University of Louisville

ThinkIR: The University of Louisville's Institutional Repository

Electronic Theses and Dissertations

5-1975

The effects of selected pesticides on ten isolates of terrestrial algae.

Martha Scheer Salk 1945-
University of Louisville

Follow this and additional works at: <https://ir.library.louisville.edu/etd>

Recommended Citation

Salk, Martha Scheer 1945-, "The effects of selected pesticides on ten isolates of terrestrial algae." (1975). *Electronic Theses and Dissertations*. Paper 1253.
<https://doi.org/10.18297/etd/1253>

This Doctoral Dissertation is brought to you for free and open access by ThinkIR: The University of Louisville's Institutional Repository. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of ThinkIR: The University of Louisville's Institutional Repository. This title appears here courtesy of the author, who has retained all other copyrights. For more information, please contact thinkir@louisville.edu.

THE EFFECTS OF SELECTED PESTICIDES ON TEN ISOLATES
OF TERRESTRIAL ALGAE

By

Martha Scheer Salk
B.A., Albion College, 1967
M.S., University of Iowa, 1969

A Dissertation
Submitted to the Faculty of the
Graduate School of the University of Louisville
in Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

Department of Biology
University of Louisville
Louisville, Kentucky

May, 1975

THE EFFECTS OF SELECTED PESTICIDES ON TEN ISOLATES
OF TERRESTRIAL ALGAE

By

Martha Scheer Salk
B.A., Albion College, 1967
M.S., University of Iowa, 1969

A Dissertation Approved on

Date

by the Following Reading Committee:

Dissertation Director

Chairman

ABSTRACT

Ten algal isolates were obtained from soil into axenic culture and placed into defined media containing from 0 to 50 ppm of DDT, 2,4-D, 2,4,5-T, rotenone, or malathion (in two purities, 95 and 99%). For nine of the isolates, growth was measured photometrically every two weeks for a period of two months. Because of the twisted growth form of the tenth isolate, a visual estimation was used to measure its growth over the same period of time.

The results of the experiments in the light were as follows:

- 1) 2,4-D and 2,4,5-T were generally neutral in their growth effects. However, both stimulated growth of two isolates, and 2,4,5-T inhibited the growth of two other isolates while 2,4-D inhibited only one.
- 2) Rotenone and DDT had mixed effects; rotenone inhibited five, stimulated three, and did not effect two isolates, while DDT inhibited four, stimulated one, and have no effect on five isolates.
- 3) Malathion was neutral toward one isolate and reduced the growth of all others.
- 4) In several cases, the isolates appeared to use the pesticides to supplement their growth. Two of the isolates were better able to withstand the effects of the pesticides and tended to be stimulated more often than the others.

None of the algae were able to use any of the pesticides as a sole carbon source when grown in defined media in the dark for two

months. Several isolates did not survive under those conditions, and none of the isolates survived in a 10 ppm DDT solution.

The effect that pesticide use may have on natural populations of soil algae is discussed.

ACKNOWLEDGMENTS

Many people have graciously and generously given of their time and knowledge to the author during the course of this study. Dr. Varley E. Wiedeman gave valuable assistance throughout, from the initial conception of the study, through its implementation, and during the preparation of this manuscript. I am particularly indebted to Mr. Leroy David Isaacs who patiently taught me the laboratory techniques which were crucial to this work and who gave me advice and assistance whenever I needed it. In addition, Dr. Hugh Spencer and Mr. Carroll Gunter assisted with the statistical analysis; Dr. Arnold Karpoff with the photography; Dr. Arland Hotchkiss with the taxonomic identifications; and Dr. Burt Monroe with a critical review of the manuscript. Dr. R. E. Deems and the American Cyanamid Company kindly supplied the 99% purity malathion sample. And finally, I wish to thank my husband, Gary, who gave me the encouragement to continue whenever I felt like quitting and who endured many dinners of soup and crackers while this paper was being researched and written.

M. S. S.

The University of Louisville

Louisville, Kentucky

August, 1974

TABLE OF CONTENTS

	Page
ABSTRACT	iii
ACKNOWLEDGEMENTS	v
LIST OF TABLES	viii
LIST OF FIGURES	ix
INTRODUCTION	1
REVIEW OF THE LITERATURE	3
Soil Algae	3
Pesticide Persistence	5
MATERIALS AND METHODS	10
RESULTS	16
Description of Algal Isolates	16
Growth of Isolates in Control Media	23
Statistical Analysis	33
2,4-D	38
2,4,5-T	51
DDT	63
Rotenone	80
Malathion	91
Dark Experiment	109
Effects on Each Alga of All Pesticides	112
DISCUSSION	118
SUMMARY AND CONCLUSIONS	130

TABLE OF CONTENTS (Cont'd)

	Page
APPENDIX I: DECOMPOSITION TIME FOR 2,4-D UNDER LAB CONDITIONS	133
APPENDIX II: DECOMPOSITION TIMES FOR 2,4-D UNDER FIELD CONDITIONS	134
APPENDIX III: ORGANISMS WHICH CAN USE 2,4-D FOR A SOLE CARBON SOURCE	135
APPENDIX IV: MICROORGANISMS INVOLVED IN AT LEAST ONE STEP OF DDT BREAKDOWN	136
APPENDIX V: DDT METABOLITES	138
LITERATURE CITED	140
VITA	162

LIST OF TABLES

		Page
TABLE I.	CLASSIFICATION AND SOURCE OF ALGAL ISOLATES USED IN THIS STUDY	17
TABLE II.	VISUAL ESTIMATIONS OF THE GROWTH OF <i>Hormidium flaccidium</i> (10) IN CONTROL MEDIA	30
TABLE III.	ANALYSIS OF VARIANCE TABLE FOR WEEK TWO	34
TABLE IV.	ANALYSIS OF VARIANCE TABLE FOR WEEK FOUR	35
TABLE V.	ANALYSIS OF VARIANCE TABLE FOR WEEK SIX	36
TABLE VI.	ANALYSIS OF VARIANCE TABLE FOR WEEK EIGHT	37
TABLE VII.	VISUAL ESTIMATIONS OF THE GROWTH OF <i>Hormidium flaccidium</i> (10) IN 2,4-D	50
TABLE VIII.	VISUAL ESTIMATIONS OF THE GROWTH OF <i>Hormidium flaccidium</i> (10) IN 2,4,5-T	61
TABLE IX.	ALGAE UNAFFECTED BY DDT AT LOW CONCENTRATIONS	67
TABLE X.	VISUAL ESTIMATIONS OF THE GROWTH OF ISOLATES 1 - 9 IN 50 PPM DDT	78
TABLE XI.	VISUAL ESTIMATIONS OF THE GROWTH OF <i>Hormidium flaccidium</i> (10) IN DDT	79
TABLE XII.	VISUAL ESTIMATIONS OF THE GROWTH OF <i>Hormidium flaccidium</i> (10) IN ROTENONE	90
TABLE XIII.	VISUAL ESTIMATIONS OF THE GROWTH OF <i>Hormidium flaccidium</i> (10) IN MALATHION 95 AND 99	107
TABLE XIV.	GROWTH OF ALGAE IN VARIOUS PESTICIDES IN THE DARK	110

LIST OF FIGURES

	Page
FIGURE 1. - Photomicrographs of the ten algal isolates 10. used in this study	19-22
FIGURE 11. - Effect of control media on growth of isolates 13. used in this study	24-29
FIGURE 14. - Effect of 2,4-D on the growth of algal 16. isolates	44-49
FIGURE 17. - Effect of 2,4,5-T on the growth of algal 19. isolates	55-60
FIGURE 20. - Effect of DDT on the growth of algal isolates 22.	72-77
FIGURE 23. - Effect of rotenone on the growth of algal 25. isolates	84-89
FIGURE 26. - Effect of malathion-95 on the growth of 28. algal isolates	95-100
FIGURE 29. - Effect of malathion-99 on the growth of 31. algal isolates	101-106
FIGURE 32. - Effect of all pesticides on the growth of 33. algal isolates	113-116

INTRODUCTION

The number of different compounds used as pesticides and the total magnitude of their use has increased dramatically since the end of World War II. Each year new pesticides are developed and registered for use, often with little knowledge of their effects on non-target organisms or their behavior in the environment. By many different pathways, some direct, many indirect, most, if not all, of these compounds reach the soil where they may effect any of the multitude of soil organisms, ranging from animals such as earthworms to microorganisms such as bacteria, fungi, and algae. Those studies which are done before registration of new pesticides usually concentrate on their effects on man and other large non-target organisms; e. g., mammals, birds, trees, etc., and exclude the microorganisms which often play a vital role in soil fertility and dynamics. Therefore, recognizing the existence of that void, this study was undertaken to determine the effects of a few selected pesticides on the growth of some soil algae. The pesticides used were selected from widely varying groups in the hope of discovering if there were differences between these groups in their effects on the soil algae. Some of the questions which this study attempted to answer were: Do the pesticides under consideration have any effect on the growth of axenic cultures of soil algae? Is there a relationship between persistence of the pesticide in the soil and its effect on the algae there? Can any of the algae use any of the pesticides as a sole carbon source, thereby, degrading it? It is hoped that the information detailed

in this dissertation will contribute to our understanding of the interactions of pesticides and soil algae.

REVIEW OF THE LITERATURE

Soil Algae

Species of diatoms, other chrysophytes, blue-green, and green algae (Waksman, 1952), as many as 1,000,000 in a gram of well-fertilized soil (Shields and Durrell, 1964), are an often overlooked part of the soil microflora. Most works on soil biology ignore them completely or mention them only briefly, and their importance is probably underestimated (Lund, 1967). Recent studies on the taxonomy of soil algae have indicated that some species are the same ones found in fresh water, but many others are unique to the soil habitat (Deason and Bold, 1960; Mattox and Bold, 1962; Chantanachat and Bold, 1962; Bischoff and Bold, 1963; Shields and Durrell, 1964; Groover and Bold, 1969; Kantz and Bold, 1969; Bold, 1970). Other recent studies have dealt with the distribution of soil algae and annual cyclic changes (Willson and Forest, 1957; Forest, et al., 1959; Potul'nitskii, 1962; Lund, 1962, 1967; Forest, 1962; Shields and Durrell, 1964; MacEntee, et al., 1972). Most of these studies emphasized the importance of combining field and laboratory culture work for the complete identification and characterization of soil algae.

Factors which determine the growth and distribution of soil algae are pH, water requirement and availability, depth in soil, and substrate (Waksman, 1952; Lund, 1962; Shields and Durrell, 1964). They are most commonly found in soil of pH 5.5 to 8.5 (Lund, 1967), but many can tolerate a wider pH range and only at extremes of pH is there any correlation between pH and flora (Lund, 1962); e. g., the green algae

being particularly abundant in acid soil (Waksman, 1952). Many of the species can survive air drying for periods of up to 10 years (Lund, 1962, 1967; Shields and Durrell, 1964; Trainor, 1970) or shorter periods at extreme temperatures (0 C for 6 days, 60 or 100 C for 1 hour, MacEntee, et al., 1972; Lund, 1962, 1967). While algae are found to a considerable depth, they usually are most abundant near the surface (Willson and Forest, 1957; Waksman, 1952; Shields and Durrell, 1964; Lund, 1967); and if buried by rain or earthworms, they can often return to the surface (Peterson, 1935, cited in Lund, 1967). If furnished with an appropriate carbon source, algae can live heterotrophically in the lab in the dark so that those found at great depths in the soil may actually be growing and multiplying there, although probably at a slower rate than in the light (Waksman, 1952; Parker, et al., 1960; Parker, 1961; Lund, 1967). (However, care must be taken in interpreting these studies since organic substrates are often less easily used in soil than in free solution [Alexander, 1964]). Little information is available on the specific nutrient needs of soil algae, but adding phosphorous and nitrogen (Lund, 1962) or manure (Waksman, 1952) will usually increase their growth while the findings on the effects of other elements are contradictory and may be more closely correlated with species. Drewes, in 1928, was the first to definitely establish the ability of *Anabaena* and *Nostoc* species to fix nitrogen (Waksman, 1952). It is now known that about one-third of the algae which can fix nitrogen are found in the soil (Lund, 1962) and are able to fix nitrogen there (Tiffany, 1951; Waksman, 1952; Shields and Durrell, 1964; Berger, 1965; Lund, 1967).

The interaction of soil algae with other organisms may vary from neutral to protocoooperation to competition (Alexander, 1964; Lund, 1967),

but only in a very few cases has a detailed analysis of the specific relationship been attempted (Parker and Bold, 1961; Parker and Turner, 1961). Some algae live more or less symbiotically with higher plants (Lund, 1962; Shields and Durrell, 1964), but they, as a group, flourish most where higher plants are sparse or absent (Lund, 1962).

Algae often serve in the initial stage in plant succession helping to form and adding energy substrates to the soil (Tiffany, 1951; Potul'nitskii, 1962; Alexander, 1964; Shields and Durrell, 1964; Berger, 1965), but there do not seem to be any specific "pioneer" algae, the most abundant adjacent ones simply moving in first (Forest, et al., 1959; Shields and Durrell, 1964). Blue-green algae are among the first organisms which grow on volcanic rock where they form an interwoven mat which stabilizes and forms a non-erodible surface which breaks the force of falling water, improves infiltration, and acts as a substrate for the germination of seeds and spores (Shields and Durrell, 1964). The algal layer, especially in arid and saline soils, is a source of humus and nitrogen, exerts a solvent action on certain soil minerals, and may change the balance of inorganic factors (Lund, 1962; Shields and Durrell, 1964). But in normal soils they do not significantly alter the structure or texture, mainly adding organic matter since either dead or alive they may act as fertilizers (Lund, 1962, 1967). While in climax communities, the algae are mainly heterotrophs using the energy fixed by the higher plants (Alexander, 1964).

Pesticide Persistence

The quantity of pesticides produced and used in the United States each year is staggering. In 1969, 1,333,377,000 pounds (514,099,800 kilograms) were produced here and it is estimated that

over 10^{10} pounds (4.536×10^9 kilograms) have been used since 1945, although there seems to be a slow decline in the use of some pesticides in the last few years (Matsumura, 1972). The over 900 compounds used here as pesticides (Crosby, 1973) may reach the soil by many different routes including direct application, drifting of spray, rainfall, wind blown dust, accidental spills, manufacturing and agricultural by-products and wastes, erosion, and residues from both living and dead plants and animals (Edwards, 1966; Westlake and Gunther, 1966; Crosby, 1973); and it is probable that pesticides are now present in at least small amounts in all parts of the world, even where never applied directly (Westlake and Gunther, 1966). Dr. Howard Reiquam of the Battelle Institute ranks pesticides as the most hazardous of 19 environmental problems due to their world wide range, extreme persistence, and complexity in terms of biological effects, social and political ramifications, air, land, and water involvement, and effects on nutrition sources, but he expects them to drop to number 15 in the future as the most persistent are eliminated (Anon., 1972).

The ultimate environmental fate of any pesticide is difficult to determine because of dilution and dispersal, chemical and biological changes, and inadequate means of detection and identification (Crosby, 1973). The main causes of deactivation are photodecomposition and volatilization from the soil surface, adsorption, leaching, and chemical and biological decomposition by oxidation, reduction, hydrolysis, or other transformations, although the complete mechanisms are not usually known (Horowitz, 1969; Crosby, 1973). Disappearance curves are generally sigmoidal, the more persistent compounds have a long period of slow disappearance followed by a period of rapid reduction (Audus, 1950;

Horowitz, 1969; Kearney, et al., 1970). The most important factor determining the stability of a pesticide in the soil is the structure of the chemical itself (Edwards, 1966; Kearney, et al., 1970; Horowitz, 1969; Alexander, 1965a; Lichtenstein, 1972). Of the soil characteristics which effect persistence, the organic matter content is the most important as it is the major adsorptive site for the chemicals (Edwards, 1966) and, therefore, determines how readily available the chemical will be to attack by microorganisms (Kearney, et al., 1967; Wolcott, 1970). Other factors influencing persistence are moisture, soil temperature, cover crops, cultivation, mode of application, formulation used, wind or air movement, and presence of microorganisms which can attack and biodegrade the pesticide (Lichtenstein, 1965, 1972; Horowitz, 1969). Due to the large number of varying effects, no specific "half-life" can be assigned to one particular pesticide (Lichtenstein, 1972).

Persistence of a pesticide in the soil may be due to its basic non-biodegradability or to its combining with another compound to become unavailable for attack (Alexander, 1965a, 1965b). Many pesticides are recalcitrant since conditions are not always proper for their biological degradation; i.e., organisms may not be present which can degrade them, the compounds may not be in a form suitable for degradation or may not be available to the microorganisms, they may not be capable of inducing formation of the appropriate enzymes for decomposition, or environmental conditions may be toxic or a growth factor missing so the degradation organisms cannot grow or the enzyme cannot work (Kearney, et al., 1967; Audus, 1964; Alexander, 1965a, 1967). Soil type, pH, cation exchange capacity, temperature, organic matter, and moisture content of the soil not only affect the availability and persistence of pesticides in the

soil directly, but also indirectly through effects on microorganisms (Kearney, et al., 1967; Wolcott, 1970; Crosby, 1973). Since different organisms have different metabolic abilities to degrade specific pesticides (Fletcher, 1960; Crosby, 1973), uncommon organisms may become prominent when pesticides are the primary carbon source as the compounds are often unusual and only broken down by a few specific species. Once a suitable population has developed after an initial dose, second and later applications of the pesticide will disappear more rapidly (Alexander, 1971).

Since there is no clear relationship between the structure of a chemical and its sensitivity to degradation, each new compound must be tested individually to find out how it will react (Moore, 1967; Horowitz, 1969). Complicating factors in predicting persistence include a difference in disappearance rates between the lab and the field, the effect of interactions of organisms since many compounds are broken down by combinations of several microbes, and presence of a second carbon compound (e. g. glucose) which can result in degradation of normally resistant compounds (Alexander, 1965b; Cripps, 1971). The latter factor called "cometabolism" or "cooxidation" appears to be widespread and may result when a foreign molecule is acted on by an enzyme which normally has a similar function, but in this case the product is not used for growth because it cannot be further metabolized or because it is toxic (Alexander, 1967, 1971).

Therefore, because of the extensive use of pesticides, their common persistence, and the inadequate knowledge of the ecology of soil algae, this study was undertaken in an attempt to determine some of the interactions between soil algae and pesticides. Since the

problem is very complex, this study is only a bare beginning which included isolating organisms from soil into axenic culture, growing them on artificial media in the lab, and testing the effects of certain pesticides on their growth under carefully controlled conditions.

MATERIALS AND METHODS

Soil samples were collected from several sites in Oldham and Jefferson Counties, Kentucky. Approximately ten grams of soil from each sample were placed in 125 ml Ehrlenmeyer flasks and covered with 50 ml of Tris-buffered inorganic medium (TBIM) (Smith and Wiedeman, 1964), which is described below.

TBIM

Chemical compound	Amount	Final concentration	
		mg/liter	µM/liter
KNO_3	20 ml of 0.1 M solution	202	2000
Na_2HPO_4	10 ml of 0.1 M solution	142	1000
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	3 ml of 0.1 M solution	64	300
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	1 ml of 0.1 M solution	14	100
Tris(hydroxymethyl)-aminomethane (TRIS)	25 ml of 0.2 M solution	606	5000

Each of the above was added to approximately 800 ml of deionized or glass-distilled water. One ml of each of the micronutrient stock solutions was then added and a final dilution to one liter made. The pH of the medium was adjusted to 7.5 with 1.0 N HCl.

Micronutrient Stock Solutions:

	Amount	Final concentration	
		mg/liter	µM/liter
I. EDTA	50 g	per liter deionized or glass-distilled water	170
KOH, 85%	31 g		470
II. H_3BO_3	11.42 g/liter	11.42	185
III. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	4.98 g/liter acidified water*	4.98	17.8

		Amount	Final concentration	
			mg/liter	μM/liter
IV.	ZnSO ₄ ·7H ₂ O	8.82 g	8.82	30
	MnCl ₂ ·4H ₂ O	1.44 g	1.44	7.3
	(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	0.87 g	0.87	4.9
	CuSO ₄ ·5H ₂ O	1.57 g	1.57	6.3
	Co(NO ₃) ₂ ·6H ₂ O	0.49 g	0.49	1.7

per liter

acidified water*

*Acidified water: 999 ml deionized or glass-distilled water
1 ml concentrated H₂SO₄

The flasks were placed in a plant growth chamber at a temperature of 22 C with a 12 hour cycle of alternating light and dark periods provided by Natur-escent¹ lights, henceforth termed standard conditions. In addition, ten-gram samples of soil were placed in the bottom halves of steril petri plates, moistened daily with deionized water, and left covered in front of a south facing window.

The algae which grew under these conditions were streaked on TBIM solidified with 1.5% agar. Unialgal isolations were made using the methods of Bold (1942), Pringsheim (1946), and Lewin (1959). The algae were isolated into axenic culture using a modification of the atomizing technique of Wiedeman, et al. (1964). Instead of ultrasonication, the algal cells were soaked for two hours in 10 ml of the following solution:

50 ml TBIM

45 ml deionized water

5 ml full strength Walgreen Justrite clear lotion detergent.

The cells were then centrifuged, washed with TBIM 10 times, and sprayed onto a sterile plate of proteose-peptone-TBIM agar. Cells

1) Duro-Lite Lamps, Inc., Fair Lawn, N.J.

which appeared to be bacteria-free after having grown for several days were inoculated into five ml of TBIM in 10 x 100 mm Pyrex, rimless test tubes and plugged with non-absorbent cotton. Approximately two months later, these cultures were streaked onto proteose peptone, nutrient, yeast extract, and malt extract agar plates, and into fluid thioglycolate to determine if they were axenic. The plates and tubes were incubated for two weeks at 37 C, and any contaminated cultures were disposed of at the end of that time. Stock cultures were grown in 10 ml of TBIM in 18 x 130 mm Pyrex, rimless test tubes plugged with non-absorbent cotton, wrapped with transparent plastic film, and secured with rubber bands. Reserve stocks were kept on TBIM and TBIM plus proteose-peptone agar slants (the latter as a check for possible bacterial contamination). All stocks were grown under standard conditions. After the isolates had grown on the slants, liquid TBIM was added aseptically to the tubes to cover about two-thirds of the exposed agar. The liquid TBIM stocks were used for all experimental inocula.

Five pesticides were selected for use covering a broad range of pesticide types. Each chemical was used at six concentrations: 0.1, 0.5, 1.0, 5.0, 10.0, and 50.0 parts per million. The pesticides used and the molar concentrations used for each were as follows:

2,4-D (2,4-dichlorophenoxy acetic acid); 0.45, 2.26, 4.5, 22.4, 45, and 226 μM ;

2,4,5-T (2,4,5-trichlorophenoxy acetic acid); 0.39, 1.96, 3.9, 19.6, 39, and 196 μM ;

rotenone ($\text{C}_{23}\text{H}_{22}\text{O}_6$); 0.25, 1.27, 2.5, 12.7, 25, and 127 μM ;

malathion (0,0-dimethyl S-bis (carboethoxy) ethyl phosphorodithioate); in two formulations, 95% and 99% purity; 0.30, 1.51, 3.0, 15.1, 30, and 151 μM ; and

DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane); 0.28, 1.41, 2.8, 14.1, 28, and 141 μ M.

A dosage of one pound per acre (1.121 kg/hectare) results in a concentration in the upper soil layer of approximately 2 to 2.5 ppm (Fletcher, 1960). Therefore, concentrations were selected which ranged proportionately around the average recommended dose of 2 to 2.5 pounds per acre. The sodium salts of 2,4-D and 2,4,5-T were used since they are more water soluble than their acid equivalents. Rotenone, DDT, and malathion were dissolved in ethanol before being added aseptically to the sterilized growth medium. Ethanol was added at a rate of 20 ml per liter of growth medium. Since DDT should not be used in an alkaline medium or with iron salts (Stecker, 1968), Bold's Basal Medium II (BBM2) minus iron as described below was used instead of TBIM for the experiments with DDT.

