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thesis entitled

MODE OF ACTION OF THE HERBICIDE

ANTIDOTE R-25788 (N,N-2,2-DICHLOROACETAMIDE)

presented by

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has been accepted towards fulfillment
of the requirements for
Ph.D.
_____degree in _____

Department of Crop and Soil Science

Major professor

Date May 15, 1978

O-7639

MODE OF ACTION OF THE HERBICIDE ANTIDOTE R-25788 (N,N-DIALLYL-2,2-DICHLOROACETAMIDE)

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirement
for the degree of

DOCTOR OF PHILOSOPHY

Department of Crop and Soil Science

1978

ABSTRACT

MODE OF ACTION OF THE HERBICIDE ANTIDOTE
R-25788 (N,N-DIALLYL-2,2-DICHLOROACETAMIDE)

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R-25788 (N,N-diallyl-2,2-dichloroacetamide) protected corn (Zea mays L.) from injury caused by two herbicide classes to which it has structural similarity, the acetanilides and the thiocarbamates. R-25788 was the most effective antidote for both acetanilide and thiocarbamate injury of six compounds tested (R-25788, R-29148, CDAA, 1,8-naphthalic anhydride, carboxin, and gibberellin GA_3). The protective effect of R-25788 was specific for corn, it did not protect four weed species treated. The structural similarity between R-25788 and the herbicides could be the basis of the protective effect; R-25788 could act as a competitive inhibitor to the herbicides at some unknown site of action specific to corn.

It has been suggested that R-25788 could prevent an herbicide induced inhibition of gibberellin synthesis or lipid synthesis. Exogenous application of gibberellin GA_3 did not prevent either acetanilide or thiocarbamate injury symptoms indicating that the herbicides do not act by simply inhibiting gibberellin synthesis. Although EPTC, a thiocarbamate herbicide, induced epicuticular wax aggregation on corn leaves which was prevented by R-25788, it did not reduce the total amount of epicuticular wax on corn leaves. This indicates that EPTC might not inhibit lipid synthesis in this plant species. Metolachlor, an acetanilide herbicide, had no observable effect on epicuticular wax

on corn.

All three acetanilide herbicides and one thiocarbamate sulfoxide tested reacted with glutathione to form herbicide-glutathione conjugates in an *in vitro*, non-enzymatic system. Since R-25788 has been reported to selectively increase the glutathione content of corn, R-25788 might protect corn from acetanilide herbicide injury by increasing the rate of herbicide metabolism to non-phytotoxic glutathione conjugates. However, because R-25788 was required to protect genetically atrazine-susceptible corn from alachlor injury but not from thiocarbamate injury it is suggested that R-25788 may protect corn from EPTC injury by increasing the rate of EPTC sulfoxidation followed by EPTC-sulfoxide conjugation. R-25788 did not protect genetically atrazine-susceptible corn from atrazine injury, indicating that R-25788 does not stimulate glutathione-*S*-transferase activity or atrazine-GSH conjugation in corn.

ACKNOWLEDGMENTS

I would like to express my appreciation to Dr. Donald Penner for his friendship, assistance, and guidance during the completion of this dissertation. I would also like to thank Dr. Matthew Zabik for his advice and the gracious use of his equipment, Dr. James Flore and Dr. Violet Wert for providing various analytical standards, and Dr. Gary Hooper and Dr. Karen Baker for their help and use of the Electron Optics Center. I am grateful to Dr. William F. Meggitt, Dr. Matthew Zabik, Dr. James Flore, and Dr. Bernard Knezek for their service as guidance committee members.

I extend a very special thanks to my wife Maria for the preparation of this manuscript.

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INTRODUCTION

The purpose of this study was to investigate the mode of action of the herbicide antidote R-25788 (N,N-diallyl-2,2-dichloroacetamide). A herbicide antidote is a compound that selectively protects crop plants from herbicide injury without protecting weeds. R-25788 is the only herbicide antidote in commercial production today and is used to protect corn from thiocarbamate herbicide injury. A comparison of the structure of R-25788 (Appendix B) to a wide variety of known herbicides reveals that its structure is similar to that of two herbicide classes, the thiocarbamates and the acetanilides (Appendix A). Because of this structural similarity it has been suggested (41) that R-25788 prevents thiocarbamate injury by acting as a competitive inhibitor to the herbicides at some unknown site of action in corn. This hypothesis was tested by examining the efficacy of R-25788 (and related compound-Appendix B) as an antidote to the acetanilide herbicides with which it also has structural similarity. It has also been reported (12) that the thiocarbamates inhibit gibberellin synthesis in corn and that R-25788 might reverse this inhibition. This hypothesis was evaluated by applying exogenous gibberellin to herbicide treated corn in an attempt to prevent herbicide injury. Thiocarbamate herbicides also inhibit lipid synthesis in various plant species (14, 17, 28, 44, 46, 47). The hypothesis that R-25788 might prevent this inhibition (45) was tested by examining the effect of both thiocarbamate and acetanilide

herbicides and R-25788 on epicuticular wax deposition on corn leaves. It has also been reported (3, 26, 27) that R-25788 increases the glutathione content of corn which increases the rate of thiocarbamate metabolism to non-toxic glutathione conjugates. Glutathione conjugation reactions with various herbicides were therefore investigated.

CHAPTER 1

POTENTIAL ANTIDOTES AGAINST ACETANILIDE HERBICIDE INJURY TO CORN (*ZEA MAYS*)

Abstract

R-25788 (2,2-Dichloro-*N*,*N*-diallylacetamide) was the most effective of six potential antidotes evaluated to counter corn (*Zea mays* L.) injury from the acetanilide herbicides alachlor, metolachlor, acetochlor, H-22234 (*N*,chloroacetyl-*N*-(2,6-diethylphenyl)glycine ethyl ester), H-26910 (*N*-chloroacetyl-*N*-(2-methyl-6-ethylphenyl)glycine isopropyl ester). The other potential antidotes in order of decreasing effectiveness were: R-29148 (2,2-dimethyl-5-methyl-dichloroacetyloxazolidine), NA (1,8-naphthalic anhydride), CDAA (2-chloro-*N*,*N*-diallylacetamide), Carboxin (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin), and gibberellin GA_3). GA_3 only partly relieved the stunting of corn caused by EPTC and metolachlor and did not prevent other herbicide injury symptoms, suggesting that the mode of action of EPTC and metolachlor is not to simply block GA_3 synthesis. R-25788 protected corn equally well from acetanilide or EPTC injury.

Introduction

Since the discovery by Hoffman (8) that 4'-chloro-2-hydroxy-imino-acetanilide selectively protected wheat (Triticum aestivum L.) from injury caused by subsequant foliar applications of barban, numerous other compounds have been examined for antidotal activity. Hoffman (9) later found that seed treatment with NA protected corn from EPTC injury. Rains and Fletchall (14) confirmed this and also reported that R-25788 selectively protected corn from EPTC and that NA protected sorghum (Sorghum bicolor (L.) Moench) from alachlor injury. However, Estin (5) could not substantiate this. Donald and Fawcett (4) reported that gibberellin (GA_3) and R-29148 were effective in preventing EPTC injury to corn, but Harvey, Chang, and Fletcher (6) reported that GA_3 and indoleacetic acid were ineffective protectants against EPTC injury to corn. Chang, Marsh, and Jennings (2) found that alachlor inhibited growth of oat (Avena sativa L.) seedlings and that pre-treatment with GA_3 overcame this inhibition. Miller and Nalewaja (12) reported that seed treatments of carboxin and R-25788 decreased wheat injury from triallate.

Chang, Stephenson, and Bandeen (1) found that R-25788 was a more effective EPTC antidote than either NA or CDAA. Lay and Casida (11) concluded that four out of 32 compounds tested, including R-25788, had superior antidotal activity, and CDAA had moderate antidotal activity against EPTC.

Dixon, Stoller, and McGlamery (3) reported that corn has widely varying tolerance to alachlor and five closely related compounds. Since Hickey and Krueger (7) reported that NA could reverse an alachlor

injury effect, corn tolerance to some of the more toxic acetanilide herbicide might be improved with this or other herbicide antidotes.

In this study several chemicals with previously reported antidote activity were evaluated for their efficacy in protecting corn from five acetanilide herbicides.

Materials and Methods

Plant culture, herbicide and antidote evaluation.

Plants for all studies except those using nutrient solution were grown in a greenhouse soil (1:1:1 soil, sand, peat) in 946-ml waxed food cups. The herbicides, R-25788, CDAA, and GA_3 were sprayed on the soil as formulated emulsifiable concentrates in an oil-in-water emulsion. The formulated emulsifiable concentrate of R-29148 was sprayed on the soil in a 50% water-50% ethanol mixture. The herbicides and the antidotes were sprayed sequentially with a link belt sprayer at 2.1 kg/cm^2 pressure in 935 L/ha spray volume and incorporated into the top 2.5 cm of soil. Carboxin and NA were applied as a 0.5% (w/w) seed treatment without binder. Five DeKalb 315A corn seeds were planted 2.0 cm deep into the soil of each cup. After planting, the cups were placed in a glass house with supplementary artificial lighting. Temperature ranged from 20° C at night to 33° C during the day. The plants in the main-herbicide-antidote and the separate CDAA-acetochlor studies were fertilized twice (6 and 12 days after planting) with a commercial fertilizer in solution testing 20:20:20 for NPK. When herbicide and gibberellin effects were examined the plants were not fertilized to avoid any possible hormone-fertilizer interactions. Post-emergence treatment of GA_3 were sprayed at 2.1 kg/cm^2 pressure in 310 L/ha spray

volume when the plants were 10 cm tall. EPTC and metolachlor were applied 24 h later in a 10 ml soil drench. The number of emerged seedlings showing visible herbicide injury were counted 10 days after planting. Twenty-one days after planing, plant heights were measured and the plants harvested, dried in a forced-air oven at 50° C for 48 h, and the dry weight determined. The data are expressed as the percent injured seedlings per cup, the average height in cm per corn plant per cup, and the average dry weight in mg per corn plant per cup. The percent injury data were converted to their arc sines for statistical analysis.

For the nutrient culture study, plants were grown in Hoagland's No. 1 solution which was changed daily. DeKalb 315A corn seeds were germinated at 24° C on filter paper in the dark for 72 h between sealed trays. Three corn seedlings were placed in slits cut in 7.5 cm diam by 1.9 cm deep foam rubber disks. The disks sat in 295-ml plastic tumblers wrapped in aluminum foil to keep out light. Technical grade EPTC and metolachlor were solubilized by adding 0.1% ethanol to each solution except for the ethanol-free control. The gibberellin (GA_3) used was the water-soluble 10% potassium salt. Plants heights were measured 9 days after transfer to the plastic tumblers.

Chemicals used.

The five antidotes used were R-25788, R-29148, CDAA (all at 1.12 kg/ha) and carboxin and NA (0.5% w/w seed treatments). These were evaluated against 13.44 kg/ha of alachlor, metolachlor and H-22234 in the first part of the study and H-26910 and acetochlor in the second part. EPTC was included as a reference. Though all of these compounds are not named as acetanilide herbicides, they all have a 2-chloroaceta-

nilide core. The CDAA-acetochlor interaction was further evaluated with CDAA at 0, 1.12, 2.24 kg/ha against acetochlor at 0, 4.48, 13.44 kg/ha in a two-way factorial experiment.

In the nutrient culture study, EPTC was added to give concentrations of 5×10^{-6}, 5×10^{-5} and 5×10^{-4} M and metolachlor at 5×10^{-6} and 5×10^{-5} M. GA_3 was tested for antidotal effects in the 5×10^{-5} M concentration of each herbicide and was given at daily increasing concentrations from 10^{-7} M on day 1 to 8×10^{-6} M on day 9. In a further herbicide-gibberellin study, the antidotal properties of formulated GA_3 was evaluated at 1.12 kg/ha against 4.48 or 13.44 kg/ha of EPTC and metolachlor on corn grown in soil culture. Both herbicide and GA_3 were applied pre-plant-incorporated in the first part of this study and postemergence in the second part. This study was designed as a two-way factorial study split between methods of application. All experiments were repeated and had five replications except for the extended rate study of the CDAA-acetochlor interaction which had four replications.

