Effect of Two Substituted Urea and Two s-triazine Type Herbicides on the Photosynthesis of *Lemna perpusilla* Torr.[1, 2]

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ABSTRACT

The effect of Prometryne, Ametryne, Diuron, and Fluometuron on the photosynthesis of the aquatic species *Lemna perpusilla* Torr. was investigated using an oxygraph with a Clark-type electrode. All four herbicides produced immediate strong inhibition of photosynthesis when applied to intact plants. Diuron was the strongest inhibitor, causing a 50% reduction of photosynthetic rate at concentrations of 3.5×10^{-7} M. Ametryne and Prometryne were less inhibitory. Fluometuron had the lowest effect on photosynthesis, producing 50% inhibition at 1.6×10^{-5} M. Experiments on long term effect showed that all herbicides tested, except Fluometuron, became severely inhibitory to *Lemna* at concentrations exceeding 1×10^{-6} M. Concentrations of these herbicides in the environment exceeding 1×10^{-6} M could constitute a risk to this and other similarly sensitive species.

INTRODUCTION

Application of herbicides for weed control is one of the modern tools employed to facilitate use and improve productivity of land. Year after year increasing amounts of these chemicals are added to the environment in an effort to control the growth of unwanted vegetation. Some widely used herbicides are fairly persistent in the environment, with the possibility of disturbing the life activities of non-target species. Overdose or inadequate use of these chemicals could result in serious degradation of the environment and unwanted ecological side effects.

In Puerto Rico, although use of herbicides is a standard weed control practice, little research has been done to determine their environmental effects. Some of the most common herbicides used in the Island belong to the substituted ureas and the s-triazines. These two groups of compounds are well known for their effects on photosynthesis. All the s-triazine herbicides are strong inhibitors of the Hill reaction and

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photophosphorylation (7). Substituted urea herbicides, such as Diuron and Fluometuron, are potent inhibitors of the Hill reaction and other photosynthetic reactions (5,6). Although the mechanism of action of these compounds is well known and their effects on specific weed species have been studied extensively, very little information is available on their effect on non-target species, especially those of tropical habitats.

The present paper is part of work conducted with the purpose of determining the effects of herbicides on non-target species. This paper reports the effects of two s-triazines and two substituted ureas on the photosynthesis of *Lemna perpusilla* Torr.

MATERIALS AND METHODS

The floating aquatic plant *Lemna perpusilla* Torr. was chosen as a representative non-target aquatic species and used as experimental material throughout the present studies. *Lemna* was cultured under autotrophic conditions as previously described (3). The four herbicides tested were: 2-(ethylamino)-4-isopropylamino-6-methylthio-s-triazine (Ametryne), 2,4-bis(isopropylamino)-6-(methylthio)-s-triazine (Prometryne), 3,(3,4-dichlorophenyl)-1,1-dimethylurea (Diuron) and 1,1-dimethyl-3-(α,α,α,trifluoro-m-toyl) urea (Fluometuron).

Photosynthesis was assayed by measuring O_2 evolution or uptake with a Clark-type electrode.[4] The apparatus consisted of a 1.75-ml water-jacketed assaying vessel connected to the recording unit of a Gilson Oxygraph Model K-IC. A temperature of 25° C was maintained by circulating water through the water jacket from a constant temperature water bath. The O_2 level in the assay vessel was adjusted as required by bubbling gaseous N_2 through the assay medium in the vessel.

Rate of O_2 evolution or consumption was determined from the slopes of the recorded tracings. Photosynthesis was expressed as micromoles O_2 evolved per milligram of chlorophyll per hour after correcting for dark reactions. Chlorophyll was determined by the method of Arnon (1).

The immediate effect of the herbicides was determinated by adding a solution of the herbicide directly into the assay vessel containing 50 *Lemna* fronds and enough assay medium to make a final volume of 1.75 ml. The composition of the medium was the same as that of the solution used to grow the species. Following addition of the herbicide, photosynthesis was immediately assayed by turning the lights on and off twice for periods of approximately 2 min. In order to compare the photosynthetic rate of control *Lemna* with those receiving different herbicide treatment,

[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.

the photosynthetic rate of control *Lemna* was calculated at 100%, and those receiving herbicide treatments were converted accordingly as shown in figure 1.

