SOIL BIOLOGICAL FACTORS INFLUENCING PYRICLOR PHYTOTOXICITY

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CHAPTER I

INTRODUCTION

Soil persistence of biotoxins and factors that determine their effectiveness are increasingly important to agriculture. Many new organic compounds are being synthesized for the control of insects, fungi, and weeds. These compounds must not only destroy undesirable or harmful forms of life but must be suitable for continued productive coexistance of beneficial organisms. Much research must be accomplished on these biotoxins before they can be cleared and marketed for general public use. The pyridine ring is a parent structure of insecticides, fungicides and herbicides that are known to be highly persistent in the soil, however, pyridine ring moiety compounds are also essential for all cellular metabolism. Even though these compounds have the same basic ring structure, their biological properties and metabolic functions vary widely. The pyridine ring is comparatively resistant to clevage, although substitutions on ring loci and condensation reactions occur readily.

The purposes of this study were: (1) to determine soil factors that influence the activity and degradation of 2,3,5-trichloro-4-pyridinol (pyriclor), and (2) to determine the effects of pyriclor with several selected soil fungi.

CHAPTER II

LITERATURE REVIEW

In recent years numerous effective biotoxins have been developed from substituted pyridine compounds. Specific examples of such compounds include 4-amino-3,4,6-trichloropicolinic acid (picloram); 2-chloro-6-(trichloromethyl) pyridine (N-Serve); O,O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate (dursban); 1-hydroxy pyridine-2-thione (omadine); and 2,3,5-trichloro-4-pyridinol (pyriclor).

Picloram, with the trade name Tordon, has proven to be one of the more effective systemic or auxin type herbicides. This chemical is comparable to 2,4-D and 2,4,5-T in toxicity effects, foilage absorption, and translocation characteristics. Under certain soil conditions, levels of picloram have been 50 per cent effective for 508 days as compared to 56 days for 2,4-D and 87 days for 2,4,5-T (11). Goring, et.al. (9) showed losses of picloram ranged from 58 to 96 per cent within one year after application and from 78 to 100 per cent within two years after application. Field trials have shown that Tordon 22K herbicide was effective for the control of Canada thistle and field bindweed at rates of 1 gallon (2 pounds) per acre for thistle and 1.5 gallons per acre for bindweed (16, 28). Rieck (22) has shown that phytotoxic levels of picloram were approximately 10 fold higher than pyriclor and 100 times higher than N-Serve. A

decrease in phytotoxicity of picloram was apparent as cation exchange capacity was increased from 2 to 14 millequivalents per 100 grams (23).

N-Serve is a nitrogen conserving agent, manufactured by Dow Chemical Company, designed to reduce nitrogen losses from ammonia fertilizers applied to soil (1). The basic biological action of N-Serve in soils was studied by Goring (8). Results show N-Serve to be highly toxic to the organisms converting ammonia to nitrite, and to have a low order of toxicity to (a) the organisms or enzyme converting urea to ammonia; (b) the organism converting nitrite to nitrate; (c) the general fungus and bacterial populations; and (d) the seedlings of many plants. Turner and Nilson (26, 24) have shown that when N-Serve is added to fertilizer applied to cotton, a crop can be grown with only one nitrogen addition and no decrease in yield. N-Serve is found to initially increase in activity with increased organic matter levels, which indicate that microorganism activity influences activation. A highly active form of this pyridine accumulated in plant materials with four grams dry plant tissue giving an equivalent response corresponding to a 5ppm level of N-Serve (23). Redemann et. al. (21) has shown that N-Serve is lost form the soil by two processes; (1) volatilization and (2) degradation to 6-chloropicolinic acid. Degradation is relatively rapid.

Dursban is an insecticide which controls a wide variety of insect pests, especially mosquitos and household and turf pests (10). In household pest control investigations, dursban at 0.5% gave excellent control of the following pest species: cockroaches (5 species), ants (9 species), carpet beetle, earwig, flea,

silverfish, spiders, and ticks (2 species) (25). Data indicate that dursban insecticide applied at routine use concentrations should not present any hazard to birds and other vertebrate wildlife (5), (18). In low volume dosage studies with dursban, Lembright (17) has shown that at 0.05 pounds per acre, mosquitos were effectively controlled in large scale California tests. At this rate there was little or no effect on non-target organisms (29).

Omadine and its salts have been reported to have pronounced antibacterial and antifungal activity. Omadine and certain of its derivatives have also been found to be inhibitory in certain plant systems (20). Chisam (4) found that sodium omadine was non-phytotoxic to oats and grain sorghum at levels below 320 ppm. Both oats and grain sorghum growth was markedly inhibited above the 320 ppm level. Evidence of phytotoxicity was more pronounced on sterilized soil than on unsterilized soil. The influence of sodium omadine had largely subsided after 56 days. Fungus bioassay of soil samples indicated that at the highest application level, small residual amounts of sodium omadine were present.

