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**Enhanced Biodegradation and Reduced
Pesticide Efficacy with Repeated Application
and Cropping**

D. D. Kaufman, N. Aharonson, J. Katan

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ENHANCED BIODEGRADATION AND REDUCED
PESTICIDE EFFICACY WITH REPEATED
APPLICATION AND CROPPING

BY

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C. ABSTRACT:

Accelerated degradation of the fungicide benomyl and of the herbicides diphenamid, EPTC and other thiocarbamates was found in Israel, in soils collected from fields with a history of application as well as in simulation systems in the laboratory. Accelerated degradation became more pronounced upon repeated applications. A small volume of soil with benomyl-history, when mixed with untreated soil conditioned the entire soil volume for accelerated degradation. Soil sterilization by gamma irradiation or by autoclave, antimicrobial agents, fungicides, soil fumigation and soil solarization controlled accelerated degradation to various degrees. Fungi capable of degrading these pesticides were isolated, enumerated, and identified from soils with and without accelerated degradation. Population levels of pesticide degrading bacteria were much higher in soils with accelerated degradation than non-history control soils. This research shows that microorganisms play a major role in the development of accelerated degradation in soil. It appears that with the pesticides studied, bacteria are the major microbial component responsible for this phenomenon. This situation is different from that found in non-history soils, and results from a shift in the populations of the pesticide degrading microbes.

In the U.S. the dissipation of several organophosphate insecticides and their metabolites was examined in the laboratory in soil samples collected from: (1) fields where specific organophosphate insecticide performance failures (PF) had been observed, and (2) adjacent virgin fence-row areas (VF) where no pesticide applications had been made. Experiments conducted with ^{14}C -insecticides revealed a more rapid and extensive evolution of $^{14}\text{CO}_2$ from PF than VF soils. Gas chromatographic analysis of solvent extracts of these soils revealed that while the degradation rate of the parent molecule was

largely unaffected by the continuous use history, their sulfoxide and sulfone metabolites were not accumulating in PF soils as they did in VF soils.

Failure of these toxic metabolites to accumulate during the degradation of these insecticides drastically reduce their efficacy in history fields.

The degradation and persistence of the herbicides alachlor and metolachlor were examined in fresh soil samples collected from field plots which had been treated yearly with alachlor and continuously cropped with soybeans or corn. Degradation of alachlor was significantly slower in soil from fields with a continuous alachlor treatment history than in untreated control soils, and slower in corn-cropped soils than in soybean-cropped soils. Similar results were obtained with metolachlor applied to these same corn-cropped soils. The relative importance of microbial vs chemical degradation of alachlor appeared to be different between alachlor history and nonhistory soils. Experimental results indicated that alachlor degradation was a mixed chemical and microbial process which may be affected by interactions with the crop plant or other agricultural practices.

D. THE OBJECTIVES OF THE RESEARCH PROJECT AND ITS SIGNIFICANCE:

Primary Goals:

The primary goal of this proposed project was (is) to investigate the phenomena of enhanced degradation of pesticides in soil which lead(s) to rapid loss of their effectiveness when they, or chemically related pesticides, were (are) too frequently applied. Emphasis was (will be) given to situations where the agricultural regimes involve frequent application of the same pesticides, e.g., under continuous cropping systems. The mechanism involved and the means for preventing or retarding the enhanced degradation (in order to extend the duration of use of the pesticide) were to (will) be explored.

More specifically:

1. Survey and characterization of "problem" or "history" soils:

The occurrence and frequency of reduced effectiveness and enhanced degradation phenomena of pesticides in field soils with various histories of pesticide application, will be determined at various locations in the U.S.A. and Israel. Soil type, cropping systems, crop management, pesticide application regimes and frequency of application, crop rotation and other features will be correlated with rates of pesticide degradation in these soils. These studies will enable us to locate the more severe problems of reduced effectiveness of pesticides which will be further investigated, and to estimate the severity of the problem of loss of pesticidal effectiveness.

2. Mechanisms of enhanced degradation:

Microbial and enzymatic investigations of pesticide biodegradation in problem soils will be conducted in an attempt to define the identity, enzymatic degradation capacity, rate of reproduction and population levels in the soil, of microorganisms involved in degradation processes. The buildup in soil of populations of pesticide-degrading microorganisms with repeated

pesticide application, will be followed in problem soils defined in Section 1. Pesticide-microbe interactions will be a major goal in our mechanisms studies, as microbes play a major role in the dissipation of many pesticides from soil. Similarly, other mechanisms related to enhanced degradation, e.g., adsorption of pesticides to soil as related to microbial degradation, will be investigated. The relationship between chemical structures of the applied pesticides and the subsequent development of the enhanced degradation phenomenon in soil will be studied in an attempt to delineate interactions between pesticides which lead to either enhanced or delayed pesticide degradation. Studies mentioned in Section 2 will be the basis for developing means for controlling the reduced effectiveness of pesticides, as specified in Section 3.

3. Controlling enhanced degradation

Our studies should ultimately lead to the development of chemical and biological assays of soil for predicting situations where enhanced degradation is more likely to develop, and therefore repeated application of pesticides should be avoided. Such "conducive" or "predisposed" soils will be identified and special pesticide and pest control regimes in these soils will be developed. Methods for preventing or controlling enhanced degradation will be developed through use of suitable crop and/or pesticide rotations, use of antimicrobial agents or enzyme inhibitors, combinations with other pesticides, and use of suitable crop management practices.

Secondary objectives:

1. Beneficial side-effects

Some of the methods for preventing enhanced pesticide degradation, which will be developed, e.g., crop rotation, reduced frequency of application of chemically related pesticides, and integrated pest control, have additional

beneficial side effects. These include: retarding the build-up of pest populations which usually occurs in monoculture; reducing the chances for occurrence of bio-types resistant to pesticides; and possibly delaying nondesirable shifts in populations of nontarget pests which may develop under repeated application of the same pesticide.

2. Reduced pesticide pollution

Many farmers who encounter failures in pest control, as happens in problem soils, frequently respond by increasing dosages or application frequencies of pesticides in the hope that this will compensate for the reduced effectiveness. Thus, by avoiding development of problem soils, we shall indirectly contribute to reduced environmental pollution.

3. Impact on studies related to persistent pesticides:

A better understanding of the mechanisms of enhanced pesticide degradation may provide tools for studying persistent pesticides whose toxic residues in the environment are of major concern.

E. RESULTS OF INVESTIGATIONS:

1. ISRAEL:

a. Accelerated Degradation of Fungicide Benomyl

Population analysis of soil-borne fungi capable of degrading MBC in MBC-history and non-history soils.

Soil-borne fungi tolerant to MBC in Martin's medium (amended with Rose Bengal) were isolated from MBC-history soil ($9 \times 10^2/\text{g}$) was significantly lower than in the non-history soil ($1.4 \times 10^3/\text{g}$). Tolerant bacterial colony forming units (CFU) were higher in the MBC-history soil ($2.6 \times 10^4/\text{g}$) compared to $1.6 \times 10^4/\text{g}$ in the non-history soil.

In order to assess the degradation capabilities of the fungi isolated, 80 colonies obtained from each soil type were randomly transferred to potato dextrose agar amended with 10 ppm MBC. Following 5 days of growth, disks of medium were removed from the area of fungal growth and assayed for MBC. Among the 80 strains tested from each soil, 70 and 65 degraded the fungicide in culture (the numbers respective of fungi isolated from MBC-history and non-history soils). Irrespective of the origin of the isolates, nearly 80% were identified as either Alternaria alternata or Ulocladium sp. One unique MBC-degrading fungus isolated from MBC-history soil was identified as Acremonium falciforme.

Nested statistical analysis based on the degradation capacity of each isolate that could degrade the fungicide showed that there was no difference in the degradation capacity of the populations, thus proving that based on our method of analysis no induction of MBC-degrading capacity could be accounted for. This analysis would not cover the possibility of a very rapid induction that might occur immediately upon contact with the fungicide (and perhaps at the early stage of the isolation process).

Degradation of MBC by mixed bacterial cultures.

In order to assess the possible role of bacteria in accelerated degradation of MBC in soil, degradation capacity of mixed bacterial cultures

derived from MBC-history and non-history was determined. Five g of the tested soils were added to a nitrogen and salts medium. Carbon source in the medium consisted of MBC (10 $\mu\text{g/ml}$) and available carbon from the soil sample. The mixed cultures were incubated (30°C) on a reciprocal shaker (140 strokes/min). Samples were taken at various time intervals and analyzed for MBC-history soil (Figure 1).

When transferred to fresh medium (containing 5 g autoclaved soil) MBC-degrading capacity fluctuated in time indicating, most likely, the presence of a microbial factor capable of repressing MBC-degrading capacity once it reaches a high level (Figure 2). We think this can be due to either direct predation or competition for an MBC-derived metabolite which the competitor cannot independently obtain from the parent compound, yet is more efficient than the degrader in utilizing as a carbon source. Fluctuations in MBC degradation are more frequent in medium amended with a high concentration of the fungicide (100 ppm). This can be due to a higher abundance of substrate and metabolite, providing for a more rapid quantitative change in the bacterial components of the culture.

When transferred to the same medium unamended with soil, degradation capacity was rapidly diminished.

MBC DEGRADATION BY MIXED CULTURES FROM
MBC-HISTORY AND NON-HISTORY SOILS

Fig. 1

DEGRADATION OF MBC BY MIXED CULTURES (10 ppm)

DEGRADATION OF MBC BY MIXED CULTURES (100 ppm)

Fig. 2

b. Accelerated Degradation of Herbicide Diphenamid

Accelerated Degradation of Diphenamid in Soils and Means for its Control

ELANA AVIDOV, NADAV AHARONSON and JAACOV KATAN

Abstract. Accelerated degradation of the herbicide diphenamid (N,N-dimethyl-2,2-diphenyl-acetamide) was investigated in Israeli soils. Repeated application of this herbicide in the soil in the laboratory enhanced its degradation. The degradation increased with increasing number of applications. After the fourth application nearly 100% of the herbicide was degraded within 5 days of incubation, whereas only slight degradation was observed during the first 25 days in a previously untreated soil. Accelerated degradation was also observed in a soil collected from a field with previous diphenamid treatments. Fumigation with methyl bromide or treating the soil with the fungicide fentin acetate (triphenyltin acetate) was effective in decreasing the accelerated degradation of diphenamid, whereas the fungicides TMTD (tetramethylthiuram disulfide) and TBZ [2-(4-thiazolyl)-benzimidazole] were only partially effective in inhibiting accelerated degradation. In the laboratory, fentin acetate prevented the degradation of diphenamid applied to previously untreated soil. Several fungi capable of degrading diphenamid were isolated from soils with or without accelerated degradation.

משרד החקלאות
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INTRODUCTION

The accelerated degradation of pesticides in soil and the accompanying loss of pest control have been demonstrated in recent years for a growing number of "soil-applied" pesticides (3, 8, 9, 12, 14, 16, 17, 18, 19). In most cases the accelerated degradation of these pesticides has been linked with previous application of the same pesticide or a structurally related one. Rahman et al. (14) and Obrigawitch et al. (12) observed reduced activity of EPTC in soils receiving annual applications and correlated their findings with more rapid degradation of the herbicide in these previously treated soils. Similar observations were reported for other pesticides such as carbofuran, metalaxyl, benomyl, iprodione and diphenamid (3, 8, 9, 16, 17, 18, 19).

Preliminary work in our laboratory indicated that repeated soil applications of diphenamid cause an increase in the chemical's degradation. Fungicides or disinfestants slowed down the degradation of certain fungicides or herbicides applied to the soil (1, 2, 11, 15, 16, 18, 19).

The present work was planned to examine the effect of repeated applications of diphenamid on its degradation and possibilities to control the phenomenon of accelerated degradation.

MATERIALS AND METHODS

Soils. A sandy soil from Rehovot (soil subgroup - Rhodoxerolf: 6% clay, 1% organic matter, 11% field moisture capacity and pH 7.5) was used. The soil was collected from a noncultivated field from the upper 5-20 cm layer and stored at 5 C until used. In one experiment (Table 1) an additional soil from Bet haGedi (soil subgroup - calciorthid: 25% clay, 19% field moisture capacity and pH 8.3) was used. This soil was collected from a commercial field, previously treated twice with diphenamid, in 1982 and 1984. For comparison, samples of untreated soil were collected from an adjacent plot. At the beginning of each laboratory experiment, the soil was sieved through a 0.5 mm screen and moistened to about 80% of field moisture capacity.

Pesticides. Analytical diphenamid was obtained from the EPA, and 50 % w.p. formulation from Palimport, Israel. TMTD, 80 % w.p. formulation was obtained from Tapazol Ltd.; fentin acetate (60 % w.p.) from Makhteshim Ltd.; Daconil (tetrachloroisophthalonitrile), 75 % w.p., and PCNB (pentachloronitrobenzene) 75 % w.p. from Palimport, all in Israel. TBZ (60 % w.p.) was obtained from E. Merck.

