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Soil Differences in the Biodegradation of Carbofuran and Trimethacarb Following Pretreatment with These Insecticides

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Pretreatment of soils with certain pesticides can accelerate the degradation rate of subsequent applications of those pesticides and structurally related analogues. This phenomenon, which is commonly referred to as enhanced biodegradation (Kaufman and Edwards 1983), has been attributed to a proliferation of inducible biological agents for which a particular molecule is a substrate. Rather than being a simple enrichment process, development of enhanced biodegradation in soils is modulated, often unpredictably, by other soil characteristics (Chapman et al. 1986a,b). For example, separate studies performed in our laboratory have shown that carbofuran (2,3-dihydro-2,3-dimethyl-7-benzofuranyl methylcarbamate) conditioned two soils for its own enhanced degradation and that of trimethacarb (isomeric mixture of 3,4,5- and 2,3,5-trimethylphenyl methylcarbamate) (Felsot 1986; Dzantor and Felsot 1989). On the other hand, only one of the soils could be conditioned by trimethacarb for the degradation of either insecticide (Felsot 1986). Since these experiments were performed sequentially rather than concurrently with each soil type, we simultaneously pretreated both soil types with carbofuran (CB) or trimethacarb (TM) and reevaluated the effects on degradation rate. We determined whether there were any differences in soil microbial biomass and bioactivity that could explain the different induction patterns in the two soils. Furthermore, we determined whether the patterns of insecticide degradation in the two soil types were related to differences in adsorption.

MATERIALS AND METHODS

The two soils were collected from experimental fields at the Illinois Natural History Survey and Univ. of Illinois. One was a silty clay loam (coded as no. 52) with the following characteristics: OC, 2.0%; CEC, 22.9 meq/100g; moisture holding capacity (MHC), 84.0%; pH, 5.0. The other soil was a silt loam (coded as no. 11), with 3.5% OC content; CEC, 25 meq/100g; MHC, 93%; pH, 7.0. The soils had received no insecticide treatments for at least 3 years prior to the experiment. Freshly collected soils were air-dried to about 20% moisture content, passed through a 2-mm mesh sieve, and stored at 5° C until used.

For the initial pretreatments, 3 kg portions of each soil type were weighed into plastic containers that were lined with plastic bags. Soil in individual

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containers was treated with granular formulations of CB (Furadan 15G) or TM (Broot 15G) at a rate of 10 mg ai/kg oven-dry soil. An untreated container of each soil type served as a control. The insecticide formulations were mixed into the soil, and soil moisture was adjusted to 40% of MHC. The loosely covered containers were incubated in a greenhouse at ambient conditions. After 60 days subsamples of the soils were analyzed and found to contain about 1% of the initially added insecticides. The soils were subsequently brought into the laboratory, partially air-dried, sieved, and stored at 5° C overnight.

For the retreatment experiments, 30-gram oven-dry equivalents of soil from each pretreatment were weighed into 250-ml Erlenmeyer flasks. Soils with pretreatments of CB and TM were treated with technical grade CB and TM, respectively. Subsamples of the no-insecticide control soil (NI) also received treatments of each of the two insecticides. To test for the development of cross-conditioning, CB-pretreated soil was treated with TM, and TM-pretreated soil was treated with CB. The insecticides were added from stock solutions of technical CB or TM in acetone to give final insecticide concentrations of 10 mg a.i./kg soil. The TM treatment consisted of equal concentrations (5 mg/kg) of each isomer. Soils treated only with acetone served as controls to correct for insecticide residue remaining from the pretreatment incubation period. After the acetone had evaporated, soil moisture was adjusted to 25% MHC, and the flasks were covered with Parafilm® and incubated at 25° C for 28 days. The soils were aerated weekly for about 10 min. Immediately after treatment, and after 7, 14, and 28 days of incubation, three flasks from each treatment were frozen (-10° C) for chemical assay, and soil from two flasks were combined in sterilized Whirl-Pak® bags and stored at 2° C for analysis of microbial biomass and bioactivity.

