Selective Mode of Action of Bensulfuron Methyl among Rice Cultivars*

Akira Ohno, Jong Yong Pyon**, Kozo Ishizuka, and Hiroshi Matsumoto

Abstract: Effect of root-applied bensulfuron methyl \([3-(4,6\text{-dimethoxypyrimidin-2-yl})-1-\[(2\text{-methoxycarbonylbenzyl)sulfonyl]\]urea}\) on growth of five rice cultivars (Oryza sativa L. cv. Milyang 30, Shingwang, Sangpung, Shinseonchalbyeo, and Nihonbare) was investigated under applied concentrations of \(10^{-6}\), \(5 \times 10^{-6}\) and \(10^{-5}\) M. Based on effects of the herbicide on growth of the plant parts (the 4th to 6th leaves and roots) which grew largely after its application, the order of sensitivity among tested cultivars to the herbicide is Nihonbare > Sangpung, Shinseonchalbyeo > Shingwang > Milyang 30. Root absorption of the \(^{14}\text{C}\)-herbicide and translocation from roots to shoots were investigated at \(10^{-6}\) and \(10^{-5}\) M concentrations. There was no clear relation between the absorption or the translocation rates and the growth inhibition. Metabolism of root-absorbed \(^{14}\text{C}\)-bensulfuron methyl was studied in four of the cultivars, excluding Shinseonchalbyeo. Milyang 30 and Shingwang had lower percentages of the parent compound in roots than Sangpung and Nihonbare; they also had higher percentages of O-demethyl bensulfuron methyl and water soluble metabolites than the latter two. In Milyang 30, bensulfuron methyl was less translocated from roots to shoots and largely degraded in roots, and these may contribute to its resistance. The low sensitivity of Shingwang may be related to its high degradation ability in roots, while in Nihonbare and Sangpung the rate of degradation of the herbicide was much lower than Milyang 30.

Key words: bensulfuron methyl, rice cultivar, absorption, translocation, metabolism

Introduction

Bensulfuron methyl \([3-(4,6\text{-dimethoxypyrimidin-2-yl})-1-\[(2\text{-methoxycarbonylbenzyl)sulfonyl]\]urea}\), a sulfonylurea herbicide, selectively controls broadleaf weeds and sedges in paddy rice field. This compound is active at an extremely low dosage of 20–50g ai/ha\(^{9}\). The mode of action of sulfonylurea herbicides has been studied intensively by Ray\(^{7}\). The reduction in growth by chlorosulfuron, one of the sulfonylurea herbicides, was closely associated with an inhibition of plant cell division. Under conditions where cell division occurred, DNA synthesis was strongly inhibited. Since that finding, the primary site of these compounds has been considered to be an inhibition of acetolactate synthase, a key enzyme for branched-chain amino acid biosynthesis\(^{8}\). Bensulfuron methyl inhibits acetolactate synthase from weeds associated
with rice cultivation\textsuperscript{10}, and this inhibition may account for the herbicidal action of the chemical.

The difference in sensitivity among rice cultivars has been reported for simetryn\textsuperscript{1-3}, benthiocarb\textsuperscript{1}, and other herbicides\textsuperscript{4}. In field tests in Thailand and Japan, \textit{japonica} type rice cultivars were generally more sensitive to bensulfuron methyl than \textit{indica} type cultivars\textsuperscript{11}. Ohno \textit{et al.}\textsuperscript{6} reported that \textit{japonica} type rice cultivars were often more susceptible than \textit{indica} type cultivars or hybrids of \textit{japonica} and \textit{indica}. Nakayama \textit{et al.}\textsuperscript{5} also reported on susceptibility differences to bensulfuron methyl. The mechanism of rice cultivar differential sensitivity to this herbicide, however, has not been investigated.

In this study, we were interested in the selective mode of action of bensulfuron methyl in Korean rice cultivars and \textit{japonica} rice cultivar ['Nihonbare']. The Korean cultivars used were selected from among many Korean types against bensulfuron methyl. First, we attempted to determine differential sensitivity of the cultivars to root applied bensulfuron methyl in a water culture. Interactions between the herbicide and rice plants were then examined from the aspects of root absorption, and the translocation and metabolism of the herbicide in the plants.

