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Growth Response of Soil Algae to Tordon 101 Mixture

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GROWTH RESPONSE OF SOIL ALGAE
TO TORDON 101 MIXTURE

A Thesis
Presented to
the Graduate Faculty
Central Washington State College

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Jon Hans Arvik
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Many persons were responsible for the completion of this program, more than can be mentioned in this brief passage; they know their role and my sincere gratitude is extended to each of them. However, the author wishes to acknowledge the assistance of Dr. Dan Willson, who served as graduate advisor, and whose patience and criticism were indeed welcome and enlightening. My appreciation is extended as well to Dr. Arnold Blomquist for his guidance into the lighter side of research during our period of mutual service, 1964-1965.

A graduate program is undertaken for the benefit of the family as well as for the student, but too often the cost becomes extreme. My sincere hope is that someday I can repay my wife and my young son for the year they spent without their husband and father.

Jon H. Arvik
Captain, USAF

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SUMMARY

The effects of a new herbicide, TORDON 101 MIXTURE^R*, were observed on soil algae in field plots and in laboratory culture of algal species selected from those isolated from the plots. Though the herbicide caused no change in the quality of the soil algal population over a period of 180 days at concentrations of 0.5, 1.0, and 2.0 gallons per acre, inhibition of a filamentous blue-green alga (Cylindrospermum licheniforme) was evidenced in vitro at concentrations above 1 ppm, becoming significantly higher at 500 and 1000 ppm. Unicellular green algae (Chlorella vulgaris and Protococcus sp.) were subjected to Tordon and one of its active ingredients, Picloram (4-amino, 3,5,6-trichloropicolinic acid), with no inhibition in any concentration up to 250 ppm. The cyanophyte and one species of the Chlorophyta were stimulated by low concentrations of Tordon.

INTRODUCTION

As increasing emphasis is placed on efficient use of cropland, improved means of elimination of weeds and extraneous brush are being developed. Many of the improvements are in the area of chemical herbicides, and more specifically, the synthetic plant growth regulators. Auxin-like in action, these chemicals efficiently and economically control the less desirable forms of vegetation, while crops are allowed to develop in a less competitive environment. As each new

*Trademark, Dow Chemical Company, Midland, Mich.

herbicide is developed, it must be examined for effects other than those desired, for though a chemical may indeed be an excellent herbicide, deleterious side-effects can prevent its commercial use. Thus, each new discovery requires extensive field and laboratory tests, including investigation of its effects on microorganisms.

Since the soil algae represent a large percentage of the soil community and their significance in the role of nitrogen fixation and as soil-forming biotic factors has been established (Smith, 1944; Fogg, 1947; Waksman, 1952; Shields, et al, 1957; Shields, 1959), it is necessary to prevent the elimination of this group of organisms from the soil due to application (or more likely, improper use) of herbicides. Several investigators have examined the effects of specific herbicides and fungicides such as Atrazine, Monuron, and Simiazin on algal forms, including studies throughout the world (Fletcher, 1960; Il'in, 1962; Kumar, 1963) as well as in the United States.

TORDON 101 MIXTURE^R (hereafter referred to as Tordon), marketed by the Dow Chemical Company as a broad-range herbicide for brush control, has proved to be quite effective in controlling undesirable vegetation (Hamaker, et al, 1963; Wiltse, 1964; Warren, 1965). Investigations of Tordon in connection with some life forms have been undertaken (Arnold, 1965; Lynn, 1965; Jackson, 1965; Dow Chem Co., 1966), but information relative to its effect on algal forms is sparse indeed (Hardy, 1965). It was the purpose of this study to examine the effects and possible toxicity of the herbicide on soil algae.

MATERIALS AND METHODS

Tordon is a mixture of two active ingredients with a surfactant, and contains 0.54 pounds of 4-amino, 3,5,6-trichloropicolinic acid (Picloram) and 2.0 pounds of 2,4-dichlorophenoxyacetic acid (2,4-D) per gallon. The herbicides are formulated as the triisopropanolamine salts, and as such are completely water-soluble. The manufacturer's shipping label for the mixture gives the following information:

ACTIVE INGREDIENTS:

4-amino, 3,5,6-trichloropicolinic acid* as the triisopropanolamine salt.....	10.2%
2,4-dichlorophenoxyacetic acid as the triisopropanolamine salt.....	39.6%

INERT INGREDIENTS..... 50.2%

ACID EQUIVALENTS:

4-amino, 3,5,6-trichloropicolinic acid.....	5.7%
2,4-dichlorophenoxyacetic acid.....	21.2%

*Known under the trade name Picloram (Dow Chemical Company, 1965).

