Mode of Herbicidal and Selective Action of DPX-F5384 between Rice and Weeds*

Shunji TAKEDA, David L. ERBES**, Philip B. SWEETSER**,
James V. HAY** and Takeshi YUYAMA

Agricultural Products Division, Du Pont Japan Limited, Tokyo, Japan

**Agricultural Products Department E.I. du Pont de Nemours
and Company, Inc. Wilmington, Delaware 19898

Introduction

Londax® (trade name of DPX-F5384) is a new broad spectrum herbicide for paddy rice. DPX-F5384 (methyl 2-[[[(4,6-dimethoxypyrimidin-2-yl)amino]carbonyl]amino]sulfonylmethyl]benzoate), the active ingredient, is highly effective for control of most annual and perennial broadleaf weeds and sedges. Other papers in this series describe these properties in detail^{6,7)}.

Both DPX-F5384 and chlorsulfuron (2-chloro-*N*-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide) are herbicidal sulfonylureas. The primary site of action for chlorsulfuron is the inhibition of acetolactate synthase, an enzyme in the pathway for the biosynthesis of branched-chain amino acids³⁾. Secondary effects include the cessation of DNA synthesis, cell division and plant growth⁴⁾. Plants such as wheat and nightshade tolerate chlorsulfuron because of their abilities to metabolize the herbicide to metabolites which are relatively weak inhibitors of acetolactate synthase^{1,2,5)}. In this paper we demonstrate that DPX-F5384 also inhibits acetolactate synthase and branched-chain amino acid biosynthesis and that rice plants can metabolically inactivate this compound.

Materials and Methods

1. Compounds

Radioactive DPX-F5384 was synthesized by New England Nuclear Corp. with ¹⁴C labels in the phenyl ring (29.8 μ Ci/mg) and the pyrimidine ring (16.6 μ Ci/mg). Metabolite I of DPX-F5384 in plant was synthesized by a two-step sequence. Coupling of 2-(methoxy-carbonyl)phenylmethylsulfonylisocyanate with 4-benzyl-oxy-6-methoxypyrimidin-2-amine in methylene chloride gave the 4-benzyl-oxy-6-methoxypyrimidin-2-yl analog of DPX-F5384. Hydrogenolysis (5% Pd-C, THF) of the benzyl-oxy analog gave metabolite I, mp 210~215° (d).

2. Rice plants

The metabolism studies and special studies were made with Japonica rice, varieties M-101 and Nihonbare. The rice was grown in a greenhouse (28 $\pm$ 4°C days, 21 $\pm$ 4°C nights) to the 2 to 2.5 leaf stage. The plants were transferred to controlled environment growth rooms (24 $\pm$ 2°C days, 20 $\pm$ 1°C nights; 16 hr days) one to three days prior to the metabolism studies.

3. Metabolism studies

a. Uptake of ¹⁴C-DPX-F5384

Metabolism studies with DPX-F5384 were made on excised leaves of rice plants at the 2~3 leaf stage. The rice leaves were cut under water and the cut ends quickly placed in 20 ml

* A part of the work was presented at the 10th conference of the Asian-Pacific Weed Science Society, 1985 and at the British Crop Protection Conference-Weeds, 1985.

vials containing 2 ml of 2 ppm ^{14}C -DPX-F5384 (made up in 1/4X Hoagland's nutrient solution). The leaves were removed from the vial after allowing one hour uptake, washed and the cut ends placed in 20 ml vials containing 5 ml of 1/4X Hoagland's solution. After one to six hours, the leaves were washed with distilled H_2O , frozen in liquid N_2 , and stored at -80°C until the extraction step. The frozen leaves were extracted with 80% cold acetone as described by Sweetser *et al.*^{2,5)}. A similar procedure was used for the metabolism of DPX-F5384 by the leaves of weeds.

