Chapter 6

Enhanced Biodegradation of Insecticides in Midwestern Corn Soils

Kenneth D. Racke[1] and Joel R. Coats[2]

[1]Environmental Chemistry Laboratory, DowElanco, 9001 Building, Midland, MI 48641–1706
[2]Department of Entomology, Iowa State University, Ames, IA 50011

An experimental strategy for the study of enhanced degradation is described based on its occurrence in Midwestern corn soils. The shift from recalcitrant chlorinated hydrocarbons to biodegradable organophosphorus and carbamate insecticides has resulted in the failure of some compounds, notably carbofuran and isofenphos, to provide adequate pest control following repeated use. Enhanced degradation of an insecticide involves its rapid degradation by a population of soil microorganisms that has adapted to beneficially catabolize it following exposure to it or a similar insecticide. For enhanced degradation to be thoroughly investigated studies must be carried out to demonstrate an increased rate of degradation in soils with prior insecticide exposure, to identify the rates and products of degradation in similar soils under controlled conditions, and to elucidate the microbiological mechanisms.

Agriculture in the Midwestern United States relies heavily on the cultivation of corn (*Zea mays*). Management of this crop in turn hinges on the successful control of a panoply of destructive pests, chief of which are soil-dwelling insect larvae of corn rootworms (*Diabrotica spp.*), cutworms (*Agrotis spp.*) wireworms (Elateridae), and grubs (*Phyllophaga spp.*). The corn rootworm is the key pest posing the greatest potential for yield reduction. It is worth noting that although corn is planted in April or May, rootworm larvae hatch and are active during an extended period from May to July. Because corn rootworms prefer to inhabit continuous corn, early control practices focused on crop rotations as a means of limiting populations (1).

Insecticidal Control of Corn Soil Pests

With the advent of synthetic organic insecticides following World War II, a new chemical control paradigm took root in corn agriculture. In the late 1940's it was discovered that soil application of

0097–6156/90/0426–0068$06.00/0

chlorinated hydrocarbon insecticides provided excellent control of these pests (2). The cyclodiene aldrin became the most widely used compound by the mid 1950's as use of these soil-applied insecticides blossomed. For example, soil insecticide use in Iowa went from nothing in 1950 to 237,000 acres in 1954 (6% of corn acres) (3). The control was so effective that a broadcast soil application in many cases provided control both that year and the next!

The perspective that "The persistent chlorinated hydrocarbons appear to be uniquely well adapted for soil use" (3) rather quickly collided with the reality of insect microevolution. The first evidence of rootworm control failures occurred in Nebraska in 1959, and by 1962 resistance of rootworms to the cyclodienes had been conclusively documented (4). The spread of this resistance during the 1960's, along with environmental scrutiny of the ecological impacts of these persistent compounds, created a demand for more environmentally sound insecticides.

Beginning in the mid to late 1960's the biodegradable organophosphorus and carbamate insecticides began to replace the chlorinated hydrocarbons as the rootworm control chemicals of choice. Diazinon, oxydisulfoton, and phorate were the first, and were quickly followed by bufencarb, trimethacarb, fonofos, and fensulfothion. In the mid to late 1970's the introduction of new chemicals continued as ethoprop, carbofuran, chlorpyrifos and terbufos came into use. Because rootworm insecticides are most commonly applied at planting, 4-8 weeks prior to rootworm egg hatch, it was early recognized that the inherently rapid degradation rate of some of these insecticides precluded their effective use (5). However, most provided effective control without the undesirable environmental impact of the chlorinated hydrocarbons. By 1973 roughly 20-30 million acres of midwest corn were treated annually with soil insecticides (6), and corn had become the leading crop for use of soil insecticides.

Enhanced Insecticide Degradation and Control Failure

Although it was realized that various environmental factors could influence rootworm control with soil-applied insecticides (7), the failures of several reliable insecticides wrought some degree of consternation within the agricultural community. As various compounds fell from common use during the 1960's and 1970's due to poor performance (diazinon, oxydisulfoton, bufencarb, trimethacarb, fensulfothion) resistance was examined as a possibility and found not to prove sufficent for explaining the observed failures (6,8). The continuing introduction of new insecticides for the corn rootworm market tended to balance out the overall effect of the losses of failure-prone products.

Research on control failures during the late 1970's and early 1980's focused on carbofuran, a compound that had earlier provided excellent control and had captured a considerable share of the rootworm insecticide market (e.g. 20% of treated acres in Iowa in 1977)(9). Although some studies found no correlation between prior use of carbofuran and an enhanced rate of carbofuran degradation (10,11) both monitoring of field residues (12-14) and laboratory degradation tests (15-17) conclusively demonstrated enhanced microbial degradation as the cause of decreased carbofuran persistence in fields with histories of carbofuran use. Thus, a

phenomenon that had first been noted with the phenoxyalkanoic herbicides during the 1940's and had languished as an academic curiosity became a primary concern in the Midwestern corn belt. See Felsot (18) for a more complete account of the recognition of carbofuran enhanced degradation.