Pyriclor was introduced by Dow Chemical Company in 1965 under the trademark Daxtron (2), (6), (15). It is a systemic herbicide which is readily absorbed and translocated by roots and foliage of grasses and broadleafs (6). Pyriclor is especially effective for the control of annual and perenniel grasses, but will also control many broadleaf weeds (15), (30). Dechlorophyllation occurs on most plant species following treatment and is reversable at sub-lethal concentrations. It has been used as a chemical defoliant and regrowth inhibitor in cotton. In three years of field tests in

Mississippi (12), pyriclor was used to prevent regrowth on defoliated cotton. Excellent defoliation and regrowth control were obtained with combination treatments of pyriclor and commercial defoliants (13).

Quackgrass was controlled by 1-4 pounds per acre pyriclor applications at 1-8 months prior to planting of corn and alfalfa (3). Oats were injured by residues of pyriclor from 2 pounds per acre applications made 20 months previously. Corn and alfalfa yields were not affected by pyriclor applications. This compound is subject to leaching from soil, however, under most soil conditions it has a long residual period.

The mode of action of pyriclor is not currently established. Killion and Frans (14) have reported that this chemical inhibits oxygen uptake and oxidative phosphorolation (27) of mitochondria isolated from hypocotyls of etiolated soybean leaves. Geronimo and Herr (7) have shown that treatment of tobacco with pyriclor resulted in disruption of chloroplast ultrastructure.

CHAPTER III

MATERIALS AND METHODS

This experiment was performed in the laboratory with a *Psammentic Paleustalf*, Eufaula sand. Proximate analysis of this soil was 90.0% sand, 7.0% silt, 3.0% clay, pH 5.7, 0.5% organic matter, 0.04% total N., exchange capacity of 2.7 m.e. with exchangeable cations of Ca 1.1, Mg 0.9, K 0.3 m.e./100 grams soil and available P 3.4 ppm.

Air dry soil, that had previously passed through an 8 mesh sieve was placed in 4 inch square plastic pots. Four hundred gram soil cultures were used in each experiment with three replications per treatment. The pyriclor used in these studies was a 19.4% commercial formulation supplied by Dow Chemical Company.

In order to evaluate the rate of pyriclor degradation under varying experimental conditions, three common plant species including Cimarron oats, OK 612 grain sorghum, and Long Green cucumbers, were screened for pyriclor sensitivity. The main objective of this screening was to determine threshold symptoms on different plants and levels of pyriclor associated with these symptoms (19). These preliminary screening studies indicated that OK 612 grain sorghum was a suitable bioassay plant due to its easily observed rates of dechlorophyllation. The most suitable pyriclor treatment levels were found to be 0.25, 0.50, 1.00, and 2.00 ppm.

Thirty-five grain sorghum seeds were planted per pot. The plants were thinned to 25 plants per pot upon reaching a height of one to two inches. The plants were grown under continuous fluorescent light of 500 foot candles from "Gro-lux" lamps at room temperature. Adequate moisture was provided to insure maximum growth. The plants were harvested when the higher treatments of pyriclor showed symptoms of necrosis. This time varied due to fluctuations in room temperature but usually ranged from 12-16 days. Above ground green weights and dry weights were recorded. The soil cultures were then air dried, remixed and replanted. The same procedures were used in subsequent plantings as in the first one.

Experiment I. Effect of soil amendments on pyriclor toxicity and degradation.

Soil Reaction: The effect of soil reaction on phytotoxicity of pyriclor was accomplished using normal Eufaula soil as the standard. Acid and alkaline Eufaula were compared to the standard. The soil was made acidic by adding twice the milliequivalents of sulfur as the exchange capacity. The soil was made alkaline by adding calcium oxide in the same manner. Normal Eufaula had a pH of 5.7. The acid Eufaula had a pH of 4.7-4.9 and the pH of the alkaline Eufaula was 8.2-8.5.

Organic Matter Additions: Organic matter additives were in the form of ground wheat straw and alfalfa meal. Composition of the alfalfa was 1.05% N, 0.26% P, 1.97% K, and 1.58% Ca. These were added to the soil as a percentage of the soil by weight. Levels used were 1% straw, 1% alfalfa, 1% straw plus 1% alfalfa, 2% straw,

2% alfalfa, 2% straw plus 2% alfalfa, and 3% straw plus 3% alfalfa. Pyriclor treatment levels and bioassay procedures were described previously. Two harvests were obtained in this experiment.

Cation Exchange Capacity: Bentonite clay was added to the soil to determine the effects of changes in cation exchange capacity on the phytotoxicity of pyriclor. The soil was altered to give cation exchange capacities of five and ten milliequivalents per 100 g. soil. Normal Eufaula with a CEC of 2.7 was used as check. Bioassay procedures and treatment levels were the same as those described previously. Two experiments were performed with two harvests per experiment.