Soil fumigation. Polyethylene bags, each containing 1 kg. soil (pretreated with diphenamid, or untreated) (Figure 2), were buried open in the field. A layer of 5 cm of soil from the field was placed on top of the open bags. Methyl bromide was applied at 50 g/m² to the preirrigated soil under a 0.1- mm-thick polyethylene cover. After 48 h the polyethylene sheets were removed and the soil was left to aerate for 48 h.

Degradation study of diphenamid after consecutive applications. Rehovot soil was treated in the laboratory in batches of 3 kg each. Diphenamid (1 mg/ml in acetone) was added dropwise to 100 g of air-dried soil and the soil was aerated to remove all traces of the solvent. The soil was then mixed with 2.9 kg of the same

soil, to give a final diphenamid concentration of 5 $\mu\text{g/g}$ soil. The soil was moistened to 80 % of field moisture capacity, kept throughout the incubation period at room temperature ($25 \pm 3^\circ\text{C}$), in plastic containers covered with thin polyethylene sheets. After 90 days of incubation, when the herbicide concentration dropped below 1 $\mu\text{g/g}$, the soil was treated once more with diphenamid following the same procedure described for the first treatment. This procedure was repeated four times (Figure 1). After each incubation period, 150 g soil, replicated three times, was withdrawn from the container and transferred to biometer flasks for degradation assessment as described below. Concomitantly, control soils (nontreated with diphenamid) were also moistened and kept in plastic container; at the end of each incubation period, soil samples were withdrawn and treated as described for diphenamid pretreated soil. In additional experiments (Figures 2-4) batches of Rehovot soil with accelerated degradation were obtained by treating the soil twice with diphenamid as described above.

Degradation of diphenamid with and without the addition of fungicides. Acetone solution of 1 mg/ml diphenamid was added dropwise to 40 g of air-dried and sieved Rehovot soil. The soil was left uncovered for 24 h in a ventilated hood to remove all traces of the solvent. The treated soil was then mixed with 110 g of the same soil to provide a sample of 150 g. Each sample was placed in a biometer flask and moistened to 80 % of field moisture capacity. The flasks were sealed with thin polyethylene sheets and incubated in the dark at 26°C . Twenty ml of distilled water was added to the side arm of the flask to maintain the moisture level of the soil. Each experiment was replicated three times. At different time intervals subsamples of 20 g each were taken from the flasks and stored at -18°C until analyzed. When fungicides were used, they were dissolved in acetone (1 mg a.i./ml), added to 40 g Rehovot soil one day after diphenamid application, aerated to remove the acetone and then mixed with 110 g of untreated Rehovot soil to provide a sample of 150 g. The final concentrations of diphenamid and the fungicides in the soil were 5 and 20 $\mu\text{g/g}$, respectively.

Diphenamid extraction and chemical analysis. Twenty g of soil was shaken in a 250 ml Erlenmeyer flask with a 150 ml mixture of water:acetone (1:1, v/v) for 5 minutes. The aqueous solution was separated from the soil and the soil was re-extracted with 100 ml petroleum ether. The two extracts were combined, shaken, and the upper petroleum ether phase was separated. The aqueous phase was re-extracted twice more

with a mixture of petroleum ether and acetone (10:3, v/v). The petroleum ether extracts were combined, dried over anhydrous Na_2SO_4 , concentrated to near dryness on a rotary evaporator, and diluted with acetone to ca 10 ml, for GLC analysis. If needed, the sample was concentrated to a smaller volume. Diphenamid in the sample was determined against analytical standards using a Varian 6000 gas chromatograph, equipped with a Varian thermionic specific detector (TSD) and a glass column (180 x 0.2 cm i.d.), packed with 3% OV-17 on 80/100 mesh Gas Chrom Q. Injector, detector and column temperatures were 220, 230 and 210 C, respectively. Nitrogen (carrier gas), hydrogen and air were used at flow rates of 30, 4.5 and 175 ml/min, respectively. Retention time of diphenamid was 4.20 min, minimal detection level was 0.005 ug/g soil, and the efficiency (recovery) of the method was $95 \pm 5\%$.

Degradation of diphenamid by fungi isolated from soil. Fungi were isolated from the soil by the standard soil dilution method (6), using 0.1 % agar for dilution. Aliquots of 0.2 ml of the dilution suspensions were spread over solidified Martin rose bengal medium (6). After 5 days of incubation at 27 C, the fungal colonies were transferred to plates with potato dextrose agar medium to which diphenamid (10 ug/ml) was added. After 10 days of incubation at 27 C, five agar pellets (13 mm in diameter, approximate total volume of media 0.4 ml) were transferred from each plate to 125 ml Erlenmeyer flasks containing a solution of 80 ml water and acetone (1:1 v/v). Diphenamid was extracted by placing the flasks for 10 min in a Sonicator ultrasonic apparatus. The aqueous phase was separated from the pellets and the pellets re-extracted in the ultrasonic apparatus with 55 ml of petroleum ether for 10 min. The two extracts were combined, shaken, and the petroleum ether phase separated. The aqueous phase was re-extracted twice more with 70 ml of petroleum ether and acetone (10:3 v/v). The three petroleum ether extracts were combined, dried over anhydrous Na_2SO_4 , concentrated on a rotary evaporator, and adjusted to ca 10 ml with acetone. The efficiency (recovery) of the extraction from the pellets was $75 \pm 5\%$. Data are presented as the mean + standard error.

RESULTS

Degradation of diphenamid after consecutive applications. Diphenamid persisted longer in soils with no prior treatment, than in previously treated soil (Figure 1). This accelerated degradation increased with

increasing number of diphenamid applications. After two applications, 30 % of the applied diphenamid (5 $\mu\text{g/g}$) was degraded within 25 days of incubation, while no degradation was observed for the single application. After the third application, 61% of the applied dose was degraded within 10 days of incubation and after the fourth application nearly 100% was degraded within 5 days of incubation. Soil samples taken after the second and fourth applications of diphenamid were incubated at 25 C for 18 months in order to determine the persistence of this phenomenon. Then diphenamid (5 $\mu\text{g/g}$) was added to these soils and its degradation determined as described above. Accelerated degradation of diphenamid in these batches of soils (after 18 months of incubation) was still the same as shown in Figure 1.

Diphenamid degradation, as tested in the laboratory in soil that was pretreated twice with diphenamid in a field at Bet haGedi, was found to be faster than in the nontreated soil obtained from an adjacent plot (Table 1).

After 10 days of incubation, in the laboratory, the concentration of the applied herbicide was 1.1 $\mu\text{g/g}$ in the previously treated soil, whereas its concentration in the nontreated soil was three fold higher.

Diphenamid degradation with or without fungicides. Two approaches were followed in this study in an attempt to control the phenomenon of accelerated degradation of the herbicide. The first approach involved the use of fumigation or fungicides for suppressing accelerated degradation in a soil with a history of application with the herbicide and which already acquired the capacity of accelerated degradation. The second approach involved the addition of fungicides to the soil with no history of diphenamid application in an attempt to delay or to avoid future development of accelerated degradation in the soil.

Figure 1. Effect of repeated application of diphenamid (5 µg/g) in the laboratory, on its degradation in Rehovot soil (Appl.=application). (Bars are the mean standard error of three replicates).

Soil fumigation with methyl bromide strongly slowed down the degradation of diphenamid in a soil that had been previously treated with this herbicide and that had acquired the capacity of accelerated degradation (Figure 2). The degradation of diphenamid in the fumigated soil was even slower than in the control soil without previous application of diphenamid. Fumigation with methyl bromide slowed down diphenamid degradation in the latter (control) soil as well. Fentin acetate was as effective as methyl bromide in slowing down the degradation of diphenamid in the pretreated soil (Figure 3). This effect on the degradation of diphenamid was less pronounced for TMTD and TBZ than for fentin acetate. Similar results with the three fungicides were obtained in another experiment repeated 12 months later.

Fentin acetate and TMTD were found effective in suppressing the degradation of diphenamid in a soil without previous application of diphenamid (Figure 4). In a separate experiment, the fungicide daconil was also effective in suppressing degradation of diphenamid in a soil without previous application of the herbicide. After 70 days of incubation, the residues of diphenamid, as percent of the applied, were 65.3 and 1.8 in soils with and without daconil, respectively. However, daconil was not effective in soils which had already developed accelerated degradation. PCNB was effective in suppressing accelerated degradation in one experiment, but failed to do so in the second experiment.

Table 1. Degradation of diphenamid in previously treated and untreated soils collected from a field near Bet haGedi.

Days after treatment ^a	Soil previously treated with diphenamid ^b	Nontreated soil ^c
Diphenamid in soil (µg/g) ^d		
0	4.00 + 0.081	4.20 + 0.04
10	1.10 + 0.040	3.30 + 0.07
20	0.13 + 0.000	0.22 + 0.02
35	0.08 + 0.004	0.15 + 0.00

^aFor the degradation assessment the soil was treated in the laboratory with diphenamid at 5 µg/g soil and incubated for 35 days at 27 C. ^bThe soil was previously treated in the field, in 1982 and 1984. ^cThe soil was collected from a noncultivated adjacent plot. ^dResults are the average of three replicates (± standard error).

In order to evaluate the effectiveness of fungicides in inhibiting development of accelerated degradation, diphenamid and the respective fungicides (fentin acetate or TMTD) were applied to a nontreated soil twice (120 day interval), as shown in Table 2. The results showed that only fentin acetate inhibited the development of accelerated degradation of diphenamid after two consecutive treatments. The concentration of diphenamid, 88 days after the second treatment with the herbicide plus the fungicide, was 44 % of the amount applied for fentin acetate, whereas TMTD was not effective in the second treatment.

Figure 2. Effect of soil fumigation with methyl bromide (MB) on the degradation of diphenamid (5 µg/g) in Rehovot soil with (P) and without (NP) accelerated degradation. (Bars are the mean standard error of three replicates).

Figure 3. Effect of three fungicides on the degradation of diphenamid (5 µg/g) in Rehovot soil with accelerated degradation (P). NP = control soil, without accelerated degradation. (Bars are the mean standard error of three replicates).

Figure 4. Effect of two fungicides on the degradation of diphenamid (5 $\mu\text{g/g}$) in a Rehovot soil without previous application of diphenamid. FA=Fentin acetate. (Bars are the mean standard error of three replicates).

Degradation of diphenamid by fungi isolated from soil. The effectiveness of certain fungicides in slowing down diphenamid degradation suggested that soil fungi might be involved in the breakdown of this herbicide. About 100 isolates from each soil were tested. A variety of fungi belonging to various genera, including *Fusarium*, *Aspergillus*, *Penicillium* and some non-sporulating fungi, which were not identified, were isolated from soils with and without a history of previous treatments. About 7% of the fungi isolates from each soil were found to be able to degrade diphenamid in culture. For example, one isolate of *Fusarium* that was isolated from a soil with a history of applications degraded 70% of diphenamid within 10 days of incubation in agar plates. However, there were no differences in the number of degraders and in the rate of degradation among the different fungi.

Degradation of diphenamid by soil bacteria (mixed culture). Five gram of nontreated and diphenamid pretreated soil were transferred to erlenmeyer flasks containing 25 ml of soil extract medium and diphenamid 10 $\mu\text{g/ml}$. The flasks were incubated at 28°C on a shaker at 115 rpm. After 6 and 12 days of incubation 2 ml aliquot was taken from each

Table 2. Effect of the fungicides TMTD and fentin acetate on the degradation of diphenamid in Rehovot soil .

Days after treatment ^a	Diphenamid	Diphenamid + TMTD ^b	Diphenamid + fentin acetate ^b
Diphenamid in soil (µg/g) ^c			
First treatment			
60	1.7 + 0.04	1.8 + 0.16	2.0 + 0.06
90	1.3 + 0.00	1.3 + 0.16	1.5 + 0.00
Second treatment ^d			
50	0.010 + 0.006	0.02 + 0.000	3.5 + 0.006
88	0.007 + 0.006	0.02 + 0.000	2.2 + 0.000

^aFor the degradation assessment the soil was treated in the laboratory with diphenamid at 5 µg/g soil and incubated for the indicated period at 27 C. ^bThe fungicides were added one day after diphenamid application, at 20 µg/g soil. ^cResults are the average of three replicates (+ standard error). ^dThe second treatment, following the same procedure, was carried out 120 days after the first treatment.

erlenmeyer and extracted with 2 ml of ethyl acetate. The extract was dried over anhydrous sodium sulphate and the residues determined using GLC. After 6 days of incubation diphenamid concentration in all the samples was unchanged, while after 12 days only 1% of the initial concentration was found in the flasks containing mixed culture of bacteria that were collected from the pretreated - problem soil.

Diphenamid concentration in the mixed cultures of bacteria from the nontreated soils was after 20 days still 80-90% of the amount applied. Thus only bacteria from the pretreated history soils showed adaptation to degrade diphenamid at much higher rates.

