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Transformation of ^{14}C-pyrimidynyloxybenzoic herbicide ZJ0273 in aerobic soils

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ABSTRACT

A soil metabolism study of propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoate (ZJ0273), a novel broad-spectrum herbicide, was carried out using ^{14}C labeled on two different rings, i.e., [*pyrimidine*-4,6-^{14}C] ZJ0273 and [*benzyl*-U-^{14}C] ZJ0273. Ultralow liquid scintillation counting and LC-MS/MS were used to identify the degradation intermediates and quantify their dynamics in aerobic soils. Four aromatic intermediates, 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoic acid (M1), 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzamido)benzoic acid (M2), 2-(4,6-dimethoxypyrimidin-2-yloxy)benzoic acid (M3), and 4,6-dimethoxypyrimidin-2-ol (M4), were identified and their identity was further confirmed against authentic standards. Analysis of metabolites suggested two degradation pathways: (1) Upon loss of the propyl group, M1 was produced via hydrolysis of propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy) benzylamino)benzoate after which the C–N bond between rings A and B was cleaved by oxidation and biochemical degradation to yield M3, which was further converted into M4 and finally mineralized to CO_2; and (2) the first step was the same as in pathway 1, but M1 first underwent a carbonylation to form M2. The C–N bond between rings A and B of M2 was cleaved by hydrolysis to yield M3. Dynamic changes in the four metabolites in aerobic soils were also investigated by HPLC coupled analysis of radioactivity of isolated peaks. After a 100-day incubation, 1.7–9.7% of applied ^{14}C was found as M1, 0.3–1.1% as M2, 14.5–20.9% as M3, and 3.7–6.7% as M4 in the soils, and pH appeared to be the most influential soil property affecting the formation and dissipation of these metabolites.

1. Introduction

Oilseed rape ranks as the third most important oilseed crop worldwide (Fu et al., 2003; Li et al., 2006; Liu et al., 2008). In China, the planting area of oilseed rape was 7.27×10^6 ha in 2005 (National Bureau of Statistics of China, 2006). In oilseed rape fields, weeds can greatly decrease rapeseed yield making weed management a great challenge for rapeseed production. However, the extensive use of massive amounts of herbicides has resulted in herbicide-resistant weeds, which has been an established fact that weeds reduce farm yields and farm income drastically. Among all other weed control practices, herbicide alone is easy prompt, most effective and economically acceptable mean (Ozair, 2010). Thus, it is essential to seek novel and highly effective herbicide substitutes for weed control in China. Propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoate (abbreviated herein as ZJ0273), is a new herbicide for weed control against pre- and post-emergence weeds in oilseed rape fields and is usually applied at 40–60 g ai/ha^2 to achieve an eradication efficiency of 80–90% (Lu et al., 2004; Tang et al., 2005). The herbicide, same as Pyribenzoxim, is classed as an acetolactate synthase (ALS) inhibiting pyrimidynyloxybenzoic herbicide due to the fact that, when applied, it is easily degraded into the biologically-active ingredient, 2-(4,6-dimethoxypyrimidin-2-yloxy)benzoic acid, which inhibits the ALS in plants. In recent years, the acreage of ZJ0273 application has quickly expanded in China, reaching 533,000 ha in 2007 (Wang et al., 2009a).

We recently carried out studies to understand the environmental behavior and fate of ZJ0273, including absorption, translocation and residue accumulation in oilseed rape plants, and formation of bound residues and their bioavailability to succeeding crops. For instance, Han et al. (2009a) found that absorption and translocation of ZJ0273 in oilseed rape plants were both acropetal and basipetal, and suggested that there was a low dietary exposure risk for humans if the recommended application rates were followed. Yang et al. (2009) detected only a trace level of ZJ0273 residue presents in rapeseed. Wang et al. (2009a) reported that bound residues from [*pyrimidine*-4,6-^{14}C] ZJ0273 and [*benzyl*-U-^{14}C] ZJ0273 formed in aerobic soils after 100-day incubation were substantially lower than the non-accumulative criteria (70%) set by the Commission of the European Communities. Han et al. (2009b) studied plant availability and phytotoxicity of soil bound

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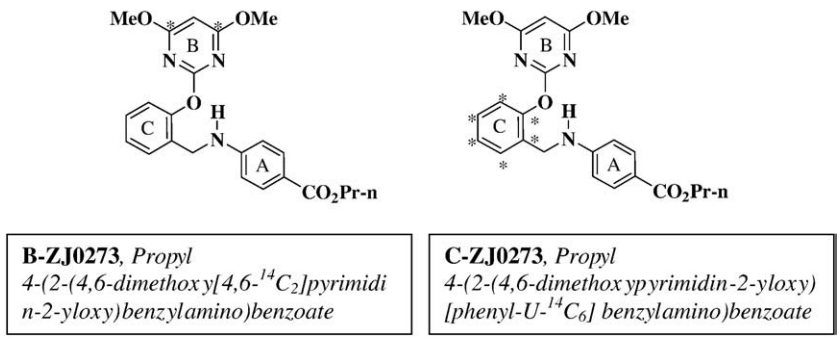

Fig. 1. Structures of radiotracers, ZJ0273 with asterisks marking the position of ^{14}C.

residues derived from ZJ0273 and found that significant inhibition was seen in rice seedlings, while no significant inhibition occurred to corn seedlings, reflecting the potential release and phytotoxicity of 2-(4,6-dimethoxypyrimidin-2-yloxy)benzoic acid under certain conditions. However, currently little is known about the metabolism and degradation pathways of ZJ0273 in soil.

