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Effects of EPTC and Acifluorfen on Sunflower

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EFFECTS OF EPTC AND ACIFLUORFEN ON SUNFLOWER

BY

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EFFECTS OF EPTC AND ACIFLUORFEN ON SUNFLOWER

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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MEL

TABLE OF CONTENTS

	Page
Introduction.	1
Review of the Literature	3
EPTC and Epicuticular Wax	3
Wax Composition	4
Cuticular Transpiration	5
Sunflower Response to Acifluorfen	5
Herbicide Interaction	6
Materials and Methods	7
Greenhouse Plant Culture and Herbicide Application. .	7
Cuticular Transpiration	7
Gravimetric Determination of Epicuticular Wax	8
Nuclear Magnetic Resonance.	8
Scanning Electron Microscopy.	9
Field Experiments 1984.	10
Field Experiments 1985.	12
Results and Discussion.	16
Cuticular Transpiration	16
Quantitative Wax Determination.	18
Scanning Electron Microscopy.	18
Field Experiments 1984.	18
EPTC-Sunflower Height and Stem Diameter	29

TABLE OF CONTENTS (continued)

Page

Acifluorfen-Sunflower Height and Stem Diameter. . . . 33

Summary 37

Literature Cited. 38

Appendix. 42

LIST OF TABLES

Table		Page
1.	Location and dates of EPTC and acifluorfen application, planting, evaluations, and plant sampling for field experiments in 1984	13
2.	Temperature and precipitation dates for a 6 month period at White, South Dakota and Toronto, South Dakota in 1984.	14
3.	Influence of EPTC on epicuticular leaf wax deposition on sunflower.	19
4.	The type of interaction with various rates of EPTC and acifluorfen on sunflower phytotoxicity at White, South Dakota	28
5.	Influence of EPTC on sunflower height at White, South Dakota.	31
6.	Influence of acifluorfen on sunflower height at White, South Dakota	33
7.	Influence of acifluorfen on sunflower height at Toronto, South Dakota	34
8.	Influence of acifluorfen on sunflower stem diameter at White, South Dakota.	35
9.	Influence of acifluorfen on sunflower stem diameter at Toronto, South Dakota.	36

LIST OF FIGURES

		Page
Figure 1.	Moisture loss through the cuticle of EPTC treated sunflower leaves.	17
Figure 2.	Nuclear Magnetic Resonance peaks for the 0.0 kg/ha and 5.04 kg/ha rates of EPTC	20
Figure 3.	SEM of adaxial surface of fresh sunflower leaves. A: greenhouse, sunflower leaf (x200); B: greenhouse, sunflwer leaf treated with 5.04 kg/ha EPTC (x200); C: field, sunflower leaf (x200); D: field sunflower leaf treated with 6.72 kg/ha EPTC (x200).	21
Figure 4.	Phytotoxicity resulting from EPTC and acifluorfen at White, South Dakota in 1984. Phytotoxicity is based on visual ratings on a scale of 0 to 100 with 0=no injury and 100=complete kill. In the equation P=phytotoxicity, E=rate of EPTC, and A=rate of acifluorfen.	27
Figure 5	Sunflower yield effects from EPTC and acifluorfen at White, South Dakota in 1984. Herbicide axes are reversed to view surface. In equation P=phytotoxicity, E=rate of EPTC, and A=rate of acifluorfen. .	30

INTRODUCTION

Sunflowers (Helianthus annuus L.) rank third in acreage among the world's oil crops (3). Approximately 185,000 ha of sunflowers are grown in South Dakota each year (32). Despite sunflower's economic importance, few herbicides are available for use in the crop and many of the herbicides commonly used on soybeans (Glycine max (L.) Merr.) and corn (Zea mays L.) will harm sunflowers. Therefore, weed infestations are a major factor confronting the sunflower grower. Wild mustard (Brassica kaber (DC.) Wheeler) and green foxtail (Setaria viridis (L.) Beauv.) commonly infest sunflower fields in North and South Dakota. According to a survey in North Dakota, 94% of the sunflower fields contained green foxtail and 75% contained wild mustard (7). Wild mustard is very competitive and one wild mustard plant/m of crop row reduces yield (22).

EPTC (S-ethyl dipropylthiocarbamate), trifluralin (a,a,a-trifluoro-2,6-dinitro-N-N-dipropyl-p-toluidine), and chloramben (3-amino-2,5-dichlorobenzoic acid) are labeled for control of annual grasses and certain broadleaf weeds in sunflower fields (34). Although herbicides are available for grass control, more effective ones are needed to control broadleaf weeds in sunflower fields. A postemergence herbicide would significantly aid weed control and could be used in conservation tillage

REVIEW OF THE LITERATURE

EPTC and Epicuticular Wax

The cuticle is defined as a protective membrane which covers the epidermal cells. Cutin and waxes are the two primary elements of the cuticle. The cutin forms the grid of the membrane and the waxes are both embedded and extruded from the membrane surface. The extruding waxes form a waxy surface bloom. Cellulose and pectin combine with the cutin as the membrane covers the outer wall of the epidermal cells (1, 8, 18).

EPTC reduces production and changes the morphology of epicuticular wax in cabbage (Brassica oleracea L.) (9, 10, 21, 36). Gentner reported that the amount of wax deposition on cabbage leaves was correlated with the application rate of EPTC (13). Wax deposition was inhibited by more than 90%. The reduction in epicuticular wax; however, is greatest on the leaves developing immediately subsequent to soil treatment. This effect was progressively less on leaves which developed on subsequent nodes (9). EPTC reduced the amount of surface wax on the navy bean (Phaseolus vulgaris) (36, 37). Wax deposition was reduced 84% from the 4.5 kg ai/ha rate of EPTC (37).

The morphological characteristics of the leaf wax bloom of various species was altered with EPTC (10, 13, 20, 21, 32, 36). Scanning Electron Microscopy (SEM) was used to view the epicuticular wax on EPTC treated corn

(21). Many methods of specimen preparation can be used and were evaluated by Parson et al (28). Aggregation of the leaf wax was observed on the EPTC treated cabbage leaf. Epicuticular wax from both a treated and non-treated cabbage leaf appeared as an interconnecting network of wax projections. This network was diminished by EPTC (20). On the leaves of control plants, Wyse found small flakes of epicuticular wax evident that did not appear on leaves treated with EPTC (37).

Epicuticular wax fine structure influences wetability (13, 16) which in turn affects retention. Herbicide penetration is directly related to droplet retention. Studies have determined the surface area of aqueous droplets on a leaf and how contact angles relate to leaf penetration (2, 5, 6, 12, 16, 27, 30). The contact angle of a droplet on a leaf containing normal quantities of wax was 148°, whereas the contact angle of the droplet on the leaf with reduced wax was 94° (13). The area under the droplet was 277% greater on the leaf with reduced wax (13). Therefore, with reduced wax, greater herbicide penetration may occur.

Wax Composition

Researchers have investigated the chemical structure of epicuticular wax of many species (11, 15, 17, 19, 33). EPTC altered the wax composition of developing cabbage leaves, but did not affect cutin composition. The

alkane, ketone and secondary alcohol content of epicuticular wax was reduced and ester content increased (11). Although relative amounts changed, homologue composition within a chemical group was not altered.

Cuticular Transpiration

Plants lose water to the atmosphere by stomatal and cuticular transpiration. Cuticular wax, environmental conditions and guard cells of the stomates regulate the relative amounts of water lost (14). At one time it was thought that cuticular wax did not play a role in transpiration (23), but had only a protective function. Researchers have since discovered that water relations are affected by epicuticular wax (29). Gentner reported that as the rate of EPTC increased, the transpiration rate of the affected leaves increased (13, 21). Wyse observed severe wind blast injury and leaf desiccation in navy beans treated with EPTC under conditions of low humidity, high winds, and limited soil moisture (37).

