Chemosphere 74 (2009) 1224–1230

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Photodegradation product of sulcotrione and the physiological response of maize (*Zea mays*) and white mustard (*Sinapis alba*)

Jaroslaw Wiszniowski [a,b,1], Alexandra Ter Halle [a], Claire Richard [a], Adnane Hitmi [c], Gérard Ledoigt [b,*]

[a] Laboratoire de Photochimie Moléculaire et Macromoléculaire, UMR CNRS-UBP 6505, Université Blaise-Pascal, 24 Avenue des Landais, 63177 Aubière Cedex, France
[b] ERTAC, UMR 547 PIAF université-INRA, Physiologie et Génétique Végétales, Université Blaise-Pascal, 24 Avenue des Landais, 63177 Aubière Cedex, France
[c] ERTAC-Laboratoire de physiologie et Biotechnologies Végétales, IUT de Clermont-Ferrand, Université d'Auvergne, 100, rue de l'Egalité, 15000 Aurillac, France

ARTICLE INFO

Article history:
Received 23 January 2008
Received in revised form 24 October 2008
Accepted 8 November 2008
Available online 27 December 2008

Keywords:
Chromone
Ecotoxicology
Herbicide
Plant stress
Sulcotrione photodegradation

ABSTRACT

One of the strategies for decreasing the consumption of herbicides consists in improving their uptake and efficiency. It was suggested that the photodegradation of herbicides due to sunlight results in a greater demand of herbicides to be introduced into the environment in order to ensure the plant protection activity. Moreover, an ecotoxicological effect of the photoproducts needs to be clarified. The physiological response of *Zea mays* and *Sinapis alba* (weed) to sulcotrione and its main photoproduct, called chromone (xanthene-1,9-dione-3,4-dihydro-6-methylsulfonyl), was evaluated under controlled conditions in a growth chamber. The dose-response effects were determined on *Z. mays* and *S. alba*. Using the sulcotrione (doses ranging from 1 to 9 mg per plant), the physiological parameters indicated a decrease of photosynthesis for the *S. alba* species while the *Z. mays* species were only slightly affected. On the contrary, the chromone had no herbicide activity on both species. The sulcotrione is known to block 4-hydroxyphenyl pyruvate dioxygenase (HPPD) enzyme. The differences between the parent herbicide and the photoproduct could be ascribed to drastic structural modifications. We have shown that the chromone probably do not block the HPPD active site.

1. Introduction

Nowadays, more and more concern is shown to the possibility of adverse effects of pesticides on human health and ecosystems. Agricultural uses of herbicide remain abundant because of the direct benefits their application generates (high agricultural yields and low labor input).

The extended risk assessment and ecotoxicological monitoring of plant protection products, including the main degradation products, is already required within the framework of existing European legislation (Plant Protection Products Directive 91/414/EEC; Water Framework Directive 2000/60/EC).

The sulcotrione (2-[2-chloro-4-(methylsulfonyl) benzoyl]-1,3-cyclohexanedione) belongs to a relatively new class of triketone herbicides, which are involve in the specific inhibition of the 4-hydroxyphenyl pyruvate dioxygenase (HPPD, EC 1.13.11.27) (Schulz et al., 1993; Secor, 1994). The decrease in carotenoid and chlorophyll contents resulted in bleaching symptom of new growing plants (Boger, 1996). Sulcotrione is quickly degraded in soil (Cherrier et al., 2004; Chaabane et al., 2005). Consequently, it has been successfully implanted in agriculture as an alternative to atrazine herbicide, which had been prohibited in France since 2003.

Recent studies have proved that exposure to sunlight can be one of the most destructive factors for pesticides following the crop treatment (Katagi, 2004). A quick phototransformation of herbicide can reduce its herbicidal activity (Mangel, 1991).

