Pesticide Effect on Growth and ^{14}C Assimilation in a Freshwater Alga[1]

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Pesticide residues are a widespread occurrence in freshwater ecosystems (1). Knowledge concerning the effect of these toxicants on phytoplankton is limited (2, 3). Any alteration of the photosynthetic process will ultimately be reflected in altered production at all trophic levels of the aquatic community.

This investigation evaluated the effects of pesticides on low density populations of a freshwater alga in terms of changes in growth and metabolism rather than death. Specifically we measured the effects of commonly used pesticides on cultures of the plankton alga Scenedesmus quadricaudata (Turpin) de Brébisson as changes in cell biomass, cell number and carbon-14 assimilation.

Materials and Methods

Unialgal cultures of the green alga Scenedesmus quadricaudata obtained from field collections were maintained in enriched liquid Allen's media (4). Subcultures for bioassay were established in Chu's culture media #10 (5) with added micronutrient solution (6) to provide trace elements. This mixture furnished a nutrient level approximating that of eutrophic lakes (7).

All cultures were maintained in a growth chamber with a mean light intensity of 1390 ft-c, with a 12-hour light cycle, and temperature of 70 ± 2°F (21 ± 1°C).

Bioassay cultures were prepared as follows: 12 liters of Chu's enriched nutrient solution were innoculated with Scenedesmus, mixed thoroughly, cell number

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determined, and then divided into 12 subcultures.

Pesticides diuron, carbaryl, 2,4-D, DDT, dieldrin, toxaphene and diazinon[3] were investigated under static conditions. Acetone was the solvent carrier. A stock solution containing 10 mg chemical per ml acetone was prepared and appropriate dilutions were added to experimental subcultures to provide initial concentrations of 0.1 and 1.0 mg/l. The reader should be cautioned that the concentrations cited will decrease rapidly with time primarily through codistillation (8, 9) and adsorption to surfaces of the containers. Control subcultures received 0.1 ml acetone per 1000 ml nutrient solution. Values throughout the paper are the means of four replicates from each experiment.

Carbon assimilation was determined the day specific pesticide tests were initiated and subsequently at 2-day intervals. A 50-ml aliquot was withdrawn from each well-mixed subculture, placed in a 125-ml glass Erlenmeyer flask, and 0.5 ml of $Na_2{}^{14}CO_3$ was added (2.3 μc/ml). Flasks were stoppered with cotton and incubated for 4 hours in a growth chamber at 21 ± 1°C. Subsequent treatment and measurement of radioactivity followed the method of Lind and Campbell (10). Carbon assimilation is expressed in terms of ^{14}C-activity per cell density and per volume of culture.

Measurements of carbon assimilation and of cell number as 4-cell units with a Sedgwick-Rafter counting cell were continued at 2-day intervals until exponential growth of algal cells terminated. At that time dry weight biomass was determined at 60°C for 24 hours for each subculture.

Results

The most conspicuous effects of pesticides on algal subcultures of *Scenedesmus quadricaudata* were found with the herbicide diuron and the insecticide carbaryl. Following the second day of treatment with diuron there was a drastic reduction in cell numbers which continued throughout the 8-day period (Figure 1). This decline was reflected in a conspicuous decrease in biomass: final dry

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[3] 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl) ethane (DDT); 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene (dieldrin); octachlorocamphene (toxaphene); O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate (diazinon); 1-naphthyl methyl-carbamate (carbaryl, Sevin®); 2,4-dichlorophenoxy-acetic acid (2,4-D); 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron).

