Herbicidal Activity and Residual States of Metolachlor in Upland Soil*

Hiroshi Sugiyama** and Katsuichiro Kobayashi**

Abstract: The residues of metolachlor [2-chloro-2'-ethyl-*N*-(2-methoxy-1-methylethyl)-6'-methylacetanilide] applied to a soybean field were determined separately as to water-soluble and water-insoluble ingredients and were found to fluctuate with precipitation. The half life of all metolachlor was calculated as 40 to 60 days in 1990 and 4 to 5 days in 1991. The half life of the water-soluble ingredients, however, was as 6 to 12 days in 1990 and 3 days in 1991. Plant growth of Italian ryegrass in soils collected periodically after metolachlor application was inhibited, and this inhibition was closely related to the concentration of the water-soluble ingredients but little to that of the water-insoluble ingredients. This confirmed that the inhibition of plant growth by soil-applied metolachlor was induced by its water-soluble ingredients, while concentration of the herbicide in the soil water fluctuated little with the amount of precipitation, as different amounts of precipitation caused little variation in activity.

Key words: metolachlor, water soluble ingredients, soil water, residue in soil, herbicidal activity

Introduction

Metolachlor [2-chloro-2'-ethyl-*N*-(2-methoxy-1-methylethyl)-6'-methylacetanilide], is widely applied to soil in annual upland weed

control. Many reports have given the adsorption, mobility, residue and performance of metolachlor in soils^{2,3,8,9,14}, its inhibitory characteristics on plant growth⁴, the effects of residue on rice¹ and influence of soil water content on metolachlor injury to corn^{10,13}. However, these reports have provided little information on the relationship between the performance of metolachlor in soil and its inhibitory activity on plant growth. In our previous reports^{11,12}, the inhibitory activities of butachlor [*N*-(butoxymethyl)-2-chloro-2', 6'-diethylacetanilide], pretilachlor [2-chloro-2', 6'-diethyl-*N*-(2-propoxyethyl)acetanilide] and mefenacet [2-(benzothiazol-2-yloxy)-*N*-methylacetanilide], were shown to be clearly related to the concentrations of water-soluble ingredients in paddy soil. It was also suggested that the inhibitory activity of soil-applied herbicides was dependent on the concentration of water-soluble ingredients in the soil.

The objectives of this study were to confirm the relationship between the inhibitory activity of metolachlor applied to upland soil on plant growth and ingredient concentration in soil water with different amounts of precipitation.

Materials and Methods

Soybean cultivation and metolachlor application

Soybean (*Glycine max* Merr. cv. 'Enrei') was cultivated following conventional cropping in an upland field of National Agriculture Research Center in Tsukuba City (light-colored andosol, total carbon: 3.29%, total nitrogen: 0.29%, phosphate adsorption coefficient: 2,241, cation

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** National Agriculture Research Center, Tsukuba, Ibaraki 305, Japan

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Table 1. Precipitation and temperature during the 60 days after application of metolachlor to the soybean field.

Application date	Soil sampling following application (days)	Precipitation (mm)	Average temperature (°C)
1990.6.22	0		
		3.5	21.7
	10	49.5	23.2
	30	83.0	26.4
	60		
	total	136.0	24.5
1991.6.26	0		
		101.5	23.8
	12	32.5	24.5
	30	154.0	24.6
	60		
	total	288.0	24.4

exchange capacity : 19.0 me/100 g, clay content : 12.0%). An emulsifiable concentrate of metolachlor (ai 45%, 'Dual') was diluted with water and applied to the soil surface with a hand sprayer immediately after the seeding of soybean. The row areas where soybean was sown were treated with more of the herbicide than the furrows. Two rates of dosage, 1.8 kg and 3.6 kg ai/ha, and a control were set up. Experiments were made with two replications on 26 m² plots in both 1990 and 1991. Soil sampling and climatic conditions are shown in Table 1.

Soil sampling

Three hundred ml of soil was periodically collected by a soil sampler with a 100 ml cylinder (diameter 5.04 cm, 5.0 cm depth) at fixed points in each plot. The collected sample was adequately mixed and the soil water content and soil volume were determined. Soil volume was calculated as follows : 5 g of fresh soil was added to a graduated cylinder containing 10 ml of distilled water. The volume was increased by 5 g of fresh soil. The average volume of the fresh soil was 3.0 ml (range : 2.5-3.3 ml). The water content in the

soil samples ranged from 31 to 40%. Leftover samples were frozen at -20°C until used for the analysis of metolachlor residue.

