1969

The Effects of Herbicides on Three Soil Inhabiting Blue Green Algal Species

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THE EFFECTS OF HERBICIDES ON THREE SOIL INHABITING BLUE GREEN ALGAL SPECIES

A Thesis

Presented to

the Graduate Faculty

Central Washington State College

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

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August, 1969

APPROVED FOR THE GRADUATE FACULTY

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ACKNOWLEDGEMENT

Many persons were helpful in the completion of this graduate study. The author wishes to thank his committee, Professors Jack Peary, Janet Lowe and Robert Gaines, for the professional advice and criticism used in the development and completion of a research topic.

A graduate program encompasses many areas of experience. One important aspect is contact with other students. Special thanks is extended to Louis Dillman, John Falkenbury, Wayne "Wino" McKenzie, Jim Southall, Fran Smyer and Mary Allen for aid in research and to Rainier Beer for moral support.

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INTRODUCTION

Since the introduction of 2, 4-D as a hormonal herbicide in the mid-1940's, the use of herbicides as a method of vegetation control has greatly increased. Widespread use has been made of herbicides in eliminating undesirable plant species from cultivated lands, grasslands, ponds, lakes, irrigation canals, reservoirs and marshes (DeVaney, 1968). A feature of herbicides which has further broadened their usage is their selectivity towards various plant groups.

The phenoxyacetic acid derivatives, 2, 4-D; 2, 4, 5-T; Silvex, are effective against dicotyledonous plants, but have little effect on monocots making them useful for removal of broadleaved vegetation in lawns and grasslands (Audus, 1964).

The chloroacetic acids, Dalapon and Trichloroacetic acid, are used primarily to control grasses, cattails, phragmites and rushes. They are also effective against white cedar, white pine and jack pine (Audus, 1964; DeVaney, 1968).

The phenylurea compounds, Diuron, Fenuron and Monuron; benzoic acid compounds, Dicamba, 2, 3, 6-TBA; and the triazines, Atrazine and Simazine, are used principally as soil sterilants effecting both annual and perennial plants (DeVaney, 1968).

A picolinic acid derivative, Tordon, has proven to be an effective brush control agent and plant defolient (Boffey, 1968; Watson and Wiltse, 1963; Ferguson, 1965).

Soil fertility has been shown to depend on an equilibrium among the diverse populations of soil microorganisms, a balance which could

possibly be disturbed by herbicides (Audus, 1964).

The phenoxy compounds are toxic to some soil microorganisms, but their susceptibility is variable. Concentrations of one and two per cent 2, 4-D will inhibit aerobic bacteria, but have no effect on facultative bacteria (Worth and McCabe, 1948). Studies by Newman and Downing (1958) indicate that gram positive bacteria are more sensitive to 2, 4-D than gram negative bacteria. Monuron, a phenyl urea compound, which affects the photosynthetic mechanism, is toxic to the chlorophycean soil algae, Stichococcus bacillaris, at concentrations of 1.0 parts per million (p.p.m.) (Raud et al, 1959).

Results of a screening test to determine the toxicity of a variety of herbicides to unialgal cultures of three green algal species indicated that they could tolerate concentrations up to 200 micrograms per milliliter without toxic effects. In one case, the growth of Chlamydomonas eugametos was increased by the herbicide, Simazine, at concentrations up to 200 micrograms per milliliter (Vance and Smith, 1969). Hale et al (1957) and Worsham and Giddens (1957) have reported a slight stimulatory effect on the general soil population by Dalapon.

The persistancy of herbicides in soils has received much attention and has been found to be dependent on leaching; chemical, photo and microbial decomposition, and on soil type and soil characteristics. The relative persistance of herbicides in soils can vary from 2-5 weeks (2, 4-D) to 1-3 years (Monuron) (Audus, 1964; DeVaney, 1968).

Few investigators, however, have been concerned with the effects of herbicides on soil algal flora. Arvik (1967) reported that Tordon

101 mixture (a mixture of Tordon and 2, 4-D) inhibited the growth of Cylindrospermum licheniforme in soil plate cultures at concentrations of 3 to 1000 p.p.m.

Of the algae inhabiting soils, certain blue-green algae have been found to contribute to soil fertility by their ability to fix atmospheric nitrogen which becomes available to higher plants upon excretion or cellular decomposition (Fogg, 1947; Allen, 1956; and Stewart, 1967). It is therefore desirable to prevent the loss of these organisms from the soil microflora by the secondary effects of herbicide usage. The object of this study was to examine the effects of varying concentrations of 2, 4-D, Dalapon, and Tordon on the growth of several nitrogen-fixing blue-green algal species obtained from soil samples and grown under culture conditions in the laboratory.

The herbicidal action of 2, 4-D is hormonal, stimulating plant growth to the extent the plant destroys itself. Some morphological and physiological effects of 2, 4-D on dicotyledonous plants include: reduction of the photosynthetic rate, acceleration of respiration, galls and tumors and production of short, thick roots and curved and twisting stems. Recommended concentrations of 2, 4-D required to control various plant species vary, but range between 2 and 30 pounds acid equivalent per acre (2.24 - 33.64 gms/m^2) (DeVaney, 1968).

Dalapon, a herbicide absorbed by both roots and leaves, acts on the growing shoots of grasses and buds of some broad-leaved species (Foy, 1961 and 1962) through protein breakdown (Andersen et al, 1962) or an alteration in protein synthesis (Mann et al, 1965). Application

rates required for the control of various grasses range from 5 to 10 pounds per acre (5.61 - 11.21 gms/m^2) (Buchholtz and Peterson, 1957).

Application of Tordon causes stem twisting and other growth effects in broad-leaved dicotyledonous plants similar to those caused by the phenoxyacetic acids through the promotion of cell expansion (Eisinger et al, 1966). Recommended rates of application range from one fourth to one pound acid equivalent per acre (.28 - 1.12 gms/m^2) (Alley, 1967).

METHODS AND MATERIALS

Isolation

The algal species used in this study were obtained from subsurface core samples collected in a grassland area about three miles west of Ellensburg, Kittitas County, Washington (T18N, R18E, S32).

