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Short Communication

Short term recovery of periphyton photosynthesis after pulse exposition to the photosystem II inhibitors atrazine and isoproturon

Martin Laviale [a,b,c,1], Soizic Morin [d], Anne Créach [a,b,c,*]

[a] Université Lille Nord de France, F-59000 Lille, France
[b] USTL, GEPV, F-59655 Villeneuve d'Ascq Cedex, France
[c] CNRS, FRE 3268, F-59655 Villeneuve d'Ascq Cedex, France
[d] Cemagref, UR REBX, 50 avenue de Verdun, F-33612 Cestas Cedex, France

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ABSTRACT

Aquatic organisms are exposed to fluctuating concentrations of herbicides which contaminate rivers following their use for agricultural or domestic purposes. The development of sensitive bioanalytical tests enabling us not only to detect the effects of those pollutants but to take into account this pattern of exposure should improve the ecological relevance of river toxicity assessment. In this respect, the use of chlorophyll fluorescence measurements is a convenient way to probe the effect of photosystem II (PSII) inhibitors on primary producers. This study was devoted to validate the combined use of two fluorescence parameters, the effective and the optimal quantum yields of PSII photochemistry (Φ_{PSII} and F_v/F_m), as reliable biomarkers of initial isoproturon (IPU) or atrazine (ATZ) toxicity to natural periphyton in a pulse exposition scenario. Φ_{PSII} and F_v/F_m were regularly estimated during a 7 h-exposure to each pollutant (0–100 μM) and also later after being transferred in herbicide-free water (up to 36 h). Our results showed that IPU was more toxic than ATZ, but with effects reversible within 12 h. Moreover, these two similarly acting herbicides (i.e. same target site) presented contrasted short term recovery patterns, regarding the previous exposure duration.

1. Introduction

Contamination of rivers by herbicides has been widely reported in agricultural as well as urbanized watersheds (Holvoet et al., 2007). Once in the aquatic environment, those substances may affect non-target photosynthetic organisms. In lotic systems, attached microalgae (i.e. periphyton), are generally exposed to low herbicides exposure, with transient peaks related with changes in hydrology (Holvoet et al., 2007). Several authors stressed out that more environmental realism, regarding both patterns of exposure (i.e. pulsed vs chronic) and biological models (i.e. single species vs community level based tests) is essential (e.g. Sabater et al., 2007). In particular, the literature investigating the relevance of the recovery time between pulses of herbicides on algal sensitivity is scarce (Gustavson et al., 2003; Tlili et al., 2008; Vallotton et al., 2008a,b, 2009). In this respect, the development of tools based on periphyton physiological response should be of great help to refine our understanding on the initial herbicides effect at the community level (Sabater et al., 2007).

The Pulse Amplitude Modulated (PAM) chlorophyll fluorescence technique is a convenient way to study in vivo the early adverse effects of herbicides on photosynthetic activity and related physiological activities (Juneau et al., 2007). Of the fluorescence parameters already validated for toxicological purposes, the effective quantum yield of the photosystem II (PSII) photochemistry (Φ_{PSII}) gives a measure of the proportion of the PSII absorbed light that is used in photochemistry and integrates all the processes downstream of PSII which are dependant of the actual test conditions, e.g. light and temperature (Baker, 2008). In comparison, the optimal quantum yield (F_v/F_m) reflects the number of functional PSII, thereby illustrating the sample physiological state (Baker, 2008). Although it offers a more accurate view than Φ_{PSII} regarding the pollutant toxicity, it is rarely used, especially for river biofilms (Dorigo and Leboulanger, 2001; Laviale et al., 2010).

In this context, the aim of this study was to validate the combined use of Φ_{PSII} and F_v/F_m for a reliable assessment of periphyton sensitivity to herbicides and its potential recovery in a pulse exposition scenario. For this purpose, we focused on the effects of the triazine atrazine (ATZ) and of the phenylurea isoproturon (IPU), two herbicides specifically designed to alter photosynthesis by

* Corresponding author at: Laboratoire GEPV, Université des Sciences et Technologies de Lille, F-59655 Villeneuve d'Ascq Cedex, France. Tel.: +33 3 20 33 60 06; fax: +33 3 20 43 69 79.
E-mail addresses: martin.laviale@univ-nantes.fr (M. Laviale), anne.creach@univ-lille1.fr (A. Créach).
[1] Present address: Laboratoire Mer, Molécules, Santé, UPRES-EA 2160, Université de Nantes, 2 rue de la Houssinière, BP 92208, Nantes Cedex 03, France.

