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Interactions between Trichlorfon and Three *Chlorophyceae*

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Trichlorfon (dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate) is one of the most popular organophosphorus insecticides in Spain and is used at very high concentrations by some farmers in an attempt to eliminate the red American crayfish (Repetto et al. 1988) that was introduced into the marshes of the Guadalquivir river and into the inland marine lake of Valencia and whose great expansion and excessive proliferation causes severe damage to the neighboring ricefields. Having in mind the ecological importance of these Spanish wetlands as stop-over areas in the Euro-African migrant birds currents, an investigation is being conducted to evaluate the potential risk of this insecticide on the freshwater microalgae, the main source of reduced C and N in aquatic ecosystems.

Previous works showed that trichlorfon treatment alters growth, cell composition, ultrastructure and physiological processes in a representative group of cyanobacteria, and that these effects are dose dependent and related to a primary mechanism of inhibition of nitrate uptake (Marco et al. 1990) or to the inhibition of N_2 fixation, when grown in N_2-fixing conditions (Orús et al. 1990). These prokaryotic algae do not contribute at all to the removal of the insecticide from the medium (E. Marco, personal communication). In this paper we report the response of three of the most representative genera of freshwater Chlorophyceae to trichlorfon, as well as its role in the removal of the insecticide from the medium.

MATERIALS AND METHODS

Axenic batch cultures of Chlorella vulgaris UAM 101 (from the Autónoma University of Madrid culture

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collection), Chlamydomonas reinhardtii SAG 11-32b and Scenedesmusquadricauda UAM 103 were grown in Tacker and Syrett medium (1972) under 150 $\mu E\ m^{-2}\ s^{-1}$ continuous illumination at 26°C. Trichlorfon, technical grade 97%, was obtained from the Spanish Customs Office (Madrid) and added to the culture medium to the final concentration of 25, 50 and 100 mg L^{-1}.

To evaluate the removal of this insecticide, 200 mL of culture medium in 0.5 L flasks were inoculated to give an initial algal concentration of 50 μg dry weight mL^{-1} and treated with 100 mg L^{-1} of trichlorfon. Control flasks without cells were prepared and kept under the same conditions and used to determine the spontaneous degradation of the insecticide. At 0 time and at regular intervals of 24 hr, 10 mL samples were removed from the control and from the culture flasks and algae were pelleted by centrifugation. The insecticide of the media was recovered with dichloromethane and transferred to ethyl ether, being 89% the percentage of recovery. This extract was used for gas chromatographic analysis, according to Anderson et al. (1966), using a Shimadzu GC-8A chromatograph equipped with a 16% GEXE 60 Cromosorb WAW 80/100 mesh column (6.35 mm I.D., 1.7 m) and a flame ionization detector. Column temperature was 80°C and detector temperature was 270°C. The retention time for trichlorfon was 3.9 min.

Growth was measured by optical density at 750 nm, in an Hitachi 150-20 spectrophotometer, and expressed as dry weight by using the corresponding dilution curves. Proteins were extracted with 1 N NaOH at 80°C for 1 h and determined according to Lowry et al. (1951). Nucleic acids were acid extracted with 0.5 N perchloric acid at 70°C for 1 h and estimated according to Ogur and Rosen (1950). Chlorophylls were extracted with 90% methanol at 90°C for 3 min and estimated spectrophotometrically (Holden 1966). Photosynthesis was estimated as the rate of O_2 evolution of 3 mL aliquots of cell suspensions in a Hansatech CB1 Clark-type oxygen electrode under 300 $\mu E\ m^{-2}\ s^{-1}$ white light. To determine the respiratory rate the O_2 uptake in darkness was recorded with the same equipment.

Data in figures and tables are the means and standard deviations from at least three independent experiments with duplicate cultures and duplicate samples within each individual experiment. The statistical significance of the data was estimated by means of a Student's t test for $p < 0.05$.

