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Effects of Pesticides on Pure and Mixed Species Cultures of Salt Marsh Pool Algae[1]

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A number of studies on the effects of pesticides and other toxicants in single species algal cultures have demonstrated that while some species show inhibition of growth and/or photosynthesis when exposed to levels as low as a few ppb (WURSTER 1968), other species are resistant to levels of several ppm or higher (UKELES 1962, MENZEL et al. 1970, DERBY & RUBER 1970).

Because of differential algal sensitivity one might predict that toxicants would have no long-term inhibitory effect on biomass and production in mixed-species cultures of algae. Instead, a shift in community composition, with the more resistant species replacing the sensitive ones might be expected. There have been few studies using several species communities comprised only of algae. MOSSER et al. (1972) found that addition of DDT or PCB reversed the outcome of competition between two species and also that neither final biomass nor productivity were affected by the toxicants.

In this study we report on the general sensitivity of five species of salt marsh pool algae to five pesticides and then proceed to examine more closely the effects of carbaryl on single species and on communities made up of three and five species in order to determine the effects on community sensitivity of varying the composition and numbers of species.

MATERIALS AND METHODS

Three diatom species, Nitzschia closterium, Amphora coffeaformis v. borealis and Amphiprora sp; a green alga, Chlorococcum sp.; and a dinoflagellate, Gonyaulax sp., were isolated and axenized from samples collected at the Parker River National Wildlife Refuge in Massachusetts (MALY 1980). All species were maintained and tested in liquid f_2 growth medium (GUILLARD & RYTHER 1962) enriched with 1 ppm sodium glycerolphosphate and 15 ppm yeast autolysate and made up to a salinity of 27 ppt with Rila

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Marine salts and distilled water. Cultures were kept in erlenmeyer flasks on a reciprocating shaker under an illumination of 350 footcandles from incandescent-supplemented flourescent lights on a 12 hr light - 12 hr dark photoperiod. Temperatures were maintained at 32° C ± 3° during the light period and 23° C ± 2° during the dark. These temperatures are within normal ranges for summer in the marsh pools.

Single species experiments were performed to assess the sensitivity of the five species of algae to three organophosphate (temephos, chlorpyrifos, and malathion) and two carbamate (propoxur, carbaryl) insecticides. In these, the organisms were exposed to 10, 2, 1 and 0.5 ppm pesticide for 96 hrs. Since the pesticide was dispersed in acetone, controls were run with appropriate acetone concentrations. Cell counts were made 0, 48, and 96 hrs after pesticide addition. The instantaneous growth rate (r) was calculated for time span 0-48 hrs and 48-96 hrs by $r = \ln (N_t/N_0)$ where N_0 is the cell density at the beginning and N_t is the density at the end of the interval. In addition, at 48 hrs, measurements of optical density were made at 660 mu and 430 mu on acetone extracts of samples taken from the cultures.

Multispecies experiments were used to assess the effect of carbaryl on the structure and function of three simple algal communities: a three-species community (E-3) composed of Nitzschia, Chlorococcum, and Amphiprora, a three-species community (E-4) of Chlorococcum, Amphora, and Amphiprora and a community composed of all five species (E-5). There were four replicates for each community experiment. In these experiments, 2 ppm carbaryl in acetone was added to experimental cultures, and acetone alone to the controls. The cultures were then allowed to grow for 21 days. Cell counts were made at 0, 2, 4, 7, 9, 14 and 21 days, although only two cultures of each community were sampled on days 4, 14, and 21. Optical density was measured, as in the single species experiments, on days 2, 9, 14, and 21. Primary production was measured on days 2 and 9 using liquid scintillation counting of ^{14}C uptake from $NaH^{14}CO_3$. This necessitated the removal of 15% of the culture volume which was replaced with fresh medium.

RESULTS AND DISCUSSION

General Screening

Our initial objective was to select a pesticide with a broad enough effect to permit it to be used as a tool for further work. Carbaryl was found to be the most generally toxic of the 5 insecticides tested (Table 1) and so it was selected for use in the remaining experiments. It should be noted that the dosage shown in Table 1 is very high, 10 ppm, also that Amphiprora was the most broadly sensitive alga followed by Chlorococcum.

Single Species Cultures

Effects of carbaryl on the instantaneous growth rate of the five algal species is reported in Table 2, and the percentages of inhibition in Table 3. These data show that Gonyaulax, resistant

to a concentration of 10 ppm, was the species least affected by the pesticide. Gonyaulax grew very slowly and gave inconsistent results. Nitzschia, second most resistant, was inhibited by 10 and 2 ppm but only for the first 48 hrs after pesticide addition. Amphiprora had lowered growth with concentrations of 10, 2, and 1 ppm, but the effect on growth lasted only 48 hrs with 2 and 1 ppm. Amphora was slightly more sensitive than the other species: growth was depressed by 10, 2, and 1 ppm, this effect persisting through the entire 96 hr testing period. Growth of Chlorococcum was depressed for 96 hrs at 10, 2, and 1 ppm of carbaryl.

