM) fluorescence technique as well as RED/NIR-light reflection measurements for detection and quantification of UV-B induced damages was evaluated in greenhouse experiments with apple seedlings (*Malus domestica* Borkh.). Photosynthetic recovery from short-term UV-B stress was assessed during 7 days after UV-B treatment with the PAM fluorometer. The exposure of apple leaves to UV-B doses in the range of 10-26 W m^{-2} for 180 minutes ($UV\text{-}B_{BE}$ dose = *5.4-14 kJ* m^{-2}) affected neither chlorophyll content nor leaf reflection. Although UV-B damage was not visually evident 2 hours after irradiation, it could be detected by PAM and LIF fluorescence techniques with equivalent success. The intensity of LIF, estimated as the integral of fluorescence spectrum, was reduced after UV-B irradiation by 19-30%. A stronger decrease in F686 compared to F740 fluorescence resulted in significantly lower F686/F740 values in all UV-B treatments.

Apple leaves displayed a strong and significant reduction in maximum fluorescence (Fm) and a slightly increase in ground fluorescence (Fo) 2 hours after UV-B treatment, as documented by PAM fluorescence measurement.

Negative linear regressions between investigated UV-B doses and selected PAM parameters were found with determination coefficients (R^2) of 0.50 for Fv, 0.48 for Fv/Fm, and 0.58 for Fv/Fo. Among the PAM and LIF parameters tested, the Fv/Fo ratio appeared most sensitive for detection of UV-B induced damages displaying greatest changes and strongest correlation with the applied UV-B doses. PAM fluorescence images of apple leaves visualised an enhanced spatial heterogeneity of photosynthetic activity with increasing UV-B dose.

The disturbance in photosynthetic functionality was followed by a continuous recovery process as indicated by restoring Fo and Fm parameters. A decline in maximum photochemical efficiency Fv/Fm from 0.80 to 0.72 and 0.43 after exposure to 20 W m^{-2} for 240 and 360 minutes ($UV\text{-}B_{BE}$ = *14.4 and 21.6 kJ* m^{-2}), respectively, was followed by recovery at 7 x 10^{-4} and 5 x 10^{-3} units per hour during the first 48 hours after UV-B treatment. The recovery curves of Fm, Fv, Fv/Fm and Fv/Fo parameters during a week after UV-B irradiation were well fitted with exponential rise to maximum function, such as: $y = y_o + a\,(1 - e^{-bx})$. However, within 7 days after exposure to UV-B light, apple leaves displayed 14% or 4% lower Fm, and 5% or 1% lower Fv/Fm values compared with control plants, indicating only a partial recovery from photoinhibition and irreversible damages in PSII.

Introduction

The stratospheric ozone depletion of at average 1.8% per decade in Europe (Stolarski et al., 1992) and consequent increases in UV-B radiation (Herman et al., 1996) in the last years stimulate investigations on assessing UV-B radiation effects on plants (Kakani et al., 2003). Enhanced UV-B radiation usually has negative impacts on photosynthetic activity (Zhao et al., 2004), growth (Gao et al., 2004) and reproductive development (Musil, 1994; Koti et al., 2004) of plants, resulting in significant reductions in crop quality and yield (Runeckles and Krupa, 1994).

At the cellular level, the major sites of UV-B impairment are chloroplasts, and PS II seems to be the most vulnerable component of thylakoid membranes (Bornman, 1989; Vass, 1997). Within electron transport chain, UV-B induced damages in Mn clusters of water-splitting enzyme complex (Renger et al., 1989; Post et al., 1996; Vass et al., 1996), Tyr-Z and Tyr-D electron donors (Vass et al., 1996), primary (Q_A) and secondary (Q_B), quinone acceptors of PSII, Q_AFe^{2+} complex and in plastoquinone (P_Q) pool have been established (Melis et al., 1992; Jansen et al., 1996; Vass et al., 1996). Besides electron transport components, also D1 and D2 protein subunits of PS II reaction centres may degrade due to UV-B radiation (Trebst and Depka, 1990; Melis et al., 1992; Friso et al., 1993; Friso et al., 1994). A protective mechanism of plants to enhanced UV-B irradiation is a rapid biosynthesis of UV-screening phenolic compounds such as flavonoids, (Fedina et al., 2006) and hydroxycinnamic acid derivates in epidermal cells; also a rapid turnover and a replacement of damaged chloroplast proteins during UV-B stress have been reported (Burchard et al., 2000; Gao et al., 2004).

Since high UV-B flux affects components of photosynthetic apparatus, chlorophyll fluorescence may be a well suited method for monitoring UV-B stress. This technique delivers fast and extensive information about the potential and current efficiency of photosynthesis, the integrity of the photosynthetic apparatus, the relative functionality of different physiological protective mechanisms and the rate of photosynthetic electron transfer (for reviews see e.g. Maxwell and Johnson, 2000; Baker and Rosenqvist, 2004)

With PAM method, fluorescence is measured at different stages of electron transport chain by applying different light sources. Recently developed PAM imaging systems allow detecting whole leaf reactions to plant stress in spatial and temporal resolution (Chaerle and Van der Straeten, 2000). In most studies with PAM fluorescence techniques on different plant species, an increased ground fluorescence Fo and a decreased maximum fluorescence Fm was observed in response to UV-B stress (Sharma et al., 1998; Vass et al., 1999; Heraud and Beardall, 2000; Gilbert et al., 2004).

Less and very controversial information is available on detection of UV-B stress by means of LIF. Recently, this method was developed as a technique of remote sensing for determination of nitrogen requirement of plants and site-specific fertilisation under field conditions (Cecchi et al., 1994; Sticksel et al., 2001). The principle behind this approach is a negative correlation established between leaf chlorophyll concentration and red/near-infra-red chlorophyll fluorescence ratio F690/F735 (Rinderle and Lichtenthaler, 1988).