The study was divided into two parts, Phase I and Phase II. Phase I included treatment of field plots with varying concentrations of Tordon. After 90 and 180 days, soil samples were collected and subjected to culture techniques permitting growth and identification of the organisms. This phase was expected to provide an indication of those algae present and their possible reaction to the herbicide. Phase II was an in-vitro study of growth responses of species selected from the list provided by Phase I. Of these, Chlorella vulgaris (Univ. Ind.

#260) and Protococcus sp. (Univ. Ind. #433) were purchased from the University of Indiana algal collection, while Cylindrospermum lich-
enforme was isolated from the test plot soil. These three algae were grown in the presence of various concentrations of Tordon. Phase I: Four plots of 0.01 acre each were selected in an area that had been a field but had not been cultivated in the last six years. The area was moderately covered with grasses and a few annual and perennial weeds, which were mowed occasionally and burned over each spring, though not during this study. The plots are located less than a mile west of the Yakima River in Kittitas County, near Ellensburg, Washington. The soil within the plots is a clay loam, with a pH of 8.2 (determined by the bicarbonate method) and containing 2.5% organic matter (Bloom, 1966). The control plot was treated only with tap water. The other plots were sprayed with Tordon concentrations of 0.5, 1.0, and 2.0 gallons per acre, respectively, on October 22, 1966.

Spraying was accomplished in the Fall of 1966 with a low-volume water-hose sprayer commonly used in home gardening. Following treatment, all plots were sprayed with water to aid penetration of the herbicide into the upper soil layer. Samples were collected in the following manner: a vertical cut in the soil was made with a shovel, then the smooth face of the cut scraped in a side-to-side manner with a sterile instrument to expose an area uncontaminated by soil from a higher plane. Three levels were sampled; A=0-1 cm, B=1-5 cm, C=5-15 cm. A core sampler described by Willson and Forest (1957) was used

to remove horizontal cores at the B and C levels, while a spoon-like device was used to sample the surface, including the duff and litter not yet incorporated into the soil. Three random points in each plot were sampled in this manner, with the soil from each level being amalgamated to provide culture material. Effort was made to reduce transfer of organisms from level to level and from plot to plot by flaming collecting equipment with a small hand torch.

In the laboratory, 5 cultures were made from the material taken from each level in each plot. The cultures were prepared by placing a piece of circular filter paper in sterile 100mm Petri dishes, then adding approximately 5 ml of a soil extract medium (Bold, 1942). After this, approximately 10 gm of the amalgamated sample material was added to each dish. The soil algae were cultured at approximately 20°C., under fluorescent lighting (Ken-Rad 40 watt Cool-White) with an intensity of 250-300 ft-candles for 16 hours each day. The cultures were examined periodically with an 80X binocular dissecting microscope, and colonies removed for identification as they appeared. Random samples of the soil were also examined microscopically to insure as complete a determination of species as possible, since they may be present as individuals or in minute colonies.

Phase II: As algal forms were identified during Phase I, they were isolated on various media, usually soil extract agar, Bristol's agar, or sterile soil. For the most part, the procedures of Pringsheim (1964) were followed with excellent results. At this point, certain

species were selected for treatment with the herbicides. Culture techniques for each of the algae were determined in order that the different species were not limited by their artificial environments while at the same time being exposed to the herbicide. For example, it was found that Cylindrospermum licheniforme and Nostoc ellipso-sporum exhibited uniform, rapid growth under more intense lighting conditions and higher temperatures than were provided in Phase I. Since the species C. licheniforme was selected for Phase II, care was taken to determine the needs of the alga in terms of light and temperature. It was illuminated with fluorescent lights at a uniform 850 ft-candles, with the temperature maintained between 25° and 30°C. The uniformity of light over the entire culture area proved to be extremely important, since the alga was observed to grow at a rate proportional to the amount of light present, in a range from less than 100 to 1,200 ft-candles. Irregularity of even a hundred foot-candles over the surface of the culture area produced a corresponding irregularity in the results of the experiment.

