Research Note

A BIOASSAY METHOD FOR DETECTING HERBICIDE CONCENTRATIONS IN WATER[1]

Herbicides are widely used for weed control on farms in Puerto Rico. Because of the rising cost of labor and diminishing mechanical cultivation as a result of fuel shortage, Puerto Rican farmers may have to increase herbicide usage. Moreover, the varied and steep topographic conditions in the humid mountain region of our farm lands are likely to aggravate herbicide runoff into lowlands and water supply areas. In a recent investigation[2] one of the authors detected low concentrations of Diuron [3 (3,4-dichlorophenyl) 1,1-dimethylurea] present in the leachate under lysimeter conditions. This finding suggests that herbicide contamination of water may be occurring in Puerto Rico. To detect adverse levels of herbicide contamination, a constant surveillance program is imperative.

Chemical analysis has long been regarded as the standard methodology to carry out a sound monitoring project. However, this methodology is rather tedious, and requires highly trained personnel and expensive equipment. Bioassay, on the other hand, is simple and easy to perform. Furthermore, bioassay is useful for the quantitative determination of herbicide runoff from experimental plots treated with a known herbicide, to see how much of the herbicide is carried off by water. In a previous study,[3] the high sensitivity of a duckweed (*Lemna perpusilla* Torr.) to low concentration of four herbicides was noted. The lower limit of detection and increased range of detectable concentration of these herbicides have not been established. The present investigation represents an endeavor toward this end in developing a bioassay method for a number of commonly used herbicides; namely; 1,1-dimethyl (α,α,α, -trifluoro-m-tolyl) urea (Fluometuron); 2,4 bis (isopropylamino) -6-(methylthio)-s-triazine (Prometryn); monosodium methanearsonate (MSMA); 2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-s- triazine (Ametryn); 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine (Atrazine); and 3, (3,4-dichlorophenyl)-1,1-dimethylurea (Diuron).

Duckweed collected from the Dorado area was used as the indicator plant. Ametryn, Prometryn, Atrazine and Diuron were tested at 0, 1.95×10^{-3}, 3.90×10^{-3}, 7.80×10^{-3}, 1.56×10^{-2}, 3.13×10^{-2}, 6.25×10^{-2}, 1.25

[1] Manuscript submitted to editorial Board February 13, 1978.

[2] Liu, L. C., Leaching of Fluometuron and Diuron in a Vega Alta soil, J. Agri. Univ. P.R. 58(4): 473–82, 1974.

[3] Liu, L. C. and Cedeño Maldonado, A., Effect of Fluometuron Prometryn, Ametryne and Diuron on growth of two *Lemna* species, J. Agri. Univ. P.R. 58(4): 483–8, 1974.

$\times 10^{-1}$, 2.50×10^{-1}, 5.0×10^{-1} and 1.00 p/m; Fluometuron was tested at 3.90×10^{-3}, 7.80×10^{-3}, 1.56×10^{-2}, 3.13×10^{-2}, 6.25×10^{-2}, 1.25×10^{-1}, 2.50×10^{-1}, 5.00×10^{-1}, 1.00 and 2.00 p/m; MSMA was tested at 1.56×10^{-2}, 3.13×10^{-2}, 6.25×10^{-2}, 1.25×10^{-1}, 2.50×10^{-1}, 5.0×10^{-1}, 1.00, 2.00, 5.00 and 1.00×10.00 p/m. Duckweed was grown autotrophically in a modified Wong and Dennis nutrient solution.[4] The composition of the nutrient solution is as follows: KH_2PO_4 2.0×10^{-3}, KNO_3 5×10^{-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} and $CoSO_4$ 3.0×10^{-6} M. The pH of the nutrient solution was

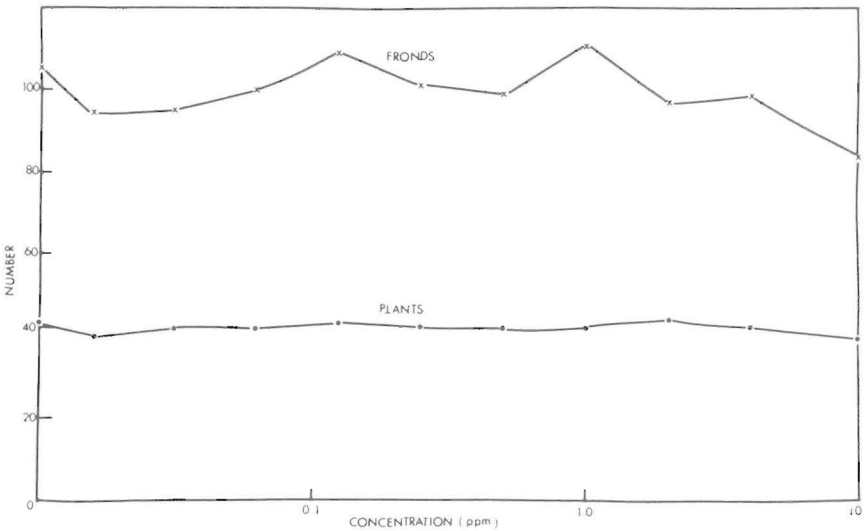

FIG. 1.—Effect of different concentration levels of MSMA on the number of fronds and plants of duckweed.

adjusted to 5 with KOH. Continuous illumination of 4.3 Klux was provided by fluorescent light. All experimental cultures were obtained from a *Lemna* stock culture maintained in exponential growth by serial transfers into fresh nutrient solution. The culture was kept in glass jars covered with petri dishes to prevent deposition of dust and evaporation. Five 3-frond duckweeds were introduced into jars containing 100 ml of different concentrations of the herbicide solution. All cultures were kept in an air-conditioned laboratory at 25 C. Each treatment was replicated four times.