Precise information on the effect of enhanced UV-B irradiation on the LIF and PAM parameters is necessary for the interpretation of remote sensing data as basis for management decisions in precision agriculture. Further studies on UV-B stress are still needed to quantify the effects of UV-B radiation on crops in order to establish dose-response relationships (Kakani et al., 2003). In order to facilitate the development of dynamic simulation models for use in UV-B and other environmental impact assessments, information on course and rate of photosynthetic recovery from UV-B stress is also required.

Due to lack of such studies, the objective of this work was to evaluate the potential of LIF and PAM fluorescence techniques for early detection and quantification of UV-B induced damages in apple leaves. Of particular interest in this work was to assess dose-response relationships and time course of photosynthetic recovery from short term UV-B stress. We hypothesize that a) the impact of low UV-B radiation on apple leaves can be detected by LIF and PAM techniques with comparable success, even if no macroscopic damage is apparent; and b) that UV-B affected plants are able to recover from UV-B stress, dependent on the dose of UV-B radiation and the degree of damage.

Materials and methods

Plant material

Apple (*Malus domestica* Borkh.) seeds were subjected to stratification for 21 days at 4 °C. After germination, seedlings were planted in pots (V=150 cm^3) with a substrate containing 50% commercial potting mixture and 50% sand and then transferred in a growth chamber. The apple seedlings were grown with a photoperiod of 14/10 h (day/night) at a temperature of 20/18±2 °C, a relative humidity of 60/70±15% and a light intensity of 100 µmol m^{-2} s^{-1}. The plants were fertilised with a Hoagland nutrient solution twice a week and supplied with water from the bottom, without wetting the leaves. For the experiments, uniformly developed apple seedlings at the stage of 5-6 fully expanded leaves were selected.

UV-B irradiation

UV-B stress was induced in an irradiation chamber with ten narrow-band (λ=311 nm) fluorescent lamps (Philips, TL 100 W/01) and ambient PAR intensity of about 100 µmol m^{-2} s^{-1}. The intensity of UV-B radiation was measured with a RM-21 spectroradiometer (Gröbel UV-Electronics, Ettlingen, Germany). In the experiment for assessment of dose-response relationships, plants were irradiated with UV-B fluxes of 10, 13, 18 or 26 W m^{-2} for 180 minutes. These resulted in a total biological effective UV-B (UV-B_{BE}) dose of 5.4, 7.0, 9.7, and 14.0 kJ m^{-2}, respectively, as weighted by Caldwell's generalised plant action spectrum (CALDWELL, 1971).
Time course of photosynthetic recovery of the apple leaves was evaluated after exposure of seedlings to 20 W m^{-2} of UV-B light for 240 min and 360 min or 14.4 and 21.6 kJ m^{-2} biological effective UV-B doses, respectively. After UV-B treatments, plants were placed in a growth chamber with 100 µmol m^{-2} s^{-1} PAR.

Chlorophyll a fluorescence

All fluorescence measurements were done on the adaxial side of the largest fully developed leaves after dark adaptation of plants for 20 minutes. Dose-response relationships were established with LIF and PAM-Imaging fluorometer (Heinz Walz GmbH, Effeltrich, Germany). Time course and rate of photosynthetic recovery from UV-B stress was evaluated by means of PAM-2000 and PAM-Imaging fluorometer (Heinz Walz GmbH, Effeltrich, Germany). The first fluorescence measurements were conducted on plants 2 hours after UV-B irradiation.

LIF

A blue light emitting diode with a maximum wavelength of 408 nm was applied for the excitation of fluorescence. The fluorescence spectra were recorded with a spectroradiometer Fieldspec™ VNIR (Analytical Spectral Devices ASD, Inc, Boulder, USA) using an integration time of 1 s. The fiber-optic detector of this spectrometer had a conical view subtending a full angle of about 25 degrees. During the fluorescence measurements, the distance between detector and object level was set to 1 cm, providing a viewing surface area of about 0.2 cm^2. From the recorded spectra, the intensities of chlorophyll fluorescence at 686 nm (F686) and 740 nm (F740), the F686/F740 ratio and the integral of fluorescence spectrum between 650 nm and 900 nm were estimated.

PAM-Imaging

An Imaging-PAM Chlorophyll Fluorometer (Walz, Effeltrich, Germany) was used to investigate the patterns of chlorophyll fluorescence and RED/NIR-light reflection of UV-B irradiated apple leaves in temporal as well as in spatial resolution. Leaf images of ground fluorescence (Fo) were recorded by the mounted CCD camera (640 x 480 pixel) after illumination of the sample with blue light (470 nm) of 0.5 µmol m^{-2} s^{-1} PAR. The CCD camera was protected from stray excitation light by long-pass filter (Schott, RG 645; Mainz, Germany) and from long-wavelength ambient light by a short-pass filter (Balzer, Calflex-X, λ<780 nm; Bingen, Gemany). Images of maximum fluorescence (Fm) were taken after a white light saturation pulse of 2400 µmol m^{-2} s^{-1} PAR for 0.6 seconds at a stage when all reaction centres of PSII are closed, i.e. primary quencher Qa are completely reduced. The variable fluorescence (Fv) was estimated as Fm-Fo, the ratio of the variable fluorescence to Fo as Fv/Fo and the maximum photochemical efficiency (Fv/Fm) as (Fm-Fo)/Fm. In addition to fluorescence images, the remissions (reflected and scattered) of red (650 nm, RED) and near-infra-red (780 nm, NIR) light were estimated from which the PAR-Absorption (ABS) as 1-RED/NIR and the NDVI as (NIR-RED)/(NIR+RED) of investigated leaves were calculated.

PAM-2000

In measurements with the portable chlorophyll fluorometer PAM-2000, leaves were illuminated with red (with a peak of 650 nm) modulated light of 0.1 µmol m^{-2} s^{-1} PAR for the estimation of ground fluorescence (Fo). This was followed by a white saturation pulse of 1800 µmol m^{-2} s^{-1} PAR for 0.6 seconds in order to assess maximum fluorescence (Fm).