The algal soil flora and vascular plants of the area had been analyzed in an earlier study (Arvik, 1967). Soil samples were placed in petri dishes, brought up to dampness with fertilized spring water, and incubated at room temperature at a light intensity of 350 ft - c (cool white, fluorescent) until algal growth appeared on the soil surface. Isolation of the individual species was accomplished by macerating a mixed algal sample on a slide, withdrawing selected filaments with a micropipette, washing them several times in sterile fertilized spring water (Table 1) to insure removal of adhering algal contaminants, after which they were pipetted into a suitable growth medium. Of the algal species isolated by this procedure, three were selected for experimental study: Nostoc muscorum (Fig. 1), Cylindrospermum licheniforme (Fig. 2), and Anabaena variabilis (Fig. 3)(Prescott, 1962).

Culturing

The preliminary medium used to grow stock cultures of algae consisted of a fertilized spring water. The spring water was obtained from a warm mineral spring located about three miles west of Lester, King County, Washington (T20N, R9E, S21).

A growth study was conducted to determine which of seven different artificial culture media (Table 2) would yield maximum growth of Nostoc muscorum. The algal inoculum was prepared by first centrifuging the culture on an International Model HN centrifuge at 3000 rpm for 30 seconds and then resuspending the algal material in distilled water. One milliliter aliquots of the algal suspension were added to flasks containing 50 ml of each sterile growth medium which had been adjusted before autoclaving to yield a final pH of 8.0 and incubated for five days at 25^{o} C at a light intensity of 350 ft - c (cool white, fluorescent). At the end of this period, the algal filaments were collected on Whatman #1 filter papers and extracted in 25 ml of methanol for 24 hours in the dark. As a measure of growth the absorbance of the various extracts was measured on a Bausch and Lomb Spectronic 20 colorimeter at 434, 580, and 668 mu. A methanol extract analyzed earlier on a Beckman DB recording spectrophotometer indicated maximum absorbance at 434 and 668 mu and minimum absorbance at 580 mu. Of the growth media analyzed, maximum growth was obtained with the medium of Hughe's et al and modified Bjalve's media. The latter medium, upon reanalysis, was chosen as the experimental growth medium. A variety of trace element solutions (Table 3) were added to the modified Bjalve's growth medium and tested as above with N. muscorum. Greatest growth was achieved with modified Bjalves solution plus Gaffron's minor element solution. This growth medium (Table 4) was used throughout the course of the study.

Aerated unialgal cultures of N. muscorum, C. licheniforme, and

A. variabilis in exponential phase of growth (Fig. 4) were used as experimental inoculum. The inoculation cultures, prepared as follows, were incubated at 25° C at a light intensity of 500 ft - c (cool white, fluorescent), in a Percival Growth Chamber (Model #WE-106) for five days.

Nostoc muscorum

Twenty-five ml aliquots of algal suspension adjusted to an optical density of 0.51 at 434 mu by the addition of sterile medium, were added to 375 ml of sterile culture medium in one liter Erlenmeyer flasks.

Cylindrospermum licheniforme and Anabaena variabilis

Seventy-five ml aliquots of algal culture, blended for 20 seconds at high speed in a Waring blender and adjusted to an optical density of 1.0 at 434 mu, were added to 325 ml of sterile medium in one liter Erlenmeyer flasks.

The growth characteristics of each species had been determined by taking optical density measurements at various time intervals and establishing the exponential period of growth.

Experimental

Five ml aliquots of each algal species adjusted to the optical densities described above for the preparation of the inoculum cultures, were added to about 120 ml of sterile medium in acid-washed 250 ml Erlenmeyer flasks, aerated, and incubated for three days at 25° C at light intensities of 250, 500, 750, and 1,000 ft - c (for N. muscorum)

or 500 ft - c (C. licheniforme and A. variabilis) in the growth chamber.

At the termination of this period herbicides were introduced into the flasks to yield concentrations of 0.0, 100, 200, 500, 1,000, 2,500, 5,000, and 10,000 (for Dalapon 15,000 and 20,000) parts per million per 125 ml of total culture solution and incubated for an additional four days. Each herbicide concentration was tested in triplicate. The herbicides used in this study, provided by the Dow Chemical Company, are as follows:

1. 2, 4-D (2, 4-dichloro phenoxyacetic acid - Na salt; lot No. 784205.
2. Tordon (88.6%K salt of 4-amino-3, 5, 6-trichloro picolinic acid). Ref. GH - 1 - 4279 - 5 - RVH.
3. Dalapon (2, 2-dichloropropionic acid - Na salt; Lot No. 091371).

At the end of this period, the cultures were filtered, extracted in methanol, and analyzed colorimetrically as described above. The influence of herbicide activity on the various algal species was determined by calculating the per cent difference between the averaged optical density measurements of the replicate herbicide and control extracts (Tables 5, 6, and 7).

TABLE 1

Composition of algal growth medium used in isolation and initial culturing of experimental algal species expressed in grams per liter of Lester warm spring water.

KNO_3	0.26
$MgSO_4 \cdot 7H_2O$	0.1
K_2HPO_4	0.1
sequestrene	0.005
soil extract (Bold, 1942)	50.0 ml

TABLE 2

Algal culture medium used in culture of Nostoc muscorum.

Bjalve's solution + 1gm/liter $NaNO_3$ (Bjalve, 1962).

Hughes et al solution (Vance, 1966).

Kratz and Meyers C solution (Kratz and Meyers, 1955).

Kratz and Meyers D solution (Kratz and Meyers, 1955).

Lazaroff and Vishniac solution + 1gm/liter $NaNO_3$ (Lazaroff and Vishniac, 1962).

Modified Hughes et al (M. M. Allen, 1968).

101 new solution (Fujiwara and Okutsu, 1959).

TABLE 3

Trace element solutions used in conjunction with modified Bjalve's growth medium in culture of Nostoc muscorum.

Allen's microelement solution (Allen, 1968).

Kratz and Meyers A_5 microelement solution (Kratz and Meyers, 1955).

Kratz and Meyers H_5 microelement solution (Kratz and Meyers, 1955).

Gaffron's minor-element solution (Zehnder and Gorham, 1960).

TABLE 4

Composition of modified Bjalve's growth medium and Gaffron's minor-element solution expressed in grams per liter.

