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Relationship between rimsulfuron degradation and microbial biomass content in a clay loam soil

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Abstract The present research was conducted to determine the relationship between the degradation of rimsulfuron and soil microbial biomass C in a laboratory-incubated clay loam soil (pH = 8.1; organic matter = 2.1%) under different conditions and at different initial dosages (field rate, 10 and 100 times the field rate). The half-life values varied between 0.4 and 103.4 days depending on temperature, soil moisture and initial dose. Evidence suggested that rimsulfuron could pose environmental risks in cold and dry climatic conditions. Significant decreases in microbial biomass C content in rimsulfuron-treated soil, compared to untreated soil, were observed initially, especially at higher temperatures and low moisture levels, but never exceeded 20.3% of that in control soil. The microbial biomass C content then returned to initial values at varying times depending on incubation conditions. The relationship between herbicide degradation and microbial biomass C content gave parabolic curves ($P < 0.005$ in all cases) under all conditions tested. Generally, maximum biomass C decrease coincided with the decrease in the concentration of rimsulfuron to about 50% of the initial dose, except at 10 °C and 100×, when biomass began to recover as early as 65–70% of the initial dose. The final equations could be useful to deduce the decrease of soil microbial biomass in relation to herbicide concentration. From the degradation kinetics of the herbicide, the time required to reach this decrease can also be calculated.

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Key words Degradation kinetics · Soil microbial biomass · Rimsulfuron · Herbicide · Pedoclimatic conditions

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 broad-leaved weeds. This compound, a highly active inhibitor of acetolactate synthase (ALS; *EC* 4.1.3.18), is the first common enzyme in the biosynthesis of branched-chain amino acids (Brown 1990) and as such can be used at very low dosages.

Rimsulfuron hydrolyses rapidly in soil under conditions of high temperature and low pH (Bassi et al. 1990). In laboratory tests performed on a sandy loam soil (pH 6.3), half-lives of 24.5 and 22.5 days under anaerobic and aerobic conditions, respectively, have been observed (Schneiders et al. 1993). On the other hand, in field experiments on a Danish light sandy soil, half-lives of 90 and 120 days for split treatment and single herbicide application, respectively, have been found (Reinke et al. 1991). These findings indicate the link between rimsulfuron persistence in soil and soil physical and chemical properties and also point to the possibility of a large range of half-lives depending on climatic conditions.

The effect of herbicide on soil biochemical properties concerning soil fertility is well known (Perucci and Scarponi 1994; Perucci et al. 1999). As most soil enzymes involved in nutrient mineralization are of microbial origin, the responses of soil microbial biomass to herbicide persistence are of particular interest.

In recent years pesticide leaching models have been developed to include the simulation of many processes such as water and solute transport through the soil profile and adsorption and degradation of pesticides in soils. As regards degradation, most models consider a

first-order approach and some include the effects of temperature and soil moisture on degradation rate. However, the models make no distinction between biotic and abiotic mechanisms in the degradation process. The elaboration of a mathematical relationship between pesticide degradation and the changes in soil microbial biomass C content could be useful to predict these changes under different pedoclimatic conditions.

The present research was conducted to ascertain the degradation of rimsulfuron in a laboratory-incubated clay loam soil under different temperature and soil moisture conditions and, subsequently, to determine the relationship between herbicide degradation and soil microbial biomass content.

Materials and methods

Chemicals and apparatus

Standard rimsulfuron was kindly supplied by DuPont de Nemours (Wilmington, Del.). 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 spectrophotometer were employed.

Soil sample preparation, contamination and recoveries

A silty clay loam soil (0–20 cm layer) taken from the mid Tiber Valley (central Italy) was used. Determination of pH, cation exchange capacity, organic C content and 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 (<2 mm) to remove plant material, soil macrofauna and stones. After sieving, the soil was homogenized for 3 h in a rotary cylinder and stored at 20 °C in the dark for 3 days (pre-incubation). Subsequently, the soil was divided into two portions: one was treated with rimsulfuron, the other was used as control.

For recovery studies, triplicate soil samples (100 g) were each contaminated with methanol solutions containing 2 μg, 20 μg and 200 μg of rimsulfuron to obtain concentrations of 20, 200 and 2000 μg kg^{-1}, corresponding to field rate (×), 10 times (10×) and 100 times (100×) the field rate. The samples were immediately 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 rimsulfuron was partitioned in $CHCl_3$ (40 ml × 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, using the following mobile phase: solvent 1, $CH_3CN:CH_3COOH$ 99.4:0.6; solvent 2, $H_2O:CH_3COOH$ 99.4:0.6. The gradient was as follows: 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}.

