Metabolism of Clomeprop in Plant Seedlings

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Clomeprop (2-(2,4-dichloro-3-methylphenoxy) propionanilide or MY-15), a selective rice herbicide which is abbreviated here as CMP, was reported to be effective for many species of broadleaf and cyperus weeds. Both clomeprop and its acid metabolite (2-(2,4-dichloro-3-methylphenoxy) propionic acid), abbreviated DMPA, were reported to exhibit herbicidal activity^{3,4)}. Clomeprop in plants might be metabolized to DMPA, the herbicidal active compound, and the herbicidal activity of this herbicide was provided from the metabolite DMPA, similar to the case of naproanilide^{2,5)}.

Clomeprop, CMP

DMPA

From the previous study, it was found that azuki bean and rice seedlings were tolerant to clomeprop and DMPA, whereas tomato and radish seedlings were susceptible⁶⁾. In order to clarify the mechanism of selectivity of clomeprop in plants, metabolism study of this herbicide and its acid metabolite was conducted in four species of plants: azuki bean, rice, tomato and radish.

Materials and Methods

Plant materials

Seedlings of azuki bean (*Azuki angularis*),

rice (*Oryza sativa* L. cv. Nihonbare), tomato (*Lycopersicon esculentum* MILL.) and radish (*Raphanus sativus* L.) were prepared as described in the previous study⁶⁾. Rice seedlings at the 3-leaf-stage of growth and 7-day-old seedlings of azuki bean, tomato and radish were used in this study.

¹⁴C herbicide solutions

Solutions used were ¹⁴C-CMP and ¹⁴C-DMPA (benzene rings in the phenoxy moieties are labeled with ¹⁴C, specific activity 13.5 and 17.6μCi/mg, respectively) at the concentration of 10⁻⁵ M dissolved in 1% acetone and 750ppm Tween 20.

Application method

Roots of the seedlings were immersed in the test solutions to 1 cm below the base of stems for 24 hr, then washed with distilled water. Thereafter, plants were transferred to herbicide-free nutrient solutions.

Metabolism study

Plants were harvested 0, 1 and 3 days after soaking and shoots separated from roots. The fresh weight of each part was determined and amounts of the parent compound and its metabolites were investigated following the steps shown in Fig.1.

Plant samples were homogenized twice in an acetone/water solution (2/1 by volume) and filtered through a membrane filter. Radioactivity of acetone/water-insoluble fractions in residues was quantified by combustion. Acetone in acetone/water extracts was evaporated, and the remainder of the aqueous solutions was then extracted twice with dichloromethane at pH below 2 in order to obtain the dichloromethane- and water-soluble fractions. Radioactivity in each

* Parts of this work were reported at the 27th annual meeting of the Weed Science Society of Japan in April, 1988.

Fig. 1. Flow chart of metabolism study of clomeprop

fraction (acetone/water-insoluble, water soluble and dichloromethane-soluble fractions) was determined using a liquid scintillation spectrometer (Beckman LS 8100).

The parent compound and metabolites in dichloromethane fractions were identified and quantified by thin layer chromatography.

In order to identify metabolites in the water-soluble fraction, these fractions were hydrolyzed by either HCl or β -glucosidase and the hydrolyzed compounds were extracted and studied by the same method as described above.

For thin layer chromatography study, the pre-coated aluminum TLC plate with concentrated zone (Merck Art 5583) was used for the dichloromethane-soluble fraction, and the developing solution was benzene: methanol: acetic acid (100: 5: 15 by volume)

Results and Discussion

All plant species tested in this experiment metabolized ^{14}C -CMP and ^{14}C -DMPA to

water-soluble and acetone/water-insoluble metabolites, but differed in the rates of metabolism.

