Morphological and Anatomical Effects of Mefenacet on the Growth of Barnyardgrass (*Echinochloa oryzicola* Vasing.)*

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Abstract: Morphological and anatomical effects of mefenacet on the growth of barnyardgrass (*Echinochloa oryzicola* Vasing.) were investigated in petri-dish and pot tests. In the petri-dish, mefenacet did not inhibit weed germination. After germination, however, leaves failed to emerge through the coleoptile, and the primary root was reduced at high concentrations, 10 and 5 ppm. At low concentrations, 0.5 and 0.1 ppm, the growth of shoot, root and coleoptile was slightly inhibited, while the mesocotyl was not affected by the herbicide. In the preemergence treatment in pots, mefenacet did not inhibit the elongation of mesocotyl or coleoptile, however, it severely retarded the growth of 1st and 2nd leaf primordia and length of the primary root. When the weed was treated at 1, 2 and 3 leaf stages, the growth of primordia of subsequent leaves was retarded, and the elongation of coronal roots was inhibited. Anatomical results showed that mefenacet clearly exerted a strong inhibitory action against meristems, but had little effect on existing plant tissue. When applied at the 2-leaf stage, the growth of subsequent leaves was strongly

retarded, as was the leaf sheath of the 3rd leaf. The upward and marginal growth of the 3rd and 4th leaves was inhibited and the growth of primordia of the 5th and 6th leaves was also severely inhibited. The growth of epidermal cell was affected and the intercellular spaces of the mesophyll did not form extensive lacunae as shown in the untreated plant. At the 3-leaf stage application, the growth of primordia of the 4th, 5th and 6th leaves was retarded and abnormal swelling of the 1st, 2nd and 3rd tillers was observed.

Key words; mefenacet, *Echinochloa oryzicola* Vasing., morphological effect, anatomical effect

Introduction

2-(2-benzothiazolyl-oxy)-*N*-methyl-*N*-phenylacetamide (mefenacet, trademark: Hinochloa®) is an herbicide of the oxyacetamide group, which is effective on annual monocotyledonous and some dicotyledonous weeds in rice fields. It is widely used to control *Echinochloa* spp. from preemergence to 3 leaf stage in combination with sulfonyleurea herbicides in Japan. In spite of many years of research, the basis for the mode of action of mefenacet remains obscure. Fedtke^{6,7)} and Hess et al.¹¹⁾ reported that mefenacet inhibited cell division and cell enlargement in both green alga and oat. The degree of inhibition of cell division was greater than that of cell enlargement. The herbicide inhibited mitotic entry rather than disrupting the mitotic sequence. The mor-

*A part of this study was presented at the 30th annual meeting of the Weed Science Society of Japan (1991).

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(Received August. 9 1999; Accepted March 14, 2000)

phological effect of mefenacet on green algal cells was similar to that observed for thiocarbamates and chloroacetamides. The results of a few morphological studies of *Echinochloa* spp. treated with mefenacet have been published^{13,23,24}.

The objectives of this study were to investigate morphological and anatomical changes in the shoots and roots of barnyardgrass caused by the herbicide.

Materials and Methods

Effect of mefenacet on germination, shoot and root growth in petri-dish test

Spikelets of barnyardgrass (*Echinochloa oryzicola* Vasing.) were offered by Okayama University and the germination rate was 80%. They were placed on filter paper in petri-dishes. Ten ml of treatment solution was added to each dish. The treatment solution was distilled water containing the herbicide at 10.0, 5.0, 1.0, 0.5 and 0.1 ppm. The herbicide was dissolved with 0.1% acetone. The control was distilled water with 0.1% acetone. The dishes were incubated for 7 days in a growth chamber at 25°C at 80% relative humidity with 12-hr light (10,000 lx) and 12-hr dark. At the end of the experiment, the length of each part of the weed on twenty spikelets was measured.

