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## EFFECT OF PARAQUAT ON MICROBIAL ACTIVITIES IN SOILS\*

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**Summary.** When paraquat was added to four different soils, nitrification was not appreciably affected but ammonification of soil organic-matter nitrogen was slightly retarded. Carbon dioxide evolution as well as oxygen consumption was used as an index of metabolic activity of soil micro-organisms and paraquat had a stimulatory effect at higher concentrations. Oxidation of added sulphur was slightly depressed. Paraquat decreased both the total mould and bacterial populations in Chehalis silt loam. After 30 days incubation the percentages of *Streptomyces* and *Penicillia* were markedly increased by most treatments but were little affected in Chehalis silt clay and Woodburn soils. Except for the temporary suppression of nitrification, paraquat had no significant influence on general microbial activities of importance to soil fertility. Some bimodal effects or toxicity inversions were observed with intermediate concentrations.

*Effet du paraquat sur les activités microbiennes dans les sols*

**Résumé.** L'addition de paraquat à quatre sols différents n'a pas affecté la nitrification de façon appréciable mais l'ammonification de l'azote de la matière organique du sol a été légèrement retardée. L'évolution du gaz carbonique et la consommation de l'oxygène ont été utilisées comme un index de l'activité métabolique des micro-organismes du sol. Le paraquat manifesta un effet stimulant aux concentrations les plus élevées. L'oxydation du soufre ajouté a été légèrement ralentie. Le paraquat réduisit les populations totales, à la fois des moisissures et des bactéries dans un limon argileux de Chehalis. Après 30 jours d'incubation les pourcentages des *Streptomyces* et de *Penicillia* furent accrus d'une façon marquée par la plupart des traitements, mais furent peu affectés dans le limon argileux de Chehalis et dans les sols de Woodburn. Sauf en ce qui concerne la suppression temporaire de la nitrification, le paraquat n'eut pas d'influence significative sur les activités microbiennes générales qui sont importantes pour la fertilité du sol. Quelques effets bimodaux ou des inversions de toxicité furent observés aux concentrations intermédiaires.

*Einfluss von Paraquat auf die Mikroorganismen-tätigkeit im Boden*

**Zusammenfassung.** Bei Zugabe von Paraquat zu vier verschiedenen Böden wurde die Nitrifikation nicht wesentlich beeinflusst, die Ammonifikation des Stickstoffs aus der organischen Bodensubstanz jedoch leicht gehemmt. Kohlendioxydabgabe und Sauerstoffaufnahme wurden als Mass für die Stoffwechselaktivität der Bodenmikroorganismen herangezogen. Hierbei hatte Paraquat in höheren Konzentrationen eine stimulierende Wirkung. Die Oxydation von Schwefel wurde leicht gehemmt. Durch Paraquat wurde die Zahl von Pilzen und Bakterien in 'Chehalis'-Lehm vermindert. Nach 30-tägiger Inkubationszeit war der Anteil an *Streptomyces* und *Penicillia* in den meisten Behandlungen stark erhöht, in 'Chehalis'-Lehm und 'Woodburn'-Böden wurden die Anteile dieser Arten jedoch nur wenig verändert. Ausser der temporären Hemmung der Nitrifikation hatte Paraquat keinen signifikanten Einfluss auf die allgemeinen mikrobiellen Aktivitäten, die für die Bodenfruchtbarkeit von Bedeutung sind. Einige bimodale Effekte oder Toxizitäts-Inversionen wurden bei mittleren Konzentrationen beobachtet.

### INTRODUCTION

Interaction between herbicides and soil micro-organisms may be of practical significance because of the possible inhibition of the microbial activities con-

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tributing to soil fertility. These organisms, chiefly bacteria, fungi and *Streptomyces*, occupy a unique position in biological cycles in terrestrial habitats and are essential for plant growth. They decompose root and crop residues, mobilize the reserves of organic nitrogen and phosphorus, carry out transformations involving a change of oxidation state and bring about many other reactions necessary for crop production. Any deleterious influence, even of a temporary nature, could have a serious effect upon crop yields.