Modified Bjalve's			
K_2HPO_4	0.8	$MgSO_4 \cdot 7H_2O$	0.2
KH_2PO_4	0.2	$CaSO_4 \cdot 2H_2O$	0.1
NaCl	0.2	$Na_2MoO_4 \cdot 2H_2O$	0.005
Ferric Citrate	0.01	$NaNO_3$	1.0
Citric Acid	0.01	Gaffron's minor-element solution	0.08 ml

Gaffron's			
H_3BO_3	3.100	$NiSO_4(NH_4)_2SO_4 \cdot 6H_2O$	0.198
$MnSO_4 \cdot 4H_2O$	2.230	$Cd(NO_3)_2 \cdot 4H_2O$	0.154
$ZnSO_4 \cdot 7H_2O$	0.287	$Cr(NO_3)_3 \cdot 7H_2O$	0.037
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.088	$V_2O_4(SO_4)_3 \cdot 16H_2O$	0.035
$CuSO_4 \cdot 5H_2O$	0.125	$Na_2WO_4 \cdot 2H_2O$	0.033
$Co(NO_3)_2 \cdot 6H_2O$	0.146	KBr	0.119
$Al_2(SO_4)_3K_2SO_4 \cdot 24H_2O$	0.474	KI	0.083

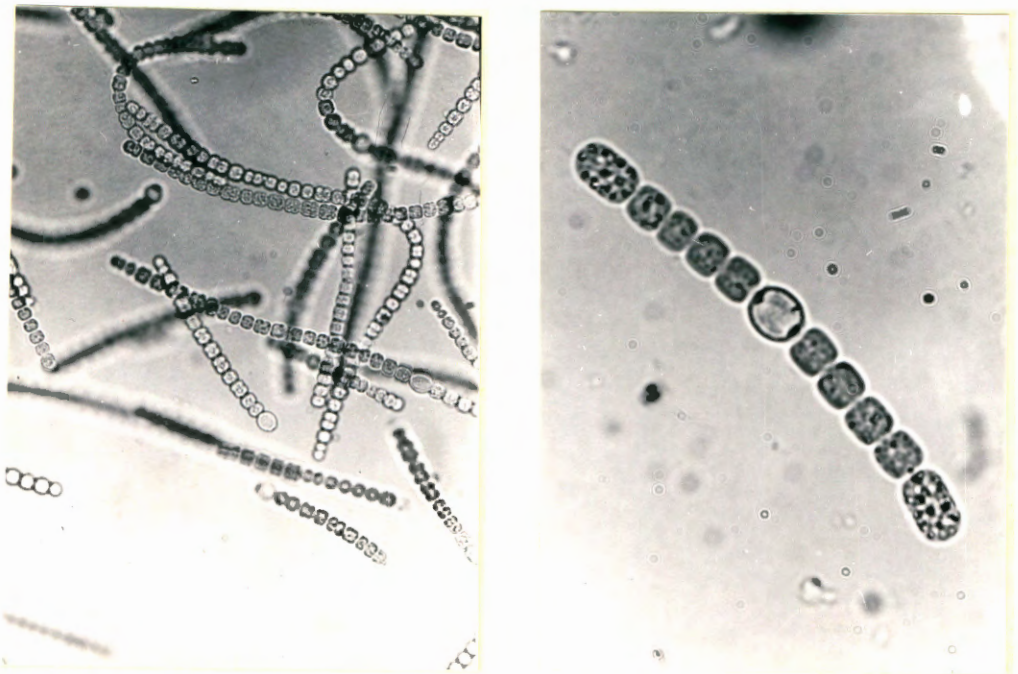

Fig. 1 - Nostoc muscorum. Right 1000 X. Note the terminal akinetes and centrally located heterocyst. Left 400 X.

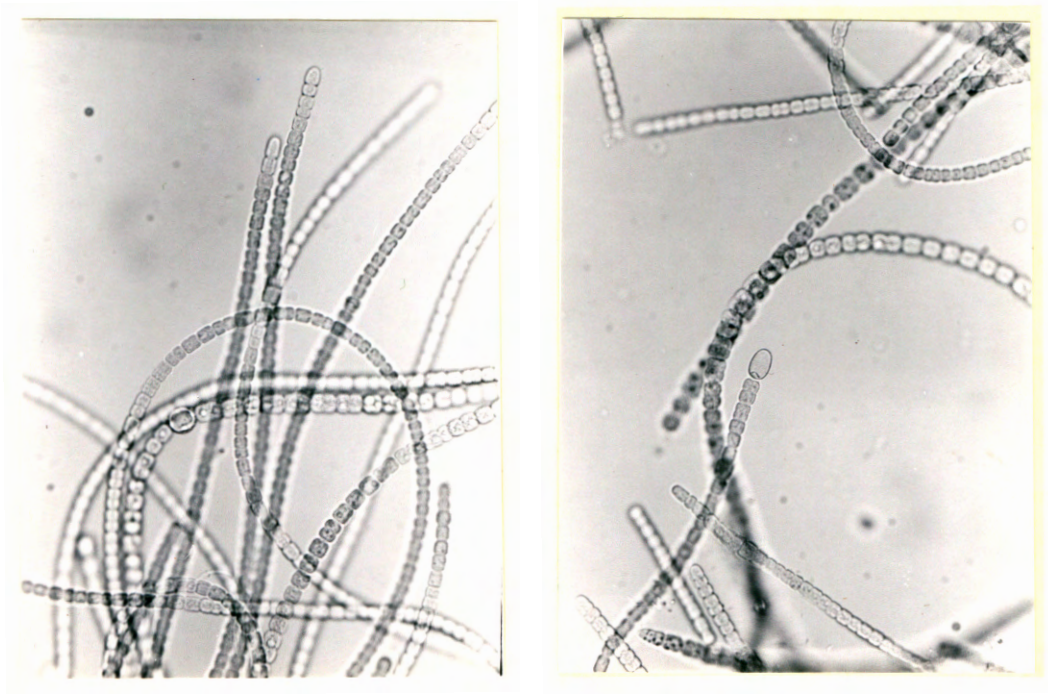

Fig. 2 - Cylindrospermum licheniforme. 400 X.