The sulcotrione photochemistry has only been described recently. The xanthene-1,9-dione-3,4-dihydro-6-methylsulfonyl (chromone) is the sulcotrione main photoproduct (ter Halle et al., 2006). This photoproduct is generated after the intramolecular cyclization of sulcotrione at the surface of cuticle wax coating the leaves, or in the aqueous medium. A field monitoring demonstrated that sulcotrione decay on maize leaves after foliar application was mainly due to photodegradation. Moreover, chromone was shown to accumulate on maize leaves (ter Halle et al. 2007).

The chromone was isolated and characterized, but its biological activity has not been evaluated so far. The physiological responses of plant exposed to the studied molecules were mainly followed *in vivo* by the chlorophyll *a* fluorescence (Maxwell and Johnson, 2000), a technique widely applied for detecting herbicide-induced perturbations in plant metabolism (Kim et al., 1999, 2002; Korres et al., 2003). The analysis was achieved by photosynthesis gas exchange measurements (Creech et al., 2004). Moreover, the plant response was examined through the analysis of physiological

* Corresponding author. Tel.: +33 04 73 40 79 08; fax: +33 04 73 40 79 42.
E-mail address: Gerard.LEDOIGT@ertac.univ-bpclermont.fr (G. Ledoigt).
[1] Present address: The Silesian University of Technology, Environmental Biotechnology Department, Akademicka 2, 44-100 Gliwice, Poland.

0045-6535/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2008.11.017

parameters known to be involved in stress responses, such as the contents of pigment and accumulation of free proline (Saladin et al., 2003). The main goal of the study was to evaluate the phytotoxic action of chromone product towards a crop plant, *Zea mays* and a weed (target plant), *Sinapis alba*.

2. Methods

2.1. Materials

The sulcotrione (98.7%) was obtained from Riedel-de-Haën, and the 2-chloro-4-methylsulfonylbenzoic acid (95%) from ACROS. The commercial formulated product had a 300 g L^{-1} active sulcotrione content.

2.2. Photochemical set up and preparation of the chromone

Photochemical experiments were carried out in Pyrex tube reactors operating in batch test mode. The irradiation device was made of six tubes of sunlamps Ducke FL 20 (20 W) fitted in a metal cylindrical enclosure. The lamps and the reactor were, respectively, put along the focal axes of a cylindrical mirror with an oval base. The fluorescence lamps emitted radiations in the range from 290 to 600 nm wavelength with the maximum at 310 nm wavelength. The cooling fan installed at the bottom allowed maintaining a stable temperature of 20 °C in the reactor.

The sulcotrione commercial formulation was used for the chromone synthesis. In order to ensure a high rate of production, the phototransformation was performed in pure acetonitrile (Sigma, first grade >99%). The commercial product was mixed up with distilled water in the ratio 1–10. Then, aliquots of product–water mixture (equivalent to 30 mg sulcotrione) were dissolved in 100 mL acetonitrile. Before the irradiation, the solution was filtered through filter paper in order to remove filamentous residues (some products of the formulation) and the filtrate was put in the photoreactor. The reaction was followed by HPLC a Water 515 HPLC pump and a 115 Gilson UV detector fitted with a reverse phase column (Nucleodur® C8 Gravity 5 μm, 250 mm × 4.6 mm Nucleodur, Macherey-Nagel). The mobile phase was a mixture of 45% water (with formic acid, 3‰ v/v; Sigma, analytical grade) and 55% methanol (v/v; Sigma, for HPLC). When the photochemical reaction was stopped, the solvent was evaporated with a rotavapor (Buchi R 210) under vacuum and the remaining residue was stored. The residues from three irradiations were subsequently pooled and purified in silica gel chromatography (Merck, 230–400 mesh) with a mixture of dichloromethane and acetone as a mobile phase (in ratio 80/20 v/v). The purity of recovered chromone was validated by ^{1}H NMR ($CDCl_3$) and by HPLC-ESI-MS analysis. The HPLC-ESI-MS analyses were performed using a Hewlett-Packard HP1100-MSD system working in positive and negative atmospheric pressure ionization. The column was a Varian Omnispher® C18 100 mm × 3.5 mm (3 mm). The ^{1}H NMR spectra was recorded on a Bruker AM 400 MHz spectrometer using tetramethylsilane (TMS) as internal standard.