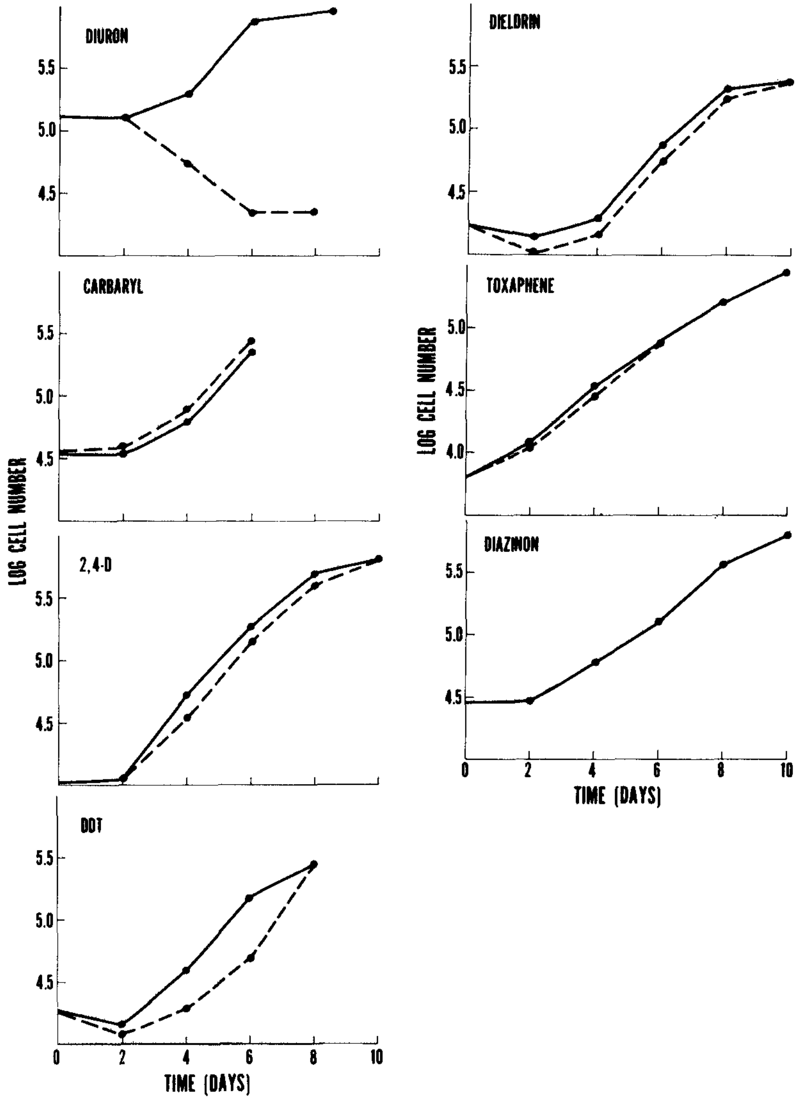

Figure 1. Effect of selected pesticides on cell number of Scenedesmus quadricaudata. Solid line represents control culture; broken line treated culture, 0.1 mg/liter pesticide. All values are means of four samples. Where coordinates are similar solid line alone is shown.

weight 0.05 mg/l in the treated subcultures as opposed to 46.8 mg/l in the controls (Table 1), and a significant suppression of carbon assimilation. Subcultures treated with both 0.1 and 1.0 mg/l diuron showed reduced carbon assimilation by approximately 90 percent within 4 days (Figure 1, Table I). Carbaryl, in contrast to diuron, stimulated cell growth concomitant with an increase in carbon assimilation (Figure 1). Cell biomass at the end of 6 days had increased 44 to 57 percent in the 0.1 and 1.0 mg/l treated subcultures as opposed to the controls (Table I). A dramatic stimulation of carbon assimilation was noted at the 2-day sampling period of the 1 mg/l culture (Table I).

The herbicide 2,4-D produced less severe metabolic changes than did diuron. At both levels of treatment, a marked decrease in cell density appeared days 4 through 8 (Figure 1). Some indication of carbon fixation stimulation beginning on day 6 was noted using the counts per cell parameter. There was a small reduction in biomass by day 10 (Table I).

The persistent organochlorine insecticides DDT, dieldrin and toxaphene all decreased cell number at all levels of treatment (Figure 1). Similarly, for each treatment, cell biomass was reduced 25 and 51 percent with DDT, 22 and 32 percent with dieldrin, but only 3 and 4 percent with toxaphene (Table I).

There was a marked polarity in carbon assimilation response to the three persistent organochlorine insecticides tested. For example, in 2-day cultures at both concentrations a 75 percent inhibition was observed with DDT, while with toxaphene there was a 450 percent increase in carbon fixation (Table I).

Dieldrin showed similar trends to both DDT and toxaphene in that an initial stimulation in carbon assimilation was observed at the 2-day interval, followed by a significant inhibition at the 6-day interval, with a final stimulation at day 10 (Table I).

