Mode of Safener Action of Dimepiperate, a Thiolcarbamate Herbicide, on Bensulfuron Methyl Activity

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Bensulfuron methyl is a sulfonylurea herbicide controlling a broad spectrum of broadleaf and sedge weeds in paddy rice. Safening effects with certain thiolcarbamate herbicides for grass control have been discovered in the process of combination products development. The results of mode of safening studies revealed that the enhancement of metabolic inactivation rate of bensulfuron methyl in plants was the basis of safening.

Among these thiolcarbamates, dimepiperate was unique in exhibiting safening action even in direct water-seeded rice. Further studies on dimepiperate effects on root-applied bensulfuron methyl indicated that rice root elongation, enhanced metabolism in roots and in shoots, were the key to the safening effects. The translocation rate of bensulfuron methyl, from roots to shoots, on the other hand, was not significantly affected by dimepiperate.

Introduction

Bensulfuron methyl (BSM) is one of the sulfonylurea herbicides invented by Du Pont company (Fig. 1) [1]. The compound is particularly selective and effective for the control of a broad spectrum of weeds in paddy rice with extremely low use rates (Table I) [2]. Mode of herbicidal and selective action studies of BSM demonstrated that this compound inhibited acetolactate synthase, a key enzyme in the pathway of branched-chain amino acid biosynthesis, and that differential plant metabolic inactivation rates between rice and sensitive weeds was the basis for selectivity (Fig. 2) [3].

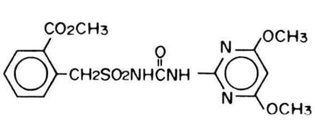

Fig. 1. Chemical structure of bensulfuron methyl (methyl = α-(4,6-dimethoxypyrimidin-2-yl carbamoylsulfamoyl)-*o*-toluate).

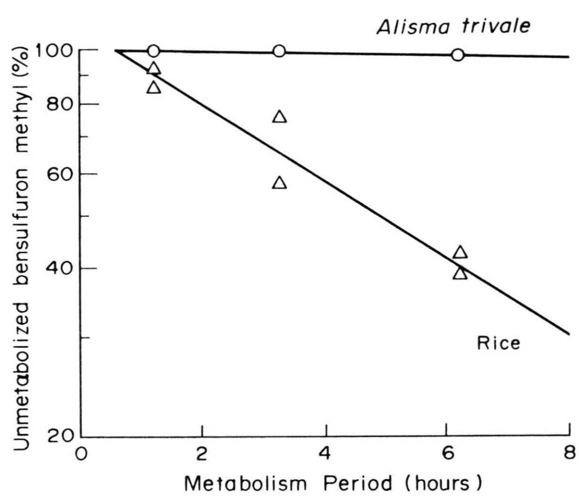

Fig. 2. Metabolism of bensulfuron methyl in rice and *Alisma trivale* leaves.

In Japan, BSM has been developed mainly in combination with grass herbicides to provide a total, season-long weed control by a single application of the herbicide product.

As the field development of BSM progressed, more precise characteristics of the compound were revealed. Some of the environmental factors affecting the BSM performance include temperature,

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Table I. Herbicidal activity and rice safety of bensulfuron methyl (greenhouse pot test).

Chemical	Rate [g ai/ha]	Timing	Rice safety V.C.	Rice safety D.W.	Eo	Weed control (V.C.) Mv	Bl	Sj	Sp	Cs
Bensulfuron methyl	25	3 DAT	0	105	0	10	10	9	9	10
	50		0	102	5	10	10	9.5	9.5	10
	100		2	90	7	10	10	10	10	10

DAT, days after transplanting; V.C., visual count (0–10 rating scale); D.W., dry weight of shoot (% of untreated check); Eo, *Echinochloa oryzicola;* Mv, *Monochoria vaginalis;* Bl, annual broadleaf weeds; Sj, *Scirpus juncoides;* Sp, *Sagittaria pygmaea;* Cs, *Cyperus serotinus.*

soil, water management and planting depth. In addition, it has been found that *indica*-type rice cultivars tend to be much more tolerant than *japonica*-type rice to BSM (Table II). Actually, it has been reported that BSM sometimes causes moderate growth stunting under adverse conditions such as high temperature, light or sandy soil, shallow transplanting, etc., although the crop damage is usually recovered later to an acceptable degree.

