水稻와 너도방동사니에서 Bensulfuron의 吸收, 移行 및 代謝

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Absorption, Translocation and Metabolism of Bensulfuron in Rice and *Cyperus serotinus* Rottb.

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ABSTRACT

Root absorption, translocation and metabolism studies of ^{14}C-bensulfuron in rice and *Cyperus serotinus* Rottb. were conducted to determine their selective mode of action. Rice absorbed a greater amount of bensulfron than *Cyperus serotinus*. The translocation rates of bensulfuron from roots into shoots were much faster in *Cyperus serotinus* than rice plants. The metabolic rate of bensulfuron was very fast in rice plants, but slow in *Cyperus serotinus* and therefore, the amount of parent bensulfuron remained in the shoots after 12 hours absorption was greater in *Cyperus serotinus* than rice. In conclusion, this studies indicated that *Cyperus serotinus* was susceptible to bensulfuron because of the slower metabolic rate and fast translocation rate, but tolerance of rice might be caused by faster metabolic rate in plants.

Key words : bensulfuron, ^{14}C-bensulfuron, absorption of bensulfuron, translocation of bensulfuron, metabolism of bensulfuron, Cyperus serotinus

INTRODUCTION

Bensulfuron(DPX-F5384, methyl-((((((4,6-dimethoxy pyrimidine-2-yl)amino)carbonyl)amino)sulfonyl)methyl)benzoate), a member of sulfonylurea herbicides, is a broad-spectrum herbicide for the control of broadleaved weeds and sedges in the paddy rice fields. As the selective herbicide for transplanting rice, bensulfuron is active at a rate as low as 20-25 grams ai/ha and has a good herbicidal activity on most annual and perennial weeds in the rice paddy fields, but can not control barnyardgrass. Therefore, bensulfuron has been used as a mixture with butachlor, mefenacet, quinclorac, and other herbicides.

The modes of action of sulfonylurea herbicides have been studied by Ray[4,5,6,7] and other scientists[8,10]. The primary site of these compounds is the inhibition of acetolactate synthase, an important enzyme in the pathway for branched-chain amino acids biosynthesis. Secondary effects include the cessation of DNA synthesis, cell division and plant growth. Bensulfuron also inhibits acetolactate synthase from weeds associated with rice. The inhibition of acetolactate synthase can account for the herbicidal action of bensulfuron in weeds. However, through the metabolism study of bensulfuron in rice leaves, Takeda et al.[9,11,12] suggest that inactivating metabolism plays an important role in

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the tolerance of rice to bensulfuron since the rice acetolacate synthase is readily not inhibited by this sulfonylurea. Takeda et al.[10] concluded that the safety of bensulfuron for rice was due to metabolism to a hydroxylated compound which did not readily inhibit rice acetolactate synthase and had no herbicidal activity.

This research was conducted to study selective mode of action of bensulfuron in rice and *Cyperus serotinus* by absorption, translocation and metabolism studies using ^{14}C-bensulfuron.

MATERIALS AND METHODS

Absorption and Translocation of ^{14}C-Bensulfuron : Rice cultivar, Samgang and *Cyperus serotinus* Rottb., a perennial sedge, were used for absorption, translocation and metabolism study using ^{14}C-bensulfuron supplied by the Du Pont Company, which was uniformly ring-labeled with a specific activity of 16.6μCi/mg. Intact 2-leaf stage rice seedlings were dipped in 500ml of 10^{-6}M ^{14}C-bensulfuron for 3, 6, 12 and 24 hours. For translocation study, after 24 hour-exposure to ^{14}C-bensulfuron at 10^{-6}M, plants were transferred to the bensulfuron-free Kasugai nutrient solution and grown for 1, 3, 5 and 7 days. After growing in the nutrient solution, plants were removed, and roots were throughly washed with distilled water and blotted dry. The plants were sectioned into shoots and roots ; dried at 90℃ oven for 24 hours ; weighed. The plants were combusted in the sample combustion system(Packard Tri-carb 306) and the radioactivity was determined both qualitatively by radioautography and quantitatively by radioassay using the liquid scintillation spectrometer(Packard Tri-carb 2000) with correction for quenching. Translocation rate was computed by the ratio of radioactivity in the shoots to that in the whole plants. Each treatment was replicated 3 times using

