Biogeochemistry **39:** 165–176, 1997.
© 1997 *Kluwer Academic Publishers. Printed in the Netherlands.*

Rimsulfuron in soil: Effect of persistence on growth and activity of microbial biomass at varying environmental conditions

COSTANTINO VISCHETTI[1], PIERO PERUCCI[2] & LUCIANO SCARPONI[1]
[1]*Centro di Studio sulla Chimica e Biochimica dei Fitofarmaci C.N.R. Istituto di Chimica Agraria dell'Università Borgo XX Giugno 72, 06121 Perugia, Italy;* [2]*Dipartimento di Produzione Vegetale, Università della Basilicata Via N. Sauro 85, 85100 Potenza, Italy*

Key words: global hydrolitic activity, rimsulfuron persistence, soil microbial biomass-C, VARLEACH model

Abstract. The research was carried out to ascertain the effect of rimsulfuron, a solfonylurea herbicide, on soil microbial biomass growth and activity. Laboratory experiments were performed in a silty clay loam soil to relate changes of soil microbial biomass-C content and global hydrolytic activity to the rimsulfuron persistence under different conditions of temperature and soil humidity. The results showed that rimsulfuron persistence depended significantly on temperature, while it remained almost unchanged by humidity changes. A range of half-life values from 3.5 to 14.8 days was found in a temperature range from 10 °C to 25 °C, with lower half-lives at higher temperature. Persistence data were processed with the VARLEACH model, in order to predict rimsulfuron persistence under different environmental conditions. On comparing treated soils with untreated soil samples, decreases in the microbial biomass-C content and increases in the global hydrolytic activity were found to be connected with rimsulfuron persistence at the various experimental conditions. These effects persisted for a short time and, they were evident earlier at higher temperature and more persistent at lower humidity. This behaviour is discussed in terms of rimsulfuron toxicity, with the consequent release of endocellular hydrolytic enzymes from the dead microorganisms. An equation was derived to calculate the microbial biomass-C content in response to the variation of rimsulfuron persistence.

Introduction

Rimsulfuron, N-[[(4,6-dimethoxypyrimidin-2-yl)amino]carbonyl]-3-(ethylsulfon-yl)-2-pyridinesulfonamide, is a sulfonylurea herbicide used on corn and potato crops to control a wide variety of grasses and broadleaf weeds. This compound is a highly active inhibitor of acetolactate synthase (ALS, EC 4.1.3.18), the first common enzyme in the biosynthesis of branched-chain amino acids (Brown 1990).

It has been found that rimsulfuron hydrolyses rapidly in soil under conditions of high temperature and low pH (Bassi et al. 1990). In laboratory tests performed in a sandy loam soil (pH 6.3) rapid degradation with half-live times

of 24.5 or 22.5 days under anaerobic or aerobic conditions, respectively, has been observed (Schneiders et al. 1993). On the other hand, in field experiments on a light sandy soil of Denmark, Reinke et al. (1991) found half-live values of 90 or 120 days for split treatment or single herbicide application, respectively. These findings evidence the link between rimsulfuron persistence in soil and soil physical and chemical properties. They also point to the possibility of a large range of half-life depending on climatic conditions.

The occurence of herbicide interferences on the biochemical soil properties related to soil fertility is well known. As most soil enzymes involved in nutrient mineralization are of microbial origin, of particular interest are the responses of soil microbial biomass to the herbicide persistence. As concerns the ALS inhibitors, Perucci & Scarponi (1994) found that the imidazolinone herbicide imazethapyr, when applied at ten-fold field rate under laboratory conditions, caused decreases of the microbial biomass-C content and dehydrogenase activity and increases of protease and catalase activity. Little information is available on the interferences of sulfonylureas with soil biochemical properties and no data have been reported on the effect of rimsulfuron on soil microbial biomass.

The main objective of this research was to evaluate the behaviour of the growth and activity of soil microbial biomass in relation to the persistence of rimsulfuron in soil under different experimental and environmental conditions. This objective was achieved by determining, at various temperatures and soil humidities, the microbial biomass-C content, the global hydrolytic activity and the degradation trends of rimsulfuron. A further aim was to develop an equation correlating changes of soil microbial biomass with persistence trends at varying experimental conditions.