Extraction of metolachlor in soil

1) Water soluble metolachlor

Thirty ml of distilled water was added to 10 g of a fresh soil sample in an Erlenmeyer flask (100 ml). The stoppered flask was vigorously shaken by autoshaker for 30 min.. The mixture of soil and water was separated through a Kiriyaama funnel with 1 g of celite 545 onto glass fiber filter paper under reduced pressure. Twenty ml of distilled water was added to the soil sediment for washing. The metolachlor contained in the filtered and washed water was combined and extracted with 10 ml of *n*-hexane after addition of 1 g of NaCl. The extraction procedure was repeated several times. The *n*-hexane layer was dehydrated on separator filter paper (Toyo 2S). This *n*-hexane layer was then evaporated to dryness and subsequently made up to 4 ml by acetone for gas chromatograph (GC) analysis.

2) Water insoluble metolachlor

Fifty ml of acetone was added to a flask containing the soil sediment which had been extracted with water as described above and the flask was allowed to stand overnight at room temperature. After vigorous shaking for 30 min., the mixture was filtered through a Kiriyaama funnel onto glass fiber filter paper. The residual soil was washed with 30 ml of acetone and the acetone solutions were combined. The acetone-soluble extracts were concentrated under reduced pressure in a water bath kept at 40°C, put into a separatory funnel, and added with 5 ml of 4% NaCl solution. Extraction by *n*-hexane was repeated 2 times. The *n*-hexane layer was separated and dehydrated with separator filter paper (Toyo 2S). The *n*-hexane was evaporated under reduced pressure and the residue was made up to 4 ml by acetone for GC analysis.

Metolachlor determination

Metolachlor was determined according to the

modified analytical method for pesticide residue⁵⁾ by GC (HP 5890) equipped with a nitrogen-phosphorus flame ionization detector. The column was 2% OV-101 on Chromosorb W (100-120 mesh). The temperatures of column, detector and injection port were 200, 300 and 280°C, respectively. Carrier gas flow rates were as follows: nitrogen 24 ml/min, air 100 ml/min and hydrogen 3 ml/min. The detectable limit of metolachlor was 0.05 ppm and the recovery from the soil fortified with metolachlor was over 80%. Standard metolachlor used for GC analysis was contributed by CIBA-GEIGY (Japan), Ltd.

Assay for inhibitory activity on plant growth

Italian ryegrass (*Lolium multiflorum* Lam. cv. 'Waseaoba') was subjected to an assay of the growth inhibitory activity of metolachlor in soil. The seeds were germinated on wet filter paper in a petri dish at 25°C for 24 hours and the uniformly germinated seeds were placed in glass tubes (2 cm diameter×7 cm depth) which were packed with the soil from the metolachlor treated upland fields. The tubes were kept for 3 days in an incubator (25°C, 4 klux) and the shoot lengths of the plant were measured. Three seeds were used per tube with 4 repetitions.

Results and Discussion

1. Residues of metolachlor in soil

The residues of metolachlor in the soil are shown in Figs. 1 and 2. On the day of application, the herbicide's water-soluble ingredients were determined as 2 µg/ml fresh soil at the rate of 1.8 kg ai/ha; at the rate of 3.6 kg ai/ha they were 5 µg/ml in 1990 and 7 µg/ml in 1991 (Fig. 1). In the same way, the water-insoluble ingredients of metolachlor were 4 µg/ml at 1.8 kg/ha and 9 µg/ml at 3.6 kg in 1990, and 3 µg/ml and 7 µg/ml in 1991 respectively (Fig. 2). The total amount of metolachlor detected with the 1.8 kg and 3.6 kg ai/ha applications, therefore, was estimated as 6 µg and 14 µg/ml in 1990, and 5 µg and 14 µg/ml in 1991, respectively. Thus the total residues of

Fig. 1. Residues of water-soluble metolachlor in the field soil.

Application rate of metolachlor: ○ 1.8 kg ai/ha,

● 3.6 kg ai/ha

* soil water was estimated as 40%

Fig. 2. Residues of water-insoluble metolachlor in the field soil.

Application rate of metolachlor: △ 1.8 kg ai/ha,

▲ 3.6 kg ai/ha

metolachlor on the day of application in the two years were almost identical.

