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INTERACTIONS OF BIPYRIDYLIUM HERBICIDES AND SOIL MICROORGANISMS

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Abstract

By enrichment and dilution-plate techniques several organisms tolerant to 5,000 ppm of diquat were isolated from seven soil samples. After two weeks incubation of the microorganisms, aliquots of the synthetic medium containing ^{14}C-diquat were spotted on silica gel plates for thin-layer electrophoresis. Autoradiographs of the plates showed three degradation products, one of which was a negatively charged compound. *Lipomyces starkeyi* was studied further to determine the effect of incubation period on the amount and types of degradation products of diquat and paraquat. Autoradiographs of these plates indicated that there were three additional radioactive spots after four-day incubation period. Results from the paraquat study were the same as from the diquat study except there were only two degradation products. Degration of the herbicides was shown to be enzymatic. Results from Warburg studis indicated that diquat decreases the oxygen uptake of actively respiring yeast cells and is not utilized as a carbon source by the resting cells. Growth chamber studies showed that the yeast which had been incubated for four days had the highest CO_2 release, and the six-day-old yeast had the greatest growth. In addition, the yeast utilized diquat as a sole source of nitrogen.

Introduction

Several reviews on the effects of bipyridylium herbicides on soil microorganisms (Bozarth, 1966; Baldwin *et al.*, 1966) and the effect of soil microorganisms on the herbicides have been reported (Funderburk, 1969; Kuwatsuka and Niki, 1976). Microorganisms have been isolated from a wide variety of soils by means of enrichment techniques (Bozarth, 1966). Some soils have been treated before sampling with the herbicides for different periods of time, and it has been found that some soil-isolated microorganisms can utilize the herbicides as a sole source of carbon or nitrogen in synthetic liquid media (Bozarth, 1966).

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Rodriguez-kabana *et al.* (1967) studied the effect of paraquat on the growth of *Rhizoctonia solani* in synthetic media and found that the effect on dry weight of the fungus became more pronounce as the concentrations of paraquat were increased from 6.25 to 100 ppm. Bozarth *et al.* (1966) studied the effect of paraquat on respiration of the isolated bacterium, *Pseudomonas sp.* by means of conventional Warburg techniques and found that the herbicide had no apparent effect on the oxygen uptake of actively respiring cells at concentrations up to 1,000 ppm. It was also found that the bacterium could not utilize paraquat as a sole carbon source. Recently, Grossbard and Davies (1976) demonstrated that paraquat decreased microbial growth in soil and pure culture. Mathur *et al.* (1976) also found that paraquat generally increased the bacterial and actinomycetal populations in an organic soil with little differences in levels of available N and P, and in the CO_2 evolution rate.

Several soil-isolated microorganisms in pure culture (Bozarth, 1966; Baldwin *et al.*, 1966; Funderburk, 1969; Anderson and Drew, 1972) have been previously shown to degrade free paraquat. Fryer *et al.* (1975) analyzed the soil samples from a long term field experiments in a sandy loam soil and concluded that paraquat may be broken down only slowly if at all in the soil under field condition. This reserarch was conducted to determine if diquat and paraquat can be degraded by soil microorganisms and to investigate the effect of diquat on the microorganisms.

Materials and Methods

Materials

The technical grade dibromid salt of diquat and dichloride salt of paraquat were used in all experiments, and all concentrations were calculated on the basis of the cation and not the salt form. When radioactive material was employed, the ^{14}C-ethylene-labeled diquat (specific radioactivity=1.0 mc/gm) and ^{14}C-methyl-labeled paraquat (specific radioactivity=0.8 mc/gm) were used. The soils used for the experiments were: (1) Cahaba Loamy Fine Sand, high in nutrients, collected from a cultivated cotton field at the Plant Breeding Station in Tallassee, Alabama, (2) sandy loam, low in nutrients, collected from a cultivated cotton field at Auburn University, and (3) sandy loam soil from five variously treated plots of the Callars rotation experiments of the Department of Agronomy and Soils, Auburn University. These plots had been rotated with cotton, winter legume, corn or oats, and crotalaria, and had received fertilizer treatments as indicated in Table 1 since 1956.

A stock culture of the soil yeast, *Lipomyces starkeyi* was obtained from the Imperial Chemical Company at Jeolett's Hill Experimental Station in

England. Strains of the genus *Lipomyces* have been isolated from soil from various countries and also from human skin (Cook, 1958).

