

772

ORIGINAL -  
DO NOT REMOVE  
FROM FILE

Interaction between Paraquat and Microbes in soils

C. M. TU AND W. B. BOLLEN

Reprinted from  
*Weed Research*  
Vol. 8, No. 1, March 1968

BLACKWELL SCIENTIFIC PUBLICATIONS  
OXFORD AND EDINBURGH

## INTERACTION BETWEEN PARAQUAT AND MICROBES IN SOILS\*

C. M. TU† AND W. B. BOLLEN‡

*Department of Microbiology, Oregon State University, Corvallis, Oregon, U.S.A.*

**Summary.** Laboratory experiments were conducted to determine if paraquat had any effect on soil micro-organisms and consequently on soil fertility.

Numbers of bacteria and moulds tended to increase with incubation time as residual paraquat decreased. Lag periods of 1-5 days were observed in the development of soil microflora and concomitant degradation of paraquat. Under controlled culture conditions, *Aerobacter aerogenes*, *Agrobacterium tumefaciens*, *Pseudomonas fluorescens* and *Bacillus cereus* were able to utilize paraquat as sole carbon and nitrogen sources in synthetic media.

Paraquat at 0.25, 0.5, 2.5 and 25 ppm cation was added to four different soils to study changes in microbial activities concomitant with decreases in herbicide concentration. Analytical recoveries were correlated inversely with clay mineral content, cation-exchange capacity and organic matter.

Mineralization of peptone nitrogen by ammonification and nitrification was stimulated by 0.25 ppm; higher rates up to 25 ppm had little influence. A bimodal effect or growth inversion was observed in several instances, with increased microbial development at intermediate concentrations. Paraquat at recommended field rates appeared to have no appreciable influence on general microbial activities of importance to soil fertility.

### *Interaction entre le paraquat et les microbes dans les sols*

**Résumé.** Des expériences de laboratoire ont été réalisées pour rechercher si le paraquat avait un effet sur les micro-organismes du sol, et, par voie de conséquence sur sa fertilité.

Le nombre des bactéries et des moisissures tend à s'accroître avec le temps d'incubation, à mesure que la teneur en paraquat résiduel décroît. Des décalages de 1 à 5 jours furent observés entre le développement de la microflore du sol et la dégradation concomitante du paraquat. Dans des conditions contrôlées de culture, l'*Aerobacter aerogenes*, l'*Agrobacterium tumefaciens*, le *Pseudomonas fluorescens* et le *Bacillus cereus* furent capables d'utiliser le paraquat comme unique source de carbone et d'azote sur un milieu synthétique.

Le paraquat à raison de 0,25, 0,5, 2,5 et 25 ppm (en cation) fut ajouté à quatre sols différents pour étudier les changements des activités microbiennes en relation avec les diminutions de la concentration de l'herbicide. Les quantités retrouvées à l'analyse furent en corrélation inverse avec la teneur en argile, la capacité d'échange des cations et la teneur en matière organique.

La minéralisation de l'azote de la peptone par ammonification et nitrification fut stimulée par 0,25 ppm de paraquat; les doses supérieures eurent peu d'influence. Un effet bimodal ou une inversion de croissance furent observés dans plusieurs cas avec un développement microbien accru aux concentrations intermédiaires. Le paraquat, aux doses recommandées pour l'application en plein champ semble n'avoir pas d'influence appréciable sur les activités microbiennes générales importantes pour la fertilité du sol.

### *Interaktion zwischen Paraquat und Mikroorganismen im Boden*

**Zusammenfassung.** In Laboruntersuchungen wurde der Einfluss von Paraquat auf Bodenmikroorganismen und damit auf die Bodenfruchtbarkeit untersucht.

Die Zahl von Bakterien und Pilzen schien mit abnehmender Residualmenge von Paraquat während der Vegetationszeit zuzunehmen. Lagphasen von 1-5 Tagen wurden bei der Entwicklung der Bodenmikroflora und dem parallel gehenden Abbau von Paraquat beobachtet. In Vitro waren *Aerobacter aerogenes*, *Agrobacterium tumefaciens*, *Pseudomonas fluorescens* und *Bacillus cereus* in der Lage Paraquat als alleinige C- und N-Quelle im Medium zu verwenden.