Repeated application of diphenamid in soil resulted in its accelerated degradation, which indicates the establishment of a degrading factor in the soil. Under laboratory conditions, half-life of this herbicide was shortened from about 50 days in previously untreated soil to less than 5 days after four applications. Accelerated degradation was also detected in soil that was pretreated under field conditions (Table 1), and under field and laboratory conditions in another study in the USA, using $^{14}\text{CO}_2$ evolution as a parameter for degradation (8). Thus, diphenamid is another pesticide which may exhibit diminishing herbicidal efficacy in soils with a history of applications.

The development of accelerated degradation might be due to a buildup of population of soil microorganisms capable of degrading the pesticide as shown in various cases (9, 13), or to the accumulation in soil of enzymes induced by the pesticide. In the present study, antimicrobial agents of different types reduced the accelerated degradation to various degrees, thus indicating the involvement of microbial processes in the accelerated degradation. Fentin acetate was very effective in suppressing diphenamid degradation in a soil which had already acquired the capacity of accelerated degradation (Figure 3), as well as in soil without previous applications. TMTD was more effective in reducing the degradation of diphenamid in previously untreated soil than in soils with previous applications. This difference indicates that repeated application of pesticides may cause both quantitative and qualitative changes in the population of soil microorganisms which degrade the pesticide. This may be due to a shift in the composition of soil microflora which leads to an increase in the population of specific groups of microorganisms. Diphenamid affects the activity of soil microorganisms and the relationships between pathogenic and nonpathogenic soil microorganisms (7). In addition to fungi (10), other groups of microorganisms apparently play a role in the degradation of diphenamid in soil. Although accumulating evidence shows that accelerated degradation is due to microbial mechanisms, other abiotic mechanisms should not be ruled out. A higher desorption capacity of diphenamid found in a soil with a history of diphenamid application may cause an increase in its availability to soil microorganisms for degradation (8).

Similar to the concept of the extenders which are antimicrobial agents (5), broad spectrum drastic biocides, e.g. methyl bromide, Vorlex, dazomet and solarization to disinfest soil (Figure 2, and references 6, 15, 16,

18), and the fungicide fentin acetate (Figure 3), were very effective in controlling accelerated degradation of various pesticides. Fungicides are cheaper and easier to apply than soil disinfestants. Fentin acetate was very effective in both preventing the development of accelerated degradation and in suppressing it in soil which had acquired this capacity. Possible explanations for the high effectiveness of this fungicide are long persistence in the soil (4) and high toxicity to the microbial degraders. Suppression of biodegradation of pesticides by fungicides and other agrochemicals was shown in other studies (2, 11, 19). However, antimicrobial agents should be used with special care in the soil, since they may also adversely affect beneficial microorganisms.

In fields where crop rotation has been abandoned, various adverse phenomena may develop: accelerated degradation and consequently loss of pesticidal efficacy, selection of biotypes resistant to the pesticides, and a rapid buildup of pest, pathogen and weed populations. An adequate crop management strategy which includes crop rotation and alternation of methods of control and of pesticides, should be the primary tool for preventing the development of accelerated degradation. If this can be achieved, the reliance on extenders and other antimicrobial agents might be much reduced.

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c. Accelerated Degradation of Thiocarbamate Herbicides in Israeli Soils with History of Application

1. Accelerated degradation of thiocarbamate herbicides in field soils

Soils were collected from fields with a history of vernolate application at three different sites in Israel. In these fields, failures in weed control was noticed or suspected. These soils were tested either in the greenhouse (using oats as a bioassay plant) or in the laboratory (using ^{14}C -EPTC) for assessing rate of degradation, as compared to untreated (nonhistory) control soils collected from adjacent sites. These studies were carried out jointly with A. Tal and B. Rubin at the Faculty of Agriculture.

Results from bioassay studies clearly show that the three soils acquired accelerated degradation for EPTC, vernolate and butylate (Fig. C-1). This was expressed in a significant reduction in the half life of each of the three herbicides in each soil. The average half life of EPTC, vernolate and butylate was shortened in the history soils by 7.6, 8.6 and 6.7 days, respectively. Cycloate on the other hand was slowly degraded and had the longest half life (38 days or more) and its degradation rate was similar in soils with or without history of application. The rate of degradation in the control soils was similar to that reported from other studies. It is also shown that cross accelerated degradation between thiocarbamate herbicides is possible.

The vernolate history and nonhistory soils were also tested for accelerated degradation by amending with ^{14}C -EPTC and measuring $^{14}\text{C}_2$ evolution as a criterion for biodegradation. Results (Fig. C-2) essentially confirmed those obtained with bioassay studies regarding accelerated degradation of EPTC in the three history soils tested. No "lag period" was observed. Netzer Sireni soil exhibited the most pronounced accelerated degradation and was used

Fig. C-1. Half life of four thiocarbamate herbicides applied at 7 ug/g to three soils with a history of vernolate application (NH) as compared to nonhistory (control) soils (H). An asterik denotes a significant ($P=0.05$) difference between H and NH. Oats were used for bioassaying the soil and determining concentration of the herbicide.

Fig. C-2. $^{14}\text{CO}_2$ evolution from soils treated with ^{14}C -EPTC (0.5 μCi) collected from three sites. NH=control soil without history of application. H=Soil with a history of vernolate application. Statistical analysis ($P=0.05$) were done separately for each incubation period.

in most of the following studies.

Accelerated degradation of EPTC was artificially induced in nonhistory soils collected from the above three sites. Thus, at 21 days intervals the soils were treated with EPTC ($10 \mu\text{g/g}$) and rate of degradation was measured by oat plant bioassays and compared for respective nonhistory soils. Results from these studies clearly showed that accelerated degradation can be induced in each of the above soils. With increasing number of applications the half life of EPTC becomes shorter. However, accelerated degradation could not be induced in a soil collected from Rehovot.

2. Microbial studies

Sterilization of soils, either by autoclave or by gamma irradiation, nullified accelerated degradation. This suggested that this phenomena might be due to the enrichment in soil of populations of microorganisms capable of degrading the herbicide, as a result of the repeated application of the herbicide. The following experiments deals with this possibility.

Total counts of bacteria, fungi, and actinomycete populations did not reveal significant differences regarding history or nonhistory soils (Fig. C-3). It should be mentioned, however, that such counts cannot distinguish between microorganisms that degrade herbicides and those lacking such a capacity. The relative density of microbial EPTC degraders in soils was estimated using enrichment mixed cultures. Samples of 50 g tested soils were incubated in flasks containing 200 ml of liquid medium containing basal salts and $50 \mu\text{g/ml}$ EPTC. Results (Fig. C-4) show accelerated degradation only when the medium was inoculated with a history soil. The antibacterial agent chloramphenicol inhibited accelerated degradation while the antifungal agent chloheximide did not. Similar results were obtained when a soil with accelerated degradation from Netzer Sireni was treated with the above two

Fig. C-3. Population densities of microorganisms in Netzer Sireni soil with (H) or without (NH) history of vernolate application.

Fig. C-4. The effect of chloramphenicol (100 ppm) and cycloheximide (100 ppm) on the degradation of ^{14}C -EPTC by microorganisms isolated from soils with (H) or without (NH) history of applications.

antimicrobial agents. These results suggest that bacteria play an important role in the accelerated degradation of EPTC. This was further confirmed using pure microbial cultures. Sixty isolates of bacteria were randomly isolated from mixed cultures originated from either Netzer Sreni history or from a nonhistory soil. Each of these isolates was separately tested for its capacity to degrade EPTC in liquid culture. Eighteen isolates of bacteria from history soil (30% of the isolates) and none of those from nonhistory soil were able to degrade the herbicide in culture. Typical pattern of degradation of EPTC by two bacteria isolates capable of degrading the herbicide and one isolate while does not degrade the herbicide is shown in Fig. C-5).

One bacterial isolate capable of degrading EPTC was tested for its capacity to degrade in culture the thiocarbamate herbicides EPTC, vernolate, butylate, and cycloate in the earlier studies. Results (Fig. C-6) show a phenomenon of cross accelerated degradation similar to that observed in soil (Figs. C-1, C-2). Again, EPTC, vernolate and butylate were more readily degraded (in that order) by the bacterial degradation than cycloate. The effectiveness of the nondegrader isolate was very low.

Accelerated degradation was induced in a nonhistory soil by inoculating it with an isolate of bacterial degrader (Fig. C-7).

3. Controlling accelerated degradation in soil

Antimicrobial treatments, e.g., soil sterilization (autoclave, gamma irradiation), soil disinfestation (fumigation, solarization), and fungicides were used in an attempt to curb accelerated degradation of EPTC in soil. Results (Tables 1 and 2) demonstrate that soil sterilization and the mercury fungicide Hg-ethyl phosphate, were very effective in controlling accelerated degradation. The other treatments exhibited various degrees of effectiveness in reducing degradation. These results further confirm the previous studies

Fig. C-5. Degradation of EPTC (50 ug/ml) in liquid culture by two isolates of bacteria.

Fig. C-6. Degradation of four thiocarbamate herbicides in liquid culture supplemented with the herbicides at 50 ug/ml, by two isolates of bacteria. ND=A nondegrader isolate which served as a control.

Table C-1. Effect of soil sterilization, solarization or fumigation on degradation of ^{14}C -EPTC (0.5 μCi) added to Netzer Sireni soil with accelerated degradation.

Soil treatment	$^{14}\text{CO}_2$ (% of applied ^{14}C -EPTC)			
	Days of incubation			
	6	12	18	24
Untreated	25a *	52a	57a	58a
Solarization	14b	28b	38b	45ab
Methyl bromide	4bc	17c	29c	39b
Metham sodium	5bc	24bc	43b	54a
Autoclave	1c	3d	5d	5c
Gamma irradiation	2c	4d	4d	4c

*In each column, values with a common letter are not significantly different ($P=0.05$).

Table C-2. Effect of antimicrobial substances or fungicides on degradation of ^{14}C -EPTC (0.5 μCi) added to Netzer Sireni soil with accelerated degradation. Concentrations (in $\mu\text{g/g}$) are given in parenthesis.

Soil treatment	$^{14}\text{CO}_2$ (% of applied ^{14}C -EPTC)			
	Days of incubation			
	6	12	18	24
Untreated	25a *	52a	57a	58a
THID (20)	12bc	31b	50ab	59a
Fentin acetate (20)	13bc	27bc	46b	56ab
Ilg-ethyl phosphate (20)	7c	11d	13d	14d
Cycloheximide (100)	19ab	51a	56a	57d
Chloramphenicol (100)	10bc	18cd	32c	48b
Dichloramide (1)	20ab	50a	54ab	57a
Diethyloate (1)	12bc	23c	26c	31c

*In each column, values with a common letter are not significantly different ($P=0.05$).

regarding the involvement of microorganisms in accelerated degradation.

Fig. C-7. Degradation of EPTC in a soil (at 10 $\mu\text{g/g}$) without history of application either uninoculated or inoculated with nondegrader bacterium (ND) or a degrader bacterium (D).

2. UNITED STATES:

a. Accelerated Degradation of Organophosphate Insecticides

The objective of this investigation was to examine the rate and mechanism of terbufos, phorate, fonofos, chlorpyrifos, and ethoprop dissipation in soils where field performance failures were apparent.

Materials and Methods

Soil samples from Midwestern corn fields where insecticide performance was in question were collected and shipped to the Soil-Microbial Systems Laboratory, Beltsville, MD. Control soil samples (no insecticide history) were also obtained from fence row areas adjacent to each of the sampled fields. Information received, location and owner of soil samples used in this investigation are recorded in Table 1. Soils received were stored at 4°C until used. Prior to use, each soil sample was sufficiently air dried to allow sieving through a No. 10 (2.0 mm) U.S. Standard sieve. All soils were characterized as to their existing soil moisture content, organic matter content, pH and soil moisture content at 75% of field capacity (Table 2).

The dissipation of phorate and terbufos, the accumulation and dissipation of their sulfoxide and sulfone degradation products, and the dissipation of chlorpyrifos, ethoprop and fonofos were examined in the various soils collected.

Soil treatments were established by treating ca 15 grams of each treatment, each soil with an acetone solution of the pesticide, thoroughly mixing the treated soil, allowing the solvent to evaporate (ca. 3 hrs), and then mixing the treated soil with the remainder of the sample (ca. 205 gms) and placing it in a 500 ml Erlenmeyer flask. The treated soils were then brought to 75% field moisture capacity with dropwise addition of water, covered with polyvinyl plastic film fastened with a rubber band and incubated

Table 1. Number, location, history, and problem description of soils used in this investigation.

