Effects of Fluometuron, Prometryne, Ametryne, and Diuron on Growth of Two *Lemna* Species[1,2]

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INTRODUCTION

Weed control through chemical means has become a standard practice in modern agriculture. Large quantities of herbicides are being applied locally to our ecosystem every year to control weeds. The phytotoxic effect of herbicides, however, often extends beyond the weed species by affecting the life and activities of non-target species. Inadequate and overdose applications of herbicides certainly would cause these chemicals to persist in the terrestrial and aquatic habitats. It is of vital importance, therefore, to know whether any of the widely used, relatively persistent herbicides are apt to cause serious degradation of our environment. Little research has been carried out in Puerto Rico to determine the effect of various herbicides on growth of aquatic non-target species. The only directly related work was that of Fromm (2) with *Lemna minor* L. The lowest lethal concentration for the said species was found to be 5×10^{-3} M for sodium chlorate, 10^{-3} M for ammonium thiocyanate and ammonium sulfamate and 5×10^{-5} M for 2,4-D. Most other studies concerning the effect of herbicides on non-target species were carried out with the green alga *Chlorella pyrenoidosa*. Gramlich and Frans (4) reported that growth inhibition response of *Chlorella pyrenoidosa* induced by naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) were best characterized by a kinetic analysis. The response of *Chlorella* to 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (Atrazine) was more adequately interpreted by a probit analysis. Using the same alga species, Zweig et al. (9) found that the number of cells was severely affected by 2,3-dichloro-1,4-benzoquinone (dichlone) and 2,3,5,6-tetrachloro-1,4-benzoquinone (chloranil) and moderately affected by 2-amino-3-chloro-1,4-naphthoquinone and naphthoquinone at a concentration of 3×10^{-5} M. Diuron and benzoquinone caused only a slight depression in cell number.

The present investigation was conducted with the purpose of determin-

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ing the effects of 1,1-dimethyl-3-(α,α,α-trifluoro-m-toyl) urea (Fluometuron), 2,4-bis(isopropylamino)-6(methylthio)-s-triazine (Prometryne), 2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-s-triazine (Ametryne) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (Diuron) on growth of floating hydrophytes, i. e. *Lemna major* L. (*Spirodela polyrrhiza*) and *Lemna perpusilla* Torr. It is expected that this study will contribute to determine the limiting concentrations of the above-mentioned herbicides that could persist in the environment without adverse effect on growth of specific non-target species.

MATERIALS AND METHODS

Lemna major L. and *Lemna perpusilla* Torr. were chosen as non-target species to determine the effects of Fluometuron,[4] Prometryne,[4] Ametryne,[4] and Diuron[4] on their growth. The former species, a clone indigenous of La Molina, Perú, was obtained from M. A. Tió, Plant Physiologist of this Station. The latter one was collected locally from the Dorado area by Woodbury, also of this Station. All four herbicides were tested at 0, 1×10^{-8}, 1×10^{-7}, 5×10^{-7}, 1×10^{-6}, 1×10^{-5} and 2×10^{-5} M. These concentrations ranged from very toxic (causing 100 percent growth inhibition) to non-toxic (causing no growth inhibition). The two *Lemna* species were grown autotrophically in a modified Wong and Dennis' nutrient solution (8). The composition of the nutrient solution is as follows: KH_2PO_4 2.0×10^{-3}, KNO_3 5.0×10^{-3}, $MgSO_4$ 2.0×10^{-3}, $Ca(NO_3)_2$ 7.0×10^{-3}, $ZnSO_4$ 1.8×10^{-6}, $MnSO_4$ 9.2×10^{-6}, Fe EDTA 38×10^{-6} (as Fe^{+++}), H_3BO_3 46×10^{-6}, Na_2MO_4 4.1×10^{-6}, $CuSO_4$ 3.2×10^{-6}, $CoSO_4$ 3.0×10^{-6}. The pH of the nutrient solution was adjusted to 6.0 with KOH. Continuous illumination of 1100–1200 fc. was provided by two to four fluorescent tubes (Sylvania-40 watt).[4] All experimental cultures were obtained from stock *Lemna* cultures maintained in exponential growth by serially transferring into fresh nutrient solution. The cultures were kept in glass jars covered with petri dishes to prevent deposition of dust and evaporation of moisture. Ten fronds of the *Lemna* species were introduced into individual jars containing 100 ml of nutrient solution. All cultures were kept in a Percival chamber or growth room at 25° C.

