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Rapid degradation of bensulfuron-methyl upon repeated application in paddy soils

XIE Xiao-mei[1], LIU Wei-ping[2], ABID Subhani[1]

(1. College of Environmental and Resource Sciences, Department of Resources Science, Zhejiang University, Hangzhou 310029, China. E-mail: xiexiaomei686@yahoo.com.cn; 2. College of Environmental and Resource Sciences, Department of Environmental Science, Zhejiang University, Hangzhou 310029, China)

Abstract: Rapid degradation of bensulfuron-methyl upon repeated application in paddy soils was studied. The results showed that the DT_{50} of bensulfuron-methyl was reduced from 16 d to 9 d in soil with one-year bensulfuron-methyl application. Rapid bensulfuron-methyl degradation was happened to previously untreated soil by addition 5% rapid bensulfuron-methyl adapted soil and was inhibited following pre-treatment with broad-spectrum antibiotic chloramphenicol. In bensulfuron-methyl adapted soil mineralisation of ^{14}C labeled bensulfuron-methyl to $^{14}CO_2$ occurred at a faster rate than with previously untreated soil. It was concluded that rapid bensulfuron-methyl degradation upon repeated application is probably linked to the adaptation of soil bacteria which can utilize bensulfuron-methyl as a source of carbon and energy.

Keywords: bensulfuron-methyl; rapid degradation; paddy soil

Introduction

The herbicide bensulfuron-methyl is a sulphonylurea (methyl-2-[[[(4, 6-dimethoxy-pyrimidin-2-yl)-aminocarbonyl]-aminosulfonyl]methyl]benzoate) used to control many broadleaf weeds in rice field (Yuyama, 1984). These factors have contributed to their high-efficiency (Beyer, 1988). In China, rice cultivation is common and bensulfuron-methyl is widely used in paddy fields to control weeds during the flooding season which can easily cause environmental pollution by direct runoff or other routes (Thompson, 1992). Continued widespread use and release of such synthetics has become common, resulting in environmental pollution (Yuyama, 1987; Beyer, 1988; Fletcher, 1993). Hence, predicting the fate of herbicides in paddy soil is necessary to prevent their toxic influences.

Rapid degradation upon repeated application has been reported for numerous pesticides (Felsot, 1989; Kaufman, 1986; Roeth, 1986) and became the subject of several symposia (Racke, 1990). In many cases rapid degradation of pesticides has been linked to elevated populations of soil bacteria capable of pesticide degradation (Karns, 1990). Other studies have elucidated the biological nature of rapid pesticide degradation. Such studies have examined whether the characteristic can be transferred between soils and the influence of microbial inhibitors (Skipper, 1986; Walker, 1993). These approaches have also been used to elucidate the biological nature of soilborne diseases (Wiseman, 1996). An alternative methodology for confirming rapid pesticide degradation in soil is to monitor the evolution of $^{14}CO_2$ from soil treated with ^{14}C-labelled pesticide. In rapid degrading soils, radiolabelled compounds are typically mineralized to $^{14}CO_2$ at a faster rate than observed from control soils (Skipper, 1990). Rapid mineralisation of radiolabelled substrates suggests utilization of the radiolabelled region of the compound as a source of carbon and/or energy.

The objectives of the present experiments were to confirm rapid bensulfuron-methyl degradation in soil upon repeated application and to elucidate the biological nature of rapid bensulfuron-methyl degradation.

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1 Materials and methods

1.1 Soils and general methods

Soils were sampled from the surface layer (0—15 cm) of three agricultural fields used for rice cultivation near Jinhua City, Zhejiang Province, China, after removal of surface water. Where appropriate, soils without history of bensulfuron-methyl application (control) were collected prior to collecting soil previously treated with bensulfuron-methyl. The soil was dried to moist condition, passed through a 2-mm sieve and stored at 4℃ prior to analyses. A subsample of the soil was taken, air-dried, ground, and analyzed for various physico-chemical characteristics (Anderson, 1993). The total organic carbon, mechanical properties, pH and bensulfuron-methyl history of the soils are listed in Table 1.