As control failures of other insecticides for the rootworm market (isofenphos, terbufos, fonofos, cloethocarb, bendiocarb) occurred, enhanced degradation began to be blithely invoked as the universal cause for many of these failures (19). With this assumption many extension agents began recommending rotations between rootworm insecticides as a possible means of avoiding this perceived problem (e.g., 20). The inference of enhanced degradation became a marketing weapon of some agrochemical companies to raise questions regarding the performance of competitor's products. It was obvious that a rational, experimental approach was needed to address the question of which insecticides had actually undergone enhanced degradation.

An Experimental Approach for the Study of Enhanced Degradation

In an effort to determine the criteria that should be used to invoke cases of enhanced degradation, an experimental approach for its study was developed that focused on laboratory investigations with field-collected soils. It was obvious that insecticide control failures were common occurrences and certainly not all due to enhanced degradation, as investigations of faulty application methods and unusual environmental conditions have shown (18). The ideal approach to the study of enhanced degradation would involve controlled field research in which pesticide persistence and control efficacy were both measured at many locations over a number of years. However, the tremendous cost in time and effort and confounding of results by environmental variables make a controlled laboratory approach desirable. The limitation of laboratory efforts focused exclusively on the soil-insecticide interaction is that they cannot fully address the additional insect-insecticide and insect-crop interactions present in the field. This means that caution must be excercised when proof of enhanced degradation is discovered in the laboratory, for this does not necessarily mean that insect control and crop yield will be adversely affected under field conditions. With this caution in mind, results of laboratory investigations can be kept in proper perspective for what they can best provide: a mechanistic understanding of soil-microbe-insecticide interactions.

Rapid Insecticide Degradation Assay

The first part of the laboratory methodology for study of enhanced degradation involved use of a rapid degradation assay by which an idea of the rate of insecticide degradation could be obtained for a large number of soils. The soils for this assay were collected during the fall from Iowa cornfields with known histories of insecticide use. In some cases the soils collected were specifically from fields in which an insecticide had been used for several years and no longer provided suitable control of soil insect pests. In these cases a separate soil was also collected from an adjacent field or from the field fencerow.

A 25-g portion of each soil was placed in a glass jar and treated with ^{14}C-insecticide at 5 μg/g. Insecticides used for this assay included those for which fields with some treatment histories could be identified: ^{14}C-carbonyl-carbofuran, ^{14}C-ring-isofenphos, ^{14}C-ring-fonofos, ^{14}C-ethoxy-terbufos, ^{14}C-ethoxy-phorate, and ^{14}C-ethyl-ethoprop. After each soil was moistened to 0.3 bar soil-moisture tension, glass vials containing 0.1 N NaOH were placed inside each jar to serve as CO_2 traps. The jars were incubated in the dark at 25°C for 1 week, during which the traps were analyzed daily for trapped $^{14}CO_2$.

Results of the rapid degradation assay (Table I) are expressed for each insecticide as cumulative mineralization in history soils (treated with that pesticide at least one previous year) and non-history soils (untreated or treated with other insecticides). All soils from fields previously treated with carbofuran exhibited a much higher mineralization rate than soils from non-history fields. Previous reports have confirmed the enhanced degradation of carbofuran, and its rapid mineralization in each carbofuran-history soil is indicative of its great susceptibility to enhanced degradation. Even the fencerow soils surrounding carbofuran-history fields exhibited increased carbofuran mineralization rates, and had apparently been contaminated by carbofuran or carbofuran-treated soil. Up to 5 years had elapsed since the last carbofuran soil application to history soils.

Table I. Effect of Soil Insecticide Use History on the Mineralization of ^{14}C-Insecticides to $^{14}CO_2$ in Soils During a 1-Week Laboratory Assay

	% Mineralization ± SE	No. of Soils
Carbofuran		
History	84.0 ± 8.0	8
Nonhistory	18.1 ± 7.6	11
History Fencerow	39.7 ± 8.2	2
Isofenphos		
History	35.9 ± 22.4	6
Nonhistory	2.4 ± 1.0	19
Fonofos		
History	20.8 ± 13.6	13
Nonhistory	9.1 ± 3.5	16
Terbufos		
History	12.5 ± 4.0	11
Nonhistory	9.7 ± 3.2	5
Phorate		
History	13.1 ± 4.1	4
Nonhistory	8.0 ± 1.3	12
Ethoprop		
History	32.7 ± 18.9	2
Nonhistory	19.9 ± 11.6	8

For isofenphos, there also was an increased mineralization rate in isofenphos-history soils. Very little mineralization occurred in

nonhistory soils, and in all cases the rate of mineralization was greater in history soils, but varied in magnitude from 5.1 to 64.4%. Some difference in the mineralization of fonofos in the history and nonhistory soils was evident, but the behavior of fonofos was much more variable in the fonofos-history soils. There seemed to be two groups of history soils: those in which the fonofos mineralization rate was quite rapid (5 soils, 24.2-46.6%) and others in which it was comparable to the non-history soils (8 soils, 8.3-14.9%).

