Journal of Applied Phycology **5**: 505–516, 1993.

Changes in yield of *in-vivo* fluorescence of chlorophyll a as a tool for selective herbicide monitoring

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Received 6 May 1993; accepted 22 June 1993

Key words: fluorescence, bioassay, PSII herbicides, algae, triazines, phenylureas

Abstract

Triazines and derivatives of phenylurea, which are often found in outdoor water samples, induce specific changes in the yield of the *in-vivo* chlorophyll *a*-fluorescence of PSII. These changes are correlated quantitatively with the concentration of the herbicides and can therefore be used to set-up a low-price monitor system. In order to detect selectively the herbicide-sensitive part of the fluorescence emission a pulse amplitude modulated fluorimeter was used. The bioassay system was optimised with respect to test organism, growing and measuring conditions. The relationship between fluorescence yield and herbicide concentrations were experimentally determined for the triazines atrazine and simazine and the phenylurea herbicide DCMU and mathematically fitted ($r = 0.99$). The I_{50}-values were 0.9 μM for DCMU, 2.2 μM for simazine and 3.3 μM for atrazine. The detection limit of about 0.5 μM clearly shows that the sensitivity of this bioassay system is too low to reach the requirements of the drinking water regulation. However, due to its insensitivity against complex water matrices, there is good hope to combine this fluorometric bioassay with a potent herbicide preconcentration method like a solid-phase extraction procedure.

Abbreviations: Chl, chlorophyll; DCMU, dichlorophenyldimethylurea, F, fluorescence, F_m, maximal fluorescence; F_o, minimal fluorescence; F_v, variable fluorescence; GC, gas chromatography; HPLC, High performance liquid chromatography; MS, mass spectroscopy; PAM, pulse-amplitude-modulated fluorometer; PSII, photosystem II; qN, non-photochemical quenching; I_{50}, concentration required for 50% inhibition of electron transport; CCCP, carbonyl cyanide m-chlorophenylhydrazon

Introduction

According to the German drinking water regulation (1986), the sum of all pesticides in drinking water must not exceed 0.5 $\mu g\, l^{-1}$. Usually, gas chromatography (GC) or high performance liquid chromatography (HPLC), both coupled with a preconcentration step, are used for the sensitive detection of a pesticide pollution (Oehmichen *et al.*, 1987; Granet *et al.*, 1988; Schlett, 1991; Reupert *et al.*, 1992). However, the chemical analysis of a water sample is expensive and very

time-consuming. Thus, the number of analyzed samples is limited. Therefore, in the last few years different biological monitoring systems have been developed, which are cheap, fast and easy-to-handle to select contaminated water samples for further chemical analysis (Bringmann & Kühn, 1978; Wiegand-Rosinus *et al.*, 1989; Weston & Robinson, 1991; Hein *et al.*, 1991). A pesticide monitoring system has to agree with the following requirements:

(1) be specific for the chemicals to be monitored;
(2) have a very high reproducibility;
(3) must work in water systems highly charged with unknown chemicals;
(4) must reach the detection limit of the European drinking water regulation.

Additionally to drinking water analysis, such a biological monitoring system can also be used for any other outdoor water sample such as ground water or surface water or – with a much lower sensitivity – as an early warning system for uncontrolled pesticide pollution ('pesticide accident').

Of major importance with respect to pesticide pollution is that group of herbicides, whose primary mechanism of action is the block of photosynthetic functions (Reupert & Plöger, 1989). Especially the most commonly used phenylureas (DCMU) and triazines (atrazine, simazine) are known to bind specifically with high affinity at the Q_B-binding site of PSII. This block of photosynthetic electron flow causes an increase of PSII-dependent chl *a*-fluorescence (for reviews see Renger, 1986; Van Reensen, 1982; Fedtke, 1982; Draber *et al.*, 1991). Thus, this herbicide-related enhancement of *in-vivo* PSII fluorescence emission can be used as a biological test system to monitor herbicidal pollution in aquatic systems.

Recently, different types of algal herbicide monitors based on *in-vivo* chl *a*-fluorescence have been introduced. Sayk and Schmidt (1986), Schmidt (1987) and Noack (1988) used an impulse fluorometer, which applies strong light pulses at a wavelength of 435 nm to a sample of *Scenedesmus subspicatus* suspended in the matrix to be tested and records the emitted light intensity at 685 nm. Using such an experimental set-up, the intensity of fluorescence is measured which depends not only on the blockage of PSII, but also on the intensity of the light source, the amount of algae and the physiological state of the cells. Therefore, these parameters have to be controlled strictly which needs a high demand of hardware. The resulting value of fluorescence intensity is then compared with that of a herbicide-free and a herbicide-saturated control. The detection limit of this set-up is about a few $mg\,l^{-1}$ and operates quasi-continuously. This equipment was optimised and tested under on-line outdoor conditions and was shown to be suitable as an early warning system (Putzicha, pers. communication). However, the signals obtained are highly susceptible to the physiological status of the cells and chemical composition of the water matrix. The reasons for changes in the fluorescence intensity which are not due to herbicide action are of a complicated physiological nature, which is briefly summarized here.