Bold's Basal Medium Modified (BBM2) (Cain, 1965)

Ten milliliters of each of the following stock solutions was added to approximately 800 ml of deionized or glass distilled water;

NaNO_3	2.5%	12.5 g/500 ml
KH_2PO_4	1.5%	7.5 g/500 ml
K_2HPO_4	1.0%	5.0 g/500 ml
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.75%	3.75 g/500 ml
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.25%	1.25 g/500 ml
NaCl	0.25%	1.25 g/500 ml

To this was added one ml of each of the micronutrient stock solutions (as described for TBIM) and a final dilution to one liter made. The pH of the resultant medium was 6.6 after autoclaving and cooling.

Each alga was grown under all the following conditions:

Controls:	TBIM
	TBIM + 2% ethanol,
	BBM2
	BBM2 - Fe,
	BBM2 - Fe + 2% ethanol.
Experimental groups:	DDT in 2% ethanol in BBM2 - Fe (6 concentrations);
	malathion, 95% purity in TBIM + 2% ethanol (6 concentrations);
	malathion, 99% purity in TBIM + 2% ethanol (6 concentrations);
	2,4-D in TBIM (6 concentrations);
	2,4,5-T in TBIM (6 concentrations); and
	rotenone in TBIM + 2% ethanol (6 concentrations).

Each experiment was run in six replications under standard conditions. Growth was measured at two week intervals for a period of two months at a wavelength of 425 nm with a Spectronic 20, and also at 12 weeks for those isolates which showed slow growth in one or more pesticides.

A further experiment was conducted in the dark. All controls and the following concentrations of pesticides were used:

rotenone, 50 and 10 ppm,
malathion, 99% purity, 50 ppm,
malathion, 95% purity, 50 ppm,
DDT, 10 ppm,
2,4-D, 50 ppm, and
2,4,5-T, 50 ppm.

Each experiment was run in six replications at 22 C in the dark for a period of two months. Growth was determined visually at the end of that period as none, trace minus minus minus, trace minus minus, or trace minus.

Photomicrographs were made of each isolate at the end of eight weeks with a Zeiss RA microscope with Nikon camera and shutter components on Kodak Panatomic X black and white film.

RESULTS

Description of the Algal Isolates

Ten isolates from soil collected in Jefferson and Oldham Counties, Kentucky were obtained in axenic culture for use in this study. Nine of the isolates are members of the Division Chlorophycophyta. The tenth, isolate 8, could not be readily identified and is, therefore, listed as unknown. This isolate gave a very weak or negative reaction when tested for the presence of starch with potassium iodine and is, therefore, possibly a member of the Division Chrysophycophyta. Since the primary purpose of this investigation was ecological rather than taxonomic, the complete identification of this alga has been reserved for those with taxonomic inclinations. It is possible that this alga is a new species or even a new genus, as the taxonomic study of soil algae is still very much in its infancy and most investigations describe one or more new species (Bold, 1970).

The other nine isolates have been identified to genus, and if the organism were particularly distinct, to species. The isolates are listed in Table 1 following the classification of Smith (1950) with a description of their source. Identifications which are tentative are so indicated by a question mark. Figures 1 - 10 are photomicrographs of each of the isolates.

As can be seen by an inspection of Figure 10, isolate 10, *Hormidium flaccidum*, has an unusual growth habit. This organism forms twisted ropes when growing in culture. Because of this, the colony develops as a tightly intertwined mat. This mat is so interwoven

TABLE I

CLASSIFICATION AND SOURCE OF ALGAL ISOLATES USED IN THIS STUDY

Classification	Isolation number	Isolation source
Division Chlorophycophyta		
Class Chlorophyceae		
Order: Ulotrichales		
Family: Ulotrichaceae		
<i>Hormidium</i> sp. ?	1	Rose bed, Southwest Jefferson County
<i>Hormidium flaccidium</i> A. Br.	10	Rose bed, Southwest Jefferson County
<i>Stichococcus chodati</i> (Bial.) Heering	2	Ant Hill, Horner Wildlife Refuge, Oldham County
<i>Stichococcus chodati</i> (Bial.) Heering	6	Rose bed, Southwest Jefferson County
Order: Chlorococcales		
Family: Chlorococcaceae		
<i>Chlorococcum</i> sp.	3	Woods, Horner Wildlife Refuge, Oldham County
<i>Chlorococcum</i> sp.	5	Sod Field, Horner Wildlife Refuge, Oldham County
<i>Neochloris</i> sp. ?	7	Tobacco Field, Horner Wildlife Refuge, Oldham County
<i>Bracteacoccus</i> sp. ?	4	Cedar Knoll, Horner Wildlife Refuge, Oldham County

TABLE I (Cont'd)

Classification	Isolation number	Isolation source
Family: Oocystaceae		
<i>Chlorella</i> sp.	9	Abandoned field, Horner Wildlife Refuge, Oldham County
Alga of unknown classification	8	Cedar Knoll, Horner Wildlife Refuge, Oldham County

FIGURE 1. Isolate 1: *Hormidium* sp. ?

FIGURE 2. Isolate 2: *Stichococcus chodati* (Bial.) Heering

FIGURE 3. Isolate 3: *Chlorococcum* sp.

FIGURE 4. Isolate 4: *Bracteacoccus* sp. ?

FIGURE 5. Isolate 5: *Chlorococcum* sp.

FIGURE 6. Isolate 6: *Stichococcus chodati* (Bial.) Heering

All figures are x 430.

FIGURE 7. Isolate 7: *Neochloris* sp. ?

FIGURE 8. Isolate 8: Unknown

FIGURE 9. Isolate 9: *Chlorella* sp.

FIGURE 10. Isolate 10: *Hormidium flaccidium* A. Br.

All figures are x 430.

that it is not possible to disperse the colony adequately to get an accurate reading with a Spectronic 20. Therefore, for isolate 10, only visual estimations of growth were possible. The 15 grades used were None, Trace minus minus minus, Trace minus minus, Trace minus, Trace, Trace plus, Fair minus, Fair, Fair plus, Good minus, Good, Good plus, Excellent minus, Excellent, and Excellent plus. Only 2 or more degrees of difference, except in T---, T--, and T-, were regarded as significant (Wiedeman, 1964).

Algal Growth in Control Media

As noted previously, five different media were used for controls. Which medium served as the control for a particular pesticide depended on whether or not the pesticide was water soluble and whether or not it could be used in an alkaline medium. No attempt was made to determine optimal pH for the growth of the isolates or to determine if there were changes in the pH of the media during the course of the experiment as can happen in culture (Wiedeman, 1964). Figures 11-13 show the growth of isolates 1-9 in all five control media, and Table II gives the visual growth measure for isolate 10 in each of them.

There was a very distinct difference in most of the isolates between growth in TBIM media (alkaline) and that in BBM2 media (acid). Only *Hormidium* sp (1) and *Hormidium flaccidium* (10) grew generally as well or better in BBM2 than in TBIM based media, with the difference being more pronounced for isolate 1 than isolate 10. These two species of *Hormidium* had limited growth in all defined media. Growth was so slow that observations were extended to 12 weeks instead of the 8 week period which was adequate for most other isolates under most conditions. Even after 12 weeks, growth was better than fair only for isolate 1 in

FIGURE 11

Effect of control media on growth of isolates 1 - 3. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for the first graph are applicable to the following two graphs.

1. *Hormidium* sp. (1)
2. *Stichococcus chodati* (2)
3. *Chlorococcum* sp. (3)

FIGURE 11 Controls

— TBIM
- - - TBIM + EtOH

— BBM2
- - - BBM2 - Fe
... BBM2 - Fe + EtOH

FIGURE 12

Effect of control media on growth of isolates 4 - 6. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 4 are applicable to the other two graphs.

4. *Bracteacoccus* sp. (4)
5. *Chlorococcum* sp. (5)
6. *Stichococcus chodati* (6)

FIGURE 12 Controls

— TBIM
- - - TBIM + EtOH
— BBM2
- - - BBM2 - Fe
... BBM2 - Fe + EtOH

FIGURE 13

Effect of control media on growth of isolates 7 - 9. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 7 are applicable to the other two graphs.

7. *Neochloris* sp. (7)
8. Unknown (8)
9. *Chlorella* sp. (9)

FIGURE 13**Controls**

— TBIM
- - - TBIM + EtOH
— BBM2
... BBM2 - Fe
... BBM2 - Fe + EtOH

TABLE II

VISUAL ESTIMATIONS OF THE GROWTH OF *HORMIDIUM FLACCIDIUM* (10)
IN CONTROL MEDIA

	Time (weeks)				
Medium	2	4	6	8	12
TBIM	T---	T-	T+	F-	F
TBIM + EtOH	T---	T---	T--	T--	T
BBM2	T---	T--	T-	F-	F
BBM2 - Fe	T---	T--	T-	F-	F
BBM2 - Fe + EtOH	T---	T--	T-	F-	F

BBM2 - Fe, while that same isolate only had a trace of growth at 12 weeks in both TBIM media. Lower growth for these isolates could be due to several factors. Mattox and Bold (1962) reported that only one of their isolates of *Hormidium* sp. grew better than fair in a completely inorganic Bristol's solution which was very similar to the BBM2 used in this study. The one isolate which grew very well in Bristol's solution was from an aquatic environment, the rest being terrestrial in origin (except those from the Culture Collection of Algae at Indiana University whose origins were unknown to Mattox and Bold). The addition of a vitamin mixture to the Bristol's solution increased the growth of many of the cultures to excellent. No attempt was made to determine which specific vitamin (or vitamins) was required. Therefore, the low growth in this study could be due to a vitamin deficiency. Also, tris in some cases is toxic to algae (Mc Lachlan, 1963) which could be the reason for the lower growth of isolate 1 in TBIM than in BBM2. Also, this *Hormidium* sp. might prefer a slightly acid rather than slightly alkaline pH for optimum growth as has been reported for some algal species (Wiedeman, 1964).

For the other eight isolates, growth was significantly depressed in BBM2 as compared to TBIM media. Three isolates, *Bracteacoccus* (4), *Neochloris* (7), and *Chlorella* (9), had such a reduced growth that observations were extended on those isolates in all BBM2 media, both control and experimental, to 12 weeks. Even after 12 weeks, growth in all but one case (*Neochloris* in BBM2 - Fe) was less than in the TBIM control media at 8 weeks. Isolate 8 (unidentified) had a difference in growth only at the 12 week observation point, with significantly more growth at that time in the TBIM media than in the BBM2. Since TBIM was

used for isolation of the algae, their better growth in that medium could simply be a result of selection for organisms which grow well under those conditions.

In most cases, the growth curves for the isolates were very similar in media with and without ethanol. In TBIM, five isolates, *Stichococcus chodati* (2), *Chlorococcum* (3) and (5), *Bracteacoccus* (4) and unidentified (8), had slightly better growth in the presence of ethanol at three or more of the sampling points. One isolate, *Neochloris* (7) had better growth in TBIM + ethanol at two sampling points and less growth at the other two. The remaining four isolates, *Hormidium* (1), *Stichococcus chodati* (6), *Chlorella* (9), and *Hormidium flaccidium* (10) had better growth in the absence of ethanol at three or more of the sampling points. This may indicate that the first groups of organisms can use ethanol to supplement their growth in the light. Even for those organisms with slightly depressed growth in ethanol, the reduction was insignificant compared to the differences between growth in TBIM and BBM2 media.

For the control media based on BBM2, there was also little difference in growth rates in most cases with and without ethanol. For *Stichococcus chodati* (2), *Chlorococcum* (3), *Bracteacoccus* (4) and *Hormidium flaccidium* (10), the growth curves in the three media were almost identical. In three other cases (*Chlorococcum* (5), *Stichococcus chodati* (6), and *Chlorella* (1)) there was a slight reduction in growth with the elimination of Fe and the addition of ethanol (BBM2 - Fe + EtOH). *Chlorococcum* (5) had nearly identical curves for growth in BBM2 - Fe with and without ethanol. For the other two isolates, there was less growth in BBM2 - Fe + EtOH than in BBM2 - Fe which was in turn

slightly less than in BBM2. Isolate 1, *Hormidium* sp., had almost identical growth in all three BBM2 media for the first eight weeks. However, at the 12 week sampling point, growth was much higher in BBM2 - Fe and slightly higher in BBM2 - Fe + EtOH than in BBM2. Isolate 8, unidentified, had a reduction in growth from BBM2 to BBM2 - Fe, but the addition of ethanol resulted in a growth curve nearly identical to that of BBM2. Finally, isolate 7, *Neochloris* sp., had better growth in BBM2 - Fe than in BBM2 which was in turn slightly better than BBM2 - Fe + EtOH. None of the differences within the BBM2 control media were as great as the growth differences between the BBM2 and the TBIM media. Therefore, it is concluded that the addition of 2% ethanol or the absence of iron salts from the media did not adversely effect the experimental organisms during the course of the experiment.

Statistical Analysis

A 9 by 6 by 7 factorial analysis of variance was made of the experimental data with the variables being respectively algae, pesticides, and concentrations. Tables III - VI are the summary tables for the analysis of variance for weeks 2, 4, 6, and 8 respectively. Examination of the F values of each table indicates that all variables are significant at least at the $p < 0.01$ level for all main effects and all interactions for each data collection period point. However, as Winer (1971) states,

In many cases which arise in practice, tests on main effects may be relatively meaningless when interactions are significantly different from zero.

Therefore, it is difficult to analyze the significance of the two way interactions and the main effects since the three way interaction is significant. This indicates a high level of statistical complexity. Thus, it cannot be said what simple effect the different algae had

TABLE III
ANALYSIS OF VARIANCE TABLE FOR WEEK TWO

Source	Error Term	F	Sum of Squares	Deg. of Freedom	Mean Square
1 Mean			3.265985	1	3.265985
2 I	R(IJK)	328.87	.9531845	8	.1191480
3 J	IJ	19.606	.3472589	5	.06945175
4 K	IK	11.456	.1437431	6	.02395718
5 IJ	IJK	6.4375	.1416953	40	.003542381
6 IK	IJK	3.8004	.1003799	48	.002091249
7 JK	IJK	11.5544	.1907437	30	.006358124
8 IJK	R(IJK)	1.5233	.1320663	240	.0005502761
9 R(IJK)			.6827394	1890	.0003612377

I = Algae

J = Pesticides

K = Concentrations

TABLE IV
ANALYSIS OF VARIANCE TABLE FOR WEEK FOUR

Source	Error Term	F	Sum of Squares	Deg. of Freedom	Mean Square
1 Mean			22.10565	1	22.10565
2 I	R(IJK)	575.8733	6.632678	8	.8290848
3 J	IJ	14.501162	1.983252	5	.3966503
4 K	IK	5.341488	.3328799	6	.05547998
5 IJ	IJK	12.0705	1.094124	40	.02735309
6 IK	IJK	4.5835	.4985601	48	.01038667
7 JK	IJK	9.8473	.6694537	30	.02231512
8 IJK	R(IJK)	1.5739	.5438679	240	.002266116
9 R(IJK)			2.721203	1890	.001439790

I = Algae

J = Pesticides

K = Concentrations

TABLE V
ANALYSIS OF VARIANCE TABLE FOR WEEK SIX

Source	Error Term	F	Sum of Squares	Deg. of Freedom	Mean Square
1 Mean			68.31825	1	68.31825
2 I	R(IJK)	851.18254	21.31157	8	2.663946
3 J	IJ	11.983752	5.199875	5	1.039974
4 K	IK	5.8064516	.8983440	6	.1497240
5 IJ	IJK	15.7500	3.471283	40	.08678204
6 IK	IJK	4.6799	1.237719	48	.02578580
7 JK	IJK	8.3078	1.373275	30	.04577582
8 IJK	R(IJK)	1.7605	1.322389	240	.005509950
9 R(IJK)			5.915161	1890	.003129715

I = Algae

J = Pesticides

K = Concentrations

TABLE VI
ANALYSIS OF VARIANCE TABLE FOR WEEK EIGHT

Source	Error Term	F	Sum of Squares	Deg. of Freedom	Mean Square
1 Mean			144.4903	1	144.4903
2 I	R(IJK)	980.61466	44.46577	8	5.558222
3 J	IJ	9.3267721	10.12167	5	2.024333
4 K	IK	2.9131615	.8347748	6	.1391291
5 IJ	IJK	16.0231	8.681816	40	.2170454
6 IK	IJK	3.5257	2.292422	48	.04775880
7 JK	IJK	5.3635	2.179599	30	.07265329
8 IJK	R(IJK)	2.3898	3.250994	240	.01354580
9 R(IJK)			10.71276	1890	.005668126

I = Algae

J = Pesticides

K = Concentrations

without also taking into consideration the effects of pesticides and concentrations on algae. Likewise, there is no simply interpretable effect for either pesticides or concentrations. The statistical analysis further indicates that the joint effects of algae by pesticides, algae by concentrations, or pesticides by concentrations cannot be considered without also considering the third variable. Thus, care must be taken in the interpretation of the independent effects due to algae, pesticides, or concentrations and the two way interactions of these variables. Further, since the interactions are significant, these data cannot be clearly extrapolated to other algae, pesticides, or concentrations. Therefore, the analysis in the following sections will concentrate on the interaction effects.

2,4-D Introduction

The selective herbicidal properties of the chlorinated phenoxy acetic acids were discovered in 1942, but were not publicized until the end of World War II (Martin, 1964). In spite of the numerous studies done since then, the reason for their herbicidal behavior is still not understood at the cellular and molecular level (Moreland, 1967). The recommended agricultural dose is less than 5 lb/A (5.604 kg/H) (Edwards, 1964). In 1968, 142,248 pounds (64,523.69 kg) of all forms of technical material of 2,4-dichlorophenoxy acetic acid (2,4-D) were sold in Kentucky (Moore, 1973), while in 1969, 68,624,000 pounds (31,127,846 kg) of 2,4-D and 2,4,5-T were produced in the United States (Matsumura, 1972). A national survey of pesticide use on croplands in 1969 found that 15.14% of 1684 sites had been treated with 2,4-D at an average level of 0.54 lb/A (0.605 kg/H). Of 188 sites tested for residues, 3 (1.6%) had detectable residues at a mean level of less than 0.01 ppm and a range

of 0.01 to 0.03 ppm (Wiersma, Tai, & Sand, 1972b). In Kentucky, 3.2% of 31 cropland sites surveyed had had 2,4-D application at an average level of 0.50 lb/A (0.56 kg/H) (Wiersma, Tai, & Sand, 1972b).

Degradation of 2,4-D is rapid and can be due to the molecule combining with other cell molecules, to cleavage of the side chain, or to ring hydroxylation or ring cleavage (Hilton, et al., 1963; Alexander, 1965b; Wright, 1971). Reported detoxification times in the lab or greenhouse range from six to seven days to 18 months and in the field from one week to over 160 days (Appendices I & II). Environmental factors which influence the persistence of 2,4-D in soil include depth, organic matter, cation exchange capacity, exchangeable calcium, moisture, free drainage value, total exchangeable bases, soil type, temperature, pH, leaching, type of forest litter, chemical formulation, presence of DDT, and presence of lime (Newman, et al., 1952; Hanks, 1946; Nutman, et al., 1945; Kries, 1947; Martin, 1946; Weaver, 1948; Brown and Mitchell, 1948; DeRose and Newman, 1948; Jorgensen and Hamner, 1948; Crafts, 1949; Norman, et al., 1950; Blackman, et al., 1951; Ogle and Warren, 1954; Bell, 1960; Burger, et al., 1962; Upchurch and Mason, 1962; Upchurch, 1966; Norris and Greiner, 1967; Boyce Thompson Institute, 1971; Montgomery, et al., 1972).

There are three stages in decomposition: immediate initial adsorption on soil colloids, a lag phase, and rapid and complete detoxification (Audus, 1951; Montgomery, et al., 1972). The actual decomposition is due to the action of microorganisms in most cases (Audus, 1949, cited in Bollen, 1961; Audus, 1950; Norman, et al., 1950), as conditions which destroy or inhibit microbes increase persistence (Audus, 1951; DeRose and Newman, 1948; Brown and Mitchell, 1948; Hernandez

and Warren, 1950, cited in Audus, 1964), and over 10 different species of bacteria, actinomycetes, and fungi have been isolated which can use 2,4-D as a sole carbon source (Appendix III). After initial decomposition, additional applications are detoxified without the long lag phase (Audus, 1951; Newman, et al., 1952; Rogoff and Reid, 1956). Also, CO_2 is evolved from soil in which 2,4-D is the only carbon source (Martin, 1946; Jensen and Peterson, 1952). Finally, adding organisms capable of detoxification to the soil causes breakdown typical of subsequent rather than initial application (Audus, 1950).

Determinations of the breakdown pathway suggest that it is not the same in all species. One frequent intermediate is 2,4-dichlorophenol (Audus, 1952; Evans and Smith, 1954; Steenson and Walker, 1957; Loos, et al., 1967), which may be further metabolized to 4-chloro, 2-hydroxyphenol (Steenson and Walker, 1957). Other reported intermediates are 6-hydroxy-2,4-dichlorophenoxyacetic acid (Evans and Smith, 1954), 2,4-dichloro-5-hydroxyphenoxyacetic acid (Woodcock, 1964; Faulkner and Woodcock, 1954, 1965), 2,5-dichloro-4-hydroxyphenoxy-acetic acid (Faulkner and Woodcock, 1964, 1965), and α -chloromuconic acid (Fernley and Evans, 1959).

Although a few cases of inhibition of microorganisms by 2,4-D at normal weed control doses (about 10 ppm or less) are known, most reports of negative effects are at concentrations much higher than ordinarily used. In some cases stimulation has been noted either at normal or even high concentrations (Smith, et al., 1945; Stevenson and Mitchell, 1945; Martin, 1946, 1963; Lewis and Hamner, 1946; Dubos, 1946; Newman, 1947; Payne and Fults, 1947; Fults and Payne, 1947;

Carlyle and Thorpe, 1947; Worth and McCabe, 1948; Norman, et al., 1950; Kratochvil, 1951; Gould and Hamstead, 1951; Warren, et al., 1951; Aldrich, 1953; Colmer, 1953; Roberts and Bollen, 1955; Johnson and Colmer, 1955a and b; Magee and Colmer, 1955; Rogoff and Reid, 1956; Walker and Newman, 1956; Bell, 1957; Frans, et al., 1957; Newman and Downing, 1958; Treater, et al., 1958; Woodford, et al., 1958; Fletcher, 1960; Whiteside and Alexander, 1960; Il'in, 1962; Mashtatov, et al., 1962; Petruk, 1964; Audus, 1964; Dean and Law, 1964; Goodman, 1965; Farm Chemicals Handbook, 1965; Upchurch, 1966; Mickovski, et al., 1968; Montgomery, et al., 1972; and the following all cited in Pimentel, 1971: Slepecky and Beck, 1950; Anderson and Baker, 1950; Hoover and Colmer, 1953; Rapoport and Cangioli, 1963; Bounds and Colmer, 1964; Arnold, et al., 1966).