Azuki bean and rice effectively metabolized ^{14}C -CMP and ^{14}C -DMPA in both shoot and root. Three days after application, detected radioactivity in dichloromethane-soluble fraction (which contained the parent compound and DMPA) in shoot and root was 33 and 40% respectively in ^{14}C -CMP treatment and 3 and 5% respectively in ^{14}C -DMPA treatment in azuki bean; and 30 and 27% in ^{14}C -CMP treatment and 19 and 9% in ^{14}C -DMPA treatment in rice (Fig. 2 and 3).

In tomato and radish, the percents of radioactivity remaining in the dichloromethane fraction were larger than those in azuki bean and rice (Fig. 2 and 3). In tomato 38% was found in shoots and 76% in roots with ^{14}C -CMP treatment and 15% and 10% respectively with ^{14}C -DMPA treatment. In radish 72% was found in shoots and 91% in roots with ^{14}C -CMP treatment, and 30 and 16%

Fig. 2. Distribution of ^{14}C derived from ^{14}C -CMP into each fraction 3 days after application.

Fig. 3. Distribution of ^{14}C derived from ^{14}C -DMPA into each fraction 3 days after application.

Table 1. Percent of parent compounds and metabolites in CH_2Cl_2 -soluble fraction derived from root applied ^{14}C -CMP and ^{14}C -DMPA in roots and shoots of azuki bean seedlings.

Plant parts	Compounds treated	DAA ²⁾	% ¹⁾ of radioactivity					Total
			CMP	DMPA	3-CH ₂ OH DMPA	UK ³⁾	Origin	
Root	^{14}C -CMP	0	92.80	0.91	0.17	1.28	0.14	95.30
		1	82.00	1.07	0.12	1.34	0.37	84.90
		3	32.43	1.77	0.57	3.08	1.75	39.60
	^{14}C -DMPA	0	—	12.62	3.39	4.60	1.79	22.40
		1	—	1.87	0.37	2.69	1.67	6.60
		3	—	1.07	0.35	2.32	1.56	5.30
Shoot	^{14}C -CMP	0	60.13	1.26	1.04	2.33	1.84	66.60
		1	38.51	1.34	1.17	1.25	2.33	44.60
		3	28.13	0.95	0.94	1.42	1.46	32.90
	^{14}C -DMPA	0	—	0.89	2.71	3.37	1.23	8.20
		1	—	0.63	1.14	0.92	0.71	3.40
		3	—	0.30	1.15	0.59	0.66	2.70

1 based on total radioactivity in either roots or shoots

2 DAA : days after application

3 consists of several unidentified metabolites

respectively with ^{14}C -DMPA treatment 3 days after application (Fig. 2 and 3).

Tables 1 to 4 show the percents of parent compounds and their metabolites in dichloromethane fraction with both ^{14}C -CMP and ^{14}C -DMPA treatments incorporated by azuki

bean, rice, tomato and radish, respectively. In both shoot and root, the main metabolites derived from ^{14}C -CMP were identified as DMPA and 3-CH₂OH-DMPA (2-(2,4-dichloro-3-hydroxymethylphenoxy) propionic acid), and from ^{14}C -DMPA was identified as

Table 2. Percent of parent compounds and metabolites in CH_2Cl_2 -soluble fraction derived from root applied ^{14}C -CMP and ^{14}C -DMPA in roots and shoots of rice seedlings.

Plant parts	Compounds treated	DAA ²⁾	% ¹⁾ of radioactivity					Total
			CMP	DMPA	3-CH ₂ OH DMPA	UK ³⁾	Origin	
Root	^{14}C -CMP	0	84.09	1.84	0.55	3.58	1.84	91.90
		1	67.80	4.25	1.22	2.05	0.61	75.94
		3	14.69	6.04	3.56	1.64	1.42	27.35
	^{14}C -DMPA	0	—	12.66	6.24	3.75	1.37	24.02
		1	—	3.08	5.17	2.33	1.56	12.14
		3	—	1.57	4.79	1.61	1.37	9.34
Shoot	^{14}C -CMP	0	63.64	5.13	2.19	5.98	7.24	84.18
		1	25.02	3.55	4.88	11.13	15.57	60.15
		3	11.50	4.52	5.46	6.88	1.59	29.95
	^{14}C -DMPA	0	—	16.57	15.13	3.14	5.14	39.98
		1	—	3.08	8.50	6.35	4.10	22.03
		3	—	1.19	7.39	5.46	5.33	19.36

