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Effects of Mosquito Control Insecticides on Nitrogen Fixation and Growth of Blue-green Algae in Natural Plankton Associations

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Nitrogen (N_2) fixation by blue-green algae is a significant source of fixed nitrogen for many natural and agricultural aquatic systems (FOGG et al. 1973). Laboratory studies have shown that pesticides used in aquatic habitats affect blue-green algal growth (BATTERTON et al. 1971; VENKATARAMAN and RAJYALAKSHMI 1972; SINGH 1973) and N_2-fixation (DASILVA et al. 1975). Field studies have also shown that insecticide applications can increase biomasses of blue-green algae (RAGHU and MACRAE 1967a; HURLBERT et al. 1972) and N_2-fixation rates (RAGHU and MACRAE 1967b). Since pesticides can thus affect nitrogen flow in aquatic systems, we investigated the effects of five mosquito control agents (temephos, propoxur, methoxychlor, Dimilin®, and methoprene)[2] on N_2-fixation and growth of blue-green algae in natural plankton associations. Effects of temephos, propoxur, and methoxychlor on various algal species have previously been reported (DERBY and RUBER 1970; POORMAN 1973; KRICHER et al. 1975).

METHODS

The algal culture techniques followed those described by HORNE and GOLDMAN (1974). On Oct. 30, 1975, a dip sample was taken from the pelagic area of Clear Lake, California (U.S.A.) and the plankton concentrations of this sample were increased ca. five-fold with additional plankton collected with a plankton net. One liter aliquots of this water were placed in 2 liter volumetric flasks. Zooplankton were not excluded from the cultures as we were attempting to simulate natural

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[2]Temephos: O,O'-(thiodi-4,1-phenylene)bis [O,O-dimethyl phosphorothioate]. Propoxur: 2-(1-methylethoxy) phenyl methylcarbamate. Methoxychlor: 1,1'-(2,2,2-trichloroethylidene)bis[4-methoxybenzene]. Dimilin®: 1-(2,6-Difluorobenzoyl)-3-(4-chlorophenyl) urea. Methoprene: (E,E)-1-methylethyl 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate.

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lake conditions. The initial sample contained ca. 8000 zooplankton per liter, with the following composition: copepod copepodids and adults (73%); copepod nauplii (6%); cladocerans (20%); Asplanchna sp. (1%). Since N_2-fixation is dependent upon adequate supplies of phosphate (STEWART and ALEXANDER 1971) and iron (EYSTER 1972) environmentally sensible quantities (50 ppb Fe^{++}; 100 ppb PO_4-P) of these nutrients were added to each flask. Control cultures were duplicated but the facilities only permitted a single culture for each pesticide treatment. Treatment responses which were outside of the 90% confidence intervals of the duplicated control cultures were considered significant. The cultures were incubated under continuous fluorescent light at intensities of 950-1250 lux and a mean temperature of 20$\pm$ 3°C. Two pesticide dosages were employed; a low dosage (20 ppb) within the normal range of field application rates, and a high dosage (500 ppb), reflecting situations where treatments would be excessive. The pesticides were added in acetone solution (1 ml). Control flasks received sham injections of acetone.

At one, three and six day intervals, duplicate subsamples were taken from each flask to measure N_2-fixation (acetylene reduction method) and Chlorophyll a (Chl. a) concentrations using methods described by HORNE and GOLDMAN (1974). Algae preserved in Lugols solution were enumerated by counting two swaths (10%) of a Sedgwick-Rafter cell. Heterocysts, the specialized cells which are the primary site of N_2-fixation in most blue-green algae, were also enumerated. Algal volumes were estimated according to Amer. Public Health Assoc. (1971; p.736).

The algae grew well during the experimental period. Mean Chl. a concentrations of the control cultures increased from 117 to 208 ppb during the six days of the experiment. N_2-fixation rates of the controls increased from 38 to 54 nmoles $\cdot l^{-1} \cdot hr^{-1}$ and heterocyst concentrations increased five-fold. (Table 1). The algal association in the cultures was dominated by the N_2-fixing species, Aphanizomenon flos aquae, which represented 76% and 94%, respectively, of the initial and final algal volumes in the control cultures. Other algae present at the start of the experiment were: diatoms (17%), Anabaena (4%), Microcystis (0.5%) and green algae (2%).