b. High-performance liquid chromatography (HPLC) of plant extracts

HPLC separations of the DPX-F5384 metabolites, acid hydrolysis products, and unmetabolized DPX-F5384 were made with a Du Pont model 850 liquid chromatograph and a Du Pont Zorbax® ODS column (6.2 × 250 mm). The chromatography conditions were: Mobile phase A = H_2O + 0.1% formic acid; B = acetonitrile + 0.1% formic acid; with a non-linear concave gradient of 5% B to 100% B in 30 minutes; flow 2.7 ml/min.; temperature 35°C . The distribution of radioactivity from the HPLC column was followed by collecting 0.5 to 1.0 ml fractions of the eluant directly into scintillation vials and the ^{14}C activity determined by scintillation counting.

Semipreparative amounts of the major rice metabolite were isolated from the acetone extracts of the rice leaves by first removing the acetone on a rotating evaporator, adjusting the pH of the aqueous solution to 7.0 and extracting twice with equal volumes of CH_2Cl_2 . The pH of the aqueous solution was lowered to 3.0 and extracted three times with equal volumes of CH_2Cl_2 . The pH 3.0 CH_2Cl_2 extracts were combined and taken to dryness under N_2 . The residue from the combined CH_2Cl_2 (pH 3.0) extracts were made up in 20% acetonitrile and the DPX-F5384 metabolite collected with isocratic HPLC conditions of 20% acetonitrile + 0.1% formic acid.

4. Preparation and assay of acetolactate synthase

The leaves from ten day old etiolated rice (M101) and other plants were used as a source of acetolactate synthase. The enzyme was prepared by the same method Ray used for etiolated pea shoots³⁾.

The assay procedure of Ray was used except that the concentration of MgCl_2 was 1 mM and 50 mM HEPES buffer (pH 7.5) replaced the phosphate buffer³⁾.

5. Herbicidal activity of metabolite I of DPX-F5384

The test was carried out using 100 cm^2 plastic pots filled with Arakida soil (alluvium clay loam, organic matter = 1.2%). After adding fertilizer (N,K,17-17, 0.5 g/pot), the seeds of rice and weeds listed in Table 5 were sown into the top soil (3 mm depth) and water was introduced to a depth of 3 cm.

Both metabolite I and DPX-F5384 (acetone solution) were applied at early postemergence to the rice and weeds (emerging ~ 0.5 leaf stage). Each treatment including untreated check was replicated two times. Thereafter, the pots were placed in a greenhouse until the final investigation.

Herbicidal activity was evaluated by 0~10 rating scale (0 = no effect, 10 = 100% killed) after 4 weeks of application.

6. Reversal of DPX-F5384 inhibition in pea roots

Pea roots were prepared and grown under conditions described by Ray³⁾. DPX-F5384 was present in the culture flasks at 5 ppb. Valine and isoleucine levels were 100 μM each.

Results

Reversal of DPX-F5384 inhibition by valine and isoleucine

DPX-F5384 (5 ppb) inhibited the growth of excised pea roots by 76% (Table 1). The addition of both valine and isoleucine (100 μM each) reversed the inhibition by DPX-F5384.

Table 1. Reversal of DPX-F 5384 growth inhibition in pea root tips by valine plus isoleucine

Condition	Average net root growth (mm)
Control	37.2 $\pm$ 3.0
Valine plus isoleucine (100 μ M each)	30.8 $\pm$ 3.9
DPX-F5384 (5 ppb)	8.8 $\pm$ 1.8
DPX-F5384 plus valine plus isoleucine	30.8 $\pm$ 2.6

Metabolism of DPX-F5384 by rice and weeds

The metabolism of DPX-F5384 by rice laves was very rapid compared to that of sensitive weeds. A plot of the log of unmetabolized DPX-F5384 in a leaf vs. time followed pseudo first order kinetics from which a metabolic half-life of the herbicide was calculated (Figure 1). The comparative metabolism of DPX-F5384 by leaves of rice and some sensitive and tolerant weeds is outlined in Table 2. Weeds sensitive to DPX-F5384 show little or no metabolism of DPX-F5384 at the levels tested. Barnyardgrass, which has intermediate tolerance to DPX-F5384, showed an intermediate metabolism, while the more tolerant sprangletop showed rapid metabolism of DPX-F5384.

