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Influence of Five Phenylurea Herbicides on the Diatom *Hantzschia* in a Sandy Loam Soil

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A moderate number of studies have been conducted on the influence of herbicides, including the phenylureas, on the growth of soil algae under in vitro conditions (McCann and Cullimore 1979). Reports of in vivo studies, in contrast, are few. This preoccupation with in vitro techniques is unfortunate in that the perceived response of an alga to a given herbicide may be quite different from that which would occur in vivo. Discrepancies may result from such factors as biological degradation of the herbicide in the soil by other members of the soil microflora (Kaufman and Kearney 1976); non-biological degradation (Crosby 1976); and leaching, volatilization (Spencer et al. 1973), and adsorption to soil particles (Adams 1973). Of the procedures which have been developed for in vivo studies, most involve the treatment of soil plots with the herbicide, followed by removal of soil samples at various intervals. The algal population in the soil samples is then evaluated, commonly by a serial dilution technique (Cullimore and McCann 1977; Calle 1970; Hugé 1970). This procedure suffers from the fact that the growth of algae in the dilution cultures is greatly influenced by the cultural conditions employed.

A procedure which obviates this problem is the implanted slide technique described by Pipe and Cullimore (1980) for examining the effects of the phenylurea herbicide diuron on soil algae. Algal growth in treated and control soils is monitored by the extent of colonization of microscope slides implanted vertically into the soils. The technique was developed using soils held in paper cups in the laboratory. In the present paper, it has been adapted to detect herbicide-induced alterations in algal populations in the field.

MATERIALS AND METHODS

Field plots (400 cm^2) were marked out in a sandy loam soil. Pairs of microscope slides (back-to-back) were implanted in each plot with 15 mm left projecting above the soil surface. Implantation took place 30 days before the treatment of the plots with the test herbicides. The test herbicides used were the pure chemical forms of: monuron (3-(4-chlorophenyl)-1,1-dimethylurea), diuron (3-(3,4-

dichlorophenyl)-1,1-dimethylurea), linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea), chlortoluron (3-(3-chloro-4-methylphenyl)-1,1-dimethylurea) and chloroxuron (3-[p-(p-chlorophenoxy)-phenyl]-1,1-dimethylurea). These formulations, dissolved in acetone, were applied in the spring by sprinkling from a pipette as evenly as possible the amounts necessary to give 1 or 5 ppm (calculated as μg herbicide per g dry soil) in the top 10 mm soil, dispersed in sufficient distilled water to moisten this depth to water holding capacity. This procedure was intended to simulate normal field application practices in which the phenylurea herbicide is applied in a water carrier, generally without soil incorporation (Anderson 1977). Three plots were treated with each herbicide level and three control plots were treated with acetone and distilled water only.

Slides were left implanted in the soil for 2, 3 and 12 month periods after treatment. After each of these incubation periods, one slide pair was removed from each of the triplicate plots. This was accomplished by excavating the slides in the surrounding clod of earth which was then carefully removed from the glass surfaces. On arrival at the laboratory, the slides were treated with molten 2% water agar by the technique of Pipe and Cullimore (1980), and the colonizing algae in the slide area corresponding to the top 10 mm soil (250 mm^2) were identified and counted. The algal counts for each set of 6 replicate slides at each treatment level were analyzed by non-parametric ranking according to the Mann-Whitney test (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The algal genera identified on slides and removed from control plots were: Arthrospira, Lyngbya, Microcoleus and Oscillatoria (Cyanophyceae or Cyanobacteria); Chlorella, Chlorococcum, Spongiochloris, and Ulothrix (Chlorophyceae); Hantzschia and Navicula (Bacillariophyceae); and Heterothrix (Xanthophyceae). Most genera were somewhat erratic in their occurrence on the slides, in terms of their presence both from one sampling occasion to the next (possible seasonal variation in algal abundance and periodic formation of resting stages unable to colonize the slides), and on replicate slides taken on the same sampling occasion (natural uneven distribution of the algae in the soil). One noticeable exception to this irregular pattern of colonization was that displayed by the pennate diatom Hantzschia. This genus was present consistently on all slides from untreated soils, and exhibited the lowest coefficients of variation between numbers on replicate slides. The reliability of Hantzschia as a slide colonizer was also noted by Pipe and Cullimore in 1980. This alga was therefore selected as an indicator organism in the present study, and all discussion will refer to it exclusively.

