Herbicidal Selectivity of Foliar-Applied Simetryne : Its Absorption, Translocation and Metabolism in Gramineous Plants*

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The triazine herbicide simetryne [2,4-bis (ethylamino)-6-(methylthio)-s-triazine], effectively controls broadleaf weeds and barnyardgrass in paddy rice. Its agricultural use has been studied extensively (1, 2, 3, 5, 6, 9, 11, 12, 16, 17). The absorption, translocation and metabolism of radioactive triazine herbicides in general have also been investigated in plants to clarify their selective mode of action. Most of the studies, however, have concentrated on 2-chloro-substituted s-triazine herbicides, such as simazine [2-chloro-4,6-bis (ethylamino)-s-triazine] and atrazine [2-chloro-4-(isopropylamino)-6-(ethylamino)-s-triazine]. Although several investigations indicated differences in absorption and translocation between tolerant and susceptible plants as the main factors contributing to selectivity of simazine and atrazine (19, 20, 22, 31), prometryne (27), terbutryne and propazine (4), many researchers have also shown a close correlation between metabolic transformation and tolerance to s-triazines. As for metabolic transformations, three reaction pathways have been found: (1) hydroxylation at the 2-position of the triazine ring (7, 8, 18, 21, 24, 28, 29), (2) dealkylation of the alkyl side chain (21, 23, 24, 31), and (3) peptide conjugation at the 2-position associated with dechlorination (14, 19, 25, 29, 30) and subsequent peptide metabolism (15, 26).

The present investigation deals with absorption, translocation and metabolism of simetryne in gramineous plants in order to attempt a clarification of its selectivity mechanism, which is not yet fully understood.

Materials and Methods

1. Plant materials

The seeds of rice (*Oryza sativa* L. cv. Nihonbare), barnyardgrass (*Echinochloa crus-galli* (L.) Beauv. var. *oryzicola* Ohwi), corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), finger millet (*Eleusine coracana* Gaertn.), and large crabgrass (*Digitaria adscendens* Henr.) were germinated in an incubator at 25°C for 2 days, the seedlings were then transferred to plastic pots containing Kasugai's nutrient solution (13). Plants were further grown in water culture for 15 days up to the 3-leaf stage in a greenhouse with controlled temperatures of 29°C at day and 22°C during the night. Imperfect leaves of rice were omitted in the leaf stage counting. Plants of this growth stage were used in all experiments.

2. ¹⁴C-Simetryne

The ¹⁴C-simetryne supplied by CIBA-GEIGY was uniformly ring-labeled with a specific activity of 17.0 μ Ci/mg. The radiochemical purity determined by thin-layer chromatography with a developing solvent of benzene : chloroform : ethyl acetate : n-propanol (2:1:2:1 v/v/v/v) was 96.5 %. Two spots other than simetryne were detected by autoradiography, but further

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purification was not attempted.

3. Foliar Application of Simetryne

Shoots of plants, rinsed at first with a surfactant solution (5000 times diluted Neoesterin®, Kumiai Chemical Industry Co., Ltd.), were dipped for one hour in an aqueous solution of simetryne at various concentrations. After such treatment, the shoots were thoroughly washed with water, the plants transferred to the nutrient solution and allowed to grow in the greenhouse. Seven days after treatment, the plants were harvested, divided into roots and shoots, and their fresh and dry weights were determined. Each experiment was replicated three times using ten plants, except that, for corn, five plants were used. The experiment was conducted in September, 1979.

4. Absorption of ^{14}C -Simetryne by Shoots

Shoots of plants were dipped in 0.41 ppm of the simetryne solution ($7.1 \mu\text{Ci/l}$) as described above. The solution was prepared by mixing it with 1% acetone. At various intervals the shoots were thoroughly washed with water and blotted with tissue paper. After being dried at 90°C for 24 hours, the shoots were combusted in a sample combustion system (Aloka ASC-113) and ^{14}C -activities determined by a liquid scintillation spectrometer (Beckman LS-8100). All samples counted were corrected for quenching and expressed as dpm. The experiments were carried out in a growth chamber maintained at a temperature of 30°C , 67 % relative humidity and a light intensity of 20 klux.

5. Translocation of ^{14}C -Simetryne in Plants

A solution containing $1 \mu\text{Ci/ml}$ of ^{14}C -simetryne dissolved in a 50 % ethanol solution was used for translocation studies. Ten microliters of this solution was applied to the middle part of the second or third leaf of the test plants. After application the treated plants were kept growing in the greenhouse for 6, 24, or 72 hours, and then prepared for autoradiography as described previously (10).