Experiment II. Effect of time on pyriclor degradation

Four hundred gram samples of Eufaula soil were weighed and placed in plastic bags. Pyriclor to make 0.25, 0.50, 1.00, and 2.00 ppm was added to the soil and soil moisture was adjusted to 20% by weight. The bags were then sealed and incubated at 25-30°C. for the prescribed period. At the end of each designated incubation period, soils were removed from the plastic bags, placed in pots, and planted to grain sorghum as described in the previous bioassay procedures.

Experiment III. Effect of pyriclor levels on growth of four fungi isolates

Four common soil inhabiting fungi; *Curvularia lunata*, *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus tamarii* were cultured on a liquid broth media containing 0, 50, 100, 200, and 400 ppm levels of pyriclor. 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 1.0. Composition of the Phillips Hart salt mixture was (percent): 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 0.025, and cobalt chloride 0.005. The media had no presynthesized growth factors and the final pH of the media was 4.0.

Forty milliliters of media were used per 500 milliliter metal capped Erlenmeyer culture flask. The media was autoclave sterilized for fifteen minutes at 15 psi. After cooling, spore inoculations and pyriclor treatments were added. Each treatment was replicated three times. The time of culture incubation for growth response determinations following inoculation and treatment was important. The time of harvest was approximately 24 hours after the untreated cultures had sporulated. Generally, the pyriclor treated cultures were much slower in initiating growth, and decreasing the length of incubation time would have indicated a greater magnitude of fungi toxicity and longer incubation time would have shown less. The incubation time for the cultures was 4-5 days at 30°C.

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

Soil amendments used in these studies influenced not only the pyriclor treated soil cultures, but also the soils without pyriclor additions. Therefore, soil treatments which did not receive pyriclor additions were used as a 100% base for a relative evaluation of toxicity within a specific soil amendment series. Yields within each specific soil amendment were expressed as "percent of check" and these data were graphed as three dimensional figures.

Because of the dessicating action of pyriclor on grain sorghum, green plant weights were used as a measure of toxicity. Oven dried plant weights were less suitable due to the small differences in dry weights of completely dead plants and of normal plants.

The following discussion of data from these experiments is presented chronologically by experiment number as presented in Chapter III, Methods and Materials.

Experiment I: Effect of soil amendments on pyriclor toxicity and degradation.

Soil Reaction: Effects of soil reaction manipulation on pyriclor phytotoxicity to grain sorghum plants are shown in Table I, Figure 1 and Figure 2. Effectiveness of pyriclor was reduced at both acid and alkaline pH levels. Growth of the first crop as percent compared to

TABLE I

EFFECT OF PYRICLOR LEVELS AT DIFFERENTIAL SOIL REACTIONS ON GROWTH OF GRAIN SORGHUM PLANTS

Soil Reaction	Pyriclor levels (ppm) 0	0.25	0.50	1.00	2.00
First crop					
Normal	100.0	106.5	83.0	58.4	50.2
Acid	100.0	109.0	105.6	93.1	68.5
Alkaline	100.0	98.0	89.8	83.9	58.6
Second crop					
Normal	100.0	86.2	85.9	73.9	65.8
Acid	100.0	109.1	104.5	89.1	67.8
Alkaline	100.0	97.2	87.9	76.7	67.5

Percent yields were calculated from sums of yields from three replicate cultures per treatment, 20 plants per plot.

	Treatment F values		C. V.
First crop	7.64	p=<.01	13.03%
Second crop	22.57	p=<.01	9.56%