In all experiments, the number of fronds of the *Lemna* species were counted at the end of the 2nd, 4th and 7th day of each transfer into fresh medium. However, only the data obtained at the end of 7th day were used

[4] Trade names are used in this publication solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico or an endorsement over other equipment or materials not mentioned.

to calculate the multiplication ratio (M.R.). The multiplication ratios of the *Lemna* species as affected by different concentrations of herbicides were calculated from the following formula (6):

$$\text{M.R.} = \frac{\log \frac{Fd}{Fo}}{d} \times 1000$$

TABLE 1.—*The effect of different herbicides on multiplication ratio of two* Lemna *species*[1]

Concentration (molarity)	Multiplication ratio[2]							
	Lemna major L.				*Lemna perpusilla* Torr.			
	Fluometuron	Prometryne	Ametryne	Diuron	Fluometuron	Prometryne	Ametryne	Diuron
2×10^{-5}	0	0	0	0	0	0	0	0
1×10^{-5}	21.9	0	0	0	31.9	0	0	0
5×10^{-6}	28.2	0	0	0	48.7	0	0	0
1×10^{-6}	55.6	8.9	4.5	0	95.6	5.7	7.0	6.9
5×10^{-7}	—	32.9	27.1	16.3	100.4	21.9	15.6	13.0
1×10^{-7}	63.3	62.7	53.6	50.7	103.7	57.5	53.1	64.4
1×10^{-8}	82.0	81.6	85.2	79.9	112.4	110.7	110.8	115.2

[1] Average of four replications per treatment.

[2] Multiplication ratio of the *major* L. control = 62.6. Multiplication ratio of the *perpusilla* Torr. control = 110.7.

where

Fd = number of fronds at day of counting

Fo = initial number of fronds

d = number of days involved

Herbicide dosage and growth relationship curves of *Lemna* species were constructed by plotting increase in frond number (percent of control) against different concentrations of herbicides. The concentrations of herbicides causing 50 percent inhibition of growth were then obtained directly from the graphs and served as criteria for phytotoxicity evaluations.

RESULTS AND DISCUSSION

The effects of different concentrations of four herbicides on the multiplication ratios of the two Lemna species are shown in table 1. Of the four herbicides tested, Fluometuron was found to be the least toxic to both *L. major* L. and *perpusilla* Torr. This is evidenced by the fairly high concentration of the chemical needed to cause 100 percent growth inhibition

(2×10^{-5} M). On the other hand, Diuron was the herbicide most toxic to *L. major* at a concentration of 1×10^{-6} M. The other two herbicides were almost equally toxic to *L. perpusilla* (table 1).

Figure 1 is graphically presented to illustrate the concentrations of the herbicides causing 50 percent growth inhibition of *L. major* (as percent of control increase in frond number measured 7 days after inoculation). The concentrations required to cause 50 percent growth inhibition of *L. major*