Chlorophyll content of leaves

For the assessment of chlorophyll content, leaf sections of 2.0 cm^2 in size were taken and stored in plastic bottles at -20 °C. The concentration of chlorophyll was determined according to BLANKE (1992) after extraction with DMSO. The absorbance of extracts was evaluated at 665 nm (A_{665}) and 647 nm (A_{647}) with a UV-VIS spectrophotometer (Perkin-Elmer, Lambda 5). The concentrations of chlorophyll a (C_a) and b (C_b) were calculated with the following equations:
$C_a = (12.7 * A_{665} - 2.79 * A_{647})$ and $C_b = (20.7 * A_{647} - 4.64 * A_{665})$.

Statistics

The experimental data were subjected to ANOVA with the SPSS statistical package (Superior Performance Software System, Version 11.0 for Windows). The 5 % probability level was accepted to indicate significant differences. Means were compared by Tukey LSD multiple range test after data were evaluated for normal distribution and variance homogeneity. Fluorescence, reflectance and chlorophyll content data presented were calculated from replicate measurements.

Results

Effect of different UV-B doses on RED/NIR-light reflection, LIF and PAM characteristics of apple leaves

After irradiation with UV-B fluxes of 10, 13, 18 or 26 W m^{-2} for 180 minutes, apple leaves did not display any visible symptoms of damage. Neither leaf chlorophyll content nor chlorophyll a/b ratio or red and near-infra-red reflection were affected by these doses (data not shown). In all treatments, apple leaves exhibited a chlorophyll content of 25 ± 3 μg cm^{-2} and ABS and NDVI values of 0.91 ± 0.01 and 0.84 ± 0.02, respectively.

As expected, both LIF and PAM fluorescence methods appeared to be well suited for an early detection of UV-B stress in apple seedlings. Exposure of apple seedlings to UV-B radiation in the range of 10-26 W m^{-2} for 180 minutes reduced the intensity of chlorophyll fluorescence, estimated by the integral of fluorescence spectrum, by 17-30% (Fig. 1). Thereby, the decline of 25-41% to 4817-3764 units in the intensity of red fluorescence (F686) was more pronounced than that of 19-34% to 6287-5160 units in near-infra-red (F740). This resulted in a significant (6-12%) reduction of F686/F740 ratio to 0.76-0.72 units in all UV-B treatments except for the level of 18 W m^{-2} (Fig. 1). A weak linear dose-effect relationship was found for the applied UV-B doses and the evaluated LIF parameters with determination coefficients (R^2) of 0.12 for the F686/F740 ratio, 0.30 for the integral of the spectrum, 0.34 for F686 and 0.38 for F740. Thus, among LIF parameters, F686 and F741 showed the strongest correlation with the applied UV-B doses. These parameters also enabled discrimination between control plants, those treated with 10-18 W m^{-2} and 26 W m^{-2} of UV-B light. However, no significant difference among LIF parameters was found between the plants irradiated with 10, 13 or 18 W m^{-2}.

Measurements with PAM-Imaging showed slight increases of Fo and significant decreases of Fm, Fv, Fv/Fm and Fv/Fo in all UV-B treatments (Fig. 2). Two hours after exposure of plants to UV-B irradiation, ground fluorescence displayed 6-10% higher values than untreated plants. Other PAM fluorescence parameters were affected more strongly and showed significant reductions of 8-35% for Fm, 12-47% for Fv, 4-20% for Fv/Fm and 18-52% for Fv/Fo as compared to control (Fm=0.651; Fv=0.517; Fv/Fm=0.793; Fv/Fo=3.873 units). Thus, the most noticeable changes were observed for Fv/Fo ratio. A linear dose-effect relationship between PAM parameters and UV-B doses was found with higher determination coefficients compared to LIF parameters of 0.44 for Fm, 0.50 for Fv, 0.48 for Fv/Fm and 0.58 for Fv/Fo. Similarly to the results obtained with LIF, significant differences in Fm related parameters were observed between control plants, those treated with 10-18 W m^{-2} or 26 W m^{-2} of UV-B light, respectively. UV-B irradiation resulted in higher heterogeneity of PAM fluorescence parameters on the surface of apple leaves, evaluated on the basis of fluorescence imaging. Images of photochemical efficiency displayed more intense reduction along the leaf veins than in intercostal regions (Fig. 3), reflecting more pronounced changes in Fm as compared to Fo values in these areas.