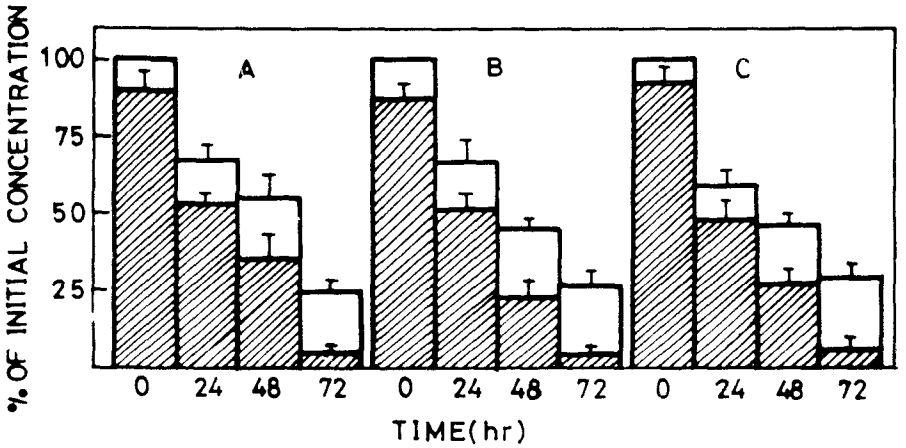

Figure 1. Dissapearance of trichlorfon from culture media without (total height of the bars) and with algae (ruled bars inserted in the blank ones). Data represented as percentage ± SD of the initial concentration of insecticide in cell-free media (100 mg L^{-1}) (A) Chlorella vulgaris (B) Chlamydomonas reinhardtii (C) Scenedesmus quadricauda.

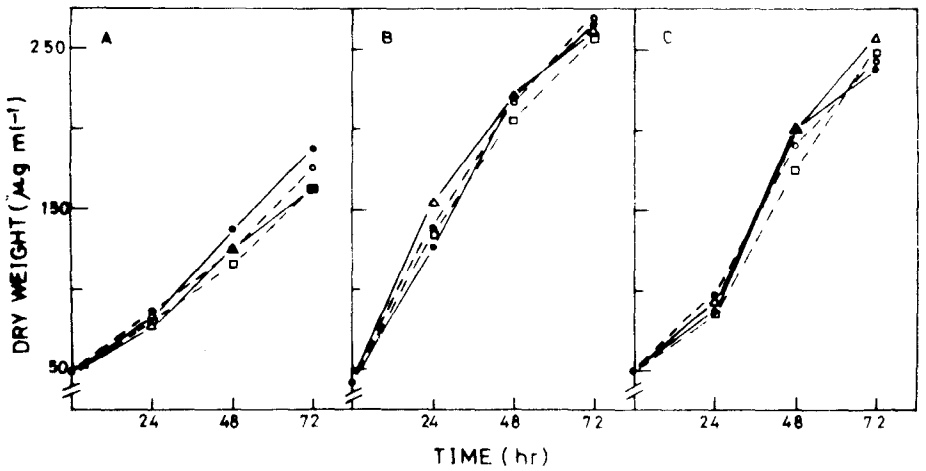

Figure 2. Growth of (A) Chlorella vulgaris (B) Chlamydomonas reinhardtii (C) Scenedesmus quadricauda in culture media without and with trichlorfon. (o) control, (●) 25 mg L^{-1}, (△) 50 mg L^{-1}, (□) 100 mg L^{-1}. Statistical analysis with Student's t test indicated no significant differences at any time of growth

RESULTS AND DISCUSSION

Figure 1 shows that the three green algae assayed contribute to the removal of trichlorfon from the medium. It also shows the rapid spontaneous degradation of the insecticide that had already been reported under different experimental conditions and will not be discussed here because our objective was the role of the algae. A considerable amount of the insecticide --approximately a 10%-- is taken up by the algae at the same time they get in contact. This suggests that this removal depends mainly on a passive mechanism of sortion to the cell surfaces, that coincides with the explanation proposed by Weinberger et al. (1983) for fenithrotion, by Lohman and Hagedorn (1985) for parathion and by Rao and Lal (1987) for malathion, every group of investigators working with different algae. It is remarkable that the three algae asssayed take up the insecticide in a comparable way. These results contrast with the fact that any of the six different cyanobacteria assayed were able to remove any trichlorfon from the medium (E. Marco, personal communication). This difference between the two algal groups can probably be due to the different structure of their cell walls. In fact, the work of Lohman and Hagedorn (1985) demonstrated that the pattern of uptake of parathion by several algae depended precisely on their specific wall layers. Measurements made at 168 hr (data not shown) indicated that approximately 10% of the initial concentration of trichlorfon was present in the cell-free control flasks but insecticide was not detectable in any of the media where algae had been grown. This is a fact of environmental significance since it indicates that some of the most aboundant genera of the freshwater phytoplankton can contribute to the detoxification of their aquatic media.

