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Effects of a Diazinon Formulation on Unialgal Growth Rates and Phytoplankton Diversity

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Diazinon and other organophosphorus insecticides are used primarily for their broad effectiveness, short persistence, and relatively low mammalian toxicity. Although these insecticides are less toxic to algae than most organochlorines (Hurlbert 1975), the extensive use of Diazinon and the subsequent exposure to aquatic communities may pose a serious threat to algal growth and population diversity. The significance of phytoplankton as primary producers as well as their ability to intrinsically alter the balance of aquatic ecosystems has warranted greater concern for the toxic effects of this widely accepted insecticide.

Few reports are available on the effects of Diazinon on nontarget aquatic organisms and even less is known about its effects on algal growth and phytoplankton diversity. Singh (1973) found that three genera of blue-green algae tolerated a Diazinon formulation at concentrations as high as 300 and 400 mg/L. Another study indicated that population densities of three species of freshwater algae were relatively unaltered by a concentration of 100 mg/L (Clegg and Koevenig 1974). Two other reports have conveyed much greater susceptibilities to Diazinon toxicity. Butler et al. (1975) demonstrated that Diazinon inhibited growth of numerous species of green and blue-green algae at concentrations of 0.01 and 0.1 mg/L. Wong and Chang (1988) studied the effects of Diazinon on the growth of the green alga, Chlamydomonas reinhardtii, and observed a reduction in growth at concentrations of 5 and 10 mg/L and complete inhibition at 20 and 40 mg/L. Studies on the effects of Diazinon on phytoplankton communities are also limited to only one known published report. Murray and Guthrie (1980) examined the effects of Diazinon on a native population of freshwater algae and observed a short term suppression of species at 5 mg/L followed by an apparent stimulatory response by Chrysophyta and Chlorophyta species at 14 days.

In light of the sparse information available on the effects of Diazinon on phytoplankton population dynamics, the objectives of this study were: 1) to determine the effects of a Diazinon formulation on the growth rates of three widely distributed species of freshwater algae, 2) to ascertain the effects of this formulation on the diversity of a natural phytoplankton assemblage.

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MATERIALS AND METHODS

Aseptic cultures of Chlorella pyrenoidosa - normal strain, Chlorella pyrenoidosa - high temperature strain, Selenastrum capricornutum, and Synechococcus leopoliensis were obtained from the University of Texas at Austin Culture Collection. The axenic cultures were transferred and maintained as such on Bold's Basal Medium (BBM) agar slants (Stein 1973).

A Diazinon formulation, Diazinon 4E, (O,O-diethyl-O-2-isopropyl-4-(methyl)-6-pyrimidinyl phosphorothioate, active ingredient 47.5%) (Ford Chemical Company) was purchased from a commercial source in Springfield, Missouri. The concentrate formulation was diluted with 95 mL of sterile medium to yield final concentrations of 1, 5, 10, 20, and 40 mg/L of original formulation. Control flasks containing no Diazinon were also maintained.

Unialgal suspensions were obtained using 10 mL of sterile BBM. Known cell concentrations were added in 1-mL aliquots to the various concentrations of Diazinon formulation. Each test alga was maintained in triplicate and grown for a period of 10 d in 250-mL foam-plugged Erlenmeyer flasks. All cultures were exposed to a continual light source from fluorescent tubes with an intensity of 2000 lux and placed on a continuous shaker at 160 rpm. Daily growth of Chlorella and Selenastrum cultures were monitored by Bright Line Hemocytometer counts. Growth of the blue-green alga, Synechococcus was monitored by daily absorbance readings at 678 nm with a Milton Roy Spectronic 1201 spectrophotometer (Milton Roy Company, Rochester, New York).

The growth rate (k) of the alga grown in each concentration, as well as the controls, was determined by the following equation (Wong and Chang 1980):

$$k = (\ln X_2 - \ln X_1) / (T_2 - T_1)$$

Where X_2 represents the number of cells/mL x 10^4 or absorbance at 678 taken at T_2; and X_1 represents the number of cells/mL x 10^4 or absorbance at 678 nm taken at time T_1.

In order to study the effects of the Diazinon formulation on a natural phytoplankton assemblage, a 2-L water sample was obtained from McDaniel Lake north of Springfield, Missouri. The unfiltered lake water was dispensed in 80-mL aliquots into 250-mL Erlenmeyer flasks. Each flask was spiked with 20 mL of BBM and 0.5 mL of $NaSiO_3$ to yield final concentrations of 10 mg/L $NaSiO_3$. Triplicate concentrations of 1, 5, 10, 20, and 40 mg/L of Diazinon formulation as well as three control flasks with no insecticide were maintained under the previously stated conditions for a period of 9 d.

During the ninth day of growth, phytoplankton were counted using a hemocytometer counting chamber. The cells were identified according to a key to freshwater algae in Prescott (1978). The mean number of genera occurring in each concentration of Diazinon formulation was recorded, and a measure of generic diversity was

determined by using Simpson's Index of Diversity as follows (Krebs, 1972):

$$D = 1 - \sum(p_i)^2$$

where D is Simpson's index of Diversity, and p_i is the proportion of individuals of genus i in each concentration of Diazinon.

RESULTS AND DISCUSSION

Table 1 summarizes the effects of the Diazinon formulation on each unialgal test. It is evident from these data that the effects vary greatly and are largely dependent upon the concentration of insecticide and algal species in question.

The marked difference in susceptibilities between the two strains of Chlorella pyrenoidosa exemplify this degree of variance. Although previous investigations on the effects of Diazinon on closely related species do not exist, this initial comparison of two strains suggests that algal species may possess an adaptive mechanism for tolerance.