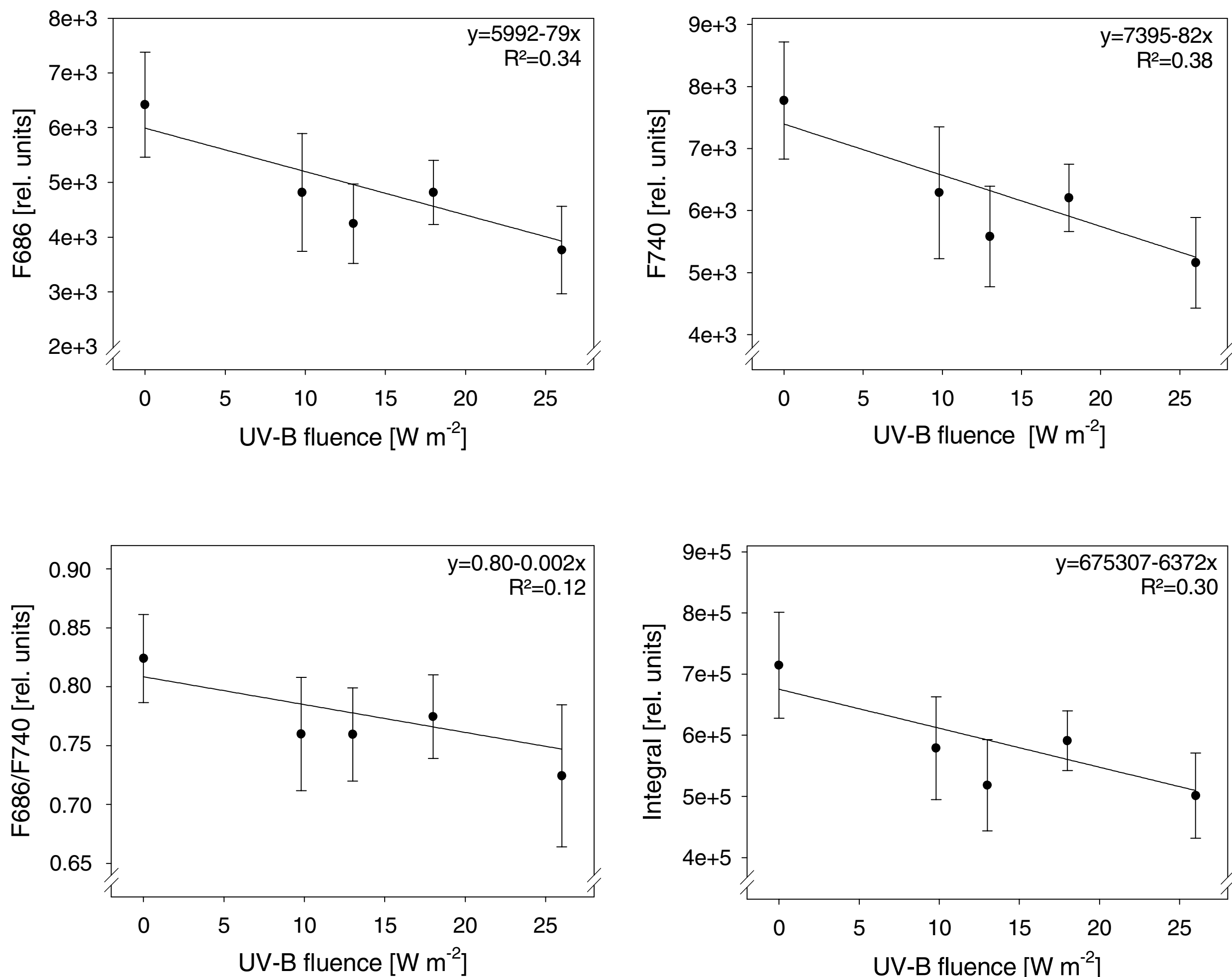

Fig. 1: Effect of different UV-B doses on LIF parameters of apple leaves. Leaves were irradiated for 180 minutes with UV-B fluencies of 10, 13, 18 or 26 W m^{-2}. Fluorescence measurements were done 2 hours after UV-B treatment. Vertical bars indicate ± standard deviation (n=15).