A new class of herbicides, the bipyridyl compounds, was first studied by Brian *et al.* (1958). Kaneshiro & Zweig (1965) found that diquat (1,1'-ethylene-2,2'-bipyridylum dibromide) at  $2 \times 10^{-4}$  moles inhibited both aerobic and anaerobic growth of *Rhodospirillum rubrum*. With photosynthetic cultures, diquat affected the synthesis of bacteriochlorophyll more than it did cell mass. Bozarth *et al.* (1965) found that several moulds, bacteria and actinomycetes tolerated relatively high concentrations of paraquat (1,1'-dimethyl-4,4'-bipyridylum dichloride), and that some activity degraded the herbicide. The studies reported here were initiated to determine changes in the microbial activity of soil populations following treatment with different rates of paraquat.

#### MATERIALS AND METHODS

The formulation\* used contained 2 lb/gal paraquat as di(methylsulphate) and a wetter, or non-ionic spreader activator.† When applied to the soil at the recommended rate of 0.5 lb/ac paraquat ion, the wetter concentration in 2 000 000 lb of soil would be approximately 0.1 ppm.

Solutions of the herbicide were mixed with portions of soil to give concentrations of 0.25 ppm, corresponding to the normal field rate, and also concentrations up to 25 ppm to determine the effects of excessive applications. All rates are in terms of the paraquat cation and were calculated for an acre—6.67 in., or 2 000 000 lb soil. Although this laboratory procedure does not exactly correspond to ideal spray practice in the field it does cover the range of concentrations that might result from irregularities in application. Some small areas may receive no herbicide while others may be heavily dosed, as by drip from foliage. In the soil most of the chemical will at first be concentrated on or near the surface but later may diffuse or leach downward, depending upon soil type and climatic conditions. For the kind of laboratory studies reported here a near approach to conditions that could occur in the field can be obtained by a range of treatments, each uniformly mixed with a sample of the soil.

Four different agriculturally important soil types were selected for study. Composite samples of three Willamette Valley soils, Chehalis silty clay loam (CSCL), Chehalis silt loam (CSL) and Woodburn silt loam (WSL) and samples of Delhi sandy loam (DSL) from near Fresno, California, were collected to a depth of 6 in. from fallow ground. The bulk samples were passed through a 10-mesh sieve. The chemical and physical properties and microbial analyses of the bulk samples are shown in Table 1.

\* Supplied by the manufacturer under the Trade Mark 'Gramoxone'.

† A mixture of alkylarylpolyoxyethylene glycols, free fatty acids and isopropanol, supplied by the manufacturer under the Trade Mark 'X-77'.



Mechanical analyses were determined by the hydrometer method (Bouyoucos, 1936); pH, with a Beckman model N glass-electrode pH meter; and organic matter by dry combustion at 1400° C (Allison, Moodie & Bollen, 1965).

Reagent grade dextrose, peptone,  $(\text{NH}_4)_2\text{SO}_4$ , and flour sulphur were employed to determine carbon dioxide evolution, ammonification, nitrification and sulphur oxidation, respectively. The required amount of the liquid was added to sufficient water to mix with 80 g portions of soil, oven-dry basis, to bring the moisture content to 50% of the water-holding capacity. The flour sulphur was first mixed with the soil. Paraquat treatments were made by adding the required amounts in dilute solution to give 0.25, 0.5, 2.5 and 25 ppm cation when mixed with the soil. Controls, with soil only, were included.

Table 1  
Characteristics of soils\*

	CSCL	CSL	WSL	DSL
Chemical analysis				
pH	6.2	6.3	6.1	6.9
Nitrogen				
$\text{NH}_4^+$ (ppm)	38	80	40	21
$\text{NO}_2^-$ (ppm)	0.32	0.70	0.20	0.03
$\text{NO}_3^-$ (ppm)	7	16	9	1
Kjeldahl (%)	0.183	0.112	0.120	0.020
Organic matter† (%)	3.93	2.55	2.67	0.55
C/N ratio	12.48	12.65	12.91	10.70
Cation-exchange capacity (m-eq/100 g)	32.4	25.3	16.1	2.7
Lime requirement (tons/ac)	0	0	1	0
Phosphate (as P) (ppm)	36	13	36	23
Sulphate (as S) (ppm)	21	5	5	10
Water capacity (%)	64	54	42	29
Moisture (%)	21.5	15.6	4.0	0.5
Ash (%)	95	96	95	99
Mechanical analysis				
Sand (%)	11	24	9	77
Silt (%)	60	51	70	11
Clay (%)	30	25	22	12
Microbial analysis				
Moulds (total) (thousands/g soil)	23	120	250	148
Mucors (%)	17	7	5	6
Penicillia (%)	51	31	45	62
Aspergilli (%)	1	3	9	2
Bacteria (total) (millions/g soil)	9	15	13	49
Streptomyces (%)	54	39	49	58

\* All data expressed on oven-dry basis.

