J. Pesticide Sci. **17**, 137–139 (1992)

Note

Microflora in Soils with Long-term Application of Paraquat

Arata KATAYAMA
and Shozo KUWATSUKA

Laboratory of Soil Science, School of Agriculture, Nagoya University, Chikusa-ku, Nagoya 464–01, Japan

(Received September 28, 1991;
Accepted December 16, 1991)

INTRODUCTION

It is generally believed that the herbicide paraquat is quickly inactivated after being incorporated into soil and becomes unavailable to microorganisms since it is tightly adsorbed to soil.[1] It is expected that the adsorbed paraquat persists indefinitely. In fields to which paraquat had been applied for a long period, however, the concentration in soil did not increase so much as expected in spite of repeated applications[2] and decreased after stopping the application.[3] These observations suggested that the paraquat adsorbed to soil was gradually degraded by microorganisms during the repeated applications. Our study was aimed to elucidate the interaction between adsorbed paraquat and microorganisms in soil during long-term application. As a preliminary experiment, we compared microflora in soils treated with paraquat for a long time and those in untreated soils.

MATERIALS AND METHODS

Ibaraki applied- and control-plot soils were collected from the plow layer of paddy fields under nonflooded conditions in May at the Research Institute of the Japan Association for Advancement of Phyto-regulator, Ushiku, Ibaraki Prefecture. In the same manner, Miyagi applied- and control-plot soils were collected from paddy fields of Miyagi Agricultural Center, Miyagi Prefecture, and Saga applied- and control-plot soils from paddy fields of Saga Agricultural Center, Saga Prefecture. The applied-plot soils had been treated with paraquat dichloride (960 g-a.i./ha, 4 *l*/ha as GRAMOXONE®) once a year for 13 years.[3] The control-plot soils had not been treated with any herbicides, where weeds had been removed manually.

The soils were passed through a 2-mm sieve and the moisture content was adjusted to 60% of the maximum water-holding capacity. The moist soils were preincubated at 30°C for 1 week to allow for equilibration. Then, microorganisms in the soils were enumerated with a dilution plate technique. A sodium albuminate agar[4] was used for bacteria and actinomycetes, and a Martin's rosebengal agar[4] for fungi. To count paraquat-tolerant microorganisms, paraquat was added to the media in the range from 1 to 1000 mg/*l*.

The paraquat-dissipation ability of soil was examined by measuring the dissipation of paraquat in various media (100 ml) inoculated with a soil suspension (0.1 mg-soil in 1 ml sterile distilled water) of Ibaraki or Saga soils. The media used were 1/10 diluted nutrient broth (Eiken Chemical, Tokyo), Czapek-dox broth[4] supplemented with yeast extract (0.5 g/*l*), paraquat N-source medium[5] and paraquat C-source medium. The paraquat C-source medium consisted of 0.5 g/*l* of K_2HPO_4, 0.5 g/*l* of $NaNO_3$, 0.2 g/*l* of $MgSO_4 \cdot 7H_2O$, 0.01 g/*l* of $FeSO_4 \cdot 7H_2O$ and paraquat dichloride (pH=7). The paraquat concentration in all media was 100 mg/*l* as paraquat dichloride. After incubation on a reciprocal shaker at 28°C for 19 or 48 days, the cultures were centrifuged at $10,000 \times g$ for 30 min, and the remaining paraquat in the supernatant was measured colorimetrically.[6] The cultures inoculated with autoclaved soil suspensions served as controls.

RESULTS AND DISCUSSION

Table 1 shows the population of microorganisms able to grow on the paraquat-containing media, designated as paraquat-tolerant microorganisms, in Ibaraki applied- and control-plot soils. With an increase in the paraquat concentration in the media, the population of microorganisms able to grow decreased. The decrease was more drastic for bacteria and actinomycetes than for fungi. There was no significant difference in the population of paraquat-tolerant microorganisms in the applied-plot and the control-plot soils. The results were also identical in the Miyagi and Saga soils.

It should be noted that microorganisms tolerant to 1000 mg/*l* paraquat were present in large numbers even in the control-plot soils to which paraquat had not been applied. This indicated that many soil microorganisms original-

Table 1 The population of microorganisms able to grow in paraquat-containing media in Ibaraki applied- and control-plot soils.

Paraquat concentration (mg/*l*)	Bacteria (10^6 cfu/g[a])		Actinomycetes (10^5 cfu/g)		Fungi (10^3 cfu/g)	
	Applied	Control	Applied	Control	Applied	Control
0	74	58	11	12	59	46
1	58	49	19	23	68	51
10	35	27	16	8	56	38
100	15	11	9	8	25	28
1000	2	2	<1	<1	12	2

[a] cfu/g: colony forming unit per g of soil on a dry-weight basis.

ly had protection mechanisms against paraquat toxicity at a fairly high concentration of 1000 mg/*l*. Reports say that two bacteria *Escherichia coli* K-12 W3110 and *Pseudomonas* sp. strain TTO1 and a yeast *Lipomyces starkeyi* (Lod & Rij) were tolerant to paraquat higher than 1000 ppm in cultures.[7] Fungi *Aspergillus niger* and *Penicillium frequentans* are also reported to have been tolerant to 2000 ppm paraquat.[8] However, no report has yet shown the abundance of paraquat-tolerant microorganisms in soil.

