EFFECTS OF HERBICIDES ON GROWTH AND SPORULATION OF <u>BIPOLARIS SOROKINIANA</u> AND ON SPOT BLOTCH DEVELOPMENT IN WHEAT SEEDLINGS

By

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CHAPTER I

INTRODUCTION

Herbicides are important in modern agriculture. High labor cost commensurate with expanded and intensified farming methods made herbicide usage essential to the production of abundant high quality food and fiber. In recent years, an increase in the amount of farmland converted to some form of reduced tillage has increased the need for chemical weed control (12). The increased use of herbicide has prompted a number of investigations of the effects of herbicides on non-target organisms; especially microorganisms that inhabit soil (4,24) and those that cause plant diseases (3,22).

The response of microorganisms to herbicides varies widely. For example, only 2 ppm of 2,4-D[1] inhibits growth of certain *Rhizobium* sp., while 2,000 ppm are required to inhibit other *Rhizobium* sp., and 40,000 ppm are required to inhibit certain fungi (26). In a Cecil sandy loam soil,

[1]See TABLE I for chemical formulae of herbicides.

TABLE I

NAMES OF HERBICIDES AND CHEMICAL FORMULAE FOR THOSE PRODUCTS MENTIONED IN THE TEXT

Common Name	Product Name	Chemical Formula
Cycloate	Ro-neet	S-ethyl N-ethyl thio-cyclohexane-carbamate.
(IPO) Propham	Chem Hoe	Isopropyl carbanilate.
MCPA	Chiptox	(4-chloro-o-tolyl) oxy) acitic acid.
MCPP	Mecoprop	2-(4-chloro-o-tolyl) oxylpropionic acid.
Pendimethalin	Stomp	N-(1-ethylpropyl)-3,4 (dimethyl-2,6 dinitro-benzeneamine.
PCB*		
	Stomp 330-D*	
	Stauffer 1607*	
2,4-DEP	Falone	Mixture of tris (2,4-dichloro-pheoxy-ethyl) phosphite and bis(2,4-dichloro-phenoxy-ethyl). phosphite.
Trifluralin	Treflan	$\alpha, \alpha,$ -trifluro-2,6-dini-tro-N,N-dipropyl-p-tolui-dine.
Monuron	Monuron	3-(p-chlorophenyl)-1,1-dimethylurea mono (tri-chloroacetate).

TABLE I (Continued)

Common Name	Product Name	Chemical Formula
2 mercaptoeth- anol.		
TCA	TCA	Trichloroacetic acid.
Bromacil	Hyvar	5-bromo-3 sec-butyl- 6-methyluracil.
Bromoxynil	Buctril	3,5-dibromo-4-hydroxy- benzonitrite.
Dicamba	Banvel	3,6-dichloro-o-anisic acid.
Dinitramine	Cobex	N^4,N^4-diethyl-α,α, -tri- fluro-3,5-dinitrotoluene 2,4-diamine.
EPTC	Eptam	S-ethyl dipropylthio- carbamate.
Diphenamid	Enide	N,N-dimethyl-2,2-diphenyl acetamide.
DNBP*		
CIPC (chloro- pham)	Chloro IPC	Isopropyl-m-chlorocarba- nilate.
Terbutryn	Igran	2-(tert-butylamino)-4- (ethylamino)-6-(methyl- thio)-s-triazine.
2,4-DB	Butoxone	4-(2,4-dichlorophenoxy)- butanoic acid.

TABLE I (Continued)

Common Name	Product Name	Chemical Formula
CDAA	Randox	N,N-diallyl-2-chloro-acetamide.
CDEC	Vegadex	2-chloroallyl diethyldi-thio carbamate.
NC 8438	Nortron	2-ethoxy-2,3 dihydro-3,3 dimethyl-5-benzofuranyl methane-sulphonate.
Glyphosate	Round up	N-(Phosphonomethyl) gly-cine.
Oryzalin	Surflan	3,5-dinitro N^4,N^4-dipro-pylsulfanilamide.
Paraquat	Gramoxone	1,1-dimethyl-4,4-bipyridi-nium ion (as dichloride salt).
2,4-D	2,4-D	(2,4-dichlorophenoxy) acetic acid.
2,4,5-T	Weedar	(2,4,5-trichlorophenoxy) acetic acid.
Silvex	2,4,5-TP	(2,4,5-trichlorophenoxy) propionic acid.
Simazine	Simazine	2-chloro-4,6-bis (ethyl-amino)-s-triazine.
Atrazine	Atrex	2-chloro-4-ethylamino-6-isopropylamino-s-triazine.
Cyanazine	Bladex	2-(4-chloro-6-(ethyl-amino)-s-triazin-2yl) amino-2-methylpropionitrite.

TABLE I (Continued)

Common Name	Product Name	Chemical Formula
Diallate	Avadex	S-(2,3-dichloroallyl) diisopropylthio carbamate.
Benefin	Balan	N-butyl-N-ethyl-$\alpha,\alpha,$ -trifluro-2,6-dinitro-p-toluidine.
Bensulide	Prefar	o,o-diisopropyl phosphorodithioate s- ester with N-(2-mercaptoethyl) benzenesulfonamide.

*Experimental chemical.

bacteria were reported to be the least sensitive to high 2,4-D concentrations (5 mg and 20 mg/g of soil) over an 11 week period actinomycetes were the most sensitive (none were isolated after three weeks), and fungi were intermediate (27). As a further example, atrazine which reportedly possesses slightly fungicidal properties (5) was conversely reported to be stimulatory to Sclerotium rolfsii Sacc. (30).

The fungus, Bipolaris sorokiniana (Sacc.) Shoemaker (syn. = Drechslera sorokiniana (Sacc.) Subram. and Jain, and Helminthosporium sativum Pammel, King and Bakke; perfect state = Cochliobolus sativus (Ito and Kurbayashi) Drechsler) is an important leaf and root pathogen of cereals (9). It has been isolated from 79 species of Gramineae in the norhtern Great Plains of North America (33). The effects of low concentrations of several pre- and post-emergence herbicides directly on B. sorokiniana and on disease symptoms induced on Poa pratenses L. have been reported by Hodges (15,16,17).

This thesis reports the effects of high concentrations of selected herbicides, especially 2,4-D and the s-triazine derivatives, atrazine and terbutryn on growth, condial production, and induced disease symptoms by B.

sorokiniana. *B. sorokiniana* will be used as the genus name and specific epithet of the fungus regardless of designations used by other writers whose works are cited.

CHAPTER II

LITERATURE REVIEW

The reported effects of herbicides on life processes of plant pathogens and the diseases they cause have been reviewed extensively by Altman and Campbell (3) and by Katan and Eshel (22). The following review includes publications regarding some herbicides that are the same, or chemically related, to herbicides used in this study, and to some unrelated ones that have been reported to strongly affect plant pathogens or the diseases they cause.

Cole and Batson (10) reported that diphenamid, used as an herbicide, decreased pre-emergence damping off but increased post-emergence damping off of tomato caused by *Pythium aphanidermatum* (Edson) Fitzp. and *Rhizoctonia solani* Kuehn. However, the difference between pre- and post-emergence damping off resulted in an increase in stand count. Growth of *P. aphanidermatum* and *R. solani* was reduced on an artificial medium containing the herbicide.

Huber *et al.* (19) showed that diuron applied at the rate of 1.12 kg/ha reduced the severity of winter wheat foot rot caused by Cercosporella herpotrichoides Fron. Initial penetration by the fungus was not affected, but host resistance appeared to be increased by the herbicide.

Jones and Williams (20) reported that paraquat suppressed mycelial growth, spore production and spore germination of Septoria nodorum Muller and S. tritici Desm.

Gunasekaran and Ahuja (13) studied the effects of herbicides (atrazine, bromacil, bromoxynil, CIPC, monuron, and paraquat) representing various groups of compounds on the mycelial growth of Phymatotrichum omnivorum (Shear) Dugg. At high concentrations all of the herbicides inhibited growth except atrazine and paraquat. In bromoxynil medium, growth occurred in the form of strands and the colonies were irregular, whereas, in the other cases the colonies were compact and circular.

Grau (11) studied the effect of dinitramine and trifluralin on growth, reproduction and infectivity of Aphanomyces euteiches Drechs. When zoospore inoculum was amended with commercial grades of the herbicides, the number of propagules were reduced and fewer zoospores germinated.