The mineralization of terbufos and phorate was not significantly greater in the corresponding history soils. Ethoprop exhibited quite variable mineralization behavior in both history and nonhistory soils, and from the small number of soils available with ethoprop history it is not possible to draw any meaningful conclusions. This corroborates earlier studies that have reported no evidence of enhanced terbufos or phorate degradation. Other reports have investigated the possible enhanced degradation of terbufos and phorate and also have found no evidence of an accelerated rate of degradation (21,22). Therefore, more intensive soil degradation studies focused on carbofuran, isofenphos, fonofos, and several related insecticides.

Cumulative plots of carbofuran mineralization in a history and a nonhistory soil are presented in Figure 1. The initially accelerating rate of $^{14}CO_2$ production, indicative of a microbial response (e.g., enzyme induction, population growth), is characteristic of enhanced degradation. Comparison of the mineralization of ^{14}C-carbonyl and ^{14}C-ring labelled carbofuran demonstrates an important consideration in this type of assay: the location of the ^{14}C label in the insecticide is critical for this type of assay to provide useful information. Although the carbonyl ^{14}C was almost completely evolved as $^{14}CO_2$, the ring ^{14}C was only slowly mineralized.

There are several complementary rapid degradation screening assays that have been effectively used in the laboratory. These include bioassay (16,23), gas chromatographic assay of persistence (17), colorimetric assay (24), and liquid medium assay (15,25). All can be useful in screening large numbers of soils for gross differences in insecticide-degrading behavior. However, it is extremely important that such assays are not used to provide the sole basis for explaining potential instances of enhanced degradation (26). In order for enhanced degradation to be truly confirmed for a given pesticide a more thorough laboratory and/or field investigation in which the microbiological aspects are clarified is required. Only then can enhanced degradation be distinguished from natural variations in soil insecticide degradation due to variation in soil properties (e.g., biomass, pH, organic content). The most useful aspect of these rapid degradation assays is in identifying suspect soils and pesticides for further investigation.

Pesticide Degradation in Companion Soils

The second component of the laboratory methodology for study of enhanced degradation involved a more intense investigation of the soil degradation of insecticides identified as suspect from the rapid degradation assay (carbofuran, isofenphos, fonofos) as well as 2 additional insecticides (cloethocarb, chlorpyrifos). The degradation