A modified procedure used by Jansen, et al (1958) was used to measure the algicidal properties of the herbicide. As a substitution for the inorganic Perlite base used by Jansen, a medium was prepared by drying soil for 24 hours at 85°C., after which the soil was crushed, sifted through an 18 mesh wire screen and autoclaved at a pressure of 16 pounds per square inch for 20 minutes to eliminate algal forms that might have appeared as contaminants. Approximately

25 gm of the soil were added to sterile 100 mm disposable Petri dishes, then the plates were irrigated with enough herbicide solution to bring the soil to saturation. This provided relatively uniform absorption of approximately 0.75 ml of solution per gram of soil. Approximately 4mm squares of the surface growth of the selected filamentous organism were placed in the center of the soil plates with a set of modified forceps. Only rapidly-growing colonies were selected as inoculant sources, with samples removed along a circumference inscribed within the circular colony growth to give some assurance that all samples were of approximately the same age. Similar results were obtained by using older colonies that had been allowed to assume the mature akinete condition; however, a 72 hour time lag occurred while the akinetes germinated. Mean diameter measurements were recorded, with growth expressed in Figure I as per cent of control growth under the same conditions. One alga, C. licheniforme, was treated in this manner. The method of Ukeles (1965) was adopted to determine the effects of the herbicides on the unicellular green algae Chlorella vulgaris and Protococcus sp. C. vulgaris was grown in synchronized culture in Bristol's solution and induced to take the Da (dark-active, the most photosynthetic stage) condition described by Morimura (Ashton, et al, 1966), then used as an inoculant for the treatment series. The inoculant was prepared by centrifuging the culture cells in a graduated centrifuge tube at approximately 1000 X gravity for one minute, then 0.1 cc of the residue was collected and the organisms

resuspended in 50 ml of the supernatant. One hundred and fifty ml of each concentration of Tordon and Picloram were prepared in 250 ml Erlenmeyer flasks, using Bristol's solution as a base. Each flask was inoculated with 1 ml of the prepared suspension of cells. The treated cultures were placed in a growth chamber at 20°C., and illuminated with a fluorescent light source of approximately 300 ft-candles on 12 hour cycles. After 10 days of incubation, turbidimetric readings were taken with a Klett Model 800-3 colorimeter, using a red filter. Samples of the suspended organisms were compared with sterile blanks of the herbicide concentrations and the results recorded in Figure III as per cent growth of the control cultures. Protococcus sp. was treated in a similar manner, except without the procedure of Morimura.

In preparation of the Tordon concentrations, the herbicide was considered to be a pure substance and mixed by volume to provide the appropriate concentrations of 0.5, 1, 3, 10, 100, and 250 ppm. The Picloram was prepared to yield the same concentrations by weight, using a Mettler Model B5 scale. Since only the pure acid form of Picloram was available, the upper limit of 250 ppm was selected due to the low solubility of this form of the herbicide in water.

RESULTS

In Phase I, the Chlorophyta were represented qualitatively (Table I), though to a lesser degree in quantity. Three species were observed with regularity without regard to season (Chlorella vulgaris,

Protococcus viridis, Chlorococcum sp. #1), one, Stichococcus bacillaris, appeared regularly later in the study and four appeared only once (Characium ambiguum, Chlorococcum sp. #2, Palmellococcus sp., Palmella mucosa). Two of this group, Protococcus and Chlorococcum sp. #1, seemed better able to grow on the filter paper near the edge of the soil than on the soil itself. Small green colonies of each alga would be observed within a few weeks of incubation, whereas they could not be located on the soil, even by thorough examination, until somewhat later. All of the other species of this group were found by random sample; none were found growing in macroscopic colonies.

Within the Cyanophyta, three species could be located in every sample at every level: Cylindrospermum licheniforme, Nostoc ellipso-sporum, and N. muscorum. One (C. majus) was found on fewer occasions, three rarely (C. catenatum, Anabaena catenula, Phormidium favosum), and four which made only single appearances were A. variabilis, Lyngbya aerugineo-caerulea, Oscillatoria tenuis, and P. autumnale.

Diatoms were present in very limited numbers; frequently, several slide preparations could be examined microscopically before even an empty frustule could be found. However, as the summer approached, the number of live organisms increased noticeably.

Figure I shows the inhibition of the filamentous alga C. licheniforme in the presence of selected concentrations of Tordon. The technique gave relatively uniform results, providing the opportunity to compare cultures of the alga in terms of macroscopic size of the

plant mass. At 1 ppm, the alga was slightly stimulated, but at all concentrations higher than that figure, growth was retarded. At 500 ppm, inhibition permitted only 79% of the control growth, and at 1000 ppm, growth of the alga was 61% of the control.