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inhibiting the photosystem II (PSII) activity (Rutherford and Krieger-Liszkay, 2001) and which are among the 10 pesticides most frequently quantified in French rivers (Agences de l'Eau). A short-term ecotoxicological bioassay was carried out to monitor the photochemical response to IPU and ATZ of natural stream biofilms. Φ_{PSII} and F_v/F_m were regularly estimated during herbicide exposure (up to 7 h) and then after transfer in herbicide-free water (up to 36 h).

2. Materials and methods

2.1. Biofilms collection and incubation conditions

As previously described (Laviale et al., 2009, 2010), natural stream periphyton was regularly sampled from glass substrata immersed in early Spring in the Stream Sensée (Nord-Pas de Calais, France) at a site slightly influenced by herbicides (<0.2 μg L^{-1} over the last 12 months, Agence de l'Eau Artois-Picardie). After 2–3 weeks of colonization, the substrata were transported to the laboratory within 1 h in cool-boxes filled with site water and transferred in dark climate (20 °C) chamber. Preliminary microscopic observations indicated that these communities were dominated by diatoms. Six randomly selected slides were used to estimate the total diatom density (cells cm^{-2}). Each biofilm was collected with a razor blade in a preservative 5% formalin solution (Formol 37%) and observed under stereomicroscope using a Nageotte counting chamber (VWR, Fonteney sous Bois, France) at 200 magnification.

Within 1 d, slides were incubated horizontally under gentle agitation in polycarbonate vessels (1 slide per vessel) containing 60 mL of filtered stream water (Whatman GF/F, VWR, Fontenay sous Bois, France) and placed in continuous light (50 μmol m^{-2} s^{-1}) provided by fluorescent tubes (36 W, Grolux, Sylvania, VWR). Incubation media containing IPU ([3-(4-isopropylphenyl)-1,1-dimethylurea], Dr. Ehrenstorfer GmbH, Augsburg, Germany) or ATZ ([2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine], Sigma Aldrich, St. Quentin Fallavier, France) were prepared as Laviale et al. (2010).

2.2. Chlorophyll fluorescence parameters

The fluorescence signals were measured with a PAM 2100 fluorometer (Walz, Effeltrich, Germany) by means of home-made systems which were consistently described elsewhere (Laviale et al., 2009, 2010). Φ_{PSII} was evaluated on several slides under ambient light (50 μmol m^{-2} s^{-1}) according to Genty et al. (1989):

$$\Phi_{PSII} = (F'_m - F_t)/F'_m \quad (1)$$

where F_t is the fluorescence steady-state level under ambient light and F'_m is the maximum level of fluorescence measured during a saturating white light pulse (0.8 s).

Other slides were transferred to complete darkness for 10 min. The minimum fluorescence (F_0) was determined after a weak (5 s) far red modulated light (735 nm). Then the maximum fluorescence (F_m) was reached by exposing the biofilm to a saturating light pulse (0.8 s). F_v/F_m was then calculated using the Genty et al. (1989) equation:

$$F_v/F_m = (F_m - F_0)/F_m \quad (2)$$

2.3. Experimental design

The biofilms were exposed for 7 h to four nominal concentrations (0.1, 1, 10 and 100 μM) of each herbicide, i.e. 20.6–20.6 × 10^3 μg IPU L^{-1} or 21.6–21.6 × 10^3 μg ATZ L^{-1}. At the end of each time of exposure, the biofilms were gently rinsed and transferred for 36 h in uncontaminated filtered stream water which was regularly replaced to limit nutrient depletion and herbicide reuptake by passive diffusion through the biofilm. Chlorophyll fluorescence was measured on independent samples: (i) at the beginning of the experiment; (ii) after 1, 3 and 7 h (Φ_{PSII}) or 3 and 7 h (F_v/F_m) of herbicide exposure and (iii) after 1, 12 and 36 h in herbicide-free water for each condition of exposure.

All experiments were performed twice and each algal sample was analyzed in triplicates. Analyses of variance (ANOVA) and Tukey's honestly significant difference (HSD) tests were performed using the *R* statistical computing environment (v 2.8.1, Ihaka and Gentleman, 1996) after checking data normality and homoscedasticity of the residuals. Φ_{PSII} and F_v/F_m were expressed as % of response of the mean value obtained from the control community at the same time of exposure or recovery; letters indicate statistically homogenous groups.