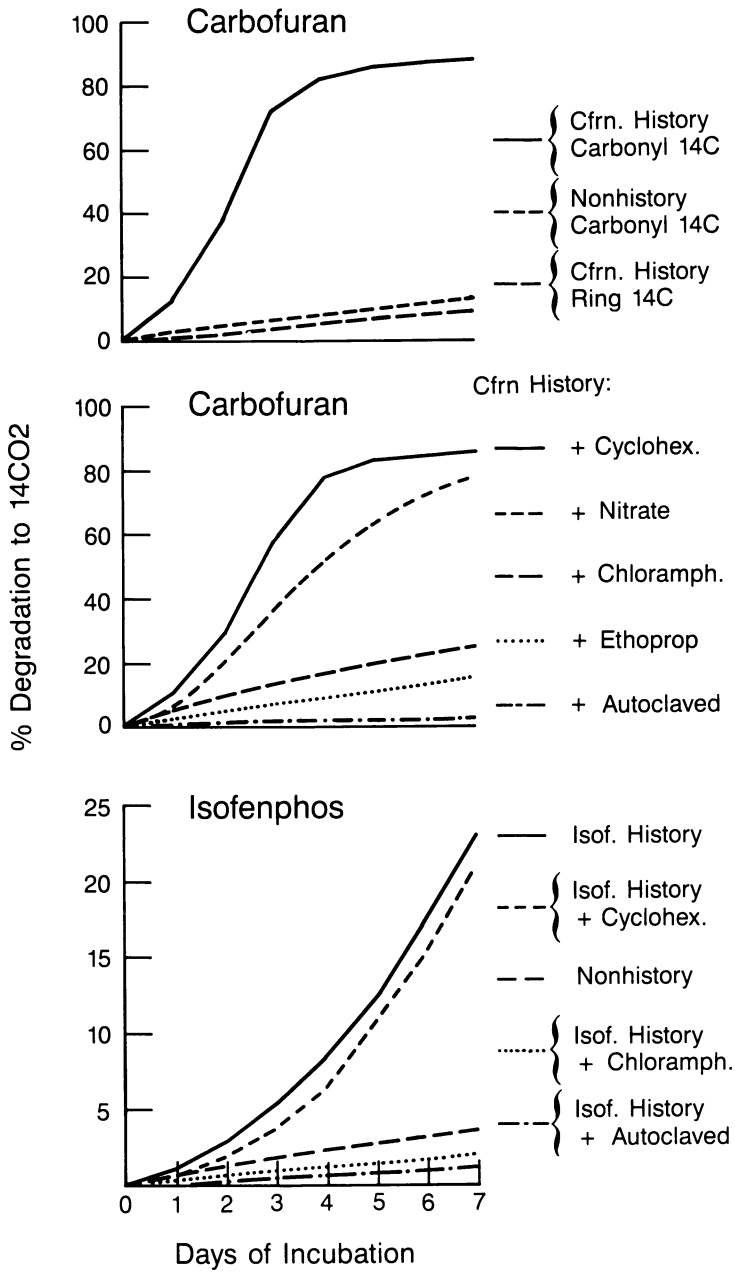

Figure 1. Mineralization of ^{14}C–labeled carbofuran and isofenphos to $^{14}CO_2$ during a one-week laboratory assay.

of these insecticides was examined in 'companion' soils. For a given insecticide this included a soil with a history of application of that insecticide and a similar soil from an untreated adjacent experimental plot or field. The history soils for carbofuran, isofenphos, and fonofos had displayed evidence of rapid insecticide degradation in the rapid degradation assay. The strategy was to examine the rates and products of degradation in treated and untreated companion soils to determine if a shift in degradative behavior had occurred.

For this study 100 g samples of soil were placed in glass jars and treated with either ^{14}C-ring-carbofuran, ^{14}C-ring-cloethocarb, ^{14}C-ring-isofenphos, ^{14}C-ring-fonofos, or ^{14}C-ring-chlorpyrifos at 5 μg/g. Soils were moistened to 0.3 bar soil moisture tension and incubated for 4 weeks at 25°C by using a flow-through incubation system in which air was periodically passed from the jars and through 0.1 N NaOH traps for monitoring of evolved $^{14}CO_2$ and maintenance of aerobic conditions (27). Soils were then extracted with organic solvents to remove extractable metabolites and the extract analyzed by thin-layer or high-pressure liquid chromatography for determining distribution of metabolites. Samples of extracted soil were combusted to determine soil-bound ^{14}C-residues.

Results of the soil pesticide metabolism study are presented in Table II, and the companion soils are listed first for each pesticide investigated. For carbofuran, there was a tremendous difference in degradation rate between the history and nonhistory soils. As oppposed to the mineralization assay, in which ^{14}C-carbonyl-carbofuran was used, this study employed ^{14}C-ring-carbofuran. Therefore, although little mineralization of the carbofuran ring occurred in any soil, there was a tremendous accumulation of soil-bound residues in the soil in which carbofuran was rapidly degraded. The enhanced degradation of carbofuran has been extensively documented (18). The behavior of cloethocarb was similar to that of carbofuran, and it too was extensively degraded in the history versus the nonhistory soil with substantial accompanying production of soil-bound residues. Cloethocarb is a carbamate insecticide that was under development for the rootworm insecticide market but was withdrawn about the same time that decreased persistence in soil after repeated use was noted (28).

Both isofenphos and fonofos were much less persistent in the history versus the nonhistory soils. A major difference between these insecticides and the carbamates was that considerable mineralization of the aromatic ring portion of the organophosphorus compounds occurred. For isofenphos, considerable quantities (15.2%) of isofenphos oxon accumulated only in the nonhistory, whereas no such accumulation was noted in the isofenphos-history soil. Several reports of the reduced persistence of isofenphos in field plots following repeated use have appeared (29,30), and isofenphos was withdrawn from the rootworm insecticide market after widespread experiences of control failure following second year applications. This is especially ironic because upon first application to soil isofenphos behaves as one of the most persistent organophosphorus insecticides (31).