Materials and Methods

Plants

To compare the tolerance of rice (\textit{Oryza sativa} L.) cultivars to bensulfuron methyl, five cultivars, Milyang 30, Shingwang, Sangpong, Shinseonchalbyeo, and Nihonbare were selected through a preliminary experiment. The former two were hybrids of \textit{japonica} and \textit{indica} and the latter three were \textit{japonica} types.

Rice seeds were germinated in darkness for 2 days at 25°C in an incubator. Uniform seedlings were selected and transferred to plastic trays. The plants were grown in Kasugai nutrient solution until the 3rd leaf stage in a growth chamber at 25°C daytime and 20°C during the night, at 55-60% relative humidity with 12 hours daylength and light intensity of 15klx.

Root application of bensulfuron methyl

Roots of intact seedlings at the 3rd leaf stage were soaked in Kasugai nutrient solution containing $10^{-5}$, $5 \times 10^{-6}$, or $10^{-6}$M of bensulfuron methyl for 4 days. The roots were washed with distilled water and then transferred to bensulfuron methyl-free Kasugai nutrient solution and grown another 6 days in a growth chamber. At harvest, plant height, leaf length and dry weight of roots and shoots were measured. The treatment was replicated 3 times using 3 plants for each replication.

Absorption and translocation of bensulfuron methyl

Uniformly $^{14}$C-labeled bensulfuron methyl at phenyl ring with a specific activity of $5.6 \times 10^5$ Bq/mg was used in the study. Roots of intact seedlings were soaked in 0.5l of $10^{-6}$ or $10^{-5}$M $^{14}$C-bensulfuron methyl for 3, 6, 24, and 48 hours and thereafter were removed and blotted dry. They were then sectioned into shoots and roots, oven dried at 90°C for 24 hours, weighed and combusted in an automatic sample combustion system (Aloka AS C-113); radioactivity was determined by a liquid scintillation spectrophotometer (LSS, Beckman LS-1801). Translocation rate was calculated by rate of radioactivity in shoots to that in whole plants. The treatment was replicated 3 times using 2 plants each.

Metabolism of bensulfuron methyl

Roots of intact seedlings were soaked in 0.8l of $1 \times 10^{-6}$M $^{14}$C-bensulfuron methyl for 6 or 24 hours. Following the absorption period the plants were removed from the solution, roots rinsed with distilled water and the plants sectioned into shoots and roots. Each treatment was duplicated using 30 plants. After determination of their fresh weight, each plant part was separately homogenized and extracted twice with 80% acetone.
radioactivity of the combined extracts and nonextractable residues was determined and then acetone in the extracts was evaporated in vacuo at 35°C. Aqueous residue was adjusted to pH 7 with ammonium hydroxide and partitioned with dichloromethane. Then the aqueous residue was adjusted to pH 3 with phosphoric acid and partitioned again with dichloromethane. The radioactivities in dichloromethane extracts at pH 7 and pH 3, and water fractions were determined by LSS. Radioactive compounds in dichloromethane at pH 7 and pH 3 were analyzed by thin-layer chromatography (TLC). For TLC analysis, aliquots of each concentrate were applied as a band to silica gel (Merck Silica gel 60 F 354, layer thickness of 250µm) and Sic18 (Whatman KC18F, layer thickness of 200µm) plates. The plates were developed with a mixture of dichloromethane/methanol/NH₄OH (144/50/6, v/v/v : for silica gel plate), or with a mixture of dichloromethane/acetonitrile/glacial acetic acid/water (150/27/2.5/0.3, v/v/v/v : for Sic18 plate). Authentic reference standards of expected plant metabolites were co-chromatographed. The nonradiolabeled standards were detected under a UV monitor. Radiolabeled compounds in TLC plates were detected by Fuji X-ray film and their bands of radioactivity were counted quantitatively by LSS.

