SOIL PARAMETERS GOVERNING PHYTOTOXICITY OF BROMACIL, A SUBSTITUTED URACIL

By

SUNIL KUMAR PANCHOLY

Bachelor of Science
University of Udaipur
Udaipur, Rajasthan, India
1964

Master of Science
University of Udaipur
Udaipur, Rajasthan, India
1966

Submitted to the faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
July, 1968

SOIL PARAMETERS GOVERNING PHYTOTOXICITY OF

BROMACIL, A SUBSTITUTED URACIL

Thesis Approved:

J. V. Lynd
Thesis Adviser

Paul W. Santelmann

N. P. Denham
Dean of the Graduate College

ACKNOWLEDGMENTS

The author is sincerely grateful to his parents, Dr. and Dr. (Mrs.) S. N. Pancholy, for their constant encouragement and support during the course of this study.

The author wishes to express his deep sense of gratitude to his major adviser, Dr. J. Q. Lynd, whose encouragement, valuable guidance and constructive suggestions made his task a success. The assistance of the other members of his graduate committee, Dr. L. W. Reed, Dr. P. W. Santelmann and Dr. G. W. Todd, is also acknowledged and appreciated.

The author wishes to express his appreciation to the Agronomy Department of Oklahoma State University for the use of their facilities and the financial assistance which made this study possible.

The writer wishes to thank Mrs. Ron Yeck for typing the final copy of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Highly effective phytotoxic pyrimidine compounds have recently been developed that are of particular interest for control of undesirable plant growth in cropped and non cropped areas. A wide range of weeds and grasses can be controlled with uracil herbicides now available. Pyrimidines are identified from the hydrolysates of nucleic acids and the synthesis, degradation and controlled activity of these compounds are of much interest to biological sciences.

It is believed that the substituted pyrimidine herbicides may act as photosynthetic inhibitors. However, little has been established regarding their specific phytotoxic effects. Soil characteristics and microbial activities are assumed to greatly influence their effectiveness, persistence and eventual fate in soils.

The objective of this study was to determine factors which govern the phytotoxicity and persistence of substituted uracil compounds in soil including the rates and magnitude of degradation with selected soil microorganisms.

CHAPTER II

LITERATURE REVIEW

Substituted pyrimidine uracil compounds having highly effective phytotoxic activity were first reported by Bucha, et al. (6) in 1962. These compounds were found to be phytotoxic to a wide variety of plants and had low mammalian toxicity (3). Specific examples of these compounds included bromacil (5-bromo-3-sec-butyl-6-methyl uracil) and isocil (5-bromo-3-isopropyl-6-methyl uracil). Toyo Koatsu AM (2-amino-4-chloro-6-methyl pyrimidine) was more recently developed as a specific nitrification inhibitor (2).

The pyrimidines are complex organic molecules with the heterocylic 2N ring configuration and may serve as substrate for a non specific segment of the microflora according to Alexander (1). This herbicide type should disappear from the soil in a relatively short period of time if suitable as a carbonaceous nutrient source for microorganisms. Biological degradation is generally desirable as repeated application of non-decomposable compounds could ultimately result in concentrated accumulations detrimental to desirable plant growth.

Holly and Roberts (9) evaluated the toxic activity of isocil on the growth of microorganisms and found that Saccharomyces cerevisiae, Meyen and Hansen, and Escherichia coli (Migula) Cast. and Chalm., were tolerant to isocil except at concentrations which approached saturation of the aqueous medium. Saccharomyces cerevisiae was insensitive at

$4X10^{-3}M$ concentrations of this herbicide. Later studies conducted with Neurospora crassa, Shear and Dodge 1298, a mutant organism that requires pyrimidine for growth, was inhibited by isocil at $1X10^{-3}M$. The growth antagonism was also apparently of little physiological significance in phytotoxicity by this substituted uracil herbicide.

Hilton et al. (7) reported that barley seedlings that were germinated and grown in "Perlite" saturated with Hoagland's solution, then treated preemergence with isocil at rates 1 to 6 pounds per acre, were not affected during the first week. However, desiccation of all shoot tissue was observed within two weeks after planting. These symptoms caused by substituted uracils were similar to those of other herbicides known to act as photosynthetic inhibitors of the Hill reaction (8).

Holly and Roberts (9) while working with small seeds such as rye grass (Lolium multiflorum) found that the uracil herbicides which characteristically inhibit photosynthesis did not usually exert their full effects on seedling plants until after the seed reserves were exhausted. It was necessary to wait 3 to 4 weeks with small grain test species before the most satisfactory results could be obtained with measurement of shoot weight.

It was observed by Moreland, et al. (12) that as a group the substituted uracils were more inhibitory than the polycyclic urea, s-triazine and N-phenyl carbamate herbicides. However, even the most active uracil was less effective than either 3-(3,4-dichloro phenyl)-1, 1-dimethyl urea (diuron) or N-(3,4-dichloro phenyl)-2-methyl pentanamide(3,4-DCPMP).

It was observed that the toxicity of these uracils is apparently related with saturated substitutents at the number-3-ring position as

more inhibitory than the compounds with unsaturated substituents on the ring. The inhibitory action of the substituted uracils on the Hill reaction was attributed to hydrogen bonding between the imino hydrogen of the nitrogen atom at the number-1-ring position and the carbonyl oxygen of the carbon atom at the number-2-ring position with the appropriate receptors at the unidentified active centers in the chloroplast (9, 12).

Factors manipulated to determine the phytotoxicity of substituted uracil herbicides were soil pH, texture and organic matter by Barnes (4). He noted that change in soil pH did not have a significant effect on uracil phytotoxicity. More toxicity was apparent with a poorly buffered sandy soil in comparision to results with a clay loam soil. However, the addition of 1 to 3 percent organic matter was found to be significant in modifying uracil phytotoxicity.

Recently pyrimidine compounds have been also established as nitrification inhibitors. Toyo Koatsu AM (2-amino-4-chloro-6-methyl pyrimidine) retarded oxidation of ammonium to nitrate when applied to soil with ammonium fertilizers.