After ten days of growth, neither C. vulgaris nor Protococcus sp. showed significant inhibition of growth as a result of treatment with the herbicides. With one exception, growth of the organisms in both Tordon and Picloram was within 2% of that of the control cultures. C. vulgaris in 0.5 ppm Tordon exhibited a slight but regular stimulation. (Figures III & IV).

DISCUSSION

The use of herbicides is extremely wide-spread. Most of the chemicals are available commercially in any quantity for lawn preparation, gardening or other uses for which weed control is desirable. Fortunately, the most widely used compounds, 2,4-D and 2,4,5,-T, alone and in combination, are subject to fairly rapid breakdown by microbial action (Audus, 1960; Fletcher, 1960; Whiteside, et al, 1960; Bollen, 1961; Theigs, 1962). Since the major active ingredient in Tordon, in terms of quantity, is 2,4-D, much of the degradation of this herbicide may be expected to parallel the breakdown of that chemical. The Picloram fraction, though lesser in quantity, is extremely active in minute amounts, and may resist microbial breakdown to a greater degree than 2,4-D. Goring (1966) suggests that the biodegradation of Tordon

in the soil depends upon (a), the quality of the soil microorganism population, (b), the quality and quantity of food material in the soil for microorganisms, and (c), temperature and moisture. He further indicates that the amounts of herbicide decomposed over a period of time are proportional to concentration, and that half-lives of Tordon in the soil can be estimated, permitting prediction of length of time a field must not be seeded with a susceptible crop.

Birk (1964), Goring, et al (1965), and Goring (1966) all report movement of Tordon through soils. Goring (1966) indicates leaching of the herbicide parallels its sorption by the soil; that is, the greater the tendency of the herbicide to be bound by a particular soil type, the less its potential movement. In addition, the herbicides may be carried alternately upward and downward through the soil by leaching and evapotranspiration. Since lateral movement of Tordon is controlled by the same processes as leaching, the herbicide may be carried with the soil moisture, being less restricted by sandy soils of low organic content than by clay soils.

In this investigation the bioassay techniques of Leasure (1964) were used to trace the movement of the herbicide. Within 72 hours after treatment, the three test plots were found to contain the herbicide in the upper 15 cm of the soil, though not below that level. Bioassay samples were also taken from the borders surrounding the plots, the results indicating that all of the herbicide was within the desired area. The clay element of the soil in which this study

was conducted effectively prevented significant vertical or lateral movement of the chemical. This lack of chemical movement was also due to the relatively low annual precipitation (less than 10"). Bioassay samples of the plots and their borders 180 days after treatment showed retention of the herbicide in the upper 30 cm of soil, with little or no lateral movement.

In the interpretation of Table I, it could not be presumed that elimination of an alga from the list previously located in the same area would be proof that the herbicide was the cause of its disappearance. Conversely, it should be stated that location of an alga at a given sampling date is proof that it was not eliminated by the concentration applied to that particular plot. In this frame of reference, comparison of the respective sampling periods reveals little indication that the herbicide is playing an active role in the determination of the quality of the soil algal population. For the period of 180 days, plus time in the culture chamber (up to 90 days), no algal form was found to be absent when it had been located with regularity earlier. Indeed, several species were located in the third sampling period where they had not been observed before. Further examination reveals eleven filamentous species of blue-green algae, seven unicellular greens, and a single filamentous green alga. Four genera of diatoms were represented, but were not included in the comparative study due to the difficulty of locating them in the early samples.

The lack of filamentous green algae was thought unusual, for in previous studies using similar techniques in a similar area, no less than four species of this type were found (Fairchild, 1964). Another striking observation was the notable absence of diatoms. Though four genera were represented, only a very few live organisms or empty frustules could be located. These were contrasted with samples of soil taken from an adjacent field, in which two species of Ulothrix and an abundance of diatoms were cultured with the techniques used in this study.

The Cyanophyta were by far the dominant algae in terms of biomass and colony numbers. Cylindrospermum and Nostoc could be located macroscopically with ease within a few weeks following incubation; over longer periods of time, these organisms would overgrow other colonies.

