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Rimsulfuron in Soil: Effects on Microbiological Properties under Varying Soil Conditions

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SUMMARY

The effects of rimsulfuron a sulfonylurea herbicide on the growth and activity of soil microorganisms under laboratory conditions was investigated in two soils.

The application rates were: 0.2, 2.0 and 20.0 mg a.i kg^{-1} soil. The lowest concentration tested was the label rate (0.2 mg a.i kg^{-1}), and the other two were ten and hundred times higher. No adverse effects on microbiological processes were observed for the label rate. Decrease in microbial biomass carbon, dehydrogenase activity, fungi and bacteria in comparison with untreated control, were found at higher rates. The magnitude of these effects were generally slight and transitory.

Keywords: Rimsulfuron; Clay soil; Sandy soil; Dehydrogenase activity; Microbial biomass carbon; Fungi; Bacteria

INTRODUCTION

Modern agriculture depends on wide variety of synthetically produced chemicals, including herbicides, insecticides, fungicides and other pesticides. Continual widespread use and release of such synthetics became an every day practice, resulting in environmental pollution. In this context, the influence of pesticides on the microbial activity of soil microorganisms was studied by some researchers.

Sulfonylureas are class of herbicides characterized by high biochemical activity at low application rates. Depending on crop type and local legalization, application rates of these herbicides range from 2 g to 150 g a.i ha^{-1}. Though the mode of action of these classes of herbicides was reported (Green and Uldrich, 1993; Baghestani et al., 2007; Sikkema et al., 2008), to date little information is available on the overall effects of either herbicide class on living part of soil.

Rimsulfuron 1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-ethylsulfonyl-2-pyridylsulfonylurea), a member of this class, is a common agricultural herbicide used to control most annual and perennial grasses and several broad-leaved weeds in maize and potato crops. Perucci and Scarponi (1996) reported the side-effects of rimsulfuron on soil microbial biomass in a clay loam soil, where rimsulfuron was applied at potato weed control rate (15 g a.i. ha^{-1}) and at a 10-fold rate, under field conditions. Dumontet et al. (1993) reported the effects of five sulfonylureas, including rimsulfuron applied at rates 10- and 100-times higher than the field rate, on the growth and respiration of some selected microbial strains.

The purpose of the present study was to examine how the herbicide rimsulfuron at normal field concentration, ten times and hundred times higher concentrations affects the microflora of two agricultural soils. The interactions established among total number of bacteria, fungal population, microbial biomass carbon, dehydrogenase activity were determined.

MATERIAL AND METHODS

Rimsulfuron 1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-ethylsulfonyl-2-pyridylsulfonylurea) technical substance, tested in the experiment is a product of DuPont Company, Switzerland.

The application rates were: 0.2, 2.0 and 20.0 mg/kg soil. The lowest concentration tested was the label rate and the other two were ten and hundred times higher.

The experiment was carried out in two agricultural soils. Various soil characteristics were determined using techniques previously described (Anonymous, 1966; Bošnjak, 1997) (Table 1).

Table 1. Characteristics of agricultural soil samples

Soil	Clay soil (Soil 1)	Sandy soil (Soil 2)
Locality	Putinci	Tavankut
Sand (%)	50	30
Silt (%)	30	40
Clay (%)	20	30
Total N (%)	0.12	0.06
Organic matter (%)	3.8	0.6
pH (H_2O)	7.1	8.2

The soil chosen for the study had never been treated with pesticide before. Soil samples were collected from the upper layer (0-10 cm), carefully dried, sieved to pass 5 mm mesh, and stored at 4°C. Before using, the soils were air-dried at room temperature for 24h. The solutions of technical substance in distilled water were prepared for each herbicide concentration. The solution concentration was pipetted to the surface of 1 kg of soil before homogenization on a rotating stirrer for 30 minutes. After homogenization by mixing, the soil was portioned out in pots. Untreated soil served as control. The experiments were conducted with four replications. The pots were kept in a controlled-environment chamber at 20 ± 2°C temperature, 50% air humidity and 12/12 h day/night photoperiod during the experiment. Soil humidity was kept at 50% field capacity. The samples for the analysis were collected 7, 14 and 30 days after rimsulfuron application.