The dissipation pattern of metolachlor in soil in 1991, however, was clearly different from that in 1990. The total residue of metolachlor in 1991 after 12 days was reduced to $0.8 \mu\text{g/ml}$ and $3.9 \mu\text{g/ml}$ in fresh soil with 1.8 kg/ai and 3.6 kg ai/ha application, respectively. Bravermann *et al.*²⁾, pointed out that metolachlor probably moves through the soil profile within the range of 0 to 7.5 cm of the soil surface and that its half life fluctuates with the precipitation, being calculated to be 60 days in a year of little rainfall but 13 days in a year of enough rainfall. Walker and Zimdahl¹⁴⁾ also reported that the persistence of metolachlor depended on properties of the soil and suggested that it was more persistent in dry land than in irrigated land, even in the same soil.

In the present study, the half life of total metolachlor at a soil depth of 0 to 5 cm was calculated from the data shown in Fig. 1 and Fig. 2 to be 40 to 60 days in 1990 and 4 to 5 days in 1991. The half life of its water-soluble ingredient, however, was computed as 6 to 12 days in

1990 and 3 days in 1991 (Fig. 1). Precipitation in 1991 totaled 288 mm, over 2 times the 136 mm in 1990 (Table 1). In 1991 recorded rainfall was 101.5 mm during 12 days following herbicide application. These findings showed that the residue of metolachlor water-insoluble ingredient in the soil was fluctuated remarkably with precipitation, whereas the water-soluble ingredients in 1990 and 1991 dissipated similarly and showed on great fluctuation. The amount of water-soluble ingredients thus appear little affected by precipitation, in contrast to the total and water-insoluble residues of the herbicide.

2. Inhibitory activity of metolachlor in soil on plant growth

Italian ryegrass was cultured in fresh soils periodically collected after metolachlor application and the amount of the residual herbicide was determined. Figure 3 shows the relationship between the amount of water-soluble metolachlor and plant growth. The regression equations of 1990(a) and 1991(b) are: $Y = 29.2545 - 20.4875 \ln X$ ($\gamma = -0.8941^{**}$), and $Y = 26.7149 - 21.8313 \ln X$ ($\gamma = -0.9311^{**}$), respectively. The regression

Fig. 3. Plant growth inhibition in ratio to the residual state of metolachlor in soil

- (a) $Y = 29.2545 - 20.4875 \ln X$ ($\gamma = -0.8941^{**}$) ($\circ$ water soluble 1990)
- (b) $Y = 26.7149 - 21.8313 \ln X$ ($\gamma = -0.9377^{**}$) ($\bullet$ water soluble 1991)
- (c) $Y = 61.2119 - 15.5089 \ln X$ ($\gamma = -0.3890$) ($\triangle$ water insoluble 1990)
- (d) $Y = 59.0330 - 36.6421 \ln X$ ($\gamma = -0.9550^{**}$) ($\blacktriangle$ water insoluble 1991)

Fig. 4. Plant growth inhibition in field soils applied with metolachlor in 1991.

1.8 kg ai/ha: —○— fresh soil, ---○--- soil after extraction of water soluble metolachlor

3.6 kg ai/ha: —△— fresh soil, ---△--- soil after extraction of water soluble metolachlor

coefficient is almost the same in the two linear equations. Regression equations for the amount of herbicide water-insoluble ingredients and plant growth are $Y = 61.2119 - 15.5089 \ln X$ ($\gamma = -0.3890$) in 1990(c) and $Y = 59.0330 - 36.6421 \ln X$ ($\gamma = -0.9550^{**}$) in 1991 (d), respectively.

The growth of Italian ryegrass in soil without metolachlor water-soluble ingredients was also tested in 1991 and compared with growth in fresh soil (Fig. 4). Growth in the soil from which these ingredients had been extracted was little inhibited, whereas growth in the fresh soil before extraction was remarkably inhibited. In both types of soil application rate and time had a

bearing on plant growth. No growth inhibition was found in soils from which water-soluble ingredients had been extracted 30 days following 1.8 kg ai/ha application or 60 days following application of 3.6 kg ai/ha (Fig. 4), although these amount of water insoluble metolachlor remained about $1 \mu\text{g/ml}$ to be matched for three times dosage of 50% inhibition on plant growth by water-soluble metolachlor (Fig. 3). Evaluation of these various results appeared to indicate that the herbicidal activity of metolachlor in the soil depended on the concentration of its water soluble ingredients but was not affected by its water-insoluble ingredients.