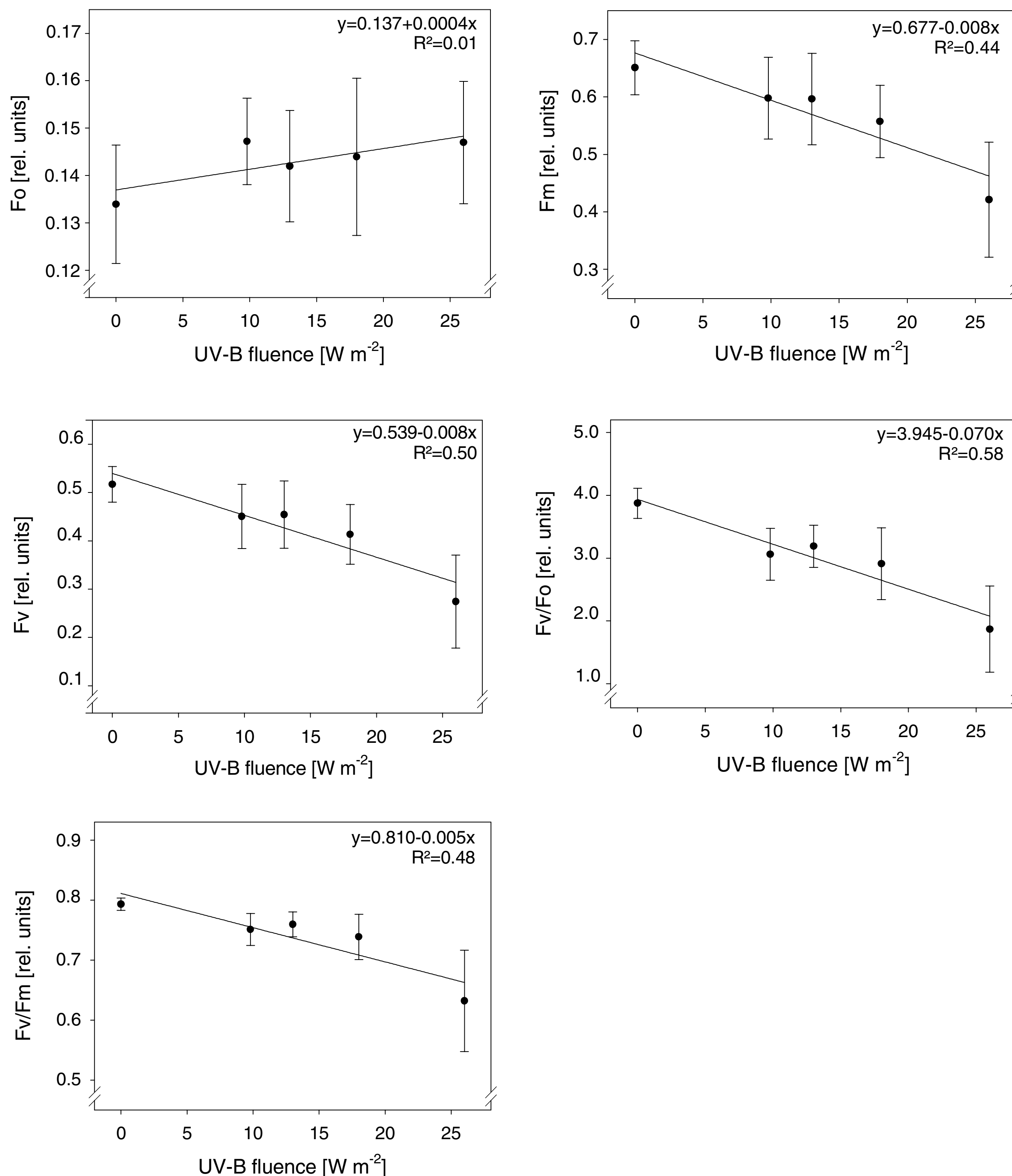

Fig. 2: Effect of different UV-B doses on PAM fluorescence parameters of apple leaves. Leaves were irradiated for 180 minutes with UV-B fluencies of 10, 13, 18 or 26 Wm^{-2}. Fluorescence readings were taken 2 hours after UV-B treatment. Vertical bars indicate ± standard deviation (n=15).

Recovery of apple leaves after UV-B stress

The temporal response of photosynthetic recovery of apple leaves was studied after irradiation with 20 W m^{-2} of UV-B light for 240 or 360 minutes (Fig. 4). Two hours after these treatments, apple leaves exhibited 14% or 24% higher Fo and 19% or 50% lower Fm values than control plants (Fo=0.276 or 0.306; Fm=1.409 or 1.460 units). These changes resulted in a decrease of Fv, Fv/Fo, and Fv/Fm, respectively, by 27%, 36% and 10% after 240 and by 69% 74% and 45% after 360 minutes of UV-B irradiation.

The disturbance in photosynthetic functionality was followed by a continuous recovery process as indicated by restoring PAM fluorescence parameters (Fig. 4). The increase of Fm related parameters was most pronounced during the first two days after exposure to UV-B light for 240 and 360 minutes with a rate of 3.2×10^{-3} and 1.1×10^{-2} units per hour for Fm, 3.2×10^{-3} and 1.0×10^{-2} for Fv, 9.8×10^{-3} and 2.4×10^{-2} for Fv/Fo, 6.7×10^{-4} and 4.8×10^{-3} for Fv/Fm, respectively. In contrast to this, ground fluorescence (Fo) did not display a rectilinear

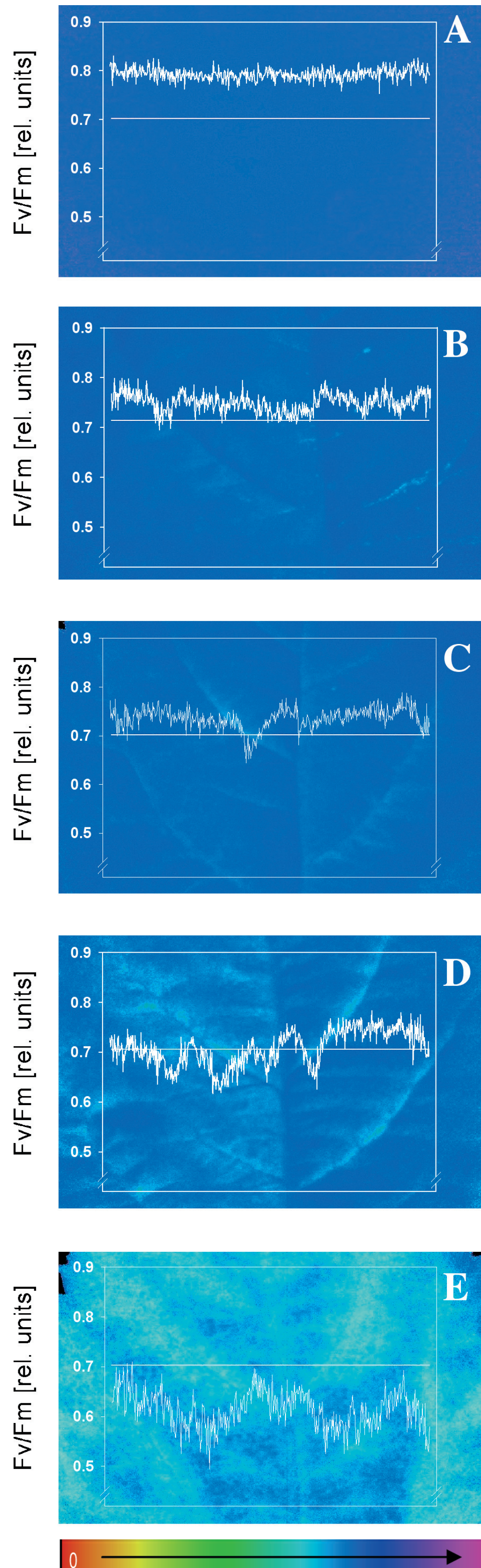

Fig. 3: Fv/Fm images of apple leaves irradiated for 180 minutes with UV-B fluencies of 0 [A], 10 [B], 13 [C], 18 [D] or 26 W m^{-2} [E]. The Fv/Fm values are displayed for each pixel in the leaf profile [white horizontal line]. Fluorescence measurements were made 2 hours after UV-B treatment.

recovery response during the first two days after UV-B treatment. The recovery curves of Fm, Fv, Fv/Fo and Fv/Fm during a week after UV-B exposure showed a good fitting with exponential rise to maximum function, such as: $y = y_o + a\,(1 - e^{-bx})$. Within 7 days after UV-B stress, apple leaves irradiated for 240 or 360 minutes still displayed lower values of Fm (by 4% or 14%), of Fv (by 5% or 18%) of Fv/Fo (by 4% or 18%) and of Fv/Fm (by 5% or 1%) compared to control (Fig. 4), indicating partial recovery from UV-B stress. The spatial heterogeneity of all PAM fluorescence parameters, as affected by UV-B stress, also decreased in the course of recovery processes (Fig. 5). However, 7 days after exposure to UV-B irradiation, apple leaves still displayed higher variability of leaf fluorescence parameters in comparison to control plants.