(1) The proportion of energy not utilised in photosynthesis and emitted as fluorescence varies very markedly depending on the plant species, light and temperature regime during growth and measurement and other factors which determine their physiological situation. This is especially true for the commonly used unicellular algae whose range of physiological reactions are tremendous (Büchel & Wilhelm, 1993).

(2) The intensity of *in-vivo* fluorescence emission consists of different fluorescence parameters: the ground fluorescence F_o, often believed to originate from LHC-complexes and independent of any photosynthetic activity, is superimposed by the so-called variable fluorescence F_v of PSII (for reviews see Govindjee & Satoh, 1986; Krause & Weis, 1991). The contribution of F_o and F_v to the totally emitted fluorescence intensity can change, but only the variable part of it is sensitive to PSII herbicides.

(3) The variable fluorescence intensity F_v is highly controlled by photosynthetic regulation phenomena, namely photochemical and non-photochemical quenching processes: When dark-adapted algae are suddenly illuminated, complex

changes of the variable fluorescence yield occur, reflecting the light-activation of the photosynthetic apparatus. This fluorescence induction kinetic (Kautsky *et al.*, 1934; Kautsky *et al.*, 1940) is mainly determined by the redox state of Q_A, the primary electron acceptor of PS II and the light-dependent energetization of the thylakoid membrane. Quenching phenomena related to the redox state of Q_A are termed 'photochemical quenching' and are sensitive to herbicide action. The energetization of the thylakoid membrane leads to the so-called 'non-photochemical quenching', which is insensitive to PSII herbicide action, but it decreases drastically the yield of variable fluorescence of PSII in favour of an increased heat emission (for review, see Krause & Weis, 1991).

Because of these considerations, a herbicide monitor system based on *in-vivo* chl *a*-fluorescence has to fulfil the following requirements:

(1) The test algae must be in well-defined physiological conditions. A screening of the most suitable test organism should select that alga, which shows highest sensitivity to PSII herbicides, but lowest susceptibility to non-photochemical quenching effects.
(2) Non-photochemical quenching which decrease the yield of variable fluorescence must be minimised during measurement by the application of short and weak light pulses instead of actinic light.
(3) To recognise selectively that part of the totally emitted fluorescence which is directly correlated with the herbicide action, the measuring device must be able to discriminate between F_o and F_v. This can be achieved only by the measurement of *fluorescence yield* instead of intensity.

Fluorescence yield measurements can be done by the use of a pulse amplitude modulated fluorometer introduced by Schreiber (Schreiber, 1986; Schreiber *et al.*, 1986) and distributed as PAM 101 by Fa. Walz (Effeltrich, Germany).

The PAM operates with a modulated measuring beam given by a high frequency light-emitting diode (LED). The fluorescence is monitored by a so-called 'selective window amplifier' assuring a highly selective recording of pulse fluorescence signals against a vast background of non-modulated light, but also light-induced scattering or reflection of daylight into the cuvette does not influence the fluorescence signal. With a constant measuring light intensity these signals reflect the fluorescence yield. The measuring beam intensity can be decreased below that level where photochemistry is driven and true F_o determinations are possible.

The theoretical basis of PAM measurements were developed for higher plants. Recently Büchel and Wilhelm (1993) have reviewed the limits of this instrument when applied to green and chromophyte algae.

In this work we present the conditions to use the PAM for herbicide monitoring. Different algal species and herbicides are tested in various matrices to find to most suitable organism. In a second paper we present another modulated fluorescence system, which uses, in contrast to the PAM system very low cell densities for the herbicide monitoring. Both systems were constructed in a way that they can be combined with a concentration device by solid phase extraction.

Material and methods

Test organisms and culture conditions (see Table 1).

In order to screen for the most suitable organism, the following algae were tested: *Chlorella fusca* (Chlorophyceae, strain C.1.1.1.10. Shihira *et* Kraus), *Scenedesmus subspicatus* Chodat (Chlorophyceae, Göttingen strain 86–81), *Chlamydomonas reinhardtii* Dangeard (Chlorophyceae,

Table 1. Media used to grow the different algal species.

