TOXICITY, SELECTIVITY, UPTAKE, DISTRIBUTION AND SITE OF ACTION OF EPTC IN CORN (*ZEA MAYS* L.) AS AFFECTED BY A HERBICIDE ANTIDOTE

by

Erasmo G. Sagaral

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APPROVED:

Chester L. Foy
C. L. Foy, Chairman

S.W. Bingham
S.W. Bingham

D D Wolf
D. D. Wolf

J. S. Coartney
J. S. Coartney

J. A. Swader
J. A. Swader

D. M. Orcutt
D. M. Orcutt

August, 1978

Blacksburg, Virginia

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I. INTRODUCTION

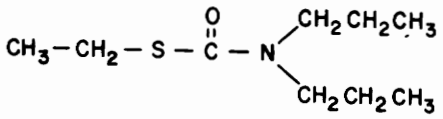
EPTC (S-ethyl dipropylthiocarbamate) was the first thiocarbamate introduced by the Stauffer Chemical Company in 1956 for use as an experimental herbicide for the control of grasses and many of the broadleaf weeds (1, 4, 8). It has many desirable characteristics including low toxicity to mammals and wildlife. EPTC also undergoes rapid degradation in the environment so it is appropriate for use in crop rotation sequences (10). Its chemical structure is shown in Figure 1. The volatile nature of the chemical resulted in highly variable weed control when it was first used as a surface applied preemergence herbicide. Soil incorporation corrected this deficiency and provided the first general use of that technique (8).

EPTC is federally registered in the United States for use in alfalfa (Medicago sativa L.), corn (Zea mays L.), cotton (Gossypium hirsutum L.), beans (Phaseolus spp.), pea (Pisum sativum L.) and other crops (13). When EPTC is applied at low rates, it can stimulate weed seed germination (5, 9). However, when high rates of this herbicide are used to control difficult weed species, severe stunting and injury of corn may result (11, 12).

The "antidote" concept of treating plants with a chemical to protect them from the injurious effects of herbicide was proposed in 1959. Hofmann (6) reported that several compounds when applied to the seed would protect wheat (Triticum vulgare L.) against damage by barban (4-chloro-2-butynl-m-chlorocarbanilate). These compounds were not developed commercially but, in 1969, a more efficient compound NA

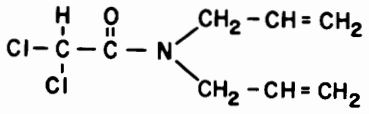
1. EPTC (Eptam)

S-ethyl dipropylthiocarbamate



2. R-25788

N,N-diallyl-2,2-dichloroacetamide



3. Eradicane — Mixture of EPTC and R-25788

Figure 1-1. Names and chemical structures of EPTC (herbicide), R-25788 (antidote) and their formulated mixture (Eradicane).

(1,8-naphthalic anhydride) was found to protect corn from EPTC injury (3, 6) as well as chloroacetanilide and dithiocarbamate herbicide injury to sorghum and molinate (S-ethyl hexahydro-1H-azepine-1-carbothioate) injury to rice (7).

In 1972, the Stauffer Chemical Company introduced R-25788 (N,N-diallyl-2,2-dichloroacetamide) which is marketed with EPTC as Eradicane and with butylate (S-ethyl diisobutylthiocarbamate) as Sutan +. It is incorporated into the soil for better effectiveness and has greater specificity for crops than does NA. This specificity allows it to be used as a formulation or tank mix in preference to seed dressing (2). R-25788 was found to be more effective than NA in reducing corn injury due to EPTC herbicide (3).

Despite considerable progress made in the use of EPTC alone, and recently in combination with the antidote R-25788, information is still inadequate to understand the responses of various corn cultivars under field conditions. Also, little information is available concerning the mechanism of tolerance and susceptibility in some corn cultivars, its effects on carbohydrate composition at different stages of plant growth and its effect on plant metabolic processes.

Objectives of the present study were to:

a) determine the response of different corn cultivars to various rates of EPTC and or antidote R-25788 under field as well as greenhouse conditions, with the aim to learning why some corn cultivars are highly tolerant and some very susceptible to EPTC despite the use of antidote.

b) determine by radiotracer techniques, the patterns of uptake distribution, translocation and metabolism of EPTC and antidote R-25788 in corn protoplasts and Chlorella cells.

c) determine the relationships between EPTC toxicity and carbohydrate contents of selected corn cultivars at different stages of growth.

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II. THE EFFECT OF ANTIDOTE R-25788 IN PROTECTING CORN FROM EPTC INJURY

Abstract. Field experiments were conducted in 1976 and 1977 at two locations in eastern Virginia to determine the effectiveness of the herbicide antidote R-25788 (N,N-diallyl-2,2-dichloroacetamide) in protecting selected corn (Zea mays L.) cultivars from EPTC (S-ethyl dipropylthiocarbamate) injury. Preplant incorporated applications were made to Othello fine sandy loam soil containing 3.1% organic matter and Dothan sandy soil with less than 1.0% organic matter. Fifteen corn cultivars were each planted in four rows at spacing of 23 x 91 cm on May 5, 1976 and May 18, 1977, respectively. Weed control and toxicity ratings were evaluated at appropriate intervals after herbicide application, and grain yields were recorded at the end of the growing season. EPTC at the rate of 6.7 kg/ha was 20 to 30% more toxic to the susceptible cultivars under sandy and lower organic soil conditions than with fine sandy loam soil. Herbicide treatments were effective in suppressing weed growth at the two experimental locations except for broadleaf dock (Rumex obtusifolius L.) which germinated soon after EPTC at 13.4 kg/ha. The 'TXS114' cultivar was found to be highly tolerant to EPTC at 6.7 kg/ha at both locations. The other corn cultivars used which were observed to be susceptible to EPTC applications, showed significant improvement in their stands without any noticeable symptoms of toxicity when the antidote R-25788 was used in combination with EPTC. The use of the antidote R-25788 in combination with EPTC in DeKalb 'XL55' and 'XL379' cultivars did not alleviate EPTC toxicity at the rate

of EPTC used. There was a more obvious advantage to using the antidote with EPTC in susceptible than in tolerant corn cultivars. EPTC applied in combination with R-25788 showed no or only slight injury to corn plants thus resulting in higher yields than herbicide treatments without the antidote. In general, earlier and more vigorous seedling establishment was observed in the Othello fine sandy loam soil (sand 53%, silt 33%, clay 14%) than in Dothan loamy sand soil (sand 81%, silt 12%, clay 6%).

INTRODUCTION

Weed control in corn (Zea mays L.) with high rates of thiocarbamate herbicides often causes severe injury to the crop. Twisting of the leaves, reduced growth, and death of the highly susceptible species are the common symptoms (15, 16, 17). Dawson (9) found that EPTC (S-ethyl dipropylthiocarbamate) caused a "zigzag pattern of kinking" of the first internode of barnyardgrass (Echinochloa crusgalli L.) and noted that the developing foliar leaves within the coleoptile were the major site of EPTC injury. Similar observations were made by Gray (10) and Appleby et al. (1) and they suggested that the root was the major site of uptake.

Gray and Weierich (11) studied the importance of root, shoot and seed exposure to the herbicidal activity of EPTC using the charcoal barrier method to prevent movement of EPTC vapor in soil. They found that exposing the roots of barley (Hordeum vulgare L.), oats (Avena sativa L.) barnyardgrass, Italian ryegrass (Lolium multiflorum L.), wheat (Triticum vulgare L.), rice (Oryza sativa L.), cotton (Gossypium hirsutum L.) and yellow nutsedge (Cyperus rotundus L.) to EPTC resulted in more injury, than shoot exposure of the same species. Seed exposure brought about variable results. EPTC applied at concentrations as low as 1 ppm to the roots in culture solution inhibited root growth of oats, barley and corn.

Apparently, there is an antagonistic interaction between EPTC and 2,4-D [2,4-dichlorophenoxy)acetic acid] in corn and sorghum (Sorghum

vulgare Pers.)(2), but the interaction is believed to be additive on dicotyledonous species (14). Chang et al. (7) found R-25788 (N,N-diallyl-2,2-dichloroacetamide) to be more effective than NA (1,8-naphthalic anhydride) in reducing corn injury due to EPTC herbicide. R-25788 in combination with EPTC eliminated injury symptoms in corn (5, 6) and greatly increased yields (12). The highest yield was obtained in mixtures of vernolate (S-propyl dipropylthiocarbamate) + R-25788 and atrazine [2,chloro-4-(ethyl-amino)-6-(isopropylamino)-s-triazine] at rates of 6 + 2 lb/A.

EPTC applied alone and in combination with R-25788 can cause more damage to corn on light soils than on heavy soils (8) and is less influenced by temperature and soil moisture (4). The depth of planting (12, 19) temperature and position of the seed at planting can affect toxicity (3). Thomas and Gray (18) found that the half life of R-25788 at 27 C was 8 days in Sorrento loam and 9 days in Felton loamy sand. At 5 C, the half life was 6.5 and 5 weeks, respectively, in the two soils. R-25788 disappeared much faster in nonautoclaved than in autoclaved soils, indicating that microbial action was responsible for the disappearance from moist soils.

In 1972, Rains and Fletchall (15) found that EPTC at 6 lb/A injured 84% of the corn plants while 8 lb/A injured 93%, however, only about 3% of the plants were injured by EPTC when the seeds were treated with 0.125% R-25788. With NA at 0.5% concentration, to treated seeds, 12% of the corn plants were injured by 6 lb/A and 9% when EPTC was applied at the rate of 8 lb/A.

Wright et al. (20) tested the effect of R-25788 on EPTC injury in 300 corn genotypes, of which there were 100 dent corn inbreds, 80 sweet corn lines, 20 Kentucky sterile lines and 100 were dent corn hybrids. The results of the study showed that dent corn inbreds were most susceptible to EPTC even if R-25788 were present. Differences in plant responses to EPTC treatment were thought to be due to genetic variation (13).

The objective of this study was to determine the response of different corn cultivars to varying rates of EPTC and antidote R-25788 under field conditions at two different locations in Virginia.

MATERIALS AND METHODS

Field experiments were conducted during the 1976 and 1977 crop seasons at two locations in eastern Virginia. The Othello fine sandy loam soil at the Holland area may be characterized as follows: sand 53.17%, silt 33.13%, clay 13.7%, pH 5.4 and O.M. 3.1%. In contrast, the Dothan loamy sand at Emporia has the following properties: sand 81.37%, silt 12.33%, clay 6.31%, pH 5.5 and O.M. 0.8%. The weed species at the two locations before and during these experiments are shown in Table II-I. Fifteen different corn cultivars obtained from various sources (Table II-2) were included in each experiment. Land preparations for the two locations were by disking and rotary tilling of the land two weeks before planting. Commercial formulations of the EPTC herbicide and the antidote R-25788 were used either alone or in combination, and treatments were sprayed on the soil surface using a

Table II-1. Common and scientific names of major weed species found before and during the conduct of the experiments at two locations in Virginia, 1976-77.

Common name	Scientific name	Location[a]	
		Holland	Emporia
1. Fall panicum	Panicum dichotomiflorum Mich.	X	X
2. Yellow nutsedge	Cyperus esculentus L.	X	X
3. Redroot pigweed	Amaranthus retroflexus L	X	X
4. Jimsonweed	Datura stramonium L.	X	-
5. Velvetleaf	Abutilon theoprasti Medic.	X	-
6. Yerba-de-tago	Eclipta alba L.	X	-
7. Carpetweed	Mollugo verticillata L.	X	X
8. Common Ragweed	Ambrosia artemisiifolia L.	X	-
9. Smartweed	Polygonum pensylvanicum L.	X	-
10. Large crabgrass	Digitaria sanguinalis L.	X	X
11. Broadleaf dock	Rumex obtusifolius L.	X	-
12. Lambsquarters	Chenopodium album L.	X	X
13. Goosegrass	Eleusine indica L.	-	X

[a] Letter X indicates presence of the weed species.

Table II-2. Corn cultivars used in the study and their sources.

Cultivar or trade name	Source of seed
1. Trojan TXS114	Trojan Seed Co., Windfall, IN
2. Trojan TXS113	" " " " "
3. Trojan TXS111	" " " " "
4. Trojan TXS115A	" " " " "
5. DeKalb XL80A	DeKalb Agric. Association, DeKalb, IL
6. DeKalb XL43	" " " " "
7. DeKalb XL379[a]	" " " " "
8. DeKalb XL22B	" " " " "
9. DeKalb XL55[a]	" " " " "
10. Dennis DS-37	Dennis Hybrids, Windfall, IN
11. Funks G-4646	A.H. Hoffman Seed Inc., Landisville, PA
12. Funks G-4525	" " " "
13. McNair RX94[a]	McNair Seed Co., Laurinburg, NC
14. Hofmeyer SX71	A.N. Hofmeyer, Williamsburg, VA
15. Todd M-98	Todd Hybrid Sales, Abbotstown, IA
16. Pioneer Brand 3780[a]	Pioneer Corn Co., Tipton, IN
17. Northrup-King PX77[a]	Northrup-King and Co., Minneapolis, MN
18. Northrup-King PX79[a]	" " " "

[a] Planted only at one location

tractor-mounted power sprayer. The volume of spray was 170 l/ha. The chemicals were incorporated into the soil immediately by means of a McClenny PTO rotary tiller operating to a depth of about 10 cm. Planting was in three or four rows at a spacing of 23 x 91 cm on May 5, 1976 at the Holland area, and May 18, 1977 at Emporia. One seed per hill was planted at a depth of 3 cm. A uniform topdressing of 45 kg/ha N fertilizer in the form of ammonium nitrate was applied to the plots. Weed control and toxicity ratings were evaluated at appropriate intervals after spraying treatments and grain yields were recorded at the end of the growing season.

In order to confirm the field performance of the various corn cultivars under greenhouse conditions, soil samples from the two locations were collected for study. Soil was mixed thoroughly and sprayed with EPTC, with and without the antidote using a laboratory sprayer. The volume of spray was the same as was used under field conditions. The soil was then mixed in plastic bags and placed in 15 cm diameter pots. Four seeds were planted in each pot and the pots arranged in split plot design in three replications.

RESULTS AND DISCUSSION

Holland study (Othello fine sandy loam soil).

Three hours after planting, there was 0.18 in of precipitation, another 0.04 and 0.02 in two and three days after planting, respectively. This condition resulted in uniform emergence of the corn seedlings. The density and stand of the various weed species in the

untreated control plots were excellent at early stages of plant growth and were indicative of good weed control effectiveness in herbicide treated plots. The toxicity and weed control ratings were evaluated 30 days after herbicide treatments and are shown in Tables II-3 and II-4. The injury symptoms were clearly noticeable when the primary leaf pushed its way through the coleoptile. The emerging leaves were twisted, which was noticeable particularly with the susceptible cultivars. The 'TXS114' cultivar was highly tolerant to EPTC at 6.7 kg/ha and only showed a slight twisting and folding of the youngest leaf at the highest rate of 13.4 kg/ha. However, where the antidote R-25788 was mixed with EPTC, there was not only excellent control of the weeds, but also the corn plants appeared healthy. Three of the DeKalb cultivars 'XL22B', 'XL43' and 'XL80A' were among the cultivars found to be highly sensitive to EPTC. The other susceptible corn cultivars were 'G-4646', 'RX94', 'M-98', 'PX79', 'SX71', 'TXS111', and 'TXS113'. Some of the moderately tolerant cultivars were 'DS-37', 'G-4525' and 'TXS115A'. Where the antidote R-25788 was used in combination with EPTC at 6.7 kg/ha, the injury symptoms usually caused by EPTC were eliminated.