In the process of studying suitable combinations, we found very interesting phenomena in greenhouse pot tests. While we observed an apparent rice injury in BSM treated plots, the rice plants treated with some of the combinations looked as healthy as the untreated ones. It was then confirmed that some of the thiolcarbamate herbicides, mainly used for barnyardgrass *(Echinochloa oryzicola)* control in paddy rice, showed clear safening effect to BSM at their normal use rates (Table III) [4]. Further studies demonstrated that this safening effect was based on the metabolic inactivation in rice plants, which was significantly enhanced by the thiolcarbamate application (Table IV) [4]. Interestingly, molinate, which did not show a clear safening as the others, did not enhance the metabolic rate.

Table II. Effects of bensulfuron methyl on direct-seeded *japonica* and *indica* rice varieties.

Variety	Rate [g ai/ha]	Application timing 0.5–1.0[a] V.C.	D.W.	1.5–2.0[a] V.C.	D.W.
Nipponbare[b]	50	5.0	59	3.0	92
	100	6.0	52	4.0	72
	150	7.0	51	5.0	65
IR-24[c]	50	2.0	90	1.0	108
	100	3.5	78	1.5	112
	150	4.0	73	2.0	102
RD-93[c]	50	2.0	97	1.0	98
	100	3.5	75	2.0	91
	150	4.0	76	2.0	93

[a] Leaf stage of rice; [b] *japonica* rice; [c] *indica* rice.
For abbreviations see Table I.

Table III. Safening of bensulfuron methyl with thiocarbamate herbicides in transplanted *japonica* rice.

Treatment	Rate [g ai/ha]	Rice safety V.C.	D.W.
Bensulfuron methyl	100	3.4	77
Bensulfuron methyl + thiobencarb	100 + 2100	1.7	97
Bensulfuron methyl + dimepiperate	100 + 3000	1.3	100
Bensulfuron methyl + CH-83	100 + 3000	1.5	95
Bensulfuron methyl + esprocarb	100 + 2800	1.5	96
Bensulfuron methyl + molinate	100 + 2400	2.5	88
Thiobencarb	2100	0	103
Dimepiperate	3000	0	105
CH-83	3000	0	95
Esprocarb	2800	0.5	98
Molinate	2400	0	98

Each value is an average of 2 to 6 tests.

Table IV. Effect of thiocarbamate pretreatments on bensulfuron methyl metabolism by rice plants.

Treatment	Rate [g ai/ha]	Metabolism, half-life [h]
Solvent control	–	8.4
Thiobencarb	2000	1.9
Dimepiperate	2000	2.6
Molinate	2000	7.9

Solvent = 20% acetone + 0.1% Tween 20.

Among these thiolcarbamate herbicides, dimepiperate shows better safener activity in practical conditions. It has been reported that dimepiperate itself sometimes gives rice growth enhancement, and possibly safens some other herbicides of different mode of action [5]. In the case of the BSM combination, dimepiperate safened BSM even in the direct water-seeded japonica rice which was generally much more sensitive to most herbicides than was transplanted rice due to more root exposure and earlier growth stage (Table V).

Further studies have been conducted to determine the mode of safening action of dimepiperate when the herbicides are absorbed by the roots.

Table V. Safening of bensulfuron methyl with dimepiperate in direct water-seeded *japonica* rice.

Treatment	Rate [g ai/ha]	Rice safety V.C.	D.W.
Bensulfuron methyl	25	2.5	82
	50	3.5	77
	100	5.0	65
Bensulfuron methyl + dimepiperate	25 + 100	0.5	102
	50 + 2000	0.8	96
	100 + 4000	2.5	85
Dimepiperate	4000	0	103

Materials and Methods

Plant material

Seeds of rice (*Oryza sativa* L. *cv.* Nihonbare) were germinated in an incubator at 25 °C for 2 days and then transferred to plastic trays and grown for 14 days. Seedlings were transferred to plastic pots and grown in Kasugai's nutrient solution up to the 2.8 leaf stages in a growth chamber controlled at 25/20 °C (day/night, 12 h photoperiod) and 70% relative humidity. The roots were then cut at 1 cm below the basal part of the stem and the cut seedlings were further grown in nutrient solution to the 3–3.2 leaf stages.

Herbicidal activities of root-applied BSM and dimepiperate

The newly formed roots of the cut seedlings were exposed to either BSM (0.41 ppm) or a BSM (0.41 ppm) plus dimepiperate (16.4 ppm) mixture in an aqueous solution containing 1% acetone. Twenty-four hours after application, the roots were washed with distilled water and the plants were transferred to a herbicide-free nutrient solution and grown for 7 more days. Both root and shoot lengths were measured every other day. Each application was replicated three times using three plants.