† (Total C by dry combustion) ÷ 0.58.

The mixtures and controls were set up in 1-pint milk bottles, which were closed with Du Pont polyethylene film 1.5 mil thick, and incubated at  $28 \pm 1^\circ \text{C}$  for appropriate periods. No restoration of distilled water during incubation was required, because the polyethylene film while quite permeable to oxygen, nitrogen and carbon dioxide, had very low permeability to water vapour.

Two replications in duplicate were used in each series of treatments with each rate of herbicide. After 15 and 30 days incubation, samples of the soil were weighed out on an oven-dry basis and added to sufficient distilled water to give 1:5 dilutions for determination of nitrate, nitrite, sulphate and pH.



Harper's (1924) phenoldisulphonic acid method for nitrates was modified by use of ammonium carbonate to remove the excess calcium hydroxide remaining in a 1:5 soil-water extract after clarification. Aliquots of the same extract were used for determination of nitrites by the diazotization method with sulphanilic acid,  $\alpha$ -naphthylamine hydrochloride and sodium acetate buffer (A.P.H.A., 1955). Sulphate was determined turbidimetrically (A.P.H.A., 1955) in the 1:5 soil-water extracts clarified as for nitrates. Readings were made on a Klett-Summerson photometer with appropriate filters and dilutions, and standard curves were used for calculating concentrations of nitrate and nitrite N, and sulphate sulphur.

Analyses for ammonium N were made by the method of Shrikhande (1941), which employs distillation of the sample with phosphate buffer at pH 7.4. Kjeldahl N was determined by the macro-method, using 10 g soil samples, 20 g Hibbard's mixture plus a Hengar selenized granule and 35 ml  $\text{H}_2\text{SO}_4$ ; digestion was continued for 1 hr after clearing. When cool, the digest was diluted, made alkaline with 70% NaOH, and 150 ml steam-distilled into 30 ml saturated boric acid solution. The absorbed ammonium was titrated with  $\text{N}/14 \text{H}_2\text{SO}_4$  after adding bromocresol green-methyl red mixed indicator.

Exchange capacity and exchangeable cations were determined by the ammonium acetate method of Schollenberger & Simon (1945). Bicarbonate-soluble phosphorus was determined by the method of Olsen *et al.* (1954).

In the soil respiration studies to show mineralization of organic matter as indicated by carbon dioxide evolution, 80 g portions of soil, oven-dry basis, were placed in 1-pint milk bottles and treated in duplicate. The herbicide was added at different rates with sufficient water to give 50% of the water-holding capacity. The bottles were then placed at random in the incubator at 28° C and connected to a manifold supplying moisture-saturated  $\text{CO}_2$ -free air at the slight pressure necessary to cause it to bubble slowly through 16 × 150 mm test tubes containing 10 ml of approximately  $\text{N}/1 \text{NaOH}$ . At intervals of 1, 2, 5, 10, 20 and 30 days incubation the tubes were replaced and the absorbed  $\text{CO}_2$  was determined by differential titration with a Beckman model K automatic titrator. Results were graphed as mean values of  $\text{CO}_2$ -C evolved per 80 g of soil (Fig. 1).

The effect of paraquat on general soil microbial activity in Delhi sandy loam was studied by determining oxygen uptake in a Warburg apparatus. Each flask received 4 g soil moistened with 0.56 ml distilled water. Paraquat was added at rates of 0, 0.25, 2.5 and 25 ppm. Treatments with 100 ppm dextrose-C were also made. To absorb  $\text{CO}_2$ , the centre well of each vessel contained 0.20 ml 10% KOH and a filter paper strip. Optimum oxygen consumption was measured at 30° C for 46 hr by stationary manometer. The cumulative values of the oxygen taken up are shown in Fig. 2.