CHAPTER III

MATERIALS AND METHODS

These studies were conducted with the two soils used by Barnes (4) with bromacil: a Psammentic Paleustalf, Eufaula sand, and a Pachic Argiustoll, Brewer clay loam. The physical and chemical analyses (5) of these soils are given in Table I. Soils used for experiments were air dried and passed through 8-mesh sieve. Three replicates were used per treatment with cultures grown in 4 inch square pots.

Experiment I. Bioassay with Soil Organic Matter Levels

Oats (Avena sativa, L.), Cimarron variety and sorghum (Sorghum vulgare, Pers.), Ok 612 variety were used for bioassay work. Cucumber Cucumis sativus, L.), Long green variety, also used to determine threshold symptoms for bromacil. Thirty seeds of oats or sorghum were planted per pot with treatment levels of 0, 0.25, 0.5, and 1 ppm active ingredient (ai) bromacil as 50% wettable powder commercial product (Hyvar-X). The herbicide was added in a water solution and mixed thoroughly in the soil. An organic matter mixture containing equal weights of ground wheat straw and alfalfa meal was added and mixed into the soil at 0, 1, 2, and 4 percent by weight. The herbicide and organic matter level treatments were combined in factorials with three replicates per treatment. The seedlings were thinned to twenty per pot after emergence.

These cultures were grown under continuous light of 500 foot

TABLE I

CHEMICAL AND PHYSICAL CHARACTERISTICS OF
EUFAULA AND BREWER SOILS

Determination	Eufaula 0-6"	Brewer 0-6"
Mechanical analysis		
% 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 (1N Ammonium acetate)
**pounds per acre (0.25N HCl)

candles from fluorescent "Grolux" lamps at room (25-30°C.) temperature for 15 days. Green weight and dry weight of the above ground plant tissue were determined at harvest. These soils were allowed to dry and then were dumped, stirred, repotted and replanted. The same procedure for growth and harvesting was used in this planting as in the previous one. Successive crops were taken in this manner till no further uracil phytotoxicity was apparent with these crops. Cucumber seeds were then planted at this level to determine threshold toxicity symptoms for the more sensitive cucumber plants. Manipulations and amendments to soils used in these studies influenced the growth of indicator plants without pyrimidine herbicide additions. Soil amendment treatments not receiving pyrimidine additions within each study were used as 100 percent base for relative evaluations of phytotoxicity and degradation for each treatment series.

Fresh or green plant weights were used as a measure of the plant response to bromacil levels. These criteria were used because of the nature of the phytotoxicity symptoms of this herbicide. Plant dry weights were not sensitive indicators of toxicity with normal growing plants yielding dry weights similar to those of completely dead plants as a result of the "delayed action" toxicity of bromacil in these bioassay studies (Fig. 1).

Experiment II. Effects of Organic Matter and Plant Nutrient Levels

Sorghum as a bioassay species was used to determine the effect of various soil factors on persistence and toxicity of bromacil. Soil factors manipulated were organic matter level and differential levels of nitrogen and phosphorus. Bromacil was used at 0, 1, 2, and 4 ppm levels uniformly in combination with all soil amendments. Soil organic

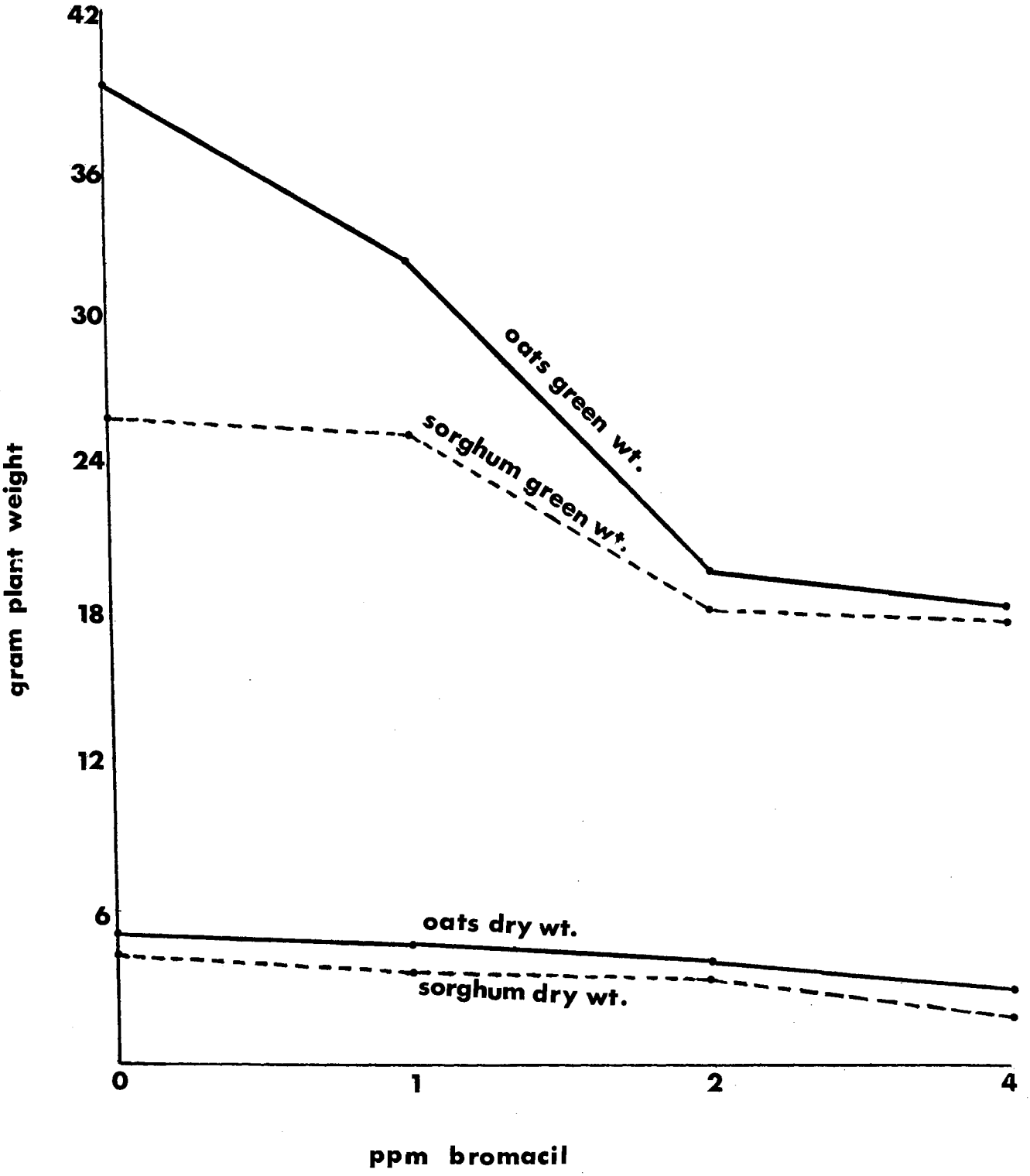

Figure 1 Relationship between fresh green weights and dry weights of above ground materials for oats and grain sorghum plants as affected by bromacil levels, Eufaula sand.