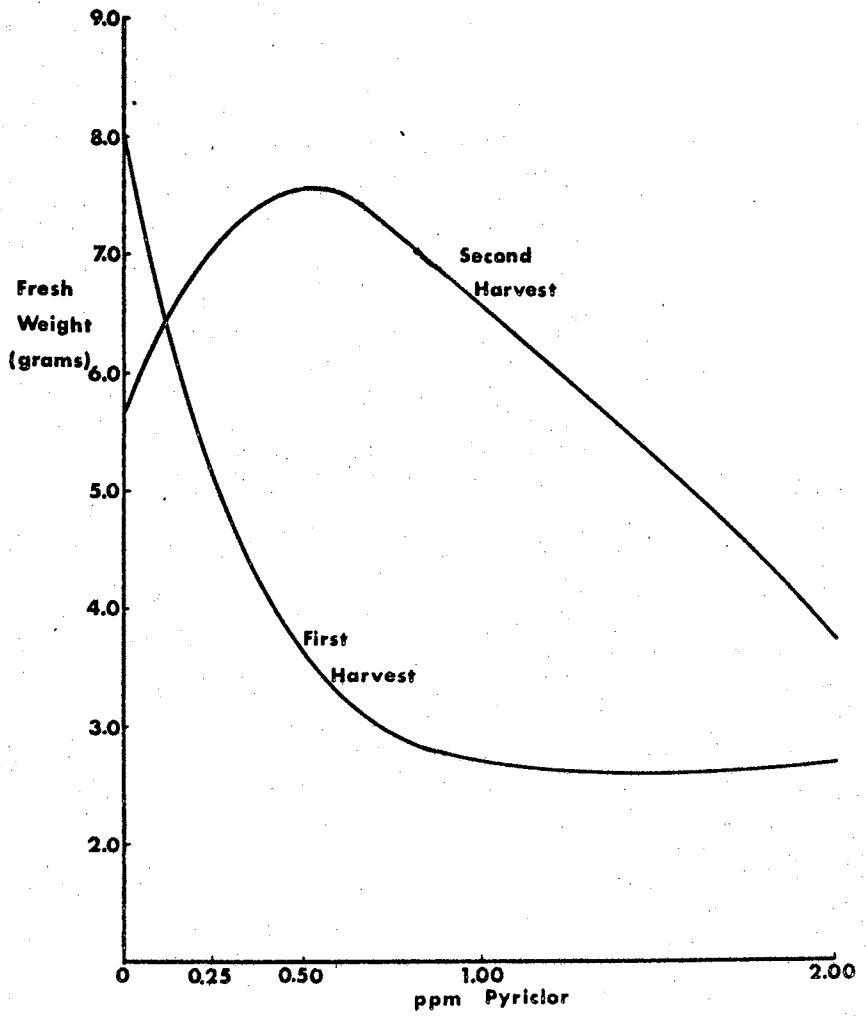

Figure 1. Effect of pyriclor levels on growth of grain sorghum plants as fresh weight of first and second harvests.

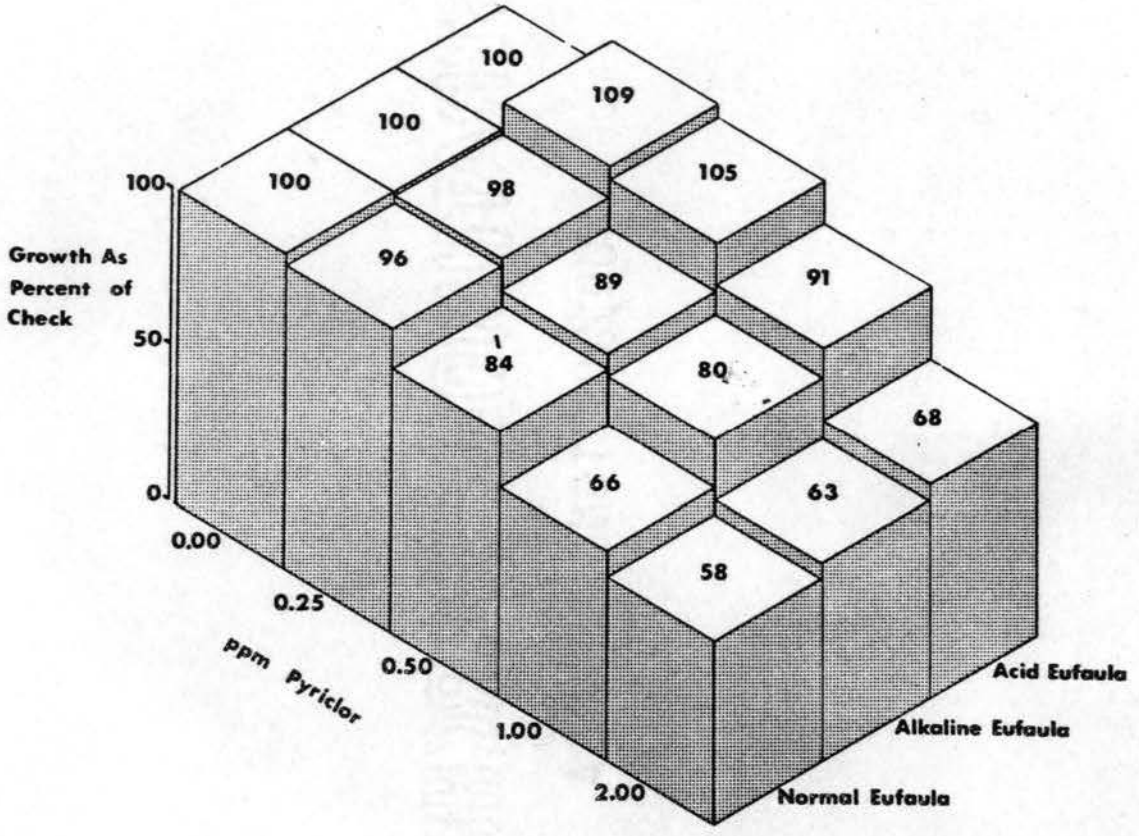

Figure 2. Effect of soil reaction on pyriclor phytotoxicity on growth of grain sorghums.

the check was 105.6, 93.1, and 68.5 with the acid soil, and 89.8, 83.9, and 58.6 with the alkaline as compared to 83.0, 58.4, and 50.2 with the normal soil at the pyriclor levels of 0.50, 1.00, and 2.00 ppm, respectively. Phytotoxicity was apparently decreased most effectively with the acid Eufaula (pH 4.7-4.9). Generally, fungi dominate the soil microbial population at strongly acid pH levels. Soil fungi isolates have demonstrated active decomposition of pyridine compounds in other studies (22). Similar results were apparent with the second crop, although there was less difference in growth apparent at the higher pyriclor levels. Increased activity of microfloral degraders in the normal soil along with decreased plant nutrient availability for the second crop may have influenced these results, Figure 1. Figure 2 presents these data as an average of both first and second crops in this study.

