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Selective Herbicidal Activity of 3,3′-Dimethyl-4-Methoxybenzophenone (Methoxyphenone, NK-049); Absorption, Translocation and Metabolism

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Absorption, translocation and metabolism of 3,3′-dimethyl-4-methoxybenzophenone (NK-049) applied to rice and barnyardgrass were examined to elucidate its selective herbicidal action between the two plants. Marked differences were observed in the rates and amounts of absorption and translocation between the rice (tolerant) and barnyardgrass (susceptible) plants. When applied on leaf surface, NK-049 readily penetrated into the leaf of barnyardgrass, while in rice, the chemical was slowly absorbed. Translocation both upward and downward from the treated zone through leaf veins were slight in both plants. When applied to roots by dipping the roots in NK-049 solution, the chemical was absorbed much more by barnyardgrass as compared with by rice and readily translocated to aerial parts. When applied to intact seeds, NK-049 was absorbed more rapidly by barynardgrass on seed weight basis, while on seed number basis rice seeds absorbed much more NK-049 than barnyardgrass seeds. Radioactivity once absorbed by seeds was translocated to the first leaves in the seedlings of both rice and barnyardgrass, but the amount of translocated radioactivity was much greater in barnyardgrass than in rice. No appreciable difference was found in the metabolism of NK-049 between the two plants. It was suggested that one of the main factors for the selective action of NK-049 is the differences in the rate and amount of absorption and translocation of the chemical between rice and barnyardgrass. Carotenoid formation in barnyardgrass was strongly inhibited by NK-049, whereas that in rice was not affected at all. It was suggested that the susceptibility of the carotenoid-biosynthesis systems of the two plants to NK-049 were greatly different from each other and this difference contributes to the selective herbicidal action of the chemical.

INTRODUCTION

A herbicide, methoxyphenone (3,3′-dimethyl-4-methoxybenzophenone, NK-049) shows a highly selective action between rice and barnyardgrass both in paddy and upland fields.[1,2] Its herbicidal activity is pronounced especially when the chemical is absorbed through young shoots of weed at emerging stages. It is available as a pre-emergence weed killer.[1,2]

The mode of herbicidal action of NK-049 was partly clarified in our previous study.[3] The chemical was shown to inhibit specifically the carotenoid biosynthesis, which leads consequently to photodecomposition of chlorophylls.

The present study deals with the absorption and translocation of NK-049 as well as its metabolism in plant tissues in order to elucidate its mechanisms of selectivity between susceptible (barnyardgrass) and tolerant (rice) plants. Since the selective activity of NK-049 was greatly dependent on the way of treatment, three different ways of application were compared in terms of herbicidal activity and selectivity.

MATERIALS AND METHODS

1. *Test Plants*

Rice (*Oryza sativa* L. c. v. Nihonbare) and barnyardgrass (*Echinocloa crus-galli* B. var. *orizicola* OHWI) were grown during the same time of the year on culture soil (Kureha soil D, Japan) in plastic pots in a green house at 28°C.

2. *Chemicals*

^{14}C–NK–049 labeled at the carbonyl carbon was used for tracer experiments. The specific activity of ^{14}C–NK–049 was 5.5 mCi/mmol with impurity less than 1%[4].

3. *Analyses*

Radioactivity was determined with a liquid scintillation spectrometer (Packard Tri-Carb 3320) in either of the following scintillator solution, A: 100 g of naphthalene, 8 g of 2,5–diphenyloxazole, 0.1 g of dimethyl–POPOP and 20 ml of ethylene glycol dissolved in 1 liter of dioxane, B: 4 g of 2,5–diphenyloxazole and 0.2 g of dimethyl–POPOP dissolved in 1 liter of toluene. Unextractable radioactivity remaining in plant tissue was measured after combustion in a sample oxidizer (Packard Tri-Carb 306). Thin layer chromatographic separation of NK–049 and the metabolites derived from it was carried out on a silica gel prelayered chromatoplate (Merck 60 F254, thickness of 0.25 mm) with a solution of *n*–hexane, ethyl acetate and acetic acid (20 : 20 : 1) as a developing solvent. Radioactivity on a chromatogram or in plant tissues was localized by autoradiography with an X–ray film (Fuji Kx, Japan). Quantitative determination of the radioactivity on the chromatogram was carried out by scraping the radioactive spots on a silica gel into a counting vial.