Among the algae collected, the greatest quantity of growth was evidenced in the upper layers of soil. Level A (0-1 cm) contained 15 species, B (1-5 cm) contained eleven, and C (5-15 cm) contained 13. These comments appear to be contradictory, unless the total number of times each species in each level was represented is considered; A=94, B=89, and C=56. Though C contained nearly as many species as A and more than B, a number of algae made fewer appearances in this level. For example, Chlorella was present twelve times in both A and B, but only twice in C. The same can be said for Chlorococcum (represented 10 times in A, 10 in B and 2 times in C), and Protococcus (11,10, and 5). Stichococcus, found only on the second and third

sampling dates, was frequently represented in A and B, but could not be found in C. These results were repeated with the Cyanophyta, the most frequent occurrences of any given alga being in the upper levels. Though two of the Chlorophyta and two Cyanophyta were found only in the C level, and four others only in A, none were represented singly in B.

The inhibition of the filamentous blue-green alga, Cylindrospermum, followed precisely the statement by Hueppe that "Every substance which in definite concentrations will kill protoplasm, will inhibit development in smaller amounts, and in still greater dilution act as a stimulant" (Bollen, 1961). At any concentration greater than 1 ppm, the alga was inhibited in its growth rate. As the concentration increased to 250 ppm, inhibition remained relatively constant, but at 500 ppm, growth was only 79% of the control. At 1000 ppm, the inhibitory effect was great enough to allow only 61% of the control growth. Though 500 and 1000 ppm are relatively high rates of application of a given active ingredient, only 10.2% of the herbicide is Picloram and 39.6% is 2,4-D, corresponding to an actual active ingredient application of 102 and 396 ppm of the salt form, respectively, at the higher test concentration.

Ukeles (1966) reports some inhibition of algal growth as a result of surfactant activity. Presumably, this factor acts in this instance, since a sufficient amount of surfactant is present in the higher concentrations to cause considerable foaming. Though this fraction of

the formulation is beneficial in weed and brush control, it may increase the activity of the herbicide relative to its toxicity to soil algae.

The single obvious deviation from the control growth in the unicellular green algae again reflects the stimulation of growth typical of small quantities of many plant growth regulators in higher plants. Since the actual active ingredient concentration in this test was 0.05 ppm Picloram and 0.19 ppm 2,4-D, it is to be expected that if minute amounts of regulator do stimulate algal growth, it would be expressed at the lower concentrations, as indeed it was. Even the highest concentration of the most active fraction of the herbicide did not cause significant inhibition of growth of the algae used in this portion of the experiment. (See Figures III & IV).

TORDON 101 MIXTURE^R is a brush control agent intended for use in a highly diluted (1 gallon of herbicide in 100 gallons of water) drenching spray rather than for uniform spreading in weed control as is the purpose of related formulations of the chemicals. Thus, the recommended amount of herbicide for a given area cannot be established with accuracy. It is doubtful that application of this mixture will be sufficiently concentrated or repeated at close enough intervals to approach the herbicide levels at which significant inhibition was observed in these tests; however, sufficient evidence is presented to suggest caution in the repeated use of this or any chemical having toxic effects against the biota of the soil community. In a time when

it is thought that any soil condition may be rectified by application of artificial materials, a gentle hand is needed to prevent serious and perhaps irreparable consequences.

Table I: Phase I. Algae located by plot, level and date.

ORGANISM	Control			0.5			1.0			2.0			freq. by level		
	I	II	III	I	II	III	I	II	III	I	II	III	A	B	C
CHLOROPHYTA:															
<u>Characium ambiguum</u> Herm.								C							1
<u>Chlorella vulgaris</u> Beyer.	ABC	AB	AB	AB	AB	AB	ABC	AB	AB	ABC	ABC	AB	12	12	4
<u>Chlorococcum</u> sp. #1	AB	A	AB	AB	AB	AB		BC	AB	AB	AB	ABC	10	10	2
<u>Chlorococcum</u> sp. #2	A												1		
<u>Palmella mucosa</u> Kuetz.								C							1
<u>Palmellococcus</u> sp.	A												1		
<u>Protococcus viridis</u> C. A. Ag.	BC	AB	AB	ABC	AB	ABC	ABC	AC	AB	AB	A	AB	11	10	5
<u>Stichococcus bacillaris</u> Naeg.			A		B	AB		AB	AB			A	5	4	
CYANOPHYTA:															
<u>Anabaena catenula</u> (Kuetz.) Born	C			B	A		A		A			A	4	1	1
<u>A. variabilis</u> Kuetz.												A	1		
<u>Cylindrospermum catenatum</u> Ralfs	A	A			B		B	A		B			3	3	
<u>C. licheniforme</u> (Bory) Kuetz.	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	12	12	12

Table I, continued.

