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Light modulated toxicity of isoproturon toward natural stream periphyton photosynthesis: A comparison between constant and dynamic light conditions

Martin Laviale[a,1], Jean Prygiel[b,c], Anne Créach[a,*]

[a] Laboratoire de Génétique et Evolution des Populations Végétales, UMR CNRS 8016, Université des Sciences et Technologies de Lille - Lille 1, F-59655 Villeneuve d'Ascq Cedex, France
[b] Agence de l'Eau Artois-Picardie, 200 rue Marceline, F-59508 Douai Cedex, France
[c] Laboratoire Processus et Bilans des Domaines Sédimentaires, UMR CNRS 8110, Université des Sciences et Technologies de Lille - Lille 1, F-59655 Villeneuve d'Ascq Cedex, France

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ABSTRACT

This study tested if a variation in light intensity, in comparison to constant light required in well-designed toxicity test, could have measurable consequences on the sensitivity of phototrophic biofilms (periphyton) to isoproturon. Two independent experiments were carried out to investigate the combined effects of light and isoproturon on the photochemical behavior of intact natural biofilms by measurements of chlorophyll fluorescence and pigment composition. Experiment 1 consisted of exposing biofilms to series of isoproturon concentrations (0–2 mg L^{-1}) for 7 h under constant light at different irradiance levels (25–300 μmol m^{-2} s^{-1}). In experiment 2, biofilms were exposed using more environmentally realistic conditions to three selected concentrations of isoproturon (2, 6 and 20 μg L^{-1}) during a 7-h-simulated daily light cycle. Our results demonstrated that light, considered here as a direct physical stressor, slightly modulated the acute toxicity of isoproturon on these diatom dominated communities. This was attributed to the fact that these two factors act specifically on the photosynthetic activity. Furthermore, it was shown that a dynamic light regime increased periphyton sensitivity to isoproturon by challenging its photoprotective mechanisms such as the xanthophyll cycle, therefore implying that traditional ecotoxicological bioassays lead to underestimate the effect of isoproturon.

1. Introduction

The use of natural freshwater for agricultural, industrial, recreational or domestic purposes has been leading to an increasing pollution of surface and ground waters with a large array of organic and inorganic micropollutants (Schwarzenbach et al., 2006). In the context of the European Water Framework Directive (WFD, 2000/60/EC) implementation, a sustained management of the aquatic ecosystems requires a reliable river toxicity assessment by developing analytical and modeling tools to probe not only the distribution and bioavailability but also the biological effects of these pollutants. In the case of herbicides, several monitoring campaigns have already consistently determined their occurrence in streams, rivers and lakes (Chèvre et al., 2008; Hoagland et al., 1996; Solomon et al., 1996). Up to now, in situ prediction of the ecological effects of toxicants on primary producers and therefore on aquatic ecosystems has been mostly based on laboratory investigations, resulting in comprehensive ecotoxicological data based on single-species tests, mainly with unicellular planktonic microalgae cultivated in standardized conditions (Nyholm and Källqvist, 1989). However these studies, which report a wide variation between species in their sensitivity to the same chemicals (Hoagland et al., 1996), lack environmental realism because they do not take into account the multiplicity of biotic (inter-specific competition, grazing, etc.) and abiotic (temperature, light, etc.) environmental factors which are potentially involved. For instance, some authors have demonstrated that the complexity of inter-specific relationships (Seguin et al., 2001; Leboulanger et al., 2001; Bérard et al., 2003; Schmitt-Jansen and Altenburger, 2007) the grazing pressure (Munoz et al., 2001) or the trophic status (Wendt-Rasch et al., 2004) may directly influence the global response of microalgae to herbicides at the community level. Other studies have pointed out that this response can depend on physiological adaptations of microalgae to environmental selective pressures such as light (e.g. Guasch and Sabater, 1998; Bérard and Benninghoff, 2001) or pollution (e.g. Bérard and Benninghoff, 2001; Dorigo and Leboulanger, 2001; Dorigo et al., 2004; Seguin et al., 2002; Bérard et al., 2003) histories. Also, the

* Corresponding author. Tel.: +33 3 20 33 60 06.
E-mail addresses: martin.laviale@univ-nantes.fr (M. Laviale), anne.creach@univ-lille1.fr (A. Créach).
[1] Present address: Laboratoire Mer, Molécules, Santé, EA 2160, Université de Nantes, 2 rue de la Houssinière, BP 92208, F-44322 Nantes Cedex 3, France.