Weed control. Fall panicum and yellow nutsedge, which were the predominant weed species in the experimental plots, were effectively controlled with EPTC at 6.7 kg/ha. The only weed species that escaped EPTC spray at 13.4 kg/ha was broadleaf dock and the reason for this resistance is probably due to its being a perennial. Some of the early weed species that had early regrowth were yerba-de-tago, redroot pigweed and jimsonweed. These weed species appeared 60 days after EPTC appli-

Table II-3. Effect of EPTC with and without the antidote R-25788 on toxicity in various corn cultivars 30 days after treatment in Othello fine sandy loam soil at Holland, Virginia, 1976.[a]

Treatment[b]	Rate (kg/ha)	Cultivar															Treatment mean[d]
		XL22B	XL43	G-4646	RX94	M-98	TXS111	PX79	SX71	TXS113	XL80A	PX77	TXS115A	G-4525	DS-37	TXS114	
1. EPTC	6.7	5.7	7.5	6.3	6.7	5.0	4.3	6.0	4.7	4.0	5.0	3.3	2.7	2.0	1.8	0.7	4.4b
2. EPTC	13.7	8.0	9.7	9.7	9.8	8.0	8.7	9.0	8.5	9.0	8.5	7.2	7.5	7.7	6.7	4.3	8.2a
3. EPT+R-25788	6.7+0.56	4.0	2.7	1.3	1.3	2.3	2.7	1.3	2.3	2.0	1.3	1.7	2.0	1.7	1.0	0.7	1.9c
4. EPTC+R-25788	13.4+1.12	4.7	1.3	2.3	1.3	2.8	2.3	1.0	1.3	1.3	1.5	1.8	1.0	1.0	1.7	0	1.7c
5. Mechanical[c]	(once)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 d
6. Untreated control		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 d
Cultivar mean[d]		3.7a	3.5ab	3.3abc	3.2abc	3.0abc	3.0bc	2.9bcd	2.8cd	2.7cde	2.7cde	2.3def	2.2ef	2.1f	1.9f	1.0g	

[a] Visual toxicity ratings are averages of three replications based on a scale of 0 = no injury and 10 = complete kill.

[b] Preplant incorporated into the soil 10 cm deep immediately before planting.

[c] Rotary tilled once with hand tractor and followed with light hoeing 40 days after planting.

[d] Cultivar means or treatment means followed by a common letter are not significantly different at the 5% level according to the Duncan's Multiple Range Test.

Table II-4. Effect of EPTC with and without antidote R-25788 on weed control in various corn cultivars 30 days after treatment in Othello fine sandy loam soil at Holland, Virginia, 1976.[a]

Treatment[b]	Rate (kg/ha)	Cultivar															Treatment mean[d]
		DS-37	TXS115A	PX77	TXS114	TXS111	TXS113	SX71	RX94	M-98	G-4646	G-4525	XL80A	XL22B	XL43	PX79	
1. EPTC	6.7	9.2	6.0	9.0	9.0	9.3	9.0	8.8	9.5	9.0	9.5	9.0	9.2	8.0	9.2	9.2	9.1b
2. EPTC	13.4	10.0	10.0	9.8	10.0	10.0	10.0	9.8	9.8	9.8	10.0	9.8	9.8	9.8	10.0	10.0	9.9a
3. EPTC+R-25788	6.7+0.56	9.2	9.2	9.2	9.3	9.3	9.3	9.3	9.3	9.0	9.2	8.5	9.0	9.2	8.2	9.7	9.1b
4. EPTC+R-25788	13.4+1.12	10.0	10.0	9.8	10.0	10.0	9.7	10.0	10.0	10.0	10.0	9.8	10.0	10.0	9.8	10.0	9.9a
5. Mechanical[c]	(once)	0	0	0	9	0	0.3	0.3	0.3	0	0	0	0.2	0	0	0.3	0.1c
6. Untreated control		0	0.2	0.2	0	0.2	0.2	0.2	0.3	0	0	0.2	0.2	0	0	0.3	0.1c
Cultivar mean[d]		6.4ab	5.9ab	6.3abc	6.4ab	6.5ab	6.4ab	6.4ab	6.6a	6.4ab	6.5ab	6.2bc	6.4ab	6.1c	6.2bc	6.6a	

[a] Weed control ratings ar e averages of three replications based on a scale of 0 = no control and 10 = complete control.

[b] Preplant incorporated into the soil 10 cm deep immediately before planting.

[c] Rotary tilled once with hand tractor followed by light hoeing 40 days after planting.

[d] Cultivar or treatment means followed by a common letter are not significantly different at the 5% level according to the Duncan's Multiple Range Test.

cation at 13.4 kg/ha. The combination of EPTC and R-25788 resulted in no reduction in weed control effectiveness of EPTC.

Yield. Yield data are presented in Table II-5. Highest yields were obtained with the cultivars 'G-4525', 'TXS115A' and 'DS-37'. The reason for the high yields however, is believed not due to resistance from EPTC toxicity, but mainly due to plant type and high yield potential coupled with good weed control. The cultivar 'TXS114' which was found highly tolerant to EPTC in this study performed well with EPTC at 6.7 kg/ha. The reason for its low yields despite the addition of antidote R-25788 is not known; however, some physiological disorders resulting from chemical antagonism, which were not visible morphologically, may have been responsible for this reduction in yield.

Emporia study (Dothan loamy sand).

The soil at Emporia is sandier and contains a lower organic matter content (0.8%) than that at the Holland area. At the time of herbicide treatment, the soil was of moderate moisture content and there was no precipitation immediately following planting. The toxicity ratings taken 30 days after herbicide application indicated significant differences among treatments (Table II-6). The most effective treatments were those of EPTC applied in combination with the antidote R-25788 for all cultivars except for new ones included in this study. Cultivars 'XL55' and 'XL379' were highly sensitive to EPTC at 6.7 kg/ha even when the antidote R-25788 was added, and the injury symptoms were similar to that of EPTC alone. When EPTC was used alone, these cultivars were almost completely killed four weeks after treatment. The

Table 11-5. Effect of EPTC with and without antidote R-25788 on yields of 15 different corn cultivars grown in Othello fine sandy loam soil at Holland, Virginia, 1976.[a]

Treatment[b]	Rate (kg/ha)	Cultivar															Treatment mean[c]
		G-4525	TXS115A	DS-37	PX77	XL22B	G-4646	PX79	TXS113	XL43	TXS111	XL80A	M-98	TXS114	PX94	SX77	
		(Grain Yield (kg/hax10^2)															
1. EPTC	6.7	40.9	62.3	40.5	38.0	33.7	38.6	40.7	47.4	42.6	41.2	34.7	37.6	38.6	32.2	37.4	41.1b
2. EPTC	13.4	51.5	38.5	35.6	41.1	39.7	11.3	25.4	27.9	10.1	35.7	32.6	20.8	35.8	15.8	22.6	29.6c
3. EPTC+R-25788	6.7+0.56	62.3	53.6	49.6	56.1	56.5	57.4	50.1	36.1	62.2	34.9	53.7	30.0	31.3	44.5	34.5	48.1a
4. EPTC+R-25788	13.4+1.12	62.1	41.8	51.4	49.0	48.3	56.9	58.6	59.6	38.4	47.1	37.2	48.0	22.9	45.5	47.9	47.7a
5. Mechanical	(once)[c]	33.2	39.4	41.5	30.3	32.2	39.4	30.6	28.8	37.8	29.1	22.1	27.9	36.5	20.0	19.5	32.2c
6. Untreated control		5.4	6.6	7.6	2.5	5.4	8.8	3.8	7.8	10.7	12.6	3.4	10.1	4.7	21.0	1.4	7.5d
Cultivar mean[d]		42.6a	40.4ab	37.9abc	36.2bc	36.0bc	35.4bcd	34.9cde	34.6cde	33.6cde	33.4cde	30.6def	30.0ef	29.8ef	26.7f		

[a] Yield of shelled corn, averages of three replications at 14% moisture.

[b] Preplant incorporated into the soil 10 cm deep immediately before planting.

[c] Rotary tilled with tractor and light hoeing 40d ays after planting.

[d] Cultivar or treatment means followed by the same letter are not signigicantly different at the 5% level according to the Duncan'a Multiple Range Test.

Table II-6. Effect of EPTC with and without the antidote R-25788 on toxicity in various corn cultivars 30 days after treatment in Dothan loamy sand at Emporia, Virginia. 1977.[a]

Treatment[b]	Rate (kg/ha)	Cultivar															Treatment mean[d]
		XL22B	XL43	G-4646	RX94	M-98	TXS111	XL55	XL379	TXS113	XL80A	3780	TXS115A	G-4525	DS-37	TXS114	
1. EPTC	6.7	9.9	9.1	9.4	8.6	6.9	9.0	10.0	9.6	8.6	8.6	9.8	6.3	8.6	4.5	2.9	8.1b
2. EPTC	13.4	10.0	9.9	10.0	9.6	9.0	9.8	10.0	10.0	9.3	9.4	9.4	8.8	9.1	6.3	7.8	8.8a
3. EPTC+R-25788	6.7+0.56	0.1	1.5	0.9	0.1	0.1	0.1	8.9	8.3	0	0	0.8	0	0.8	0	0	1.4c
4. EPTC+R-25788	13.4+1.12	1.4	2.0	1.8	0.5	0.3	0.1	9.1	8.1	0.4	0.3	0	0.3	1.0	0	0	1.7c
5. Mechanical[c]	3 Times	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 d
6. Untreated control		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 d
Cultivar mean[d]		3.6bcd	3.8b	3.7bc	3.1de	2.7fg	3.2de	6.3a	6.0a	3.0e	3.0ef	3.3cde	2.5g	3.1cde	1.8h	1.8cde	

[a] Visual toxicity ratings are averages of four replications based on a scale of 0 = no injury and 10 = complete kill.

[b] Preplant incorporated into the soil 10 cm deep immediately before planting.

[c] Rotary tilled three times with hand tractor followed by light hoeing as required to maintain weed free plots.

[d] Cultivar or treatment means followed by a common letter are not significantly different at the 5% level according to the Duncan's Multiple Range Test.

first and second leaves which were able to come out earlier from their coleoptiles were severely distorted, brittle and hard. Examination of the roots showed a significant reduction in growth compared to the untreated controls which showed extensive growth. The death of susceptible plant cultivars was possibly due to exhaustion of the carbohydrates resulting from reduced uptake of nutrients and water from the soil.

Weed control. Control of weeds in this area was much longer than that observed at the Holland experiment because regrowth first appeared only after about 60 days in plots treated with EPTC at 6.7 kg/ha. In plots treated with EPTC at 13.4 kg/ha, weeds appeared at about 80 days. Similar result was found in plots where EPTC was combined with the antidote R-25788. Some of the weed species which were earliest to appear were carpetweed, lambsquarters and pigweed. Table II-7 shows results of treatments taken late in the crop growing season.

Yield. The yield data in the Dothan loamy sand soil at Emporia area were not given much importance in this study because, during the ear-initiation stage, the area was exposed to severe drought which affected the yield.

Greenhouse study.

Results of the greenhouse work confirmed the field observations which showed that EPTC was 20 to 30% more toxic to the corn seedlings grown at the Dothan loamy sand at Emporia where the organic matter content is less than (1.0%) than in Othello fine sandy loam soil at Holland (3.1%) (Table II-8). In contrast, the effectiveness of the

Table II-7. Effect of EPTC with and without R-25788 on weed control in various corn cultivars 75 days after treatment in Dothan loamy sand at Emporia, Virginia, 1977.[a]

Treatment[b]	Rate (kg/ha)	Cultivar															Treatment mean[d]
		DS-37	TXS115A	3780	TXS114	TXS111	TXS113	XL55	RX94	M-98	G-4646	G-4525	XL80A	XL22B	XL43	XL379	
1. EPTC	5.7	9.0	9.0	7.5	9.0	9.5	10.0	9.0	9.5	9.5	9.0	9.0	10.0	9.0	8.0	8.0	8.4c
2. EPTC	13.4	9.5	8.0	9.0	10.0	9.0	9.5	9.0	10.0	10.0	9.5	10.0	10.0	9.0	9.5	9.5	8.5bc
3. EPTC+R-25788	6.7+0.56	9.5	9.5	9.5	9.5	10.0	9.5	9.5	9.0	10.0	10.0	10.0	10.0	10.0	9.5	9.5	9.4ab
4. EPTC+R-25788	13.4+1.12	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	9.5	10.0	9.5	10.0	9.5	10.0	9.0	9.5a
5. Mechanical[c]	3 Times	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0c
6. Untreated control		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 d
Cultivar mean[d]		9.1ab	8.8abc	8.3cd	9.3a	8.8abc	8.9abc	8.1d	9.2ab	9.0abc	8.6bcd	8.9abc	9.1ab	8.5bcd	8.9abc	8.6abcd	

[a] Weed control ratings are averages of four replications based on a scale of 0 = no control and 10 = complete control.

[b] Preplant incorporated into the soil 10 cm deep immediately before planting.

[c] Rotary tilled three times with hand tractor and followed with light hoeing as required to maintain weed free plots.

[d] Cultivar or treatment means followed by a common letter are not significantly different at the 5% level according to the Duncan's Multiple Range Test.

Table II-8. Comparative injury responses of four cultivars grown under two soil types in the greenhouse evaluated 30 days after treatment.[a]

Treatment[b]	Rate (kg/ha)	Soil Type: Othello Fine Sandy Loam (Cultivar)				Soil Type: Dothan Loamy Sand (Cultivar)			
		TXS114	DS-37	SL43	XL55	TXS114	DS-37	XL43	XL55
1. EPTC	6.7	0	1.5	4.0	7.0	0	3.0	7.0	9.0
2. EPTC	13.4	2.5	6.0	8.0	10.0	4.0	8.0	10.0	10.0
3. EPTC+R-25788	6.7+0.56	0	0	2.0	6.0	0	0	4.0	9.0
4. EPTC+R-25788	13.4+1.12	0	2.0	8.0	10.0	1.0	4.0	8.0	10.0
5. Untreated control		0	0	0	0	0	0	0	0

[a] Visual toxicity ratings are averages of four replications based on a scale of 0 = no injury and 10 = complete kill. Injury was based on twisting of leaves, hardening or stunted growth.

[b] Treatments were incorporated into the soil 10 cm deep immediately before planting. Pot sizes were 15 cm in diameter.

herbicide in controlling the weeds persisted 20 to 30% longer in the Dothan loamy sand than in the Othello soil. Figure II-1 shows the injury symptoms of 'XL55' and 'TXS114' corn cultivars treated with EPTC at 6.7 kg/ha and 13.4 kg/ha, respectively. The 'TXS114' cultivar which had been observed previously to be highly tolerant to EPTC under field conditions was found to show some abnormality symptoms in the greenhouse due to EPTC treatment. This was observed at about 60 days after planting when the temperature in the greenhouse was below normal. It is believed then that the toxicity may be the result of EPTC being converted to some other forms more toxic to the corn or that the formation of adventatious roots within a confined space in a 15-cm pot may have contributed to a more rapid uptake from the soil.