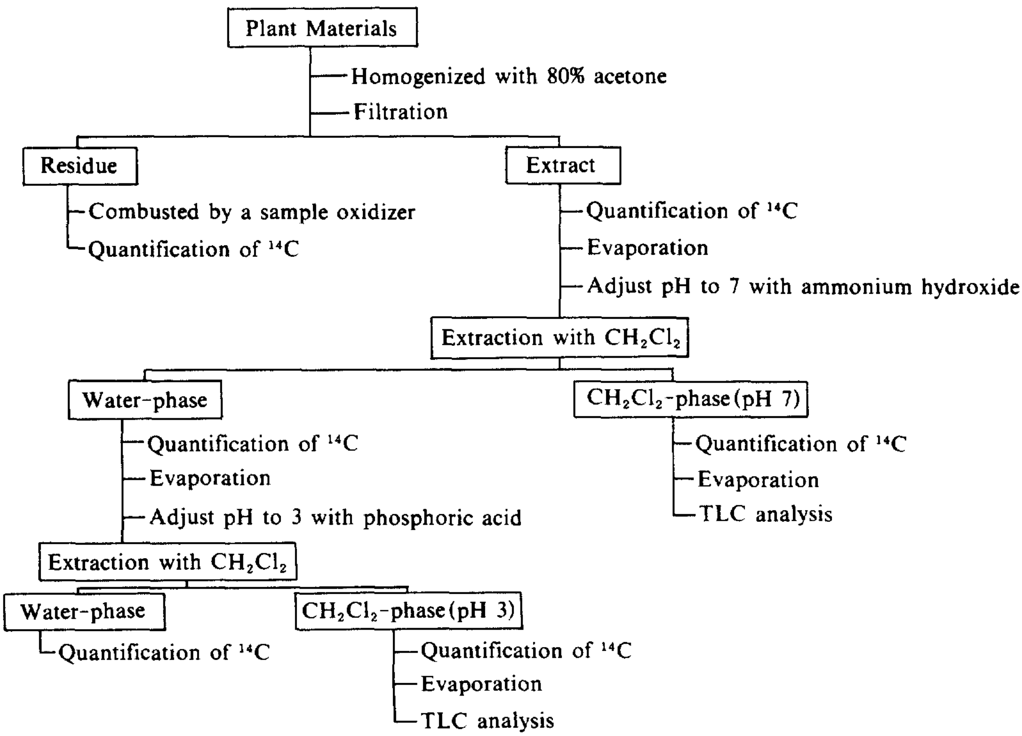

Fig. 1. Flow digram of extraction, separation and quantification of ^{14}C-bensulfuron and its metabolites in shoots and roots of rice plants and *Cyperus serotinus*.

2 plants.

Metabolism of ^{14}C-Bensulfuron in Plants : For metabolism study of bensulfuron in plants, roots of intact seedlings were dipped in 10^{-6}M ^{14}C-bensulfuron for 12 and 24 hours. Following the absorption periods, the plants were removed from the solution, rinsed with distilled water and sectioned into roots and shoots and weighed fresh weight. Each treatment was duplicated using 30 plants. Each plant part was separately homogenized and extracted 2 times with 80% acetone as illustrated in Fig. 1. The radioactivity of the combined extracts and non-extractable residues were determined and then their acetone extracts were evaporated *in vacuo* at 30℃. Water extract was adjusted to pH 7 by addition of ammonium hydroxide and extracted with dichloromethane. The extract was partitioned into dichloromethane and water. Water extract was adjusted to pH 3 by addition of phosphoric acid and extracted with dichloromethane. The extract was partitioned into dichloromethane and water. The radioactivity of dichloromethane extracts at pH 7 and pH 3 and water extract was determined by liquid scintillation spectrometry and the extracts of dichloromethane at pH 7 and pH 3 were further assayed by thin-layer chromatography. Chromatograms were obtained by developing pH 7-extract with a mixture of methylene chloride/methanol/concentrated ammonia(144/50/6, v/v/v), and pH 3-extract with a mixture of methylene chloride/acetonitrile/glacial acetic acid/water(150/27/2.5/0.5, v/v/v/v) in the room temperature(15℃). The standard metabolites supplied by the Du Pont Company were delveloped on the same conditions of samples to identify the metabolites and the parent compound in plants.