Total culturable microorganisms were counted by a soil dilution plate technique using tryptic soy agar for bacteria and Czapek agar for fungi. The inoculated agar plates (three replicates) were incubated at 28°C for 3 days for bacteria and 5 days for fungi, before the colonies were counted.

Microbial biomass C was determined by the fumigation-extraction method (Vance et al., 1987). The samples were fumigated with non-alcohol chloroform under moist conditions for 24h. After incubation, carbon was extracted with a 100 ml 0.5 M solution of potassium sulphate (K_2SO_4) and its content determined by titration with 0.0333 M solution of Mohr salt ($(NH_4)_2Fe(SO_4)_2$) in the presence of phenylantranil acid as the indicator. Non-fumigated samples were extracted under the same conditions. Microbiological biomass carbon (MBC) was calculated by expression: MBC = C extracted x 0.33 (Jenkinson et al., 1979), where C was extracted from fumigated and non-fumigated samples. The results are presented in μg C g^{-1} soil.

Soil dehydrogenase activity was determined by method reported by Tabatabai (1982). The soil samples were prepared by incubation with triphenyltetrazolium chloride (TTC) under moist conditions at 37°C for 24h. Determination of triphenylformazan (TPF), which is derived from triphenyltetrazolium chloride (TTC) as a product of enzyme activity was done spectrophotometricaly. Measurements were done at 485 nm wavelength and enzyme activity given as μg TPF g^{-1} soil.

Statistical evaluation: the obtained data were analyzed using ANOVA and the means were separated by Duncan's multiple range test. In all analyses, the level of significance was at least $P< 0.05$.

RESULTS AND DISCUSSION

The effects of rimsulfuron on soil microbiological and biochemical variables tested are shown in Tables 2-5.

At the field rate for maize weed control corresponding 0.2 mg a.i. kg^{-1} no effects were observed. The lack of interference with soil biological processes would suggest that rimsulfuron has little or no harmful effect on soil microbes when it is applied at the field rate in soil. In agreement with the reports of Perucci and Scarponi (1994, 1996), this finding can be attributed to the low toxicity of rimsulfuron on soil microbes or to soil processes, such as the adsorption of small amounts of herbicide on clay or soil organic matter, which limits the effects of agrochemicals on the soil microbial biomass. This phenomenon is of primary importance especially when small amounts of a herbicide are employed in soil weed control (Schneiders et al., 1993). In addition, the low persistence time makes difficult to determine any indirect effects on soil microflora mainly for herbicides, such as rimsulfuron, applied at very low field rates (Metzger et al., 1996; Sabatini et al., 1998; Accinelli et al., 2002).

To minimize the influence of soil processes and thereby highlight the potential side-effects of rimsulfuron on non-target microorganisms, two further herbicide rates were employed 10 and 100 times higher than field rate. The 10 times higher field rate is recommended in laboratory tests to assess the side effects of pesticides on soil microflora (Sommerville, 1987), while the 100 times higher field rate was chosen since in this soil rimsulfuron has very short half-life (Schneiders at al., 1993).