Optical density measurements generally paralleled cell count data and so are not included here. One significant exception was Chlorococcum which showed optical density inhibition at 10 ppm but not at 2 or 1 ppm although cell counts were depressed at these dosages. This may indicate that cell growth continued at lower pesticide concentrations, but without cell division. We have no cell measurements to confirm this.

Table 1. Sensitivities of the algal species to 5 insecticides given at a dosage of 10 ppm. Instantaneous growth rate (r) for the interval 0-48 hrs is tested for statistically significant deviation between pesticide and control cultures.

	PESTICIDE				
Algae	Temephos	Propoxur	Chlorpyrifos	Malathion	Carbaryl
Amphiprora	**[a]	***	***	**	**
Amphora					**
Nitzschia					***
Chlorococcum		***	**		**
Gonyaulax			*		

a. *, **, *** signify respectively at 0.9, 0.95, 0.99 levels of confidence (t-test) that the mean instantaneous growth rate of the experimental culture is suppressed relative to its control.

Table 2. Effect of varying concentrations of carbaryl on the instantaneous growth rate (r) of single species cultures. Pesticide treated cultures are composed with the adjacent acetone (AC) controls for the time intervals 0-48 and 48-96 hrs.

	AC	10 ppm	AC	2 ppm	AC	1 ppm	AC	0.5 ppm
Amphiprora								
0-48	0.254[a]	-0.293***[b]	0.190	0.048***[d]	0.281[f]	0.103**[g]	0.160[e]	0.064[e]
48-96	0.418	0.156*	0.156	0.170[d]	0.222[d]	0.198[e]	0.231[e]	0.348[e]
Amphora								
0-48	0.314[c]	-0.116***[d]	0.378[c]	-0.054***	0.575	0.005***	0.348	0.101
48-96	0.507[c]	0.190***[d]	0.327[c]	0.246	0.522	-0.056***	0.625	0.382
Nitzschia								
0-48	0.497	-0.296***	1.025	0.318**	0.808	0.724*	Not Done	
48-96	0.565	0.714	0.181	0.637*	0.449	0.400		
Chlorococcum								
0-48	0.957	0.238***	0.701	0.421***	0.368	0.193**	0.641	0.758
48-96	0.483	-0.354***	0.454	0.035**	0.526	0.241**	0.545	0.388
Gonyaulax								
0-48	0.191	-0.110	0.230	0.001	0.015	0.254	Not Done	
48-96	0.196	-0.018	0.069	0.230	-0.074	-0.143		

a. Values are obtained by $\ln N_t/N_0$ for the 2 day intervals where N_0 and N_t are the cell densities at the starting time and the finishing time for each interval.

b. *, **, and *** signify respectively .9, .95 and .99 levels of significance by t-test. All values are means of 3 replicate cultures except those marked c, d, e, f and g which indicate respectively 4, 5, 6, 8 and 9 replications.

Table 3. Percentage inhibition[a] of growth rates of single-species cultures by carbaryl

	Carbaryl Concentrations			
Algae	10 ppm	2 ppm	1 ppm	0.5 ppm
Amphiprora				
0-48	>100***[b]	75***	63**	60
48-96	63*	-9	11	-51
Amphora				
0-48	>100***	>100***	99***	71
48-96	62***	25	>100***	39
Nitzschia				
0-48	>100***	69**	10*	
48-96	-26	-352*	11	
Chlorococcum				
0-48	75***	40***	48**	-18
48-96	>100***	92**	54***	29
Gonyaulax				
0-48	41			
48-96	76			

a. Measured by $\% = \left(1 - \frac{r \text{ pesticide}}{r \text{ acetone control}}\right)(100)$. Inhibition above 100% means that numbers were declining in experimental cultures. Negative values mean experimental growth rates exceeded control rates.

b. *, **, *** signify respectively 0.9, 0.95, 0.99 levels of significance by t-test.

Multispecies Cultures

In the three-species experiment (E-3), densities of Nitzschia and Chlorococcum are not statistically different from the controls but Amphiprora density is inhibited (Table 4). In the three-species experiment (E-4), Amphiprora numbers are again inhibited, Chlorococcum is enhanced while Amphora is unaffected except for an interval during the middle of the experiment's duration. In the five-species experiment (E-5), Amphiprora is once again inhibited, Gonyaulax disappears, Amphora is somewhat inhibited during the middle of the experiment and Nitzschia and Chlorococcum are enhanced (Table 4).

Taken all together, on the last day of these experiments Nitzschia and Chlorococcum numbers are enhanced, Amphora is inhibited in one experiment and enhanced in the other, Amphiprora is severly inhibited, and Gonyaulax is excluded.

Table 4. Effect of carbaryl at 2 ppm on cell densities in multispecies cultures over a 21 day period.