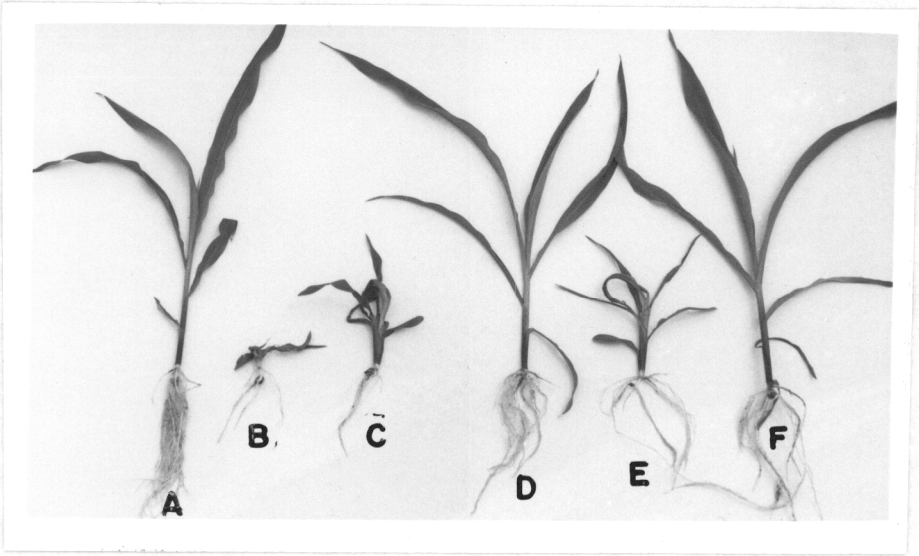

Figure II-1. Injury symptoms in corn cultivars resulting from EPTC treatment. (A) 'XL55' control, (B)'XL55' treated with EPTC (6.7 kg/ha), (C) 'XL55' treated with EPTC + R-25788 (6.7 + 0.56 kg/ha), (D) 'TXS114' control, (E) 'TXS114' treated with EPTC (13.4 kg/ha) and (F) 'TXS114' treated with EPTC + R-25788 (13.4 + 1.12 kg/ha).

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III. COMPARATIVE UPTAKE, DISTRIBUTION, AND TRANSLOCATION OF EPTC AND ANTIDOTE R-25788 IN TOLERANT AND SUSCEPTIBLE CULTIVARS OF CORN

Abstract. The study on uptake, distribution, and translocation of ^{14}C-EPTC (S-ethyl-1-^{14}C dipropylthiocarbamate) and the antidote R-25788 (N,N-diallyl-2,2-dichloroacetamide) were conducted in corn (Zea mays L.). Results indicated differential uptake and distribution of the labeled EPTC in corn with 'XL55', a highly susceptible corn cultivar absorbing about 20% more than 'TXS114', a highly tolerant cultivar after 12 hr of incubation. The movement of accumulated ^{14}C was toward the actively growing portions of the shoots and root tips of seedlings. The embryo of 'TXS114' accumulated about 48% of the total uptake compared to 60% in the 'XL55' cultivar. The combination of antidote R-25788 with EPTC did not significantly reduce the total uptake by seeds of susceptible cultivars, but rather seemed to stimulate uptake by the more tolerant 'TXS114' and 'DS-37' cultivars during the incubation period. Apparently, R-25788 conjugated or otherwise delayed movement of ^{14}C toward the shoot by concentrating ^{14}C radioactivity in the seed of highly tolerant compared to shoot of susceptible cultivars. The movement of ^{14}C to shoot in 'XL55' appeared to be unaltered by R-25788. Therefore, the susceptibility of 'XL55' is apparently due to its relatively high uptake and site of accumulation.

INTRODUCTION

Uptake and movement of herbicides vary considerably according to the nature of the chemical and plant species. Many herbicides show an accumulation pattern which can determine their effects on plants. In studies with EPTC (S-ethyl-N,N-dipropylthiocarbamate), Fang and Theisen (4) indicated that the sulfur labeled EPTC accumulated in the above-ground portions of beans (Phaseolus sp.) peas (Pisum sativum L.) and corn (Zea mays L.)

Gray (5) showed that EPTC was rapidly translocated upward throughout the leaves and stems. He indicated that substantial radioactive CO_2 was evolved when the alkyl groups attached to sulfur and nitrogen were labeled with carbon 14. He identified the radioactive metabolites as amino acids, fatty acids, organic plant acids, and sugars. Yamaguchi (13) found ^{35}S-EPTC to be very mobile. When used as vapor on leaves, the labeled EPTC was absorbed and translocated symplastically in corn. When applied to the roots ^{35}S-EPTC was readily absorbed and distributed apoplastically into the foliage. Nalewaja et al. (9) found radioactive accumulation of ^{14}C-EPTC in alfalfa for five days to be twice the accumulation in two days.

Weinberg and Castelfranco (11) treated cotyledons of 6-day old ethiolated cucumber (Cucumis sativus L.) with EPTC and found an accumulation of 35% to 45% more photochlorophyll and 30 to 40% more glycolipids than in the control.

Prendeville et al. (10) investigated species differences in the site of shoot uptake and tolerance. Using pots with wax barriers to separate treated and untreated soil, they tested barley (Hordeum vulgare L.), wheat (Triticum vulgare L.), oats (Avena sativa L.) and sorghum (Sorghum vulgare L.). Results indicated that wheat, barley and oats were severely injured when treated at the coleoptile internode. Exposure of the shoot above this zone did not affect growth. Sorghum was severely injured regardless of the shoot zone exposed. This shoot absorbed twice the amount of EPTC absorbed by wheat. Laboratory studies with ^{14}C-butylate (S-ethyl diisobutyl-thiocarbamate) by Wright and Rieck (12) showed that the resistant hybrid 'Pioneer 3030' absorbed less ^{14}C-butylate and metabolized the herbicide to $^{14}CO_2$ than did the other variety 'PAG644'. Chang et al. (3) claimed that more of the $^{14}CO_2$ was released from plants treated with ^{14}C-EPTC and R-25788 (N,N-diallyl-2,2-dichloroacetamide) than with EPTC alone. However, they suggested that these differences do not seem large enough to fully explain the effect on the plant. In contrast, Carringer et al. (2) found no effect of R-25788 on $^{14}CO_2$ evolution from carbonyl-^{14}C-EPTC-treated corn. Treatment with 1 ppm of R-25788 increased production of water soluble and unextractable products from EPTC and increased the rate of disappearance of soluble ^{14}C radioactivity.

Murphy et al. (8) found that corn 'DeKalb XL374' seedlings grown in soil treated with (2-^{14}C)-R-25788 liberated 6% of the absorbed radioactivity as $^{14}CO_2$ and other volatile radioactive

products during a 10-day test. They found the metabolite of R-25788 to include N-allyl-2,2-dichloroacetamide; N,N-diallyl-glycomide; N,N-diallyloxamic acid and the glycoside of N,N-diallylglycolamide.

Leavitt and Penner (7) did not find EPTC and R-25788, whether used alone or in combination, to change the amount of epicuticular wax deposited upon corn leaf surfaces. However, they found that EPTC used alone changed the physical arrangement of wax on the corn leaf surfaces with the formation of large aggregates of epicuticular wax. The antidote R-25788 used alone caused no such effect. When R-25788 was used in combination with EPTC, the EPTC induced aggregation of epicuticular wax was prevented.

It was found by Wright and Rieck (12) that R-25788 increased the metabolism of butylate in corn hybrids. Similarly, Guneyli as cited by Blair et al. (1) found increased absorption of ^{14}C-EPTC by a single variety of corn seedling and stimulated EPTC metabolism due to R-25788, possibly by activating the enzyme system that breaks down EPTC. This is identical to the mechanism suggested by Lay et al. (6) for R-25788.

The purpose of this study was to investigate varietal differences, effect of the antidote R-25788 on uptake, distribution and translocation of ^{14}C-EPTC in tolerant, medium and susceptible corn cultivars.

MATERIALS AND METHODS

Effect of duration of incubation on ^{14}C-EPTC accumulation. A total of sixty seeds from each cultivar were soaked in distilled water for 24 hr to provide a uniform moisture level for each seed, then air-dried for 12 hr. The seeds were then incubated at different intervals in solution made up of ^{14}C-EPTC (specific activity 1.3μCi/mM) added to technical EPTC to form 12 x 10^{-5}M final concentration. This was compared to a similar solution where EPTC at 12 x 10^{-5}M was combined with R-25788 at 1 x 10^{-5}M or a ratio of 12:1 of final concentration. The initial activity of ^{14}C-EPTC solution was 98,320 cpm/ml while the combination gave 98,680 cpm/ml. A solution (6 ml) was used to incubate 10 seeds of each cultivar in glass vials. After 12, 24 and 48 hr of incubation, seeds were washed with distilled water and air-dried. The seeds were combusted with the Intertechnique 4101 Sample Oxidizer and the $^{14}CO_2$ collected were assayed by scintillation spectrometry. The amount of uptake by the various corn cultivars are expressed in cpm/ mg dry wt of seed used.

Effect of seed coat on ^{14}C accumulation. In order to determine the initial distribution of ^{14}C-EPTC in tolerant as well susceptible corn cultivars, seeds of 'TXS114', a highly tolerant cultivar, 'DeKalb XL55' a highly sensitive cultivar, were used. Also, seeds of moderate susceptibilities were included for comparison. The seeds were soaked for 24 hr in distilled water, after which the pericarp or seed coat of some seeds were peeled off. They were then air-dried

and incubated as before to 4 ml of 12 x 10^{-5}M final concentration of ^{14}C-EPTC. The radioactivity of the solution was 125,330 cpm/ml. Three days after incubation, seeds were washed thoroughly with tap water and then sectioned into endosperm, embryo, and seed coat for intact seeds, while embryo and endosperm for those without seed coats. The separated plant parts were air-dried, oxidized, and radioactivity determined as previously. ^{14}C accumulation in various sections of corn cultivars used are reported in cpm/mg dry weight and expressed in percent.

Autoradiography. Seeds of each corn cultivar incubated as above were made to grow in 60 ml test tubes containing vermiculite moistened with distilled water. The seedlings were kept at room temperature of 25 $\pm$ 2 C and illuminated from above with fluorescent light at intensity of 14 ueinsteins.m^2.sec^{-1}.

Effect of R-25788 on ^{14}C accumulation and distribution. Five seeds of each cultivar were germinated in petri dishes (100 x 15 cm) lined with two layers of Whatman no. 1 filter paper for a period of five days. A solution of ^{14}C-EPTC mixed as before at 12 x 10^{-4}M final concentration and a combination of ^{14}C-EPTC and R-25788 at 12 x 10^{-4}M + 1 x 10^{-4}M final concentration were used to incubate corn seeds. A solution (10 ml) was used to incubate the seeds at room temperature under dark conditions. The seedings were washed thoroughly and then sectioned into seed, root, and shoot. These sections were oxidized and radioactivity determined as before.

Distribution of ^{14}C in seedlings with and without seed coat. Intact

seeds of two corn cultivars were soaked as before and then seed coat of some were removed. After incuabting the seeds for five days in 5 ml of ^{14}C-EPTC at 6 x 10^{-5}M final concentration in glass vials, they were transferred to 60 ml test tubes containing vermiculite and moistened with distilled water and maintained at room temperature as before. A combination of ^{14}C-EPTC and antidote R-25788 at 6 x 10^{-5} + 1 x 10^{-5}M final concentration was used for comparison. After growing the seedlings for six days, they were washed thoroughly with tap water and then sectioned into seed, 1 cm of root tips, internode (mesocotyl) and the portion above the coleoptilar node as the shoot. The term seed as used here was the remnant after the root and shoot of seedlings were removed. The air-dried seedling sections were oxidized and determined for radioactivity as previously.

^{14}C translocation. Seedlings of corn were grown in vermiculite in the greenhouse until they were in two to three leaf stage. Healthy seedlings from 'TXS114' and 'XL55' cultivars were selected. The roots were washed thoroughly and then each seedling was transfered to 20 ml test tubes containing 0.5 strength Hoagland solution. After 12 hr the solution was replaced entirely with new solution containing ^{14}C-EPTC in 15 ml of 12 x 10^{-5}M final concentration. The plants were removed after 48 hr, washed and dried at 35 C for three days. After pressing, the dried seedlings were autoradiographed using the Gaf Medical X-ray film 25 x 30/10 x 12 in for three weeks.

RESULTS AND DISCUSSION

Effect of duration of incubation. ^{14}C uptake by corn seeds are shown in Table III-1. The average weight of the seeds used in this study indicated that 'TXS114', a tolerant cultivar was about 5% heavier than 'XL55', a susceptible corn cultivar. In total uptake, 'XL55' accumulated 20% more ^{14}C than 'TXS114' 12 hr after incubation when the ^{14}C-EPTC was used alone. A combination of antidote R-25788 and ^{14}C-EPTC showed a slight reduction of about 7% uptake in 'XL55' cultivar, but not with 'TXS114'. Both cultivars accumulated ^{14}C at an almost linear rate with time, with 'XL55' accumulating about 35% more in radioactivity than what was accumulated in 12 hr. After 48 hr, two of the cultivars accumulated the same amount of radioactivity, except for 'TXS114' and 'XL43'. The result has shown no significant reduction in total uptake with the addition of R-25788 after 48 hr of incubation.

Effect of seed coat on ^{14}C accumulation. Seeds with and without their seed coats showed differential response in total uptake of the ^{14}C after 72 hr of incubation with the labeled EPTC. The intact seeds of 'TXS114' showed lower total uptake of ^{14}C compared to seeds of 'XL55', a highly susceptible cultivar (Table III-2). The results then suggest that seed coats were not preventing the entry of ^{14}C into the seed, but maybe slowing it down. Distribution of ^{14}C in germinating intact seeds, showed that 60% of the ^{14}C accumulated in the embryo of the cultivar 'XL55' compared to 48%

Table III-1. Effect of incubation time on uptake of ^{14}C-EPTC with and without antidote R-25788 by corn seeds.[a]

Treatment[b]	Concentration (M)	Cultivar	Seed weight (mg/seed)	Time of incubation (hr)		
				12	24	48
				(cpm/mg dry wt)		
^{14}C-EPTC	12×10^{-5}	TXS114	293	49.8	69.6	82.8
	"	DS-37	251	64.2	81.0	99.0
	"	XL43	325	49.8	64.2	85.2
	"	XL55	278	62.4	80.4	97.2
^{14}C-EPTC + R-25788	12×10^{-5} + 1×10^{-5}	TXS114	296	55.2	69.6	93.0
	"	DS-37	257	78.4	84.6	105.0
	"	XL43	318	50.4	64.8	84.0
	"	XL55	269	58.2	73.8	100.2

[a] Values are averages of four seeds combusted for each cultivar.

[b] Ten seeds incubated in 6 ml of treatment solution in sealed glass vials after soaking the seeds in distilled water for 24 hours and air-drying for 12 hours.

Table III-2. Distribution of ^{14}C in seedlings of tolerant and susceptible corn cultivars with and without seed coats after 72 hours incubation.[a]

Cultivar	Seed coat[b]	Total uptake (Cpm/mg dry wt)	Distribution of absorbed ^{14}C (% of total)		
			Endosperm	Embryo	Seed coat
TXS114	+	133.3	39	48	13
"	-	142.2	53	47	-
DS-37	+	155.7	21	63	16
"	-	139.0	37	63	-
XL43	+	121.0	24	57	19
"	-	157.8	39	61	-
XL55	+	222.2	23	60	17
"	-	162.7	41	59	-

[a] Values are averages of four seedlings incubated in 4 ml of ^{14}C-EPTC at 12 x 10^{-5}M final concentration.

[b] Intact seeds (+) or seed coat removed (-)

in the tolerant cultivar 'TXS114'. A similar distribution in seeds without seed coats of ^{14}C was observed. Seeds without seed coats showed an increased accumulation of ^{14}C in the endosperm. Intact 'XL55' seeds accumulated 24% more of ^{14}C than 'TXS114' cultivar. The 'DS-37', a moderately tolerant cultivar, showed 63% of ^{14}C to accumulate in the embryo for both intact and those without seed coats. The tolerance of this cultivar to EPTC appeared to be due to its high carbohydrate content in seeds (78%) compared to 56.7% in 'XL55'.