4. *Application of NK–049 to Plants*

The chemical was applied in the following three different ways: (A) *Application to leaf surface*; Two μl (0.2 μg) of 50% ethanolic solution of ^{14}C–NK–049 was applied in 1 cm width on the surface of a second leaf of the 3-leaf stage plants. (B) *Application to roots*; The plants at 2 or 3-leaf stage were rooted up and their roots were dipped in 2 ppm ^{14}C–NK–049 solution after being carefully washed with tap water. (C) *Application to intact seeds*; One gram of dry seeds were dipped in a serial concentration of ^{14}C–NK–049 solution for several hours as indicated in the legend of each experiment.

The applied zone of the leaves or roots were washed first with acetone and then with distilled water to remove surface residues. Roots and shoots were, then, separated and dried at 80°C for 2 hr and weighed. Absorbed or translocated radioactivity in each part of the plant tissues was determined with a liquid scintillation spectrometer. A portion of the treated seeds and an aliquot of the remaining cultivating solution were analyzed to determine the absorbed ^{14}C–NK–049. The rest of the seeds were germinated on sea sand moistened with distilled water under 12-hours light and dark cycle in a green house at 28°C for several days, and the translocated radioactivity, chlorophylls and carotenoid content of the leaves were determined. Pigment analysis was carried out according to the method of Arnon[5] for chlorophylls, and for carotenoids by Davies' method.[6]

5. *Metabolism of NK–049 in Plant Tissue*

Rice and barnyardgrass seeds dipped in NK–049 solution for 24 hr were germinated on a sea-sand bed as described above. The first leaves were harvested, extracted by Abdel-Wahal's methods,[7] and then, fractionated into three fractions, acetone-chloroform soluble fraction, water soluble fraction and unextractable residues. The radioactivity in each fraction was determined by the procedures described above.

RESULTS

1. *NK–049–induced Chlorosis in Rice and Barnyardgrass*

Figure 1 shows a selective action of NK–049 between rice and barnyardgrass as measured in terms of chlorophyll contents. Perfect chlorosis occurred in barnyardgrass treated at the concentration of 50 ppm, while in rice, no chlorosis occurred unless the concentration reached above 200 ppm. The selectivity index estimated from the ratio of the concentrations to reduce the chlorophyll content to 50% of

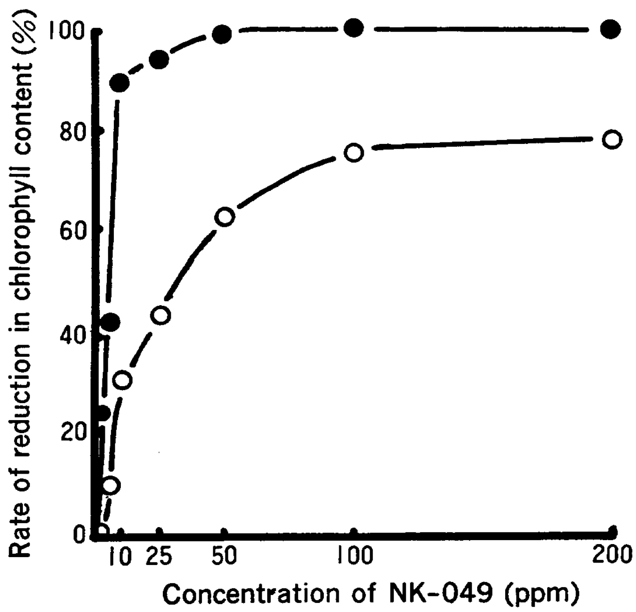

Fig. 1 Reduction of chlorophyll content in rice and barnyardgrass treated with NK–049.

o Rice, • Barnyardgrass

Rice and barnyardgrass seeds treated with NK–049 were germinated and grown for 6 days on a sea-sand bed at 28°C in a green house under cyclic illumination of 12 hr.

the original level was 5 to 7.

2. Absorption and Translocation of ^{14}C–NK–049 Applied to Leaf Surface

Table 1 shows the amounts of NK–049 absorbed from leaf surface by rice and barnyardgrass. NK–049 was more rapidly absorbed by barnyardgrass than by rice. During the first 6 hours after the application, 72% of the radioactivity was absorbed by barnyardgrass, whereas only 59% of the radioactivity was found in rice even after 48 hours. The absorption by barnyardgrass was very fast especially in the earlier stages after application. Decrease in total radioactivity during application was presumed to be due to photodecomposition and/or volatilization of ^{14}C–NK–049. Autoradiogram of the plants treated by ^{14}C–NK–049 on leaves indicated that the radioactivity was slightly translocated both upward and downward from the treated zone through leaf veins both in rice and barnyardgrass (Fig. 2).