Sunflower Response To Acifluorfen

While wild mustard control was excellent, sunflower injury occurred when a rate of 0.28 kg ai/ha of acifluorfen was applied to sunflower plants in the 4 leaf stage (24). However, acifluorfen applied at 0.07 kg ai/ha in the 4-8 leaf stage of sunflower caused only 1% crop injury and gave 83% wild mustard control (25, 26).

Herbicide Interaction

Under field conditions, plants pretreated with herbicides that inhibit epicuticular wax could be more sensitive to postemergence herbicide applications (10, 13). EPTC at planting predisposed corn to increased crop injury from later applications of paraquat (1,1'-dimethyl -4,4'-bipyridinium ion) (21). EPTC induced epicuticular wax aggregation caused increased paraquat uptake. Another study reported an increase in toxicity in EPTC treated cabbage plants from a postemergence application of DNBP (4,6-dinitro-o-scc- butylphenol) (13). A similar response was observed when EPTC pretreated pea (Pisum sativum L.) plants were treated with diallate (S-(2,3-dichloroallyl) diisopropylthio-carbamate). These plants became increasingly sensitive to the herbicidal action of foliarly applied propanil (3',4'-dichloropropionanilde [N-(3,4-dichlorophenyl) propionamide] (31). EPTC treated plants may also have less resistence to pathogens. Wyse reported that navy bean seedlings grown in soil treated with EPTC were more susceptible to root rot caused by (Fusarium solani (Mart.)) (35, 36).

Previous research suggests that EPTC affects the epicuticular wax of several species, as well as preconditioning plants to additional crop injury from later applications of a postemergence herbicide. Acifluorfen is effective for postemergence wild mustard

control; however, a phytotoxic response from sunflowers is evident at higher rates of application.

MATERIALS AND METHODS

Greenhouse Plant Culture and Herbicide Application

Sunflower plants were grown in soil which had been treated with EPTC at rates of 0.0, 1.68, 3.36, 5.04, and 6.72 kg ai/ha. EPTC was sprayed with a greenhouse pot sprayer onto a sandy loam soil with a pH of 7.1 and organic matter content of 3.6%. The treated soil was mixed with a Y tube soil blender and then transferred to 946 ml waxed disposable pots, which had been partially filled with untreated soil. Treated soil overlayed untreated soil in each pot at a depth of 7.62 cm. Three sunflower seeds were planted in each pot at a depth of 3.81 cm. The plants were grown in a greenhouse supplemented with artificial lighting and with a temperature of 20° C to 32° C.

Cuticular Transpiration

The effect of EPTC on the rate of transpiration through the cuticle was determined by measuring the rate of water loss from excised sunflower leaves. When the sunflower plants were in the 6-leaf stage, leaves 5 and 6 were excised. The area of the leaves was measured with an automatic area meter. Lannolin was applied to the cut petiole and the stomates were allowed to close as evident

by slight wilting and visual inspection under a microscope. After stomatal closure was complete, weight loss was attributed to cuticular transpiration. Values are the average of two experiments with four replications of each treatment and with 6 leaves harvested per replication.

Gravimetric Determination of Epicuticular Wax

When the sunflower plants were in the 6-leaf stage, the fifth and sixth leaves were removed, placed in the bottom of a 1 liter beaker, and washed three times for 10 s each time with 100 ml of chloroform. The area of the leaves was measured with an automatic area meter (Lambda Instruments). The chloroform-epicuticular wax extract was filtered through Whatman No. 1 filter paper into preweighed evaporating tins. After the solvent was evaporated for 18 h at room temperature, the tins were reweighed, and the weight of epicuticular wax per cm^2 leaf area was calculated. Values are the average of two experiments, four replications per treatment, with 12 leaves harvested per replication.

Nuclear Magnetic Resonance

The effect of EPTC on the chemical structure of epicuticular wax was determined by Nuclear Magnetic Resonance (NMR). When sunflower plants were in the

6-leaf stage, leaves five and six were excised and immersed in carbon tetrachloride for 30 s to remove surface wax. Only leaves from the control and 5.04 kg ai/ha were used. The leaves were removed from the carbon tetrachloride and then the carbon tetrachloride was evaporated. The remaining residue was brought up to 2 ml volume with deuterized chloroform and placed in a NMR tube. Changes in leaf wax structure were evaluated with a Perkin Elmer R12B Nuclear Magnetic Resonance Spectrophotometer.

Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) was used in all observations of leaf fine structure. Scanning electron micrographs were made with a International Scientific Instruments Super III A SEM of the adaxial surface of fresh sunflower leaves. Micrographs were made of the fifth leaf when plants were in the 6-leaf stage. Only leaves from the control, 5.04 (greenhouse) and 6.72 (field) kg ai/ha rates of EPTC were used. Rectangular leaf pieces (4 X 8 mm) were cut from each leaf at one side of the midvein near the leaf center. The leaf pieces were glued abaxial surface down with metallic glue to the top of an aluminum SEM stub. The stubs were then placed in the SEM and micrographs taken on Polaroid type 55 film at 10 kv acceleration potential. Micrographs

were taken within 15 minutes from the time the leaf pieces were cut.

Field Experiments 1984

In 1984, field studies were conducted near Toronto and White, South Dakota. The experimental design was a randomized complete block with four replications. Plot size was 2.3 by 30.5 m and plots were arranged in a 5 X 5 factorial.

EPTC was applied preplant at 0.0, 1.68, 3.36, 5.04 and 6.72 kg ai/ha with an experimental plot sprayer equipped with four Tee Jet 8002 flat fan nozzles spaced 51 cm apart. The boom was held 46 cm above the soil surface to provide uniform application over an area 2 m wide. The nozzles delivered 187 l/ha spray solution at 276 kPa pressure and ground speed of 4.83 km/hr. The herbicide was incorporated into the soil immediately after application by making two passes with a field cultivator set to a depth of 7.6 cm.

The sunflower variety 'Stauffer 3100' was planted to achieve a population of 61,700 plants/ha in rows spaced 76 cm apart. Carbofuran (2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) was banded on the rows for insect control.

When the sunflowers were in the 6-8 leaf stage at Toronto and the 6-leaf stage at White, acifluorfen was applied factorially at 0.0, 0.071, 0.14, 0.28, and 0.56

kg ai/ha with the same sprayer nozzle size, carrier volume and speed as above. The combination of 0 rates of both herbicides constituted the untreated control.

Stem diameter, height, and visual crop injury ratings were taken 2 weeks after the treatment of acifluorfen. The herbicide application, planting, evaluation and sampling dates are shown in Table 1. Injury ratings were based on a scale of 0 to 100, with 0 indicating no visual injury and 100 representing total death of the crop. Using a caliper, stem diameter at the base was measured on 10 randomly chosen plants per plot. Accuracy of the measured value was to 0.01 cm. Ten randomly chosen plants within each plot were chosen for height measurement. Sunflower yields were determined by hand harvesting a measured area, threshing with a plot thresher, weighing the sample and converting it to kg/ha. The meteorological data were evaluated for both locations and are contained in Table 2.

The soil at the Toronto location was an Estelline silt loam (Pachic Udic Haploborolls, fine-silty over sandy mixed) with a pH of 6.5; organic matter content of 3.9%; and sand, silt, and clay content of 19, 63, and 18%, respectively. The soil at the White location was a Kranzburg silt loam (Udic Haploborolls, fine-silty, mixed) with a pH of 6.5; organic matter content of 5.6%; and

sand, silt, and clay content of 17, 58, and 25%, respectively.

Field Experiments 1985

Two field studies were conducted on the South Dakota State University Agronomy Farm in 1985. The experiments were arranged in a randomized complete block design with four replications. Plot size was 2.3 by 15.2 m.

EPTC was applied preplant at 0.0, 1.68, 3.36, 5.04 and 6.72 kg ai/ha with an experimental plot sprayer equipped with four Tee Jet 8002 flat fan nozzles spaced 51 cm apart. The boom was held 46 cm above the soil surface to provide uniform application over an area 2 m wide. The nozzles delivered 187 l/ha spray solution at 276 kPa pressure and ground speed of 4.83 km/hr. The herbicide was incorporated immediately after application by making two passes with a field cultivator set to a depth of 7.6 cm.