Absorption and translocation of [^{14}C]BSM in rice

Roots of the cut seedlings were exposed to either 0.41 ppm of a [^{14}C]BSM (phenyl-labeled: 227.6 Bq/ml) or a [^{14}C]BSM plus dimepiperate mixture solution as described above. Following the designated absorption period, plants were removed from the solution and the roots were washed with distilled water. Then the plants were sectioned into roots and shoots, and dried at 80 °C for 72 h. After measurement of weights, plants were combusted in a sample combustion system (Aloka ASC-113) and ^{14}C radioactivity was determined by a liquid scintillation spectrometer (LSS: Beckman LS-8100). Translocation rate was calculated by the ratio of ^{14}C radioactivity in shoots to that in whole plants.

Metabolism of [^{14}C]BSM in roots and shoots

Roots of the cut seedlings were exposed to the radioactive herbicide solution as described above. Following the designated absorption periods, the roots were washed with distilled water, sectioned into shoots and roots, and weighed. Each plant section was separately homogenized and extracted twice with 80% acetone. Residual radioactivity in the non-extracted sample was determined by combustion and LSS. Acetone was removed from the combined extracts. The resulting aqueous extract was adjusted to pH 7 with 28% ammonium hydroxide solution and extracted twice with dichloromethane. The aqueous fraction was then adjusted to pH 3 with 25% phosphoric acid and again extracted twice with dichloromethane. The radioactivity in each extract was determined by LSS. The two dichloromethane fractions and the aqueous fraction were concentrated to near dryness *in vacuo* at 30–40 °C, respectively. These concentrated dichloromethane and water fractions were then dissolved in a small volume of dichloromethane for the former and methanol for the latter for respective analysis by thin layer chromatography (TLC). TLC was performed with two developing solvent systems: 1. Plates with Merck 25 µm silica gel 60 F254 developed by a mixture of dichloromethane:methanol:ammonium hydroxide (144:50:6, v/v/v), and 2. Whatman 200 um KC 18F developed by a mixture of dichloromethane: acetonitrile:acetic acid:water (150:27:25:0.3, v/v/v/v). Radiolabeled metabolites were identified by cochromatography with authentic reference standards. The radioactive spots on the developed TLC plates were scraped off and their radioactivities were determined by liquid scintillation counting.

Results and Discussion

Safening of BSM with dimepiperate on rice plants

The effects of root-applied BSM, alone or in combination with dimepiperate, on shoot and root elongation of rice seedlings were studied (Fig. 3). When the seedlings were cultivated in a medium containing BSM alone, the growth of roots was severely inhibited while only slight inhibition was observed in shoots. Dimepiperate significantly reduced the inhibition of root elongation caused by 0.41 ppm of BSM. It seems that rice plants with 1 cm cut roots used in this study are more sensitive to BSM than intact plants, and the inhibitory effect of BSM on the root growing portion, where cell division is active, is well detectable. The growth inhibition of BSM and the safening effects of dimepiperate may therefore be more clearly observed on the plants with 1 cm cut roots than when intact plants are used.

Effect of dimepiperate on absorption and translocation of [^{14}C]BSM in rice seedlings

Results on absorption of [^{14}C]BSM by rice seedlings are shown in Fig. 4 and 5. When roots of seedlings were exposed to BSM solution, ^{14}C concentration was found higher in the roots than in the shoots at every exposure time. Addition of dimepiperate caused a decrease in the rate of absorption of BSM by roots (Fig. 5): the absorption rate was about 1.7 times higher when [^{14}C]BSM was applied alone than when applied with dimepiperate. The decrease in absorption rate was estimated to reduce BSM activity clearly on the plant growth. This differential absorption may be involved partly in the safening mechanism [4]. There were no differences detected in the rate of translocation of [^{14}C]BSM from roots to shoots between BSM alone and in combination with dimepiperate (Fig. 6).