Materials and methods

Chemicals and apparatus

Rimsulfuron (N-//(4,6-dimethoxypyrimidin-2-yl)amino/carbon-yl/-3-(ethylsulfonyl)-2-pyridinesulfonamide) was kindly supplied by DuPont de Nemours Inc. (Wilmington, USA). Fluorescein diacetate (3′,6′-diacetylfluorescein, FDA) was obtained from Aldrich-Chemie (Steinheim, Germany)

A Perkin-Elmer Series 410 HPLC, equipped with an LC 95 UV detector at wavelength 254 nm and with a C8 column (4.6 mm i.d. per 25 cm length) and a Varian model Cary 210 double grating spectropho-tometer, were employed.

Model and model parameters

The VARLEACH model is a new version of the CALF model, described in detail by Nicholls et al. (1982). The degradation subroutine in the model

(PERSIST) was changed by Walker (1987) to include the sequence of equations used in the earlier persistence models of Walker (1974) and Walker & Barnes (1981) that account for moisture and temperature effects on degradation. In the model, moisture effects on degradation were characterized using the empirical equation:

$$H = AM^{-B} \tag{1}$$

(where H is the half-life at moisture content *M*, and *A* and *B* are constants). Temperature effects are characterized using the Arrhenius equation. Other data required are soil moisture contents at field capacity (-10 KPa) and at a soil water stress of -200 KPa, the average bulk density of the soil, and the initial soil moisture content. Also required are weather data (daily rainfall, evaporation from an open water surface, and maximum and minimum air temperature) for the period of the experiment. Simulation conditions are reported in Table 1.

Soil sample preparation

A silty clay loam soil (0–20 cm layer) taken from the middle Tiber Valley (central Italy) was used. Determination of pH, cation exchange capacity, organic carbon content, particle size distribution, were performed according to ASA-SSSA methods (Page 1982; Klute 1986); field capacity and wilting point were determined according to Cavazza (1981) (Table 1).

A suitable amount of moist soil was air dried and sieved at 2 mm to remove plant material, soil macrofauna and stones. After sieving, the soil was homogeneized for 3 hours in a rotary cylinder and stored at 20 °C in the dark for 3 days (pre-incubation). Subsequently, the soil was subdivided into two portions: one (2.5 kg) was used as the control, the other (4.5 kg) was treated with rimsulfuron as follows: 10 ml of a water/methanol (80/20) solution, containing 225 mg of rimsulfuron, was added to a subportion (100 g) of soil; after evaporation of water/methanol solution at room temperature, the subportion was incorporated back into the remaining 4.4 kg of soil by homogenization in a rotary cylinder for 4 hours. For the control soil, 100 g of soil subportion was treated with 10 ml of the water/methanol (80/20) solution and, after evaporation, incorporated back into the remaining 2.4 kg of soil.

Trials

Three trials were carried out at the following conditions: 25 °C and 75% of field capacity (25 °C – 75% fc), 25 °C and 33% of field capacity (25 °C – 33% fc), and 10 °C and 75% of field capacity (10 °C – 75% fc).

Table 1. Simulation conditions.

Rimsulfuron properties		
Water solubility ($mg.l^{-1}$)		1,500
Application ($g.ha^{-1}$)		150
Degradation constants	A	55.35
	B	−0.97
	E ($J.mol^{-1}$)	80,000
Soil properties		
pH∗		8.1
Organic matter(%)∗		2.1
CEC ($meq.100g^{-1}$)∗		18
Sand (%)∗		23.5
Silt (%)∗		47.5
Clay (%)∗		29.0
Bulk density ($kg.dm^{-3}$)		1.3
Field capacity (% w/w)		30
Wilting point (% w/w)		10
Water content at −200KPa (% w/w)		16

Climate

	Rain + irrigation (mm)		Mean monthly temperature (°C)	
	1984	1990	1984	1990
Jan	80.2	53.2	2.5	4.2
Feb	132.3	14.8	3.3	9.5
Mar	57.8	21.4	6.3	10.9
Apr	73.6	98.8	10.5	10.6
May	162.3	29.2	12.8	16.7
June	76.2	57.6	18.3	17.9
July	32.0	56.6	21.8	21.0
Aug	47.2	47.2	24.4	22.8
Sept	78.2	55.4	19.3	18.5
Oct	62.8	88.1	12.7	13.8
Nov	16.2	12.6	8.3	7.3
Dec	20.2	28.7	6.8	5.6
Total	839.0	563.6		

Site	
Altitude (m)	170
Longitude (degree)	42

(∗) The Model does not need these entries.

