Bull. Environ. Contam. Toxicol. (1992) 49:750–756

Environmental Contamination and Toxicology

Effect of the Herbicide Diquat on the Growth of Microalgae and Cyanobacteria

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Diquat is a contact herbicide widely used for the control of aquatic plants. It is reportedly rapidly degraded by light and inactivated through absorption onto sediments (Langeland and Warner 1986). The mode of action of diquat as a competitive inhibitor of photosynthetic electron transport does, however, make it potentially lethal to a wide variety of non-target species of primary producers, including phytoplankton and benthic algae (Dodge 1971).

Reports of the toxicity of diquat to phytoplankton and benthic algae are variable. A 1980 review of diquat effects reported that it was toxic to many algal species at concentrations of 10 to >100 mg L^{-1} (Halter 1980). These concentrations are, however, well in access of the maximum recommended application rate of 2.94 mg L^{-1}. At doses of 2.94 mg L^{-1}, or below, there are a number of reports of significant inhibition of growth and survival of phytoplankton (Birmingham and Coleman 1983; Butler 1977; Newbold 1975; Robson et al. 1976; Thomas et al. 1973; Zweig et al. 1968). Based on their study of two cyanobacteria (Anabaena flos-aquae and Anacystis nidulans) and two eucaryotic algae (Navicula pelliculosa and Chlorella vulgaris), Birmingham and Coleman (1983) hypothesized that the former group may be more sensitive to diquat than the latter.

This paper describes the effect of the herbicide diquat on the growth and survival of nine species of phytoplankton and one benthic cyanobacterium, representing six phyla.

MATERIALS AND METHODS

The species of phytoplankton tested were: (1) cyanobacteria (Cyanophyceae), (a) Anabaena flos-aquae -

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Brebisson (ATCC 22664, American Type Culture Collection), (b) Lyngbya wollei (local benthic isolate, Florida), (c) Microcystis aeruginosa Lemmermann (ATCC 22663); (2) green algae (Chlorophyceae), (a) Chlorella vulgaris Beijerinck (ATCC 11468), (b) Selenastrum capricornatum Printz (ATCC 22662); (3) diatoms (Bacillariophyceae), (a) freshwater diatom Navicula sp. (local isolate, Florida), (b) Skeletonema costatum (Greville) Cleve (SKEL Clone, Bigelow Laboratory for Ocean Sciences); (4) euglena (Euglenophyceae), Euglena gracilis Klebs (UTEX 753, University of Texas Culture Collection, Austin); (5) cryptomonad (Cryptophyceae), Cryptomonas ozolini Skuja (UTEX LB2194); (6) Crysophyte (Chrysophyceae), Ochromonas danica Pring Sheim (UTEX L1298).

Artificial culture media was prepared for each species according to its particular needs. A majority of the species tested were grown on a modified Hoaglands consisting of the following constituents, in mg L^{-1}, Na_2EDTA, 30; NaCl, 20; KCl, 60; Cl_2*2H_2O, 370; THAM, 400; $MgSO_4*7H_2O$, 100; K_2HPO_4, 20; KNO_3, 200; $Na_2SiO_3*5H_2O$, 4.0; H_3BO_3, 34.2; N_2HCO_3, 40; $MnCl_2*4H_2O$, 4.3; $ZnCl_2$, 0.31; $CuSO_4$, 0.03; $CoCl_2*6H_2O$, 0.01; $FeSO_4*7H_2O$, 4.0; $Na_2MoO_4*2H_2O$, 1.3; Thiamine, 0.20; B^1_2, 0.02; Biotin, 0.02. Ochromonas danica and Euglena gracilis were grown on species specific media described by Starr and Zeikus (1987). Skeletonema costatum was grown on enriched artificial seawater media (Starr and Zeikus 1987). Diatom medium was supplemented with sodium silicate (100 mg L^{-1}). Growth irradiance was 100 μmol photons $m^{-2}s^{-1}$. Temperature was maintained at 28 C for the cyanobacteria and Chlorella, and 25 C for the remainder of the species, except Skeletonema, which was maintained at 21 C. pH for the cyanobacteria and Skeletonema media was set at 8.2, using THAM buffer (5 mM). The remainder of the species were run at pH 7.0, using HEPES buffer (5 mM).