	Medium	Reference
C. fusca	N_5b medium	Strotmann, 1966
C. reinhardtii	N_5b medium	Strotmann, 1966
S. subspicatus	N_5b medium	Strotmann, 1966
E. gracilis	*Euglena* medium	Wiessner, 1968
C. ovata	Desmidiaceen medium	Schlösser, 1982
P. tricornutum	Bristol medium	Starr, 1987

Göttingen strain 11–32a and 11–32b), *Euglena gracilis* Klebs (Euglenophyceae, Göttingen strain 1224–5/25), *Cryptomonas ovata* Ehr. var. *palustris* Pringsheim (Cryptophyceae, Göttingen strain B 979–3) and *Phaeodactylum tricornutum* (Bacillariophyceae, freshwater strain, UTEX 640).

Batch cultures were grown in a suspension volume of 200 ml at 18 °C under continuous illumination of 15 μmol photon $m^{-2} s^{-1}$ white light. With the exception of one experiment, in which the growing time was varied, cells were harvested after fourteen days by centrifugation (3500 **g**, 5 min) and resuspended into culture medium containing 0.05 M $KHCO_3$. Experiments were carried out using 250 μl cell suspension with a chlorophyll content of 250 mg l^{-1} unless otherwise specified. Chlorophyll determination was performed in 80% acetone (Ziegler & Egle, 1965) for chl *a*/*b* containing algae and in 90% acetone (Jeffrey & Humphrey, 1975) for chl *a*/*c* containing algae.

Fluorescence induction kinetic (see Fig. 1)

All fluorescence measurements were made using a PAM fluorimeter (H. Walz, Effeltrich, FRG). After a dark adaptation period of 5 min (*Chlorella fusca*, *Scenedesmus subspicatus*, *Chlamydomonas reinhardtii*) and 15 min (*Euglena gracilis*, *Cryptomonas ovata*, *Phaeodactylum tricornutum*), respectively, F_o was determined by applying only the modulated measuring beam with an intensity of 0.05 μmol photon $m^{-2} s^{-1}$. $(F_v)_m$ was determined with a pulse of saturating white light (600 ms, 1000 μmol photon $m^{-2} s^{-1}$) given by a flash lamp (Schott, KL 1500). In order to measure only the fluorescence response of PSII without driving photosynthesis and the generation of non-photochemical quenching, short, non-saturated light pulses (600 ms, 100 μmol photon $M^{-2} s^{-1}$) were applied with a time intervall of 30 s for 15 min. The herbicide-sensitive fluorescence enhancement of PSII was induced by adding 2.5 μl of defined herbicide solutions. In the control sample the herbicide solution was substituted by the herbicide solvent ethanol.

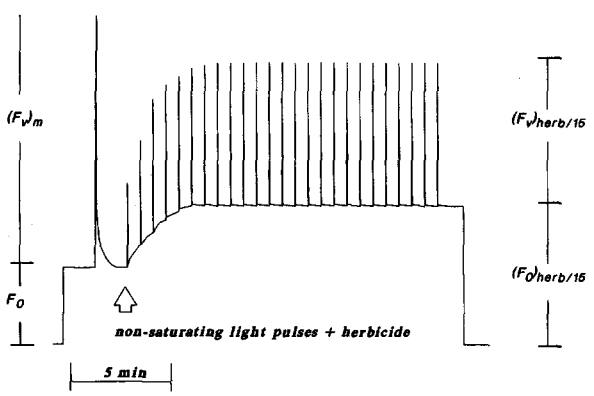

Fig. 1. Fluorescence induction kinetic with ground fluorescence F_0, maximum variable fluorescence $(F_v)_m$, variable fluorescence measured 15 min after herbicide addition $(F_v)_{herb./15}$, and ground fluorescence 15 min after herbicide addition $(F_0)_{herb./15}$.

The following herbicides were used for the measurements: DCMU (3-(3,4-dichlorophenyl)-1,1dimethylurea), atrazine (2-ethylamino-4-chloro-6-isopropylamino-1,3,5-triazine) and simazine (2,4-bis(ethylamino)-6-chloro-1,3,5-triazine) from Serva, Heidelberg, FRG. They were dissolved in ethanol at 10^{-3} M and stored at 4 °C until needed.

Matrix effects

2.5 μl of different uncoupling reagents, namely 2 mM and 4 mM NH_4Cl, 2 mM $(NH_4)_3PO_4$, 10 μM CCCP, and 10 μM Nigericin ($C_{40}H_{67}O_{11}Na$) were added to the poisoned cell suspension to study their effect on the fluorescence yield of PSII. To test the influence of complex matrices algae cells were suspended in water samples from river Rhine and Moselle.