The sunflower variety `Stauffer 3100' was planted to achieve a population of 61,700 plants/ha in rows spaced 76 cm apart. The plant material from the field experiments was used for cuticular transpiration, nuclear magnetic resonance, and gravimetric cuticular wax experiments.

Table 1. Location and dates of EPTC and acifluorfen application, planting, evaluations, and plant sampling for field experiments in 1984.

Location	Application Dates	Planting Dates	Evaluation Dates	Plant Sampling
White	May 11, 1984[a] June 29, 1984[b]	May 20, 1984	July 13, 1984	October 3, 1984
Toronto	May 12, 1984[a] June 29, 1984[b]	May 20, 1984	July 14, 1984	October 7, 1984

[a]Date EPTC was applied

[b]Date acifluorfen was applied

Table 2. Temperature and precipitation data for a 6 month period at White, South Dakota and Toronto, South Dakota in 1984.

	Temperature (C°)		Precipitation (mm)	
	White	Toronto	White	Toronto
April	6.3	6.7	85.9	83.3
May	11.7	12.9	77.7	65.0
June	18.3	19.0	215.4	201.2
July	21.0	21.7	55.1	30.0
August	21.2	22.6	41.7	27.7
September	15.5	13.6	46.5	90.2
Total	--	--	522.0	497.4
Mean	14.7	16.1	87.0	82.9

At location one, the soil was a Vienna loam (Udic Haploborolls, fine-loamy, mixed) with a pH of 7.7; organic matter content of 3.7%; and sand, silt, and clay content of 37, 39, and 24%, respectively. The soil at location two was a Lismore silty clay loam (Pachic Udic Haploborolls, fine-loamy, mixed) with a pH of 6.5%; organic matter content of 4.5%; and sand, silt, and clay content of 37, 39, and 24%, respectively.

RESULTS AND DISCUSSION

Cuticular Transpiration

No significant differences in water loss from sunflower leaves were observed in field experiments (data not shown). My results are similar to those reported by Leavitt et al (20).

However, in the greenhouse, EPTC increased cuticular transpiration (Figure 1). A linear response over time occurred for both the 5.04 and the 1.68 kg ai/ha rates of EPTC. Leaves treated with 6.72 kg/ha transpired linearly with a greater rate of moisture loss until reaching a maximum, where the moisture loss tapered off. The cuticular resistance decreased as the rate of EPTC increased.

The difference in the results between the field and greenhouse experiments could be that less herbicide volatilization occurred in the greenhouse and thus more herbicide was available to the plant. Greater herbicide availability magnified the effects of EPTC in the greenhouse experiments. Also, the difference could be that the plants naturally produced more wax in the field than in the greenhouse. Plants grown in the field could have been stressed and could have produced enough wax to prevent water loss.

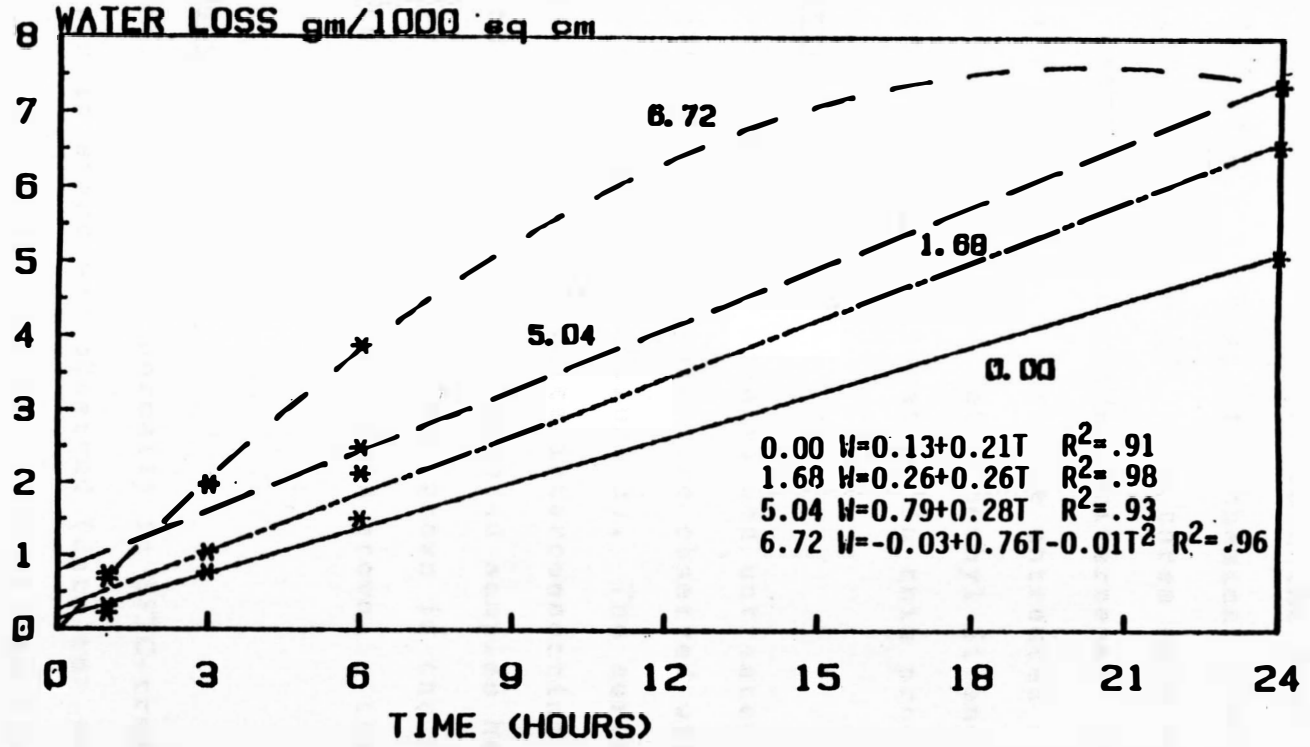


Figure 1. Moisture loss through the cuticle of EPTC treated sunflower leaves.

Quantitative Wax Determination

The effect of EPTC on epicuticular wax deposition is shown in Table 3. EPTC significantly reduced the amount of epicuticular leaf wax, at both the 5.04 and the 6.72 rates. EPTC did not change the chemical structure of the epicuticular wax (Figure 2). The three main peaks on the graph represent the flavonoids, triterpenes, ketones, fatty acids, and aliphatics. Both the untreated and the 5.04 kg/ha peaks are similar. Tetra Methyl Silene (TMS) was the standard used for calibration in this procedure.

Scanning Electron Microscopy

Sunflower leaves from treated and untreated plants grown in the greenhouse and field were observed with a scanning electron microscope (Figure 3). The surfaces of the untreated leaves have definite interconnecting ridges formed by epicuticular wax. The treated samples have less ridge formation. Leaves from plants grown in the field were similar to the leaves from plants grown in the greenhouse.

Field Experiments 1984

The sunflowers emerged normally in EPTC-treated soil and no reduction in stand was observed (data not shown). Two weeks after acifluorfen was applied, the sunflowers were evaluated for crop injury and a significant interaction between EPTC and acifluorfen was observed at

Table 3. Influence of EPTC on epicuticular leaf wax dispostion on sunflower.

Treatment	Rate	Wax Weight[a]
	(kg/ha)	(ug/1000 cm^2)
Control		30.9 A
EPTC	1.68	26.3 AB
EPTC	3.36	24.7 AB
EPTC	5.04	22.2 B
EPTC	6.72	21.2 B

[a]Values with the same letter on them are not significantly different at the 5% level using the Waller-Duncan k-ratio t-test (k=100).