2.3. Plant materials and herbicide treatment

The seeds of *Z. mays* cv. Bangui and *S. alba*, were kindly provided by Limagrain and the Botanical Gardens of Clermont-Ferrand, respectively. The seeds of each plant were grown up in a greenhouse (30/20 °C, day/ night and under 16-h photoperiod). At the three-leaf stage, the plants were transferred in single plant pots (95 cm^2) in substitute soil with Humustar substrate. The plants were used at the 5–6 leaf stage, corresponding to the treatment with sulcotrione in agriculture.

The short-term effect (within 5–8 days) of the chromone, on the physiology of maize (*Z. mays*) and white mustard (*S. alba*), was evaluated in a growth chamber. The following doses were used: 1, 3 and 9 mg per plant. Parallel studies were undertaken using sulcotrione and formulated sulcotrione.

It was assumed that maize population density was 100 000 per ha and application rate amounts to 300 g of active substance (sulcotrione) per ha. It corresponds to a concentration of 3 mg of active substance per plant (or 30 mg m^{-2}). In the present studies 3–4 replicates (plants) for each concentration of active substance were used in a greenhouse; it corresponded to plant density in the field of maize. For instance, 4 plants were arranged per 400 cm^2 and were sprayed with 12 mg of active compounds solution. It ensured application dose of 3 mg per plant.

The sulcotrione, commercial form and chromone solutions were prepared in acetone and diluted with distilled water including non-ionic surfactant (Tween 20) (Kim et al., 2002). The final concentrations of acetone and Tween 20 in the solutions were 50% and 0.1%, respectively. The plants were sprayed out with solutions containing water, acetone and Tween 20 (as a control) or with solutions containing different concentrations of tested molecules, acetone and Tween 20. Then the plants were transferred into a controlled chamber with a 16 h-photoperiod and an irradiance of 250 μmol m^{-2} s^{-1} of photosynthetically active radiations (PAR) provided by sodium lamps. The PAR measurement was carried out using a quantum sensor LI-COR, Lincoln, NE, USA at the plant level. Day/night temperatures were 28 ± 2 °C/20 ± 2 °C and the relative humidity was 50 ± 10%. Plants were watered daily to maintain high water contents in the soil profile (above 40%).

2.4. Chlorophyll content

The carotenoid and chlorophyll contents were estimated according to Lichtenthaler and Welburn (1983) after an extraction with acetone (80% v/v). Three or four replicates (plants) for each concentration were tested.

2.5. Chlorophyll a fluorescence

The chlorophyll *a* fluorescence transients were determined *in vivo* using a pulse amplitude modulated fluorometer (FMS1, Hansatech Instruments Ltd., Norfolk, UK). The measurements were carried out on the adaxial side of the second and third youngest leaves. Six measurements per plant were performed. The method was applied 1, 2, 3, 5 and/or 8 days after the herbicide treatment. The fluorescence parameters such as the maximum quantum yield (F_v/F_m) and the quantum efficiency (ΦPSII) were determined in the dark-adapted (after 6 h) and the light-adapted (after 5 h) leaves, respectively (Genty et al., 1989; Maxwell and Johnson, 2000). The fluorescence readings of F_v/F_m were carried out in a dark room under safe light (green light). The maximal fluorescence (F_m) was obtained with a saturating flash (1 s, 13 000 μmol m^{-2} s^{-1}).

2.6. Gas exchange/photosynthesis

After eight days of treatment with the chromone, the sulcotrione or the commercial product, the net photosynthetic rates (P_n) were measured using a handle-care infrared gas analyser (LI-Cor Model 6400, Lincoln, NE, USA). The apparatus was equipped with a clamp-on leaf bowl that exposed 6 cm^2 of leaf area. The light, temperature and humidity parameters were 400 μmol m^{-2} s^{-1}, 25 ± 2 °C and 30%, respectively. The CO_2 was maintained at a constant level of 360 μmol mol^{-1}. The test was performed with 9 mg per plant dose at eight days. Four to six measurements were carried out for each concentration.