Soil No.	Farmer-Location	Treatment history by year					Problem description
		1985	1984	1983	1982	1981	
OP-85-45/46*	Kellog, Iowa	Counter	Counter	Counter	Counter	Furadan	-lodged corn, severe root pruning
OP-85-129/130	Fred Awe Rt #1 Shullsburg, Wisconsin 53586	Counter	Lorsban	Counter	Counter	Counter	Plants 1000 acres corn, used Lorsban, Thimet, Counter and had problems with all compounds.
OP-85-133/134	Bob Weber Rt #2 Knightstown, Indiana 46148	Counter	Counter	Counter	Counter	Dyfonate	Starting to see reduced performance
OP-85-135/136	Kinney Bros. Farm Kokomo, Indiana 46901	Counter	Counter	Amaze	Counter	Furadan	Severe lodging, 1983 Amaze failure
OP-85-43/44	Paul Engel Edelstein, Illinois	Counter	Counter	Counter	Counter	Counter	Counter failure this year, one field last year
OP-85-25/26	Fred Wells Dixon, Coop. Dixon, Iowa	Dyfonate	Dyfonate	Dyfonate	Dyfonate	Dyfonate	100% goosenecked, root rating of 5
OP-85-27/28	Bob Dahms Dixon Coop. Dixon, Iowa	Dyfonate	Lorsban	Counter	Lorsban	Lorsban	50% goosenecked

*Odd number is problem soil. Even number is fence row control.

Counter = terbufos
 Lorsban = chlorpyrifos
 Dyfonate = fonofos
 Furadan = carbofuran

Table 2. Chemical-physical characteristics of soils used in this investigation.

Soil No.	% Organic matter	pH	Soil type
OP-85-45*	2.5	5.9	silt loam
OP-85-46	2.5	5.3	silt loam
OP-85-129	2.36	5.6	loam
OP-85-130	2.80	5.9	loam
OP-85-135	2.4	5.5	loam
OP-85-136	2.7	5.8	loam
OP-85-43	2.6	5.4	loam
OP-85-44	2.6	5.6	loam
OP-85-25	1.5	5.6	sandy loam
OP-85-26	2.3	5.7	sandy loam
OP-85-27	2.2	5.7	silt loam
OP-85-28	2.4	5.8	silt loam
OP-85-133	1.38	5.5	sandy loam
OP-85-134	1.77	5.4	sandy loam

at 25°C in the dark. Each treatment was replicated three times. A ten gram sample of soil was removed from each treatment and replication at 0, 1, 2, 3, and 4 weeks after treatment, placed in a scintillation vial and stored at -18°C until analyzed.

All soil samples were extracted by the method of Aharonson et al. (1986). Briefly, each soil sample (10 gm dry weight) was placed in a 250 ml Erlenmeyer flask and extracted with an acetone water mixture (75 ml:75 ml) on an oscillating shaker (200 rpm) for 30 minutes. The soil suspension was allowed to settle over night and a 50 ml aliquot of the supernatant was extracted sequentially in a 250 ml separating funnel with (a) hexane, 50 ml; (b) hexane, 50 ml/acetone, 20 ml; and (c) hexane, 50 ml/acetone, 20 ml. The combined extracts were concentrated with a low pressure evaporator to less than 5 ml, transferred to screw-cap test tubes and brought back up to 5 ml with hexane. One ml of this concentrated extract was then dried over anhydrous sodium sulfate and used for GC analyses.

A Perkin-Elmer Sigma 2B Gas Chromatograph equipped with a NPD detector, column 6', 1/4" ID, loaded with 3% OV-17 on 80/100 mesh Gas Chrom Q, carrier gas, hydrogen at 10 kpa, helium at 60 kpa, and air at 175 kpa. The injector and detector temperatures were set at 220°C, and column temperature at 200°C. Peak height was used for quantification. Attenuation was set at 1. Terbufos, terbufos sulfone, phorate, phorate sulfone, fonofos, chlorpyrifos, and ethoprop were all analyzed with the same parameters with excellent peak identification. Terbufos sulfoxide and phorate sulfoxide determinations were made by oxidation of the sulfoxide to the corresponding sulfone by the method of Chapman et al. (1980). One ml of the concentrated extract was diluted with 9 ml of hexane, and oxidized with 20 ml of aqueous saturated potassium permanganate solution by shaking in a 50 ml screw-cap test tube for 15

minutes. The upper hexane layer was separated and dehydrated with anhydrous sodium sulfate, brought to a standard volume and injected into the GC. The recoveries of terbufos, terbufos sulfoxide, terbufos sulfone, phorate, phorate sulfoxide, phorate sulfone, chlorpyrifos, ethoprop and fonofos at zero times were greater than 99%.

The degradation of ethyl- ^{14}C -terbufos and methylene- ^{14}C -terbufos was examined in soil samples obtained from Kellogg, Iowa. Fifty grams (dry weight basis) of each soil sample were treated with terbufos to a final concentration of 3.5 ppm. Soils were treated by applying the terbufos solution dropwise over the soil surface, allowing the solvent to evaporate, thoroughly mixing the sample by shaking and stirring and placing in a biometer flask (Bartha and Pramer 1965). The soils were then watered to a final moisture content of 75% of field capacity (as determined by a Star pressure plate extractor, Soil Moisture Equipment, Santa Barbara, Calif.). The side arm of each biometer flask was then charged with 10 ml of 0.1N NaOH to absorb evolved CO_2 . $^{14}\text{CO}_2$ evolution was determined every two days by withdrawing the 10 ml of NaOH and replacing it with fresh NaOH. One ml subsamples of the spent NaOH solution from each flask were mixed with 10 ml of Ready-Solv HP Scintillation cocktail (Beckman Instruments Inc., Fullerton, CA 92634) and ^{14}C content determined by a 1219 Spectral Liquid Scintillation Counter (LKB Wallac, Finland). Each treatment had three replications and the experiment was repeated twice.

All ^{14}C soil treatments were sacrificed after a 16-day incubation period and extracted by the method of Chapman et al. (1982). Briefly, each 50 gram sample was extracted with 100 ml acetone by shaking on an oscillating shaker (30 rpm) for 1 hr. The acetone extract was separated from the soil by vacuum filtration through a Buchner funnel fitted with Whatman No. 1 filter paper. The soil residue on the filter paper in the funnel was then rinsed with an

additional 25 ml of acetone. The combined filtrate was then transferred quantitatively to a 250 ml separatory funnel and extracted with 100 ml of chloroform. The lower chloroform-acetone phase was retained and appropriate aliquots were partitioned with 10% acetone in hexane mixture and concentrated for analyses by GLC as described previously. Relative concentrations of terbufos, terbufos sulfoxide and terbufos sulfone were determined as described in preceding sections. The remaining chloroform-acetone extract was concentrated by evaporation. Forty ml of the concentrated extract was applied to 60 F254, precoated (thickness 0.25 mm; 20 x 20 cm, Merck) thin-layer chromatography (TLC) plates and developed with the solvent system used by Laveglia and Dahm (1975). Each TLC plate was developed in the first direction with toluene; and after drying, in the second direction with methanol:chloroform:toluene (10:95:95); and after drying, again in the original direction with nitromethane:acetone:toluene (26:65:10). Immediately after their final drying the plates were exposed to direct-exposure x-ray film (DEF-5, 8x10 inch, Eastman Kodak Co., Rochester, N.Y.) for 2 weeks. After development processing, the images on the x-ray film were mapped and compared with authentic metabolite standards. All radioactive spots were scraped from the plates, dissolved in Ready-Solv HP scintillation cocktail and counted to determine relative distribution of ^{14}C in metabolites.

Results

Degradation of terbufos and the appearance and disappearance of its sulfoxide and sulfone were examined in several performance failure (PF) and virgin fencerow (VF) pairs of soils. Examples of the results obtained are listed in Table 3. Terbufos degraded rapidly in each of the three soils to less than 50% of its original concentration in the initial week of incubation, and to approximately 10% of its original concentration in 2 weeks. Terbufos

Table 3. Dissipation of terbufos and appearance and dissipation of its metabolites in PF & VF soils from Shullsburg, WI (A), Knightstown, IN (B), and Kokomos, IN (C), (Original concentration applied was 1.5 ppm)

Soil source	Product	Soil types	ppm at weeks after treatment*				
			0	1	2	3	4
A	terbufos	PF	1.226a	0.301a	0.052a	0.024a	0.022a
		VF	1.423a	0.360a	0.151a	0.048a	0.027a
	t. sulfoxide	PF	0.055a	0.627a	0.085b	0.042b	0.009b
		VF	0.078a	0.872a	1.413a	1.023a	0.703a
	t. sulfone	PF	0.069a	0.060a	0.000b	0.000b	0.000b
		VF	0.043a	0.081a	0.146a	0.153a	0.243a
B	terbufos	PF	1.402a	0.443a	0.170a	0.057a	0.040a
		VF	1.351a	0.390a	0.142a	0.051a	0.040a
	t. sulfoxide	PF	0.104a	0.864a	0.960a	0.802a	0.390a
		VF	0.134a	0.802a	1.145a	1.015a	0.671a
	t. sulfone	PF	0.045a	0.097a	0.173a	0.103b	0.075b
		VF	0.018a	0.094a	0.191a	0.260a	0.331a
C	terbufos	PF	1.311a	0.593a	0.200a	0.094a	0.044a
		VF	1.500a	0.425a	0.121b	0.063a	0.060a
	t. sulfoxide	PF	0.087a	0.710a	0.144b	0.093b	0.043b
		VF	0.160a	0.880a	0.725a	0.872a	0.565a
	t. sulfone	PF	0.018a	0.052b	0.000b	0.010b	0.000b
		VF	0.026a	0.122a	0.131a	0.226a	0.241a

* Data in the same column, same metabolite, and same PF & VF soils followed by the same letter are not significantly different at 5% level using Duncan's multiple range test.

was converted to terbufos sulfoxide which accumulated to more than 60-80% of the total residues in all of the VF soils. Terbufos sulfoxide failed to accumulate in either of the A or C PF soils. Although the degradation of the terbufos sulfoxide was also somewhat faster in the B-PF soil than the B-VF soil, the difference was not significant at the 5% level using Duncan's multiple range test. (This soil was described in the soils table as, "starting to see reduced performance.") Terbufos sulfoxide was degraded to terbufos sulfone which accumulated from 5% of the total metabolites at time 0 to nearly 20% by the 4th week in VF soils. Terbufos sulfone either was degraded rapidly or did not accumulate in all PF soils as it did in VF soils.

Tables 4, 5 and 6 contain results obtained when the degradation of terbufos, phorate, chlorpyrifos, fonofos and ethoprop were examined in the terbufos problem soil from Shullsburg, WI. The results obtained with terbufos (Table 4) are again similar to those described in Table 3, i.e., not much of an effect on terbufos degradation, but a significant difference in the formation and accumulation of both the terbufos sulfoxide and sulfone. The pattern obtained with phorate (Table 5) was somewhat different in that the phorate sulfoxide did not accumulate appreciably in either the PF or VF soils. Phorate sulfone accumulated in the VF soil, but was not even detectable in the PF soil. The degradation of fonofos (Table 6) did not appear to differ between the terbufos PF or VF soils. The degradation of ethoprop actually was faster in the VF soil than in the PF soil, whereas the degradation of chlorpyrifos was significantly more rapid in the terbufos PF than VF soil. These results indicate that some cross degradation may occur between terbufos and phorate, and that either cross degradation or a multiple problem situation could develop with terbufos and chlorpyrifos. This soil had an application of chlorpyrifos (Lorsban) in the year preceding its last treatment with terbufos

Table 4. Dissipation of terbufos in terbufos history soil from Shullsburg, WI.

Insecticide or metabolite	Soil type	Weeks after treatment*				
		0	1	2	3	4
terbufos	PF	2.269a	0.963a	0.407a	0.194a	0.138b
	VF	2.294a	0.900a	0.341b	0.156a	0.210a
t. sulfoxide	PF	0.239a	1.197b	0.316b	0.258b	0.113b
	VF	0.336a	1.747a	1.753a	1.732a	1.748a
t. sulfone	PF	0.068a	0.034b	0.000b	0.000b	0.000b
	VF	0.068a	0.177a	0.211a	0.304a	0.370a

* Data in the same column with the same product and same set of PF & VF soils followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Table 5. Dissipation of phorate in terbufos history soil from Shullsburg, WI.

Insecticide or metabolite	Soil type	Weeks after treatment*				
		0	1	2	3	4
phorate	PF	1.942a	0.108a	0.048a	0.041a	0.029a
	VF	1.694	0.091a	0.041a	0.021a	0.021a
p. sulfoxide	PF	0.453a	0.160b	0.000a	0.000a	0.000a
	VF	0.331a	0.420a	0.064a	0.000a	0.000a
p. sulfone	PF	0.000b	0.000b	0.000b	0.000b	0.000b
	VF	0.135a	1.851a	1.993a	2.050a	2.025a

* Data in the same column with the same product and same set of PF & VF soils followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Table 6. Dissipation of chlorpyrifos, fonofos, and ethoprop in terbufos history soil from Shullsburg, WI.

