Bull. Environ. Contam. Toxicol. 30, 344–350 (1983)

Effect of Pesticides and PCBs on Budding Rates of Green Hydra

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Pesticides play a significant role in increasing agricultural productivity and controlling arthropod-borne diseases. Since World War II, pesticide use has increased; in 1980, over a billion pounds were applied in the United States. The evidence regarding the impact of these chemicals on aquatic ecosystems consists mainly of acute toxicity data. However, subtle, sublethal effects, especially on lower invertebrates, have generally been neglected, although such effects must be both common and important and play a vital role in the overall impact of a toxicant on the environment. Research has been done on the effects of pesticides and PCBs on the reproduction of cladocerans (MAKI & JOHNSON 1975) and amphipods (NEBEKER & PUGLISI 1974). This study will attempt to elucidate the effects of some pesticides and of PCBs on the budding rate and budding efficiency of a lower aquatic invertebrate, the green hydra (Chlorohydra viridissima).

The experimental animal, C. viridissima, was chosen for several reasons. Mass culturing techniques have been developed (LOOMIS 1953) enabling studies to be done in the laboratory under controlled conditions, and adult hydra growth normally occurs as asexual budding, which results in the formation of new individuals rather than an increase in adult size. This means that counting the number of hydranths in a growing clone on successive days provides a measure of their growth rate. As this growth rate is logarithmic under uniform environmental conditions and can continue indefinitely, hydra growth may be quantitated and, as hydra are diploblastic and possess no vascular interior areas, all cells are exposed to environmental pollutants. In addition, C. viridissima has intracellular algal symbionts, which have been investigated by other workers (OSCHMAN 1967; COOK 1972). Thus, since algae-free Chlorohydra are obtainable, it is possible to note the effects of algae on growth when under stress from toxic chemicals.

MATERIALS AND METHODS

Initial stocks of *Chlorohydra viridissima* were obtained from Ward's Natural Science Establishment, Inc. (Rochester, NY) who indicated they were *Hydra viridis* (synonymous with *C. viridissima*). Stock cultures were maintained using techniques modified from LENHOFF & BROWN (1970). All animals reproduced asexually. Hydra were kept at 20°C in constant temperature incubators in 'M' solution (MUSCATINE 1961). Illumination consisted of one 20-watt white flourescent lamp, 21 cm above the bottom of the culture dishes (providing approximately 110 foot candles) with a 12-h photoperiod. Stock cultures were fed daily an excess of newly-hatched nauplii of the brine shrimp, *Artemia salina*. These were hatched from dried, disinfected cysts in Instant Ocean® (Aquarium Systems Inc., Eastlake, OH) and then rinsed in distilled water and 'M' solution. One hour after feeding, hydra culture medium was decanted and replaced with fresh medium. Initial stocks of albino *Chlorohydra* were obtained by growing normal individuals in 10^{-6} DCMU (3-[3,4-dichlorophenyl] -1,1-dimethylurea) with high light intensity consisting of a 150-watt incandescent lamp 15 cm from the cultures. The dieldrin used was recrystallized (Shell Chemical Company, New York, NY). Toxaphene was the technical grade of chlorinated camphene manufactured by Hercules, Inc., Wilmington, DE (Batch X-16189-49). The fungicide PMA (phenylmercury acetate) was obtained from K & K Laboratories, Inc., Painview, NY (Batch #17412) and the PCB tested was Aroclor 1254 made by Monsanto Co., St. Louis, MO (Lot #KA630). Atrazine (Batch #100117), 2,4,5-T, and DDT were obtained from Aldrich Chemical Co., Milwaukee, WI.

Pesticide concentrations used were below the solubility in water as indicated by GUNTHER et al. (1968). Sublethal concentrations of these toxicants were derived by trial with aid from toxicity data on other invertebrates. In each treatment, four uniform hydra were placed in separate depressions of a 12-depression porcelain spot plate. Uniform hydra were classified as animals bearing one small bud which had been starved for one day. Each depression held 1 mL of culture medium and 5 μL of pesticide in acetone or 5 μL of acetone, for the control, were added to each depression. The "no treatment" group received neither pesticide nor acetone. For each of 21 days, each hydra was fed 2 freshly-hatched brine shrimp, and after feeding, old medium was removed and fresh medium added, along with appropriate pesticides. Each day, the number of detached buds was recorded and removed. Student "t" tests were used for calculating all significance levels. Due to the fact that multiple comparisons were made, if the error probability was to be controlled to 5%, we had to operate at the 0.05/52 = 0.00096 level. Because this is so low, the per-comparison P is shown next to each treatment and all P values given in the text are on a per-comparison basis.

