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Toxicity of Fluridone in Algal Bioassays

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As pesticide usage in agricultural practices has increased in recent years, the influence of these chemicals on bacterial and algal activities involved in biological processes is of concern. A number of methods have been described for quantitatively assessing the toxic effects of environmental pollutants on algae (American Public Health Association, 1975; EPA, 1978; Trevors et al., 1983). Recent research has focused on the effect of pesticides on nontarget microorganisms involved in nutrient and mineral cycling, and secondly, on organisms that can serve as biological indicators for assessing toxicants.

In particular, algae have been extensively used as bioassay organisms (Scherer, 1979). Scenedesmus spp. are widely distributed in freshwater and soils (Bold and Wynne 1978), and Anabaena spp. are primarily found in aquatic environments where they are capable of fixing dinitrogen. The use of algal growth rates and/or activity measurements (such as N_2-fixation) are useful in assessing the toxic effect of pollutants on algae. Both batch culture growth and nitrogen fixation measurements are simple to perform. Since growth is a summation of all cellular metabolism, any inhibition of growth reflects toxic effects on a number of metabolic processes. Also, the use of growth rates allows one to observe if the bioassay organism has the capability of recovering from the toxic effect, over extended periods of time. There is the disadvantage that as the algal cells increase in number, the concentration of toxicant per cell decreases from the original value.

Acetylene-reduction (an estimate of N_2-fixation) is also a useful activity to assess the toxic effect of environmental pollutants (Tam and Trevors, 1981). It allows an ecologically important process to be studied for its response to toxicants.

Fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone is a relatively new herbicidal compound active at low concentrations (0.3 to 2.4 kg of active ingredient/ha) (Walrep and

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Taylor 1976). It can be used to control broadleaf weeds and annual grasses, and is reported to be more effective in a preemergent than a postemergent application (Waldrep and Taylor 1976). It has also been used as an aquatic herbicide at low application rates to kill aquatic vascular plants (West et al. 1979). However, very little information is available on the effects of fluridone on bioassay organisms such as algae. In the present study, the potentially inhibitory effects of fluridone on algal growth and nitrogen-fixation are reported.

MATERIALS AND METHODS

Scenedesmus quadricauda was grown in 500 ml Bellco sidearm flasks containing 50 ml of medium composed of: $NaNO_3$, 1.5 g; K_2HPO_4, 0.04 g; $MgSO_4 \cdot 7H_2O$, 0.075 g; $CaCl_2 \cdot 6H_2O$, 0.04 g; citric acid 0.006 g; EDTA, 0.001 g; $NaHCO_3$, 0.02 g; ferric citrate, 0.006 g; 999 ml distilled water, and 1 ml of a trace element solution consisting of $CuSO_4 \cdot 5H_2O$, 19.6 mg; $ZnSO_4 \cdot 7H_2O$, 44 mg; $CoCl_2 \cdot 6H_2O$, 20 mg; $MnCl_2 \cdot 4H_2O$, 36 mg; $Na_2MoO_4 \cdot 2H_2O$, 12.6 mg; H_3BO_3, 618.4 mg; and 1000 ml distilled water. The pH was adjusted to neutrality and the medium sterilized by autoclaving. The flasks were incubated at 22°C with shaking at 180 rpm under cool fluorescent tubes with an 18 h light and 6 h dark cycle. Fluridone (Lily Research Laboratories, Green Field, Indiana) was added as a 50% wettable powder suspended in sterile distilled water. Growth was determined spectrophotometrically by measuring the absorbance at 550 nm. In one series of experiments, the fluridone was added at zero time. In a second series of experiments, the herbicide was added after the algal cultures had been actively growing for 6 days, and reached an absorbance of about 0.10.

Anabaena cylindrica was grown in the liquid medium previously described with the $NaNO_3$ omitted. After 14 days growth, the cells were harvested by centrifugation at 3,000 x g for 10 min at 20°C. The cells were washed once in sterile growth medium and resuspended in 20 ml of the same medium. One ml aliquots of the Anabaena cell suspension were added to 9 ml of sterile N-free medium contained in 50 ml Erlenmeyer flasks. Appropriate concentrations of fluridone were added with a micropipette. The flasks were capped with serum stoppers and pure acetylene added to provide a 10 kPa atmosphere, after an equal volume of the gas phase had been removed. At appropriate intervals, gas samples (0.2 ml) were withdrawn with a gas tight syringe and analyzed for acetylene (C_2H_2) and ethylene (C_2H_4) using gas chromatography as previously described by Tam and Trevors (1981). All acetylene reduction data are the means of duplicate flasks and were corrected for temperature and flask volume.

RESULTS AND DISCUSSION

Fluridone has been reported to be a slow-acting, translocated herbicide that appears to inhibit chlorophyll synthesis (Waldrep and Taylor 1976). At concentrations ranging from 0.5 µg/ml to 10 µg/ml, it completely inhibited the growth of S. quadricauda over a 15-day period (Fig. 1). This suggested that the herbicide was

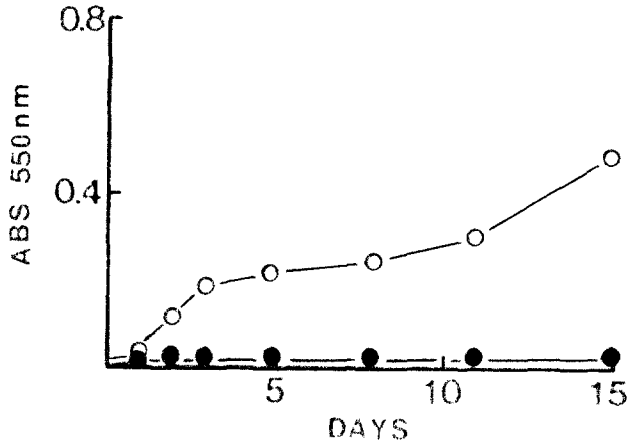

Figure 1. Effect of fluridone on growth of S. quadricauda. Fluridone concentrations were added at zero time. Initial cell number was about 1 x 10^4 cells/ml. Control, no fluridone (○); fluridone added at 0.5, 1, 5, and 10 µg/ml completely inhibited growth (●).