matter levels varying in nitrogen-carbon ratio were combined with Eufaula soil. Organic matter addition consisted of an equal mixture of wheat straw and alfalfa meal by weight. Composition of these materials as percent on dry weight basis as N, P, K, and Ca respectively were: ground wheat straw: 0.39, 0.03, 0.48, and 0.06; alfalfa meal, 2.40, 0.20, 2.20 and 0.44 These additions were mixed with soil at 0, 1, 2, and 4 percent by weight. Sorghum plants were harvested after 15 days and both green and dry weight determined.

Nitrogen as ammonium nitrate and phosphorus as monobasic calcium phosphate were applied in combinations at levels of 0, 100, 200 and 400 ppm to determine the effect of plant nutrient interactions on the persistence and toxicity of bromacil. Three crops were harvested in this study with green and dry plant weights taken.

Experiment III. Effects of Bromacil and Growth Factors on Various Soil Fungi

Ten soil inhabiting fungi: Aspergilli: tamarii, niger, flavus, and oryzae, Curvularia lunta, Mucor pusillus, Trichoderma viride, Pencillium funiculosum, Penicillium brevicompactum, and Myrothecium verucaria were cultured on liquid broth media with five bromacil levels for five to eight days. The basic medium (14) in grams per liter consisted of sucrose 100, citric acid 2, potassium sulfate 1, ammonium nitrate 3, and inorganic 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 0.51, potassium iodide 0.08, copper sulfate 0.03, zinc chloride 0.25 and cobalt chloride 0.005.

Forty milliliters of the medium were used in 500 ml Erlenmeyer

flask for each culture. Three replications were used per treatment. Autoclave sterilization at 15 pounds per square inch for twenty minutes was used and upon cooling all cultures were inoculated with spore suspensions of the respective organisms and incubated for six days.

Following incubation, the fungi mycelial pads were removed with forceps, washed, dried at 100 degrees centigrade for 24 hours and weighed. Bromacil levels used during this experiment were 0, 250, 500, 1000 and 5000 ppm. Fungi responses were determined as pad weight in grams and growth was evaluated in direct proportion to that of the corresponding control cultures.

From these studies an Aspergillus niger isolate was found to be the most consistent sensitive organism of those tested to bromacil toxicity. This A. niger selection was used in the culture studies with bromacil interactions and growth factor amendments. The minimum level of nitrogen in the culture medium in combination with bromacil levels, required for growth response of A. niger was determined using the same base medium detailed previously. Levels of nitrogen included 0, 250, 500, 1000, and 2000 ppm supplied as ammonium nitrate. Bromacil was initially used at 0, 125, 200, 250, 400, 500, 800, 1000, 1500, 1600, 1700, 1800, 1900 and 2000 ppm.

Various growth factor additives including thiamine hydrochloride, riboflavin, pyridoxine base, vitamin B-12 (cyancobalamin), yeast extract, peptone, folic acid and glutathione were used at 0, 1000 and 2000 ppm to offset bromacil toxicity on the growth of A. niger. Bromacil was applied at levels of 0, 1000, 1600, and 1800 ppm. Fungus pad weights were taken after drying at 100° centigrade for 24 hours.

Experiment IV. Effects of Bromacil and Toyo Koatsu AM on Soil Nitrification

Bromacil and Toyo Koatsu AM were applied to Eufaula soil cultures at levels of 0, 10, and 100 ppm with and without 200 ppm nitrogen as urea. Determinations were made for extractable ammonia, nitrite and nitrate nitrogen after soil incubation at 30^{o} centigrade at 5, 10, and 15 day interval.

CHAPTER IV

RESULTS AND DISCUSSION

The discussion of results from these studies is presented chronologically by experiment numbers as indicated in Chapter III, Methods and Materials. The initial soil factor experiments confirmed results reported by Barnes (4) indicating little consistent influence of soil pH and soil texture with bromacil toxicity. Soil organic matter and soil fertility levels appeared to be dominant influencing parameters, however.

Experiment I. Bioassay with Soil Organic Matter Levels

Results of bromacil phytotoxicity to four oat plantings grown in soil cultures treated with organic matter amendments are shown in Table II and Figure 2. Bromacil significantly inhibited plant growth at higher levels for the first three crops decreasing with successive planting until no further toxicity was noted in the fourth crop. Visual symptoms of toxicity accelerated with growth time on the affected plants. All plants appeared normal for the first week. The affected plants then started drying and curling first at youngest tissue. Plants growing at the lethal herbicide level were dead following ten to twelve days of normal growth. Oats were found to be more sensitive than sorghum at equivalent levels of bromacil: 0.25, 0.5, and 1 ppm. Oat growth without bromacil was quadratic to organic matter increases initially and probably resulted from an initial nutrient imbalance from