Insecticide	Soil type	Weeks after treatment*				
		0	1	2	3	4
chlorpyrifos	PF	5.562a	2.393b	0.946b	0.715b	0.498b
	VF	5.394a	3.672a	2.544a	2.377a	1.866a
fonofos	PF	2.827a	2.096a	1.812a	1.972a	1.885a
	VF	2.798a	2.036a	1.819a	1.970a	1.682a
ethoprop	PF	1.685a	1.212a	0.805a	0.745a	0.438a
	VF	1.914a	1.040a	0.746a	0.486b	0.279b

* Data in the same column with the same insecticide and same set of PF & VF soils followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

(counter).

Table 7 contains data obtained on the degradation of terbufos, fonofos, and chlorpyrifos in a terbufos history soil from Edelstein, IL. In this instance the rate of terbufos degradation was significantly more rapid in the PF than VF soils by the second, third and fourth weeks. A more rapid degradation of the terbufos sulfoxide and failure of the sulfone to accumulate or form were noted in the terbufos PF soil. Chlorpyrifos degradation is significantly more rapid in the terbufos PF soil than in the VF soil. A significant difference in the rate of fonofos degradation was observed at the third and fourth week incubation periods.

Terbufos, chlorpyrifos and fonofos degradation was examined in a fonofos history soil obtained from Dixon, IA (Table 8). The degradation of fonofos occurred at a significantly more rapid rate in the fonofos PF than the VF soil. With exception of one sampling period, no statistically significant differences were observed in the rate of chlorpyrifos, terbufos, or terbufos sulfoxide in this soil. Terbufos sulfone formulation was significantly less in the PF soil in comparison to the VF soil; however, this effect may have resulted from the slower degradation of terbufos in the PF soil than in the VF soil.

The dissipation of terbufos, chlorpyrifos and fonofos was also examined in a soil having a history of all three organophosphate insecticides (Table 9). The dissipation of chlorpyrifos occurred more rapidly in this history (PF) soil than in its corresponding control (VF). Although small differences were apparent in the rates of terbufos and terbufos sulfoxide dissipation in these soils the differences were not statistically significant at the 5% level. There was no significant effect on fonofos degradation in this soil.

Table 10 contains results obtained when terbufos sulfoxide and phorate

Table 7. Degradation of terbufos, fonofos, and chlorpyrifos in terbufos history soil from Edelstein, IL.

Insecticide or metabolite	Soil type	Weeks after treatment*				
		0	1	2	3	4
terbufos	PF	3.957a	2.475a	1.281b	0.493b	0.281b
	VF	4.153a	2.527a	1.990a	1.230a	1.017a
t. sulfoxide	PF	0.918a	1.648a	0.717b	0.216b	0.124b
	VF	0.743a	1.519a	2.241a	1.993a	2.286a
t. sulfone	PF	0.000a	0.025a	0.000b	0.000b	0.000b
	VF	0.000a	0.025a	0.039a	0.082a	0.135a
chlorpyrifos	PF	4.510a	2.084b	0.930b	0.726b	0.582b
	VF	4.662a	3.559a	2.897a	2.823a	2.603a
fonofos	PF	2.688a	2.021a	1.876a	1.527b	1.148b
	VF	2.834a	2.005a	2.024a	1.832a	1.887a

* Data in the same column with the same metabolite and same PF & VF set of soils followed by the same letter are not significantly different at 5% level using Duncan's multiple range test.

Table 8. Dissipation of terbufos, fonofos, and chlorpyrifos in a fonofos history soil from Dixon, IA.

Insecticide or metabolite	Soil type	Weeks after treatment*				
		0	1	2	3	4
terbufos	PF	3.710a	1.961a	1.358a	0.623a	0.115a
	VF	3.873a	1.847a	0.901a	0.408a	0.071b
t. sulfoxide	PF	0.547a	1.584a	2.782a	2.265a	2.748a
	VF	0.632a	1.723a	2.965a	2.511a	2.357a
t. sulfone	PF	0.000a	0.039a	0.107b	0.147b	0.269b
	VF	0.000a	0.115a	0.266a	0.310a	0.415a
chlorpyrifos	PF	4.130a	2.035a	1.120a	0.825a	0.634a
	VF	4.043a	2.194a	1.119a	0.859a	0.567a
fonofos	PF	3.288a	1.578a	0.470b	0.179b	0.105b
	VF	3.024a	1.939a	1.742a	1.375a	1.342a

* Data in the same column with the same metabolite and same PF & VF set of soils followed by the same letter are not significantly different at 5% level using Duncan's multiple range test.

Table 9. Dissipation of terbufos, fonofos, and chlorpyrifos in a fonofos-chlorpyrifos-terbufos history soil from Dixon, IA.

Metabolites	Soil type	Weeks after treatment*				
		0	1	2	3	4
terbufos	PF	3.141a	1.485a	0.702a	0.221a	0.116a
	VF	3.441a	1.543a	0.776a	0.408a	0.162a
t. sulfoxide	PF	0.745a	1.175a	0.351a	0.084b	0.062a
	VF	0.622b	1.511a	0.412a	0.283a	0.168a
t. sulfone	PF	0.000a	0.039a	0.000a	0.000a	0.000a
	VF	0.000a	0.045a	0.000a	0.000a	0.000a
chlorpyrifos	PF	3.455a	2.050b	0.628b	0.570b	0.420b
	VF	4.060a	2.521a	1.148a	0.830a	0.608a
fonofos	PF	2.118a	2.091a	1.769a	1.484a	1.198a
	VF	2.143a	1.773a	1.503b	1.506a	1.207a

* Data in the same column with the same metabolite and same PF & VF set of soils followed by the same letter are not significantly different at 5% level using Duncan's multiple range test.

Table 10. The dissipation of terbufos sulfoxide and phorate sulfone in terbufos history and nonhistory soils obtained from Metamoro, IL.

Metabolites	Soil type	Weeks after treatment*				
		0	1	2	3	4
terbufos sulfoxide	PF	3.807a	2.808a	0.757b	0.305b	0.171b
	VF	3.794a	2.645a	2.253a	1.974a	1.665a
t. sulfone	PF	0.013a	0.523a	0.144b	0.064b	0.060b
	VF	0.000a	0.507a	0.664a	0.792a	0.721a
phorate sulfone	PF	6.648a	6.353a	0.962b	0.464b	0.320b
	VF	6.299a	6.387a	3.682a	2.758a	2.688a

* Data in the same column with the same metabolite and same PF & VF followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

sulfone were introduced directly into terbufos history and nonhistory soils. Terbufos sulfoxide was converted to the sulfone in both PF and VF soils during the first week of incubation, but thereafter both are degraded more rapidly in the PF soil than in the VF soil. After the first week of incubation the phorate sulfone is also more rapidly degraded in the PF than in the VF soil.

The degradation of ethyl- ^{14}C -terbufos and methylene- ^{14}C -terbufos was examined in terbufos history and nonhistory soils obtained from Kellogg, IA. Beginning with day 4-6 a more rapid evolution of $^{14}\text{CO}_2$ occurred from PF than VF treated soils with both ^{14}C -labels (Figure 1). This difference is more pronounced with the ethyl- ^{14}C label than with the methylene- ^{14}C label. Table 11 illustrates the distribution of original ^{14}C applied in products recovered and identified after a 16-day incubation period. Differences in distribution of ^{14}C activity are evident between both PF and VF soils and the ethyl- ^{14}C and methylene- ^{14}C labels. Less terbufos, terbufos sulfoxide and terbufos sulfone are observed in PF soils than in VF soils. Two additional metabolites detected only with the methylene- ^{14}C label are present in greater abundance in PF than VF soils. These materials are tentatively identified as CL 99843 and CL 99875 by chromatographic comparison with standards supplied by American Cyanamid personnel. Both CL 99843 and CL 99875 are hydrolytic products ultimately derived from terbufos (Figure 2). At least theoretically CL 99843 would not be derived from the hydrolysis of terbufos sulfone. Since in PF soils the terbufos sulfone either does not accumulate or does not form it would seem most likely that the CL 99843 is derived from an alternate pathway involving the hydrolysis of the terbufos sulfoxide (CL 94301) by the pathway indicated in Figure 2. Product CL 99875 may be derived from either CL 99843 or CL 99844. The absence of CL-99844 from TLC analyses and the presence of CL 99843 would suggest that CL 99875 is derived from CL 99843.

Table 11. Distribution of ^{14}C from ethyl- ^{14}C -terbufos treated history and nonhistory soils obtained from Kellog, IA.

Metabolites	ethyl- ^{14}C -terbufos		methylene- ^{14}C -terbufos	
	PF	VF	PF	VF
---- % of original ^{14}C radioactivity ----				
Origin spot	6.30	1.95	6.40	1.95
terbufos	0.00	3.00	2.85	6.70
t. sulfoxide	10.20	42.40	11.45	42.84
t. sulfone	0.00	12.00	6.83	10.00
CL99843	0.00	0.00	10.00	1.66
CL99875	0.00	0.00	13.93	2.36
$^{14}\text{CO}_2$	78.00	22.00	35.00	17.00
Soil bound residues	5.50	18.65	13.54	17.49

Figure 1. Percent of original ^{14}C -(ET) and methylene- ^{14}C -terbufos (MT) evolved as $^{14}\text{CO}_2$ from treated history (PF) and nonhistory (VF) soils obtained from Kellogg, IA.

Figure 2. Proposed degradation pathways of terbufos (counter) in history and nonhistory soil.

A preliminary experiment was conducted to assess which segment of the soil microbial population was most active in terbufos degradation. Selective inhibitors were added to terbufos history soil to control fungi (cycloheximide, 1000 ppm) and actinomycetes and bacteria (chloramphenicol and streptomycin, 1000 ppm each). A heat treated sample (105°C for 48 hrs) was used to reduce the population of all three groups of organisms. The results of this experiment are listed in Table 12. The failure of cycloheximide to cause any inhibition in $^{14}\text{CO}_2$ evolution, and the success of a mixture of streptomycin and chlormycetin in strongly suppressing $^{14}\text{CO}_2$ evolution would indicate that soil fungi are not significantly involved in terbufos degradation and that degradation is primarily the result of bacterial and/or actinomycete activity.

Summary and Conclusions

The microbial degradation of terbufos was examined in several soils where insecticide performance problems had been observed. A more rapid decomposition of terbufos, per se, was observed in only one of seven soils examined and described herein (Table 7). A graphic plot of the data obtained with this soil (Figure 3) illustrates that while the differences between the two soils are statistically significant, the actual differences are sufficiently small that they may not totally account for a reduced performance of terbufos. Significant differences were observed in the formation and/or dissipation of terbufos sulfoxide and sulfone in all soils, however. The relative toxicity of terbufos and its sulfoxide and sulfone products has been demonstrated by others (Chapman and Harris, 1980; Sutter and Tollefson, 1987; and Solheim, 1982). These investigators have demonstrated that terbufos sulfoxide and/or terbufos sulfone may be as toxic, if not more toxic than the parent terbufos to various stages of the corn rootworm. A plot of the

Table 12. Effect of selective soil microbial inhibitors and dry heat (105°C, 48 hr) on degradation of ethyl-¹⁴C-terbufos in a terbufos history soil obtained from Kellog, IA.

Treatment	% original ¹⁴ C recovered as ¹⁴ CO ₂ on day indicated							
	2	4	6	8	10	12	14	Total
None	1.83	4.56	8.21	8.04	8.31	8.32	11.43	50.70
Actidione	1.60	4.79	9.17	8.27	8.02	7.91	10.67	50.43
Chloramphenical + streptomycin	0.77	0.96	0.84	0.70	0.73	0.83	1.54	6.34
Dry heat	0.87	0.64	0.76	0.77	0.88	0.64	0.17	4.73

Figure 3. Dissipation of terbufos from terbufos problem and virgin soil (plot of data from Table 7).

terbufos, terbufos sulfoxide, and terbufos sulfone data (Figure 4) reveals more clearly the differences in accumulation of these residues in problem and virgin soils. A plot of their combined residues (terbufos + t. sulfoxide + t. sulfone) (Figure 5) provides a more plausible explanation for reduced performance of terbufos in some soils.

Some capability for cross-degradation of organophosphate insecticides was observed in several of the organophosphate history soils. Although phorate degradation per se was not affected by a terbufos history, phorate sulfoxide and sulfone were strongly affected (Table 5). Chlorpyrifos degradation was significantly affected in all three of the terbufos history soils it was tested in. Two of these soils, however, had a history of at least one previous treatment with chlorpyrifos. An effect of terbufos history on fonofos degradation was noted only after the third and fourth week of incubation (Table 7) in one soil and was not affected in a second soil (Table 8). Fonofos degradation occurred more rapidly in a fonofos history soil (Table 8). Although terbufos and terbufos sulfoxide were not affected by a fonofos history the formation and/or dissipation of terbufos sulfone was affected (Table 8).

