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Effects of Fluometuron on Embryonic Development and Hatching of *Fasciola hepatica's* Miracidia

Frederick A. Christian and Twintillia M. Tate

Department of Biological Sciences and the Health Research Center, Southern University, Baton Rouge, LA 70813

Fasciola hepatica (sheep liver fluke) is a common parasite of herbivorous mammals and humans in many parts of the world including the United States (CHANDLER & READ 1961). Its increasing infections in many mammals and man have become one of the most important and serious economic and medical concerns to our urban and agricultural societies. The reason for this increase in Fasciola hepatica infections is not known.

An approach to elucidating the cause of increases in Fasciola hepatica infections is to experimentally determine the effects of commonly used pesticides on the physiological characteristics of non-target organisms as Fasciola hepatica. The pesticide of choice here is fluometuron (1,1-dimethyl-3-(2,2,2-trifluro-m-tolyl)urea (HAYES 1975; WARE 1978) which is a urea herbicide for weed control in cotton and sugarcane. It is known to inhibit the Hill reaction which involves the oxygen evolution site in the photosystem II. The inhibition prevents the formation of ATP and NADPH which are necessary for CO_2 fixation (CRAFT 1979; CORBETT 1974). This pesticide was selected because of its herbicidal effects in plants and their relationship in the life cycle of Fasciola hepatica. The infective stage (metacercariae) encysts on vegatation (plants) and is eaten by the definitive host. Both the intermediate snail host (Lymnea sp.) and the definitive hosts (herbivores and omnivores) utilize plants as their source of food.

The present paper contains results of the effects of varying concentrations (0-100 ppm) of fluometuron on the hatching of Fasciola hepatica's miracidia.

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MATERIALS AND METHODS

Condemned livers were supplied by Roucher's Meat Market in Plaquemine, Louisiana. The flukes were removed from the bile ducts of the liver and placed in 0.85% saline. Each fluke was removed from the saline and placed in distilled water where they were teased for eggs. After removal of the eggs from the uterus they were washed three times in distilled water to remove all debris. A supply of eggs was maintained in the refrigerator at 4°C.

Approximately 100 eggs were transferred to culture dishes with varying concentrations of fluometuron (0-100 ppm). Cultures of eggs in distilled water at pH 7.0 served as controls. Distilled water was used for diluting the pesticide. The cultures of eggs were maintained at 25 ± 2°C. Observation of the embryonic development was carried out daily (GUTTOWA 1976; MOCZON 1978). Records were kept at the time of appearence of visible developmental changes in the eggs and of the onset of hatching. After the period of hatching, calculations were made of the average hatching rate in each culture. Fasciola hepatic'a eggs were exposed for a total of 30 days with 20 days being the most significant period (STPICZYNSKA et al. 1978). The process was continued to see if more hatching would occur over a longer period. The experiments were conducted in 10 replications. Statistical analysis performed on experimental data included: Two-way analysis of variance, correlation (Linear regression) (RUSSELL et al. 1981) and LC_{50} was calculated by probit analysis.

RESULTS AND DISCUSSION

Approximately 100 Fasciola hepatica eggs were exposed to varying concentrations of fluometuron (0-100 ppm). Figure 1. shows the

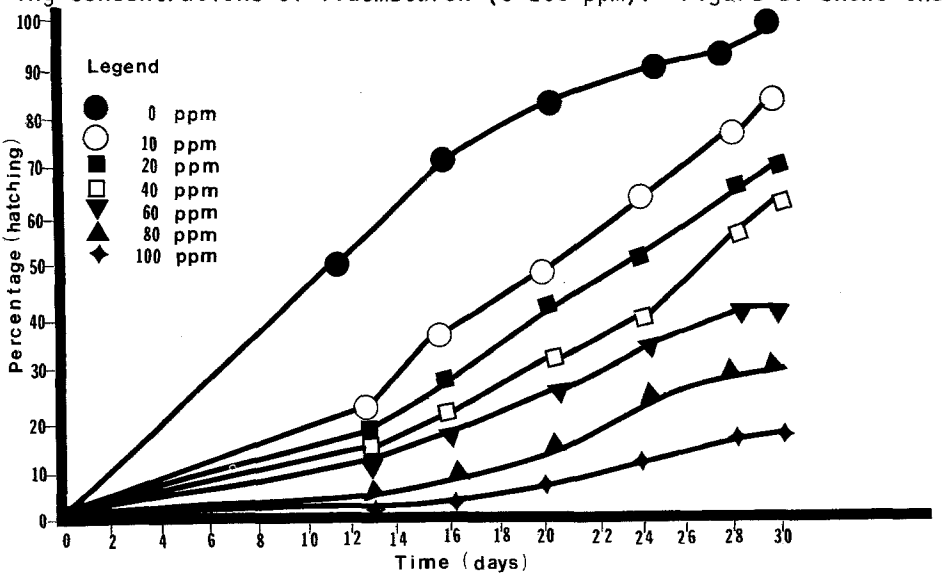

Figure 1. Effects of Fluometuron on Hatching of Fasciola hepatica's miracidia.

effects of fluometuron on hatching (inhibition of hatching) of Fasciola hepatica's miracidia. Hatching decreased with each increase in concentration; but, from 0-40 ppm there is very little inhibition. At 60 ppm and above there is a sharp decrease in the percentage of hatching and continues up to 100 ppm where there is very little hatching occurring. Since, fluometuron shows most of its inhibitory effects at high concentrations 60 ppm and above; it should be used at these concentrations to control hatching of miracidia.