**Results**

1. **Growth response of rice cultivars to bensulfuron methyl**

Effect of root applied bensulfuron methyl to growth of the rice plants was evaluated by lengths of the 4th, 5th and 6th leaves, and the dry weights of roots and shoots (Table 1). Degrees of growth inhibition were shown as

<table>
<thead>
<tr>
<th>Rice cultivar</th>
<th>BSM conc.(M)</th>
<th>Leaf length</th>
<th>Dry weight (per 3 plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4th (%)</td>
<td>5th (%)</td>
<td>6th (%)</td>
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<td>(cm)</td>
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<td></td>
<td>Shoot (%)</td>
<td>Root (%)</td>
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<td></td>
<td>(g)</td>
<td>(g)</td>
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<tr>
<td>Milyang</td>
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</tr>
<tr>
<td>30</td>
<td>0</td>
<td>9.7a</td>
<td>12.7a</td>
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<td></td>
<td>10⁻⁶</td>
<td>9.1b</td>
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<td></td>
<td>5×10⁻⁶</td>
<td>8.5c</td>
<td>8.0d</td>
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<tr>
<td>Shing-wang</td>
<td>0</td>
<td>13.1a</td>
<td>16.0a</td>
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<tr>
<td></td>
<td>10⁻⁶</td>
<td>11.6b</td>
<td>9.1b</td>
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<td></td>
<td>5×10⁻⁶</td>
<td>11.4c</td>
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<td>Sang-pung</td>
<td>0</td>
<td>17.6a</td>
<td>20.3a</td>
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<tr>
<td></td>
<td>10⁻⁶</td>
<td>14.9b</td>
<td>10.7b</td>
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<td>5×10⁻⁶</td>
<td>13.3c</td>
<td>7.3d</td>
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<td>Shin-seonchal-</td>
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<td>18.5a</td>
</tr>
<tr>
<td>byeo</td>
<td>10⁻⁶</td>
<td>16.8b</td>
<td>11.2b</td>
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<td>15.4c</td>
<td>7.2d</td>
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<tr>
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<td>20.5a</td>
</tr>
<tr>
<td></td>
<td>10⁻⁶</td>
<td>15.6b</td>
<td>7.4b</td>
</tr>
<tr>
<td></td>
<td>5×10⁻⁶</td>
<td>13.8c</td>
<td>7.1d</td>
</tr>
</tbody>
</table>

Means followed by the same alphabets in columns of each cultivar are not significantly different at 5% level by Duncan's multiple range test.

Percentage indicates ratio of determined value of herbicide treated plants to determined value of nontreated plants in each rice cultivar.
a percent of control.

The effect of bensulfuron methyl was investigated on each leaf. At the treatment with the herbicide, the 4th leaf had emerged a little. In the $10^{-6}$ M treatment, Milyang 30 had only slight 4th leaf damage, while other cultivars tested sustained $10\sim15\%$ growth retardation. In the $5\times10^{-6}$ M treatment, the growth inhibition of Sangpung and Nihonbare was about 25%, greater than the other 3 cultivars. In the $10^{-5}$ M treatment, the rate of inhibition of Nihonbare was 40%, and damage to other cultivars was nearly the same as in the $5\times10^{-6}$ M treatment. In the 5th leaf length, Milyang 30 showed the least inhibition among all cultivars: 19% in the $10^{-6}$ M treatment. The 5th leaf of Nihonbare was injured the most with a rate of inhibition of 64% in the $10^{-6}$ M treatment. In $5\times10^{-6}$ M, the inhibition degree of Nihonbare was a remarkably large 93%, while, in contrast, Milyang 30 growth was only slightly inhibited by the same treatment; even in the $10^{-5}$ M treatment, the rate of inhibition of Milyang 30 was 47%. Strong inhibition occurred in Sangpung and Shinseonchalbyeo at treatments of $5\times10^{-6}$ M and $10^{-5}$ M, and the 5th leaf of Nihonbare did not emerge at all with the $10^{-5}$ M treatment. Milyang 30 was the only cultivar among the rice plants treated of which the 6th leaf emerged.