Table 1 Characteristics of the soil used

Soil	Total organic carbon, g/kg	pH (H_2O)	Clay, g/kg	Silt, g/kg	Sand, g/kg	Years of bensulfuron-methyl application
Field A	15.3	4.74	160	562	278	1998—2001
Field B	16.3	4.87	128	599	273	None
Field C	17.5	4.75	141	594	265	1996—2001

Bensulfuron-methyl degradation kinetics were determined under laboratory conditions (60% water-holding capacity (WHC), 25℃). Bensulfuron-methyl was supplied by DuPont De Nemours S. p. a. Agricultural Products, with a purity level of 99.4%. ^{14}C bensulfuron-methyl (23.3 MBq/kg with a purity level of 95%) was supplied by Institute of Nuclear Energy Utilization of Chinese Academic Agricultural Sciences. The remaining of bensulfuron-methyl in soil extracted as Wu *et al*. described (Wu, 2000) and determined by a high-pressure liquid chromatograph (HPLC) equipped with UV detector at 224 nm wavelengths. HPLC separation was performed on a C_{18} reversed-phase column, using a mixed solution as mobile phase (mixed with (v/v) 60% CH_3CN, 35% H_2O and 5% of 0.085 % H_3PO_4 solution). The flow rate was 1 ml/min and injection volume was 10 μl.

Ten *cyperus compressus L*. seeds were sown into the remaining soil in each container. Following 7 days incubation at 25℃, seeds were recovered and *cyperus compressus L*. mortality recorded.

1.2 Repeated bensulfuron-methyl application under laboratory conditions

Soil(150 g, oven dry equivalent) collected from field A(3 years bensulfuron-methyl application) was weighed into 64 plastic containers fitted with loose fitting lids. One half of the containers were treated with 1.0 mg/kg bensulfuron-methyl, the remaining containers were treated with an equivalent amount of water. Following mixing, samples were incubated at 25℃ for 56 d. All soil samples were then treated with 1.0 mg/kg bensulfuron-methyl and mixed thoroughly. After treatment, four replicate containers of soil from each treatment were assayed by HPLC and *cyperus compressus L*. bioassay to determine remaining of bensulfuron-methyl, sampling and seeding times were at 0, 1, 3, 7, 14, 21, 28, 49 d.

1.3 Effect of a single field application of bensulfuron-methyl on subsequent bensulfuron-methyl degradation

Four plots(10 × 2 m) within field B(previously untreated with bensulfuron-methyl) were treated with bensulfuron-methyl(0.113 kg/hm^2). Four replicate control plots remained untreated. Fourteen months later, each plot was sampled approximately 0.5 kg by composting three samples in different sites. Bensulfuron-methyl(1.0 mg/kg) degradation kinetics was assayed by HPLC and *cyperus compressus L*. bioassay as described above.

1.4 Antibiotics, antifungal and heat sterilization

Rapid bensulfuron-methyl degrading soil and a previously untreated soil were collected from adjacent fields(B and C respectively, Table 1). Four replicate soil samples from each field(150 g) were treated with either chloramphenicol(50 mg/kg) or cycloheximide(50 mg/kg), were heat sterilized(3 h at 121℃), or remained untreated. After incubation at 25℃ for 3 d, soil samples were treated with 1.0 mg/kg bensulfuron-methyl. In contrast to other experiments, bensulfuron-methyl was incorporated by shaking the intact containers of soil to minimize contamination of heat treated soil samples. Bensulfuron-methyl degradation was monitored by HPLC in soil samples at 7 and 14 d after addition of bensulfuron-methyl. This experiment was repeated and the results averaged. Data were analyzed statistically according to Completely Randomized Design using CoStat Software (CoStat Statistical Software, 1990).

1.5 Transfer of rapid bensulfuron-methyl degradation

Soil samples were collected as for the previous experiment from fields B and C. Soil was removed from containers containing control soil (field B) and replaced with rapid bensulfuron-methyl degrading soil(filed C) to give final percentages of 0%, 1%, 5%, 10% and 100%(w/w) rapid bensulfuron-methyl degrading soil. Soil in each container was then mixed thoroughly and treated with bensulfuron-methyl(1.0 mg/kg). Soil subsamples(25 g) were taken at 7 and 14 d after treatment and analysed by HPLC. Data were subjected to analysis of variance using CoStat Software.

