RESISTANCE TO DDT OF A FRESHWATER ALGA[1]

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ABSTRACT

The amount of chlorophyll, the oxygen evolution in the light, and the oxygen uptake in the dark by *Chlamydomonas reinhardtii* Dangeard are unaffected by exposure to 100–1,000 ppb DDT for 16–96 hours at 18°–22°C in inorganic basal medium with and without acetate in the medium. The growth rate and final cell density of *Chlamydomonas reinhardtii* Dangeard were identical in control and experimental cultures exposed to 1000 ppb DDT in the growth medium for nine days at 22°C.

INTRODUCTION

The chlorinated hydrocarbon, DDT (1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane) and its metabolites, DDE and DDD, have contaminated the world's food chains and have affected many animal species (Wurster, 1969). The ability of algae to accumulate DDT (Södergren, 1968) and the subsequent concentration of DDT in organisms at higher trophic levels (Wurster, 1969) are well known. The direct effects of DDT on algae and higher plants, however, have not been studied extensively.

The fact that DDT inhibits photosynthesis and growth in some marine algae has been demonstrated by Wurster (1968) and Menzel *et al.* (1970). As in higher plants (Chapman and Allen, 1948; Lawler and Rogers, 1967; Hayes, 1959), the inhibition varies among species. Lazaroff and Moore (1967) reported that the growth of some species of freshwater algae was inhibited by exposure to DDT in laboratory cultures. Bishop (1947) reported that the relative abundance of some freshwater algae was affected by dusting or spraying DDT on small ponds. Bishop's qualitative data preclude any definitive conclusions, but in terms of trends of relative abundance during the summer months, three categories of responses can be identified: 1) decrease in relative abundance of two algal taxa (*Synura*, Dinophyceae) in the DDT-treated pools, 2) increase in relative abundance of one taxa (Chroococcaceae), and 3) no difference in relative abundance of algae between treated and untreated ponds. The taxa recorded included diatoms, *Scenedesmus*, *Chlamydomonas*, *Euglena*, and *Trachelomonas*.

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Algae, like other lipid-containing systems, have the ability to concentrate the lipid-soluble chlorinated hydrocarbons, which include DDT. For example, *Cladophora* sp. had concentrated DDT to 96 ppm by three days after direct spraying (Meeks, 1968) and *Cladophora gracilis* had accumulated 0.083 ppm DDT from lower ambient concentrations of DDT in an estuary (Woodwell, *et al.* 1967). Södergren (1968) has presented direct evidence for the rapid uptake and accumulation of DDT by *Chlorella* sp. in experimental cultures. The fact that freshwater algae can accumulate DDT from their aquatic habitat and that DDT occurs commonly in most major river systems in the United States (Weaver, *et al.* 1967; Breidenbach, *et al.* 1967) raises numerous questions about the potential ecological consequences of DDT on freshwater ecosystems. This study was designed to investigate the effects of DDT on the growth, photosynthesis, respiration, and chlorophyll concentration of a freshwater alga in laboratory culture.

MATERIALS AND METHODS

Chlamydomonas reinhardtii, Dangeard strain 89 (+) (Starr's Collection of Algae, Bloomington, Indiana, U.S.A.) was grown on two media, one with and the other without acetate (Sager and Granick, 1953). Recrystallized commercial DDT (mp 106–107.5°C) was dissolved in 95% ethyl alcohol and added to the algal media to yield concentrations of 100 and 1,000 ppb DDT; under the conditions of these experiments, the ratio of cells to DDT ranged from 100 to 4,000 cells/μg DDT. Cells for all experiments were obtained from stock cultures initiated and maintained under standard conditions, which had been determined to yield cells in the exponential growth phase after four days in acetate medium and five days in non-acetate medium. These stock cultures were innoculated to yield 10^5 cells/ml in 50 ml of medium in 125 ml flasks, which were rotated continuously at 60 r.p.m. at 22°C and 6500 lux.

Growth was determined by daily cell counts of algal cells grown in acetate medium with and without 1,000 ppb DDT. Growth experiments were initialized with an algal cell concentration of 10^5/ml and a DDT concentration of 1000 ppb. The DDT was added as a 5μl aliquot of a 1% DDT ethanolic solution to each experimental flask containing 50 ml of medium and algal cells. A 5μl aliquot of ethanol only was added to each control flask. Duplicate flasks for controls and DDT treatments were illuminated with fluorescent lamps (6,500 lux) at 22°C and rotated at 60 rpm to ensure homogeneous chemical conditions in all culture flasks.