Dinitramine was more effective than trifluralin in reducing root rot of pea caused by A. euteiches because the latter was adsorbed more readily to the soil particles than the former. Consequently, the concentration of trifluralin in the soil solution was lowered.

Hodges (17) evaluated different concentrations of the pre-emergence herbicides; benefin, bensulide, DCPA, and siduron for their effect on conidial production, conidial germination, germ tube growth, and primary branching of germ tubes by *B. sorokiniana*. All herbicides at all concentrations inhibited germination, and either inhibited germ tube growth or had no growth promoting effect.

The effects of temperature and herbicides used for turf maintenance (NC 8938, benefin, bensulide, and 2-mercaptoethyl) on radial growth of *Drechslera cynodontis* Nelson, *P. aphanidermatum*, *R. solani*, and *Sclerotinia homoeocarpa* Bennett were investigated by Karr *et al*. (21). Growth was inhibited by most herbicide concentrations at all temperatures. At 35 C, growth of *R. solani* was stimulated by very low concentrations of bensulide.

Katan and Eshel (22) suggested four possible mechanisms through which a pesticide may increase or decrease a disease; a) effect on pathogen virulence, b) effect on pathogen growth and reproduction, c) effect on host susceptibility, and d) effect on relationships with other microorganisms. In one experiment by Katan and Eshel (22), damping off of pepper by R. solani along with diphenamid contributed to the disease increase. The mechanisms involved in the increase were analyzed by studying the effect of diphenamid on the pathogen, the host, and the soil microorganisms. Natural soil was amended with 0.2% glucose and the utilization of glucose and the respiration by the soil microorganisms in the initial period, but later increased respiration. Glucose utilization was also suppressed.

Herbicides that could be used as pre- or post-emergence weed control agents in forage on pasture crops were tested by Bain (6) for their effect on growth of Rhizoctonia sp., Sclerotium rolfsii, and S. bataticola Taub. in culture, Herbicides tested were; 4-(2,4-DB), DNPB, DNPB + Falone, IPC, diuron, PCB, CIPC, CDEC, CDAA, Stauffer 1607 and cyclohexanon. One method used to assess the reaction of the fungi to the chemicals was accomplished by placing herbicide treated filter paper discs on solidified agar in petri dishes. Immediately thereafter, discs 6 mm in diameter and 1 to 2 mm thick cut from actively growing fungi on PDA (Potato Dextrose Agar) medium were inverted on the paper discs. Inhibition of growth indicated fungistatic activity of the herbicides.

Rodriguez-Kabana *et al*. (32) studied the effect of the herbicide EPTC on the growth of S. rolfsii in liquid and soil cultures. In modified Czapek's solution, mycelial production was inhibited by all concentrations tested. A corresponding decline in utilization of glucose, NO_3-N, and inorganic phosphate occurred. An increase in titrable activity of the culture medium at the two highest herbicide concentrations, and an increase in the ratio regulating glucose consumed in inorganic phosphate uptake, indicated a possible action of the herbicides in the respiratory cycle of the fungus.

The interaction between *Pythium* sp. and Stomp 330 D (pendimethalin) was studied by Abdulla and Manci (1). Pendimethalin reduced the colony diameter of *Pythium* sp. In submerged cultures, the mean dry weight consistently decreased with increases in herbicide concentration. The rate of sporangial development at concentrations ranging between 100 and 700 ppm was not changed significantly; whereas, higher doses (800 to 1,000 ppm) significantly suppressed sporangial formation. On the other hand, the herbicide stimulated oogonial production at 100 ppm. An increase of the herbicide from 300 to 400 ppm caused a slight reduction in numbers of oogonia. Further increase in concentration (between 700 and 1,000 ppm) greatly inhibited oogonial production.

According to Wilkinson and Lucas (36) three methods can be used to investigate the effects of certain herbicides on the growth of fungi. These are: measurement of hyphal extensions across agar plates; measurement of hyphal extension across sterilized plant material; and manometric techniques.

Four points emerged from these studies. First, there was no stimulation of fungal growth by selected herbicides. Second, herbicides interfered with fungal growth by suppressing spore germination, inhibiting the rate of linear hyphal extension, and by inducing abnormalities in growth habit and patterns of spore production. Third, some herbicides, i.e., linuron and paraquat, were more fungitoxic than others, such as MCPA and simazine, to a range of organisms. Fourth, fungi differed in their sensitivity to different herbicides. Together these factors indicated that interactions in soil are complex and that metabolic pathways of microorganisms often differ.

Harris and Grossbard (14) found that the growth rates of four isolates of _S_. _nodorum_ were inhibited on a medium amended with a range of concentrations of Gramoxone W (paraquat) and glyphosate. All isolates were more sensitive to glyphosate than to paraquat, and differences between isolates were smaller than those between herbicides. Conidiogenesis was markedly reduced on Czapek-dox V-8 agar and PDA containing glyphosate at 80 and 160 ppm. Paraquat had no significant effect on the number of conidia produced at all concentrations in either medium. The effect of various concentrations of herbicides on germination was studied by growing the fungus on amended media. Conidia were washed and transferred onto water agar contained in petri dishes. Percentage of conidia which germinated after 12 hours of incubation was reduced by glyphosate but was unaffected by paraquat. Their experiment demonstrated the anti-fungal activity of both herbicides, especially glyphosate. Transfer of residual herbicides may occur through conidia grown on herbicide amended agar since germiantion was subsequently impaired on water agar not containing herbicide.

Hodges (16) evaluated four chloro-phenoxy and one benzoic acid (dicmba) post-emergent herbicides for their influence on development of leaf spot caused by *B.sorokiniana* on *Poa pratensis*. Leaf spot development was severly inhibited at a concentration of 10^{-3} M of 2,4-D, 2,4,5-T and MCPP, but was unaffected at a concentration of 10^{-12} M and 10^{-9} M of 2,4-D and 2,4,5-T. By contrast, MCPP and dicamba at the same concentrations increased leaf spot severity.

Further work by Hodges (15) showed that the synthetic auxinlike post-emergent herbicides, 2,4-D, 2,4,5-T, 2,4,5-TP, dicamba, and MCPP, at 10^{-3} M concentration prohibited conidial production of _B_. _sorokiniana_. Conidial germination was not affected by any herbicide at concentrations of 10^{-12} to 10^{-4} M. Germtube growth and primary branching of germ tubes were stimulated by most herbicides at concentrations of 10^{-12} to 10^{-4} M. Conidial production was inhibited by 2,4,5-T and MCPP at all concentrations. Dicamba and 2,4,5-T caused significant increase in conidial production at low concentrations and a significant decrease at high concentrations. Only 2,4-D increased conidial production at higher concentration. Germ tube growth was stimulated by the herbicides at low concentrations.

Cycloate has been used as a pre-plant treatment for sugar beet fields in Colorado and in other intermountain states in which sugar beets are grown. Altman (2) found that linear growth of _R_. _solani_, after 72 hours at 20 C, was significantly less in different nutrient concentrations of PDA amended with cycloate from 10 to 100 ug/ml, than in controls without cycloate. Colonization of sterilized sugar beet seeds by *R. solani* also was less with 8, 16, and 32 ug/g cycloate than in controls.

CHAPTER III

MATERIALS AND METHODS

Source and Maintenance of Fungal Cultures

A culture of _B_. _sorokiniana_ was obtained from the USDA Wheat Research Laboratory at Oklahoma State University. The _B_. _sorokiniana_ culture previously had been demonstrated to cause spot blotch and damping-off of wheat seedlings.

Source and Concentration of Herbicides

Six herbicides commonly used to control weeds in wheat; glyphosate (Round up, Monsanto Agri. Prods. Co., St. Louis, MO 63166), oryzalin (Surflan, Elanco Prods. Co., Indianapolis, IN 46206), 2,4-D (2,4-D, Diamond Shamrock Corp., Cleveland, OH 44114), cyanazine (Bladex, Shell Chem. Co., Houston TX 77001), Atrazine (Aatrex, CIBA Geigy Corp., Greensboro, NC 27409) and terbutryn (Igran, CIBA GEIGY Corp., Greensboro, NC 27409) were obtained from Dr. T. F. Peeper, Department of Agronomy, Oklahoma State University. Depending upon the experiment, the herbicides were used in concentrations equivalent to the recommended per hectare rate of active material applied in 49 gallons (187.0 L) of water (Table II) on dilution thereof. When used in agar media, they were added prior to autoclaving. In all tests, the herbicides were used singly, i.e. they were neither mixed nor combined in any combination.