Colonization of slides in control plots by Hantzschia generally increased throughout the sampling period. Mean cell numbers per slide were 68, 140 and 900 for the 2, 3 and 12 month implantation periods respectively. Low numbers early in the sampling period may probably be accounted for by an adjustment period, during which the algae became physically reorganized following disruption of the soil structure by insertion of the slides, and began to colonize the glass.

Colonization in herbicide treated soils was significantly reduced by some concentration of each of the applied phenylureas (Table 1). Diuron, monuron and chloroxuron affected colonization at 1 ppm, suggesting that they were more toxic to the diatom than were chlortoluron and linuron. Diuron appeared to be the most toxic, most data being significantly different from corresponding control data at the 0.01 level. A similar scale of toxicity was observed for Hantzschia and also for Chlorella in related studies using a heavy clay soil (Pipe 1982).

Table 1. Colonization by Hantzschia of slides in sandy loam soil treated with phenylurea herbicides[a]

Soil treatment[b]		Implantation period (months) 2	3	12
Diuron	1	22	4**	5**
	5	10**	8**	7**
Monuron	1	133	24*	28**
	5	98	114	40**
Chloroxuron	1	100	157	42*
	5	71	257	38*
Chlortoluron	1	206	66	74
	5	38	15	38**
Linuron	1	94	100	122
	5	28	55	18**

[a] Colonization expressed as percentage of that in corresponding untreated plots.
[b] Figures indicate herbicide concentration (ppm).
* Significantly different from corresponding control data at 0.05 level.
** Significantly different from corresponding control data at 0.01 level.

Of particular interest is the apparent extended duration of the adverse effects of the herbicides on colonization (Table 1). This could well be a reflection of the long-term persistence of the herbicides in the soil. This phenomenon is well documented for the phenylureas (Anderson 1977; Fryer and Evans 1968). Using the same sandy loam soil as that employed in the present study, Smith and Emmond (1975) found that 33% of a 2.2 kg/ha spring application of linuron was still present in soil samples analyzed 6 months after treatment. A similar degree of persistence may be assumed for the phenylureas in the present study. Levels of herbicide residues present 12 months after application would probably be comparable with those expected at 6 months, since the period between these two occasions coincided with the winter months. The extremely low temperatures affecting the study area (Saskatchewan prairie belt) would effectively prevent microbial degradation, the chief means of inactivation of the phenylureas (Anderson 1977), during this period.

The apparent slow response of Hantzschia to most of the herbicides (Table 1) may have been a function of differences in availability of the herbicide during the sampling period. The phenylureas are known to become strongly adsorbed to soil organic matter (Grover 1975). In this state, they may be virtually unavailable to soil microorganisms (Hill and Wright 1978). The extent of adsorption and desorption is partly governed by soil moisture. Moisture levels during the summer following application were low (mean 3%). The availability of the herbicides to the Hantzschia in the present study may have been greatest in the spring, 12 months after application, as a result of desorption in response to higher soil moisture levels at run-off.

While the data presented in Table 1 clearly indicate a considerable reduction in the numbers of cells of Hantzschia able to colonize slide surfaces following herbicide treatment of the soil, it should be emphasized that mortality of the cells is not necessarily indicated. Instead, the herbicides may have affected the ability of the diatom to move towards the slides and adhere to them prior to potential colonization. Some evidence that this may be the case was provided by Pipe (1982), who showed that levels of linuron as low as 0.01 ppm arrested the locomotion of Hantzschia without affecting its viability. The consequences of sub-lethal doses of herbicides on diatoms and other soil algae should not be underestimated. Diatoms are known to move downwards in the soil during periods of prolonged drought (Patrick 1977). The impaired motility mentioned above may therefore result in the inability of certain algae to survive adverse conditions in the soil. Sub-lethal herbicide doses may accordingly be potentially as harmful to the soil algal flora as are higher directly lethal doses. Impairment of the activities of the soil algal flora by any means can impede the contribution these microorganisms can make to the fertility of the soil.

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