TABLE II

SOIL ORGANIC AMENDMENT AMELIORATION EFFECTS ON BROMACIL PHYTOTOXICITY WITH FOUR SUCCESSIVE OAT PLANTINGS, EUFAULA SAND

Bromacil ppm	% O.M.	Oat Plantings 1	2	3	4	Σ
		Grams Fresh Weight[1]				
0	0	9.28	4.20	4.81	4.70	22.99
0	1	12.31	14.52	5.50	4.56	36.89
0	2	9.66	14.81	6.63	5.05	36.15
0	4	8.06	11.05	6.29	5.34	30.74
0.25	0	4.20	3.02	3.47	4.44	15.13
0.25	1	10.22	13.93	4.76	5.05	33.96
0.25	2	9.73	11.86	6.36	5.64	33.59
0.25	4	7.98	8.49	6.79	5.76	29.02
0.50	0	2.20	4.15	2.83	2.89	12.07
0.50	1	3.89	3.83	3.30	4.35	15.37
0.50	2	5.40	4.44	5.15	5.10	20.09
0.50	4	8.15	9.14	5.62	6.02	28.93
1.00	0	2.66	3.44	1.90	2.71	10.71
1.00	1	3.86	7.35	1.14	5.37	17.72
1.00	2	6.22	4.65	6.95	6.15	23.97
1.00	4	6.34	8.53	7.01	6.36	28.24
Σ		110.16	127.41	78.61	79.49	395.57
F values[2]						
Treatment		8.51	15.97	23.20	7.14	
Bromacil level		23.28	13.62	22.00	4.52*	
O. M. level		8.97	37.16	80.00	31.42	
Bromacil X O.M.		3.45*	12.02	5.00	2.00*	
C.V. %		26.20	22.22	14.36	12.11	

[1]Yields are total fresh weights of three replicate cultures

[2]All F values are statistically significant at .005 level except those designated * (non significant)

organic matter decomposition.

Bromacil levels without organic matter decreased oat growth drastically initially. The 0.25 ppm level lost toxicity with each succeeding planting. The 0.5 and 1 ppm level maintained apparent phytotoxicity throughout the four plantings with no organic amendments.

In general, each additional increment of organic matter resulted in decreased bromacil toxicity, particularly at the 0.5 and 1 ppm herbicide level in all four plantings. However, total yields were highest with the 0 and 0.25 ppm bromacil-organic matter combinations substantiating the indication that some phytotoxicity was still present at the higher herbicide levels without organic matter additions.

Similar trends were also noted with two successive crops of sorghum as shown in Table III. Visual symptoms of toxicity accelerated with growth time. However, these symptoms were similar to those in oats but never so severe. Bromacil level of 0.25 ppm was almost ineffective when compared with no herbicide treatment. The 0.5 and 1 ppm levels inhibited growth of the first sorghum crop markedly. With the second crop, phytotoxicity at these same levels was greatly reduced.

Organic amendment addition at 2 and 4 percent by soil weight not only reduced the bromacil phytotoxicity but also resulted in higher fresh weight in comparison to the no herbicide treatment. Again the total yields were highest with the 0 and 0.25 bromacil-organic matter combination.

Cucumber grown after sorghum in the same pots showed varied degree of specific chlorosis. Characteristic leaf patterns were observed even in young cotyledonary leaves. This was essentially a palmate type of veinal chlorosis with gradual spreading transition from yellow to green

TABLE III

SOIL ORGANIC AMENDMENT AMELIORATION EFFECTS ON BROMACIL PHYTOTOXICITY WITH TWO SUCCESSIVE SORGHUM PLANTINGS, EUFAULA SAND

Bromacil ppm	% O.M.	Sorghum Plantings 1	2	Σ
		Grams Fresh Weight[1]		
0	0	6.36	6.01	12.37
0	1	6.91	6.44	13.35
0	2	7.31	7.46	14.77
0	4	7.33	9.21	16.54
0.25	0	5.98	6.17	12.15
0.25	1	6.70	6.49	13.19
0.25	2	7.09	9.17	16.26
0.25	4	7.45	9.54	16.99
0.50	0	3.50	5.66	9.16
0.50	1	5.85	5.80	11.65
0.50	2	6.25	6.60	12.85
0.50	4	6.68	8.79	15.47
1.00	0	2.25	3.12	5.37
1.00	1	3.55	5.83	9.38
1.00	2	6.63	7.05	13.68
1.00	4	7.25	8.11	15.36
Σ		97.09	111.45	208.54
F values[2]				
Treatment		10.25	13.27	
Bromacil level		16.62	14.71	
O. M. level		23.87	53.80	
Bromacil X O.M.		3.62	1.27*	
C.V. %		13.86	11.15	

[1]Yields are total fresh plant weights of three replicate cultures

[2]All F values statistically significant at .005 level except those designated * (non significant)

tissue eventually resulting in the death of the leaves after about seven days (Fig. 3). These plants were similarly affected with residual activity from all bromacil levels but symptoms were slightly less severe at the 0.25 ppm level. However, diminution of toxicity with organic matter was less obvious due to toxicity with extreme sensitive nature of the cucumbers to sorghum and oats. These symptoms contrast markedly to threshold symptoms for other herbicide compounds (11, 13).

Experiment II. Effects of Organic Matter and Plant Nutrient Levels

Organic amendments addition modified the bromacil phytotoxicity at all levels, as shown in Table IV. Organic matter addition at 1, 2, and 4 percent of soil weight was found to be significant in reducing bromacil phytotoxicity to sorghum crop. However, organic matter reduced phytotoxicity less in the first and second crops. In third and fourth crop it not only reduced the bromacil phytotoxicity but also resulted in higher green weight of plants. Bromacil at 1, 2, and 4 ppm remained active for all the four crops with decreasing phytotoxicity apparent with time. Addition of 1, 2, and 4 ppm of bromacil resulted in about 50%, 25% and 20% yield in comparison to control. Highest total yields were obtained with no bromacil and 4% organic matter.

Soil fertility manipulations in terms of nitrogen and phosphorus showed significant effect on sorghum treated with 1, 2, and 4 ppm of bromacil as shown in Tables V and VI. Nitrogen was found to be highly effective in reducing the bromacil phytotoxicity when applied to soil at the rate of 100, 200, and 400 ppm as ammonium nitrate. Bromacil was less effective at these same concentrations when applied in combination with various levels of nitrogen. Nitrogen remained effective for two crops of sorghum equally well. However, phosphorus additions did not