Discussion

The exposure of apple seedlings to UV-B radiation of 10-26 W m^{-2} for 180 minutes affected neither leaf chlorophyll content nor chlorophyll a/b ratio or ABS and NDVI. Damages induced by UV-B irradiation were not visually evident 2 hours after irradiation; however, they could be successfully detected both by PAM and LIF fluorescence techniques. With both fluorescence techniques, significant differences were found between control plants, those treated with 10-18 W m^{-2} or 26 W m^{-2} of UV-B light, respectively. However, neither PAM nor LIF allowed discrimination between plants irradiated with 10 W m^{-2}, 13 W m^{-2} or 18 W m^{-2} of UV-B light (Figs. 1, 2).

In our study apple leaves showed a decreased LIF after UV-B treatment. Similar results were obtained for peanut leaves in experiments conducted by MINEUCHI et al. (2001), in which intensities of red and near-infra-red fluorescence, induced by Ar laser (351-364 nm), decreased when increasing UV-B dose from 50 kJ m^{-2} to 150 kJ m^{-2}. Since a strong reduction in the intensity of emitted fluorescence is common for photoinhibition (LAWLOR, 2001), such a response of leaves may indicate a photoinhibitory effect of UV-B light or damages in the photosynthetic membranes similar to those induced by photoinhibition. However, a decrease in LIF intensity is not always observed in response to UV-B stress. In the study of MIDDLETON et al. (1996), UV-B treatment at 340 nm reduced intensity of leaf chlorophyll fluorescence in an UV-B sensitive cucumber cultivar, but did not affect chlorophyll fluorescence intensity in leaves of an insensitive cultivar. KRIZEK et al. (2001) reported significantly decreased chlorophyll fluorescence in cucumber leaves if LIF was excited by the wavelength of 380 nm, no effect by excitation with 280 nm and increased intensity with broad-band (300-400 nm) excitation source centred at 360 nm. SUBHASH et al. (1995) found an increase in the intensity of chlorophyll fluorescence of *Salvia splendens* L. leaves by fluorescence excitement with 337 nm and no significant changes by excitement with 458 nm. Such discrepancy in the results may be explained by distinct differences in absorption and penetration depth of excitation light of different wavelengths in leaf profiles. Another possible reason might be a different magnitude of damage caused by UV light (from photoinhibition to chlorophyll breakdown) due to previous growing conditions and/or leaf age. In addition, a long-term exposure to UV-B irradiation as well as a prolonged period between UV-B treatment and fluorescence measurements may also facilitate recovery processes in leaves, accompanied by reconstitution of chlorophyll fluorescence. In our study, apple seedlings were grown at low light intensity, and this could enhance photoinhibition effects and fluorescence quenching due to UV-B irradiation. Different extent in decrease of red and near-infra-red fluorescence resulted in a significant reduction of F686/F740 ratio in the UV-B treatments. The decrease in red/near-infra-red ratio has been already described for the shade-adapted leaves exposed to PAR with intensity higher than 1000 µmol m^{-2} s^{-1} (AGATI et al., 1995). In the experiments of SUBHASH et al. (1995), prolonged UV-B irradiation in combination with PAR, only slightly affected F685/F730

