SOIL PARAMETERS AND FUNGI ISOLATES INFLUENCING PHYTOTOXICITY AND DEGRADATION OF A METHYL MERCAPTO TRIAZINE HERBICIDE

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CHAPTER I

INTRODUCTION

The substituted symmetrical triazine herbicides have shown promise in control for a wide spectrum of weeds in crop production and for non-crop areas having different environmental and edaphic conditions. This herbicide family is generally recommended as a pre-emergence treatment, however, their use as post-emergence contact, lay-by and soil sterilent is not uncommon. Added attention must be given to the problems of detoxification and soil residues as use of the s-triazines increases.

The s-triazines possess some properties common to other herbicide families. The mode of action of the s-triazines appears to be similar to that of the substituted ureas. The first symptoms of plant injury for both herbicide families include chlorosis, interference with CO_2 assimilation and sugar formation, and inhibition of the Hill reaction. Some s-triazines have a unique property of being detoxified by enzyme systems within certain plant species.

The purpose of this study was to manipulate soil factors that affect biological transformations and activity of 2,4-bis(isopropylamino)-6-methylmercapto-s-triazine in soil and establish rates and magnitudes of degradation. Included in this study were experiments designed to determine the effects of prometryne on selected common soil fungi isolates and establish rates and magnitudes of phytotoxic diminution.

CHAPTER II

LITERATURE REVIEW

In recent years the s-triazines were found to be highly effective pre-emergence herbicides and some are also extremely active as post-emergence sprays. The s-triazine, 2,4-bis(isopropylamino)-6-methyl-mercapto-s-triazine (prometryne) is used as a selective herbicide for control of broadleaf weeds and grasses in cotton and selected grass species grown for seed (16)[1]. Applications may be made pre-emergence, post-emergence directed, or lay-by in cotton. Prometryne has considerable contact activity when used with a surfactant, therefore, post-emergence treatments must be directed.

Numerous investigations in recent years have shown the biological, physical, and chemical properties of the s-triazines, atrazine and simazine. However, to date, little research has been conducted on these same properties for propazine, prometone, and prometryne.

Holstun and McWhorter (11) showed that chloro-triazine derivatives were low in selectivity on cotton and low to moderate in activity against brachiaria and crabgrass. Amino derivatives were generally high in selectivity, but all were very low in activity. Alkylamino substituents were present in active, inactive, selective, and non-selective compounds. Only methyl mercapto or methoxy derivatives had

[1]Figures in parenthesis refer to Literature Cited.

both high activity on weeds and low activity on cotton. As post-emergence sprays the methyl mercapto derivatives were found to be more active than the methoxy derivatives (21). Sheets and Crafts (20) showed that the chloro derivatives with a single methyl-ethyl or isopropyl substituent on each amino group were more toxic than those with a single substituent on one amino group and two substitutions on the other.

Triazines exhibit practically no influence on germinating seeds (7). The effect is mainly on the growing plant, however, some of the more soluble triazines (chloro-, methoxy-, and methyl mercapto-) have remarkable activity on established vegetation.

Herbicides become non-phytotoxic or disappear from soil by a variety of means. Some of the more important are microbial breakdown, leaching, plant uptake, volatilization, chemical breakdown, photodecomposition, and soil adsorption.

Detoxification of the s-triazines has been shown to occur within both resistant and susceptible plant species (6). When corn and cucumbers were treated with simazine-C^{14} it was found that the cucumbers produced more carbon^{14} dioxide than did the corn (6). Since simazine-C^{14} was uniformly labeled in the triazine ring, the evolution of $C^{14}O_2$ must have resulted from splitting the ring, indicating complete decomposition of the basic structure of simazine. The fact that cucumbers metabolized simazine at a more rapid rate than corn illustrates that toxicity is not associated with the inability to metabolize the herbicide. These findings agree with those of Whitenberg (25) which showed prometryne, like other s-triazines, is accumulated by the lysigenous glands of cotton. Carbon dioxide collections indicated that cotton was unable to metabolize the triazine ring of prometryne after a 4-week

period. The present results suggest that polyphenols may serve as one of the binding or complexing agents for prometryne (25). This complexing is apparently necessary since prometryne was not metabolized and should result in more phytotoxic effects. Gysin (7), however, has shown plants that have high peroxidase activity are relatively resistant to simazine and other chloro-triazines. In determining the metabolism of atrazine and simazine by corn, Montgomery and Freed (18) found that the concentration of total C^{14} in the plants decreased after about 30 days. It was also shown that only trace amounts, if any, of the materials remained unchanged in the plant. Hamilton (8) has shown that the content of benzoxazinone derivatives were directly related to the ability of excised corn roots to metabolize simazine to form hydroxysimazine and another minor metabolite.

Kearney et al. (13) found simazine metabolites from Aspergillus fumigatus differed from the simazine metabolites from plants. They proposed a new pathway of metabolism which does not involve the hydroxy analog reported to occur in higher plants. Menzie (17) and Gysin (7) proposed that some soil microorganisms appear to degrade prometryne by oxidation of the methylmercapto group to a sulfoxide or sulfone. However, Whitenberg (25) suggests the oxidation to hydroxypropazine is also a possibility.

The evolution of carbon dioxide over a 30-day period was utilized by Burnside et al. (4) to determine whether simazine influenced the over-all activity of soil microorganisms. They found no significant effect on carbon dioxide evolution from soil samples even though application rates up to 4096 ppm were used, indicating that microbial activity in soils was not markedly inhibited or stimulated by simazine.

Deactivation of simazine occurred under conditions conducive to microbial growth, but the rate of breakdown was fairly slow (4). Most of the deactivation occurred after the fourth month indicating that an adaptation period was required by the microorganisms involved. The phytotoxicity of 4 ppm of simazine in non-sterile Waukegan silt loam soil at field capacity was reduced 90 percent during 10 months of incubation at 85° F. No deactivation of simazine occurred in sterile or frozen soil. Gysin (7) found that fungi present in soils with organic matter were able to break down the chloro-triazines, however, it was not established whether these fungi were able to use a considerable part of the nitrogen of simazine and similar compounds for their growth.

The methylmercapto analog of propazine, prometryne, and the methylmercapto analog of atrazine, ametryne, have a shorter residual activity in general than do the other s-triazines. As shown by Gysin (7) the higher solubility of the mercapto-triazines cannot be used to explain the rapid breakdown because alkoxy-triazines have both a higher solubility and persistence.

Adsorption of the s-triazines onto soil colloids accounts for a significant loss of toxicity. Burnside and Behrens (2) have shown that soil high in organic matter and/or clay content caused reduced simazine phytotoxicity as compared to soil low in these substances. Talbert and Fletchall (22) have shown similar results with five s-triazines, however, they found adsorption of prometone and prometryne was less closely associated with percent organic matter than the chloro-triazines. The clay content appeared to be the factor affecting the adsorption of prometone and prometryne. Harris and Sheets (9) have shown that montmorillonite appeared to have more influence on the adsorption of

simazine than did kaolinite and illite. Harris and Warren (10) have shown atrazine and monuron were easily desorbed by repeated leaching with water, however, the bentonite released the two herbicides more readily than did the muck. They have also shown adsorption by bentonite was much greater at 0° C than at 50° C, but adsorption by the muck was the same at both temperatures.

The amino groups of the s-triazines have been shown (20) to produce weak basic properties and can be removed from solutions by a cation exchange resin but not by an anionic resin, indicating a net positive charge. Nearpass (19) proposed that simazine was adsorbed onto soils by proton association.

Leaching appears to be an important avenue of dissipation for the s-triazines and substituted ureas (3). It was also shown (19) that simazine appeared to leach deeper in limed soil as opposed to unlimed soil.

Burnside and Behrens (2) have shown that with higher pH values simazine was more phytotoxic than at the lower pH values, indicating more breakdown in the acid soil. Confirming results were also shown by Nearpass (19) when liming reduced the decomposition of simazine as did intermittent drying. However, Talbert and Fletchall (22) and Harris and Warren (10) indicated that pH was negatively correlated to adsorption rates, but Nearpass (19) has reported conflicting results. Research has shown that hydrolysis of simazine is rapid in strong acid solutions (21).

Jordon, et al. (12) showed, by changes in ultraviolet absorption spectra, that simazine, atrazine, and ametryne will photodecompose in ultraviolet light or sunlight. Similar results were shown by Comes and

Timmons (5) when simazine and atrazine were exposed to sunlight on soil surfaces for 25 days even when the surface soil temperatures did not exceed 120° F.

The volatilization among the s-triazines is now known to be influenced by molecular structure and by the degree to which they are adsorbed to soil (14). Prometone appeared to be the most volatile. Simazine and propazine were the most stable compounds. Prometryne was found to be more volatile from a wet soil than from a dry one. Kearney et al. (14) indicated that part of the difference due to moisture level at the time of application could be attributed to the water solubility of the various s-triazines, to the depth of penetration, and to differences in adsorption of the herbicides to soil binding sites.

Research concerned with herbicide and fertilizer interactions has led to much conflicting data. Adams (1) found that, in general, increasing the phosphorus level of the soil would increase plant injury. Adding simazine reduced the amount of phosphorus required to produce injury which was accredited to salt injury. Upchurch et al. (23) have shown with results of field, greenhouse, and controlled environment chamber studies that simazine phytotoxicity was not affected by soil phosphorus over wide phosphorus levels. Evidence was also presented that indicates simazine did not have any direct effect on calcium, magnesium, potassium, and phosphorus content of cotton shoots when the cotton was grown in soils varying widely in $Ca(H_2PO_4)2$ content.

CHAPTER III

MATERIALS AND METHODS

Two soils were used in this investigation: A Psammentic Paleustalf, Eufaula sand and a Pachic Argiustoll, Brewer clay loam. The chemical and physical epipedon properties of these two soils are shown in Table I.