6. Metabolism of Simetryne

Shoots of rice and barnyardgrass were dipped in 2.4 ppm of herbicide solution containing $20.4 \mu\text{Ci}/500 \text{ ml}$ of ^{14}C -simetryne for 1.5 hours. After this treatment they were washed with water, and the plants were transferred into a nutrient solution for the designated periods. Sixty shoots of rice and barnyardgrass were then homogenized in 50 ml of 90 % methanol and re-extracted twice with 90 % methanol. ^{14}C -Radioactivity of the extracts was determined by a liquid scintillation spectrometer. The combined methanol extracts were concentrated *in vacuo* at 35°C and spotted on thin-layer chromatographic plates coated with silica gel (Merck Kieselgel F 254, 0.2 mm in layer thickness). Chromatograms were developed with a mixture of benzene:chloroform:ethyl acetate:n-propanol (2:1:2:1 v/v/v/v, solvent system I), the upper phase of a mixture of n-butanol:acetic acid:water (5:1:4 v/v/v, solvent system II), a mixture of chloroform:methanol:formic acid:water (75:20:4:2 v/v/v/v, solvent system III) and a mixture of hexane:ethyl acetate (2:8 v/v, solvent system IV). Extracts were further partitioned with equal volumes of dichloromethane and water. Aliquots of the organic and aqueous fractions were also used for determining ^{14}C -radioactivity after being concentrated and chromatographed. The flow sheet of extraction and determination of simetryne and its metabolite is shown in

Fig. 1. Location of the radioactive spots on thin-layer chromatograms were surveyed by autoradiography, and identified by a comparison with R_f values of authentic compounds. The radioactive spots were then scraped off the plates, and the material placed into scintillation vials for determination of radioactivity. Radioactivity of non-extractable residues was determined by the sample combustion method.

Fig. 1. Flow sheet of qualitative and quantitative determination of simetryne and its metabolites in shoots of rice and barnyardgrass.

Results and Discussion

1. Effects of Foliar-Applied Simetryne on Plant Growth

Seven days after treatment finger millet and large crabgrass were almost killed at concentrations exceeding 0.23 mM, as shown in Fig. 2. Rice, wheat, corn and barnyardgrass, however, were not, even at the highest concentration used, but showed some symptoms typical of simetryne injury: wilting or rolling up, which started at the tips of the treated leaves. The extent of damage, however, was very different among these plant species, barnyardgrass being the most severely damaged. The effects on growth exhibited by simetryne are also shown in Fig. 2. It was observed that simetryne was quite selective among gramineous plants, rice being the most tolerant, and finger millet, large crabgrass and barnyardgrass the most susceptible species. Wheat and corn showed

Fig. 2. Effect of foliar-applied simetryne on growth of gramineous plants.

Fig. 3. Absorption of ^{14}C -simetryne by shoots of rice and barnyardgrass.

Fig. 4. Autoradiographs (right 4 plates) and photographs (left 4 plates) of rice and barnyardgrass treated with ^{14}C -simetryne.

Droplets of ^{14}C -simetryne solution were applied to the second or third leaf as indicated by arrows. The photographs indicate 2 rice plants (right) and 2 plants of barnyardgrass (left).

A. 6 hours after treatment. D. 72 hours after treatment.

B. 24 hours after treatment.

C. 48 hours after treatment.

intermediate tolerance.

Rice and barnyardgrass were chosen for further studies of absorption, translocation and metabolism, because both species grow competitively in the same ecological environment and were different in their response to simetryne.

2. Absorption of ^{14}C -Simetryne by Shoots of Rice and Barnyardgrass

The results of the absorption studies using shoots of rice and barnyardgrass are shown in Fig. 3. The rates of absorption were very different between the two plant species, the concentration of simetryne in shoots of barnyardgrass was 1.9 to 2.6 times higher than that in rice. There was also a good correlation between the rate of absorption by shoots and the effect on plant growth. The differential absorption by shoots appears to be one of the factors determining the herbicidal selectivity of foliar-applied simetryne.

3. Translocation of ^{14}C -Simetryne in Rice and Barnyardgrass

The translocation of ^{14}C -simetryne within the second and third leaf of rice and barnyardgrass is shown in Fig. 4. The autoradiographs demonstrated that absorbed simetryne moved acropetally at an extremely high rate in both plant species though it was not clear whether simetryne or its metabolites were translocated. Twenty-four or 48 hours after treatment, a little basipetal movement was observed. There were no differences in direction or rate of translocation in rice and barnyardgrass; thus, translocation was not a factor in determining selectivity of foliar-applied simetryne.