Insecticide residues in the soils were extracted twice by stirring with 90 ml of ethyl acetate, and parent CB and TM were determined directly by GLC using a nitrogen-phosphorus detector (Dzantor and Felsot 1989). Differences among treatments for mean insecticide concentration on each sampling day were resolved by analysis of variance (n=3).

Bacterial numbers were estimated by the plate dilution frequency assay (Harris and Sommers 1968) using soil extract agar (Lockhead 1940), and fungi were enumerated by the soil dilution pour plate method using rose-bengal streptomycin agar (Martin 1950). The initial sizes of CB-degrader populations in NI, CB, and TM pretreated soils were estimated by a modification of the ^{14}C-most probable number method (Lehmicke et al. 1979), using radiolabeled CB as a carbon source in mineral salts medium (Dzantor and Felsot 1989).

The effect of insecticide treatment and retreatment on soil bioactivity was measured as a function of the activities of soil dehydrogenase, urease and amidase enzymes. Dehydrogenase activity was measured as triphenylformazan (TPF) formed when 5 g of soil was incubated for 24 h at 30° C with 2,3,5-triphenyl-tetrazolium chloride (TTC) (Lewis et al. 1978). TPF was extracted with methanol and color intensity was measured at 485 nm using a Perkin Elmer Lambda 4 UV/Vis spectrophotometer. Urease and amidase activities were measured as the release of NH_3 when soil was

incubated with urea and formamide, respectively (Zantua and Bremner 1975; Frankenberger and Tabatabai 1980). Ammonia released from urea was determined by the method of Douglas and Bremner (1970), and NH_3 released from formamide was measured by the sodium phenoxide method (Technicon Autoanalyzer II. Method No. 696.82W). All enzyme activities were expressed as the mean of triplicate determinations after correction for duplicate controls that were incubated without the respective substrate.

Adsorption of CB and the two isomers of TM in soil 11 and 52 was determined by modified batch equilibration at a single concentration, 10 mg a.i./kg. Twenty grams of oven-dried soil were shaken in glass centrifuge bottles with 100 mL of each insecticide in 0.01 M $CaCl_2$. After centrifugation at 4000 x g, the volume of the supernatant was measured and extracted by passage through a Baker SPE C_{18} cartridge and eluted with 3 mL of methylene chloride followed by 3 mL of ethyl acetate. The soil was extracted by shaking twice with 50 mL of ethyl acetate. Concentrations of insecticides in each phase were determined by GLC as described above. The concentration of insecticide in the saturated soil following centrifugation was corrected for the concentration remaining in the retained water. Soil distribution coefficient (Hamaker 1972) was calculated as the ratio of corrected concentration in soil (mg/kg) to concentration in 0.01 M $CaCl_2$ (mg/L).

RESULTS AND DISCUSSION

After one week of incubation in soil 52, less than 10% of the applied CB was recovered in soils that had been previously treated with either CB or TM, compared to about 89% in the NI-pretreated soil (Figure 1A). Within 2 weeks, CB had practically disappeared from both CB- and TM-pretreated soils. Carbofuran-degrading activity appeared to have been induced in NI soils between 7-14 days. During this period, CB had decreased by 62% compared to only 11% during the first week of incubation.

As previously observed by Dzantor and Felsot (1989), pretreatment of soil 52 with either CB or TM promoted the accelerated degradation of a subsequent TM treatment (Figure 1B). Harris et al. (1984) also observed enhanced biodegradation of TM after pretreatment of a sandy loam with CB. In general, TM was more persistent than CB in analogous soil treatments, and TM-degrading activity developed more slowly than CB-degrading activity in NI soils (Figure 1A,B).

Induction of insecticide-degrading activity in soil 11 contrasted sharply with the patterns in soil 52. In soil 11, CB induced its own enhanced degradation (Figure 1A) and that of TM (Figure 1B), but CB-degrading activity did not develop in NI-pretreated soil during 28 days of incubation. Pretreatment of soil 11 with TM did not alter the rate or extent of degradation of subsequent applications of CB or TM when compared to NI soil. (Figure 1A,B).