We found no effect with diazinon on cell number, photosynthesis, or biomass over the 10-day study (Figure 1, Table I).

Discussion

The soil sterilant and herbicide diuron was the most toxic to *Scenedesmus* of the substances tested. This pesticide is subject to partial detoxification (11) by bacterial organisms. Evidence of this may be in the leveling off on days 6 to 8 of the steady decline in cell numbers (Figure 1). On the other hand, the slight effect of the herbicide 2,4-D on algal growth indicates that

TABLE I

Percentage differences cell number, carbon assimilation and biomass between treated cultures and controls at 0, 2, 4, 6, 8, and 10 days. Carbon assimilation expressed as unit volume = cpm^{14}C per 50 ml, and as unit count = cpm^{14}C per cell. *=significant difference (p = 0.05, N=4).

Concentration			Time in days					
Pesticide	mg/l	Item	0	2	4	6	8	10
Diuron	0.1	Cell number	- 1	- 3	-72*	-96*	-97*	
		Unit volume	-97*	-96*	-98*	-99*	-99*	
		Unit count	-96*	-95*	-89*	-75*	-62*	
		Biomass					-99	
	1.0	Cell number	- 1	- 7*	- 7*	-96*	-97*	
		Unit volume	-96*	-96*	-97*	-99*	-99*	
		Unit count	-96*	-95*	-90*	-91*	-71*	
		Biomass					-99	
Carbaryl	0.1	Cell number	+ 3	+ 8*	+19*	+23*		
		Unit volume	+ 1	+36*	+30*	+17*		
		Unit count	- 2	+27*	+10	- 5		
		Biomass				+144		
	1.0	Cell number	+ 1	+ 4	+64*	+32*		
		Unit volume	+ 7	+148*	+66*	+96*		
		Unit count	+ 4	+141*	+ 1	+56*		
		Biomass				+157		
2,4,-D	0.1	Cell number		- 3	-36*	-23*	-15*	- 3
		Unit volume	- 7	0	-30	- 5	- 2	- 1
		Unit count		+ 3	+10	+24*	+16*	+ 2
		Biomass						-12
	1.0	Cell number		- 1	-30*	-19*	-16*	- 5
		Unit volume	- 5	+10	-37	- 2	+ 7	+ 7
		Unit count		+12	-11	+30*	+28*	+12
		Biomass						-11
DDT	0.1	Cell number	- 6	-14*	-52*	-66*	- 1	
		Unit volume	-18	-76*	-70*	-67*	- 5	
		Unit count	-12	-72*	-37*	+28	- 4	
		Biomass					-25	
	1.0	Cell number	- 2	-15*	-51*	-74*	- 5*	
		Unit volume	-18	-78*	-66*	-70*	-11	
		Unit count	-15	-72*	-29*	+18	- 6	
		Biomass					-51	

Table I. Continued

Pesticide	Concentration mg/l	Item	Time in days 0	2	4	6	8	10
Dieldrin	0.1	Cell number		-32*	-23*	-26*	-17*	- 9
		Unit volume	+ 5	+ 1	-20*	-42*	-25	+35
		Unit count		+38*	0	-22*	+ 4	+ 5*
		Biomass						-22
	1.0	Cell number		-37*	-29*	-38*	-23*	-11
		Unit volume	+ 3	+ 1	-33*	-51*	-32*	+33
		Unit count		+46*	- 3	-20*	-10	+51*
		Biomass						-32
Toxaphene	0.1	Cell number		- 6	-13*	- 1	+ 6	+ 1
		Unit Volume		+52	+10	- 5	+ 1	- 3
		Unit count		+48*	+26	- 9	- 7	- 3
		Biomass						- 3
	1.0	Cell number		-26*	-14*	-22*	- 5	-19*
		Unit volume		+311*	+120*	+41*	- 8	+ 8
		Unit count		+453*	+157*	+76*	0	+30*
		Biomass						- 4
Diazinon	0.1	Cell number		+ 5	+ 4	+ 4	+ 2	+ 1
		Unit volume	+ 7	+ 3	+ 5	+ 1	- 1	- 1
		Unit count		- 1	+ 1	- 3	- 3	- 1
		Biomass						0
	1.0	Cell number		+ 5	+ 1	+ 4	+ 2	+ 1
		Unit volume	+ 1	+11	+ 7	+ 1	- 1	- 1
		Unit count		+ 6	+ 2	- 3	- 3	- 1
		Biomass						- 2

not all herbicides affect this algal species similarly.