EFFECT OF EPTC ON LEAF WAX CHEMISTRY

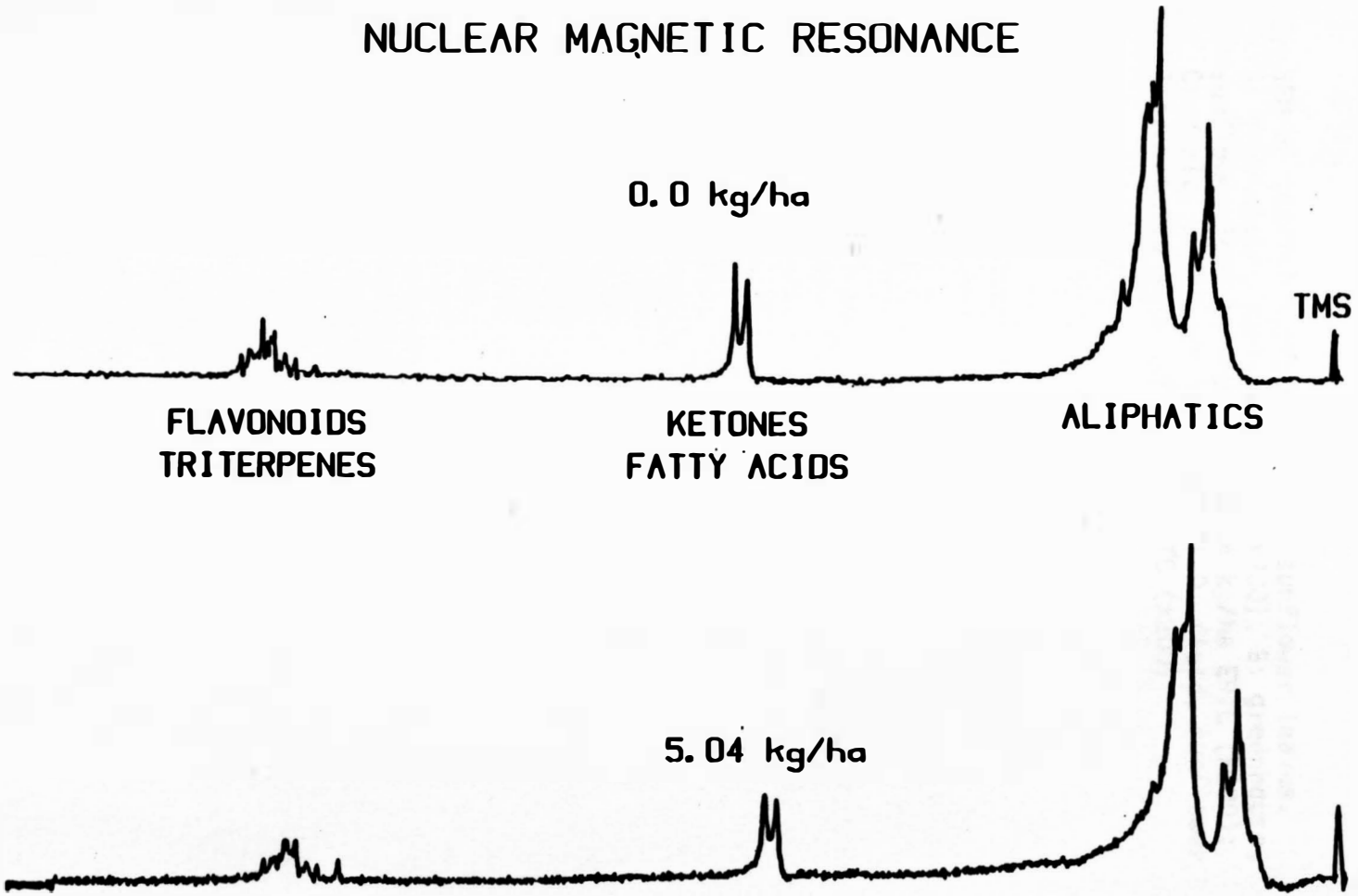
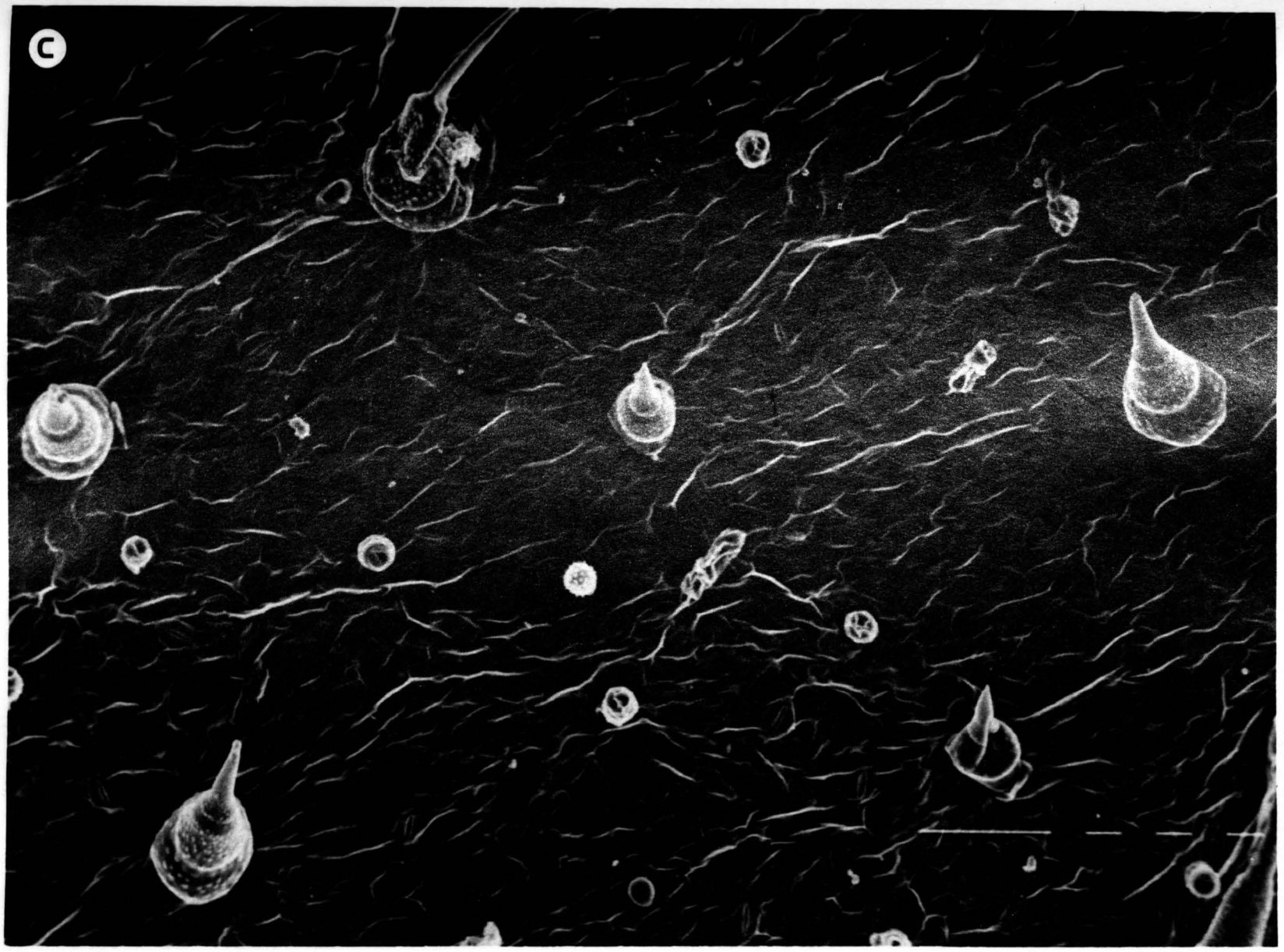


Figure 2. Nuclear Magnetic Resonance peaks for the 0.0 kg/ha and 5.04 kg/ha rates of EPTC

Figure 3. SEM of adaxial surface of fresh sunflower leaves. A: greenhouse, sunflower leaf (x200); B: greenhouse, sunflower leaf treated with 5.04 kg/ha EPTC (x200); C: field, sunflower leaf (x200); D: field, sunflower leaf treated with 6.72 kg/ha EPTC (x200).