Determination of F_v

The herbicide-sensitive fluorescence yield of PSII was defined as follows:

$$\text{fluorescence yield} = \frac{(F_v)_{herb/15}}{f} - \text{control value.}$$

The variable fluorescence of PSII was calculated 15 min after addition of the herbicide solution (= $(F_v)_{herb./15}$). To reduce the variability between different measurements, $(F_v)_{herb./15}$ was divided by a correction factor f, which is the ratio between the maximum variable fluorescence $(F_v)_m$ and the ground fluorescence F_o of the dark adapted, non-poisoned cell suspension. The resulting value is diminished by the control value, in which the herbicide solution is substituted by the herbicide solvent ethanol.

The detection limit is calculated as $\bar{x} + 3\Delta x$, with $\bar{x}$ as the mean of the control samples and delta x the error of mean on a 95% confidence interval.

Results

Beside the fact that the fluorometric detection of PSII herbicides with unicellular algae needs a maximum sensitivity, it is the reliability, which is the determinant factor for such a biological monitor system. Thus, to reach a minimum error probability, it has to be optimised and standardised, not only with respect to growth and measuring conditions, but also with respect to the test organism and its physiological state.

Screening

Figure 2 shows the sensitivity of different algae against DCMU. Their different behaviour originate mainly from non-photochemical quenching processes (qN), which decreases the herbicide sensitive part of PSII fluorescence emission in an unspecific and non-reproducible way. Although these disturbing regulation phenomena are intended to be avoided using light pulses short enough to prevent a whole-chain electron transport, the various algae species differ largely with respect to light utilisation capacity and the extent of photosynthetic regulation phenomena like qN. Whereas the green algae *Chlorella fusca* showed a high herbicide sensitivity over the whole DCMU concentration range, both strains of *Chlamydomonas reinhardtii* and *Scenedesmus subspicatus* produced remarkably lower fluorescence signals due to increased qN. Only the brackish water alga *Phaeodactylum tricornutum* (Bacillariophyceae) showed a higher fluorescence yield than *Chlorella fusca* at concentrations below 1 μM, at higher DCMU concentrations, however, the fluorescence yield is strongly quenched. Since *Euglena gracilis* (Euglenophyceae) could not be concentrated by centrifugation without loss of activity and the reproduction rate of *Cryptomonas ovata* (Cryptophyceae) was too low, *Chlorella fusca* proved to be the most suitable test organism, not only according to a maximum sensitivity, but also according to easy handling and a high rate of reproduction. For these reasons, all other experiments were carried out with *Chlorella fusca*.

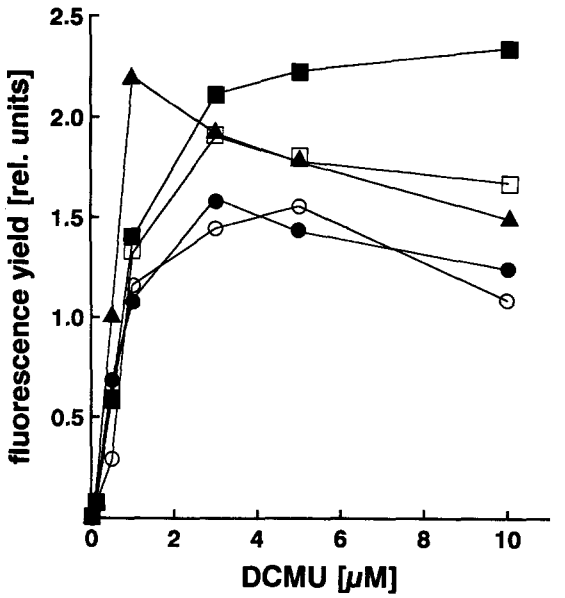

Fig. 2. Sensitivity of *Chlorella fusca* (■), *Phaeodactylum tricornutum* (▲), *Chlamydomonas reinhardtii* 11–32a (●), *Chlamydomonas reinhardtii* 11–32b) (○) and *Scenedesmus subspicatus* (□) to DCMU.

Physiological state of the cells

Samuelsson and Öquist (1977) clearly showed that the relative increase of algal chl a-fluorescence after addition of the PSII-herbicide DCMU

is closely related to the photosynthetic capacity changing during the growth period. Additionally, Senger and Frickel-Faulstich (1974) have pointed out that DCMU inhibition can be greatly affected by differences in the permeability of the cell membrane at different stages of cell cycle. Therefore, the physiological state of the algal cells used for the fluorometric assay is of great importance for the reproducibility and the sensitivity of an algal herbicide monitor system based on *in-vivo* chl a-fluorescence. Thus, the relation between the chl a-fluorescence yield and the age of the cell suspension, which is correlated with its physiological behaviour, is examined and the results are shown (Fig. 3). Although there is no linear correlation between the age of the cell suspensions and the fluorescence yield after herbicide action, the results clearly show that the age of the cells is important for sensitive and reproducible results. Optimal results are obtained with 14 day-old cell suspensions (exponential phase), with a typical cell density of $1-2 \times 10^7$ cells ml^{-1} and a chlorophyll content of 15–20 mg l^{-1}.