\* Technical Paper No. 2317, Oregon Agricultural Experiment Station, Corvallis.

† Portion of a thesis submitted by the senior author in partial fulfillment of the requirement for the Ph.D. degree in Microbiology, Oregon State University. Present address: Research Institute, Canada Department of Agriculture, London, Ontario.

‡ Professor of Microbiology, Oregon State University, Corvallis, Oregon.

Um die Veränderung der mikrobiellen Aktivität zusammen mit der parallel gehenden Verminderung der Herbizidkonzentration zu untersuchen wurden 0,25, 0,5, 2,5 und 25 ppm Paraquat (Kation) vier verschiedenen Böden zugegeben. Die analytisch gefundenen Rückstandsmengen waren mit dem Tongehalt, der Kation-Austauschkapazität und der organischen Substanz negativ korreliert.

Die Mineralisation von Pepton-N durch Ammonifizierung und Nitrifizierung wurde durch 0,25 ppm Paraquat stimuliert; höhere Aufwandmengen bis zu 25 ppm hatten nur geringe Wirkung. Mehrmals wurde ein bimodaler Effekt oder eine Wachstumsinversion beobachtet, mit verstärkter mikrobieller Entwicklung bei mittleren Konzentrationen. In der empfohlenen Aufwandmenge hatte Paraquat offensichtlich keinen nennenswerten Einfluss auf solche mikrobiellen Aktivitäten, die für die Bodenfruchtbarkeit von Bedeutung sind.

## INTRODUCTION

Herbicides applied to soils may be decomposed by zymogenous organisms, decomposed chemically or photochemically, adsorbed by soil colloids in active or inactive form or volatilized. The relative importance of these factors will be determined by the nature of the herbicide, the composition of the soil and environmental conditions. Application of certain organic compounds to the soil may retard specific organisms or stimulate others which can utilize the applied material to support proliferation, thus leading to the disappearance of the chemical. Baldwin (1964) and Baldwin, Bray & Geoghegan (1966) found that many gram-positive bacteria in pure culture could effectively reduce 1,1'-dimethyl-4,4'-bipyridylium dichloride (paraquat) to the blue free radical, while *Corynebacterium fascians* (K 324) and *Clostridium pasteurianum* (K 219) destroyed it, both aerobically and anaerobically. *Corynebacterium fascians* (K 324) after 4 weeks training was able to grow in nutrient broth and in dextrose broth containing 10 000 ppm bipyridylium ion.

Rodriguez-Kabana, Curl & Funderburk (1966) studied the effect of paraquat on the growth of *Rhizoctonia solani* in Czapeck's liquid solution with 1 g/l of yeast extract, and found that inhibition of the fungus became more pronounced as the concentration of paraquat was increased. An initial lag or adaptation period of 10-15 days was followed by accelerated growth. The following investigation was conducted to study the interactions between soil micro-organisms and paraquat.\*

## MATERIALS AND METHODS

Microbial degradation of paraquat under controlled cultural conditions and the course of decline as revealed by chemical analysis was correlated with concomitant changes in microbial numbers, ammonification and nitrification in four soil types.

Inocula of *Pseudomonas fluorescens*, *Bacillus cereus*, *Aerobacter aerogenes*, *Agrobacterium tumefaciens* and two soil diphtheroids were introduced into synthetic media to determine whether they could utilize paraquat as a source of carbon or nitrogen.

The basal medium (Ayers, Rupp & Johnson, 1919) to which filter-sterilized

\* As di(methylsulphate); supplied by the manufacturer under the Trade Mark 'Gramoxone'. The formulation used contained a wetter supplied by the manufacturer under the Trade Mark 'X-77'.

paraquat at 10, 100 and 1000 ppm was added as the sole carbon source had the following composition:

NaNH <sub>4</sub> HPO <sub>4</sub> ·4H <sub>2</sub> O	1.0 g
KCl	1.0 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2 g
Brom-cresol purple	0.01 g
Distilled water	1000 ml
Adjusted to pH 7.0 with HCl solution	

The bacteria were first grown on nutrient agar slopes at 30° C for 18 hr. The resulting growth was scraped from the surface of the agar and suspended in 15 ml sterile normal saline solution prepared with distilled water. The organisms were washed three times by centrifugation and finally resuspended in 15 ml of normal saline. Three drops (approximately 0.25 ml) of each suspension were then added to tubes of the synthetic medium and also to control tubes containing only the basal medium. Tests were made in quadruplicate.