Results and Discussion

The compounds evaluated in the herbicide-antidote study varied significantly in their antidotal properties. R-25788 was the most effective; it countered visible injury caused by five acetanilide herbicides as well as EPTC (Tables 1 and 2). The height and dry weight of corn treated with R-25788 or R-29148 in combination with all six herbicides was not significantly different from that of the corn treated with either antidote alone. NA was also an effective antidote, although it was weak against H-22234 (Tables 1 and 2). CDAA was less effective as an antidote, and corn plants treated with it in combination with

metolachlor, H-22234, and H-26910 were taller than with the herbicides alone but significantly shorter than plants treated with CDAA alone or the controls (Tables 1 and 2). The antidotal properties of CDAA were not increased by increasing the rate from 1.12 to 2.24 kg/ha (Table 3). Carboxin had little or no antidotal activity on any of the herbicides tested, including EPTC.

The antidotes caused some corn injury. As shown in Table 2, the R-25788, R-29148, CDAA, and carboxin used alone significantly decreased the dry weight of corn below that of the control, but only R-29148 had the same effect shown in Table 1. CDAA, which has partial antidotal properties, also has herbicidal properties and can damage wheat and ryegrass seedlings (Jaworski (10) as well as corn (Chang et al. (1).

Of the herbicides used, alachlor was the least phytotoxic to corn, although it did decrease plant height and dry weight at the rate used (Table 1). Metolachlor was more phytotoxic than alachlor but not as phytotoxic as EPTC. Acetochlor and H-26010 had approximately equal phytotoxicity (Table 2). H-22234 was significantly more phytotoxic than EPTC (Table 1).

In the nutrient culture study, the 0.1% ethanol added to increase the solubility of the herbicides inhibited corn growth (Table 4). The corn plants treated with EPTC and metolachlor were usually shorter than the controls containing ethanol and the ethanol-free control (Table 4). Both herbicides caused greater growth inhibition at increasing rates.

GA_3 increased corn growth sufficiently to overcome the stunting caused by EPTC and metolachlor as compared to the ethanol control (Table 4) but it did not prevent herbicide injury symptoms such as leaf

stunting, leaf rolling and twisting, and stem swelling. Furthermore, the GA_3 did not fully overcome the herbicide-induced growth inhibition as the plants receiving the combination were shorter and lighter than those receiving only the GA_3 (Tables 4 and 5). Since exogenously added GA_3 did not overcome the injury symptoms caused by EPTC and metolachlor, it is doubtful that the mode of action of these two herbicides is merely the blocking of GA_3 synthesis. Although GA_3 may partially prevent plant stunting due to EPTC and metolachlor injury, its failure to prevent the other injury symptoms severely limits its effectiveness as an antidote.

The simplest proposed mode of antidotal action is that the antidote inhibits the action of the herbicide because of structural similarity (Stephenson et al.,(15). If so, R-29148 should be a more effective acetanilide antidote than R-25788 because its oxazolidine ring more closely resembles the phenyl ring of the herbicides than does the allyl side chains of R-25788.

The dichloroacetamides, R-25788 and R-29148, were more effective in preventing acetanilide herbicide injury to corn than was the single monochloroacetamide, CDAA. This is in agreement with Pallos et al. (13) who reported that the dichloroacetamides were more effective thiocarbamate antidotes than the monochloroacetamides.

In conclusion, R-25788 was the most effective antidote for acetanilide herbicide injury tested, and R-29148 and NA also had good antidotal activity. CDAA only partially overcame acetanilide herbicide injury, and carboxin and GA_3 had little or no antidotal activity.

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Table 1 - Effects of six potential antidotes on corn injury from 13.44 kg/ha of alachlor, metolachlor, H-22234 and EPTC.[a]

Main effects	Herbicides	Antidotes	Corn injury (%)	Plant ht (cm)	Plant dry wt (mg/plant)
i) antidotes		none	50 d	30 a	296 a
		R-25788	1 a	54 e	515 c
		R-29148	0 a	51 d	441 b
		CDAA	14 b	42 b	437 b
		Carboxin	39 c	29 a	290 a
		NA	5 ab	46 c	448 b
ii) herbicides	none		2 a	54 e	509 e
	alachlor		3 a	50 d	467 d
	metolachlor		16 b	42 c	407 c
	H-22234		45 d	27 a	276 a
	EPTC		26 c	35 b	365 b
Interactions	none	none	0 a	55 jkl	550 kl
	alachlor		8 a	48 fg	440 fghij
	metolachlor		48 b	31 de	313 de
	H-22234		100 d	5 a	65 a
	EPTC		92 c	8 a	114 ab
	none	R-25788	4 a	54 hijkl	472 fghijk
	alachlor		0 a	52 ghijk	507 hijk
	metolachlor		0 a	54 hijkl	531 ijkl
	H-22234		0 a	55 ijkl	534 ijkl
	EPTC		0 a	55 ijkl	532 ijkl

Table 1 - (continued)

Main effects	Herbicides	Antidotes	Corn injury (%)	Plant ht (cm)	Plant dry wt (mg/plant)
	none	R-29148	2 a	51 ghijk	427 fgh
	alachlor		0 a	49 gh	434 fghi
	metolachlor		0 a	49 gh	422 fgh
	H-22234		0 a	52 ghijk	457 fghijk
	EPTC		0 a	49 gh	466 fghijk
	none	CDAA	4 a	58 l	599 l
	alachlor		0 a	56 jkl	528 ijkl
	metolachlor		0 a	47 fg	467 fghijk
	H-22234		60 b	16 b	197 bc
	EPTC		8 a	35 e	392 def
	none	Carboxin	0 a	56 kl	536 jkl
	alachlor		9 a	44 f	398 efg
	metolachlor		49 b	22 c	218 c
	H-22234		82 c	9 a	99 a
	EPTC		54 b	14 b	200 bc
	none	NA	0 a	51 ghij	468 fghijk
	alachlor		0 a	50 ghi	496 ghijk
	metolachlor		0 a	51 ghij	487 fghijk
	H-22234		26 a	28 d	305 d
	EPTC		0 a	49 gh	487 fghijk

[a] Means within columns for each main effect and for interactions with similar letters were not significantly different at the 5% level by Duncan's Multiple Range Test.

Table 2 - Effects of six potential antidotes on corn injury from 13.44 kg/ha of H-26910, acetochlor and EPTC. [a]

Main effects	Herbicides	Antidotes	Corn injury (%)	Plant ht (cm)	Plant dry wt (mg/plant)
i) antidotes		none	54 b	30 a	400 a
		R-25788	11 a	50 d	571 c
		R-29148	10 a	45 c	486 b
		CDAA	44 b	38 b	425 ab
		Carboxin	50 b	30 a	354 a
		NA	27 a	46 c	594 c
ii) herbicides	none		8 a	54 c	649 b
	H-26910		45 c	33 a	391 a
	acetochlor		51 c	41 b	443 a
	EPTC		26 b	31 a	404 a
Interactions	none	none	1 a	55 ij	788 h
	H-26910		76 fg	16 ab	228 a
	acetochlor		88 g	35 cd	371 abcd
	EPTC		51 def	13 a	215 a
	none	R-25788	8 ab	54 ij	597 fg
	H-26910		8 ab	51 hij	532 defg
	acetochlor		10 abc	49 ghi	568 efg
	EPTC		17 abcd	48 fghi	589 fg
	none	R-29148	3 a	49 ghi	520 cdefg
	H-26910		6 ab	43 defgh	443 bcdefg
	acetochlor		17 abcd	44 efgh	437 bcdef
	EPTC		14 abc	45 fgh	543 efg

Table 2 - (continued)

Main effects	Herbicides	Antidotes	Corn injury (%)	Plant ht (cm)	Plant dry wt (mg/plant)
	none	CDAA	10 abc	54 ij	583 ij
	H-26910		69 efg	32 c	409 bcde
	acetochlor		71 efg	43 defg	427 bcdef
	EPTC		27 abcd	22 b	279 ab
	none	Carboxin	10 abc	55 ij	613 g
	H-26910		68 efg	14 a	211 a
	acetochlor		77 fg	36 cde	353 abc
	EPTC		46 cde	14 ab	241 a
	none	NA	18 abcd	57 j	794 h
	H-26910		42 bcde	40 def	526 defg
	acetochlor		43 bcde	42 defg	502 cdefg
	EPTC		18 abcd	43 efgh	556 efg

[a] Means within columns for each main effect and for interactions with similar letters were not significantly different at the 5% level by Duncan's Multiple Range Test.

Table 3 - Effects of CDAA on corn injury from acetochlor.[a]

	CDAA kg/ha	Acetochlor kg/ha	Corn injury %	Plant ht cm	Plant dry wt mg/plant
Main effects	0		55 b	44 a	377 a
i) CDAA	1.12		18 a	47 b	404 ab
	2.24		15 a	47 b	421 b
ii) acetochlor		0	3 a	53 c	465 c
		4.48	36 b	45 b	390 b
		13.44	49 c	40 a	346 a
Interaction	0	0	4 a	54 d	484 d
		4.48	68 c	42 b	349 ab
		13.44	93 d	35 a	298 a
	1.12	0	0 a	54 d	470 d
		4.48	26 b	45 bc	383 bc
		13.44	28 b	42 b	348 ab
	2.24	0	4 a	52 d	442 cd
		4.48	14 ab	48 c	428 cd
		13.44	26 b	42 b	393 bc

a. Means within columns for each main effect and for interaction with similar letters were not significantly different at the 5% level by Duncan's Multiple Range Test.

Table 4 - Effects of EPTC or metolachlor, with and without GA_3, on height of plants grown in nutrient solution. [a]

Herbicides	Plant ht, cm	
	- GA_3	+ GA_3 (1.12 kg/ha)
None	31 f	
None + ethanol 0.1%	21 d	38 g
EPTC + ethanol 0.1%		
5×10^{-6} M	13 b	
5×10^{-5} M	14 b	20 d
5×10^{-4} M	9 a	
Metolachlor + ethanol 0.1%		
5×10^{-6} M	21 d	
5×10^{-5} M	17 c	28 e

a. Means within columns with similar letters were not significantly different at the 5% level by Duncan's Multiple Range Test.

Table 5 - Effects of GA_3 on corn injury caused by metolachlor and EPTC [a]

Method of Application	Herbicide kg/ha	GA_3 kg/ha	Plant ht cm	Plant dry wt mg/plant
Preplant Incorporated	None	0	49 e	417 g
		1.12	61 f	489 h
EPTC				
4.48		0	20 b	224 cd
4.48		1.12	32 c	300 e
13.44		0	6 a	95 a
13.44		1.12	16 b	188 bc
Metolachlor				
4.48		0	26 c	252 d
4.48		1.12	37 d	346 f
13.44		0	18 b	172 b
13.44		1.12	26 c	253 d
Post-emergence [b]	None	0	55 e	412 bc
		1.12	63 f	484 d
EPTC				
4.48		0	33 b	348 b
4.48		1.12	45 cd	391 bc
13.44		0	22 a	254 a
13.44		1.12	23 a	265 a
Metolachlor				
4.48		0	40 bc	363 b
4.48		1.12	48 d	440 cd
13.44		0	25 a	238 a
13.44		1.12	37 b	378 bc

a. Means within columns with similar letters were not significantly different at the 5% level by Duncan's Multiple Range Test.

b. Herbicides given as soil drench and GA_3 as foliar spray when plants were 10cm tall.

CHAPTER 2

PROTECTION OF CORN (*ZEA MAYS*) FROM ACETANILIDE HERBICIDAL INJURY WITH THE ANTIDOTE R-25788

Abstract

The antidote R-25788 (*N*,*N*-diallyl-2,2-dichloroacetamide) protected corn (*Zea Mays* L. 'DeKalb 315A') seedlings from injury caused by the acetanilide herbicides, alachlor (2-chloro-2',6'-diethyl-*N*-(methoxymethyl) acetanilide), metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide), H-22234 (*N*-chloroacetyl-*N*-(2,6-diethylphenyl)glycine ethyl ester), and H-26910 (*N*-chloroacetyl-*N*-(2-methyl-6-ethylphenyl)glycine isopropyl ester) in a grenhouse study. R-25788, however, did not protect four weed species tested. R-25788 only partially protected corn from injury caused by acetochlor (2-chloro-*N*-(ethoxymethyl)-6'-ethyl-*o*-aceto-toluidide). R-25788 was an effective antidote whether applied preemergence, preplant-incorporated, or as a tank mix. Injury symptoms caused by EPTC (*S*-ethyl dipropylthiocarbamate) and the acetanilide herbicides were similar; both caused leaf twisting and rolling, and at high rates leaves failed to emerge through the coleoptile.