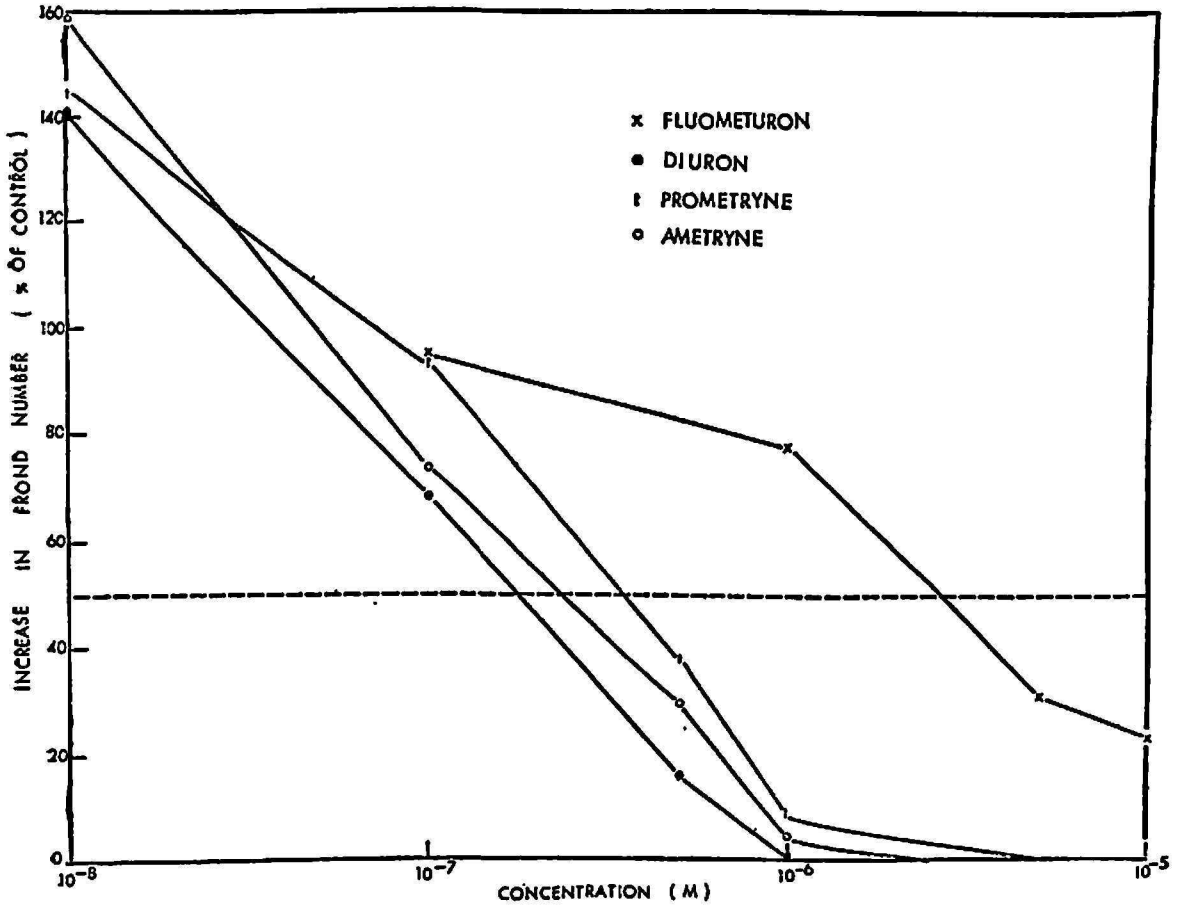

FIG. 1.—Effect of different herbicides on the frond number of *Lemna major*. (Control was 28.68.)

were as follows: Fluometuron, 2.6×10^{-6} M; Prometryne, 3.5×10^{-7} M; Ametryne, 2.30×10^{-7} M; and Diuron, 1.75×10^{-7} M. Figure 2 is similarly presented to demonstrate the concentrations of the four herbicides causing 50 percent growth inhibition of *L. perpusilla*. These concentrations were as follows: Fluometuron, 2.05×10^{-6} M; Diuron, 6.4×10^{-8} M; Prometryne, 5.4×10^{-8} M; and Ametryne, 4.6×10^{-8} M. It is worthy to note that, in the case of *L. major* the Fluometuron concentration causing 50 percent growth inhibition was approximately 10-fold that of the other herbicides. Similarly, the Fluometuron concentration causing 50 percent

growth inhibition of *L. perpusilla* was approximately 25 to 35 times higher than that of the other three herbicides.

The findings that these herbicides at slightly higher concentrations inhibited growth of both *Lemna* species could be attributed to the known effect of triazine and substituted urea herbicides on inhibition of Hill reaction and consequent suppression of growth (1,3,5,7). It is interesting to note from the present study that some growth stimulation as compared to the control occurred at the concentration of 1.0×10^{-8} M of the four