Organic Matter: The effects of wheat straw and alfalfa in various combinations as organic matter amendments on pyriclor toxicity to grain sorghum is illustrated in Table II and Figure 3. These combinations provided widely varying C:N ratios that should influence soil microbiological activity. All organic matter amendments resulted in decreased pyriclor phytotoxicity except 2% straw, 1% alfalfa-straw, and 2% alfalfa-straw combinations. With the higher herbicide level treatments, the largest growth depressions occurred with the 1% alfalfa-straw and the 2% alfalfa-straw amendments. However, at the 0.25 ppm pyriclor level, the wheat straw and alfalfa combinations resulted in higher growth than with 0 ppm pyriclor (check). Improved soil conditions for plant growth with rapid degradation of the pyriclor could be expected. Alfalfa alone seemed to decrease toxicity

TABLE II

EFFECT OF PYRICLOR LEVELS AT DIFFERENT ORGANIC MATTER AMENDMENTS ON THE GROWTH OF GRAIN SORGHUM PLANTS

Organic Matter Amendments	Pyriclor levels (ppm)				
	0	0.25	0.50	1.00	2.00
	Percent of check				
Normal	100	86.2	85.4	75.8	65.8
1% Straw	100	87.6	89.8	72.2	62.6
1% Alfalfa	100	88.4	87.3	84.5	67.3
1% Each	100	103.8	91.4	66.9	56.2
2% Straw	100	84.2	79.5	67.9	57.2
2% Alfalfa	100	90.4	90.2	83.9	81.5
2% Each	100	102.3	92.2	73.4	57.6
3% Each	100	103.8	95.4	80.5	69.1

Percent yields were calculated from the sums of yields from three replicate cultures per treatment.

	Treatment F values		C. V.
First harvest	13.08	p=<.01	9.90%
Second harvest	10.28	p=<.01	13.70%

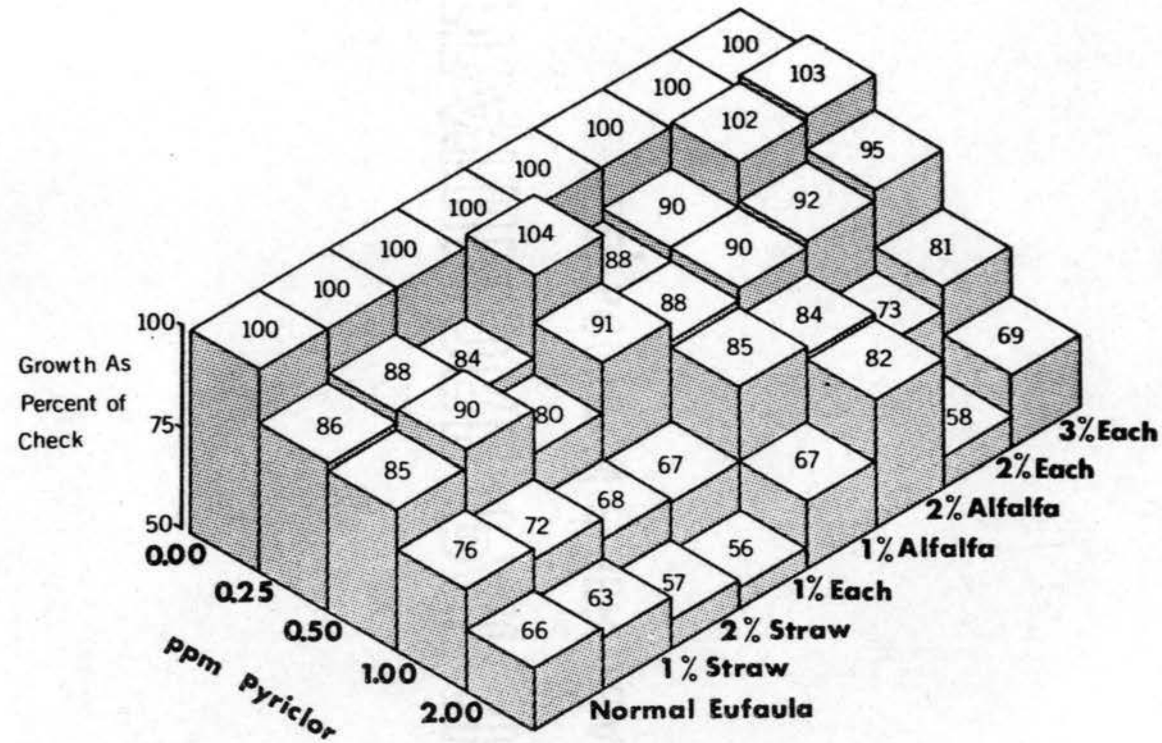

Figure 3. Effect of wheat straw and alfalfa organic matter additions on pyriclor phytotoxicity to grain sorghums.