Introduction

Rains and Fletchall (10) reported that R-25788 selectively protected corn from EPTC injury and Meggitt et al. (9) and Chang et al. (2) confirmed this. Chang et al. (3) found that R-25788 significantly reduced the phytotoxicity of ten of twenty-two herbicides tested on corn; six of these were thiocarbamates. They reported that R-25788 was more effective against EPTC, vernolate (S-propyl-dipropylthiocarbamate), and butylate (S-ethyl diisobutylthiocarbamate) than alachlor. Chang et al. (3) suggested that different sites of action and (or) different structure-activity relationship might explain the differential antidotal activity of R-25788 to the different herbicide groups.

Stephenson and Chang (12) synthesized 25 dichloroacetamide analogues of R-25788 and tested them for antidotal activity against five thiocarbamate herbicides, including EPTC. The R-25788 analogue that was the best antidote to each of the thiocarbamate herbicides was the one with side chains identical to the respective herbicides. They speculated that the dichloroacetamides were competitive inhibitors at the site of thiocarbamate herbicide action.

The purpose of this study was to investigate more extensively the antidotal properties of R-25788 by comparing its antidotal effect on varying rates of five acetanilide herbicides and by testing varying rates of R-25788 against the same herbicides, by examining different methods of antidote application, and to determine if the antidotal properties of R-25788 against acetanilide herbicides were selective for corn.

Materials and Methods

Plant culture and chemical treatment evaluation.

The soil used in all experiments was a greenhouse soil (1:1:1 soil, sand, peat). Plants for the study combining R-25788 with various rates of herbicides were grown in 473-ml waxed cups. Plants for the study of various rates of R-25788 and study of the method of application were grown in 946-ml waxed cups. Plants for the selectivity study were grown in 15.2 by 30.4 by 5.1 cm styrofoam trays. Formulated emulsifiable concentrates of the herbicides and the R-25788 were sprayed on the soil surface sequentially with a link belt sprayer at 2.16 kg/cm^2 pressure with 935 L/ha spray volume, and incorporated into the soil separately in all but the study of method of application. In the method of application research some treatments were made preemergence and some combinations of H-22234 with R-25788 were sprayed as a tank mix. Incorporation was done by mixing the top 2.5 cm of soil in each group or tray. Soil in all cups for control plants was stirred similarly, although no chemicals were incorporated. Five corn seed were planted 2.0 cm deep into the soil of each group or tray. Fifteen seeds of pigweed (*Amaranthus retroflexus* L.), barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), yellow foxtail (*Setaria lutescens* (Weigel) Hubb.), and green foxtail (*Setaria viridis* (L.) Beauv.) were planted 0.28 cm deep in the trays for the selectivity study. After planting, the cups or trays were placed in a greenhouse supplemented with artificial lighting to give a 14-h day. The temperature ranged from 25 C at night to 33 C during the day. All plants were fertilized twice (6 and 12 days after planting) with a commercial 20:20:20 fertilizer. The number of emerged seedlings showing visible herbicide injury were counted 10 days after planting for all but

the selectivity study. In the study to compare the injury from EPTC and metolachlor, the plants were harvested and photographed 10 days after treatment. In the selectivity study the number of emerged seedlings injury by herbicides was determined 21 days after planting. In all the other studies plant ht was measured and the plants harvested 21 days after planting. The plants were dried in a forced air oven at 50 C for 48 h and the dry wt determined. The data are expressed as the percent injured corn seedlings, the average ht in cm per corn plant and the average dry wt in mg per corn plant. The percent injury data were converted to their arc sines for statistical analysis.

Chemical application.

The efficacy of a high rate (1.12 kg/ha) of R-25788 was tested against five rates (0.0, 2.24, 4.48, 6.72, 13.44 kg/ha) of the five acetanilide herbicides and one thiocarbamate herbicide. The study was divided into two parts and both parts repeated with a three-way factorial design. In the first part of this study, the herbicides used were EPTC, alachlor, metolachlor, and H-22234. In the second part, the herbicides H-26910 and acetochlor were applied.

To determine the rate of R-25788 necessary to protect against a high rate (13.44 kg/ha) of the herbicide, five rates of R-25788 (0.0, 0.14, 0.28, 0.56, 1.12 kg/ha) were used. This study was a two-way factorial design. In the method of application study, the herbicide H-22234, and the antidote R-25788 were applied alone and in combination, both preemergence and preplant-incorporated in a completely randomized design.

The study on selectivity examined the antidotal effect of R-25788 against acetanilide herbicides between corn and four weed species. This study was a two-way factorial design and was repeated using acetochlor

with the same results (data not presented). Because the injury symptoms of the acetanilide herbicides and EPTC were very similar, the relative injury was compared photographically. Metolachlor was chosen because it was similar to EPTC in the rates required to cause identical symptoms.

The data presented are the means of two experiments with five replications per experiment except for the selectivity study which had four replications.

Results

R-25788 effectively protected corn from injury caused by five acetanilide herbicides tested (Tables 1, 2). The corn receiving herbicide plus R-25788 showed significantly less visible injury and had greater ht and dry wt than corn that did not receive the antidote (Tables 1, 2). R-25788 was as effective in protecting corn from acetanilide injury as it was in protecting corn from EPTC (Table 1).

The various herbicides examined differed significantly in their phytotoxicity (Tables 1, 2). Alachlor was least injurious and did not decrease either corn ht or dry wt significantly at rates less than 13.44 kg/ha, although it did cause significant visible injury when rated at 10 days (Table 1). Metolachlor, acetochlor, and EPTC all significantly decreased corn ht at 4.48 kg/ha (Tables 1 and 2). Metolachlor and acetochlor also significantly decreased corn dry wt and increased visible injury occured with metolachlor and acetochlor at 4.48 kg/ha, and EPTC at 6.72 kg/ha (Table 1). H-22234 and H-26910 also caused significant injury to corn. Both of these herbicides significantly decreased corn ht at only 2.24 kg/ha (Tables 1, 2). R-25788 alone had no signi-

ficant effect on corn (Table 3).

Different rates of R-25788 were required to protect against injury from the 13.44 kg/ha rate of the different herbicides. A rate of 0.14 kg/ha R-25788 was needed to provide protection from 13.44 kg/ha of alachlor, metolachlor, and EPTC (Table 3). R-25788 applications of 0.56 kg/ha completely prevented the phytotoxic effects of H-22234 and H-26910 (Table 3). In one study R-25788 at 1.12 kg/ha prevented visible injury caused by acetochlor but did not prevent the decrease in both ht and dry wt caused by acetochlor at 13.44 kg/ha (Table 2). In another study visible injury caused by 13.44 kg/ha acetochlor was also not prevented by R-25788 (Table 3).

H-22234 alone caused less corn injury if applied preemergence rather than preplant incorporated, 64 vs 87%, respectively (Table 4). R-25788 applied sequentially or as a tank mix, protected corn from H-22234 injury (Table 4). R-25788 prevented H-22234 injury whether both were applied preemergence or preplant incorporated (Table 4).

The protective effects of R-25788 against injury from acetanilide herbicides were selective for corn (Table 5). None of the four weed species tested, pigweed, barnyardgrass, yellow foxtail, or green foxtail showed any evidence of being protected from H-26910 injury by R-25788.

Identical injury symptoms were evident on corn treated with EPTC and all the acetanilide herbicides tested, though the rates required to produce symptoms of the same intensity varied widely. Because of their similar rate responses, EPTC and metolachlor were compared photographically for injury symptoms with and without R-25788 at 1.12 kg/ha (Figures 1, 2). The injury symptoms common to both classes of herbicide include leaf rolling and twisting at low rates, extremely distorted

leaves and enlarged stems at higher rates, and complete failure of the first true leaves to emerge through the coleoptile at the highest rates examined.

Discussion

The antidote R-25788 effectively protected corn from acetanilide herbicide injury. A prior report indicating that R-25788 was less effective as an acetanilide herbicide antidote than an EPTC antidote was based on comparison between EPTC and alachlor (3). Since alachlor is not as phytotoxic to corn as EPTC, it does not provide a good basis for comparison.

Since the plants in all of these studies were grown in the greenhouse for only 21 days, it is not certain if the protective effects of R-25788 to acetanilide herbicides can be extended to an entire growing season in the field. The results of these greenhouse studies indicate that H-22234, H-26910, and to a lesser degree metolachlor may cause sufficient crop injury that the protection offered by R-25788 cauld be beneficial for corn under field conditions.

The similarity between EPTC and the acetanilide herbicide injury symptoms, as well as the efficacy of R-25788 as an antidote for symptoms of both, could be easily explained if both classes of herbicides have similar mode of action. The simplest proposed mode of action of R-25788 is that the antidote inhibits the action of the herbicide because it is structurally similar to them (11). While it is true that R-25788 resembles EPTC, it resembles the acetanilide herbicides much less. However, all of the herbicides, including EPTC, contain a carbamate linkage as does R-25788. They all have electronegative groups attached to the

carbonyl carbon; i.e., S-ethyl for EPTC, 2-chloromethyl for R-25788, and chloromethyl for the five acetanilide herbicides. Therefore, their chemical reactivities toward certain carbamoyl acceptors such as glutathione may be the same. It has also been proposed that EPTC is metabolized after activation by conjugation to glutathione (1, 5, 7, 8). There are reports that acetanilide herbicides may also form glutathione conjugates. Frear and Swanson (4) reported that propachlor (2-chloro-N-isopropylacetanilide) and barban (4-chloro-2-butynyl m-chlorocarbanilate) reacted with glutathione in vitro. They also reported that propachlor, barban, and alachlor were inhibitors of glutathione conjugation with triazine herbicides. Lamoreaux et al. (6) have isolated a glutathione conjugate of propachlor from corn. If R-25788 increases the glutathione content and the glutathione-S-transferase activity in corn and if the thiocarbamate and acetanilide herbicides are both metabolized by conjugation with glutathione, then R-25788 could increase the rates of metabolism of both classes of herbicides and explain the antidote mode of action.

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Figure 1 - Corn injury symptoms caused by pre-plant-incorporated applications of EPTC and their prevention by R-25788. a) EPTC 0.0 kg/ha, b) EPTC 4.48 kg/ha, c) EPTC 13.44 kg/ha, d) EPTC 0.0 kg/ha plus R-25788 1.12 kg/ha, e) EPTC 4.48 kg/ha plus R-25788 1.12 kg/ha, f) EPTC 13.44 kg/ha plus R-25788 1.12 kg/ha. Ruler scale is in inches.