ORGANISM	Control			0.5			1.0			2.0			freq. by level			
	I	II	III	I	II	III	I	II	III	I	II	III	A	B	C	
<u>C. majus</u> Kuetz.	BC	AB	B	A	AB	B	AB	C		A		AB	6	7	2	
<u>Lyngbya aerugineo-caerulea</u> (Kuetz.) Gom.									A				1			
<u>Nostoc ellipsosporum</u> (Desmaz.) Raben.	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	12	12	12	
<u>N. muscorum</u> C. A. Ag.	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	ABC	12	12	12	
<u>Oscillatoria tenuis</u> C. A. Ag.						C									1	
<u>Phormidium autumnale</u> (C. A. Ag.) Gom.						C									1	
<u>P. favosum</u> (Bory) Gom.	A		B	B	B	C	B	A		AC	B	B	3	6	2	
CHRYSOPHYTA:																
<u>Hantzschia</u> sp.																
<u>Navicula</u> sp.																
<u>Nitzschia</u> sp.																
<u>Pinnularia</u> sp.																
													TOTALS	94	89	56

Sampling dates:

I=Oct. 20, 1966, prior to treatment

II=Jan. 22, 1967, 90 days after treatment

III=April 22, 1967, 180 days after treatment

Figure I: Growth of Cylindrospermum licheniforme in the presence of TORDON 101 MIXTURE