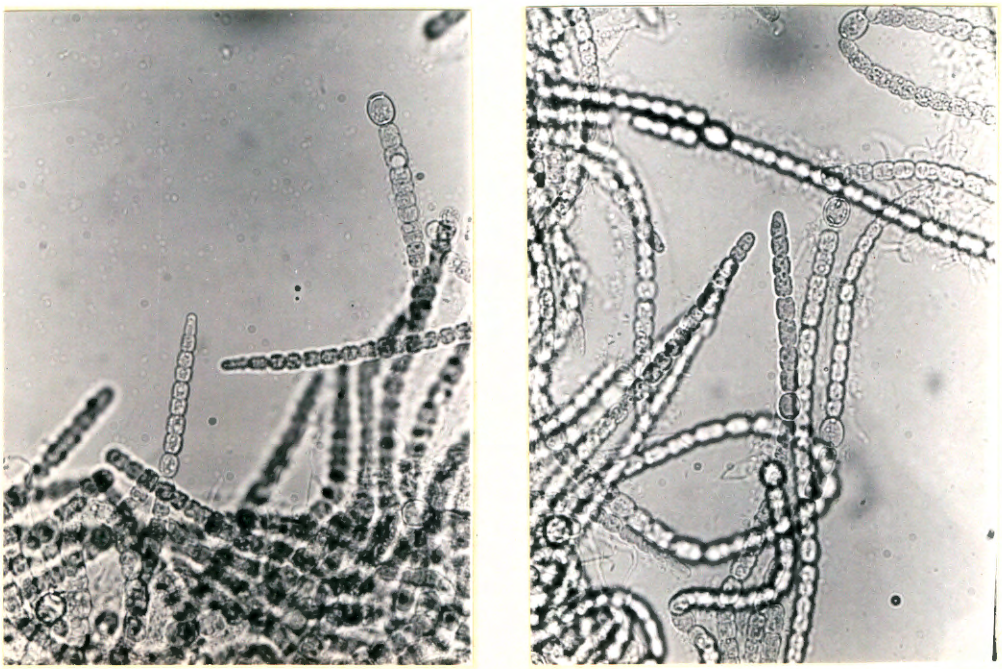

Fig. 3 - Anabaena variabilis. 400 X.

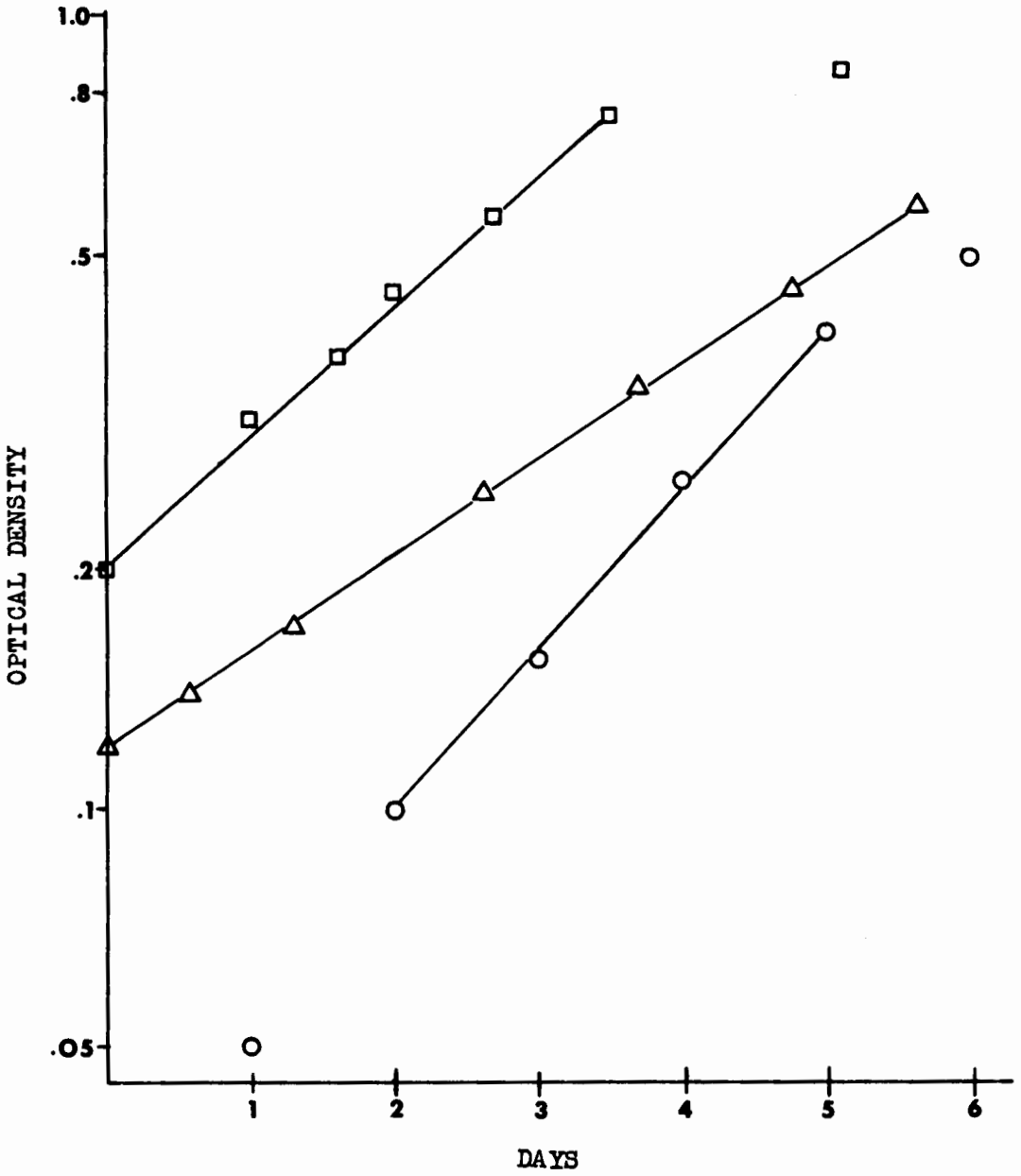

Fig. 4 - Exponential phases of growth of Nostoc muscorum (O), Cylindrospermum licheniforme (Δ), and Anabaena variabilis (□) grown at 25°C and 500 ft-c and measured colorimetrically at 434 mu.

RESULTS

The herbicides, 2, 4-D, Dalapon and Tordon, at the concentrations used were toxic to all three of the soil blue-green algal species studied. The effects of the herbicides on the growth of the algal species are listed in tables 5, 6, and 7.

Nostoc muscorum and Cylindrospermum licheniforme were killed in concentrations of 2, 4-D at 5000 p.p.m. and above (Fig. 5). Cultures of the two species in concentrations of 5000 and 10,000 p.p.m. became yellow within 12 hours and one hour, respectively, and after four days yielded extracts with little or no absorbance. Cultures of Anabaena variabilis were initially somewhat more tolerant of the herbicide, requiring 48 hours for the yellowing to occur at a concentration of 5000 p.p.m. Of the three species in the lower concentrations of 2, 4-D, C. licheniforme displayed the greatest inhibition. After four days incubation, concentrations of 500 p.p.m. resulted in 82% less growth than the control cultures, whereas N. muscorum and A. variabilis yielded 31% and 35% less growth, respectively.