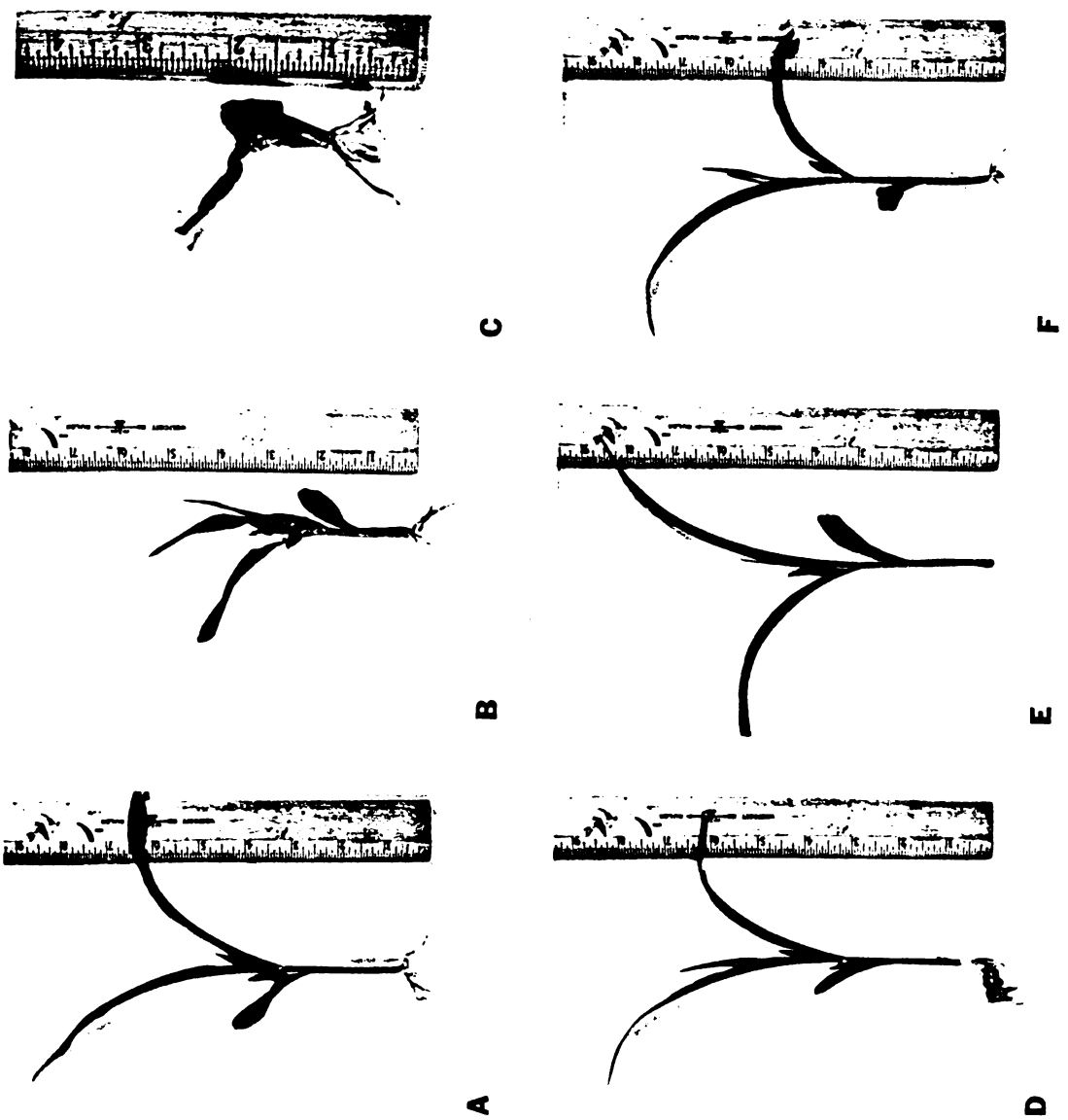
C
F
B
E
A
D

Figure 2 - Corn injury symptoms caused by pre-plant-incorporated applications of metolachlor and their prevention by R-25788. a) metolachlor 0.0 kg/ha, b) metolachlor 4.48 kg/ha, c) metolachlor 13.44 kg/ha, d) metolachlor 0.0 kg/ha plus R-25788 1.12 kg/ha, e) metolachlor 4.48 kg/ha plus R-25788 1.12 kg/ha, f) metolachlor 13.44 kg/ha plus R-25788 1.12 kg/ha. Ruler scale is in inches.

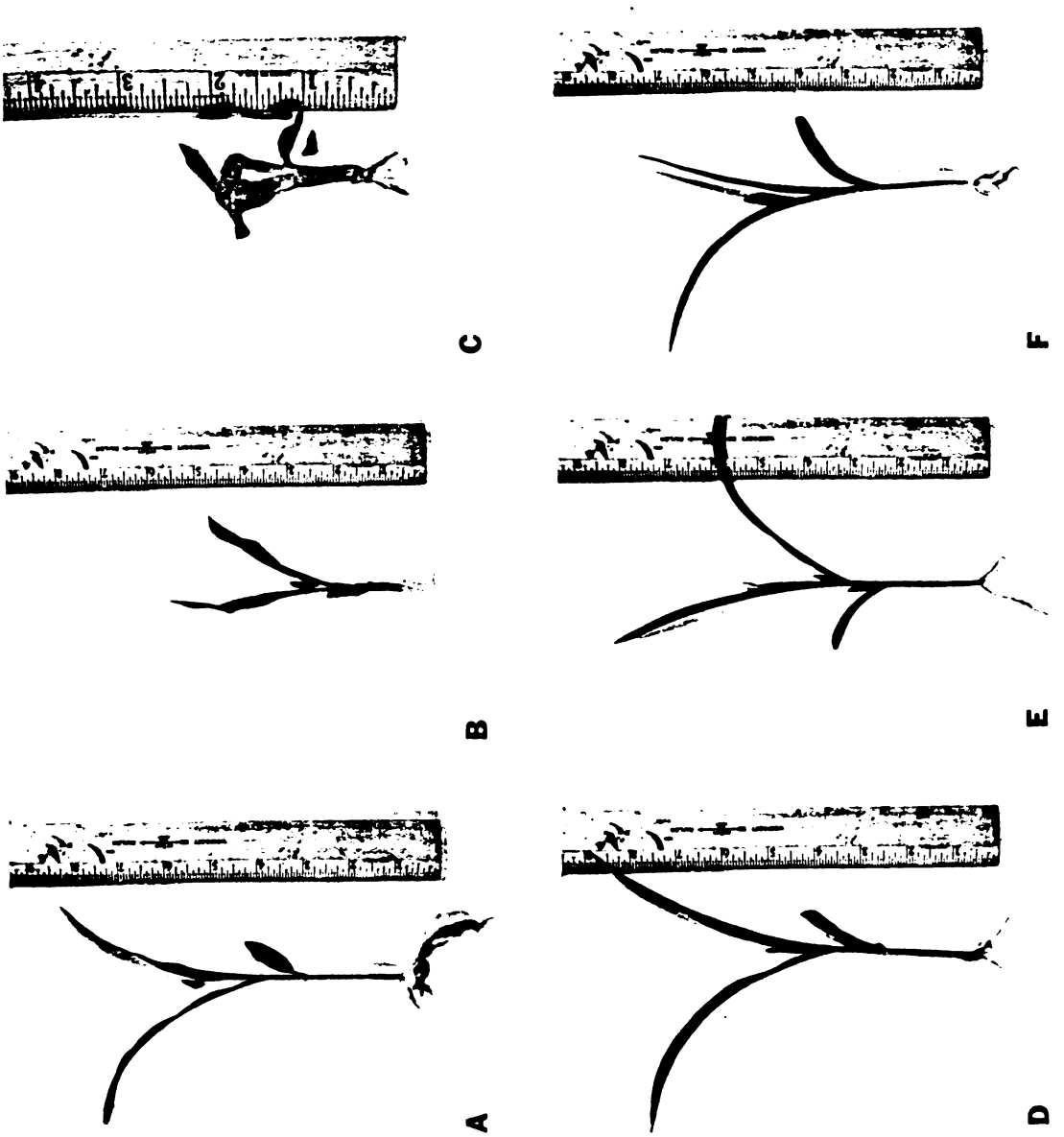
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Table 1 - Protective effect of 1.12 kg/ha R-25788 against corn injury from three acetanilide and one thiocarbamate herbicide applied at five rates.[a]

Effect to be compared	Herbicide	Rate (kg/ha)	R-25788 (kg/ha)	Corn injury %	Plant ht (cm/plant)	Shoot dry wt (mg/plant)
Main effect of herbicide	H-22234			36.6 c	32.7 a	383 a
	Metolachlor			22.1 b	41.1 b	465 b
	Alachlor			18.1 ab	46.3 c	543 d
	EPTC			14.0 a	40.7 b	524 c
Main effect of rate		0.0		2.0 a	47.7 e	564 d
		2.24		14.2 b	44.4 d	538 d
		4.48		24.8 c	40.7 c	489 c
		6.72		30.5 c	36.1 b	440 b
		13.44		42.0 d	31.9 a	363 a
Main effect of antidote			0.0	42.2 b	34.4 a	446
			1.12	3.2 a	45.9 b	512 b
Interaction	H-22234	0.0	0.0	10.0 ab	45.9 jklm	543 fghijk
			1.12	0.0 a	46.4 jklmn	550 ghijk
		2.24	0.0	59.1 ef	28.6 e	427 cde
			1.12	4.2 a	46.0 jklm	502 defghij
		4.48	0.0	84.0 h	16.6 bc	249 b
			1.12	3.8 a	46.1 jklm	496 defghij
		6.72	0.0	97.5 i	6.8 a	99 a
			1.12	4.0 a	42.8 ijk	460 cdefgh
		13.44	0.0	98.1 i	6.3 a	110 a
			1.12	5.3 a	41.1 hij	400 cd

Table 1 - (continued)

Effect to be compared	Herbicide	Rate (kg/ha)	R-25788 (kg/ha)	Corn injury %	Plant ht (cm/plant)	Shoot dry wt (mg/plant)
Interaction	Metolachlor	0.0	0.0	2.5 a	48.4 lmn	596 jklm
			1.12	2.0 a	45.8 jklm	452 cdefg
		2.24	0.0	24.3 abcd	45.2 ijklm	581 jklm
			1.12	2.0 a	47.4 klmn	509 defghijk
		4.48	0.0	36.1 cd	36.1 fg	430 cdef
			1.12	2.0 a	48.2 klmn	538 efghijk
		6.72	0.0	57.0 ef	33.3 f	405 cd
			1.12	9.0 a	43.3 ijkl	464 cdefghi
		13.44	0.0	84.5 h	18.1 cd	194 ab
			1.12	2.0 a	44.9 ijklm	480 cdefghij
	Alachlor	0.0	0.0	0.0 a	49.4 mn	590 jklm
			1.12	0.0 a	46.3 jklmn	549 ghijk
		2.24	0.0	12.3 ab	45.4 ijklm	556 ghijk
			1.12	3.5 a	46.2 jklmn	497 defghij
		4.48	0.0	35.4 cd	47.8 klmn	548 ghijk
			1.12	14.2 abc	47.5 klmn	580 ijklm
		6.72	0.0	41.8 de	47.6 klmn	624 klm
			1.12	2.5 a	46.5 klmn	547 ghijk
		13.44	0.0	66.5 fg	40.4 ghi	448 cdefg
			1.12	4.5 a	45.7 jklm	494 defghijk
	EPTC	0.0	0.0	0.0 a	51.6 n	678 m
			1.12	1.3 a	47.8 klmn	554 ghijk
		2.24	0.0	7.0 a	49.1 mn	671 lm
			1.12	1.3 a	47.2 klmn	563 ghijkl
		4.48	0.0	21.0 abcd	37.3 fgh	582 jklm
			1.12	1.8 a	46.4 jklmn	491 defghij
		6.72	0.0	32.5 bcd	21.7 d	369 c
			1.12	0.0 a	47.3 klmn	552 ghijk
		13.44	0.0	75.3 gh	13.3 b	213 b
			1.12	0.0 a	45.7 jklm	569 hijklm

Table 1 - (continued)

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a. Means within columns for each main effect and for interaction followed by a common letter or letters are not significantly different at the 5% level as judged by the Duncan's Multiple Range Test.

Table 2 - Protective effect of 1.12 kg/ha R-25788 against corn injury from two acetanilide herbicides applied at five rates.[a]

Effect to be compared	Herbicide	Rate (kg/ha)	R-25788 (kg/ha)	Corn injury %	Plant ht (cm/plant)	Shoot dry wt (mg/plant)
Main effect of herb.	H-26910			27.1 a	49.2 b	666 a
	Acetochlor			27.1 a	39.9 a	649 a
Main effect of rate				1.3 a	55.3 d	962 c
		2.24		15.0 b	49.2 c	698 b
		4.48		29.5 c	43.8 b	621 b
		6.72		38.4 c	40.8 b	569 b
		13.44		51.1 d	33.7 a	439 a
Main effect of R-25788			0.0	49.1 b	38.0 a	601 a
			1.12	5.1 a	51.1 b	715 b
Interaction	H-26910	0.0	0.0	0.0 a	56.0 g	1112 g
			1.12	2.5 a	53.7 fg	782 def
		2.24	0.0	23.5 a	43.4 cd	696 bcdef
			1.12	0.0 a	48.5 defg	682 bcdef
		4.48	0.0	62.5 cd	24.0 b	451 bc
			1.12	2.5 a	52.2 efg	790 def
		6.72	0.0	75.4 de	21.7 b	429 b
			1.12	5.0 a	49.5 defg	759 cdef
		13.44	0.0	96.7 f	4.1 a	141 a
			1.12	2.5 a	46.2 def	651 bcde

Table 2 - (Continued)

Effect to be compared	Herbicide	Rate (kg/ha)	R-25788 (kg/ha)	Corn injury %	Plant ht (cm/plant)	Shoot dry wt (mg/plant)
	Acetochlor	0.0	0.0	2.5 a	56.8 g	961 efg
			1.12	0.0 a	54.6 fg	993 fg
		2.24	0.0	30.0 ab	50.5 defg	679 bcdef
			1.12	6.7 a	54.3 fg	734 bcdef
		4.48	0.0	50.8 bc	42.4 cd	566 bcd
			1.12	2.0 a	56.4 g	676 bcdef
		6.72	0.0	61.3 cd	43.8 cde	538 bcd
			1.12	12.0 a	48.5 defg	553 bcd
		13.44	0.0	87.8 ef	37.9 c	438 bc
			1.12	17.7 a	46.8 def	526 bcd

[a] Means within columns for each main effect and for interactions followed by a common letter or letters are not significantly different at the 5% level as judged by the Duncan's Multiple Range Test.

Table 3 - Protective effects of five rates of R-25788 against corn injury from five acetanilide and one thiocarbamate herbicide applied at 13.44 kg/ha.