Herbicide Effect on Growth of *B. sorokiniana* in Agar Media

Bipolaris sorokiniana was grown in Czapek-dox agar medium with and without added herbicides (concentrations of the herbicides were equivalent to field application rate, and depending upon the herbicide, ranged from 4,790 to 11,980 ppm. Growth was measured in separate experiments by determining the dry weight of mycelium and by radial extension of hyphae from the inoculum.

Measurement of Growth by Dry Weight of Mycelium

For each of the six herbicides, 12 glass petri dishes containing Czapek-dox agar medium amended with the herbicide were prepared. An additional 12 dishes containing Czapek-dox agar but without herbicides were used as controls.

TABLE II

HERBICIDES AND BASIS OF RATES USED TO CHALLENGE GROWTH, RESPIRATION, AND AND ASEXUAL REPRODUCTION OF *BIPOLARIS SOROKINIANA*

Common Name	Trade Name	Formulation rate/ha (kg)	Active Ingredient[a] kg/ha	g/L	ppm
Glyphosate	Round up (41%)	2.19	0.89	4.79	4,790
Oryzalin	Surflan W (75%)	1.49	1.11	5.99	5,990
Cyanazine	Bladex W (80%)	2.80	2.22	11.98	11,980
Atrazine	Atrex W (90%)	2.49	2.22	11.98	11,980
2,4-D	2,4-D (67.9%)	1.65	1.11	5.99	5,990
Terbutryn	Igran W (80%)	2.80	2.22	11.98	11,980

a/ Concentration (ppm) of herbicides based on kilograms of active ingredient per hectare used in 187 L of water.

Each set of amended and non-amended media were divided into three sets of 4 and inoculated at the center with B. sorokiniana. Inoculum consisted of 4 mm diam plugs of agar and mycelium cut from actively growing regions of stock cultures. Cultures were incubated at 25 $\pm$ 0.1 C in the dark.

Four days after inoculation, mycelium was harvested from four replicate dishes of each herbicide amended medium, and from corresponding replicate dishes of control medium. Two additional harvests were made similarly 7 and 10 days after inoculation, respectively. At harvest, cultures were autoclaved at 120 C and 1.05 kg/cm^2 for 15 minutes to melt the agar. Mycelial mats were lifted from the melted medium, and placed on previously weighed, oven dried (70 C) filter papers (9 cm diam). Filter paper and mycelial mats were oven dried for 20 hours and weighed.

Effects of Herbicides on Respiration of B. sorokiniana

Soil was obtained from the upper 10 cm of the McGruder plots on the Oklahoma State University Agronomy farm. (The McGruder plots have been sown with winter wheats for about 100 successive years.) Ten samples were taken from within a 1 m^2 area. Tests conducted by the soil and water service laboratory, Oklahoma State University, indicated that the samples had a pH of 6.2 and contained nitrate nitrogen, phosphorous, potassium, calcium, and magnesium in amounts equivalent to 4, 25, 296, 2, 524, and 634 lbs per acre, respectively.

The soil was dried and sifted through a 60 mesh screen. Two grams of sifted soil was placed in each of six 250 ml flasks and pasteurized with no pressure in an autoclave at 80 C for 1 hour. Then 1.00 g of oryzalin, 1.66 g of atrazine, 3.04 ml of glyphosate, and 1.10 ml of 2,4-D were measured which was equivalent to field application rate for 125 ml water. From each 125 ml solution, 20 ml were transfered to each of the flasks containing 2 g of soil. Flasks containing the soil solution and herbicide were again pasteurized for 1 hr at 80 C.

Inoculum of B. sorokiniana was maintained in Czapek-dox broth medium on a reciprocatory platform shaker (New Brunswick Scientific Co. Model R-2). Two week old cultures, consisting of the mycelial mats along with conidia spores were ground in a Waring Blendor. Conidial concentration was counted by hemacytometer (Bright line, American Optical Co. Buffalo, N.Y.). Concentration was 2.5 X 10^6 conidia and mycelial fragments of whole cells per ml. One-half ml of inoculum was added to each flask of soil containing herbicides and to a control flask containing soil but no herbicide. A second control flask contained only soil suspension. All flasks were shaken as uniformly as possible to assure that the inoculum was in contact with the herbicides and the soil particles. After 4 days, 4 ml samples were removed from each flask and the respiratory activity of each was measured with a Gilson respirometer. This device is a combination of manometer and micrometer with a digital scale that can be manually operated to indicate directly any change of gas volume in microlitres. Potassium hydroxide (2 N) was used as a CO_2 trapping agent in the system. Readings were taken every 15 minutes until a total of eight readings were obtained for each culture. At this time most of the manometers indicated there was no further significant change in the volume of CO_2 being absorbed.

Growth and Sporulation of _B_. _sorokiniana_ on Water Agar Medium Amended With Atrazine, Terbutryn, and 2,4-D 0.01 and 0.001 of Applied Field Concentrations

Atrazine, terbutryn, and 2,4-D were added to water agar before autocalving at rates equivalent to 1 X 10^{-2} and 1 X 10^{-3} of the concentrations applied in the field. Specifically, these concentrations in the medium were 120 and 12 ppm for atrazine, 120 and 12 ppm for terbutryn, and 60 and 6 ppm of 2,4-D. The media were poured into petri dishes and inoculated with B. sorokiniana on an agar plug removed from the edge of a growing stock culture growing on PDA. Each treatment (herbicide/concentration) consisted of 20 petri dish cultures. The cultures were grown for 6 days at 23 C under continuous light of 21 microeinsteins/m^2 per sec.

Growth of each culture was determined as the mean of two measurements of the colony diameter taken at right angles to each other.

Conidial production by B. sorokiniana on the herbicide amended water agar was estimated from four plugs 1.2 cm in diam (4.52 cm^2 of agar surface) taken from individual plates of 31 day old cultures. Each four plug sample was placed in 2 ml of distilled water in vials. The vials were pressed against a vortex mixer to dislodge the conidia from the conidiophores. A drop (approximately 20 micro-liter) of each conidial suspension was deposited on a hema-cytometer slide and number of conidia per unit area of agar medium was calculated. Eight separate counts were made of conidia produced on each medium.

Effect of Atrazine, Terbutryn, and 2,4-D on Root, Shoot, And Disease Development in Seedlings of TAM W-101 Wheat Cultivar Uninoculated And Inoculated With *B. sorokiniana*

Prior to determining the effects of atrazine, terbutryn, and 2,4-D on root shoot development of wheat seedlings and their reaction to inoculation with *B. sorokiniana* a preliminary test was performed to derive the most optimum concentration for each herbicide in agar that would provide measurable responses. From this test, concentrations of 24 ppm of atrazine, 12 ppm of terbutryn and 0.6ppm of 2,4-D were determined to be the best suited for the study. These concentrations represented, in the above order, 2×10^{-3}, 1×10^{-3}, 1×10^{-4} of the recommended concentrations for field applications in 187 L (49 gal) of water per hectare (20 gal/A).

One hundred ml of water agar media containing separately each herbicide in the concentration mentioned above, and a water agar control medium without the herbicide, was poured into 64 small aluminum pans measuring 5.0 X 8.5 X 3.5 cm in width, length, and height using Medjo's technique (25).

Seed of the wheat cultivar TAM W-101 were surface sterilized by soaking in 95% ethyl alcohol for 2 min, washed four times in deionized water, and placed on wet filter paper in petri dishes for 48 hr to germinate. Using clean forceps, eight of the germinated seed were placed on the surface of the medium in each pan in such a manner that the radicle of each fit into a small hole in the medium formed with the points of the forceps.

The pans were placed in an 8 X 8 latin-square design on water soaked burlap in a metal box and covered with transparent polyethylene film. The box was maintained in a closed room at approximately 20 C in an alternating 12 hr dark and light regime of 75 microeinsteins/m^2/sec.

On the 10th, 11th, and 12th day after placing the germinating seed on the herbicide amended and control media, the developing seedlings in one pan of each treatment per replication were inoculated with _B. sorokiniana_ conidia suspended in 100 ml of distilled water (about 1,000 to 2,000 conidia per ml) to which a drop of Tween 20 had been added.