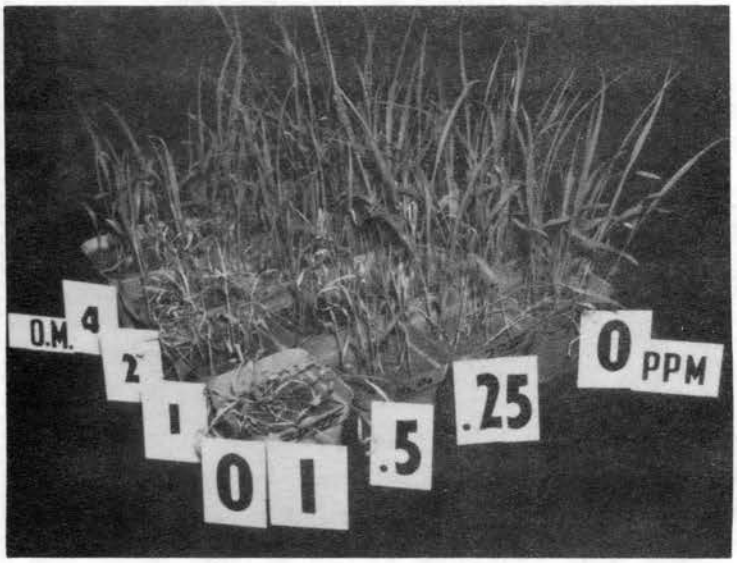

Figure 2 Growth interactions of oat plants in factorial combinations with ppm bromacil levels and per cent soil organic matter amendment.

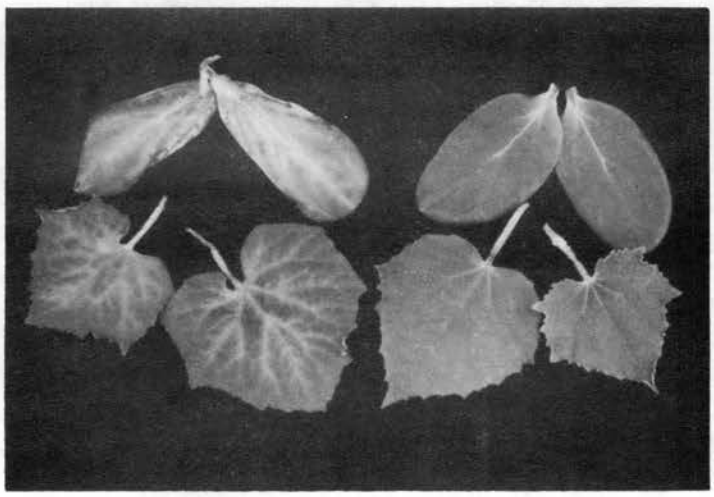

Figure 3 Normal leaves compared with threshold chlorotic foliar patterns of cucumbers induced with bromacil residues, Eufaula sand.

TABLE IV

SOIL ORGANIC AMENDMENT AMELIOGRATION EFFECTS ON BROMACIL PHYTOTOXICITY WITH FOUR SUCCESSIVE SORGHUM PLANTINGS EUFAULA SAND

Bromacil ppm	% O.M.	Sorghum Plantings 1	2	3	4	Σ
		Grams Fresh Weight[1]				
0	0	7.49	7.60	3.72	1.85	20.66
0	1	6.20	8.19	4.62	2.64	21.65
0	2	6.03	8.13	5.27	3.14	22.57
0	4	6.06	8.27	5.69	4.71	24.73
1	0	2.69	4.96	1.82	1.36	10.83
1	1	1.21	5.46	2.88	1.66	11.21
1	2	1.89	5.79	2.98	1.96	12.62
1	4	2.99	5.97	3.78	2.54	15.28
2	0	1.32	1.80	1.29	0.71	5.12
2	1	1.97	2.50	2.48	0.95	7.90
2	2	3.13	3.03	2.83	1.60	10.59
2	4	4.01	3.64	4.47	1.79	13.91
4	0	1.03	1.45	1.18	0.72	4.38
4	1	1.43	2.04	1.58	1.08	6.13
4	2	2.27	2.54	2.13	1.34	8.28
4	4	1.80	2.93	4.00	2.02	10.75
Σ		51.52	74.30	50.72	30.07	206.61
F values[2]						
Treatment		73.00	33.15	10.15	8.75	
Bromacil levels		318.50	161.33	23.65	24.50	
O.M. levels		15.50	6.16	23.65	16.00	
Bromacil X O. M.		15.50	0.33*	2.50*	1.00*	
C.V. %		13.08	15.57	22.84	27.67	

[1]Yields are total above ground plant material from three replicate cultures

[2]All F values statistically significant at the .005 level except those designated * (non significant)

TABLE V

INTERACTION EFFECTS OF NITROGEN, PHOSPHORUS, AND BROMACIL LEVELS WITH PHYTOTOXICITY TO SORGHUM PLANTS, EUFAULA SAND

NH_4NO_3	$CaH_4(PO_4)_2 \cdot 2H_2O$	Bromacil level ppm				
N ppm	P ppm	0	1	2	4	Σ
		Grams Fresh Weight				
0	0	7.65	6.71	5.40	2.02	21.78
0	100	7.71	7.19	6.19	1.93	23.02
0	200	7.95	7.58	6.52	2.71	24.76
0	400	8.05	6.94	5.38	3.41	23.78
100	0	8.50	7.42	6.69	3.95	26.56
100	100	8.13	7.81	6.96	3.72	26.62
100	200	8.29	7.96	7.48	3.66	27.39
100	400	8.64	8.40	7.59	4.52	29.15
200	0	9.19	8.35	7.08	5.88	30.50
200	100	8.93	8.28	6.98	5.60	29.79
200	200	9.55	9.13	8.09	6.90	33.67
200	400	9.83	9.20	8.51	5.85	33.39
400	0	10.45	9.63	9.15	8.46	37.69
400	100	11.02	10.65	9.92	9.13	40.72
400	200	10.86	10.64	10.69	9.78	41.97
400	400	11.15	11.04	10.77	10.13	43.09

Yields are total plant material from three replicates of first crop.