Effect to be compared	Herbicide (13.44 kg/ha)	Rate R-25788 (kg/ha)	Corn injury (%)	Plant ht (cm/plant)	Shoot dry wt (mg/plant)
Main effect of herbicide	Control		0.6 a	48.9 d	669 c
	H-22234		38.5 c	32.8 a	450 b
	Metolachlor		19.4 b	40.4 bc	488 b
	Alachlor		13.4 b	43.0 c	594 c
	H-26910		35.4 c	32.2 a	353 a
	Acetochlor		52.9 d	38.9 b	416 ab
	EPTC		13.5 b	40.5 bc	472 b
Main effect of rate R-25788		0.0	63.6 c	23.3 a	333 a
		.14	22.2 b	40.6 b	476 b
		.28	16.5 ab	42.6 b	587 d
		.56	11.4 a	45.9 c	565 cd
		1.12	10.4 a	45.3 c	499 bc
Interaction	Control	0.0	0.0 a	47.5 ijkl	591 fghijk
		.14	0.0 a	47.3 ijkl	712 ijk
		.28	0.0 a	51.4 l	738 k
		.56	0.0 a	50.1 kl	732 jk
		1.12	3.1 a	48.1 ijkl	574 efghijk
	H-22234	0.0	88.1 f	10.3 a	170 ab
		.14	41.8 cd	29.7 cde	358 abcdef
		.28	38.8 bcd	33.8 ef	658 hijk
		.56	17.5 ab	44.3 hijkl	554 defghijk
		1.12	6.3 a	46.0 hijkl	512 cdefghijk

Table 3 - (continued)

Effect to be compared	Herbicide (13.44 kg/ha)	Rate R-25788 (kg/ha)	Corn injury (%)	Plant ht (cm/plant)	Shoot dry wt (mg/plant)
	Metolachlor	0.0	73.8 e	18.4 b	346 abcde
		.14	10.3 a	47.0 hijkl	523 cdefghijk
		.28	7.5 a	45.8 hijkl	589 efghijk
		.56	3.1 a	45.5 hijkl	519 cdefghijk
		1.12	2.5 a	45.3 hijkl	463 cdefgh
	Alachlor	0.0	42.3 d	31.3 de	614 ghijk
		.14	16.2 a	47.8 ijkl	524 cdefghijk
		.28	6.3 a	47.0 hijkl	598 fghijk
		.56	0.0 a	44.5 hijkl	725 jk
		1.12	2.5 a	44.5 hijkl	507 cdefghijk
	H-26910	0.0	88.4 f	6.6 a	141 a
		.14	39.3 bcd	29.5 cde	288 abc
		.28	19.4 abc	36.1 efg	320 abcd
		.56	12.6 a	46.3 hijkl	475 cdefghi
		1.12	17.2 ab	42.3 ghijk	543 defghijk
	Acetochlor	0.0	97.2 g	25.8 cd	295 abc
		.14	39.9 cd	43.6 hijk	469 cdefghi
		.28	43.4 d	40.5 fghi	537 defghijk
		.56	42.7 d	41.6 ghij	380 bcdefg
		1.12	41.2 cd	43.1 ghijk	399 bcdefg

Table 3 - (continued)

Effect to be compared	Herbicide (13.44 kg/ha)	Rate R-25788 (kg/ha)	Corn injury (%)	Plant ht (cm/plant)	Shoot dry wt (mg/plant)
	EPTC	0.0	55.5 d	23.1 bc	171 ab
		.14	7.8 a	39.4 fgh	455 cdefgh
		.28	0.0 a	43.5 hijk	669 hijk
		.56	4.3 a	48.9 jkl	569 efghijk
		1.12	0.0 a	47.8 ijkl	494 cdefghij

[a]Means within columns for each main effect and for interactions followed by a common letter or letters are not significantly at the 5% level as judged by the Duncan's Multiple Range Test.

Table 4 - Protective effects of 1.12 kg/ha R-25788 applied as a tank mix with, or sequentially to, H-22234 applied pre-plant incorporated and pre-emergence at 13.44 kg/ha.[a]

Rates (kg/ha)		Application combination method			Corn injury %	Plant ht (cm/plant)	Shoot dry wt (mg/plant)
H-22234	R-25788	H-22234	R-25788	R-25788			
0.0	0.0	-	-	-	0.0 a	52.3 e	613 c
0.0	1.12	-	ppi	-	0.0 a	45.9 cd	502 bc
0.0	1.12	-	pre[e]	-	2.0 a	51.0 e	448 b
13.44	0.0	ppi[d]	-	-	87.1 c	12.3 a	123 a
13.44	1.12	ppi	ppi	seq[b]	7.8 a	43.4 c	426 b
13.44	1.12	ppi	ppi	tank mix[c]	2.0 a	48.4 de	509 bc
13.44	0.0	pre	-	-	64.0 b	22.2 b	242 a
13.44	1.12	pre	pre	seq	0.0 a	48.3 de	512 bc
13.44	1.12	pre	pre	tank mix	5.2 a	48.2 de	449 b

[a] Means within columns followed by a common letter or letters are not significantly different at the 5% level as judged by Duncan's Multiple Range Test.

[b] seq+ H-22234 and R-25788 applied sequentially, H-22234 first.

[c] Tank mix = H-22234 and R-25788 combined in same solution for application.

[d] ppi designates preplant incorporation of the herbicide.

[e] pre designates preemergence application of the herbicide.

Table 5 - A comparison of the protective effects of 1.12 kg/ha R-25788 against injury from H-26910 between corn and four weed species.

Herbicide	Rate (kg/ha)	R-25788 (kg/ha)	Corn injury %	Pigweed (control)	Barnyard grass (control)	Y. Foxtail (control)	G. Foxtail (control)
H-26910	0.0	0.0	0.0 a[a]	0.0[b]	0.0	0.0	0.5
		1.12	0.0 a	0.0	0.5	1.0	0.0
	4.48	0.0	27.5 a	2.5	2.5	3.0	4.0
		1.12	0.0 a	2.0	2.5	3.0	4.0
	13.44	0.0	75.0 b	4.0	3.5	3.0	4.0
		1.12	0.0 a	4.0	3.5	3.0	4.0

[a] Means followed by a common letter or letters are not significantly different at the 5% level as judged by Duncan's Multiple Range Test.

[b] Rating 0 to 4 (0 = no injury, 4 = death).

CHAPTER 3

PREVENTION OF EPTC-INDUCED EPICUTICULAR WAX AGGREGATION ON CORN (ZEA MAYS) WITH R-25788

Abstract

Scanning electron micrographs showed that EPTC (S-ethyl-dipropyl-thiocarbamate) caused an aggregation of the epicuticular wax layer of corn (Zea Mays L.). R-25788 (2,2-dichloro-N,N-diallylacetamide) prevented this aggregation when applied in combination with EPTC. Neither EPTC, metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methyl)ethyacetamide), nor R-25788 changed the weight of chloroform extractable epicuticular wax on corn leaves. EPTC apparently does not block lipid synthesis in corn as it does in other plant species. Thus R-25788 does not protect corn from EPTC or metolachlor by overcoming such a block, but EPTC did affect wax arrangement on the leaf surface and caused an increase in the cuticular transpiration of corn and predisposed corn to injury from subsequent postemergence applications of paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride). R-25788 protected corn against these deleterious effects.

Introduction

Thiocarbamate herbicides such as EPTC have been reported to inhibit epicuticular wax deposition on peas (Pisum sativum L.) (8), sicklepod (Cassia obtusifolia L.) (9), cabbage (Brassica oleracea var. Capitata) (3, 4, 5) and navy bean (Phaseolus vulgaris L.) (12). This inhibition has been associated with EPTC-induced predisposition of navy bean to root rot caused by Fusarium solani (Mart) Appel (12, 13). EPTC has also been reported to inhibit lipid synthesis in isolated spinach (Spinacia oleracea L.) chloroplasts (11). R-25788, when applied in combination with EPTC, prevented this inhibition of lipid synthesis (10). Since R-25788 is an effective antidote to corn injury caused by EPTC (2, 6, 7), it was suggested that the general mode of action of R-25788 is to overcome an EPTC-induced inhibition of lipid synthesis (10). The purpose of this research was to investigate the effects of EPTC on epicuticular wax deposition on corn, to examine the interaction of R-25788 with any EPTC effect found, and to test whether any EPTC effect could predispose corn to subsequent herbicide stress injury. Metolachlor was included in the study since it has been reported that R-25788 protects corn from metolachlor injury as effectively as it protects EPTC (6).

Materials and Methods

Plant culture and chemical application.

In all studies the plant material was corn (Zea mays L. 'Pioneer 3780') grown in a greenhouse soil (1:1:1, soil:sand:peat). All herbicides and R-25788 used were commercial formulations without any additional surfactant. The plants for the epicuticular wax extraction, cuticular

transpiration, and scanning electron-micrograph (SEM) studies were grown in 5 x 14.5 x 29 cm styrofoam trays placed outside (between the greenhouses) on a rough gravel bed (to prevent roots from growing out of the trays). The soil in the styrofoam trays was treated with a commercial N-P-K fertilizer (20:20:20) before herbicide application or planting.

Before planting the corn 2.0 cm deep, EPTC and/or R-25788 in the first study and metolachlor and/or R-25788 in the second study were sprayed on the surface of the soil on the trays with a link belt sprayer (2.11 kg/cm^2 pressure; 935 L/ha spray volume). The chemicals were incorporated into the top 2.5 cm of soil. When R-25788 was applied in combination with one of the herbicides, the herbicide was applied and incorporated first. The epicuticular wax extraction, cuticular transpiration, and SEM studies were two-way factorial in design. EPTC at 0.0, 1.68, 3.36, or 6.72 kg/ha or metolachlor at 0.0, 1.68, or 3.36 kg/ha were applied in combination with 0 or 1.12 kg/ha R-25788. These studies consisted of six replications per treatment, five of which were used for wax extraction and cuticular transpiration measurements. The sixth was used for Scanning Electron Microscopy (SEM). The experiment combining metolachlor and R-25788 had five replications per treatment and was not repeated since the results were of minor interest. The seeds were planted 2.0 cm deep in 946-ml waxed cups for the EPTC, R-25788 paraquat interaction study and the plants grown in a greenhouse with supplemental lighting (16 hr day). The temperature ranged from 22 C at night to 30 C during the day. This study was three-way factorial in design with four replications combining EPTC at 0.0, 3.36, or 6.72 kg/ha, R-25788 at 0.0 or 1.12 kg/ha, and paraquat at 0.0 or 0.56 kg/ha. Immediately after planting, the EPTC and R-25788 were applied in 50 ml of solution soil

drench. When the corn was 12 days old (15 cm tall), the paraquat was sprayed on the leaves with the link belt sprayer (2.11 kg/cm^2 pressure; 935 L/ha volume). The non-absorbed paraquat was washed off the leaves 24 hr later. Forty-eight hr after paraquat treatment visual injury ratings were taken, and five days later the fresh weight per plant was measured. Except for the experiment with metolachlor, all data presented are the means of two experiments.

Wax extraction, cuticular transpiration, SEM, and GLC.

When the corn plants were in the fifth leaf stage, the leaf blades from the third oldest leaves were removed for epicuticular wax extraction. Upon removal, the leaf blade area was measured with an automatic area meter (Lambda Instruments). Three hundred cm^2 of blades were placed in the bottom of a 1-L measuring cup and washed twice with glass-distilled chloroform (once with 150 ml for 30 sec., once with 100 ml for 15 sec). Both washes were combined. A 75-ml aliquot of the chloroform-wax mixture was filtered through Whatman #1 filter paper into pre-weighed 80-ml aluminum pans. After the chloroform was evaporated for 18 hr, the pans were re-weighed and the weight of epicuticular wax per cm^2 leaf area calculated. The cuticular transpiration was measured on the fourth oldest leaf of the same plants. The leaf blades were harvested, their leaf area measured with the automatic area meter and cut into three 10 cm long sections. The incisions were covered with lanolin to prevent water loss. Three leaves were placed into 10-cm-dia aluminum pans, weighed, allowed to transpire for 45 hr in an exhaust hood, and then re-weighed. The water loss per cm^2 leaf area was calculated. Scanning electron micrographs were taken of the adaxial surface of 4 by 8 mm leaf pieces of the leaf blade of the third leaf (taken approximately 1/3 of the distance from

sheat to blade tips to the side of the mid vein) as previously described (5). A sample of the chloroform wax solution was analyzed by gas-liquid chromatography (GLC) for the effects of the chemical treatment upon the major corn wax components. The solvent from a 10-ml aliquot of the solution of chloroform wax was evaporated under nitrogen and the wax subsequently redissolved in 1 ml fresh chloroform. Over 60% of the detected area was in one peak with a retention time of 6.8 min and was identified as 1-dotriacontanol. This identification was made by comparison of the retention time of the unknown to the retention times of a homologus series of known standards. It has been previously reported that 1-dotriacontanol is the major constituent of the corn epicuticular waxes (1). The GLC system used was as previously described except column temperature was 280 C (5).