Pretreatment of the soils with granular insecticides and retreatment with technical-grade insecticides had no consistent effects on bacterial and fungal counts or significant effects on soil enzyme activities (Table 1). For example, bacterial counts were 10-fold higher in CB- and TM-pretreated

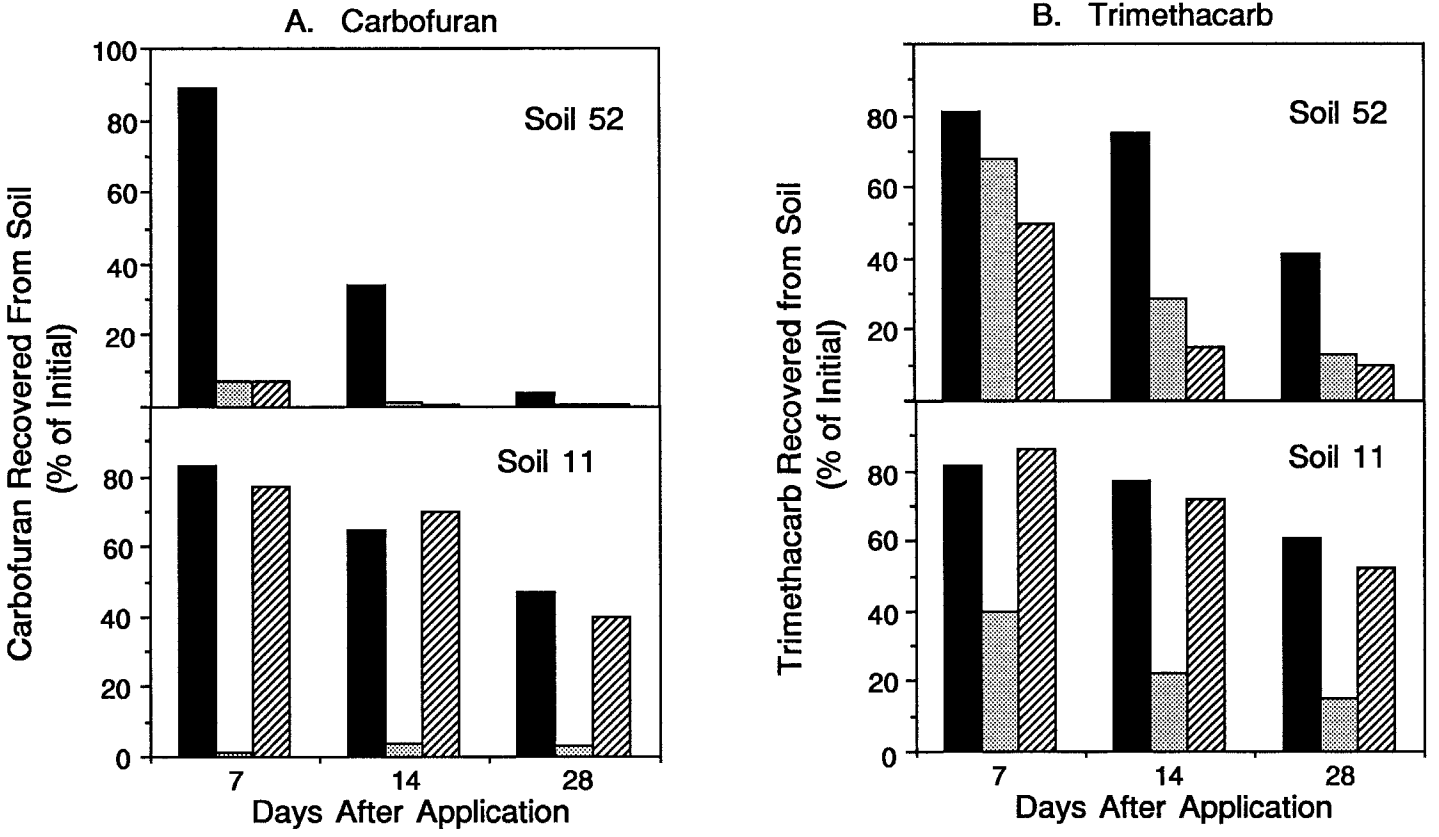

Figure 1. Recovery of carbofuran (A) and trimethacarb (B) from soil 52 and soil 11 following pretreatment with no insecticide ( ), carbofuran ( ), or trimethacarb ( ).

soil 11 than in the NI-pretreated soil; in soil 52, however, bacterial counts were highest in NI-pretreated soil. When soil types were compared, only amidase activities were significantly higher in soil 11 than in soil 52 throughout a three week monitoring period.