Chlorophyll concentration

The chlorophyll concentration of the cell suspension during the measurement is of fundamental significance, because it represents the amount of PSII reaction centers available for the herbicide action. Despite the fact that the ratio of blocked centers to the total amount of PSII reaction centers will theoretically get worse with increasing chlorophyll concentration, it is clearly shown (Fig. 4) that the fluorescence yield of a DCMU-poisoned cell suspension rise considerably with the chlorophyll concentration, ranging from 5 mg l^{-1} in the minimum to a maximum effect at 250 mg l^{-1}.

Temperature effect

Experiments with the herbicide atrazine showed the nessecity to control not only the measuring temperature, but also the storage temperature of the cell suspension. Lowering the storage temperature from 25 °C to 4 °C led to a considerably higher fluorescence yield of the poisoned sample

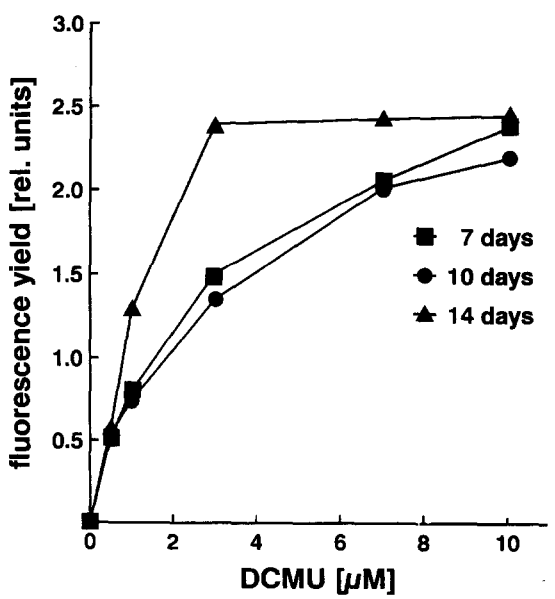

Fig. 3. Influence of growing time on the sensitivity of *Chlorella fusca* to DCMU.

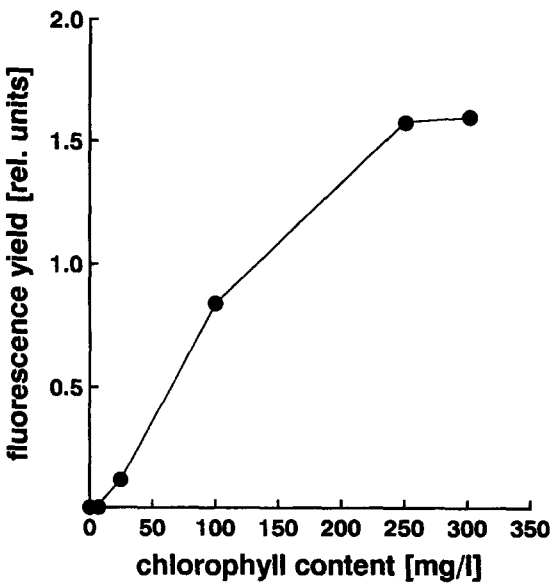

Fig. 4. Influence of different chlorophyll concentrations on the fluorescence yield of *Chlorella fusca* poisoned with 10^{-6} M DCMU.

(Fig. 5). This phenomenon is already described and still under discussion in the literature (Janssen, 1988; van Wijk, 1990; Janssen, 1992; Huner, 1992; Buman *et al.* 1992a, 1992b) but, nevertheless, it is very useful to achieve a maximum sensitivity.