Six series of polypropylene boxes (three of 14 boxes each, containing 100 g of control soil, three of 21 boxes each, containing 200 g of treated soil) were used. For each of experimental conditions a series of 14 boxes for

control and a series of 21 boxes for treated soil were prepared. During the experiment, soil moisture was controlled daily by adding suitable amount of sterile water when necessary. At different days after treatment, two control boxes and three treated boxes were removed to perform residue determination, microbial biomass-C content evaluation and FDA-hydrolysis assays. All the analytical determinations were carried out during time periods of 56 days for the trial at 10 °C – 75% fc, 42 days for the trial at 25 °C – 33% fc and 28 days for the trial at 25 °C – 75% fc.

Rimsulfuron residue determination

The determination of rimsulfuron residues in soil was performed according to Schneiders et al. (1993) with slight modifications. Subportions of soil samples (100 g) were extracted three times with 100 ml of a CH_3CN/H_2O (2/1) mixture. After centrifugation at 2500 rpm for 10 min, the extracts were combined, the pH was adjusted to 2.5 and the rimsulfuron was partitioned in $CHCl_3$ (40 ml x 3 times). The $CHCl_3$ phase was evaporated to dryness, rinsed with 1 ml of the H_2O/CH_3CN (1/2) mixture and submitted to analysis by HPLC, employing the following mobile phase: solvent 1, CH_3CN/CH_3COOH 99.4/0.6; solvent 2, H_2O/CH_3COOH 99.4/0.6. Gradient: solvent 1/solvent 2 – 20/80 initial; 35/65 in 5 min; 50/50 in 10 min. The flow rate was 1 ml min^{-1}. Under these conditions the retention time of rimsulfuron was 14.0 min, the limit of detection 10 ng and the sensitivity of the method 0.005 mg kg^{-1}.

Microbial biomass-C content

Soil microbial biomass was evaluated using the method of Sparling & West (1988). Duplicate samples (20 g) of the control and treated soils were fumigated with ethanol-free $CHCl_3$. After removal of $CHCl_3$, soil moisture was adjusted to 60% W.H.C. Fumigated and unfumigated soil samples were extracted with 0.5 M $K_2SO_4$and organic C quantified by an oxidation with 0.0667 M $K_2Cr_2O_7$ and subsequent back-titration of the unreduced dichromate. The biomass-C content was estimated as follows: biomass-C = 2.64 Ec, where Ec is the difference between the organic carbon extracted from the fumigated and unfumigated treatments (Vance et al. 1987).

Hydrolysis of fluorescein diacetate (FDA-hydrolysis)

The FDA-hydrolysis rate was estimated by determining the hydrolysed FDA according to Swisher and Carroll's method (1980). FDA was dissolved in acetone and stored as a stock solution at −20 °C. A 0.5 ml of fluorescein diacetate solution was added to 5 g of soil in 100 ml phosphate buffer (60 mM;

Table 2. Times (days) to disappearance of 50% (DT50) and 90% (DT90) of rimsulfuron in soil under different temperature and soil moisture conditions.

Experimental conditions	DT50	DT90
25 °C 75% fc	3.5	10.9
25 °C 33% fc	7.3	23.7
10 °C 75% fc	14.8	48.0

pH 7.6), and the mixture was incubated at 25 °C on a rotary shaker. Hydrolysis of FDA was stopped after 2 h by adding acetone, and the concentration of hydrolysed FDA was determined at 490 nm after removal of the soil by centrifugation.

Results and discussion

Considering the layer of soil sampling (0–20 cm) and the soil bulk density (1.5 g cm^{-3}), the rimsulfuron application was at 10 times the suggested agronomical rate. This dosage was chosen to better highlight the effect on soil microbial biomass growth and activity. This massive dosage is advised and usually adopted to assay effects of pesticides on non-target microrganisms (Sommerville 1987).