For the effect on dry weight of shoots; the only cultivar suffering slight damage by $10^{-6}$ M bensulfuron methyl was Milyang 30, others had remarkable damage. In the $5\times10^{-6}$ M treatment, degrees of inhibition ranged from 31 to 35 in all cultivars. In the $10^{-5}$ M treatment, Milyang 30 and Sangpung were less damaged than the other 3 cultivars.

For the effect on dry weight of roots, inhibition degree of Milyang 30 was again the least of all cultivars in the $10^{-6}$ M treatment followed by Shingwang. In the $5\times10^{-6}$ M treatment, rates of growth inhibition of Milyang 30 and Shingwang were about 40% but other cultivars were inhibited about 50%. In the $10^{-5}$ M treatment, Milyang 30 was inhibited the least, to a degree about the same level as in the $5\times10^{-6}$ M treatment. Shingwang was inhibited more strongly in the $10^{-5}$ M treatment than in $5\times10^{-6}$ M, but its degree of inhibition was still less than other

![Fig. 1. Absorption of $^{14}$C-bensulfuron methyl by roots of rice cultivars at the 3rd leaf stage.](image-url)
cultivars except Milyang 30.

2. Absorption and translocation of \(^{14}\text{C}-\text{bensulfuron methyl} \)

Absorption of \(^{14}\text{C}-\text{bensulfuron methyl} \) by roots and translocation to shoots were studied with 2 concentrations, \(10^{-6}\text{M}\) and \(10^{-5}\text{M}\).

2-1 Absorption

Figure 1 shows results of the absorption experiment as absorption values which were obtained by dividing the absorbed amount of \(^{14}\text{C}\)-radioactivity in plants by dry weight of roots, the organs of absorption.

With \(10^{-6}\text{M}\), no great difference in absorption among the 5 rice cultivars was noted at 3 hours, and at 6 hours Nihonbare had a less absorption value than other cultivars. At 24 hours, the absorption values of Shingwang and Milyang 30 were larger than the other 3 cultivars. At 48 hours, Nihonbare showed the largest absorption value among all cultivars, with Shingwang and Milyang 30 next.

With \(10^{-5}\text{M}\), there was no great difference in absorption value among the 5 cultivars. That of Nihonbare was less than the others at 6 hours and Sangpung was less than the others at 24 hours. At 48 hours, Shingwang showed the largest absorption value among all cultivars and the next largest was Nihonbare.

2-2 Translocation

Figure 2 shows the translocation rate, which was obtained by dividing the amount of \(^{14}\text{C}\)-radioactivity in shoots by the amount in whole plants.

At \(10^{-6}\text{M}\), Nihonbare showed a higher translocation rate than other cultivars after 3 hours. At 6 hours, Milyang 30 showed a lower rate than others. At 24 hours, the translocation rate of Nihonbare was the highest value among all cultivars, and at 48 hours. Nihonbare and Shinseonchalbyeo showed nearly a 40% rate. The translocation rate of Sangpung increased rapidly from 24 hours and its value was over 30%, similar to Shingwang. Milyang 30 showed the lowest translocation rate among the 5 cultivars.

With \(10^{-5}\text{M}\), no great difference in translocation rate among the 5 cultivars was observed at 3 hours. At 6 hours, the rate of Sangpung was higher than other cultivars, but at 24 hours it had decreased and was the lowest among the five. At this time, Shingwang showed the highest rate. At 48 hours, the translocation rate of Sangpung rapidly increased rapidly from 24 hours and its value was over 30%, similar to Shingwang. Milyang 30 showed the lowest translocation rate among the 5 cultivars.

![Fig. 2. Rate of translocation from roots to shoots of \(^{14}\text{C}-\text{bensulfuron methyl} \) in rice cultivars at the 3rd leaf stage.](image-url)
increased and reached about 40%; Shingwang showed the next highest and the rate of Nihonbare was about 30%. The rates of Shinseonchalbyeo and Milyang 30 did not greatly increase.

3. Metabolism of $^{14}$C-bensulfuron methyl

Degradation of $^{14}$C-bensulfuron methyl in rice plants was studied, in an experiment in which $10^{-6}$ M of the herbicide was absorbed by roots for either 6 or 24 hours.