In contrast to the other insecticides intensely investigated, chlorpyrifos showed no decrease in persistence in soil with a history of chlorpyrifos use. This corroborates earlier laboratory and field

Table II. Recovery of Parent Insecticides and Degradation Products From Companion Insecticide-History and Untreated Soils After a 4-Week Laboratory Incubation *

Insecticide/ Soil History	Parent Pesticide	Extractable Metabolites	Soil-Bound	$^{14}CO_2$
	^{14}C Recovered in % of Applied			
Carbofuran				
Untreated Soil	56.0	6.5	29.5	5.8
Carbofuran Soil	0.8	1.9	71.8	14.0
Cloethocarb Soil	1.6	2.2	76.9	11.0
Isofenphos Soil	46.4	3.0	39.6	9.3
Cloethocarb				
Untreated Soil	67.0	2.0	22.8	8.1
Cloethocarb Soil	6.4	0.1	73.9	18.8
Carbofuran Soil	24.0	2.1	43.9	15.3
Isofenphos Soil	45.6	1.2	35.8	10.2
Isofenphos				
Untreated Soil	62.8	16.1	9.2	10.0
Isofenphos Soil	12.9	3.6	23.6	52.4
Fonofos Soil	76.3	13.5	8.0	4.7
Carbofuran Soil	74.9	12.4	7.3	4.5
Fonofos				
Untreated Soil	50.3	1.8	27.2	19.3
Fonofos Soil	2.1	1.2	33.7	51.2
Isofenphos Soil	35.3	3.0	37.3	25.6
Carbofuran Soil	60.4	1.7	20.8	15.0
Chlorpyrifos				
Untreated Soil	57.1	6.0	17.1	15.2
Chlorpyrifos Soil	55.2	5.2	8.1	26.7
Fonofos Soil	33.9	32.6	18.6	9.1
Carbofuran Soil	55.8	13.4	15.4	10.9

*Adapted in part from (22,27,28)

studies in which the persistence of chlorpyrifos has not been affected by repeated field applications (21,32).

It is at this point that too many studies of enhanced insecticide degradation conclude. Evidence has been generated that in soils, plots, or fields selected for study the pesticide exhibits decreased persistence when it has been repeatedly applied. These companion soils have often been selected as a result of observed field failures, with 'control' soil collected from an adjacent fencerow, plot, or field. But does the comparison of rates of insecticide degradation in history and non-history soils represent sufficient evidence for proving that an insecticide has undergone enhanced degradation? We believe not for several reasons. First, it is recognized that there is a tremendous amount of natural variation in the rate of pesticide degradation between soils due to such environmental factors as organic matter content, pH, texture, and moisture. For example, Laskowski et al. (33) reported that there was

between a 2- and 36-fold difference in the degradation rate of 12 pesticides in different soil types. Merely because soil from one field displays a much more rapid rate of degradation than an adjacent one does not mean that the microbial adaptation for enhanced degradation has occurred. Second, by definition, enhanced microbial degradation is a microbial process, and unless it is demonstrated that the microbial community is involved in the rapid degradation observed, it is impossible to distinguish it from abiotic mechanisms of pesticided degradation. Third, current understanding of enhanced degradation recognizes that the mechanism of enhanced microbial degradation involves an inducible microbial adaptation for pesticide catabolism (18,34). It has been demonstrated that the level of total microbial biomass in soil can sometimes greatly influence the rate of pesticide degradation (35). Should the term 'enhanced degradation' be used to describe the increased rate of degradation seen in soils from fields that have greater levels of microbial biomass (e.g. manured fields)? It is evident from these considerations that for enhanced degradation to be truly characterized, the microbiological aspects of the phenomenon must be investigated.

Microbiology of Enhanced Insecticide Degradation

A first step in the laboratory methodology regarding the microbiology of enhanced degradation involved determination of microbial involvement in the rapid insecticide degradation noted in some history soils. The strategy employed was to expose soils with suspected enhanced degradation to various antimicrobial treatments and monitor any inhibition of insecticide degradation. The rapid insecticide degradation assay in which evolution of $^{14}CO_2$ was used as an indicator of degradation proved ideal for such an investigation. Samples of soil for the mineralization assay were either autoclaved to destroy total microbial activity, treated with 100 μg/g of chloramphenicol to inhibit soil bacteria, or treated with 100 μg/g of cycloheximide to inhibit soil fungi prior to ^{14}C-insecticide application. To investigate reports that the insecticide ethoprop interfered with enhanced carbofuran degradation and that soil microorganisms might be utilizing carbofuran as a nitrogen source, soil samples pretreated with 25 μg/g of ethoprop or sodium nitrate were also incubated.