The suspension was sprayed on the seedlings to run-off with an 'airless' paint sprayer. The seedlings were kept in the chamber and moistened daily by spraying with tap water.

Seven days after the first inoculation, the seedlings were evaluated for response to the herbicides and for reactions to _B_. _sorokiniana_. The length of the longest root, the length of the sheath of the cotyledonary leaf (from the point of origin at the seed to the auricle), & the length of the lamina of the cotyledonary leaf were used as parameters of herbicides induced effects on growth. Number of lesions and percent necrosis in the cotyledonary leaves were used to evaluate response to _B_. _sorokiniana_. Lesions of approximately 2 mm or more in diam were counted and percent necrosis was estimated visually.

CHAPTER IV

RESULTS

Effects of Herbicides on Growth and Conidial production of *B. sorokiniana*

Measurement of Growth by Dry Weight of Mycelium

Measurement of growth by dry weight of mycelium showed that atrazine, 2,4-D, terbutryn, glyphosate, oryzalin, and cyanazine at field rate concentrations reduced the growth of *B. sorokiniana* significantly (Figures 1,2,3,4) in order of increasing inhibitory effect the herbicides ranked as follows: 2,4-D, terbutryn, atrazine, cyanazine, oryzaline, glyphosate.

Effect of Herbicides on Respiration of *B. sorokiniana*

The volume of displaced liquid in the manometer of a Gilson respirometer was taken as a measure of oxygen uptake by *B. sorokiniana* growing in sterile soil. The

Figure 1. Growth Rates of *Bipolaris sorokiniana* in Czapek-dox Agar Medium Alone and Amended With 2,4-D and Terbutryn at Applied Field Rate Concentration.

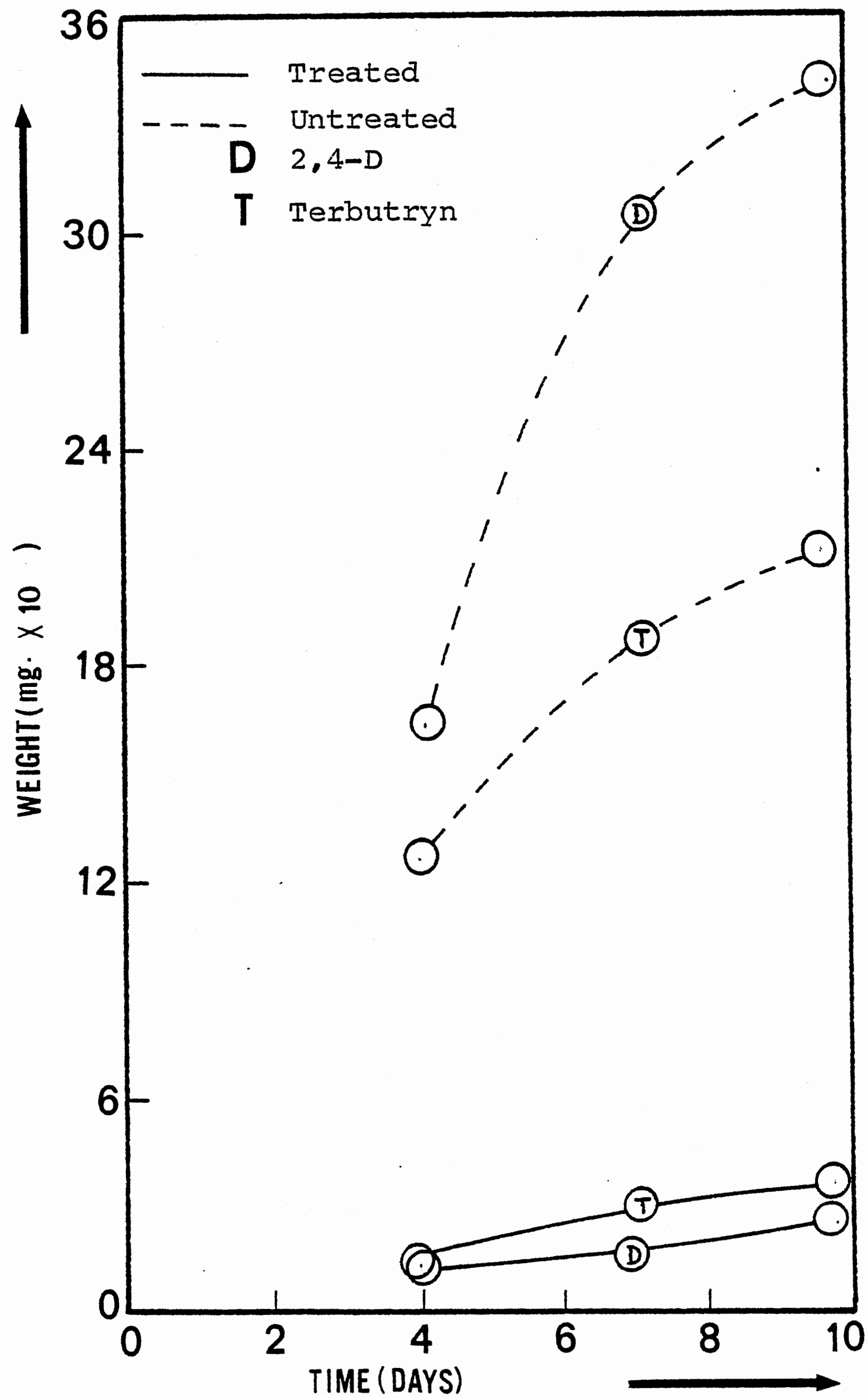
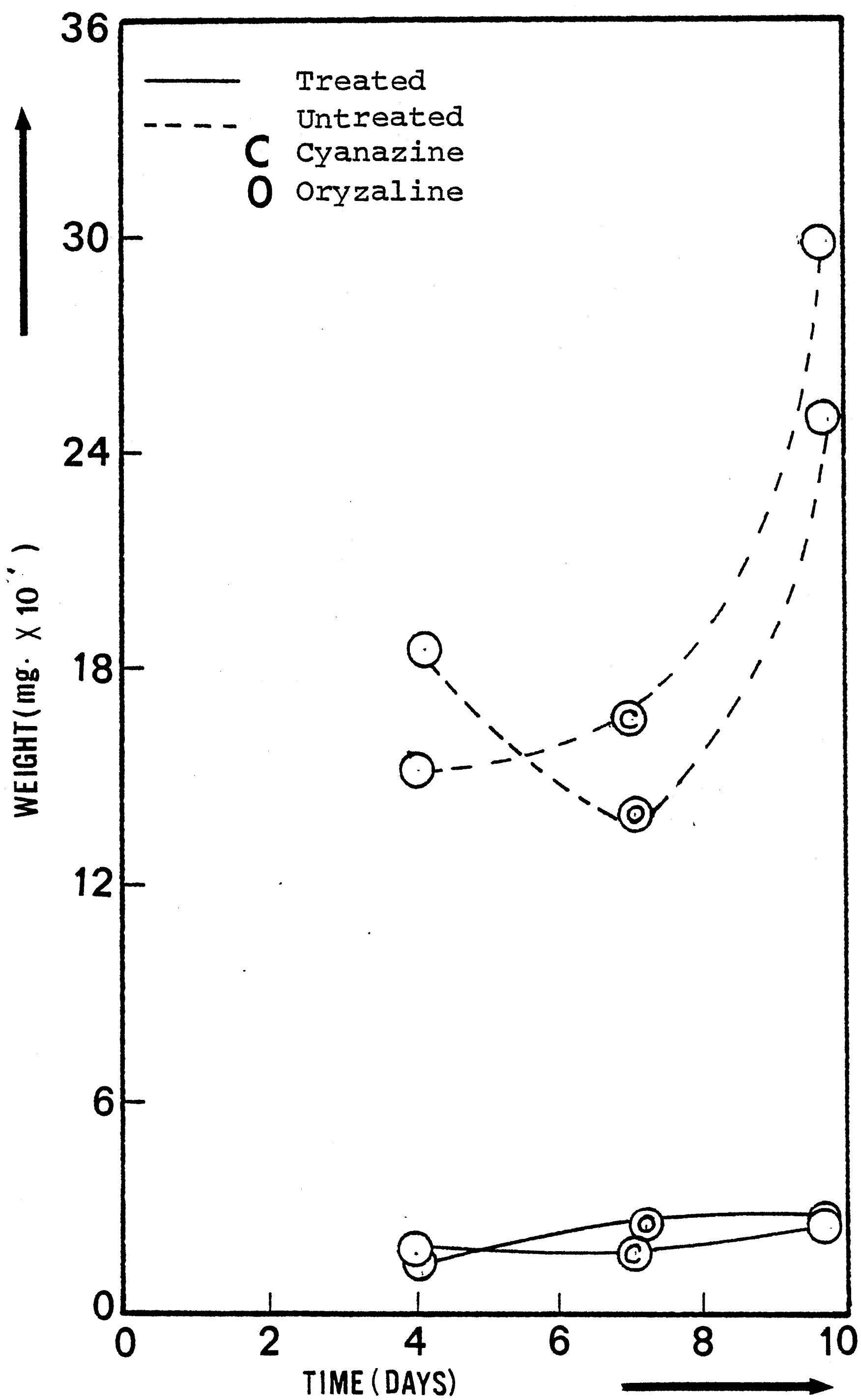
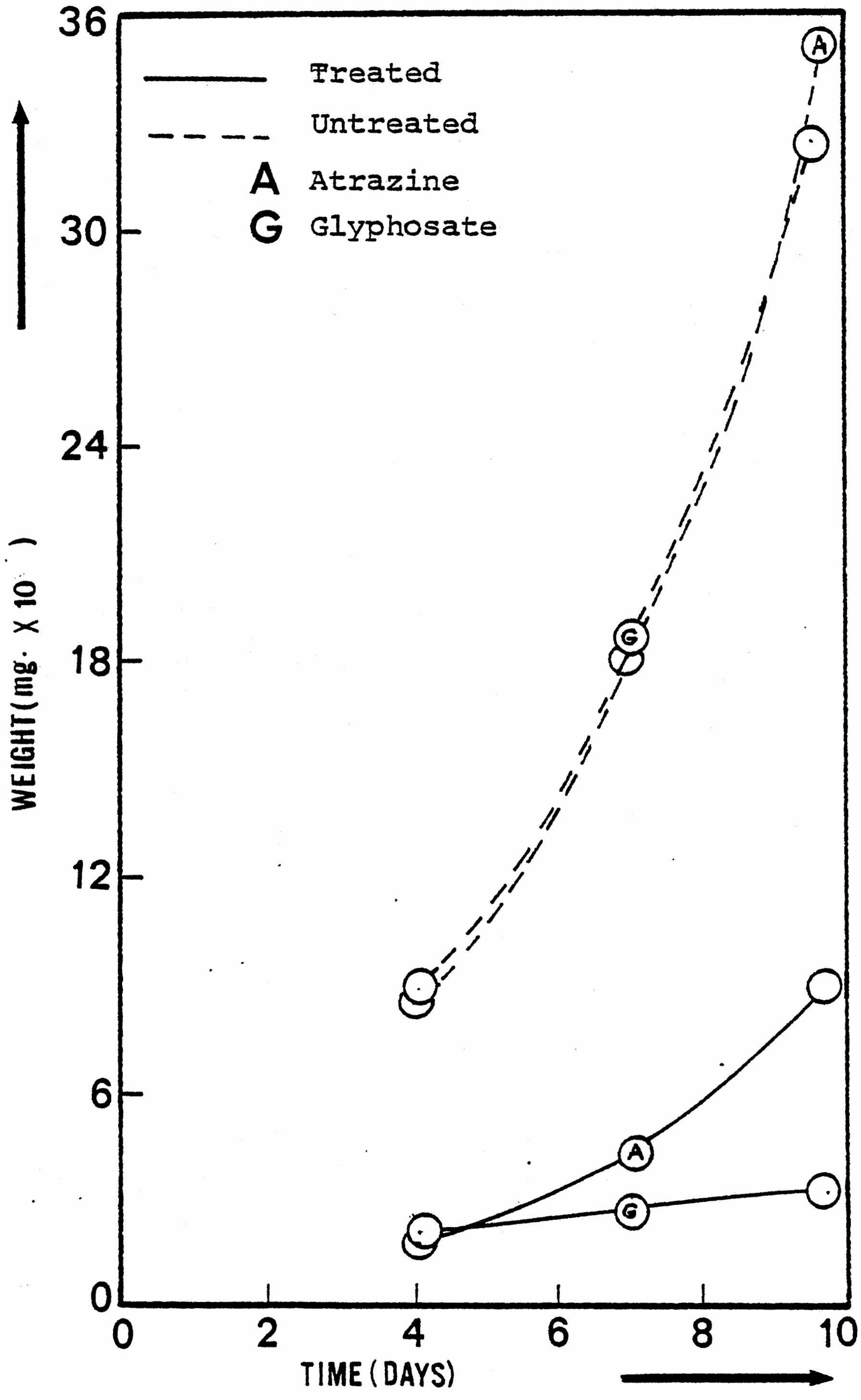