In most cases, studies of 2,4-D and algae also find negative effects only at high concentrations. In sea water, 1.0 ppm had little effect on productivity of phytoplankton (Butler and Springer, 1964, cited in Loosanoff, 1965); while four hours exposure to 1.0 ppm did not decrease phytoplankton growth (Butler, 1963, cited in Pimentel, 1971). Growth of *Nitzschia palea* was inhibited by 2 ppm 2,4-D at three days, but from 7-21 days was the same as the control; *Cylindrospermum licheniforme* had reduced growth at three days, but not at 7 - 21 days; and four other algae tested in unialgal cultures (*Microcystis aeruginosa*, *Scenedesmus obliquus*, *Gomphonema parvulum*, and *Chlorella varigata*) were not affected at any time up to three weeks (Palmer and Maloney, 1955). At 20 ppm and up, *Anabaena* was significantly inhibited (inhibition increasing with concentration); *Chlorella* was inhibited by 5 - 100 ppm (inhibition increasing with concentration), with 1 ppm reducing dry weight but having no other significant influence; 1 - 50 ppm caused

marked growth inhibition of *Oedogonium*; and 10 ppm reduced growth of *Vaucheria* to 74% of controls; while, in some cases, 1 ppm of 2,4-D promoted growth (Kim, 1961). *Chlorella pyrenoidosa* respiration was stimulated at moderate concentrations (about 35 ppm) (Erickson, et al., 1955; Wedding, et al., 1954) and inhibited at high concentrations (Wedding, et al., 1954). At $2 \times 10^{-3} \text{M}$, photosynthesis was almost completely inhibited at pH 3.10 and 4.10, but was stimulated at pH 7.0 and above, while cells in $9.35 \times 10^{-5} \text{M}$ and higher were progressively decolorized (Wedding, et al., 1954). At pH 4.49, a $3.02 \times 10^{-3} \text{M}$ concentration of 2,4-D was needed to produce complete photosynthesis inhibition (Erickson, et al., 1955). Exposure to high concentrations (10^{-2}M) at 10 C for 4 hours did not kill this alga (Wedding, et al., 1959). No toxic effects of up to 200 ppm technical grade 2,4-D were found on axenic cultures of *Scenedesmus quadricauda*, *Chlamydomonas*, and *Chlorella pyrenoidosa* (Vance and Smith, 1969), while 250 ppm had no effect on *Microcystis aeruginosa* (Fitzgerald and Skoog, 1952). For a period of 40 hours, 1000 ppm of 2,4-D had no inhibition at pH 5.1, 6.0, or 7.4 to axenic cultures of *Chlorella pyrenoidosa* (Tomisek, et al., 1957). Finally, eight algae isolated from waste stabilization ponds had a maximum tolerance to 2,4-D of 1.0 mM, with the other 31 isolates having a maximum tolerance at 2.0 mM. None of the 39 isolates could grow at 10 or 20 mM, and one isolate (*Chlorella* 23) did not survive at 2.0 mM. Two isolates (*Dictyosphaerium* 380 and *Scenedesmus* 410) grew better at 2.0 mM than in the controls (Wiedeman, 1964).

2,4-D Results

At the concentrations used in this study, the basic effect of 2,4-D was neutral or stimulatory. A careful examination of Figures 14

to 16 and Table VII reveals that in most cases the growth curves are not greatly different at high or low concentrations of 2,4-D than those for the control. For example, *Stichococcus chodati* (2) shows a typical neutral effect. The growth curve for the control is situated in the midst of the curves for the 2,4-D concentrations, and there is no systematic arrangement of the curves for the experimental concentrations. This same pattern is evident for *Bracteacoccus* (4), *Chlorococcum* (3) and (5), *Stichococcus chodati* (6), unidentified (8), and *Hormidium flaccidum* (10). *Chlorella* (9) had an apparent reduction in growth at the higher concentrations (5 ppm and above) at both the six and eight week sampling points with little or no reduction at lower concentrations. *Hormidium* (1) and *Neochloris* (7) have definite stimulation of growth by 2,4-D, especially at the later sampling points. In both cases, growth at the final sampling point is greatly increased in one of the lower concentrations, with the rest of the concentrations clustered at a point midway between that and the control. It is possible that these two isolates are able to use 2,4-D to some extent to increase their growth in the light. Graph 10 in Figure 16 shows the overall interaction of isolates 1-9 with 2,4-D and confirms the original observation that the basic effect is neutral.

These results agree quite well with the general finding of past experiments that 2,4-D has negative effects on algae only at high concentrations, usually much higher than those used in this study. The only genera used in this experiment which had been reported on previously were *Chlorella* and *Chlorococcum*. The one reference to *Chlorococcum* (Wiedeman, 1964) reported that five isolates of that species grew as well in 2.0 mM 2,4-D (442 ppm) as they did in the control, but they could not grow in 10.0 mM (2210 ppm). Of the five isolates, two had the same growth

FIGURE 14

Effect of 2,4-D on the growth of isolates 1 - 3. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for the first graph are applicable to the following two graphs.

1. *Hormidium* sp. (1)
2. *Stichococcus chodati* (2)
3. *Chlorococcum* sp. (3)

FIGURE 14 2,4-D

50 ppm
10 ppm
5 ppm
1 ppm

0.5 ppm
0.1 ppm
0 ppm (Control)

FIGURE 15

Effect of 2,4-D on the growth of isolates 4 - 7. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 4 are applicable to the following three graphs.

4. *Bracteacoccus* sp. (4)
5. *Chlorococcum* sp. (5)
6. *Stichococcus chodati* (6)
7. *Neochloris* sp. (7)

FIGURE 15 2,4-D

— 50 ppm
- - - 10 ppm
... 5 ppm
- . - . 1 ppm

- - - 0.5 ppm
- . - . 0.1 ppm
— 0 ppm (Control)

FIGURE 16

Effect of 2,4-D on the growth of isolates 8 and 9 and on a composite of isolates 1 - 9. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 8 are applicable to the following two graphs.

8. Unknown (8)
9. *Chlorella* sp. (9)
10. Composite of isolates 1 - 9

FIGURE 16 2,4-D

50 ppm
10 ppm
5 ppm
1 ppm

0.5 ppm
0.1 ppm
0 ppm (Control)

TABLE VII

VISUAL ESTIMATIONS OF THE GROWTH OF *HORMIDIUM FLACCIDIUM* (10)
IN 2,4-D

Concentration	Weeks				
	2	4	6	8	12
50 ppm	N	T--	T-	F	F+
10 ppm	N	T--	T	F	F+
5 ppm	T---	T-	T	F	F+
1 ppm	T---	T--	T	F-	F
0.5 ppm	N	T--	T	F	F+
0.1 ppm	T---	T--	T-	F	F+
0 ppm	T---	T-	T	T-	F

at all concentrations up to 2.0 mM while the other three were stimulated slightly by all concentrations up to 2.0 mM. The present study found no stimulation of growth even at concentrations comparable to those used by Wiedeman which is probably the result of using isolates belonging to different species or different physiological strains of similar species.

There have been a number of reports of the effects of 2,4-D on *Chlorella*, both on unspecified species and in a few cases on identified species. These reports vary from no toxic effects at moderate to high concentrations, to growth inhibitions in some cases at low concentrations. Studies which measured photosynthesis and respiration found stimulation of *Chlorella* at moderate and inhibition at high concentrations, while other studies reported no negative effects at concentrations close to those used in this study (Tomisek, et al., 1957; Wedding, et al., 1954; Vance and Smith, 1969; Wiedeman, 1964). This study found a growth inhibition at concentrations of 5 ppm and higher, but only after a period of six weeks. This agrees with the work of Kim (1961) who found that *Chlorella* had growth inhibited by 5-100 ppm, with 1 ppm causing only a reduction in dry weight (which was not measured in this study). The differences between some of the previous studies and the present report may be due to different algae used, to different media used, or to different experimental conditions, with the different organisms being the most likely reason.

2,4,5-T Introduction

The auxin type herbicide most effective against woody plants is 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) which has a recommended usage level of 6 lb/A (6.725 kg/H) or less (Blackman, et al., 1951; Fletcher, 1960; Martin, 1964; Farm Chemicals Handbook, 1965; Moore,

1967). In 1968, 38,356 pounds (17,398.3 kg) of technical material of all forms of 2,4,5-T were sold in Kentucky (Moore, 1973). A national survey of pesticides used on croplands in 1969 (1684 sites) found that 0.18% of the sites had been treated with 2,4,5-T at an average application of 0.83 lb/A (0.93 kg/H) (Wiersma, Tai, & Sand, 1972b).

Soil decomposition is much slower than for 2,4-D (DeRose, 1946; Audus, 1951; Alexander, 1965b; Walker, 1967), but follows the same basic pattern of adsorption, a long lag phase, and then rapid detoxification (Audus, 1951). The disappearance rate of a second application after detoxification of the first has been reported to be both without a long lag (Audus, 1951) and as long as the initial detoxification (Newman, et al., 1952; Newman and Downing, 1958). There is also a controversy over whether the decomposition is due to microorganisms. Whiteside and Alexander (1960) reported that 2,4,5-T was not broken down by microbes, and no organism which can decompose it has been isolated into pure culture (Newman and Downing, 1958; Walker, 1967). However, those conditions which favor the growth of microbes decrease the persistence of 2,4,5-T in the soil (including soil type, moisture, dose, temperature, and organic matter) (DeRose and Newman, 1948; Norman, et al., 1950; Blackman, et al., 1951; Audus, 1951; Boyce Thompson Institute, 1971). In addition, a bacterial poison, 0.01% sodium azide, destroyed the detoxifying ability of soil which had been able to breakdown 2,4,5-T (Audus, 1951). However, if microorganisms are responsible for the decomposition, they are not the same ones which detoxify 2,4-D (Audus, 1951; MacRae and Alexander, 1965).

Persistence varies greatly both under lab or greenhouse conditions and in the field, but is generally in terms of several

months. Some of the times cited for disappearance in the lab were: over three months (Whiteside and Alexander, 1960); more than 47, 124, and 205 days (different soil types) (Alexander and Aleem, 1961); 270 days (Audus, 1951); 147 days and 11 months (different concentrations) (DeRose and Newman, 1948); and over 15 months (stored in dry soil) (Weaver, 1948). Persistence in the field was reported to be two to five weeks (Klingman, 1961, cited in Pimentel, 1971); over 93 days (DeRose and Newman, 1948); 19 weeks (Newman, et al., 1952); five months (75-100% loss) (Matsumura, 1972); six to seven months (Alexander, 1965b); and 12 months (95% disappearance) (Edwards, 1964).

At concentrations normally used for weed control, reports of negative effects on microorganisms are uncommon (Kratochvil, 1951; Magee and Colmer, 1955; Roberts and Bollen, 1955; Newman and Downing, 1958; Fletcher, 1960; Whiteside and Alexander, 1960; Bounds and Colmer, 1964, cited in Pimentel, 1971). Only at very high concentrations are inhibitions noted (Newman, 1947; Magee and Colmer, 1955; Roberts and Bollen, 1955; Johnson, et al., 1956; Fletcher, 1960; Whiteside and Alexander, 1960), with 2,4,5-T, in general, appearing to be more toxic than 2,4-D (Magee and Colmer, 1955; Johnson, et al., 1956). In some cases, stimulation of soil organisms has even been reported (Roberts and Bollen, 1955; Newman and Downing, 1958).

Studies on the effect of 2,4,5-T on algae generally agree with those on other microorganisms: i. e., adverse effects only at concentrations far exceeding the normal dose for weed control (about 10 ppm). Exposing phytoplankton to 1 ppm of 2,4,5-T for 4 hours did not decrease productivity (Butler, 1963, cited in Pimentel, 1971). A commercial preparation at 2 ppm was toxic to *Microcystis aeruginosa* at three and

seven days (but not at 14 and 21 days), stimulated *Cylindrospermum licheniforme* at three days, and was non-toxic to four other algae (*Scenedesmus obliquus*, *Chlorella variegata*, *Gomphonema parvulum*, and *Nitzschia palea*) at three and seven days and to all six unialgal cultures at 14 and 21 days (Palmer & Malovey, 1955). Concentrations up to 200 µg/ml (200 ppm) of technical grade material had no toxic effects on axenic cultures of *Scenedesmus quadricauda*, *Chlamydomonas eugametos*, and *Chlorella pyrenoidosa* for a period of four days (Vance and Smith, 1969). Finally, the effect of 2,4,5-T at concentrations of 0.02 to 20 mM was tested on 39 axenic algal cultures isolated from waste-stabilization ponds (Wiedeman, 1964). None of the isolates survived in concentrations of 10 or 20 mM; two isolates (*Chlorella*, 23 and *Ankistrodesmus*, 340) did not tolerate 2.0 mM (511 ppm); and two could only tolerate a maximum of 0.1 mM (25.5 ppm) (*Chlorella*, 293 and 370). Two other isolates were stimulated by 2.0 mM (*Dictyosphaerium*, 380 and *Scenedesmus*, 410).

2,4,5-T Results

The effect of 2,4,5-T depended on the isolate under consideration, ranging from stimulation to neutrality to inhibition. *Stichococcus chodati* (2) and (6), *Bracteacoccus* (4), unidentified (8), *Chlorella* (9), and *Hormidium flaccidum* (10) were all basically unaffected by 2,4,5-T at the concentrations used as can be seen by an examination of Figures 17-19 and Table VIII. Two isolates, *Hormidium* (1) and *Neochloris* (7), were stimulated by 2,4,5-T, especially at the later sampling points. Both *Chlorococcum* isolates (3) and (5) were inhibited by the higher concentrations (10 and 50 ppm), but were only slightly effected at lower concentrations. In no case was there total inhibition of growth of any isolate. Figure 19-10 showing the combined effect of 2,4,5-T across isolates 1-9

FIGURE 17

Effect of 2,4,5-T on the growth of isolates 1 - 3. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 1 are applicable to the other two graphs.

1. *Hormidium* sp. (1)
2. *Stichococcus chodati* (2)
3. *Chlorococcum* sp. (3)

FIGURE 17 2,4,5-T

— 50 ppm
- - - 10 ppm
... 5 ppm
- . - 1 ppm

— 0.5 ppm
- - - 0.1 ppm
— 0 ppm (Control)

FIGURE 18

Effect of 2,4,5-T on the growth of isolates 4 - 7. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 4 are applicable to the other three graphs.

4. *Bracteacoccus* sp. (4)
5. *Chlorococcum* sp. (5)
6. *Stichococcus chodati* (6)
7. *Neochloris* sp. (7)

FIGURE 18**2,4,5-T**

— 50 ppm
- - - 10 ppm
... 5 ppm
- . - . 1 ppm

- - - 0.5 ppm
... 0.1 ppm
— 0 ppm (Control)

FIGURE 19

Effect of 2,4,5-T on the growth of isolates 8 - 9 and a composite of isolates 1 - 9. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 8 are applicable to the other two graphs.

- 8. Unknown (8)
- 9. *Chlorella* sp. (9)
- 10. Composite of isolates 1 - 9

FIGURE 19 2,4,5-T

— 50 ppm
- - - 10 ppm
... 5 ppm
- - - 1 ppm

- - - 0.5 ppm
- - - 0.1 ppm
— 0 ppm (Control)

TABLE VIII

VISUAL ESTIMATIONS OF THE GROWTH OF
HORMIDIUM FLACCIDIUM (10) IN 2,4,5-T

Concentrations	Weeks				
	2	4	6	8	12
50 ppm	N	T---	T--	T	T+
10 ppm	N	T--	T-	T	F
5 ppm	T---	T--	T-	T+	F
1 ppm	T---	T--	T	F-	G-
0.5 ppm	N	T--	T-	T+	F-
0.1 ppm	T---	T-	T-	F-	F+
0 ppm	T---	T-	T	F-	F

verifies the basic neutrality of 2,4,5-T to soil algae at the concentrations tested.

This study generally agrees with past work, finding negative effects rarely and mainly at higher concentrations. An earlier report on five isolates of *Chlorococcum* sp. (Wiedeman, 1964) found that they could tolerate concentrations up to 2.0 mM (511 ppm), but could not survive at 10.0 mM. Only one of those five isolates was stimulated and two were slightly inhibited (but not significantly) by concentrations comparable to those used in this study. A number of studies reported on effects of 2,4,5-T on *Chlorella* sp. Palmer and Maloney (1955) found no toxic effects of 2 ppm over a period of 21 days, while Vance and Smith (1969) reported no toxic effects of technical grade chemical at concentrations up to 200 ppm. Wiedeman (1964) worked with six isolates of *Chlorella* sp. and found varying tolerances to different concentrations of 2,4,5-T. None of the isolates could tolerate a concentration of 10.0 mM (about 2554 ppm), one grew as well in 2.0 mM (511 ppm) as it did in the control, three others could grow as well as in the control at concentrations only up to 0.2 mM (51 ppm), while the other two isolates had growth equal to the control at a maximum concentration of 0.1 mM (25 ppm). It is possible that more inhibiting effects might have been found in this study if concentrations higher than 50 ppm had been used, but it was desired to keep this study within the limits that would most commonly be encountered by the organisms in nature.

A comparison of the graphs for 2,4-D and 2,4,5-T for isolates *Chlorococcum* (3) [Figures 14-3 & 17-3] and (5) [Figures 15-5 & 18-5] shows that they are more susceptible to 2,4,5-T than to 2,4-D. Close inspection of the graphs for those isolates in 2,4-D shows that both

are inhibited by 2,4-D to some extent at the final sampling point with inhibition much less evident at the earlier periods. This may indicate that the *Chlorococcum* isolates are sensitive to both 2,4-D and 2,4,5-T, but more sensitive to the latter. This agrees with Wiedeman (1964) who also found that the *Chlorococcum* isolates he tested were more sensitive to 2,4,5-T than to 2,4-D. *Chlorella* isolate (9) shows the opposite reaction, being more sensitive to 2,4-D than to 2,4,5-T. But even the inhibition due to 2,4-D is not as prominent as that due to the other pesticides tested. In the case of 2,4,5-T there appears to be a slight inhibition at the higher concentrations, but this is not as obvious as that with 2,4-D. It appears likely that the addition of one chlorine atom, changing 2,4-D to 2,4,5-T, can have a large influence on the effects on soil algae.

DDT Introduction

In 1874, Othmar Zeidler first described 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane, commonly called DDT; but it was not until 1939, that Paul Müller recognized its value as an insecticide (Farm Chemicals Handbook, 1965). Since that time the total amount used may be as high as 10^6 short tons ($9,078 \times 10^5$ metric tons) (Moore, 1967), and residues are often found in the soil of over 100 ppm (Wiersma, Mitchell, and Stanford, 1972). In 1972, the use of DDT in the U. S. was halted for crops (with three minor exceptions), for nonhealth applications, and for health purposes without a prescription (Ruckelshaus, 1972). The governmental findings leading to that decision stated that the risk from continued use outweighed the benefits since suitable, but less persistent, substitutes were available. The specificity of DDT may be due to its high penetrability through insect cuticle but not through vertebrate skin

(Martin, 1964), or it may be due to differences in rates of degradation (O'Brien, 1966). Its precise mode of action is unknown, but it appears to interfere with nerve transmission (O'Brien, 1966, 1967). DDT is nonselective and often kills the natural enemies of the pest it is applied to control, thereby causing an increase in the undesired pest (Wurster, 1973).

Shortly after DDT use began on a large scale, it became apparent that it persisted in soils for many years, even decades. Since the late 1940's, reports have shown that even a single application will persist for many years, that repeated applications accumulate to amounts much higher than amounts applied in any one year, that it is seldom found in quantity in the soil below plow depth, that it is concentrated by organisms in the food chain, and that DDT and its residues are found in soils where they have never been directly applied (Smith, 1948; Chisholm, et al., 1950 and 1955; Foster, 1951; Fleming and Maines, 1953; Allen, et al., 1954; Ginsburg and Reed, 1954; Ginsburg, 1955; Lichtenstein, 1957; Lichtenstein and Schulz, 1959; Lichtenstein, et al., 1960; Taschenberg et al., 1961; Clore, et al., 1961, cited in Alexander, 1965b; Roberts, et al., 1962; Wheatley, et al., 1962; Bridges, et al., 1963; Edwards, 1963, 1964; Woodwell and Martin, 1964; Harris, et al., 1966; Nash and Woolson, 1967; Woodwell, et al., 1967; Woodwell, 1967; Cole, et al., 1967; Chacko and Lockwood, 1967; Ko and Lockwood, 1968a; Dimond, et al., 1970; Kearney, et al., 1970; Cox, 1970a, 1970b; Lichtenstein, et al., 1971; Matsumura, 1972; Brown, 1972; Tarrant, et al., 1972; Wiersma, Tai and Sand, 1972a; Wiersma, Mitchell, and Stanford, 1972; Menzie, 1972; Yule, 1973, and the following, all cited in March, 1965: Randolph, et al., 1960; MacPhee, et al., 1960; Gallaher and Evans, 1961).

Factors which influence DDT persistence in the soil include soil type, temperature, ultraviolet light, moisture, amount of mixing into soil, plowing, and organic matter (Foster, 1951; Fleming and Maines, 1953; Eno, 1958; Lichtenstein and Schulz, 1959 and 1961; Lichtenstein, et al., 1960).

Although first speculated on almost 20 years ago (Jones, 1956, cited in Walker, 1967), evidence has only accumulated in the last 10 years to show that DDT can be degraded by microorganisms. No single microbe is known which can break it down all the way to CO_2 , H_2O , and chlorine, but it appears that several can break it down by acting in sequence. None can use DDT or any of its reduction products as a sole carbon source, so breakdown must be a form of cometabolism (Chacko, et al., 1966; Wedemeyer, 1967b; Alexander, 1971; Focht, 1972; Pfaender and Alexander, 1972). Organisms which have been shown to be involved in at least one step of the breakdown are listed in Appendix IV. The main metabolite is IDE (DDD), but other breakdown products have been identified and are listed in Appendix V. Some of the breakdown steps require the absence of air, others its presence (Pfaender and Alexander, 1971; Focht, 1972; Alexander, 1972; Focht and Alexander, 1971; Wedemeyer, 1966, 1967a, and 1967b). If microorganisms can break it down, why does DDT persist and accumulate in the soil? This may be because of the need for alternating anaerobic and aerobic conditions, or more likely because organisms which break it down are not abundant and they have no selective advantage in decomposing it since they cannot use it as a carbon source (Pfaender and Alexander, 1972; Alexander, 1972; Chacko, et al., 1966; Focht, 1972).

DDT has been reported to have effects on higher plants varying from negative to neutral to stimulation (Chapman and Allen, 1948;

Appleman and Sears, 1946; Gould and Hamstead, 1951; Thurston, 1953; Chisholm, et al., 1955; Harcourt and Cass, 1955; Clower and Matthyse, 1954; Eno and Everett, 1958; Eder, 1963; Bridges, et al., 1963; Lichtenstein, et al., 1962; Lawler and Rogers, 1967; National Academy of Sciences, 1968).

The effects of DDT on microorganisms and their processes are usually reported to be neutral. Negative effects are normally only found at very high concentrations, while a few cases of stimulation have even been reported (Appleman and Sears, 1946; Wilson and Choudhri, 1946; Smith and Wenzel, 1947; Fults and Payne, 1947; Gould and Hamstead, 1951; Bollen, et al., 1954a and 1954b; Eno, 1968; Eno and Everett, 1958; Pathak, et al., 1961, cited in Marth, 1965; Bartha, et al., 1967; Ko and Lockwood, 1968b; Ledford and Chen, 1969; Tarrant, et al., 1972; Payne and Fults, 1947; Eder, 1963; MacRae and Vinchx, 1973; Roberts and Bollen, 1955; the following all cited in Bollen, 1961; Martin, et al., 1959; Jones, 1956; and Braithwaite, et al., 1958).

Since DDT has a very low water solubility (about 1.2 ppb. [Bowman, et al., 1960]), studies on its effects on phytoplankton are often at concentrations which might be found in water, but which are far below amounts normally used for insect control. Algae which have been reported to be unaffected or only mildly affected by DDT under such conditions are listed in Table IX. However, algae can accumulate DDT passively and rapidly to a level several hundred times higher than the concentration in which they are growing, and surveys of algae isolated from nature have found many with high DDT concentrations in their cells (Rice and Sikka, 1973; Keil and Priester, 1969; Gregory, et al., 1969; Woodwell, et al., 1967; Cox, 1970a and 1970b; Vance and Drummond, 1969).