Identifying degradation intermediates and characterizing metabolism pathways in soil is critical for risk evaluation of new agrochemicals. The use of ^{14}C isotope labeling is often a preferred approach for intermediate identification (Primus et al., 1997; Pascal-Lorber et al., 2004; Cao et al., 2006; Chang et al., 2007). However, different labeling positions of ^{14}C on the same molecule may give vastly different information. For instance, previously Wang et al. observed that O–N-type Smiles rearrangement in ZJ0273 caused the detachment of ^{14}C label in some degradation products due to metabolism (Wang and Guo, 2004; Wang et al., 2005). Therefore, ZJ0273 labeled with ^{14}C on different aromatic rings was employed in this study, to provide additional information and verification of experimental results. This paper is the first report on soil metabolism of ZJ0273 under aerobic conditions that describes the degradation pattern of ZJ0273, using multi-position ^{14}C-labels and HPLC analysis coupled with mass spectrometry detection.

2. Experimental section

2.1. Chemicals

The two radiolabeled compounds, propyl 4-(2-(4,6-dimethoxy [4,6-^{14}C]pyrimidin-2-yloxy)benzylamino)benzoate (B-ZJ0273, radiochemical purity 99.4%; chemical purity 98.1%; specific activity 3.77×10^7 Bq/mmol) and propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)[*phenyl*-U-^{14}C] benzylamino)benzoate (C-ZJ0273, radiochemical purity 98.9%; chemical purity 98.1%; specific activity 3.74×10^7 Bq/mmol) (Fig. 1) and five non-labeled standards of ZJ0273 (Formula Weight, FW 423) and its metabolites, 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoic acid (M1, FW 381), 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzamido)benzoic acid (M2, FW 395), 2-(4,6-dimethoxypyrimidin-2-yloxy) benzoic acid (M3, FW 276), and 4,6-dimethoxypyrimidin-2-ol (M4, FW 156) were synthesized at the Institute of Nuclear Agricultural Sciences, Zhejiang University, China and Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences (Yang et al., 2005, 2008, 2009). HPLC grade methanol was purchased from Burdick & Jackson (MI, USA). All other reagents and common chemicals were analytical grade.

2.2. Test soils

Three agricultural soils (S_1, red clayey soil; S_2, fluvio-marine yellow loamy soil; S_3, coastal saline soil) were used for the incubation experiment using both ^{14}C labels. The soils were taken from the surface layer (0–15 cm) of fields from different quarters of Zhejiang Province, China. The soils were air dried, mixed, and passed through a 1-mm sieve before use. Some basic physicochemical characteristics of the soils were determined using standard methods (Nelson and Sommers, 1982; Gee and Bauder, 1986) and may be found in Wang et al. (2009a).

2.3. Incubation, extraction and pretreatment

The incubation experiment using multi-position ^{14}C-labels in aerobic soils followed the procedure given in our previous study (Wang et al., 2009a). Briefly, incubation flasks were connected to a series of air-tight test tubes, which allowed for the scrubbing of CO_2 from the inlet air, and for maintaining constant soil moisture, and entrapment of volatiles and $^{14}CO_2$. During each sampling period, the subsamples of incubated soils (10.0 g, air-dried weight equivalent) were removed for determination and identification of degradation products. Soil subsamples (10.0 g) were consecutively extracted with solvents of decreasing polarity, from 0.01 M $CaCl_2$, to acetonitrile/water (9:1, v/v), methanol, and finally dichloromethane (Mordaunt et al., 2005) until no further radioactivity was detected in the centrifugation supernatant. The 0.01 M $CaCl_2$ extract was adjusted to pH 3.0 and the solution was extracted with dichloromethane (1:1, v/v) three times. The dichloromethane phases were combined, concentrated under vacuum on a rotary evaporator (Eyela SB-1000, Eyela Co., Tokyo, Japan) at 40 °C until dry and then combined with the extracts from the other three steps. The combined mixture of extracts was concentrated to 1.0 mL on the rotary evaporator at 40 °C and centrifuged again for 15 min before HPLC analysis.

2.4. Analytical HPLC with ULLSS

The analysis was conducted on a Waters HPLC system, using a Waters 996 photodiode array (PDA) detector at 254 nm and 301 nm, and a Diamonsil C_{18} column (5-μm, 250×4.6 mm, Dikma Technologies Inc., CA, USA) with a C_{18} guard column (30×4.6 mm, Dikma Technologies). The column temperature was maintained at 30 °C. The mobile phase for analysis was composed of water and methanol, both containing 0.1% acetic acid and the elution was achieved using a gradient program (min/%A (water + 0.1% acetic acid): 0/80, 0–40/25, 40–80/25, 80–90/0) at a flow rate of 1.0 mL min^{-1}. The sample injection volume was 20 μL. The fractions were collected by a programmable fraction collector. The ^{14}C activity of the eluate was detected every minute by a Quantulus 1200 ultralow liquid scintillation spectrometer (ULLSS, Wallac, Turku, Finland), to precisely determine the accumulation of degradation products.

2.5. LC-MS analyses

To elucidate the structures of the intermediates, LC-MS/MS analysis was carried out on a Micromass Quattro micro API™ with a HPLC detector and triple quadrupole mass analyzer for determining mass-to-charge ratio (m/z) for a wide variety of analytes (Waters, Milford, MA, USA). Control of the instruments and calculation was made using MassLynx V4.1 software (Waters). The instrument was operated in positive ESI ionization mode. Operating conditions were optimized by constantly introducing a