1 based on total radioactivity in either roots or shoots

2 DAA : days after application

3 consists of several unidentified metabolites

Table 3. Percent of parent compounds and metabolites in CH_2Cl_2 -soluble fraction derived from root applied ^{14}C -CMP and ^{14}C -DMPA in roots and shoots of tomato seedlings.

Plant parts	Compounds treated	DAA ²	% ¹ of radioactivity					Total
			CMP	DMPA	3- CH_2OH DMPA	UK ³	Origin	
Root	^{14}C -CMP	0	95.67	0.58	0.19	0.68	0.10	97.22
		1	90.68	1.09	0.48	0.65	0.13	93.03
		3	69.75	2.69	1.70	1.28	0.35	75.77
	^{14}C -DMPA	0	—	20.63	2.64	2.36	0.26	25.89
		1	—	3.27	2.22	1.86	0.16	7.51
		3	—	4.88	3.43	1.75	0.34	10.40
Shoot	^{14}C -CMP	0	58.15	7.16	4.34	1.51	1.16	72.32
		1	34.32	7.33	9.30	1.55	0.96	53.46
		3	20.65	4.16	11.08	1.15	1.42	38.46
	^{14}C -DMPA	0	—	15.25	3.50	2.53	0.70	21.98
		1	—	7.40	4.72	2.20	0.47	14.79
		3	—	5.63	6.62	1.42	1.17	14.84

1 based on total radioactivity in either roots or shoots

2 DAA : days after application

3 consists of several unidentified metabolites

3- CH_2OH -DMPA in all 4 species although the amounts produced were different.

In azuki bean (Table 1), the amount of root absorbed ^{14}C -CMP decreased rapidly in both roots and shoots. Three days after application there remained, respectively, only

32.43 and 28.13% of the total radioactivity. Only a small amount (less than 2%) of the metabolites DMPA and 3- CH_2OH -DMPA derived from ^{14}C -CMP was found in both plant parts. With ^{14}C -DMPA treatment, the initial compound DMPA was metabolized

Table 4. Percent of parent compounds and metabolites in CH_2Cl_2 -soluble fraction derived from root applied ^{14}C -CMP and ^{14}C -DMPA in roots and shoots of radish seedlings.

Plant parts	Compounds treated	DAA ²	% ¹ of radioactivity					Total
			CMP	DMPA	3- CH_2OH DMPA	UK ³	Origin	
Root	^{14}C -CMP	0	97.58	0.43	0.06	0.55	0.04	98.66
		1	94.64	0.68	0.11	0.68	0.09	96.20
		3	87.70	1.81	0.39	1.26	0.27	91.43
	^{14}C -DMPA	0	—	20.91	7.86	10.57	1.18	40.52
		1	—	7.88	6.73	7.03	0.49	22.13
		3	—	5.04	6.25	4.31	0.55	16.15
Shoot	^{14}C -CMP	0	69.30	7.31	3.74	2.98	1.70	85.03
		1	60.03	10.35	4.30	3.18	1.75	79.61
		3	48.90	10.82	7.00	3.31	2.09	72.12
	^{14}C -DMPA	0	—	24.15	16.35	8.25	1.87	50.62
		1	—	10.87	19.93	7.54	0.90	39.24
		3	—	7.26	15.65	5.86	0.98	29.75

1 based on total radioactivity in either roots or shoots

2 DAA : days after application

3 consists of several unidentified metabolites

Table 5. Concentration of CMP and DMPA in roots and shoots of plant seedlings at various times after the root application of ^{14}C -CMP or ^{14}C -DMPA.