Air-dried soil that had previously passed through an 8 mesh sieve was placed in 4 inch square plastic pots, except in Experiment X where 2½ inch round styro-cups were used. Four hundred gram soil cultures were used in each experiment except Experiment X where 200 gram cultures were used. All treatments were replicated three times.

A commercial formulation of prometryne was used which consisted of an 80 percent wettable powder.

Experiment I. Effect of Soil Reaction

Varying soil reactions of the Eufaula soil in this experiment were obtained by adding calcium oxide and elemental sulfur in amounts equal to twice the cation exchange capacity of the soil. By using the treated soils and untreated Eufaula along with 1:1 ratios between the untreated soil and the two treated soils resulted in a total of five soil reaction levels.

The pots were planted with approximately 30 Cimarron oat (Avenia sativa) seeds which were later thinned to uniform stands of 25 seedlings

TABLE I

CHEMICAL AND PHYSICAL PROPERTIES OF EUFAULA AND BREWER EPIPEDONS AS DETERMINED BY LABORATORY ANALYSES

Determination	Eufaula 0-6"	Brewer 0-6"
Particle Size Distribution		
% Sand	90.0	27.1
% Silt	7.0	44.6
% Clay	3.0	29.3
Textural Class	Sand	Clay Loam
pH	5.7	6.3
% Organic Matter	0.5	3.1
% Total Nitrogen	0.04	0.11
Cation Exchange Capacity*	2.7	14.6
Exchangeable Cations*		
Calcium	1.1	9.5
Magnesium	0.9	3.1
Potassium	0.3	0.5
Available Phosphorus**	6.8	6.4

*m.e. per 100 grams soil (1 N Ammonium acetate)

**Pounds per acre (0.25 N HCl)

per pot. Prometryne levels of 0, 1.5, 3, and 5 ppm were applied to the soil as solutions at the time of planting. The seedlings were grown under continuous light of 500 foot candles furnished by flourescent "Grolux" lamps and maintained at room temperature. At the end of 14 days, the above-ground plant parts were harvested and dry weights taken. The soils were air-dried, mixed, and replanted with oats. The same growing and harvesting procedures were used in this harvest as in the previous one. The second planting was harvested at the end of 19 days.

Experiment II. Effect of Cation Exchange Capacity

Brewer clay loam and washed silica sand were used in this experiment to furnish varying textural differences. The six mixtures gave cation exchange capacities of 3.7, 7.3, 9.1, 11.0, 12.8 and 14.6, milliequivalents per 100 grams of soil. The bioassay procedures as described in Experiment I were followed in this experiment. The first harvest was made at the end of 10 days, the second at the end of 15 days.

Experiment III. Effect of Prometryne on Nine Fungi Isolates

Nine common soil inhabiting fungi; Alternaria tenius, Curvularia lunta, Trichoderma viride, Penicillium funiculosium, Paecilomyces varioti, and the Aspergilli: flavus, niger, tamarii, and oryzae were cultured on a liquid broth media containing 0, 10, 100, 500, and 1000 ppm levels of prometryne. The basic media in grams per liter consisted of: sucrose 100, ammonium nitrate 1.5, potassium sulfate 1.5, inorganic salt mixture (Phillips-Hart) 1.5, and citric acid 1. Composition of the salt mixture (percent) was: dipotassium phosphate 32.2, calcium

carbonate 30.0, sodium chloride 16.7, magnesium sulfate 10.2, monocalcium phosphate 7.5, ferric citrate 2.75, manganese sulfate .51, potassium iodide .08, copper sulfate .03, zinc chloride .025, and cobalt chloride .005.

Forty milliliters of media were used in 500 milliliter cotton stoppered Erlenmeyer flasks. Each treatment was replicated three times. These flasks were autoclave sterilized at 15 psi for 20 minutes. Spore suspensions and prometryne treatments were added after sterilization. Cultures were incubated at 30° C for 5 days. Following incubation, the mycelium pads were removed from the flasks, washed, oven-dried at 100° C for 24 hours, and weighed.

Experiment IV. Effect of Prometryne on Four Fungi Isolates

Four soil inhabiting fungi; Aspergilli: niger, flavus, tamarii, and oryzae were grown on the same media as described in Experiment III. Prometryne levels of 0, 2000, 4000, 6000, 8000, and 10000 ppm were added after sterilization. The bioassay procedures were the same as used in Experiment III.

Experiment V. Effect of Prometryne and Sulfur on Fungi Isolates

Four soil fungi; Curvularia lunta, Aspergilli: niger, tamarii, and flavus were cultured on a nutrient broth media containing 0, 10, 100, 500, and 1000 ppm levels of prometryne. A sulfur-free media was used in this study which, in grams per liter, consisted of: sucrose 100, ammonium nitrate 4, magnesium chloride 2, citric acid 1, potassium chloride 1, ammonium phosphate .8, calcium phosphate .5, ferrous chloride .042, copper acetate .007, and zinc chloride .004. In

addition to the basic media four sulfur levels were established; 0, .1, .5, and 1 milligram sulfur per culture as sodium sulfate. These cultures were grown on 40 milliliters of media autoclave sterilized at 15 psi for 20 minutes. After cooling, spore suspensions and prometryne at various levels were added. The bioassay procedures were the same as used in Experiment III.

Experiment VI. Effect of Prometryne on Two Fungi Isolates

Two soil fungi; Aspergillus niger and Curvularia lunta were cultured on the same basic media as described in Experiment V, however, no sulfur was available to the organisms except the sulfur which was contained in the prometryne and carrier. The amount of sulfur contained in prometryne is approximately 13.28 percent. LeBaron (15) has estimated the amount of sulfur in prometryne 80 W, other than in the structure itself, was about .5 percent. Prometryne levels of 0, 10, 100, 500, 1000, 2000, 5000, and 10000 ppm were added to the forty milliliters of media after sterilization. The bioassay procedures were the same as used in Experiment III.

Experiment VII. Effect of Fungi Isolates on Degradation

Four Aspergilli: niger, flavus, oryzae, and tamarii were cultured on a media and under conditions described in Experiment III, however, the prometryne levels were 0, 25, 50, and 100 ppm. The residual media and mycelium pads were retained for bioassay. The fungi pads were screened through an 8 mesh sieve and incorporated into the Eufaula soil. The herbicide levels were 0, 2.5, 5, and 10 ppm after addition to the soil. Cimarron oats were planted and thinned to 25 seedlings per pot.

The bioassay procedures were the same as used in Experiment I. Two harvests were taken; the first at the end of 11 days, the second at the end of 24 days.

Experiment VIII. Effect of *Aspergillus niger* on Degradation

Aspergillus niger was cultured on the sulfur-free media described in Experiment V. Sulfur was supplied to this media at three levels; 0, .1, and .5 milligrams sulfur per culture as sodium sulfate. The herbicide levels in the flasks were 0, 25, 50, and 100 ppm. After incorporation into the soil these herbicide levels were reduced to 0, 2.5, 5, and 10 ppm. The residual media and mycelium pads were retained and used for bioassay as described in Experiment VII. Only one harvest was taken at the end of 18 days.

Experiment IX. Effects of Time, Temperature, and Organic Matter

Twelve hundred grams of Eufaula soil were weighed and placed in 4000 gram capacity plastic bags. Organic matter additions of 0, 1, and 2 percent were supplied by equal mixtures of ground wheat straw and alfalfa meal and thoroughly incorporated into the soil. Prometryne levels of 0, 1.5, 3, and 5 ppm were added to the soil as solutions and soil moisture levels were adjusted to 20 percent by weight. The plastic bags were sealed and placed in their respective incubators. Soil temperatures of 10, 20, and 30 degrees Centigrade were maintained by three thermostatically-controlled incubators.

Incubation time in weeks were 0, 4, 6, 8, 10, 12, and 14. At the end of each incubation time interval, the soils to be bioassayed were removed from their respective incubators, air-dried, stirred, and

placed in 400 gram pots replicated three times. Cimarron oats were planted and thinned to 25 seedlings per pot.

TABLE II

CHEMICAL COMPOSITION OF ORGANIC MATERIALS USED FOR SOIL AMENDMENTS

Organic Materials	%N	%P	%K	%Ca
Wheat Straw	0.39	0.03	0.48	0.06
Alfalfa Meal	1.05	0.26	1.97	1.58

Incubation times of 0, 4, 6, 8, 10, 12, and 14 weeks were harvested at the end of 13, 24, 22, 19, 22, 23, and 24 days respectively. The harvesting and weighing procedures were the same as used in previous experiments.

Experiment X. Reactions With Adsorptive Amendments

Eight grams of the adsorptive amendments; wheat straw, alfalfa meal, oak sawdust, activated charcoal, cation exchange resin, and anion exchange resin were placed in 200 milliliter Erlenmeyer flasks replicated three times. These additions, had they been incorporated into 400 gram soil cultures, would have represented 2 percent of the soil weight. Soil prometryne levels of 0, 5, 10, and 15 ppm in 100 milliliter solutions were added to the six amendments. Suspensions were

hand-shaken eight times periodically during a 48-hour reaction time. Amendments were separated from the prometryne solutions by filtration. The solutions were applied to 400 grams of Eufaula soil and planted with Cimarron oats which were thinned to stands of 25 seedlings per pot. Growing and harvesting procedures were the same as described in previous studies. There were two harvests taken on this study; the first, 16 days, and the second, 23 days after planting.

The amendment materials were added to 200 grams of Eufaula soil and planted with Cimarron oats which were thinned to stands of 15 seedlings per pot. These additions represented four percent of the soil weight. Soil herbicide levels were 0, 10, 20, and 30 ppm. Growing and harvesting procedures were the same as used before. Two harvests were taken on this study; the first, 16 days, and the second, 25 days after planting.

Statistical significance of treatment F values and coefficients of variation were determined using conventional analysis of variance procedures for all experimental results in these studies.