3. Results and discussion

3.1. Short-term effects of herbicides

Fluorescence measurements were carried out on periphytic communities before contamination and then after 1, 3 and 7 h of exposure. F_v/F_m and Φ_{PSII} estimated on the controls were stable all along the experiment with mean values of 0.69 ± 0.03 (95% Confidence Interval) and 0.65 ± 0.01 respectively, indicating that the diatoms, which dominated communities (1.4 × 10^5 ± 0.1 × 10^5 cells cm^{-2}), were in good physiological state (Baker, 2008; Laviale et al., 2009, 2010).

In treated biofilms, the established concentration–effect relationships indicate that both herbicides strongly inhibited these parameters ($p < 0.001$, Fig. 1). Effects on Φ_{PSII} were significant within 1 h ($p < 0.001$) and remained stable thereafter ($p \geqslant 0.07$), whatever the concentrations tested (Fig. 1 A and B). IPU was 10-fold more toxic than ATZ with a total inhibition of Φ_{PSII} at 1 μM IPU and 10 μM ATZ, respectively (Fig. 1A and B). This falls within the range of values reported for periphyton in comparable short term bioassays based on chlorophyll fluorescence measurements (Guasch et al., 2003; Schmitt-Jansen and Altenburger, 2007,

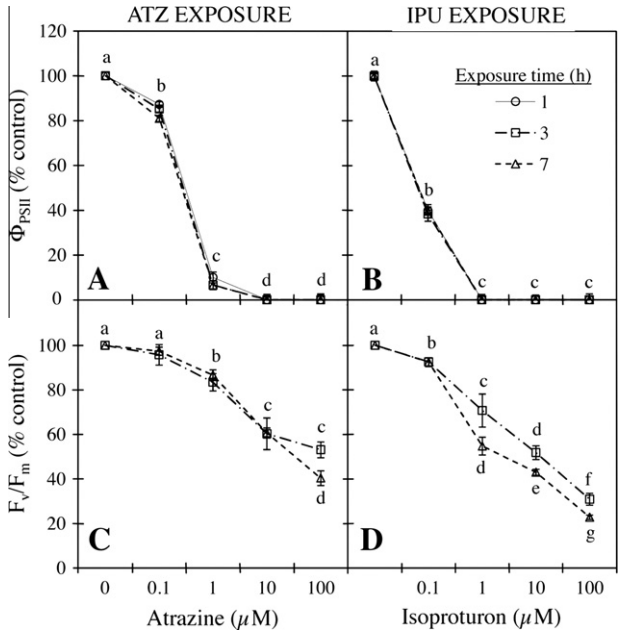

Fig. 1. Mean values (±95% CI, $n = 6$) of Φ_{PSII} (A and B) and F_v/F_m (C and D) estimated after 1 (○), 3 (□) or 7 h (△) of exposition to 0–100 μM of ATZ (A–C) or IPU (B and D).

2008; Laviale et al., 2010). In comparison, the F_v/F_m drop was less pronounced and maximal only after the 7 h-exposure period whatever the concentration tested ($p < 0.01$, Fig. 1C and D), decreasing to 23% and 46% of the controls when exposed to 100 μM IPU and ATZ, respectively. Although less sensitive than Φ_{PSII}, this contrasts with previous work where no important changes of F_v/F_m were revealed over a 24-h-incubation with ATZ and IPU (Dorigo and Leboulanger, 2001).