Introduction of the herbicide, Tordon, into the algal cultures produced yellowing of the cultures at the two highest concentrations (Fig. 6). The algal species reacted more adversely to Tordon in comparison to 2, 4-D. Extracts of the three algal species which had been incubated in concentrations of 2500 p.p.m. were nearly devoid of absorbance. At 5000 and 10,000 p.p.m. absorbance was essentially zero. C. licheniforme reacted the most severely to the herbicide, yielding at a concentration of 500 p.p.m. 90% less growth than the controls, whereas,

N. muscorum and A. variabilis had 55% and 65% less growth, respectively.

The algal species were the least inhibited by the herbicide, Dalapon, requiring concentrations of 10,000 p.p.m. to completely inhibit A. variabilis and C. licheniforme and 20,000 p.p.m. for a similar inhibition of N. muscorum (Fig. 7). Cultures of A. variabilis and C. licheniforme in concentrations of 10,000 p.p.m. became yellow within two hours after the introduction of the herbicide. The less sensitive cultures of N. muscorum required 24 hours for the yellowing to become apparent at the above concentrations. Inhibition of the three algal species in the lower concentrations of Dalapon were similar, the reduction of growth ranging from 36% to 45% at 500 p.p.m.

The above results are based on absorbance measurements of the methanol extracts taken at 434 mu on a Bausch and Lomb Spectronic 20 instrument. Herbicide inhibition based on absorbance at 668 mu yielded results comparable to those above. Results based on absorbances at 580 mu were in some cases variable but generally were comparable to those based on absorbance wavelengths of 434 and 668 mu (Tables 5 - 7).

At the termination of the four day herbicide-incubation period, the algal material was examined microscopically for evidence of any changes in cell morphology. No observable changes, other than loss of pigmentation, were noted in the three algal species in all the herbicide concentrations tested.

The effects of 2, 4-D and Tordon on the growth of N. muscorum incubated under various light intensities are listed in Table 8.

In 2, 4-D concentrations up to 10,000 p.p.m., growth of N. muscorum was slightly more inhibited at the higher light intensities

of 750 and 1000 ft - c than at the lower light intensities of 250 and 500 ft - c. Addition of Tordon, at concentrations up to 1000 p.p.m., to cultures of N. muscorum resulted in a distinct inhibition of growth at the two lower light intensities. Reduction in growth between the lower and higher light intensities differed by 9% to 22% at the lower herbicide concentrations.

Table 5 - Effect of 2,4-D on the growth of Nostoc muscorum, Cylindrospermum licheniforme, and Anabaena variabilis at 500 ft-c and 25°C as determined by optical density measurements of methanol extracts at 434, 580, and 668 mu. Percent difference between control and experimental optical densities are indicated below.

Conc. p.p.m.	N. muscorum						C. licheniforme						A. variabilis		
	434		580		668		434		580	668			434	580	668
000	1.3	1.5	.15	.16	.75	.80	.47	.28	.02	.15	.11		2.0	.24	1.05
100	1.0 77%		.12 80%		.60 80%		.42 89%		.02 100%	.14 93%			1.79 90%	.21 88%	.95 90%
200	.95 73%		.09 60%		.47 63%		.37 79%		.02 100%	.12 80%			1.5 75%	.18 75%	.82 78%
500	.90 69%		.08 53%		.42 56%		.13 28%		.01 50%	.05 33%			1.3 65%	.15 63%	.72 69%
1000	.85 65%	1.0 67%	.08 53%	.08 50%	.40 53%	.40 50%	.10 21%	.06 21%	.01 50%	.04 27%	.03 27%		1.0 50%	.11 46%	.52 50%
2500		.19 13%		.01 6%		.06 8%		.02 7%	.00 0%		.01 9%		.52 26%	.05 21%	.26 25%
5000		.05 3%		.00 0%		.02 3%		.00 0%	.00 0%		.00 0%		.02 1%	.00 0%	.01 1%
10,000		.01 1%		.00 0%		.00 0%		.00 0%	.00 0%		.00 0%		.01 .5%	.00 0%	.00 0%

Table 6 - Effect of Tordon on the growth of Nostoc muscorum, Cylindrospermum licheniforme, and Anabaena variabilis at 500 ft-c and 25°C as determined by optical density measurements of methanol extracts at 434, 580 and 668 mu. Percent difference between control and experimental optical densities are indicated below.

Conc. p.p.m.	N. muscorum						C. licheniforme			A. variabilis		
	434		580		668		434	580	668	434	580	668
000	1.5	1.3	.15	.12	.73	.60	.72	.06	.30	2.3	.20	.96
100	.95 63%		.09 60%		.48 66%		.35 49%	.04 67%	.16 53%	2.0 87%	.17 85%	.82 85%
200	.85 57%		.07 47%		.37 51%		.30 42%	.02 33%	.13 43%	2.0 87%	.17 85%	.82 85%
500	.68 45%		.05 33%		.26 36%		.07 10%	.01 17%	.03 10%	.80 35%	.07 35%	.35 36%
1000	.33 22%		.02 13%		.12 16%		.03 4%	.00 0%	.02 7%	.20 9%	.02 10%	.10 10%
2500		.16 12%		.02 17%		.11 18%	.02 3%	.00 0%	.00 0%	.05 2%	.01 5%	.03 3%
5000		.01 1%		.00 0%		.00 0%	.01 1%	.00 0%	.00 0%	.01 0%	.00 0%	.00 0%
10,000		.00 0%		.00 0%		.00 0%	.00 0%	.00 0%	.00 0%	.00 0%	.00 0%	.00 0%

Table 7 - Effect of Dalapon on the growth of Nostoc muscorum, Cylindrospermum licheniforme, and Anabaena variabilis at 500 ft-c and 25°C as determined by optical density measurements of methanol extracts at 434, 580 and 668 mu. Percent difference between control and experimental optical densities are indicated below.