Results obtained in experiments using ^{14}C -terbufos indicate that an alternate degradation pathway may result in a more rapid degradation of terbufos sulfoxide with little or no sulfone formation occurring. Tests with selective microbial inhibitors indicate that soil bacteria and/or actinomycetes may be primarily responsible for terbufos degradation in a terbufos history soil.

Figure 4. Dissipation and accumulation of terbufos (counter), t. sulfoxide, and t. sulfone from counter problem and virgin soil (plot of data for 11-7)

Figure 5. Plot of combined residue dissipation of terbufos, t. sulfoxide and t. sulfone (data from Table 7).

b. Enzyme Activity in Soils Showing Enhanced Degradation of Organophosphate Insecticides

The susceptibility of certain methylcarbamate insecticides to enhanced biodegradation in soil (Harris et al. 1984; Kaufman et al. 1985) has resulted in the increased use of organophosphate insecticides for corn (Zea mays L) rootworm (Diabrotica sp.) control. However, numerous reports from the field and the literature have indicated that soil applied organophosphates have failed to control the target pest successfully even with repeated applications (Horng and Kaufman 1987), and may also be susceptible to enhanced biodegradation (Horng and Kaufman, 1987; Gauger et al. 1984; MacDonald et al. 1984; Chapman et al. 1986; Read 1983; Sethunathan and Pathak 1972; Barik et al. 1979; Racke, D. D., and Coats, J.R., 1987; Harris et al. 1988). To study this problem more fully, several sets of soil samples were obtained from cooperators who reported that application of an organophosphate failed to protect corn from corn rootworm (Diabrotica sp.) in 1985. The purpose of the study was to examine the enzyme activity in these soils to determine whether a certain class of enzymes as opposed to a specific enzyme may be elevated in the soils. The results, in turn, would suggest a possible mechanism of control or provide a means of predicting when soils will exhibit the enhanced biodegradation of organophosphates.

Materials and Methods

Twenty-one pairs of soil samples were taken from various fields in the Midwestern U. S. by cooperators. Each pair of soils consisted of a sample from a cropped field which experienced failure of protection from corn rootworm as indicated by lodging, goosenecking, root pruning, etc. and a sample of soil from the edge of the field (fence row) which had not been cropped or had not received an application of insecticide. The samples were

taken according to a protocol developed by us which consisted of sampling the field in several places down to a depth of 15 cm and placing the composite sample in a plastic bag and bucket provided. The samples were sent to our laboratory where they were stored at 4C until used. Table 13 lists the samples analyzed in this study, their locations, soil type and their insecticide application history as provided by the cooperator. The blanks in the history of each soil set is a result of either an insecticide not being applied (some cooperators reported participation in the PIK program in 1983) or incomplete information obtained from the cooperator.

Samples were screened through a 2 mm sieve prior to enzyme analysis. The enzymes assayed were acid phosphomonoesterase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2), alkaline phosphomonoesterase (orthophosphoric monoester phosphohydrolase EC 3.1.3.1), phosphodiesterase (orthophosphoric diester phosphohydrolase EC 3.1.4.1), phosphotriesterase, an unclassified enzyme that hydrolyzes tris-*p*-nitrophenol phosphate (Eivazi and Tabatabai 1977) and dehydrogenase. The methods used were those outlined by Tabatabai (1982) and Eivasi and Tabatabai (1977). Phosphatase enzyme activities were expressed as mg *p*-nitrophenol kg⁻¹ soil hr⁻¹ and dehydrogenase activity was expressed as mg triphenyl formazan kg⁻¹ soil 24 h⁻¹. All other analytical data on the soils including soil type determination were performed by the Iowa Testing Laboratories, Eagle Grove, IA. Paired *t* tests were used to calculate the significant differences between the pairs.

Table 13. Location of samples, soil type, and reported insecticide history of cropped fields

Soil Set Design.	Location	Soil Type	Insecticide History ^a				
			-----Year-----				
			1985	1984	1983	1982	1981
3	Clinton, IA	loam	Chlor.	Chlor.	- ^b	-	Fon.
5	Clinton IA	silt loam	Fon.	-	-	Fon.	Fon.
7	Clinton IA	silt loam	Fon.	Fon.	-	Fon.	Fon.
23	Clinton IA	-	Fon.	Fon.	-	Terb.	Terb.
25	Dixon IA	silt loam	Fon.	Fon.	Fon.	Fon.	Fon.
31	Hillsboro ND	clay loam	Terb.	Phor.	-	-	-
33	Chillicarthe IL	sandy loam	Fon.	Fon.	-	-	Fon.
35	Edelstein IL	silt loam	Terb.	Chlor.	Chlor.	-	Chlor.
37	Sparland IL	silt loam	Terb.	Chlor.	Chlor.	Fon.	Fon.
41	Sparland IL	silty clay loam	Terb.	Chlor.	Chlor.	-	Fon.
43	Edelstein IL	silty clay loam	Terb.	Terb.	Terb.	Terb.	Terb.
47	Rock Isle IL	silt loam	Chlor.	Chlor.	Chlor.	Chlor.	Chlor.
59	Rock Isle IL	silt loam	Terb.	Terb.	Fon.	Fon.	-
71	Rock Isle IL	-	Chlor.	Chlor.	-	Chlor.	Chlor.
77	Manchester IA	loam	Terb.	Terb.	-	Fon.	-

99	Francisville IN	loamy sand	Terb.	Fura.	Terb.	Fura.	Terb.
103	Elgin NE	silt loam	Chlor.	Terb.	Terb.	Terb.	Terb.
111	Mt. Herec WI	Silt loam	Phor.	Terb.	PIK.	Phor./ Terb.	Phor./ Terb.
113	Monroe WI	silt loam	Fon.	Fon.	PIK	Fon.	Fon.
117	Barreweid WI	silt loam	Terb.	Terb.	PIK.	Terb.	Terb.
127.	Darlington WI	silt loam	Terb.	Phor.	PIK.	Phor.	Phor.

^aKey: Chlor = chlorpyrifos, Fon. = fonofos, Fura. = furadan, Phor. = phorate, Terb. = terbufos, PIK. = government "payment in kind" - fallow

^bInsecticide not applied or history not provided by cooperator.

The dissipation of fonofos and terbufos was examined in two of the soils. Soil treatments were established by heating 15 grams of each soil with an acetone solution of the pesticide, thoroughly mixing the treated soil, allowing the solvent to evaporate, and then mixing the treated soil with the remainder of the sample (205 gms) and placing it in a 500 ml Erlenmeyer flask. The soils were then brought to 75% field moisture capacity with dropwise addition of water, covered with polyvinyl plastic film fastened with a rubber band and incubated at 25°C in the dark. Each treatment was replicated three times. A ten g sample of soil was removed from each treatment and replicated at 0, 1, 2, 3, and 4 weeks after treatment, placed in a scintillation vial and stored at -18°C until analyzed.

All soil samples were extracted by the method of Aharonson et al. (1986). Briefly, each soil sample (10 gm dry weight) was placed in a 250 ml Erlenmeyer flask and extracted with an acetone water mixture (75 ml:75 ml) on an oscillating shaker (200 rpm) for 30 minutes. The soil suspension was allowed to settle over night and a 50 ml aliquot of the supernatant was extracted sequentially in a 250 ml separating funnel with (a) hexane, 50 ml; (b) hexane, 50 ml/acetone, 20 ml; and (c) hexane, 50 ml/acetone, 20 ml. The combined extracts were concentrated with a low pressure evaporator to less than 5 ml, transferred to screw-cap test tubes and brought back up to 5 ml with hexane. One ml of this concentrated extract was then dried over anhydrous sodium sulfate and used for GC analyses.

A Perkin-Elmer Sigma 2B Gas Chromatograph equipped with a NPD detector, column 6', 1/4" ID, loaded with 3% OV-17 on 80/100 mesh Gas Chrom Q, carrier gas, hydrogen at 10 kpa, helium at 60 kpa, and air at 175 kpa. The injector and detector temperatures were set at 220°C, and column temperature at 200°C. Peak height was used for quantification. Attenuation was set at 1. Terbufos,

terbufos sulfone, and fonofos were all analyzed with the same parameters with excellent peak identification. Terbufos sulfoxide determinations were made by oxidation of the sulfoxide to the corresponding sulfone by the method of Chapman et al. (1980). One ml of the concentrated extract was diluted with 9 ml of hexane, and oxidized with 20 ml of aqueous saturated potassium permanganate solution by shaking in a 50 ml screw-cap test tube for 15 minutes. The upper hexane layer was separated and dehydrated with anhydrous sodium sulfate, brought to a standard volume and injected into the GC. The recoveries of terbufos, terbufos sulfoxide, terbufos sulfone, and fonofos at zero times were greater than 99%.

Results and Discussion

Table 14 contains the enzyme activities and the ratio of the activities to the dehydrogenase activity. Ratios were calculated because soils vary in their biological activity and their organic matter content and comparing enzymatic activity between soils without considering their varying biological activities could lead to incorrect conclusions. A comparison of phosphatase activity in many of the soils from the cropped field to the fence row soil indicated that the highest enzyme activities were in the fence row samples. The general biological activity in the fence row soils was higher, however, as recorded by the dehydrogenase activity and organic matter contents of these soils.

Calculating the ratios of the phosphatase activities to that of dehydrogenase activity in the same soil gave a more accurate picture of the phosphatase enzyme activity in all the samples. In most cases the activities as calculated by the ratios were greater in the cropped field sample than the fence row sample. In some instances the reverse was true such as in sample pairs 37 and 47. Although the basic structure of the organophosphates would

Table 14. pH, CEC and enzyme activities on cropped (A) and fence row (B) soils and the ratio of phosphatase activity to dehydrogenase activity.

Soil set	pH	CEC	% Org. Mat.	-----Enz Units/g soil-----					-Ratio of phosphatase:dehydros-			
				Acid Phos	Alkal Phos	Diest	Triest	Dehyd	Racid	Ralkal	Rdiest	Rtriste
3A ^a	6.7	31.5	4.1	368.	506.	138.	21.1	31.9	11.5 ^b	16.0 ^b	4.33	.662
3B	6.8	28.3	3.7	385.	642.	181.	26.2	66.3	5.81	9.69	2.73	.395
5A	5.3	15.6	2.7	582.	83.1	31.2	30.1	16.5	36.1 ^b	5.23	1.95	1.89 ^b
5B	7.0	21.5	3.5	288.	508.	154.	49.4	56.1	5.17	9.09	2.76	.887
7A	6.7	18.7	2.7	328.	214.	98.9	26.2	37.3	8.85 ^b	5.76	2.66	.705
7B	7.5	14.9	2.9	245.	454.	151.	30.1	81.4	3.04	5.71	1.88	.374
23A	7.2	18.6	1.4	187.	354.	123.	32.4	12.7	15.2 ^b	28.4 ^b	9.89 ^b	2.59 ^b
23B	7.0	14.9	2.4	291.	539.	193.	24.3	97.6	2.99	5.54	1.99	.250
25A	7.2	23.1	1.3	203.	263.	88.3	34.9	5.41	39.0 ^b	50.8 ^b	16.8 ^b	6.78 ^b
25B	6.3	19.2	2.9	507.	530.	172.	32.7	39.6	13.0	13.5	4.41	.842
31A	7.7	41.1	3.1	123.	296.	85.0	19.3	30.0	4.10	9.84	2.83	.642
31B	7.7	40.7	3.3	138.	364.	102.	21.2	21.6	6.38	16.9 ^b	4.70	.982
33A	7.1	21.8	2.1	81.8	236.	84.3	32.8	27.7	2.98	8.57	3.06	1.19
33B	7.3	11.2	2.0	98.3	366.	88.4	29.2	35.1	2.92	10.9	2.63	.866
35A	6.4	23.2	3.2	349.	217.	85.6	27.3	19.5	17.9 ^b	11.2	4.40	1.40
35B	6.6	26.5	3.8	377.	435.	142.	32.5	35.4	10.7	12.3	3.99	.916
37A	7.1	22.4	2.4	132	211.	69.9	26.2	21.4	6.19	9.84	3.27	1.22
37B	6.8	18.8	3.5	453.	513.	167.	27.8	44.0	14.5 ^b	16.3 ^b	5.37	.878
41A	6.6	23.9	3.0	440	199.	80.0	28.8	22.5	20.0 ^b	9.06	3.65	1.33 ^b
41B	7.0	26.1	4.0	364.	362.	130.	24.2	52.0	7.03	6.99	2.51	.469
43A	5.6	19.2		613.	117.	160.	26.2	14.4	43.3 ^b	8.12	11.3	1.84 ^b
43B	7.4	24.0	3.0	374.	327.	543.	22.4	23.9	17.0	14.6 ^b	24.5 ^b	1.06
47A	6.8	22.9	3.4	451.	370.	120.	34.7	35.9	12.6	10.3	3.36	.969
47B	6.8	24.4	3.5	414	343.	120.	38.2	26.1	16.1	13.4	4.72	1.47
59A	5.7	24.0	3.7	531.	205.	61.3	31.0	21.6	25.3 ^b	9.79	2.96	1.47
59B	5.8	23.5	4.8	529.	451.	110.	51.2	58.0	9.13	7.78	1.90	.882
71A	7.2	20.9	2.2	195.	193.	81.1	21.3	18.4	10.7	10.6	4.44	1.17
71B	7.2	20.1	2.1	271.	353.	98.2	24.2	29.0	9.35	12.2	3.39	.834
77A	6.6	16.6	2.8	294.	194.	68.2	27.3	32.5	9.07	5.97	2.10	.841
77B	6.8	21.6	4.1	449.	663.	173.	29.5	91.7	4.92	7.24	1.89	.322