Diazinon was the only compound tested which had no effect on algal cell number, biomass or ^{14}C uptake. Lichtenstein et al. (12) has reported degradation of diazinon by both biological and chemical means.

There are reports of detrimental effects of pesticides on marine phytoplankton. Wurster (2) has demonstrated a reduction in photosynthesis caused by DDT. In our study with the freshwater alga Scenedesmus, DDT also suppressed the rate of carbon assimilation. In addition we found reduction in total biomass and in cell number. Butler (13) and Ukeles (14) showed carbaryl to be toxic to marine phytoplankton. However, we found a marked stimulation of cell growth and carbon fixation in Scenedesmus. Perhaps this effect was the result of an increased N source arising from the degradation of carbaryl. Hydrolysis of the ester linkage followed by successive decarboxylation and oxidative demethylation of the N-methyl carbamic acid moiety would release NH_3 and formic acid (15).

The literature on acute toxicity of pesticides is extensive for fishes (16) but is limited for algae. The studies show major differences in acute toxicity in fishes between persistent carbamates and organophosphates (carbaryl, diazinon). This difference is also conspicuous in our study of the freshwater alga Scenedesmus. While DDT, toxaphene and dieldrin generally caused a decrease in cell number, biomass and carbon assimilation, carbaryl and diazinon either had no effect or stimulated carbon fixation and cell division.

This study emphasizes that one must consider long term chronic effects of pesticides in the ecosystem in terms of plant life as well as animal species. Observations on Scenedesmus suggest that small additions of persistent organochlorine insecticides may cause a reduction in population density and a suppression of carbon fixation. Even though recovery in cell number (but not biomass) seems to have occurred (Figure 1, Table I), one can postulate a more drastic effect on lowered production at all trophic levels of repeated entrance of a pesticide in the freshwater ecosystem. Similarly, repeated exposure of the aquatic community to carbaryl could lead to increased algal blooms with subsequent reinforcement of production at all trophic levels.

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References

1. Henderson, C., W. L. Johnson and A. Inglis. Pesticides Monit. J. 3, 149 (1969).
2. Wurster, C. F., Jr. Science 159, 1474 (1968).
3. Menzel, D. W., J. Anderson and A. Randtke. Ibid. 167, 1724 (1970).
4. Allen, M. B. Arch. Mikrobiol. 17, 34 (1952).
5. Chu, S. P. J. Ecol. 30, 284 (1942).
6. Hutner, S. H., L. Provasoli, A. Schatz and C. P. Haskins. Proc. Amer. Phil. Soc. 94, 152 (1950).
7. Fogg, G. E. Algal Cultures and Phytoplanktonic Ecology, p. 6 (1965), University of Wisconsin Press, Madison.
8. Bowman, M. C., F. Acree, Jr., C. H. Schmidt and M. Beroza. J. Econ. Entomol. 52, 1038 (1959).
9. Acree, F., Jr., M. Beroza and M. C. Bowman. J. Agr. Food Chem. 11, 278 (1963).
10. Lind, O. T. and R. S. Campbell. Limnol. Oceanog. 14, 787 (1969).
11. Murray, D. S., W. L. Rieck and J. Q. Lynd. Weed Science 17, 52 (1969).
12. Lichtenstein, E. P., T. W. Fuhremann and K. R. Shulz. J. Agr. Food Chem. 16, 870 (1968).
13. Butler, P. A. U. S. Fish Wildlife Serv. Circ. 167 (1963) pp. 12-20.
14. Ukeles, R. Appl. Microbiol. 10, 532 (1969).
15. Hassan, A., S. M. A. D. Zayed and F. M. Abdel-Hamid. Biochem. Pharmacol. 15, 2045 (1966).
16. Johnson, D. W. Trans. Amer. Fish. Soc. 97, 348 (1968).