RESULTS AND DISCUSSION

Absorption and Translocation of ^{14}C-Bensulfuron : The concentrations of ^{14}C-activity after absorption of ^{14}C-bensulfuron were increased with the increase of exposure time from 3 hours to 48 hours in rice and *Cyperus serotinus* (Fig. 2). The

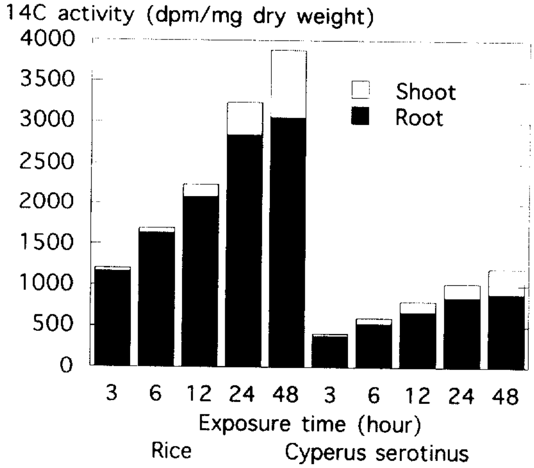

Fig. 2. Absorption of ^{14}C-bensulfuron(10^{-6}M) by roots of rice and *Cyperus serotinus* at the two leaf stage over time.

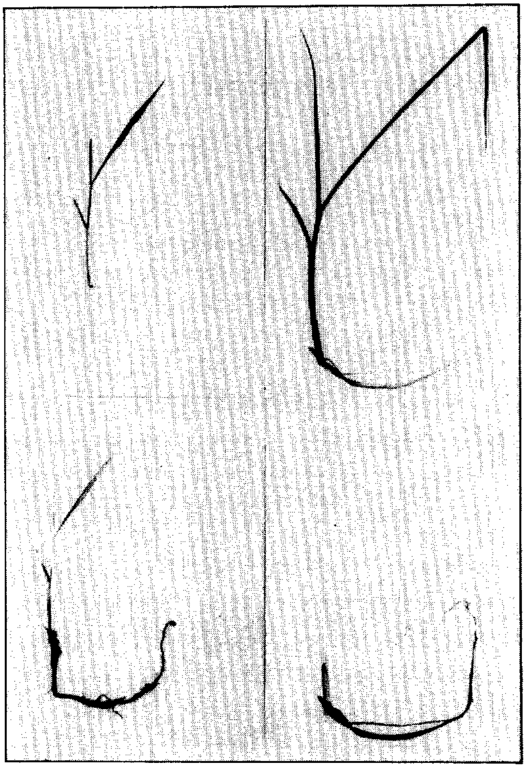

Fig. 3. Absorption and distribution of ^{14}C-bensulfuron in rice and *Cyperus serotinus*. Plant and corresponding radioautograph after root application of 10^{-6}M for 24 hour. Plant top, radioautograph bottom, rice left, *Cyperus serotinus* right.

concentrations of ^{14}C-activity after absorption was higher in roots than shoots and were much greater in rice than *Cyperus serotinus*. Radioautographs of treated plants suggested that more extensive distribution of ^{14}C occurred in rice than *Cyperus serotinus* (Fig. 3).

The translocation rates of ^{14}C derived from ^{14}C-bensulfuron from the roots into the shoots after 24 hour's root absorption at 10^{-6}M of ^{14}C-bensulfuron increased rapidly after transferring plants from ^{14}C-bensulfuron solution to the bensulfuron-free solution, and the rates shown were generally higher in *Cyperus serotinus* than rice plants. One day after treatment, the rates were not increased, but just kept this balance until 7 days after treatment (Fig. 4).