To relate the rimsulfuron effect on soil microbial biomass to its persistence in soil it was necessary to ascertain the degradation kinetics of the rimsulfuron at the various experimental conditions. In Figure 1, rimsulfuron degradation in soil at 10 °C – 75% fc, 25 °C – 33% fc and 25 °C – 75% fc, is reported as the logarithm of residual concentration versus time. The degradation followed first order kinetics at all temperature-soil moisture combinations. The times to 50% (DT50) and 90% (DT90) of herbicide disappearance ranged from 3.5 days to 14.8 days, and from 10.9 days to 48.0 days, respectively (Table 2). Persistence was greatest at the lower humidity and temperature, in any case it failed to reach very high values, even at the most severe of the conditions tested.

Although rimsulfuron is highly unstable in aqueous medium (Bassi et al. 1990), its persistence in soil, measured under laboratory conditions, has been found to vary according to soil characteristics and temperature and humidity conditions (Palm et al. 1990; Schneiders et al. 1993). The DT50 and DT90 values found in our experiment fall within the range of this literature. On the other hand, when rimsulfuron persistence, was investigated in field experiments, it has been found to vary over a wider range: a DT50 of 5.6 days was found by Schneiders et al. (1993) in a sandy clay loam soil site in USA,

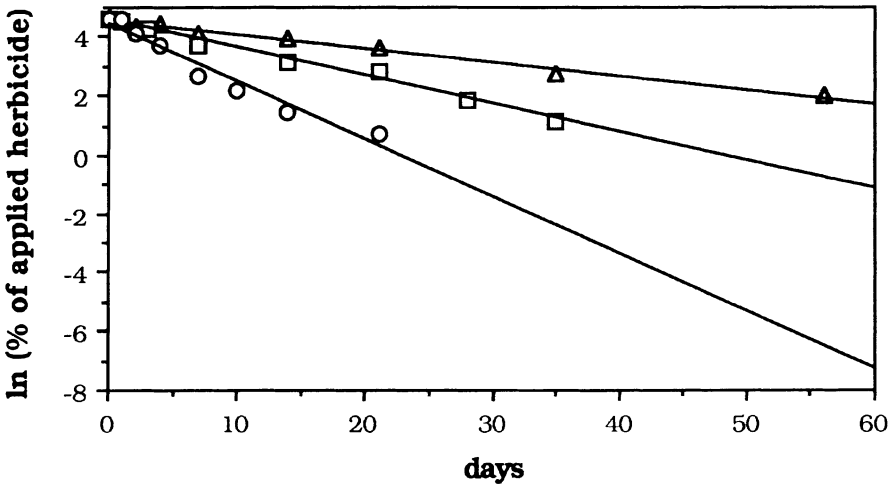

Figure 1. Degradation of rimsulfuron in soil at 10 °C – 75% fc (△), 25 °C – 75% fc (○) and 25 °C – 33% fc (□).

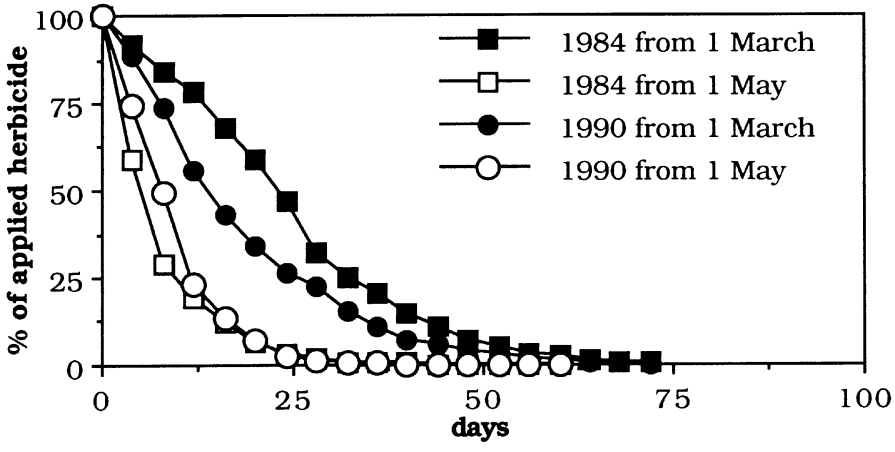

Figure 2. Simulation of rimsulfuron persistence in a central Italy scenario, under climatic conditions of the two most extreme years available (out of 20) and supposing two treatment times.

while a DT50 of 120 days was found by Reinke et al. (1991) in a light sandy soil in Denmark.