CHAPTER IV

RESULTS AND DISCUSSION

Detailed data from all experiments are presented within the tables in the Appendix. Soil amendments and manipulations in these studies influenced the indicator plants without prometryne additions. The indicator plant yields from amendments not receiving prometryne additions were used as 100 percent base for evaluating relative phytotoxicity and degradation within each amendment series. Yields from prometryne treatments within each series were then expressed as "percent of check." Three dimensional figures were then used to graph the results showing values as percent of check.

Fungi responses are shown in three dimensional figures as dry pad weights in grams. Fungi yields were shown in grams rather than percent of check to show responses to sulfur levels as well as prometryne treatments.

Experiment I

Results of soil reaction manipulations and prometryne levels are shown in Table III. Degradation was increased, or herbicide activity was less effective, at acid soil pH ranges. The apparent trend from the highest amount of herbicide activity to the lowest amount of herbicide activity was shown to be from alkaline soil reaction to acid soil reaction respectively.

TABLE III

EFFECT OF SOIL pH ON PROMETRYNE PHYTOTOXICITY

Treatments		Harvests			
ppm Prometryne	Soil pH	First	Second	Total	Percent Check**
		Avenia sativa Growth*			
0	5.60	.88	1.49	2.37	100
1.5	"	.71	1.48	2.19	90
3	"	.51	.95	1.46	61
5	"	.50	.67	1.17	51
0	5.65	.86	1.43	2.29	100
1.5	"	.72	1.39	2.11	91
3	"	.59	.80	1.39	63
5	"	.52	.82	1.34	59
0	5.90	.95	1.26	2.21	100
1.5	"	.69	1.41	2.10	92
3	"	.58	.95	1.53	68
5	"	.53	.67	1.20	54
0	7.00	.88	1.54	2.42	100
1.5	"	.46	1.03	1.49	60
3	"	.42	.72	1.14	48
5	"	.46	.69	1.15	48
0	7.45	1.08	1.48	2.56	100
1.5	"	.42	.86	1.28	49
3	"	.54	.75	1.29	51
5	"	.45	.66	1.11	43

C.V. = 9.16% F: Treatment = 33.53***, Herbicide = 174.68***, pH = 7.93***, and Herbicide X pH = 6.75.

*Sum of three replications; dry weight in grams

**Ratio of untreated to treated sums

***Significant at .005

Experiment II

The influence of cation exchange capacity (CEC) on prometryne phytotoxicity is shown in Table IV. Yield levels of indicator plants generally increased with increased exchange capacity. Apparently, cation exchange capacity had little effect on prometryne phytotoxicity.

Experiment III

Prometryne levels up to 1000 ppm generally did not inhibit or stimulate fungi growth as shown in Table V, however, a small depression in growth was apparent with all fungi in this study. An inhibitory response to the prometryne was shown by Aspergillus flavus and Aspergillus niger, however, a stimulatory response was shown by Alternaria tenius. No response was shown by Aspergillus tamarii, Aspergillus oryzae, Curvularia lunta, Trichoderma viride, Penicillium funiculosium, and Paecilomyces varioti.

Experiment IV

Prometryne levels up to 10000 ppm did not inhibit or stimulate A. tamarii growth. A shown in Table VI, A. niger growth was stimulated while that of A. flavus and A. oryzae was inhibited.

As shown in Tables V and VI, prometryne was apparently non-toxic to these fungi isolates at lower levels or levels associated with normal agricultural rates, however, the higher herbicides rates caused mixed fungi responses including suppressed growth, stimulated growth, or no apparent increase or decrease in growth.

TABLE IV

EFFECT OF CATION EXCHANGE CAPACITY ON PROMETRYNE PHYTOTOXICITY

Treatments		Harvests			
ppm Prometryne	CEC	First	Second	Total	Percent Check**
		Avenia sativa Growth*			
0	14.6	.58	1.04	1.62	100
1.5	"	.45	.92	1.37	85
3	"	.41	.89	1.30	80
5	"	.38	.77	1.45	71
0	12.8	.61	.94	1.55	100
1.5	"	.40	.91	1.31	84
3	"	.32	.82	1.14	73
5	"	.34	.91	1.25	81
0	11.00	.61	.67	1.28	100
1.5	"	.46	.64	1.10	86
3	"	.41	.56	.97	75
5	"	.27	.67	.94	73
0	9.1	.64	.74	1.38	100
1.5	"	.46	.58	1.04	75
3	"	.40	.73	1.13	82
5	"	.39	.67	1.06	77
0	7.3	.56	.60	1.16	100
1.5	"	.37	.61	.98	85
3	"	.39	.69	1.08	94
5	"	.42	.71	1.13	97
0	3.7	.58	.74	1.32	100
1.5	"	.39	.56	.95	81
3	"	.38	.56	.94	71
5	"	.32	.38	.70	53

C.V. = 13.75% F: Treatment = 5.29***, Herbicide = 17.71***, CEC = 10.75***, and Herbicide X CEC = .96.

*Sum of three replications; dry weight in grams

**Ratio of untreated to treated sums

***Significant at .005

TABLE V

EFFECT OF FIVE PROMETRYNE LEVELS ON NINE SELECTED SOIL FUNGI ISOLATES

Fungus	ppm Prometryne 0	10	100	500	1000	C.V.	F
	Fungus Growth*						
Aspergillus flavus	2.16	2.10	1.93	2.00	1.85	10.39%	4.52**
Aspergillus niger	3.00	3.12	2.98	3.00	2.82	2.89%	4.67**
Aspergillus tamarii	2.37	2.32	2.29	2.19	2.24	5.44%	.91
Aspergillus oryzae	1.92	2.26	1.91	1.87	1.81	9.38%	2.61
Alternaria tenius	1.87	2.00	1.96	1.81	1.82	3.45%	4.79**
Curvularia lunta	1.80	1.87	1.90	1.78	1.80	9.91%	.26
Trichoderma viride	1.80	1.62	1.66	1.70	1.66	9.18%	.59
Penicillium funiculosium	2.27	2.44	2.31	2.20	2.17	12.76%	.40
Paecilomyces varioti	1.64	1.66	1.62	1.49	1.65	5.82%	1.16

*Sum of three replications; dry weight in grams

**Significant at .05

TABLE VI

EFFECT OF SIX PROMETRYNE LEVELS ON FOUR SELECTED SOIL FUNGI ISOLATES

Fungus	ppm Prometryne 0	2000	4000	6000	8000	10000	C. V.	F
				Fungus Growth*				
Aspergillus niger	1.97	2.04	2.06	2.23	2.31	2.19	5.45%	3.73**
Aspergillus flavus	2.83	2.28	2.45	2.21	2.26	2.09	6.38%	8.92**
Aspergillus tamarii	1.89	1.87	1.87	1.86	1.80	1.78	10.05%	.16
Aspergillus oryzae	2.68	1.72	1.64	1.66	1.63	1.51	8.80%	22.29**

*Sum of three replications; dry weight in grams

**Significant at .05

Experiment V

As shown in Figures 1, 2, 3, and 4, and Table XI, all four fungi apparently utilized the methyl mercapto moiety for their sulfur source at the lower sulfur levels (.0 and .1 mgm. S/culture). The order of increasing utilization was Curvularia lunta, Aspergilli: tamarii, niger and flavus. The amount of sulfur contained in the prometryne other than the structure itself was estimated to be .025 mgm. S/culture when the prometryne level was 1000 ppm. This amount is far less than the .1 mgm. S/culture as sodium sulfate, however, with all four fungi there was more growth at the 1000 ppm prometryne level without additional sulfur than was obtained at the 0 ppm prometryne level with .1 mgm. S/culture. This indicates that sulfur from the methyl mercapto moiety was utilized. The growth of three fungi increased with higher sulfur level combinations in the order of increasing growth, Aspergilli: tamarii, niger, and flavus. However, with Curvularia lunta there was a slight decrease in growth with higher sulfur levels (Figure 4), indicating less ability to adapt to the herbicide concentrations or more sensitive to herbicide toxicity.

Experiment VI

With prometryne levels up to 10000 ppm but without sulfur additions very significant growth increases of both A. niger and Curvularia lunta were obtained as shown in Figure 5 and Table XII. When a direct source of sulfur is not available to these two fungi, they are apparently very tolerant to the prometryne. Utilization of the herbicide and carrier for their complete sulfur source is indicated.