99A	7.1	20.0	3.5	249.	386.	136.	29.1	61.6	4.11	6.37	2.25	.484
99B	7.3	31.4	3.0	185.	438.	121.	33.1	58.8	3.14	7.45	2.06	.563
103A	6.7	21.9	2.6	368.	295.	90.9	27.1	25.1	14.8	11.9	3.67	1.08
103B	6.7	23.1	1.9	343.	232.	79.0	27.1	20.5	17.3	11.6	3.95	1.34
111A	6.4	19.8	2.0	270.	450.	112.	30.4	37.3	7.46	12.4	3.10	.847
111B	6.6	18.0	2.7	404.	317.	104.	37.3	38.9	10.4	8.19	2.68	.962
113A	7.0	17.2	2.8	265.	307.	109.	39.6	31.2	8.67	10.0	3.57	1.31 ^b
113B	7.0	19.7	3.6	414.	601.	164.	35.1	89.6	4.72	6.92	1.90	.408
117A	7.1	27.4	3.5	367.	590.	149.	25.0	91.6	4.01	6.45	1.63	.273
117B	7.3	27.4	3.8	433.	720.	175.	25.8	133.	3.26	5.51	1.32	.194
127A	6.7	18.6	2.6	349.	228.	398.	24.7	40.7	8.63	5.62	9.95	.610
127B	6.4	17.4	2.4	455.	422.	576.	30.1	49.0	9.38	8.64	11.8	.614

^aA denotes cropped field soil, and B denotes fence row soil from same location.

^bIndicates significant diff. at 5%.

suggest that a phosphotriesterase may be involved in the biodegradation (Fig 6), 43% of all the cropped soils and two thirds of the cropped soils treated with fonofos in 1985 were significantly higher in acid phosphatase activity (Table 15).

Other variables considered in the analysis of factors effecting enzyme activity were pH, CEC, and soil type. None appeared to be correlated with phosphatase enzyme activities. All the soils were similarly classified as loams and the pH values were in a relatively narrow range while the enzyme activities varied greatly. Cation exchange capacity (CEC) correlated somewhat with the organic matter content which was corrected for by using the ratio method. Therefore the increased enzyme activity found in the soils was most closely related to the insecticide application history.

Several of the soils tested in this study were also used in a previous study to determine if the enhanced degradation could be duplicated in the laboratory (Horng and Kaufman, 1987). Soil pairs 25 and 43 were amended with terbufos or fonofos at 5 mg kg^{-1} (dry weight basis) and monitored for the disappearance of the respective insecticides. Significantly faster degradation of fonofos occurred in cropped soil 25A as compared to the fence row soil (25B) (Table 16). In soil 43, terbufos degraded more rapidly in the cropped soil (43A) (Table 17) than in the fence row soil (43B). Most but not all the soils from the previous survey had similar laboratory biodegradation data which correlated to the observation in the field that a particular insecticide failed to protect the crop against the target organism.

Accelerated degradation of organophosphates correlated with an increased phosphatase activity. A direct relationship between the two factors, however, cannot be concluded at this time. The structure of each insecticide would

Fonofos

Phorate

Terbufos

Chlorpyrifos

Figure 6. Chemical structures of chlorpyrifos, terbufos, phorate, and fonofos.

Table 15. Percentage of the total number of cropped soils which had significantly higher phosphatase enzyme activities (ratios) than the fence row soils.

Insecticide treatment	No. of samples	Enzyme			
		Racid	Ralkal	Rdiest	Rtriest
-----%					
Chlorpyrifos	4	25	25	0	0
Terbufos	10	40	0	0	20
Fonofos	6	67	33	17	67
Phorate	1	0	0	0	0
Combined, all treatments	21	43	14	5	29

Table 16. Dissipation of fonofos in soil obtained from Dixon, Iowa.

Soil Sample	Weeks after treatment				
	0	1	2	3	4
Cropped	104.2	50.0	14.9	5.7	3.3
Fence row	95.8	61.4	55.2	43.6	42.5

Table 17. Dissipation of terbufos and its metabolites in soil obtained from Edelstein, Ill.

Insecticide or metabolite	Soil Sample	Weeks after treatment				
		0	1	2	3	4
Terbufos	cropped	81.2	50.8	26.3	10.1	5.8
	fence row	84.8	51.7	40.6	25.1	20.8
Terbufos sulfoxide	cropped	18.8	33.8	14.7	4.4	2.5
	fence row	15.2	31.0	45.8	20.4	46.7
Terbufos sulfone	cropped	0	0.5	0	0	0
	fence row	0	0.5	0.8	1.7	2.8

Total toxicant	cropped	100	85.1	41.0	14.5	8.3
	fence row	100	83.2	87.2	47.2	70.3

suggest that a phosphotriesterase would be the only enzyme to degrade the original compounds. Acid phosphatase would not attack compounds with the diethyl groups in place. Increased phosphatase enzyme activity could be a result of increased production of the enzyme by microorganisms in response to inhibition caused by the insecticide due the presence of the dithiol groups. Inhibition would result in a decreased end product and subsequent stimulation of enzyme production. Perucci et al. (1988) found that application of the acetanilide herbicides (metolachlor and alachlor resulted in a temporary increase in phosphatase (acid and alkaline phosphomonoesterase, phosphodiesterase, and phosphotriesterase) activity of soil. The application of the atrazine caused a decrease of phosphotriesterase activity 10 days after application. Increases in activity were considered to be the product of microbial lysis that resulted from the application of the herbicides. Various combinations of these herbicides have also been applied to the soils used in this investigation and the extent to which these effects may have confounded the observations reported herein is not known. Whatever the reasons for the increased activity recorded in our studies, phosphatase enzyme analysis may prove useful in locating fields which are susceptible to enhanced biodegradation of organophosphates. Further research will be directed towards increased acid phosphatase activity and the threshold levels of enzyme activity which may predict a potential, enhanced biodegradation problem.

c. Acylanilide Persistence in Continuously Cropped Soils

Bailey and Coffey (1985) demonstrated that repeated application of the acylanilide fungicide metalaxyl in California avacodo soils resulted in an accelerated or enhanced level of biodegradation of metalaxyl and a concomitant loss of fungicidal efficacy in these soils. Although informal questions and concerns had arisen concerning soils continuously treated with the herbicides alachlor and metolachlor, the investigators were not familiar with any research documenting the accelerated biodegradation of these herbicides in continuously treated field soils. The objective of this experiment was to examine the degradation rates of the acylanilide herbicides alachlor and metolachlor and the fungicide metalaxyl in soils which had a continuous history of their use and to determine whether or not their persistence in soil was influenced by repeated usage in the same soil.

Materials and Methods

The degradation and persistence of the herbicides alachlor and metolachlor were examined in the laboratory in fresh soil samples collected from two sets of Beltsville and Frederick field plots which had been treated yearly with alachlor and continuously cropped for 5 years with either soybeans or corn. Degradation and persistence were determined by treating sieved soil samples with acetone solutions of the herbicides, thoroughly mixing the treated soil after evaporation of the acetone, watering the soil to 70% of field capacity, and incubating the samples in the dark at 26°C. At regular intervals each soil sample was subsampled and the subsamples stored at -18°C until analyzed. After thawing the soil samples were extracted with a water-acetone (1:1, v/v) mixture which was then partitioned with hexane and the hexane concentrated and analyzed on a gas chromatograph equipped with a thermionic specific detector. Chemical structures of the acylanilide pesticides examined in this

investigation are shown in Figure 7.

Alachlor

Metalaxyl

Metolachlor

Propachlor

Figure 7. Chemical structure of the acylanilide herbicides, alachlor, metolachlor, and propachlor, and the fungicide metalaxyl.

Results

Soil from a cherry orchard with a history of metalaxyl treatments

(Background information: This soil was being treated by applying approximately 8 oz material per square meter around base of trees 3-4 times per year for approximately 4 years) was found to degrade metalaxyl faster than the non-treated control soil (Fig. 8). The differences in rate of degradation between the metalaxyl-treated soil and the non-treated soil were less than that described earlier by Bailey and Coffey (1985) for some other soils. However, since the treated and non-treated soils were stored in the laboratory for several months prior to the analysis, both soils were treated once more to 10 $\mu\text{g/g}$ of metalaxyl. As can be seen from Fig. 8, the additional treatment strongly enhanced the degradation of metalaxyl in the previously treated soil. The degradation of the fungicide in the non-treated control soil was not affected by this additional treatment. The results so far may suggest that the acquired enhanced degradation of metalaxyl may decline with time.

Metolachlor was added to the metalaxyl history soil, but its degradation was not changed (Table 18). The rate of metolachlor degradation was the same in the metalaxyl-treated and in the non-treated soils.

An attempt to isolate from the metalaxyl history soil specific microorganisms capable of rapidly degrading this chemical was not successful. Moreover, when non-treated soil was mixed with either 3% or 10% of the metalaxyl history soil the enhanced degradation capability was slowly suppressed by the non-treated soil (Table 19). The microorganisms responsible for the faster degradation of metalaxyl were not capable of building up their population even when added to a sterile (gamma-irradiated) soil. We have shown before that, for example, 1% of carbendazim history soil was sufficient to acquire rapid degradation to a non-treated soil. The results as shown in

Figure 8.

Table 18. Degradation of metalaxyl and metolachlor in soils that were previously treated with metalaxyl and in nontreated control. Results presented as ug pesticide/gram soil.

Days after treatment	Metalaxyl		Metolachlor	
	Metalaxyl history soil	No previous metalaxyl treatment	Metalaxyl history soil	No previous metalaxyl treatment
1	9.3	9.4	12.1	12.3
11	2.1a*	8.1b	11.6	11.6
20	0.3a	8.5b	11.1	10.8
46	--	5.5	8.0	7.5

* On each date, numbers followed by different letters are significantly different ($p=0.05$) according to Duncan's multiple range test.

Table 19. Degradation of metalaxyl in gamma-irradiated and non treated control soils to which 3% and 10% of metalaxyl history soil was added. The mixed soils were incubated for one week prior to the addition of 12 $\mu\text{g/g}$ of the fungicide. Results are presented as % of applied, each number is the average of three replicates.

Days after addition of metalaxyl	gamma-irradiated control soil mixed with metalaxyl history soil		Nontreated control soil mixed with metalaxyl history soil		Nontreated control soil	Metalaxyl history soil
	3%	10%	3%	10%		
0	100	100	100	100	100	100
12	84	78	70	62	84	25
26	62	54	58	51	65	1

Table 19 suggested that such an effect could not be produced with metalaxyl. These findings may lead to the conclusion that the degraders of metalaxyl do not compete so well with other microorganisms and therefore the buildup of such degraders in a metalaxyl history soil may be slow and may easily be reversed.

More evidence to support this hypothesis is presented in Table 20. Two soils were treated in the laboratory three times with metalaxyl but failed to develop any enhancement in their degradation of metalaxyl. It should be pointed out that with other soil pesticides such as benomyl, diphenamid, carbofuran, butylate, iprodione and others, only one or two treatments in the laboratory were sufficient to enhance their degradation.

The effect of repeated applications of acylanilide herbicides on their degradation was examined in a local Beltsville soil and a soil obtained from Frederick, Maryland.

The Beltsville and Frederick soils were sampled from two field experiments that were identical in their design and part of a larger study related to conservation tillage. The Beltsville soil was collected in the fall (September 18, 1985) and the Frederick soil was collected in the spring (March 27, 1986). The field experiments included 10 different herbicide treatments and two untreated controls. Soil samples were collected from four corn and soybean plots that were treated with the same herbicide mixtures for five consecutive years:

<u>Crop</u>	<u>Treatments</u>	<u>Rates (lb/A)</u>		
Corn	Untreated control	--		
Corn	Alachlor, Atrazine, Paraquat	2.5	2.5	0.5
Soybean	Untreated control	--		
Soybean	Alachlor, Metribuzin, Paraquat	2.0	0.38	0.5

Table 20. Degradation of alachlor, metolachlor and metalaxyl in a soil with a history of two previous treatments of the respective pesticide. All the treatments were carried out in the laboratory on two soils. The results are presented as % of applied. Each number represent the average of three replicates.