The rates of oxygen evolution and oxygen uptake were determined by Warburg manometry. Cells were concentrated by centrifugation to yield a 2 ml suspension of 4–15 x 10^7 cells/flask. The pCO_2 was maintained at 0.8% with a diethanolamine-bicarbonate buffer contained in the center well of the reaction vessel; light intensity was adjusted to $4{,}300 \pm 100$ lux under each reaction vessel. The cells used for these manometric determinations were grown in two concentrations of DDT, 100 ppb and 1,000 ppb. At the lower concentration (100 ppb), the calculated ratio of cells to DDT was 100 cells/μg DDT, which is equivalent to the cell/DDT ratio at which Wurster (1968) reported 50% inhibition of the ^{14}C-$NaHCO_3$-uptake by *Skeletonema costatum*, an alga similar in size to *Chlaymdomonas reinhardtii*. For these experiments the *C. reinhardtii* sample was grown in 5 liters of inorganic basal medium with and without DDT at 18°C. for 16–19 hours, by which time the mean cell density was 10^4 cells/ml; the medium was agitated continuously by bubbling with filtered compressed air. At the conclusion of the manometric determinations, the cell number and chlorophyll *a* of the contents of the Warburg flask were determined. The method of Arnon (1949) was used to determine chlorophyll *a* concentration. At the higher concentration of 1,000 ppb DDT, the cells were grown in 50 ml of medium with and without DDT at 22°C for four days, at which time the ratio of cells to the initial DDT concentration was 4,000 cells/μg DDT (4 x 10^6 cells/ml).

RESULTS

Effects on Photosynthesis and Respiration

The data in Table 1 indicate that under the above conditions DDT causes no significant alteration of the rate of oxygen consumption in the dark or of the net photosynthetic evolution of oxygen in the light. The amount of chlorophyll per cell was similarly unaffected by exposure to DDT at a ratio of 100 cells per μg DDT (Table 1). Chlorophyll determinations were not made for the experiments in which the number of cells per μg DDT ratio was 4000. Although not reported in Table 1, the results obtained with cells grown in non-acetate medium at a ratio of 4,000 cells/μg DDT were identical to those reported for cells grown in acetate medium.

TABLE 1

Oxygen exchange in *Chlamydomonas reinhardtii* in acetate medium with and without DDT.

Cells/μg DDT	Cells/ml	DDT ppb	O_2 Uptake		O_2 Production	
			Control	DDT	Control	DDT
			(μl O_2 hr^{-1} $cells^{-7}$)			
4,000	4×10^6	1000†	17.86	17.57	20.62	22.47
			18.38	15.52	22.34	20.82
			14.58	13.92	23.87	18.84
			14.05	13.66	22.80	20.90
			17.78	19.39	38.33	44.46
			17.01	18.54	42.93	44.36
			16.58	19.41	40.69	34.45
			14.55	19.02	39.52	34.05
			21.84	22.86	33.89	51.38
			20.86	22.45	38.06	45.41
			24.74	25.35	45.74	50.01
			24.69	27.29	49.57	47.76
			17.85	17.46	27.70	37.98
			18.33	17.06	31.32	34.69
		Mean	18.51	19.25	34.10	36.26
		(±se)	(0.91)	(1.07)	(2.51)	(3.10)
100	1×10^4	100	13.99	13.27	21.27	24.98
			13.91	15.33	33.04	29.31
			16.30	16.40	28.86	34.70
			10.61	10.17	16.21	14.17
			10.51	11.18	15.82	12.60
		Mean	13.06	13.27	23.04	23.15
		(±se)	(1.12)	(1.17)	(3.45)	(4.30)

	μg. Chlorophyll *a* $cells^{-7}$	
	Control	DDT
	26.1	30.8
	25.1	24.1
	23.4	24.6
	16.3	11.1
	20.1	19.3
Mean	22.2	22.0
(±se)	1.8	3.3

†Duplicate flasks for seven experiments.

Effects on Growth

Daily cell counts using a Levy Counting chamber yielded coefficients of variation of 8.8% at high cell concentrations and 24.6% at low cell concentrations. The results of these experiments (fig. 1) failed to show any effect of DDT on the rate of cell division or on the final number of cells produced after nine days of growth. Qualitative observations of the cultures revealed no differences with respect to cell motility or pigmentation.