1.6 $^{14}CO_2$ evolution from soil treated with ^{14}C-labelled bensulfuron-methyl

Rapid bensulfuron-methyl degrading soil(field C) and previously untreated soil(field B) was weighed into disposable plastic containers and was treated with ^{14}C labeled bensulfuron-methyl(9×10^4 Bq/kg). After mixing, the soil in each container was divided into 25 g samples and placed into 250 ml HPDE bottles with screw closures. Evolved CO_2 and $^{14}CO_2$ in each bottle was trapped in vials containing 2 ml of 0.5 mol/L NaOH. CO_2 traps were changed weekly and $^{14}CO_2$ was determined by liquid scintillation following addition of scintillation fluid(2 ml, Ultima Gold XR, Canberra). Bottles of soil were removed at times from 0 to 49 days after treatment.

2 Results and discussion

2.1 Rapid bensulfuron-methyl degradation upon repeated application

Bensulfuron-methyl degraded more rapidly in soil previously treated with this same herbicide. Rapid degradation was evident in soil with a history of bensulfuron-methyl application treated 49 d earlier and maintained under laboratory conditions(Fig. 1a). Also, rapid bensulfuron-methyl degradation was evident in soil with only one previous exposure to bensulfuron-methyl 14 months earlier. This was also evident in soil assayed 1 year after multiple annual bensulfuron-methyl applications(Figs. 2—4). The results in Fig. 1 demonstrate that a single prior treatment with bensulfuron-methyl to a soil with no prior bensulfuron-methyl history can substantially reduce the persistence of a subsequent application. In this study the DT_{50}(time taken for 50% of the applied pesticide to degrade) of bensulfuron-methyl was reduced from 16 d to 9 d. As bensulfuron-methyl is rapidly metabolized within weeds compared to the time taken for the weeds to be controlled, continued bensulfuron-methyl absorption from soil is required for efficacious weed control. Therefore as rapid bensulfuron-methyl degradation shortens the time period of weed exposure, this phenomenon is the likely cause of poor bensulfuron-methyl performance upon repeated application. Hole and Powles(Hole, 1997) reported similar finding that carbetamide fails to control weeds when applied to soils with any carbetamide application history and that reduced efficacy can be observed after a single prior carbetamide application.

Cyperus compressus L. bioassay results(Figs. 1b) confirmed the HPLC data that bensulfuron-methyl degraded more rapidly in soil previously treated with bensulfuron-methyl. At time points from 0 to 21 d after bensulfuron-methyl treatment the *cyperus compressus L*. bioassay demonstrated clear differences in the bensulfuron-methyl degradation kinetics in both soils examined. There is a high correspondence between the HPLC chemical assay and the bioassay(Fig. 1). However, from 21 d onwards there is a poor correspondence between the chemical and bioassay techniques in soil treated initially under laboratory conditions (Fig.1). This anomaly may have been due to the sampling process which may have stimulated biological activity and hence bensulfuron-methyl degradation in these samples. Adsorption of bensulfuron-methyl onto the soil, thus reducing bioavailability, could also explain the observed results(Cavanna, 1998).

2.2 Biological nature of rapid bensulfuron-methyl degradation

Experiments were conducted to study the biological nature of rapid bensulfuron-methyl degradation in soil. Bensulfuron-methyl degradation in both rapid bensulfuron-methyl degrading soil and control soil was significantly($P = 0.05$) affected by pretreatment with anti-microbial agents and heat sterilization(Fig.2). Following pre-treatment of rapid bensulfuron-methyl degrading soil with the broad-spectrum antibiotic chloramphenicol, the percentage of bensulfuron-methyl remaining after 7

d was not significantly different($P = 0.05$) to that recovered from the control soil(81%—86%). However, between 7 and 14 d, bensulfuron-methyl degraded rapidly in with 21% of the applied bensulfuron-methyl remaining at 14 d after treatment. Pre-treatment of control soil (previously untreated with bensulfuron-methyl) with chloramphenicol resulted in 74% of the applied bensulfuron-methyl remaining after 14 days, which was not significantly different($P = 0.05$) from that observed in heat sterilized soil. Studies demonstrating partial inhibition of rapid degradation by chloramphenicol have been reported previously(Walker, 1990; 1993). Chloramphenicol may not completely suppress the bensulfuron-methyl degrading population, or chloramphenicol resistant microorganisms may rapidly be selected such that only partial suppression of bensulfuron-methyl degradation is observed.