Changes in the soil microflora were determined by analysing samples of soil from the  $\text{CO}_2$  respiration experiment at 30 days. Soil dilution plate technique was applied by adjusting soil dilutions to give about forty microbial colonies per plate with Martin's (1950) medium for moulds, and about 100 colonies per plate with sodium albuminate agar (Waksman & Fred, 1922) for bacteria and *Streptomyces*. All dilutions were plated in triplicate. Counts were made as soon after



incubation as colonies could be differentiated. The arithmetic means of the triplicate counts were used to calculate numbers of micro-organisms per g soil expressed on an oven-dry basis.

## RESULTS AND DISCUSSION

Each soil had good ammonifying power (Table 2). The influence of paraquat varied, showing no pattern consistent with rate. Effects on ammonification of native organic matter were slightly depressive except in Chehalis silt loam at 2.5 ppm and in Delhi sandy loam soils. Paraquat at all rates had no influence on nitrification of the soil's native organic N in Chehalis silty clay loam but markedly increased nitrification of ammonium sulphate (Table 3). As evident

Table 2  
Ammonification\* in different soils after 5 days treatment with paraquat

Treatment		CSCL		CSL		WSL		DSL	
Nitrogen† source	Paraquat (ppm)	pH	NH <sub>4</sub> <sup>+</sup> -N (ppm)	pH	NH <sub>4</sub> <sup>+</sup> -N (ppm)	pH	NH <sub>4</sub> <sup>+</sup> -N (ppm)	pH	NH <sub>4</sub> <sup>+</sup> -N (ppm)
None	None	6.2	43	6.2	40	6.0	23	6.7	7
	0.25	6.2	13	6.2	20	6.0	13	6.8	7
	0.50	6.3	20	6.2	20	6.0	12	—	—
	2.50	6.2	20	6.1	70	6.1	15	6.8	7
	25.00	—	—	—	—	—	—	6.7	11
Peptone	None	7.2	547	6.8	717	7.1	733	8.9	697
	0.25	7.3	527	7.5	653	6.9	800	8.7	713
	0.50	7.2	620	7.6	657	7.2	663	—	—
	2.50	7.2	570	7.7	713	7.1	752	8.9	664
	25.00	—	—	—	—	—	—	8.9	703

\* Nitrogen liberated as NH<sub>4</sub><sup>+</sup>-N.

† Added to give 1000 ppm peptone-N.

from the data for NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N, ammonification of the native soil N was extensive. This was not so with the other soils, which showed low nitrifying capacity. Paraquat definitely depressed nitrification in the Chehalis silt loam. Less extensive nitrification of the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> occurred in the untreated sandy soil and addition of the herbicide increased it slightly. The effect of paraquat on nitrification of the native soil N during the incubation period for the respiration study also was negligible. The low rate of nitrification based on total nitrogen is not unusual for soils low in organic matter.

The sulphur-oxidizing power of the Chehalis soils and Delhi sandy loam ranged from approximately 12 to 28% (Table 4). However, in the Woodburn soil it was less than 9%. Generally speaking, paraquat slightly decreased the oxidation of added sulphur, especially in Chehalis silt loam. The effect was less obvious with the Delhi and Woodburn soils. In the silty clay loam paraquat at 0.5 ppm had almost negligible effect while 0.25 and 2.5 ppm were definitely depressive, indicating a possible bimodal or inversion effect.

The effect of paraquat on 0.25, 0.5 and 2.5 ppm on carbon dioxide evolution in the soil respiration experiments is presented in Fig. 1. The influence of the various treatments is shown as changes in total mg CO<sub>2</sub>-C evolved from 80 g