3. Absorption and Translocation of ^{14}C–NK–049 Applied to Roots

When ^{14}C–NK–049 was applied in a cultivating solution, the chemical was absorbed from roots and then translocated to the upper parts of the plants with the time elapsed, as shown in Fig. 3. The rate of absorption of ^{14}C–NK–049 by roots was much higher in barnyardgrass than in rice. In rice, the absorbed radioactivity mostly remained in the roots and was slightly translocated into the leaves during 48 hours after the application. On the contrary, more than 40% of the radioactivity absorbed by the roots of barnyardgrass was rapidly translocated into the upper leaves and the remaining 60% was retained in the roots. These results were confirmed by the autoradiogram of such treated plants as shown in Fig. 2.

Table 1 Absorption of radioactivity by rice and barnyardgrass after foliar application of ^{14}C–NK–049.

Time (hours)	Distribution of radioactivity (%)					
	Rice			Barnyardgrass		
	Surface wash	Leaf	Total	Surface wash	Leaf	Total
0.5	93.9	11.0	104.9	70.1	30.0	100.1
1	85.7	15.1	100.8	54.7	47.6	101.3
3	73.5	27.9	101.4	22.9	63.3	86.2
6	47.0	33.8	80.8	11.7	71.9	83.6
9	36.9	41.1	78.0	9.7	72.4	82.1
24	18.5	52.3	71.8	7.1	62.2	69.3
48	13.3	58.8	72.1	5.1	61.7	66.8
72	11.4	55.5	66.9	2.2	62.7	64.9
96	6.9	53.7	60.6	1.0	55.4	56.4