The plant number and frond numbers were counted at the end of 3rd, 5th and 7th day after transfer to the medium containing the herbicide.

[4] Wong, K. F. and Dennis, D. T., Aspartokinase in *Lemna minor* L., Plant Physiol. 51: 327–31, 1973.

For the lower concentrations of herbicides, effects on the duckweed count became evident on the seventh day. Therefore, only the data obtained at the end of 7th day were used as criteria for constructing standard bioassay curves. The standard bioassay curve of each herbicide was constructed by the plotting of the number of plants and fronds of duckweed against different concentrations of the herbicide.

Of the six herbicides tested, MSMA was least phytotoxic to duckweed. This phenomenon is shown by failure of the increasing MSMA concentration in reducing the number of plants and fronds of duckweed (fig. 1). The highest MSMA concentration of 10 p/m caused only a slight reduction in plant and frond counts. On the other hand, Ametryn and Diuron

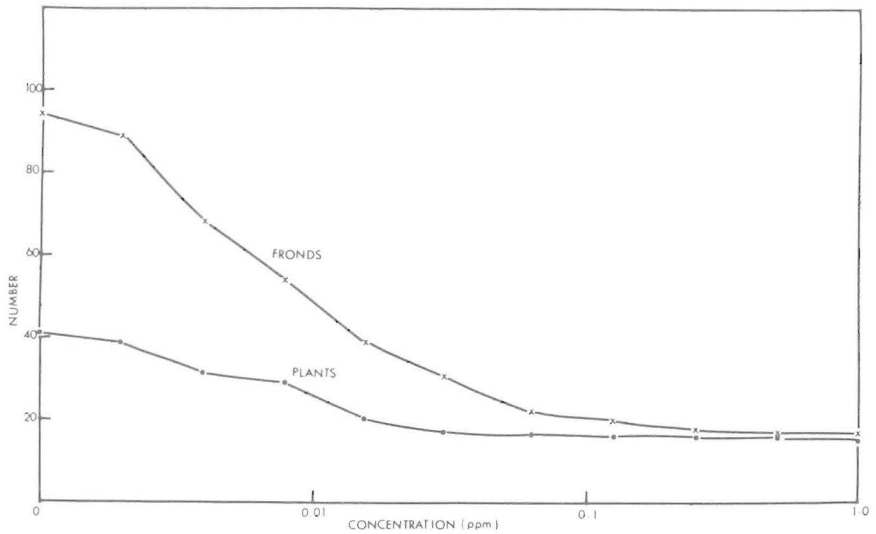

FIG. 2.—Effect of different concentration levels of Ametryn on the number of fronds and plants of duckweed.

were found to be highly toxic to duckweed. Both herbicides, at the lowest concentration tested (1.95×10^{-3} p/m), produced inhibitory effects on the number of plants and fronds. A representative standard bioassay curve of Ametryn is presented in figure 2. The lower limit of detection and the increased range of detectable concentration of Ametryn were from 1.95×10^{-3} to 6.25×10^{-2} p/m, when the frond count of duckweed was used as a criterion. However, the lower limit of detection and increased range of detectable concentration of Ametryn were further narrowed down from 1.95×10^{-3} to 3.13×10^{-2} p/m when the plant count was used for evaluation. The lower limit and increased detectable concentration of the other four herbicides were similarly obtained (table 1).

TABLE 1.—*The lower limit of detection and increased range of detectable concentration of five herbicides using duckweed as a bioassay indicator*

Herbicides	Concentration range in frond count	Concentration range in plant count
	P/m	*P/m*
Ametryn	$1.95 \times 10^{-3} - 6.25 \times 10^{-2}$	$1.95 \times 10^{-3} - 3.13 \times 10^{-2}$
Atrazine	$7.80 \times 10^{-3} - 1.00$	$3.13 \times 10^{-2} - 2.50 \times 10^{-1}$
Diuron	$1.95 \times 10^{-3} - 6.25 \times 10^{-2}$	$1.95 \times 10^{-3} - 3.13 \times 10^{-2}$
Fluometuron	$6.25 \times 10^{-2} - 2.00$	$1.25 \times 10^{-1} - 1.00$
Prometryn	$3.90 \times 10^{-3} - 1.25 \times 10^{-1}$	$1.25 \times 10^{-1} - 1.00$

In general, the frond count of duckweed appeared to be a better criterion than plant count for detecting lower limits of detection and increasing the range of detectable concentrations of the five herbicides. The extreme sensitivity of duckweed to the five herbicides tested suggests this bioassay method may be used to indicate whether the water collected in some farm areas contains any phytotoxic herbicide, whose concentration may be determined further by chemical method. Additional use of the bioassay method may be developed for synergism among herbicides themselves and between herbicides and other pesticides.

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