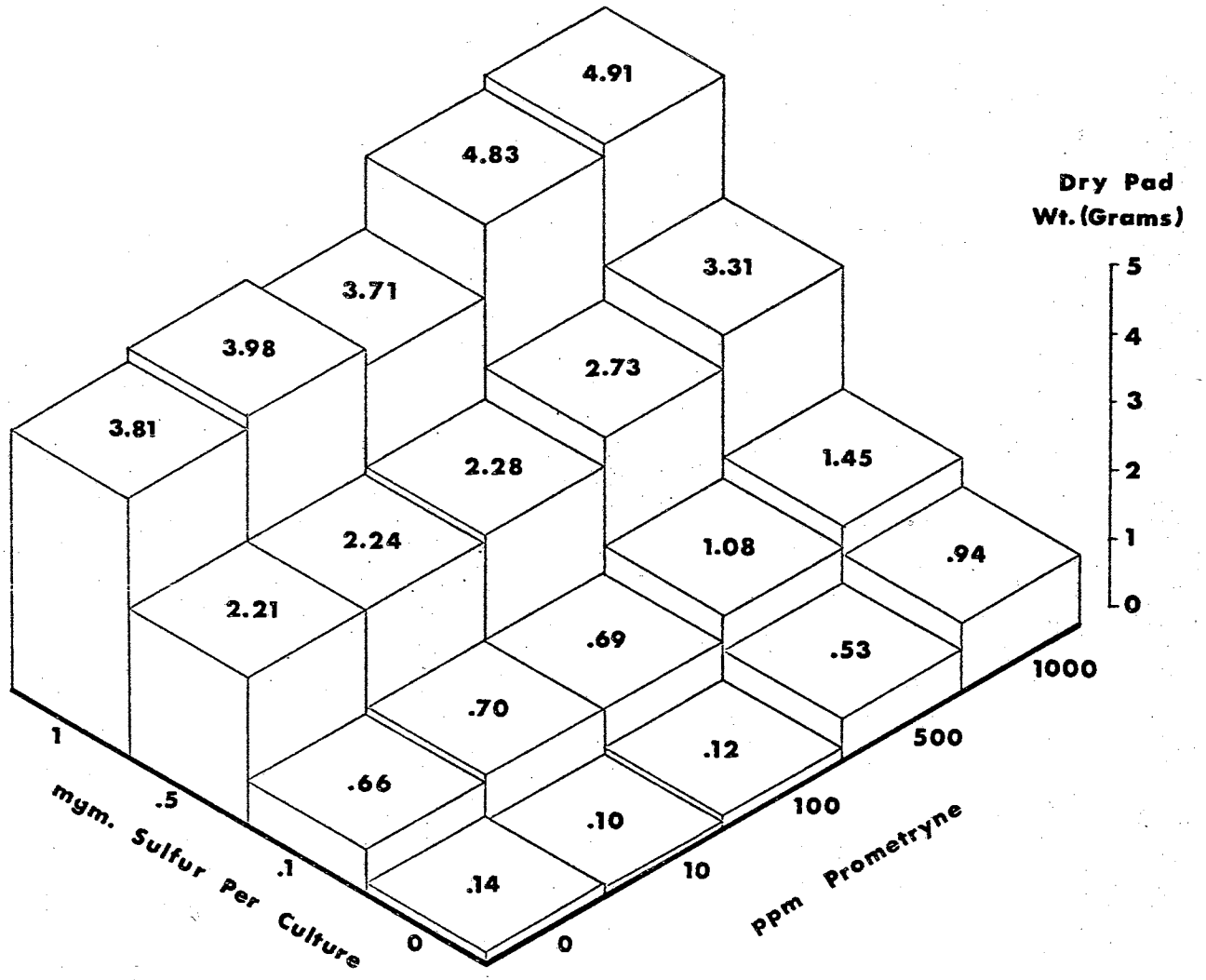

Figure 1. Effect of Prometryne and Sulfur on Aspergillus niger

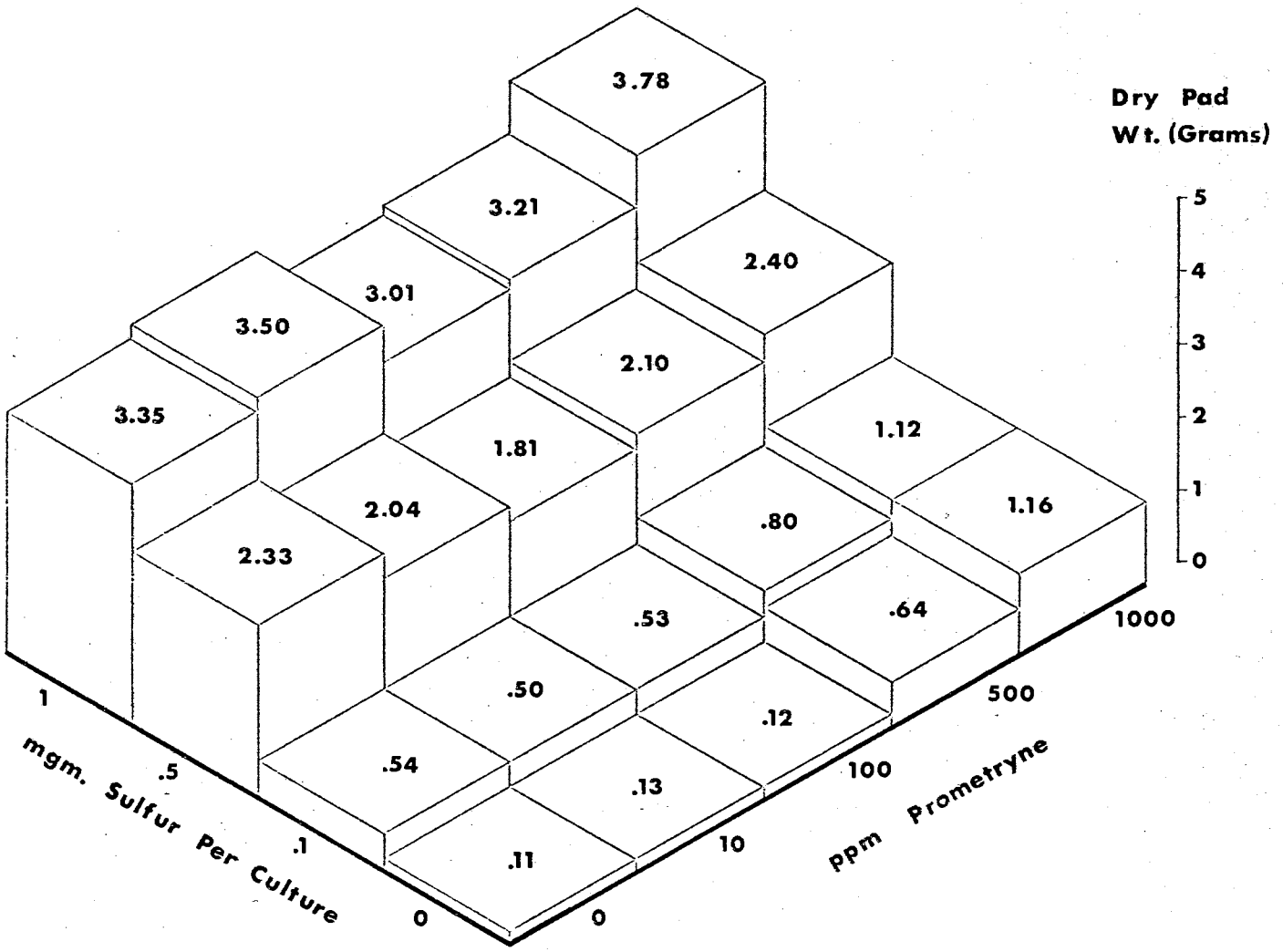

Figure 2. Effect of Prometryne and Sulfur on Aspergillus tamarii

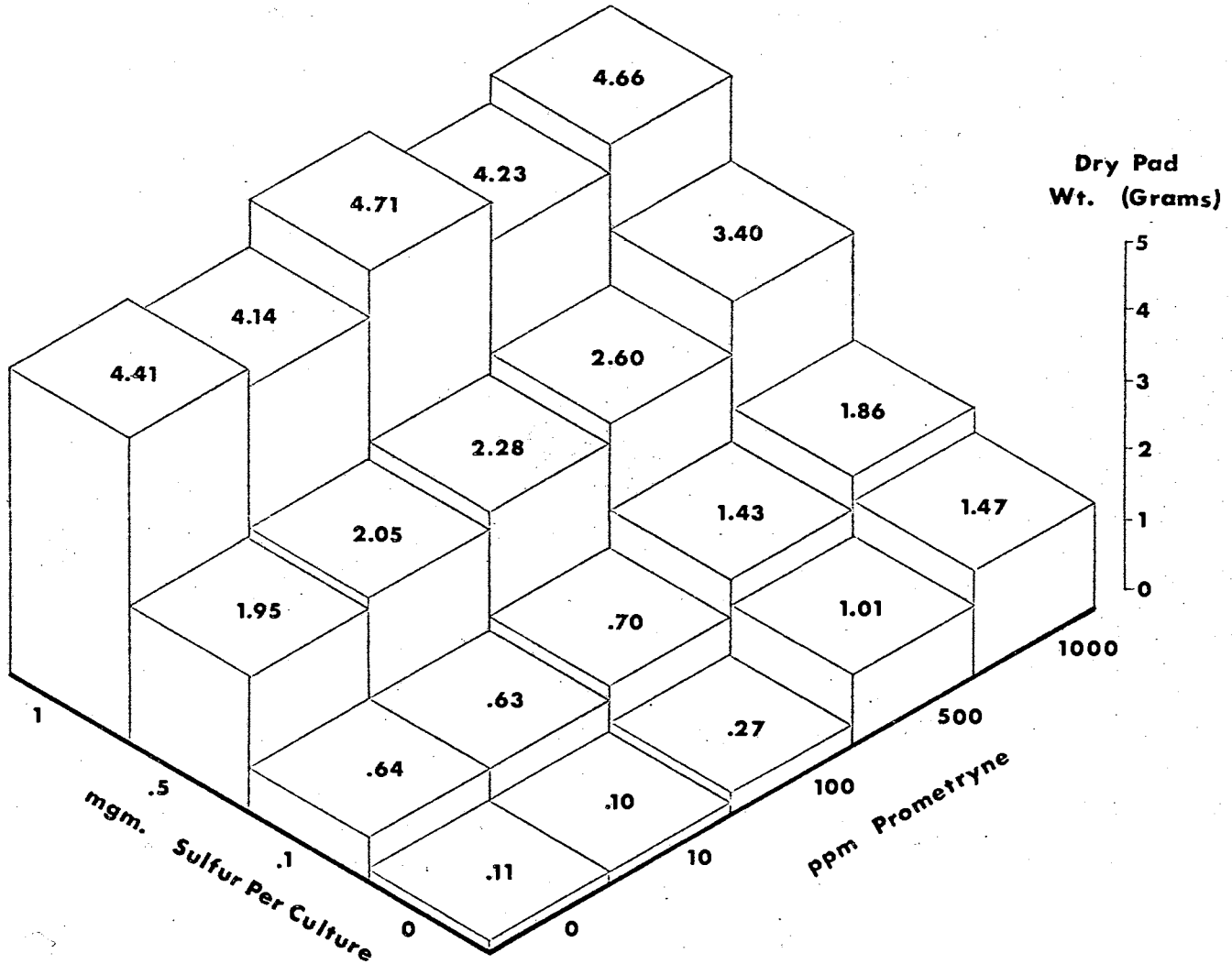

Figure 3. Effect of Prometryne and Sulfur on Aspergillus flavus

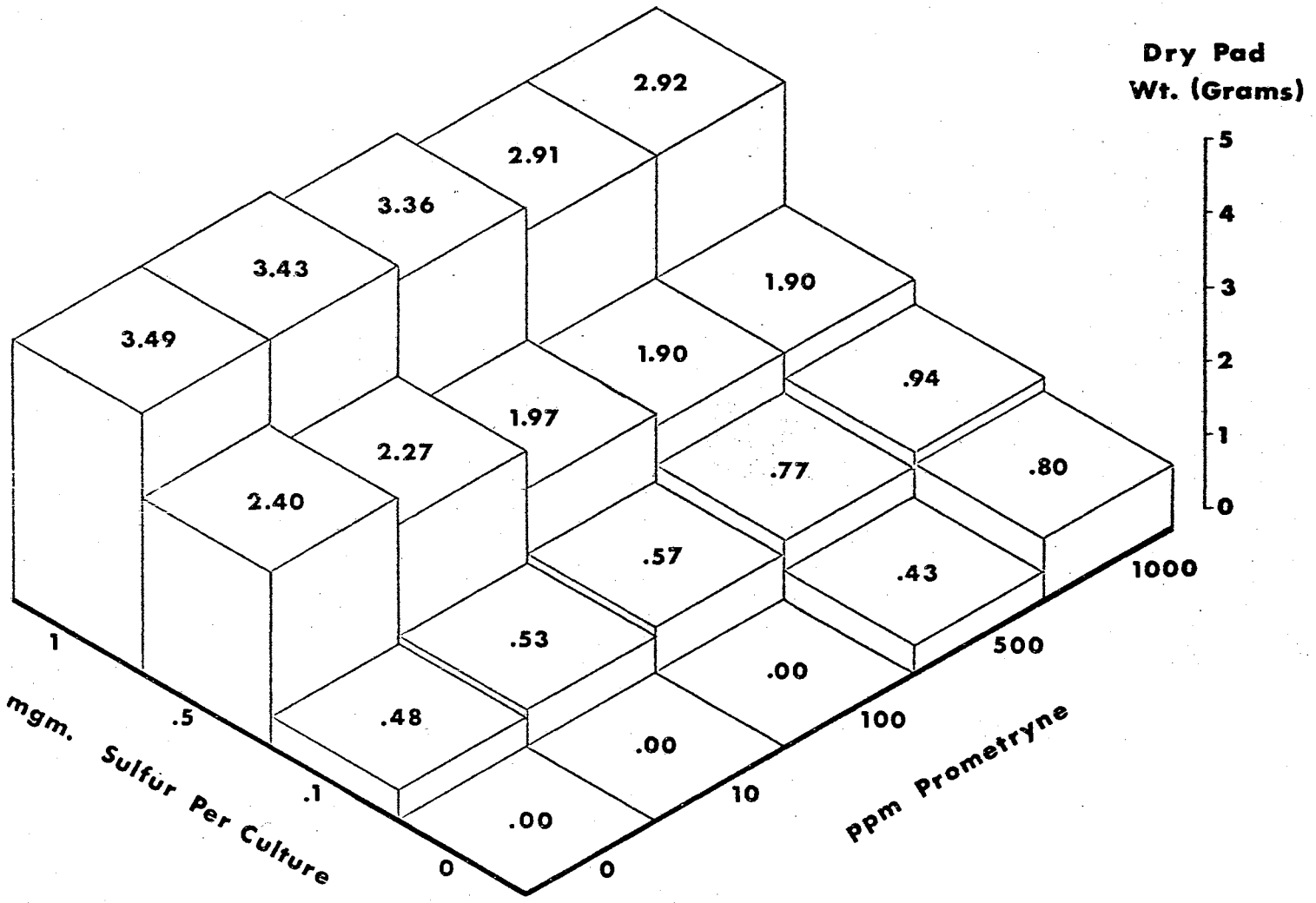

Figure 4. Effect of Prometryne and Sulfur on Curvularia lunta

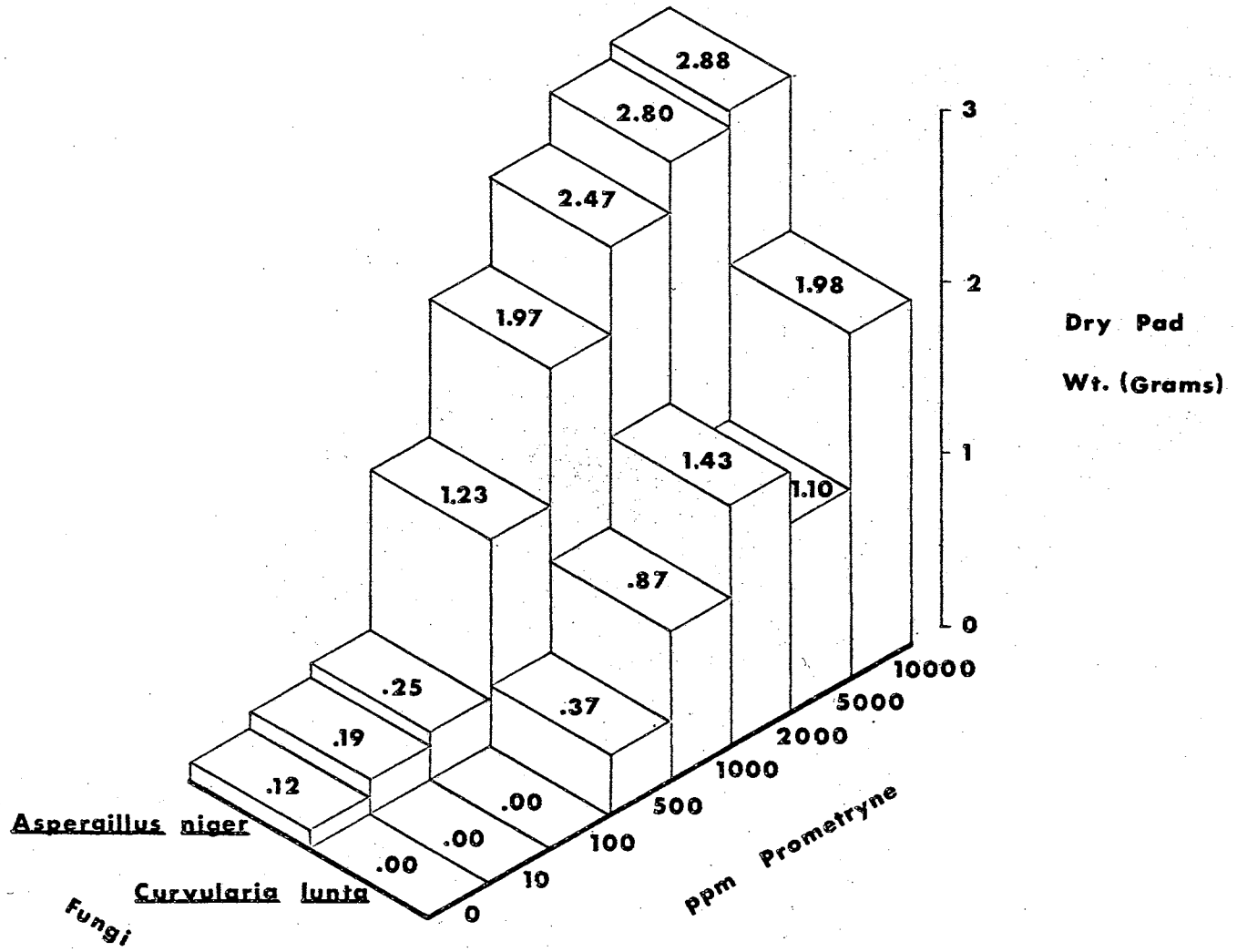

Figure 5. Effect of Prometryne on Two Soil Fungi in the Absence of Inorganic Sulfur

Experiment VII

In general, the sterile media amendments increased growth over the check, probably due to the media nutrients. Fungi pads from four Aspergilli: niger, flavus, oryzae, and tamarii were non-phytotoxic, indicating complete metabolism of the prometryne or no uptake of the prometryne as shown in Table VII. The residual media from A. niger and A. tamarii was also non-phytotoxic indicating near complete degradation of the prometryne. Phytotoxicity was apparent with the residual media of A. flavus and A. oryzae, indicating incomplete degradation of the prometryne.

Experiment VIII

Soil amendments of sterile media, residual media and residual pad increased plant growth over herbicide only additions, indicating degradation by A. niger. As shown in Table VIII, the residual pad apparently was non-phytotoxic even at the highest sulfur and prometryne levels. Both sterile and residual media having levels of .5 mgm. S/culture were more toxic than the media having lower sulfur levels, indicating more degradation occurring in the media containing lower levels of readily available sulfur. A. niger apparently utilized the methyl mercapto moiety of the prometryne for its sulfur source since adequate inorganic sulfur was not present. In general, the sterile media was more phytotoxic than the residual media, indicating that A. niger isolates are more capable of prometryne degradation than the total soil population. However, this lower degradation rate by the total soil population could be due to a needed adaptation period or competition between the soil microorganisms themselves.