The study of the toxicity of trichlorfon indicates that the three Chlorophyceae assayed are quite resistant to this pesticide. It did not affect their growth, as shown in Figure 2, in spite of using concentrations of insecticide far higher than those occurring under natural conditions. Neither was growth affected in experiments conducted with 300 mg L^{-1} and 144 hr of exposure. However, species of Chlorella (Ukeless 1962), Chlamydomonas (Cain and Cain 1984) and Scenedesmus (Ordog 1979) different to those used in this work have been reported to be sensitive to concentrations lower than 100 mg L^{-1}, although complementary experiments to understand the mode of action of the insecticide on such organisms were not presented in these papers. The only studies of which we are aware which have examined the effect of trichlorfon on cyanobacteria are our own recent works

Table 1. Concentration of the major nitrogenous compounds after 72 h of growth without/with trichlorfon*

ALGA	Insecticide mg L^{-1}	Protein	Nucleic acid	Chlorophyll
		$\mu g\ mg^{-1}$ dry weight of algae		
Chlorella vulgaris	0	427±38	66.3±4.2	28.2±2.5
	25	475±41	69.1±3.7	31.5±1.7
	50	472±52	70.0±5.3	32.0±2.1
	100	514±36**	76.3±5.5**	30.9±3.3
Chlamydomonas reinhardtii	0	612±48	81.9±6.7	42.2±3.6
	25	602±36	86.2±2.8	47.3±4.2
	50	596±24	83.3±5.2	44.4±3.1
	100	607±42	90.0±5.9	44.6±2.8
Scenedesmus quadricauda	0	579±56	103.2±6.5	57.1±4.2
	25	599±42	100.4±4.2	56.6±3.3
	50	567±38	93.0±8.5	52.2±4.0
	100	579±45	88.2±4.9**	48.0±3.1**

Table 2. Photosynthesis and respiration (evaluated as O_2 exchange) after 72 h of growth without/with trichlorfon*. Rates are based on dry weight (dw) of algae.

ALGA	Insecticide mg L^{-1}	Photosynthesis	Respiration
		μmol O_2 evolv/consum mg^{-1} dw h^{-1}	
Chlorella vulgaris	0	2.04±0.22	1.26±0.14
	25	2.46±0.31	1.44±0.12
	50	2.22±0.25	1.26±0.09
	100	2.22±0.18	1.48±0.15
Chlamydomonas reinhardtii	0	6.06±0.50	2.04±0.19
	25	5.34±0.46	1.92±0.08
	50	6.06±0.37	2.04±0.06
	100	5.28±0.61	2.04±0.22
Scenedesmus quadricauda	0	4.86±0.38	2.10±0.07
	25	3.66±0.37**	1.98±0.16
	50	3.48±0.25**	2.10±0.20
	100	3.12±0.33**	1.98±0.12

* Data are the means and SD of three independent experiments with duplicate cultures and duplicate samples

**Significant reduction or stimulation over control ($P<0.05$)

with Anabaena PCC 7119 (Marco et al. 1990; Orús et al. 1990) and with a selected group of other cyanobacteria (unpublished results), that have shown that the sensitivity of this group of microalgae to trichlorfon is due to a primary inhibition of nitrate uptake and of nitrogen fixation. In order to check whether or not trichlorfon induced the same kind of alteration in green algae, we examined the cellular content of the main nitrogenous compounds (Table 1). These results discard a negative effect of the insecticide on nitrate uptake since no decrease of proteins (the major nitrogenous fraction) appeared in any case. Nevertheless, a slight decrease on the nucleic acids and chlorophylls content was recorded in Scenedesmus quadricauda grown with the highest concentration of insecticide tested (100 mg L^{-1})

Many organophosphorus insecticides have been reported to affect photosynthesis of algae (Lal and Shivaji 1984). To our knowledge, the effect of trichlorfon on algal photosynthesis had not been previously investigated by other authors. Our results indicate that photosynthetic O_2 evolution of Scenedesmus quadricauda is affected from the lowest concentration of insecticide assayed (25 mg L^{-1}) but is unaltered in Chlorella vulgaris and Chlamydomonas reinhardtii, even at 100 mg L^{-1}. This is once more indicative of the high degree of tolerance of these green algae to trichlorfon, since photosyntyhesis is the physiological process the most sensitive to different toxicants. The respiratory rate --that is usually increased under conditions of stress-- was not affected either by trichlorfon treatment.

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