Table 1. *Treatments received by soil plots*[1]

Plot	Fertilizer	Legume
A	LPK	Legume
C	O	No legume
2	LKN	Legume
8	LKN	Legume (P from triple super-no sulfur)
10	LPKN	Legume plus minor elements

(1) L is 2,000 pounds dolomite on all three tiers first year and afterward each three years.
P is 200 pounds P_2O_5 applied to cotton crop.
K is 200 pounds K_2O applied to cotton crop.
N is 120 pounds N from ammonium nitrate or urea applied to cotton and corn and 60 pounds N from nitrate of soda applied to oats.
Minor elements (all broadcast at turning for cotton) 5 pounds copper sulfate, 10 pouns manganese sulfate, 10 pounds selenite, 15 pounds zinc sulfate, and 0.5 pound sodium molybdate.

Isolation of diquat-tolerant microorganisms from soils

Forty-two 250-ml Erlenmeryear flasks containing 50 ml of liquid medium, described in Table 2, were stoppered with foam plugs and autoclaved for 20 minutes at 20 psi. After cooling, six flasks were inoculated with 2 gm soil from

Table 2. *Modified Czapek's sucrose agar for culture of soil fungi and bacteria*[1]

Chemicals	Concentration (g.)
K_2HPO_4	1.000
$MgSO_4 \cdot 7H_2O$	0.500
KCl	0.500
$FeSO_4 \cdot 7H_2O$	0.010
Sucrose	30.000
$ZnSO_4 \cdot 7H_2O$	0.010
$MnSO_4 \cdot H_2O$	0.006
Agar[2]	15.000
Water	1,000.000

(1) This medium was used for substrated nitrogen nutrient, in which the pH of the medium solution for bacteria culture was 6.5 and the pH for fungi culture was 4.0.
(2) Agar was omitted for liquid culture.

the seven different sources described previously and incubated for two weeks at 30°C.

The cultures from each soil source were then thoroughly mixed, and 0.2 ml of the suspension was added to each of 42 petri dishes containing agar medium, as described in Table 2. These plates were incubated for two weeks at 30°C. Microorganisms that appeared as colonies on the plates were then transferred to three petri dishes containing the same medium but to which had been added 500 ppm diquat.

The microorganisms which grew on 500 ppm diquat were transferred to agar medium containing 1,000 ppm diquat, and the organisms which grew on these plates were transferred to plates containing 5,000 ppm diquat. Each of the four organisms which grew best on this last medium was transferred to separate flasks containing 50 ml of liquid medium (described in Table 2) and 5,000 ppm diquat. These cultures were incubated for two weeks at 30°C.

Degradation of radioactive diquat and paraquat by the microorganisms

Twenty-one flasks with the good medim or poor growth of soil microorganisms from enriched culture procedures were chosen for this study. Two flasks per soil sample, each containing 50 ml of liquid medium (Table 2), with 4,960 ppm unlabled and 40 ppm radioactive diquat were inoculated with 0.2 ml of a cell suspension of each microorganism. One additional flask was treated in a similar manner but was not inoculaed; this served as a check for nitrogen utilization. The flasks were then incubated for two weeks at 30 C. After a two-week incubation period, the culture solutions were frozen, then removed and ground with a mortar and pestle. The solutions were then centrifuged for 20 minutes at 15,000 xg. The supernatant was decanted and saved. Five milliliters of distilled water were added to each residue, and this was frozen, ground, and centrifuged as previously described. The solutions were condensed under vacuum refrigeration to about 5 ml, and a 10-μl aliquat from each of the condensed solutions was spotted on plates for thin-layer electrophoresis as described by Pate and Funderburk (1965). Parameters for electrophoresis were 250 microns of silica gel on 8-x-10-inch glass run in two per cent lutidine and four per cent glacial acetic acid in water at 500 volts for 1.5, 2.0, or 2.5 hr. The plates were then exposed to Kodak "no screen" x-ray film for approximately two weeks after which the film was developed.

Aliquots were taken from the culture solutions before, during, and after the experiment was completed and were radioassayed in a Bechman liquid scintillation spectrometer.

The yeast, *L. starkeyi*, was used to determine the effect of incubation period on the amount and kinds of degradation products of diquat and paraquat.

This involved growing the yeast in liquid medium containing 40 ppm of radioactive diquat or paraquat for 2, 4, 8, and 16 days (Table 3). After the prescribed time periods, the cultures were frozen, ground, centrifuged, condensed, and radioassayed as previously described. An aliquot of 10 μl of 5,000 ppm diquat or paraquat from each treatment was spotted on thin-layer plates coated with silica gel-G for thin-layer electrophoresis (TLE) or spotted on thin-layer plates coated with plain cellulose (250 microns in thickness) for thin-layer chromatography (TLC). The TLE plates were developed one dimensionally in n-butanol, water, and glacial acetic acid (4:2:1, v/v/v). The plates were also exposed to "no screen" x-ray film for approximately two weeks and developed.