4. Metabolism of ^{14}C -Simetryne in Shoots of Rice and Barnyardgrass

Results on ^{14}C -simetryne metabolism in shoots of rice and barnyardgrass over periods of 0 to 72 hours after foliar application are given in Figs. 5 and 6. Information on the chromatographic behavior of simetryne and simetryne-related authentic compounds is summarized in Tables 1 and 2.

In rice, a time study showed that radioactivities in dichloromethane-soluble fractions decreased with time, while in water-soluble fractions they remarkably increased over a period, reaching a maximum 24.5 % of total radioactivity 24 hours after treatment. Methanol-insoluble residues increased up to 19.8 % 72 hours after treatment.

In barnyardgrass, radioactivities found in dichloromethane-soluble fractions also decreased with time but the radioactivity increase in the water-soluble fractions was lower than that in rice (17.1 % 24 hours after treatment). Methanol-insoluble residues

Fig. 5. Metabolism of ^{14}C -simetryne applied to rice shoots.

Fig. 6. Metabolism of ¹⁴C-simetryne applied to barnyardgrass shoots.

increased to only 8.0 % 72 hours after treatment, which concentration was small compared to that of rice.

It was thus concluded that rice was more active than barnyardgrass in metabolizing simetryne to water-soluble or methanol-insoluble compounds.

The dichloromethane-soluble fractions were further investigated in detail by thin-layer chromatography. The concentrations of unchanged simetryne which represented the major compound in dichloromethane-soluble fractions decreased with time in both species, and little difference in their rates of decrease was detected. In barnyardgrass, 2-amino-4-(ethylamino)-6-(methylthio)-s-triazine (mono-dealkylated simetryne) was produced as a major metabolite by N-dealkylation (29.5 % in 24 hours). In rice, however, this metabolic route was not observed to be of major importance.

Both species showed the presence of unknown I and other dichloromethane-soluble metabolites. Although none of these metabolites has been identified, they were considered to be more polar than mono-dealkylated simetryne. We assume that they represent precursors of

Table 1. R_f values of authentic compounds determined by thin-layer chromatography.

Authentic compounds	Solvent systems*			
	I	II	III	IV
2,4-bis (ethylamino)-6-methylthio-s-triazine (simetryne)	0.86	0.84	0.96	0.71
2-amino-4-ethylamino-6-methylthio-s-triazine	0.63	0.79	0.95	0.41
2,4-diamino-6-methylthio-s-triazine	0.33	0.72	0.91	0.20
2,4-bis (ethylamino)-6-hydroxy-s-triazine	0	0.50	0.65	0
2-amino-4-ethylamino-6-hydroxy-s-triazine	0	0.40	0.45	0
2-amino-4,6-dihydroxy-s-triazine	0			0

- * I. Benzene-chloroform-ethyl acetate-n-propanol (2 : 1 : 2 : 1, v/v/v/v)
II. n-Butanol-acetic acid water (5 : 1 : 4, v/v/v, upper phase)
III. Chloroform-methanol-formic acid-water (75 : 20 : 4 : 2, v/v/v/v)
IV. Hexane-ethyl acetate (2 : 8, v/v)

Table 2. R_f values of dichloromethane-soluble and water-soluble fractions of rice and barnyardgrass by thin-layer chromatography.

Dichloromethane-soluble fraction of rice				
Solvent systems				Identification
I	II	III	IV	
0.84	0.85	0.70		Simetryne
0.63	0.79	0.41		Mono-dealkylated simetryne
0.30		0.07		Unknown I
0	*	0		Others
Dichloromethane-soluble fraction of barnyardgrass				
Solvent systems				Identification
I	II	III	IV	
0.84	0.82	0.71		Simetryne
0.63	0.76	0.41		Mono-dealkylated simetryne
0.31		0.06		Unknown I
0	*	0		Others
Water-soluble fraction of rice				
Solvent systems				
I	II	III	IV	
0	*	*	0	
Water-soluble fraction of barnyardgrass				
Solvent systems				
I	II	III	IV	
0	*	*	0	

* This fraction did not give a clear spot because of tailing. For solvent systems see Table 1.

water-soluble metabolites because they were found in the early steps of metabolism.

These results indicated that both rice and barnyardgrass metabolized simetryne, but by different patterns of metabolism. Rice effectively converted simetryne to water-soluble compounds or insoluble plant residues, whereas barnyardgrass was mainly converted into mono-dealkylated simetryne.