TABLE VII

EFFECT OF FOUR FUNGI ISOLATES ON PROMETRYNE PHYTOTOXICITY

ppm Prometryne	Treatments Amendment	Harvests First	Second	Total	Percent Check**	C. V. F
			Avenia sativa Growth*			
0	Check	.57	.83	1.40	100	8.22%
2.5		.40	.74	1.14	81	38.5***
5		.33	.48	.81	58	
10		.35	.43	.78	56	
0	Sterile Media	.40	1.39	1.79	100	6.83%
2.5		.28	1.34	1.52	85	63.4***
5		.38	1.11	1.49	83	
10		.29	.48	.77	43	
0	*A. niger* Res. Pad	.59	.75	1.34	100	11.36%
2.5		.63	.69	1.32	98	4.07
5		.49	1.01	1.40	104	
10		.50	1.23	1.73	129	
0	*A. niger* Res. Media	.39	.61	1.00	100	22.71%
2.5		.34	.66	1.00	100	1.47
5		.39	.80	1.19	119	
10		.18	.62	.80	80	
0	*A. flavus* Res. Pad	.61	1.26	1.87	100	10.04%
2.5		.57	1.18	1.75	94	1.61
5		.57	1.11	1.68	90	
10		.49	1.08	1.57	84	
0	*A. flavus* Res. Media	.21	1.13	1.34	100	18.12%
2.5		.15	1.06	1.31	98	29.97***
5		.13	.46	.59	44	
10		.03	.30	.33	25	
0	*A. oryzae* Res. Pad	.55	1.24	1.79	100	10.45%
2.5		.61	1.53	2.24	125	3.58
5		.70	1.59	2.29	128	
10		.57	1.39	1.96	109	
0	*A. oryzae* Res. Media	.22	1.37	1.59	100	23.50%
2.5		.30	1.14	1.44	91	8.08***
5		.31	.94	1.25	79	
10		.21	.34	.54	34	
0	*A. tamarii* Res. Pad	.58	.96	1.54	100	13.48%
2.5		.48	1.07	1.55	101	4.89
5		.56	1.28	1.84	119	
10		.39	1.80	2.19	142	
0	*A. tamarii* Res. Media	.08	1.27	1.35	100	17.32%
2.5		.15	1.33	1.48	110	6.40
5		.16	1.41	1.57	116	
10		.12	.71	.83	61	

*Sum of three replications; dry weight in grams

**Ratio of untreated to treated sums

***Significant at .025

TABLE VIII

EFFECT OF ASPERGILLUS NIGER AND SULFUR LEVELS ON PROMETRYNE PHYTOTOXICITY

ppm Prometryne	Media Addition				Harvest First	Percent Check**
					Avenia sativa Growth*	
0			0		.74	100
2.5			"		.65	87
5			"		.69	93
10			"		.55	74
0	Ster.	Med.	.0	mgm S/Cult.	1.12	100
2.5	"	"		"	1.08	96
5	"	"		"	1.20	107
10	"	"		"	1.13	101
0	"	"	.1	"	1.22	100
2.5	"	"		"	1.20	98
5	"	"		"	1.18	97
10	"	"		"	1.25	103
0	"	"	.5	"	1.19	100
2.5	"	"		"	1.36	114
5	"	"		"	1.19	100
10	"	"		"	.59	50
0	Res.	Med.	.0	"	1.10	100
2.5	"	"		"	1.34	123
5	"	"		"	1.33	122
10	"	"		"	.95	87
0	"	"	.1	"	1.12	100
2.5	"	"		"	1.24	110
5	"	"		"	1.12	100
10	"	"		"	.77	69
0	"	"	.5	"	1.36	100
2.5	"	"		"	1.44	106
5	"	"		"	1.34	99
10	"	"		"	.94	70
0	Res.	Pad	.0	"	1.31	100
2.5	"	"		"	1.14	87
5	"	"		"	1.09	83
10	"	"		"	1.01	77
0	"	"	.1	"	1.06	100
2.5	"	"		"	1.06	101
5	"	"		"	1.08	102
10	"	"		"	1.22	115
0	"	"	.5	"	1.21	100
2.5	"	"		"	1.28	106
5	"	"		"	1.28	106
10	"	"		"	1.28	106

C.V. = 9.39% F: Treatment = 13.66***

*Sum of three replications; dry weight in grams

**Ratio of untreated to treated sums

***Significant at .005

Experiment IX

Incubation time, incubation temperature, and soil organic matter amendments influenced prometryne phytotoxicity as shown in Figures 6, 7, and 8 and in Table XIII.

Added soil organic matter decreased phytotoxicity below the check, but, the one percent amendment was more effective than the two percent amendment. Amendment effectiveness in reducing phytotoxicity indicates that the organic matter increased the activity of the soil microorganisms and/or the organic matter adsorbed the prometryne, making it unavailable and non-phytotoxic.

With increased soil incubation temperatures a decrease in phytotoxicity was apparent, indicating an increase in soil microorganism activity.

Increased time of incubtation up to 8 weeks decreased phytotoxicity, but, after 8 weeks of incubation, a decrease in indicator plant growth was apparent. This decrease in growth could be attributed to the organic matter being decomposed and releasing its nutrients or the adsorbed prometryne was released from the decomposing organic matter.

Experiment X

Adsorption by organic materials is highly active as shown in Table IX. All extracts from the amendments, with the exception of straw, were less active than the selective anion and cation exchange resins, indicating that the prometryne was adsorbed from solution by these amendments. Apparently the anion exchange resin was the most active.