Trials

Treatments were as follows: 25 °C and 75% of field capacity (FC), 25 °C–33% FC, 10 °C–75% FC, 10 °C–33% FC, each at three initial concentrations equivalent to field rate for a corn crop (25 g ha^{-1}) (×), and 10× and 100× the field rate; and 40 °C–75% FC and 40 °C–33% FC each at 10× and 100×. For each of the three concentrations, a methanol solution of rimsulfuron (10 ml) was added to 100 g of soil. After evaporation of methanol at room temperature, this soil was incorporated back into the remaining soil by homogenization in a rotary cylinder for 4 h. For the control soil, 100 g of the soil was treated with methanol (10 ml) without herbicide and, after evaporation, incorporated back into the remaining soil.

For each of the experimental conditions a series of boxes for treated and control soil were prepared, each containing 200 g of soil. During the experiment, soil moisture was maintained at constant levels by daily monitoring and sterile water was added when necessary. At different days after treatment, two control boxes and a treated box for each temperature-soil moisture combination were removed to determine residual rimsulfuron and microbial biomass C content. Each trial was continued until the biomass C content returned to approximately the initial value.

Table 1 Soil properties

pH(H_2O)	8.1
Organic matter (%)	2.1
CEC (mEq 100 g^{-1})	18.0
Sand (%)	23.5
Silt (%)	47.5
Clay (%)	29.0
Bulk density (kg dm^{-3})	1.3
Field capacity (%, w/w)	30.0
Wilting point (%, w/w)	10.0

Determination of rimsulfuron residues and microbial biomass C content

Rimsulfuron was extracted from soil and determined as described in the recovery section. Soil microbial biomass was evaluated using the fumigation extraction (FE) method (Sparling and West 1988). Duplicate samples (20 g) of the treated and control soils were fumigated with ethanol-free $CHCl_3$. After removal of $CHCl_3$, soil moisture was adjusted to 60% water-holding capacity (WHC). Fumigated and unfumigated soil samples were extracted with 0.5 M K_2SO_4 and organic C quantified by oxidation with 0.0667 M $K_2Cr_2O_7$ and subsequent back-titration of the unreduced dichromate. Biomass C content was estimated as follows: Biomass C = 2.64 Ec, where Ec is the difference between the organic C extracted from the fumigated and unfumigated treatments (Vance et al. 1987).

Results and discussion

Recoveries of rimsulfuron from soil at three initial concentrations are reported in Table 2. They can be considered satisfactory and varied from 84.4% at 20 μg kg^{-1} to 97.4% at 2000 μg kg^{-1}. However, care must be taken when a concentration of 20 μg kg^{-1} is used for recovery trials, due to the sensitivity of the analytical method (5 μg kg^{-1}) and the extreme data variability, since this concentration suggests that the reliability of soil degradation data at the initial concentration of 20 μg kg^{-1} could be very low. This is confirmed by the results obtained in degradation experiments at field rate where the data variability at all conditions tested is very high (differences up to 100% between twin-sample measurements). Bearing this in mind, the degradation parameters ($t_{1/2}$) reported in Table 3 repre-

Table 2 Recoveries (%) of rimsulfuron from soil at three initial concentrations

Concentration ($\mu g\ kg^{-1}$)	Sample			Mean ± SD
	1	2	3	
20	57.4	114.1	81.7	84.4 ± 28.4
200	91.5	96.2	98.3	96.5 ± 3.5
2000	97.8	99.2	95.3	97.4 ± 2.0

sent a low reliability at field rate. For this reason and to provide a higher reliability of degradation data, rimsulfuron degradation at 10× and 100× was followed. The use of these concentrations is further justified by the fact that 10× is recommended in laboratory tests to determine the side-effects of pesticides on soil microflora (Sommerville 1987) and 100× was chosen since in a previous study (Vischetti et al. 1997) rimsulfuron was found to have a very short half-life in the same soil type.

The degradation parameters ($t_{1/2}$) reported in Table 3 were calculated from the best fit lines of the logarithm of residual concentration versus time, at various temperatures and soil moistures. Half-life values ranged from 0.4 to 103.4 days. Although rimsulfuron is highly unstable in an aqueous medium (Bassi et al. 1990), its persistence in soil, measured under laboratory conditions, has been found to vary according to soil characteristics as well as temperature and humidity (Palm et al. 1989; Schneiders et al. 1993). Rimsulfuron persistence under field conditions varied within a wider range of a DT50 of 5.6 days in a sandy clay loam soil site in the United States (Schneiders et al. 1993) and a DT50 of 120 days in a light sandy soil in Denmark (Reinke et al. 1991). The half-life values in our experiment fall within the range reported in the literature. At the same initial dosage and soil moisture, half-life values of rimsulfuron increased 5- to 15-fold both from 40 °C to 25 °C and from 25 °C to 10 °C. The effect of initial dose on degradation did not seem to be significant from× to 10×, while from 10× to 100× the effect of the concentration proved to be relevant only at 10 °C. Indeed at this temperature, the half-life values doubled with both soil moisture treatments (Table 3).