effects more than straw alone, but this could also result from the higher nutrient content of alfalfa. Additions of straw alone appeared to result in higher pyriclor toxicity than alfalfa alone with growth depression at 2% straw greater than with the 1% straw addition. At the lower pyriclor treatment levels, less toxicity was apparent with the second harvest than within the first. However, as herbicide levels increased, higher toxicity was exhibited with the first crop, both in reduced growth and plant chlorosis.

Cation Exchange Capacity: The effects of variations of soil cation exchange capacity on the phytotoxicity of pyriclor to grain sorghum are presented in Table III and Figure 4. Data from this study indicate that a high cation exchange capacity very slightly decreased pyriclor toxicity. This was apparent in both the first and second crops with pyriclor phytotoxicity reduced by cation exchange capacities of 5 and 10 m.e./100g. at 0.50, 1.00, and 2.00 ppm pyriclor levels. This response may have resulted from an increase in deactivation of the herbicide by increased adsorption of the soil colloids. The 10.0 m.e. was generally more effective than the 5.0 m.e. C.E.C. Less contrast with the second crop may be the result of soil biological degradation with all treatments and reduced plant nutrient availability for the second crop. Average plant response from both crops is shown in Figure 4.

Experiment II. Effect of time on pyriclor degradation.

Time of incubation influenced pyriclor phytotoxicity as shown in Table IV and Figure 5. Generally, pyriclor was apparently affected by time of soil incubation, depending upon the initial herbicide level.

TABLE III

EFFECT OF PYRICLOR LEVELS AT DIFFERENTIAL CATION EXCHANGE CAPACITIES ON GROWTH OF GRAIN SORGHUM PLANTS

Cation Exchange Capacity	Pyriclor levels (ppm)				
	0	0.25	0.50	1.00	2.00
First crop	Percent of check				
2.7	100	86.2	85.9	73.9	65.8
5.0	100	101.3	99.0	85.8	66.4
10.0	100	96.8	102.3	81.6	77.5
Second crop					
2.7	100	106.5	83.0	58.4	50.2
5.0	100	84.6	86.3	58.3	55.8
10.0	100	98.5	84.3	73.8	53.2

Percent yields were calculated from sums of yields from three replicate cultures per treatment, 20 plants per pot, two plant harvests per crop.

	Treatment F values		C. V.
First crop	17.35	p=<.01	11.65%
Second crop	16.99	p=<.01	11.30%