Characterization of the DPX-F5384 metabolite in rice

Metabolism studies with (14 C-phenyl) and (14 C-pyrimidine) labeled DPX-F5384 indicate that rice leaves rapidly metabolize DPX-F5384 to one major metabolite which still contains both the phenyl and pyrimidine rings. The rice metabolite was isolated by semipreparative HPLC and characterized as the 4-hydroxy analog of DPX-F5384. These studies suggest that the metabolism of DPX-F5384 takes place by hydroxylation of one of the methoxy groups, giving an unstable $-OCH_2OH$ intermediate which decomposes to the hydroxy pyrimidine, Metabolite I (Figure 2).

Proof of the structure of Metabolite I was the following:

A. Metabolite I gave identical HPLC reten-

Figure 1. Metabolism of DPX-F5384 in rice and water plantain leaves. Section 3 of material and methods describes the method used in this experiment (rice, Δ ; water plantain, $\circ$)

Table 2. Metabolism of DPX-F5384 by rice and weed leaves

Plant material*	Metabolism Half-life (hours)
Rice (Japonica M-101)	2.6—9.0
Rice (Japonica Nihonbare)	2.6—4.2
Rice (Indica Star Bonnette)	1.1—5.8
Waterplantain (<i>A. trivale</i>)	>50
Arrowhead (<i>S. latifolia</i>)	>50
Sedge (<i>Cyperus difformis</i>)	>50
Sedge (<i>Scirpus mucronatus</i>)	>50
Barnyardgrass (<i>E. crus-galli</i>)	12—50
Sprangletop (<i>Leptochloa dubia</i>)	1.5

* The age of the plants was: rice-13 to 18 days old; water plantain-28 to 34 days old; arrowhead-10 to 15 days old; sedges-6 to 8 weeks old; barnyardgrass-14-19 days old; sprangletop-19 days old.

tion times as the authentic I on two columns and 4 different mobile phases.

- B. Mass spectrum (E.I.) of Metabolite I and the authentic compound gave similar fragmented ions.
- C. Acid hydrolysis of Metabolite I and the authentic compound gave the same products (2-amino-4-hydroxy-6-methoxy-pyrimidine and 2-aminosulfonylmethyl benzoic acid, methyl ester) while acid hydrolysis of DPX-F5384 gave the same benzoic acid but a different heterocycle (2-amino-4,6-

Figure 2. Metabolism of DPX-F5384 by rice leaves

dimethoxypyrimidine).

Effect of DPX-F5384 on acetolactate synthase

DPX-F5384 was an effective inhibitor of the acetolactate synthases from rice, barnyardgrass, *Cyperus*, and peas (Table 3). Fifty per cent inhibition in the 30 min fixed time assay was reached with 7 to 25 ppb DPX-F5384. By contrast, the metabolite of DPX-F5384 produced by rice produced 50% inhibition at 19900 ppb (Table 4).

Table 3. Effect of DPX-F5384 on the acetolactate synthase from several plants

Source of acetolactate synthase	I ₅₀ (ppb)
Japonica rice (M101)	6.9 ± 0.7
Barnyardgrass	8.8 ± 2.5
<i>Cyperus esculentus</i>	9.5 ± 1.3
Pea (Alaska)	24.7 ± 6.4

Table 4. I₅₀ values (rice acetolactate synthase) for DPX-F5384 and metabolite I from rice

Compound	I ₅₀ (ppb)
DPX-F5384	6.9 ± 0.7
Metabolite I	19900. ± 5100.