Table 3. *Medium used for Lipomyces starkeyi study*

Chemical	Concentration (g.)
KH_2PO_4	1.00
KCl	0.20
$MgSO_4 \cdot 7H_2O$	0.20
$FeSO_4 \cdot 7H_2O$	0.01
Sucrose	3.00
Water	1,000.00

In an attempt to study chemical or enzymatic degradation of dipyridyls by yeast, the seven-day-old culture of *L. starkeyi* was boiled five minutes and then treated with 40 ppm of radioactive diquat or paraquat. This culture was further incubated for eight days at 30°C.

Effect of diquat on the respiration of ***Lipomyces starkeyi***

Five hundred ml of the medium (described in Table 3) were placed in 2,800-ml flasks, stoppered, and autoclaved. The medium was then inoculated with a dilute cell suspension of *L. starkeyi* and incubated for four days at 30°C.

Oxygen uptake by actively respiring cells was measured by conventional Warburg techniques (Umbreit *et al.*, 1964). Cells were harvested by centrifugation at 2,000 xg for 10 minutes at 10°C. The main compartment of the Warburg flask received 2.8 ml of liquid medium containing the cells (30 mg dry cells per ml). The center well received 0.2 ml of 2N KOH, and the sidearm received 1.0 ml of diquat dibromide solution to give the desired final concentration (40, 100, 1,000, and 5,000 ppm diquat) in the main compartment of the flask after tipping. Flasks were allowed to equilibrate for 10 minutes at which time the readings were begun, and after 10 additional minutes the contents

of the sidearms were tipped into the main compartment. Temperature of the water bath was maintained at 30°C.

Oxygen uptake by resting yeast cells was also measured using Warburg techniques. Cells were harvested by centrifugation at 2,000 xg for 10 minutes, washed twice with 0.05 M phosphate buffer (pH 6.5) and resuspended in the buffer to give about 100 mg dry cells/ml cell suspension. The center well of the Warburg flasks received 0.2 ml of 2 N NaOH; 2.8 ml of the cell suspension were placed in the main compartment; and the sidearm received 1.0 ml of substrate (1.2 per cent sucrose or 6,000 ppm diquat). Temperature of the water bath was maintained at 30°C, and 10 minutes after equilibration the contents of the sidearms were tipped into the main compartment. Readings were taken at 10-minute intervals for seven hours.

In both Warburg experiments, all treatments were run in duplicate, and each experiment was repeated at least twice.

Utilization of diquat by ***Lipomyces starkeyi***

The following basic medium was used in this study: 1.00 g KH_2PO_4, 0.2 g KCl, 0.20 g $MgSO_4$, 0.01 g $FeSO_4$, and 1,000 ml distilled water. In addition, the basic medium was supplemented with: 1.0 g NH_4NO_3 plus 3.0 g sucrose (N+C) as the complete medium for *L. starkeyi*; 3.0 g sucrose (−N+C) was used as the minus nitrogen medium; 3.0 g sucrose plus 2,300 ppm diquat (−N+C+D) was used to study diquat utilized as a nitrogen source; 1.0 g NH_4NO_3 (N−C) was used as the minus carbon medium; and 1.0 g NH_4NO_3 plus 1,500 ppm diquat (N−C+D) was used to study diquat utilized as a carbon source. In these five different treatments, 1,500 ppm diquat was equivalent to 3.0 g sucrose as a carbon source, and 2,300 ppm diquat to 1.0 g NH_4NO_3 as a nitrogen source.

Sixty ml of the medium were placed in 300-ml Nephelo culture flasks, stoppered, and autoclaved. The medium was then inoculated with cells of the yeast and incubated at 28°C. The optical density (O.D.) of the culture was determined with a Bausch and Lomb Spectronic 20 Spectrophotometer at 450 nm, at intervals of 0, 2, 4, 6, 8, 10, 12, and 14 days after inoculation. Dry weight of the cells was obtained by comparing the O.D. of the culture with that in the standard growth curve of the yeast cells. The standard curve was made by determining the relationship of O.D. to dry weight of a cell suspension of the yeast. In this procedure, two, 20-ml aliquots of cell suspension were filtered through Whatman No. 1 filter paper, and the cells were dried in an oven at 100°C over night, then weight. The O.D. of the cell suspensions was also determined with a Bausch and Lomb Spectronic 20 Spectrophotometer at 450 nm.

CO_2 production by ***Lipomyces starkeyi***

The 500-ml flasks were fitted with two-hole, No. 7 rubber stoppers. A

short piece of glass tubing filled with loosely packed cotton was inserted into each hole to serve as a filter when the flasks were latter attached to a vacuum assembly. Each of the flasks contained 100 ml of yeast medium (Table 3) and was autoclaved fos 20 minutes. After cooling, the flasks were inoculated aseptically with the yeast cells by means of a wire loop.