Table 2. Microbiological biomass carbon (μg C g^{-1} soil) in two soils in the presence of rimsulfuron

Soil	Soil 1			Soil 2		
	Days after application					
Rimsulfuron (mg a.i. kg^{-1})	7	14	30	7	14	30
0.0	172.1a	170.3a	174.2a	157.3a	154.2a	156.8a
0.2	171.6a	173.1a	168.1a	149.2a	152.8a	147.5a
2.0	159.6b	159.6b	166.5a	145.0a	146.2a	152.4a
20.0	152.4b	156.8b	165.4b	140.4b	139.8b	150.5a

a – means of variants were separated by Duncan's multiple range test (P<0.05)

Under various experimental conditions decrease in the biomass-C content varies during the experiment and the decreases were depended on soil type, rates of application and exposure time (Table 2). In soil 2 no significant effects were observed for concentration 2.0 mg a.i. kg^{-1}. At each soil the maximum decrease was always found at the highest application rate and decrease ranged from 4.0-11.4%. The maximum decrease value was 11.4 % (soil 1, 20.0 mg a.i. kg^{-1}, 7 days after application). However, these effects were transitory, because all the variables tested showed a tendency to the control values. There are also other reports on the activity of different pesticides in relation to biomass carbon. Perucci and Sacrponi (1994, 1996), for example, found that the effect of rimsulfuron and imazethapyr on biomass carbon depended on soil moisture. Under reduced moisture, the harmful activity of rimsulfuron lasted 36 hours, but as long as 72 hours under high moisture. Similar findings were reported also by Wardle and Parkinson (1991), as well as by Rath et al. (1998), in experiments investigating 2.4-D and glyphosate. Startton and Stewart (2002) recorded harmful effects of glyphosate on soil biomass and respiration in Canadian coniferous forests. Finally, Radivojević et al. (2008) observed transitory effects of atrazine on soil biomass-C.

Table 3. Dehydrogenase activity (μg TPF g^{-1} soil) in two soils in the presence of rimsulfuron

Soil	Soil 1			Soil 2		
	Days after application					
Rimsulfuron (mg a.i. kg^{-1})	7	14	30	7	14	30
0.0	156.3a	152.7a	155.9a	128.6a	125.3a	129.1a
0.2	150.6a	153.1a	149.6a	117.6a	121.2a	123.5a
2.0	145.8a	145.9a	151.8a	120.5a	116.4a	124.8a
20.0	138.0b	140.8a	154.2a	98.9b	101.1b	111.8b

a – means of variants were separated by Duncan's multiple range test (P<0.05)

Soil dehydrogenases are generally considered reliable measures of microbial oxidative activity and testing of soil dehydrogenase is necessary to evaluate the side-effects of pesticides on soil microorganisms (Sabatini et al., 1998; Accinelli et al., 2002). At both soils (soil 1 and soil 2) no significant effects were observed for concentration 2.0 mg a.i. kg^{-1}. Dehydrogenase activity was found to be inhibited by 20.0 mg rimsulfuron treatment in soil 2. The maximum decrease value was 23.1% (soil 2, 20.0 mg a.i. kg^{-1}, 7 days after application) (Table 3). The experimental data are consistent with results reported by other authors on the effect of different pesticides on this enzyme. Dinelli et al. (1998) and Accinelli et al. (2002) reported that sulfonylurea herbicides at a rate up to 20 mg kg^{-1} inhibited dehydrogenase acitivity. The results Radivojević et al. (2008) showed a decreased activity of dehydrogenase under all atrazine concentrations (8.0, 40.0 and 80.0 mg a.i. kg^{-1}) from the 1st to the 30th day after atrazine application. The decrease ranged: 12.5-18.2% for 8.0 mg concentration, 4.8-24.8% for 40.0 mg, and 6.6-39.6% for 80.0 mg.