F values statistically significant at .005 level unless designated *, non significant

Treatment = 25.83, bromacil level = 160.16, nitrogen level = 240.80, phosphorus level = 11.50, bromacil X nitrogen = 44.50, bromacil X phosphorus = 2.00*, nitrogen X phosphorus = 3.33, bromacil X nitrogen X phosphorus = 5.00, C.V. % = 9.33

TABLE VI

INTERACTION EFFECTS OF NITROGEN, PHOSPHORUS, AND BROMACIL LEVELS WITH PHYTOTOCITY TO SORGHUM PLANTS, EUFAULA SAND

NH_4NO_3	$CaH_4(PO_4)_2 \cdot 2H_2O$	Bromacil level ppm				
N ppm	P ppm	0	1	2	4	Σ
		Grams Fresh Weight				
0	0	7.46	7.17	6.06	4.24	24.93
0	100	7.26	7.78	6.43	5.90	27.37
0	200	8.16	7.75	6.81	6.08	28.80
0	400	8.85	8.58	7.22	6.60	31.25
100	0	7.94	8.27	7.74	7.23	31.18
100	100	9.24	9.08	8.33	8.29	34.94
100	200	9.38	9 29	8.51	7.50	34.68
100	400	9.54	8.54	8.86	8.29	35.23
200	0	10.07	9.49	9.21	8.71	37.48
200	100	10.09	9.55	9.24	9.00	37.88
200	200	10.30	9.72	9.55	8.46	38.03
200	400	11.22	10.46	9.75	9.30	40.73
400	0	11.65	11.67	11.47	10.60	45.39
400	100	11.82	11.36	11.41	11.37	45.96
400	200	12.07	11.88	11.45	11.54	46.94
400	400	12.30	11.93	11.95	11.17	47.35

Yields are total plant material from three replicates of second crop.

F values statistically significant at .005 level unless designated *, non significant.

Treatment = 32.70, bromacil level = 142.20, nitrogen level = 322.20, phosphorus level = 32.22, bromacil X nitrogen = 17.50, bromacil X phosphorus = 10.50, nitrogen X phosphorus = 11.75, bromacil X N X P = 7.8, C.V. % = 6.53.

have the obvious effects obtained with nitrogen levels, although differences were found to be statistically significant.

Interactions of bromacil and nitrogen, nitrogen and phosphorus, nitrogen, phosphorus and bromacil were found to be significant. While bromacil and phosphorus interactions were nonsignificant with the first crop, they were significant in the second.

Results with bromacil and nitrogen culture levels with Aspergillus niger growth discussed later in Experiment III and IV confirmed somewhat the apparent ability of an available nitrogen source for the plant to offset bromacil phytotoxicity.

Experiment III. Effects of Bromacil and Growth Factors on Various Soil Fungi

Bromacil up to 1000 ppm apparently did not inhibit or stimulate the growth of following soil inhabiting fungi: Aspergilli: flavus, oryzae, tamarii, Curvularia lunta, Mucor pusillus, Tricoderma viride, Penicillium funiculosum, Penicillium brevicompactum, and Myrothecium verucaria. However, bromacil at 1500 ppm inhibited approximately 50% of A. niger mycelium growth even though 500 ppm of nitrogen as ammonium nitrate was included. However, no growth was attained at the 1700, 1800, 1900, and 2000 ppm bromacil levels with different combinations of 125, 250, and 500 ppm nitrogen. There was less inhibition of mycelium growth as the levels were increased from 125 to 500 ppm (Table VII).

This response of this organism with these bromacil X nitrogen interactions indicated an apparent inability to utilize the pyrimidine ring nitrogen. Bromacil also apparently provides some block in the ability of A. niger to utilize other nutrients in metabolism. Nitrogen sources for this organism could be offset at the lower herbicide levels

TABLE VII

INTERACTIONS OF INTERMEDIATE NITROGEN AND BROMACIL LEVELS WITH ASPERGILLUS NIGER GROWTH

Bromacil ppm	NH_4NO_3 ppm N 0	125	250	500	Σ
		Grams Dry Mycelium			
0	0	3.07	3.77	4.56	11.40
500	0	2.44	3.37	4.15	9.96
1000	0	2.25	2.86	3.51	8.62
1500	0	2.21	2.22	3.05	7.48
1600	0	2.08	2.20	2.82	7.10
1700	0	0	0	0	0
1800	0	0	0	0	0
1900	0	0	0	0	0
2000	0	0	0	0	0
Σ		12.05	14.42	18.09	44.56

Yield figures are sums of three replicate cultures per treatment.

F values statistically significant at .005 level, except designated as *, non significant.

Treatment = 52.00, bromacil level = 131.22, nitrogen level = 151.32, bromacil X nitrogen = 13.33, C.V. % = 29.26

with ammonium nitrate. No response with urea was obtained in these studies when used in place of ammonium nitrate as the nitrogen source.

Level of nitrogen supplied in A. niger cultures was found to be the single most important factor in determining apparent bromacil toxicity at various concentrations. Table VIII shows the effect of nitrogen additions from 0 to 2000 ppm to these cultures treated with varying concentration of bromacil ranging from 0 to 2000 ppm. It was observed that nitrogen at 500 ppm could offset the effect of bromacil levels up to 1500 ppm. However, no fungus growth was obtained at 2000 ppm bromacil even when 2000 ppm of nitrogen was supplied in form of ammonium nitrate. Nitrogen levels higher than 500 ppm apparently were not effective for influencing increased growth of A. niger at bromacil levels below 1500 ppm.

Thiamine hydrochloride, riboflavin, pyridoxine base, folic acid, and glutathione included in culture media at 1000 and 2000 ppm did not offset bromacil toxicity as indicated with A. niger proliferation. However, yeast and peptone stimulated growth of this fungus when applied separately at 1000 and 2000 ppm in combination with 1000, 1600 and 1800 ppm bromacil, Tables IX and X. Both yeast and peptone, respectively, applied at 1000 ppm not only reduced the bromacil toxicity but also apparently served as a potential source of various nutrient factors for increased fungus growth.

There was little difference between the cultures containing 1000 ppm bromacil and no bromacil when combined with a simultaneous application of 1000 ppm of yeast or peptone. However, mycelium growth decreased as the levels of bromacil were increased beyond 1000 ppm. With yeast as well as peptone the treatments with the application of 125 ppm

TABLE VIII

INTERACTIONS OF HIGH NITROGEN AND BROMACIL LEVELS WITH ASPERGILLUS NIGER GROWTH

NH_4NO_3	Bromacil ppm					
ppm N	0	500	1000	1500	2000	Σ
			Grams Dry Mycelium			
0	0	0	0	0	0	0
500	4.08	3.83	3.41	2.23	0	13.55
1000	4.06	3.87	3.35	1.49	0	12.77
1500	4.07	3.90	3.16	2.56	0	13.69
2000	4.05	3.85	3.59	2.44	0	13.93
Σ	16.26	15.45	13.51	8.72	0	53.94

Yield figures are sums of three replicate cultures per treatment.