Results of these assays are presented in Figure 1. With carbofuran, isofenphos, and fonofos (not shown), sterilization by autoclaving totally inhibited rapid pesticide degradation in history soils. By itself this seems to indicate that the rapid degradation is microbially mediated. However, autoclaving also destroys immobilized enzymes present in soil, and it has been shown that these enzyme systems are responsible for the extremely rapid organo-phosphorus insecticide degradation observed in some soils (36). In order to better demonstrate the involvement of the soil microbial community in pesticide degradation, it is desirable to use antimicrobial treatments that are more selective than autoclaving. Although the antibacterial compound chloramphenical greatly inhibited the degradation of these insecticides, the antifungal compound cycloheximide did not. Thus, this type of assay can provide information not only on the microbial nature of the phenomenon but also on the microbial groups involved. The primary role of the soil

bacteria in the enhanced degradative process has been well documented (15,27,34). It is worth noting that an excess supply of nitrate did not affect carbofuran degradation, but the insecticide ethoprop apparently inhibited the rapid degradation of carbofuran through some unknown mechanism. Use of sterilized soil assays has commonly been used to distinguish enhanced degradation from abiotic degradation (15,29).

A second step in the study of the microbiology of enhanced degradation involved an attempt to enumerate the microbial population in soil capable of beneficially catabolizing these insecticides. This is a critical step because it distinguishes between mere cometabolism and microbially beneficial catabolism. A most-probable-number (MPN) assay, by which microbial numbers are statistically estimated from a series of dilutions, was used to determine levels of microorganisms capable of metabolizing carbofuran, isofenphos, or fonofos in soils displaying rapid pesticide degradation. MPN techniques have been extensively utilized for the study of microorganisms in aquatic systems and the basic methodology described by Somerville et al. (37) was used. The basal salts medium in which microbial growth was assayed contained either isofenphos or fonofos as a sole carbon source, or carbofuran as a sole carbon and nitrogen source.

Results with isofenphos were quite conclusive. In nonhistory soils there were no microorganisms detected that could catabolize isofenphos as a sole carbon source, whereas in soils with an enhanced rate of isofenphos degradation between 6,000 and 12,000 isofenphos-degrading microorganisms were present per gram of soil. A similar population of isofenphos degraders could be induced in untreated soil by pretreatment with 100 ppm of isofenphos. At higher isofenphos pretreatment levels increases in the total number of soil bacteria as measured by standard plate count methods could be detected. Thus, the numbers of total bacteria in isofenphos-history and untreated soils increased 20-fold and 7-fold in response to a 5,000 μg/g isofenphos application, respectively. In the case of fonofos, no population of fonofos-catabolizing microorganisms could be detected in the soils with rapid rates of fonofos degradation. This indicates that the rapid degradation of fonofos in these soils may be unrelated to any actual microbial adaptation and thus may not involve enhanced degradation. In the case of carbofuran, both soils displaying an enhanced rate of carbofuran degradation and untreated soils contained significant populations of carbofuran-metabolizing microorganisms. In general, the numbers of carbofuran degraders was greater in carbofuran-history soils displaying an enhanced rate of carbofuran degradation (150,000-458,000/g soil) than in untreated soils (23,000-183,000/g soil). This implies that there may be qualitative differences in the rate at which soil microorganisms can degrade a given insecticide as well as quantitative differences in populations of degraders. Although work on estimating the populations of phenoxyalkanoic herbicide degraders and carbamothioate herbicide degraders in soil has shown higher population levels in soils displaying enhanced rates of herbicide degradation (38,39), in some cases no significant difference in population levels has been noted (40).

A third step in the study of the microbiology of enhanced degradation involved isolation of pure cultures of insecticide-

degrading soil microorganisms. The strategy was to inoculate a minimal microbial medium with either isofenphos supplied as the sole carbon source, or carbofuran supplied as the sole carbon and nitrogen source, and then isolate individual colonies from the medium for further characterization and identification. Two bacterial strains were isolated from soil displaying an enhanced rate of isofenphos degradation, an *Arthrobacter sp.* and a *Pseudomonas sp.* (22,27). The *Arthrobacter* proved to be the most effective degrader of isofenphos and could utilize it or its hydrolysis products, isopropyl salicylate and salicylate, as sole carbon sources for growth while mineralizing the aromatic ring (22). An earlier study of enhanced isofenphos degradation had resulted in the isolation of a *Corynebacterium sp.* from isofenphos-history soils that could mineralize isofenphos (41). Repeated efforts to isolate carbofuran-degraders failed to identify microorganisms that could grow on a minimal medium containing carbofuran. Although an early study resulted in isolation of a *Pseudomonas sp.* that had some capability to degrade carbofuran in culture (15), the only carbofuran-catabolizing microorganism successfully isolated from carbofuran-history soil was an *Achromobacter sp.* that utilized carbofuran as a sole nitrogen source (42).

It seems likely that there are a variety of soil microorganisms, primarily bacteria, that can adapt for insecticide catabolism and thus mediate enhanced degradation. By itself, isolation of an insecticide-catabolizing microorganism from soil is not sufficient evidence of enhanced degradation, but coupled with data on increased rates of degradation in history soils it does complete the evidence necessary to substantiate this phenomenon. One of the additional bonuses that results from isolation of these insecticide-degrading microorganisms is the opportunity to study the enzymology and genetics of the process.