RESULTS AND DISCUSSION

After three to six days, temephos, methoxychlor and the high dosages of methoprene and propoxur

significantly stimulated N_2-fixation rates of the algae (Fig. 1). Final fixation rates in the high concentration of temephos were nearly 500% of control values, and the N_2-fixation rate in the high concentration of methoprene was over 900% of the controls. In several cases, increases in N_2-fixation rates were accompanied by small but significant increases in Chl. *a* (Fig. 1). Changes in N_2-fixation rates were usually accompanied by concomitant changes in heterocyst to vegetative cell ratios of *Aphanizomenon* (Table 1), indicating that the changes in N_2-fixation rates were not simply a function of varying algal biomasses. N_2-fixation rates and Chl. *a* concentrations of Dimilin® treated cultures were not significantly different from the controls. However, continuing experiments with Dimilin® (James Roth, personal communication) have yielded results consistent with those which we found for the other pesticides.

Multiple regression analysis indicated that N_2-fixation rates were most strongly correlated with *Aphanizomenon* heterocyst concentrations ($r = .969$: other tested variables were the final biomasses of each algal taxon, heterocyst to vegetative cell ratios, and Chl. *a* concentrations). Addition of *Anabaena* heterocyst concentrations to the equation increased the multiple correlation coefficient to .981, indicating that 96% (coefficient of multiple determinism) of the variability in N_2-fixation among the treatments could be explained by variations in heterocyst numbers.

While N_2-fixation was markedly affected by many of the pesticides, the mode(s) of action of these compounds are difficult to assess with our experimental design. However, some insights may be gained by an understanding of heterocyst formation in blue-green algae. Heterocyst formation is induced by a need for fixed nitrogen (i.e. NH_4^+, NO_3^-) (FOGG, 1949). Under normal conditions, heterocyst numbers and N_2-fixation activity show a close parallel (KULASOORIYA et al. 1972). However, if the nitrogenase enzyme system responsible for fixation is inactivated, a decrease in the N_2-fixation rate per heterocyst (NF:Het) will occur since the algae will continue to produce heterocysts even though N_2 is not being fixed (FAY, 1973).

Mean N_2-fixation rates in treatments receiving Dimilin® and the low dosage of methoprene were lower (though not significantly) than in control cultures. However, NF:het ratios in these treatments were essentially unchanged from controls (Table 1), indicating that these compounds are not directly affecting the nitrogenase enzyme system. Stimulation of N_2-fixation occurred in many treatments. However, only the low dosage of methoxychlor caused an increase in the NF:Het