Availability of paraquat as a nitrogen source was determined similarly, using 10, 100 and 1000 ppm. The basal synthetic medium (Ayers *et al.*, 1919) contained dextrose as the carbon source:

K <sub>2</sub> HPO <sub>4</sub>	1.0 g
NaCl	0.2 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2 g
Dextrose	10.0 g
Distilled water	1000 ml
Adjusted to pH 7.0 with HCl solution	

All tubes were incubated at 28° C and observed daily during a period of 3 weeks for turbidity and development of a yellow colour indicating acid production. In doubtful cases, a loopful of sediment and medium was smeared on a clean glass slide, gram stained and examined under the microscope.

A series of 35 g, oven-dry basis, portions of each of four soils, Chehalis silty clay loam (CSCL), Chehalis silt loam (CSL), Woodburn silt loam (WSL) and Delhi sandy loam (DSL) (Tu & Bollen, 1968), were treated in two replications in duplicate with paraquat at the recommended field rate of 0.5 lb and at 1, 5 and 50 lb/ac cation calculated on the basis of 2 000 000 lb soil per 6.67 in.-ac to assess the concomitant effect of paraquat toxicity in soil. In one series, peptone at 1000 ppm N was added as nitrogen source for ammonification and nitrification. The experiment was set up in 1-pint milk bottles. After moisture had been adjusted to 50% of the water-holding capacity, the bottles were covered with Du Pont polyethylene film of 1.5 mil thickness and randomly incubated at 28° C. Appropriate controls were included. To allow time for the analytical work required, the series of treatments with each soil were made at different periods.

Samples of one replication were removed 0, 1, 5, 10 and 20 days after treatment for determination of pH, ammonium, nitrite and nitrate N using methods described in the previous paper (Tu & Bollen, 1968). Samples of 5 g from the control soils were immediately analysed to determine numbers of moulds; using

rose bengal-streptomycin agar (Martin, 1950), and for bacteria and *Streptomyces*, using sodium albuminate agar (Waksman & Fred, 1922).

The remainder of the soil sample was dried for 24 hr at 105° C for later determination of paraquat. The analytical method was that of Baldwin (1964) and Calderbank & Yuen (1965), modified slightly as follows:

Thirty g of the oven-dried soil were refluxed for 6 hr in a flat-bottom boiling flask with 60 ml 18 N H<sub>2</sub>SO<sub>4</sub>. After cooling, the mixture was diluted to 500 ml, filtered with suction on Whatman No. 5 filter paper, diluted to slightly less than 1 N acidity, and passed through 3.5 g of Dowex 50 W-XB (100–200 mesh) cation-exchange resin which had been prewashed with 25 ml saturated NaCl and 50 ml water. After adsorption on the resin the column was washed with 100 ml 1 N HCl, 100 ml of 2.5% NH<sub>4</sub>Cl and finally with 50 ml water. The paraquat was eluted with saturated NH<sub>4</sub>Cl into a 50 ml volumetric flask at a flow rate of about 1 ml/min. Difficulty was encountered in preparation of a straight base line for the bipyridylum standard curve until 0.2% sodium dithionite (Baldwin, 1964) was substituted for the 1% sodium dithionite used by Calderbank & Yuen (1965).

After shaking, a 15 ml aliquot of the eluate was transferred to a 25 × 10 mm test tube, 3 ml of 0.2% sodium dithionite in 1 N NaOH solution was added, followed by mixing. Within 5 min after adding the sodium dithionite, the concentration of bipyridylum ion was estimated by measuring the optical density in a Cary model 11 recording spectrophotometer, using a 50 mμ path cylindrical cell at 392, 396 and 400 mμ against a blank containing the same amount of dithionite solution in saturated NH<sub>4</sub>Cl solution.

The optical density of the sample at 396 mμ ( $A_{396}^*$ ) required correction by subtracting the means of the readings at 392 mμ ( $E_{392}$ ) and 400 mμ ( $E_{400}$ ) from the optical density at 396 mμ.

$$A_{396}^* = E_{396} - \frac{E_{400} + E_{392}}{2}$$

The bipyridylum content in ppm was then calculated as described by Calderbank & Yuen (1965). A standard curve showing paraquat ion concentration *v.* optical density was plotted from results obtained with a series of concentrations of paraquat dichloride recrystallized from a 98% technical grade.