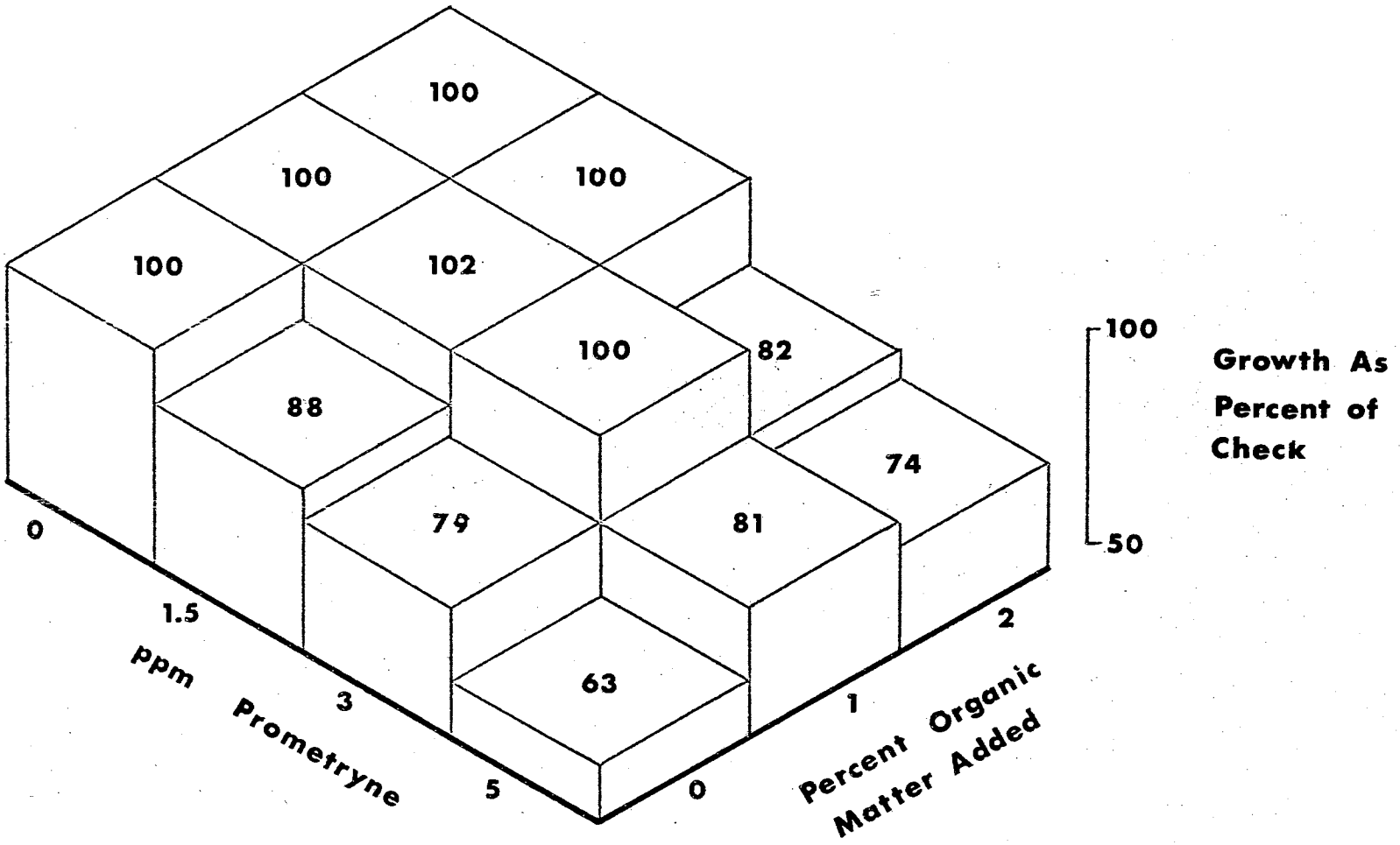

Figure 6. Effect of Soil Organic Matter on Prometryne Phytotoxicity

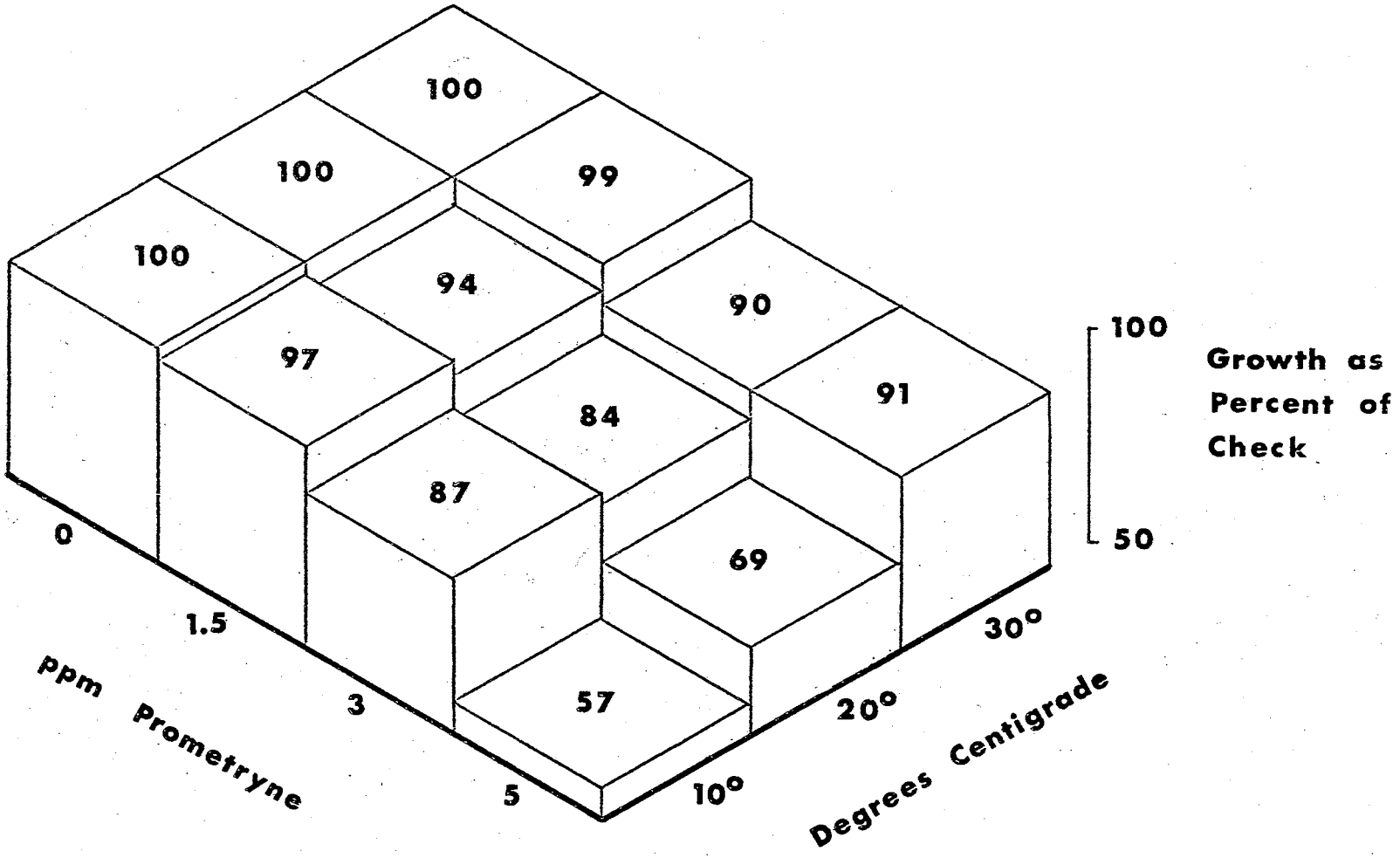

Figure 7. Effect of Incubation Temperature on Prometryne Phytotoxicity

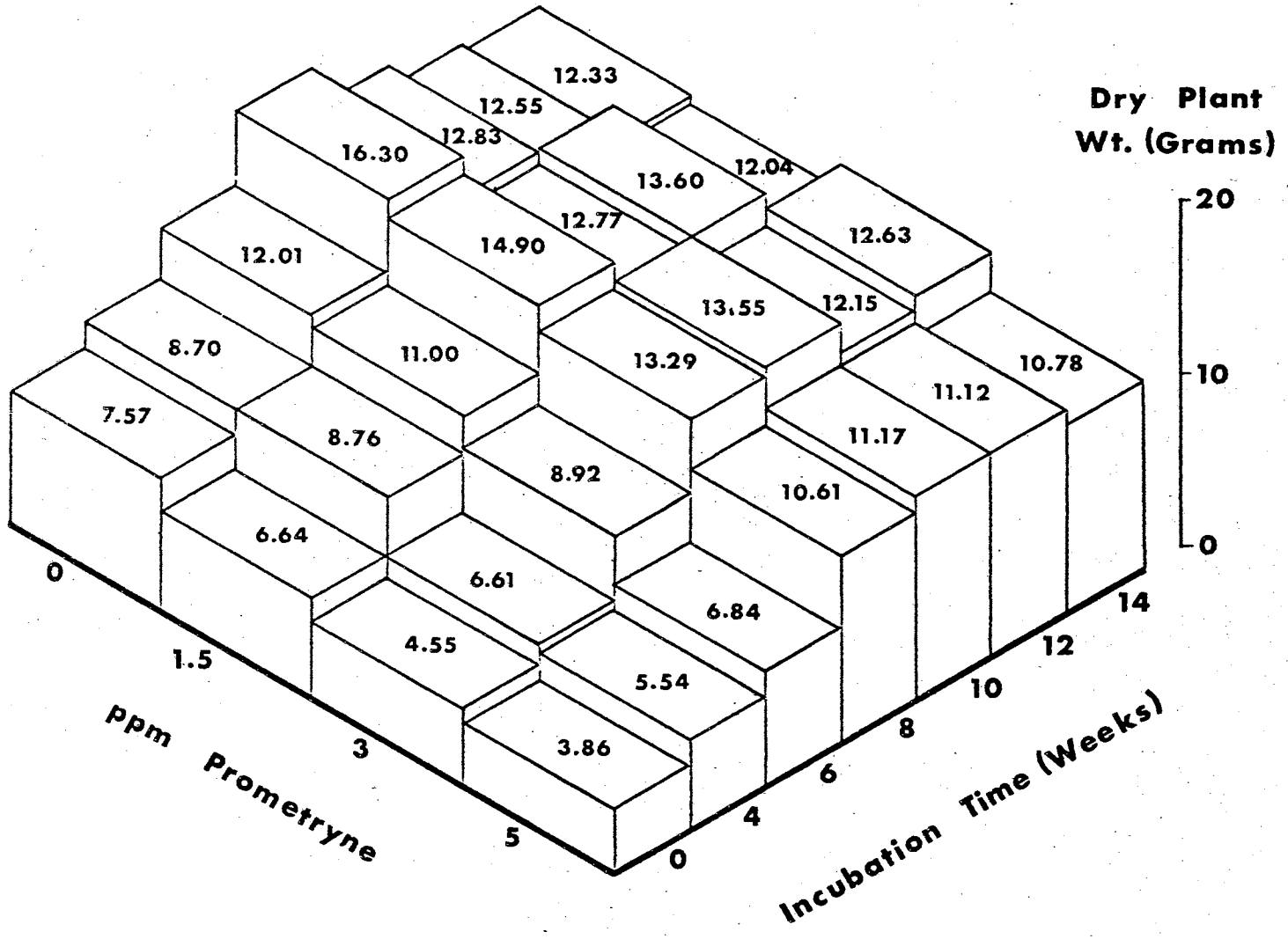

Figure 8. Effect of Incubation Time on Prometryne Phytotoxicity

TABLE IX

EFFECT OF EXTRACT SOLUTIONS FROM ADSORPTIVE AMENDMENTS ON PROMETRYNE PHYTOTOXICITY

Treatments		Harvests			
ppm Prometryne	Solutions From Amendments	First	Second	Total	Percent Check**
		Avenia sativa Growth*			
0	Check	.64	.87	1.51	100
5	"	.54	.70	1.24	82
10	"	.52	.59	1.01	74
15	"	.47	.53	1.00	66
0	2% Straw	.98	.89	1.87	100
5	"	.93	.96	1.89	101
10	"	.72	.74	1.46	78
15	"	.77	.77	1.54	82
0	2% Alfalfa	1.09	.86	1.95	100
5	"	.90	.89	1.79	92
10	"	.92	.87	1.79	92
15	"	1.02	.87	1.89	97
0	2% Sawdust	.86	.74	1.60	100
5	"	.94	.83	1.77	110
10	"	.86	.83	1.69	105
15	"	.83	.85	1.68	104
0	2% Charcoal	.90	.79	1.69	100
5	"	1.01	.82	1.83	109
10	"	1.02	.75	1.77	105
15	"	.85	.82	1.67	100
0	2% Anion Resin	.92	.74	1.66	100
5	"	.95	.75	1.70	103
10	"	.68	.70	1.38	84
15	"	.58	.58	1.16	70
0	2% Cation Resin	.94	.97	1.91	100
5	"	.98	1.01	1.99	104
10	"	.98	.89	1.87	98
15	"	1.02	.93	1.95	103

C.V. = 7.13% F: Treatment = 39.05***, Herbicide = 9.95***, Amendments = 39.05***, and Herbicide X Amendments = 3.50***.

*Sum of three replications; dry weight in grams

**Ratio of untreated to treated sums

***Significant at .005

As shown in Table X during decomposition these amendments released phytotoxic substances or prometryne at different rates. Sawdust, straw, and alfalfa meal decreased indicator plant yields below the check, indicating less adsorptive powers as compared to the other amendments.