Results and Discussion

Neither EPTC nor metolachlor, applied alone or in combination with R-25788, caused any significant deviation from the control in amount of epicuticular wax extracted from corn leaf blade surfaces (Tables 1 and 2). However, the highest rate of EPTC plus R-25788 did result significantly less epicuticular wax than did the lower rates of EPTC plus R-25788 (Table 1). Since EPTC did not decrease epicuticular wax deposition on corn, it may not decrease lipid synthesis in corn as it does in other plant species. Since the antidote R-25788 protects corn from injury by high rates of EPTC, the mode of action of R-25788 apparently is not to overcome an inhibition of lipid synthesis caused by either EPTC or metolachlor. Cuticular transpiration was increased by EPTC when applied alone but not by EPTC in combination with R-25788 (Table 1).

Metolachlor had no effect on cuticular transpiration whether applied alone or with R-25788 (Table 2). The increase in cuticular transpiration caused by EPTC, and its reversal by R-25788, without concomitant changes in the amount of epicuticular wax on the leaf surface can only be explained by a change in either the chemical composition or the distribution (fine structure) of the wax on the corn leaf surface. Analysis of the epicuticular wax by GLC did not reveal any obvious effects of EPTC or R-25788 treatment on any of the unidentified minor components. Analysis of the major wax component (1-dotriacontanol) by GLC revealed a slight inhibition by EPTC that was not prevented by R-25788 (Table 3), thus eliminating the possibility that R-25788 reversal of EPTC-induced changes in chemical wax composition could account for the transpiration effect. However, scanning electron micrographs of corn leaves (Fig. 1) showed that EPTC caused definite aggregation of the epicuticular wax upon the surface. When R-25788 was applied with EPTC, the epicuticular wax layer appeared normal (Figure 1). The increase in cuticular transpiration caused by EPTC and its prevention by R-25788 can be explained by these observations. By inducing aggregation of the epicuticular wax, it appears that EPTC causes areas of the underlying cuticle layers to be relatively more exposed, leading to increased water loss. Elimination of the aggregates by combining EPTC with R-25788 eliminates the increase in transpiration. Though it is difficult to estimate amounts of epicuticular wax from the micrographs, the treatments do not appear to differ in amount of epicuticular wax despite the differences in distribution.

EPTC treatments at planting predisposed corn to increased damage from later application of paraquat (Table 4). R-25788 prevented this

predisposition. Apparently EPTC-induced epicuticular wax aggregation caused increased uptake of paraquat. R-25788 eliminated the predisposition effect by eliminating the aggregation effect.

In conclusion, EPTC caused a change in the distribution of epicuticular wax on corn leaf surface wich R-25788 prevented. R-25788 prevented EPTC-induced increases in cuticular transpiration and predisposition to paraquat injury.

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Table 1 - Influence of EPTC and R-25788 on epicuticular wax deposition and cuticular transpiration of corn leaves.

EPTC rate	Weight of epicuticular wax on corn[a]		Cuticular transpiration in 45 h[b]	
	R-25788		R-25788	
	0.0 kg/ha	1.12 kg/ha	0.0 kg/ha	1.12 kg/ha
(kg/ha)	(ug/cm^2)	(ug/cm^2)	(mg H_2O/cm^2)	(mg H_2O/cm^2)
0.0	54.2 abc[c]	55.6 bc	8.7 a	8.1 a
1.68	62.2 bc	67.1 c	8.1 a	7.4 a
3.36	61.8 bc	57.6 bc	10.7 b	7.1 a
6.72	49.7 ab	40.8 a	10.7 b	7.2 a

[a] Measured on the leaf blade of the third oldest corn leaf at fifth leaf stage of the plant.

[b] Measured on the leaf blade on the fourth oldest corn leaf at fifth leaf stage of the plant.

[c] Values with the same letter or letters under main heading are not significantly different at the 5% level using the Duncan's Multiple Range Test.

Table 2 - Influence of metolachlor and R-25788 on epicuticular wax deposition and cuticular transpiration of corn leaves.

Metolachlor rate	Weight of epicuticular wax on corn[a]		Cuticular transpiration in 45 h[b]	
	R-25788		R-25788	
	0.0 kg/ha	1.12 kg/ha	0.0 kg/ha	1.12 kg/ha
(kg/ha)	(ug/cm^2)	(ug/cm^2)	(mg H_2O/cm^2)	(mg H_2O/cm^2)
0.0	51.9 a[c]	46.2 a	10.5 a	9.7 a
1.68	41.2 a	50.8 a	9.6 a	9.8 a
3.36	52.6 a	47.6 a	9.8 a	8.9 a

[a] Measured on the leaf blade of the third oldest corn leaf at fifth leaf stage of the plant.

[b] Measured on the leaf blade of the fourth oldest corn leaf at fifth leaf stage of the plant.

[c] Values with the same letter or letters under each main heading are not significantly different at the 5% level using the Duncan's Multiple Range Test.

Table 3 - Effect of EPTC and R-25788 on the major corn epicuticular wax component, 1-dotriacontanol.

EPTC rate (kg/ha)	Peak area/cm^2 (% of control) R-25788	
	0.0 kg/ha	1.12 kg/ha
0.0	100 cd[a]	106 d
1.68	108 c	84 b
3.36	89 bc	78 ab
6.72	67 a	76 a

[a] Values with the same letter or letters are not significantly different at the 5% level using Duncan's Multiple Range Test.

Table 4 - Predisposition of corn to injury from postemergence-applied paraquat by EPTC and R-25788 applied at planting.

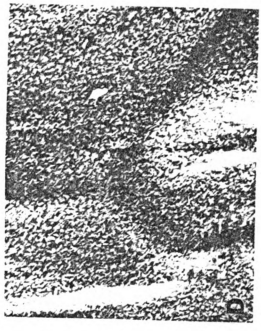
Treatment	Rate (kg/ha)	Corn injury		Fresh Weights	
		Paraquat rate		Paraquat rate	
		0.0 kg/ha (rating)[a]	0.56 kg/ha (rating)	0.0 kg/ha (g/plant)	0.56 kg/ha (g/plant)
Control		0.0	1.5	1.6 def	1.4 bcd
R-25788	1.12	0.0	1.5	1.9 g	1.4 bcd
EPTC	3.36	0.0	3.3	1.7 efg	0.7 a
EPTC + R-25788	3.36 + 1.12	0.0	1.6	1.8 fg	1.2 b
EPTC	6.72	0.0	3.7	1.3 bc	0.5 a
EPTC + R-25788	6.72 + 1.12	0.0	1.7	1.9 g	1.5 cde

[a] Injury ratings (0 = no injury, 4 = death).

[b] Values with the same letter or letters under each main heading are not significantly different at the 5% level using Duncan's Multiple Range Test.

Figure 1. Scanning electron micrographs of corn leaf surfaces showing the epicuticular wax aggregation induced by EPTC and its prevention by R-25788. (a) Control 2000x (b) R-25788 (1.12 kg/ha) 2000x (c) EPTC (6.72 kg/ha) 2000x (d) EPTC (6.72 kg/ha) + R-25788 (1.12 kg/ha) 2000x


B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CHAPTER 4

THE IN VITRO CONJUGATION OF GLUTATHIONE AND OTHER THIOLS WITH ACETANILIDE HERBICIDES AND EPTC SULFOXIDE AND THE ACTION OF THE HERBICIDE ANTIDOTE R-25788

Abstract

Non-enzymatic reaction in vitro of ^{3}H-labeled glutathione (GSH) with ^{14}C-alachlor, ^{14}C-metolachlor, ^{14}C-H-22234, and ^{14}C-EPTC sulfoxide formed dual labeled GSH-herbicide conjugates. GSH did not conjugate in this system with the herbicides buthidazole, atrazine, EPTC or the herbicide antidote R-25788. Alachlor also conjugated with the thiol containing compounds cysteine, dithiothreitol, and coenzyme A but not with methionine, acetyl CoA, mercaptoethanol, or ethanethiol. The alachlor-GSH conjugation reaction yielded more product with increasing pH (over pH 6.0) indicating that the reactive species of GSH is the GS^{-} ion. Through the GSH-acetanilide conjugation reaction had a low yield at physiological pH it could be the basis for the protection of corn from acetanilide herbicide injury by R-25788. Because R-25788 was required to protect atrazine-susceptible corn from alachlor injury but not from thiocarbamate injury it is suggested that R-25788 may protect corn from EPTC injury by increasing the rate of EPTC sulfoxidation followed by subsequent EPTC sulfoxide-GSH conjugation. R-25788 did not protect genetically atrazine-susceptible corn from atrazine injury, indicating that R-25788 does nor stimulate glutathione-S-transferase activity or atrazine GSH conjugation in corn.

Introduction

In vitro, non-enzymatic conjugation of glutathione (glutamylcysteinyl-glycine) (GSH) with three fungicides was reported by Seigel (17) in 1970. Atrazine (2-chloro-4(ethylamino)-6-(isopropylamino)-*s*-triazine)-GSH conjugates have been isolated from sorghum (*Sorghum vulgare* Pers.) leaf pieces by Lamoreaux *et al.* (9), and a glutathione-*S*-transferase that catalyzes GSH-atrazine conjugation has been identified in corn (*Zea mays* L.), sorghum and sugarcane (*Saccharum officianarum* L.) by Frear and Swanson (6). GSH-*S*-transferases that catalyze the conjugation of GSH with fluorodifen (*p*-nitrophenyl-*a*-*a*-*a*-trifluoro-2-nitro-*p*-tolyl ether) have also been isolated from peas (*Pisum sativum* L.) and peanuts (*Arachis hypogaea* L.) (7). Although Lay and Casida (11) reported a GSH-*S*-transferase from corn roots that catalyzed the conjugation of GSH with EPTC (*S*-ethyl dipropylthiocarbamate) sulfoxide, Carringer *et al.* (2) disputed the existence of this enzyme and reported that the GSH-EPTC sulfoxide conjugation proceeded *in vitro* non-enzymatically in buffer only. Both Lay and Casida (11), Lay et al. (12) and Carringer et al. (3) reported that the herbicide antidote R-25788 (*N*,*N*-diallyl-2,2-dichloroacetamide) increased the GSH content of corn and hypothesized that this GSH increase could cause an increased rate of EPTC detoxification by forming increased GSH-EPTC sulfoxide conjugation (after initial EPTC sulfoxidation) and thereby explain the mode of action of this antidote. Leavitt and Penner (13) have recently reported that R-25788 also protects corn from five acetanilide herbicides as effectively as it protects corn from EPTC. The acetanilide herbicide analog, chloroacetamide, readily conjugates non-enzymatically with certain thiol compounds, including GSH (14, 15).

The GSH conjugate of propachlor (2-chloro-N-isopropylacetanilide) has also been isolated from corn and a non-enzymatic GSH-propachlor conjugation reaction described (10). Preliminary experiments failed to find a GSH-S-transferase responsible for GSH-acetanilide herbicide conjugation; therefore, the objective of this study were to a) characterize the non-enzymatic conjugation of GSH and other thiols with the three acetanilide herbicide alachlor (2-chloro-2',6'-diethyl-N-(metoxymethyl) acetanilide), metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide), and H-22234 (N-chloroacetyl-N-(2,6-diethylphenyl)glycine ethyl ester) and the herbicide derivative EPTC sulfoxide, and b) to determine whether the mechanism for the protective action of R-25788 was the same for thiocarbamate and acetanilide herbicide by using a GSH-S-transferase deficient inbred corn line.

Material and Methods

Reagents and Equipment.