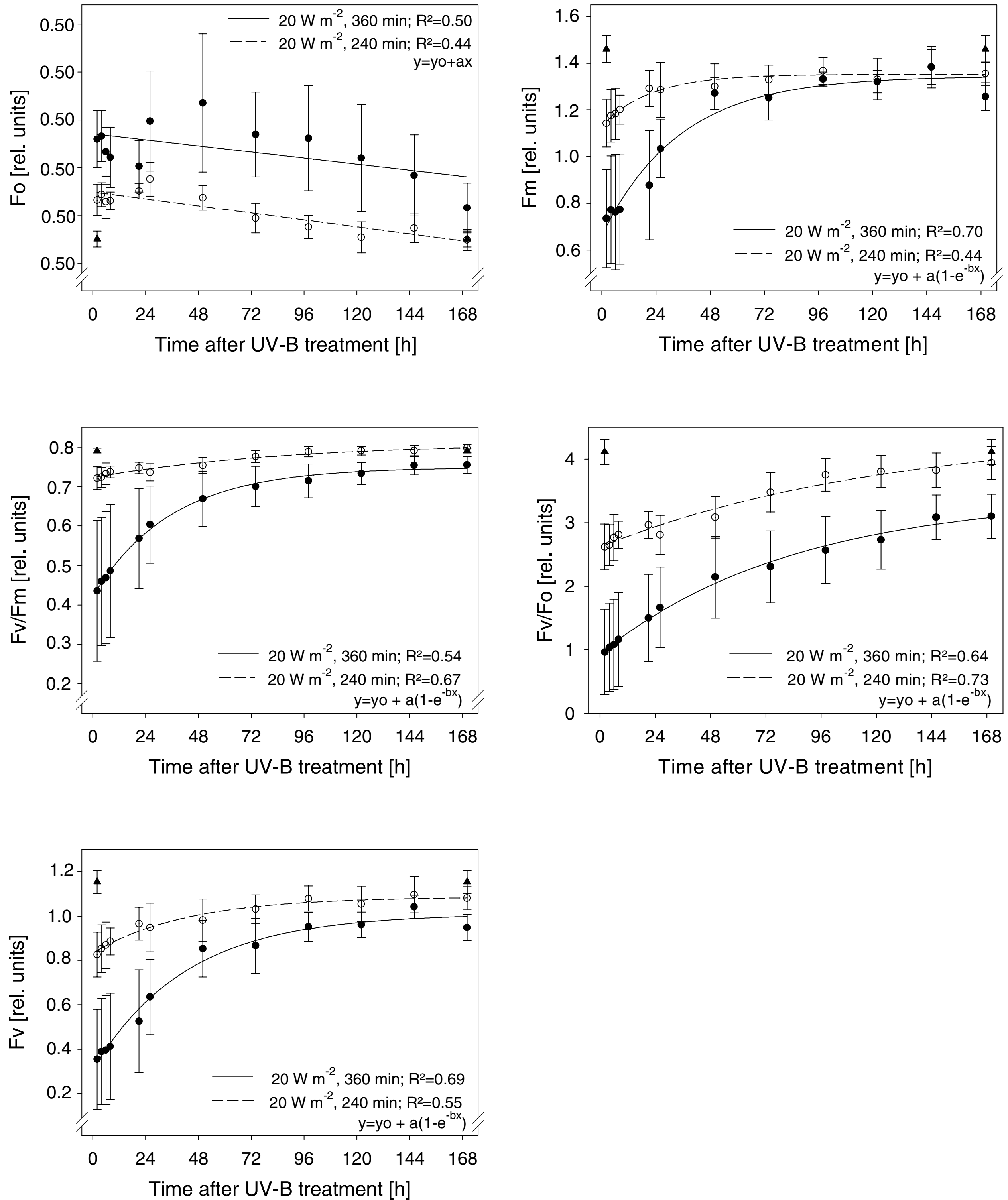

Fig. 4: Parameters of pulse amplitude modulated chlorophyll fluorescence of apple leaves before (triangle symbol) and after (circle) exposure to UV-B light. Leaves were irradiated for 240 (open symbols) or 360 minutes (closed symbols) with UV-B fluence of 20 Wm^{-2}. Vertical bars indicate ± standard deviation (n=8).

ratio in mature and young leaves of *Salvia splendens* L. In the study on cucumber plants of MIDDLETON et al. (1996), the reduction of leaf chlorophyll content in response to UV-B stress was accompanied by an increase of red/near-infra-red ratio. No UV-B effects were detected on this ratio in cucumber leaves with slightly decreased chlorophyll a content in the experiments of KRIZEK et al. (2001) by LIF excitation with 380 nm.

The negative correlation between leaf chlorophyll content and F686/740 ratio can be used for determination of the nitrogen status of plants (TARTACHNYK and RADEMACHER, 2003) and site-specific fertilisation under field conditions (CECCHI et al., 1994; STICKSEL et al., 2001). However, results of our and previous investigations (AGATI et al., 1995) indicate that this ratio may be significantly affected by UV-B or PAR radiation of high intensity also when no changes in leaf

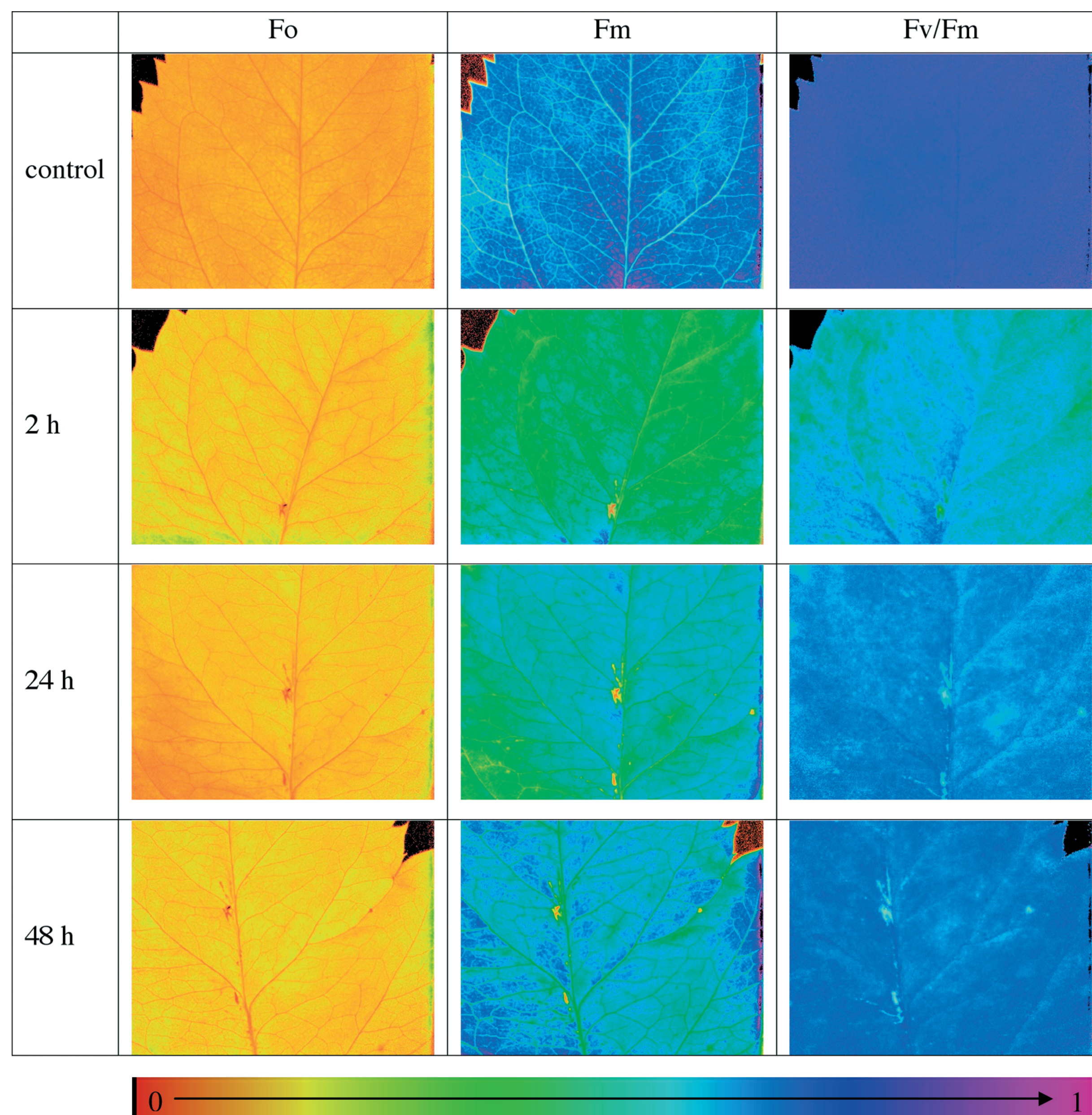

Fig. 5: PAM fluorescence images of apple leaves 2, 24 and 48 h after UV-B irradiation with 20 W m^{-2} for 240 minutes compared to control.