The remarkable difference in the main metabolic pathway was not reflected in the rate of simetryne degradation. The concentration of unchanged simetryne was found to be higher in barnyardgrass than in rice due to the former plant's higher rate of absorption. Although the significance of mono-dealkylated simetryne in causing phytotoxicity has not been defined, it was concluded that differences in rates of absorption by shoots and in metabolic pathways may contribute to selectivity of foliar-applied simetryne.

5. Absorption of ^{14}C -Simetryne by Shoots of Gramineous Plants

Since differences in the rates of absorption were considered to be an important factor in determining selectivity, absorption of ^{14}C -simetryne was investigated using gramineous plants other than rice and barnyardgrass. The results are shown in Fig. 7. Rates of absorption by shoots greatly differed among plants, large crabgrass and barnyardgrass absorbing much higher rates than the other plant species. Rice, wheat, corn and finger millet were almost identical. It was felt probable that the susceptibility of large crabgrass and barnyardgrass was mainly due to their high rate of absorption. It was also noted that finger millet, which is fairly susceptible to simetryne, showed an exceptionally slow rate of absorption comparable to that of tolerant plants. The reason for this particular sensitivity must be further studied.

When considering absorption, translocation and metabolism, we concluded that the selectivity of simetryne after foliar application was mainly due to the differential absorption by shoots, and a differential pathway of metabolism.

Fig. 7. Absorption of ^{14}C -simetryne by shoots of gramineous plants.

—Δ— Large crabgrass, —▲— Wheat,
 —○— Barnyardgrass, —●— Rice,
 —□— Finger millet, —■— Corn.

Summary

The selectivity pattern of foliar-applied simetryne [2,4-bis (ethylamino)-6-(methylthio)-s-triazine] was studied in six gramineous plant species. Their tolerance to simetryne could be described by the following sequence: rice (*Oryza sativa* L.) > wheat (*Triticum aestivum*

L.) > corn (*Zea mays* L.) > barnyardgrass (*Echinochloa crus-galli* Beauv.) > large crabgrass (*Digitaria adscendens* Henr.) > finger millet (*Eleusine coracana* Gaertn.). In order to clarify the selectivity mechanism of simetryne, its absorption, translocation and metabolism in rice (tolerant) and barnyardgrass (susceptible) were investigated. The rates of absorption of ^{14}C -simetryne by shoots were much higher in barnyardgrass than in rice, but few differences were observed in translocation and degradation rates. The main metabolic pathway, however, was remarkably different. The investigation on absorption of ^{14}C -simetryne was then further extended to six gramineous plant species. The rates of absorption were remarkably different among these plants, susceptible plants exhibiting much higher rates than tolerant species. It was concluded that the selectivity of foliar-applied simetryne was mainly due to species related differences in the rates of absorption, and in metabolic pathways.