Effect of mefenacet on shoot and root growth in pot test

The twenty spikelets were planted 2 cm deep in 1000 cm² pots containing clay soil, and the pots were put in the greenhouse. The temperatures in the greenhouse were controlled at 25±3°C and water depth was kept at 3 cm on the soil surface during the test period. Mefenacet at the rate of 1000g ai/ha was applied to the pots at the growth stages of preemergence, 1, 2 and 3 leaves of the weed. The length of shoots and roots was measured at the end of the 10 days experi-

mental period. Growth progression and herbicidal symptoms appearing on the weed were evaluated every day for 21 days after application.

For anatomical observation, treated and untreated plants were harvested 10 days after herbicide application. The plants were fixed with FAA (formalin: acetic acid: ethanol=1:1:18) solution for further investigation. Fixed materials were dehydrated in an ethanol: tertiary butanol series, embedded in paraffin, sectioned at 10 µm, and double stained with safranin and Delafield's hema-toxylin.

Results and Discussion

Effect of mefenacet on germination, shoot and root growth in petri-dish test

Mefenacet did not inhibit the germination of barnyardgrass (Table 1). After germination, however, growth of shoot and primary root was inhibited. Shoot length was inhibited by 13.1% at 10.0 ppm and by 16.9% at 5.0 ppm. At the same concentrations, primary root length was inhibited by 37.6% and 54.5%, respectively. The length of coleoptile was slightly reduced. Shoot growth was influenced by the herbicide more than the primary root, while the length of mesocotyl was not affected. The growth inhibition induced by mefenacet was different from those of pronamide and cinmethylin, which are more active in the roots than in the shoots^{1,5}.

Morphological effect of mefenacet on shoot and root growth in pot test

When the weed was treated with mefenacet at preemergence, the growth of mesocotyl and coleoptile was not affected, but the growth of 1st and 2nd leaves and the primary root were severely inhibited (Table 2). No visible injury symptoms on plants were observed until 3 days after treatment with the herbicide. The first symptoms were observed at 5 days, and were inhibition of the elonga-

Table 1. Effect of mefenacet on the growth of shoot and root of barnyardgrass*.

Mefenacet Concentration (ppm)	Shoot	Length (mm)		Primary root	Germination rate (%)
		Coleoptile	Mesocotyl		
0.0	57.1±0.7*	14.0±0.1	1.0±0.1	43.1±1.5	80
10.0	7.5±0.4	11.0±0.2	1.0±0.1	16.2±0.9	76
5.0	9.7±0.2	12.2±0.2	1.0±0.1	23.5±1.1	82
1.0	38.0±1.9	12.2±0.2	1.0±0.1	29.8±1.1	74
0.5	53.1±1.0	12.5±0.1	1.0±0.1	43.2±2.3	80
0.1	55.1±1.7	12.8±0.2	1.0±0.1	51.4±2.8	80

* : Spikelets were immersed in 0.1% acetone solutions containing the designated amounts of the herbicide. Data were obtained 7 days after the beginning of immersion.

** : Each value is the mean of four replications±SE.

Table 2. Effect of mefenacet on the growth of barnyardgrass by preemergence application*.

Treatment	Primary root	Mesocotyl	Length (mm)**		
			Coleoptile	1st leaf	2nd leaf
untreated	31.0±2.2	21.7±1.7	12.7±0.7	40.3±3.1	49.8±8.3
mefenacet	12.1±4.8	21.7±1.2	12.5±1.3	9.3±2.9	2.5±1.1

* : Spikelets sown in pots were treated with the herbicide at 1000 g ai/ha, Data were obtained 10 days after treatment.