A

B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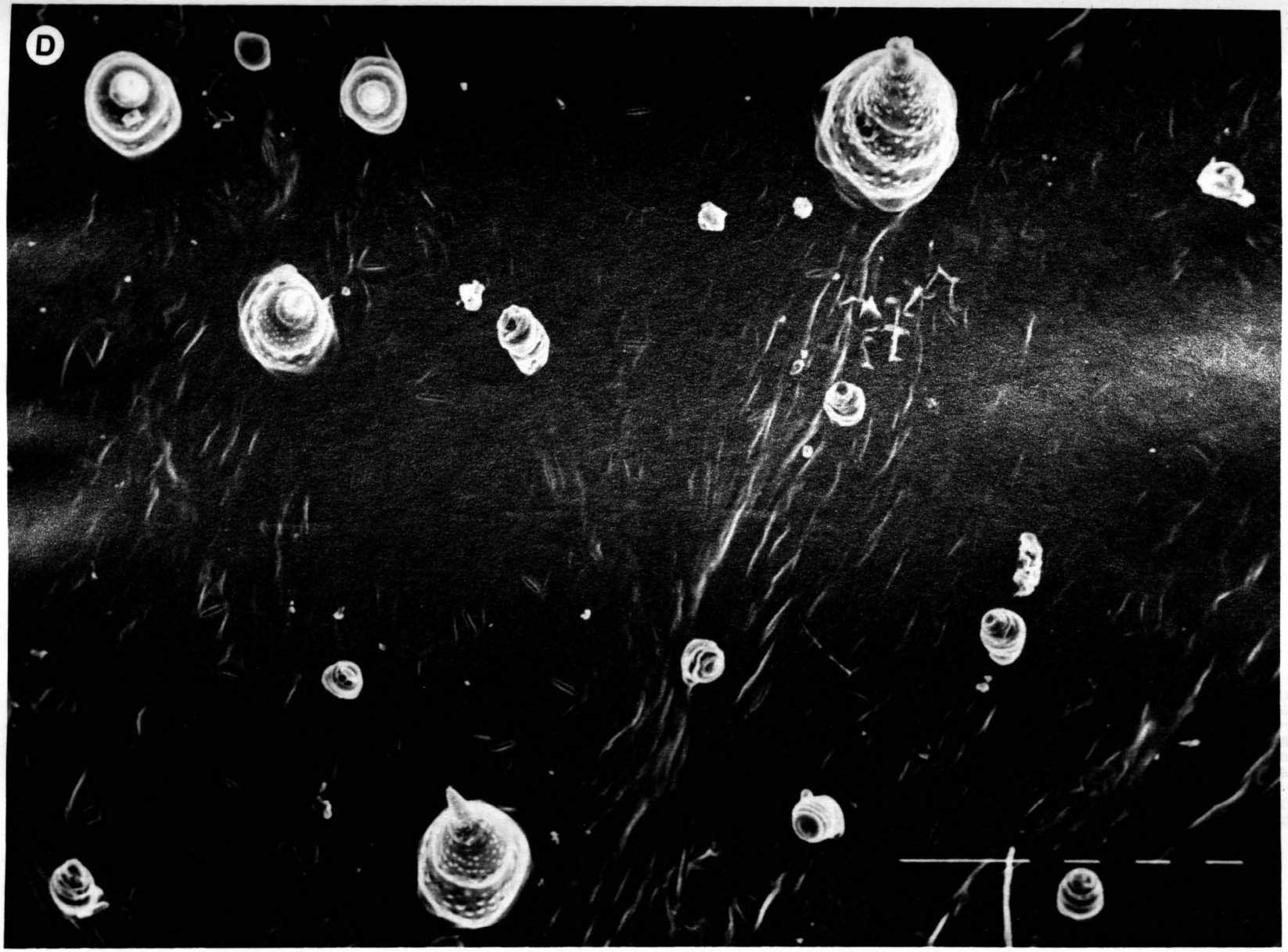
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Toronto and White. At White the multiple regression equation fitted to the data accounted for 91% of the variation in phytotoxicity (Figure 4). As the combined rates of EPTC and acifluorfen were increased, the percentage of phytotoxicity was increased in a synergistic response. This synergism is evident in the upward slope from both herbicde axes. When Colby's Method for interactions is used, the synergism is also apparent (4) (Table 4). The expected values are calculated by:

$$E = X + Y - \frac{(X * Y)}{100}$$

X = observed value with herbicide A at rate p
Y = observed value with herbicide B at rate q
E = expected value with herbicides A plus B at rates p and q

The difference between the observed value and the expected value are presented in Table 4 for two combinations of EPTC and acifluorfen. The difference at the rates of 1.68 and 0.56 kg/ha of EPTC and acifluorfen respectively, was an increase of 1.5. The difference at the 3.36 and 0.56 kg/ha rate was 16.5, a 15 unit increase. This increase indicates a even stronger synergistic response between EPTC and acifluorfen.

At Toronto, EPTC had little effect on phytotoxicity caused by acifluorfen. This anomaly between the interactions at White and Toronto could be due to the stage of growth at which the sunflowers were sprayed with