Compounds treated	DAA*	Radioactivity (dpm/mg fresh weight)							
		azuki bean		rice		tomato		radish	
		CMP	DMPA	CMP	DMPA	CMP	DMPA	CMP	DMPA
^{14}C -CMP	0	1025.0	10.1	900.9	19.7	1148.7	7.0	1137.6	5.0
	1	552.0	7.2	385.0	24.1	493.9	5.9	516.8	3.7
	3	196.0	10.7	47.4	19.5	127.6	4.9	225.9	4.7
^{14}C -DMPA	0	—	49.8	—	55.1	—	51.3	—	38.3
	1	—	6.3	—	12.5	—	7.4	—	8.5
	3	—	4.1	—	5.9	—	7.4	—	3.5
^{14}C -CMP	0	8.0	0.2	49.7	4.0	45.9	5.7	8.1	0.9
	1	8.3	0.3	12.6	1.8	19.2	4.1	10.3	1.8
	3	8.3	0.3	6.7	2.6	6.5	1.3	10.2	2.3
^{14}C -DMPA	0	—	0.4	—	20.1	—	21.9	—	29.0
	1	—	0.2	—	2.6	—	10.8	—	12.5
	3	—	0.1	—	0.8	—	7.2	—	8.7

* DAA : days after application

rapidly, much more than CMP. Within 3 days, the remaining absorbed DMPA was only 1.07 and 0.30% in root and shoot, respectively. The metabolite 3- CH_2OH -DMPA was

found in only small amounts.

In rice (Table 2), similar to the case of azuki bean, absorbed ^{14}C -CMP and ^{14}C -DMPA were rapidly converted in both plant

Table 6. Metabolites in water-soluble fractions derived from root applied ^{14}C -CMP and ^{14}C -DMPA in azuki bean seedlings (after HCl acid hydrolysis).

			Radioactivity (%) ¹⁾					
Plants Parts	Compounds treated	DAA ²⁾	Re-extracted CH ₂ Cl ₂ -soluble fraction					Water- ⁴⁾ soluble fraction
			DMPA	3-CH ₂ OH -DMPA	5-OH- DMPA	UK ³⁾	Origin	
Root	¹⁴ C-CMP	0	0.69	0.83	0.03	0.12	0.05	1.48
		1	4.71	2.91	0.09	0.21	0.13	4.45
		3	31.76	9.88	0.37	2.08	0.80	11.51
	¹⁴ C-DMPA	0	13.98	19.54	0.74	2.38	1.31	32.45
		1	15.64	26.26	0.90	3.39	1.37	36.62
		3	17.85	28.74	1.04	2.98	1.73	33.46
Shoot	¹⁴ C-CMP	0	1.53	11.13	0.34	0.76	0.40	17.74
		1	3.22	17.29	0.66	0.94	0.58	31.21
		3	4.55	25.08	0.87	1.98	0.74	32.18
	¹⁴ C-DMPA	0	2.31	33.89	0.99	2.75	1.47	49.59
		1	2.36	38.78	1.13	1.42	0.62	51.39
		3	2.75	42.90	1.33	2.70	1.17	44.55

1) based on total radioactivity in either roots or shoots

2) DAA : days after application

3) consists of several unidentified metabolites

4) water-soluble fraction after hydrolysis

parts. CMP was decreased to 14.69 and 11.5 % in root and shoot, respectively, within 3 days and metabolite DMPA was found in amounts of 6.04 and 4.52% in root and shoot, which were larger than those in azuki bean. When ^{14}C -DMPA was used as the initial compound, DMPA was metabolized rapidly, and less than 2% remained in both plant parts 3 days after application.

In tomato and radish, ^{14}C -CMP and ^{14}C -DMPA were metabolized more slowly than in azuki bean and rice (Table 3 and 4) .