Autoradiography. A comparison of autoradiographs of seedlings grown from seeds incubated for 72 hr in ^{14}C-EPTC Solution are shown in Figures III-1 and III-2. The dark image indicate distribution of ^{14}C absorbed by seeds was accumulated more in coleoptile and shoot than in other sections of the seedlings. High accumulation in shoot indicates rapid movement of ^{14}C from seed toward the site of action which are the shoots and root tips.

Effect of R-25788 on ^{14}C accumulation and distribution. The effect of incubating seeds of various corn cultivars with ^{14}C-EPTC showed increasing uptake with varietal susceptibility. The 'TXS114' cultivar accumulated much less than in 'XL55', a susceptible cultivar (Table III-3). This is different from earlier observations where there was only slight difference in total uptake. The reason for this may be due to the constant contact of growing seedlings with the solution of ^{14}C-EPTC in horizontal position in petri dishes. The results however, confirmed earlier observations which showed

Table III-3. Distribution of ^{14}C in sections of corn seedlings as affected by incubating seeds in ^{14}C-EPTC with and without R-25788 for 5 days in petri dishes.[a]

Treatment[b]	Concentration (M)	Cultivar	Total uptake (cpm/mg dry wt)	Distribution of absorbed ^{14}C(% of total)		
				Seed[c]	Root	Shoot
1. ^{14}C-EPTC	12×10^{4}	TXS114	13.2	7.2	50.3	42.4
2. "	"	DS-37	21.5	9.0	38.0	21.5
3. "	"	XL43	19.4	21.5	27.7	59.8
4. "	"	XL55	26.3	16.6	24.8	58.6
5. ^{14}C-EPTC + R-25788	12×10^{-4} 1×10^{-4}	TXS114	13.8	22.5	27.5	49.9
6. "	"	DS-37	24.4	21.0	36.0	43.5
7. "	"	XL43	14.5	17.0	4.4	78.6
8. "	"	XL55	24.5	16.5	22.3	61.2

[a] Values are averages of five seedlings from each cultivar germinated in 10 ml treatment solution (final concentration).

[b] Corn germinated in dark under room temperature at 25 ± 2 C.

[c] Seed remnant after growth of root and shoot.

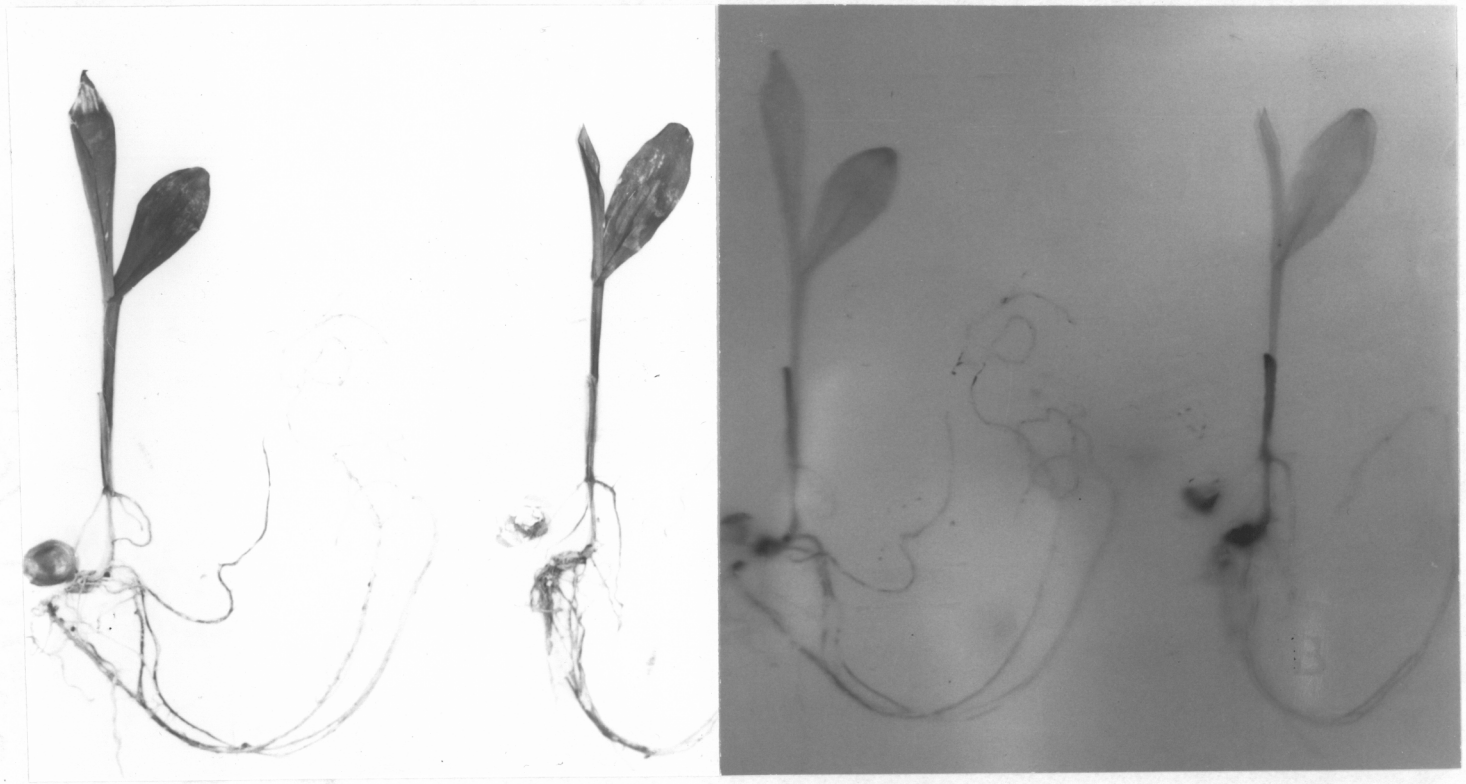

Figure III-1. Autoradiographs of corn cultivar 'XL55', a hybrid that is highly susceptible to EPTC, six days after emergence in vermiculite. Seeds were incubated, with seed coat (A) and without seed coat (B), for 72 hr in ^{14}C-EPTC solution at 12 x 10^{-5}M final concentration (125,330 cpm/ml).

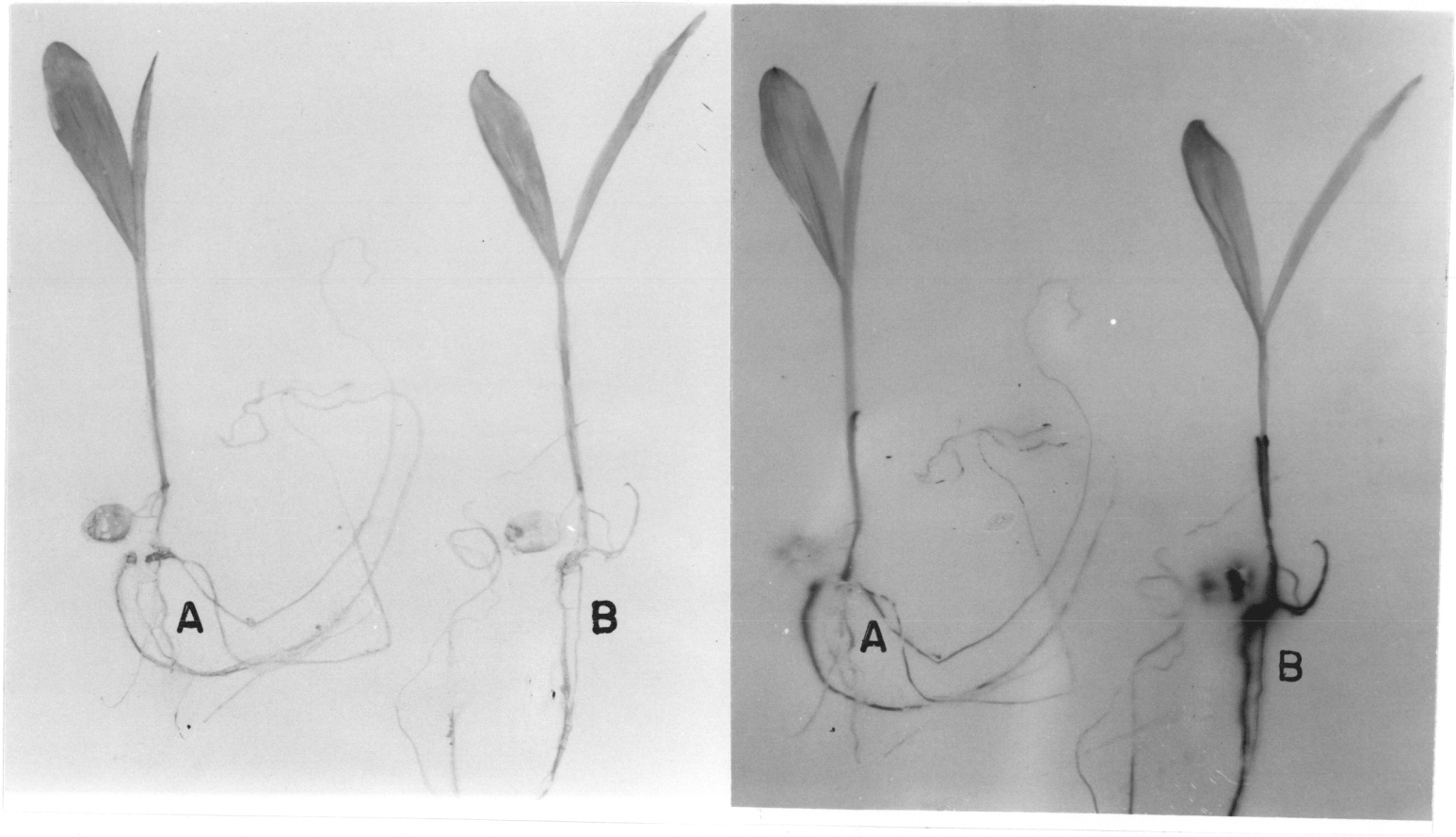

Figure III-2. Autoradiographs of corn cultivar 'TXS114', a hybrid that is highly tolerant to EPTC, six days after emergence in vermiculite. Seeds were incubated, with seed coat (A) and without seed coat (B), for 72 hr in ^{14}C-EPTC solution at 12 x 10^{-5}M final concentration (125,330 cpm/ml).

that the shoot accumulated more than the root or seed in susceptible cultivars. EPTC in combination with R-25788, has shown a slight reduction in total accumulation with susceptible cultivars, but not with highly tolerant ones. It is then suggested that the reason why susceptible cultivars are readily injured with EPTC treatment, is possibly due to the relatively higher accumulation of EPTC in shoot and in root tips. The result shown in Table III-3 would indicate concentration of ^{14}C in seeds of tolerant cultivars when ^{14}C-EPTC was applied in combination with R-25788, possibly the result of conjugation with carbohydrates in seeds.

Distribution of ^{14}C in seedlings with and without seed coat. ^{14}C-EPTC whether used alone or in combination with antidote R-25788 for incubating seeds of corn are readily absorbed by seeds (Table III-4). The compound is highly mobile in the plant and accumulates to a large extent in the shoot and the root tips. The total uptake of ^{14}C by seeds (intact) of 'XL55' incubated for five days in ^{14}C-EPTC and then grown for six days in vermiculite was 30% higher than the 'TXS114' cultivar. Distribution of ^{14}C- is fairly similar to trends discussed previously. The 'TXS114' cultivar accumulated 15% more than 'XL55'. Low total uptake in intact seeds suggests a function of the pericarp over longer periods of incubation. Combination of R-25788 with EPTC has shown some advantage in uptake when used to incubate susceptible 'XL55' compared to 'TXS114' cultivar in this study.

^{14}C translocation. Both 'TXS114' and 'XL55' corn cultivars grown

Table III-4. Uptake and distribution of ^{14}C in tolerant and susceptible corn cultivars with and without seed coat during seed incubation in solution.[a]

Treatment	Cultivar	Seed Coat[b]	Total Uptake (cpm/mg dry wt)	Distribution of Absorbed ^{14}C (% of total)				
				Seed[c]	Root-tip	Root	Internode	Shoot
A. ^{14}C-EPTC $6x10^{-5}$	TXS114	X	301.9	29	38	5	13	15
	TXS114	-	1097.4	5	78	2	4	11
	XL55	+	429.2	25	31	11	18	15
	XL55	-	938.1	10	73	4	8	5
B. ^{14}C-EPTC $6x10^{-5}M$+ R-25788 $1x10^{-5}M$	TXS114	+	413.1	16	60	8	7	9
	TXS114	-	440.6	16	56	6	12	10
	XL55	+	470.5	41	10	5	25	19
	XL55	-	341.6	27	51	7	11	4

[a] Values are averages of four seedlings harvested from each treatment.

[b] Seeds incubated for five days in 5 ml final concentration and grown for six days with intact seed coat (+) or without seed coat (-)

[c] Seed remnants after elongation of roots and shoots.

in 0.5 uCi of ^{14}C-EPTC at 12 x 10^{-5}M final concentration in nutrient solution showed rapid absorption by roots and translocation in shoots after 48 hr of culture in nutrient solution. The dark image shown in root tips (Figure III-3) in both cultivars indicated that ^{14}C is concentrated in actively growing portions of the plant. The high concentration in the root tips may possibly affect cell division thus slowing root growth.

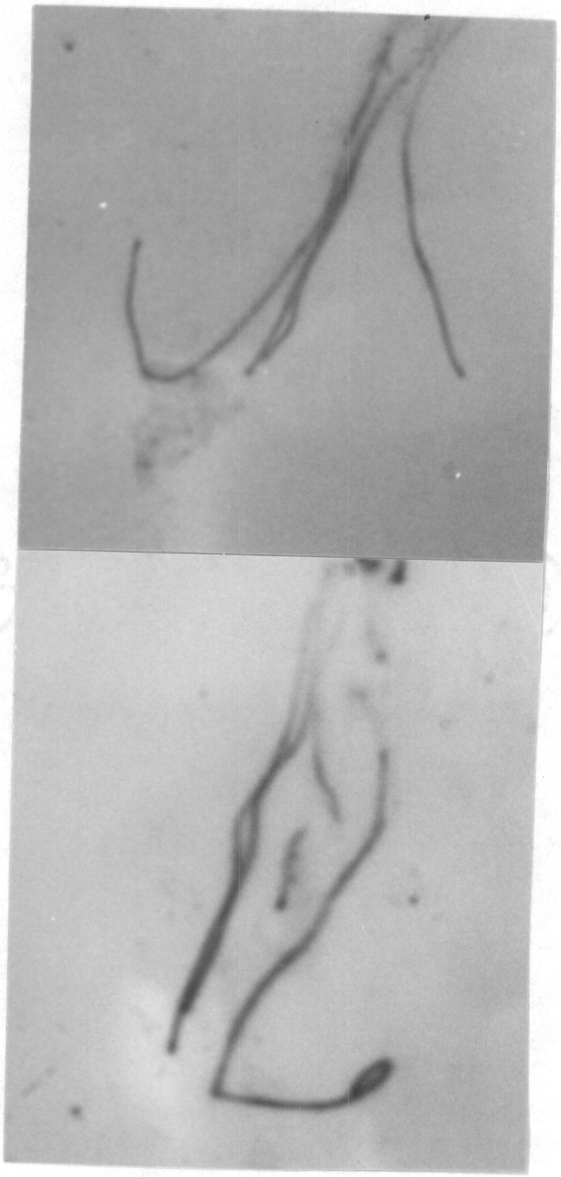

Figure III.3. Autoradiographs of corn seedlings 'TXS114' (above) and 'XL55' (below) after 48 hr in nutrient solution containing 0.5 uCi of ^{14}C-EPTC added to technical EPTC to a final concentration of 12 x 10^{-5}M solution in a greenhouse study. Note concentration of labeled materials in the root tips.