The concentrations of paraquat in the applied soils used in this study ranged from 6 to 18 mg/kg-soil.[3] This range of concentrations in the applied soils was too low to affect the population of paraquat-tolerant microorganisms in the applied soils.

The paraquat-dissipation ability did not differ in the applied-plot and the control-plot soils when examined in Saga and Ibaraki soils. Compared to autoclaved control cultures, more than 85% of paraquat dissipated only in the paraquat N-source medium inoculated with Saga control-, Ibaraki applied- and Ibaraki control-plot soils. Such dissipation was not always observed when experiments were repeated, which suggested that there was a small population of paraquat-degrading microorganisms in the soils. The degrading microorganisms in cultures of the three soil suspensions showing dissipation ability were enriched and isolated as reported in previous paper,[9] except for that the paraquat dichloride concentration was 100 mg/*l*. From each of the three soils, a yeast was isolated, which completely degraded paraquat as the sole source of nitrogen. The three strains were all identified as *Lipomyces* sp. by the morphological feature of asci developed from active buds and other taxonomic experiments.[10] It has been reported that *Lipomyces starkeyi* degraded paraquat completely in a pure culture.[5] No other paraquat degrader was isolated from the cultures in this study, although various bacteria and fungi have been reported as paraquat degraders.[8,9,11-13] The paraquat-degrading microorganisms in Ibaraki applied- and control-plot soils were enumerated by the most probable number method using paraquat N-source medium. The population was less than 100 cells/g-soil in both plot soils, which was the detection limit of the method used. These results suggested that *Lipomyces* spp. were present both in the applied- and control-plot soils but at very low population levels.

In our experiments the population of paraquat-tolerant microorganisms in the applied-plot soils was not significantly different from that in the control-plot soils. The paraquat dissipation by soil microorganisms was not enhanced, nor the paraquat-degrading microorganisms was enriched in the applied-plot soils. The long-term application of paraquat had no effect on microflora in these soils.

ACKNOWLEDGMENTS

We thank ICI Agrochemicals for their offer of paraquat dichloride and the reference culture of *Lipomyces starkeyi*. Our thanks are also due to Mr. C. Maruyama, Mr. Y. Matsuoka and Mr. Y. Furuya for their technical assistances.

REFERENCES

1) J. D. Fryer, R. J. Hance & J. W. Ludwig: *Weed Res.* **15**, 189 (1975)
2) R. J. Hance, T. H. Byast & P. D. Smith: *Soil Biol. Biochem.* **12**, 447 (1980)
3) J. Kanazawa (Nihon Shokubutsu Chosetsuzai Kenkyukai): *Shokucho* **24**, 388 (1990) (in Japanese)
4) H. Kondo & K. Kato: "Experimental Methods for Soil Microorganisms," ed. by Soil Microbiological Society of Japan, Yokendo, Tokyo,

pp. 21–26, 1975 (in Japanese)
5) J. R. Anderson & E. A. Drew: *J. Gen. Microbiol.* **70**, 43 (1972)
6) A. Calderbank & S. H. Yuen: *Analyst* **90**, 99 (1965)
7) R. J. G. Carr, R. B. Bilton & T. Atkinson: *Appl. Environ. Microbiol.* **52**, 1112 (1986)
8) S. N. Smith, A. J. E. Lyon & I. Bin Sahid: *New Phytol.* **77**, 735 (1976)
9) Y. Imai & S. Kuwatsuka: *J. Pesticide Sci.* **14**, 475 (1989)
10) J. Lodder: "The Yeasts, A Taxonomic Study," 2nd Ed., North-Holland Publishing Company, Amsterdam, London, pp. 379–402, 1970
11) B. C. Baldwin, M. F. Bray & M. J. Geoghegan: *Biochem. J.* **101**, 15p (1966)
12) R. G. Burns and L. J. Audus: *Weed Res.* **10**, 49 (1970)
13) C. M. Tu and W. B. Bollen: *Weed Res.* **8**, 38 (1968)

要　約

パラコート長期連用土壌中の微生物相

片山新太，鍬塚昭三

日本植物調節剤研究協会研究所，佐賀県農業試験場および宮城県農業センターの3か所の水田圃場のパラコート連用区と無施用区の間で微生物相が比較された．0から1000 mg/*l* のパラコートを含む培地上で生育する微生物数には連用区・無施用区土壌間で差がなかった．パラコート濃度の増加につれ生育する菌数，とくに細菌数と放線菌数が減少した．無施用区土壌中にも1000 mg/*l* のパラコート濃度に耐性の菌が多く存在した．パラコートを唯一の窒素源とする培地に佐賀連用区土壌，茨城連用区・無施用区土壌の懸濁液を接種した場合にのみ，培地中のパラコートが有意に減少した．これらの培養液からパラコート分解能を有する *Lipomyces* 属酵母が単離された．パラコート分解菌数は茨城連用区・無施用区土壌中ともに100個/g土壌以下であった．土壌微生物相に対するパラコート長期連用の影響はないものと推察された．