A simulation to evaluate rimsulfuron persistence in a typical Italian scenario was set up. The PERSIST program of the VARLEACH model was employed because of its ascertained good performance in studies on herbicide persistence in soil (Walker 1978; Vischetti 1995). For this reason, the

data obtained in laboratory experiments together with soil properties and the climatic conditions of the two most extreme years available (out of 20) were used in the model. Figure 2 shows the results of simulation performed at the two treatment times, which correspond to the cropping periods of potato and maize.

As can be seen, model simulation confirms that persistence varies according to different climatic conditions in the scenario considered. In fact, DT50 and DT90 values ranging from 4.0 to 27.6 days and from 16.4 to 38.4 days, respectively were found. In addition, it is noteworthy that the differences in persistence are more pronounced within the same year for different treatment times than between two years for the same treatment times. This might be due to the higher temperature and soil moisture variations recorded at different months within the same year, than at the same months in the two different years. Therefore, it becomes evident that environmental behaviour of a pesticide strictly depends on treatment times. Indeed, this must be borne in mind when the herbicide is registered for several crops grow in different seasons.

The values of DT50 and DT90 found in the laboratory experiment, as well as those calculated by the simulating model, do not seem to pose great risks of carryover.

Pesticide effects on soil microflora are well known (Sommerville 1987) and, in addition, herbicide interference on soil biochemical parameters involved in soil fertility have been observed following treatments with other herbicide ALS inhibitors (Dumontet et al. 1993; Perucci & Scarponi 1994), On this account, also rimsulfuron can interfere with the growth and activity of soil microbial biomass, the extent of which depends on the different herbicide persistence. Therefore, the evaluation of the side effect of rimsulfuron on no-target microorganisms was achieved by the determination of microbial biomass-C content. In addition, the global hydrolytic activity (FDA-hydrolysis rate) was also assayed because it is considered suitable for testing the overall microbial activity in soil (Vekemans et al. 1989). These assays were carried out for a period of approximately 5-fold the rimsulfuron half-life in each experimental condition.

The responses of the microbial biomass-C content and global hydrolytic activity to the herbicidal treatment are shown in Figure 3(a, b, c). Significant decreases in microbial biomass-C content and increases in FDA-hydrolysis rate were observed compared to untreated soil. The decreases of microbial biomass-C content did not exceed 12.4% and increases of FDA-hydrolysis rate 11.8%. These effects were generally more promptly shown at the higher temperature and they were more persistent at the lower humidity. The significance began to lessen at the 7th day in the experiment performed at 25 °C –