## RESULTS AND DISCUSSION

With 10 ppm paraquat as the sole carbon source *Aerobacter aerogenes*, *Agrobacterium tumefaciens*, *Bacillus cereus* and *Pseudomonas fluorescens* were able to develop detectable growth in 21 days. At higher concentrations the herbicide apparently was toxic, no growth appearing up to 21 days. The appearance of turbidity at 21 days, but not before, indicated either very slow growth or a previous adaptation period.

Paraquat served well as a sole nitrogen source for certain bacteria. *Bacillus cereus* grew more rapidly with this source than did the other species. Only the soil diphtheroid 15a was not able to develop. Moreover, the growth with 100

\* Personal communication from Dr V. H. Freed, Department of Agricultural Chemistry, Oregon State University.

ppm paraquat showed that, except for the diphtheroid, the compound was non-toxic to the organisms tested. Good growth of *Aerobacter aerogenes*, *Agrobacterium tumefaciens*, *Bacillus cereus* and *Pseudomonas fluorescens* with 10 ppm paraquat as the nitrogen source suggests that these organisms may break it down in the soil.

The amount of paraquat recovered from the eluate in the analytical procedure does not represent the total amount in the sample taken for analysis because there are losses at the various stages of the assay. This amount has to be corrected by applying a factor dependent on the percentage recovery.

Different minerals have different adsorption and desorption properties (Harris & Warren, 1964). Adsorption is greater in soils high in organic matter and high in clay. The structure of the clay is partially destroyed by boiling with strong acid and the binding sites thus removed. The only effective means of displacing bipyridylum herbicides even from very sandy soil when they are present in relatively low concentrations, is to reflux for several hours with 9-18 N sulphuric acid. Baldwin (1964) recommended 12 N H<sub>2</sub>SO<sub>4</sub> at the rate of 10:1 v/w. The recovery obtained from the four soil types was far lower than by using 18 N H<sub>2</sub>SO<sub>4</sub> with 2:1 ratio. The percentage recovery appeared to be correlated with the properties of the four soils (Tu & Bollen, 1968, Table 1). Using this procedure, it was possible to recover quantitatively 1 ppm of the bipyridylum ion.

Table 1  
Recovery of paraquat added to different soils before incubation

Soil	Total carbon (%)	Clay (%)	Cation exchange capacity (m-eq/100g)	Paraquat added (ppm)				
				0	0.25	0.50	2.50	25.00
				Recovery (%)				
CACL	2.28	29.57	32	0	118	82	75	—
CSL	1.48	24.69	25	0	129	96	91	—
WSL	1.55	21.45	16	0	151	107	99	—
DSL	0.72	12.00	3	0	159	—	101	77

Before the method was routinely applied a series of recovery tests was carried out on samples treated with varying amounts of recrystallized paraquat. Routine application of this method gave 75-159% recovery in the soils, based on the concentrations added (Table 1). Recovery was higher from soil of lower adsorptive capacity, which depends upon amount and type of clay and organic matter. Thus, the increase in order of recovery was from the Chehalis silty clay loam to the Delhi sandy loam. The silty clay loam and Chehalis silt loam contains mostly montmorillonitic and illitic clays (Harward, Theisen & Evans, 1962) which are more adsorptive than kaolinite, the predominant clay in Woodburn silt loam. Our recovery values of more than 100% are attributable to analytical limitations of the method. The recovery values for 0.25 ppm added paraquat varied with the soil and ranged from 118 to 159% (Table 1). At higher rates of paraquat the recoveries were usually less than 100%, probably for reasons already mentioned. In the present work we consider these variations to be of minor importance.



Paraquat recoveries from the four soil types after 20 days incubation are shown in Table 2. In general, the amounts found, referred to the applied concentration (Table 2) decreased with time, the decreases being proportionally greater for higher concentration. Because the adsorption process rapidly proceeds to completion (Baldwin, 1964), decreases in herbicide concentrations after 1 day can be attributed partly to microbial decomposition. The decreases were greater in the Chehalis silty clay loam and Woodburn silt loam.