Curve fitting and I_{50}-determination

After optimising both the measuring and growth conditions, ten independent series of experiments were carried out to determine the *in-vivo* inhibitory potency of the PSII herbicides DCMU, atrazine and simazine expressed as I_{50}-values. The experimental data were used to calculate the mathematical relationship between fluorescence yield and the herbicide concentration. The mathematical descriptions are given in formulae 1–3.

$$y = \frac{2.4 \times [\text{DCMU}]^{1.69}}{0.84 + [\text{DCMU}]^{1.69}}, \quad (1)$$

$$y = \frac{2.3 \times [\text{Atrazine}]^{1.27}}{4.6 + [\text{Atrazine}]^{1.27}}, \quad (2)$$

$$y = \frac{2.2 \times [\text{Simazine}]^{1.48}}{3.2 + [\text{Simazine}]^{1.48}}, \quad (3)$$

with [herbicide] in [μmol l^{-1}]

The calculated relationship for each herbicide fitted well to the experimentally determined data (Fig. 6a–c). These dose-response curves showed a slight sigmoidal behaviour which is already described for isolated chloroplasts (Fedtke, 1982; Siderer *et al.*, 1984). The inhibition constants I_{50} was calculated from the mathematical functions. As expected, DCMU had the highest affinity to the PSII of *Chlorella fusca*, shown by the lowest I_{50}-value of 0.9 μM. In contrast to the I_{50}-values measured in thylakoid suspensions or in isolated chloroplasts (Fedtke, 1982) the *in vivo* inhibition constant of simazine ($I_{50} = 2.2$ μM) indicated a higher affinity of PSII to simazine than to the more hydrophilic atrazine ($I_{50} = 3.3$ μM). The detection limit was 0.3 μM for DCMU, 1.0 μM for atrazine and 0.5 μM for simazine. The relative error of this measuring system varied between $\pm 10\%$ at higher herbicide concentrations and rose up to $\pm 40\%$ near the detection limit.

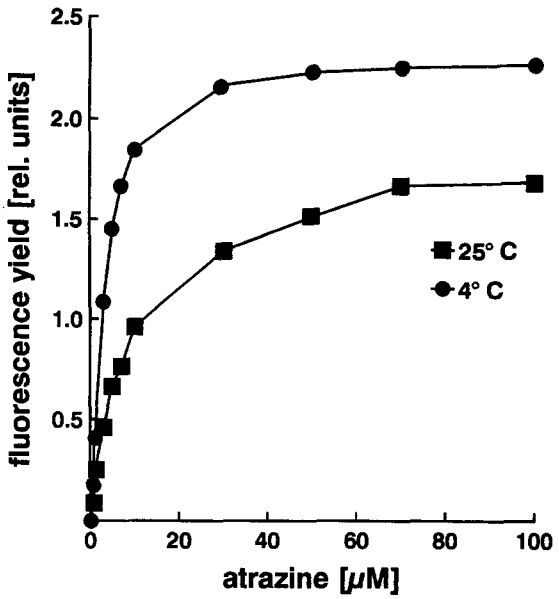

Fig. 5. Influence of the storage temperature on the fluorescence yield of *Chlorella fusca* poisoned with atrazine.

Matrix effects

In order to test the sensitivity of the bioassay system to several chemicals which might impair the fluorescence behaviour, different uncouplers were added to the cell suspension in relatively high amounts. As expected, there were no detectable influences on the variable fluorescence of PSII with the exception of CCCP (Table 2). In order to test a more complex matrix, containing a great amount of unknown organic and inorganic compounds, *Chlorella* cells were suspended into natural water collected from the Rhine and the Moselle. Since the concentration of PSII herbicides are not used to exceed 1–2 nM, there should be no effect on the fluorescence yield. Indeed, there is no interference between these complex matrices and the herbicide-induced fluorescence emission of PSII (Fig. 7).