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IV. EFFECTS OF EPTC AND ANTIDOTE R-25788 ON GERMINATION AND TOTAL NONSTRUCTURAL CARBOHYDRATE CONTENTS IN CORN

Abstract. EPTC (S-ethyl dipropylthiocarbamate) applied at a concentration of 10^{-7}M promoted elongation growth of both roots and shoots in 'TXS114' corn cultivar, whereas higher concentration (up to 10^{-3}M) were progressively inhibitory. Elongation growth of roots and shoots of 'XL55' a susceptible corn cultivar, was inhibited by all levels of EPTC. The shoots were more sensitive than roots, with inhibition beginning at the 10^{-5}M concentration. This result demonstrates the sensitivity of 'XL55' to EPTC at early stages of germination and confirms that R-25788 (N,N-diallyl-2,2-dichloroacetamide) only benefited the tolerant 'TXS114' cultivar. The total nonstructural carbohydrate (TNC) contents of corn grains were strongly correlated with their injury responses to EPTC treatments. The TNC contents in grains of 'TXS114' cultivar was about 18% more than 'XL55', a susceptible cultivar. Rapid depletion of TNC in shoots of 'XL55' by 35 and 63% at 15 and 45 days after EPTC treatment, respectively, was one of the factors responsible for death of the corn plants. The application of R-25788 two days before EPTC increased the TNC contents of both roots and shoots of 'TXS114' while increases were observed only in shoots of 'XL55' cultivar.

INTRODUCTION

The use of preplant applications of EPTC (S-ethyl dipropyl thiocarbamate) herbicide for weed control in corn (Zea mays L.) often results in good to excellent weed control. When applied at low rates, EPTC can stimulate weed seed germination (7, 13). However, when high rates of EPTC are used to control difficult weed species, severe stunting and injury of corn may result (20, 21).

EPTC applied alone and in combination with R-25788 (N,N-diallyl-2,2-dichloroacetamide) can cause more damage to corn on light soils than on heavy soils (5) and is less influenced by temperature and soil moisture (1). Pallos et al. (18) applied 0.1% R-25788 by weight to corn seeds and they found complete protection from EPTC injury at 6.7 kg/ha. Also, a mixture of EPTC and R-25788 applied to the soil before the seeds were planted provided much better results without any reduction in weed control. Rains and Fletchall (20) found that EPTC at 6 lb/A injured 84% of the corn plants while 8 lb/A resulted in a 93% injury rate. However seed treatment with 0.125% R-25788 resulted in only 3% of the plants being injured by EPTC. Elliott and Purnell (6) reported results of three years in which EPTC + R-25788 was incorporated prior to corn planting. They found EPTC to give excellent control of Agropyron repens and Johnsongrass (Sorghum halepense L.). A combination of EPTC and R-25788 at a ratio of 12:1 increased tolerance of corn

without affecting the control of weeds (2). Wright et al. (24) tested the effect of R-25788 on EPTC injury to 300 corn genotypes, of which 100 were dent corn inbreds, 80 were sweet corn lines, 20 were Kentucky male sterile lines and 100 were dent corn hybrids. The results indicated that dent corn inbreds were more susceptible even if R-25788 was present.

Murphy et al. (16) found that corn cultivar 'XL374' seedlings grown in soil with (2-^{14}C) R-25788 liberated 6% of the absorbed radioactivity as CO_2 and other volatile radioactive products during a 10-day test. Leavitt and Penner (14) found that EPTC and R-25788 used alone or in combination did not change the amount of epicuticular wax deposited upon corn leaf surfaces. However, they observed that EPTC alone changed the physical arrangement of wax on the corn leaf surfaces with formation of large aggregates of epicuticular wax. When R-25788 was used in combination with EPTC, the EPTC-induced aggregation of epicuticular wax was prevented. The resistance factor in corn may also be involved in corn tolerance to EPTC. Palmer and Crogan (19) reported that a natural product in corn, the 2-glucoside of 2,4-dihydroxy-7-methoxy-4-benzoxazine-3-One, is capable of inactivating triazine herbicides. Castelfranco and Brown (3) proposed the mechanism for the dechlorination of simazine in corn. The amount of benzoxazinone hydroxysimazine is strongly correlated with resistance (4, 9, 10, 17).

The nonstructural carbohydrates (TNC) are immediate products of photosynthesis and are the main sources of energy used for plant

dry matter increases and growth (23). The status of this important energy source in growing corn plants are unknown. It was then the objective of this investigation to determine if EPTC applied alone or in combination with the antidote R-25788 has any effect on germination and TNC contents of various corn cultivars that earlier were identified under our field experiments to be highly tolerant or susceptible to EPTC treatments.

MATERIALS AND METHODS

Germination study. Five seeds of each 'TXS114' and 'XL55' corn cultivars identified earlier as being tolerant or susceptible to the application of EPTC were germinated in separate petri dishes (100 x 15 mm) each lined at the bottom with two layers of the Whatman No. 1 filter paper. Treatments were arranged in randomized complete block design and replicated three times. Treatments consisted of EPTC used at various concentrations (Figures IV-1, IV-2 and IV-3) and applied alone or in combination with the herbicide antidote R-25788. The antidote was either mixed with the EPTC and applied at the same time, six hours before or six hours after EPTC. Each treatment (10 ml) was used and the seeds were germinated in dark at room temperature of 25 $\pm$ 2C for six or seven days. The length of roots and shoots were measured for comparison.

Preparation of plants. A randomized complete block design replicated four times was used to start the growth of corn seedlings in the greenhouse. Six seeds of each of the corn cultivars 'TXS114',

'DS-37', 'XL43' and 'XL55' were grown in a 15 cm diameter plastic pots using the Othello fine sandy loam soil (sand 53%, silt 33%, Clay 14% and O.M. 3.%). However only five uniform seedlings were allowed to continue to grow in the pots. No fertilizer was added to the soil. A supplemental lighting for the 16-hr day period was provided using the high pressure sodium lamps with intensity of 140 ueinsteins.m^{2}.sec^{-1}. Fifteen days after planting, seedlings from two pots representing each of the treatments were harvested. The shoots were clipped at the soil line and were freeze-dried for five days. The dried samples were ground to fine sizes using the cyclone mill. The same procedure was followed at 45 days.

Effect of EPTC with and without antidote. In order to determine the influence of herbicide antidote R-25788 on the carbohydrate (TNC) composition of seedlings treated with EPTC, a study was carried out in the greenhouse with the same procedure described previously. Treatments consisted of EPTC at 6.7 kg/ha; EPTC 6.7 + R-25788 at 0.56 kg/ha, and R-25788 at 0.56 kg/ha applied alone. An untreated control was maintained for comparison. Plants were harvested 30 days after planting and the shoots and roots were analyzed separately for TNC composition.

Effect of time of R-25788 application. Seedlings were started as previously in the greenhouse, except that where the antidote R-25788 at 0.56 kg/ha was applied before EPTC, the R-25788 was sprayed first to the soil containing the seeds and was stored in enclosed plastic bags until the EPTC was applied. After spraying the soil

with EPTC the soil was then thoroughly mixed in the bag and transferred to pots. A similar procedure was used when EPTC treatments were made at 1, 2 and 3 days after R-25788 application. The harvested corn seedlings were washed and separated into roots and shoots for the TNC analyses.

Total nonstructural carbohydrates(TNC). The TNC were determined according to the methods of Wolf and Ellmore (22, 23). Two hundred milligrams of the finely ground materials were digested with 10 ml of distilled water in a waterbath for 30 min at 100 C. After cooling, 10 ml of buffer, consisting of sodium acetate and acetic acid adjusted to pH 4.5 and 10 ml of the enzyme clarase 900 at 0.05% were added. Samples were incubated at 37 C for two days in the oven, washed, and brought to 100 ml in volumetric flask. Each of the samples (3 ml) were collected in cups and analyzed for TNC using a Technicon Auto Analyzer.

RESULTS AND DISCUSSION

Germination study. In general, the primary roots of all treatments were longer than the shoots (Figure IV-1). Applications of the EPTC at 10^{-7}M stimulated growth in both roots and shoots in 'TXS114' cultivar, and showed to be inhibitory with increasing levels of EPTC. The 'XL55', a susceptible cultivar, had their roots and shoots showing a steady decline in growth at all levels of EPTC, with the shoots decreasing at much faster rate than roots beginning at 10^{-5}M concentration. These results demonstrate the

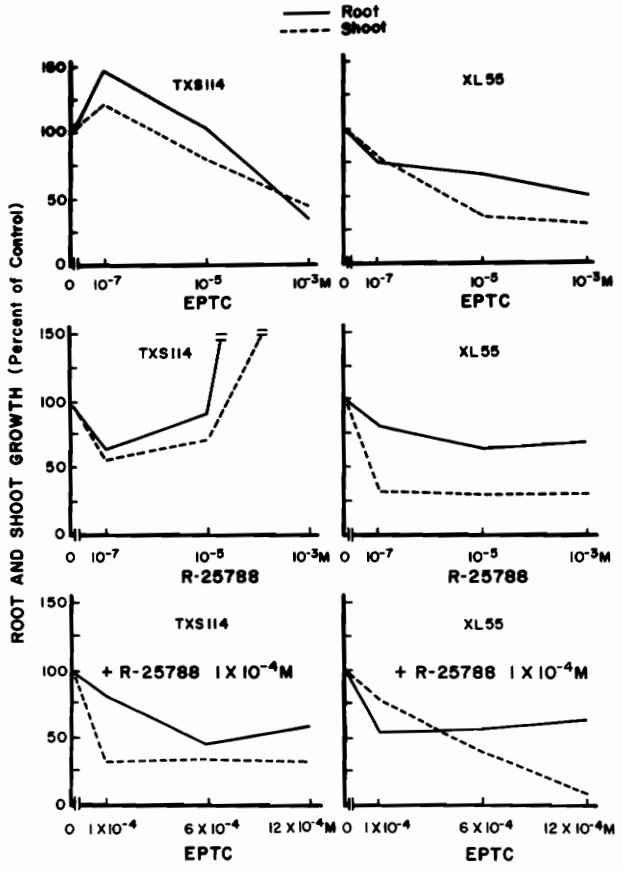

Figure IV-1. Effect of EPTC with and without the antidote R-25788 on root and shoot growth of 'TXS114', a highly tolerant corn cultivar, and 'XL55', a highly susceptible cultivar, after 7 days of treatment. Seeds were incubated in 10 ml solution in the dark at 25 $\pm$ 2 C temperature.

sensitivity of 'XL55' to EPTC at early stages of seed germination. R-25788 at 10^{-3}M concentration resulted in a strong stimulation of root and shoot elongation, especially in cultivar 'TXS114'. The growth of 'TXS114' which was stimulated by R-25788 when applied alone at high concentration (Figure IV-1) was not affected when combined with EPTC.

In Figure IV-2, the advantage in combining R-25788 with EPTC on growth appears unclear insofar as response of roots and shoots are concerned. However, root growth of 'XL55' seemed stimulated in growth of their roots than the 'TXS114' when EPTC was applied together with the antidote. This is possibly due to annullment by R-25788 of peroxidase activity caused by EPTC (8) or detoxification of EPTC by antidote R-25788 (11, 12).

Effect of EPTC and antidote R-25788 on total nonstructural carbohydrates (TNC). The four corn cultivars included in this study showed varying TNC contents in their grains (Table IV-1), and their injury responses to EPTC treatments seemed to be strongly correlated with TNC composition in their shoots. The grains of 'TXS114', a highly tolerant cultivar had 69.4% of TNC contents compared to 'XL55', a highly sensitive variety to EPTC treatment of 56.7% or a difference of 18%. It appears then that the initial TNC composition of corn seeds at the early stages of plant growth seem essential in providing the mechanism for resistance by corn plants against the injurious effects of EPTC application. Rapid depletion of TNC in the 'XL55' cultivar by 35 and 63% at 15 and 45

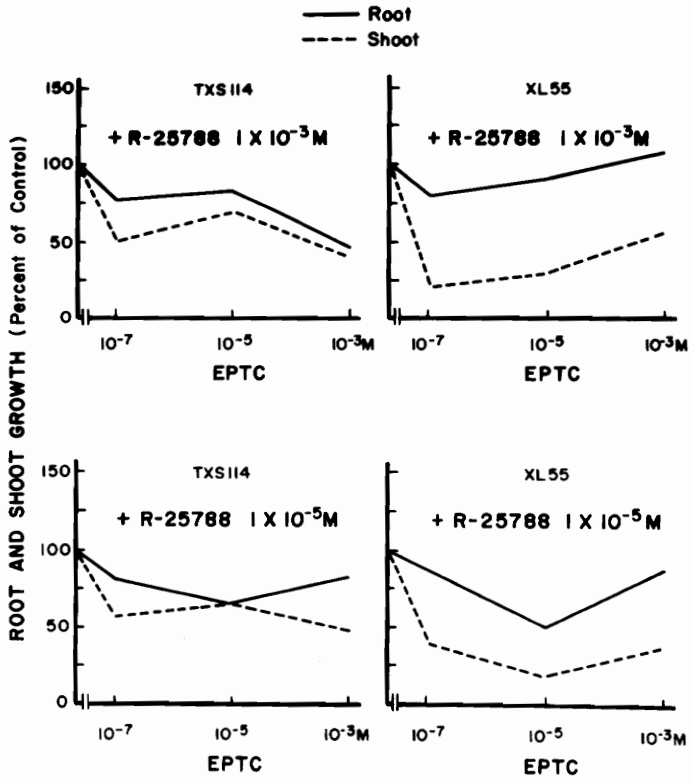

Figure IV-2. Effect of rate of antidote R-25788 on EPTC injury to shoot and root of tolerant 'TXS114' and susceptible 'XL55' corn cultivars 6 days after treatment in 10 ml solution in the dark.

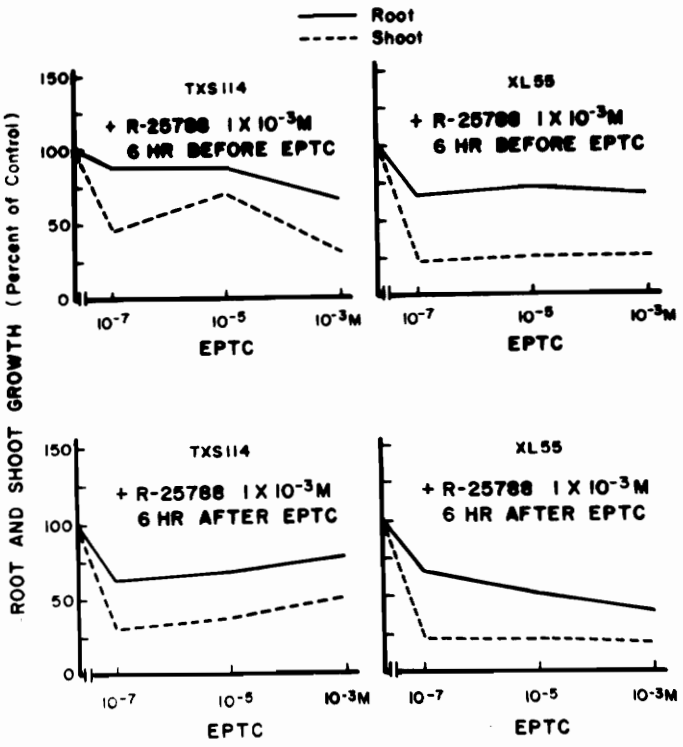

Figure IV-3. Effect of time of applying antidote R-25788 on EPTC injury to root and shoot of tolerant 'TXS114' and susceptible 'XL55' corn cultivars 6 days after treatment in 10 ml solution in the dark.

days after treatment, respectively, was the cause for the death of the corn plants (Table IV-1). The low TNC composition in seed of the 'XL55', may then be one of the factors responsible for its susceptibility to EPTC treatment since germination and early plant growth establishment are to a large extent dependent on the stored food available in the seeds. Also, the low TNC content in the seed may reduce conjugation of EPTC in seed, thus increasing the possibility of EPTC concentration immediately moving toward the site of action in corn shoot.