The average number of eggs hatching on day 20 from each concentration was used to determine the relationship between hatching and increasing concentrations in Figure 2. Results show that they were closely related $r=0.79$ and $P<0.01$. For each concentration a

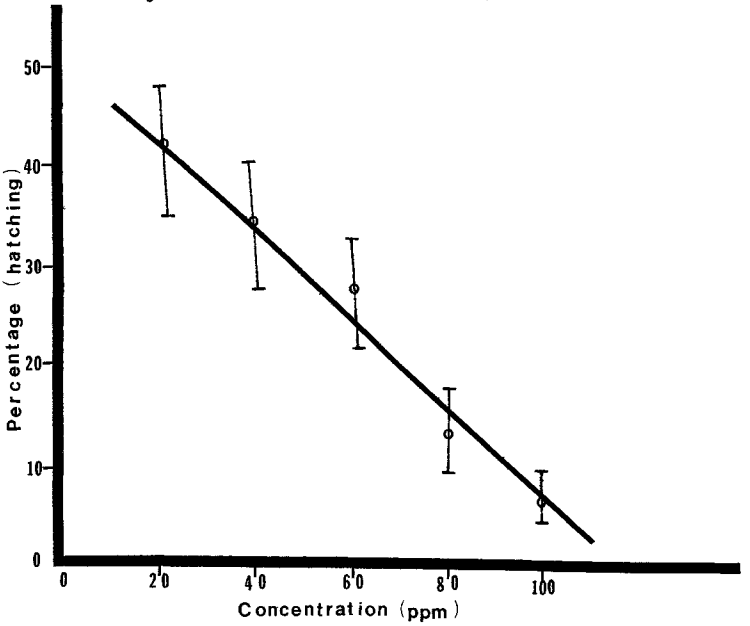

Figure 2. A regression analysis of percent hatching versus the concentration (ppm) at which Fasciola hepatica's eggs was exposed. Standard error is represented by bars (I) $r=0.79$ and $P<0.01$.

standard error of the mean was also plotted. This correlation shows that for each increase in concentration there is a decrease in the percentage of hatching.

The average percentage of Fasciola hepatica's eggs hatching in varying concentrations (0-100 ppm) of fluometuron was shown in Table I. The percentage of hatching on days 13, 16, 20 and 30 was analysed. Each increase in concentration showed a decrease in the percentage of hatching. Statistical analysis performed on these data included two-way analysis of variance which gave the level of significance and correlation (Linear regression) which gave the r-values. Results show that on day 13 the r-value was 0.56 and

was not significant. Day 16, r=0.87; day 20, r=0.79; day 30, r= 0.77. Days 16, 20 and 30 were very significant $P < 0.01$. The LC (I) (Lethal concentration) values were calculated and represented in Table 2. The total incubation time was 30 days. The 95% Fiducial limits were also calculated. The lethal concentration for 25 percent inhibition was 29 ppm; 50% = 57 ppm; 75% = 86 ppm and 100% = 160 ppm. Inhibition increases as concentrations increase.

TABLE 1

AVERAGE PERCENTAGE OF Fasciola hepatica's EGGS HATCHING IN FLUOMETURON

DAYS	CONTROL	CONCENTRATION (PPM)					R-VALUE
		20	40	60	80	100	
0	—	—	—	—	—	—	—
13	33	19	17	9	3	0	$0.56^{n.s.}$
16	69	37	24	17	9	2	0.87**
20	89	43	31	27	17	8	0.79**
30	96	72	67	37	33	17	0.77**

n.s. = not significant

** = $P < 0.01$

TABLE 2

PROBIT ANALYSIS ON DOSE

LC (I) PERCENTAGE PROBABILITY	EXPOSURE TIME (DAYS)	LC (I) VALUE (PPM)	95 PERCENT FIDUCIAL LIMITS	
			LOWER	UPPER
25	30	29	17	38
50	30	57	48	69
75	30	86	73	106
100	30	160	130	200

On the basis of inhibition of hatching, the pesticide fluometuron can be used as a controlling agent for the parasite (Fasciola hepatica) only at high concentrations, 50 ppm and above. To determine the total contribution of this pesticide on the eradication of Fasciola hepatica in the environment, further studies should be done to determine the effects of fluometuron on the intermediate snail host and other developmental stages of Fasciola hepatica.

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