The differences in concentration of organic matter, clay content and the cation-exchange capacity in the four soils offer an explanation for the differences in residual concentrations of herbicide found. Possibly some other physico-chemical factors of the soil may influence the recoveries, especially after incubation. It seems unlikely that moisture variation during incubation could be responsible because the containers were tightly covered with 1.5 ml polyethylene film, which is permeable to air but not to water vapour.

Table 2  
Effect of paraquat on ammonification and nitrification of 1000 ppm added peptone nitrogen in four soils after different periods of incubation

Soil	Paraquat added (ppm)	Paraquat found (ppm)				Ammonification (%)				Nitrification (%)			
		1 day	5 days	10 days	20 days	1 day	5 days	10 days	20 days	1 day	5 days	10 days	20 days
CSCL	0	0	0	0	0	43	58	62	63	9	4	12	35
	0.25	0.19	0.16	0.23	0.22	39	52	55	68	10	5	13	38
	0.50	0.36	0.28	0.33	0.31	48	57	58	57	9	5	17	37
	2.50	1.74	1.55	1.41	1.36	50	64	52	65	8	5	10	38
CSL	0	0	0	0	0	53	68	65	54	2	3	3	4
	0.25	0.23	0.24	0.22	0.20	54	66	74	60	2	3	3	12
	0.50	0.52	0.40	0.35	0.38	45	59	74	51	2	2	2	4
	2.50	2.44	1.92	2.00	1.98	51	69	69	55	2	2	3	8
WSL	0	0	0	0	0	32	53	55	66	0	0	2	5
	0.25	0.24	0.20	0.23	0.19	27	48	54	51	0	0	2	5
	0.50	0.40	0.36	0.56	0.34	28	54	52	47	0	0	2	4
	2.50	1.62	2.05	1.94	1.83	32	49	45	59	0	0	2	4
DSL	0	0	0	—	0	40	70	—	57	0	0	—	0
	0.25	0.25	0.26	—	0.24	49	71	—	49	0	0	—	0
	2.50	2.54	2.01	—	1.91	43	66	—	49	0	0	—	0
	25.00	23.60	22.00	—	21.00	47	70	—	43	0	0	—	0

Bailey & White (1964) found that the adsorption and desorption of bipyridyls by soil was correlated with the organic matter content and that adsorption increased as pH decreased.

Ammonification and nitrification of native organic matter did not show any appreciable changes. The transformations of added peptone are summarized in Table 2.

Paraquat has no marked or consistent influence on ammonification or nitrification; the increases and decreases shown are considered relatively minor. In Chehalis silty clay loam some depressions by paraquat appeared, more so during the early stages of incubation. Some apparent bimodal effects mentioned in the previous paper (Tu & Bollen, 1968) are again evident, 0.5 ppm being depressive while 0.25 and 2.5 ppm seem slightly stimulating. In all cases

ammonium accumulated because ammonification is more rapid and less sensitive than nitrification to environmental factors, especially acid, alkaline or anaerobic conditions. In acid environments, as in the Woodburn silt loam, nitrification proceeds slowly even in the presence of an adequate supply of substrate. Paraquat effected no significant changes in pH, which in a few cases varied from the controls by only 0.1 or 0.2 during incubation. However, ammonium production from the added peptone increased pH by 1-2 units. This favoured nitrification in the acid soils, especially in CSCL, but in the Delhi soil (pH 6.8) which has low buffer capacity, ammonium production changed the pH to 8.8 after 1 day's incubation and to 9.0 at 20 days. This prevented nitrification, which is inhibited, especially by free ammonium, above pH 8.

The paraquat adsorbed on montmorillonite clay apparently is not available to the ammonifiers, nitrosifiers, and nitrifiers. The small amount adsorbed on the surface of montmorillonite clay particles, although it may be available to the microbes, is not significantly inhibitory to the soil microflora. This is in agreement with the desorption studies of bipyridylum by Weber, Perry & Upchurch (1965) and Weber & Scott (1966). They found that when the herbicide was adsorbed on montmorillonite clay, only 5% was removed by four extracts with 1 M BaCl<sub>2</sub>, as compared to 80% when the herbicide was adsorbed on kaolinite clay and treated in the same fashion.