Carbon dioxide evolved from the flasks was measured by the technique described by Davis *et al.* (1976). In this method, the inoculation flasks were connected to a continuous aeration apparatus in which CO_2 was swept from the culture flasks by CO_2-free air and the CO_2 evolved from the cultures was trapped in 1 N NaOH. Sampling was performed at 2, 4, 6, 8, 10, 12, and 14 days after inoculation by removing two 10-ml aliquots of solution for each CO_2-collection trap. Each aliquot was delivered into a 125-ml Erlenmeyer flask, and 20 ml of a normal solution of $BaCl_2$ was added. Carbon dioxide absorbed by the alkali was determined by titration with a standard 0.5 N HCl solution to a phenolphthalein end point. At the termination of the CO_2 experiment, the O. D. and dry weight of the cell suspensions from the culture flasks were determined as previously described.

An additional CO_2 experiment was initiated which involved the use of radioactive diquat. In this study, 100 ppm radioactive diquat was added to the medium and the NaOH solution as well as the culture were radioassayed.

Results

Isolation of diquat-tolerant microorganisms from soil

The diquat-enriched soil contained a number of fungi and bacteria, indicating that diquat did not have any apparent depression on the growth of soil microorganisms. Species of *Fusarium*, *Penicillium*, *Aspergillus*, and an unidentified bacterium grew well in the presence of 5,000 ppm diquat. *Fusarium* sp. grew well on the modified Czapek's agar with 100 ppm streptomycin, but the unidentified bacterium did not.

Microscopic studies showed that diquat-tolerant microorganisms became abnormal in shape and/or size and thick cell walls were formed.

Fusarium sp. (isolate-A-12-5) grew well on the modified Czapek's agar with 0.5 per cent yeast extract, and the colonies of this organism were white. A milky ring about 2 to 4 mm in diameter was observed beneath colonies of *Fusarium* sp. (isolate-M-3-4) which appeared very similar to isolate-A-12-5; however, better growth was observed, and a purple ring of 1 to 2 mm diameter appeared beneath colonies of this organism.

The unidentified bacterium grew very slowly on the modified Czapek's sucrose agar with 0.5 per yeast extract, and this organism grew in small milky colonies spaced closely together.

Degradation of ^{14}C-labeled diquat by the microorganisms

The radioactivity of the sterile control culture was the same as that of the culture inoculated with the microorganism cells for two weeks. This indicates that none of the degradation products were lost as volatile compounds.

Figure 1 is a picture of autoradiogrphs of thin-layer electrophoresis (TLE) plates spotted with aqueous solutions of ^{14}C-labeled diquat in which the soil microorganisms had been grown. At least three radioactive compounds other than diquat were present in the cultures. Two of the metabolites moved toward the cathode, like diquat, but at a slower rate. The other metabolite moved toward the anode at a rapid origin, indicating that probably the diquat or some radioactive compound was adsorbed to something in the culture extract which prevented it from moving on the plates.

In the time course study, it was found that there was no apparent degradation after two days, but after four days there were three additional radioactive spots besides the diquat on the TLE plates (Fig. 2), and two on TLE plates (Fig. 3). For paraquat, at the four-day incubation period, there were two additional radioactive spots on both the TLE plates (Fig. 4) and the TLC plates (Fig. 5). The 8- and 16-day autoradiographs were similar to the one for the four-day incubation period. Again, there was a large amount of radioactive material remaining at the origin which showed the possibility of another degradation product with a neutral charge or adsorption. The amount of radioactive diquat or paraquat on 16-day autoradiographs was loss than that on 4- or 8-day autoradiographs, indicating the possibility of additional degradation (Figs. 2 and 4). Of the three main spots on the plates (Fig. 2), other than diquat, one spot moved approximately one half the distance of diquat toward the negative pole, the second one moved a short distance toward the positive pole. This indicated that two of the degradation products were negatively charged compounds. The spot that moved the shortest distance on the plates showed an irregular movement; i.e., this spot moved toward the negative pole and often toward the sides of the plate (Fig. 2). This was also found on the plates for paraquat, but no positively charged spot was found (Fig. 4).

One of the two degradation products of diquat found on TLC plates had an Rf of 0.98 and the other had an Rf of 0.7. The intensity of the spots of the metabolites on the TLC autoradiographs was the highest after an eight-day incubation period. This may be explained by the hypothesis that these products were further degraded into volatile compounds which were lost as CO_2 and/or NH_3 in the air (Fig. 3).