**Table 3**  
Effect of paraquat on nitrification in different soils

Treatment		CSCL				CSL				WSL				DSL		
Nitrogen* source	Paraquat (ppm)	pH	15 days $NH_4^+$ (ppm)	$NO_3^-$ (ppm)	30 days† $NO_3^-$ (ppm)	pH	15 days $NH_4^+$ (ppm)	$NO_3^-$ (ppm)	30 days† $NO_3^-$ (ppm)	pH	15 days $NH_4^+$ (ppm)	$NO_3^-$ (ppm)	30 days† $NO_3^-$ (ppm)	pH	15 days $NH_4^+$ (ppm)	$NO_3^-$ (ppm)
None	None	6.0	70	16	23	6.1	8	25	29	5.9	7	26	37	6.7	9	8
	0.25	6.0	67	15	23	6.2	40	26	22	5.9	25	26	43	6.7	14	8
	0.50	6.0	87	21	33	6.2	30	28	26	5.9	3	29	44	—	—	—
	2.50	6.0	57	9	30	6.2	28	25	29	5.9	4	20	42	6.7	17	7
	25.00	—	—	—	—	—	—	—	—	—	—	—	—	6.6	18	8
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	None	5.2	147	36	—	5.6	90	49	—	5.8	170	7	—	5.4	159	5
	0.25	5.2	103	89	—	5.7	123	14	—	5.8	180	2	—	5.6	169	9
	0.50	5.2	120	153	—	5.7	173	28	—	5.9	170	4	—	—	—	—
	2.50	5.2	120	155	—	5.7	132	25	—	5.9	190	0	—	5.4	147	15
	25.00	—	—	—	—	—	—	—	—	—	—	—	—	5.4	165	14
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

\* (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> added to give 200 ppm N.

† Nitrification of native nitrogen source after addition of paraquat.



soil except for the Warburg study with the Delhi soil in which only 4 g per flask were used. The measurements of oxygen consumption (Fig. 2) due to the effect of herbicide on the soil microflora give the same general results as the release of  $\text{CO}_2$ . A comparison of the  $\text{CO}_2$  output and  $\text{O}_2$  uptake of the untreated soil, and soil plus glucose shows that each of the soil types harboured an active microflora; response of respiratory activity to the glucose addition was vigorous.

Although initially depressive, paraquat increased  $\text{CO}_2$  evolution from the Chehalis silty clay loam, with 0.5 ppm more effective than the other rates. Similar results were obtained in the other soils, but in the sandy loam it was effective only at the higher rates. After 30 days incubation a pattern was observed between respiration and the oxidation of native organic N in that additional increases in microbial numbers and activity could result to some extent from the utilization of degradation products and bipyridylum ion.

The changes in pH induced by paraquat were minor. The other treatments especially sulphur, lowered pH, especially in the lighter soils.

Table 4  
Sulphur oxidation in different soils 30 days after treatment with paraquat

Treatment		CSCL		CSL		WSL		DSL	
Sulphur*	Paraquat (ppm)	pH	$\text{SO}_4^{--}\text{-S}$ (ppm)	pH	$\text{SO}_4^{--}\text{-S}$ (ppm)	pH	$\text{SO}_4^{--}\text{-S}$ (ppm)	pH	$\text{SO}_4^{--}\text{-S}$ (ppm)
None	None	6.1	33	6.2	24	5.8	25	6.6	9
	0.25	6.3	33	6.2	28	5.8	20	6.6	9
	0.50	6.3	29	6.2	30	5.8	20	—	—
	2.50	6.3	43	6.2	37	5.8	20	6.5	10
	25.00	—	—	—	—	—	—	6.5	18
Sulphur	None	4.9	275	4.7	219	5.9	87	3.9	116
	0.25	5.0	218	4.8	146	5.8	59	3.8	121
	0.50	4.9	266	4.7	140	5.8	70	—	—
	2.50	4.9	234	4.6	131	5.9	51	3.8	119
	25.00	—	—	—	—	—	—	3.9	116

\* Added to give 1000 ppm S.

In the Chehalis silty clay loam paraquat at all rates increased total moulds and percentage of *Penicillia* while decreasing mucors. Bacteria and *Streptomyces* were little changed. In Chehalis silt loam, mould counts were decreased but mucors increased at the expense of *Penicillia* while bacteria and *Streptomyces* were only slightly influenced.