Treated
Untreated
D 2,4-D
T Terbutryn
36
30
24
18
12
6
0
WEIGHT (mg · X 10)
0
2
4
6
8
10
TIME (DAYS)

Figure 2. Growth Rates of _Bipolaris_ _sorokiniana_ in Czapek-dox Agar Medium Alone and Amended With Oryzalin and Cyanazine at Applied Field Rate Concentration.



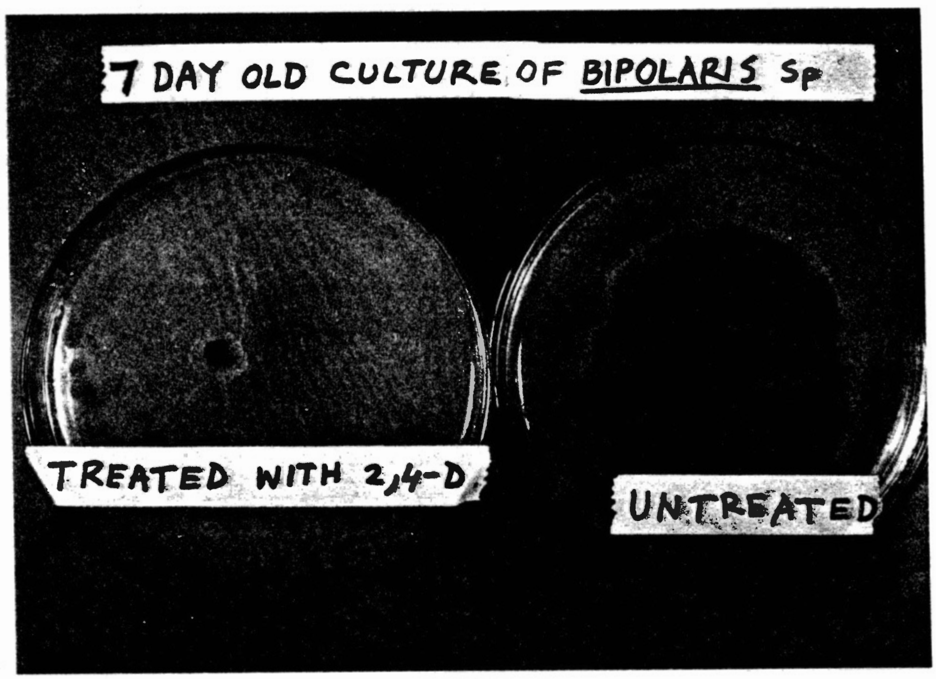
Treated
Untreated
C Cyanazine
O Oryzaline
WEIGHT (mg. X 10⁻¹)
36
30
24
18
12
6
0
0
2
4
6
8
10
TIME (DAYS)

Figure 3. Growth Rates of <u>Bipolaris sorokiniana</u> in Czapek-dox Agar Medium Alone and Amended With Glyphosate and Atrazine at Applied Field Rate Concentration.



36
30
24
18
12
6
0
WEIGHT (mg. X 10)
Treated
Untreated
A Atrazine
G Glyphosate
0
2
4
6
8
10
TIME (DAYS)

Figure 4. Seven Day Old _Bipolaris_ _sorokiniana_ Growing on 2,4-D Treated and Untreated Czapek's-dox Agar Medium.