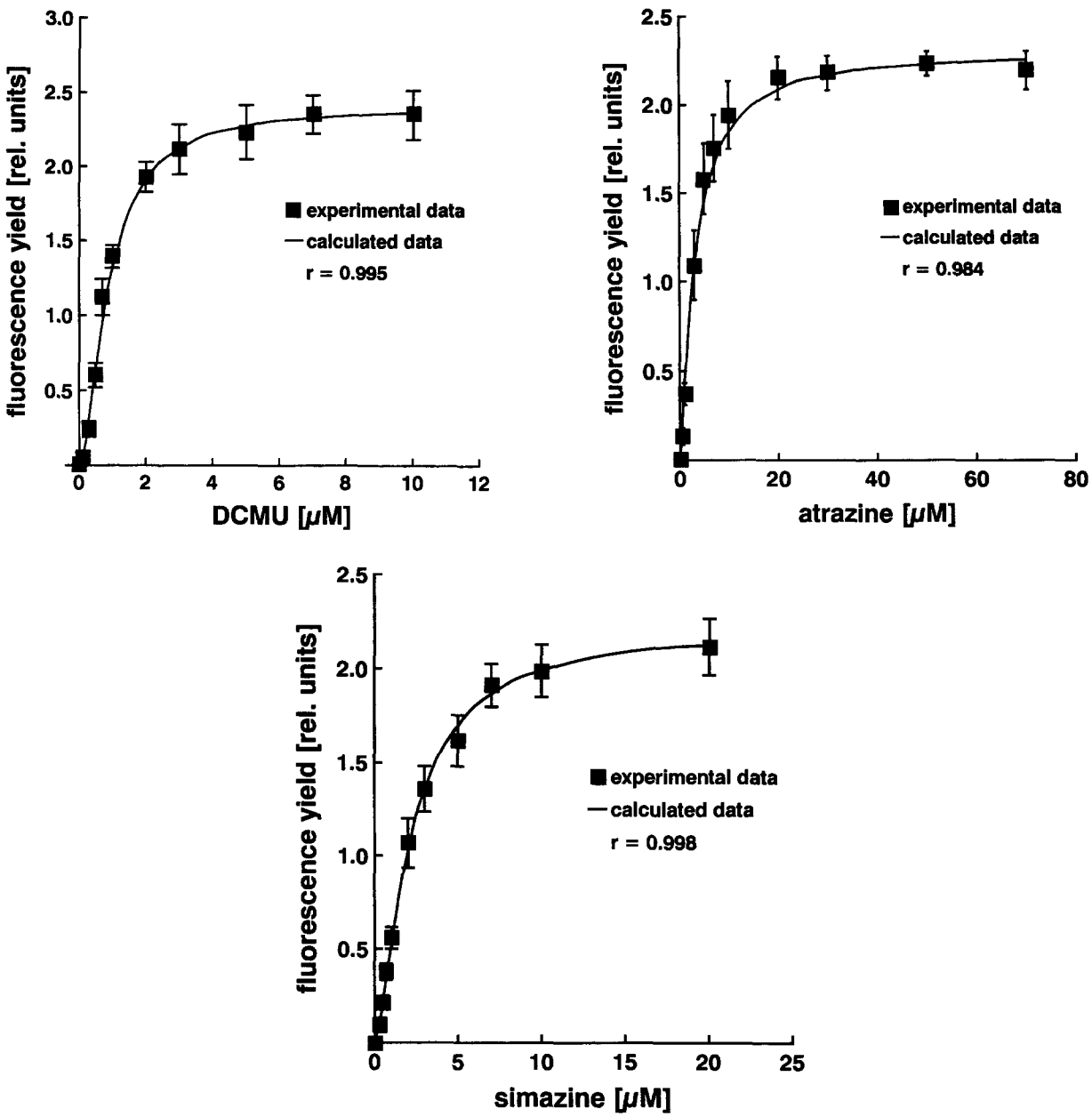

Fig. 6. Comparison between the experimental and calculated signal-response curves for DCMU (6A), atrazine (6B) and simazine (6C) obtained with *Chlorella fusca*. The symbols are means of ten independent experiments and the vertical bars on symbols represent 95% confidence interval.

Discussion

In applied water technology the green alga *Scenedesmus subspicatus* is often used as a test organism to monitor phytotoxic substances in outdoor samples (Schmidt, 1983; Schmidt, 1987; Noack, 1988). But this organism is not suitable for an algal herbicide monitor based on *in-vivo* chl *a*-fluorescence, because it showed a strong non-photochemical quenching effect lowering the sensitivity to PSII herbicides. Therefore, it was decided to use *Chlorella fusca* as test organism. With

Table 2. Effect of several uncouplers on the yield of variable fluorescence F_v of PSII poisoned with 10 μM DCMU.

Uncoupler	Concentration (mmol l^{-1})	Fluorescence yield (rel. units)
Conytol	–	2.26
Nigericin	0.01	2.25
CCCP	0.01	1.55
NH_4Cl	2.0	2.22
NH_4Cl	4.0	2.35
30 min preincubation		
$NH_4H_2PO_4$	2.0	2.37

the PAM system the obtained fluorescence yield signals which are correlated with the herbicide concentration fitted very well with the calculated mathematical function. This is concomitant with a good reproducibility and a very low susceptibility against uncouplers in the matrix. Specifically, concentrations of ammonium high enough to inactivate photosynthesis and respiration do not impair the test system. In addition, the preliminary tests on the influence of undefined matrices of outdoor water samples from the rivers Rhine and Moselle did not have any effect on the fluorescence signal. These results reveal good evidence that our test system selectively records that specific part of fluorescence that is altered by the herbicide action. But despite this high selectivity the sensitivity is not high enough to reach the limit of the drinking water regulation of the European Community which is 0.5 $\mu g\, l^{-1}$ for the sum of all pesticides. The detection limit of about 0.5 μM or 100 $\mu g\, l^{-1}$ demands for a potent preconcentration procedure. The development of a solid-phase extraction method which can be combined with the PAM measuring system is now under investigation and positive results will be reported in near future.