Early effects of IPU and ATZ were significant within 1 h, supporting toxikinetic studies which reported an inhibition of periphyton photosynthetic activity within minutes with negligible additional toxicity after 1 h (Gustavson et al., 2003; Legrand et al., 2006; Schmitt-Jansen and Altenburger, 2007; Laviale et al., 2010). This can be ascribed to a fast uptake of these two organic molecules which can penetrate into the algal cells by passive diffusion (Nikkilä et al., 2001; Weiner et al., 2004) independently of the water chemistry (Guasch et al., 2003; but see Knauer et al., 2007). Once into the cell, the two molecules bind with a high specificity to a unique niche on the D1 protein of the PSII reaction center where they replace the plastoquinon Q_B (Rutherford and Krieger-Liszkay, 2001). Higher toxicity of IPU compared to ATZ could be related to different specific affinities with their target site (Bérard et al., 2003, and references therein). Because Q_B is an essential mobile electron carrier, its substitution by herbicides leads to slow down the PSII electron flow, then drastically inhibiting photosynthetic efficiency (i.e. Φ_{PSII}). The resulting light energy that is no longer used in photochemistry could induce the production of reactive oxygen species (ROS) which are responsible for loss of PSII activity and D1 protein degradation (Rutherford and Krieger-Liszkay, 2001). In normal conditions, damaged D1 is degraded and replaced by a newly synthesized protein. However, urea and triazine herbicides may perturb this damage–repair cycle (Zer and Ohad, 1995). In our study, F_0 and especially F_t increased with increasing herbicide concentration (data not shown), suggesting that a significant part of this excitation light was remitted as fluorescence (Dorigo and Leboulanger, 2001; Dorigo et al., 2004; Dewez et al., 2008). Alternatively, several physiological mechanisms (i.e. nonphotochemical quenching) may help in dissipating excess energy through harmless thermal radiations (Baker, 2008). The onset of these processes is indicated by the observed decrease in maximal fluorescence (F_m and F_m') with increasing herbicide concentration (data not shown). However, PSII inhibitors can perturb, at least indirectly, these mechanisms (Fai et al., 2007). The production of photoprotective carotenoids, especially those of the xanthophyll cycle, can be inhibited by IPU (Laviale et al., 2010). The time lag in decrease of F_v/F_m exposed to high concentrations of ATZ or IPU suggests an overexcitation of the photosystems, thereby altering sensitive parts of the cellular machinery including the photosynthetic apparatus and, consequently, the overall physiological state of the cell (Fai et al., 2007; Laviale et al., 2010). However, the reasons for stress-induced decreases in F_v/F_m are often complex, and their identification need to consider jointly changes during herbicide exposure but also kinetics of recovery.

3.2. Recovery in herbicide-free water

During the 36 h of the recovery period, Φ_{PSII} and F_v/F_m values estimated on control biofilms slightly decreased (0.55 ± 0.04 and 0.63 ± 0.03 after 12 h, respectively). Due to technical reasons (i.e. overnight experiment), water was less frequently renewed, thereby leading to a potentially stressful lack of nutrients. Considering the samples previously exposed to IPU or ATZ, a significant recovery was observed within 1 h: for instance, Φ_{PSII} values increased from 0% to 80% of the control while F_v/F_m values increased from 55% to 85% for communities pre-exposed to 1 μM

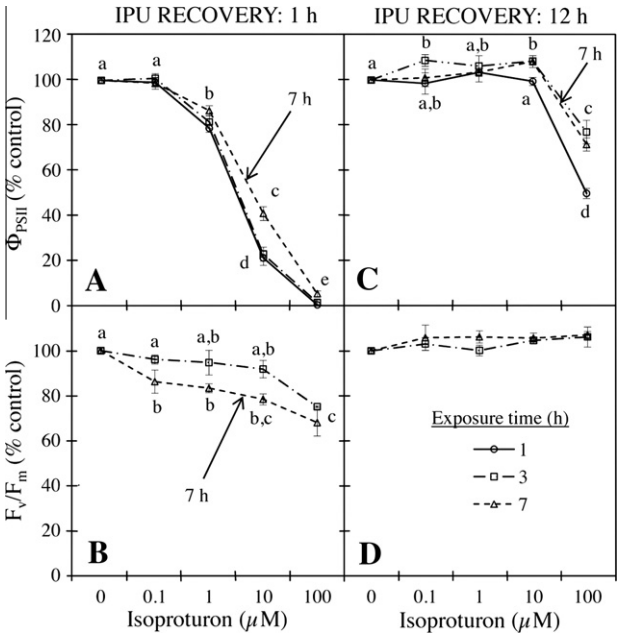

Fig. 2. Mean values (±95% CI, $n = 6$) of Φ_{PSII} (A–C) and F_v/F_m (B–D) estimated after 1 (A and B) and 12 h (C and D) in herbicide-free water for periphyton communities previously exposed to 0–100 μM IPU during 1 (○), 3 (□) or 7 h (△).