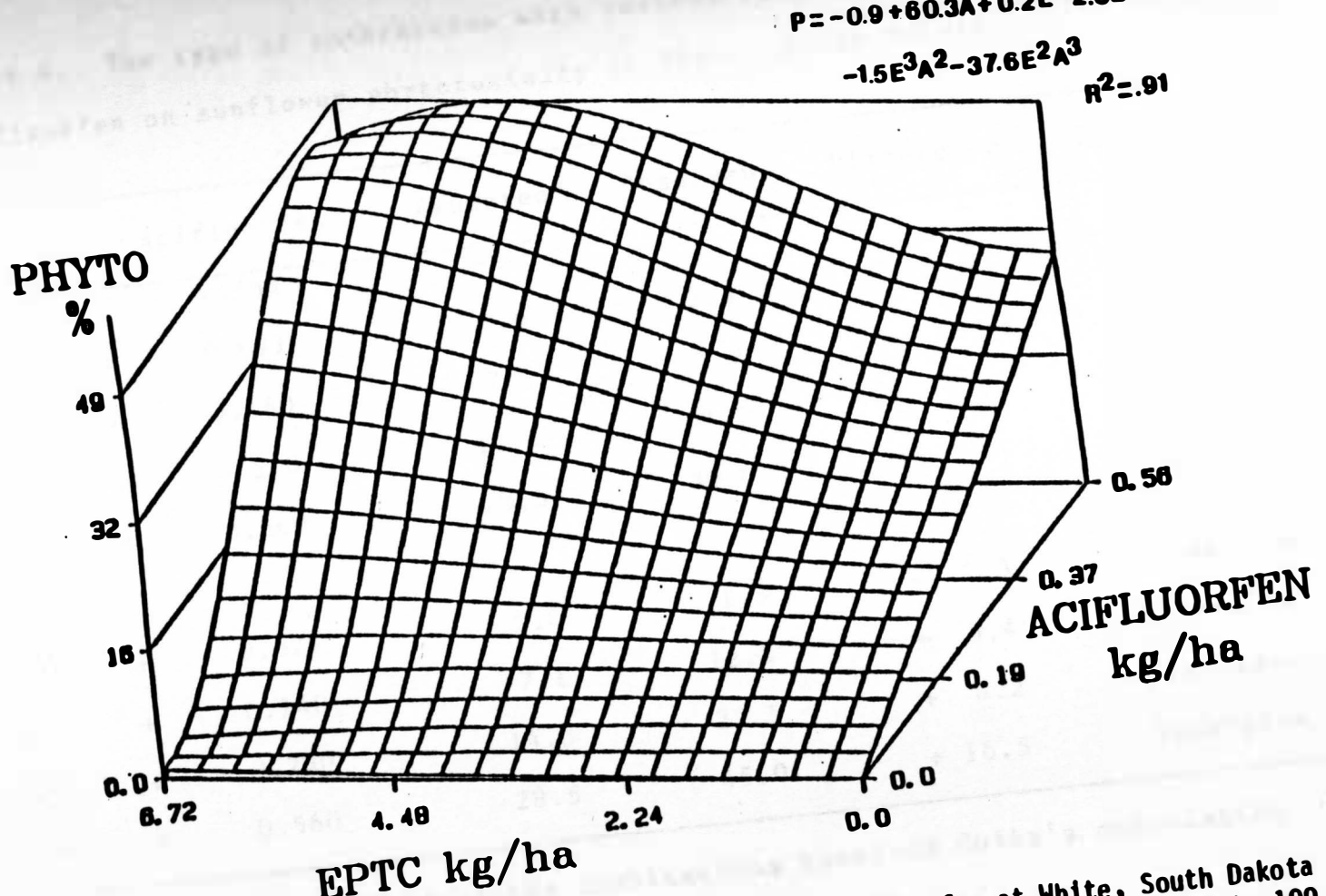

Figure 4. Phytotoxicity resulting from EPTC and acifluorfen at White, South Dakota in 1984. Phytotoxicity is based on visual ratings on a scale of 0 to 100 with 0=no injury and 100=complete kill. In the equation, P=phytotoxicity E=rate of EPTC, and A=rate of acifluorfen.

Table 4. The type of interaction with various rates of EPTC and acifluorfen on sunflower phytotoxicity at White, South Dakota.

EPTC		Acifluorfen	Expected[a]	Observed	Difference	Interaction
kg/ha		kg/ha	-%-	-%-		
1.68	+	0.071	3.6	2.5	- 1.1	Antagonism
1.68	+	0.140	7.3	5.0	- 2.3	Antagonism
1.68	+	0.280	14.3	17.5	+ 3.2	Synergism
1.68	+	0.560	28.5	30.0	+ 1.5	Synergism
3.36	+	0.071	3.6	1.3	- 2.3	Antagonism
3.36	+	0.140	7.1	12.5	+ 5.4	Synergism
3.36	+	0.280	14.3	22.5	+ 8.2	Synergism
3.36	+	0.560	28.5	45.0	+ 16.5	Synergism

[a]Expected response of herbicide combinations based on Colby's calculation (4).

acifluorfen. The sunflowers were in the 6-leaf stage when treated at White and 6 to 8-leaf stage when treated at Toronto. Flore et al reported, the reduction in epicuticular wax caused by EPTC is less intense with each new set of leaves (9). If so, larger sunflowers would not be as susceptible to acifluorfen, as was the case at the Toronto location.

A significant interaction occurred for sunflower yield by the combinations of EPTC and acifluorfen at White (Figure 5). This interaction was similar in response to the interaction which occurred for phytotoxicity. Herbicide axes are reversed in the yield surface response for viewing of the surface. The combined maximum rates of both herbicides resulted in the lowest yields and highest phytotoxicity rating. Acifluorfen reduced sunflower yield to a greater degree than did EPTC. This is apparent with a larger acifluorfen term in the multiple regression equation. The correlation between phytotoxicity and yield ($R=-.72$) demonstrates that as crop injury increases sunflower yield decreases.

EPTC - Sunflower Height And Stem Diameter

Sunflower height was significantly affected by EPTC at White (Table 5). The three highest rates apparently caused sunflower predisposition to acifluorfen. When EPTC was averaged across acifluorfen, height was significantly less than the sunflowers from the control. Sunflower

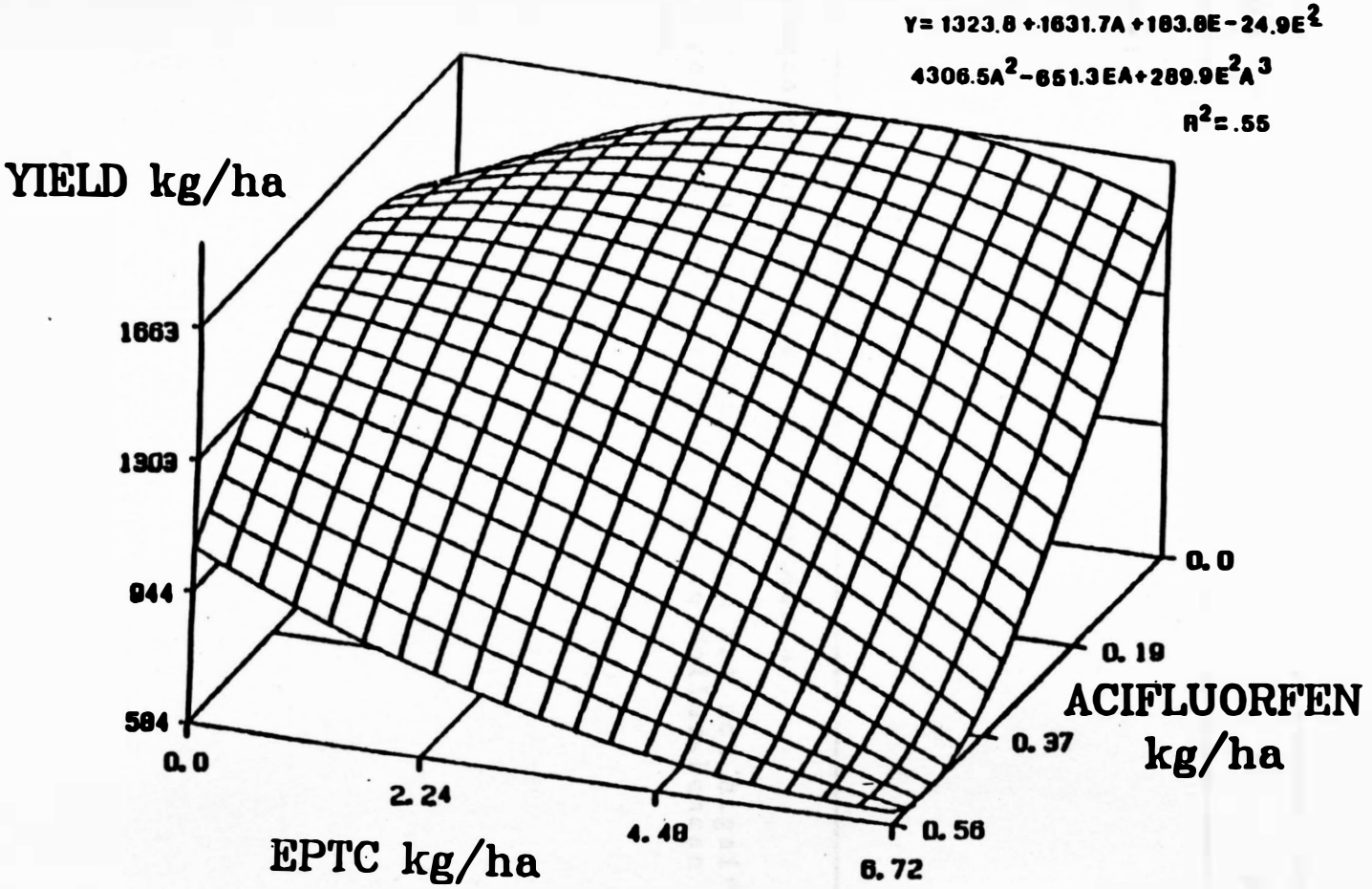

Figure 5. Sunflower yield effects from EPTC and acifluorfen at White, South Dakota in 1984. Herbicide axes are reversed to view surface. In equation, P=phytotoxicity, E=rate of EPTC, and A=rate of acifluorfen.

Table 5. Influence of EPTC on sunflower height at White, South Dakota.

Treatment[a]	Rate	Height[b]
	(kg/ha)	(cm)
Control		62.2 A
EPTC	1.68	59.5 AB
EPTC	3.36	53.2 C
EPTC	5.04	54.7 BC
EPTC	6.72	54.0 C

[a]Averaged across acifluorfen treatments.