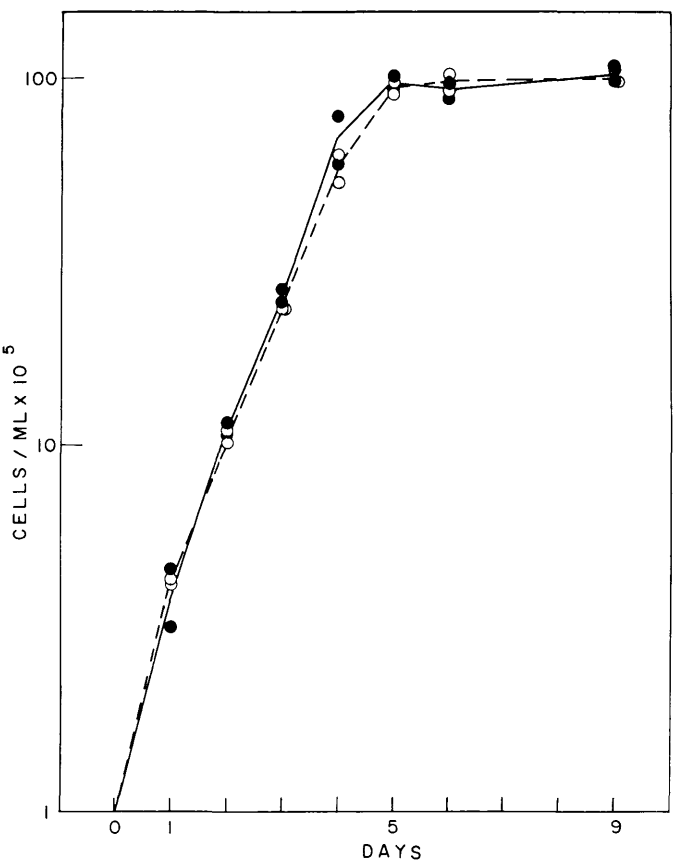

FIGURE 1. Growth of *Chlamydomonas reinhardtii* in acetate medium at 22° C. in the presence and absence of DDT, which was added initially at a concentration of 1,000 ppb. Solid circles represent results with DDT; open circles represent control results.

DISCUSSION

Menzel *et al.* (1970) reported that, of the four species of marine algae tested for the effect of DDT on ^{14}C-uptake, one, *Dunaliella tertiolecta*, was unaffected. *Dunaliella* is related morphologically to *Chlamydomonas*, from which it can be distinguished by its relatively thinner cell wall (Fritsch, 1935). The structural similarity of these genera is reflected also in the resistance to inhibitory effects of DDT, as reported by Menzel (1970) and in this paper. *Chlamydomonas reinhardtii* is an extremely hardy species, with the ability to live in organically polluted water (Palmer, 1962). Palmer (1969) ranked *Chlamydomonas* third in a list of the 60 most pollution-tolerant genera of algae, and the species, *C. rein-*

hardtii, was ranked 45th in pollution tolerance in a list of 80 algal species. The demonstrated resistance of this species to DDT is probably a factor in the remarkable capability of this species to survive under polluted conditions.

The addition of a freshwater alga to the list of algae that are metabolically unresponsive to DDT strengthens the hypothesis that the ecological consequence of DDT in the environment is not simply one of toxicity. Potentially the characteristic seasonal succession of phytoplanktonic and zooplanktonic organisms could be severely altered by a substance which is toxic to some species but not to others. In combination with the well-documented deleterious effects of inorganic enrichment on the diversity and abundance of planktonic organisms (National Academy of Science, 1969), the additional stress of organic insecticides like DDT may result in even greater perturbations. It remains to be determined whether ambient environmental concentration of DDT will cause the same responses observed in laboratory cultures, in which relatively higher concentrations of DDT have been employed. Although evidence from natural ecosystems has not been presented, the demonstrated insensitivity of a common freshwater alga in the laboratory suggests that similar results would be expected in natural ecosystems.

More freshwater species must be tested, but the ecological implications of the data collected at present are serious. As in the marine habitat (Menzel *et al.*, 1970), varying effects of DDT on algae can be expected in freshwater. Species like *Chlamydomonas reinhardtii*, unaffected by DDT (and probably by other pollutants), will be expected to displace less tolerant species in polluted situations and thereby reduce or alter the species composition of such freshwater ecosystems.

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