Coleoptile elongation of the oat, *Avena sativa* L. cv. 'Victory' was significantly inhibited at a metolachlor concentration of 1×10^{-6} M when treated for 48 hours⁴⁾. In the present study, Italian ryegrass shoot elongation was inhibited by 50% at $0.3 \mu\text{g}$ of water-soluble ingredients in 1 ml of fresh soil (Fig. 3). This inhibition seemed to correspond to oat growth, since $0.3 \mu\text{g/ml}$ of metolachlor was roughly estimated to be 2×10^{-6} M in the soil water.

There has been no information on the herbicidal activity of metolachlor metabolites, although the metabolism of the herbicide by soil microbes has been clarified^{6,7)}. The herbicidal activity of metolachlor metabolites in soil on plant growth was not examined in the present study; however, the results obtained here confirmed that the herbicidal activity is primarily governed by its water-soluble ingredients in soil. In flooded paddy soils, the herbicidal activity of acetanilide herbicides to rice plant was dependent on the concentration of water-soluble ingredients and was dominated by the soil properties^{11,12)}. It thus can be postulated that the herbicidal activity of an herbicide is actually caused by the concentration of the herbicide in soil water in both upland and paddy soil. It is important to emphasize that the total amount of metolachlor residue in soil is not as much influenced by properties of

the soil as by environmental factors, whereas the herbicide's concentration in soil water is dominated by the soil properties and environmental factors have little impact.

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畑土壌におけるメトラクロールの残留形態と殺草活性

杉山 浩*・小林勝一郎*

摘 要

大豆播種直後に施用したメトラクロール [2-

chloro-2'-ethyl-N-(2-methoxy-1-methyl-ethyl)-6'-methylacetanilide] の土壌中の残留を水可溶態および水不溶態とに分けて測定し殺草活性との関連を調べた。土壌中の水不溶態メトラクロール量は1990年と1991年で変動したが (Fig. 2), 水可溶態メトラクロール量は降雨による影響が小さく (Fig. 1), それは降水量の差によると考えられた (Table 1)。生土壌容積あたりのメトラクロール総量 (水不溶態および水可溶態メトラクロールの和) の半減期は1990年では40日～60日, 1991年では4日

* 農林水産省農業研究センター

～5日であった。しかしながら水可溶態メトラクロールの半減期は1990年では6日～12日、1991年では3日であり総量の半減期に比べ、年次間の変動がきわめて小さかった。メトラクロールの土壌施用後、定期的に採取した土壌の殺草活性を調べた。1990年および1991年の水可溶態メトラクロール濃度と殺草活性との相関係数はともに高く、回帰係数も同様の値を示した(1990: $Y=29.2545-20.4875 \ln X$ ($\gamma=-0.8941^{**}$), 1991: $Y=26.7149-21.8313 \ln X$ ($\gamma=-0.9377^{**}$))。しかし、水不溶態メトラクロール濃度は、1991年の場合には殺草性との相関係数は高かったが、1990年は相関係数は低い値を示し、また両年の回帰係数は著しく異なっていた(1990: $Y=61.2119-15.5089 \ln X$ ($\gamma=0.3890$), 1991: $Y=59.0330-36.6421 \ln X$ ($\gamma=-0.9550^{**}$)) (Fig. 3)。メトラクロールを処理した生土壌と水可溶態メトラ

クロール除去土壌との殺草性を比べた結果、前者の土壌では1.8 kg ai/haの場合には30日後でも活性を示したが、後者の土壌は処理当日および12日後の場合でも殺草活性は生土壌に比べて著しく劣り、30日以降では殆ど殺草性を示さなかった (Fig. 4)。以上の結果から、土壌処理したメトラクロールの殺草活性は土壌中における本剤の総量でなく、水可溶態メトラクロール濃度に依存していることが確かめられた。また、土壌中における本剤の水可溶態および水不溶態量は降水量によって変動するが、土壌水分中のメトラクロール濃度の変動は小さいので、降水量が異なっても殺草活性は、殆ど変化しないものと思われる

キーワード: メトラクロール, 水可溶態メトラクロール, 土壌水分, 土壌残留, 殺草活性