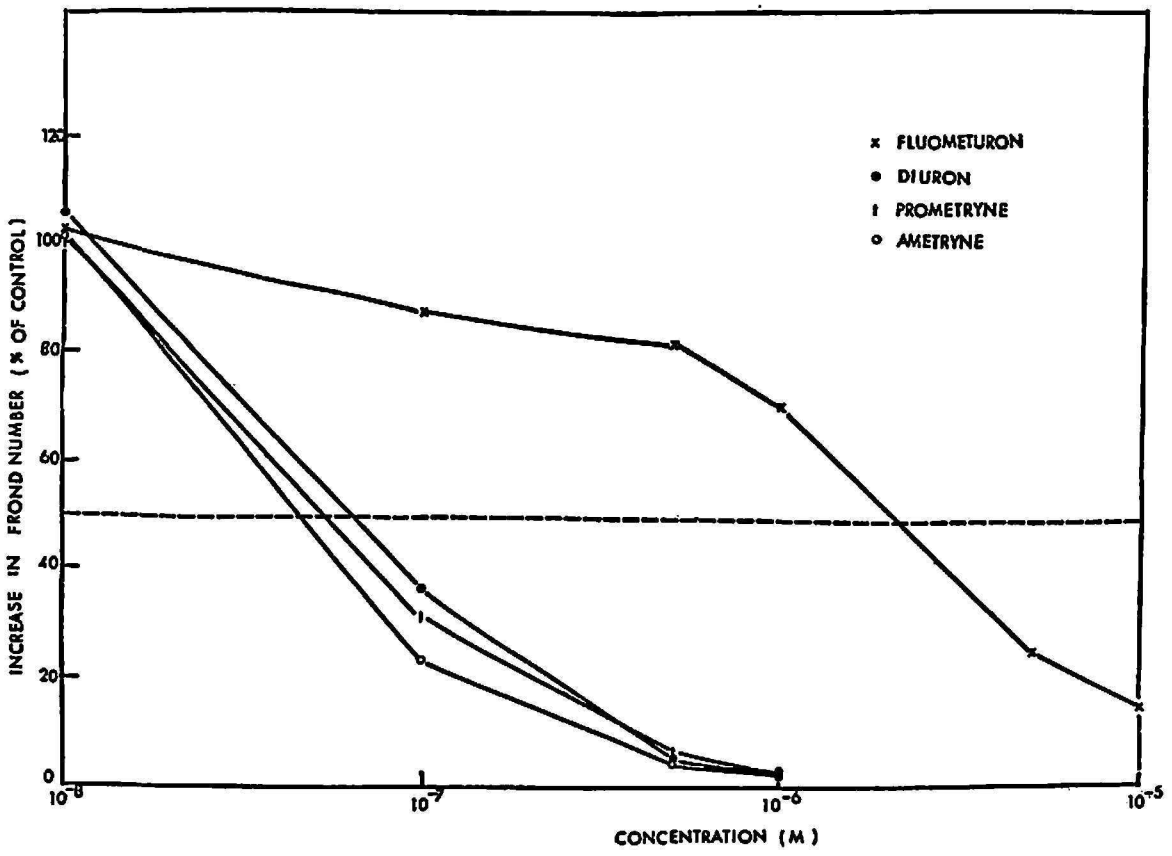

FIG. 2.—Effect of different herbicides on the frond number of *Lemna perpusilla*. (Control was 50.00.)

herbicides. The growth stimulation was particularly pronounced with *L. major* and not as evident for *L. perpusilla*. On the basis of our finding that the four herbidices at micromolar concentration adversely affected the growth of the two *Lemna* species, caution should be taken not to permit their build up in the environment so as to pose potential hazard to aquatic non-target species.

SUMMARY

The effect of the herbicides Fluometuron, Prometryne, Ametryne and Diuron on the autotrophic growth of the aquatic species *Lemna major* L.

and *L. perpusilla* Torr. was determined. Fluometuron was the least toxic chemical to both species. Diuron was the most toxic herbicide to *L. major*, Ametryne most toxic to *L. perpusilla*. Concentrations of herbicides causing 50 percent growth inhibition of *L. major* were as follows: a) Fluometuron, 2.6×10^{-6} M; b) Prometryne, 3.5×10^{-7} M; c) Ametryne, 2.30×10^{-7} M; and d) Diuron, 1.75×10^{-7} M. Concentrations of herbicides causing 50 percent growth inhibition of *L. perpusilla* were as follows: a) Fluometuron, 2.05×10^{-6} M; b) Diuron, 6.4×10^{-8} M; c) Prometryne, 5.4×10^{-8} M; and d) Ametryne, 4.6×10^{-8} M. These herbicides applied at micromolar concentrations proved to be phytotoxic to both *Lemna* species tested.

RESUMEN

Se determinó el efecto de los herbicidas Fluometuron, Prometryne, Ametryne y Diuron sobre el crecimiento autotrófico de las especies acuáticas *Lemna major* L. y *Lemna perpusilla* Torr. El Fluometuron resultó menos tóxico a ambas especies de *Lemna*. El Diuron fué el herbicida más tóxico a *L. major*, mientras Ametryne fué el más tóxico a *L. perpusilla*. Las concentraciones del herbicida requeridas para causar 50 por ciento de inhibición del crecimiento de *L. major* fueron las siguientes: a) Fluometuron, 2.6×10^{-6} M; b) Prometryne, 3.5×10^{-7} M; c) Ametryne, 2.30×10^{-7} M. Las concentraciones necesarias para causar 50 por ciento de inhibición del crecimiento de *L. perpusilla* fueron las siguientes: a) Fluometuron, 2.05×10^{-6} M; b) Diuron, 6.4×10^{-8} M; c) Prometryne, 5.4×10^{-8} M y d) Ametryne, 4.6×10^{-8} M. Se concluyó que la aplicación de estos herbicidas a concentraciones micromolares constituye un riesgo para las dos especies de *Lemna* sometidas a tratamiento.

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