Long term effects of the herbicides were studied by adding the appropriate concentration of herbicide solution to *Lemna* cultures maintained in flasks containing 100 ml of growth medium. The cultures were transferred every 2 days to fresh medium containing herbicide and kept under continuous light (400 fc) until assayed the sixth day after treatment. Photosynthesis was measured by removing 50 fronds and 1.75 ml of medium from the culture flasks and assaying as stated for immediate effects.

TABLE 1.—*Immediate effect of different concentrations of the herbicides Fluometuron, Diuron, Ametryne, and Prometryne on the photosynthetic rate of* **Lemna perpusilla** *Torr.*

Concentration (Molar)	Fluometuron		Diuron		Ametryne		Prometryne	
	A[1]	B[2]	A	B	A	B	A	B
0[3]	14.53	100	18.03	100	16.74	100	14.39	100
1×10^{-5} [4]	7.75	54	0	0	0	0	0	0
8.5×10^{-6}	9.08	63	0	0	0	0	0	0
5.7×10^{-6}	14.17	98	0	0	0	0	0	0
2.85×10^{-6}	17.93	123	0	0	0	0	5.24	36
2.50×10^{-6}	14.59	100	0	0	0	0	6.11	43
1×10^{-6}	14.33	99	6.32	35	7.73	46	10.47	73
5×10^{-7}	14.44	99	8.27	46	12.22	73	13.06	91
1×10^{-7}	13.19	91	11.93	66	15.79	94	15.41	107

[1] A = Micromoles of O_2 evolved per milligram of chlorophyll per hour.

[2] B = Percent of control rate.

[3] Values for control are average of eight observations.

[4] Values for the different treatments are the average of four observations.

RESULTS AND DISCUSSION

The immediate effect of different concentrations of the herbicides on the photosynthesis of *L. perpusilla* is shown in table 1. A concentration of 5.7×10^{-6} M of Prometryne and concentrations as low as 2.5×10^{-6} M of Ametryne and Diuron caused complete inhibition of photosynthetic activity of intact *Lemna* plants. This indicates the relatively low concentrations of these herbicides that are capable of interacting very rapidly with the photosynthetic mechanism of the test plant. Ametryne and Prometryne are known to produce rapid contact injury when applied to sensitive species (7). Fluometuron was the least inhibitory of the compounds tested, and concentrations as high as 1×10^{-5} M caused only partial inhibition of photosynthesis in these experiments.

Figure 1 illustrates the concentration of the different herbicides causing 50% inhibition of photosynthesis. If the curves are used as an indication of relative toxicity, then Diuron, causing 50% inhibition at 3.5×10^{-7} M, was the most toxic; and Fluometuron, causing 50% inhibition at 1.6×10^{-5} M, the least toxic of all four herbicides tested. Ametryne and Prometryne were also strong inhibitors of photosynthesis in this species, causing 50% inhibition at 8.4×10^{-7} M and 2.5×10^{-6} M, respectively. If these results are compared with previous findings (3) it is found that for each compound the concentration causing 50% inhibition of growth is lower than that causing 50% inhibition of photosynthesis. In addition, the order of toxicity is different

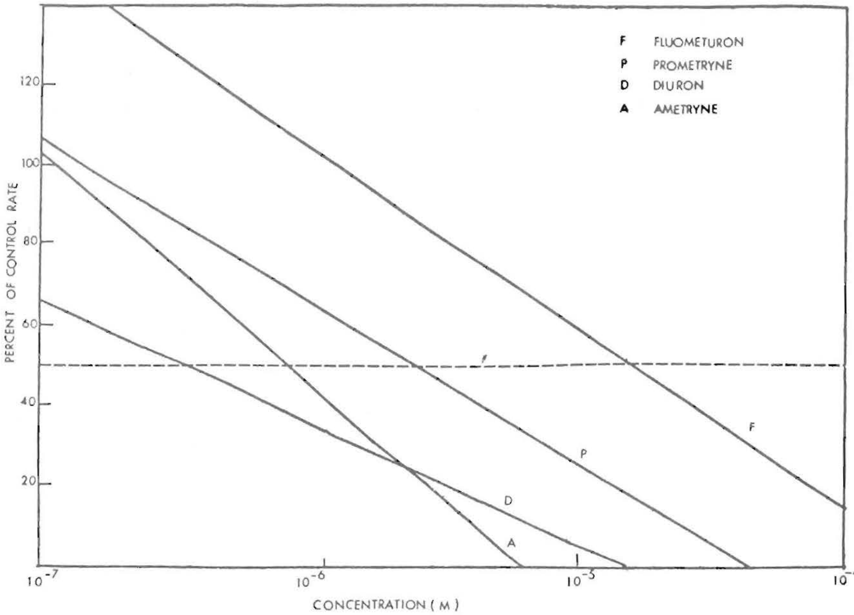

FIG. 1.—Comparative effect of different herbicides on the photosynthetic rate of *Lemna perpusilla* Torr.

for both processes, Ametryne being most inhibitory to growth and Diuron, to photosynthesis. For both processes, however, Diuron and Ametryne are the strongest inhibitors, an indication that inhibition of growth is a reflection of the more direct damage of these herbicides to photosynthesis.

