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Effect of 2,4-D on Electron Transport System (ETS) Activity and Respiration in Soil

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Research on the effects of environmental toxicants on microbial activities in soil has focused on the toxic and non-toxic effects on nontarget soil microorganisms (TAM & TREVORS 1981, TREVORS 1982), and secondly on the microbial species responsible for the degradation of environmental toxicants (BOLLAG et al. 1968, EVANS et al. 1971).

The herbicide, 2,4-dichlorophenoxyacetate (2,4-D) has been widely used in agricultural and forestry practices. It displays moderate persistence in soils (AUDUS 1964) with the effective phytotoxic applications disappearing within one month.

Electron transport system (ETS) activity can be measured using the quantitative reduction of 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) to iodonitrotetrazolium formazan (INT-formazan) in soils (TREVORS et al. 1982). This assay measures soils, the ETS activity of a large part of the microbial community in soils, and may be useful for initially assessing the effects of environmental toxicants on microbial activity in soil over a short period of time. The present report describes the effects of 2,4-D on ETS activity and respiration in soil.

MATERIALS AND METHODS

A sandy loam soil and a loam soil were collected from the top 10 cm of two agricultural fields at Guelph, Ontario, Canada. The soils were passed through a 2-mm mesh sieve and used immediately for ETS activity and respiration measurements. The soil characteristics (Table 1) were determined using methods previously described by TAM & TREVORS (1981) and TREVORS (1982).

A 1.0-g sample of each soil was placed in separate 16 x 150 mm sterile glass test tubes. The soil treatments received 0.2 mL of the appropriate concentration of 2,4-D (Amchem Products Inc., Ambler, PA.) which was prepared by dissolving the appropriate amount in distilled water and filter sterilizing the solution by passing it through a 0.20 μm average pore size membrane. Each tube received 0.1 mL of a 0.4% (w/v) aqueous solution of filter sterilized INT (Aldrich

Chemical Co., Milwaukee, WI). The amount of liquid added adjusted the soil moisture content to 60%, providing for maximum aerobic microbial activity. The test tubes were loosely capped and incubated at 20°C in the dark for 24 h, at which time 10 mL of methanol was added to each tube. The contents were vortexed for 1 min and then filtered through a Whatman No. 5 filter. The INT-formazan in the methanolic extract was measured spectrophotometrically at 480 nm against a methanol extract of soil containing no INT. The INT-formazan concentration was calculated from a standard curve of INT-formazan (Sigma Chemical Co., St. Louis, Mo.) in methanol. A detailed description of the procedure has been described elsewhere by TREVORS et al. (1982)

The effect of 2,4-D on O_2 consumption and CO_2 evolution in the same soils was also measured using the gas chromatography technique described by TAM & TREVORS (1981) and TREVORS et al. (1982). All trials were carried out in triplicate. Statistical analysis was performed using a two-tailed Student's t-test at P = 0.05 level on an Apple II plus microcomputer. The statistical program used was obtained from Edu-Ware Services, Inc., Agoura, CA.

RESULTS AND DISCUSSION

The effect of 2,4-D on ETS activity in two different soil types is presented in Table 2. ETS activity was not significantly inhibited in the sandy loam soil by 2,4-D concentrations ranging from 10 to 200 µg/g soil. However, in the loam soil, ETS activity was significantly inhibited by 2,4-D concentrations as low as 10 µg/g. The significant decrease in activity was observed in soil samples treated with 2,4-D concentrations ranging from 10 to 200 µg/g.

Oxygen consumption and CO_2 evolution in the sandy loam soil treated with the same concentrations of 2,4-D revealed that soil respiration generally was not affected by the 2,4-D (Table 3). Statistical testing showed that only the 50 µg 2,4-D/g soil treatment stimulated O_2 consumption. Concentrations above and below this treatment caused no significant inhibition or stimulation of O_2 consumption. CO_2 evolution was only stimulated by the 25 and 200 µg 2,4-D/g soil treatment. Oxygen consumption in the loam soil was stimulated by the 25 ug 2,4-D/g soil treatment. CO_2 evolution in the loam soil was not significantly inhibited or stimulated by 2,4-D concentrations from 10 to 200 µg/g soil. The results of this study suggested that the INT technique for measuring ETS activity may be a useful bioassay for assessing the effects of environmental chemicals on microbial activities in soil. For example, the inhibitory effects of 2,4-D on soil ETS activity clearly show a good dose-response relationship in the loam soil (Table 2). The same concentrations of 2,4-D produced somewhat erratic results with higher variability when soil respiration was measured in the presence of 2,4-D concentrations ranging from

10 to 200 μg/g soil. The slight stimulation of microbial activities by low concentrations of environmental chemicals, followed by inhibition at high concentration is not unusual.

The lack of an inhibitory effect by 2,4-D on ETS activity in the sandy loam soil, and the inhibition of activity in the loam soil clearly suggest that 2,4-D can bring about very different responses from the soil microbial populations. This clearly emphasizes the need for assessing the behaviour of environmental chemicals in different soil types over a large concentration range. Also, it may be necessary to use several methods to assess toxic and non-toxic effects of environmental chemicals. This is clearly demonstrated in this study, where ETS activity is a more sensitive assay than soil respiration.

Table 1. Characteristics of the soil

Characteristic	Sandy loam	Loam
pH (in H_2O)	7.7	7.2
Total carbon (%)	3.2	3.4
Sand (%)	78	35
Silt (%)	10	26
Clay (%)	12	39
Water holding capacity	0.49 mL/g	0.65 mL/g
No. heterotrophs	1.22×10^7/g	1.57×10^6/g

Values are expressed on a per g dry weight of soil basis, where applicable.

Table 2. Effect of 2,4-D on ETS activity in a sandy loam and clay loam soil

2,4-D Concentration (μg/g)	ETS activity[a] (μg INT-formazan/g)	
	Sandy loam	Loam
Control (0)	39.5±0.73	37.3±2.5
10	44.3±3.7	25.1±1.0[b]
25	41.7±1.6	21.3±1.2[b]
50	33.3±4.2	20.8±0.25[b]
75	38.8±3.5	20.5±0.76[b]
100	38.5±0.96	18.5±0.95[b]
200	35.8±1.8	16.3±0.76[b]

[a] Mean ± S.E.M. (n = 3)
[b] Significantly different from the control at 95% level.
Sterile controls of the above treatments displayed no ETS activity.

Table 3. Effect of 2,4-D on O_2 uptake and CO_2 evolution in soil

2,4-D Concentration (μg/g)	O_2 consumption (μmoles/g)[a]		CO_2 evolution	
	Sandy loam	Loam	Sandy loam	Loam
Control	1.7±2.1	13.0±1.0	1.8±0.32	0.96±0.14
10	6.9±4.7	18.0±4.4	2.5±0.45	0.94±0.12
25	10.2±6.4	17.9±1.0[b]	2.9±0.32[b]	1.03±0.72
50	10.6±1.6[b]	13.5±0.87	2.6±0.32	0.83±0.19
75	6.2±3.8	18.8±3.7	2.8±0.27	0.84±0.12
100	0 ±0	13.9±1.3	3.1±1.1	0.88±0.05
200	1.1±1.3	15.5±2.7	2.9±0.25[b]	1.1 ±0.15

[a]Mean ± S.E.M. (n = 3).
[b]Significantly different from the control at 95% level.
Sterile controls of the above treatments displayed no respiratory activity.

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