** : Each value is the mean of twenty plans±SE.

tion of the 1st leaf and a dark green coloration in the coleoptile. Then, discoloration appeared at the base of the coleoptilar node and progressed upward. Within 9–10 days, necrosis appeared near the base of the coleoptile and the plant died. In the present experiment, mefenacet had little effect on the growth of mesocotylar roots, but the growth of primary root was inhibited, though root tip swelling did not occur. The growth of primary root seems to be indirectly influenced through the herbicidal damage to the shoot. The symptoms on the root were similar to those of chloroacetamide and thiocarbamate herbicides⁹⁾ and different from those of dinitroanilines, *N*-phenylcarbamates and chlorthal-dimethyl, which inhibit root elongation but also induce substantial root tip swelling^{9,10,19)}. Another effect was a clear bending of the 1st leaf from the base of the coleoptile. The leaf was repulsed to the coleoptile by

marginal growth of the leaf blade. This symptom was also observed on plants treated with metolachlor¹⁸⁾ and di-allate¹⁷⁾.

When the weed was treated at 1 leaf stage, the 1st leaf and coleoptile engthened and the growth was little affected by the herbicide. However, growth of the 2nd leaf sheath and 3rd leaf was severely inhibited²⁴⁾. Within 4 days, the 2nd leaf was stunted and discoloration appered at the base, and then the basal part caused necrosis at 7–10 days. Mefenacet killed the plants within 14 days after treatment.

At 2- and 3- leaf stage treatments, the growth of the subsequent 3rd and 4th leaves, and the 4th and 5th leaves were retarded by the herbicide, respectively. Coronal roots were also inhibited in length and number (Table 3).

The growth of subsequent leaves of barnyardgrass after the application is inhibited

Table 3. Effect of mefenacet on the growth of barnyardgrass seedlings by the applications at 1, 2 and 3 leaf stages*.

Appli. Timing	Treatment	Length (mm)**					Coronal root	
		1st leaf	2nd leaf	3rd leaf	4th leaf	5th leaf	No.	Length(mm)
1 leaf stage	untreated	48.0±6.4	134.3±15.6	70.0±13.9	—	—	2.1±0.32	0.1± 7.8
	mefenacet	41.7±7.0	67.8± 8.4	10.9± 1.8	—	—	1.4±0.5	10.1± 7.9
2 leaf stage	untreated	—	148.0±13.8	187.0±15.3	213.0±22.3	—	6.2±0.9	91.1±16.5
	mefenacet	—	145.7±13.5	96.9±27.9	21.3±11.9	—	3.9±0.6	42.9±12.5
3 leaf stage	untreated	—	—	208.1±19.9	260.7±29.5	305.5±20.9	8.8±1.2	135.2±21.1
	mefenacet	—	—	192.6±19.9	156.3±31.7	40.2±12.9	6.4±0.5	73.4±15.3

* : Barnyardgrass seedlings were treated with the herbicide at 1000 g ai/ha at 1, 2 and 3 leaf stages.
Data were obtained 10 days after treatment.
** : Each value is the mean of twenty plants±SE.

by butachlor²⁾ and benthicarb²⁰⁾. However, these herbicides have little effect on root growth of the weed. From the results, mefenacet seemed to inhibit the root growth more than butachlor and benthicarb.

Anatomical effect of mefenacet on growth of shoot and root

With preemergence application, the 1st sign of influence by the herbicide was in the coleoptilar node (Fig. 1). The upward and marginal growth of the 1st and 2nd leaves were inhibited and their elongation was longitudinally inhibited. Growth of coleoptile and mesocotyl, which had already been formed in the caryopsis, was not influenced. These symptoms were similar to those observed in plants treated with metolachlor⁴⁾.

