HERBICIDE EFFECTS IN SOIL

# Effect of Certain Herbicides on Rate of Nitrification and Carbon Dioxide Evolution in Soil

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Bactericidal effects of certain herbicides applied to arable soils may possibly be as important as their herbicidal effects. This study was undertaken to determine the effects of  $\alpha$ -chloro-N,N-diethylacetamide, 2-chloroallyl diethyldithiocarbamate, isopropyl-N-(3-chlorophenyl) carbamate, and an amine salt of 2,4-D on microbial activity in an incubated soil. Normal field rates of these herbicides had little effect on nitrification and carbon dioxide evolution. Higher rates inhibited nitrification but increased carbon dioxide evolution from the soil. Herbicidal selectivity among the organisms was indicated.

**S** OIL MICROORGANISMS are influenced by pre-emergence herbicides. The nature and extent of this influence vary according to the type of microorganism (2, 6, 10, 15-17), kind of herbicide (2, 4,11, 13), and concentration of herbicide (2, 7, 8, 12, 13, 17). Many previous studies have dealt with 2,4-D and its derivatives, however, the introduction of new herbicides warrants further investigation.

In this paper some effects of  $\alpha$ chloro-N,N-diethylacetamide (CDEA), 2 - chloroallyl diethyldithiocarbamate (CDEC), isopropyl-N-(3-chlorophenyl) carbamate (CIPC), and the triethanolamine derivative of 2,4-D on nitrate accumulation and carbon dioxide evolution in Brookston silty clay loam are reported.  $\alpha$ -Chloro-N,N-diethylacetamide and 2-chloroallyl diethyldithiocarbamate, recently developed by Monsanto Chemical Co., have shown a high degree of preemergence activity (3, 5, 9).

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### Materials and Methods

Two separate experiments were conducted: one to study the effects of the four previously mentioned herbicides on nitrate accumulation in incubated soil, and the other to study the effects of the same herbicides on general microbial activity as measured by carbon dioxide evolution. For the nitrification experiment, three replications of each treatment were prepared for each of nine sampling periods. Fifty-gram samples of air-dried Brookston silty clay loam (Table I) were placed in 2-ounce, wide-

#### Table I. Properties of Brookston Silty Clay Loam

pH (1 to 1 soil-water ratio)	6.1
Water-holding capacity at $1/3$	
atm., %	38.3
Organic matter content, $\%$	11.8
Exchange capacity, meq./100 g.	39.3
Nitrate nitrogen content, p.p.m.	16.0

mouthed powder jars. Herbicides were applied at rates of 6, 24, and 96 pounds per acre except for 2,4-D, which was applied at 2, 8, and 32 pounds per acre. These rates were figured on the basis of surface area of soil, the smallest rate of each herbicide being comparable to recommended field application.

The herbicides were applied to the surface of the soil in sufficient volume of water to bring the soil to 38% moisture by weight. The aqueous solution of herbicide also contained ammonium sulfate in sufficient concentration to add 60 p.p.m. of ammonium nitrogen to the soil. The jars were stoppered loosely with cotton plugs and incubated at  $30^{\circ}$  C. During incubation the soils were periodically adjusted to 38% moisture.

Nitrates were extracted at each testing period by shaking a sample of each treatment with 100 nnl. of distilled water. Five-milliliter aliquots of the filtrate were dried in evaporating dishes and nitrate nitrogen in the residue was determined by the phenoldisulfonic acid method as described by Prince (1.4).



Figure 1. Effect of certain herbicides on accumulation of nitrates in Brookston silty clay loam

A	2,4-D	
	Check	
	O 2 lb./acre	
	× 8 lb./acre	
	⊙ 32 lb./acre	
В	Isopropyl-N-{3-chloro-	
	phenyl) carbamate	
С	2-Chlorocllyl diethyldi-	
	thiocarbamate	
D	N,N-diethylacetamide	
	Check	
	6 lb./acre	
	imes 24 lb./acre	
	⊙ 96 lb./acre	

 $\int LSD_{a}$  for 64-day data (P = 0.05) = 25 p.p.m.

In the carbon dioxide evolution experiment, 100-gram samples of Brookston silty clay loam were placed in 250-ml. Erlenmeyer flasks. The soil was treated with the same herbicides, rates, and manner of application as in the nitrification experiment. Three replications of each treatment were prepared. Immediately after treatment, the flasks were attached to an aspiration apparatus and incubated at 28° C. Carbon dioxide evolved from the soil was collected in sodium hydroxide and determined volumetrically.

## **Results and Discussion**

The results of the nitrification experiment are presented in Figure 1. Although nitrate accumulation in the 2pounds-per-acre treatment of 2,4-D generally paralleled that of the check, the 8- and 32-pounds-per-acre rates showed a marked inhibition of nitrate accumulation-especially during the first half of the incubation period (Figure 1). These results with 2,4-D are generally in agreement with those of other investigators (7, 8, 13). Figure 1(B, C, D)shows that, when applied at the same effective herbicidal equivalent, 2-chloroallyl diethyldithiocarbamate has about the same degree of effect as 2,4-D, isopropyl-N-(3-chlorophenyl) carbamate somewhat more inhibitive, and  $\alpha$ -chloro-N,N-diethylacetamide much more inhibitive to nitrate accumulation than is 2.4-D.