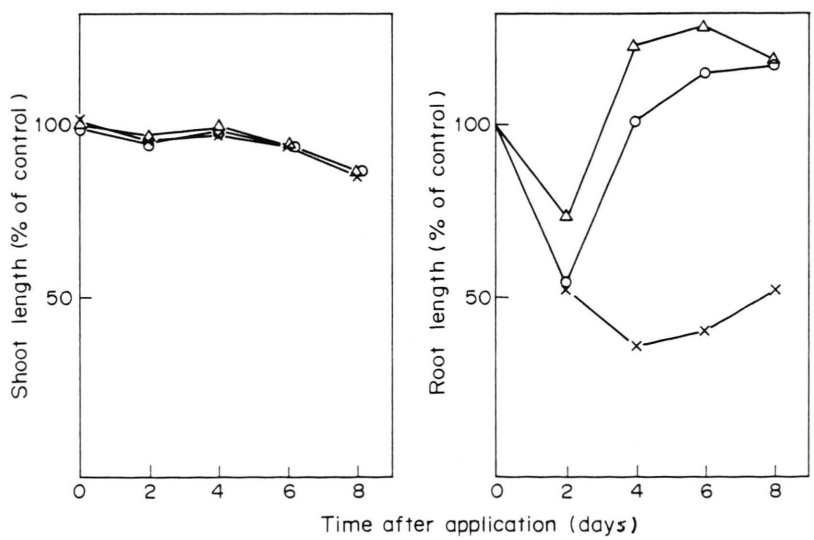

Fig. 3. Effect of bensulfuron methyl and dimepiperate on rice shoot and root elongation. ×, bensulfuron methyl (0.41 ppm); ○, bensulfuron methyl + dimepiperate (0.41 ppm + 16.4 ppm); △, dimepiperate (16.4 ppm).

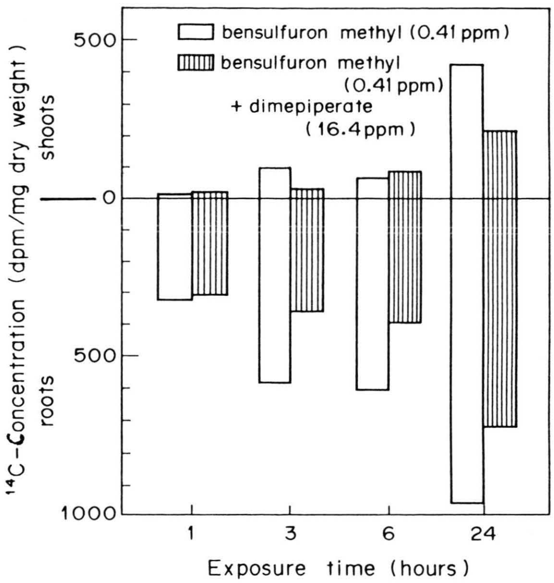

Fig. 4. ^{14}C concentration in shoots and roots.

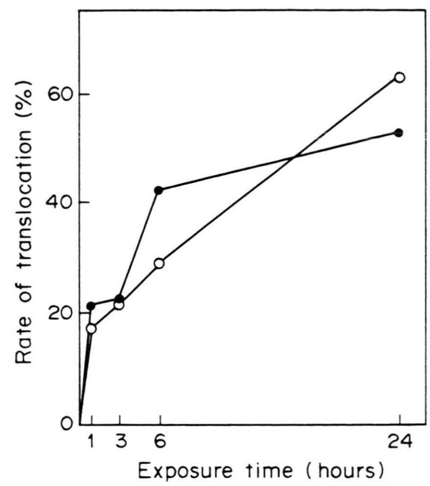

Fig. 6. Translocation of [^{14}C]bensulfuron methyl from roots to shoots. ○, bensulfuron methyl (0.41 ppm); ●, bensulfuron methyl (0.41 ppm) + dimepiperate (16.4 ppm).

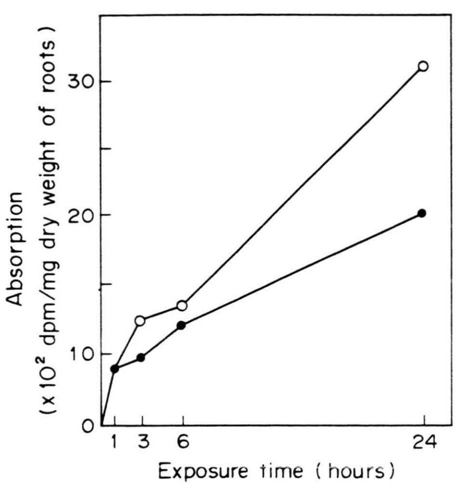

Fig. 5. Absorption of [^{14}C]bensulfuron methyl by roots of rice. ○, bensulfuron methyl (0.41 ppm); ●, bensulfuron methyl (0.41 ppm) + dimepiperate (16.4 ppm).