7 DAY OLD CULTURE OF BIPOLARIS Sp
TREATED WITH 2,4-D
UNTREATED

mean of the last (8th) reading of four replications showed that 2,4-D displaced 16.2 ul, glyphosate 14.1 ul, oryzalin 21.4 ul and terbutryn 29.6 ul, while that of the control was 49.1 ul. These results indicated that glyphosate had the greatest effect on respiration followed by 2,4-D, oryzalin and terbutryn (Table III and Figure 5). The amount of respiration in the control (untreated culture) increased commensurate with time from the initial 15 min to 120 min when the experiment terminated. A similar pattern occured in flasks containing terbutryn except the rate of CO_2 evolution was lower than in the control flasks. For oryzalin and glyphosate, the rate of CO_2 evolution began decreasing about 75 to 90 min after the experiment began, and appeared to have reached a near maximum after 120 min. The evolution of CO_2 in 2,4-D treated increased between 15 and 30 min, then dropped to near zero at 45 min, and then increased again to a near maximum after 120 min. The reason for the drop in CO_2 evolution at 45 min is unknown.

Growth and Sporulation of _B. sorokiniana_ on Water Agar Medium Amended With Atrazine, Terbutryn, and 2,4-D at 10^{-2} and 10^{-3} of Applied Field Concentrations

Colony diameter of B. sorokiniana on agar medium

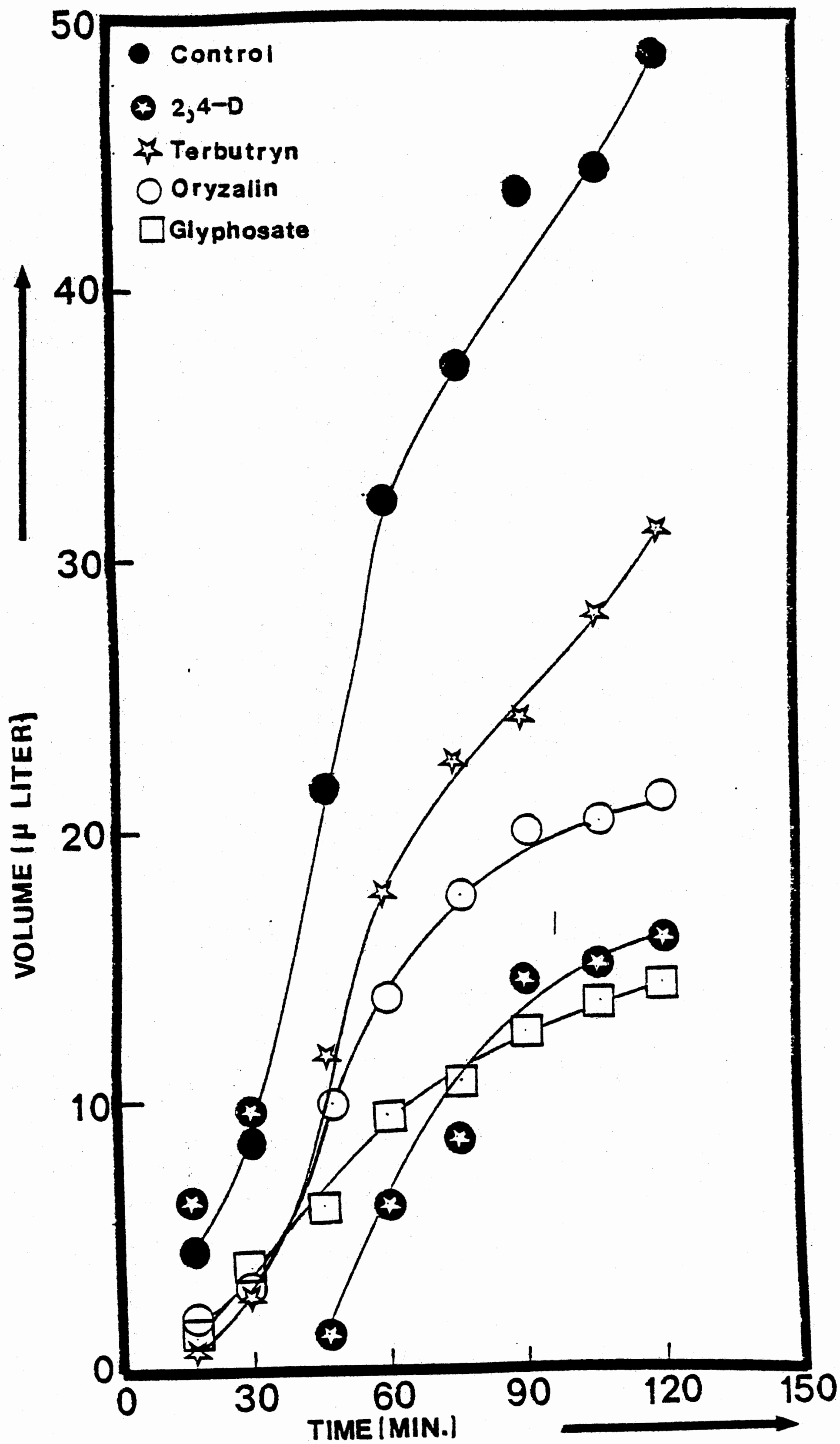
TABLE III

THE EFFECT OF FOUR HERBICIDES IN STERILE FIELD SOIL SUSPENSION ON RESPIRATION OF BIPOLARIS SOROKINIANA

Herbicide[b]	Manometer Reading (ul Displacement in 120 min)[c]		
	Initial	Final	Difference
Terbutryn	200	170.4	29.6
2,4-D	200	183.8	16.2
Glyphosate	200	185.9	14.1
Oryzalin	200	171.0	24.0
Control	200	150.9	49.1

a/ Two g of soil per 10 ml of sterile distilled water.
b/ Herbicides concentrations were: terbutryn, 11,980 ppm; 2, 4-D, 5,990 ppm glyphosate, 4,790 and oryzalin, 5,990 ppm.
c/ Readings shown are the mean of four replications. A total of eight readings were taken every 15 min but only the final reading is shown.

Figure 5. The Rate of Respiration of *Bipolaris sorokiniana* in the Presence of Four Herbicides at Applied Field Rate Concentration in a Sterile Soil Suspension Without any Herbicide.



Control
2,4-D
Terbutryn
Oryzalin
Glyphosate
VOLUME (μ LITER)
TIME (MIN.)
0
10
20
30
40
50
0
30
60
90
120
150

Colony diameter of *B. sorokiniana* on agar medium amended with herbicide at two concentrations was measured after six days to determine the inhibitory effect of each. Except for atrazine and 2,4-D, both at 10^{-3} concentration of applied field rate, all of the herbicides at 10^{-2} and 10^{-3} concentrations significantly reduced the colony diameter (Table IV).

Conidial production per cm^2 of colony surface after 25 days was significantly reduced compared to the control (Table IV) by each herbicide at 1×10^{-2} concentration of field rate. Conidia produced on the control medium were 15, 420, and 1.8 times more abundant than in the media containing atrazine, terbutryn and 2,4-D respectively.

Effect of Atrazine, Terbutryn, and 2,4-D on Root, Shoot, and Disease Development in Seedlings of TAM W-101 Wheat Cultivar Uninoculated And Inoculated With *B. sorokiniana*

Roots of seedlings grown in medium amended with either atrazine or 2,4-D significantly reduced the length of root growth compared to the control and terbutryn amended (Table V and Figure 6). Roots of all plants grown in media amended

TABLE IV

COLONY DIAMETER AND NUMBER OF CONIDIA OF BIPOLARIS SOROKINIANA GROWING ON HERBICIDE AMENDED MEDIA

Herbicide	Concentration in ppm & Fraction of Field rate Concentration (in parenthesis)	Mean Diam of Colony (cm)	Least Significant Difference	Mean Number of Conidia[a] (cm^{-2})	Least Significant Difference
Atrazine	120 (10^{-2})	3.97	0.35	300.9	H.S.D.[d]
	12 (10^{-3})	7.40		3,296.5	
Terbutryn	120 (10^{-2})	2.17	0.29	10.9	H.S.D.
	12 (10^{-3})	6.42		1,672.6	
2,4-D	60 (10^{-2})	6.47	N.S.D.[b]	2,500.0	1.11
	6 (10^{-3})	7.72	N.S.D.[c]	2,278.8	N.S.D.
Control		7.56		4,623.0	

a/ Mean number of conidia was determined from 8 samples per medium replicated 5 times.
b/ N.S.D. = Not significant difference. H.S.D. = Highly significant difference.
c/ All treatments at all concentrations were significantly different from that of control except for atrazine and 2,4-D both at 10^{-3} field rate concentration.
d/ LSD was not calculated for a difference in conidial production in atrazine concentrations of 10^{-2} and 10^{-3}, nor in terbutryn concentrations of 10^{-2} and 10^{-3}, because an analysis of variance indicated that the differences were highly significant.