Cross-Adaptations for Enhanced Degradation

A final consideration of enhanced degradation of soil insecticides in the cornbelt is the specificity of the adaptation that leads to enhanced microbial degradation. It has been noted that in some cases an insecticide is degraded rapidly in soil from a field to which it has never been applied before, but a similar insecticide has. The specificity of enhanced degradation can be studied at all levels from field persistence behavior to interactions with microbial enzymes. Cross-adaptations seem to be especially characteristic of enhanced carbamate insecticide degradation. Carbofuran degraded rapidly in a soil previously exposed to cloethocarb, while the rate of cloethocarb degradation was somewhat enhanced in soil with carbofuran history (Table II). A number of related carbamate insecticides were degraded in soil repeatedly treated with carbofuran (17), and the degree of cross-adaptation for carbamate insecticide degradation in soil has been shown to depend on structural similarity. Likewise, the carbofuran-catabolizing *Achromobacter sp.* isolated from carbofuran-history soil could utilize a number of carbamate insecticides as sole nitrogen sources (42). The adaptation for enhanced degradation of organophosphorus insecticides, however, appears to be much more specific. Neither fonofos nor chlorpyrifos was rapidly degraded in isofenphos-history soil, and only fonofos was

degraded rapidly in fonofos-history soil (Table II). No cross-adaptations for enhanced degradation of organophosphorus insecticides were noted in soil displaying enhanced isofenphos degradation, and the *Arthrobacter sp.* isolated could metabolize only isofenphos in pure culture and did not metabolize or cometabolize other organophosphorus insecticides (22).

It is likely that the specificity of enhanced degradation depends on the microbial metabolic use of the insecticide. Hydrolysis of any N-methylcarbamate insecticide yields methylamine as one product, and if the bacteria are utilizing this product as a nitrogen source, the ability to hydrolyze carbamate insecticides in general would be advantageous (42). However, microbial degradation of organophosphorus insecticides as carbon sources proceeds via initial hydrolysis and secondary metabolism of the aromatic phenolic metabolites. Because the hydrolytic metabolites of organophosphorus insecticides tend to be somewhat unique, this might explain the high specificity of the enhanced degradation of both isofenphos (27) and diazinon (43).

Towards an Experimental Definition of Enhanced Degradation

In summary, the enhanced degradation of an insecticide involves its rapid degradation by a population of soil microorganisms that has adapted to beneficially catabolize it following exposure to it or a similar insecticide. This enhanced rate of degradation may or may not result in failure of the compound to control the target pest depending on environmental conditions. For enhanced degradation to be thoroughly investigated studies must be carried out to demonstrate an increased rate of degradation in soils with prior insecticide exposure, to identify the rates and products of degradation in similar soils under controlled conditions, and to elucidate the microbiological aspects of the phenomenon including the identification of an adapted, insecticide-catabolizing microbial population. Using these criteria, only carbofuran and isofenphos have been sufficiently experimentally scrutinized so as to characterize their enhanced degradation in soil. Some evidence suggests the possibility that other insecticides may also have undergone enhanced degradation, but further study is required to provide sufficient evidence.

Literature Cited

1. Krysan, J. L.; Miller, T.A. Methods for the Study of Pest Diabrotica; Springer-Verlag; New York, NY, 1986.
2. Hill, R. E.; Hixson, E.; Muma, M. H. J. Econ. Entomol. 1948, 41, 392-401.
3. Lilly, J. H. Ann. Rev. Entomol. 1956, 1, 203-222.
4. Ball, H. J; Weekman, G. T. J. Econ. Entomol. 1962, 55, 439-441.
5. Apple, J. W.; Walgenbach, E. T.; Knee, W. J. J. Econ. Entomol. 1969, 62, 1033-1035.
6. Chio, H.; Chang, C. S.; Metcalf, R. L.; Shaw, J. J. Econ. Entomol. 1978, 71, 389-393.
7. Turpin, F. T.; Dumenil, L. C.; Peters, D. C. J. Econ. Entomol. 1972, 65, 1615-1619.