of IPU (Fig. 2A and B). After 12 h, Φ_{PSII} and F_v/F_m values were comparable to the controls whatever the herbicide and the incubation concentration tested (Figs. 2C, D, 3C and D), except for those previously exposed to the highest IPU concentration (100 μM) which only fully recovered after 36 h (data not shown). A comparable recovery after a severe alteration of the photosynthetic activity was observed for communities pre-exposed to hexazinone (Schneider et al., 1995), metribuzin (Gustavson et al., 2003) and IPU (Laviale et al., 2010). Most of IPU and ATZ were probably reversibly bound to their target sites, thus being rapidly diluted in uncontamined water (Nikkilä et al., 2001; Tlili et al., 2008). The rapid recovery of the fluorescence parameters suggests that the response of periphyton to environmentally realistic herbicide concentrations (<1 μM) was linked to a down-regulation of PSII rather than to photosynthetic damages, which would reduce photosynthesis for longer time, as also observed with short-term light stress (Baker, 2008). Nevertheless, this apparent reversible effect on the photosynthetic activity at the community level does not preclude exclusion of sensitive species over longer time scale (Gustavson et al., 2003).

The duration of exposure influenced differently the algal community recovery regarding the herbicide tested. After 1 h in herbicide-free water, the longer biofilms were previously incubated with IPU, the higher the Φ_{PSII} values ($p < 0.001$, Fig. 2A), whereas F_v/F_m presented an opposite trend ($p < 0.001$, Fig. 2B). In the case of ATZ, the Φ_{PSII} recovery decreased with increasing exposure ($p < 0.05$, Fig. 3A) while the fastest F_v/F_m recovery was observed for the 7 h pre-exposed biofilms ($p < 0.001$, Fig. 3B). It is suggested here that the recovery following IPU exposure was based on a reduced number of but more efficient functional PSII reaction centers while the response to ATZ tended to increase active but lower efficient reaction centers. It is noteworthy that considering F_v/F_m in combination with Φ_{PSII} gave us a better insight into the periphyton response to ATZ and IPU in comparison to Φ_{PSII} measurements alone. However, elucidating the mechanisms behind these contrasted recovery strategies remains an open question to be addressed.

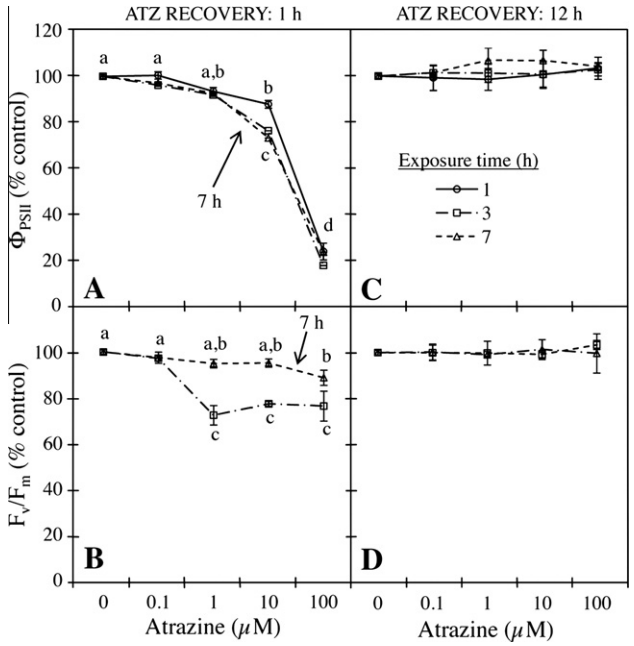

Fig. 3. Mean values (±95% CI, $n = 6$) of Φ_{PSII} (A–C) and F_v/F_m (B and D) estimated after 1 (A and B) and 12 h (C and D) in herbicide-free water for periphyton communities previously exposed to 0–100 μM ATZ during 1 (○), 3 (□) or 7 h (△).

4. Conclusion

The fluorescence parameters Φ_{PSII} and F_v/F_m provided useful information making them suitable complementary biomarkers of toxicity for PSII inhibitors to be employed in future ecotoxicological bioassays. We showed that two herbicides sharing the same mode of action (i.e. same target site), but one (IPU) being more toxic than the other (ATZ), may present opposed short term recovery patterns regarding the exposure duration. From these results, it appears that considering only one of these parameters would have led to a partial view of periphyton photochemical response to these herbicides, thus causing some limitations for interpretation of these differential effects. Although these observations cannot be extrapolated to less specifically acting compounds, awareness to these differences may help in our future studies which are intended to improve our understanding of the responses and recovery potential of periphytic communities exposed to cocktails of pesticides with similar or different modes of action, such as expected in streams.

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