TABLE IX
ALGAE UNAFFECTED BY DDT AT LOW CONCENTRATIONS

<u>Algal species</u>	<u>Conditions</u>	<u>Reference</u>
<i>Chlorella</i> sp.		Wilson and Choudhri, 1946
<i>Agmenullum quadruplicatum</i>	100 ppb DDT	Boush and Batterton, 1972
<i>Anacystis nidulans</i>	100 ppb DDT	Boush and Batterton, 1972
<i>Dunaliella tertiolecta</i>	80 ppb	Bowes, 1972
<i>Cyclotella nana</i>	80 ppb, short lag before division started	Bowes, 1972
<i>Thalassiosira fluviatilis</i>	80 ppb, short lag before division started	Bowes, 1972
<i>Amphidinium carteri</i>	80 ppb, short lag before division started	Bowes, 1972
<i>Coccolithus huxleyi</i>	80 ppb, short lag before division started	Bowes, 1972
<i>Porphyridium</i> sp.	80 ppb, short lag before division started	Bowes, 1972
Phytoplankton culture (mainly <i>Chlorella</i> , <i>Chlamydomonas</i> , other common species, and zooplankton)	1.0, 0.1, 0.05, 0.01 ppm	Loosanoff, et al., 1957
<i>Dunaliella tertiolecta</i>	100 ppb	Menzel, et al., 1970
<i>Coccolithus huxleyi</i>	100 ppb	Menzel, et al., 1970
<i>Chlamydomonas reinhardtii</i>	0.2-20 ppm	Morgan, 1972
<i>Cylindrospermum licheniforme</i>	2 ppm, commercial preparations unialgal cultures	Palmer and Maloney, 1955
<i>Scenedesmus obliquus</i>	2 ppm, commercial preparations unialgal cultures	Palmer and Maloney, 1955
<i>Chlorella variegata</i>	2 ppm, commercial preparations unialgal cultures	Palmer and Maloney, 1955

TABLE IX (Cont'd)

<u>Algal species</u>	<u>Conditions</u>	<u>Reference</u>
<i>Nitzschia palea</i>	2 ppm, commercial preparations unialgal cultures	Palmer and Maloney, 1955
<i>Microcystis aeruginosa</i>	2 ppm (toxic at first)	Palmer and Maloney, 1955
<i>Gomphonema parvulum</i>	2 ppm (reduced growth slightly at first)	Palmer and Maloney, 1955
<i>Anacystis nidulans</i>	1 ppm	Gregory, et al., 1969
<i>Scenedesmus obliquus</i>	1 ppm	Gregory, et al., 1969
<i>Euglena gracilis</i>	1 ppm	Gregory, et al., 1969
<i>Microcystis aeruginosa</i> , <i>Anabaena cylindrica</i> , <i>Scenedesmus quadricauda</i> , and <i>Oedogonium</i> sp. in unialgal cultures	up to 1.0 ppm	Vance and Drummond, 1969

There are several reports that DDT might upset a natural balance. In some cases, the changes were concluded to be no greater than those caused by climate or other ecological factors (Bishop, 1947; DeKoning and Mortimer, 1971). Others reported that the effects might influence the algal population composition in nature (Lazaroff and Moore, 1966; Woodwell, et al., 1967; Mosser, et al., 1972; Batterson, et al., 1972). Shane (1948) described a bloom of *Synedra* sp. in a reservoir due possibly to stimulation by a DDT spray or to a reduction in the number of zooplankton grazers because of the DDT spray. A pond treated with 0.02 ppm DDT had a disappearance of *Chara* sp. and *Spirogyra* sp. within two months with the regrowth the following summer less than 20% of the previous year's high (Bridges, et al., 1963).

Negative effects on algae have been reported at low, normal, and high concentrations of DDT. Butler (1963, cited in Pimentel, 1971) found that phytoplankton communities exposed to DDT at 1 ppm in the lab for four hours had a reduction in productivity of 77.2%. Stadynek (1967, cited in Vance and Drummond, 1969) reported a significant reduction in ^{14}C assimilation by *Scenedesmus quadricauda*. Wurster (1968) found that DDT as low as a few ppb reduced photosynthesis in lab cultures of four species of marine algae in axenic culture and also a natural mixed phytoplankton community. *Chlorella* grown in less than 0.3 ppb DDT for three days had great morphological changes which disappeared when the DDT was removed (Södergren, 1968). The LD_{100} for *Anabaena cylindrica* is between 15 and 20 ppm DDT in unialgal culture, while for *Microcystis aeruginosa*, *Scenedesmus quadricauda*, and *Oedogonium* sp. it is over 20 ppm (Vance and Drummond, 1969). At the end of four days, DDT at 100 ppm reduced the growth of *Scenedesmus* and *Euglena*, but not *Chlorella* and *Ankistrodesmus* and varying pH did not change the effect of DDT on

Chlorella (Christie, 1969). Growth of *Monochrysis lutheri* was reduced 75% by 0.60 ppm DDT, while *Protococcus* sp. growth was reduced 50%, *Dunaliella euchlora* growth was reduced 25%, and *Chlorella* sp. and *Phaeodactylum tricorutum* growth was reduced very little by concentrations up to 1.0 ppm DDT (Ukeles, 1962). Cell division of *Skeletonema costatum* was blocked after two to three divisions by 100 ppb DDT, while division of *Cyclotella nana* was slowed (Menzel, et al., 1970). Another report found that 80 ppb DDT delayed cell division of *Skeletonema costatum* for nine days, after which it continued at the normal rate (Bowes, 1972). At 250 ppm, *Coccolithus huxleyi* and *S. costatum* had lag phases of two and ten days respectively before normal cell division began. The only morphological differences cited were that cells of *S. costatum* in lag phase looked "unhealthy". Finally, a wide range in tolerance of waste stabilization pond algae to DDT was reported (Wiedeman, 1964). Four isolates (*Pediastrum*, 161 and 265, and *Chlorella*, 185e and 370) had no tolerance for even 0.02 mM, the lowest concentration used. One isolate (*Chlorococcum*, 155) grew quite well at the highest concentration (20 mM), while 11 others showed at least some growth at that concentration. Four other isolates (*Chlamydomonas*, 29, *Stigeoclonium*, 96, and *Chlorococcum*, 78 and 292) grew as well in 10.0 mM as they did in the controls.

DDT Results

DDT is a most perplexing compound to work with. Its solubility in water is very low, so it must be dissolved in a solvent before being added to the growth medium. Unfortunately, at the highest concentration used in this study, 50 ppm, the DDT precipitated when added to the medium. This precipitate scattered light and made it impossible to obtain accurate readings with the Spectronic 20 at that concentration. Therefore, only

visual readings are given for the growth of the isolates in 50 ppm DDT (Table X), but these follow the pattern for other concentrations for each isolate. For isolates 1 through 9, graphs of the effect of DDT on the growth curves, Figures 20-22, are given as usual for concentrations of 10 ppm and below where there was not the problem of interference from the DDT precipitate. Table XI gives visual estimations of growth for isolate 10. Because of the slow growth of several of the isolates in BBM2, observations were made additionally at 12 weeks for algae 1, 4, 7, 8, 9, and 10. The general effect of DDT was neutral, although in several cases there was a wide spread in the growth levels, especially at the final sampling point. The following isolates were either uneffected or only mildly effected: *Hormidium* (1), *Chlorococcum* (3) and (5), *Stichococcus chodati* (6) and unidentified (8). *Bracteacoccus* (4) and *Neochloris* (7) show a growth pattern with inhibition at the higher concentrations and either no effect or a slight stimulation at the lowest DDT concentrations. *Chlorella* (9) showed a neutral effect at the 2, 4, and 6 week sampling points, but a stimulation by DDT at the 8 and 12 week points. Finally, *Stichococcus chodati* (2) and *Hormidium flaccidium* (10) had depressed growth at all concentrations of DDT. Figure 22-10 shows the effect of DDT on the total of isolates 1-9. This indicates a rather mixed reaction over all with little systematic effect.

These results are comparable to those in the literature which found widely varying effects of DDT depending upon the organism being examined. Only *Chlorella* and *Chlorococcum* of the algae used in this study have been previously reported. Wiedeman (1964) described the effects of concentrations of DDT up to 20 mM (7090 ppm) on five isolates of *Chlorococcum*. One of these isolates grew as well in the highest concentration as in the controls (although the maximum growth was found

FIGURE 20

Effect of DDT on the growth of isolates 1 - 3. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 1 are applicable to the other two graphs.

1. *Hormidium* sp. (1)
2. *Stichococcus chodati* (2)
3. *Chlorococcum* sp. (3)

FIGURE 20 DDT

10 ppm
5 ppm
1 ppm

0.5 ppm
0.1 ppm
0 ppm (Control)

FIGURE 21

Effect of DDT on the growth of isolates 4 - 7. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 4 are applicable to graphs 5 and 7.

4. *Bracteacoccus* sp. (4)
5. *Chlorococcum* sp. (5)
6. *Stichococcus chodati* (6)
7. *Neochloris* sp. (7)

FIGURE 21 DDT

10 ppm
5 ppm
1 ppm
0.5 ppm
0.1 ppm
0 ppm (Control)

FIGURE 22

Effect of DDT on the growth of isolates 8 and 9 and a composite of isolates 1 - 9. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 8 are applicable to the other two graphs.

- 8. Unknown (8)
- 9. *Chlorella* sp. (9)
- 10. Composite of isolates 1 - 9

FIGURE 22 DDT

TABLE X

VISUAL ESTIMATIONS OF THE GROWTH OF ISOLATES 1 - 9
IN 50 PPM DDT

Isolate #	Weeks				
	2	4	6	8	12
1	T---	T---	T--	T-	T+
2	T---	T--	T-	T	
3	T--	T--	T-	T+	
4	N	T---	T---	T---	T---
5	T---	T--	T-	T	
6	T---	T--	T-	T	
7	N	N	N	N	T-
8	N	N	N	T--	F-
9	N	T---	T---	T--	T

TABLE XI

VISUAL ESTIMATIONS OF THE GROWTH OF
HORMIDIUM FLACCIDIUM (10) IN DDT

Concentration	Weeks				
	2	4	6	8	12
50 ppm	N	N	T---	T-	T
10 ppm	N	N	T---	T-	T+
5 ppm	N	N	T--	T	T
1 ppm	N	T---	T--	T	F
0.5 ppm	N	T---	T--	T	F-
0.1 ppm	T---	T--	T--	T+	F
0 ppm	T---	T--	T-	T+	F

in concentrations of 1.0 and 2.0 mM). Three other isolates could survive in the highest concentration, with greatly reduced growth, and two of those isolates could grow as well in 10.0 mM (3545 ppm) as in the control. The final isolate had a growth equal to the control at a maximum concentration of 2.0 mM (709 ppm). In four of the five cases, growth was stimulated by at least one of the concentrations, most commonly 1.0 and 2.0 mM. The results at the concentrations used in this study are comparable to those from Wiedeman (1964).

A number of studies have mentioned the effects of DDT on *Chlorella* sp. The reported results vary greatly. Less than 0.3 ppb DDT caused great morphological changes, but no growth effects (Södergren, 1968); while from 1.0 ppm to 100 ppm did not reduce growth even with varying pH's (Ukeles, 1962; Christie, 1969). Finally, Wiedeman (1964) reported that two of the six isolates of *Chlorella* which he investigated did not grow as well in the lowest concentration used (0.02 mM or 7.1 ppm) as they did in the control. The other four isolates were inhibited at concentrations of either 0.2 or 1.0 mM (70.9 or 354.5 ppm), and none of the isolates was stimulated by any concentration used. Two other studies have also reported little or no effect of DDT on *Chlorella* at low concentrations (Wilson and Choudhri, 1946; Falmer and Maloney, 1955). The present study found neutral effects for the first six weeks of observation and a stimulation for weeks eight and 12. This stimulation may not have been noticed in earlier studies since most of the studies reported in the literature were of much shorter duration (or of unspecified duration). However, the neutral effects for the first six weeks agree with most previous studies.

Rotenone Introduction

Rotenone is a botanical or natural compound with a biosynthesis similar

to that of the isoflavonoids (Fukami and Makajima, 1971) and the following structure:

It is extracted from the dried roots of *Derris elliptica* in southeast Asia and Indonesia and *Lonchocarpus* spp. in South America, Peru being the chief source (Farm Chemicals Handbook, 1965). The crude extract from the "tubaroot" includes a number of other derivatives, some of which are not insecticidal (O'Brien, 1967). This extract has long been used as a fish poison by Malayan natives; and in 1848, T. Oxley suggested that it might be useful as an insecticide against leaf-eating caterpillars (Fukami and Nakajima, 1971). In 1912, Nagai isolated the active ingredient from tubaroot naming it rotenone; and in 1961, Miyano, Kobayashi, and Matsue first synthesized it. In addition to rotenone, 10 related compounds are known from plants of the legume family, some of which also have insecticidal properties.

Rotenone is short lived as an insecticide (O'Brien, 1967), breaking down on exposure to heat or light to at least 20 different, mainly noninsecticidal compounds, the major toxic one being 6 α β , 12 α β -rotenolone (Cheng, et al., 1972). Toxicity is almost completely lost in as short a period as 10 days due to decomposition or other chemical changes and not to absorption and translocation in plants (Pagan and Morris, 1953).

Rotenone becomes firmly bound in the mitochondria (Yamamoto, 1969) and acts by inhibiting the coupled oxidation of NADH₂ and the reduction

of cytochrome b, thereby blocking oxidation of all substrates oxidized by the NAD system (glutamate, α -ketoglutarate, pyruvate, etc.), but not interfering with the oxidation of succinate (O'Brien, 1967). The reason for its high toxicity to insects, fish, and pigs, but low toxicity to most mammals is still unknown, but differences in degradation rates have been proposed as a possible explanation.

Little research has been done on the effects of rotenone on nontarget species as its use has been greatly curtailed since the introduction of many synthetic insecticides in the last 30 years. Only 2,254 pounds (1022.4 kg) of the technical material were sold in Kentucky in 1968 (Moore, 1973). Rotenone does not leave harmful residues on vegetable crops (Farm Chemicals Handbook, 1965) and has no effect on cucumbers (Harcourt and Cass, 1955).

Several studies have noted effects on aquatic microorganisms. Hooper (1948) found a decrease in the number of protozoans after treating a lake with derris root (cited in Cope, 1965). Hoffman and Olive (1961) noted that rotenone at a concentration of 1.0 ppm applied to a lake reduced the number of entomostraca, rotatoria, and protozoa. Kiser, Donaldson, and Olson (1963) treated a shallow lake with 0.5 ppm rotenone and reported a complete removal of the open water zooplankton species for three months, a more gradual and shorter disappearance of the shore edge species, and only a reduction in numbers of those species in the dense weed patches. Of 42 cladoceran and copepod species in that lake none was permanently eliminated. A second lake was treated with 1 ppm of rotenone which penetrated to the thermocline at 30 feet (9.144 m) in six hours killing Cladocera and Copepoda as it sank. The zooplankton did not return to their preapplication level for several months after the

lakes had become non-toxic to fish and been restocked. None of these studies made any mention of the effects of rotenone on phytoplankton or other plants.

Rotenone Results

The most common effect of rotenone on algal growth was inhibition, but it was also neutral in some cases and stimulatory in others (Figures 23-25 and Table XII). These isolates which were definitely inhibited include *Stichococcus chodati* (2), *Chlorococcum* (3) and (5), *Bracteacoccus* (4), and unidentified (8). In several cases the pattern of inhibition was quite marked, with a gradual increase in growth from the highest concentrations to the control. That pattern is particularly apparent for isolates 3, 4, and 5. *Chlorella* (9) and *Hormidium flaccidum* (10) (Figure 25-9 and Table XII) were unaffected by rotenone as growth was almost identical at all concentrations and in the control. For the remaining three isolates, there was some stimulatory effect. *Stichococcus chodati* (6) was stimulated only at 10 and 50 ppm with the other concentrations being very close to the control. *Neochloris* (7) was stimulated by all concentrations considerably at eight weeks, slightly at six weeks, and hardly at all at weeks two and four. *Hormidium* (1) was stimulated by all concentrations slightly at four weeks and considerably at the six, eight, and 12 week periods. Since rotenone is known to break down in the light to several other compounds, it is possible that those organisms which are stimulated by its presence are actually not responding to the rotenone but to one of its breakdown products since the stimulation occurs only at the later observation times when breakdown is likely to have occurred. Figure 25-10 indicates the overall effect of rotenone across all algae studied. It confirms the above discussion indicating that rotenone

FIGURE 23

Effect of rotenone on the growth of isolates 1 - 3. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 1 are applicable to the other two graphs.

1. *Hormidium* sp. (1)
2. *Stichococcus chodati* (2)
3. *Chlorococcum* sp. (3)

FIGURE 23 Rotenone

— 50 ppm
- - - 10 ppm
... 5 ppm
- · - 1 ppm

- - - 0.5 ppm
- · - 0.1 ppm
— 0 ppm (Control)

FIGURE 24

Effect of rotenone on the growth of isolates 4 - 7. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 4 are applicable to the other three graphs.

4. *Bracteacoccus* sp. (4)
5. *Chlorococcum* sp. (5)
6. *Stichococcus chodati* (6)
7. *Neochloris* sp. (7)

FIGURE 24**Rotenone**

FIGURE 25

Effect of rotenone on the growth of isolates 8 and 9 and a composite of isolates 1 - 9. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 8 are applicable to the other two graphs.

- 8. Unknown (8)
- 9. *Chlorella* sp. (9)
- 10. Composite of isolates 1 - 9

FIGURE 25 Rotenone

— 50 ppm
- - - 10 ppm
... 5 ppm
- . - 1 ppm

- . - 0.5 ppm
... 0.1 ppm
— 0 ppm (Control)

TABLE XII

VISUAL ESTIMATIONS OF THE GROWTH OF
HORMIDIUM FLACCIDIUM (10) IN ROTENONE

Concentration	Weeks				
	2	4	6	8	12
50 ppm	N	N	N	T---	T+
10 ppm	N	N	T---	T--	T
5 ppm	N	T--	T-	T+	F-
1 ppm	N	T---	T--	T-	T
0.5 ppm	N	T---	T--	T-	T
0.1 ppm	N	T---	T--	T-	T+
0 ppm	N	T---	T---	T-	T+

is more inhibitory than stimulatory to the isolates. The control is basically at a higher level than the experimental conditions, with the highest concentration being lower than the others.

Since no previous work has been reported on rotenone and algae, no comparison of the present study and past studies can be made. The general finding of an inhibition of zooplankton appears to be similar to the prevalent negative effect on growth which was found in this study.

Malathion Introduction

O,O-dimethyl S-bis [(carboethoxy)ethyl] phosphorodithioate, commonly called malathion, is a short-lived synthetic organophosphate pesticide (O'Brien, 1966). It was introduced in the early 1950's (Haller, 1952) and has had increased use in recent years as a replacement for many of the persistent organochloride pesticides, 51,467 pounds (23,345.4 kg) being sold in Kentucky in 1968 (Moore, 1973). The organophosphate insecticides as a group act by inhibiting acetylcholinesterase and, thereby, disrupting nerve function (Casida, 1974). Early studies (Metcalf and March, 1953) indicated that they were transformed *in vivo* by oxygen and enzymes to their active forms, as the highly purified insecticides *in vitro* did not greatly inhibit insect cholinesterase. Malathion has a low toxicity for mammals, 1375 mg/kg being the acute oral LD₅₀ for rats (Farm Chemicals Handbook, 1965), while 100 ppm in the diet is considered safe for man (Spiller, 1961); and it is, therefore, used in preference to others of this group which are more toxic to humans.

In soil outdoors, malathion is very short-lived, but it may be more persistent in soil indoors (Spiller, 1961). There is no evidence of its volatilization from soils (Harris and Lichtenstein, 1961), but degradation there apparently by chemical hydrolysis is directly related to

adsorption and pH (Menzie, 1972). A national survey of croplands in 43 states (1684 sites) reported that 7.54% of the sites had been treated with malathion at an average rate of 0.17 lb/A (0.19 kg/H) (Wiersma, Tai, and Sand, 1972b). Of 66 samples tested for residues, only 2 (3%) had traces of malathion at rates of 0.04 and 0.36 ppm. Other reports on persistence included a 75-100% loss in bioactivity in one week (Kearney, et al., 1969); a persistence of only two days (Laygo and Schulz, 1963, cited in Pimental, 1971); an 85% reduction in three days and a 96.6% loss in eight days when applied at five lb/A (about 3.2 ppm) (Lichtenstein and Schulz, 1964); and even with very heavy applications (76.6 lb/A [85.86 kg/H] the first year and 16 lb/A [17.9 kg/H] the second and third years) complete disappearance at the end of one year (Roberts, et al., 1962). Wiersma, Mitchell, and Standford (1972) sampling onion fields for malathion residues reported that 13.6% had been treated with an average of 2.86 lb/A (3.21 kg/H), but found no residues either in the soil or in the onions grown in those fields.

Phytotoxicity to higher plants varies with the species from none (Haller, 1952; Roberts, et al., 1962; Spiller, 1961) to moderate (Haller, 1952; Clower and Matthyse, 1952; Stafford, 1954; Haviland and Highland, 1955; and Lichtenstein, et al., 1962) to outright killing (Clower and Matthyse, 1954); and the effects include lower yields (Gojmerac, 1957) and reduced germination (Starks and Lilly, 1955; Strong, et al., 1959, and Gojmerac, 1956 and 1957).

There appears to be little negative effect of malathion on soil fungi and bacteria, possibly because most spraying is done when the fungi are not active (Spiller, 1961). *Trichoderma viride*, a soil fungus, and *Pseudomonas* sp., a soil bacterium isolated from heavily sprayed northern Ohio soils, could metabolize malathion (Matsumura and Boush, 1966) while autoclaved soil did not break it down. Certain colonies of *T. viride*

from a culture collection had a very marked ability to cause the breakdown of malathion through the action of carboxylesterase(s). Degradation of malathion added to soil at 150 and 1500 ppm was indicated by a high level of CO_2 production and was increased by the addition of glucose (Bartha, et al., 1967). An initial inhibition of nitrification decreased with time as the chemical was either detoxified or a resistant nitrifying population developed.

Reports of the effects of organophosphates on algae are few. Methyl parathion at 3 ppb applied to a lake three times between June 19 and August 7 caused a reduction in zooplankton followed by an *Anabaena* bloom which had not occurred the previous year when there was no treatment (Cook and Connors, 1963). Hurlbert, et al., (1972) reported a rapid increase in phytoplankton and a reduction in herbivores after treating several ponds with Dursban at 0.025 and 0.25 lb/A (0.028 and 0.28 kg/H). They found blooms of blue-green algae in four ponds and a diatom in a fifth pond and concluded that the changes in species were likely due to a reduction in grazing. Malathion at 7.25 ppm inhibited growth of *Euglena gracilis* by 48.9% in the light and 19.2% in the dark (Moore, 1970). Inhibition was less at lower concentrations and 0.15 ppm stimulated growth in the dark by 11.4%. Christie (1969) reported on the effects of malathion on several species of algae from waste stabilization ponds. After four days 100 mg/l caused growth effects ranging from complete inhibition of *Ankistrodesmus* to a 2/3 reduction for *Scenedesmus* and a very slight reduction for *Chlorella* to an increase of 2-1/2 times for *Euglena*. After seven days at the same concentration growth of *Chlorella* was greatly stimulated while *Scenedesmus*, *Euglena*, and *Schroderia* had growth reductions of 1/6, 1/3 and 1/2 respectively. Further experiments were carried out with axenic cultures of *Chlorella pyrenoidosa* in a defined medium. The pH of that

medium influenced the effect of 100 mg/l over a period of seven days; at pH 9.0, growth was reduced for days one to five, but only slightly depressed by day seven; while at pH 6.0, growth reduction was very slight for all seven days. Lower concentrations, 0-10 mg/l, at pH 6.0 resulted in less than a 10% growth reduction for *Chlorella*. The general conclusion was that malathion was capable of altering the composition of a mixed algal community from waste stabilization ponds, but would not display a persistent inhibitory effect.

Malathion Results

Two different purities of malathion were used in this investigation. The first was 95% purity, the technical grade. This is the form that is sold for public use, either diluted or mixed with an inert chemical. The other, 99% purity, was laboratory grade. Figures 26-31 and Table XIII show that the majority of the isolates were adversely effected by both forms of malathion with some very dramatic reductions in the growth curves, but no complete inhibition of growth at the concentrations used. *Stichococcus chodati* (2), *Chlorococcum* (3) and (5), *Bracteacoccus* (4), and unidentified (8) show a definite reduction in growth in both malathion 95 and 99. In some cases, isolates 2 and 4 in particular, there is a systematic drop in growth as the concentration of malathion is increased, while in other cases the presence of even a small amount of pesticide reduces growth and additional pesticide does not decrease growth further. In both purities, *Stichococcus chodati* (6) has reduced growth at the first three sampling points, but at the last time period grows as well in at least one of the experimental conditions as in the control. This may indicate an initial suppression which is overcome as the pesticide is either degraded or tied up in the cells. *Hormidium* (1), *Neochloris* (7), and

FIGURE 26

Effect of malathion - 95 on the growth of isolates 1 - 3. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 2 are applicable to graph 3.