It was also found that the movement of the degradation products for paraquat on TLC plates was almost the same as for diquat. However, the

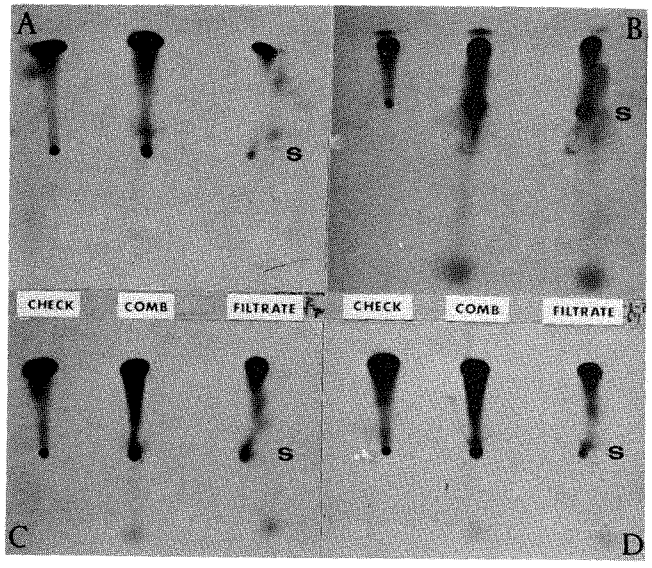

Fig. 1. Autoradiographs of thin-layer electrophoresis plates spotted with the filtrates of (A) *Fusarium* sp. (isolate-A-12-2); (B) bacterium (isolate-2-5-1); (C) *Fusarium* sp. (isolate-M-3, 4); and (D) *L. starkeyi* growing in culture media containing ^{14}C-labeled diquat (S indicates starting point; top is cathode; botton is anode).

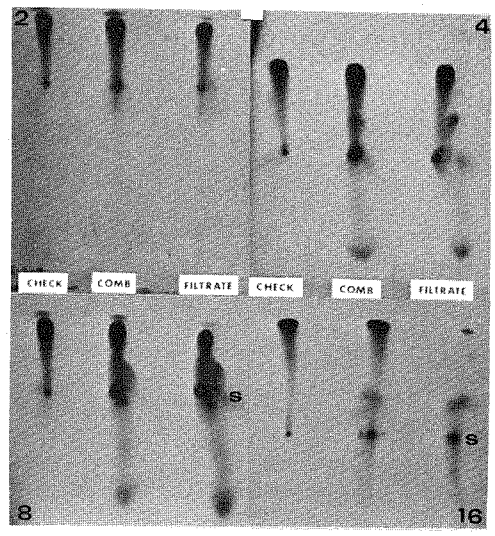

Fig. 2. Autoradiographs of TLE plates spotted with filtrates from cultures of *L. starkeyi* that contained ^{14}C-labeled diquat, after 2, 4, 8, and 16 days incubation (top is cathode; bottom is anode).

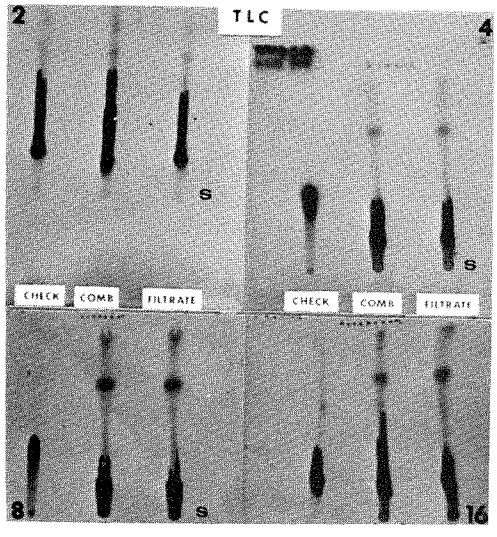

Fig. 3. Autoradiographs of thin-layer chromatography plates spotted with filtrates from cultures of *L. starkeyi* that contained radioactive diquat after 2, 4, 8, and 16 days incubation.

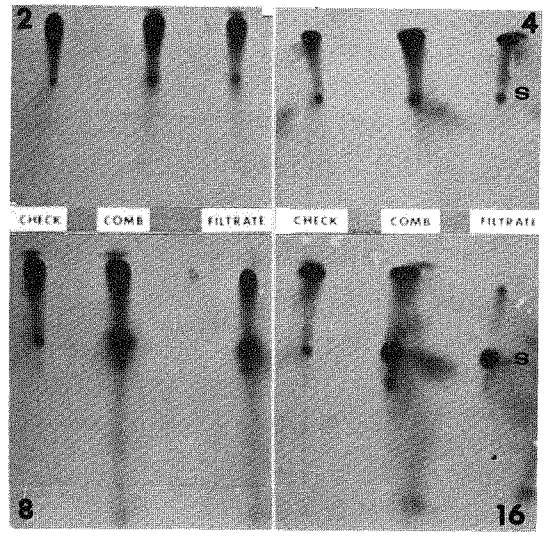

Fig. 4. Autoradiographs of TLE plates spotted with filtrates from cultures of *L. starkeki* that contained radioactive paraquat after 2, 4, 8, and 16 days incubation (top is cathode; bottom is anode).