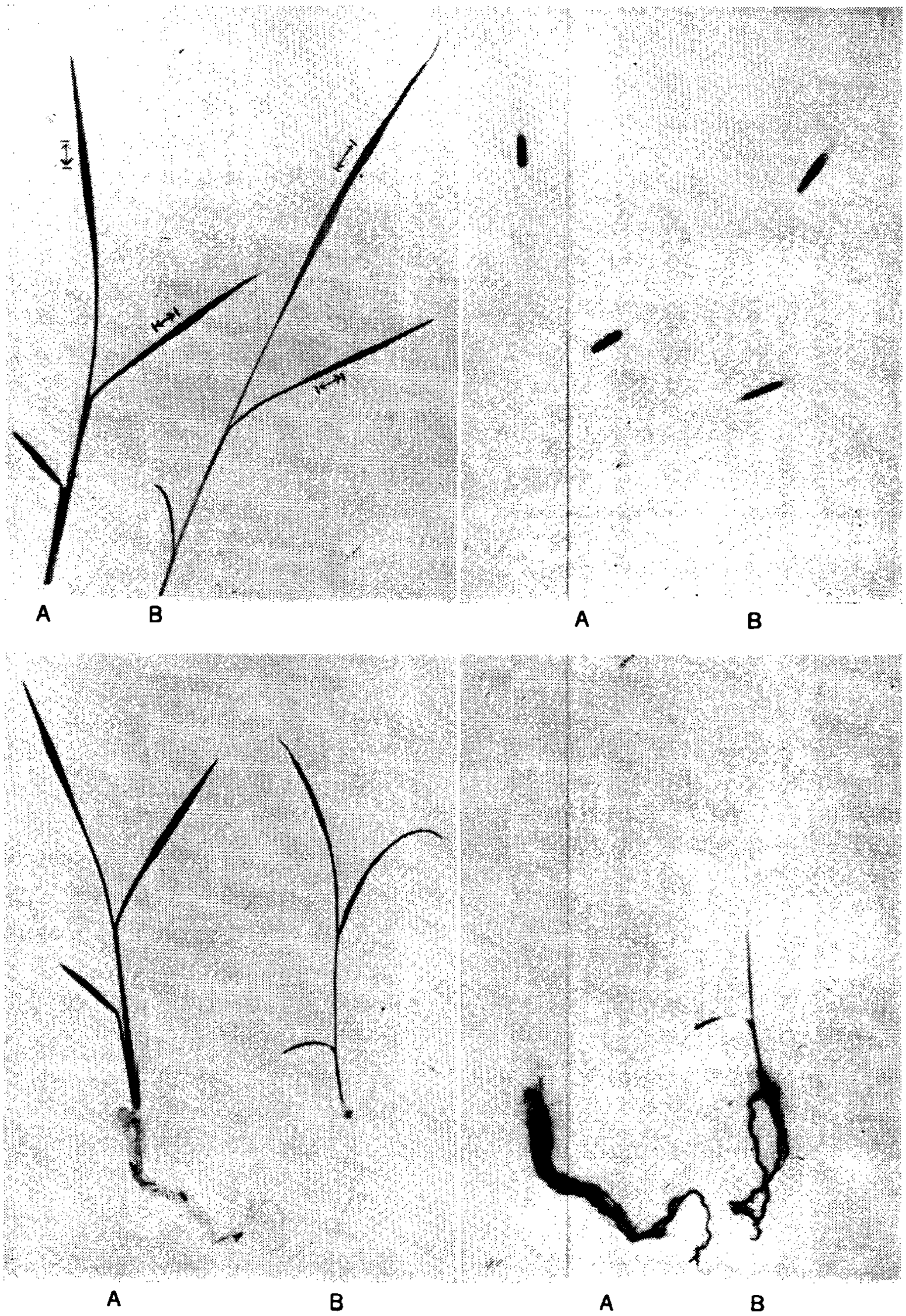

Fig. 2 Autoradiograms (right) and photographs (left) of rice and barnyardgrass treated with ^{14}C–NK–049.
A; Rice, B; Barnyardgrass.
upper: NK–049 was applied to the zones indicated by arrows for 24 hr.
lower: NK–049 was applied to the roots for 6 hr.

4. Absorption and Translocation of ^{14}C–NK–049 by Intact Seeds

NK–049 was absorbed by intact seeds of both barnyardgrass and rice and accumulated in them as much as 9 fold and 2 to 3 fold, respectively, against the concentration of the dipping solution after 24-hours of application (Table 2). On seed weight basis, barnyardgrass seeds absorbed 3 to 4 fold of radioactivity as compared with rice, while the radioactivity absorbed by a single rice seed was 4 to 7 fold much more than that by a single seed of barnyardgrass. Translocation of the radioactivity once accumulated in seeds to the

Table 2 Absorption and translocation of ^{14}C–NK–049 by intact seeds of rice and barnyardgrass.

	Barnyardgrass								Rice							
	NK–049 content in dipping solution (ppm)															
	0	0.5	1.0	5.0	25.0	50.0	100.0	200.0	0	0.5	1.0	5.0	25.0	50.0	100.0	200.0
NK–049 content in seed* μg/g seed (fresh weight)		4.5	9.4	45.1	218.2	397.0	818.9	1300.0		1.4	3.1	17.2	52.9	98.8	222.6	569.1
NK–049 content in seed* ng/seed		7.5	15.7	76.8	363.7	661.7	1364.8	2166.7		38.8	86.1	550.0	1469.4	2744.4	6183.3	15808.3
Concentration ratio**		9.0	9.4	9.2	8.7	7.9	8.2	6.5		2.8	3.1	3.4	2.1	2.0	2.2	2.8
NK–049 content in leaf* μg/g leaf (fresh weight)			0.8	2.2	11.7	22.8	33.2	51.4		0.1	0.3	1.0	2.6	3.4	8.7	9.4
NK–049 content in leaf* ng/leaf			3.0	8.3	32.3	69.0	86.3	137.0		2.0	3.7	9.4	31.4	37.3	69.5	75.8
Translocation ratio***			19.1	10.8	8.9	10.4	6.4	6.3		5.2	4.3	1.7	2.1	1.4	1.1	0.5
Chlorophyll content μg/g leaf (fresh weight)	1160.0		951.5	650.7	55.7	17.8	6.1	0.0	449.1		457.7	448.8	418.0	396.7	372.0	367.9
Carotenoid content μg/g leaf (fresh weight)	96.0		94.9	91.3	17.2	6.6	3.6	3.6	51.4		44.8	48.0	47.9	47.8	47.9	45.0

Both plant seeds were dipped in serial concentrations of NK–049 solution for 24 hr and then the absorbed radioactivity was measured. The dipped seeds were germinated and grown for 6 days and the first leaves were analyzed to determine the translocated radioactivity, chlorophylls and carotenoids.