chlorophyll content occur. This has to be taken in account for management decisions in remote sensing technologies in order to avoid misinterpretation of plant nutritional status when referring to F686/F740 ratio.

In respect to PAM fluorescence, apple leaves displayed a strong and significant reduction in maximum fluorescence and an increase in ground fluorescence after UV-B treatment. A similar tendency was reported by LARKUM and WOOD (1993) for sea grasses and phytoplankton and GILBERT et al. (2004) for barley and tomato leaves. In these studies, among PAM parameters variable fluorescence Fv displayed strongest changes in comparison to control due to concomitant increase in Fo and decrease in Fm values and was therefore postulated as the most sensitive parameter for monitoring UV-B stress. Derived from the fluorescence signals of PS II antenna complexes Fo and reaction centres Fm, variable fluorescence Fv reflects the balance of energy between these units and is related to the efficiency with which this energy is used by the photochemical processes (LAWLOR, 2001). In present work, admittedly, normalised Fv/Fo ratio showed most noticeable changes in response to UV-B stress and the strongest correlation with applied doses (Fig. 2) demonstrating better suitability than Fv for an early detection of this stress and better evaluation of dose-effect responses of plants. Similar to the results of GILBERT et al. (2004), who did not find any differences in Fv values of barley leaves irradiated with lower UV-B intensities, e.g. 1.9 and 3.8 W m^{-2}, a discrimination of UV-B doses in the range of 10-18 W m^{-2} was not possible when referring to Fv/Fo ratio in our experiment. However, fluorescence images of apple leaves visualised an enhanced heterogeneity of estimated parameters with increasing UV-B doses. Images of photochemical efficiency displayed more intense reduction alongside the leaf veins (Fig. 3) indicating higher susceptibility of these leaf areas to oxidative UV-B stress. The higher variability of chlorophyll fluorescence within the leaf after UV-B exposure was also reported by KRIZEK et al. (2001) suggesting heterogeneous states in the photosynthetic apparatus due to localized differences in chloro-

phyll concentration and photosynthetic rate. Two-photon fluorescence imaging of intact chloroplasts also showed more random spatial distribution of fluorescence compared with untreated samples (LUKINS et al., 2005).

Of particular interest in this study was to examine the time course of recovery process in apple leaves after UV-B treatment. Our results indicate that disturbance in photosynthetic functionality was followed by a continuous recovery process as indicated by increased maximum fluorescence. This increase was mostly pronounced during the first two days after termination of UV-B stress. The rate of photosynthetic recovery, estimated by Fv/Fm, appeared faster in the treatment with longer exposure to UV-B (Fig. 4). This result is consistent with the KOK (1956) model, which assumes a dynamic interaction between damage and repair with repair being proportional to the pool size of inactivated targets. HERAUD and BEARDALL (2000), taking into account lincomycin sensitivity of fluorescence parameters in *Dunaliella,* assumed that rate of recovery or repair is dependent on chloroplast-encoded protein synthesis. The fast rate of repair processes during the first hours after UV-B exposure has to be considered for a reliable assessment of damages induced by UV-B. However, also irreversible destructions in PS II may occur. For example, primary barley leaves in the study of GILBERT et al. (2004) displayed continuing inhibition of PS II after exposure to 1.9 W m^{-2} for 4 hours. CHOW et al. (1992) reported no recovery of Fv/Fm within 3 days after UV irradiation in pea plants.

In our study, the recovery curves of Fm, Fv, Fv/Fm and Fv/Fo parameters throughout 7 days after UV-B treatment were well fitted with an exponential rise to maximum function. Such kinetics of recovery processes resembled those found in the study of HERAUD and BEARDALL (2000) for *Dunaliella tertiolecta* cells exposed to UV-B radiation of 4.9 W m^{-2} for 30 minutes. As in our experiment, the relaxation kinetic of Fv/Fm was well described by an equation $y = y_o + a(1 - e^{-bx})$. Fv/Fm values in the experiment of HERAUD and BEARDALL (2000) returned to a control level within 270 min after UV-B irradiance. Complete recovery of Fv/Fm values was also observed on cucumber plants within 24 hours after ceasing UV-B treatment with 1.17 W m^{-2} for 8 hours (HUNT et al., 1996). In our study, fluorescence parameter values of apple leaves did not completely restore to the level of control plants during 7 days after irradiation indicating only partial recovery from applied UV-B treatments. So far, the magnitude of repair processes after UV-B stress seems to be dependent on UV-B doses, plant species and physiological state of leaves and may range from irreversible damages, partial and complete recovery.

Conclusion

In conclusion, the exposure of apple leaves to a short-term UV-B stress affected neither leaf chlorophyll content nor RED/NIR-light reflection, but could be well detected by PAM and LIF technique. Significantly reduced fluorescence intensities and F686/F740 ratio as well as high spatial heterogeneity of photosynthetic performance after UV-B stress have to be taken into account for the using of PAM and LIF in remote sensing. Furthermore, our results indicate that the rate of recovery seems to be dependent on UV-B dose and can be well described by an exponential rise to maximum function.

Acknowledgements

The study was funded by the Deutsche Forschungsgemeinschaft (DFG-Graduiertenkolleg 722).

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