1. *Hormidium* sp. (1)
2. *Stichococcus chodatii* (2)
3. *Chlorococcum* sp. (3)

FIGURE 26 Malathion-95

— 50 ppm
 - - - 10 ppm
 . . . 5 ppm
 - - - 1 ppm

- - - 0.5 ppm
 . . . 0.1 ppm
 — 0 ppm (Control)

FIGURE 27

Effect of malathion - 95 on the growth of isolates 4 - 7. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 4 are applicable to the other three graphs.

4. *Bracteacoccus* sp. (4)
5. *Chlorococcum* sp. (5)
6. *Stichococcus chodati* (6)
7. *Neochloris* sp. (7)

FIGURE 27**Malathion-95**

— 50 ppm
- - - 10 ppm
... 5 ppm
- . - 1 ppm

- - - 0.5 ppm
... 0.1 ppm
— 0 Control

FIGURE 28

Effect of malathion-95 on the growth of isolates 8 and 9 and a composite of isolates 1 - 9. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 8 are applicable to the other two graphs.

8. Unknown (8)
9. *Chlorella* sp. (9)
10. Composite of isolates 1 - 9

FIGURE 28 Malathion-95

— 50 ppm
- - - 10 ppm
... 5 ppm
- . - 1 ppm

- - - 0.5 ppm
- . - 0.1 ppm
— 0 ppm (Control)

FIGURE 29

Effect of malathion-99 on the growth of isolates 1 - 3. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 2 are applicable to graph 3.

1. *Hormidium* sp. (1)
2. *Stichococcus chodati* (2)
3. *Chlorococcum* sp. (3)

FIGURE 29 Malathion-99

FIGURE 30

Effect of malathion-99 on the growth of isolates 4 - 7. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 4 are applicable to the other three graphs.

4. *Bracteacoccus* sp. (4)
5. *Chlorococcum* sp. (5)
6. *Stichococcus chodati* (6)
7. *Neochloris* sp. (7)

FIGURE 30 Malathion-99

— 50 ppm
- - - 10 ppm
... 5 ppm
- . - 1 ppm

— 0.5 ppm
- - - 0.1 ppm
— 0 ppm (Control)

FIGURE 31

Effect of malathion-99 on the growth of isolates 8 and 9 and a composite of isolates 1 - 9. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 8 are applicable to the other two graphs.

8. Unknown (8)
9. *Chlorella* sp. (9)
10. Composite of isolates 1 - 9.

FIGURE 31 Malathion-99

— 50 ppm
- - - 10 ppm
... 5 ppm
- - - 1 ppm

- - - 0.5 ppm
... 0.1 ppm
— 0 ppm (Control)

TABLE XIII

VISUAL ESTIMATIONS OF THE GROWTH OF
HORMIDIUM FLACCIDIUM (10) IN MALATHION-95 AND 99

Malathion-95		Weeks				
Concentration	2	4	6	8	12	
50 ppm	N	T---	T--	T--	T--	
10 ppm	N	T---	T---	T--	T-	
5 ppm	N	T---	T--	T-	T-	
1 ppm	N	T---	T--	T--	T-	
0.5 ppm	N	T---	T--	T--	T-	
0.1 ppm	N	T---	T--	T-	T-	
0 ppm	N	T---	T---	T-	T+	
Malathion-99		Weeks				
Concentration	2	4	6	8	12	
50 ppm	N	T---	T--	T	T+	
10 ppm	N	T--	T-	T+	F	
5 ppm	N	T---	T-	T	T	
1 ppm	N	T--	T--	T-	T	
0.5 ppm	N	T--	T-	F	F+	
0.1 ppm	N	T--	T-	T+	F	
0 ppm	N	T---	T---	T-	T+	

Hormidium flaccidium (10) are in general unaffected by malathion. All have a slight depression in growth at the first sampling period, but by the second data point growth in at least one concentration is equal to the control and by six or eight weeks, there are no significant differences. The only case of a significant difference in the response of an isolate to the different purities is for *Chlorella* (9). In that case, malathion 95 does not cause a reduction in the growth, but the malathion 99 does. The growth reduction in the malathion 99 is particularly striking at the six and eight week sampling points.

These results appear to agree with the literature reports which found varying effects depending on species. The only genus used in this study which had been reported on before was *Chlorella*. In that case, Christie (1969) reported that *Chlorella pyrenoidosa* was inhibited more by 100 ppm of malathion at pH of 9.0 than at a pH of 6.0, but that by day seven, the difference was no longer significant. It is possible that the differences found in this study between the effects of malathion 95 and 99 may be due to a slightly different pH of the medium or to changes in the pH of the medium during growth. But since this study was over a much longer period than that of Christie, a definite comparison cannot be made. Further research is needed to clarify this point and investigate probable causes.

Figures 28-10 and 31-10 indicate the overall effect of malathion on all isolates tested. It confirms the negative effects described above, with the control having a curve much above that of any of the experimental concentrations, and the highest concentrations being lower than the intermediary concentrations. One striking difference between the two figures is that there is a significant gap between the 10 and 50 ppm concentrations for malathion 99, but an overlap for these curves for

malathion 95. This may indicate a difference between the two purities at higher concentrations, with manufacturing impurities masking the effect of the 95% purity.

Dark Experiment-Results

The purpose of this experiment was to determine if any of the isolates could use any of the pesticides as a sole carbon source. Since the amount of plant mass in any isolate was very small, growth was measured only visually. The results are shown in TABLE XIV. In the control groups, the presence of ethanol did not result in any systematic growth increase. It is difficult to determine if the results indicated in TABLE XIV are true growth or simply the ability of the isolates to survive, but not divide, in those conditions in the dark, the latter being the more likely. Parker (1961) reported heterotrophic growth of several algae isolated from soil both in defined media with added sugars and in Texas soil-water flasks when a bacterium also isolated from the same soil was present. In the latter case, growth was much slower than in the glucose solution. At the end of 14 months, there was a 16-fold increase in algal cells over the original, indicating an average of only four divisions of each of the original cells. Therefore, it is possible that even if the algae used in this study could grow at a very slow rate in the pesticides in the dark, the two month incubation time would not have been long enough to show that. The concentrations of sugars used in Parker and in other studies on heterotrophic growth (Wiedeman, 1964; Wiedeman and Bold, 1965) are always much higher than that of the pesticides used in this study so that might also be a factor in the present lack of heterotrophic growth. One result of this experiment which is readily evident upon inspecting TABLE XIV is that none of the algae could survive in 10 ppm of DDT in

TABLE XIV

GROWTH OF ALGAE IN VARIOUS PESTICIDES IN THE DARK

Pesticide	Isolate #									
	1	2	3	4	5	6	7	8	9	10
Rotenone, 10 ppm	N	T--	N	T--	N	N	T---	N	N	N
Rotenone, 50 ppm	N	T--	N	T--	N	T---	T---	N	N	N
Malathion-99, 50 ppm	N	T--	T---	T--	N	N	T--	N	T---	T---
Malathion-95, 50 ppm	N	T--	T---	T--	N	T--	T--	N	T---	T---
DDT, 10 ppm	N	N	N	N	N	N	N	N	N	N
2,4,5-T, 50 ppm	N	T--	T---	T--	T---	T--	T---	T---	T---	T---
2,4-D, 50 ppm	N	T--	T---	T--	T---	T--	T--	N	T---	T---
Controls:										
TBIM	T---	T--	T---	T--	T---	T--	T---	N	N	N
TBIM-EtOH	N	T--	T---	T--	N	T--	T--	N	T---	T---
BBM ₂	N	T--	N	T--	T---	T--	T--	N	T---	N
BBM ₂ - Fe	N	T--	T---	T--	T---	T--	T---	N	T---	T---
BBM ₂ - Fe + EtOH	T---	T--	T---	T--	T---	T--	T--	N	T---	N

the dark, even though they all could grow in this concentration in the light. This could not be due to the BBM2 medium or any of its variants as most of the isolates could survive in one or more of them.

No algae were found in this study which were able to use any of the pesticides as a sole carbon source and grow in the dark. It is, however, possible that an investigation geared primarily at isolating algae that could so use them would be successful. This is particularly likely for the herbicide 2,4-D which has been found to be readily broken down and used as a carbon source by many bacteria, fungi, and actinomycetes (Appendix III). Because of the similarity between blue-green algae and bacteria (Stanier, et al., 1971), a search for an alga capable of breaking down 2,4-D or other pesticides would probably most profitably start with representatives of that group isolated from soil which had been sprayed at some time with the pesticide in question. Another group which might provide an alga which can utilize pesticides as a sole carbon source is the chlorococcalean algae (Division Chlorophycophyta, Order Chlorococcales, Family Chlorococcaceae) since that group exhibited a great degree of heterotrophy in past studies (Parker, 1961).

Although none of the isolates grew heterotrophically in the dark with the tested pesticides, there were some cases of stimulation of growth in the light. Wiedeman (1964) and Wiedeman and Bold (1965) described five ways in which organisms responded to supplemental carbon sources. The organisms in this study would fall in either their category 2 (obligate autotrophs needing both light and air for growth) or category 3 (obligate phototrophs which can grow in light either aerobically or with air excluded), a distinction which cannot be made on the basis of the present study. It is possible that the organisms in this study which

were stimulated by one or more of the pesticides fall into category 3. That is, they can use the pesticide as a carbon source to supplement the natural carbon supply from carbon dioxide or can perhaps use it as a sole carbon source if air is excluded, as long as light is present, but cannot grow in the absence of light even in the presence of the stimulatory pesticide. A second possibility is that the isolates which are enhanced by the pesticides in the light are able to use them as a supplemental source of carbon in a form of cometabolism, but cannot use them as a sole carbon source in the dark. Further study will be needed to determine if either of these hypotheses is correct.

Results - Overall Effects of All Pesticides on Each Isolate

Figures 32 and 33 show the average effects for each isolate for all six pesticides and give a good indication of the general reaction of each alga to pesticides. *Stichococcus chodati* (2), *Chlorococcum* (3) and (5), *Bracteacoccus* (4), and *Chlorella* (9) are all definitely inhibited by the addition of pesticides to the growth medium. Although the degree of inhibition varies, these organisms all show a definite pattern with lower growth in all concentrations of pesticides. In the case of isolates 3, 4 and 5, the inhibition is particularly orderly with the growth curves for 10 and 50 ppm falling below those for the other pesticide concentrations which in turn are lower than that for the controls. *Stichococcus chodati* (6) and unidentified (8) have patterns which are indefinite, not having any obvious order to the different concentrations and the controls. This is a particularly interesting difference between isolates 2 and 6 which have been tentatively identified as the same species. Finally, *Hormidium* (1) and *Neochloris* (7) have a pattern which indicates an overall stimulation in growth due to the presence of pesticides. In both cases this is

FIGURE 32

Interactions of all pesticides on the growth of isolates 1 - 4.
Abscissa - Time in weeks; Ordinate - Optical density as measured on
the Spectronic 20 at 425 nm. The numbers for graph 2 are applicable
to graphs 3 and 4.

1. *Hormidium* sp. (1)
2. *Stichococcus chodati* (2)
3. *Chlorococcum* sp. (3)
4. *Bracteacoccus* sp. (4)

FIGURE 32 Interactions

— 50 ppm
- - - 10 ppm
... 5 ppm

- · - · - 1 ppm
- · - · - 0.5 ppm
- · - · - 0.1 ppm
— 0 ppm (Control)

FIGURE 33

Interactions of all pesticides on the growth of isolates 5 - 9. Abscissa - Time in weeks; Ordinate - Optical density as measured on the Spectronic 20 at 425 nm. The numbers for graph 5 are applicable to graphs 6, 7, and 9.

5. *Chlorococcum* sp. (5)
6. *Stichococcus chodati* (6)
7. *Neochloris* sp. (7)
8. Unknown (8)
9. *Chlorella* sp. (9)

FIGURE 33 Interactions

— 50 ppm
- - - 10 ppm
... 5 ppm
- - - 1 ppm
- - - 0.5 ppm
- - - 0.1 ppm
— 0 ppm (Control)

more marked at the later data points which might indicate an ability of these organisms to overcome any initial inhibition due to the pesticides and then use them to supplement their growth.

DISCUSSION

This section will be divided into four parts covering: 1) stimulation due to certain pesticides; 2) analysis of growth effects of each pesticide; 3) the possible natural interactions of these pesticides and soil algae; and 4) suggestions for further research.

Stimulation Effects

The stimulation of growth of some isolates by four of the five pesticides tested (only malathion did not increase the growth of any isolates) is interesting since it was shown that none of the algae can use the pesticides as a sole carbon source in the dark. Other workers have also reported increased growth in some cases with the pesticides used. Since the tubes in which the isolate were growing were not shaken, it is probable that the amount of CO_2 in the media was the limiting factor in growth. It is known that suboptimal photosynthesis rates in higher plants are often the result of a lack of CO_2 (Salisbury and Ross, 1969), and it is possible that this situation occurred in the tubes in the present experiment toward the end of the daily 12 hour light period. Since the stimulation of growth is most pronounced at the later sampling periods, there might be a greater deficiency of CO_2 as the number of photosynthesizing cells increased. Wiedeman (1964) and Wiedeman and Bold (1965) have shown that some algae can utilize supplemental carbon sources under varying conditions of light and air. Organisms which could grow either aerobically or with air excluded but only in the light were labeled obligate phototrophs. Wiedeman (1964) reported a photometabolic process for many of the algae he studied when they were grown in glucose, mannose,

fructose, sodium acetate, and casein hydrolysate with exogenous carbon dioxide excluded. It is possible that the organisms in this study which were stimulated by the pesticides are obligate phototrophs, being able to take advantage of the supplemental carbon source (pesticide or ethanol) to increase their growth when the available CO₂ has been depleted or reduced to a very low level, but only in the presence of light. Further studies with CO₂ excluded or studies following the breakdown of the pesticides in the culture medium could clarify this point.

It is interesting to note that isolates 1, *Hormidium* sp., and 7, *Neochloris* sp., were both stimulated by 2,4-D, 2,4,5-T and rotenone, and were unaffected by malathion. The only pesticide toward which they reacted differently was DDT, with 1 being unaffected and 7 being inhibited by the highest concentrations used. These 2 isolates appear to be the most resistant to pesticides and would be the best species to test for possible pesticide degradation. Further studies of the means by which they are able to use a variety of pesticides to enhance their growth and to avoid the negative effects often found with other isolates could provide further insights into the pesticide-soil algae interaction.

Growth Effects

The five different pesticides used in this study fall into the following general sequence from least inhibitory to most inhibitory toward the growth of the isolates used in this study: 2,4-D, 2,4,5-T, DDT, rotenone, and malathion. Discussion of the possible mechanisms for these effects will follow in that order.

2,4-D

As early as 1950, 2,4-D was known to effect many plant processes (Norman, et al., 1950; Freeland, 1950; Van Overbeek, et al., 1951; Wort

and Cowie, 1953). However, in spite of 30 years of work, the mode of action of 2,4-D and the reason for its specificity for dicots over monocots is still not completely understood (Moreland, 1967; Salisbury and Ross, 1969). The current theory is that 2,4-D and other auxin type herbicides act at the nuclear level by enhancing synthesis of RNA and protein and, therefore, tissue proliferation. The extra synthesis induced by these herbicides might preclude normal cell development and function, thereby, being the basis for herbicidal action by upsetting the natural metabolic balances resulting in uncontrolled metabolism and death of the plant (Hilton, et al., 1963; Moreland, 1967; Key, 1963; Key, et al., 1960; Salisbury and Ross, 1969; Chrispeels and Hanson, 1962). However, many responses are observable within a few minutes of application, a period too short for RNA synthesis. Therefore, it may be that auxins and auxin-like herbicides act in more than one way (Moreland, 1967; Salisbury and Ross, 1969). Even if this hypothesis is correct for the mode of action of 2,4-D and other auxin-type herbicides, it does not account for 2,4-D's specificity for broad leaf plants. It is possible that auxin selectivity is associated with cyclic AMP which is known to be involved in the physiological responses of animal hormones which are often exceedingly specific (Salisbury and Ross, 1969). It is also possible that differential selectivity of 2,4-D is due to differential penetration, translocation, and inactivation rates in susceptible and resistant plants.

This study found that nine of the ten algae tested were either unaffected or stimulated by 2,4-D. From the preceeding discussion, it would appear than 2,4-D either does not penetrate into the algal cell or if it does, is rapidly detoxified there. Since 2,4-D is an auxin-mimicing herbicide, and auxins in general have little effect on algae, even though at least some algae are known to contain IAA (Salisbury and Ross, 1969),

it is not too suprising that 2,4-D is basically neutral toward algae. The one isolate which was inhibited, *Chlorella* (9), may be more sensitive to auxins and may not be able to breakdown those compounds readily when they enter the cell or to keep them from entering.

The stimulation of two isolates, *Hormidium* (1) and *Neochloris* (7), is interesting since it was shown that neither can use 2,4-D as a sole carbon source in the dark. Other workers have also reported stimulation of algal growth in some cases in the presence of 2,4-D. Respiration of *Chlorella pyrenoidosa* was stimulated at concentrations comparable to those used in this study (about 35 ppm, Erickson, et al., 1955), while Wedding, et al., (1954) reported a stimulation of photosynthesis in the same algae at pH of 7.0 and above (comparable to the pH of the TBIM medium in this study) at 2,4-D concentrations of $2 \times 10^{-3} \text{M}$ (442 ppm). Wiedeman (1964) reported that two isolates, *Dictyosphaerium* 380 and *Scenedesmus* 410, grew better at 2.0 mM 2,4-D than they did in the control. Therefore, it is possible that the stimulation in the present study was due to similar mechanisms with the increase in growth due to increased photosynthesis and respiration.

2,4,5-T

The general effects of 2,4,5-T in this study were found to be only slightly different than those of 2,4-D, two isolates being inhibited by 2,4,5-T (*Chlorococcum* (3) and (5)) while only one was inhibited by 2,4-D. 2,4,5-T, like 2,4-D, is one of the chlorophenoxy herbicides and is, therefore, similar to naturally occurring auxins. Structurally, the only difference between 2,4-D and 2,4,5-T is that the latter has an additional chlorine atom at the number 5 position of the benzene ring in place of an hydrogen. This small structural difference, however, has a large

physiological difference. 2,4,5-T is very highly toxic toward woody plant species and is commonly used to remove undesired shrub growth. Because of a dioxin which results as a manufacturing impurity, 2,4,5-T was suspended from use around the home, near water, on food crops, and in South Vietnam in April, 1970. This dioxin is supposed to be very toxic to humans and other organisms. Since the 2,4,5-T used in this study was a purified form and not that generally sold in the market place, these dioxins should not have had any effect on the results reported here.

A comparison of Figures 14-16 and 17-19 shows that two isolates, *Chlorococcum* (3) and (5) are more susceptible to 2,4,5-T than to 2,4-D. The inhibition at concentrations of 10 and 50 ppm is quite obvious in both isolates in 2,4,5-T. The probable mechanism which results in inhibition due to 2,4-D in the algae is also probably acting in the case of 2,4,5-T so the analysis described above will not be repeated.

The isolates which were stimulated by 2,4-D, *Hormidium* (1) and *Neochloris* (7), were effected in the same way by 2,4,5-T. Isolate 1 has a greater stimulation due to 2,4,5-T while 7 is more effected by 2,4-D. The stimulation may be a result of photometabolism of 2,4,5-T under conditions of low CO₂ as was discussed in the first section of the discussion. The only reports on the effects of 2,4,5-T on physiological processes is that 1 ppm did not decrease productivity of a phytoplankton sample over a period of four hours (Butler, 1963, cited in Pimentel, 1971). Therefore, the effect on photosynthesis or other physiological processes is uncertain.

DDT

Although the insecticidal properties of DDT have been known for over 35 years, an understanding of its mechanism and selectivity at a

cellular level is still far from complete. Its primary effect is on the central nervous system (Moore, 1967), apparently in increasing the potassium permeability of the axons of the sensory nerves (O'Brien, 1967). The effects it has on plants may be related, but cannot be exactly the same since plants have no nerves! Reports on the effects of DDT at the cellular level with higher plants have shown a significant respiration reduction (Lichtenstein, et al., 1962) and a reduction in the light reaction of photosynthesis in susceptible varieties of barley (Lawler and Rogers, 1967). The latter reaction to DDT was controlled by a single major gene, susceptibility being dominant to resistance. With algae, DDT has been reported to reduce productivity of phytoplankton communities (Butler, 1963, cited in Pimentel, 1971); reduce C^{14} assimilation (Stadynk, 1967); reduce photosynthesis (Wurster, 1968); and block or delay cell division (Menel, et al., 1970; Bowes, 1972). Other studies have reported morphological changes and both growth stimulations and growth reductions from varying concentrations of DDT.

The present study found varying effects due to DDT depending upon the isolate under consideration. The inhibition found in four of the isolates tested here could be due to any of the mechanisms discussed above. However, the actual basis for this negative effect cannot be determined from the present study. The difference in susceptibility between the isolates, especially striking between the two isolates tentatively identified as *Stichococcus chodati* (2 and 6), is probably due to either differential breakdown rates or to differential effects within the cell. Resistance is probably not due to ability to keep the molecule from entering the cell since it has been shown that uptake is passive and very rapid even with dead cells (Södergren, 1968).

Rotenone

Rotenone acts by inhibiting the coupled oxidation of NADH_2 and the reduction of cytochrome b, a pathway common to all aerobic cells. It is not surprising then that it had negative effects on the growth of half of the isolates tested in this study. What is most surprising is that it was neutral toward two isolates and stimulated the growth of three others. Rotenone could have lost its effectiveness due to breakdown from exposure to light within the early part of the experiment. Some of the breakdown products are also toxic, and it may be that the isolates most affected are those that are inhibited both by rotenone and its toxic breakdown products. Those algae which were not effected may have a resistance to the breakdown products or may be able to modify them to other nontoxic chemicals. Two of the three isolates which were stimulated by rotenone were the same ones which were stimulated by 2,4-D and 2,4,5-T (*Hormidium* (1) and *Neochloris* (7)). These two isolates appear to be particularly well adapted to using supplemental carbon sources to increase their growth when light is present. The third isolate which was stimulated by rotenone was *Stichococcus chodati* (6) while the other isolate tentatively identified as the same species, (2), had a definite inhibition in growth due to rotenone. This difference may be due to difference in the physiological responses of the two strains. Finally, the pattern of stimulation of isolate 6 is interesting since it is stimulated only by the two higher concentrations and not by the lower ones indicating a possible threshold level for stimulation. Further studies will be necessary to determine the reason for the patterns of stimulation and the difference in response of two similar isolates.

Malathion

Malathion, an organophosphate, had the strongest effect of any of the pesticides tested, inhibiting seven isolates, being neutral to three and stimulating none. The effect of inhibition is quite systematic, with the highest concentrations inhibiting most and successively lower concentrations being less inhibitory. The organophosphates exert their inhibitory action in animals by reacting with cholinesterase (O'Brien, 1967). Specifically, the OH^- of a serine at the active site of cholinesterase combines with the phosphorous of the organophosphate, forming a covalent bond between the enzyme and the pesticide. Since esterases are common in algal cells, malathion likely exerts a negative effect on algae by tying up and inactivating essential esterase enzymes. This would explain the systematic effect regularly seen in this study. At higher concentrations more enzymes would be tied up with a greater disruption of the intermediary metabolism of the cells as a consequence. Organophosphates are activated *in vivo* when S-alkyl isomerization occurs (a CH_3 - group moves from an oxygen atom to the sulfur atom. This makes the phosphorous more positive than it is in the original form, and it is able to react more rapidly with esterases.) Another change which can take place *in vivo* is the complete replacement of the sulfur atom by oxygen. (For malathion, the resulting sulfurless compound is called malaaxon and is a more potent cholinesterase inhibitor than malathion). Christie (1969) found that at least some of the C^{14} malathion added to an axenic culture of *Chlorella pyrenoidosa* was converted to malaaxon. Therefore, a similar conversion may have occurred in the algae in this study which were inhibited by malathion.

Carboxyesterases and phosphatases are known in mammals and insects which degrade malathion to malathion acid (which can be further cleaved to

malathion diacid) or to dimethyl phosphorothioate. Degradations of this type may also be possible in algae and may account for the few isolates which were found to be unaffected by malathion. Certain compounds are known which can, however, tie up the enzymes which are responsible for this hydrolysis and, therefore, increase the toxicity.