Treatments with other ALS inhibitors have proved to produce an effect on soil biochemical parameters involved in soil fertility (Dumontet et al. 1993; Perucci and Scarponi 1994; Perucci et al. 1999). Consequently, rimsulfuron can influence soil microbial biomass content, the extent of which depends on its degradation.

Significant decreases in microbial biomass C content, compared to untreated soil, were observed initially, but never exceeded 20.3% of that in control soil. This effect was more pronounced at higher temperatures and at the lowest soil moisture level. The microbial biomass C content returned to initial values at varying times depending on incubation conditions. The relationship between herbicide degradation and microbial biomass C content gave parabolic curves ($P < 0.005$ in all cases) under all conditions tested (Figs. 1–3).

From the vertices of the parabolas it is possible to deduce the peak values of biomass C content decrease and the corresponding rimsulfuron concentrations (Table 4). At the time of maximum decrease in biomass C content, the concentration of rimsulfuron was generally about 50% of the initial dose, except for at 10 °C–75% FC-100× and 10 °C–33% FC-100×, where the recovery of the microbial biomass began at 70.4% and 65.1% of rimsulfuron persistence, respectively.

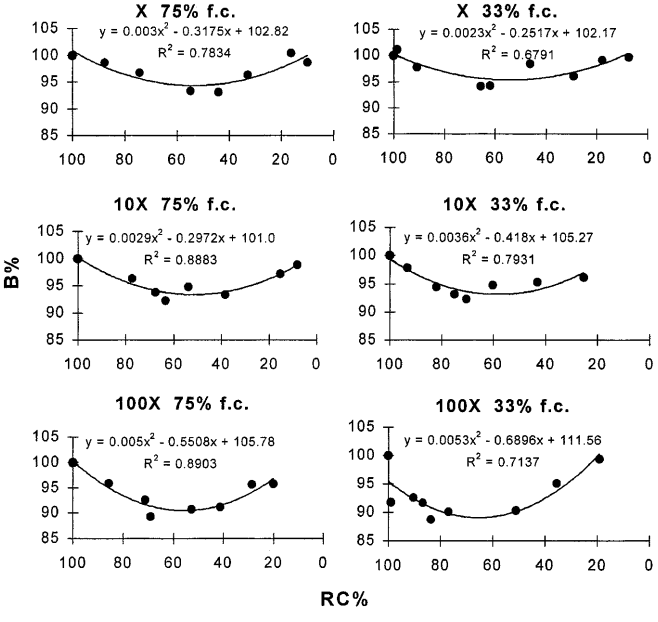

Fig. 1 Correlation between biomass C content and persistence of rimsulfuron at 10 °C (*B%* percent of biomass C content vs control; *RC%* percent of rimsulfuron concentration vs initial dosage)

Table 3 Half-life values ($t_{1/2}$, days) for rimsulfuron in soil at different temperatures, soil moistures and initial dosages

Initial dosage	Incubation condition					
	10 °C–75% FC	10 °C–33% FC	25 °C–75% FC	25 °C–33% FC	40 °C–75% FC	40 °C–33% FC
×	16.6	50.2	3.4	5.6	–	–
10×	15.6	54.2	3.7	7	0.5	0.6
100×	34.7	103.4	6.0	9.1	0.4	0.6

Table 4 Biomass decrease (%) versus control and corresponding rimsulfuron concentration (%) vs initial dosage (*bd* biomass depression, *rc* rimsulfuron concentration

Initial dosage	Incubation condition											
	10 °C–75% FC		10 °C–33% FC		25 °C–75% FC		25 °C–33% FC		40 °C–75% FC		40 °C–33% FC	
	bd	rc	bd	rc	bd	rc	bd	rc	bd	rc	bd	rc
×	5.6	52.9	4.7	54.7	6.7	59.9	7.6	50.9	–	–	–	–
10×	9.1	49.0	6.9	58.1	6.6	51.2	7.9	50.2	16.5	50.3	11.0	49.2
100×	9.3	70.4	10.9	65.1	11.8	52.9	10.3	55.2	20.3	50.8	18.5	52.1