[b]Values with the same letter on them are not significantly different at the 5% level using the Waller-Duncan k-ratio t-test (k=100).

height, associated to EPTC, was not affected at the Toronto location. The difference experienced between White and Toronto are probably due to the different growth stages at which acifluorfen was applied. Stem diameter was evaluated and
EPTC had no effect at any location.

Acifluorfen - Sunflower Height and Stem Diameter

Acifluorfen significantly reduced height of sunflowers at both White and Toronto (Tables 6 and 7). Maximum reduction was 44% and 25% at White and Toronto respectively. The sunflowers were taller throughout the growing season at Toronto than at White.

Stem diameter was reduced by acifluorfen at both White and Toronto (Tables 8 and 9). As with height, stem diameter was affected most at the White location with a reduction of 25%.

The decrease in stem diameter may increase insect injury. The sunflower head clipper (Haplorhyncites aeneus) infested plots where stem diameters were smaller but did not infest plots with larger stems. Therefore, acifluorfen treated sunflowers may be more susceptible to insect damage or lodging.

Table 6. Influence of acifluorfen on sunflower height at White, South Dakota.

Treatment[a]	Rate	Height[b]
	(kg/ha)	(cm)
Control		69.8 A
Acifluorfen	0.07	66.8 A
Acifluorfen	0.14	59.5 B
Acifluorfen	0.28	48.4 C
Acifluorfen	0.56	39.0 D

[a]Averaged across EPTC treatments.

[b]Values with the same letter on them are not significantly different at the 5% level using the Waller-Duncan k-ratio t-test (k=100).

Table 7. Influence of acifluorfen on sunflower height at Toronto, South Dakota.

Treatment[a]	Rate	Height[b]
	(kg/ha)	(cm)
Control		82.8 A
Acifluorfen	0.07	80.4 B
Acifluorfen	0.14	78.8 B
Acifluorfen	0.28	73.2 C
Acifluorfen	0.56	62.1 D

[a]Averaged across EPTC treatments.

[b]Values with the same letter on them are not significantly different at the 5% level using the Waller-Duncan k-ratio t-test (k=100).

Table 8. Influence of acifluorfen on sunflower stem diameter at White, South Dakota.

Treatment[a]	Rate	Stem Diameter[b]
	(kg/ha)	(cm)
Control		1.28 A
Acifluorfen	0.07	1.23 AB
Acifluorfen	0.14	1.21 AB
Acifluorfen	0.28	1.10 C
Acifluorfen	0.56	0.96 D

[a]Averaged across EPTC treatments.

[b]Values with the same letter on them are not significantly different at the 5% level using the Waller-Duncan k-ratio t-test (k=100).

Table 9. Influence of acifluorfen on sunflower stem diameter at Toronto, South Dakota.

Treatment[a]	Rate	Stem Diameter[b]
	(kg/ha)	(cm)
Control		1.24 A
Acifluorfen	0.07	1.22 A
Acifluorfen	0.14	1.22 A
Acifluorfen	0.28	1.21 A
Acifluorfen	0.56	1.05 B

[a]Averaged across EPTC treatments.

[b]Values with the same letter on them are not significantly different at the 5% level using the Waller-Duncan k-ratio t-test (k=100).

SUMMARY

EPTC increased cuticular transpiration on plants grown under greenhouse conditions; however, under field conditions cuticular transpiration was not affected. The leaf epicuticular wax was reduced due to the EPTC application. However, no change in chemical structure was evident in the wax.

Field studies indicated a significant interaction between EPTC and acifluorfen at White. The combination of both herbicides increased crop injury and decreased yield synergistically. At Toronto, this interaction was not as apparent as at the White location. This difference is probably due to the different growth stages at which acifluorfen was applied.

The influence of EPTC significantly reduced sunflower height at White but not at Toronto when averaged across the acifluorfen rates. This difference in height is again probably due to the different growth stages. Acifluorfen reduced stem diameter and height at both locations.

This research gives evidence that EPTC affects epicuticular wax on sunflower leaves and EPTC may predispose sunflowers to additional injury from acifluorfen applied at an early growth stage.

LITERATURE CITED

1. Baker, E. A., R. F. Batt, and J. T. Martin. 1964. Studies on plant cuticule: VII. The Nature and determination of cutin. Ann. Appl. Biol. 53:59-65.

2. Cantliffe, D. J., and G. E. Wilcox. 1972. Effect of surfactant on ion penetration through leaf wax and a wax model. J. Amer. Soc. Hort. Sci. 97:360-363.

3. Carter J. F., Editor. 1978. Sunflower Science and Technology. Agronomy 19:482.

4. Colby, S. R. 1967. Calculating synergistic and antagonistic responses of herbicide combinations. Weeds 15:20-22.

5. Currier, H. B., and C. D. Dybing. 1959. Foliar Penetration of herbicides - review and present status. Weeds 7:195-213.

6. Darlington, W. A., and J. Barry. 1965. Effects of chloroform and surfactants on permeability of apricot leaf cuticle. J. Agr. Food. Chem. 13:76-80.

7. Dexter, A. G., J. D. Nalewaja, and J. A. Buchli. 1979. Weed survey in North Dakota. North Central Weed Contr. Conf. Proc. 34:79.

8. Fernandes, A. M. S., E. A. Baker, and J. T. Martin. 1964. Studies on plant cuticle: VI. The isolation and fractionation of cuticular waxes. Ann. Appl. Biol. 53:43-58.

9. Flore, J. A., and M. J. Bukovac. 1973. Pesticide effects on the plant cuticle: I. Response of Brassica oleracea L. to EPTC as indexed by epicuticular wax production. J. Amer. Soc. Hort. Sci. 99(1):34-37.

10. Flore, J. A., and M. J. Bukovac. 1976. Pesticide effects on the plant cuticle: II. EPTC effects on leaf cuticle morphology and composition in Brassica oleracea L. J. Amer. Soc. Hort. Sci. 101:586-590.

11. Flore, J. A., and M. J. Bukovac. 1978. Pesticide effects on the plant cuticle: III. EPTC effects on the qualitative composition of Brassica oleracea L. leaf cuticle. J. Amer. Soc. Hort. 103:297-301.

12. Flore, J. A., and M. J. Bukovac. 1981. Pesticide effects on the plant cuticle: IV. The effect of EPTC on the permeability of cabbage, bean and sugar beet cuticle. J. Amer. Soc. Hort. Sci. 106:189-193.

13. Gentner, W. A. 1966. The influence of EPTC on external foliage wax deposition. Weeds 14:27-31.

14. Grncarevic, M., and F. Radler. 1967. The effect of wax components on cuticular transpiration - model experiments. Planta 75:23-27.

15. Holloway, P. J. 1966. Thin layer chromatography in the study of natural waxes and their constituents. Chromatog 25:336-346.

16. Holloway, P. J. 1969. The effects of superficial wax on leaf wettability. Ann. Appl. Biol. 63:145-153.

17. Johnson, D. A., M. L. Tonnet, and R. A. Richards. 1984. Estimation of epicuticular wax amounts in wheat using wide-line proton magnetic resonance. Crop Sci. 24:679-682.

18. Juniper, B. E., and C. E. Jeffree. 1983. Plant surfaces. p. 93. Edward Arnold. Bedford Square, London.

19. Kolattukudy, P. E., and L. Brown. 1974. Inhibition of cuticular lipid biosynthesis in Pisum sativum by thiocarbamates. Plant Physiol. 53:903-906.

20. Leavitt, J. R. C., D. N. Duncan, D. Penner, and W. F. Meggitt. 1978. Inhibition of epicuticular wax deposition on cabbage by ethofumesate. Plant Physiol. 61:1034-1036.

21. Leavitt, J. R. C., and D. Penner. 1978. Prevention of EPTC-induced epicuticular wax aggregation on corn Zea mays with R-25788. Weed Sci. 27:47-50

22. Lilleboe, D. 1983. Mustard: Presistent sunflower foe. The Sunflower 9(2):32-33.

23. Mueller, L. E., P. H. Carr, and W. E. Loomis. 1954. The submicroscopic structure of plant surfaces. Amer. J. Bot. 41:593-600.