In tomato 3 days after application, percents of CMP were 69.75 and 20.65%, and of metabolite DMPA were 2.69 and 4.16%, respectively, in roots and shoots. Three days after application of ^{14}C -DMPA, the parent DMPA remained 4.88 and 5.63% in root and shoot. In radish, percents of CMP and metabolite DMPA were found much higher than other species (CMP : 87.7% and 48.9% in roots and shoots, and DMPA : respectively 1.81% and 10.82%). The amount of parent DMPA applied as ^{14}C -DMPA was also high

in both parts (5.04 and 7.26% in roots and shoots, respectively).

In shoots of plants treated with ^{14}C -CMP, high concentration of the parent CMP remained in all plant species, but the amount of metabolite DMPA found was rather small, especially in azuki bean (Table 5). With ^{14}C -DMPA treatment, it was found that DMPA in shoot of azuki bean and rice was metabolized more rapidly than those in tomato and radish. It might be concluded that in all species, CMP was metabolized to a phytotoxic DMPA, and DMPA was detoxified to non-phytotoxic metabolites. The rapid detoxification of DMPA in both azuki bean and rice may be the reason these species are tolerant to this herbicide. In contrast, in tomato and radish, although the conversion of CMP to DMPA was rather fast, the metabolism of DMPA was quite slow ; this may explain the susceptibility of the plants.

These results are similar to the report that ^{14}C -CMP was rapidly metabolized to DMPA, 3- CH_2OH -DMPA, water-soluble and insol-

Table 7. Metabolites in water-soluble fractions derived from root applied ^{14}C -CMP and ^{14}C -DMPA in rice seedlings (after HCl acid hydrolysis).

			Radioactivity (%) ¹⁾					
Plants parts	Compounds treated	DAA ²⁾	Re-extracted CH ₂ Cl ₂ -soluble fraction					Water- ⁴⁾ soluble fraction
			DMPA	3-CH ₂ OH-DMPA	5-OH-DMPA	UK ³⁾	Origin	
Root	¹⁴ C-CMP	0	0.81	2.25	0.07	0.41	0.06	2.12
		1	3.01	7.70	0.25	1.45	0.28	5.55
		3	8.81	16.94	0.69	4.32	0.72	27.11
	¹⁴ C-DMPA	0	11.33	16.41	0.97	5.43	1.61	20.00
		1	10.94	17.80	0.68	4.30	4.00	24.93
		3	10.50	19.57	1.43	6.21	2.07	24.26
Shoot	¹⁴ C-CMP	0	2.49	7.17	0.16	0.69	0.07	4.06
		1	3.31	13.15	0.65	1.92	0.08	17.39
		3	4.88	24.21	0.72	4.22	0.31	29.18
	¹⁴ C-DMPA	0	10.47	21.68	0.46	3.02	0.39	18.60
		1	9.36	23.97	0.62	2.34	0.26	31.61
		3	8.87	28.96	0.80	2.85	0.38	27.00

1) based on total radioactivity in either roots or shoots

2) DAA : days after application

3) consists of several unidentified metabolites

4) water-soluble fraction after hydrolysis

Table 8. Metabolites in water-soluble fraction derived from root applied ^{14}C -CMP and ^{14}C -DMPA in tomato and radish seedlings at 3 days after application (after HCl acid hydrolysis).

