Research Note

EFFECT OF PHOTOSYNTHETIC INHIBITOR HERBICIDES ON NITRATE REDUCTASE ACTIVITY OF NON-TARGET SPECIES[1, 2]

Some of the most common chemicals used in Puerto Rico for weed control belong to two of the major classes of herbicides, namely the substituted ureas and the s-triazines. These compounds are well known photosynthetic inhibitors, deleterious to the growth of both target and non-target species. Recently, Klepper[3] reported that these photosynthetic inhibitors are also harmful to plants by blocking some of the steps involved in the reduction and assimilation of nitrate. However, the extent and mechanism of damage to the nitrate reduction processes have not been fully clarified. Since nitrate is one of the most important sources of nitrogen to plants under most environments, it is expected that any chemical which impairs nitrate reduction represents a hazard to the life activities of plant communities.

One of the key enzymes involved in nitrate reduction in plants is nitrate reductase. Present in the cytoplasm, this enzyme catalyses the reduction of nitrate to nitrite through reactions involving the reducing power of the cell. Because of the key role of nitrate reductase, any inhibition of this enzyme by chemicals present in the environment, such as herbicides, could produce detrimental effects on the ecosystem.

The present investigation was conducted for the purpose of determining the effects of some widely used photosynthetic inhibitor herbicides on the activity of nitrate reductase in *Lemna major* L. (*Spirodela polyrrhiza*), a representative non-target species. The plant material was grown under autotrophic conditions as previously described.[4] The 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-tolyl) urea (Fluometuron). The treatment consisted of inoculating *Lemna* plants in nutrient solution containing different concentrations of herbicides. Each herbicide concentration was replicated four times. Two days after inoculation a sample of 20 fronds (leaves) was removed from the herbicide solution and assayed. Nitrate

[1] Manuscript submitted to Editorial Board May 9, 1978.

[2] This investigation was conducted with the cooperation of the Puerto Rico Nuclear Center, Mayagüez, P.R.

[3] Klepper, L. A., Inhibition of nitrate reduction by photosynthetic inhibitors, Weed Science, 23:188–190, 1975.

[4] Liu, L. C. and Cedeño-Maldonado, A., Effects of Fluometuron, Prometryne, Ametryne, and Diuron on growth of two *Lemna* species. J. Agri. Univ. P. R. 58:483–488, 1974.

reductase activity was assayed in vivo by a combination of the methods of Harper and Hageman[5] and Shen.[6] The assay solution contained 10 μM potassium phosphate buffer, pH 7.5, 0.2 M KNO_3. Five ml of this solution in 15 ml test tubes were used for each sample. The *Lemna* fronds, after being punctured three of four times with a syringe needle to facilitate infiltration, were submerged in test tubes containing the assay solution and infiltrated under vacuum. The infiltrated samples were incubated for 1 hour at 30° C in a shaking water bath in darkness. The reaction was terminated by the addition of 1 ml of 1% sulphanilamide in 1.5 *N* HCl. Five minutes later, 1 ml of 0.02% N-(1-naphtyl)-ethylenediamine-dihydrochloride in water was added to each sample. The samples were immediately shaken, and after 10 min color development the optical density of the samples at 540 nm was recorded. Results obtained are summarized in table 1.

TABLE 1.—*Effect of herbicides on nitrate reductase activity in* Lemna major *L.*

Herbicide molar concentration	Activity as per cent of control rate[1]			
	Ametryne	Diuron	Fluometuron	Prometryne
	%	%	%	%
0	100	100	100	100
1×10^{-9}	101	98	102	100
1×10^{-8}	143	123	139	151
5×10^{-7}	87	127	216	98
7.5×10^{-7}	100	90	148	105
1×10^{-6}	71	88	124	102
2.5×10^{-6}	65	81	133	87
5×10^{-6}	62	67	110	75
1×10^{-5}	35	53	92	68

[1] Control rate: 6.025×10^{-3} micromoles of nitrate reduced per gram of fresh weight.

For each of the herbicides there was an optimum concentration at which its influence on nitrate reductase activity was maximum. At concentrations below or above the optimum, this activity was reduced. Fluo-meturon and prometryne, the herbicides producing the highest increases in enzymatic activity, were the least inhibitory at high concentrations. Ametryne, causing 65% inhibition of enzyme activity at 10^{-5}M, was the strongest inhibitor tested. Although the effects of the herbicides were not related to the chemical nature of the compounds, the concentrations at which effects occurred were. The triazines showed maximum increase of enzyme activity at 10^{-8}M, whereas the ureas were most

[5] Harper, J. E. and Hageman, R. H., Canopy and seasonal profiles of nitrate reductase in soybeans (*Glycine maxima* L. Merr.). Plant Physiol. 49:146–154, 1972.

[6] Shen, T. C., The induction of nitrate reductase and the preferential assimilation of ammonium in germinating rice. Plant Physiol. 44:1650–1655, 1967.

effective at 5×10^{-7}M. Since the concentrations of these herbicides causing significant inhibition in nitrate reduction are much higher than those causing similar inhibition of growth and photosynthesis,[3,7] it is unlikely that inhibition of nitrate reduction is a primary mechanism of toxicity of these compounds. However, the great increase in nitrate reductase activity produced at very low concentrations could have serious ecological implications. In earlier studies, similar concentrations of these chemicals brought about significant increases in the growth and photosynthesis of *Lemna major* and similar species.[3,7] It is possible that a similar situation occurring in a natural environment would bring about an excessive growth of some species detrimental to the ecosytem. This could happen particularly in water habitats, where herbicides are most likely to accumulate and where species that multiply profusely, such as algae and duckweeds, exist.

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[7] Cedeño Maldonado, A. and Liu, L. C., The effect of two substituted urea and two s-triazine type herbicides on the photosynthesis of *Lemna perpusilla* Torr., J. Agri. Univ. P. R. 60:369–374, 1976.