F values statistically significant at .005 level, except shown as *, non significant.

Treatment = 343.22, bromacil level = 993.22, nitrogen level = 810.00, bromacil X nitrogen = 66.66, C.V. % = 8.33

TABLE IX

PEPTONE AND AMMONIUM NITRATE LEVEL INTERACTIONS WITH BROMACIL ON ASPERGILLUS NIGER GROWTH

NH_4NO_3 ppm N	Peptone level ppm	Bromacil level ppm 0	1000	1600	1800	Σ
		Grams Dry Mycelium				
0	0	0	0	0	0	0
0	1000	1.67	1.52	0.94	0.81	4.94
0	2000	2.11	2.08	2.00	1.35	7.54
125	0	3.08	2.42	1.78	0.95	8.23
125	1000	3.65	3.13	2.25	1.90	10.93
125	2000	3.56	3.22	3.17	1.50	11.45
Σ		14.07	12.37	10.14	6.51	43.09

Yields figures are sums of three replicate cultures per treatment
F values statistically significant at .005 level except when designated *, non significant.

Without N: Treatment = 83.33, bromacil level = 20.00, peptone level = 406.66, bromacil X peptone = 5.00*
C.V. % = 16.16

With N: Treatment = 50.00, bromacil level = 112.00, peptone level = 50.00, bromacil X peptone = 20.00
C.V. % = 2.58

TABLE X

YEAST EXTRACT AND AMMONIUM NITRATE LEVELS INERACTIONS WITH BROMACIL ON ASPERGILLUS NIGER GROWTH

NH_4NO_3 ppm N	Yeast extract ppm	Bromacil level ppm 0	1000	1600	1500	Σ
		Grams Dry Mycelium				
0	0	0	0	0	0	0
0	1000	.72	.63	.65	.60	2.60
0	2000	1.50	1.12	1.06	.94	4.62
125	0	3.43	2.55	1.46	.74	8.18
125	1000	3.33	3.17	3.04	2.39	11.93
125	2000	4.21	3.54	3.04	2.63	13.42
Σ		13.19	11.01	9.25	7.30	40.75

Yield figures are sums of three replicate cultures per treatment. F values statistically significant at .005 level except when designated *, non significant.

Without N: Treatment = 45.00, bromacil level = 5.00*, yeast level = 225.00, bromacil X yeast = 2.00*

With N: Treatment = 282.00, bromacil level = 51.00, yeast level = 74.00, bromacil X yeast = 16.00

nitrogen as ammonium nitrate resulted in reducing bromacil toxicity more than the treatments which did not receive the additional nitrogen. Very similar trends were observed, when cyancobalamin (B-12) was added to A. niger cultures in combination with various concentrations of bromacil as shown in Table XI.

The organic nitrogenous materials used in these cultures may be termed as components containing presynthesized growth factors. Peptone and yeast extract provide amino acids, vitamins, nucelic acids, and other essential growth factors in somewhat a heterogenous combination. The growth responses attained with these materials indicated promise for seeking single growth factors that have similar effects on culture yields with A. niger at various bromacil levels. Of the separate materials used, cyancobalamin (vitamin B-12) was most promising in these studies. A. niger yields were slightly less at 1000 ppm cyancobalamin than this same level with yeast or peptone. Both yeast and peptone additions with 125 ppm N as ammonium nitrate resulted in higher yields than those with the equivalent levels of cyancobalamin. This further indicated favorable effects from combinations of some presynthesized factors within these heterogenous materials that may offset bromacil toxicity to the fungus.

Experiment IV. Effect of Bromacil and Toyo Koatsu AM on Soil Nitrification

Bromacil did not have a significant effect on soil nitrification when applied at 10 and 100 ppm along with 200 ppm of urea nitrogen as shown in Table XII. The determination of nitrite plus nitrate made at 5, 10, and 15 days intervals indicated no inhibition of nitrification. However, a slight retardation was noted initially with 100 ppm bromacil

TABLE XI

CYANCOBALAMIN (B-12) AND AMMONIUM NITRATE LEVEL INTERACTIONS WITH BROMACIL ON ASPERGILLUS NIGER GROWTH

NH_4NO_3 ppm N	B-12 level ppm	Bromacil level ppm 0	1000	1600	1800	Σ
			Grams Dry Mycelium			
0	0	0	0	0	0	0
0	1000	1.68	1.00	0.56	0.38	3.62
125	0	2.65	1.41	0	0	4.06
125	1000	2.34	1.61	0.50	0.23	4.68
Σ		6.67	4.02	1.06	0.61	12.36

Yield figures are sums of three replicate cultures per treatment. F values statistically significant at .005 level except when designated *, non significant

Without N: Treatment = 120.00, bromacil level = 60.00, B-12 level = 450.00, B-12 X bromacil = 80.00, C.V. % = 18.00

With N: Treatment = 370.00, bromacil level = 850.00, B-12 level = 20.00, B-12 X bromacil = 2.00*, C.V. % = 8.33

TABLE XII

EFFECTS OF BROMACIL AND TOYO KOATSU AM LEVELS ON SOIL NITRIFICATION OF UREA AT 200 ppm N, EUFAULA SAND

Pyrimidine level ppm	Increase in NO_2+NO_3 with urea over paired soil samples with no urea addition			
	Days incubation			
	5	10	15	Σ
(Bromacil)				
0	31	26	24	81
10	23	26	22	71
100	18	25	25	68
(Toyo Koatsu AM)				
0	39	22	16	77
10	23	20	6	49
100	22	6	0	28

Figures are means from three replicate cultures.
F values are significant at the .005 level except designated as *, non significant.

Bromacil: Treatment = 4.14*, bromacil = 4.75*, days = 0.46*, bromacil X days = 5.68*, C.V. % = 10.62

Toyo Koatsu AM: Treatment = 181.44, Toyo Koatsu AM = 258.65, days = 424.88, AM X days = 21.20.

application.