Conc. p.p.m.	N. muscorum			C. licheniforme			A. variabilis		
	434	580	668	434	580	668	434	580	668
000	1.5 1.3 1.5	.12 .14	.68 .60 .70	.28	.02	.11	2.0	.24	1.05
200	1.3 87%	.10 84%	.57 84%	.20 71%	.02 100%	.08 73%	1.79 90%	.22 92%	.95 90%
500	.91 60%	.07 58%	.41 60%	.18 64%	.01 50%	.07 64%	1.09 55%	.12 50%	.52 52%
1000	.76 50%	.06 46%	.34 50%	.15 54%	.01 50%	.06 55%	.92 46%	.10 41%	.46 44%
2500	.32 25%	.03 25%	.13 22%	.12 43%	.01 50%	.05 45%	.80 40%	.09 38%	.41 39%
5000	.25 .30 19% 20%	.02 .02 17% 14%	.09 .10 15% 14%	.06 21%	.01 50%	.02 18%	.75 38%	.08 33%	.38 36%
10,000	.27 18%	.01 7%	.08 11%	.01 4%	.00 0%	.00 0%	.04 2%	.00 0%	.01 1%
15,000	.22 15%	.01 7%	.06 9%	.00 0%	.00 0%	.00 0%	.02 1%	.00 0%	.00 0%
20,000	.03 2%	.00 0%	.01 1%	.00 0%	.00 0%	.00 0%	.01 .5%	.00 0%	.00 0%

TABLE 8 - Effects of 2, 4-D and Tordon on the growth of Nostoc muscorum incubated at 25° C under varying light intensities as determined by optical density measurements of methanol extracts at 434 mu. Percent differences between control and experimental optical densities are indicated below.

Conc.	250 ft - c				500 ft - c				750 ft - c				1000 ft - c			
p.p.m.	2, 4-D		Tordon		2, 4-D		Tordon		2, 4-D		Tordon		2, 4-D		Tordon	
000	1.3	1.5	1.4	1.3	1.3	1.5	1.5	1.3	1.2	1.1	1.3		1.2	.85	.93	.90
100	1.0 77%		1.0 71%		1.0 77%		.95 63%		.85 71%		1.1 85%		.85 71%		.84 90%	
200	.97 75%		.90 64%		.95 73%		.85 57%		.83 69%		.92 71%		.81 68%		.75 81%	
500	.86 66%		.60 43%		.90 69%		.68 45%		.79 66%		.75 58%		.75 63%		.70 75%	
1,000	.86 66%	1.0 67%	.27 19%		.85 65%	1.0 67%	.33 22%		.73 61%	.68 62%	.32 25%		.74 62%	.53 62%	.32 34%	
2,500		.16 11%		.16 12%		.19 13%		.16 12%		.19 17%	.18 14%			.19 22%		.08 9%
5,000		.03 2%		.01 1%		.05 3%		.01 1%		.01 1%	.01 1%			.01 1%		.01 1%
10,000		.01 1%		---		.01 1%		.00 0%		.01 1%	---			.01 1%		---

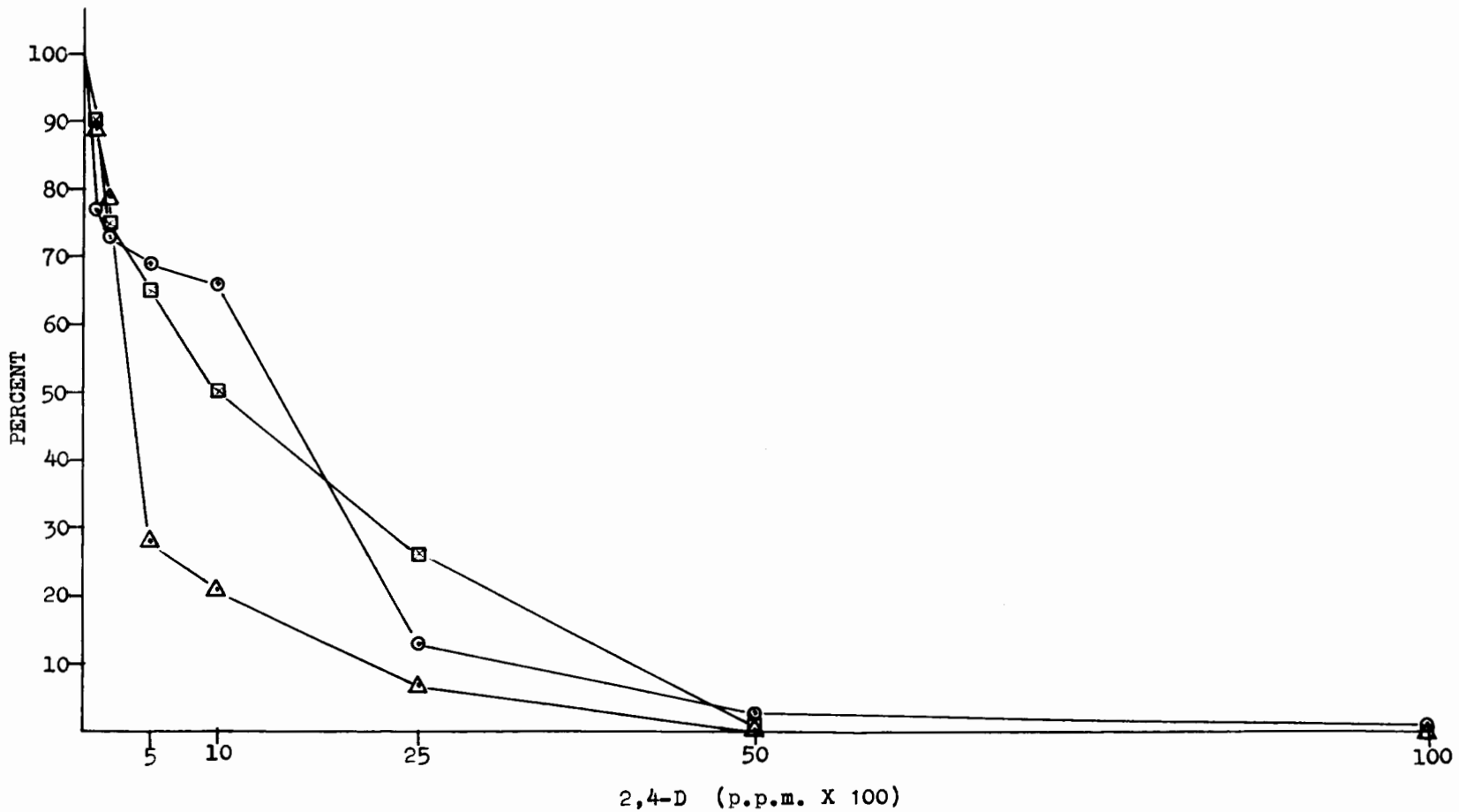

Fig. 5 - Effects of 2,4-D on Nostoc muscorum (O), Cylindrospermum licheniforme (Δ), and Anabaena variabilis (□) expressed as percent difference between optical densities (434 mu) of control and experimental methanol extracts

