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Vertical distribution in soil column and dissipation in soil of benzoylurea insecticides diflubenzuron, flufenoxuron and novaluron and effect on the bacterial community

Yu-Ling Hsiao, Wei-Hao Ho, Jui-Hung Yen *

Department of Agricultural Chemistry, National Taiwan University, No. 1, Roosevelt Road, Sec. 4, Taipei 10617, Taiwan

HIGHLIGHTS

- The three benzoylureas were dissipated in soils mainly by microorganisms.
- Flufenoxuron had the most severe impact on soil microorganisms.
- A large amount of benzoylureas adsorbed in the upper layer of soil columns.
- The use of flufenoxuron and novaluron influences soil bacterial communities.

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ABSTRACT

Benzoylurea insecticides are used for prevention and eradication of household or field pests. However, few studies have investigated their distribution and dissipation in soils and the effects on the soil microbial community. We examined the dissipation and vertical distribution of diflubenzuron, flufenoxuron and novaluron and their effects on bacterial diversity in two soils in Taiwan. The dissipation of the three benzoylureas was concentration dependent. The half-life of 1, 10 and 50 mg kg^{-1} concentration was from 3.0 to 45.9, 52.1 to 433.2 and 27.7 to 533.2 d, respectively. The proportion of residual benzoylureas in sterilized soils remained up to 83% at the end of the incubation, which implied that the dissipation was mainly by microorganisms. All three benzoylureas were not detected below 10 cm in soil column experiments. Comparison of initial pesticides concentrations (50 mg kg^{-1}), diflubenzuron was detected at <1%. However, flufenoxuron and novaluron remained at >30% and 50% in Pu and Wl soil, respectively after leaching for 70 d. Furthermore, the presence of flufenoxuron and novaluron at 5- to 10-cm depth led to greater change in bacterial community diversity in Pu than Wl soil.

1. Introduction

Benzoylurea insecticides inhibit the synthesis of chitin (the major structural component of arthropod exoskeletons) during insect development. Diflubenzuron, flufenoxuron and novaluron are halogenated benzoylureas and effective stomach and contact insecticides. Diflubenzuron is often used to control field insects such as tobacco cutworm (*Spodoptera litura*), tea stem borer (*Casmara patrona*) and *Brevicoryne brassicae* (Verloop and Ferrell, 1977). It is also used in public health applications against mosquito and noxious fly larvae. Flufenoxuron is used as a foliar spray. Depending on the crop and the country of use, 1–4 foliar applications per season are recommended for crops at rates equivalent to 0.036–0.22 lb ai/A/application. Flufenoxuron is also used as a public hygiene insecticide mainly against insects such as cockroaches and fleas (Clarke and Jewess, 1990). Novaluron is registered as an insecticide for food crops (e.g., soya, maize, pome fruit, citrus and potato) and ornamental plants in a number of countries (Cutler and Scott-Dupree, 2007). The World Health Organization assessed diflubenzuron and novaluron for use as mosquito larvicides in drinking-water containers and as a vector control in drinking water, particularly to control dengue fever because they exhibit low toxicity for mammals and other non-target organisms (WHO, 2008a,b).

The half-life of benzoylureas in the environment varies by type of medium such as soils, water or vegetables (Mansager et al., 1979; Seuferer et al., 1979; Antón et al., 1993; Mabury and Crosby, 1996; Pal et al., 2008). The half-life of diflubenzuron ranges from hours to months (Mabury and Crosby, 1996), that of novaluron in tropical soils ranges from 11 to 59 d (Pal et al., 2008), and flufenoxuron does not readily break down in the environment (ACP,

* Corresponding author. Tel.: +886 2 33664813; fax: +886 2 23620105.
E-mail address: sonny@ntu.edu.tw (J.-H. Yen).

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1994). Most studies have focused on the physical or chemical dissipation of these benzoylureas (Mansager et al., 1979; Antón et al., 1993; Marsella et al., 2000), not the direction of long-term leaching in soils or the possibility of microbial-mediated degradation and the effects of the benzoylureas on microorganisms in soils.

The diversity of the microbial community in soil is an important issue in modern soil microbiology and one of the main indicators of toxic effects of pesticides in agriculture. The diversity of soil bacterial communities has been investigated with methods to isolate and culture microorganisms (Gelsomino et al., 1999). Recent advances in molecular biology have allowed for developing techniques that no longer require the isolation and culture of bacteria to therefore reduce the associated bias. Molecular biological methods have been used to study the diversity and ecology of microorganisms in natural environments for decades and PCR–DGGE has also been developed to understand the diversity of bacteria in environment since the mid-1990s (Felske et al., 1996; Muyzer and Smalla, 1998; Wang et al., 2004, 2009; Chen et al., 2009; Yen et al., 2009).

In this study, we investigated the dissipation and distribution of three benzoylureas in soils and used molecular biology methods to study their effect on the microbial populations in two soil samples in Taiwan.