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References

- 1) ARAI M., S. FURUYA and M. MIYAHARA: Proc. Crop Sci. Japan **36**, 310~314 (1967).
- 2) ARAI M., and S. FURUYA: Proc. Crop Sci. Japan **36**, 315~319 (1967).
- 3) ARAKAWA K. and K. NODA: Weed Res. (Japan) **15**, 48~55 (1973).
- 4) DUDEK C., E. BASLER and P.W. SANTELMANN: Weed Sci. **21**, 440~443 (1973).
- 5) FURUYA S. and T. KATAOKA: Weed Res. (Japan) **8**, 22~28 (1969).
- 6) FURUYA S. and T. KATAOKA: Weed Res. (Japan) **11**, 20~24 (1971).
- 7) HAMILTON R.H. and D.E. MORELAND: Science **135**, 373~374 (1962).
- 8) HAMILTON R.H.: J. Agr. Food Chem. **12**, 14~17 (1964).
- 9) ICHIZEN N.: Weed Res. (Japan) **21**, 81~83 (1976).
- 10) ISHIZUKA K., I. TAKASE, K. TAN and S. MITSUI: Agric. Biol. Chem. **37**, 1307~1316 (1973).
- 11) ISHIZUKA K., K. FUKUCHI, K. KOBAYASHI and H. HYAKUTAKE: Weed Res. (Japan) **24** (Suppl.), 19~20 (1979).
- 12) KANAYAMA K., S. EGUCHI and M. MIYAHARA: Weed Res. (Japan) **22** (Suppl.), 132~134 (1977).
- 13) KASUGAI S.: J. Sci. Soil & Manure **13**, 669 (1939).
- 14) LAMOUREUX G.L., R.H. SHIMABUKURO, H.R. SWANSON and D.S. FREAR: J. Agr. Food Chem. **18**, 81~86 (1970).
- 15) LAMOUREUX G.L., L.E. STAFFORD, R.H. SHIMABUKURO and R.G. ZAYLSKIE: J. Agr. Food Chem. **21**, 1020~1030 (1973).
- 16) LEE S. and M. MIYAHARA: Weed Res. (Japan) **22** (Suppl.), 129~131 (1977).
- 17) MIYAHARA M. and S. EGUCHI: Weed Res. (Japan) **23** (Suppl.), 12~14 (1978).
- 18) MONTGOMERY M.L. and V.H. FREED: J. Agr. Food Chem. **12**, 11~14 (1964).
- 19) ROBINSON D.E. and D.W. GREENE: Weed Sci. **24**, 500~504 (1976).
- 20) ROETH F.W. and T.L. LAVY: Weed Sci. **19**, 93~97 (1971).
- 21) ROETH F.W. and T.L. LAVY: Weed Sci. **19**, 98~101 (1971).
- 22) SHEET T.J.: Weeds **9**, 1~13 (1961).
- 23) SHIMABUKURO R.H.: J. Agr. Food Chem. **15**, 557~562 (1967).
- 24) SHIMABUKURO R.H.: Plant Physiol. **42**, 1269~1276 (1967).
- 25) SHIMABUKURO R.H., H.R. SWANSON and W. C. WALSH: Plant Physiol. **46**, 103~107 (1970).
- 26) SHIMABUKURO R.H., W.C. WALSH, G.L. LAMOUREUX and L.E. STAFFORD: J. Agr. Food Chem. **21**, 1031~1036 (1973).
- 27) SIKKA H.C. and D.E. DAVIS: Weed Sci. **16**, 474~477 (1968).
- 28) THOMPSON L. Jr., J. M. HOUGHTON, F.W. SLIFE and H.S. BUTLER: Weed Sci. **19**, 409~412 (1971).
- 29) THOMPSON L. Jr.: Weed Sci. **20**, 153~155 (1972).

30) THOMPSON L. Jr.: Weed Sci. 20, 584~587 (1972).

31) WHICHMANN J. R. and W. R. BYRNES: Weed Sci. 23, 448~453 (1975).

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シメトリンの茎葉処理における選択作用機構：イネ科植物に おけるシメトリンの吸収・移行・代謝

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摘 要

シメトリンの茎葉処理における選択作用機構について検討した。まず水耕法で3葉期まで達した6種のイネ科植物の茎葉部をシメトリン水溶液に1時間浸漬し、その後の生育を調査した。その結果、シメトリンは用いた濃度内で供試植物間に選択性を示し、イネの抵抗性が高く、ついでコムギ、トウモロコシ、タイヌビエ、メヒシバ、シコクビエの順であった。

^{14}C -シメトリンの吸収・移行・代謝を、シメトリン抵抗性のイネと比較的感受性の高いタイヌビエについて比較検討した。茎葉部からの吸収は両植物間で大きな差がみられ、タイヌビエの吸収速度はイネの1.9~2.6倍であった。また吸収されたシメトリンの移行は両植物間で差は認められず、オートラジオグラフィーの結果、両植物とも速やかな求頂的移行がみられ、その後時間の経過とともにわずかな求基的移行がみられた。シメトリンの代謝は、イネでは水可溶画分および未抽出残渣画分への変化が大であるのに対し、タイヌビエでは、シメトリンのN-エチル基が脱エチル化したモノ脱アルキル体が多く生成され、両植物間でシメトリンの主要な代謝経路は異なると考えられた。ただしシメトリンの分解速度には大きな差はみられなかった。しかし、タイヌビエの吸収速度がイネよりも著しく大であったので、親化合物の体内残存濃度はタイヌビエがイネより高い結果となった。さらに前述の6種のイネ科植物の ^{14}C -シメトリンの茎葉部からの吸収を検討した。吸収速度は感受性の高いメヒシバが最も大きく、ついでタイヌビエで、他の4種はほとんど同じ程度であった。一般に感受性の高い植物は吸収速度が大きかったが、シコクビエに例外性がみられた。

これらの結果から、シメトリンは茎葉処理においてイネ科植物間に選択性を有し、これは茎葉部からの吸収速度の差に起因し、これに体内代謝の差が加わった結果生じると考えられた。