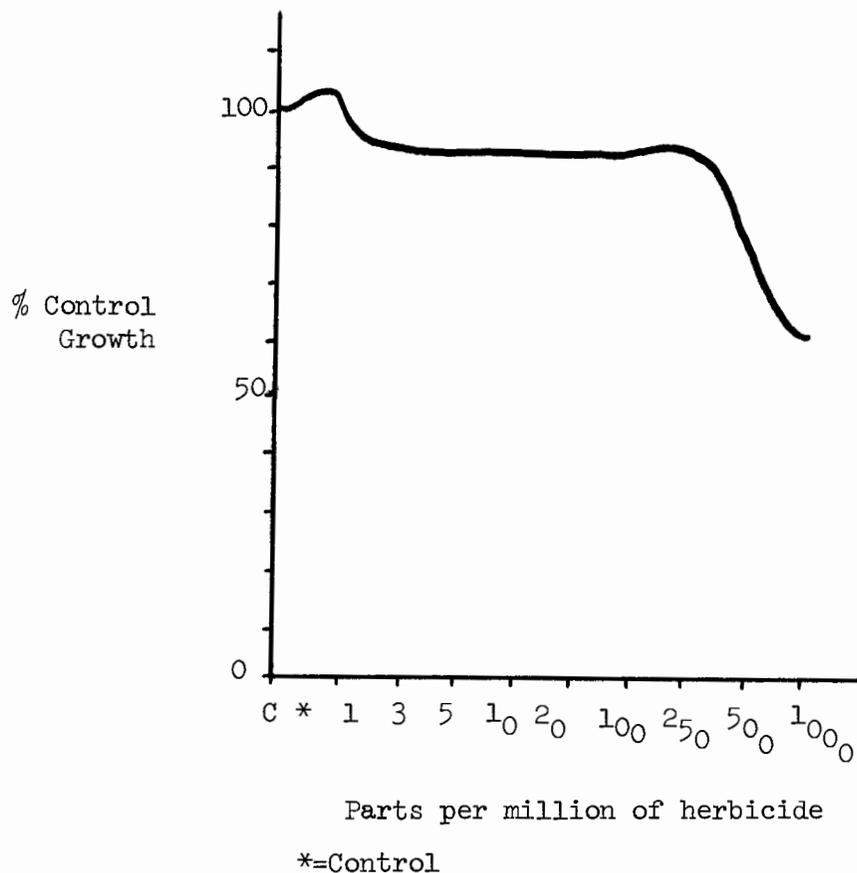


Figure II: Typical Growth Curve of Cylindrospermum licheniforme

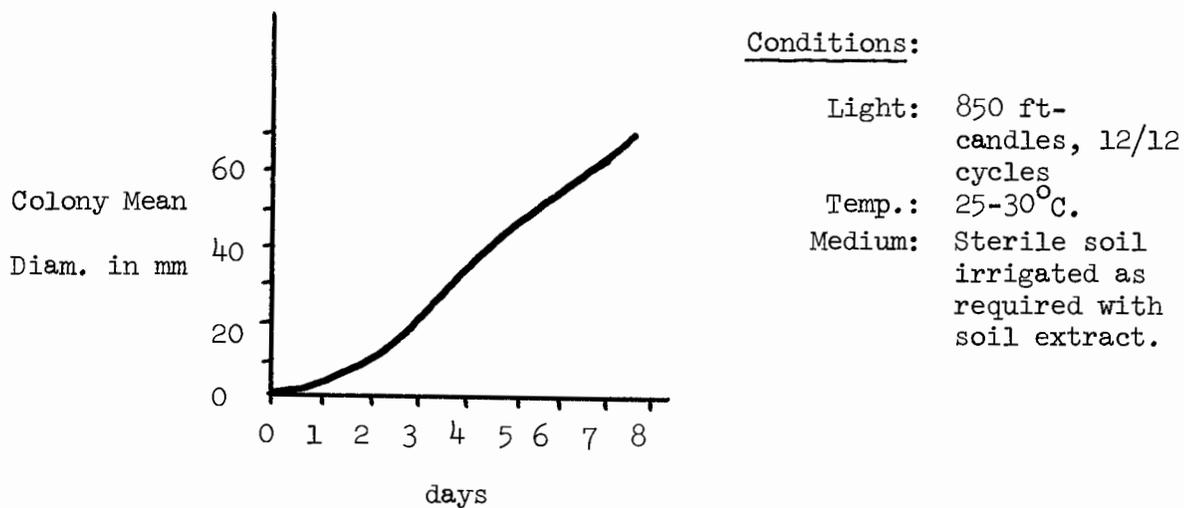


Figure III: Growth of Chlorella vulgaris in the presence of TORDON 101 MIXTURE and Picloram.