Long term effects of low concentrations of the herbicides are shown in table 2. The results follow the same pattern obtained for the immediate effects, with Diuron and Fluometuron having the greatest and lowest toxicity, respectively. At concentrations exceeding 5×10^{-6} M, all herbicides except Fluometuron were strongly inhibitory. However, in these experiments a concentration of 1×10^{-6} M of diuron, the strongest photosynthetic inhibitor tested, did not inhibit completely the photosynthetic activity of *Lemna* after 6 days of exposure. In previous experiments the growth of *Lemna* was not inhibited completely under similar conditions (3). It is clear from these experiments that even with the most toxic herbicides tested, concentrations exceeding 1×10^{-6} M have to be employed in order to completely inhibit photosynthesis and growth in this species.

It is interesting to note that sub-lethal herbicide concentrations, e.g. 1×10^{-8} M, of all four chemicals tested resulted in increased rates of photosynthesis. Increased growth rates at these concentrations were noted in an earlier work (3). Other studies have shown increases in enzyme activity and crop productivity after treatment with sub-lethal concentrations of s-triazines (2,4).

TABLE 2.—*Long term effect of different concentrations of the herbicides Fluometuron, Diuron, Ametryne, and Prometryne on the photosynthetic rate of* **Lemna perpusilla** *Torr.*[1]

Concentration (Molar)	Fluometuron		Diuron		Ametryne		Prometryne	
	A[2]	B[3]	A	B	A	B	A	B
0[4]	11.79	100	13.25	100	11.57	100	12.64	100
5×10^{-5} [5]	10.61	90	0	0	.25	2	3.66	29
1×10^{-6}	14.98	127	.96	7	4.97	43	7.96	63
5×10^{-7}	12.81	109	6.16	47	8.42	73	10.87	86
1×10^{-7}	16.07	136	13.19	99	9.83	85	12.33	98
1×10^{-8}	15.00	127	14.17	107	14.60	126	13.90	110

[1] The long term effect was determined 6 days after treatment with the herbicides.
[2] A = Micromoles of O_2 evolved per milligram of chlorophyll per hour.
[3] B = Percent of control rate.
[4] Values for control are average of eight observations.
[5] Values for treatments are average of four observations.

These data and previous results show that concentrations of Prometryne, Ametryne, and Diuron exceeding 1×10^{-6} M are severely toxic to *Lemna*, a representative aquatic non-target species. Toxicity is, at least in part, the result of a direct inhibition of photosynthesis. Accumulation of these compounds at micromolar concentrations in sites away from their point of application could result in detrimental effects to the life activities of susceptible non-target species.

RESUMEN

Se investigó el efecto de los herbicidas Prometryne, Ametryne, Diuron y Fluometuron a varias concentraciones sobre el proceso fotosintético de la especie acuática *Lemna perpusilla* Torr., como parte de un estudio para determinar el efecto de estas substancias sobre especies que medran fuera del área tratada y que indirecta o accidentalmente fuesen afectadas par los herbicidas. Los cuatro herbicidas usados causaron una fuerte inhibición fotosintética inmediatamente después de ser aplicados a plantas intactas de *Lemna*. El

Diuron fue el mayor inhibidor, causando a concentraciones de 3.5×10^{-7} M, un 50% de reducción en la tasa de fotosíntesis. El Fluometuron tuvo el menor efecto sobre la fotosíntesis, al causar un 50% de inhibición a 1.6×10^{-5} M. Los experimentos sobre los efectos a largo plazo demonstraron que todos los herbicidas usados, exceptuando el Fluometuron, causan una toxicidad severa a concentraciones mayores de 1×10^{-6} M. Concentraciones en exceso de 1×10^{-6} M de estos herbicidas en el ambiente podrían constituir un riesgo tanto para ésta como para otras especies susceptibles.

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