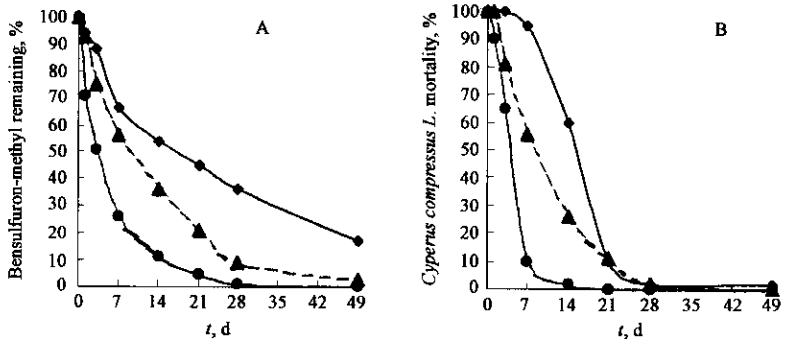

Fig. 1 Bensulfuron-methyl degradation kinetics as monitored by HPLC (A) and bioassay (B) in soil pretreated with bensulfuron-methyl and control soil(◆ control; ▲ single bensulfuron-methyl pretreated; ● three years bensulfuron-methyl pretreated)

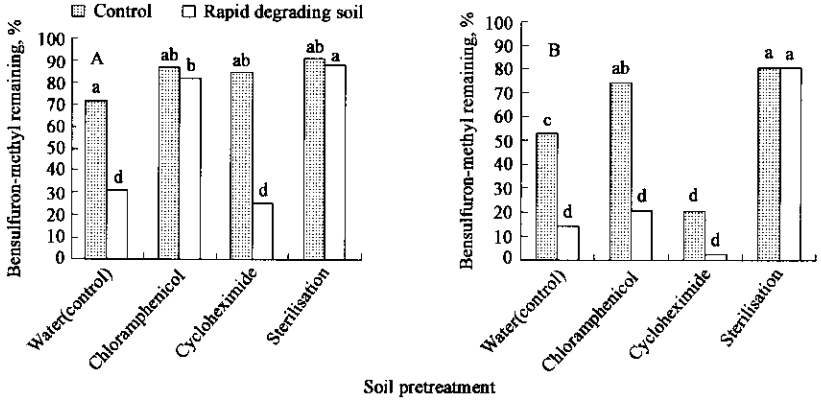

Fig. 2 Effect of treatment with choramphenicol, cycloheximide or heat sterilisation upon bensulfuron-methyl degradation in rapid bensulfuron-methyl soil and compared to soil previously untreated with bensulfuron-methyl. Treatment means labeled with the same letter are not significantly different ($P = 0.05$) (A: 7 d after treatment; B: 14 d after treatment)

Pre-treatment of rapid bensulfuron-methyl adapted soil with the broad-spectrum antifungal agent cycloheximide did not significantly increase the percentage of bensulfuron-methyl remaining at any time point. However, pre-treatment of the control soil with cycloheximide resulted in a significantly($P = 0.05$) lower percentage of bensulfuron-methyl recovered after 14 d. The cause of rapid bensulfuron-methyl degradation in this soil following cycloheximide pre-treatment is not clear from this study. These results indicated strongly responsible for the rapid bensulfuron-methyl degrading capacity of previously treated soils.

The phenomenon of rapid bensulfuron-methyl degradation was readily transferred to a control soil by addition of small percentages of rapid degrading soil. Addition of 10% rapid bensulfuron-methyl degrading soil to the control soil significantly($P = 0.05$) reduced the amount of bensulfuron-methyl remaining after 7 d(Fig. 3). Addition of 5%, or more rapid bensulfuron-methyl degrading soil to previously untreated soil resulted in significantly($P = 0.05$) decreased bensulfuron-methyl remaining after 14 d. This result supports the suggestion that rapid bensulfuron-methyl degradation is biologically based and demonstrates the potential spread of the herbicide degrading microorganisms through physical soil transfer(Walker, 1996).