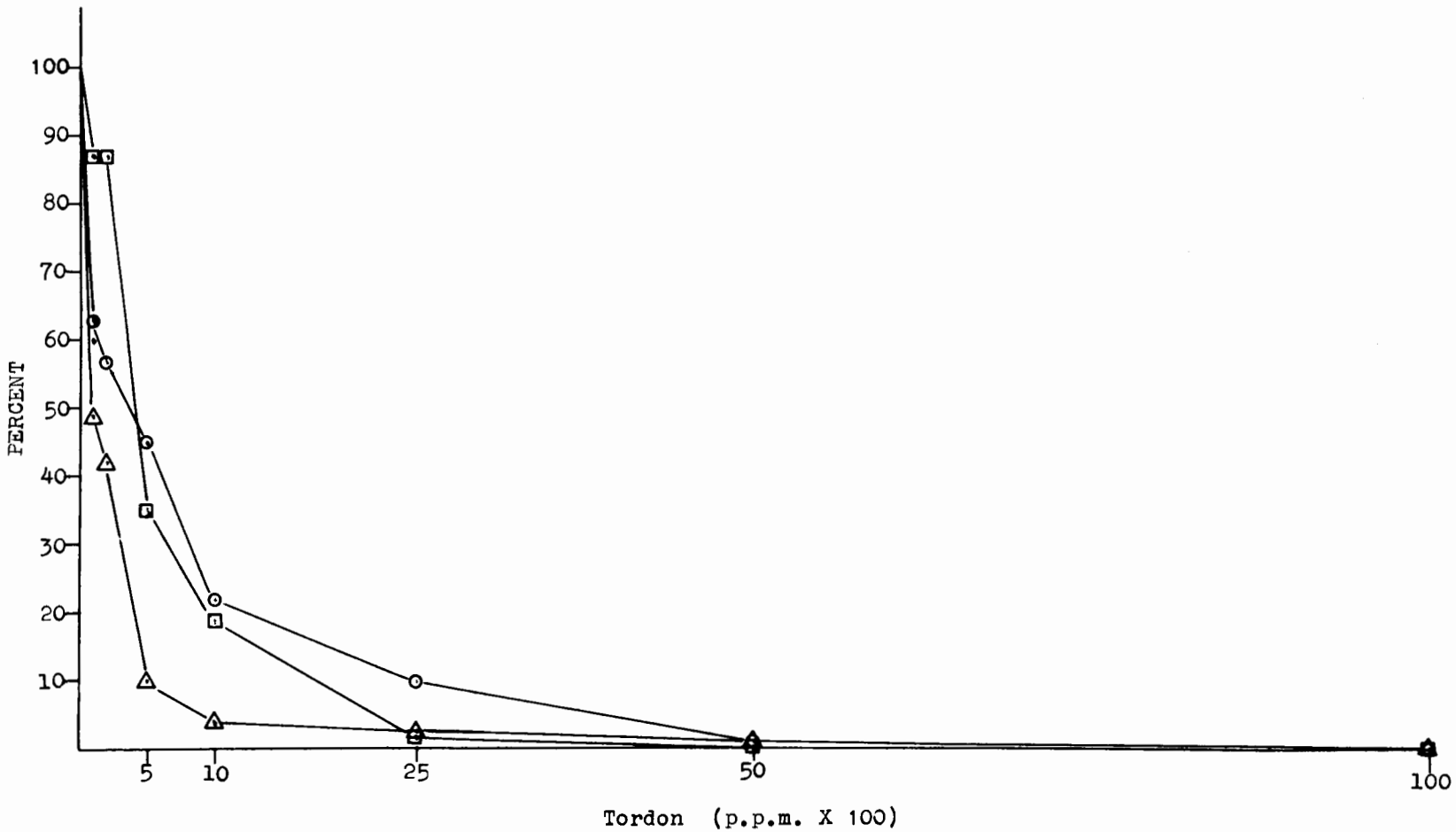

Fig. 6 - Effects of Tordon on Nostoc muscorum (O), Cylindrospermum licheniforme (Δ), and Anabaena variabilis (□) expressed as percent difference between optical densities (434 mu) of control and experimental methanol extracts