When the weed was applied at 2 leaf stage, the growth of leaves after the application was strongly retarded. The sheath of the 3rd leaf was retarded and the upward and marginal growth of the 3rd and 4th leaves was altered (Fig. 2). The primordia of the 5th and 6th leaves were also severely inhibited. The growth of epidermal cell of the 3rd leaf was restricted and the intercellular spaces of the mesophyll did not form extensive lacunae as shown in the untreated plant. The symptoms were similar to those for α -chlor-oacetamides^{3,12)}. But the effects by mefenacet were different from those caused by DBN,

Fig. 1. Photomicrographs of longitudinal (upper) and cross (lower) sections of shoot apices of barnyardgrass. Untreated (Left) and treated with mefenacet (Right) at preemergence application.
A: Shoot apex, C: Coleoptile, 1: 1st leaf, 2: 2nd leaf

Fig. 2. Photomicrographs of longitudinal (upper) and cross (lower) sections of shoot apices of barnyardgrass. Untreated (Left) and treated with mefenacet (Right) at 2-leaf stage application.

A: Shoot apex, 1: 1st leaf, 2: 2nd leaf, 3-6: leaf primordia
T: Tiller

which inhibited the elongation of mesocotyl and induced coleoptilar node swelling^{21,22)}. On

the other hand, appearance of the shoot apex was not affected and the 1st and 2nd leaves did not differ anatomically from those in an untreated plant.

At 3-leaf stage treatment, the primordia of 4th, 5th and 6th leaves were affected and abnormal swelling of the 1st, 2nd and 3rd tillers was observed (Fig. 3). The elongation of 1st and 2nd internodes was reduced. The vascular bundles were altered in number and cell arrangement. These are similar to the symptoms observed in some grass herbicides^{14,15,16)}. In addition, branched roots from coronal roots and their root hair were reduced by mefenacet as already reported for butachlor²⁾.

In conclusion, it is proposed that mefenacet retards the growth of *Echinochloa* spp. by inhibiting both cell division and elongation in meristematic areas. The effects cause reductions in length of young leaves, number and length of coronal roots, and leaf sheaths and blades. Tiller inhibition was also observed. The reduction and disruption of the vascular system may be important in causing severe retardation of leaves, internodes, and apical development.

Acknowledgements: The authors are grateful to all staff in their laboratory for their generous assistance.

摘 要

メフェナセットがタイヌビエの生長に及ぼす 外部及び内部形態的影響

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メフェナセットがタイヌビエの生長に及ぼす外部及び内部形態的影響をシャーレ及び温室ポット試験で調査した。シャーレ試験において、本剤はヒエの発芽を阻害しないが、新葉の生長を抑制し、新葉は葉鞘から抽出できなかった。また種子根の生長も、10及び5 ppmの濃度では阻害された。一方、0.5及び0.1ppmの低濃度では、本剤のシュート、種子根並びに子葉鞘の伸長に対する影響は少なく、また中

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Fig. 3. Photomicrographs of longitudinal sections of shoot apices of barnyardgrass. Untreated (Left) and treated with mefenacet (Right) at 3 leaf stage application.

A: Shoot apex, C: Coleoptile, T1, T2 and T3: 1st, 2nd and 3rd tillers

胚軸の伸長はほとんど阻害しなかった。発生前処理のポット試験では、中胚軸及び子葉鞘の伸長に対する影響はほとんど認められなかった。しかし本剤は第1葉及び第2葉の伸長を強く抑制した。また種子根長も無処理区に比べて短くなった。しかし子葉鞘及び中胚軸の伸長の抑制は認められなかった。1, 2, 3葉期処理では、各時期に伸長が著しい新葉の生長が強く抑制され、また冠根の伸長も阻害された。内部形態的観察では、本剤の処理により生長が著しい分裂組織に影響が強く現れ、既に生長している組織については形態的にほとんど影響が見られなかった。メフェナセツトを2葉期に処理すると、処理後に生長する新葉が強く阻害された。すなわち、第3葉の葉鞘、第3葉及び第4葉の上方並びに周辺成長、第5及び第6葉の原基の成長がそれぞれ阻害された。メフェナセツトを3葉期に処理すると、第4葉、第5葉並びに第6葉の生長が抑制され、第1, 第2並びに第3分げつは強く抑制され、異常な肥大が観察された。

キーワード:メフェナセツト、タイヌビエ、外部形態

的影響、内部形態的影響

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