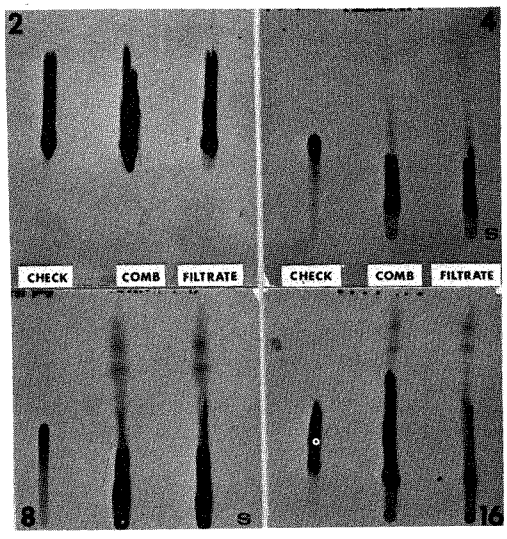

Fig. 5. Autoradiographs of thin-layer chromatography plates spotted with filtrates from cultures of *L. starkeyi* that contained radioactive paraquat, after 2, 4, 8, and 16 days incubation.

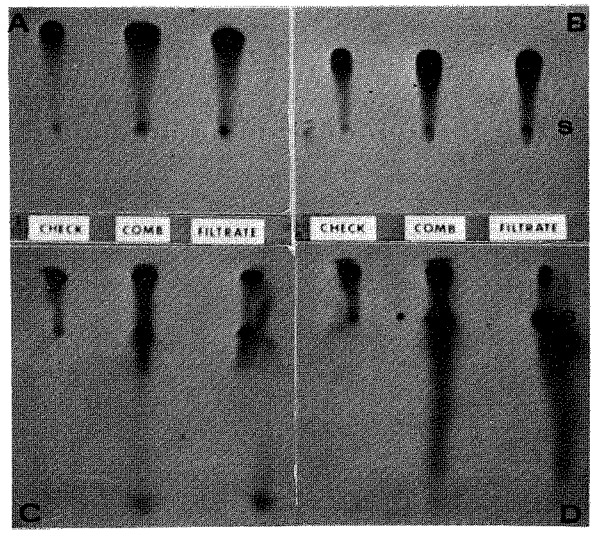

Fig. 6. Autoradiographs of TLE plates spotted with the filtrates of (A) boiled, or (C) unboiled cultures of *L. starkeyi* containing radioactive diquat, and (B) boiled, or (D) unboiled cultures of the yeast containing radioactive paraquat.

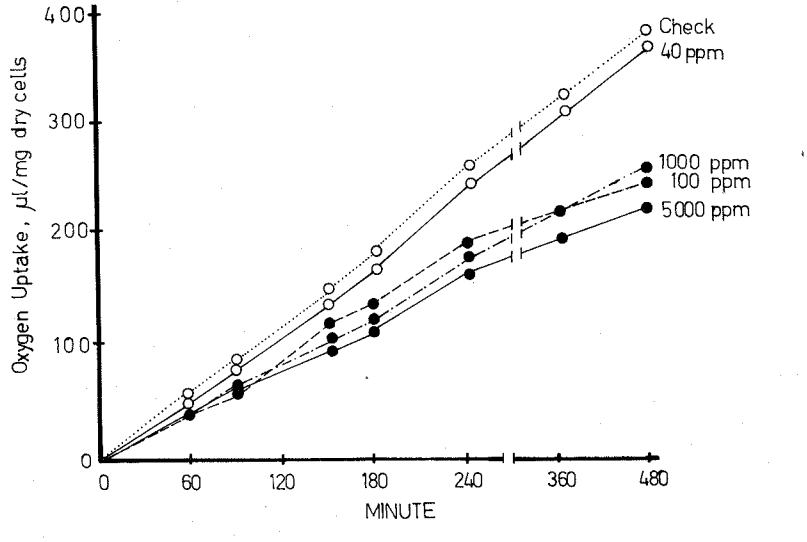

Fig. 7. Effect of diquat on oxygen uptake of actively respiring cells of *L. starkeyi*.

degradation products of paraquat had Rf values of 0.74 and 0.87 and diquat had Rf values of 0.70 and 0.98 (Fig. 3 and 5).

These studies showed that the degradation products of diquat and paraquat probably have at least a negatively charged compound (Fig. 2 and 4). However, it has not been previously demonstrated that microorganisms or ultraviolet light degraded diquat and/or paraquat into negatively charged compounds (Funderburk and Bozarth, 1967; Funderburk, 1969). This could be a result of this compound being highly mobile and, therefore, easily lost from the TLE plates if the electrophoresis was run for other than a short period of time. For this reason, in these experiments, the TLE plates were run for different periods of time: 1.5, 2.0, and 2.5 hr. It was found that two-hour autoradiographs showed the best resolution of the degradation products.