L-(glycine-2-^{3}H)-glutahione (specific activity, s.a. 2500mCi/mM) was purchased from New England Nuclear. Non-labeled GSH, L-cysteine, DL-dithiothreitol, and 2-mercaptoethanol, were purchased from Sigma Chem Company. Oxidized glutathione was prepared by bubbling O_2 through a solution of reduced GSH for 30 min. Coenzyme A (lypholized) was purchased from Nutritional Biochemicals Company, acetyl CoA from Schwarz/Mann, and ethanethiol from Eastman Organics. Formulated, technical, and uniformly ^{14}-C ring labeled alachlor (s.a. 1.7 mCi/mM) were donated by Monsanto Corp. Technical and uniformly ^{14}C-ring labeled metolachlor (s.a. 4.5 mCi/mM) as well as formulated and uniformly ^{14}C-ring labeled atrazine (s.a. 2.1 mCi/mM) were donated by Ciba-Geigy Corp. Technical and

carbonyl ^{14}C-labeled H-22234 (s.a. 1.2 mCi/mM) were donated by Hercules Corp. Formulated butylate (S-ethyl-diisobutyl thiocarbamate), formulated and carbonyl labeled ^{14}C EPTC (s.a. 1.33 mCi/mM) and formulated and technical R-25788 were donated by Stauffer Chemical Co. Labeled (^{14}C labeled) buthidazole (3-(5-(1,1-dimethylethyl)-1,3,4-thiadizol-2yl-4-hydroxy-1-methyl-2-imidazolidinone) (s.a. 12.7 mCi/mM) was donated by Velsicol Chem. Corp. ^{14}C-labeled EPTC sulfoxide (s.a. 1.33 mCi/mM) was synthesized from the ^{14}C carbonyl labeled EPTC by the method of Lay and Casida (11). All other chemicals used were reagent grade. Buffers were made in sterilized, de-aerated, distilled water, to an ionic strength of 0.1 M by the method of Cherry (5). Liquid scintillation spectrometry (lsc) was done by a Packard Tri-Carb^R Model 3320 liquid scintillation spectrometer with separate channels for ^3H, ^{14}C, and 233 Ra external standard. The scintillation cocktail used was Aqueous Counting Scintillant^R from Amersham. Mixtures were lypholized on a Vertis^R model lypholizer. The thin-layer cromatograph (TLC) system used was: Silica gel 60 or 60 F pre-coated TLC plates (E. Merck^R) developed in buthanol: acetic acid: water 30:10:15, and visualized with either autoradiography (Kodak No-Screen^R X-Ray film), ninhydrin spray reagent (16), nitroprusside (sodium) spray reagent (20), or dividing the plate into 1 x 2 cm blocks each block into scintillation vials for lsc.

Reaction of thiols with herbicides.

The reaction between ^3H-GSH and ^{14}C-acetanilide herbicide was studied by adding the following, in the order given, to a 100 x 12 mm screw top culture tube, mixed, and allowed to react for 3 h under a nitrogen atmosphere in a 30 C water bath: 1 ml phosphate buffer pH 7.0, 100 nmoles ^{14}C-acetanilide herbicide (alachlor, metolachlor, H-22234

each diluted to .07 uCi/100 nM with non-labelled herbicide) in 10 ul ethanol, 1300 nmoles GSH in 0.2 ml phosphate buffer, and o.4 nmoles ^{3}H-GSH in 50 ul 0.05 N acetic acid (approx. 1.0 uCi). The reaction was stopped by freezing the mixtures in a dry-ice acetone bath and then lypholized. The residue was extracted with 0.5 ml methanol, and 100 ul samples were applied to the TLC plates, developed, and visualized. These experiments were also repeated without ^{3}H-GSH. The specific activity of the ^{14}C-acetanilide herbicide and the ^{3}H-GSH in the reaction mixtures was approximately equal so that any conjugate formed containing 1 mole of GSH residue per mole of herbicide residue would have near equal amounts of ^{3}H and ^{14}C dpm. The pH experiments contained 1 ml of the following buffers: acetate pH 4.6, phosphate pH 6.0, phosphate pH 7.0, phosphate 8.0 or tris-HCl pH 8.6, plus 100 nm ^{14}C-alachlor and 1,000 nm GSH in one experiment and 10,000 nm GSH in another. The reaction mixture for the alachlor-thiol experiments was identical to the acetanilide-GSH experiment except only non-labeled thiols were used at a concentration of 1000 nm per reaction mixture. The reaction between GSH and other herbicides was studied by substituting the following for the acetanilide herbicides in the standard reaction mixture: 100 nmoles technical R-25788, 100 nm ^{14}C-buthidazole (1.3 u Ci), 38 nmoles ^{14}C-, 38 nmoles ^{14}C-EPTC sulfoxide, and 23 nm ^{14}C atrazine, all in 10 ul ethanol (except the buthidazol which was in 20 ul ethanol). Results from all experiments were expressed as the percent of ^{14}C recovered from the TLC plate as conjugate as compared to the total amount of ^{14}C recovered per spot.

Plant culture for herbicide-antidote response study.

Atrazine susceptible, glutathione-S-transferase deficient corn inbred GTLL2 (18) was grown in a greenhouse mix soil (1:1:1 sand:peat:

soil) in 946 ml waxed cups in a greenhouse supplemented with artificial lighting (16 h day) with a maximum temperature of 38 C and a minimum of 30 C. The response of this inbred to the herbicides EPTC, butylate, alachlor, and atrazine, alone or in combination with R-25788, was measured in three experiments. All herbicides and R-25788 were applied preplant-incorporated with 2.1 kg/cm^2 pressure in 935 l/ha spray volume with a link belt sprayer. When the herbicides were applied in combination with R-25788, the herbicides were applied and incorporated first and the R-25788 applied and incorporated 15 min later. After 4 weeks, corn heights and dry weights were measured. Only plant heights are reported. The dry weight results were similar.

Results and Discussion

The GSH conjugates of alachlor, metolachlor, and H-22234 were identified on TLC plates as spots with both ^{3}H and ^{14}C co-chromatographing in near equal relative abundance (Table 1). These spots reacted with ninhydrin (wich reacts with the free amino group in the GSH and therefore visualize both conjugated and non-conjugated GSH), did not react with nitroprusside (which reacts with free thiol groups and therefore visualizes unconjugates GSH only), and did not co-chromatograph with any of the original reactants (Table 1). Failure of the dual-labeled conjugate to react with nitroprusside indicates that the site of conjugation was the sulfur of the GSH. The presence of ^{14}C in all three conjugates indicates that the phenyl ring of the herbicides was mantained in the conjugate since both alachlor and metolachlor were phenyl labeled. The carbonyl carbon was also mantained in the conjugate since the H-22234 was carbonyl labeled. Based on this evidence the proposed

structure of the GSH-acetanilide herbicide conjugate was formulated (Figure 1). Although there were no significant differences between the amounts of conjugate formed by the three acetanilide herbicides (Table 1), the trend in amounts formed is the opposite of their relative toxicity to corn (13) (i.e., alachlor less than metolachlor less than H-22234).

The pH dependence of the alachlor-GSH conjugation reaction can be seen in Table 2. Except for acetate buffer at pH 4.6, the amount of conjugate formed in vitro increased with increasing pH to almost 100% when 10,000 nmoles of GSH were used in tris-HCl buffer pH 8.6. The pK of the sulfhydryl group of GSH has been reported as 8.66 (1). This means that the reactive species of GSH is the GS^- ion as previously reported for GSH-chloroacetamide conjugation (15). The anamalous behavior in acetate buffer at pH 4.6 could be the result of a different reaction mechanism, or the alachlor may be suitable at the low pH.

Alachlor also conjugated with cysteine, dithiothreitol, and coenzyme A (Table 3). No appreciable conjugate formation was detected with methionine, acetyl CoA, mercaptoethanol, or ethanethiol. Although R-25788 has been reported to increase GSH content of corn, (2, 11, 12), the autors are unaware of any reports on the effect of R-25788 on the concentration in corn of other thiols such as cysteine or coenzyme A.

No conjugation product of GSH with R-25788 could be detected (Table 4). GSH also did not conjugate in vitro with other chemicals buthidazole, EPTC, or atrazine. However, GSH conjugated with the EPTC-sulfoxide with 60.3% of the recoverable ^{14}C found in the conjugate (Table 4). These results support the conclusion of Carringer et al. (2) that EPTC sulfoxide conjugates non-enzymatically with GSH.

Although the physiological significance of the GSH-acetanilide

herbicide conjugation is unknown *in vivo*, it occurs at physiological pH. The reported stimulation of GSH synthesis by R-25788 (3, 11, 12), coupled with GSH-acetanilide conjugation, could explain the protective action of R-25788 against the acetanilide herbicides in corn. Similar rationale has been used to explain the protection of corn from EPTC injury (3). The possibility that R-25788 could have the same mode of action in preventing acetanilide and thiocarbamate herbicide injury was investigate by examining the response of inbred corn line, GT112, to both herbicide classes. This inbred is glutathione-*S*-transferase deficient and atrazine susceptible (18). As shown in Table 5, the two thiocarbamate herbicides EPTC and butylate caused no inhibition of growth in this corn genotype, whereas alachlor and atrazine did. The growth inhibition by alachlor was prevented by R-25788 but the inhibition by atrazine was not. In two other genotypes of corn, normal (DeKalb XL 316) and thiocarbamate susceptible (DeKalb XL 306), atrazine at 6.72 kg/ha did not inhibite growth (data not presented). Butylate at 3.36 kg/ha inhibited the growth of the thiocarbamate susceptible corn. EPTC at 6.76 kg/ha and alachlor at 4.48 and 6.72 kg/ha inhibited the growth of both genotypes. R-25788 prevented injury from all butylate, EPTC and alachlor to both genotypes. Since the inbred corn line, GT112, responded differently to thiocarbamates and alachlor, the metabolism of these two herbicide groups must differ. Whatever rendered this genotype thiocarbamate tolerant did not protect it from alachlor injury, and furthermore the action of R-25788 to prevent alachlor injury was not required for the prevention of EPTC injury. Therefore, not only is the metabolism of the two herbicide classes different, but the protective effect of R-25788 must have a different basis. Differences in GSH conjugation could result from the

requirement that EPTC be converted to its sulfoxide prior to GSH conjugation which is not required for alachlor. Furthermore, EPTC sulfoxide formed three times as much GSH conjugate *in vitro* in these experiments as alachlor, indicating that the GSH content in corn could be relatively more important for acetanilide detoxication than for EPTC detoxication. Casida et al. (4) reported that corn was injured by EPTC at 3.4 kg/ha but could tolerate EPTC sulfoxide applications of 27 kg/ha without damage. EPTC sulfoxide was more toxic to other plants than EPTC, however. Thus corn can readily detoxify EPTC sulfoxide without prior R-25788 treatments to raise the GSH content. R-25788 could therefore protect corn from EPTC injury only by increasing the rate of EPTC sulfoxidation. Increased rate of EPTC sulfoxide metabolism by increased GSH conjugation would only be secondary. Since the acetanilide herbicides do not react as readily with GSH as EPTC sulfoxide nor require prior activation in order to react with GSH, R-25788 may prevent acetanilide herbicide injury by simply increasing the GSH content of corn. The differential response of the GT112 inbred corn line to both herbicides could be explained by ease of GT112 sulfoxidation of EPTC, which then reacts with the natural GSH levels; GSH levels, however, not high enough to protect the genotype from alachlor unless R-25788 raises them. Failure of R-25788 to protect this inbred corn line from atrazine injury indicates that R-25788 does not stimulate glutathione-*S*-transferase activity or the rate of atrazine-GSH conjugation in corn.

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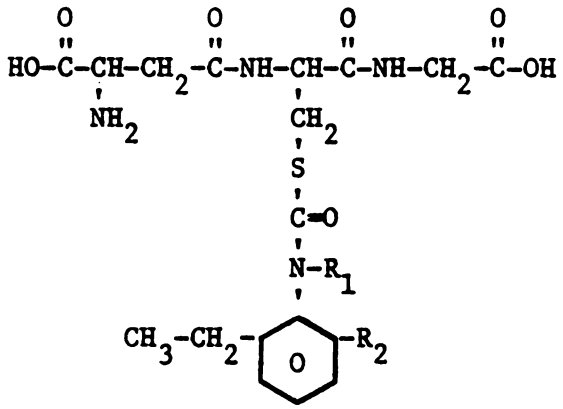
Table 1 - Reaction of GSH with three acetanilide herbicides.