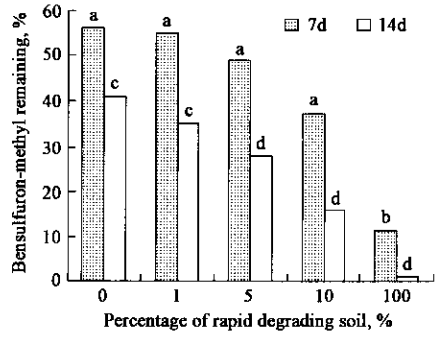

Fig. 3 Effect on bensulfuron degradation of adding rapid bensulfuron degrading soil to previously untreated control soil. Treatment means labelled with the same letter are not significantly different ($P = 0.05$)

Evolution of $^{14}CO_2$ from soil treated with ^{14}C-labelled bensulfuron-methyl was relatively faster from bensulfuron-methyl adapted soil than

untreated soil (Fig. 4). In addition, the cumulative amount of $^{14}CO_2$ evolved after 49 d was higher from rapid bensulfuron-methyl degrading soil(56%) than from soil previously untreated with bensulfuron-methyl (21%). A large proportion of the $^{14}CO_2$ evolved from the rapid bensulfuron-methyl degrading soil(approximately two-thirds) was evolved during the period of rapid bensulfuron-methyl degradation which was from 0 to 7 d after addition (Fig. 4). Rapid mineralisation of ^{14}C-labelled bensulfuron-methyl suggests that this herbicide is being mineralized as a source of carbon and/or energy by soil microorganisms.

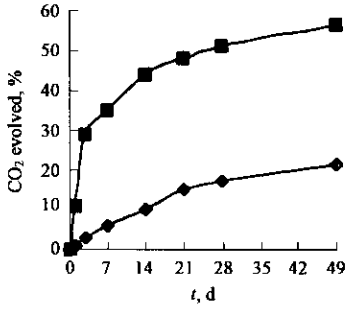

Fig.4 Mineralisation of ^{14}C-labelled bensulfuron-methyl to $^{14}CO_2$ in rapid bensulfuron-methyl degrading soil and previously untreated soil (◆ control; ■ rapid degrading soil)

Our objectives were to confirm whether enhanced bensulfuron-methyl degradation occurs upon repeated application to China paddy soils and to establish the biological nature of this degradation. The results presented herein establish that repeated applications of the herbicide bensulfuron-methyl degrade at a faster rate than the initial application (Fig.1). Rapid degradation of bensulfuron-methyl in soil was correlated with a loss in biological activity against the indicator species(Fig. 1b). These combined chemical and biological assay results strongly suggested that the inadequate efficacy of bensulfuron-methyl, when applied repeatedly in the field, is a result of rapid soil degradation. Rapid soil degradation has been reported for numerous soil applied pesticides (Racke, 1990), however only limited reports concerning carbetamide have been published(Hole, 1997). The three different types of experiments presented here provide clear evidence that rapid bensulfuron-methyl degradation is mediated by an adapted soil microflora. Similar experiments have been used to implicate microbial action as being responsible for other soil phenomena, including rapid degradation (Walker, 1990; Wiseman, 1996). Chloramphenicol slowed rapid bensulfuron-methyl degradation and strongly suggests that increased bacterial activity endows rapid bensulfuron-methyl degradation. However, the unusual response of the control soil following antifungal treatment may suggest complex interactions between microbial classes within the soil. The advent and more extensive use of DNA based techniques may allow such complexities to be elucidated. DNA based techniques could also provide rapid predictive assessment of a soils degradative potential and the likely efficacy following pesticide treatment.

3 Conclusions

The results presented in this study establish that rapid degradation of the herbicide bensulfuron-methyl will reduce the ability to control weeds in field upon repeated application. Experiments documented herein demonstrate that rapid bensulfuron-methyl degradation is a biological phenomenon, linked to soil microorganisms, probably bacteria, capable of bensulfuron-methyl degradation for metabolic gain.

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