* Values are calculated from radioactivity.

** Rate of NK–049 content in seed (μg/g seed) to NK–049 content of dipping solution.

*** Percentage of NK–049 content in leaf (ng/leaf) to NK–049 content in seed (ng/seed).

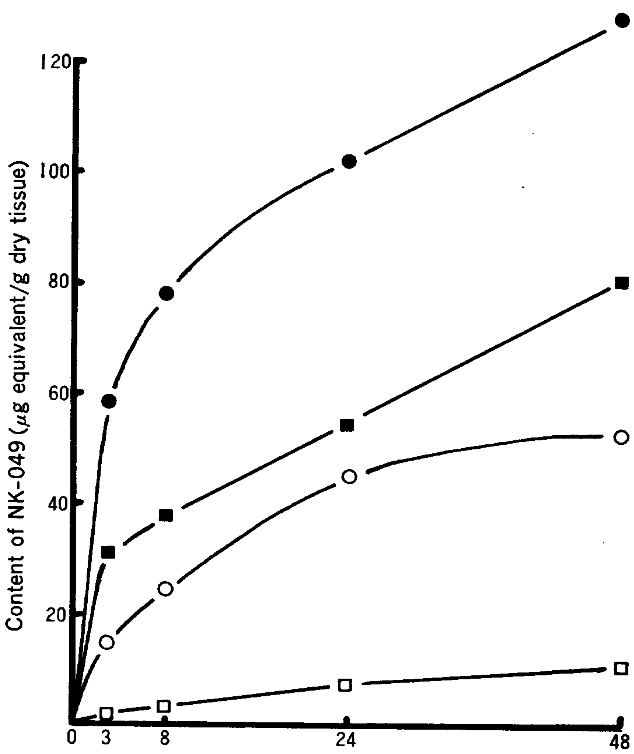

Fig. 3 Absorption and translocation of radioactivity by the root application of rice and barnyardgress.
□ leaves, and ■ roots of rice; ○ leaves, and ● roots of barnyardgrass

first leaves during germination was also investigated. The amount of the chemical translocated from seed to leaf was about 5 times more in barnyardgrass than in rice, when expressed on fresh-weight basis, whereas the amount was almost identical between the two test plants, when expressed on the basis of the number of leaves.

Table 2 also shows the relation between the amount of translocated radioactivity to the contents of chlorophylls and carotenoids in the first leaves as an index for the degree of chlorosis caused by the chemical. In barnyardgrass, appreciable chlorosis was induced when the concentration of NK-049 was higher than 5 ppm in the dipping solution, and 40 ppm or 2 ppm in the seeds or in the first leaves, respectively. Perfect chlorosis was effected by the concentration about 10 times higher than these values. In rice, on the other hand, no chlorosis was observed even at the high concentration of 200 ppm in the dipping solution.

5. Metabolism of ^{14}C-NK-049 in Plant Tissue

Chemical transformation of NK-049 in the first leaf of rice and barnyardgrass during germination was studied to make clear if the metabolic degradation is related to the selectivity between rice and barnyardgrass. Table 3 shows the percentages of radioactivity recovered in acetone-chloroform soluble fractions, water soluble fractions and residues. The radioactivity recovered in water soluble fractions increased rapidly with the age of plants, while the radioactivity recovered in acetone-chloroform soluble fractions decreased. The radioactivity recovered in the acetone-chloroform soluble fractions was identified to be NK-049 on a thin layer chromatogram. This rapid transformation of radioactivity from acetone-chloroform soluble form to water-soluble form indicates that the chemical was rapidly transformed to some water-soluble materials both in rice and barnyardgrass, although the chemical structures of the water-soluble metabolites have not yet been identified. The rate and the amount of transforma-

Table 3 Distribution of radioactivity in the first leaves of rice and barnyardgrass treated with radioactive NK-049 to the seeds before germination.

	Distribution of radioactivity (%)		
	Age of plants (days)		
	3	6	10
Barnyardgrass			
Acetone-chloroform soluble fraction	65.0	32.6	26.2
Water soluble fraction	29.4	59.4	66.7
Residue	5.5	8.1	7.2
Rice			
Acetone-chloroform soluble fraction	56.1	35.3	16.5
Water soluble fraction	35.5	57.9	69.7
Residue	8.5	6.9	13.8

Barnyardgrass and rice seeds were dipped in 25 ppm and 200 ppm ^{14}C-NK-049 solution for 24 hr, respectively, then germinated on a sea-sand bed at 28°C.

tion, however, was not much different between these two plants. The detoxication by metabolic processes does not seem to be closely related to the selective toxicity of this chemical between the two plants.