Therefore, it is probable that the inhibitory effect of malathion is due to its combining with essential esterases in the algal cells. Those organisms which are not effected are probably better able to convert malathion to nontoxic compounds or to decouple the enzyme-insecticide bond. That no cases of stimulation were found indicates that none of the isolates studied were able under any conditions to profit from the presence of malathion, even if it were degraded.

Natural Interactions

As S. J. L. Wright has stated (1971):

The microbiologist usually aims to study herbicide degradation under defined conditions, using pure microbial cultures and pure chemicals rather than commercial formulations. Such conditions are often so far removed from those in the soil environment as to render laboratory studies quite artificial. This is an unfortunate admission for physiological-ecological investigations and indicates that information obtained from *in vitro* experiments cannot necessarily be projected with certainty to the field where the microbial population and environment are infinitely more complex.

The factors which must be considered for a full study of the effects of a given pesticide on a given species include; 1) the properties of the pesticide; 2) the manner of its application; 3) the extent to which the species contacts the pesticide; 4) the response of the species (acute and chronic toxicity); 5) information on the factors which control the population size of the species in the absence of the pesticide; and 6) toxicity information on the pesticide's effects on any species which control the given species in the absence of the pesticide. If all this

information were known, then a hypothesis could be made on the effect the pesticide may have on the species in nature (Moore, 1967). Since the present study does not include all these factors, the caution of Wright must be kept in mind during the speculation which follows.

Of the five pesticides tested in this study, the two with the most harmful effects, rotenone and malathion, are also the ones which are least persistent. (However, DDT, the most persistent pesticide, is nearly as inhibitory). Therefore, the effects of these two pesticides, although they may be temporarily drastic, may not be long lasting. But it must be remembered that even sublethal effects may have a potentially great ecological importance. In addition, there are indirect effects which a pesticide may have on any one species; delayed toxic effects, reduction of habitat, and removal of symbionts, competitors, and grazers (Moore, 1967). Because none of these pesticides had a uniform effect across all the algae tested, they could alter the species composition of a mixed soil population and have some of the indirect effects noted above. As early as 1953, Aldrich suggested that 2,4-D might change the composition of microorganism populations with repeated applications by encouraging the growth of organisms which can decompose 2,4-D and use it as a carbon source while at the same time reducing the populations of other organisms which are susceptible to its effects. Cook and Connors (1963) and Hurlbert, et al. (1972) reported changes in natural species composition following application of different organophosphates. Christie (1969) found that malathion altered the species composition of a mixed algal community associated with waste stabilization ponds. Similarly the presence of DDT caused changes in species proportions in a mixed culture of two algal species (Mosser, et al., 1972) and in natural algal populations (Lazaroff and Moore, 1966; Woodwell, et al., 1967; Batterson, et

al., 1972; Shane, 1948; and Bridges, et al., 1963). Kiser, et al. (1963) and Hooper (1948, reported in Cope, 1965) found reductions in zooplankton in lake environments due to the presence of rotenone. So the present pesticides, even when there are no direct effects, may change the species composition of the soil algae community.

It appears from this study that none of the algal isolates by themselves would be able to break down the pesticides in the soil. It is, however, possible that the algae could in concert with other microorganisms cause a change in structure of the chemicals which would lead to their gradual breakdown and disappearance. Alexander (1965b) indicates that microorganisms cannot always detoxify and decontaminate soils at a sufficient rate to keep the level of even readily biodegradable pesticides low. And since some of these chemicals are modified in various ways by different microbes, they may not have the same toxicities in every soil. Studies, therefore, must also focus on all potential detoxification products and how they effect soil microorganisms.

Therefore, although it is difficult to say with great certainty from this study alone, it is probable that the pesticides tested would have an effect in nature on the species tested and would alter the ecological balance of the soil microflora.

Suggestions for Further Research

This study, like most research, while answering a few questions has raised many new ones. Efforts should be made to expand the present study to include organisms of different algal groups, especially the blue-greens and diatoms which are common members of the soil microflora. Also, work should be done using mixed populations to determine what effects the pesticides have on diversity of the soil flora and the ef-

fects that interactions of more than one organism isolated from the same habitat can have on the pesticides. Further work could be done using technical grades of the pesticides studied here to determine if there are differences due to the presence of manufacturing impurities. A concerted effort should be made, particularly among the blue-green algae, to find isolates which can use pesticides, especially 2,4-D as a sole carbon source. Along this line, a dark experiment of 12-14 month duration could be performed to determine if any of the isolates could use the compounds as a sole carbon source in the dark, but at a very slow rate. Tests could be undertaken with the organisms which were stimulated by the pesticides to determine if they are actually breaking down any of the pesticides to any extent. Other studies could evaluate the ecological effects of pesticide interaction (potentiation). Also, a study to determine the mechanisms of resistance of certain isolates would be informative. Finally, detailed morphological and physiological studies may find effects due to the presence of pesticides which were not apparent from the present study.

While this study was only a beginning, it has shown that there are definite interactions between soil algae and pesticides which have not been considered before. These interactions have been found even at concentrations normally used as pesticidal levels and not only at the very high levels often used in studies of this type. Future studies could involve soil scientists, biochemists, bacteriologists, mycologists, and phycologists working together to determine the ultimate fate of pesticides in the environment and their effect on the soil microflora.

SUMMARY AND CONCLUSIONS

A study of the effects of five different pesticides on soil algae was made. This project involved isolating into axenic culture ten algae from soil collected in Oldham and Jefferson Counties, Kentucky, and then determining the effects of different concentrations of each pesticide on the growth curve of each isolate over an eight or 12 week period, and also determining if any of the isolates could use any of the pesticides as a sole carbon source.

The algae isolated into unialgal, bacteria-free (axenic) culture were, with one possible exception, members of the Division Chlorophycophyta. Isolate 8, still unidentified, gave a very weak or negative response for starch when tested with potassium iodine. It is, therefore, possible that isolate is a member of the Xanthophycophyta. Two basic growth media were used, TBIM for most of the experiments and BBM2 for the experiments with DDT. All organisms, except for #1, grew better in TBIM than in BBM2, possibly because that was the medium used for isolation purposes or because the algae were better able to grow in an alkaline than an acid medium.

Statistical analysis revealed that the three way interaction of pesticide-algae-concentrations was significant at all data collection points. Therefore, the two way interactions of pesticide-algae, pesticide-concentration, and algae-concentrations and the main effects must be interpreted with caution. Due to the significant interactions, the results of this study cannot be generalized to other algae, pesticides, or concentrations.

The results of each pesticide in the light may be summarized as follows:

1) 2,4-D was basically neutral, inhibiting one isolate, stimulating two others, and showing neutral effects toward the other seven.

2) 2,4,5-T was also basically neutral: it inhibited the growth of two algae, stimulated two others, and had no effect on the growth of the other six.

3) DDT had mixed effects: negative effects were found with four isolates, stimulation with one, and neutrality with the other five.

4) Rotenone also had mixed effects: inhibiting five isolates, stimulating three, and having no effect on the other two.

5) Malathion was used in two purities, 95% and 99%, the first being technical and the second laboratory grade. Except for isolate 9, the results were the same with both purities. There were six cases of inhibition, three cases of neutrality, and none of stimulation. Isolate 9 was inhibited by one of the purities but not the other.

The results of the dark experiment showed that none of the isolates could use any of the pesticides as a sole carbon source to sustain growth in the dark at a level comparable to that in the light. At least some of the isolates could, however, survive for a period of eight weeks in all the pesticides except DDT. At the end of eight weeks there were no visible traces of algae in any of the tubes containing DDT.

Other observations and results can be summarized as follows:

1) In at least some cases, the isolates could use the pesticides to supplement their growth. This is probably a result of phototrophy since they could not use any of the pesticides to support their growth in the dark.

2) Isolates 1, *Horridium* sp., and 7, *Neochoris* sp., are best able to withstand the presence of pesticides and to use them to supplement their growth.

3) Although four of the pesticides in this study (all but DDT) are rapidly broken down in the soil, repeated applications could still have an effect on the soil microflora. Changes in the proportions of different algal species could occur as a result of stimulation of certain organisms and inhibition of others.

4) Finally, suggestions for further studies were made.

APPENDICES

APPENDIX I

DECOMPOSITION TIME FOR 2,4-D UNDER LAB CONDITIONS

Time for Decomposition	Conditions	Reference
3-8 weeks	wet and dry soil	Jorgensen and Hamner, 1948
2 weeks - 18 months	wet and dry storage	Mitchell and Marth, 1946
6 weeks		Hans, 1946
120 days	lake water	Aly and Faust, 1964
3 to over 7 months (95% disappearance)	different soils	Montgomery, et al., 1972
24 hours (81-85%)	lake muds previously adapted	Aly and Faust, 1964
2-3 weeks		Jensen and Peterson, 1952
10 days		Whiteside and Alexander, 1960
6-7 days		Bell, 1957
less than 13 weeks		Kries, 1947
8, 11, or 21 days; 6-7 days	varying concentrations; retreatment	Newman and Thomas, 1950
23, 26, or 94 days	different soils	Alexander and Aleem, 1961
less than 8 weeks	greenhouse, unleached	DeRose, 1946
14 days		Audus, 1951
11 or 15 months	dry storage, different concentrations	Weaver, 1948
67 days	in greenhouse	DeRose and Newman, 1948
40, 80, or 160+ days	different dosages and soil pHs	Martin, 1946
over 7 weeks		Taylor, 1947
over 6 months	organisms from activated sludge	Schwartz, 1967

APPENDIX II

DECOMPOSITION TIMES FOR 2,4-D UNDER FIELD CONDITIONS

Time for decomposition	Conditions	Reference
less than 93 days		DeRose and Newman, 1948
over 45 days		Weaver, 1948
less than 80 days		DeRose, 1946
1 month		Matsumura, 1972
4-6 weeks	95% disappearance	Edwards, 1964
1 or 4 weeks or longer	different soils and doses	Ogle and Warren, 1954
6 weeks; 5 weeks	untreated soil; soil treated previous year	Newman, et al., 1952
1-4 weeks		Klingman, 1961 (cited in Pimentel, 1971)
4-18 weeks		Hernandez and Warren, 1950 (cited in Pimentel, 1971)
2 or 6 weeks	different concentrations	Cope, et al., 1970

APPENDIX III

ORGANISMS WHICH CAN USE 2,4-D AS A SOLE CARBON SOURCE

Organism	Reference
<i>Bacterium globiforme</i> type	Audus, 1950, 1951; Audus and Symonds, 1955
<i>Rhizobium meliloti</i>	Nilsson, 1957 (cited in Audus, 1964)
<i>Flavobacterium aquatile</i>	Jensen and Peterson, 1952; Jensen, 1960 (cited in Audus, 1964)
<i>F. peregrinum</i>	Steenson and Walker, 1957, 1958
<i>Achromobacter</i> sp.	Bell, 1957, 1960; Steenson and Walker, 1957, 1958
<i>Pseudomonas</i> sp.	Fernley and Evans, 1959
<i>Aspergillus niger</i>	Woodcock, 1964
<i>Corynebacterium</i> sp.	Rogoff and Reid, 1956
<i>Mycoplasma</i> sp.	Walker and Newman, 1956
<i>Penicillium</i> sp.	Mickovski, et al., 1968
<i>Scopulariopsis brevicaulis</i>	Mickovski, et al., 1968
<i>Fusarium neoceras</i>	Mickovski, et al., 1968
Unidentified:	
Gram-negative bacterium, looked like <i>Corynebacterium</i>	Jensen and Peterson, 1952
Gram negative, motile rod	Evans and Smith, 1954
Bacterium	Loos, et al., 1967

APPENDIX IV

MICROORGANISMS INVOLVED IN AT LEAST ONE STEP OF DDT BREAKDOWN

Microorganism	Reference
Yeast	Kallmand and Andrews, 1963; and Ledford and Chen, 1969
Lake plankton	Miskus, et al., 1965
<i>Proteus vulgaris</i>	Barker, et al., 1965
<i>Nocardia</i> sp.	Chacko, et al., 1966
<i>Streptomyces aureofaciens</i>	Chacko, et al., 1966
<i>S. cinnamoneus</i>	Chacko, et al., 1966
<i>S. viridochromogenes</i>	Chacko, et al., 1966
<i>S. albus</i>	Chacko, et al., 1966
<i>S. antibioticus</i>	Chacko, et al., 1966
<i>Escherichia coli</i>	Wedemeyer, 1966
<i>Klebsiella pneumoniae</i>	Wedemeyer, 1966
<i>Aerobacter aerogenes</i>	Wedemeyer, and Kearney, et al., 1969
23 plant pathogenic and saprophytic bacteria	Johnson, et al., 1967
<i>Trichoderma viride</i>	Matsumura and Boush, 1968; and Patil, et al., 1970
Gastrointestinal microflora of the rat	Braunberg and Beck, 1968
<i>Geotrichum</i> sp.	Ledford and Chen, 1969
Rumen microorganisms	Fries, et al., 1969
<i>Hydrogenomonas</i> sp.	Focht and Alexander, 1970, 1971; Focht, 1972; Alexander, 1972; Pfaender and Alexander, 1972
<i>Pseudomonas</i> sp.	Patil, et al., 1970
<i>P. aeruginosa</i>	Cope, 1965
<i>P. fluorescens</i>	Cope, 1965

APPENDIX IV (Cont'd)

Microorganism	Reference
<i>Bacillus</i> sp.	Patil, et al., 1970
<i>Micrococcus</i> sp.	Patil et al., 1970
<i>Mucor alternans</i>	Anderson, et al., 1970
<i>Monilaceae</i> sp.	Focht, 1972
<i>Arthrobacter</i> sp.	Pfaender and Alexander, 1972
Unidentified soil microbe	Patil, et al., 1970
Gram positive rod	Ledford and Chen, 1969

APPENDIX V

DDT METABOLITES

Metabolites	Reference
Dicofol	Matsumura and Boush, 1968; Anderson, et al., 1970 Patil, et al., 1970
TDE (DDD) (2,2-bis(p-chlorophenyl)-1,1-di- chloroethane)	Ledford and Chen, 1969 Ko and Lockwood, 1968b Kallman and Andrews, 1963 Miskus, et al., 1965 Barker, et al., 1965 Wedemeyer, 1966, 1967b Chacko, et al., 1966 Johnson, et al., 1967 Guenzi and Beard, 1967 Braunberg and Beck, 1968 Fries, et al., 1969 Johnson, et al., 1972 Anderson, et al., 1970 Patil, et al., 1970 Matsumura and Boush, 1968 Ott and Gunther, 1965 Matsumura, et al., 1971 Menzie, 1972 Pfaender and Alexander, 1972
DDE (1,1-dichloro-2,2-bis(p- chlorophenyl)ethylene)	Anderson, et al., 1970 Matsumura and Boush, 1968 Wedemeyer, 1967b Ott and Gunther, 1965 Matsumura, et al., 1971 Menzie, 1972 Pfaender and Alexander, 1972
DDA (2,2-bis-(p-chlorophenyl)acetate)	Menzie, 1972 Wedemeyer, 1967b Patil, et al., 1970 Anderson, et al., 1970
DBP (4,4'-dichlorobenzophenone)	Menzie, 1972 Anderson, et al., 1970 Pfaender and Alexander, 1972 Wedemeyer, 1967b, 1967a
DDDE	Ott and Gunther, 1965
DDNS (1-bis(p-chlorophenyl)-ethane)	Matsumura et al., 1971
p-chlorobenzoic acid	Menzie, 1972

APPENDIX V (Cont'd)

Metabolite

Reference

Kelthane

Menzie, 1972

1,1-bis(p-chlorophenyl)ethane

Anderson, et al., 1970

p-chlorophenylacetic acid

Alexander, 1972
 Focht and Alexander, 1970, 1971
 Pfaender and Alexander, 1972

DDMS (1-chloro-2,2-bis(p-chloro-phenyl)ethane)

Pfaender and Alexander, 1972
 Wedemeyer, 1967b

DDMU (1-chloro-2,2-bis(p-chloro-phenyl)ethane)

Wedemeyer, 1967b

DDNU (unsym-bis(p-chlorophenyl)ethylene)

Wedemeyer, 1967b

DPM (Dichlorophenylmethane)

Wedemeyer, 1967a

DBH (Dichlorobenzhydrol)

Wedemeyer, 1967a

Phenylacetic acid

Focht and Alexander, 1971

2-phenyl-3,3,3-trichloropropionic acid

Focht and Alexander, 1971

LITERATURE CITED

- Aldrich, Richard J. 1953. Herbicides: Residues in soil. J. Agr. Food Chem. 1: 257-260.
- Alexander, M. 1964. Biochemical ecology of soil microorganisms. Ann. Rev. Microbiol. 18: 217-252.
- _____. 1965a. Biodegradation: Problems of molecular recalcitrance and microbial fallibility. Advan. Appl. Microbiol. 7: 35-80.
- _____. 1965b. Persistence and biological reactions of pesticides in soils. Proc. Soil Sci. Soc. Am. 29: 1-7.
- _____. 1967. The breakdown of pesticides in soils. Pages 331-342 in N. C. Brady, ed. Agriculture and the quality of our environment. AAAS, Washington, D. C.
- _____. 1971. Microbial ecology. Wiley, New York. 511 pp.
- _____. 1972. Microbial degradation of pesticides. Pages 365-383 in Fumio Matsumura, G. Mallory Boush, and Tomomasa Misato, eds. Environmental toxicology of pesticides. Academic Press, New York.
- _____, and M. I. H. Aleem. 1961. Effect of chemical structure on microbial decomposition of aromatic herbicides. J. Agr. Food Chem. 9: 44-47.
- Allen, Norman, R. L. Walker, L. C. Fife, R. D. Chisholm, Louis Koblitsky, J. F. Bullock, C. R. Hodge, and E. E. Hall. 1954. Persistence of BHC, DDT, and toxaphene in soil and the tolerances of certain crops to their residues. U. S. Dept. Agr. Tech. Bull. # 1090. 19 pp.
- Aly, Osman M. and Samuel D. Faust. 1964. Studies on the fate of 2,4-D and ester derivatives in natural surface waters. J. Agr. Food Chem. 12: 541-546.
- Anderson, G. R. and C. O. Baker. 1950. Some effects of 2,4-D in representative Idaho soils. Agron. J. 42: 456-58, cited in Pimentel, 1971.
- Anderson, J. P. E., E. P. Lichtenstein, and W. F. Whittingham. 1970. Effect of *Mucor alternans* on the persistence of DDT and dieldrin in culture and in soil. J. Econ. Ent. 63: 1595-99.
- Anonymous. 1972. Battelle scientist rates pesticides at top of 19 environmental hazards. Air/Water Pollution Report 10: 14.

- Appleman, M. D. and O. H. Sears. 1946. Effect of DDT upon modulation of legumes. J. Amer. Soc. Agron. 38: 545-50
- Arnold, W. R., P. W. Santelmann, and J. Q. Lynd. 1966. Picloram and 2,4-D effects with *Aspergillus niger* proliferation. Weeds 14: 89-90, cited in Pimentel, 1971.
- Audus, L. J. 1949. The biological detoxification of 2,4-dichlorophenoxyacetic acid in soil. Plant and Soil. 2: 31.
- Audus, L. J. 1950. Biological detoxification of 2,4-dichlorophenoxyacetic acid in soils: Isolation of an effective organism. Nature 166: 365.
- _____. 1951. The biological detoxification of hormone herbicides in soil. Plant and Soil 3: 170-192.
- _____. 1952. The decomposition of 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid in the soil. J. Sci. Food Agr. 3: 268-274.
- _____. 1964. Herbicide behavior in soil. Pages 163-206 in L. J. Audus, ed. The physiology and biochemistry of herbicides. Academic Press. New York. 555 pp.
- _____. and K. V. Symonds. 1955. Further studies on the breakdown of 2,4-dichlorophenoxyacetic acid by a soil bacterium. Ann. Appl. Biol. 42: 174-182.
- Barker, P. S., F. O. Morrison, and R. S. Whitaker. 1965. Conversion of DDT to DDD by *Proteus vulgaris*, a bacterium isolated from the intestinal flora of a mouse. Nature 205: 621-622.
- Bartha, Richard, R. P. Lanzilotta, and David Pramer. 1967. Stability and effects of some pesticides in soil. Appl. Microbiol. 15: 67-75.
- Batterson, J. C., G. M. Boush, and F. Matsumura. 1972. DDT: inhibition of sodium chloride tolerance by the blue-green alga *Anacystis nidulans*. Science 176. 1141-1143.
- Bell, G. R. 1957. Some morphological and biochemical characteristics of a soil bacterium which decomposes 2,4-dichlorophenoxyacetic acid. Can. J. Microbiol. 3: 821-840.
- _____. 1960. Studies on a soil *Achromobacter* which degrades 2,4-dichlorophenoxyacetic acid. Can. J. Microbiol. 6: 325-337.
- Berger, Kermit C. 1965. Sun, Soil, and survival: An introduction to soils. U. of Oklahoma Press, Norman. 371 pp.
- Bischoff, Harry W. and Harold C. Bold. 1963. Phycological Studies. IV. Some soil algae from Enchanted Rock and related algal species. Univ. of Texas Publ. No. 6318.

- Bishop, E. L. 1947. Effects of DDT mosquito larviciding on wildlife. Part III. The effects on the plankton population of routine larviciding with DDT. Public Health Rep. 62: 1263-1268.
- Blackman, G. E., W. G. Templeman, and D. J. Halliday. 1951. Herbicides and selective phytotoxicity. Ann. Rev. Plant. Physiol. 2: 199-230.
- Bold, H. C. 1942. The cultivation of algae. Bot. Rev. 8: 70-138.
- _____. 1970. Some aspects of the taxonomy of soil algae. N.Y. Acad. Sci., Annals. 175: 601-616.
- Bollen, Walter B. 1961. Interactions between pesticides and soil microorganisms. Ann. Rev. Microbio. 15: 69-92.
- _____, H. E. Morrison, and H. H. Crowell. 1954a. Effect of field and laboratory treatments with BHC and DDT on nitrogen transformations and soil respiration. J. Econ. Ent. 47: 307-312.
- _____, _____, and _____. 1954b. Effect of field treatments of insecticides on numbers of bacteria, *Streptomyces*, and molds in the soil. J. Econ. Ent. 47: 302-306.
- Bounds, H. C. and A. R. Colmer. 1964. Resistance of *Streptomyces* to herbicides. Sugar Bull. 42: 274-76, cited in Pimentel, 1971.
- Boush, G. M. and J. C. Batterton. 1972. Ecological aspects of pesticide-microbial relationship. Pages 401-421 in Fumio Matsumura, G. Mallory Boush, and Tomomasa Misato, eds. Environmental toxicology of pesticides. Academic Press, New York.
- Bowes, Gerald W. 1972. Uptake and metabolism of 2,4-bis(chlorophenyl)-1,1,1-trichloroethane (DDT) by marine phytoplankton and its effect on growth and chloroplast electron transport. Plant Physiol. 49: 172-176.
- Bowman, M. C., F. Acree, and M. K. Corbett. 1960. Solubility of carbon-14 DDT in water. J. Agr. Food Chem. 8: 406.
- Boyce Thompson Institute for Plant Research, Inc. 1971. Interaction of herbicides and soil microorganisms. Water Pollution Control Research Series # 16060 DMP.
- Braithwaite, B. M., A. Jane, and F. G. Swain. 1958. J. Aus. Inst. Agr. Sci. 24: 155-157, cited in Bollen, 1961.
- Braunberg, R. C. and Vivian Beck. 1968. Interaction of DDT and the gastrointestinal microflora of the rat. J. Agr. Food Chem. 16: 451-453.
- Bridges, W. R., B. J. Kallman, and A. K. Andrews. 1963. Persistence of DDT and its metabolites in a farm pond. Trans. Am. Fisheries Soc. 92: 421-427.