Days after	Alachlor		Metolachlor		Metalaxyl	
	History	Non- treated	History	Non- treated	History	Non- treated
0	100	100	100	100	100	100
17						
33	57	42	69	61	38	30
60						

The rate of degradation of the acylanilide herbicides in non-treated soil was found to be in the order of propachlor, alachlor and metolachlor (Table 21). Metalaxyl rate of degradation was somewhat between alachlor and metolachlor.

Five years of repeated applications of alachlor in corn and soybean plots did not result in an enhanced degradation rate of either alachlor or metolachlor. On the contrary, the degradation of alachlor was significantly slower in the treated plots when compared to the non-treated controls (Fig. 9 and Table 22). Since in practice corn fields are treated with more than one herbicide, the plots that were studied in this experiment were treated for 5 years with alachlor, atrazine and paraquat, which is a common practical combination. Thus, the slower degradation of alachlor in the treated soil could be attributed to the effect of either one of herbicides or to their combined effect. However, results from another experiment, which will be discussed later, supported these findings - showing that repeated alachlor applications results in a slower alachlor degradation rate (Table 25).

The degradation of alachlor was slower in corn than in soybean which suggested that in addition to the herbicide effect, the type of crop may affect the rate of alachlor degradation. The crop effect is well demonstrated in Table 23. It can be seen that alachlor was degraded more slowly in the non-treated corn soil than in the non-treated soybean soil. The slower degradation of alachlor in corn has been observed and confirmed in two experiments (Fig. 9 and Table 23). The two experiments were identical in their design but were carried out in different soils and at different seasons. The same effect of slower degradation in corn soil with a history of alachlor treatments was found for metolachlor in two separate experiments (Table 22). It should be pointed out that the herbicide butylate, which belongs to a

Table 21. Degradation in soil of propachlor, alachlor, metolachlor and Metalaxyl. Results presented as % of amount applied.*

Days after treatment	Propachlor	Alachlor	Metolachlor	Metalaxyl
0	100**	100	100	100
10	37	--	94	86
20	10	--	88	90
42	--	34	61	59

* Initial concentrations in the soil were; 6.8, 9.5, 12.3, and 9.4 $\mu\text{g/g}$ for propachlor, alachlor, metolachlor and metalaxyl, respectively.

**Results for propachlor were obtained from a different experiment, in which the rate of degradation of alachlor was found almost the same as in this experiment.

Table 22. The effect of 5 years of repeated application of alachlor on the degradation of metolachlor in two soils. The results presented as % of applied*.

Days after treatment	Frederick soil		Beltsville soil	
	Alachlor history	Non-history control	Alachlor history	Non-history control
0	100.0	100.0	(0) 100.0	100.0
29	38.7	26.9	(26) 55.3	41.5
51	25.8	17.2	(47) 26.0	20.0

* The concentration in soil at time 0 was about 10 $\mu\text{g/g}$.

Table 23. The effect of corn, soybean and repeated alachlor treatments on the degradation of alachlor in Frederick soil. Results are presented as % of alachlor applied.

Days after treatment	Corn		Soybean	
	Treated 5 years with alachlor	Nontreated control	Treated 5 years with alachlor	Nontreated control
0	100.0 *	100.0	100.0	100.0
9	46.2	42.0	30.7	31.7
16	30.2a**	22.2b	14.4bc	13.4c
26	14.5a	8.4b	6.0bc	4.9c

* Initial concentration of alachlor in the soil was 15-17 $\mu\text{g/g}$.

**On each date, numbers followed by different letters are significantly different ($p=0.05$) according to Duncan's multiple range test.

different class of chemicals, was affected differently when added to the same soils. Its degradation was faster in the alachlor-treated soil and was slowest in the soybean control soil.

The shape of the degradation curves, as shown in Fig. 9 indicated that alachlor degraders were affected by the repeated herbicide applications. Degradation of alachlor in the previously treated soils followed first order kinetics whereas the degradation in the non-treated soils could be characterized by a lag phase of about 10 days and a subsequent faster degradation which is typical for microbial degradation. This assumption was further supported by data obtained on the degradation of alachlor in gamma-irradiated and non-treated soils (Table 24). In this experiment gamma-irradiation of the soil retarded alachlor degradation to about 50% of that found for the non-irradiated soil. The results indicate that degradation of alachlor was a mixed chemical and microbial process and that interactions from pesticides or other agricultural practices may affect any one of these processes. In another experiment two non-treated soils, one from California and the other from Maryland, were treated twice in the laboratory with three acylanilide pesticides. The results in Table 25 summarizes the effect of these two treatments on the degradation of each pesticide when applied once more to the same soil (third application). The degradation of all the three pesticides was slower in the previously treated soils. These findings confirmed the results obtained from the two field experiments in which alachlor was applied for 5 consecutive years. An unexpected behavior was found for metalaxyl. It has been shown by Bailey and Coffey (1985) and also in our research that repeated applications of metalaxyl in avocado enhanced its rate of degradation, whereas in this experiment the degradation of metalaxyl was even more slowly than in the untreated control. The difference

Figure 9. The effect of repeated alachlor treatment on its degradation in the Beltsville Soil. On days 27 and 50 differences in degradation between the controls and their respective treated plots were significant ($p = 0.05$) according to Duncan's multiple range test. X---X = alachlor treated soils; Δ --- Δ = non-treated control soils.

Table 24. Degradation of alachlor in non-treated and gamma-irradiated soils, presented as $\mu\text{g/g}$ and as percent of amount applied.

Days after treatment	gamma-irradiated soil						Non-treated control					
	$\mu\text{g/g}$			% of applied			$\mu\text{g/g}$			% of applied		
0	5.0	4.5	3.1	100	100	100	14.2	13.2	9.3	100	100	100
	Mean 4.21			100%			Mean 12.20			100%		
41	3.3	3.2	2.2	66.0	71.1	70.3	5.7	5.1	4.0	40.1	38.6	43.0
	Mean 2.90			68.9%			4.93			40.4%		

Table 25. Degradation of alachlor, metolachlor and metalaxyl in a soil with a history of two previous treatments of the respective pesticide. All the treatments were carried out in the laboratory on two soils. The results are presented as % of applied*. Each number represent the average of three replicates.

Days after	Alachlor		Metolachlor		Metalaxyl	
	History	Non-treated	History	Non-treated	History	Non-treated
0	100	100	100	100	100	100
17						
33	57	42	69	61	38	30
60						

*The amounts added to the soil on each treatment were 10 $\mu\text{g/g}$ soil.

between the two experiments was that the enhanced degradation was detected in an avocado orchard while our results were obtained with soils from a cherry orchard that were treated and stored in the laboratory throughout the whole experiment. The results in Table 25 summarizes the degradation of metalaxyl in soil that was obtained from Hagerstown, Maryland. The results for the non-treated California soil were about the same as those described in Table 25.

Summary and Conclusions

The degradation of alachlor and metolachlor were examined in both metalaxyl and alachlor history soils. Degradation of alachlor and metolachlor was not affected by the metalaxyl history soil despite the fact that metalaxyl degradation was accelerated by this history. In contrast, metolachlor and alachlor degradation was slower in alachlor history soils. This difference, although affected by crop, occurred in both soybean and corn-cropped soils at two different locations. The actual cause of this effect is not presently known, but appears to be associated with the repeated usage of alachlor on the same soils. Additional work is needed to clarify the mechanism of this effect.

d. Characterization of Agricultural Chemical Use Patterns and Soil Chemical/Physical Properties of Soils Where Enhanced Pesticide Degradation Occurs

During the course of this investigation an attempt was made to correlate agricultural chemical use patterns with soil chemical and physical properties in soils where pesticide performance problems were attributed to enhanced biodegradation. The objective was to establish whether or not there were any other major factors which might contribute to the phenomenon of enhanced biodegradation. During the 1984, 1985, and 1986 growing season more than 400 soil samples were obtained from cooperators from fields where pesticide failures were being observed. Samples submitted were required to have a minimum data history of 4 years in which all chemicals (pesticides, fertilizers, conditioners, etc.), tillage practices and crops grown were known. Samples received were submitted to appropriate bioassays to confirm persistence problems, and to a chemical/physical analysis for soil pH, % organic matter, sand, silt and clay, cation exchange capacity, soil moisture at 1/3 bar moisture, base saturation etc. All data have been, or are being submitted to computer recording and analyses.

This aspect of this project proved to be a massive undertaking which will require some time (as available) before completion. While this aspect of this study was initiated during this investigation, it is not considered an integral part of the investigation, but is related to it. It did not require funding by this agreement. It will be completed and published as time of the principal investigator permits.

Preliminary interpretation of available data, however, indicate that the primary causal factor or agricultural practice contributing to development of problem soils is the continued use of the same chemicals in the same soil.

Some indications that specific chemicals are more prone to this phenomenon are also apparent. Other contributing factors to a lesser degree involve cropping sequence, and/or chemical sequence.

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F. DESCRIPTION OF COOPERATION:

All senior and junior investigators in the USA and Israel were in close contact throughout the research study. There was a continuous exchange of information between the laboratories and the investigators exchanged visits in both countries. The work in the USA was directed towards the effect of repeated soil application of several acylanilide herbicides and organophosphate insecticides, whereas the work in Israel emphasized the development of accelerated degradation with fungicides (benomyl) and herbicides. Dr. D. D. Kaufman visited Israel twice and Drs. N. Aharonson and J. Katan visited the USA twice and worked with Dr. Kaufman for extended periods of time. Oded Yarden and Elana Avidov, both Ph.D. students with Dr. Aharonson and Dr. Katan visited D. D. Kaufman. O. Yarden worked with D. D. Kaufman for three months. Joint publications were produced throughout the joint research - the results of our studies were jointly presented in International meetings.

G. EVALUATION OF RESEARCH ACHIEVEMENTS AND EMPHASIZING THE BENEFIT TO AGRICULTURE:

Accelerated degradation is a relatively new problem causing a great concern to the agricultural community since it poses a threat to several major soil-applied pesticides. Our study provides additional and new information regarding the occurrence and distribution of this phenomena in different soils in two countries. Unfortunately our studies show that apparently accelerated degradation is more widespread than had been known in terms of number of pesticides involved and the diversity of chemical groups to which these pesticides belong. Moreover, "multi-problem" (accelerated degradation of several pesticides in the same soil) soils were also detected. In Israel, accelerated degradation was detected for the first time by our group within the framework of this study.

Additional findings which have both fundamental and applicative significance were also shown. Accelerated degradation lasts in the soil for several years. It is affected by the crop history and it is not limited to certain soil types. Even small amounts of the pesticides, or drift from aerial pesticide applications, or contamination from "infested soil" may be sufficient to induce accelerated degradation. The role of microorganisms in accelerated degradation was investigated. Soils with accelerated degradation differ in the composition and the quantity of the microbial degraders as well as in the pathway of degradation. The importance and the involvement of bacteria in the accelerated degradation of certain pesticides as a result of a shift in their populations or in their metabolism, was demonstrated.

Our studies also dealt with developing means for the control of accelerated degradation through the use of antimicrobial processes. Our studies affected and motivated other scientists from various disciplines. The

awareness of extension workers as well as of farmers, to the possibilities that failures in pest control may result from accelerated degradation was increased. The results of our work are now taken into consideration in planning pest control strategies. For example, the recommendation to minimize repeated application of pesticides of the same chemical group, or to avoid monoculture whenever it is possible, became stronger.

Throughout the course of this investigation all three of the lead scientists maintained an active contact with pesticide chemical companies whose chemicals were being affected, as well as with agricultural extension and university personnel also concerned with this problem. The senior investigator, himself made more than 55 presentations at both national and international symposia, universities, extension and grower meetings and industrial meetings and workshops on this problem. The senior investigator worked particularly close for several years with midwestern entomologists involved in the North Central Region (NCR-46) technical committee on Corn Rootworm Control. This committee was responsible for investigating corn rootworm problems and developing pest control strategies which were subsequently used by extension workers and farmers to facilitate control of this pest. Knowledge gained in this research project thus was put to use almost immediately. Information obtained in this research was instrumental in identifying and verifying the cause of reduced efficacy of corn rootworm insecticides and in devising alternative pest control measures. The two basic control measures ultimately endorsed by most NCR-46 scientists involved 1. Crop rotation (because it interrupts the pests life cycle); and 2. Chemical rotation (chemical class to chemical class, i.e., methylcarbamates to organophosphates and back). In ancillary preliminary experiments not described herein, the scientists demonstrated that while using different

pesticides within the same chemical class generally perpetuated the problem of enhanced degradation, rotation of the pesticide to a new chemical class frequently improved pest control performance beyond what might normally be expected. Preliminary results indicate that at least a part of this improved performance of alternative chemical classes results from a mutual inhibition of microbial degradation of one chemical class by the other. Little, if anything, is known about how long use of such technology would continue to be successful, however. Throughout their investigations of this problem, the investigators frequently became aware of specific situations where pesticide performance might be improved through more judicious use of the right chemicals in the right sequence.

H. PUBLICATIONS:

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