DISCUSSION

As to the mechanisms for selectivity of herbicides, much study has been made on the differences in morphology, and absorption, translocation and metabolism of herbicides between susceptible and tolerant plants. In some cases, the selective toxicity of a herbicide has been shown to depend on the differences in sensitivity to the herbicide of various metabolic systems which normally operate in growing plants. In the present study, we attempted to clarify the mechanism of selective activity in the herbicidal activity of NK-049. The selectivity index of NK-049 between rice and barnyardgrass as estimated from the ratio of the NK-049 concentrations to reduce chlorophyll content to 50% was 5 to 7. This index was obtained both for root and seed applications. Marked differences were observed between the two plants in the rate of absorption by foliar application and in the rates and amounts of absorption and translocation by root and/or seed application. These results suggests that the differences in absorption and translocation of NK-049 between rice and barnyardgrass are evidently the dominant factors to cause the selective effect on both plants. Little difference, however, was found in the rate of metabolism of NK-049 in both test plants. Almost all the radioactivity found in acetone-chloroform soluble fractions was coincided with the authentic sample of NK-049 on a *tlc* and no appreciable metabolites were detected. This indicates that the metabolic degradation of NK-049 has very little to do with the selective response of the two plant species to NK-049. It has been shown that the metabolites of NK-049 in plants have less herbicidal activity compared with NK-049.[8]

These data obtained by applying NK-049 in three different ways confirmed that the NK-049 was effective for pre-emergence and pre-transplanting treatment in paddy and upland fields as reported in the previous papers.[1,2] In the present study, the differences in the rates and amounts of absorption and translocation of NK-049 by seed and root applications were pronounced enough, even if the chemical was applied to both susceptible and tolerant plants at the same growth stages. In field applications, the chemical is to be applied to plants and weeds at different growth stages from each other, where barnyardgrass is still at the stage of seed or just emerged, while rice have grown up to some extent. Such difference in the growing stage will enhance the selectivity between the two plants. Above conception was proved by the field examination of this chemical.[9]

As reported previously,[3] the primary action of NK-049 was presumed to be the inhibition of carotenoid biosynthesis. The contents of carotenoids and NK-049 in the first leaves of rice and barnyardgrass suggested that the carotenoid-biosynthesis systems in the two species of plants do not seem to have the same susceptibility to NK-049. Carotenoid biosynthesis in barnyardgrass was highly susceptible to NK-049, whereas that in rice was less susceptible. Carotenoid content in barnyardgrass decreased remarkably when NK-049 concentration was more than 5 ppm, while in rice, almost no decrease was observed at the concentration of 200 ppm. More than 40 times higher concentrations are required to inhibit the carotenoid synthesis in rice than in barnyardgrass. This result suggests that there exists another factor for the selectivity of NK-049, namely a difference in sensitivity of the enzymes involved in carotenoid biosynthesis between different plant species.

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要　約

3,3′-ジメチル-4-メトキシベンゾフェノン（メトキシフェノン，NK-049）の選択殺草機構：吸収，移行および代謝について

藤井保男，黒川隆史，山口　勇，見里朝正

NK-049 の選択的殺草機構を解明する目的で，^{14}C-NK-049 の吸収，移行およびその代謝について，感受性植物としてタイヌビエを，抵抗性植物として，イネを用いて検討した．葉面，根部および種子処理のいずれにおいても，吸収速度，吸収量ならびに処理部からの薬剤の移行性は，タイヌビエのほうが著しく大きいことが認められた．とくに，根部から茎葉部への移行性と種子から発芽第一葉への移行性とがヒエにおいて大きかった．植物体内における NK-049 の代謝には，両植物の間に大きな差が認められなかった．したがって NK-049 のイネとヒエ間の選択的殺草作用の一因として，薬剤の吸収速度と吸収量の差，ならびに移行性の差が挙げられ，代謝による影響は少ないものと推定された．また，NK-049 の第一次作用点と思われるカロテノイド生合成過程にも，両植物の間に NK-049 に対する感受性の差の大きいことが認められた．