RESULTS

Effects on Budding Rate

Tables 1 through 3 show the effects of the various treatments on hydra budding rates with both normal and symbiont-free forms. The slopes of the regression lines of the test groups were compared to the combined slopes of the control and to treatment groups (Table 1). This was possible as there was no evidence of any differences between these groups, in the case of either normal or albino animals ($P = 0.83$ and 0.87, respectively). For normal *C. viridissima*, 1 ppm PMA appeared to have the most marked effect (Table 1) on the reduction of the budding rate ($P<0.0001$), followed by 5 ppm atrazine ($P<0.001$). PCB (20 ppb) reduced the budding rate of normal polyps to a lesser extent ($P<0.01$) while PMA was the only chemical to show a significant effect ($P<0.01$) in reducing the budding rate of albino *C. viridissima*. Albino individuals in the control, no treatment, dieldrin (100 ppb) and toxaphene (4 ppb) groups all exhibited lower budding rates than corresponding normal animals ($P<0.01$, Table 2). These lowered growth rates in the albino groups (compared with corresponding normals) are in accordance with the findings of MUSCATINE & LENHOFF (1965). They noted that when normal and albino *C. viridissima* were fed one shrimp per day, the average growth rate of the albinos was significantly lowered, but when fed to repletion, growth rates were the same. This supports the theory that the symbiotic algae favorably influence growth of *C. viridissima* under stress. On the other hand, the present study indicated that when *C. viridissima* was given limited food and was under stress from toxicants such as PMA, PCB and atrazine as demonstrated by lowered budding rates (Table 1), there was no significant difference ($P>0.05$) between the budding rates of normal vs. albino hydra (Table 2). Only in the groups where the evidence for pesticide effects was very slight (dieldrin, toxaphene) and in the control and no treatment groups, did the algae appear to benefit the hydra in terms of helping maintain a constant growth rate. Therefore, there was evidence that the expected benefit from endosymbiotic algae was eliminated by some of these chemicals. The decreased rates observed could also have been due to changes in the hydra's physiology caused by the presence of the toxicants. Such changes could have been the result of direct effects of the chemicals on morphogenesis or pesticide-induced energy losses.

Table 1. Comparison of Budding Rates of Test Groups vs. Control and No Treatment Groups Combined. Hydra were fed 2 shrimp per day.

Treatment	x_1-x_2	99% Confidence Limits[1/]	t	p
No Treatment				
normal	0.00993	(-0.16,0.18)	0.22	0.83
albino	0.00649	(-0.14,0.15)	0.17	0.87
100 ppb Dieldrin				
normal	0.0696	(-0.046,0.19)	1.91	0.085
albino	0.0283	(-0.060,0.12)	1.02	0.33
4 ppb Toxaphene				
normal	0.0186	(-0.11,0.15)	0.45	0.66
albino	0.0296	(-0.056,0.11)	1.10	0.30
1 ppb PMA				
normal	0.265	(0.15,0.38)	7.60	$<10.^{-4}$**
albino	0.155	(0.038,0.27)	4.20	0.0018**
20 ppb PCB				
normal	0.149	(0.016,0.28)	3.55	0.0052**
albino	-0.0019	(-0.088,0.084)	-0.071	0.45
5 ppm 2,4,5-T				
normal	0.0729	(-0.13,0.27)	1.16	0.27
albino	0.0559	(0.058,0.17)	1.56	0.15
5 ppm Atrazine				
normal	0.207	(0.076,0.34)	5.00	0.00054**
albino	-0.0396	(-0.16,0.085)	1.01	0.34