Reactants[a] TLC[b] peaks	DPM[c] ^3H	DPM[c] ^{14}C	RXN with Nin.[d]	RXN with Nitro[e]	Co-Chromat. with	Identif.	$\%^{14}C$ in conj.[f]
^{14}C-alachlor + ^3H-GSH							
Rf = .10	3,200	------	+	-	GSSG		18.6% a
Rf = .30	19,100	------	+	+	GSH		
Rf = .47	1,700	1,700	+	-	----	GS-alach.conj.	
Rf = .76	------	9,400	-	-	alachlor		
^{14}C-metolachlor + ^3H-GSH							
Rf = .10	2,200	------	+	-	GSSG		7.1% a
Rf = .30	18,800	------	+	+	GSH		
Rf = .47	1,100	800	+	-	----	GS-metol.conj.	
Rf = .76	------	17,700	-	-	metolach.		
^{14}C-H-22234 + ^3H-GSH							
Rf = .10	2,600	------	+	-	GSSG		6.4% a
Rf = .30	21,000	------	+	+	GSH		
Rf = .47	1,200	1,100	+	-	----	GS-H-22234 conj.	
Rf = .76	------	21,500	-	-	H-22234		

a. ^{14}C-alachlor and ^{14}C-metolachlor uniformly ring labelled, ^{14}C-H-22234 carbonyl labelled, GSH-(2-^3H-glycine) labelled.
b. TLC system used: Silica gel 60, E. Merck, in BuoH, AcoH, H_2O 30:10:15
c. The specific activity of each reactant was approx. equal (0.7 mCi/mMole) so that any conjugate formed would have approx. equal dpm of each labelled isotope.
d. Ninhydrin used to visualize both conjugated and non-conjugated GSH.
e. Nitroprusside used to visualize conjugated GSH only.
f. These data from a separate experiment combining ^{14}C herbicide with non-labelled GSH, replicated three times. Means followed by the same letter are not significantly different as judged by Duncan's Multiple Range Test (5% level).

Figure 1 - Structure of GS-acetanilide herbicide conjugates.



for GS-alachlor conjugate

R_1= CH_3-O-CH_2-

R_2= CH_3-CH_2-

for GS-metolachlor conjugate

R_1= $CH_3-O-CH_2-\underset{CH_3}{CH}-$

R_2= CH_3-

for GS-H-22234 conjugate

R_1= $CH_3-CH_2-\overset{O}{\overset{\|}{C}}-CH_2-$

R_2= CH_3-CH_2-

Table 2 - The pH dependence of the GSH-alachlor conjugation reaction.

pH	1,000 nm GSH	10,000 nm GSH
4.6	18.5 b[1]	43.6 b
6.0	7.4 a	8.9 a
7.0	17.0 ab	38.5 b
8.0	26.4 b	85.5 c
8.6	76.5 c	99.1 d

[1]Means within columns followed by a common letter or letters are not significantly different at the 5% level as judged by the Duncan's Multiple Range Test.

Table 3 - Reaction of alachlor with other thiols.

Reactants	Rf of conjugate[1]	% of extractible ^{14}C in conjugate
^{14}C-alachlor + Cysteine	0.51	11.7% a[2]
+ Dithiothreitol	0.35	9.1% a
+ Coenzyme A	0.36	3.0% a
+ Methionine	N.R.[3]	--
+ Acetyl CoA	N.R.	--
+ Mercaptoethanol	N.R.	--
+ Ethane thiol	N.R.	--

[1]TLC system used: Silica gel 60F, E. Merck, in BuOh, AcOh, H_2O 30:10:15

[2]Means followed by the same letter or letters are not significantly different at the 5% level as judged by Duncan's Multiple Range Test.

[3]N.R. = no reaction

Table 4 - Reaction of GSH with various herbicides.

Reactions	Rf of Conjugate[1]	% of extractable ^{14}C in conjugate
^{3}H-GSH + R-25788	N.R.	--
+ ^{14}C-Buthidazole	N.R.	--
+ ^{14}C-EPTC	N.R.	--
+ ^{14}C-EPTC S=O	0.38	60.3%
+ ^{14}C-Atrazine	N.R.	--

[1]TLC system: Silica gel 60F, E. Merck: BuOh, AcOh, H_2O 30:10:15

Table 5 - Response of atrazine suscrptible, GSH-S-transferase deficient corn inbred line GT-112 to butylate, EPTC, alachlor, and atrazine in three experiments.

Exp.	Herbicide	(rate) (kg/ha)	Corn Height R-25788 0.0 kg/ha (cm)	Corn Height R-25788 1.12 kg/ha (cm)
(1)	Control		21.7 c[1]	23.7 c
	Butylate	(3.36)	23.4 c	22.1 c
	Alachlor	(4.48)	13.9 b	23.5 c
	Atrazine	(6.72)	6.9 a	8.9 a
(2)	Control		41.0 b	34.5 b
	EPTC	(6.72)	39.0 b	36.6 b
	Atrazine	(6.72)	14.0 a	17.4 a
(3)	Control		26.2 b	24.2 ab
	Alachlor	(6.72)	20.8 a	26.3 b

[1] Means within experiments followed by a common letter or letters are not significantly different at the 5% level according to Duncan's Multiple Range Test.

CHAPTER 5

Summary

The herbicide antidote R-25788 protects corn from herbicide injury by either acting as a competitive inhibitor to the herbicide, by increasing the rate of herbicide detoxification by glutathione conjugation, by increasing the rate of thiocarbamate sulfoxidation, or by some combination of these hypothesis. R-25788 does not protect corn by preventing herbicide induced inhibitions of gibberellin synthesis or lipid synthesis in corn. The competitive inhibitor hypothesis is supported by the observation that the antidote is similar in structure to both the acetanilide and thiocarbamate herbicides and protects corn from both. The hypothesis that R-25788 could protect corn by increasing the rate of glutathione-herbicide conjugation is supported by the rapid conjugation reaction that occures between glutathione and acetanilide herbicides or thiocarbamate sulfoxides *in vitro*.

However, since R-25788 was required to protect atrazine-susceptible corn from alachlor injury but not from thiocarbamate injury, the mode of action of R-25788 could differ between the acetanilide and the thiocarbamate herbicides. It is therefore suggested that R-25788 might increase the rate of sulfoxidation of thiocarbamates to their non-phytotoxic sulfoxide derivates. The driving force responsible for increased thiocarbamate sulfoxidation, however, could be increased thiocarbamate sulfoxide-glutathione conjugation.

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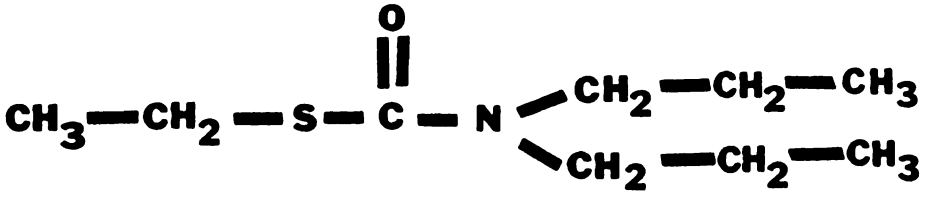
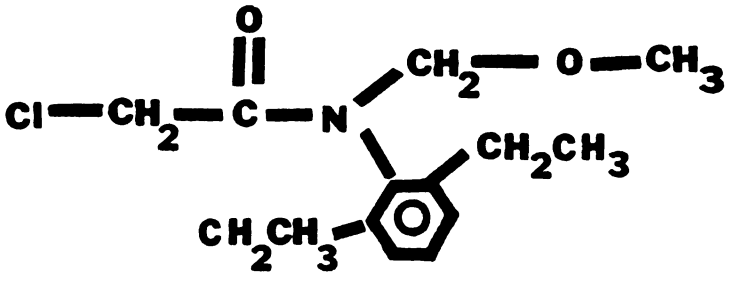
APPENDICES

Appendix A - Structures of one thiocarbamate and five acetanilide herbicides referred to in the text.

EPTC



ALACHLOR



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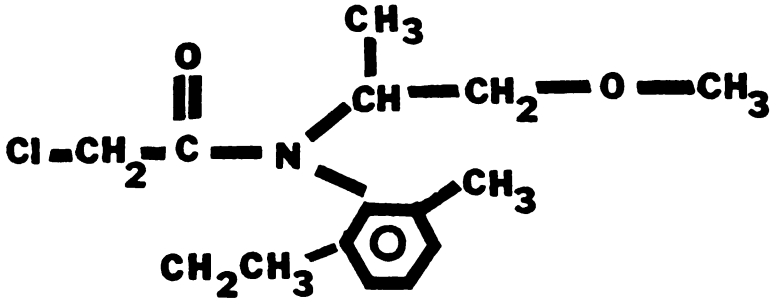

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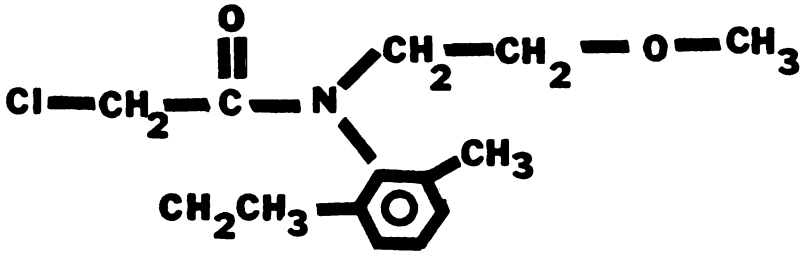
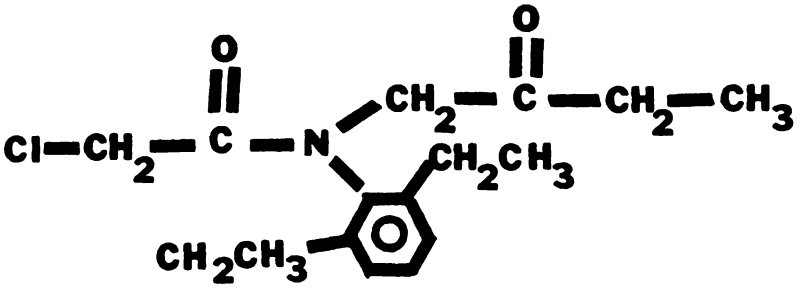


H - 22234



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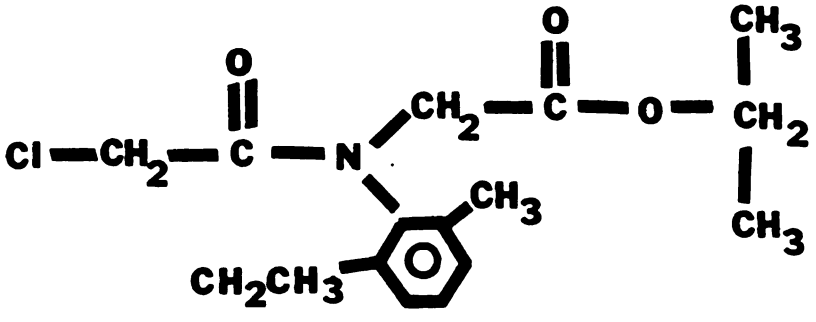
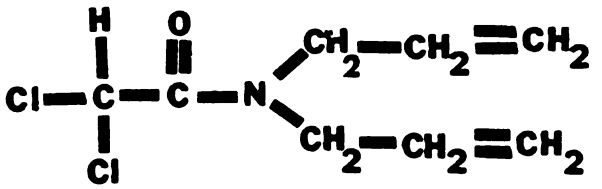


Appendix B - **Structures of five compounds screened as potential antidotes to acetanilide herbicide injury to corn.**

R-25788



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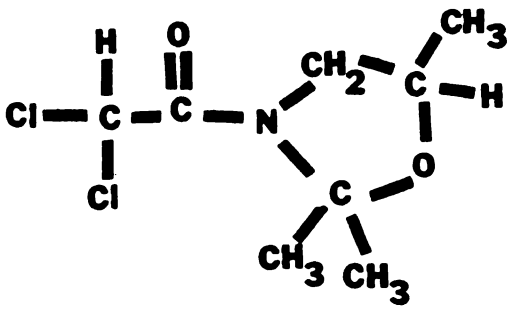

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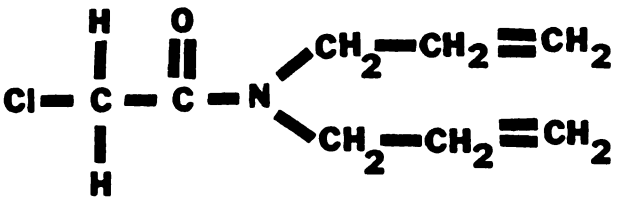
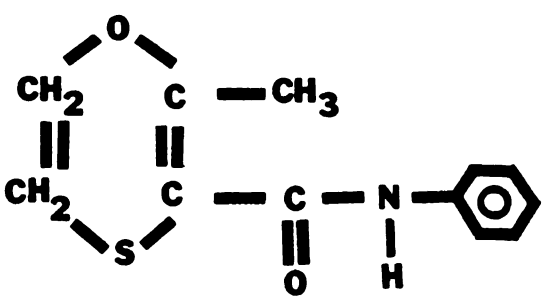


CARBOXIN



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