- Brown, John R. 1972. DDT in the agricultural soil and human tissue in Norfolk County, Ontario. Pages 551-571 in A. S. Tahori, ed. Fate of pesticides in environment. Volume VI of Proceedings of the Second International IUPAC Congress of Pesticide Chemistry. Gordon and Breach Science Publishers, New York. 572 pp.
- Brown, J. W. and J. W. Mitchell. 1948. Inactivation of 2,4-D in soils as affected by soil moisture, temperature, manure, and autoclaving. Bot. Gaz. 109: 314-323.
- Burger, K., I. C. MacRae, and M. Alexander. 1962. Decomposition of phenoxyacetic acids. Proc. Soil Sci. Soc. Am. 26: 243.
- Butler, P. A. 1963. Commercial fisheries investigations. Pages 11-25 in Pesticide-Wildlife Studies. U. S. Fish. Wildl. Serv. Circ. 167, cited in Pimentel, 1971.
- _____ and P. F. Springer. 1964. Pesticides - a new factor in coastal environments. Trans. 28th N. Am. Wildlife Natural Resources Conf. 1964: 387-390, cited in Loosanoff, 1965.
- Cain, Brother Joseph. 1965. Nitrogen utilization in 38 freshwater Chlamydomonad algae. Can. J. Bot. 43: 1367-1378.
- Carlyle, R. E. and John D. Thorpe. 1947. Some effects of ammonium and sodium 2,4-dichlorophenoxyacetates on legumes and the rhizobium bacteria. J. Am. Soc. Agron. 39: 929-936.
- Casida, John E. 1964. Esterase inhibitors as pesticides. Science 146: 1011-1017.
- Chacko, C. I. and J. L. Lockwood. 1967. Accumulation of DDT and dieldrin by microorganisms. Can. J. Microbiol. 13: 1123-1126.
- _____, _____, and Matthew Zabik. 1966. Chlorinated hydrocarbon pesticides: Degradation by microbes. Science 154: 893-895.
- Chantanachat, Srisumon and Harold C. Bold. 1962. Phycological Studies. II. Some algae from arid soils. Univ. Texas Publ. No. 6218.
- Chapman, R. K. and T. C. Allen. 1948. Stimulation and suppression of some vegetable plants by DDT. J. Econ. Ent. 41: 616-623.
- Cheng, Hong-Ming, Izuru Yamamoto, and John E. Casida. 1972. Rotenone photodecomposition. J. Agr. Food Chem. 20: 850-956.
- Chisholm, D., A. W. MacPhee, and C. R. MacEachern. 1955. Effects of repeated applications of pesticides to soil. Can. J. Agr. Sci. 35: 433-439.
- Chisholm, R. D., L. K. Koblitzy, J. E. Fahey, and W. E. Westlake. 1950. DDT residues in soil. J. Econ. Ent. 43: 941-42.

- Chrispeels, M. J. and J. B. Hanson. 1962. The increase in ribonucleic acid content of cytoplasmic particulates of soybean hypocotyl induced by 2,4-dichlorophenoxyacetic acid. *Weeds* 10: 123-125.
- Christie, A. E. 1969. Effects of insecticides on algae. *Water Sew. Works.* 116: 172-176.
- Clore, W. J., W. E. Westlake, K. C. Walker, and V. R. Boswell. 1961. Residual effects of soil insecticides on crop plants. *Wash. Agr. Expt. Sta. Bull.* 627, 9 pp, cited in Alexander, 1965b.
- Clower, D. F. and J. G. Matthysse. 1954. Phytotoxicity of insecticides in mist concentrate type formulations. *J. Econ. Ent.* 47: 735-738.
- Cole, H., D. Barry, D. E. H. Frear, and A. Bradford. 1967. DDT levels in fish, streams, stream sediments, and soil before and after DDT aerial spray application for fall cankerworm in northern Pennsylvania. *Bull. Environ. Contam. Toxicol.* 2: 127-146.
- Colmer, Arthur R. 1953. The action of 2,4-D upon the *Azotobacter* of some sugarcane soils. *Appl. Microbiol.* 1: 184-187.
- Cook, S. F. Jr. and J. D. Connors. 1963. The short-term side effects of the insecticidal treatment of Clear Lake, Lake County, California in 1962. *Ann. Entomol. Soc. Amer.* 56: 819-824.
- Cope, Oliver B. 1965. Agricultural chemicals and fresh-water ecological systems. Pages 115-127 in C. O. Chichester, ed. *Research in pesticides.* Academic Press, Inc. New York.
- Cope, Oliver B., Edward M. Wood, and George H. Wallen. 1970. Some chronic effects of 2,4-D on the bluegill (*Lepomis macrochirus*). *Trans. Am. Fish. Soc.* 99: 1-12.
- Cox, James. L. 1970a. DDT residues in marine phytoplankton: Increase from 1955 to 1969. *Science* 170: 71-73.
- _____. 1970b. Low ambient level uptake of ^{14}C -DDT by three species of marine phytoplankton. *Bull. Env. Con. Toxi.* 5: 218-221.
- Crafts, A. S. 1949. Toxicity of 2,4-D in California soils. *Hilgardia* 19: 141-158.
- Cripps, R. E. 1971. The microbial breakdown of pesticides. Pages 255-266 in G. Sykes and F. A. Skinner, eds. *Microbial aspects of pollution.* Academic Press, Inc. New York.
- Crosby, Donald G. 1973. The fate of pesticides in the environment. *Ann. Rev. Plant Physiol.* 24: 467-492.
- Dean, A. C. R. and H. S. Law. 1964. The action of 2,4-dichlorophenoxyacetic acid on *Eacterium lactis aerogenes* (*Aerobacter aerogenes*). *Ann. Bot.* 28: 703-710.

- Deason, T. R. and H. C. Bold. 1960. Phycological Studies. I. Exploratory studies of Texas soil algae. Univ. of Texas Pub. No. 6022.
- DeKoning, H. W. and D. C. Mortimer. 1971. DDT uptake and growth of *Euglena gracilis*. Bull. Env. Com. Toxi. 6: 244-248.
- DeRose, H. R. 1946. Persistence of some plant growth regulators when applied to the soil in herbicidal treatments. Bot. Gaz. 107: 583-589.
- _____ and A. S. Newman. 1948. The comparison of the persistence of certain plant growth regulators when applied to soil. Soil Sci. Soc. Amer. Proc. 12: 222-226.
- Dimond, John B., Glenn Y. Bilyea, Raymond E. Kaduvce, A. Stanley Getchell, and John A. Blease. 1970. DDT residues in robins and earthworms associated with contaminated forest soils. Can. Ent. 102: 1122-1130.
- Dubos, R. J. 1946. Inhibition of bacterial growth by auxins. Proc. Soc. Exp. Biol. and Med. 63: 317-319.
- Eder, F. 1963. On the phytotoxicity of insecticides (in German, English summary). Phytopathol. Z. 47: 129-174.
- Edwards, C. A. 1963. Persistence of insecticides in the soil. New Scientist 19: 282-284.
- _____. 1964. Effects of pesticide residues on soil invertebrates and plants. Pages 239-261 in Ecology and the industrial society. Fifty Symp. Brit. Ecol. Soc. Blackwell, Oxford.
- _____. 1966. Insecticide residues in soils. Residue Rev. 13: 83-132.
- Eno, Charles F. 1958. Insecticides and the soil. J. Agr. Food Chem. 6: 348-351.
- Eno, C. F. and P. H. Everett. 1958. Effects of soil applications of 10 chlorinated hydrocarbon insecticides on soil microorganisms and the growth of Stringless Black Valentine beans. Proc. Soil Sci. Soc. Amer. 22: 235-238.
- Erickson, L. C., R. T. Wedding, and B. L. Brannaman. 1955. Influence of pH on 2,4-dichlorophenoxyacetic acid and acetic acid activity in *Chlorella*. Plant Physiol. 30: 69-74.
- Evans, W. C. and B. S. W. Smith. 1954. The photochemical inactivation and microbial metabolism of the chlorophenoxyacetic acid herbicides. Proc. Biochem. Soc., Biochem. J. 57: xxx.
- Farm Chemicals Handbook. 1965. Edited by Gordon Berg. Meister Publishing Co. Willoughby, Ohio. 456 pp.

- Faulkner, J. K. and D. Woodcock. 1964. Metabolism of 2,4-dichlorophenoxyacetic acid ('2,4-D') by *Aspergillus niger* van Tiegh. *Nature* 203: 865.
- _____ and _____. 1965. Fungal detoxification. Part VII. Metabolism of 2,4-dichlorophenoxyacetic and 4-chloro-2-methylphenoxyacetic acids by *Aspergillus niger*. *J. Chem. Soc.* 1965: 1187-1191.
- Fernley, H. N. and W. C. Evans. 1959. Metabolism of 2,4-dichlorophenoxyacetic acid by soil *Pseudomonas*: Isolation of α -Chloromuconic acid as an intermediate. *Biochem. J.* 73: 22P.
- Fitzgerald, G. P., G. C. Gerloff, and F. Skoog. 1952. Studies on chemicals with selective toxicity to blue-green algae. *Sewage and Ind. Wastes* 24: 888-896.
- Fleming, Walter E. and Warren W. Maines. 1953. Persistence of DDT in soils of the area infested by the Japanese beetle. *J. Econ. Ent.* 46: 445-449.
- Fletcher, W. W. 1960. The effect of herbicides on soil micro-organisms. Pages 20-62 in E. K. Woodford and G. R. Sagar, eds. *Herbicides and the soil*. Blackwell Scientific Publications, Oxford. 88 pp.
- Focht, D. D. 1972. Microbial degradation of DDT metabolites to carbon dioxide, water, and chloride. *Bull. Env. Con. Toxi.* 7: 552-556.
- _____ and M. Alexander. 1970. DDT metabolites and analogs: ring fission by *Hydrogenomonas*. *Science* 170: 91-92.
- _____ and _____. 1971. Aerobic cometabolism of DDT analogs by *Hydrogenomonas* sp. *J. Agric. Food Chem.* 19: 20-22.
- Forest, Herman S. 1962. Analysis of the soil algal community. *Trans. Am. Microsc. Soc.* 81: 189-198.
- _____, Dan. L. Willson, and Robert B. England. 1959. Algal establishment on sterilized soil replaced in an Oklahoma prairie. *Ecology* 40: 475-477.
- Foster, A. C. 1951. Some plant responses to certain insecticides in the soil. *U. S. D. A. Circ.* 862. 41 pp.
- Frans, R. E., E. F. Lind, and W. E. Loomis. 1957. Kinetics of growth inhibition by herbicides. *Plant Physiol.* 32: 301-307.
- Freeland, R. O. 1950. Effects of 2,4-D and other growth substances on photosynthesis and respiration in *Anacharis*. *Bot. Gaz.* 111: 319-324.
- Fries, G. R., G. S. Marros, and C. H. Gordon. 1969. Metabolism of o,p'- and p,p'-DDT by rumen microorganisms. *J. Agr. Food Chem.* 17: 860-862.

- Fukami, Hiroshi and Minoru Nakajima. 1971. Rotenone and the rotenoids. Pages 71-97 in Martin Jacobson and D. G. Crosby, eds. Naturally occurring insecticides. Marcel Dekker, Inc. New York. 585 pp.
- Fults, J. L. and M. G. Payne. 1947. Some effects of 2,4-D, DDT and Colorado 9 on the bacteria *Rhizobium leguminosarium* Frank in the root nodules of the common bean. Amer. J. Bot. 34: 245-248.
- Gallaher, P. J. and L. Evans. 1961. Preliminary investigations on the penetration and persistence of DDT under pasture. New Zealand J. Agr. Research 4: 466, cited in Marth, 1965.
- Ginsburg, J. M. 1955. Accumulation of DDT in soils from spray practices. J. Agr. Food Chem. 3: 322-325.
- _____ and J. P. Rood. 1954. A survey of DDT-accumulation in soils in relation to different crops. J. Econ. Ent. 47: 467-474.
- Gojmerac, W. L. 1956. Effects of insecticides on germination and emergence of sugar beets under greenhouse conditions. J. Econ. Ent. 49: 710-711.
- _____. 1957. Phosphates as soil insecticides for the control of sugar root maggot. F. A. O. Plant Protect. Bull. 5: 59-60.
- Goodman, R. N. 1965. Effect of pesticides from land drainage in farm pond waters. Pages 129-133 in C. O. Chichester, ed. Research in pesticides. Academic Press, New York.
- Gould, E. and E. O. Hamstead. 1951. The toxicity of cumulative spray residues in soils. J. Econ. Ent. 44: 713-717.
- Gregory, Wesley W., Jr, John K. Reed, and L. E. Priester, Jr. 1969. Accumulation of parathion and DDT by some algae protozoa. J. Protozool. 16: 69-71.
- Groover, Robert D. and Harold C. Bold. 1969. Phycological Studies. VIII. The taxonomy and comparative physiology of the Chlorosarcinales and certain other edaphic algae. Univ. Texas Pub. No. 6907.
- Guenzi, W. D. and W. E. Beard. 1967. Anerobic degradation of DDT to DDD in soil. Science 156: 1116-1117.
- Haller, M. L. 1952. Malathon - A coined name for the insecticidal chemical O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate. J. Econ. Ent. 45: 761-762.
- Hanks, R. W. 1946. Removal of 2,4-dichlorophenoxyacetic acid and its calcium salt from 6 different soils by leaching. Bot. Gaz. 108: 186-191.
- Harcourt, D. G. and L. M. Cass. 1955. Toxicities of various insecticides to cucumber. Can. J. Agr. Sci. 35: 19-26.

- Harris, C. R. and E. P. Lichtenstein. 1961. Factors affecting the volatilization of insecticidal residues from soils. *J. Econ. Ent.* 54: 1038-1045.
- _____, W. W. Sans, and J. R. Miles. 1966. Exploratory studies on occurrence of organochlorine insecticide residues in agricultural soils in southwestern Ontario. *J. Agr. Food Chem.* 14: 390-403.
- Haviland, E. E. and H. A. Highland. 1955. The phytotoxicity of malathion to poinsettias. *J. Econ. Ent.* 48: 326.
- Hernandez, T. P. and G. F. Warren. 1950. *Proc. Am. Soc. Hort. Sci.* 56: 287-293, cited in Audus, 1964.
- Hilton, J. L., L. L. Jansen, and H. M. Hull. 1963. Mechanism of herbicide action. *Ann. Rev. Plant Physiol.* 14: 353-384.
- Hoffman, Dale A. and John R. Olive. 1961. The effects of rotenone and toxaphene upon plankton of two Colorado reservoirs. *Limnol. Oceanog.* 6: 219-222.
- Hooper, F. F. 1948. The effect of derris root (rotenone) upon plankton and bottom fauna organisms of a small Minnesota lake. *Proc. Minn. Acad. Sci.* 16: 29-32, cited in Cope, 1965.
- Hoover, M. E. and A. R. Colmer. 1953. The action of 2,4-D, TCA, and sodium chlorate on the microorganisms of Sharkey Clay. *Sugar Bull.* 31: 416-418, cited in Pimentel, 1971.
- Horowitz, M. 1969. Evaluation of herbicide persistence in soil. *Weed Res.* 9: 314-321.
- Hurlber, Stuart H., Mir S. Mulla, and Harold R. Willson. 1972. Effects of an organophosphorus insecticide on the phytoplankton, zooplankton, and insect populations of freshwater ponds. *Ecol. Mono.* 42: 269-299.
- Il'in, A. M. 1962. Concerning the effect of the herbicide 2,4-D on soil microorganisms. *Microbiology* 30: 855-856. (translated from Russian).
- Jensen, H. L. 1960. *Acto. Agric. Scand.* 10: 83-103, cited in Audus, 1964.
- _____, and H. I. Peterson. 1952. Detoxification of hormone herbicides by soil bacteria. *Nature* 170: 39-40.
- Johnsen, R. E., C. S. Lin, and K. J. Collyard. 1972. Influence of soil amendments on the metabolism of DDT in soil. Pages 139-156 in A. S. Tahori, ed. *Fate of pesticides in environment. Volume VI of Proceedings of the Second International IUPAC Congress of Pesticide Chemistry.* Gordon and Breach Science Publishers, New York, 572 pp.

- Johnson, B. Thomas, Robert N. Goodman, and Herbert S. Goldberg. 1967. Conversion of DDT to DDD by pathogenic and saprophytic bacteria associated with plants. *Science* 157: 560-561.
- Johnson, Emmett J. and Arthur R. Colmer. 1955a. The effect of herbicides on soil microorganisms. I. The effect of 2,4-dichlorophenoxyacetic acid on some phases of the nitrogen metabolism of *Bacillus cereus*. *Appl. Microbiol.* 3: 123-126.
- _____, and _____. 1955b. The effect of herbicides on soil microorganisms. II. The effect of 2,4-dichlorophenoxyacetic acid on some phases of the nitrogen metabolism of *Pseudomonas fluorescens* and the microorganisms of a soil suspension. *Appl. Microbiol.* 3: 126-128.
- Johnson, Mary K., Lyman A. Magee, and Arthur R. Colmer. 1956. Some factors affecting the respiratory response of *Azobacter* to 2,4-D and related compounds. *Appl. Microbiol.* 4: 109-113.
- Jones, L. W. 1956. *Bull. Utah Agric. Exp. Sta.* 390: 1-17, cited in Walker 1967 and Bolen, 1961.
- Jorgensen, Carl J. C. and Charles L. Hamner. 1948. Weed control in soils with 2,4-dichlorophenoxyacetic acid and related compounds and their residual effects under varying environmental conditions. *Bot. Gaz.* 109: 324-333.
- Kallman, Burton, J. and Austin K. Andrews. 1963. Reductive dechlorination of DDT to DDD by yeast. *Science* 141: 1050-1051.
- Kantz, Thomas and Harold C. Bold. 1969. *Phycological Studies. IX. Morphological and taxonomic investigations of Nostoc and Anabaena in culture.* Univ. Texas Publ. No. 6924.
- Kearney, P. C., C. I. Harris, D. D. Kaufman, and T. J. Sheets. 1965. Behavior and fate of chlorinated aliphatic acids in soils. *Advan. Pest. Con. Res.* 6: 1-30.
- _____, D. D. Kaufman, and M. Alexander. 1967. Biochemistry of herbicide decomposition in soils. Pages 318-342 in A. D. McLaren and G. H. Peterson, eds. *Soil biochemistry.* Dekker. New York. 509 pp.
- _____, R. G. Nash, and A. R. Isensee. 1970. Persistence of pesticide residues in soils. Pages 54-67 in Morton W. Miller and George G. Berg, ed. *Chemical Fallout. Current research on persistent pesticides.*
- _____, E. A. Woolson, J. R. Plimmer, and A. R. Isensee. 1969. Decontamination of pesticides in soils. *Res. Rev.* 29: 137-149.
- Keil, Julian E. and Lamar E. Priester. 1969. DDT uptake and metabolism by a marine diatom. *Bull. Env. Con. Toxi.* 4: 169-173.

- Key, J. L. 1963. Studies on 2,4-D induced changes in ribonucleic acid metabolism in excised corn mesocotyl tissue. *Weeds* 11: 177-181.
- _____, J. B. Hanson, and R. F. Bils. 1960. Effect of 2,4-dichlorophenoxyacetic acid application on activity and composition of mitochondria from soybeans. *Plant Physiol.* 35: 177-183.
- Kim, Won Kyum. 1961. Influences of several plant growth substances on 4 species of algae. Ph. D. dissertation, University of North Carolina.
- Kiser, R. W., J. R. Donaldson, and P. R. Olson. 1963. The effect of rotenone on zooplankton populations in freshwater lakes. *Trans. Amer. Fish. Soc.* 92: 17-24.
- Klingman, G. C. 1961. Weed control as a science. John Wiley & Sons, Inc., New York 421 pp., cited in Pimentel, 1971.
- Ko, W. H. and J. L. Lockwood. 1968a. Accumulation and concentration of chlorinated hydrocarbon pesticides by microorganisms in soil. *Can. J. Microbiol.* 14: 1075-1078.
- Ko, W. H. and J. L. Lockwood. 1968b. Conversion of DDT to DDD in soil and the effect of these compounds on soil microorganisms. *Can. J. Microbio.* 14: 1069-1073.
- Kratochvil, D. E. 1951. Determination of the effect of several herbicides on soil microorganisms. *Weeds* 1: 25-31.
- Kries, O. H. 1947. Persistence of 2,4-dichlorophenoxyacetic acid in soil in relation to content of water, organic matter, and lime. *Bot. Gaz.* 108: 510-525.
- Lawler, P. D. and L. H. Rogers. 1967. Effect of DDT on photosynthesis in certain varieties of barley. *Nature* 215: 1515.
- Laygo, E. R. and J. T. Schulz. 1963. Persistence of organophosphate insecticides and their effects on microfauna in soils. *Proc. N. Dakota Acad. Sci.* 17: 64-65, cited in Pimentel, 1971.
- Lazaroff, N. and R. B. Moore. 1966. Selective effects of chlorinated insecticides on algal populations. *J. Phycol. Suppl.* 2: 7-8.
- Ledford, R. A. and J. H. Chen. 1969. Degradation of DDT and DDE by cheese microorganisms. *J. Food Sci.* 34: 386-388.
- Lewin, R. A. 1959. The isolation of algae. *Rev. Alg.* 4: 181-197.
- Lewis, R. W. and C. L. Hamner. 1946. Effect of 2,4-D on some microorganisms. *Mich. Agr. Exp. Sta. Quart. Bul.* 29: 112-114.
- Lichtenstein, E. P. 1957. A survey of DDT accumulated in midwestern orchard and crop soils treated since 1945. *J. Econ. Ent.* 50: 545-547.

- _____. 1965. Problems associated with insecticidal residues in soils. Pages 199-203 in C. O. Chichester, ed. *Research in Pesticides*. Academic Press, New York.
- _____. 1972. Persistence and fate of pesticides in soils, water, and crops: significance to humans. Pages 1-22 in A. S. Tahori, ed. *Fate of pesticides in environment*. Volume VI of *Proceedings of the Second International IUPAC Congress of Pesticide Chemistry*. Gordon and Breach Science Publishers, New York. 572 pp.
- _____, L. J. DePew, E. L. Eshbaugh, and J. P. Gleesman. 1960. Persistence of DDT, aldrin, and lindane in some Midwestern soils. *J. Econ. Ent.* 53: 136-142.
- _____, T. W. Fuhremann, and K. R. Schulz. 1971. Persistence and vertical distribution of DDT, lindane, and aldrine residues, 10 and 15 years after a single application. *J. Agr. Food. Chem.* 19: 718-721.
- _____, W. F. Millington, and G. T. Cowley. 1962. Effect of various insecticides on growth and respiration of plants. *J. Agr. Food. Chem.* 10: 251-256.
- _____ and K. R. Schulz. 1959. Persistence of some chlorinated hydrocarbon insecticides as influenced by soil types, rate of application and temperature. *J. Econ. Ent.* 52: 124-131.
- _____ and _____. 1961. Effect of soil cultivation, soil surface, and water on the persistence of insecticidal residues in soils. *J. Econ. Ent.* 54: 517-522.
- _____ and _____. 1964. The effects of moisture and micro-organisms on the persistence and metabolism of some organophosphorous insecticides in soils, with special emphasis on parathion. *J. Econ. Ent.* 57: 618-627.
- Loos, M. A., R. N. Roberts, and M. Alexander. 1967. Phenols as intermediates in the decomposition of phenoxyacetates by an *Arthrobacter* species. *Can. J. Microbiol.* 13: 679-690.
- Loosanoff, Victor L. 1965. Pesticides in sea water and the possibilities of their use in mariculture. Pages 135-145 in C. O. Chichester, ed. *Research in pesticides*. Academic Press, Inc., New York.
- _____, J. E. Hanks, and A. E. Ganaros. 1957. Control of certain forms of zooplankton in mass algal cultures. *Science* 125: 1092-1093.
- Lund, J. W. G. 1962. Soil. Pages 759-770 in Ralph A. Lewin, ed. *Physiology and biochemistry of algae*. Academic Press, Inc., New York. 929 pp.