Table 5. Plant response to metabolite I of DPX-F5384

Compound	Rate g a.i./ha	Herbicidal activity					
		Rice	Eo	Cd	Mv	Sj	Bl
Metabolite I	2000	0	0	0	0	0	0
	4000	0	0	0	0	0	0
DPX-F5384	25	1	4	10	10	8.5	10

Rating: 0–10 scale

Rice: Nihonbare,

Cd: *Cyperus difformis*,

Sj: *Scirpus juncooides*,

Eo: *Echinochloa oryzicola*,

Mv: *Monochoria vaginalis*,

Bl: Annual broadleaves

Herbicidal activity of metabolite I of DPX-F5384

As shown in Table 5, plant response studies with the metabolite I indicated that it was not herbicidally active up to 4000 g ai/ha while DPX-F5384 even at 25 g a.i./ha provided high herbicidal activity on sensitive weeds in the paddy.

Effect of several sulfonylureas on rice acetolactate synthase

TAKEDA *et al.*⁶⁾ found that sulfonylureas with an -O- or -CH₂- bridge between the sulfonyl moiety and the aryl group (DPX-R5582, DPX-F5390 and DPX-F5384) were selective toward rice while those without this spacer group

Table 6. I_{50} values for several sulfonylureas on rice acetolactate synthase

Sulfonylurea	A	B	Y	X	Z	I_{50} (ppb)
DPX-W4189	Cl	—	N	-OCH ₃	-CH ₃	11.9 ± 1.3
DPX-T 6376	-COOCH ₃	—	N	-OCH ₃	-CH ₃	6.7 ± 2.0
DPX-W4515	Cl	—	C	-OCH ₃	-OCH ₃	1.5 ± 0.5
DPX-F 5390	Cl	-CH ₂ -	C	-OCH ₃	-OCH ₃	441.0 ± 93.0
DPX-R 5582	Cl	-O-	C	-OCH ₃	-OCH ₃	2.5 ± 0.8
DPX-T 6375	-COOCH ₃	—	C	-OCH ₃	-OCH ₃	2.4 ± 0.2
DPX-F 5384	-COOCH ₃	-CH ₂ -	C	-OCH ₃	-OCH ₃	6.9 ± 0.7
DPX-R 6514	-COOCH ₃	-O-	C	-OCH ₃	-OCH ₃	1.5 ± 0.5

The acetolactate synthase from etiolated shoots of M101 rice was partially purified by ammonium sulfate precipitation (25 to 50 % cut) and assayed with the sulfonylureas for 30 min. at pH 7.5 with 1 mM MgCl₂, 0.5 mM thiamin pyrophosphate, 20 mM pyruvate, and 10 μ M flavin adenine dinucleotide.

(DPX-W4515 and DPX-T6375) exhibited very low or no selectivity. With the exception of DPX-F5390, all of these compounds are very effective inhibitors of rice acetolactate synthase (Table 6).

Discussion

DPX-F5384 strongly inhibits the growth of excised pea roots at 5 ppb (Table 1). This inhibition is reversed by the addition of valine and isoleucine suggesting that the primary site of DPX-F5384 action may be the inhibition of the biosynthesis of branched-chain amino acids. This idea is further supported by the observation that DPX-F5384 inhibits acetolactate synthase from peas at the ppb level (Table 3). DPX-F5384 is also a potent inhibitor of the acetolactate synthases from weeds associated with rice. The inhibition of acetolactate synthase can account for the her-

bicidal action of DPX-F5384 in weeds.