Effect of EPTC with and without the antidote R-25788 on TNC. The TNC composition of roots and shoots of the corn cultivars taken from the untreated control plants showed moderate differences at 30 days (Table IV-2). A reduction in TNC was found in the roots of 'XL43' cultivar and to both roots and shoots of 'XL55' treated with EPTC at 6.7 kg/ha. The combination of EPTC at 6.7 and R-25788 at 0.56 kg/ha indicated no substantial change in TNC contents to three of the cultivars studied. The growth of 'XL55' treated with a combination of EPTC and R-25788 were slightly better than plants treated with EPTC alone. This improvement in growth resulted in increase of TNC contents of both roots and shoots.

Effect of R-25788 application on TNC. The application of antidote for 2 days prior to EPTC treatment resulted in an increase in TNC contents in both roots and shoots of the 'TXS114' cultivar, whereas in the 'XL55' cultivar only shoots increased. When R-25788 was used after EPTC treatment, a reduction in TNC contents of shoots

Table IV-1. Total nonstructural carbohydrates (TNC) of corn shoots as affected by EPTC application at 15 and 45 days after treatment.[a]

Treatment[b]	Rate (kg/ha)	Cultivar	% TNC of grains	Toxicity rating	Time after EPTC application (days)	
					15	45
					(% TNC)	
EPTC	6.7	TXS114	-	1.0	19.3	21.6
Control	-	TXS114	69.4	0	19.3	23.6
EPTC	6.7	DS-37	-	3.0	14.6	8.9
Control	-	DS-37	78.0	0	12.7	26.9
EPTC	6.7	XL43	-	6.0	14.8	7.5
Control	-	XL43	58.2	0	13.9	27.6
EPTC	6.7	XL55	-	9.0	7.5	8.9
Control	-	XL55	56.7	0	11.5	24.1

[a] Values are averages of four plants grown in the greenhouse using Othello fine sandy loam. Two hundred milligrams of tissue were used for analysis.

[b] Preplant incorporated into soil 10 cm deep immediately before planting.

[c] Visual score of toxicity rating: 0 means no injury such as twisting or folding of leaves and 10 is complete kill.

Table IV-2. Total nonstructural carbohydrates (TNC) composition of corn seedlings grown under greenhouse conditions at 30 days after treatment.[a]

Treatment[b]	Rate (kg/ha)	Cultivar							
		TXS114		DS-37		XL43		XL55	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
		(% TNC)		(% TNC)		(% TNC)		(% TNC)	
Control		21.3	19.1	21.3	17.7	21.6	23.0	20.4	19.6
EPTC	6.7	23.1	22.2	22.9	20.1	19.8	17.6	16.7	16.0
EPTC + R-25788	6.7 + 0.56	22.1	22.1	21.6	20.3	23.0	18.7	20.1	17.5
R-25788	0.56	24.7	22.7	23.2	21.0	24.2	23.2	20.8	19.0

[a] Values are averages of four plants grown in Othello fine sandy loam soil. Two hundred milligrams of tissue were used for analysis.

[b] Preplant incorporated into the soil 10 cm deep immediately before planting.

of 'TXS114' cultivar was noted. However, TNC contents in roots appeared to increase when R-25788 was applied three days after EPTC, indicating that R-25788 countered the injurious effects of EPTC (Table IV-3). The detoxification of EPTC appeared to be stimulated if the antidote R-25788 was applied after EPTC, possibly the result of some enzymes being activated or otherwise antidote stimulating metabolic plant processes.

Table IV-3. Total nonstructural carbohydrates (TNC) of corn seedlings as affected by time of application of antidote R-25788 either before or after EPTC treatment at 6.7 kg/ha.

Time of antidote application relative to EPTC treatment	Cultivar			
	TXS114		XL55	
	Shoot	Root	Shoot	Root
	(% TNC)		(% TNC)	
3 DBE	24.7	18.8	21.3	14.5
2 DBE	21.9	17.8	16.8	13.3
1 DBE	19.4	20.0	18.6	14.8
0	20.0	16.7	15.5	15.5
1 DAE	17.0	10.8	16.1	12.8
2 DAE	15.1	14.0	14.6	14.8
3 DAE	14.1	17.8	13.6	16.3
Untreated control	18.6	16.7	17.4	14.0

[a] Values are averages of four plants grown in Othello fine sandy loam soil in the greenhouse. The plants were harvested 25 days after EPTC treatment and 200 mg of tissue were used for analysis.

[b] EPTC was applied at a uniform dosage of 6.7 kg/ha and R-25788 was applied at 0.56 kg/ha either DBE (days before EPTC) or DAE (days after EPTC).

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V. METABOLIC SITES AFFECTED BY EPTC AND ANTIDOTE R-25788 IN SINGLE CELLS

Abstract. Effects of EPTC and the antidote R-25788 (N,N-diallyl-2,2-dichloroacetamide) on photosynthesis, respiration, and RNA, protein and lipid synthesis were studied utilizing Chlorella sorokiniana Shihira and Kraus and isolated mesophyll protoplasts from corn (Zea mays L.). EPTC at 2 x 10^{-4}M concentration inhibited photosynthetic fixation of $^{14}CO_2$ in Chlorella cells by about 50% after 2 hr incubation; whereas, 1.2 x 10^{-4}M EPTC caused about 30% inhibition in corn protoplasts. At 2 x 10^{-4}M antidote R-25788 combined with 2 x 10^{-6}M EPTC, there was a stimulation in photosynthetic activity, indicating reversal of the inhibitory effects caused by EPTC in Chlorella cells. EPTC at 2 x 10^{-8}M moderately stimulated the respiratory activity of Chlorella cells, but inhibited by about 32% when the concentration was increased to 2 x 10^{-4}M. In corn protoplasts, EPTC at 1.2 x 10^{-4}M concentration caused an inhibition of approximately 30% after 3 hr of incubation. There was reduction in EPTC inhibition by 11% when R-25788 at 10^{-5}M was combined with EPTC at 12 x 10^{-5}M. EPTC at 2 x 10^{-4}M inhibited RNA synthesis by 22% after 3 hr of incubating Chlorella cells. A combination of EPTC and R-25788 at 2 x 10^{-4}M each, showed a strong stimulation of RNA synthesis compared to when EPTC was used alone. At the rates used in corn protoplasts, the effects, of EPTC were of stimulation rather than inhibition after 12 hr

of incubation. Protein synthesis in Chlorella was inhibited by 13% when EPTC was applied at 2 x 10^{-4}M after 10 hr of incubation. The combination of R-25788 and EPTC at 2 x 10^{-4} each, resulted in increased incorporation of ^{14}C-leucine compared to either one used alone. Where R-25788 was applied at 2 x 10^{-6}M and EPTC at 2 x 10^{-4}M, a reduction by 18% of ^{14}C-leucine incorporation into Chlorella cells was observed. In corn protoplasts, there was slight inhibition of protein synthesis when EPTC was used at 6 x 10^{-5}M concentration. Lipid synthesis was inhibited by more than 50% in Chlorella cells when EPTC was used at 2 x 10^{-4}M concentration after 1 hr of incubation. A similar result was obtained in corn protoplasts, i.e. lipid synthesis was inhibited by 23% after 2 hr of incubation with EPTC at 12 x 10^{-5}M. However, when R-25788 at 1 x 10^{-5}M concentration was combined with EPTC, there was a reduction in lipid synthesis inhibition due to EPTC by 20% in isolated corn protoplasts. A reduction of about 50% was found with R-25788 at 2 x 10^{-4}M combined with 2 x 10^{-6}M of EPTC in Chlorella cells. This suggests that the primary site of action of EPTC is the inhibition of lipid synthesis. Furthermore, the results indicate that the main action of antidote R-25788 in cells was the reversal of the inhibitory effects caused by EPTC on the metabolic processes in plants.

INTRODUCTION

One of the advantages of using single cells in studying the metabolic sites of action of any given herbicide is avoiding the problem of inherent anatomical barriers present in higher plants. The procedure allows rapid and uniform exposure of cells (8). The herbicide will have equal possibility to get in contact with every cell. Ashton et al. (3) pointed out that single cell preparations allow the rapid establishment of known concentrations of the herbicide at the cellular level. The use of protoplasts from higher plants are ideal for metabolic studies (9, 11, 17, 18). EPTC inhibits both phosphorus uptake and oxygen consumption (2). At a concentration of 10^{-3}M, lipid, RNA, protein synthesis and photosynthesis are inhibited 94%, or more, whereas respiration is inhibited 76% in comparison to the controls (3). Wilkinson and Ashley (20) reported 90% reduction in incorporation of MVA-2^{14}C in total kaurenoids in wheat with 250 ppbw of EPTC. Metabolism of kaurene to kaurenoic acid was reduced by 250 ppbw EPTC resulting in accumulation of kaurene. They concluded that since the kaurenoids are gibberellin precursors, their inhibition may account for the toxicity observed in plants exposed to EPTC. Carringer et al. (5) studied EPTC metabolism in corn utilizing carbonyl-^{14}C-EPTC and propyl-1-^{14}-C-EPTC. They found five metabolites in the water-soluble fraction. Amino acid analysis indicated that one water-soluble metabolite was a

glutathione conjugate (S-N,dipropylcarbanyl glutathione) and suggested that three other metabolites may be degradation products of glutathione conjugate.

Lotlikar et al. (14) suggested that EPTC at 10^{-3}M severely inhibited oxidative phosphorylation of cabbage mitochondria. Winely and San Clemente (22) reported that EPTC exerted an uncoupling effect on oxidative phosphorylation linked to nitrate oxidation in cell-free extracts of Nitrobacter agiles. Apparently, EPTC does not affect electron transport because $NADH_2$ oxidase activity was not affected by the addition of 1.57×10^{-3}M of EPTC.

Beste and Schreiber (4) concluded that the 2,4-D enhanced synthesis of D-RNA and TB-RNA in the presence of EPTC was the basis of antagonism of the two compounds.

Moreland et al. (16) observed that both EPTC at 6×10^{-4}M and CDEC (2-chloro-allyldiethyldithiocarbamate) at 2×10^{-4}M inhibited the development of α amylase activities of germinating barley seeds. They found that ^{14}C labeled leucine, ATP, or orotic acid, moderately inhibited ATP incorporation into RNA by EPTC and of leucine incorporation into protein by CDEC in corn mesocotyl and soybean hypocotyl sections possibly the result of differences in their sites of action.

Mann et al. (15) found that CDEC and EPTC inhibited ^{14}C-1-leucine incorporation into protein segments of barley coleoptile of hemp sesbania (Sesbania exaltata) hypocotyls. At 2 and 5 ppm, EPTC inhibited this reaction 38 and 22%, respectively, in barley, and

14 and 11%, respectively, in hemp sesbania.

Lay et al. (13) and Lay and Casida (12) suggested that R-25788 may induce more rapid detoxification of thiocarbamate sulfoxides brought about by increasing the rate of carbamoylation of glutatathione-S-transferase activity. They suggested that the lesser protection in other species may be due to lower levels of detoxifying enzymes and cofactor in these species compared with corn.

Harvey et al. (10) reported that R-25788 annulled the increase in peroxidase activity and lignin deposition caused by EPTC. R-25788 alone, reduced peroxidase activity by 20-30% although it had no effect on peroxidase activity in vitro. It was observed by Wright et al. (23) that R-25788 increased metabolism of butylate in corn hybrids and suggested that differential response of corn compared to grasses may be due to differences in their sites of action.

It was the objective of this study to determine the effects of EPTC and antidote R-25788 on five major metabolic processes in plants utilizing Clorella sorokiniana cells and the isolated mesophyll protoplasts from corn plant.

MATERIALS AND METHODS

Chlorella sorokiniana Shihira and Kraus. The cells were cultured according to the procedure described by Cedeno-Maldonado and Swader (6) and chlorophyll was determined according to Arnon's technique (1) by extracting the chlorophyll with 80% acetone. The reaction

mixture contained 7.2 x 10^{-6} cells/ml with chlorophyll ranging from 3.08 to 5.2 micrograms.

Corn mesophyll protoplasts. Corn hybrid DeKalb 'XL22B' was used in this study. The plants were grown in I:1 peat-soil mix under a 16-hr photoperiod using the high pressure sodium lamps at intensity of 140 ueinsteins.m^{-2}.sec^{-1}. Light intensity was measured with a Lambda quantum meter Model LI-170. The protoplasts isolations were carried out using the method described by Earle et al. (8) with slight modifications. The youngest fully expanded leaves at 15-20 day-old plants were washed with distilled water and then blotted between paper towels. The midribs were removed and the leaves cut into 0.5 cm sections. Four grams of the cut leaves were macerated in 250-ml Erlenmeyer flask in 50 ml of driselase enzyme (Kyowa Hakko Kogyo Co. LTD.). The flask was placed in waterbath oscillating at 60 times/min at temperature of 25 C. After 4 hr, 30 ml of 0.5 M sorbitol + 10 mM $Cacl_2$ at pH 5.8 was added. At the end of 5 hr the tissue was filtered through 3 layers of nylon filter. This was then centrifuged at 160 x g for 5 min and the cells were washed 3 times with 0.5 M Sorbitol + 10 mM $Cacl_2$ and finally suspended in sorbitol and calcium solution.

Photosynthesis. Photosynthesis was determined by the methods of Jensen et al. (11) and Ashton et al. (2) with modifications. Chlorella cells (8 ml) were incubated with 6 uCi of $NaH^{14}CO_3$ (specific activity 46 mCi/mM) in 6 mM $NaH^{12}CO_3$ and finally treating 400 ul cells in 10 ml test tubes. The test tubes were

stoppered placed in a waterbath with 60 oscillations per min and the temperature was maintained at 25 C. The light source was a fluorescent lamp mounted above supplied an intensity of 14 ueinsteins.m^2.sec^{-1} for the duration of the incubation period. The cells were put on Whatman filter paper #1 and acidified with 1 ml of formic acid. After air-drying, the discs were placed in plastic vials, scintillation fluid was added, and radioactivity was determined with Beckman LS 250 scintillation spectrometer. Photosynthesis was determined as cpm/mg chlorophyll.

Respiration. This study was conducted using the method of Ashton et al. (3) with modification. Chlorella cells (8 ml) were incubated with 6 uCi D-glucose UL-^{14}C (specific activity of 12.7 mCi/mM) and transfers of 400 ul were made in 10 ml test tubes. The incubation was done as described previously except that an aluminum foil was used to cover the tubes under dark condition. The released $^{14}CO_2$ was trapped in 3 M KOH contained in Whatman filter paper #1. The radioactivity was determined by scintillation spectrometry, and respiration as cpm/mg chlorophyll.

RNA synthesis. The methods of Francki et al. (9) and Ashton et al. (3) were used to determine RNA synthesis. Chlorella cells (8 ml) were incubated with 10 uCi uracil-2-^{14}C (specific activity 55 mCi/mM) and then 400 ul transfers were treated in 10 ml test tubes. The tubes were incubated as before in light. A 12% TCA (2 ml) containing 30 mM uracil were added after the incubation

period and then test tubes were kept at 4°C for 12 hr. The reaction medium was filtered through glass fiber filter and RNA precipitated by ice cold 10% TCA adding two times with 10 ml each. This was followed by washing two times with 80% ethanol, twice with acetone and diethyl ether. The discs were put in plastic vials, air-dried, and radioactivity determined by scintillation spectrometry. The RNA synthesis was calculated as cpm/mg chlorophyll.