It was demonstrated that the culture of *L. starkeyi*, which had been treated to 100 C, was unable to degrade diquat and paraquat (Fig. 6). This suggests that the degradation of diquat and parpquat by this yeast is an enzymatic rather than a non-enzymatic chemical process.

In these experiments, at least three microorganisms isolated from various soils, were found to be capable of degrading diquat, and the yeast, *L. starkeyi*, was also shown to attach diquat and paraquat. Bipyridylium herbicide residues reaching the soil can apparently be dissipated through the metablic processes of the complex soil microflora.

Effect of diquat on the respiring cells of ***Lipomyces starkeyi***

The results of the effect of diquat on oxygen uptake by actively respiring yeast cells are summarized in Fig. 7. This graph indicates that oxygen uptake of the yeast cells after 480 minutes was depressed 3, 35, 31, and 42 per cent at concentrations of 40, 100, 1,000, and 5,000 ppm diquat, respectively. In this study, 40 ppm diquat had no apparent effect on the oxygen uptake, but there was moderate inhibition of oxygen uptake at diquat concentrations of 100 to 5,000 ppm, especially during the 240 to 280 minute period. At concentrations of 100 to 5,000 ppm diquat, there no large differences in the amount of inhibition. This could be due to the fact that the same concentration of herbicide reaches the site of action in all cases.

Figure 8 shows that the rate of oxygen uptake by *L. starkeyi* with diquat or sucrose as the sole carbon source. The rate of oxygen uptake for sucrose was higher than for diquat or endogenous respiration. This indicates that resting cells of the yeast were readily able to utilize diquat as a carbon source.

From these studies, it appears that the extracellular enzymes could have been limited from the cell suspension of the yeast since respiration studies were conducted after the cells had been transferred several times in washing

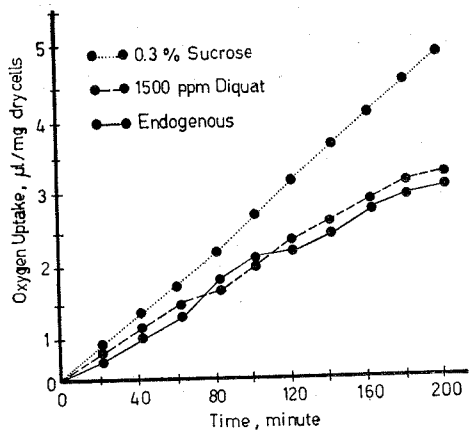

Fig. 8. Oxygen uptake by *L. starkeyi* with diquat as a sole carbon source.

procedures. These data are agreeable to Bozarth's studies (1966) with a bacterial isolate that degraded paraquat.

Utilization of diquat by Lipomyces starkeyi

Figure 9 shows that the number of dry cells of *L. starkeyi* is almost proportional to its optical density. Fig. 10 is a graph showing the effect of nitrogen and carbon sources on the rate of growth of *L. starkeyi* incubated at 28°C. This graph indicates that growth of the yeast cells was promoted when diquat was used as a sole source of nitrogen. In this treatment of −N+C+D (see

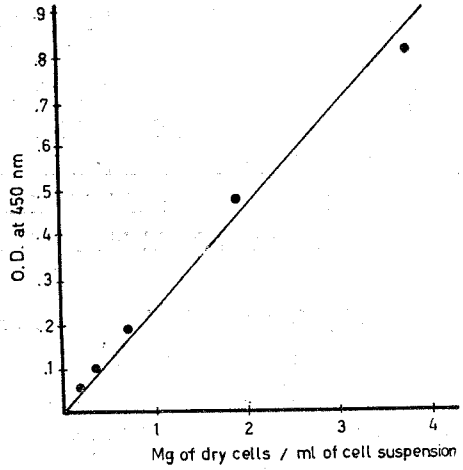

Fig. 9. Standard growth curve of *L. starkeyi*.

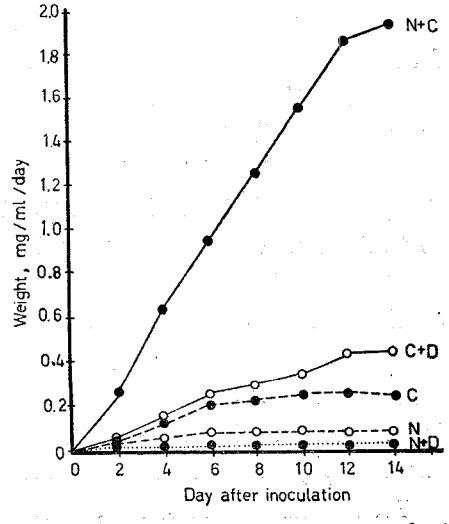

Fig. 10. Growth curve of *L. starkeyi*: N = nitrogen; C = carbon; D = diquat.

material and methods), the rate of growth after four days was more then in the −N+C treatment. This promotion of growth with diquat as the nitrogen source is clearly shown in the graph after six-day growth. Diquat was not utilized by the yeast as a sole source of carbon as shown in the treatment of N−C+D. There was very little growth of the yeast when diquat was substituted as the carbon source in the medium. The yeast grew rapidly in the complete medium.