2. Materials and methods

2.1. Standard materials and reagents

Analytical standards of the benzoylurea insecticides diflubenzuron (99.9%) and flufenoxuron (98.3%) were from Riedel-de Haën (Germany), and novaluron (99.6%) was from the Institute of Organic Industrial Chemistry (Poland). The physical and chemical properties of the three benzoylurea insecticides were listed in Table 1. Acetonitrile and acetone were high-performance liquid chromatography (HPLC) grade and purchased from Merck (Germany). Ultrapure water was obtained in the laboratory by a Milli-Q water purification system (Millipore, Billerica, MA). All other chemicals and solvents were of analytical grade and from commercial sources.

2.2. Soil samples

Samples of two soils representing different physicochemical properties and climatic environments were collected from two sites in Taiwan. The physical and chemical properties of the soils are in Table 2. The Pu soil was from the Taoyuan District Agricultural Research and Extension Station of Taiwan. The Wl soil was from the Hualian District Agricultural Research and Extension Station of Taiwan. The Pu soil was acidic loam and the Wl soil was alkaline sandy loam. These test soils, known to be free of benzoylurea insecticide residues, were collected randomly from the surface layer (0–20 cm). Each soil sample was divided into two portions: one was air-dried at room temperature then sieved through a 2-mm sieve for pesticide-degradation and column-leaching experiments and the other was kept at 4 °C as fresh soil for PCR experiments.

2.3. Incubation of pesticides in soils

Incubation experiments were as described (Wang et al.) with modification. Stock solutions of pesticides were prepared in acetone with analytical standards of the benzoylurea insecticides (10 mg mL^{-1}). Working solutions (0.1, 1 and 5 mg mL^{-1}) of the pesticides were also prepared in acetone from the stock solutions. Approximately 10 g of air-dried soil for incubating the three pesticides was placed into 50-mL plastic centrifuge tubes. Samples were incubated at 25 ± 1 °C in the dark with 100 μL of working solutions containing approximately 10–500 μg diflubenzuron, flufenoxuron and novaluron. After soil was allowed to air-dry (~10 min) for the solvent to completely evaporate, the soil was mixed thoroughly and then adjusted the soil moisture content to 60% of water holding capacity of the test soils. The final concentration of diflubenzuron, flufenoxuron and novaluron in soils was approximately 1, 10 and 50 mg g^{-1} soil (dry weight), respectively. During the incubation, soils were periodically sampled and placed in the freezer (−20 °C) for storage. The moisture content was regularly checked by weighing and kept constant with the addition of deionized water. To determine the microbial-mediated degradation of pesticides, soils were sterilized by autoclaving three times at 121 °C (1 atm.) for 30 min to eliminate microbial activity, then incubated with stock solutions of the pesticides. The final concentration was 10 mg g^{-1} soil (dry weight). All soil incubation experiments were carried out in triplicate.

2.4. Column-leaching experiment

The experiment was performed according to the OECD guidelines (OECD, 2004) and continued for 70 d. Polyvinylchloride tubes (6.5 × 48 cm) were used to pack the soil columns. To support the soil, filter papers (Whatman Grade No. 1; 125 mm diam.) and wire mesh cloth (0.145 cm) were held in place above the bottom of the column to prevent soil washout. About 2 kg soil was placed in the columns at even intervals (five intervals, 400 g each) with consistent knocking to minimize variation and to prevent excess pores among soil particles and between columns. After packing, the columns were put into a tank filled with 20 L of isocratic solution (0.01 N $CaCl_2$) to pre-wetted from bottom to top until the soil was saturated by capillary action to avoid air pocket forming. Thereafter the soil columns were allowed to equilibrate and the excess water is drained off by gravity. 3.25 mg of insecticides were spiked into dried soil (10 mg). After soil was allowed to air-dry (~10 min) for the solvent to completely evaporate, the soil was added onto the surface of each soil column. Therefore the application rate of these insecticides approximately equals 50 times the recommended application rate. The columns were fixed vertically and then infiltrated with diflubenzuron, flufenoxuron and novaluron. Isocratic solution (0.01 N $CaCl_2$), 100 mL, was added daily to the top of the column slowly to simulate the average daily rainfall in rainy season in Taiwan and allowed to infiltrate into the soils thoroughly to prevent anaerobic situation. Effluent was collected from the bottom of the columns for analysis of pesticide residue by HPLC.

2.5. Soil sampling and extraction

The column soil was equally sectioned into parts every 5 cm after leaching. Soil (50 g) of every section was randomly sampled and analyzed for pesticides and the remaining soil was refrigerated at 4 °C for assessment of bacterial communities. Soil samples were placed in a 250-mL Erlenmeyer flask and extracted with 50 mL

Table 1
Physical and chemical properties of the three benzoylurea insecticides.

Benzoylurea insecticides	Molecular formula	Vapor pressure (mPa, 25 °C)	K_{OW} (log P)	Melting point (°C)	Solubility (mg L^{-1} H_2O, 25 °C)
Diflubenzuron	$C_{14}H_9ClF_2N_2O_2$	1.2×10^{-4}	3.89	228	0.08
Flufenoxuron	$C_{21}H_{11}ClF_6N_2O_3$	6.5×10^{-9}	4.00	169–172	0.00152
Novaluron	$C_{17}H_9ClF_8N_2O_4$	1.6×10^{-2}	4.30	176.5–178	0.003