Protein synthesis. The procedures of Francki et al. (9) and that of Ashton et al. (3) were modified by incubating 8 ml of Chlorella cells with 5 uCi L-leucine-^{14}C (specific activity 310 mCi/mM). The procedure for incubation was similar as before except that the reactions were stopped by the addition of 2 ml of 12% TCA containing 50 mM L-leucine and then keeping the tubes for 24 hr at 4 C. The precipitated proteins were collected as previously discussed with RNA synthesis. Protein synthesis was calculated as cpm/mg chlorophyll.

Lipid synthesis. The methods of Francki et al. (9) and Ashton et al. (3) were modified in determining lipid synthesis as affected by EPTC with and without the herbicide antidote R-25788 in Chlorella cells. Incubation was started by adding 4 uCi of sodium acetate-^{14}C (specific activity 98 mCi/mM) to 8 ml of Chlorella cells. After the incubation period as described previously, 2 ml of 0.35 m H_2SO_4 and 0.05 m HCOOH were added and incubated for 1 hr. The cells were centrifuged at 2,000 x g for 10 min and then supernatant removed by aspiration. Four ml of chloroform/methanol

(2:1 v/v) mixture was added and the tubes were stoppered and left for 12 hours at room temperature. Two milliliters of distilled water was added and the tubes were centrifuged at 2000 x g for 7 min. The top layer was removed by suction and this procedure was repeated three times. The cells were then filtered through glass fiber filter discs and washed twice with chloroform/ methanol (2:1 v/v). The lipid fraction was air-dried in glass vials and the radioactivity determined by adding 10 ml of scintillation fluid. Lipid synthesis was calculated as cpm/mg chlorophyll.

The procedures for determining photosynthesis, respiration, RNA synthesis, protein and lipid synthesis for isolated corn mesophyll protoplasts were similar to those used in Chlorella cells except that 100 ul of the protoplasts were used for each reaction.

RESULTS AND DISCUSSION

Photosynthesis. At 2×10^{-4}M, EPTC inhibited photosymthesis by about 50% after 2 hr of incubation. A similar trend was observed with the antidote R-25788 which caused an inhibition of 30% at 2×10^{-6}M and increased to about 38% at 2×10^{-4}M. At the same concentration, EPTC inhibited photosynthesis more than the R-25788. The combination of R-25788 with EPTC showed a different response. At 2×10^{-4}M of the antidote and 2×10^{-6}M of EPTC, photosynthetic activity was strongly stimulated (Figure V-1). However, the

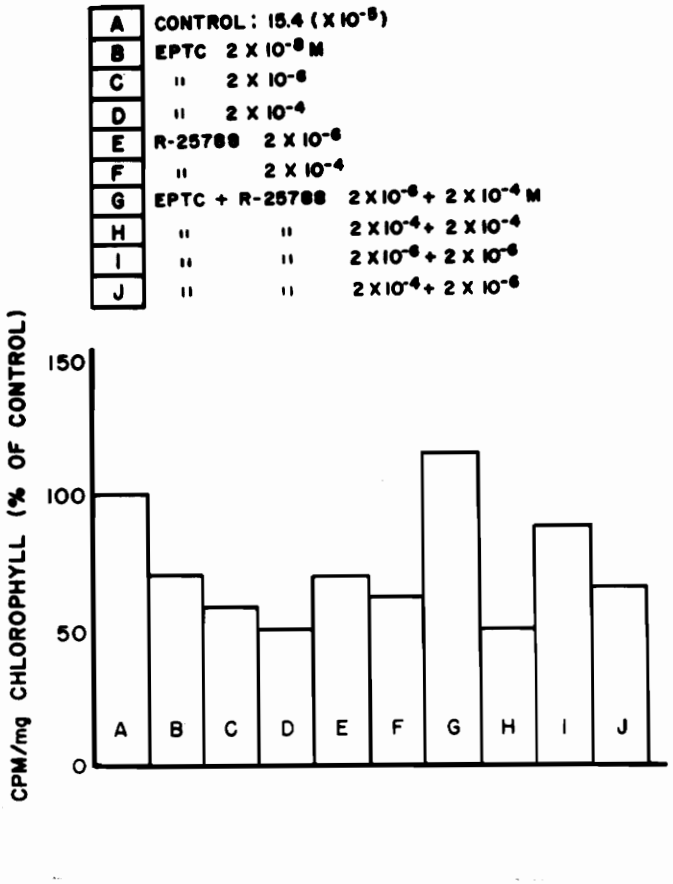

Figure V-1. Influence of EPTC and antidote R-25788 on photosynthetic fixation of $^{14}CO_2$ in Chlorella cells after 2 hr of incubation.

reaction rates in the presence of 2×10^{-4}M EPTC plus 2×10^{-4}M R-25788 was the same as with 2×10^{-4}M EPTC alone. R-25788 used at 2×10^{-6}M in combination with EPTC at 2×10^{-6}M, resulted in 30% increase in fixation of $^{14}CO_2$ in Chlorella cells after 2 hr of incubation compared to EPTC alone. It seems then that a certain ratio of antidote to EPTC is necessary for the antidote R-25788 to reverse partially, or completely, the inhibitory effects of EPTC.

The incubation of corn protoplasts with EPTC for 3 hr at 12×10^{-5}M concentration showed an inhibition of approximately 30% (Figure V-2). When applied at the above rate and combined with R-25788 at 10^{-5}M, or a ratio of 12:1, inhibition of about 23% occured possibly the result of cellular shock. However, after 12 hr incubation the treatment caused a stimulation by more than 200%.

Respiration. EPTC at 2×10^{-8}M moderately stimulated respiratory activity in Chlorella cells and inhibited by about 32% when the concentration was increased to 2×10^{-4}M. The use of R-25788 at 2×10^{-6}M only showed slight change in release of the $^{14}CO_2$ under dark respiration in Chlorella cells. It appeared from this study that R-25788 at 2×10^{-6}M in combination with EPTC at 2×10^{-6}M concentration can reverse the inhibitory effects of EPTC alone by as much as 40% in respiratory activity of Chlorella cells.

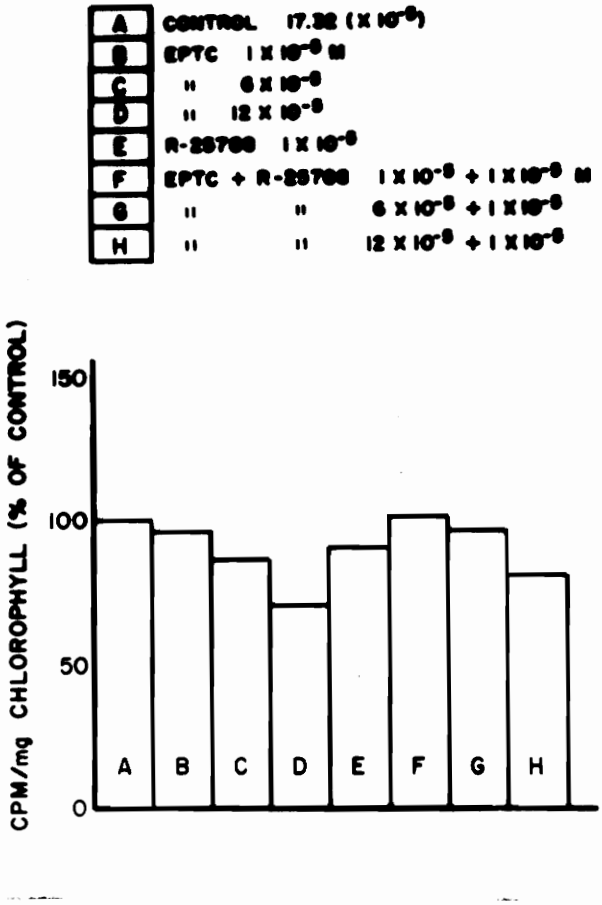

Figure V-2. Influence of EPTC and R-25788 on photosynthetic fixation of $^{14}CO_2$ in corn protoplasts after 3 hr of incubation.

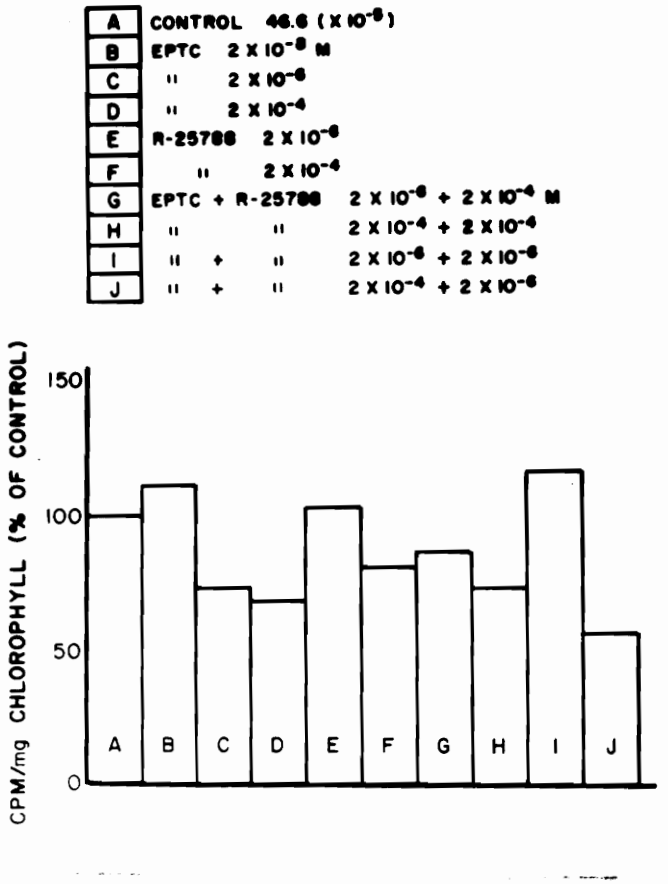

Figure V-3. Effect of EPTC and antidote R-25788 on release of $^{14}CO_2$ during dark respiration after 20 hr of incubation in Chlorella cells.

EPTC at 12 x 10^{-5}M concentration applied to protoplasts isolated from corn leaves, has shown inhibition by approximately 30% after 3 hr and then caused stimulation in release of $^{14}CO_2$ by 13% after 24 hr of incubation (Figure V-4). When R-25788 at 10^{-5}M was combined with EPTC, at 12 x 10^{-5}M, there was inhibition by 19% after 3 hr. However, after 12 hr this inhibition was no longer significant compared to controls.

RNA synthesis. The effect of EPTC on RNA synthesis was one of stimulation at low level of application (Figure V-5). RNA synthesis was stimulated by about 80% at 2 x 10^{-8}M EPTC in 3 hr, but was inhibited by 22% when EPTC was increased 2 x 10^{-4}M. The R-25788 at 2 x 10^{-4}M resulted in stimulation of ^{14}C-uracil incorporation into Chlorella cells by 13% after 20 hr of incubation. The combination of R-25788 and EPTC at 2 x 10^{-4}M each, showed a strong stimulation in ^{14}C-uracil incoporation into cells by about 70% compared to EPTC used alone.

The result on corn protoplasts treated with EPTC alone showed stimulation in incorporation of ^{14}C-uracil into cells after 12 hr of incubation (Figure V-6).

Protein synthesis. EPTC at 2 x 10^{-6}M stimulated ^{14}C-leucine incorporation into Chlorella cells by 17%, and inhibited by 13% when the rate was increased to 2 x 10^{-4}M after 10 hr of incubation. (Figure V-7). This result supports the findings by Mann et al.

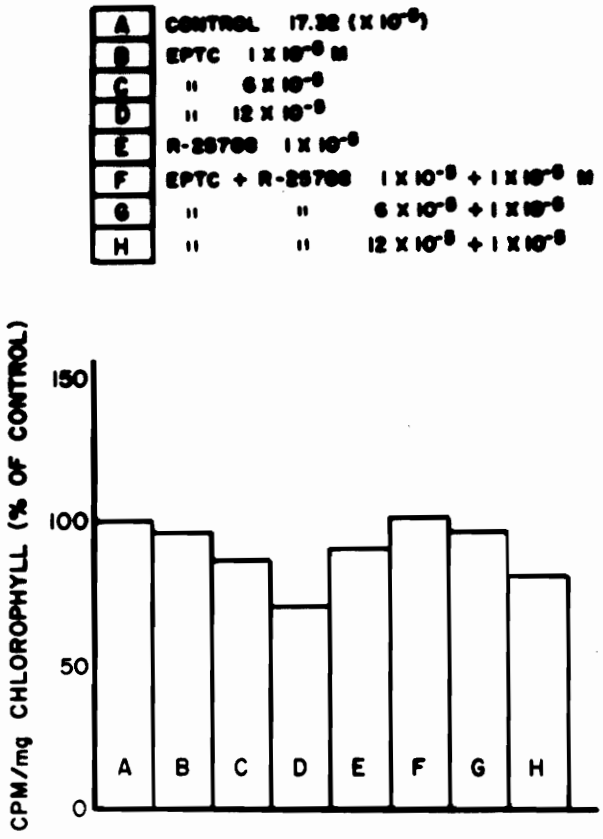

Figure V-4. Influence of EPTC and R-25788 on release of $^{14}CO_2$ during dark respiration in corn protoplasts after 3 hr of incubation.

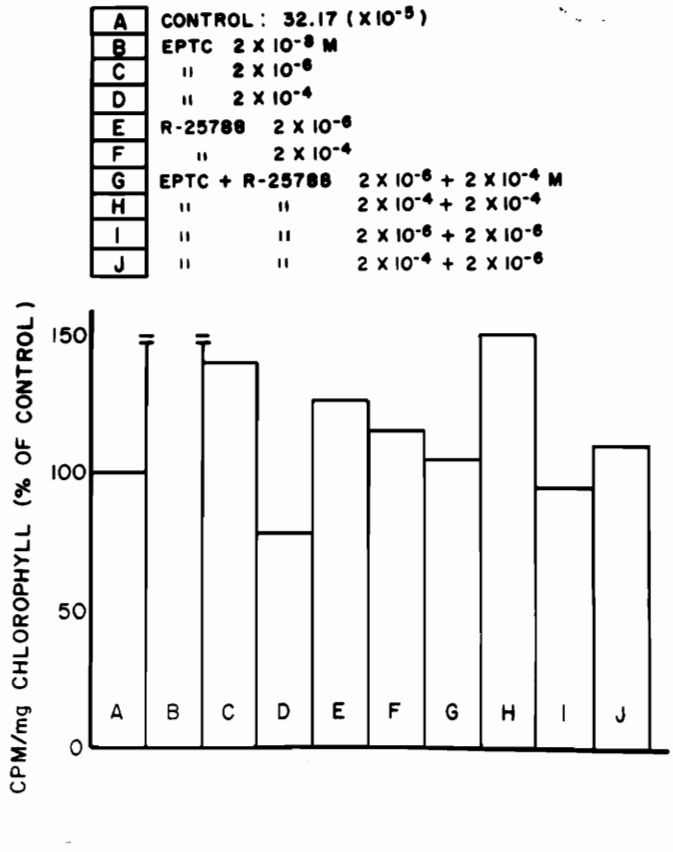

Figure V-5. Incorporation of ^{14}C-uracil into TCA insoluble products as affected by EPTC and antidote R-25788 in Chlorella cells after 20 hr of incubation.