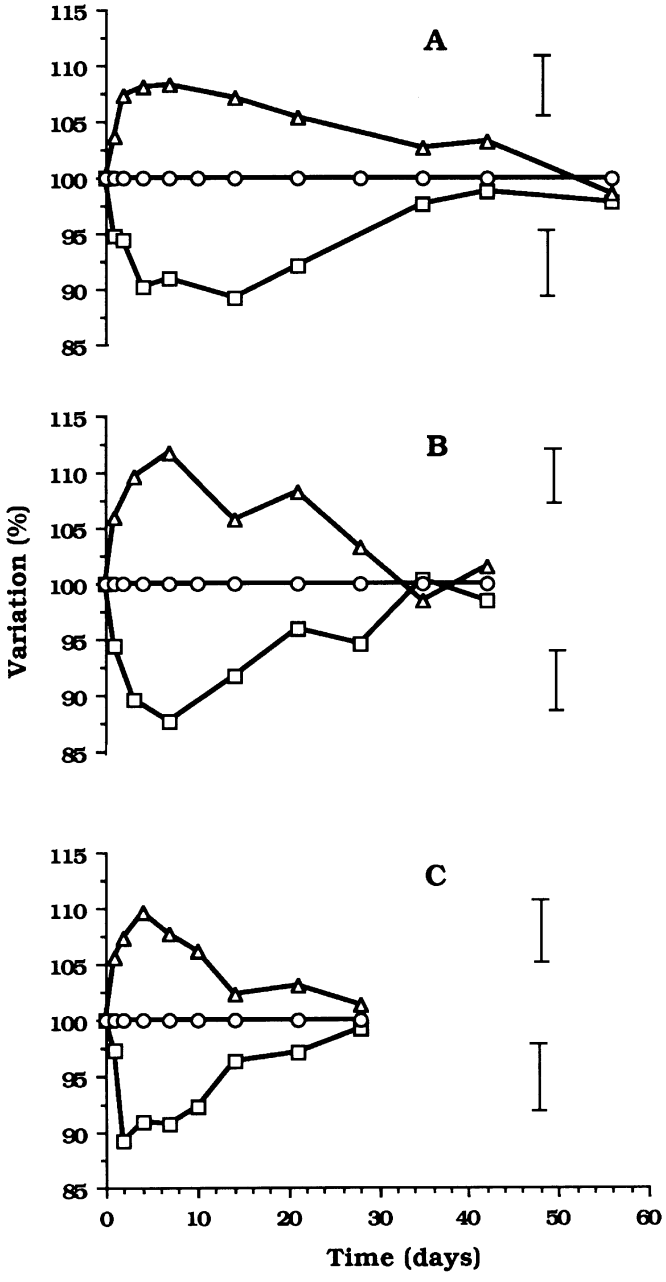

Figure 3. Variation of biomass-C content (□) and FDA-hydrolysis rate (△) at 10 °C – 75% fc (A), 25 °C – 33% fc (B) and 25 °C – 75% fc (C); (control (○) = 100%). Bars represent standard error.

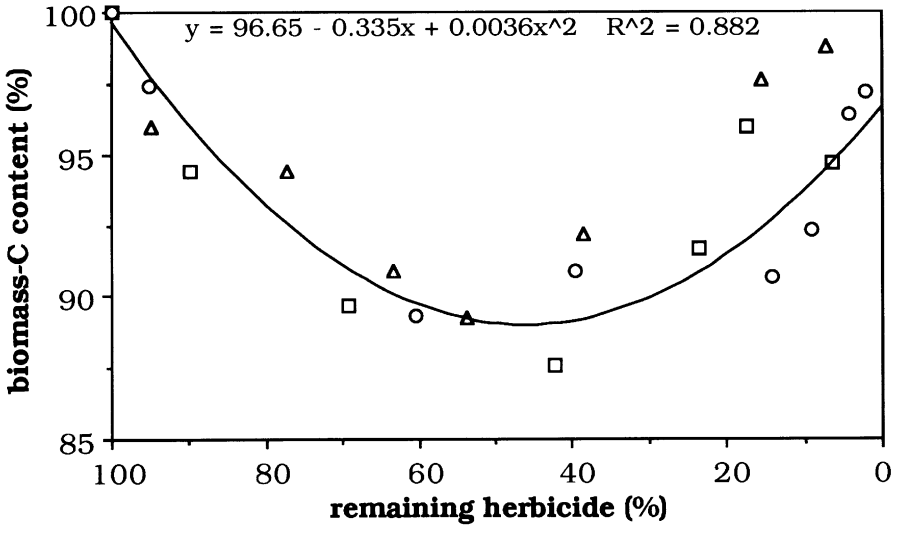

Figure 4. Correlation between biomass-C content and persistence of rimsulfuron at 10 °C – 75% fc (△), 25 °C – 75% fc (○) and 25 °C – 33% fc (□).

75% fc, at the 14th day in the experiment performed at 25 °C – 33% fc and at the 21th day in the experiment performed at 10 °C – 75% fc.

As the microbial biomass-C levels decreased the FDA-hydrolysis rate increased; thus a toxic effect of rimsulfuron causing the death of some microorganisms may be hypothesized. This hypothesis supports a recent study showing that some sulfonylureas, including rimsulfuron, dramatically reduced the growth of many microbial strains (Dumontet et al. 1993). Following the cell lysis of death microorganisms a release of endocellular enzymes occurs and, since hydrolases are the most abudant enzymes of microbial origin, a temporary increase of FDA-hydrolysis could result.

All experimental data concerning the microbial biomass-C content and rimsulfuron residues in soil have been utilzed to draw a relationship between these parameters (Figure 4). This gave a parabolic relationship which describes how the herbicide residues affect soil biomass content. At 100% of applied herbicide a depressing effect begins, though it is initially negligible because of the short time interval from treatment. The trend indicates the maximum level of microbial biomass depression at 46.0% of applied herbicide. Thereafter the depression begins to decrease, reaching negligible levels below 20% of applied herbicide, after which the recovery of microbial biomass rises to initial level.