Toyo Koatsu AM applied at 10 and 100 ppm along with 200 ppm urea nitrogen significantly retarded the oxidation of ammonium nitrogen to nitrite and nitrate at 5, 10, and 15 day intervals. Rate of nitrification was reduced to 20% of total after 15 days of AM application at the rate of 10 ppm. A complete check in nitrification was observed with 100 ppm of Toyo Koatsu AM after 15 days.

The pyrimidine configuration of bromacil apparently was not detrimental to normal activities of the soil nitrification organisms in these studies as compared to the known pyrimidine nitrification inhibitor Toyo Koatsu AM.

CHAPTER V

SUMMARY AND CONCLUSION

The objective of this study was to determine soil factors affecting phytotoxicity of bromacil and the magnitude and rate of degradation. Various soil factors manipulated included the addition of organic amendment, and variation in nitrogen and phosphorus levels. Oats, sorghum, and cucumber plants were used in bioassay studies. Ten soil inhabiting fungi were screened with media containing various levels of bromacil and nitrogen to establish degradation rates and toxicity. Pyrimidines and other compounds were included to offset the toxicity of bromacil to Aspergillius niger. Comparative soil nitrification studies were made with bromacil and Toyo Koatsu AM.

Principal soil factors found to decrease bromacil activity were level of soil organic amendment and level of available soil nitrogen. Avena sativa, L. and Sorghum vulgare, Pers. were sensitive bioassay plants to bromacil phytotoxicity. Cucumis sativus, L. developed specific chlorotic patterns indicative of threshold toxicity levels. Fungi species not inhibited at 1000 ppm bromacil levels were: Aspergilli: oryzae, flavus, tamarii, Curvularia lunta, Mucor pusillus, Trichoderma viride, Penicillium funiculosum, Myrothecium verucaria, and Penicillium breviocompactum. Aspergillus niger was sensitive to bromacil and selected pyrimidine compounds did not modify bromacil toxicity to this fungus. Yeast extract, peptone, and cyancobalamin at 1000 ppm reduced

bromacil toxicity significantly.

The pyrimidine configuration of bromacil apparently was not deterimental to normal activities of the soil nitrification organisms in these studies as compared to the known pyrimidine nitrification inhibitor, Toyo Koatsu AM.

LITERATURE CITED

1. Alexander, Martin. 1964. Introduction to Soil Microbiology. John Wiley and Sons, New York, N. Y.

2. Anonymous. 1966. Toyo Koatsu AM nitrification inhibitor. Technical Bull. No. 1. Toyo Koatsu Industries, Inc., Tokyo, Japan.

3. Annonymous. 1967. Your guide for chemical weed and brush control in the Southwestern states. E. I. Du Pont De Nemours and Co., Houston, Texas.

4. Barnes, D. L. 1965. Biometrics of pyridylium photosynthate inhibitors with Phaseolus vulgaris foliar tissues and Chlorella pyreniodosa. M. S. Thesis. Oklahoma State University.

5. Black, C. A., D. D. Evans, J. L. White, L. E. Ensminger and F. E. Clark. 1965. Methods of Soil Analysis, Part 1 and 2. Amer. Soc. of Agro., Inc., Madison, Wisconsin.

6. Bucha, H. C., W. E. Cupsry, J. E. Harrod, H. M. Loux and L. M. Ellis. 1962. Substituted uracil herbicides. Science 137: 537-538.

7. Hilton, J. L., T. J. Manaco, D. E. Moreland and W. A. Genter. 1964. Mode of action of substituted uracil herbicides. Weeds 12: 129-131.

8. Hoffman, C. E., J. W. McCracken and P. B. Sweetsen. 1964. Effect of substituted uracil herbicides on photosynthesis. Nature 202 (4932):577-578.

9. Holly, K. and H. A. Roberts. 1963. Persistence of phytotoxic residues of triazine herbicides in soil. Weed Res. 3:1-10.

10. Lynd, J. Q., C. E. Rieck and P. W. Santelmann. 1966. Soil components determining bensulide phytotoxicity. Agron. J. 58: 508-510.

11. Lynd, J. Q., Charles Rieck, Don Barnes, Don Murray, and Paul Santelmann. 1966. Indicator plant aberrations at threshold soil herbicide levels. Agro. J. 59:194-196.

12. Moreland, D. E., W. A. Genter, J. L. Hilton and K. L. Hill. 1959. Studies on the mechanism of herbicidal action of 2-chloro-4, 6-bis(ethylamino)-s-triazine. Plant Physiol. 34:432-435.

13. Rieck, C. E. and J. Q. Lynd. 1967. Parameters of chlorinated pyridine phytotoxicity to Robinia pseudoacacia. Agron. J. 59:507-509.

14. _____. 1966. Soil parameters in degradation sequences of chlorinated pyridine biocides. M. S. Thesis. Oklahoma State University.

VITA

Sunil Kumar Pancholy

Candidate for the Degree of

Master of Science

Thesis: SOIL PARAMETERS GOVERNING PHYTOTOXICITY OF BROMACIL, A SUBSTITUTED URACIL

Major Field: Agronomy

Biographical:

Personal Data: Born in Udaipur, Rajasthan, India, July 28, 1945, son of Sobhagya N. and Nalini Pancholy.

Education: Graduated from Raj. Coll. Agri., Udaipur, Rajasthan, India, in July, 1961; received the Bachelor of Science degree from University of Udaipur with a major in Agriculture in July, 1964; received the Master of Science degree from University of Udaipur, with a major in Agronomy in July, 1966; graduate study at Oklahoma State University, February, 1967 to July, 1968.

Experience: Employed by Rajasthan College of Agriculture, as a milk distributing agent, 1963 to 1964; Recipient of Junior Fellowship of Counsil of Scientific and Industrial Research, New Delhi, India, 1966. Research Assistant at Indian Agriculture Research Institute, New Delhi, July 1966 to January 1967; part-time lab technician, February, 1967 to August, 1967 at Oklahoma State University; Graduate Research Assistant, Oklahoma State University, September, 1967 to July, 1968.

Member of: Indian Society of Agronomy, Indian Society of Soil Science, Young Farmers Association, American Society of Agronomy, and Soil Science Society of America.