- _____. 1967. Soil algae. Pages 129-147 in A. Burges and F. Raw, eds. Soil biology. Academic Press, Inc., New York. 532 pp.
- McLachlan, J. 1963. Some effects of Tris(hydroxymethyl)aminomethane on the growth of *Haematococcus pulvialis* Flotow. Can. J. Bot. 41: 35-40.
- MacEntee, Frank J., S. J. Sister Gervasis Schreckenberg, SMIC, and Harold C. Bold. 1972. Some observations on the distribution of edaphic algae. Soil Sci. 114: 171-179.
- MacPhee, A. W., D. Chisholm and C. R. MacEachern. 1960. The persistence of certain pesticides in the soil and their effect on crop yields. Can. J. Soil Sci. 40: 59, cited in March, 1965.
- MacRae, I. C. and M. Alexander. 1965. Microbial degradation of selected herbicides in soil. J. Agr. Food Chem. 13: 72-76.
- _____ and E. Vinckx. 1973. Effect of lindane and DDT on populations of protozoa in a garden soil. Soil Biol. Biochem. 5: 245-247.
- Magee, Lyman A. and Arthur R. Colmer. 1955. The effect of herbicides on soil microorganisms. III. The effect of some herbicides on the respiration of *Azotobacter*. Appl. Microbiol. 3: 288-292.
- Marth, Elmer H. 1965. Residues and some effects of chlorinated hydrocarbon insecticides in biological material. Res. Rev. 9: 1-89.
- Martin, H. 1964. The scientific principles of crop protection. 5th ed. St. Martin's Press, New York. 376 pp.
- Martin, James P. 1946. The hormone weed killer 2,4-D. Calif. Citrograph 31: 248, 264-265.
- _____. 1963. Influence of pesticide residues on soil microbiological and chemical properties. Res. Rev. 4: 96-129.
- _____, R. B. Harding, G. H. Connell, and L. D. Anderson. 1959. Influence of five annual field applications of organic insecticides on soil biological and physical properties. Soil Sci. 87: 334-338.
- Mashtatov, S. M., E. S. Gurinovich, T. G. Zimenko, and I. V. Kabailova. 1962. Effect of herbicides on the soil microflora. Microbiology 31: 67-70, (Translated from the Russian).
- Matsumura, Fumio. 1972. Current pesticide situation in the United States. Pages 33-60 in Fumio Matsumura, G. Mallory Boush, and Tomomasa Misato, eds. Environmental toxicology of pesticides. Academic Press, New York. 637 pp.
- _____ and G. M. Boush. 1966. Malathion degradation by *Trichoderma viride* and a *Pseudomonas* species. Science 153: 1278-1280.

- _____ and _____. 1968. Degradation of insecticides by a soil fungus, *Trichoderma viride*. J. Econ. Ent. 61: 610-612.
- _____, K. C. Patil, and G. M. Boush. 1971. DDT metabolized by microorganisms from Lake Michigan. Nature 230: 325-326.
- Mattox, Karl R. and Harold C. Bold. 1962. Phycological Studies. III. The taxonomy of certain ulotrichacean algae. Univ. of Texas Publ. No. 6222.
- McLachlan, J. 1963. Some effects of Tris(hydroxymethyl)aminomethane on the growth of *Haematococcus pluvialis* Flotow. Can. J. Bot. 41: 35-40.
- Menzel, D. W., J. Anderson, and A. Randtke. 1970. Marine phytoplankton vary in their response to chlorinated hydrocarbons. Science 167: 1724-1726.
- Menzie, Calvin M. 1972. Fate of pesticides in the environment. Ann. Rev. Entomol. 17: 199-222.
- Metcalf, R. L. and R. B. March. 1953. Further studies on the mode of action of organic thionophosphate insecticides. Ann. Entomol. Soc. Am. 46: 63-74.
- Mickovshi, Miladin, Alan Seaman, and Malcolm Woodbine. 1968. The effect of the hormone herbicides 2,4-D, MCPA, and MCPB on soil fungus flora. J. Sci. Agr. Res. 21: 33-42.
- Miskus, Raymond P., Deanna P. Blair, and John E. Casida. 1965. Conversion of DDT to DDD by bovine rumen fluid, lake water, and reduced porphyrins. J. Agr. Food Chem. 13: 481-483.
- Mitchell, J. W. and P. C. Marth. 1946. Germination of seeds in soil containing 2,4-dichlorophenoxyacetic acid. Bot. Gaz. 107: 408-416.
- Montgomery, M. L., D. Klein, R. Goulding, and V. H. Freed. 1972. Biological degradation of pesticide wastes. Pages 117-125 in A. S. Tahori, ed. Fate of pesticides in the environment. Volume VI of Proceedings of the Second International IUPAC Congress of Pesticide Chemistry. Gordon and Breach Science Publishers, New York. 572 pp.
- Moore, E. Edsel. 1973. Pesticide sales and usage in Kentucky - 1968. Pest. Mont. J. 6: 379-387.
- Moore, N. W. 1967. A synopsis of the pesticide problem. Adv. Ecol. Res. 4: 75-129.
- Moore, Richard B. 1970. Effects of pesticides on growth and survival of *Euglena gracilis* Z. Bull. Env. Com. Toxic. 5: 226-230.
- Moreland, D. E. 1967. Mechanisms of action of herbicides. Ann. Rev. Pl. Phys. 18: 365-386.

- Morgan, Janet R. 1972. Effects of Aroclor 1242 (a polychlorinated biphenyl) and DDT on cultures of an alga, protozoan, dephnid, ostracod, and guppy. *Bull. Envir. Con. and Toxic* 8: 129-137.
- Mosser, Jerry L., Nicholas S. Fisher, and Charles F. Wurster. 1972. Polychlorinated biphenyls and DDT alter species composition in mixed cultures of algae. *Science* 176: 533-535.
- Nash, R. G. and E. A. Woolson. 1967. Persistence of chlorinated hydrocarbon insecticides in soils. *Science* 157: 924.
- National Academy of Sciences. 1968. Effects of pesticides on fruit and vegetable physiology. Volume 6 of Principles of plant and animal pest control. 90 pp.
- Newman, A. S. 1947. The effect of several plant growth regulators on soil microorganisms and microbial processes. *Soil Sci. Soc. Am. Proc.* 12: 217-221.
- _____ and C. R. Downing. 1958. Herbicides and the soil. *J. Agr. Food Chem.* 6: 352-353.
- _____ and J. R. Thomas. 1950. Decomposition of 2,4-dichlorophenoxyacetic acid in soil and liquid media. *Proc. Soil Sci. Soc. Am.* 14: 160-164.
- _____, _____, and R. L. Walker, 1952. Disappearance of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid from soil. *Soil Sci. Soc. Am. Proc.* 16: 21-24.
- Nilsson, P. E. 1957. *K. Lantbruks-Hogskol. Ann.* 23: 219-253, Cited in Audus, 1964.
- Norman, A. G., C. E. Minarik, and R. L. Weintraub. 1950. Herbicides. *Ann. Rev. Plant Physiol.* 1: 141-168.
- Norris, Logan A. and David Greiner. 1967. The degradation of 2,4-D in forest litter. *Bull. Environ. Contam. Toxic.* 2: 65-74.
- Nutman, P. S., H. G. Thornton, and N. J. Quastel. 1945. Inhibition of plant growth by 2,4-dichlorophenoxyacetic acid and other plant growth substances. *Nature* 155: 498-500.
- O'Brien, R. D. 1966. Mode of action of insecticides. *Ann. Rev. Ent.* 11: 369-402.
- _____. 1967. *Insecticides: Action and Metabolism.* Academic Press New York. 332 pp.
- Ogle, R. E. and G. F. Warren. 1954. Fate and activity of herbicides in soils. *Weeds* 3: 257-273.
- Ott, D. E. and F. A. Gunther. 1965. DDD as a decomposition product of DDT. *Res. Rev.* 10: 70-84.

- Pagan, C. and M. P. Morris. 1953. Derris deposits: Fate of deposits on young bean plants. *J. Agr. Food Chem.* 1: 580-581.
- Palmer, C. M. and T. E. Maloney. 1955. Preliminary screening for potential algicides. *Ohio J. Sci.* 45: 1-8.
- Parker, B. C. 1961. Facultative heterotrophy in certain soil algae from the ecological viewpoint. *Ecology* 42: 381-386.
- _____ and H. C. Bold. 1961. Biotic relationships between soil algae and other microorganisms. *Am. J. Bot.* 48: 185-197.
- _____, _____, and T. R. Deason. 1960. Facultative heterotrophy in some Chlorococcacean algae. *Science* 133: 761-763.
- _____ and B. L. Turner. 1961. "Operational niches" and "community-interaction values" as determined from *in vitro* studies of some soil algae. *Evolution* 15: 228-238.
- Pathak, A., N. H. Shankar, and K. S. Awasthi. 1961. Effect of some pesticides on available nutrients and soil microflora. *J. Ind. Soc. Soil Sci.* 9: 197, cited in Marth, 1965.
- Patil, K. C., F. Matsumura, and G. M. Boush. 1970. Degradation of endrin, aldrin, and DDT by soil microorganisms. *Appl. Microbio.* 19: 879-881.
- Payne, Merle G. and Jess L. Fults. 1947. Some effects of 2,4-D, DDT, and Colorado 9 on root nodulation in the common bean. *J. Amer. Soc. Agron.* 39: 52-55.
- Petersen, J. B. 1935. Studies on the biology and taxonomy of soil algae. *Dansk. Bot. Ark.* 8: 1-183, cited in Lund, 1967.
- Petruk, G. F. 1964. Influence of herbicides on the heterotrophic microorganisms of ponds. *Microbiology* 33: 901-904. (Translated from Russian).
- Pfaender, Frederic K. and Martin Alexander. 1972. Extensive microbial degradation of DDT *in vitro* and DDT metabolism by natural communities. *J. Agr. Food Chem.* 20: 842-846.
- Pimentel, David. 1971. Ecological effects of pesticides on non-target species. Executive Office of the President. Office of Science and Technology. Superintendent of Documents. Washington, D. C. 220 pp.
- Potul'nitskii, P. M. 1962. Seasonal dynamics of soil algae in relation to plant cover. *Microbiology*, 31: 92-95. (Translated from Russian).
- Pringsheim, E. G. 1946. Pure cultures of algae, their preparation and maintenance. Univ. Press. Cambridge.

- Randolph, N. M., R. D. Chisholm, L. Koblitsky, and J. C. Gaines. 1960. Insecticide residues in certain Texas soils. Texas Agr. Expt. Sta. MP-447, cited in Marth, 1965.
- Rapoport, E. H. and G. Cangioli. 1963. Herbicides and the soil fauna. *Pedobiologia* 2: 235-238, cited in Pimentel, 1971.
- Rice, C. P. and H. C. Sikka. 1973. Uptake and metabolism of DDT by 6 species of marine algae. *J. Agr. Food Chem.* 21: 148-152.
- Roberts, Jack E. and Walter B. Bollen. 1955. A microplating method for soil molds and its use to detect some effects of certain insecticides and herbicides. *Appl. Microbiol.* 3: 190-194.
- _____, R. D. Chisholm, and L. Koblitsky. 1962. Persistence of insecticides in soil and their effects on cotton in Georgia. *J. Econ. Ent.* 55: 153-155.
- Rogoff, M. H. and J. J. Reid. 1956. Bacterial decomposition of 2,4-dichlorophenoxyacetic acid. *J. Bact.* 71: 303-307.
- Ruckelshaus, William D. 1972. Consolidated DDT hearings: Opinion and order of the administrator. *Fed. Regis.* 37: 13369-13376.
- Salisbury, Frank B. and Cleon Ross. 1969. Plant physiology. Wadsworth Publishing Company, Inc. Belmont, California. 747 pp.
- Schwartz, Henry J., Jr. 1967. Microbial degradation of pesticides in aqueous solutions. *J. Water Pollu. Con. Fed.* 39: 1701-1714.
- Shane, M. S. 1948. Effect of DDT spray on reservoir biological balance. *J. Amer. Water Works Ass.* 40: 333-336.
- Shields, L. M. and L. W. Durrell. 1964. Algae in relation to soil fertility. *Bot. Rev.* 30: 92-128.
- Slepecky, R. A. and J. V. Beck. 1950. The effect of 2,4-dichlorophenoxyacetic acid on nitrification in soil. *Soc. Am. Bacteriol. Proc.* 50: 17-18, cited in Pimentel, 1971.
- Smith, Gilbert M. 1950. The fresh-water algae of the United States. Second Edition. McGraw-Hill Book Company. New York. 719 pp.
- Smith, M. S. 1948. Persistence of DDT and BHC in soils. *Ann. Appl. Biol.* 35: 494.
- Smith, Nathan R., Virginia T. Dawson, and Marie E. Wenzel. 1945. The effect of certain herbicides on soil microorganisms. *Soil Sci. Soc. Amer., Proc.* 10: 197-201.
- _____, and Marie E. Wenzel. 1947. Soil microorganisms are affected by some of the new insecticides. *Soil Sci. Soc. Amer. in Proc.* 12: 227-233.

- Smith, R. L. and V. E. Wiedemann. 1964. A new alkaline growth medium for algae. *Can. J. Bot.* 42: 1582-1586.
- Södergren, A. 1968. Uptake and accumulation of C^{14} -DDT by *Chlorella* sp. (Chlorophyceae). *Oikos* 19: 126-138.
- _____. 1971. Accumulation and distribution of chlorinated hydrocarbons in cultures of *Chlorella pyrenoidosa* (Chlorophyceae). *Oikos* 22: 215-220.
- Spiller, D. 1961. A digest of available information on the insecticide malathion. *Adv. Pest. Control Res.* 4: 249-335.
- Stadynk, L. 1967. The effects of selected pesticides upon carbon uptake and growth in phytoplankton. Master's Thesis, University of Missouri, Columbia, Mo., cited in Vance and Drummond. 1969.
- Stafford, E. M. 1954. Use of parathion to control olive scale. *J. Econ. Ent.* 47: 287-295.
- Stanier, R. Y., R. Kunisawa, M. Mandel, and G. Cohen-Bazire. 1971. Purification and properties of unicellular blue-green algae (Order Chroococcales). *Bacteriol. Rev.* 35: 171-205.
- Starks, K. J. and J. H. Lilly. 1955. Insecticide seed treatment of soybeans in relation to phytotoxicity and seed-corn maggot control. *J. Econ. Ent.* 48: 538-543.
- Stecher, Paul G., ed. 1968. The Merck Index. Merck and Co., Inc. Rahway, New Jersey.
- Steenson, T. I. and N. Walker. 1957. The pathway of breakdown of 2,4-dichloro- and 4-chloro-2-methyl-phenoxyacetic acid by bacteria. *J. Gen. Microbiol.* 16: 146-155.
- _____ and _____. 1958. Adaptive patterns in the bacterial oxidation of 2,4-dichloro- and 4-chloro-2-methyl-phenoxyacetic acid. *J. Gen. Microbiol.* 18: 692-697.
- Stevenson, E. C. and J. W. Mitchell. 1945. Bacteriostatic and bactericidal properties of 2,4-dichlorophenoxyacetic acid. *Science* 101: 642-644.
- Strong, R. G., D. E. Sbur, and G. R. Pieper. 1959. Effect of several pesticides on the germination of 3 varieties of flower seeds. *J. Econ. Ent.* 52: 355.
- Swets, W. A. and R. T. Wedding. 1964. The effect of dinitrophenol and other inhibitors on the uptake and metabolism of 2,4-dichlorophenoxyacetic acid by *Chlorella*. *New Phytologist* 63: 55-72.
- Tarrant, R. F., D. G. Moore, W. B. Bollen, and B. R. Loper. 1972. DDT residues in forest floor and soil after aerial spraying, Oregon - 1965-68. *Pest. Mon. J.* 6: 65-72.

- Taschenberg, E. F., G. L. Mack, and F. L. Ganbrell. 1961. DDT and copper residues in a vineyard soil. *J. Agr. Food Chem.* 9: 207-209.
- Taylor, D. L. 1947. Growth of field crops in soil treated with chemical growth-regulators. *Bot. Gaz.* 108: 432-445.
- Teater, R. W., J. L. Mortensen, and P. F. Pratt. 1958. Effect of certain herbicides on rate of nitrification and carbon dioxide evolution in soil. *J. Agr. Food Chem.* 6: 214-216.
- Thurston, R. 1953. The effect of some soil characteristics on DDT phytotoxicity. *J. Econ. Ent.* 46: 545-550.
- Tiffany, L. H. 1951. Ecology of freshwater algae. Pages 243-312 in G. M. Smith, ed. *Manual of phycology. Chronica Botanica.* Waltham, Massachusetts. 375 pp.
- Tomisek, Arthur, Mary R. Reid, William A. Short, and Howard E. Skipper. 1957. Studies on the photosynthetic reaction. III. The effects of various inhibitors upon growth and carbonate-fixation in *Chlorella pyrenoidosa*. *Plant Physiol.* 32: 7-10.
- Trainor, F. R. 1970. Survival of algae in a desiccated soil. *Phycologia* 9: 111-113.
- Ukeles, R. 1962. Growth of pure cultures of marine phytoplankton in the presence of toxicants. *Appl. Microbiol.* 10: 532-537.
- Upchurch, R. P. 1966. Behavior of herbicides in soil. *Res. Rev.* 16: 47-85.
- _____ and D. Mason. 1962. The influence of soil organic matter on the phytotoxicity of herbicides. *Weeds* 10: 9-14.
- Vance, B. D. and W. Drummond. 1969. Biological concentration of pesticides by algae. *J. Amer. Water Works Ass.* 61: 360
- _____ and David L. Smith. 1969. Effects of 5 herbicides on 3 green algae. *Tex. J. Sci.* 20: 329-337.
- VanOverbeek, J., Rene Blondeau, and Vesta Horne. 1951. Difference in activity between 2,4-dichlorophenoxyacetic acid and other auxins, and its significance in herbicidal action. *Plant Physiol.* 26: 687-696.
- Waksman, Selman A. 1952. *Soil microbiology.* John Wiley and Sons, Inc. New York. 345 pp.
- Walker, N. 1967. Soil micro-organisms and plant protection chemicals. Pages 493-503 in A. Burges and F. Raw, eds. *Soil biology.* Academic Press, London. 532 pp.
- Walker, R. L. and A. S. Newman. 1956. Microbial decomposition of 2,4-dichlorophenoxyacetic acid. *Appl. Microbiol.* 4: 201-206.

- Warren, J. R., F. Graham, and G. Gale. 1951. Dominance of an actinomycete in a soil microflora after 2,4-D treatment of plants. *Phytopathology* 41: 1037-1039.
- Weaver, Robert J. 1948. Contratoxification of plant growth-regulators in soils and on plants. *Bot. Gaz.* 109: 276-300.
- Wedding, R. T., L. C. Erickson, and M. K. Black. 1959. Influence of 2,4-D on solute uptake by *Chlorella*. *Plant Physiol.* 34: 3-10.
- _____, _____, and B. L. Brannaman. 1954. Effect of 2,4-D on photosynthesis and respiration. *Plant Physiol.* 29: 64-69.
- Wedemeyer, Gary. 1966. Dechlorination of DDT by *Aerobacter aerogenes*. *Science* 152: 647.
- _____. 1967a. Biodegradation of dichlorodiphenyltrichloroethane intermediates in dichlorodiphenyl acetic acid metabolism by *Aerobacter aerogenes*. *Appl. Microbiol.* 15: 1494-1495.
- _____. 1967b. Dechlorination of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane by *Aerobacter aerogenes*. *Appl. Microbiol.* 15: 569-574.
- Westlake, William E. and Francis A. Gunther. 1966. Occurrence and mode of introduction of pesticides in the environment. Pages 110-121 in *Adv. Chem. Ser.* 60: Organic pesticides in the environment. Am. Chem. Soc. Washington, D. C. 309 pp.
- Wheatley, G. A., J. A. Hardman, and A. H. Strickland. 1962. Residues of chlorinated hydrocarbons insecticides in some farm soils in England. *Plant Pathology* 11: 81-90.
- Whiteside, Jean S. and M. Alexander. 1960. Measurement of microbiological effects of herbicides. *Weeds* 8: 204-213.
- Wiedeman, Varley E. 1964. Some aspects of algal ecology in a waste-stabilization pond system. Ph.D. Dissertation, University of Texas.
- _____ and H. C. Bold. 1965. Heterotrophic growth of selected waste-stabilization pond algae. *J. Phycol.* 1: 66-69.
- _____, P. L. Walne, and F. R. Trainor. 1964. A new technique for obtaining axenic cultures of algae. *Can. J. Bot.* 42: 958-959.
- Wiersma, G. B., W. G. Mitchell, and C. L. Stanford. 1972. Pesticide residues in onions and soil - 1969. *Pest. Monit. J.* 5: 345-347.
- _____, H. Tai, and P. F. Sand. 1972a. Pesticide residues in soil from eight cities - 1969. *Pest Monit. J.* 6: 126-129.

- _____, _____, and _____. 1972b. Pesticide residue levels in soils, FY 1969 - National soils monitoring program. *Pest Monit. J.* 6: 194-228.
- Willson, Dan and Herman S. Forest. 1957. An exploratory study on soil algae. *Ecology* 38: 309-313.
- Wilson, J. K. and R. S. Choudhri. 1946. Effects of DDT on certain microbiological processes in the soil. *J. Econ. Ent.* 39: 537-538.
- Winer, B. J. 1971. Statistical principles in experimental design. Second edition. McGraw-Hill Book Company, New York. 907 pp.
- Wolcott, A. R. 1970. Retention of pesticides by organic materials in soils. Pages 128-138 in *Pesticides in the soil: Ecology, degradation, and movement. Proceedings of a symposium, Michigan State University, Feb., 1970.*
- Woodcock, D. 1964. Microbial degradation of synthetic compounds. *Ann. Rev. Phytopathol.* 2: 321-340.
- Woodford, E. K., K. Holly, and C. C. McCready. 1958. Herbicides. *Ann. Rev. Plant Physiol.* 9: 311-358.
- Woodwell, George M. 1967. Toxic substances and ecological cycles. *Sci. Am.* 216: 24-31.
- _____ and F. T. Martin. 1964. Persistence of DDT in soils of heavily sprayed forest stands. *Science* 145: 481-483.
- _____, Charles F. Wurster, Jr., and Peter A. Isaacson. 1967. DDT residues in an East Coast estuary: A case of biological concentration of a persistent pesticide. *Science* 156: 821-824.
- Wort, D. J. and L. M. Cowie. 1953. Effect of 2,4-dichlorophenoxyacetic acid on phosphorylase, phosphatase, amylase, catalase, and peroxidase activity in wheat. *Plant Physiol.* 28: 135-139.
- Worth, Winfield A., Jr. and Anne M. McCabe. 1948. Differential effects of 2,4-D on aerobic, anaerobic, and facultative anaerobic microorganisms, *Science* 108; 16-18.
- Wright, S. J. L. 1971. Degradation of herbicides by soil microorganisms. Pages 233-254 in G. Sykes and F. A. Skinner, eds. *Microbial aspects of pollution.* Academic Press, New York.
- Wurster, Charles F. Jr. 1968. DDT reduces photosynthesis by marine phytoplankton. *Science* 159: 1474-1475.
- _____. 1973. DDT proved neither essential nor safe. *BioScience* 23: 105-106.

- Yamamoto, Izuru. 1969. Mode of action of natural insecticides. Res.
Rev. 25: 161-174.
- Yule, W. N. 1973. Intensive studies of DDT residues in forest soil.
Bull. Env. Con Tox. 9: 57-64.

VITA

Martha Scheer Salk, first child of Frederick August Scheer, Jr. and Alice Lepay Scheer, was born in Detroit, Michigan on April 16, 1945. She attended public elementary and secondary schools in Dearborn, Michigan, and graduated from Edsel Ford High School in June, 1963. She matriculated at Albion College, Albion, Michigan, the following September and graduated in June, 1967, with highest honors and departmental honors in biology. While at Albion, she received many academic honors including membership in the Albion College Fellows, the highest academic honorary on the Albion campus, and was elected to the Beta of Michigan chapter of Phi Beta Kappa. In February, 1967, she was married to Gary Clive Salk. She received her Master of Science degree in botany from the University of Iowa in February, 1969 where she was the recipient of an NDEA Title IV Fellowship for the period from September, 1967 to August, 1968. From September, 1969 to May, 1970 she taught general biology parttime at Dutchess Community College in Poughkeepsie, New York. She enrolled at the University of Louisville in January, 1971 and received her Ph.D. from that institution in May, 1975.

Permanent address: Gilbert Associates, Inc.

Reading, Pennsylvania