Baldwin *et al.* (1966) stated that *L. starkeyi* isolated from soils could utilize paraquat as a sole source of nitrogen. In this case, the degradation pathways of diquat and paraquat by the yeast are probably similar. When the chemicals are completely metabolized by the yeast, the end products of both of the dipyridyls are carbon dioxide and nitrate (Funderburk, 1969).

CO_2 production by Lipomyces starkeyi

The rate of carbon dioxide evolution of *L. starkeyi* at two-day intervals is illustrated in Fig. 11. This graph shows that cells of the yeast were most active during the two to four day period as shown by CO_2 evolution. There was a progressive decrease in CO_2 production after a four-day incubation. These data, when compared with those from the degradation studies, indicate that degradation of diquat occurred after the yeast cells reached the most active stage of growth and metabolism.

There was no $^{14}CO_2$ evolved from the culture. In addition, there was no radioactivity lost from the culture after a two-week incubation period. These

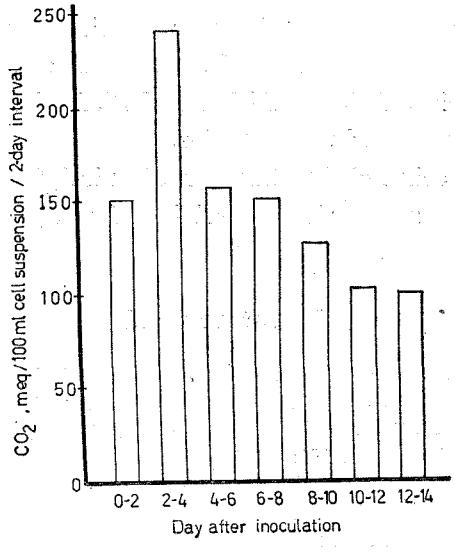

Fig. 11. The rate of CO_2 production by *L. starkeyi*.

indicate that none of the degradation products such as $^{14}CO_2$ were lost as volatile compounds.

Discussion

Results of several phases of this investigation showed that diquat had very little effect on the growth activities of several microorganisms even at rates up to 5,000 ppm. Several studies also showed that diquat was degraded in synthetic media by several microorganisms. However, one should not forget that under practical conditions this chemical is bound rather tightly to various soil particles and is probably not readily available to any biological system (Kuwatsuka and Niki, 1976).

The degradation pathway of diquat by *L. starkeyi* could possibly be different from that of photochemical degradation. Slade and Smith (1967) showed that tetrahydro-oxo-pyridopyrazinium cation is one of the major photochemical degradation products of diquat. The authors found that *L. starkeyi* could not utilize diquat as a sole source of carbon (Fig. 8) but could use it as a source of nitrogen (Fig. 10). If nitrogen is made available from the parent compound, then the organism would have to degrade the compound beyond tetrahydro-oxo-pyridopyrazinium cation.

Further studies are needed to identify the degradation products of diquat and to determine their biological activity in order to prove the phenomena suggested by these experiments.

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雙嘧啶殺草劑與土壤微生物之相互作用

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利用增殖及稀釋過程，從幾種土壤樣品中，分離幾種較普通之土壤微生物這些被分離之土壤微生物可以生長於含有 5,000 ppm diquat 之培養基。若讓這些微生物生長於含有 ^{14}C-diquat 之培養液，經兩週培養後，將少量培養液滴在矽膠板後，用薄層電泳及放射性同位素圖法，證明有三種不相同之土壤微生物可以分解 diquat。Diquat 分解物之一爲帶負電荷之化合物。

利用 *Lipomyces starkeyi*（一種土壤酵母菌）研究培養時間，影響其對 diquat 及 paraquat之分解物之量及種類；經二天培養後，diquat 未被分解，但經 4，8，及 16 天分別培養後，共有三種分解物。研究 paraquat 所得之結果，僅有二種分解物。*L. starkeyi* 係利用酵素分解 diquat 及 paraquat。

利用 Warburg 法，結果顯示 diquat 能減少酵母菌吸收氧氣之速率，同時酵母不能利用 diquat 作爲碳素之來源。利用生長箱法，則酵母經四天培養後，放出二氧化碳之速率爲最大，但經六天培養後，生長速率爲最高。同時酵母可以利用 diquat 作爲氮素之來源。