This approach could be useful in order to evaluate the real consequences of rimsulfuron persistence on the soil biochemical and microbiological properties in some way involved in soil fertility. The validity of this relationship

should be ascertained through further experiments performed at wider intervals of temperature and soil humidity.

Acknowledgements

The research was supported by National Research Council of Italy, Strategic Project “Land and Environment”.

References

Bassi A, Bencivelli A, Fabiani GP, Gamberini C, Massasso W, Salomone MC & Turchiarelli V (1990) DPX-E9636 – Nuovo graminicida di post-emergenza del mais ad ampio spettro e ridotta persistenza. Atti Giornate Fitopatologiche 1: 125–132

Brown HM (1990) Mode of action, crop selectivity and soil relations of the sulfonylureas herbicides. Pestic. Sci. 29: 263–281

Cavazza L (1981) Fisica del terreno agrario. UTET, Firenze (Italy), pp 203–295

Dumontet S, Perucci P, Scopa A & Ricciardi A (1993) Solfonylureas: Preliminary study on the effect on selected microbial strains and soil respiration. Soil Sci (Trends in Agric. Sci.) 1: 193–198

Klute A (1986) Methods of soil analysis. Part 1. Second edition, American Society of Agronomy, Inc. Soil Science Society of America, Madison, Wisconsin, USA

Nicholls PH, Walker A & Baker RJ (1982) Measurement and simulation of the movement and degradation of Atrazine and Metribuzin in a fallow soil. Pestic. Sci. 12: 484–494

Page AL (1982) Methods in soil analysis. Part 2. Second edition, American Society of Agronomy, Inc. Soil Science Society of America, Madison, Wisconsin, USA

Palm HL, Liang PH, Fuesler TP, Leek GL, Strachan SD, Swinchatt ML & Wittenbach VA (1989) New low-rate sulfonylureas for postemergence weed control in corn. Proc. Bringhton Crop Protection Conference, Weed 1: 23–28

Perucci P & Scarponi L (1994) Effects of the herbicide imazethapyr on soil microbial biomass and various soil enzyme activities. Biol. Fertil. Soils 17: 237–240

Reinke H, Rosenzweig A, Claus J, Kreidi M, Chisholm C & Jensen P (1991) DPX-E9636, experimental sulfonylurea herbicide for potatoes. Brighton Crop Protection Conference – Weeds 4C-3: 445–451

Schneiders GE, Koeppe MK, Naidu MV, Horne P, Brown AM & Mucha CF (1993) Fate of rimsulfuron in the environment. J. Agric. Food Chem. 41: 2404–2410

Sommerville (1987) Perspective on side-effects testing. In: Sommerville L & Greaves MP (Eds) Pesticide Effects in Soil Microflora (pp 5–13). Taylor & Francis, London.

Sparling GP & West A (1988) A direct extraction method to estimate soil microbial C: Calibration *in situ* using microbial respiration and 14C labelled cells. Soil Biol. Biochem. 20: 337–343

Swisher R & Carroll GC (1980) Fluorescein diacetate hydrolysis as an estimator of microbial biomass on coniferous needle surfaces. Microbiol. Ecol. 6: 217–226

Vance ED, Brookes PC & Jenkinson DS (1987) An extraction method for measuring soil microbial biomass-C. Soil Biol. Biochem. 19: 703–707

Vekemans X, Godden B & Pennockx MJ (1989) Factor analysis of the relationships between several physico-chemical and microbio-logical characteristics of some Belgian agricultural soils. Soil Biol. Biochem. 21: 53–58

Vischetti C (1995) Measured and simulated persistence of imazethapyr in soil. Bull. Environ. Contam. Toxicol. 54: 420–427

Walker A (1974) A simulation model for prediction of herbicide persistence. J. Environ. Qual. 3: 396–401
Walker A (1978) Simulation of the persistence of eight soil-applied herbicides. Weed Res. 218:305–313
Walker A & Barnes A (1981) Simulation of herbicide persistence in soil: a revised computer. Pestic. Sci. 12: 123–132
Walker A (1987) Evaluation of a simulation model for prediction of herbicide movement and persistence in soil. Weed Res. 27: 143–152