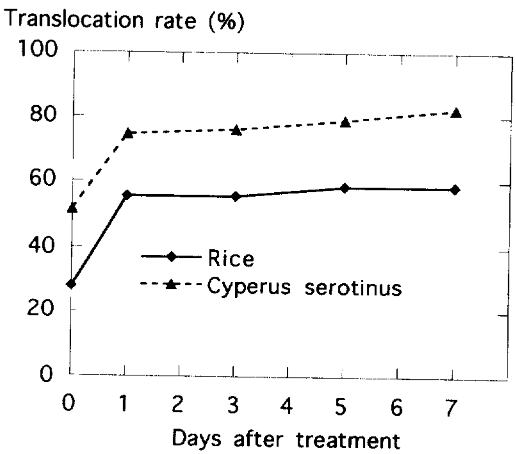

Fig. 4. Translocation of ^{14}C-bensulfuron in rice and *Cyperus serotinus* at the two leaf stage after 24 hour absorption by roots.

Considering the results of absorption and translocation studies, the rice plants, showing the high tolerance to bensulfuron, were greater in the absorption quantity, but lower in translocation rates. The *Cyperus serotinus*, susceptible species to bensulfuron, showed lower absorption and higher translocation rates of ^{14}C-bensulfuron. In the studies of absorption of ^{14}C-bensulfuron, Pyon *et al.*[3], Ohno *et al.*[2] and Guh *et al.*[1] reported that the tolerant rice cultivars had greater absorption than susceptible cultivars, and these reports were similar to this study which showed the greater amount of absorption in rice plants. It may be considered that the tolerant species kept the activity without phytotoxity, so that they absorbed more bensulfuron than the susceptible species. Yuyama *et al.*[13,15] reported that there was more ^{14}C-bensulfuron remaining in the roots than in the shoots and it was the same with this study. The translocation rates were higher in the tolerant species, which were the same as Pyon *et al*[3]. The difference in selectivity among the rice and weed species does not account for the different absorption but the translocation rate from the roots into the shoots may be one of the factors partially affecting the differential sensitivity to bensulfuron.

Metabolism of ^{14}C-Bensulfuron in Plants: The distributions of ^{14}C-activity derived from root-applied ^{14}C-bensulfuron in dichloromethane-soluble fraction were shown in Fig. 5 (rice) and Fig. 6 (*Cyperus serotinus*). In rice plants, 12 hours after absorption, most of the bensulfuron remained in roots where about 50% of it metabolized to metabolite A (ODM-bensulfuron) and metabolite B (sunfonamide) and a small amount of metabolite C (homosaccharin). After 48 hours of absorption, metabolism of bensulfuron was further developed. The matabolic rate was much slower in *Cyperus*

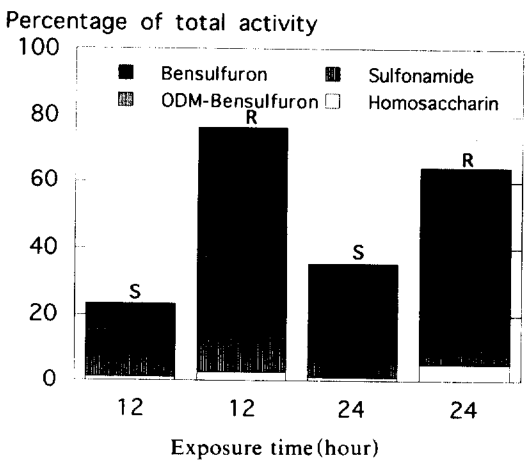

Fig. 5. Distribution of ^{14}C-radioactivity derived from root-applied ^{14}C-bensulfuron in dichloromethane-soluble fractions in rice (S : shoot, R : root).