A series of sixty 500-mL batch culture flasks were set up for testing the effects of diquat on each species. Each flask contained 400 mL of sterile medium. Ten different concentrations of diquat were tested, i.e., 0.000, 0.012, 0.023, 0.046, 0.092, 0.184, 0.368, 0.735, 1.470, and 2.940 mg L^{-1}, with three replicate flasks per concentration. 2.94 mg L^{-1} was determined to be the maximum application rate under field conditions, i.e. when 4.4 kg ha^{-1} of diquat cation is applied to plants growing in water 15 cm deep. Equal amounts of actively growing phytoplankton were then inoculated into each flask. Flasks were then incubated in illuminated constant temperature water baths at the growth irradiance and temperature levels described above. Five-mL aliquots of culture were withdrawn from

each bottle on a daily basis for 10 d. This sample was used to determine cell number and chlorophyll concentration. Chlorophyll a was determined by fluorometry according to Standard Methods (APHA 1985). Cell number was determined using an Elzone Cell Scope (Particle Data, Inc., Elmhurst, Illinois) with a 60 μm oriface tube. Cell number determination was not practical for all of the species tested. For the purpose of presentation 3- and 7-d culture data were used in this paper to compare the effects of diquat.

Linear regression analysis (SAS statistical program 6.03 GLM, SAS Institute Inc., Cary, North Carolina) was used for 3- and 7-d data sets to determine significant ($P<0.05$) diquat effects. Log transformed and non-transformed data models were used to develop regression models. Models with the best fit (highest R^2) regressions were used to interpret inhibitory effects. All of the tests generated significant models except those with Skeletonema costatum. All of the models used exhibited R^2 values >0.80.

RESULTS AND DISCUSSION

The regression relationships were generated between diquat concentration and predicted growth yield values for the diquat concentrations which produce 0, 25, 50 75 and 100 % inhibition of growth over control for 3- and 7-d old cultures (Table 1). The three cyanobacteria, A. flos-aquae, L. wollei, and M. aeruginosa, all exhibited sensitivity to diquat well within the range of diquat concentrations used in these tests. The results for the heterocystous species A. flos-aquae coincide with those obtained by Birmingham and Coleman (1983), showing strong inhibition (>90%) at 0.3 mg L^{-1}. The unicellular cyanobacterium M. aeruginosa responded similarily to A. flos-aquae. The benthic, filamentous, species L. wollei exhibited slightly less sensitivity to diquat than the planktonic forms of cyanobacteria. In general these results indicate that cyanobacteria are sensitive to diquat at concentrations as low as ten times less than the maximum recommended dose.

The two planktonic green algae tested, C. vulgaris and S. capricornutum, responded very differently to diquat. As reported by Birmingham and Colman (1983), C. vulgaris was resistant to diquat at levels up to 3 mg L^{-1}. This study, however, revealed increased diquat inhibition with extended incubation period, i.e., 7 days exposure to 0.3 to 0.4 mg L^{-1} resulted in 50% reduction of chlorophyll and cell number over control. This result is more in line with the observations of

Table 1. Predicted levels of diquat for different levels of growth inhibition.

Species	D[1]	Y[2]	Percent of Inhibition/ Diquat, μg L^{-1}			
			0%	25%	50%	100%
Anabaena flos-aquae	3	Ch[3]	0	31	42	>735
	3	C#[4]	0	24	53	>184
	7	Ch	0	67	130	>735
	7	C#	0	80	138	>735
Lyngbya wollei	3	Ch	<23	76	145	>735
	7	Ch	<92	125	175	>735
Microcystis aeruginosa	3	Ch	0	30	65	>170
	7	Ch	0	23	53	>260
Chlorella vulgaris	3	Ch	<2940	>2940	-	-
	3	C#	<735	1200	>2940	-
	7	Ch	<92	260	410	-
	7	C#	<92	154	307	-
Selenastrum capricornutum	3	Ch	<24	43	73	>1472
	3	C#	<24	44	96	>1472
	7	Ch	<13	18	26	>1995
	7	C#	<24	32	47	>759
Navicula sp.	3	Ch	<12	16	19	>368
	7	Ch	<93	99	106	>368
	7	C#	<93	107	127	>368
Skeletonema costatum	3	Ch	>2940	>2940	-	-
	7	Ch	368	794	2940	-
Euglena gracilis	3	Ch	<735	1454	>2940	-
	3	C#	<735	1259	>2940	-
	7	Ch	<735	1824	>2940	-
	7	C#	<735	1531	>2940	-

[1] Day of harvest, [2] measure of biomass, [3] chlorophyll a, [4] cell density.