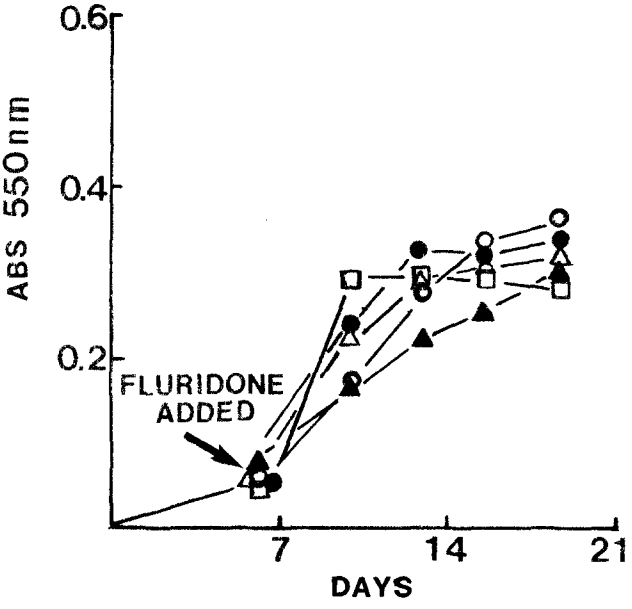

Figure 2. Effect of fluridone on growth of S. quadricauda. Fluridone was added after 6 days of culture growth had occurred. Initial cell number was about 1 x 10^4 cells/ml. Control (○); 0.5 µg/ml (●); 1 µg/ml (△); 5 µg/ml (▲); 10 µg/ml (□).

Table 1. Effect of fluridone on N_2-fixation (acetylene reduction) by A. cylindrica

Fluridone (µg/ml)	Incubation (h)	C_2H_4 evolved[a] (nmoles/flask)	Percent inhibition
Control	4	148.3	Control
0.5		166.0	0
1.0		141.5	4.6
5.0		132.0	11.0
10.0		137.7	7.1
EC_{50} = 70.2 µg/ml, 95% limits = 31.2 - 158.3			
Control	24	758.3	Control
0.5		420.6	44.5
1.0		614.9	18.9
5.0		384.8	49.3
10.0		215.0	71.6
EC_{50} = 3.3 µg/ml, 95% limits = 2.1 - 5.1			
Control	96	7967.5	Control
0.5		8058.1	0
1.0		7273.4	8.7
5.0		3923.4	50.8
10.0		2746.4	65.5
EC_{50} = 5.6 µg/ml, 95% limits = 4.9 - 6.4			

[a] Mean of duplicate experiments

Controls were set at 100% activity (0% inhibition)

EC_{50} (effective concentration inhibiting activity 50%) was estimated using an Apple II plus microcomputer and a probit analysis program based on the method described by Hubert (1980).

highly toxic at relatively low concentrations. Extended incubation of the algal cultures for 15 days did not allow the organism to recover from the initial toxic effect. The same organism was also grown for 6 days in the absence of fluridone until the culture reached an absorbance of 0.10. The cultures were then exposed to the same range of fluridone concentrations (Fig. 2). At concentrations from 0.5 to 10 µg/ml, the fluridone did not display any significant toxic effect on algal growth. Although the toxicant had no marked effect on the actively growing algae (Fig. 2), the cells may bioaccumulate the toxicant and as a result allow its entry into the food chain.

Fluridone toxicity to acetylene reduction activity by A. cylindrica was variable and dependent upon the concentration and period of time after the application. For example, after 4 h, the highest inhibition observed was 11.0% (Table 1). Since fluridone is a slow acting herbicide, the 4-hour period gave variable toxicity results. The EC_{50} was estimated to be 70.2 µg fluridone/ml. After 24 hours, N_2-fixation was inhibited 44.5% by the same treatment. However, at 96 h no inhibition was caused by the 0.5 µg/ml concentration. It would appear that the cells were able to adapt to this relatively low concentration, thus allowing N_2-fixing activity to return to normal levels similar to the control. A similar trend was also displayed to a lesser extent with the 1.0 µg/ml fluridone concentration. In this experiment, the EC_{50} value at 96 h may be a better estimate of toxicity. A good dose-response relationship was observed with increasing concentrations. The EC_{50} value after 96 h was estimated to be 5.6 µg fluridone/ml. These findings also suggested that the period of time between the initial application and the analysis was very important. This may be significant with slow acting toxicants, and bioassay organisms that need a period of time to adapt to the test system.

The present investigation suggests that fluridone may be toxic to algal growth and N_2-fixation at concentrations between 0.5-10 µg/ml. Recovery from the toxic effect was not apparent when S. quadricauda was incubated for an extended perod of time. Also, actively growing cultures of S. quadricauda were relatively insensitive to the herbicide compared to cultures exposed to the toxicant at the beginning of the bioassay.

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