Experimental Species Groups	Days After Inception 0	2	4	7	9	14	21
Experiment E-3							
Amphiprora	0.9[a]	0.3**[b]	0.2**	0.2***	0.1***	0.2***	0.1
Nitzschia	0.8	0.7	1.4	0.6	0.6	0.6	1.3
Chlorococcum	1.1	0.8	0.8	1.2	0.8	0.6	3.0
Experiment E-4							
Amphiprora	1.1	0.3*	0.3	0.1**	0.1**	0.1*	0.1***
Amphora	1.2	0.9	1.0	0.4**	0.5***	0.5	1.5
Chlorococcum	1.0	0.8	0.9	0.6	1.5	4.0***	2.2
Experiment E-5							
Amphiprora	0.9	0.5*	0.3	0.1**	0.1**	0.1***	0.1**
Amphora	0.8	0.7	0.5**	0.4	0.4	1.5	0.6
Nitzschia	1.4	1.1	2.3	2.6	3.2***	1.8	3.4
Chlorococcum	1.0	0.8	1.0	1.7	1.5	2.5	1.1
Gonyaulax	c	c					

a. Numbers are the ratio of the cell density in the experimental to that of the control culture.

b. *, **, *** indicate respectively 0.9, 0.95, 0.99 levels of significance.

c. Gonyaulax disappeared in this experiment.

In comparing the results from single and multispecies cultures experiments, it becomes apparent that one cannot predict the effect of carbaryl on components of multispecies cultures solely from its effects in single-species cultures. All four species are inhibited by carbaryl at 2 ppm in single-species cultures, but in multispecies cultures only two are inhibited. Also, the rank order of species sensitivity differs between the two experimental designs. Amphiprora is more sensitive in the multispecies communities and Chlorococcum is more sensitive in single-

species cultures (Table 5). Finally, even as results from single species experiments are poor predictors of multiple species results at the same time point of the experiments (2 and 4 days), the 2 and 4 day results are not necessarily good indicators of longer term outcomes (Tables 4 and 5).

Table 5. Comparison of relative effects of carbaryl on the algae in monocultures vs. multispecies cultures.

	Single Species Exp'ts.		Multispecies Exp'ts.		
Algae	2 days	4 days	2 days	4 days	21 days
Amphiprora	–[a]	0	–,–,–	–,–,–	–,–,–
Amphora	–	0	0,0	0,–	+,0
Nitzschia	–	–	0,0	+,+	+,+
Chlorococcum	–	–	0,0,0	0,0,0	+,+,0
Gonyaulax	0	0	b	b	b

a. –, 0, + signify respectively that the alga, treated with 2 ppm carbaryl, is performing worse, the same as, or better than its control at the indicated period of time.

b. Gonyaulax disappeared from both experiments and controls in the one multispecies experiment in which it was used (E-5).

The order of sensitivity in the multispecies cultures is negatively correlated with r, the instantaneous growth rate: the two species most inhibited by pesticide in the multispecies cultures, Amphiprora and Amphora, had considerably lower growth rates in all single species cultures (Table 2). It appears that inhibition by the pesticide left the slower growing species at an even greater disadvantage in competition for the diminishing supply of nutrients. Amphiprora resumed normal growth in single species cultures in 96 hrs, but in multispecies cultures remained depressed for as long as 21 days. In the multispecies communities the other species were all initially depressed by addition of pesticide but only to a slight degree which was not statistically significant (Tables 4 and 5). Gonyaulax, the slowest growing alga was eliminated in the multispecies situation both in experimentals and in controls. Larger differences between the carbaryl and control communities occurred later in the experiments.

Community Effects

Addition of carbaryl altered the species composition of all three communities to varying degrees. One three-species community (E-3) was least changed by pesticide addition since the

numbers of only one of the species, Amphiprora, were significantly altered. The other three-species (E-4) and the five-species community was affected to a greater extent, with the densities of three species changed in each. The degree to which the communities are affected does not appear to be a function of species diversity. Most mathematic models predict that in a community comprised on one trophic level, in which competition is the primary species interaction, stability will decrease with increasing diversity (Phillips 1973, Maynard Smith 1974). In this study the characteristics of species composing the community (growth rate, pesticide tolerance) are more important than is the community diversity.

Table 6. Effect of carbaryl at 2 ppm on community productivity measured by total ^{14}C uptake in total ^{14}C uptake from $NaH^{14}CO_3$ in multispecies experiments.

	Treatment	
	Acetone Control	Carbaryl
Experiment E-3[a]		
Day 2	9880[b]	6930
Day 9	12713	16744
Experiment E-4		
Day 2	11030	5370**[c]
Day 9	10660	9802
Experiment E-5		
Day 2	9990	5980**
Day 9	15336	23206

a. See text or Table for species complex used.

b. uptake in counts per minute

c. ** signifies a 0.95 level of significance in the difference between experimentals and controls.

Community productivity was inhibited early in the experiments (day 2) but this effect was transient and by day 9 this measure of community function had returned to normal (Table 6). Optical density (not shown) showed a similar trend but was a less sensitive measure of the effect. This recovery is less a function of the number of species in the community than it is of the high growth rate and replacement capacities of those species which happen to be present. In the present study which species were able to play this replacement role was more a function of their capacity to

reproduce quickly than their absolute resistance to carbaryl. The productivity of the plankton algae of salt marsh pools should show only transient inhibition to carbaryl input. Some species are very likely to become inhibited but identifying which ones before the fact requires an examination of competitive interactions as well as of single-species responses.

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