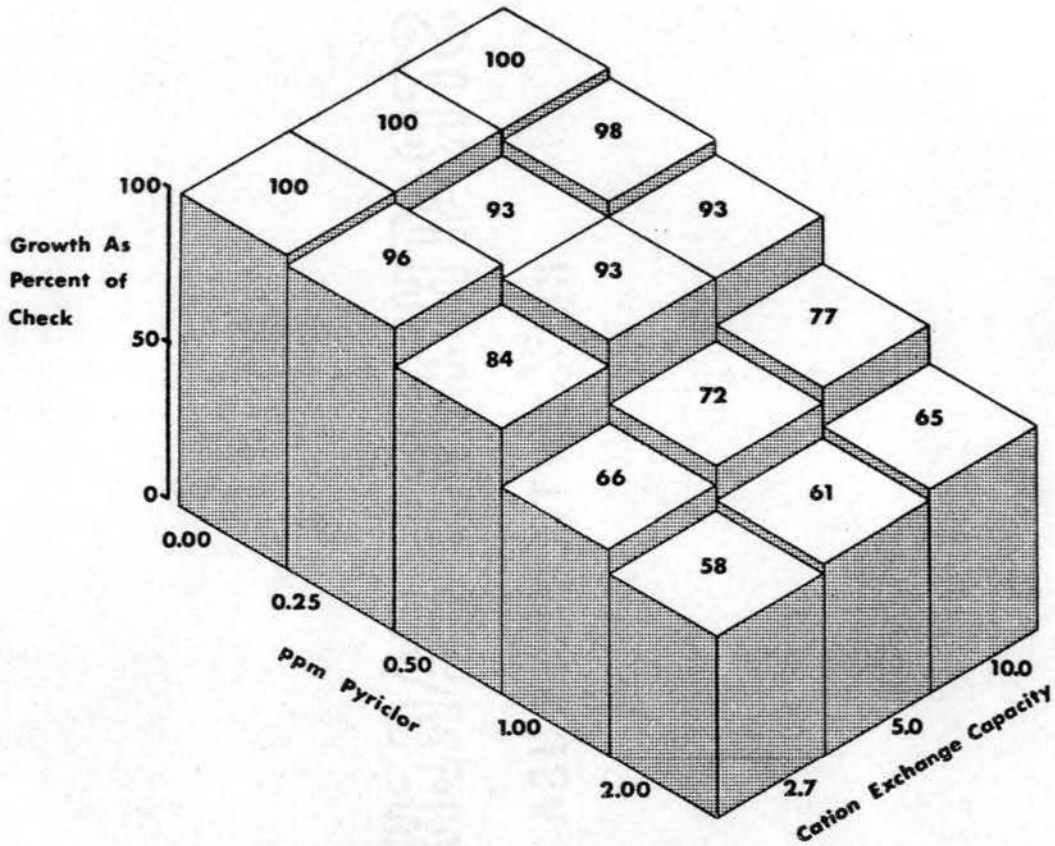

Figure 4. Effect of soil cation exchange capacity on pyriclor phytotoxicity to grain sorghums.

TABLE IV

EFFECT OF SOIL INCUBATION TIME ON PYRICLOR LEVEL PHYTOTOXICITY TO GRAIN SORGHUM

Soil incubation (weeks)	Initial pyriclor level (ppm)				
	0	0.25	0.50	1.00	2.00
	Percent of check				
0	100	90.2	65.1	39.4	15.3
2	100	71.8	60.7	35.0	17.6
4	100	68.0	57.0	44.4	23.0
6	100	72.8	55.8	48.7	28.2
8	100	73.4	58.7	56.0	36.7
10	100	81.0	63.8	64.2	42.1
12	100	101.1	92.4	87.3	50.2

Percent yields were calculated from sums of three replicates per treatment, 20 plants per pot.

	Treatment F values		C. V.
2 weeks	16.86	p=<.01	23.75%
4 weeks	3.69	n.s.	27.67%
6 weeks	32.33	p=<.01	12.00%
8 weeks	13.58	p=<.01	23.08%
10 weeks	46.04	p=<.01	7.85%
12 weeks	50.54	p=<.01	7.81%

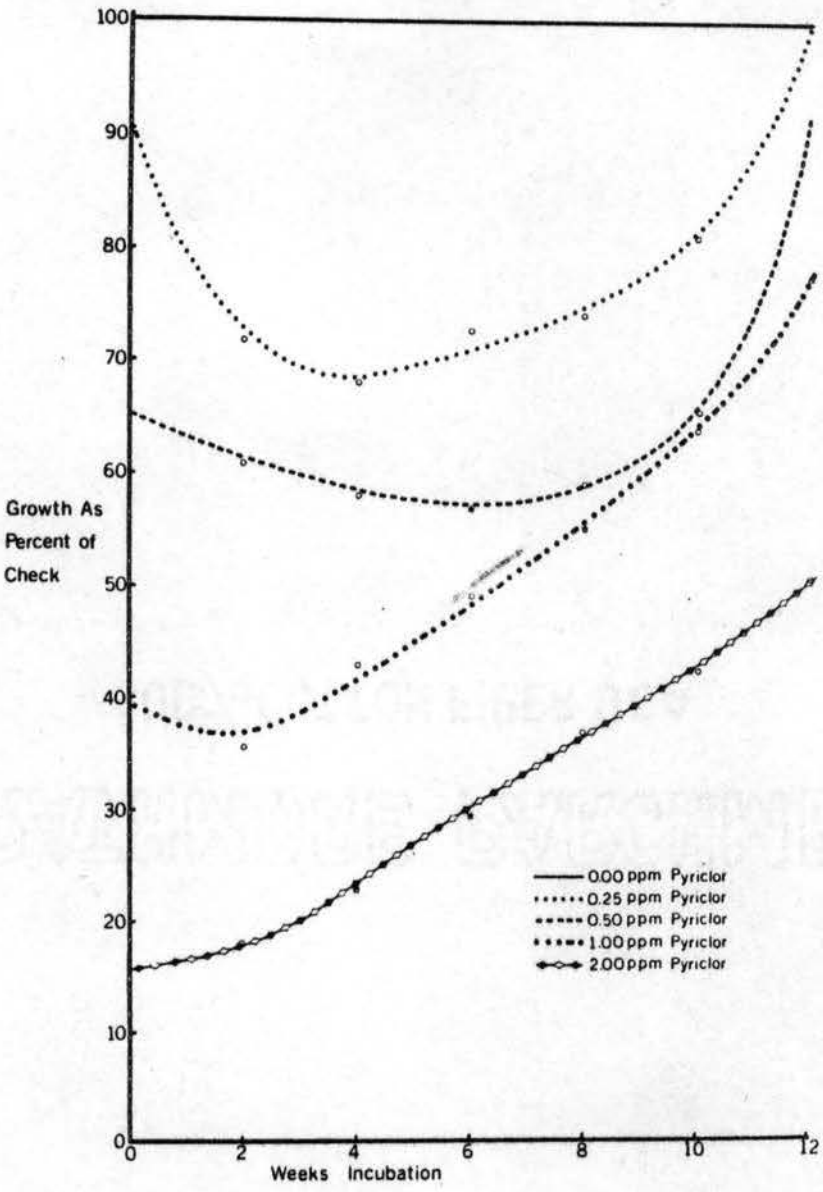

Figure 5. Effect of soil incubation time on pyriclor phytotoxicity to grain sorghums.