Table 1. Estimates of bacterial and fungal populations and selected enzyme activities on day 0 in soils pretreated with carbofuran and trimethacarb.

Soil and pretreatment[1/]	Microbial biomass/g ods			Enzyme Activity[2/]		
	Plate counts					
	Bacteria (cells x 10^8)	Fungi (cfu x 10^3)	^{14}C-MPN of CB degraders (cells x 10^7)	Dehydrogenase (TPF)	Amidase (NH_4^+-N)	Urease (NH_4^+-N)
52 NI	8.4	5.0	0.2	11 (8)	84 (4)	494 (35)
52 CB	1.6	5.4	2.2	25 (13)	89 (3)	538 (30)
52 TM	1.0	12.0	0.8	9 (3)	72 (5)	400 (50)
11 NI	1.7	13.0	6.8	28 (9)	128 (5)	461 (18)
11 CB	17.0	1.4	>20.0	61 (19)	122 (3)	494 (33)
11 TM	22.0	2.3	11.4	101 (71)	115 (13)	462 (58)

1/ See text for soil and treatment designations.

2/ Values are means of three replicates followed by the standard deviation; values are expressed as µg product formed per gram oven-dry soil.

The enrichment of either soil type for CB-degrader populations was evidenced by higher MPN estimates of CB degraders in CB-pretreated soils than in TM- and NI-pretreated soils (Table 1). In both soil types, the sizes of CB-degrader populations in TM-pretreated soils were numerically higher than those found in NI-pretreated soils. Although, the estimate of CB degraders in TM-pretreated soil 11 was more than an order of magnitude higher than in TM-pretreated soil 52, there was no induction for CB degradation in TM-pretreated soil 11 (Figure 1A).

Another factor that may partially explain the differences in degradation rates and conditioning between soil 11 and 52 is the differences in adsorption of the insecticides. The distribution coefficient for CB was similar in both soil types, but the distribution coefficient for the isomers of TM were 2.5 to 4 times larger in soil 11 than in soil 52 (Table 2). In other research the degradation rate of various pesticides in different soil types was inversely related to adsorption potential, which suggested an "inhibitory" effect of adsorption on biodegration (Iwata et al. 1973, Moshier and Penner 1978, Burkhard and Guth 1981, Felsot et al. 1982, Steinberg et al. 1987). The measured distribution coefficients for TM suggested that the insecticide was less bioavailable in soil 11 than in soil 52 and therefore did not stimulate an increase in TM- or CB-specific degraders. On the other hand, the rapid degradation of TM in soil 11 following pretreatment with CB, which had similar bioavailability in both soil types, suggested that CB-degraders had the potential to rapidly metabolize TM.

Table 2. Adsorption of carbofuran and trimethacarb.

Insecticide	Distribution Coefficient, K_d (L/kg) soil 52	soil 11	K_d Ratio soil 11: soil 52
carbofuran	0.71	0.83	1.17
3,4,5-TM	0.97	3.93	4.05
2,3,5-TM	1.12	2.91	2.60

In conclusion, the results illustrated differences in induction patterns in two soil types that were not reflected by traditional measurements of microbial biomass and bioactivity. Increases in populations of specific pesticide-degrading microorganisms have been widely used to explain enhanced biodegradation in pretreated soils (Racke and Coats, 1987; Hendry and Richardson, 1988; Mueller et al. 1989), but our results showed that this characteristic alone may not be enough to predict or explain this process in some soils. The results also suggested that the relationship between adsorption and biodegradation needs further research. A complete understanding of the interactions in soil between microbiological and physicochemical factors is crucial to explaining the phenomenon of enhanced biodegradation.

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