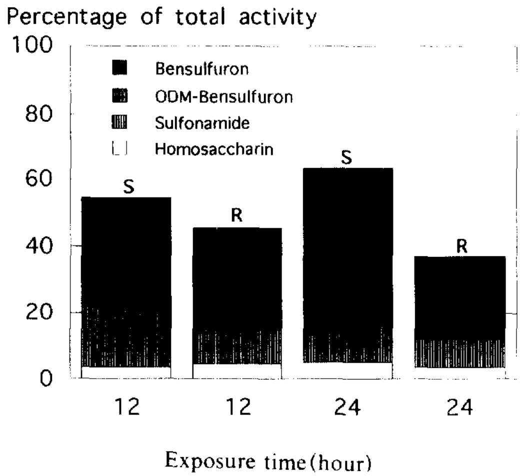

Fig. 6. Distribution of ^{14}C-radioactivity derived from root-applied ^{14}C-bensulfuron in dichloromethane-soluble fractions in *Cyperus serotinus* (S : shoot, R : root).

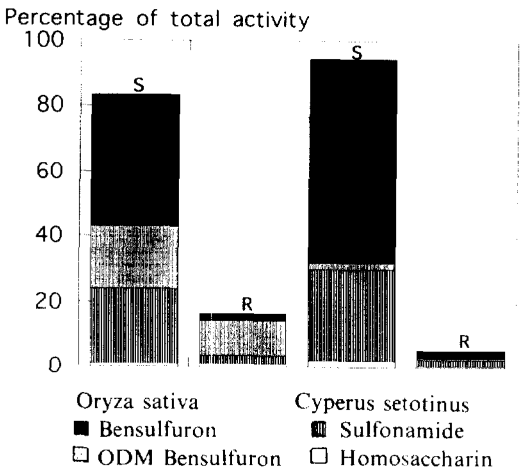

Fig. 7. Distribution of ^{14}C-radioactivity 24 hour after root application of ^{14}C-bensulfuron for 24 hour in dichloromethane-soluble fractions in rice and *Cyperus serotinus* (S : shoot, R : root).

serotinus than in rice plants and thus the least amount of bensulfuron was metabolized and remained more in *Cyperus serotinus*. *Cyperus serotinus* contained the greater amount of parent compound in plants, especially in shoots compared to rice. After 24 hours application of bensulfuron, *Cyperus serotinus* contained most of the parent compound in shoots but rice plants contained a relatively small amount of parent benusulfuron. After plants were exposed in ^{14}C-bensulfuron for 24 hours and transferred to bensulfuron-free solution for 24 hours, ^{14}C-activity was mostly distributed in roots in both species and metabolic rate in rootswas much slower in *Cyperus serotinus* than rice, and thus the ratio of parent bensulfuron to metabolites was higher in *Cyperus serotinus* than rice(Fig. 7).

We may suggest that the differential selectivity to bensulfuron between rice and *Cyperus serotinus* may be directly related to different metabolizing ability in plants and partially a differential translocation rate of bensulfuron into shoots. Pyon *et al*.[3] reported that the parent compound was low in tolerant cultivar, but high in susceptible cultivars. Also, the ratio of bensulfuron to metabolites were higher in roots than in shoots in many other reports[2,3,13,15]. Takeda *et al*.[9,10,11] found that the high tolerance of plant species to bensulfuron was related to faster inactiviting metabolism in rice compared to sensitive weeds and the metabolic half life of bensulfuron in rice leaves ranged from 2-9 hours, whereas that of weeds showed longer than 50 hours since these weeds have little or no plant metabolism.

In conclusion, this studies indicated that *Cyperus serotinus* was very sensitive to bensulfuron because of slower metabolic rate and faster translocation rate. On the other hand, the tolerance of rice may be caused by the ability of rice plants to metabolize bensulfuron to inactive metabolites at a rate much faster in rice than *Cyperus serotinus*.

摘　要

水稻와 너도방동사니간의 Bensulfuron 選擇性 機作을 밝히고자 ^{14}C-bensulfuron을 供試하여 植物體간 Bensulfuron의 吸收, 移行 및 代謝를 硏究하였다.