24. Nalewaja, J. D., and S. D. Miller. 1981. Postemergence weed control in sunflower. No. Cent. Weed Contr. Conf. Res. Rep. 38:119-120.

25. Nalewaja, J. D., and S. D. Miller. 1982. Postemergence grass and boradleaf weed control in sunflower. No. Cent. Weed Contr. Conf. Res. Rep. 39:159.

26. Nalewaja, J. D., and S. D. Miller. 1982. Postemergence wild mustard control in sunflower. No. Cent. Weed Contr. Conf. Res. Rep. 39:160-161.

27. Norris, R. F. 1974. Penetration of 2,4-D in relation to cuticle thickness. Amer. J. Bot. 61:74-79.

28. Parsons, E., B. Bole, D. J. Hall, and W. D. E. Thomas. 1974. A comparative survey of techniques for preparing plant surfaces for the scanning electron microscope. Journal of Microscopy 101:59-75.

29. Schieferstein, R. M., and W. E. Loomis. 1959. Development of the cuticular layers in angiosperm leaves. Amer. J. Bot. 46:625-635.

30. Schonherr, J. 1976. Water permeability of isolated cuticular membranes: The effect of cuticular waxes on diffusion of water. Planta 131:159-164.

31. Still, G. G., D. G. Davis, and G. L. Zander. 1970. Plant epicuticular lipids: Alteration by herbicidal carbamates. Plant Physiol. 46:307-314.

32. United States Department of Agriculture. 1984. Agricultural Statistics 1984. United States Government Printing Office p 124.

33. Wilkinson, R. E., and W. S. Hardcastle. 1969. EPTC effects on sicklepod petiolar fatty acids. Weed Sci. 17:335-338.

34. Wrage, L. J., and W. E. Arnold. 1985. Chemical weed control in sunflower 1985. Fact Sheet 525SF.

35. Wyse, D. L., W. F. Meggitt, and D. Penner. 1976. Effect of herbicides on the development of root rot on navy bean. Weed Sci. 24:11-15.

36. Wyse, D. L., W. F. Meggitt, and D. Penner. 1976. Herbicide-root rot interaction in navy bean. Weed Sci. 24-16-21.

37. Wyse, D. l., W. F. Meggitt, and D. Penner. 1976. The interaction of attrazine and EPTC on navy bean. Weed Sci. 24:5-10.

APPENDIX

LIST OF APPENDIX TABLES

Table		Page
A-1	Analysis of variance for epicuticular wax weight on sunflower leaves from the treatment of EPTC.	45
A-2	Analysis of variance for cuticular transpiration on EPTC treated sunflower leaves after a 1 h time period	45
A-3	Analysis of variance for cuticular transpiration on EPTC treated sunflower leaves after a 3 h time period	46
A-4	Analysis of variance for cuticular transpiration on EPTC treated sunflower leaves after a 6 h time period	46
A-5	Analysis of variance for cuticular transpiration on EPTC treated sunflower leaves after a 24 h time period.	47
A-6	Analysis of variance for sunflower yield resulting from the interaction of EPTC and Acifluorfen at White, South Dakota	47
A-7	Analysis of variance for sunflower phytotoxicity resulting from the interaction of EPTC and Acifluorfen at White, South Dakota.	48
A-8	Analysis of variance for sunflower height resulting from EPTC at White, South Dakota	48
A-9	Analysis of variance for sunflower height resulting from Acifluorfen at White, South Dakota.	49
A-10	Analysis of variance for sunflower height resulting from Acifluorfen at Toronto, South Dakota.	49
A-11	Analysis of variance for sunflower stem diameter resulting from Acifluorfen at White, South Dakota.	50

LIST OF APPENDIX TABLES (continued)

Table		Page
A-12	Analysis of variance for sunflower stem diameter resulting from Acifluorfen at Toronto, South Dakota.	50

Table A-1. Analysis of variance for epicuticular wax weight on sunflower leaves from the treatment of EPTC.

Source	Degree of Freedom	Sum of Squares	F-Value
Replications	3	0.000014	0.11
Treatments	4	0.000470	2.66*
Error	32	0.001414	

*Significant at the 5% level.

Table A-2. Analysis of variance for cuticular transpiration on EPTC treated sunflower leaves after a 1 h time period.

Source	Degree of Freedom	Sum of Squares	F-Value
Replications	3	0.0594	1.04
Treatments	4	0.9600	12.63**
Error	32	0.6083	

**Significant at the 1% level.

Table A-3. Analysis of variance for cuticular transpiration on EPTC treated sunflower leaves after a 3 h time period.

Source	Degree of Freedom	Sum of Squares	F-Value
Replications	3	0.1922	0.32
Treatments	4	3.8641	4.82*
Error	32	6.4197	

*Significant at the 5% level.

Table A-4. Analysis of variance for cuticular transpiration on EPTC treated sunflower leaves after a 6 h time period.

Source	Degree of Freedom	Sum of Squares	F-Value
Replications	3	0.7315	0.62
Treatments	4	10.7315	6.83*
Error	32	12.6062	

*Significant at the 5% level.

Table A-5. Analysis of variance for cuticular transpiration on EPTC treated sunflower leaves after a 24 h time period.

Source	Degree of Freedom	Sum of Squares	F-Value
Replications	3	0.3275	0.25
Treatments	4	14.5145	8.44**
Error	32	13.7653	

**Significant at the 1% level.

Table A-6. Analysis of variance for sunflower yield resulting from the interaction of EPTC and Acifluorfen at White, South Dakota.

Source	Degree of Freedom	Sum of Squares	F-Value
Replications	3	1755449.37	3.10*
Treatments	16	7421200.38	2.46**
Error	48	9060851.22	

*Significant at the 5% level.
**Significant at the 1% level.

Table A-7. Analysis of variance for sunflower phytotoxicity resulting from the interaction of EPTC and Acifluorfen at White, South Dakota.

Source	Degree of Freedom	Sum of Squares	F-Value
Replications	3	750.00	0.25
Treatments	16	32880.00	1.85*
Error	48	53400.00	

*Significant at the 5% level.

Table A-8. Analysis of variance for sunflower height resulting from EPTC at White, South Dakota.

Source	Degree of Freedom	Sum of Squares	F-Value
Replications	3	1898.75	13.80**
Treatments	4	1243.56	5.46**
Error[a]	12	683.80	
Error	48	2202.0	

**Significant at the 1% level.
[a]Appropriate error term associated with the treatment.

Table A-9. Analysis of variance for sunflower height resulting from Acifluorfen at White, South Dakota.

Source	Degree of Freedom	Sum of Squares	F-Value
Replications	3	1898.75	13.80**
Treatments	4	13283.86	67.47**
Error[a]	12	590.70	
Error	48	2202.0	

**Significant at the 1% level.
[a]Appropriate error term associated with the treatment.

Table A-10. Analysis of variance for sunflower height resulting from Acifluorfen at Toronto, South Dakota.

Source	Degree of Freedom	Sum of Squares	F-Value
Replications	3	2230.96	7.33**
Treatments	4	7986.46	45.31**
Error[a]	12	528.74	
Error	48	4870.56	

**Significant at the 1% level.
[a]Appropriate error term associated with the treatment.

Table A-11. Analysis of variance for sunflower stem diameter resulting from Acifluorfen at White, South Dakota.

Source	Degree of Freedom	Sum of Squares	F-Value
Replications	3	0.01254	5.74**
Treatments	4	0.19640	57.97**
Error[a]	12	0.01016	
Error	48	0.03493	

**Significant at the 1% level.
[a]Appropriate error term associated with the treatment.

Table A-12. Analysis of variance for sunflower stem diameter resulting from Acifluorfen at Toronto, South Dakota.

Source	Degree of Freedom	Sum of Squares	F-Value
Replications	3	0.00466	2.16
Treatments	4	0.07719	26.00**
Error[a]	12	0.00891	
Error	48	0.03456	

**Significant at the 1% level.
[a]Appropriate error term associated with the treatment.