Figure 3 shows percents of $^{14}$C-compounds which presented in roots and shoots after 6 hours. More than 90% of $^{14}$C-compounds were detected in root parts of all cultivars. The amount of non-degraded parent compound in roots was about 45% in Milyang 30 and Shingwang, while in Sangpung and Nihonbare it was more than 55%. The metabolic product found in the largest amount in roots was sulfonamide; there was no great difference detected among all cultivars, with the percents of 23-27%. The total amount of O-demethyl bensulfuron methyl, an unidentified compound and metabolites in the water soluble fraction in Milyang 30 and Shingwang were larger than in Sangpung and Nihonbare.

At the 24 hour exposure, percents of $^{14}$C-compounds detected in shoots were 19%, 26%, 29% and 29% in Milyang 30, Shingwang, Sangpung and Nihonbare, respectively (Fig.4). Percents of parent compound in roots were 29% and 26% in Milyang 30 and Shingwang, respectively, and less than the about 35% in Sangpung and Nihonbare. Percent of sulfonamide was around 19% in each cultivar. Percents of O-demethyl bensulfuron methyl and metabolites in the water soluble fraction in roots were larger in Milyang 30 and Shingwang than in Sangpung and Nihonbare. Percent of non-extracted $^{14}$C-radioactivity was larger in Milyang 30 than in other cultivars. At 24 hours, rate of the parent compound in shoots was less that in roots in all cultivars. The greatest radioactivity in metabolites was found as sulfonamide, similar to the finding in roots. Percent of sulfonamide in Milyang 30 was larger than in other cultivars. Percents of O-demethyl bensulfuron methyl and metabolites of the water soluble fraction in Sangpung and Nihonbare

![Fig. 3. Distribution of $^{14}$C-bensulfuron methyl in each fraction of roots and shoots at the 3rd leaf stage of rice plants after 6 hours of exposure and details of the CH$_2$Cl$_2$ fraction analyzed by TLC.](image)
were larger than in Milyang 30 and Shingwang, a different tendency than seen in roots, while percents of homosaccharin in Milyang 30 and Shingwang were larger than in Sangpung and Nihonbare.

**Discussion**

Bensulfuron methyl was applied to roots of rice plants at the 3rd leaf stage. At this time, the 4th leaves were already developed, and the 5th leaves were just prior to emergence. Differential sensitivity among rice cultivars was investigated in the growth inhibition of the 5th leaf and roots which were directly exposed to the herbicide. The growth and development of the 6th leaf was only in Milyang 30. Considering the effects on growth of the plant parts which grew largely after the application of the herbicide. Milyang 30 showed the least degree of inhibition in both shoots and roots (thus a resistant cultivar), while the largest inhibition degree was showed in Nihonbare (a susceptible cultivar). Considering the sensitivity among cultivars treated in shoots (the 4th, 5th, 6th leaf length and dry weight of shoots) and in roots (dry weight of roots), order of the sensitivity in shoots was Nihonbare > Sangpung > Shinseonchalbyeo > Shingwang > Milyang 30, and order of the sensitivity in roots was Nihonbare > Shinseonchalbyeo > Sangpung > Shingwang > Milyang 30. Putting together the results of the growth test, it is concluded that sensitivity to bensulfuron methyl among the rice cultivars is in the order of Nihonbare > Sangpung, Shinseonchalbyeo > Shingwang > Milyang 30.

Nihonbare, the most sensitive cultivar, showed lower absorption value than other cultivars at 6 hour exposure time, while at 48 hours its value was higher than others. Milyang 30 and Shingwang, resistant cultivars, also showed large absorption value. In 10^{-5}M concentration, the Nihonbare value was lower at 6 hours, while at 48 hours it had a value second only to Shingwang. Milyang 30, the most resistant cultivar, on the other hand, showed the lowest absorption value.

Consequently, there was no distinct relationship between degrees of sensitivity and rates of absorption because the tendencies of absorption in 10^{-6}M and 10^{-5}M differed among the treated cultivars.

