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Effect of Selected Herbicides on Bacterial Growth Rates[1]

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Specific growth rate constants were used to evaluate the effects of selected herbicides on *Erwinia carotovora*, *Pseudomonas fluorescens*, and *Bacillus* sp. Comparison of growth rate constants permitted the identification of either stimulatory or inhibitory effects of these substances. *E. carotovora* was inhibited by 6,7-dihydrodipyrido(1,2-a:2′-c)pyrazinediium (diquat) and 4-hydroxy-3,5-diiodobenzonitrile (ioxynil) at 25 μg/ml; 1,1′-dimethyl-4,4′-bipyridinium (paraquat) at 50 μg/ml; and pentachlorophenol (PCP) at 10 μg/ml. *P. fluorescens* was inhibited by paraquat and PCP at 25 μg/ml and by 4-amino-3,5,6-trichloropicolinic acid (picloram) at 50 μg/ml. Stimulation of *P. fluorescens* was observed with 4-(methylsulfonyl)-2,6-dinitro-*N*,*N*-dipropylaniline (nitralin) at 25 μg/ml. The *Bacillus* species was inhibited by diquat (25 μg/ml), ioxynil (10 μg/ml), and paraquat and PCP (5 μg/ml). No significant effect of 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine (atrazine), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron), α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine (trifluralin), or 1,1-dimethyl-3-(α,α,α-trifluoro-*m*-tolyl)urea (fluometuron) on growth rates of the bacteria was observed at 25 and 50 μg/ml.

Reviews by Audus (1), Bollen (2), Fletcher (4, 5), and Smith and Fletcher (6) indicate that most herbicides used at recommended field rates do not significantly change soil microbial populations. However, repeated applications of the same herbicide for several years may result in population changes (3) and may alter basic metabolic processes of the organism. The objective of our study was to determine the growth-rate constants of individual bacteria grown in liquid culture in the presence and absence of selected herbicides.

The herbicides tested in this study were obtained from the manufacturers as analytical standards and were recrystallized from the appropriate solvent before use. Depending on the solubility characteristics, solutions of each chemical were prepared in redistilled ethanol, acetone, or water. The chemicals tested and their common designation are: 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine(atrazine; 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron); 6,7-dihydrodipyrido(1,2-a:2′,1′-c)-pyrazinediium ion (diquat); 1,1-dimethyl-3-(α,α,α-trifluoro-*m*-tolyl)urea (fluometuron); 4-hydroxy-3,5-diiodobenzonitrile (ioxynil); 4-(methylsulfonyl)-2, 6-dinitro-*N*, *N*-dipropylaniline (nitralin); 1,1′-dimethyl-4,4′-bipyridinium ion (paraquat); pentachlorophenol (PCP); 4-amino-3,5,6-trichloropicolinic acid (picloram); and α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine (trifluralin).

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Pseudomonas fluorescens and *Erwinia carotovora* were obtained from Carolina Biological Supply, Burlington, N.C. The *Bacillus* species was isolated from a soil plot which had received repeated applications of trifluralin. Growth curves were determined in a simple salts medium (Na_2HPO_4, 10.5 g/liter; KH_2PO_4, 4.5 g/liter; NH_4Cl, 1.0 g/liter; NaCl, 5.0 g/liter; $MgSO_4 \cdot 7H_2O$, 0.27 g/liter; $CaCl_2$, 0.03 g/liter), hereafter referred to as glucose-simple salts medium with 0.1% (w/v) glucose.

Growth flasks (250-ml Bellco side arm) were inoculated with a 4.4-ml inoculum of the organism giving a final volume of 40.4 ml and were shaken at 30 C in water bath for 108 oscillations per min (WCLID model C-2156). Optical density (OD) at 600 nm was determined at regular time intervals in a Bausch and Lomb Spectronic 20. The OD_{600} values from the growth curve were converted into dry weight values from a standard dry weight curve for each organism. The slope of the straight-line segment of the plot of log dry weight versus

TABLE 1. *Growth responses*[a] *of Erwinia carotovora, Pseudomonas fluorescens, and Bacillus sp. to selected herbicides*

Herbicide	*Erwinia carotovora*		*Pseudomonas fluorescens*		*Bacillus* sp.	
	Minimal response concn (μg/ml)	Response (%)	Minimal response concn (μg/ml)	Response (%)	Minimal response conc (μg/ml)	Response (%)
Diquat	25	40.8[b]	50	0	25	100[b]
Ioxynil	25	71.0[b]	50	0	10	29.0[c]
Paraquat	50	25.5[b]	25	43.7[b]	5	100[b]
PCP	10	29.6[c]	25	65.8[c]	5	100[b]
Picloram	50	0	50	28.8[c]	50	0
Nitralin	50	0	25	+22.0[c]	50	0

[a] All responses to the herbicides listed herein were inhibitory, except the response of *P. fluorescens* to nitralin which was stimulatory.
[b] Significant at the 1% level.
[c] Significant at the 5% level.

time was determined with a Statistical Interpretal Language (STIL) computer program for least squares determinations and regression analysis. The specific growth rate constant (k) was then calculated from $k = m$ (2.303), where m is the slope of the straight-line segment. The mean experimental and control values were compared statistically for least significant differences between the two values. Results are presented as either percentage of inhibition or stimulation at the minimal herbicide response concentration.

Inhibition of the growth rate of *E. carotovora* (Table 1) was observed with diquat, ioxynil, paraquat, and PCP. No response was observed with nitralin or picloram. The growth rate of *P. fluorescens* (Table 1) was inhibited by paraquat, PCP, and picloram. Inhibition of the growth rate of the *Bacillus* sp. (Table 1) was observed with diquat, ioxynil, paraquat, and PCP. Inhibition of the growth of *B. megaterium* and *B. subtilis* by ioxynil and bromoxynil (3,5-dibromo-4-hydroxy-benzonitrile) has previously been reported (7).

An apparent stimulatory effect on *P. fluorescens* was observed in the presence of nitralin (Table 1). This may reflect a utilization or alteration of the compound by the bacterium.

Atrazine, trifluralin, cotoran, and diuron did not significantly affect the growth rate of any test organism. These chemicals are relatively nonpolar in nature, and thus they may be unable to penetrate the bacterial cell or to inhibit any membrane or cell wall surface activities.

In situations in which growth of the specific organism was not completely inhibited by the test chemical, long lag phases followed by growth were observed. This may have been due to the adaptation of the organism to the herbicide or to mutant selection. *E. carotovora* in the presence of ioxynil, PCP, and diquat; *P. fluorescens* in the presence of ioxynil, paraquat, diquat, PCP, and trifluralin; and *Bacillus* in the presence of ioxynil and diquat exhibited this type of response.

The technique reported in this study utilized a growth rate constant which is characteristic of the conditions of the growth of the bacterium in liquid culture. These values were used to evaluate the effects of selected herbicides in terms of stimulatory or inhibitory responses. Results obtained in this study are being investigated further in terms of adaptation to, and degradation of, specific compounds.

Herbicides used in this study were provided by the following companies: Amchem Products, Inc.; Chevron Chemical Co.; CIBA Agrochemical Co.; Dow Chemical Co.; E. I. du Pont de Nemours and Co.; Elanco Products Co.; Geigy Agricultural Chemical Co.; and Gulf Research and Development Co.

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