With 0.25 ppm pyriclor, growth depression was most pronounced after four weeks; at 0.50 ppm relative growth was lower after six weeks incubation; at 1.00 ppm, reduced growth was most apparent after 2 weeks incubation and at 2.00 ppm pyriclor, plant growth improved with each additional increase of soil incubation time. After approximately six weeks soil incubation there was a marked increase in plant weights of all treatments, including 0 ppm pyriclor (check), but with growth expressed as percent of check remaining generally the same as with less time of soil incubation. This could have been due to organic matter decomposition releasing nutrients for plant use. After 8 weeks incubation, weights of grain sorghum from the check soils were approximately the same as growth after 2 and 4 week incubation periods. However, less toxicity was apparent with the pyriclor treated soils. With the highest pyriclor level of 2.00 ppm, herbicide toxicity after 12 weeks incubation was roughly one half the apparent initial toxicity resulting with no soil incubation.

Nonlinear response curves with time of soil incubation may be explained by the numerous biological interactions that influence rates of herbicide degradation and plant nutrient availability. The numerous influences on plant growth from soil microbial activities and organic matter transformations are of a dynamic, ever changing system. Consistent linear trends are not usually encountered in soils.

Experiment III. Effect of pyriclor levels on growth of four fungi isolates.

Pyriclor levels up to 400 ppm inhibited fungi growth as illustrated in Table V. *A. tamarii* and *A. flavus* were not markedly

TABLE V

EFFECT OF PYRICLOR LEVELS ON GROWTH OF FOUR SELECTED SOIL FUNGI ISOLATES

Organism	Pyriclor levels (ppm) 0	50	100	200	400
	Percent of check				
Aspergillus flavus	100	92.3	84.4	94.7	30.4
Curvularia lunata	100	72.4	57.3	23.7	12.3
Aspergillus tamarii	100	81.4	78.3	84.3	20.5
Aspergillus niger	100	71.9	59.7	23.0	18.5

Yield figures are dry pad weight expressed as a percent of check with three replicate cultures per treatment.

	F value		C. V.
A. flavus	45.135	p=<.01	9.130%
C. lunata	15.270	p=<.01	29.810%
A. tamarii	11.931	p=<.01	20.930%
A. niger	35.918	p=<.01	18.100%

inhibited at 200 ppm pyriclor levels or below, but growth was suppressed with 400 ppm pyriclor. These two fungal isolates have also demonstrated high tolerance for pyridal biotoxins in previous studies. An inhibitory response to pyriclor at all treatment levels was shown by *C. lunata* and *A. niger* with a near linear decrease in growth as pyriclor levels were increased. Pyriclor was more effective at decreasing growth of *C. lunata* followed by *A. niger* and *A. tamarii* with *A. flavus* showing the least response to pyriclor levels. This *A. flavus* isolate apparently also has a remarkable adaptive capability to degrade other biotoxins in addition to being an aflatoxin synthesizer.

CHAPTER V

SUMMARY AND CONCLUSIONS

The objectives of this study were: (1) to determine soil factors that influence the activity and degradation of 2,3,5-trichloro-4-pyridinol (pyriclor), and (2) to determine the effects of pyriclor with several selected soil fungal species. Grain sorghum was used as the bioassay plant due to its easily observable rate of dechlorophyllation which provided an estimate of pyriclor activity in soil cultures. Soil factors investigated in this study included soil reaction, organic matter amendments, cation exchange capacity, and soil incubation time. In addition four selected soil fungi isolates, including *Aspergillus niger*, *Aspergillus flavus*, *Curvularia lunata*, and *Aspergillus tamarii* were studied for their response to pyriclor applications.

Soil factors found to decrease pyriclor activity were: acid and alkaline soil reactions, high organic matter addition as alfalfa meal, increasing cation exchange capacity, and incubation time to 12 weeks. Soil factors found to increase pyriclor phytotoxicity, or to have no effect were: addition of wheat straw alone as organic matter, and incubation periods of 0 to 4 weeks at levels of 0.25, 0.50, and 1.00 ppm pyriclor.

Pyriclor levels up to 400 ppm were toxic to all four fungi species. Pyriclor was more effective at decreasing growth of

C. lunata followed by *A. niger* and *A. tamarii* with *A. flavus* showing the least response to pyriclor levels.

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