The major factor responsible for the lack of phytotoxicity of the sulfonylurea herbicide, chlorsulfuron, to cereal crops and tolerant weeds is the ability of these plants to metabolize chlorsulfuron to inactive products^{2,5}. Our studies on the metabolism of DPX-F5384 by rice leaves suggest that inactivating metabolism also plays an important role in the tolerance of rice to DPX-F5384 since rice acetolactate synthase is readily inhibited by this sulfonylurea (Table 3). The major metabolite, Metabolite I, shows very low herbicidal activity and is relatively weak as an inhibitor of rice acetolactate synthase (Table 4,5). To inhibit the rice enzyme by 50% the concentration of metabolite I must be nearly 3000 times greater than DPX-F5384. Kinetic studies on the rate of DPX-F5384 metabolism by isolated rice leaves indicates that metabolism is

rapid. The metabolic half life of DPX-F5384 in the rice leaf ranges from 2~9 hours depending upon the age of the leaf and the growth conditions. There is little or no metabolism of DPX-F5384 by sensitive broadleaf weeds such as water plantain (*Alisma trivale*) and arrowhead (*Sagittaria latifolia*) (Table 2).

A difference in safety on rice plants was also found among sulfonylurea compounds that are potent inhibitors of the acetolactate synthase⁶⁾ (Table 6). The cause of such different selectivities is that sulfonylureas with spacer group seem to be more easily inactivated than those without a bridge.

Summary

DPX-F5384 is the active ingredient in the rice herbicide Londax®. Growth inhibition promoted by DPX-F5384 in pea roots can be reversed by addition of valine and isoleucine, suggesting inhibition of the pathway for branched-chain amino acid biosynthesis. This compound readily inhibits an enzyme in this pathway, acetolactate synthase. Although acetolactate synthase from rice is also inhibited by DPX-F5384, the rice plant is tolerant due to its ability to metabolically demethylate DPX-F5384 to a relatively weak inhibitor of

its acetolactate synthase.

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DPX-F5384 の除草作用と選択作用機構

武田俊司・湯山 猛 デュボンジャパン農薬事業部

D. L. ERBES・P. B. SWEETSER・J. V. HAY デュボン農薬事業部

摘 要

DPX-F5384 の除草作用と選択作用機構を明らかにするために、本化合物の生育阻害作用に対するバリンとイソロイシンの影響、植物におけるバリンとイソロイシンの生合成経路の初期段階を触媒するアセトラクテート合成酵素 (ALS) に対する阻害、および本化合物に耐性のイネと感受性雑草における代謝について比較検討した。

1) 5 ppb の濃度の DPX-F5384 を含む培地に、バリンとイソロイシンの各 100 μ M を加え、切除したエンドウの根を生育させた。DPX-F5384 のみの培地では、エンドウの根の生育は強く抑制されたが、バリンとイソロイシンを加えた培地では、DPX-F5384 による生育抑制はほぼ完全に解除された (Table 1)。

2) ^{14}C -phenyl と ^{14}C -pyrimidine 標識 DPX-F5384 を用いて、イネと雑草の葉による代謝を検討した。イネ葉による本化合物の代謝は急激であり、代謝半減期はイネで 2.6～9 時間、雑草では 50 時間以上を示しその差は極めて顕著であった。さらに、イネ葉による主要な代謝物は、ピリミジン部分の 4-位のメトキシ基の水酸化によって生ずる 4-hydroxy 類縁化合物で、この代謝物の ALS に対する活性は、親化合物の約 1/3000 程度であり、除草活性も 4000 g ai/ha の施用量で全く認められなかった。

3) DPX-F5384 はイネ、タイヌビエ、カヤツリグサおよびエンドウの ALS の働きを強く阻害した。ALS の活性を 50% 阻害する濃度は 7～25 ppb の範囲にあり、生育抑制を示す濃度とほぼ同水準で作用した。また、本化合物とその関連化合物のイネの ALS に対する影響を調べた。その結果、DPX-5390 以外のスルホニルウレア化合物は低濃度で ALS の活性を阻害した。

4) 以上の結果から、DPX-F5384 の除草作用機構は、植物の分岐鎖アミノ酸の生合成経路の初期段階における酵素、アセトラクテート合成酵素を阻害することによって、バリンとイソロイシンの生合成を妨げることを明らかにした。さらにまた、本化合物のイネと雑草間の高い選択性は、両者の代謝速度の著しい差異が重要な役割を果していることを示唆した。