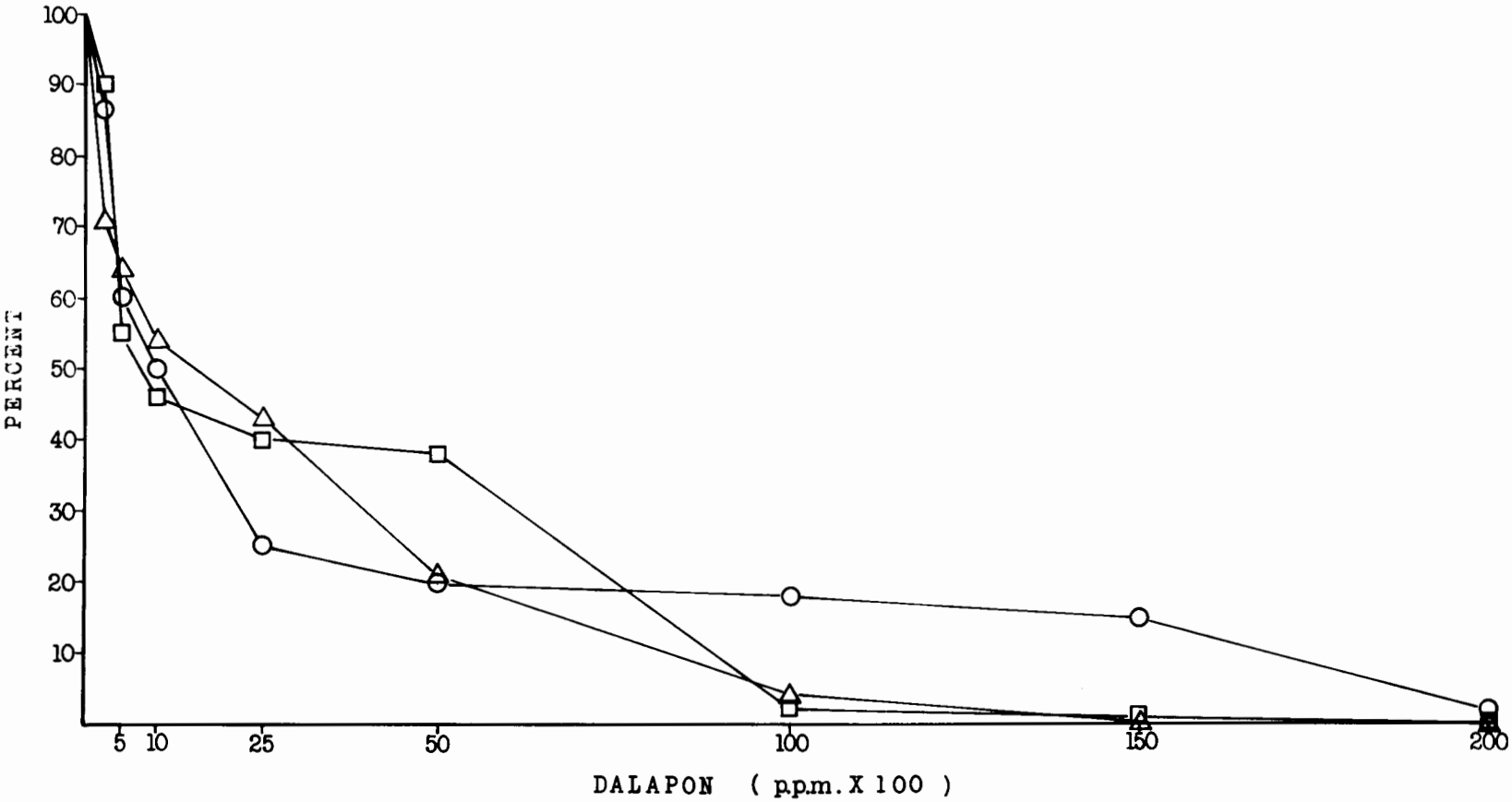

Fig. 7 – Effects of Dalapon on Nostoc muscorum (O), Cylindrospermum licheniforme (△), and Anabaena variabilis (□) expressed as percent difference between optical densities (434mu) of control and experimental methanol extracts

DISCUSSION

Although the three blue-green algal species were inhibited by the herbicides, 2, 4-D, Dalapon and Tordon, their respective degrees of inhibition varied with the herbicide and concentration used. A study by Vance and Smith (1969) of herbicidal action on several unicellular green algae indicated that different organisms react differently to various herbicides. The inhibiting and/or growth stimulating concentrations varied with each species and herbicide. In their investigation, 2, 4-D was not toxic to the algae tested, however, in this study 2, 4-D was inhibitory to all three species.

2, 4-D has been reported to vary in toxicity towards Chlorella pyrenoidsa cultured at different pH values or at one pH in various concentrations (Wedding et al, 1954). Wedding and Erickson (1957) also found that C. pyrenoidsa inhibition was greater at pH values below neutral. The pH of the cultures in this study ranged around 8, the pH of the soil from which the algae were isolated. Temperature has also been reported to be a factor in herbicide inhibition. Swets and Wedding (1964) showed that the amount of 2, 4-D absorbed by C. pyrenoidsa increased at the lower temperature in a range from 1^{o} to 25^{o} C. In this investigation the cultures were maintained at 25^{o} C, a temperature generally favoring the growth of most blue-green algae.

Another strain of C. pyrenoidsa (TX-7-11-05) proved to be resistant to 2, 4-D which suggests physiological heterogeneity

occuring within or between populations of C. pyrenoidsa. Whether or not algae can develop a resistance to herbicides upon repeated sub-lethal applications is unknown.

The results of this study suggest that light intensity may be a factor in herbicide inhibition. 2, 4-D in cultures of Nostoc muscorum at light intensities of 750 and 1000 ft - c slightly increased the herbicide inhibition. Tordon in cultures of N. muscorum was distinctly more inhibitory at the lower light intensities, 250 and 500 ft - c.

Dalapon has been reported by Anderson et al (1962) and Redemann and Hamaker (1954) to cause protein denaturation. Audus (1964) suggested that tolerant plant species may be those capable of detoxifying the protein degradation products. Of the three herbicides tested Dalapon was the least toxic. N. muscorum required a concentration of 20,000 p.p.m. to attain complete inhibition. The reduced toxicity of of Dalapon may be due to utilization or decomposition by the algae. Some soil fungi and bacteria have the ability to decompose Dalapon in the soil or utilize it as an energy source (Warren, 1964).

Tordon increased inhibition at the lower concentrations and was the most toxic herbicide tested. Its action has been reported to be similar to 2, 4-D (Eisenger et al, 1966). Of the three algal species, Cylindrospermum licheniforme was inhibited the most by 2, 4-D and Tordon. The concentrations of 2, 4-D and Tordon required for complete or nearly complete inhibition of growth were also identical, 5000 p.p.m.

Fogg (1956) states that the property of nitrogen fixation in blue-green algae closely parallels growth. Presumably the herbicide inhibition of the algae in this study was paralleled by a reduction in their nitrogen fixing capability. An investigation to determine the direct effect of herbicidal activity on the nitrogen fixing mechanism is needed to clarify this. Any herbicide induced decrease of nitrogen fixation would seriously impair soil fertility.

SUMMARY

The effects of three herbicides on the growth of three nitrogen-fixing, blue-green algal species, Nostoc muscorum, Cylindrospermum licheniforme and Anabaena variabilis from soil isolation cultures were studied in the laboratory. The herbicides, 2, 4-D, Dalapon and Tordon, were applied at concentrations ranging from 100 to 20,000 parts per million.

The herbicides at the concentrations used were all toxic to the three algal species. Addition of the higher concentrations to the cultures resulted in yellowing within several hours after application. At the lower concentrations C. licheniforme displayed the greatest intolerance to 2, 4-D and Tordon. The three species were all about equally inhibited in the lower concentrations of Dalapon, but at the higher range N. muscorum was slightly more tolerant. The influence of light intensity on 2, 4-D and Tordon inhibition was examined using the species, N. muscorum. The growth of this species in 2, 4-D was slightly more inhibited at light intensities of 750 and 1000 ft - c. At the lower light intensities of 250 and 500 ft - c, application of Tordon resulted in a distinct inhibition of growth.

Studies by Fogg indicate that nitrogen fixation in certain blue-green algae is related to their growth. From this, it is evident that the herbicidal effects on soil microfloral growth should be examined since their presence contributes significantly to soil fertility.

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