The stimulatory responses exhibited by Selenastrum capricornutum at 1 and 5 mg/L were very similar to those exhibited by the normal strain of Chlorella. However, the effects of Diazinon on Selenastrum show that at high concentrations the growth of a particular species may be thoroughly suppressed. As Table 1 indicates, the higher concentrations of 10, 20, and 40 mg/L all inhibited the growth of this species. At 40 mg/L growth was entirely suppressed.

Although the growth rates of the blue-green alga, Synechococcus leopoliensis, convey a high degree of tolerance, a suppression of growth was actually observed at 40 mg/L as evidenced by the extended lag phases of all replicates. Since the growth rates were determined from measurements taken during active log phase, the respective k value does not take into account this suppressive response. This inhibition followed by an apparent stimulation in growth suggests two possible conclusions: 1) The Diazinon was chemically degraded by means of hydrolysis to yield a less toxic compound; or 2) The alga required an induction period to synthesize enzymes essential for the metabolism of Diazinon.

The rate of hydrolysis of Diazinon is largely dependent upon the pH of the solution (Bartsch 1973). Gomaa et al. (1969) examined the rate of degradation in water under varying conditions and observed a half-life of only 12 h at a pH of 3.1. In contrast, at a pH of 7.4 Diazinon exhibited a half-life of 184 d. The pH of the media used in this investigation was 7.1; therefore, it is not probable that the apparent stimulatory response was due entirely to the hydrolysis of the formulation. The observed lag phases may be more adequately explained by a period of time required for the synthesis of metabolic enzymes (Fogg 1965). However, confirmed evidence of an enzyme induction period as well as the uptake and metabolism of Diazinon by Synechococcus does not exist.

Table 1. Growth rates of algal species grown in various concentrations of diazinon formulation.

Species	Concentration (mg/L)	Growth Rate* (k/day)
Chlorella pyrenoidosa		
Normal Strain	0	0.82 ± 0.04
	1	1.23 ± 0.32
	5	0.93 ± 0.05
	10	0.88 ± 0.05
	20	0.83 ± 0.11
	40	0.77 ± 0.09
Chlorella pyrenoidosa		
High Temperature Strain	0	1.18 ± 0.08
	1	1.14 ± 0.11
	5	1.07 ± 0.15
	10	1.04 ± 0.13
	20	0.89 ± 0.02
	40	0.84 ± 0.04
Selenastrum capricornutum		
	0	0.88 ± 0.09
	1	0.91 ± 0.09
	5	0.88 ± 0.07
	10	0.77 ± 0.07
	20	0.56 ± 0.15
	40	NG**
Synechococcus leopoliensis		
	0	0.38 ± 0.01
	1	0.37 ± 0.01
	5	0.37 ± 0.02
	10	0.33 ± 0.03
	20	0.32 ± 0.01
	40	0.47 ± 0.04

* Each value indicates a mean of three replicates ± standard error.
** NG = No Growth

The results of the diversity study illustrate the potential for profound impacts on phytoplankton population dynamics. The results specifically show an inverse relationship between the formulation concentration and species diversity (Table 2). The inhibition of the blue-green algal component was particularly apparent. Species of Oscillatoria, Anabaena, and Raphidiopsis were suppressed by all concentrations and were completely

Table 2. Phytoplankton abundance and diversity (D) of cultures exposed to diazinon.

Genus	Formulation Concentration (mg/L)					
	0	1	5	10	20	40
Chlorella	96*	62	62	86	91	134
Oscillatoria	55**	45	19	15	3	0
Dictyosphaeropsis	21	18	13	35	49	16
Ankistrodesmus	23	10	5	4	4	4
Scenedesmus	8	13	6	3	0	0
Anabaena	3**	1	2	0	0	0
Synedra	5	7	6	13	9	0
Franceia	5	6	4	4	21	19
Raphidiopsis	6	5	0	0	0	0
Chlorococcum	4	3	1	1	1	1
Spirulina	2	0	0	0	0	0
Crucigenia	0	0	1	1	0	0
Tabellaria	1	0	0	0	0	0
Total Genera	12	10	10	9	7	5
Diversity	0.75	0.78	0.74	0.65	0.65	0.39

* Each value represents cell number x 10^4 and indicates a mean of three replicates.

** Values for Oscillatoria and Anabaena reflect the mean number of filaments.

inhibited at 40 mg/L. Similar observations were made by Murray and Guthrie (1980) in which a blue-green algal component was suppressed by a Diazinon concentration of 5 mg/L (active ingredient).

Two genera of green algae, Chlorella and Franceia, were stimulated by all concentrations. An interesting observation was the similarity between this response by Chlorella and the effects on the growth rates of the normal strain of Chlorella pyrenoidosa. As previously suggested, these responses may be due to the cells' ability to readily metabolize the insecticide or the presence of a mechanism for tolerance. However, the stimulation of Chlorella and Franceia is probably coupled with the subsequent lack of competition for available nutrients.

The occurrence of Diazinon in wells and natural ecosystems has been recorded at concentrations of 0.04 to over 1 µg/L (Frank et al. 1987; Sievers and Fulhage 1989; U.S.G.S. 1988). Although these levels are far below the exposures used in this investigation, it is possible that comparable levels could exist in lakes and streams proximal to direct applications. However, extensive monitoring has previously focused on the more persistent organochlorine insecticides; therefore, valid assessments of the actual risks to phytoplankton communities are limited. The results of this investigation indicate that further research and monitoring are essential in order to accurately assess these potential risks and to appropriately surmise the subsequent ramifications to the overall quality of freshwater ecosystems.

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