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pattern of herbicide exposure including the possible interaction between toxicants in complex mixtures (Altenburger et al., 2004; Knauert et al., 2008) and the intensity and timing of the exposure (Tlili et al., 2008; Vallotton et al., 2008, 2009) cannot be discarded. On the other hand, the physicochemical conditions of water (i.e. dissolved organic carbon, oxygenation, etc.) can act indirectly by influencing the bioavailability (Nikkila et al., 2001; Knauer et al., 2007) and persistence (Graham et al., 1999) of contaminants. That is why experiments which better mimic field conditions have to be developed (Rohr et al., 2006), enabling us to improve accuracy in the extrapolations from laboratory bioassays to responses in natural systems at the community level (Cairns, 1983). Studies including phototrophic biofilms (also known as periphyton) are particularly recommended because they are major contributors to carbon fixation and nutrient cycling in most of the fluvial systems and are also key targets for herbicide contamination because of their ecophysiological similarities with terrestrial plants (Sabater et al., 2007; Wetzel, 2005). Since the work of Blanck and Wängberg (1988), there has been an increasing literature reporting the development of high-throughput methods allowing the assessment of structural (i.e. species succession) and functional (i.e. photosynthesis, etc.) changes in periphytic algae pertubated by toxicants (for review, see Sabater et al., 2007). Some of these studies focused on environmental factors that could modulate the effect of herbicides (Sabater et al., 2007). Among these factors, light plays a potential major role because it is a prerequisite for photosynthetic processes and is highly variable in situ on temporal and spatial scales (Hill, 1996). Some long term studies have already examined the relationship between light history and toxicity of atrazine to periphyton communities collected at different seasons and different stream sites differing in light regime due to riparian vegetation (i.e. opened and shaded sites). These authors demonstrated that higher sensitivity of periphyton to this herbicide was linked to higher light conditions prevailing during the colonization period (Guasch et al., 1997; Guasch and Sabater, 1998; Guasch et al., 1998, 2003). In contrast to these long term studies, the existing literature investigating the interactive short-term effects of light and pollutants on algae is scarce (Cho et al., 2008; Cleuvers et al., 2002; Gavis et al., 1981; Millie et al., 1992; O'Neal and Lembi, 1983; Petersen and Kusk, 2000; Wängberg and Blanck, 1988). Only one study has described joint effects of these two factors on photosynthesis and growth inhibition, but only by focusing on constant light levels (Millie et al., 1992). It is known nevertheless that light fluctuates from limiting to excessive level on diurnal and even shorter timescale due to the occurrence of clouds or movements of the streamside vegetation (Hill, 1996). Light fluctuations could have measurable consequences on the physiological responses of algae, formally known as photoacclimation (Falkowski and Laroche, 1991), which were consistently described in a previous work for natural stream periphytic communities in field conditions (Laviale et al., 2009). To our knowledge, no one has yet tried to find out the potential effect of the diurnal variability of light encountered by algae in the field on herbicide toxicity.

In this context, the aim of this study was to estimate the effect of isoproturon, a widespread photosystem II (PSII) inhibitor listed as a priority substance in the WFD, on the photosynthesis of periphyton exposed to different light conditions. An experimental setup of increasing complexity was devoted to study the combined effects of light and isoproturon on the photochemical behavior of natural biofilms by measurements of chlorophyll fluorescence and pigment composition. The algae were not only exposed to different intensities of constant light, but a daily cycle was simulated as close as possible to in situ conditions to test whether an additional physiological stress for the algae induced by dynamically changing light intensities could have measurable consequences to their sensitivity toward isoproturon.

Table 1
List of endpoints studied and nominal isoproturon concentrations ($\mu g\,L^{-1}$) tested during experiments 1 and 2 (i.e. in constant and dynamic light, respectively).

	Nominal isoproturon concentrations ($\mu g\,L^{-1}$) tested
Experiment no. 1: constant light	
F_v/F_m	0–2–20–200–2000
Φ_{PSII}	0–2–6–12–20–60–120–200–2000
NPQ	0–2–20–200–2000
Experiment no. 2: dynamic light	
F_v/F_m	
Φ_{PSII}	
NPQ	0–2–6–20
Pigments	

2. Materials and methods

2.1. Biofilms and water collection

The study area is located in the end section of the Stream Sensée, upstream near the town of Douai (Nord-Pas de Calais, France; 50°19′32″N, 3°4′6″E). This is an eutrophic site (see Laviale et al., 2009 for a detailed description) which is known to be slightly influenced by agricultural activities: the highest values for the most abundant herbicides (isoproturon, atrazine and diuron) detected monthly during the previous 12 months before biofilms collection were lower than $0.2\,\mu g\,L^{-1}$ (data from Agence de l'Eau Artois-Picardie). Natural stream phototrophic biofilms were collected twice a week from June to July by means of glass slides (76 mm × 26 mm) that were placed in racks equipped with floats. The racks were immersed vertically and parallel to the water flow, 15 cm below the surface. After 2–3 weeks of colonization, the glass substrata were transported to the laboratory within 1 h in coolboxes filled with site water and transferred in dark climate (20 °C) chamber, until the exposure experiments were initiated.

2.2. Experimental design

Two independent experiments were carried out. Experiment 1 consisted of exposing biofilms to series of nominal isoproturon concentrations between 0 and $2\,mg\,L^{-1}$ (for details see Table 1) for 7 h under constant light at different irradiance levels (25, 50, 100, 200 and 300 $\mu mol\,m^{-2}\,s^{-1}$) which were chosen following standard test protocols (Nyholm and Källqvist, 1989). Chlorophyll fluorescence was measured at the beginning and then after 1, 3 and 7 h of isoproturon exposure. In experiment 2, biofilms were exposed to 2, 6 and $20\,\mu g\,L^{-1}$ of isoproturon (nominal concentrations) over a daily light cycle which was simulated by progressive fluctuation of irradiance over 7 h. Preliminary in situ measurements of the light intensity have been made in order to determine the relevant range of irradiance to be used: 0–1000 $\mu mol\,m^{-2}\,s^{-1}$ with 20 successive steps of 15 or 30 min (Fig. 1). Due to methodological reasons, the chosen light period (7 h) was significantly shorter than in the field (around 10 h) but it was the same in experiments 1 and 2. Several glass slides were collected for fluorescence measurements and pigment analysis at simulated dawn, mid-morning, zenith, mid-afternoon and sunset (5, 350, 1000, 350 and 5 $\mu mol\,m^{-2}\,s^{-1}$, respectively). At the end of the simulated daily light cycle, all the communities were gently rinsed and transferred in uncontaminated filtered stream water for 12 h in complete darkness and a last measurement of the fluorescence signals was performed.

2.3. Incubation conditions

First, the bottoms of the slides were cleaned of any algae. Each slide was incubated horizontally in a polycarbonate vessel (VWR, Fontenay sous Bois, France) containing 60 mL of filtered