Changes in the microbial population with the different concentrations of paraquat were neither appreciable nor consistent. Numbers of moulds and bacteria tended to increase with incubation time, as could be expected. *Streptomyces* percentages in Chehalis silty clay loam and in the Woodburn soil gradually increased from an initial depression to approximately the 0-day values. Changes in the principal fungal genera were minor and therefore disregarded. Most of the microbes in the soil adapted in 1-5 days. The apparent 'inversion' phenomenon or bimodal effect noted in the nitrogen transformation was also represented in the changes in microbial numbers.

Changes in microbial types as well as numbers following any partial soil sterilization by herbicide treatments will result because the dead cells of soil organisms provide a readily available energy material which results in increased microbial activity. After these energy sources are depleted, some of the organisms will then attack less available carbon sources, perhaps including the herbicide.

Because the composition of soils varies so greatly, organic matter may be responsible for adsorption in some soils and clay minerals in others. Moreover an adsorbed herbicide might be released by microbial secretion of organic compounds that could exchange with the adsorbed herbicide, thus making it available to the soil microflora. While many of the micro-organisms present in the soil may not be able to utilize the original herbicide, it is possible that they can metabolize one or more products of partial degradation.

#### CONCLUSION

Soil type as determining cation-exchange capacity, amount and kind of clay, and content of organic matter, influences analytical recovery of added paraquat.



Incomplete recoveries after incubation are attributed in part to adsorptive properties of these soil factors, and in part to microbial degradation of the herbicide.

Excessive as well as normal rates of application of paraquat added to the soils studied produced no serious changes in microbial activities important to soil fertility.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the helpful advice of Dr C. R. Harris, Head, Pesticide Section, Research Institute, Canada Department of Agriculture London, Ontario, in the preparation of this paper.

This investigation was supported in part by the Chevron Chemical Company, Ortho Division, Richmond, California.

## REFERENCES

- AYERS, S. H., RUPP, P. & JOHNSON, W. T. (1919) A study of the alkali-forming bacteria in milk. *Bull. U.S. Dep. Agric.*, 782.
- BAILEY, G. W. & WHITE, J. L. (1964) Soil-pesticide relationships. Review of adsorption and desorption of organic pesticides by soil colloids with implications concerning pesticide bioactivity. *J. agric. Fd Chem.*, 12, 324-332.
- BALDWIN, B. C. (1964) Paraquat and the soil. In: *Report of progress to October 1964*, pp. 18. California Chemical Co., San Francisco, California.
- BALDWIN, B. C., BRAY, M. F. & GEOGHEGAN, M. J. (1966) The microbial decomposition of paraquat. *Biochem. J.*, 101, 15.
- CALDERBANK, A. & YUEN, S. H. (1965) An ion-exchange method for determining paraquat residues in food crops. *Analyst, Lond.*, 90, 99-106.
- HARRIS, C. I. & WARREN, G. F. (1964) Adsorption and desorption of herbicides by soil. *Weeds*, 12, 120-126.
- HARWARD, M. E., THEISEN, A. A. & EVANS, D. D. (1962) Effect of iron removal and dispersion methods on clay mineral identification by X-ray diffraction. *Proc. Soil Sci. Soc. Am.*, 26, 535-541.
- MARTIN, J. P. (1950) Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. *Soil Sci.*, 69, 215-232.
- RODRIGUEZ-KABANA, R., CURL, E. A. & FUNDERBURK, H. H. JR. (1966) Effect of four herbicides on growth of *Rhizoctonia solani*. *Phytopathology*, 56, 1332-1333.
- TU, C. M. & BOLLEN, W. B. (1968) Effect of paraquat on microbial activities in soils. *Weed Res.*, 8, 28-37.
- WAKSMAN, S. A. & FRED, E. B. (1922) A tentative outline of the plate method for determining the number of microorganisms in the soil. *Soil Sci.*, 14, 27-28.
- WEBER, J. B., PERRY, P. W. & UPCHURCH, R. P. (1965) The influence of temperature and time on the adsorption of paraquat, diquat, 2,4-D and prometon by clays, charcoal, and anion-exchange resin. *Proc. Soil Sci. Soc. Am.*, 29, 678-688.
- WEBER, J. B. & SCOTT, D. C. (1966) Availability of a cationic herbicide adsorbed on clay minerals to cucumber seedlings. *Science, N.Y.*, 152, 1400-1402.

(Received 4th July 1967)