Plant parts	^{14}C Compounds treated	Radioactivity (%) ¹⁾					
		Re-extracted CH_2Cl_2 -soluble fraction					Water- ³⁾ soluble fraction
		DMPA	3- CH_2OH -DMPA	5-OH-DMPA	UK ²⁾	Origin	
<u>Tomato</u> Root	CMP	7.14	3.58	—	0.62	0.17	5.38
	DMPA	47.65	8.27	—	2.24	0.50	14.05
Shoot	CMP	18.07	20.16	—	2.02	0.47	18.94
	DMPA	40.79	20.31	—	1.29	0.17	21.49
<u>Radish</u> Root Shoot	CMP	1.59	0.38	0.02	0.17	0.05	1.30
	DMPA	12.77	21.69	0.57	2.17	0.62	20.12
	CMP	2.74	8.35	0.53	1.26	0.03	10.81
	DMPA	11.98	31.81	0.76	1.25	0.39	22.48

1) based on total radioactivity in either roots or shoots

2) consists of several unidentified metabolites

3) water-soluble fraction after hydrolysis

uble metabolites in shoot and rhizome of *Cyperus serotinus* studied by KOBAYASHI *et al.*¹⁾.

In root, although the remainder of both CMP and DMPA was rather high (because a large amount of absorbed herbicide was still in the root), no toxic symptom of roots was clearly exhibited.

In order to study the detoxification pathway, metabolites in water-soluble fractions were identified. After hydrolyzation, the main compounds in dichloromethane-soluble fractions were 3- CH_2OH -DMPA and DMPA in all 4 species of plants (Table 6, 7 and 8) ; and 3- CH_2OH -DMPA was found in more quantity than DMPA.

From these results, it might be concluded that CMP in plants was hydrolyzed at the acylamide bond and resulted in DMPA (the herbicidal active compound). In order to detoxify DMPA, a methyl group in the phenyl ring of DMPA might be hydroxylated resulting in 3- CH_2OH -DMPA. Both DMPA and 3- CH_2OH -DMPA could be conjugated with plant metabolites and transferred to water-soluble fraction. The conversion rates of CMP to DMPA and then to water-soluble metabolites were different among plant species, and governed the tolerance or suscep-

tibility to this herbicide.

Summary

Azuki bean, rice, tomato and radish were able to metabolize CMP to non-phytotoxic metabolites, but the conversion rates differed among plant species. Azuki bean and rice effectively metabolized CMP and DMPA to water-soluble and acetone/water-insoluble metabolites, whereas in tomato and radish the metabolism rates were much slower, especially at the step of the conversion of DMPA to non-phytotoxic metabolites. The tolerance of azuki bean and rice and the susceptibility of tomato and radish to the herbicides might be explained by the different metabolism rates.

The hydrolyzation at the acylamide bond of CMP resulted in DMPA (the herbicidal active compound) and the hydroxylation of methyl group in the phenyl ring resulted in 3- CH_2OH -DMPA. Thus the conjugations of both DMPA and 3- CH_2OH -DMPA with plant metabolites are the probable pathways of metabolism of CMP in these plant species.

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各種植物体におけるクロメプロップの代謝

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クロメプロップとその酸アミド結合加水分解代謝物(それぞれ CMP と DMPA と略称)のアズキとイネ(抵抗性)およびトマトとダイコン(感受性)幼植物体中代謝を調べた。¹⁴C 標識 CMP と DMPA とを用い、24時間根部より吸収させた後経日的に試料を採取した。ジクロロメタン可溶画分、水可溶画分およびアセトン/水不溶画分に夫々分画し、加水分解反応と TLC 等を組み合わせて定性定量を行った(第1図)。4植物種とも親化合物を代謝し、代謝産物の種類には差異は認められなかったが、代謝速度に植物種間差が認められた(第1~4表)。

CMP および DMPA から殺草活性の無い化合物への変化の速度は、アズキおよびイネ体内ではトマトおよびダイコンに比べて著しく大であった(第5表, 第2, 3図)。特に DMPA の体内保留濃度がアズキとイネでは著しく小であった(第5表, 第2, 3図)。CMP の代謝産物として、DMPA の他にフェニール環側鎖のメチル基が水酸化された化合物(3-CH₂OH-DMPA)が見出された。DMPA および 3-CH₂OH-DMPA の抱合物が主たる解毒物質として同定された(第6, 7, 8表)。