TABLE X

EFFECT OF ADSORPTION AMENDMENTS ON PROMETRYNE PHYTOTOXICITY

Treatments		Harvests			
ppm Prometryne	Amendments	First	Second	Total	Percent Check**
		Avenia sativa Growth*			
0	Check	.42	.50	.92	100
10	"	.23	.51	.74	80
20	"	.28	.44	.72	78
30	"	.27	.26	.53	58
0	4% Straw	.54	.70	1.24	100
10	"	.24	.60	.84	68
20	"	.31	.46	.77	62
30	"	.30	.40	.70	56
0	4% Alfalfa	.72	1.24	1.96	100
10	"	.55	1.27	1.82	93
20	"	.46	.46	.92	47
30	"	.42	.86	1.28	65
0	4% Sawdust	.52	.76	1.28	100
10	"	.30	.55	.85	66
20	"	.24	.49	.73	57
30	"	.27	.35	.62	48
0	4% Charcoal	.44	.66	1.10	100
10	"	.51	.80	1.31	119
20	"	.51	.74	1.25	114
30	"	.46	.57	1.03	94
0	4% Anion Resin	.48	.81	1.29	100
10	"	.49	.63	1.12	87
20	"	.40	.55	.95	74
30	"	.30	.59	.89	69
0	4% Cation Resin	.11	.13	.24	100
10	"	.10	.11	.21	88
20	"	.11	.12	.23	96
30	"	.16	.10	.26	108

C.V. = 9.82% F: Treatment = 67.67***, Herbicide = 79.22***, Amendments = 224.11***, and Herbicide X Amendments = 13.56***.

*Sum of three replications; dry weight in grams

**Ratio of untreated to treated sums

***Significant at .005

CHAPTER V

SUMMARY AND CONCLUSIONS

The objectives of this study were to determine soil factors affecting prometryne phytotoxicity and degradation rates with two soils, Brewer and Eufaula. Variables in these studies included soil reaction, cation exchange capacity, organic matter, soil incubation time, and soil incubation temperature. Nine soil inhabiting fungi were grown on media containing various levels of prometryne to establish degradation rates and toxicity levels of the prometryne. Soil fungi were used in other studies having sulfur and prometryne combinations to determine prometryne degradation and/or utilization.

Soil factors shown to decreased prometryne activity were: acid soil pH ranges, increased cation exchange capacity, organic matter amendments, increasing incubation temperature, and increasing incubation time up to 8 weeks. In addition to the organic amendments increasing the biological activity of the soil, it was also found that organic amendments are highly active in adsorption.

Fungi species not inhibited by additions of 1000 ppm of prometryne included: Aspergilli: tamarii, oryzae, and niger, Curvularia lunta, Trichoderma viride, Penicillium funiculosium, and Paecilomyces varioti. Prometryne levels of 10000 ppm inhibited the growth of A. flavus and A. oryzae, whereas, A. niger was stimulated while A. tamarii showed no response. Prometryne additions of 1000 ppm were utilized as a complete

sulfur source by Curvularia lunta and Aspergilli: niger, tamarii, and flavus, indicating the sulfur came from the methyl mercapto moiety of the prometryne. Curvularia lunta and A. niger utilized 10000 ppm prometryne for a complete sulfur source without toxic effects. Bioassay of residual pads and media indicated degradation of prometryne by Aspergilli: niger, tamarii, flavus, and oryzae. No toxicity was apparent within the mycelial pads of all organisms. However, the residual medium of the Aspergilli: flavus and oryzae was toxic, indicating a lack of complete degradation. A. niger degraded prometryne at a more rapid rate when an available source of sulfur was not furnished, indicating induced degradation can be accomplished with reduced sulfur level of the growth media.

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APPENDIX

TABLE XI

EFFECT OF PROMETRYNE AND SULFUR ON FUNGUS PADS

Fungus	Mgm. S Per Culture	ppm Prometryne 0	10	100	500	1000
		Weight*				
Aspergillus niger	.0	.14	.10	.12	.53	.94
" "	.1	.66	.70	.69	1.08	1.45
" "	.5	2.21	2.24	2.28	2.73	3.31
" "	1.0	3.81	3.98	3.71	4.83	4.91
C.V. = 4.45% F: Treatment = 960.22**, Herbicide = 277.44**, Sulfur = 5681.33**, and Herbicide X Sulfur = 7.44**.						
Aspergillus tamarii	.0	.11	.13	.12	.64	1.16
" "	.1	.54	.50	.53	.80	1.12
" "	.5	2.33	2.04	1.81	2.10	2.40
" "	1.0	3.35	3.50	3.01	3.21	3.78
C.V. = 11.16% F: Treatment = 136.68**, Herbicide = 27.55**, Sulfur = 317.11**, and Herbicide X Sulfur = 2.95**.						
Aspergillus flavus	.0	.11	.10	.27	1.01	1.47
" "	.1	.64	.63	.70	1.43	1.86
" "	.5	1.95	2.05	2.28	2.60	3.40
" "	1.0	4.41	4.14	4.71	4.23	4.66
C.V. = 9.34% F: Treatment = 195.32**, Herbicide = 64.88**, Sulfur = 1125.66**, and Herbicide X Sulfur = 6.30**.						
Curvularia lunta	.0	.00	.00	.00	.43	.80
" "	.1	.48	.53	.57	.77	.94
" "	.5	2.40	2.27	1.97	1.90	1.90
" "	1.0	3.49	3.43	3.36	2.91	2.92
C.V. = 6.70% F: Treatment = 425.67**, Herbicide = 5.08**, Sulfur = 2582.58**, and Herbicide X Sulfur = 25.58**.						

*Sum of three replications; dry weight in grams

**Significant at .01

TABLE XII

EFFECT OF PROMETRYNE ON TWO FUNGI ISOLATES

ppm Prometryne	Aspergillus niger	Curvularia lunta
	Pad Weights*	
0	.12	.00
10	.19	.00
100	.25	.00
500	1.23	.37
1000	1.97	.87
2000	2.47	1.43
5000	2.80	1.10
10000	2.88	1.98
C.V. = 31.23%	Treatment F = 27.47**	

*Sum of three replications; dry weight in grams

**Significant at .005

TABLE XIII

EFFECTS OF TIME, TEMPERATURE, AND ORGANIC MATTER ON PROMETRYNE PHYTOTOXICITY

ppm Prometryne	Treatments Soil Variable	Weeks of Incubation 0	4	6	8	10	12	14	Total
				Avenia sativa Growth*					
0	B-10°, 0	.93	1.06	1.02	1.60	1.08	1.19	1.13	8.01
1.5		.77	.82	.93	1.32	1.11	1.26	1.07	7.28
3		.41	.47	.48	.86	1.13	1.28	1.53	6.16
5		.41	.55	.47	.58	.54	.81	.84	4.20
0	B-10°, A-1%	.77	.79	1.22	1.93	1.47	.94	1.65	8.77
1.5		.68	.97	1.04	1.81	1.28	1.44	1.29	8.51
3		.59	1.06	1.26	1.83	1.55	1.33	1.55	9.17
5		.34	.67	.52	.67	.92	1.01	.87	5.00
0	B-10°, A-2%	.82	.84	1.31	2.02	1.52	1.59	1.51	9.61
1.5		.76	1.02	1.22	2.17	1.43	1.53	1.48	9.61
3		.52	.81	.62	1.34	1.43	1.42	1.30	7.44
5		.54	.85	.77	.69	.92	.94	.90	5.61
0	B-20°, 0	.93	1.04	1.38	1.67	1.25	1.40	1.19	8.86
1.5		.77	.63	1.02	1.25	1.09	1.34	1.21	7.31
3		.41	.48	.44	1.65	1.27	1.28	1.26	6.79
5		.41	.38	.44	.68	.64	.98	.78	4.31
0	B-20°, A-1%	.77	.91	1.36	1.88	1.40	1.44	1.34	9.10
1.5		.68	1.01	1.40	1.80	1.58	1.67	1.52	9.66
3		.59	1.01	1.20	1.70	1.64	1.39	1.46	8.99
5		.34	.68	.54	1.54	1.52	1.51	1.46	7.59
0	B-20°, A-2%	.82	1.12	1.47	1.97	1.76	1.50	1.44	10.08
1.5		.76	.93	1.26	1.59	1.66	1.68	1.57	9.45
3		.52	.84	.67	1.48	1.63	1.22	1.33	7.69
5		.54	.48	.67	1.42	1.39	1.60	1.49	7.59
0	B-30°, 0	.93	.96	1.34	1.61	1.46	1.22	1.22	8.74
1.5		.77	1.10	1.27	1.29	1.20	1.27	1.03	7.93
3		.41	.53	1.40	1.46	1.35	1.11	1.10	7.36
5		.41	.50	1.08	1.44	1.49	1.30	1.33	7.55
0	B-30°, A-1%	.77	1.03	1.39	1.75	1.61	1.58	1.53	9.66
1.5		.68	1.31	1.22	1.78	1.65	1.69	1.40	9.73
3		.59	.96	1.38	1.63	1.72	1.52	1.44	9.24
5		.34	.93	1.50	1.90	1.76	1.65	1.45	9.53
0	B-30°, A-2%	.82	.96	1.53	1.89	1.28	1.68	1.59	9.75
1.5		.76	1.00	1.64	1.88	1.77	1.72	1.48	10.25
3		.52	.45	1.45	1.33	1.84	1.61	1.67	8.87
5		.41	.51	.85	1.71	1.98	1.30	1.65	8.41

A = Organic Matter, B = Temperature°C, O = Check, D = Time and E = Herbicide Levels.
F Values: Treatment = 30.15**, A = 265.50**, B = 163.40** D = 670.50**, E = 267.50**, B x D = 16.65**, A x D = 14.90**, D x E = 15.65**, A x B = 3.25**, B x E = 36.65**, A x E = 18.35**, A x B x D = 3.20**, B x D x E = 10.95**, A x D x E = 8.05**, A x B x E = 1.35 and A x B x D x E = 1.50**. C.V. = 11.35%.

*Sum of three replications; dry weight in grams

**Significant at .025

VITA

Donald Scott Murray

Candidate for the Degree of

Master of Science

Thesis: SOIL PARAMETERS AND FUNGI ISOLATES INFLUENCING PHYTOTOXICITY AND DEGRADATION OF A METHYL MERCAPTO TRIAZINE HERBICIDE

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Personal Data: Born in Beaver, Oklahoma, May 3, 1944, the son of Glen E. and Betty Murray.

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