1. Bensulfuron 吸收量은 줄기보다 뿌리에서 월등히 많았으며 너도방동사니보다 水稻에서 현저하게 높았다.

2. 뿌리로 부터 줄기로의 Bensulfuron 移行速度는 水稻보다 너도방동사니에서 빨랐으며 移行量도 많았다.
3. Bensulfuron의 代謝速度는 水稻에서는 현저하게 빨랐으나 너도방동사니에서는 매우 느린 경향이었다.
4. 따라서 水稻의 耐性은 빠른 代謝速度에 주로 基因하며 부분적으로 느린 移行速度와도 관련이 있으며 너도방동사니의 感受性은 느린 代謝速度와 빠른 移行速度와 관련이 있을 것으로 思料되었다.

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LITERATURE CITED

1. Guh, J.O., J.Y. Pyon and K. Ishizuka. 1988. Diffrential absorption and translocation of bensulfuron-methyl between selected rice cultivars. Korean J. Weed Sci. 8(1) : 45-52
2. Ohno, A., K. Ishizuka, T. Yuyama and S. Takeda. 1986. Activity and absorption of DPX-F5384 among rice cultivars. Weed Res. Japan 31 Suppl. : 109-110.
3. Pyon, J.Y., A. Ohno, K. Ishizuka and H. Matsumoto. 1987. Selective mode of action of root-applied bensulfuron-methyl among rice cultivars. Proc. 11th APWSS Conf. : 99-107.
4. Ray, T.B. 1980. Studies on the mode of action of DPX-4189. Proc., British Crop Protection Conf.(Weeds) 1 : 7-14.
5. Ray, T.B. 1982. The mode of action of chlorosulfuron-A new herbicide for cereals. Pesticide Biochem. & Physiol. 17 : 10-17.
6. Ray, T.B. 1982. The mode of action of chlorosulfuron : The lack of direct inhibition of plant DNA synthesis. Pesticide Biochem. & Physiol. 18 : 262-266.
7. Ray, T.B. 1984. Site of action of chlorosulfuron-Inhibition of valine and isoleucine biosynthesis in plants. Plant Physiol. 75 : 827-831.
8. Scheel, D. and J.E. Casida. 1985. Sulfonylurea herbicides : Growth inhibition in soybean cell suspension cultures and in bacteria correlated with block in biosynthesis of valine, leucien or isoleucine. Pesticide Biochem. & Physiol. 23 : 398-412.
9. Takeda, S., T. Yuyama, R.C. Ackerson, R. C. Weigel, R.F. Sauers, W. Neal, D.G. Gibian and P.K. Tseng. 1985. Herbicidal activities and selectivity of a new rice herbicide DPX-F5384. Weed Res. Japan 30 : 284-289.
10. Takeda, S., D. Erbes, P.B. Sweetser, J.V. Hay and T. Yuyama. 1986. Mode of herbicidal and selective action of DPX-F5384 between rice and weeds. Weed Res. Japan 31 : 157-163.
11. Takeda, S., T.B. Sweetser, D.L. Erbes and T. Yuyama. 1985. Mode of selectivity of Londax herbicide(DPX-F5384) in paddy rice. Proc. 10th APWSS Conf. 1 : 156-161.
12. Takeda, S., T. Yuyama, R.C. Ackerson and R.C. Weigel. 1985. Selection of rice herbicide from several sulfonylurea compounds. Weed Res. Japan 30 : 278-283.
13. Yuyama, T., R.C. Ackerson and S. Takeda. 1987. Uptake and distribution of bensulfuron methyl(DPX-F5384) in rice. Weed Res. Japan 32 : 173-179.
14. Yuyama, T., S. Takeda, H. Watanabe, T. Asami, S. Peudpaichit, J.L. Malassa and P. Heiss. 1984. DPX-F5384, A new broad spectrum rice herbicide. Proc., 17th Indonesian Weed Sci. Soc. Conf. : 89-94.
15. Yuyama, T., S. Takeda and R.C. Ackerson. 1987. Uptake and distribution of bensulfuron methyl(DPX-F5384) in paddy rice. Proc. 11th APWSS Conf. 1 : 145-151.