TABLE V

MEAN LENGTH OF ROOTS, SHEATHS OF FIRST LEAVES AND BLADES OF FIRST LEAVES OF UNINOCULATED AND INOCULATED (BIPOLARIS SOROKINIANA) WHEAT SEEDLINGS (cv. TAM W-101) GROWN IN WATER AGAR AND WATER AGAR AMENDED WITH THREE HERBICIDES[b]

	Parameter[a]		
Treatment[c e]	Root Length (cm)	Length of First Leaf Sheath (cm)	Length of First Leaf (cm)
Control	14.9 a	3.5 a	10.2 a
Control + F[d]	13.8 ab	3.6 ab	9.5 a
Terbutryn	12.8 cb	3.3 bc	7.9 b
Terbutryn + F	11.5 c	3.1 c	7.8 b
Atrazine	1.7 d	2.2 c	4.9 d
Atrazine + F	2.1 d	2.6 d	5.4 d
2,4-D	1.9 d	3.3 bc	7.0 bc
2,4-D + F	1.7 d	3.2 c	6.6 c

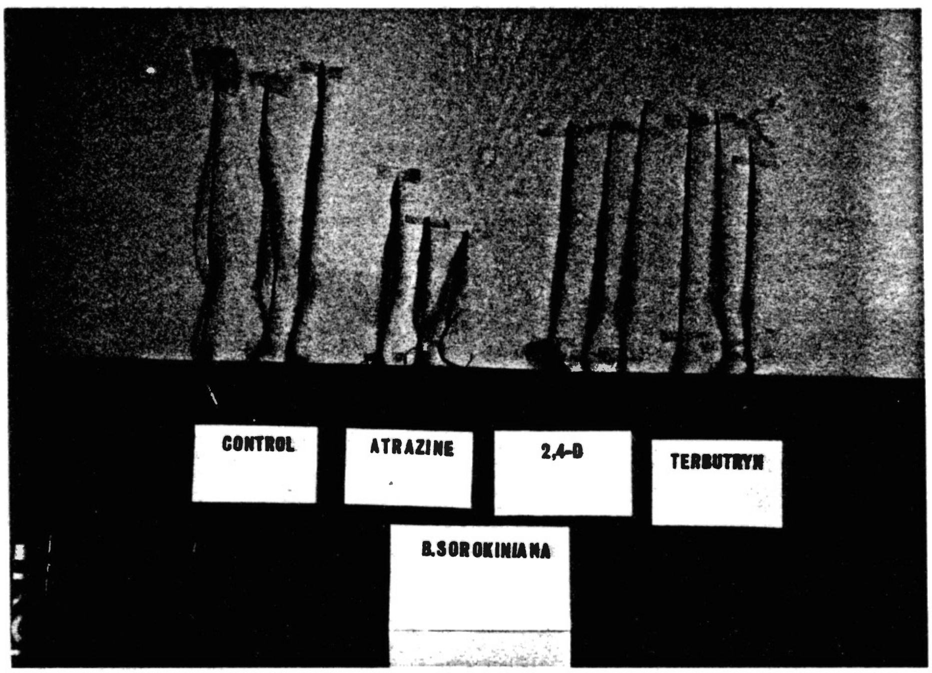
a/ LSD for root length = 1.9, LSD for first leaf sheath length = 0.2, and LSD for first leaf length = 1.0

b/ Means followed by the same letter are not significantly different (P = 0.05)

c/ Each value represents the mean of 40 seedlings per treatment.

d/ F = B. sorokiniana. e/ The herbicide concentrations were: 2,4-D. 0.6 ppm; terbutryn, 12 ppm; and atrazine, 24 ppm.

Figure 6. Roots and Inoculated Cotyledonary Leaves of Wheat Cultivar TAM-101 of Untreated and 2,4-D, Terbutryn and, Atrazine Treated Plants.


CONTROL
ATRAZINE
2,4-D
TERBUTRYN
B.SOROKINIANA

with 2,4-D were very short, thickened, and had densely crowded root hairs. In addition small callus-like nodules developed along the root and especially at the tips. Nodules often possessed chlorophyll. This stubby root system was firmly attached to medium, necessitating a much greater force to pull the plants from the medium than required for plants grown in other agar media. Roots of plants in the atrazine medium were also much shorter than those grown in the control and terbutryn containing medium. Unlike those grown in the 2,4-D and the other herbicide amended media, the roots grown in the medium amended with atrazine did not exhibit normal geotropism but generally grew in twisted configuration just below, on, and above the surface of the medium. Roots produced by plants grown in the terbutryn amended medium were only slightly shorter than those grown in the control medium and were normal in appearance. Atrazine and 2,4-D decreased root length significantly from those of the control and terbutryn. Significant differences in root length developed between seedlings grown in terbutryn and control media. No significant difference in root length between seedlingsgrown in atrazine and 2,4-D media were found. Differences in root length between inoculated and uninoculated plants grown in each medium, including the control, were not significant.

Sheath lengths of the first leaves of both inoculated and uninoculated plants grown in each herbicide amended medium were significantly shorter than those of uninoculated plants grown in the control medium. Among the herbicides, atrazine was the most inhibitory to leaf sheath elongation of inoculated plants, its effect differing significantly from those of terbutryn and 2,4-D which were similar.

The lengths of the laminae of the first leaves from both inoculated and uninoculated plants grown in each herbicide medium differed significantly from each other and from those grown in the control medium except for 2,4-D. There was no significant difference between lengths of the laminae from inoculated and uninoculated plants grown within an single medium. Under the conditions of this test, atrazine was the most inhibitory to leaf elongation.

Among the measured responses, i.e., root length, first leaf sheath length, and first leaf laminae length, none could be attributed to an interaction of herbicide and infection by B. sorokiniana.

The mean nembers of lesions in the leaves of inoculated plants grown in the control and 2,4-D media were significantly greater than in plants grown in terbutryn and atrazine media (Table VI). Successively, mean lesion numbers in leaves of plants grown in 2,4-D and terbutryn were similar (0.05 level), but both were significantly greater than the numbers produced on leaves of plants grown in atrazine medium.

The percent of necrotic tissue in the inoculated first leaves of plants grown in the control, 2,4-D, and terbutryn media did not vary significantly (avg = 58%), but was approximately five times as great as that (12%) which developed on leaves of plants grown in the atrazine medium (Table VI).

TABLE VI

MEAN NUMBER OF LESIONS AND PERCENT NECROSIS OF THE FIRST LEAVES OF WHEAT SEEDLINGS (cv. TAM-W-101) GROWN IN WATER AGAR AND WATER AGAR AMENDED WITH THREE HERBICIDES, & INOCULATED WITH *BIPOLARIS SOROKINIANA*

Treatment[a]	Parameter[bcd] Number of Lesion	Percent Necrosis
Control + F	4.4 a	55 a
2,4-D + F	3.8 ab	54 a
Terbutryn + F	3.3 b	65 a
Atrazine + F	2.1 c	12 b

a/ F = *B. sorokiniana*.
b/ Each value represents the mean of 40 seedling per treatment.
c/ L.S.D for Lesion number is 0.97, L.S.D for percent necrosis = 16.0 at the 5% confidence level
d/ Herbicide concentrations that produced a visible effect on the seedlings were used. The concentrations were: 2,4-D, 0.6 ppm; terbutryn, 12 ppm; and atrazine, 24 ppm.

CHAPTER V

DISCUSSION

Herbicides have been reported to inhibit growth or reproduction of plant pathogens in most studies, indicating that their toxicity is not limited to higher plants. Atrazine, in fact, has been reported to be slightly fungicidal but the test organisms were not named (5). Although many plant pathogens tolerate high concentrations of herbicides, and it has been shown by Heitefus *et al*. (see citation 3) that uneven distribution results in soil pockets where actual concentrations of a chemical may be considerably higher than intended. Most studies of herbicide activity against microorganisms involve concentrations near those applied at the soil surface and then assumed to be evenly distributed to arbitrary depths for various periods of time (23).