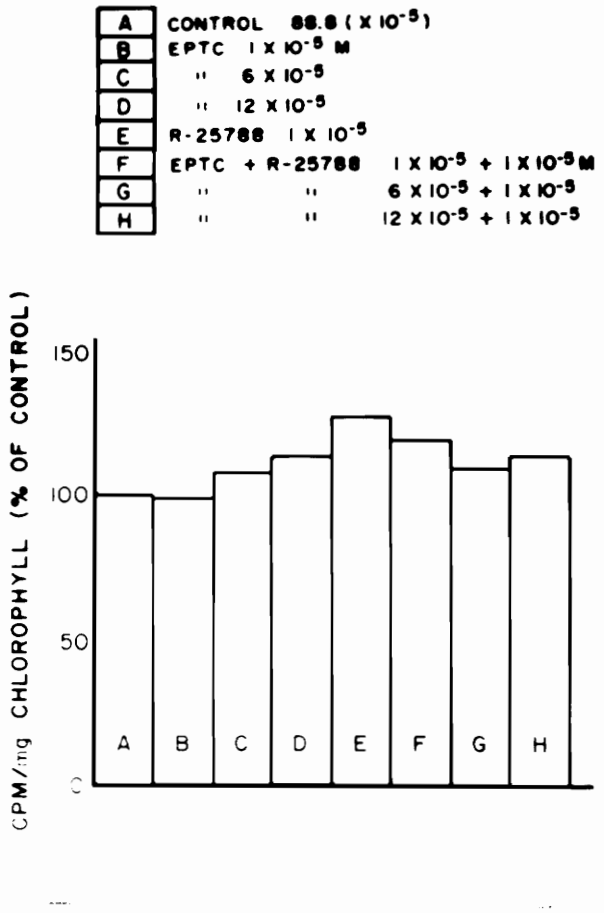

Figure V-6. Incorporation of ^{14}C-uracil into TCA insoluble products as affected by EPTC and combination with antidote R-25788 in corn protoplasts after 12 hr of incubation.

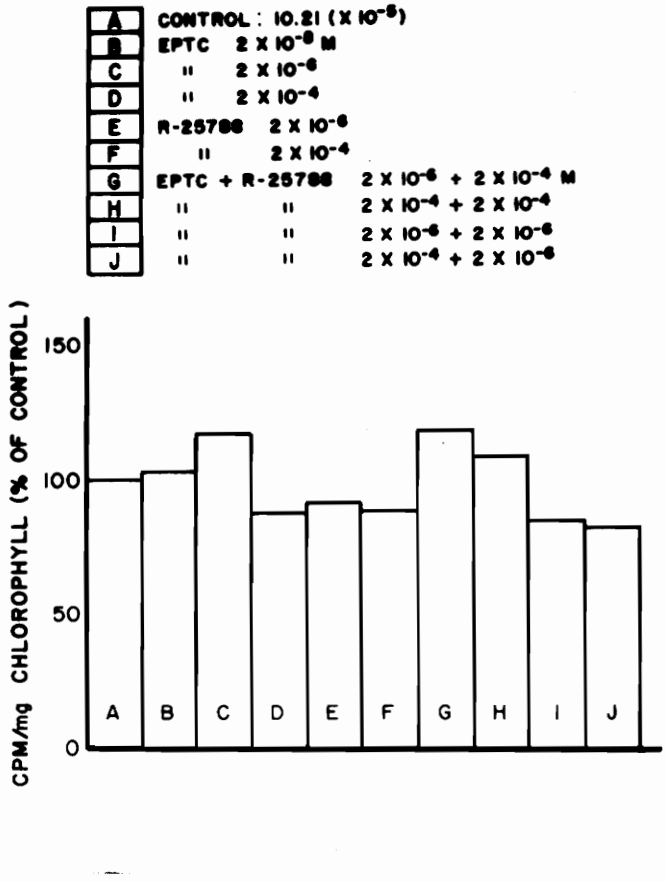

Figure V-7. Incorporation of ^{14}C-leucine into TCA insoluble products as affected by EPTC and antidote R-25788 in Chlorella cells after 10 hr of incubation.

(15) which showed that at 2 and 5 ppm of EPTC, there was inhibition of ^{14}C-1-leucine incorporation by 38 and 22%, respectively in barley and 14 and 11%, respectively, in sesbania. The R-25788 at the two rates used showed moderate inhibition in incorporation of ^{14}C-leucine into cells. The combination of R-25788 and EPTC at 2 x 10^{-4}M each, resulted in reversal of EPTC inhibition. The lower rate of R-25788 at 2 x 10^{-6}M combined with EPTC at 2 x 10^{-4}M showed no change in EPTC effect on protein synthesis.

In corn protoplasts, there was only moderate effect of EPTC inhibiting the incorporation of ^{14}C-leucine into the cells (Figure V-8). In fact at early incubation, there was stimulation with EPTC treatment in protein synthesis.

Lipid synthesis. EPTC at 2 x 10^{-4}M concentration caused an inhibition of ^{14}C-acetate incorporation into chlorella cells by more than 50% after 1 hr of incubation (Figure V-9). This was the first metabolic process detected in cells to be inhibited by EPTC treatment at all levels of application. The other metabolic processes included in this study were first of stimulation at early period of incubation and then becoming inhibitory to cells at longer periods. This results then suggest that lipid synthesis inhibition is the primary site of action of EPTC and that inhibition of the other metabolic process are only secondary effects resulting possibly from lipid inhibition. This supports the findings by Ashton et al. (3) and Wilkinson and Ashley (20). The R-25788 at 2 x 10^{-6}M showed a strong stimulation of lipid synthesis and at

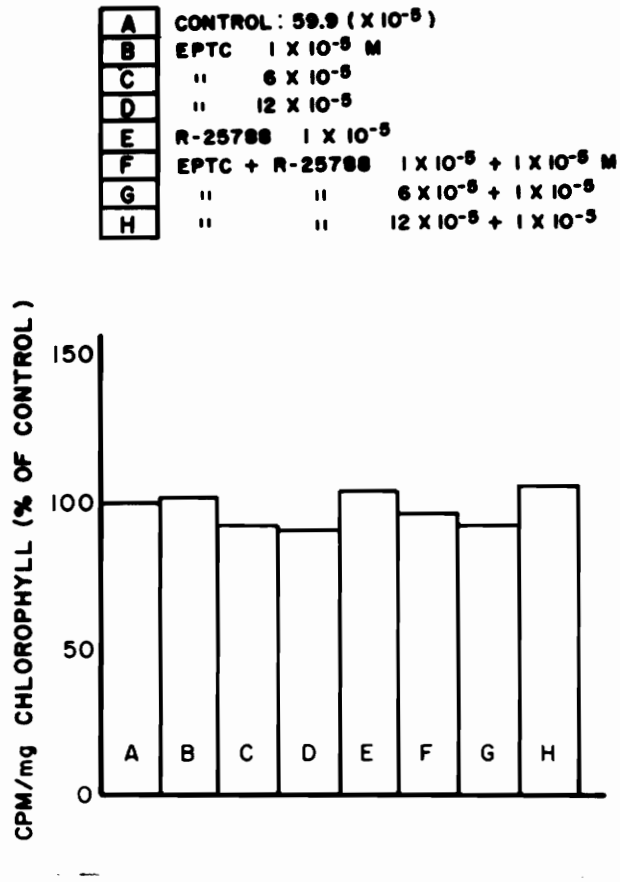

Figure V-8. Incorporation of ^{14}C-leucine into TCA insoluble products as affected by EPTC and combination with R-25788 in corn protoplasts after 12 hr of incubation.

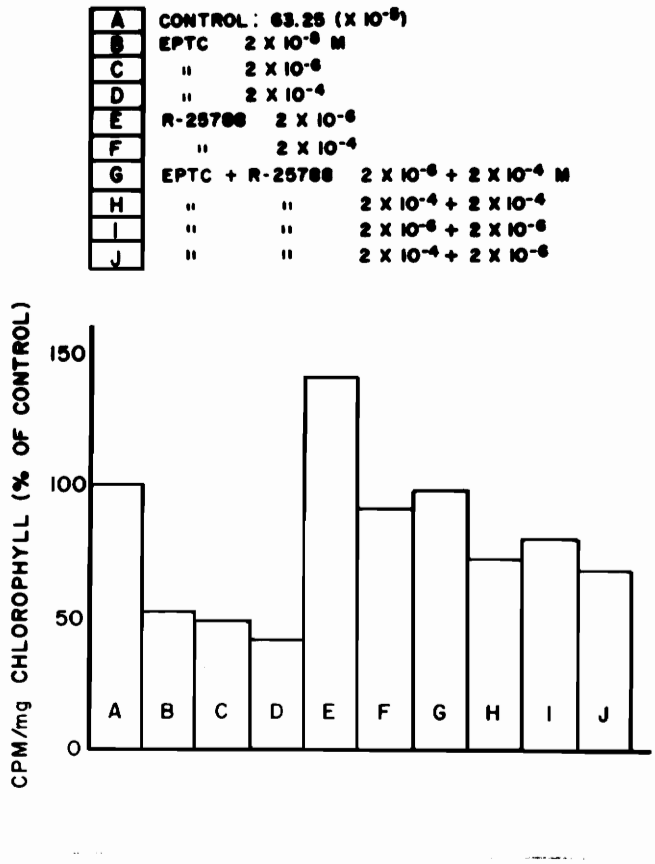

Figure V-9. Incorporation of ^{14}C-acetate into lipid products as affected by EPTC and antidote R-25788 after 1 hr of incubation in Chlorella cells.

2×10^{-4}M, it caused inhibition of ^{14}C incorporation into cells by 7%. The combination of R-25788 at 2×10^{-4}M and EPTC at 2×10^{-4}M showed a reduction by 31% of inhibition compared to when EPTC was used alone. This reversal in EPTC inhibition of lipid synthesis may be the primary action of R-25788 because this reaction happened after 1 hr of incubation.

In isolated corn cells, there was a lag in cell response to EPTC treatment for 1 hr. However after 2 hr with EPTC applied at 12×10^{-5}M, there was inhibition in the incorporation of ^{14}C in cells by about 23%. The R-25788 in combination with EPTC at 10^{-5}M, resulted in 20% reduction of inhibition caused by EPTC used alone (Figure V-10). The reason for possible detoxification of EPTC were suggested earlier (7, 10, 12, 19, 20, 21).

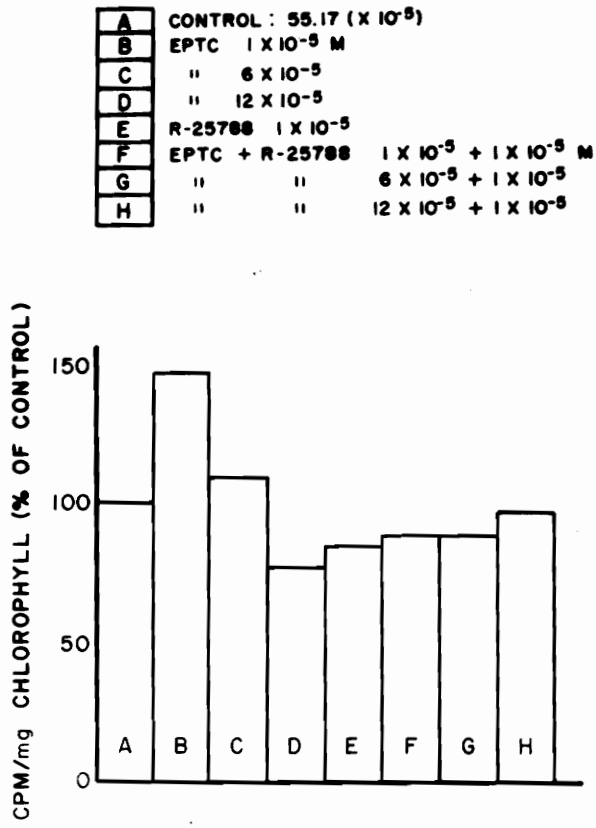

Figure V-10. Incorporation of ^{14}C-acetate into lipid products as affected by EPTC and R-25788 in corn protoplasts after 2 hr of incubation.

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VITA

The author, Erasmo G. Sagaral, was born on June 2, 1936 at Catarman, Camiguin, Philippines. He attended College at Central Mindanao State University and received his Bachelor of Science degree in agriculture with major in agricultural engineering in 1959. Immediately after graduation, he was employed as a farm mechanics teacher at Bohol Agricultural College, Bilar, Bohol. In 1962, he transferred to Central Mindanoa State University as a vocational agriculture teacher. He began his graduate studies in 1965 at the University of the Philippines College of Agriculture at Los Baños, Laguna, on a research scholarship provided by the International Rice Research Institute (IRRI). In 1968, he received his Master of Science degree in Agronomy. He returned to his teaching job as instructor of agronomy at Central Mindanoa State University and in 1969, he resigned from government service to join the College of Agriculture at Xavier University as assistant professor and acting head of Agronomy. In the same year, he was awarded an eight months research fellowship by the Southeast Asia Treaty Organization (SEATO) which enabled him to travel and observe rice production in 12 Asian countries. He went back to school in the fall of 1965 at Virginia Polytechnic Institute and State University, Department of Plant Pathology and Physiology on a Graduate Research Assistantship. In that same year, he was promoted to the rank of associate professor at Xavier University College of Agriculture, Cagayan De Oro City, Philippines.

TOXICITY, SELECTIVITY, UPTAKE, DISTRIBUTION AND SITE OF ACTION OF EPTC IN CORN (*ZEA MAYS* L.) AS AFFECTED BY A HERBICIDE ANTIDOTE

by

Erasmo G. Sagaral

(ABSTRACT)

Some factors affecting toxicity, selectivity, uptake distribution and site of action of EPTC (S-ethyl dipropydithiocarbamate) as affected by R-25788 (N,N-diallyl-2,2-dichloroacetamide) were investigated in field, greenhouse and laboratory experiments.

EPTC at 6.7 kg/ha was 20 to 30% more toxic to various corn cultivars when applied to sandy and lower organic matter (0.8%) soil than with fine sandy loam soil (O.M. 3.1%). The 'TXS114' cultivar was more tolerant than 'XL55' to EPTC treatment. The seeds of 'XL55' absorbed 20% more EPTC than the seeds of 'TXS114' after 12 hr of incubation in ^{14}C-EPTC. Movement of ^{14}C was toward the actively growing portions of root and shoot. Embryo of "TXS114' accumulated 48% of total uptake compared to 60% in 'XL55' cultivar. Apparently, herbicide antidote R-25788 conjugated or otherwise delayed movement of ^{14}C toward the shoot by concentrating ^{14}C

in seed of highly tolerant compared to shoot of 'XL55', a highly susceptible cultivar. Therefore, susceptibility of 'XL55' is apparently due to its relatively high uptake and site of accumulation.

The total nonstructural carbohydrate (TNC) in seeds of 'TXS114' was 18% more than 'XL55' and this differences strongly correlated with their injury responses to EPTC.

Lipid synthesis was inhibited by more than 50% in Chlorella cells when EPTC was used at 2×10^{-4}M concentration after 1 hr of incubation. Similar result was found in corn protoplasts which was inhibited by 23% after 2 hr of incubation with EPTC at 12×10^{-5}M, suggesting that primary site of action of EPTC is inhibition of lipid synthesis. The other metabolic processes investigated were possibly secondary effects resulting from lipid synthesis inhibition because their inhibitory actions were noticeable only after several hours of cell incubation.

The antidote R-25788 combined with EPTC caused a reduction in lipid synthesis inhibition by 31 and 20%, respectively in both Chlorella and corn protoplasts. It is then suggested that the primary action of antidote R-25788 in cells is reversal of the inhibitory effects caused by EPTC on metabolic processes in plants.