Milyang 30 showed lower translocation...
rate than other cultivars and this was correl-ative with less sensitivity of the shoots. Translocation rates of other cultivars, how-

Milyang 30 and Shingwang had a lower rate of the parent compound in roots than Sangpung and Nihonbare at 6 hour and 24 hour exposure times. In addition, Milyang 30 and Shingwang had a higher rate of O-demethyl bensulfuron methyl and water soluble metabolites than did Sangpung and Nihonbare. These results correlated with the inhibition degree of dry weight of roots. In shoots, Milyang 30 showed a lower rate of the parent compound than the other three rice cultivars at both 6 and 24 hour exposure times. Rate of the parent compound in shoots did not greatly differ among Shingwang, Sangpung and Nihonbare.

On the basis of the growth inhibition in plant parts developing after application of the herbicide, and on the roots which were directly exposed to the herbicide, it is con-

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根部施用ベンズルフロンメチルのイネ品種における吸収、移行および代謝

大野 哲・高橋 鍾英*・石塚靖造・松本 宏

要約

スルフィニルウレア系の水稲除草剤ベンズルフロンメチルは、イネ品種間で感受性差異のあることが知られており、その原因がイネに内在する性質によると示唆されている。本報は、韓国で行なわれた広範なイネ品種の選抜試験で特徴的な反応を示した、Milyang 30, Shingwang, Sangpung, Shinsonechalbyeo の4品種と、日本品種を用いてベンズルフロンメチルに対する感受性、吸収、移行および分解代謝について、水耕法による根部処理により、比較検討を行なった。

生育実験は、まずイネ根部を10^{-6}, 5×10^{-6} および10^{-5} M の薬液を含む水栽培液に4日間浸漬した。その後薬液を含まない水栽培液に移し、さらに6日間生育させた。処理開始から10日目に茎葉、根部の乾燥重と、4薬、5葉および6葉の長さを測定した。薬剤処理後に大きく成長して行く部分に着目してベンズルフロンメチルに対する品種間の感受性差異をみると、日本晴）Sangpung)＝Shinsonechalbyeo/Shingwang)≥Milyang 30 の順に感受性が高かった（第1表）。次に^{14}C-ベンズルフロンメチルを用いて吸収力と根部から茎葉部への移行率を、10^{-6} Mおよび10^{-5} M の2濃度で検討した。最も感受性の高かった日本晴は、2濃度とも吸収6時間で他の品種に比べ低い値を示し、48時間では高い値を示した。一方、感受性の低かった Shingwang, Milyang 30 は、10^{-6} M では供試品種の中で高い値を示した。しかし10^{-5} M では、Shingwang は10^{-6} M 同様高い値を示したのに対し、Milyang 30 は低い値を示した（第1図）。移行率では、Milyang 30 は10^{-6} M, 10^{-5} M とも他品種に比べ低い値を示したが、Milyang 30 に次いで感受性の低かった Shingwang は10^{-6} M であまり高い値を示さず、10^{-5} M では5品種のなかで高い値を示した。また日本晴は Shingwang とは逆の関係を示した（第2図）。以上のことにより、吸収力、移行率ともに感受性の品種間差異を説明する大きな要素ではないと考えられた。^{14}C-ベンズルフロンメチルの代謝実験は、Shinsonechalbyeo を除いた4品種について行なった。根部を10^{-6} M の水溶液に6時間および24時間浸漬し、植物体中の^{14}C-化合物を抽出、分離、同定した。その結果、感受性の低かった品種では、根部においてO-脱メチルベンズルフロンメチルおよび水溶性物質の生成の大きなことが明らかとなった（第3, 4図）。

以上、生育実験におけるベンズルフロンメチルに対するイネ品種の感受性差異を、薬剤の吸収、移行および分解代謝から考察すると、最も感受性の低かった Milyang 30 では、その感受性の低い原因として根部から茎葉部への移行率が小さかったこと、および根部での分解能が他品種に比べ大きかったことが考えられた。また、Milyang 30 に次いで感受性の低かった Shingwang では、その原因として根部での分解能の大きなことが考えられた。