Some species of *Rhizobium* tolerate up to 2,000 ppm of 2,4-D and the inhibition of some fungi require concentrations as high as 40,000 ppm (26). Altman (2) reported that *Rhizoctonia solani* grew better in a medium supplemented with any of 25 common herbicides. In each instance, *R. solani* grew better in a medium supplemented with 1, 100, and 1000 ppm than in unsupplemented medium. Some growth occurred at 10,000 ppm with 12 of the 25 herbicides and complete inhibition occurred at 10,000 ppm with only two of them.

High concentrations (field spray application rates) of atrazine, cyanazine, 2,4-D, glyphosate, oryzalin, and terbutryn in Czapek-dox agar medium suppressed growth (dry weight) of *B. sorokiniana* in the current study. But in no instance was any fungicidal activity indicated.

Respiratory rates, measured by CO_2 evolution, has been used to measure the effect of chemicals on the biomass in soils (8, 24, 26). In this study CO_2 evolution from *B. sorokiniana* introduced into sterile sandy loam soil amended with field rates of glyphosate, 2,4-D, and terbutryn was 29, 32, and 60% respectively, of the control culture in herbicideless soil. This reaction indicated that the physiochemical nature of the soil did not substantially alter, or contradict, effects of the herbicides indicated by growth responses in the amended agar medium. Using CO_2 evolution as a measure of activity of soil microbes, Chandra et al. (8) reported that 5 and 100 ppm of simazine decreased metabolic activity for at least 28 days. Works by Teater *et al.* (35) indicated that 2,4-D at the rate of 32 lb/A increased CO_2 evolution while 2 lb/A decreased it.

Frequently, reports of herbicide activity against plant pathogens appear cotradictory. Rodriguez-Kabana _et al_. (32) demonstrated in greenhouse studies that 40 ppm of EPTC in liquid culture inhibited growth of _Sclerotium rolfsii_ but 10 and 40 ug/g of soil promoted growth of the pathogen. Similarly, atrazine at 40 and 80 ppm reduced mycelial growth of _Trichoderma_ sp., _Geotrichum_ sp., and _Fusarium_ sp. in liquid medium. Growth of _Trichoderma_ sp. was stimulated during one week of incubation, but was suppressed or reduced after two weeks.

Colony diameter and conidia per cm^2 produced by _B. sorokiniana_ on water agar and water agar amended with atrazine (120 and 12 ppm), terbutryn (120 and 12 ppm), and 2,4-D (60 and 6 ppm), equivalent in concentrations to 10^{-2} and 10^{-3} of field application concentration was studied. Colony diameters did not differ significantly from the control in 12 ppm of atrazine or 6 ppm of 2,4-D, but was significantly reduced by terbutryn. A slight stimulatory effect (2.1%) of 2,4-D over the control was noted. A stimulatory effect on _B. sorokiniana_ by 2,4-D has been reported previously by Hsia and Christensen (18) and on growth and germtube development by Hodges (17).

The apparent lack of an effect by atrazine at 10^{-3} of field application concentration (12 ppm) on growth of _B._

sorokiniana is in agreement with a report by Rodriguez-Kabana *et al.* (30) that atrazine at and below 40 ppm had no effect on growth of *S. rolfsii*. These authors reported further that atrazine enhanced growth of *Trichoderma viride* in all concentrations tested (8, 20, 40, and 80 ppm). Later, Rodriguez-Kabana and Curl (30) stated that 40 and 80 ppm retarded mycelial growth of *Fusarium oxysporum* f. sp. *vasinfectum*.

Conidial production was considerably below that of the control in all herbicide amended water agar cultures. At 10^{-2} and 10^{-3} of applied field rate concentrations, respectively, conidial production per cm^2 as a percent of the control was 7.0 and 71.0 for atrazine, 54.0 and 49.0 for 2,4-D and 0.20 and 36.0 for terbutryn. Thus, increased conidial production response to a ten-fold decrease in herbicide concentration was 180 times in terbutryn, 10 times in atrazine, and none (or possibly stimulated) in 2,4-D. Hodges (15) reported that 2,4-D at high concentrations (22.1 - 0.02 ppm) stimulated conidial production of *B. sorokiniana*. At 221 ppm conidial production was significantly less than the control, while at 0.002 to 0.000002 ppm production did not differ from the control.

An attempt was made in this study to determine whether symptoms of *B. sorokiniana* infection of leaves of wheat seedlings subjected to sublethal doses of the herbicides 2,4-D, terbutryn, and atrazine differed from those not so subjected. Seedlings were grown in water agar containing the herbicides at concentrations sufficient to cause measurable differences in growth responses while the cotyledonary leaves remained viable for symptoms of B. sorokiniana infection to develop. At the concentrations selected, it is probable that the seedlings would have died eventually from continued exposure to the herbicides.

Compared to seedlings grown in the control medium, all of the herbicides significantly decreased the root length, leaf sheath length, and leaf blade length. Root length was decreased significantly less by terbutryn (14%) than by atrazine (87%) and 2,4-D (87%). Leaf sheath length was decreased by a stastically similar amount (17%) for each herbicide. Leaf blade length was decreased most by atrazine (52%) and by statistically similar amounts (27%) by 2,4-D and terbutryn. Although these differences in plant response were noted, they should not be compared directly. Herbicidal activity of atrazine and terbutryn derives primarily from their interference with chlorophyll production, while 2,4-D activity derives from its auxin-like growth stimulating properties. In this experiment, terbutryn was used at 12 ppm while atrazine was used at 24 ppm, because in a preliminary test terbutryn at 24 ppm, almost completely inhibited seedling development.

Comparison of data between inoculated and uninoculated seedlings indicated that the combined effect of infection by B. sorokiniana and the herbicides on the measured plant growth parameters were not significantly different from that produced by the herbicide alone. This was not unexpected since the seedling leaves nearly had reached their maximum length at the time of inoculation.

Significantly fewer lesions developed on leaves of seedlings grown in atrazine and terbutryn amended agar than on seedling grown in the control medium. Lesions on seedlings grown in 2,4-D and terbutryn amended agar, however, were not significantly different. Percent necrosis of infected leaves were significantly less (80%) for seedlings in atrazine amended agar than in the other media. Though terbutryn did not act as adversely on growth of the wheat seedlings as did 2,4-D and atrazine, but the percent necrosis of infected leaves was highest in seedlings grown in terbutryn amended agar.

No reasonable explanation could be deduced for the reduction in disease symptoms relative to atrazine and infection by B. sorokiniana. At equivalent concentrations, conidial production by B. sorokiniana was higher and colony diameter greater in water agar amended with atrazine than in water agar amended with terbutryn.

These results tend to substantiate a report by Heitefuss and Bödendofer (see citation 3) that eyespot of wheat (*Cercosporella herpotrichoides*) and powdery mildew (*Erysiphe graminis*) was significantly reduced by urea and triazine (simazine) herbicides. They concluded that the fungitoxic potential of triazine and urea, as indicated by *in vitro* studies, was not sufficient to explain the reduction of disease caused by *C. herpotrichoides*.

Results reported for 2,4-D in this thesis neither support nor contradict the work of Hsia and Christensen (18) which indicated that wheat grown in soil treated with 2,4-D was more heavily infected with *B. sorokiniana* than that in control plots. They suggested that susceptibility was due to a predisposing effect on the host plants rather than an increase in virulence of the pathogen. Richardson (29) reported that 2.5 - 40 ppm of 2,4-D did not affect the growth of barley seedlings but reduced root rot caused by *B. sorokiniana*.

CHAPTER VI

SUMMARY

Herbicides are used to kill weeds in wheat cultivation, especially where some form of reduced tillage is practiced. B. sorokiniana is an important pathogen of wheat which may attack both roots and foliage. Therefore, the effects that herbicides may have directly upon B. sorokiniana and the disease it causes, are important to those engaged in various facets of wheat production.

Growth of B. sorokiniana was measured by dry weight, and by respiration rate in sterile soil.

All herbicides reduced growth, sporulation and respiration of B. sorokiniana at high concentrations, at low concentration (6 ppm) of 2, 4-D colony diameter was slightly increased.

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VITA 2

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Master of Science

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