However, some other problems have to be mentioned. Our results give evidence that the physiological state of the cells is a critical parameter for the sensitivity of the system. Especially, the age of the cells and the temperature during the storage between different experiments have to be controlled. On the other hand, it is advantageous for the application in routine water control that

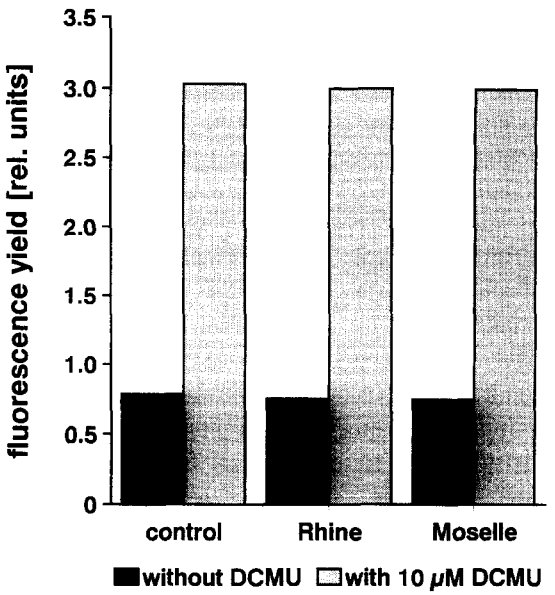

Fig. 7. Fluorescence yield of *Chlorella fusca* suspended in culture medium, Rhine water and Moselle water.

neither the composition of the medium nor the supply with CO_2 are critical parameters, so that the physiological state of the cells can be defined without special growth devices.

If one compares the I_{50}-values for DCMU, atrazine and simazine with the dissociation constants of 30–100 nM for phenylureas and triazines found by other others using isolated thylakoids (Tischer & Strotmann, 1977; Shochat *et al.*, 1982; Chow *et al.*, 1990; Jursinic *et al.*, 1991), it seems to be clear that there is some unspecific binding of these herbicides to other cell compartments as already described for leave fragments (Haddad *et al.*, 1992), spinach protoplasts (Buman *et al.*, 1992b), isolated thylakoid membranes (Tischer & Strotmann, 1977; Laasch, 1981) or intact algae cells (Laasch, 1981). Additionally, *Chlorella* possesses thick multilayered cell walls highly loaded with hydrophobic hydrocarbon chains (Derenne *et al.*, 1992) which may retain significant amounts of herbicides. A hint for this interpretation comes from the significant higher sensitivity of *Phaeodactylum* lacking this type of cell wall.

A second reason of this discrepency may be related to the high chlorophyll content during

measurement. The increase of chlorophyll has the consequence that at a given concentration of herbicide its absolute amount per PSII is reduced. Therefore, at high cell densities the advantage of the strong fluorescence signal is counterpoised by the fact that only a small portion of the cells is poisoned. From a theoretical viewpoint it is impossible to predict the best cell density for an optimal signal/noise ratio depending on the chlorophyll content on the one hand and on the percentage of poisoned PSII reaction centers at a given herbicide concentration on the other. With the presently available instrumentation a high cell density seems to be optimal for our measurements. But, the signal/noise ratio could be improved by a modified PAM fluorimeter. The LED as pulse modulated light source is for technical reasons restricted to the red spectral region, which make it impossible to measure the fluorescence signal in its maximum emission band at 685 nm. Schreiber *et al.* (1993) presented now a modified PAM fluorometer which employs Xe-flashes for measuring light with a greater flexibility in both excitation and emission wavelengths. Due to a greater sensitivity it should be possible to monitor variable fluorescence even in extremely diluted samples under conditions where the ratio PSII reaction centres per herbicide is low.

The signal/noise ratio is not only limited by the measuring light source but also by the sensitivity of the PIN-detector diode. The replacement of this diode by a well fitted photomultiplier would significantly increase the sensitivity of the system. Such an instrumentation was realised by Duval *et al.* (1993) and the corresponding results will be published elsewhere.

In conclusion, a new type of algal herbicide monitor is now in perspective which is based on the measurement of changes in the yield of variable chl *a*-fluorescence of PSII and which gives hope to work in combination with a solid-phase extraction procedure. Such a system would monitor the most commonly used phenylurea- and triazine herbicide at concentrations according to the European drinking water regulation.

Acknowledgements

The financial support by the Commission of the European Community (STEP 0045-C) and the Deutsche Forschungsgemeinschaft (Graduiertenkolleg 'Kreisläufe, Austauschprozesse und Wirkungen von Stoffen in der Umwelt', University of Mainz) are gratefully acknowledged.

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