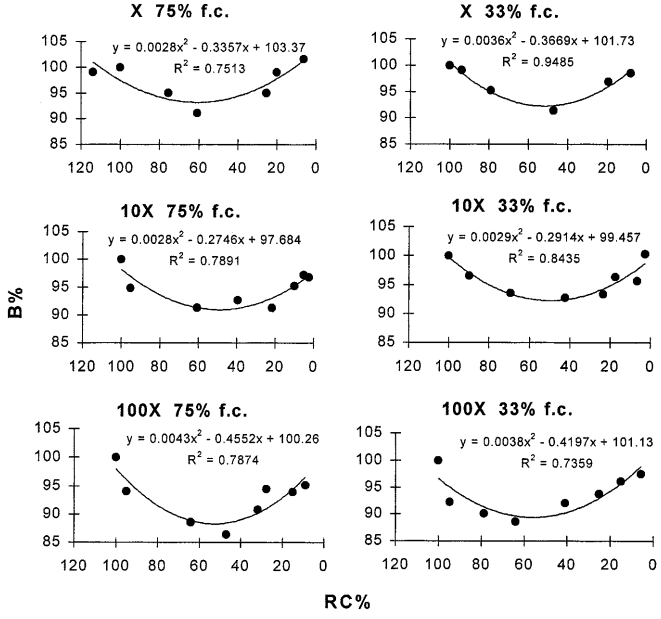

Fig. 2 Correlation between biomass C content and persistence of rimsulfuron at 25 °C (*B%* percent of biomass C content vs control; *RC%* percent of rimsulfuron concentration vs initial dosage)

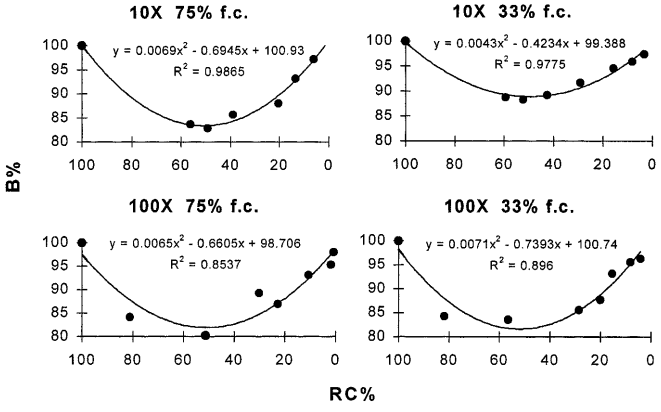

Fig. 3 Correlation between biomass C content and persistence of rimsulfuron at 40 °C (*B%* percent of biomass C content vs control; *RC%* percent of rimsulfuron concentration vs initial dosage)

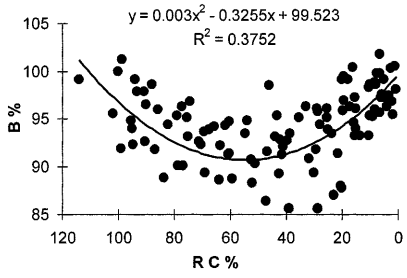

Fig. 4 Correlation between biomass C content and persistence of rimsulfuron in all conditions tested (*B%* percent of biomass C content vs control; *RC%* percent of rimsulfuron concentration vs initial dosage)

This can probably be attributed to the low temperature, which may have reduced microbial biomass activity and the toxic effect of the pesticide. Therefore, the time necessary to reach 65–70% of initial pesticide concentration was long enough to enable the biomass to adapt to the presence of the pesticide. At 100×, half-life values were twice that at × and 10×, the degradation time increased and microbial biomass recovery began when the pesticide concentration was still higher than 50% of the initial dosage. At 25 °C and 40 °C, a low effect of initial dosage on herbicide degradation was observed, probably because the effect of the higher temperature prevailed.

The equations in Figs. 1–3 may be useful in order to deduce the trend of soil microbial biomass in relation to herbicide concentration. The times to reach such a state can be calculated only if the degradation kinetics of the herbicide are known. From these equations it is possible to observe that the entity of microbial biomass decreases and the trend of the parabolic curves are similar, independent of initial concentrations. This means that the equations derived at 10× and 100× are useful to predict the soil microbial biomass content following a treatment with rimsulfuron. The parabolic curve constructed using the data from all the conditions tested (Fig. 4) confirms this finding ($P < 0.001$). Therefore, the relationships presented here could help in modelling soil microbial biomass behaviour after herbicide treatment.

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