The stimulating effect of intermediate concentrations, in contrast to the inhibitions obtained with lower as well as higher rates, is similar to results obtained in several investigations with fungicides (Kaars Sijpesteijn & VanDer Kerk, 1954; Kaars Sijpesteijn, Janssen & VanDer Kerk, 1956; 1957). Montgomery & Shaw (1943) found an inversion or polymodal effect with treatments of from 1 to 10 000 ppm of thiuram and dithiocarbamate fungicides. Kaars Sijpesteijn & VanDer Kerk (1954) showed that histidine or imidazole derivatives could antagonize the toxicity of dithiocarbamate to spores of *Penicillium italicum* and *Aspergillus niger* when sown on glucose agar containing various concentrations, and that growth inversion zones occurred at certain concen-



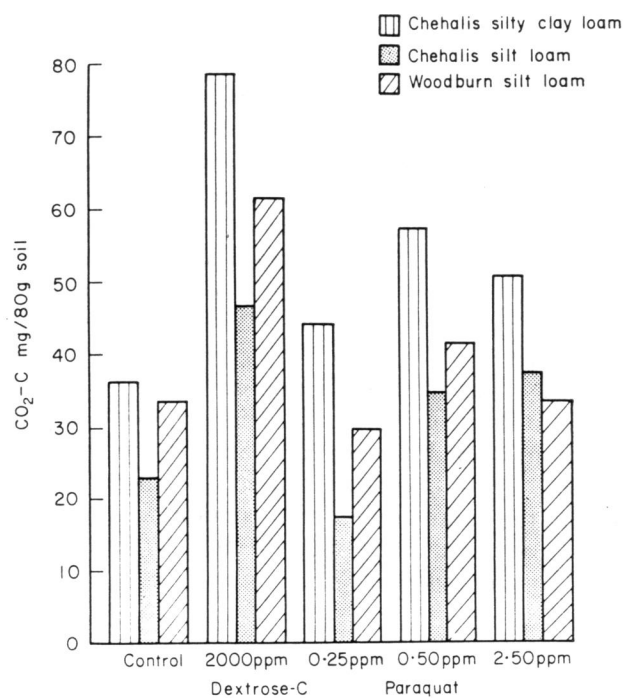


FIG. 1. Effect of paraquat on CO<sub>2</sub> evolution from different soils after 30 days incubation.

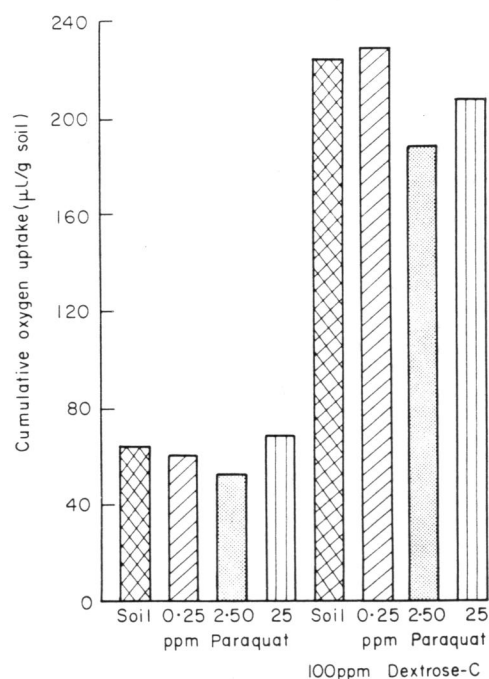


FIG. 2. Effect of paraquat in a manometric study of Delhi sandy loam after 46 hr incubation.



trations. Kaars Sijpesteijn *et al.* (1956; 1957) also showed that metal ions functioned as chelating agents in the fungitoxic action of dithiocarbamate and as components of the enzyme molecules involved. Metalloenzymes might therefore be competitively inhibited by the bipyridylium ion (Vallee & Hock, 1955). The observed inversion phenomenon with microbial growth, ammonification, nitrification, sulphur-oxidation and CO<sub>2</sub> production could also be due to an antagonistic metabolite of the herbicide as well as to chelating agents.

Possible antagonistic, additive and synergistic effects of wetters and other additives in pesticide formulations should not be overlooked. Preliminary studies with three of the soils previously mentioned (CSCL, CSL, WSL) were made with the wetter 'X-77' at 1 and 100 ppm, concentrations far in excess of any resulting from usual field applications. Except in one instance (WSL) where at 100 ppm it depressed CO<sub>2</sub> evolution, the wetter had only an insignificant influence on microbial numbers and activities in the soil.

From the results of this investigation it is concluded that if paraquat is used in accordance with the manufacturer's instructions for purposes of weed control, there will be no consequent effects of the herbicide on soil micro-organisms and their activities important to soil fertility.

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