Table 4. Number of total bacteria (10^6) in two soils in the presence of rimsulfuron

Soil	Soil 1			Soil 2		
	Days after application					
Rimsulfuron (mg a.i. kg^{-1})	7	14	30	7	14	30
0.0	192.3a	185.6a	189.7a	115.6a	107.3a	112.6a
0.2	190.4a	187.2a	191.4a	111.2a	109.5a	113.8a
2.0	180.5a	175.3a	184.0a	107.9a	102.4a	110.6a
20.0	120.3b	110.6b	160.5b	82.6b	85.1b	93.7b

a – means of variants were separated by Duncan's multiple range test ($P<0.05$)

Table 5. Number of fungi (10^4) in two soils in the presence of rimsulfuron

Soil	Soil 1			Soil 2		
	Days after application					
Rimsulfuron (mg a.i. kg^{-1})	7	14	30	7	14	30
0.0	26.5a	21.3a	24.7a	7.5a	8.3a	9.2a
0.2	22.8a	23.5a	25.1a	8.0a	7.1a	8.8a
2.0	15.3b	13.8b	21.5a	4.9b	4.5b	7.9a
20.0	7.5c	6.2c	16.3b	2.6c	3.2c	5.0b

a-means of variants were separated by Duncan's multiple range test ($P<0.05$)

In both soils (soil 1 and soil 2) no significant effects on soil bacteria were observed for concentration of 2.0 mg rimsulfuron. The presence of rimsulfuron at 100 time higher concentration in both soils negatively affected the populations of bacteria. At each soil the maximum decrease was always found at the highest application rate and decrease ranged from 15.4-40.4%. The maximum decrease value in soil 1 was 40.4% (20.0 mg a.i. kg^{-1}, 14 days after application) and in soil 2 28.5% (20.0 mg a.i. kg^{-1}, 7 days after application) (Table 4).

The fungal populations were significantly decreased in the presence 2.0 and 20.0 mg of rimsulfuron in both soils, and the degree of the inhibition increased as the concentration of the herbicide increased (Table 5). Similar results were reported for other herbicides. In such a way, metribuzin reduced total microflora, fungi, cellulolytics, nitrifiers at temperature of 10, 20 and 30°C (Radivojević et al., 2003). While, metsulfuron-methyl had pronounced effect on microbial community in different soils (Ahtianen et al., 2003; Zabaloy et al., 2008). Ratcliff et al. (2006) recorded minor changes in bacterial community after application 100x field rate of glyphosate. However, the effects obtained in our studies may be influenced by the degree of soil disruption and also by the species of the fungi present in the soil. Several studies (Metzger et al., 1996; Perucci and Scarponi, 1996; Startton and Stewart, 2002; Ratcliff et al., 2006) on soil microflora have shown that soil characteristics may modify the effect of pesticides on microbial numbers and their biological activity. This is in accordance with our research.

CONCLUSIONS

The response to the herbicidal treatments of the microbiological parameters tested evidence that rimsulfuron has no adverse effect on soil microorganisms when it is applied at field rates. Changes of those parameters were observed particularly at the higher rate but they were minor and transitory. Our study stresses the role of herbicide rate, exposure time and type of soil in determining the influence of changes on soil microorganisms.

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Rimsulfuron u zemljištu: uticaj na mikrobiološke parametre u različitim tipovima zemljišta

REZIME

Na dva tipa zemljišta, u laboratorijskim uslovima, ispitivan je uticaj herbicida rimsulfurona iz grupe sulfonilurea na brojnost i aktivnost zemljišnih mikroorganizama.

Rimsulfuron je primenjen u koncentracijama 0,2, 2,0 i 20,0 mg a.s. na kilogram zemljišta. Najmanja ispitivana koncentracija (0,2 mg) odgovarala je količini koja se preporučuje za primenu, a druge dve su deset i sto puta veće od preporučene.

Kada je primenjen u preporučenoj količini, rimsulfuron nije imao uticaja na ispitivane mikrobiološke parametre. Smanjenje mikrobiološke biomase ugljenika, dehidrogenazne aktivnosti, brojnosti gljiva i bakterija utvrđene su kod većih koncentracija rimsulfurona. Intenzitet ovih promena je, uglavnom, bio slab i prolaznog karaktera.

Ključne reči: Rimsulfuron; glinovito zemljište; peskovito zemljište; dehidrogenaza; mikrobiološka biomasa ugljenika; gljive; bakterije