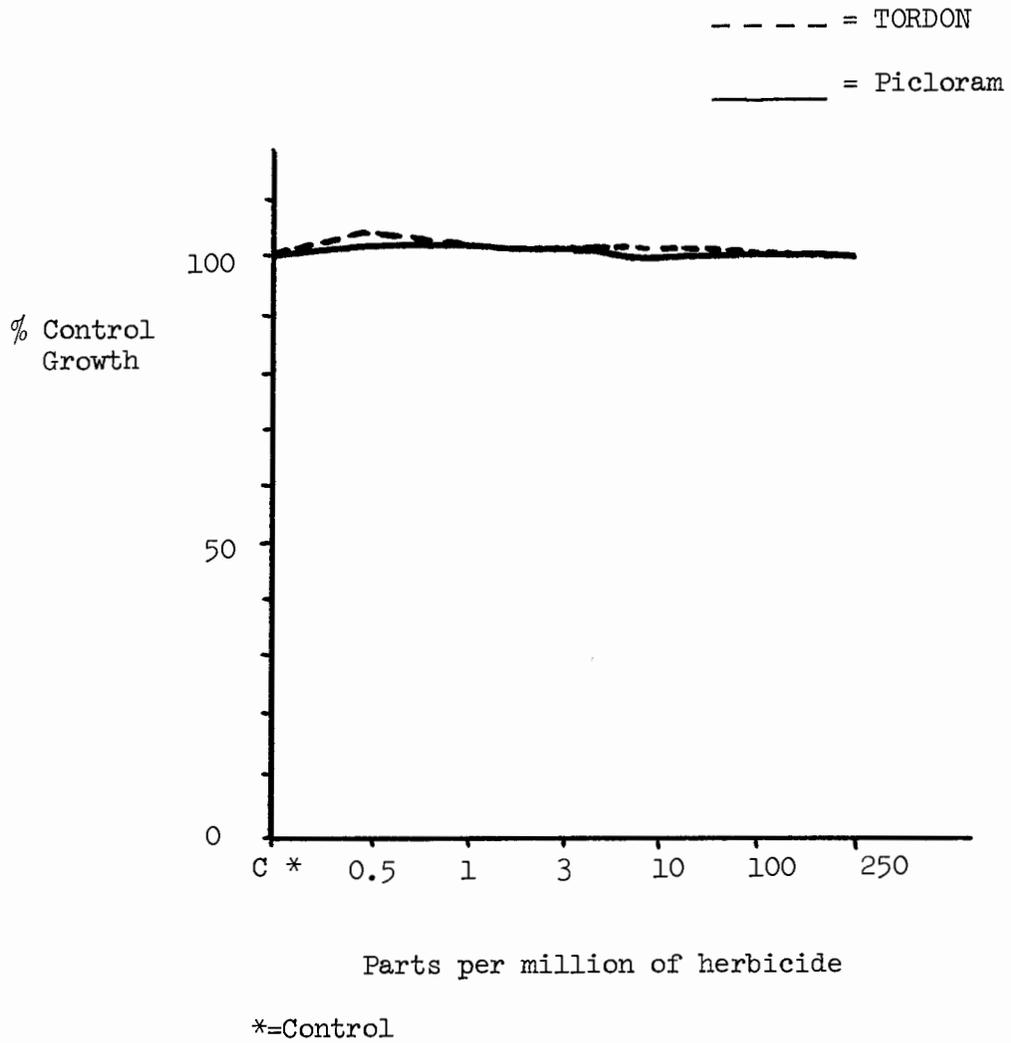
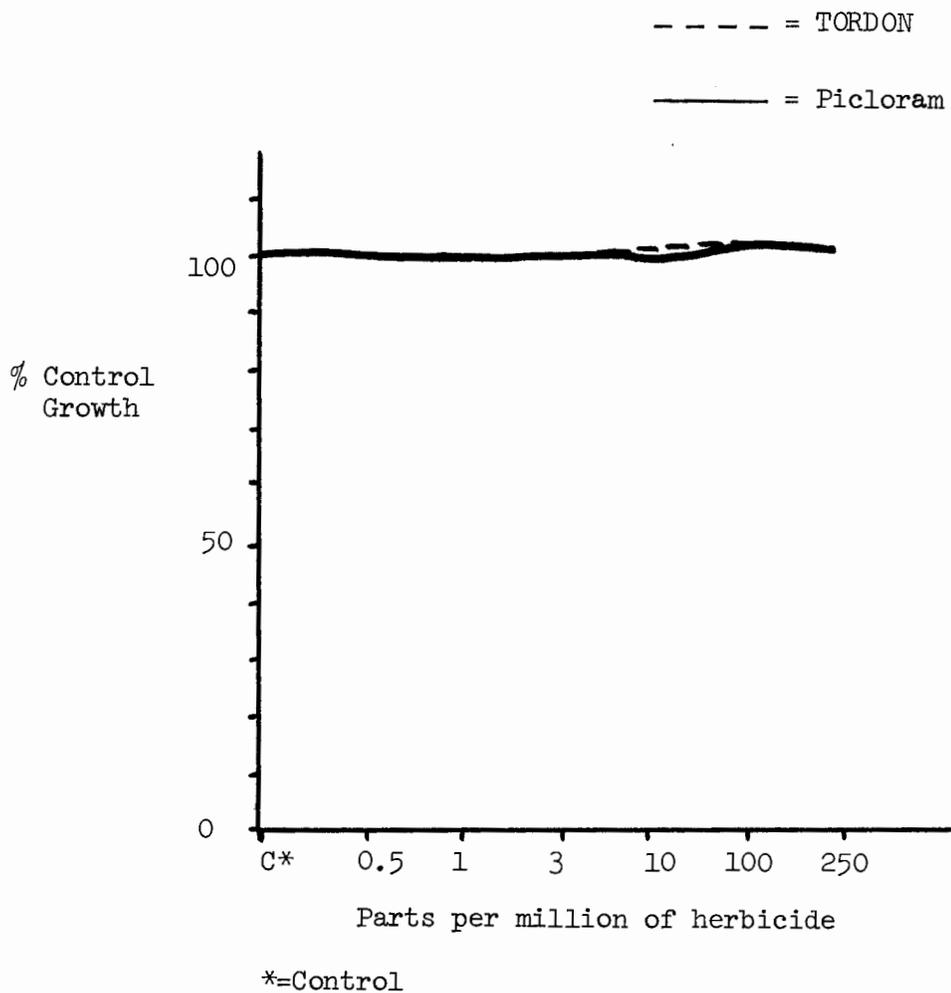


Figure IV: Growth of Protococcus sp. in the presence of TORDON 101 MIXTURE and Picloram.



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