** P<0.01 per comparison
1/ Degrees of Freedom equal ten in all cases except "No Treatment", which were six.

Effects on Budding Efficiency

Budding efficiency was determined by the number of shrimp, and therefore calories, required to produce one bud. For normal C. viridissima (Table 3), 1 ppm PMA and 5 ppm atrazine significantly decreased the budding efficiency. This means that in these groups, fewer buds were produced per ingested shrimp ($P<0.0001$). When cultured in either of these pesticides, normal hydra used approximately 50% more shrimp to produce a bud. PMA also had this effect on albino hydra. The same treatment which exhibited little evidence of lowered budding rates (no treatment, control, dieldrin, toxaphene), ate significantly more shrimp per bud produced for albino animals vs. normals. All other albino groups showed no significant loss in budding efficiency when compared to their normal counterparts (each $P<0.05$). For all treatments where meaningful effects of these chemicals on hydra were noted, the budding rates were reduced.

Table 2. Comparison of the Budding Rates of Normal vs. Albino C. viridissima. Hydra were fed 2 shrimp per day.

Treatment	x_1-x_2	99% Confidence Limits	t	p
No Treatment	0.217	(0.0825,0351)[1/]	5.99	0.00098**
Control	0.220	(0.047,0.39)	4.71	0.0034**
100 ppb Dieldrin	0.178	(0.054,0.30)	5.31	0.0018**
4 ppb Toxaphene	0.230	(0.072,0.39)	5.39	0.0017**
1 ppb PMA	0.109	(-0.064,0.28)	2.34	0.058
20 ppb PCB	0.0678	(-0.099,0.23)	1.51	0.18
5 ppm 2,4,5-T	0.202	(-0.13,0.54)	2.25	0.06
5 ppm Atrazine	-0.0276	(-0.25,0.20)	-0.45	0.67

** $P<0.01$ per comparison
1/ Degrees of Freedom equal six in all cases.

Table 3. Budding Efficiencies (Shrimp/Bud and Bud/Shrimp Ratios)

	Shrimp/Bud[1/]	P(vs. control + no treatment groups combined)	P(normal) vs (albino)
No Treatment			
normal	2.93±0.19	0.714 vs. control	0.0036**
albino	4.12±0.48	0.942 only	
Control			
normal	2.85±0.37	0.0045**	
albino	4.14±0.45		
100 ppb Dieldrin			
normal	3.31±0.37	0.0486*	0.0039**
albino	4.35±0.27	0.372	
4 ppb Toxaphene			
normal	3.00±0.32	0.547	$<10^{-3}$**
albino	4.49±0.31	0.176	
1 ppb PMA			
normal	4.42±0.70	0.000242**	0.0529
albino	5.97±1.09	0.00149**	
20 ppb PCB			
normal	3.71±0.71	0.0146*	0.229
albino	4.25±0.39	0.657	
5 ppm 2,4,5-T			
normal	3.57±1.42	0.205	0.157
albino	5.18±0.91	0.0191*	
5 ppm Atrazine			
normal	4.45±0.79	0.000428**	0.291
albino	3.86±0.61	0.398	

* P<0.01
** P<0.05
1/ Bud to Shrimp ratio is reciprocal of Shrimp to Bud.

In summary, sublethal concentrations of several pesticides (DDT, dieldrin, toxaphene, PMA, 2,4,5-T, atrazine) plus PCB (Aroclor 1254) were tested for their effects on the growth rates, budding rates and budding efficiencies of normal and albino *Chlorohydra viridissima*, the green hydra. On a food regime of two *Artemia* nauplii per day, 1 ppb PMA and 5 ppm atrazine significantly reduced the budding rate of normal *C. viridissima*. When cultured in the presence of either of these pesticides, normal *C. viridissima* used approximately 50% more calories to produce a bud. There was evidence that the expected benefit from the endosymbiotic algae in terms of budding rate was élimi-nated by some of the chemicals tested (20 ppb PCB, 5 ppm 2,4,5-T, 5 ppm atrazone).

Acknowledgements. Supported by the Division of Research, College of Agricultural and Life Sciences, University of Wisconsin, and in part by a research grant from the Sea Grant program, University of Wisconsin.

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Accepted January 3, 1983