Table 1. Continued

Species	D	Y	0%	25%	50%	100%
Cryptomonas ozolini	3	Ch	0	14	35	92
	7	Ch	<13	15	19	>92
	7	C#	<12	38	75	>175
Ochromonas danica	3	Ch	<13	18	23	>368
	3	C#	<12	65	136	>368
	7	Ch	<23	42	76	>282
	7	C#	<23	67	124	>368

Cullimore (1975) who observed 50% inhibition of the growth of C. vulgaris and Chlamydomonas terricola at 0.1 and 0.5 mg L^{-1} diquat, respectively. In contrast to C. vulgaris, S. capricornutum was highly sensitive to diquat. Both cell number and chlorophyll were 50% lower than control at concentrations of less than 0.1 mg L^{-1}. These results indicate that green algae are not less sensitive to diquat than cyanobacteria as a group, although individual species appear to be resistant to the herbicide. Cullimore (1975), for example, reported no inhibition of the growth of Chlorella pyrenoidosa, C. ellipsoidea, Coccomyxa subellipsoidea, or Stichococcus bacillaris at 10 mg L^{-1} diquat. Conversely, Thomas et al. (1973) showed strong inhibition of C. pyrenoidosa growth at 1 mg L^{-1} diquat using microbial plaque assay methods. Beyond these unicellular forms of green algae, a number of large filamentous species of green algae have been shown to be sensitive to diquat at concentrations below 1 mg L^{-1}, like Cladophora glomerata, and Rhizoclonium hieroglyphicum (Robson et al. 1976).

The two planktonic diatoms tested in this study, the freshwater species Navicula sp. and the marine species S. costatum, manifested very different responses to diquat. Navicula sp. was strongly inhibited by diquat, with 75% reduction of chlorophyll over control, after three days, at concentrations of 0.03 mg L^{-1}. This contrasts with the lack of inhibition observed for Navicula pelliculosa at 0.3 mg L^{-1} by Birmingham and Coleman (1983). The marine diatom S. costatum proved to be resistant to diquat concentrations below 2.94 mg L^{-1}. This result must be interpreted with caution, however, in lieu of the possibility that marine medium may chemically interfere with the action of diquat.

The remaining three species of phytoplankton tested in this study were members of phyla not previously studied

Table 2. Predicted level of diquat, $\mu g\ L^{-1}$, yielding 50% inhibition of growth, EC50, at three days, measured in terms of chlorophyll a.

Species	Division	EC50[1]	95% Conf. Interval
Navicula sp.	Diatom-Freshwater	19	16-23
Ochromonas danica	Crysophyte	23	15-32
Cryptomonas ozolini	Cryptophyte	35	27-42
Anabaena flos-aquae	Cyano-bacteria	42	35-49
Microcystis aeruginosa	Cyano-bacteria	65	27-100
Selanastrum capricornutum	Chlorophyte	73	62-77
Lyngbya wollei	Cyano-bacteria	145	81-205
Euglena gracilis	Euglenoid	>2940	-
Chlorella vulgaris	Chlorophyte	>2940	-
Skeletonema costatum	Diatom-Marine	>2940	-

for diquat inhibition. The cryptomonad Cryptomonas ozolini and the chrysophyte Ochromonas danica both proved to be very sensitive to diquat, with 50% inhibition of growth at diquat concentrations below 0.1 mg L^{-1}. In contrast, the euglenoid species Euglena gracilis was relatively resistant to diquat, exhibiting only 25% inhibition of growth at diquat concentrations of 1.5 mg L^{-1}.

In general, the results of this study indicate that there is a wide range of susceptibility to diquat among the species tested (Table 2). Seven of the ten phytoplankton species tested exhibit pronounced sensitivity to diquat, 50% inhibition of growth, at concentrations below 0.15 mg L^{-1}. These results, combined with those of previous investigators do not, however, manifest any broad phylogenetic pattern in susceptibility to diquat.

Acknowledgments. This research was funded by a grant from the Chevron Chemical Company. We thank Drs. William Haller and Ken Langeland for their advice and review of the manuscript. This publication is part of the Florida Agricultural Experiment Stations Journal Series No. R-01263.

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Received February 5, 1992; accepted May 5, 1992.