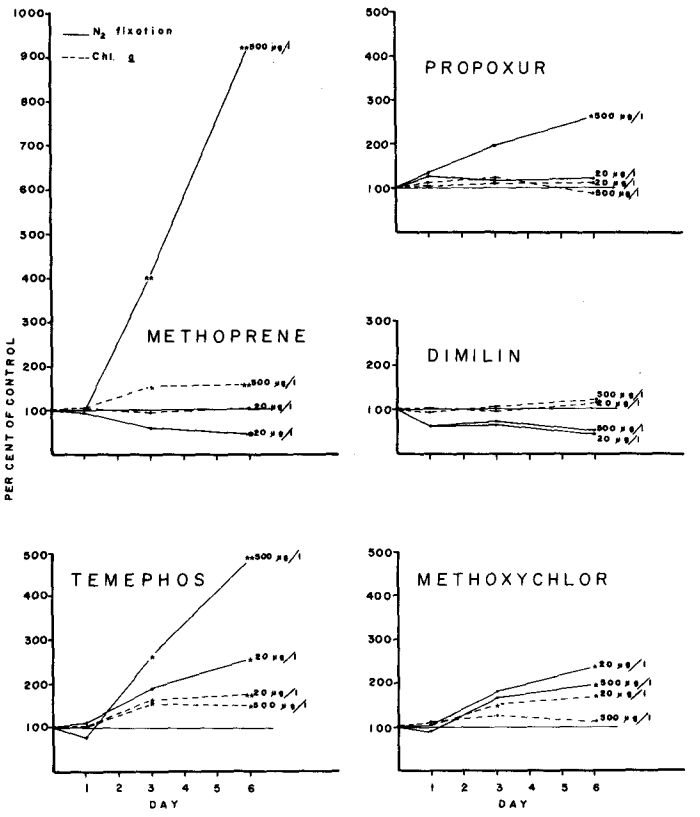

Figure 1. Nitrogen fixation rates (solid lines) and Chlorophyll a concentrations (dashed lines) in each of the pesticide treatments. Coefficients of variation of the duplicated determinations of Chlorophyll a and nitrogen fixation from the individual flasks averaged 5 and 6%, respectively. Asterisks indicate values which lie outside of the 90% (*) and 95% (**) confidence intervals of the replicated control flasks (n=2; 1 d.f.).

TABLE 1

Heterocyst concentrations, heterocyst:vegetative cell frequency (as %), N_2-fixation rates and N_2-fixation:Heterocyst (NF:Het) ratios for the initial sample and for the control and pesticide treatments on the final day of the experiment.

		Heterocysts				N_2-Fixation	
		Aphanizomenon		Anabaena		Rate	NF:Het
		No./ml	%	No./ml	%	pmole/ml·hr	pmole/het·hr
Control A		1084	.3	68	4.1	59	.051
Control B		680	.3	14	2.7	49	.071
Temephos	20 ppb	1615	.6	442	5.7	140	.068
	500 "	2592	.6	833	2.0	264	.077
Methoxychlor	20 "	765	.2	196	6.6	130	.135
	500 "	1402	.2	578	3.3	106	.054
Propoxur	20 "	744	.2	204	2.3	66	.069
	500 "	1700	.5	68	3.0	142	.080
Methoprene	20 "	468	.1	26	2.2	24	.048
	500 "	7522	.9	935	1.1	500	.059
Dimilin®	20 "	255	.1	76	4.6	23	.069
	500 "	552	.1	34	1.8	27	.046
Initial Sample		102	.1	85	.1	38	.203

ratio (Table 1). This NF:Het ratio was, however, still less than that of the initial algal sample (Table 1), indicating that this ratio is within the natural limits of the algae.

The reasons for increased fixation rates in the other pesticide treatments is also unclear. Decreased grazing pressure due to zooplankton mortalities has been suggested as a factor increasing blue-green algal growth (RAGHU and MACRAE 1967a; HURLBERT et al. 1972) and N_2-fixation rates (RAGHU and MACRAE 1967b). However, in our experiment, changes in Aphanizomenon heterocyst frequencies in those treatments which were affected (Table 1) indicate that fixation was effected chemically, rather than solely by grazing pressure. The insecticides may have directly induced heterocyst formation with concomitant increases in N_2-fixation rates. In this regard, DASILVA et al. (1975) demonstrated that pesticides can stimulate N_2-fixation rates of blue-green algae grown in pure culture. Alternatively, pesticide induced zooplankton mortalities in our study may have reduced nitrogen regeneration (through excretory products) and consequently NH_4^+ levels in the medium. Blue-green algae may have responded to this limitation by fixing N_2 at higher rates. The interrelationship of zooplankton and N_2-fixation in cultures exposed to Dimilin® are currently being investigated.

Regardless of whether pesticides act directly on the algae or indirectly through actions upon other organisms, our study and those of RAGHU and MACRAE (1967a,b) indicate that normally used dosages of pesticides can markedly affect N_2-fixation rates and growth of blue-green algae, and consequently the nitrogen flow in aquatic systems. The significance of this effect depends upon the particular aquatic habitat to which the pesticides are applied. Certainly an increase of N_2-fixation in a rice field would be desirable. Conversely, higher rates of N_2-fixation and growth of blue-green algae in a recreational lake or potable water supply would have a detrimental effect on water quality.

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