8. Felsot, A.S.; Steffey, K.L.; Levine, E.; Wilson, J.G. J. Econ. Entomol. 1985, 78, 45-52.
9. Jennings, V.; Stockdale, H. Herbicides and Soil Insecticides Used in Iowa Corn and Soybean Production, 1977; Iowa State University Cooperative Extension Service: Ames, IA, 1978.
10. Ahmad, N.; Walgenbach, D. D.; Sutter, G. R. Bull. Environ. Contam. Toxicol. 1979, 23, 572-574.
11. Gorder, G. W.; Tollefson, J. J.; Dahm, P. A. Iowa State J. Res. 1980, 55. 25-33.
12. Greenhalgh, R.; Belanger, A. J. Agric. Food Chem. 1981, 29, 231-235.
13. Felsot, A. S.; Wilson, J. G.; Kuhlman, D. E.; Steffey, K. L. J. Econ. Entomol. 1982, 75, 1098-1103.
14. Newton, J.P. M.S. Thesis, University of Nebraska, Lincoln, 1978.
15. Felsot, A. S.; Maddox, J. V.; Bruce, W. Bull. Environ. Contam. Toxicol. 1981, 26, 781-788.
16. Read, D. C. Agric. Ecosyst. Environ. 1983, 10, 37-46.
17. Harris, C. R.; Chapman, R. A.; Harris, C.; Tu, C. M. J. Environ. Sci. Health 1984, B19, 1-11.
18. Felsot, A. S. Ann. Rev. Entomol. 1989, 34, 453-476.
19. Annonymous Ag. Consult. Fieldman 1984, 40, 14.
20. Doersch, R. E.; Doll, J. D.; Wedberg, J. L.; Grau, C. R.; Harvey, R. G.; Kenney, J. E. Pest Control in Corn 1983; University of Wisconsin-Extension Service: Madison, WI; p. 36.
21. Harris, C. R.; Chapman, R. A.; Tolman, J. H.; Moy, P.; Henning, K.; Harris, C. J. Environ. Sci. Health 1988, B23, 1-32.
22. Racke, K. D.; Coats, J. R. J. Agric. Food Chem. 1988. 36, 193-199.
23. Wilde, G.; Mize, T. J. Econ. Entomol. 1984, 13, 1079-1082.
24. Chapman, R. A.; Moy, P.; Henning, K. J. Environ. Sci. Health 1985, B20, 313-320.
25. Niemczyk, H. D.; Chapman, R. A. J. Econ. Entomol. 1987, 80, 880-882.
26. Kaufman, D. D.; Katan, Y.; Edwards, D. F.; Jordan, E. G. In Agricultural Chemicals of the Future; Hilton, J. L., Ed.; Beltsville Symposia in Agricultural Research No. 8; U. S. Dept. of Agriculture: Totowa, MD, 1985; pp 437-451.
27. Racke, K. D.; Coats, J. R. J. Agric. Food Chem. 1987, 35, 94-99.
28. Racke, K. D.; Coats, J. R. J. Agric. Food Chem. 1988, 36, 1067-1072.
29. Abou-Assaf, N.; Coats, J. R.; Gray, M. E.; Tollefson, J. J. J. Environ. Sci. Health 1986, B21, 425-446.
30. Chapman, R. A.; Harris, C. R.; Moy, P.; Henning, K. J. Environ. Sci. Health 1986, B21, 269-276.
31. Felsot, A. J. Environ. Sci. Health 1984, B19, 13-27.
32. Racke, K. D.; Coats, J. R.; Titus, K. R. J. Environ. Sci. Health 1988, B23, 527-539.
33. Laskowski, D. A.; Swann, R. L.; McCall, P. J.; Bidlack, H. D. Residue Rev. 1983, 85, 139-147.
34. Kearney, P. C.; Kellogg, S. T. Pure Appl. Chem. 1985, 57, 389-403.
35. Frehse, H.; Anderson, J. P. E. In Pesticide Chemistry: Human Welfare and the Environment; Miyamoto, J., Ed.; Int. Union Pure Appl. Chem.: Pergamon Press, Oxford, England, 1983; pp 23-32.

36. Getzin, L. W.; Rosefield, I. J. Agric. Food Chem. 1966, 16, 598-601.
37. Somerville, C. C.; Monti, C. A.; Spain, J. C. Appl. Environ. Microbiol. 1985, 40, 726-734.
38. Fournier, J. C.; Codaccioni, P.; Soulas, G. Chemosphere 1981, 10, 977-984.
39. Mueller, J. G.; Skipper, H. D.; Lawrence, E. G.; Kline, E. L. Weed Sci. 1989, 37, 424-427.
40. Moorman, T. B. Weed Sci. 1988, 36, 96-101.
41. Murphy, J. J.; Cohick, A. D. Abstr. 40th Nat. Meet. Entomol. Soc. Am., 1985, paper 121.
42. Karns, J. S.; Mulbry, W. W.; Nelson, J. O.; Kearney, P. C. Pestic. Biochem. Physiol. 1986, 25, 211-217.
43. Forrest, M.; Lord, K. A.; Walker, N.; Woodville, H. C. Environ. Pollut. 1981, A24, 93-104.

RECEIVED February 1, 1990