Effect of dimepiperate on metabolism of [^{14}C]BSM in rice

Metabolic rates of root-applied [^{14}C]BSM in roots and shoots were traced in a time course. In the shoots, 52, 51 and 72% of incorporated BSM was found as metabolites after 3, 6 and 24 h exposure, respectively. The major metabolite was detected as methyl-(4-hydroxy-6-methoxypyrimidin-2-yl carbamoylsulfamoyl)-*o*-toluate (metabolite A) which had been identified as a major metabolite in excised rice leaves by Takeda *et al.* (Fig. 7) [3]. In the roots, a large part of ^{14}C radioactivity was detected in unchanged BSM (Fig. 7), and the metabolite A was also found with methyl-(aminosulfonyl)-*o*-toluate (metabolite B), 1H-2,3-benzothiazin-4-(3H)-one 2,2-dioxide (metabolite C) and other unknown water-soluble metabolites. The metabolism of the absorbed BSM was remarkably enhanced by dimepiperate addition, and the metabolites A, B and C and others were produced in a greater quantity (Fig. 7). These facts may indicate that the action of dimepiperate in increasing the metabolic rate of BSM in roots is one of the major factors for its safening effect on root-applied BSM. Not only in the major metabolite A but also the minor B, C and others were found in rice roots. This suggests that the metabolism of root-applied BSM in rice may be conducted in a different way from that which is foliar-applied (Fig. 8).

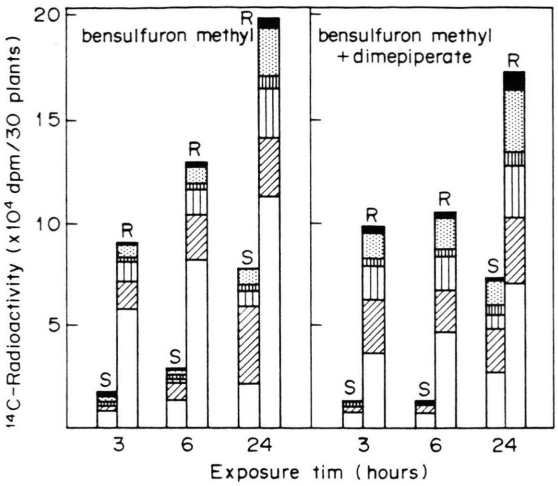

Fig. 7. Effect of dimepiperate on metabolism of bensulfuron methyl by rice. □, bensulfuron methyl; ▨, metabolite A; ▥, metabolite B; ▮, metabolite C; ▩, others (including water-soluble metabolites); ■, residue; S, shoot; R, root.

Conclusion

In the above studies on dimepiperate effects on root-applied BSM, rice root elongation, due mainly to decreased BSM absorption through the roots and enhanced BSM metabolism in roots as well as in shoots, was clearly observed. On the other hand, the translocation of BSM from roots to shoots was not significantly affected by addition of dimepiperate.

It can therefore be concluded that the major factors determining the safening action of dimepiperate when applied simultaneously with BSM are its ability to inhibit BSM absorption by roots of rice and to increase the rate of BSM metabolic inactivation in rice roots as well as in shoots.

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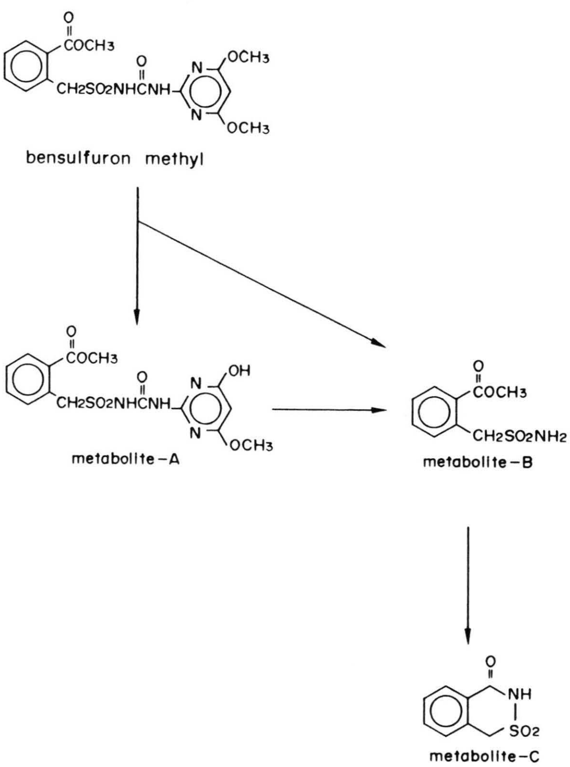

Fig. 8. Assumed pathway of root-applied bensulfuron methyl in rice.

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