The 96-pounds-per-acre treatment of  $\alpha$ -chloro-N, N-diethylacetamide essentially prohibited nitrate accumulation until near the end of the incubation period. Even the normal field rate of 6 pounds of  $\alpha$ -chloro-N, N-diethylacetamide per acre suppressed nitrate accumulation at all sampling periods during the incubation. However, the magnitude of the effects are probably not great enough to prohibit the use of this compound as a selective herbicide.

Nitrate accumulation appeared to be affected less by herbicidal treatments during the last half of the incubation. The rate of increase of nitrates in the treated soils was much greater than in the check soil between 32 and 48 days but less between 48 and 64 days. The over-all rate of increase of nitrates in the treated soils during the last 32 days was about the same as in the check. The herbicides were probably losing some of their effectiveness against nitrate accumulation after about 30 days.

2,4-D, isopropyl-*N*-(3-chlorophenvl) carbamate,  $\alpha$ -chloro-N,N-diethylacetamide, and 2-chloroallyl diethyldithiocarbamate all had a "stimulative" effect on carbon dioxide evolution. As compared to the untreated soil (Table II), all treatments produced significant increases in carbon dioxide evolution except the 2 and 8 pounds of 2,4-D and the 6 pounds of 2-chloroallyl diethyldithiocarbamate per acre. Isopropyl-*N*-(3-chlorophenyl) carbamate and  $\alpha$ -chloro-N,N-diethylacetamide brought about significant increases in carbon dioxide evolution at all rates. The 96-pounds-per-acre rate of  $\alpha$ -chloro-N,N-diethylacetamide appeared to be reaching bactericidal quantity. This same treatment also caused the greatest reduction in the accumulation of nitrate.

The inverse relationship between

Table II. Effect of 2,4-D, Isopropyl-N-(3-chlorophenyl) Carbamate, 2-Chloroallyl Diethyldithiocarbamate, and  $\alpha$ -Chloro-N,N-diethylacetamide on Total Carbon Dioxide Evolution from Brookston Silty Clay Loam

	Mg. C Lost/100 G. of Air-Dry Soil in 33 Days				
Treatment, Lb./Acre	Mean of three replicates	Deviation from check			
Check	97.0				
2,4-D					
2 8 32	94.9 104.6 108.3	$   \begin{array}{r}     -2.1 \\     +7.6 \\     +11.3^{a}   \end{array} $			
CIPC					
6 24 96	$   \begin{array}{r}     108.1 \\     121.0 \\     125.0   \end{array} $	$+11.1^{a}$ +24.0 <sup>a</sup> +28.0 <sup>a</sup>			
CDEC					
6 24 96	99.2 105.8 113.4	$+ 2.2 + 8.8^{a} + 16.4^{a}$			
CDEA					
6 24 96	112.5 120.0 106.7	$+15.5^{a}$ +23.0 <sup>a</sup> + 9.7 <sup>a</sup>			

LSD for treatment means (P = 0.05) = 8.8.

<sup>a</sup> Significant difference.

nitrate accumulation and carbon dioxide evolution in this study is of some significance. Although a decrease in either the number or activity of nitrifying organisms was indicated, this was probably not the sole cause of variation in nitrate content in the various treatments. Any "stimulative" effects that these compounds might have on soil microorganisms, such as increased production of microbial tissue, would bring about immobilization of nitrogen as indicated by a reduction in nitrate accumulation. The small, but significant, increase in carbon dioxide evolution could probably not account for the decrease in nitrate accumulation, however.

Several investigators have noted increased activity of certain microorganisms in the presence of 2,4-D, and some have suggested its utilization by various organisms as a source of carbon (7, 72). Undoubtedly, only specialized groups of organisms would be capable of attacking a given herbicide. These herbicides must serve important functions other than merely being a source of food, however, as the increased amount of carbon evolved as carbon dioxide was considerably greater than the amount of carbon added in the four herbicidal compounds tested. An additional "stimulative" effect may result from herbicidal selectivity. When added to the soil, herbicides probably cause bacteriostatic action on some organisms, allowing more tolerant organisms to multiply more rapidly. This increased activity in an otherwise resting population would cause a temporary net increase in carbon dioxide evolution as organisms in an active state of reproduction and growth have much increased respiration rates.

Application of the foregoing theory to the results of this investigation leads to the conclusion that 2,4-D, isopropyl-N-(3-chlorophenyl) carbamate,  $\alpha$ -chloro-N,N-diethylacetamide, and 2-chloroallyl diethyldithiocarbamate exhibit a bacteriostatic effect on some organisms, including nitrifying organisms, but allow others to multiply undisturbed. Extremely high rates may be bacteriostatic to all organisms. In vitro studies (17) have demonstrated the selectivity of 2,4-D to microorganisms.

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