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Characterization of Microorganisms in Soils Exhibiting Accelerated Pesticide Degradation

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Many agricultural pesticides are soil incorporated and will interact with non-target soil organisms including those microorganisms able to degrade pesticidal compounds. Generally, significant degradation of a pesticide occurs shortly after the pesticide has contacted and exerted its toxic effect upon the target pest. Thus, microbial degradation may be the single most important factor in preventing the buildup in soils of pesticides that could cause potential environmental problems. Unfortunately, certain pesticides may be degraded so rapidly by soil microorganisms that the effectiveness of the pesticides on the target pest is greatly reduced or even eliminated. Such phenomena have been recognized as accelerated microbial degradation (Kaufman and Edwards 1982). Accelerated degradation is predominantly associated with certain chemical classes of pesticides and their continuous use. Cabofuran, a carbamate soil insecticide, has in numerous instances provided inadequate control of corn rootworm after repeated use (Felsot et al. 1981). Also, some thiocarbamate herbicides have not given adequate grass weed control after repeated use (Wilson 1984; Obrigawitch et al. 1983). The continuous use of certain classes of pesticides may lead to selection of soil microorganisms that tolerate or readily metabolize them.

There are a number of studies that, in general, implicate the involvement of adapted soil microbial populations in accelerated pesticide degradation (Obrigawitch et al. 1983; Rahman and James 1983; Skipper et al. 1986). Some investigations have resulted in the identification of microbial isolates which are apparently responsible for the accelerated degradation of individual pesticides (Felsot et al. 1981; Lee 1984; Ou et al. 1982; Williams et al. 1976). Less attention has been given to the interrelationships of soil-applied pesticides and the microorganisms in soils receiving continuous, annual applications of pesticides. The predominance of specific microbial groups in these soils may be due to the ability to utilize or to increase the production of certain constitutive enzymes for immediate metabolism of the pesticide compounds.

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Decreased frequencies of other microbial groups might be a result of sensitivity of these organisms to the applied pesticides. Little, if any, published information exists on these aspects of the microbial ecology of soils exhibiting accelerated pesticide degradation. This study examined the response of microbial populations in soils after incorporation of the herbicides alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide], butylate (S-ethyl diisobutylthiocarbamate) or EPTC (S-ethyl N,N-dipropylthiocarbamate) and the insecticides carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl), cloethocarb [2-(2-chloro-1-methoxy)-ethoxyphenyl-N-methylcarbamate], isophenfos [1-methyl-2-((ethoxy)(1-methyl-ethyl) aminophosphinothioyl) oxy) benzoate] or terbufos[S-(((1,1-dimethylethyl) thio) methyl) 0,0-dimethyl phosphorodithioate]. Data are also presented on the influence of these pesticides on the activity of selected microbial enzymes.

MATERIALS AND METHODS

Field studies were conducted at two sites (Andrew and Buchanan counties, MO) in 1984 and 1985. The first site was in a field planted to continuous corn (Zea mays) 1981 - 83 and exhibited corn rootworm control problems in 1982 and 1983. The second site was in continuous corn during 1980 - 85 on which EPTC failed to control grass weeds in 1981 after which the grower discontinued use of this herbicide. A split-block design was employed with four insecticides and three herbicides treatments and non-treated controls replicated three times in plots consisting of four rows (15.2 m long with 76-cm row widths). Herbicides were applied in liquid formulation and thoroughly incorporated into the soil prior to planting.

Alachlor [3.4 kg active ingredient (a.i.)/ha], butylate (7.5 kg a.i./ha) and EPTC (7.5 kg a.i./ha) were applied at recommended rates. Granular insecticides were applied at planting (May 17, 1984 and May 15, 1985) in an 18 cm band over the row at a rate of 0.75 g/m (1.12 kg a.i./ha). Plots were planted to corn in a direction perpendicular to the direction of the previously applied herbicides.

Soil samples were collected weekly intervals for seven weeks from the date of pesticide application. The samples, consisting of cores (8.9 cm diam.) obtained from the upper 10 cm of the profile within the two center rows of each plot, were placed in sterile plastic bags using aseptic techniques and transported to the laboratory. The samples were ground using a food processor to pass a 2-mm screen and stored at 0°C until assayed within 14 days. Viable microorganisms were isolated from duplicate samples of each treatment. The samples were diluted serially (10-fold steps) in distilled water and transferred aseptically into duplicate petri plates. Appropriate agar medium (12 to 15 ml) at 40°C was added to each plate. Bacteria and actinomycetes were isolated on egg albumin agar, pH 6.8, and fungi on rose bengal plus streptomycin agar, pH 6.5 (Wollum 1982). All plates were incubated at 25°C for 10 to 14 days before enumeration and examination. Representative

fungi developing on the plates were subcultured on Czapek-Dox agar, pH 7.2 (Wollum 1982) and identified according to Domsch et al.(1980). Bacteria and actinomycetes were subcultured on nutrient agar, pH 6.8 (Wollum 1982), characterized based on standard procedures and classified as far as possible according to Bergey's Manual (Buchanan and Gibbons 1974).

To assess the sensitivity of selected microorganisms to the pesticides, technical grade formulations of each insecticide and herbicide were dissolved in acetone at concentrations of 100 ppm (w/v). Sterile paper discs (13 mm diam.) were saturated with pesticide solutions, air dried and placed on Czapek-Dox or nutrient agars (Wollum 1982) previously seeded with a fungal, bacterial or actinomycete isolate. After three days of incubation at 25°C, the plates were examined for zones of inhibition around around each disc. Data were averaged for the four replications.

Selection for assay of the microbial enzymes, alkaline phosphomonoesterase, phosphodiesterase and rhodanese (thiosulfate-cyanide sulfurtransferase), was based on their possible importance in the degradative pathways of organophosphate and carbamate insecticides and thiocarbamate herbicides (Kaufman 1967; MacDonald et al. 1983). Individual isolates of bacteria and actinomycetes were cultured in a mineral salts broth and fungi in a modified Czapek-Dox broth (Wollum 1982) supplemented with technical grade herbicide or insecticide (100 ppm, w/v). After incubation for 2, 7 and 14 days for bacteria, actinomycetes and fungi, respectively, the cultures were passed through a Seitz filter (0.1 um). The culture filtrates were assayed for enzymatic activities based on the procedures of Tabatabai (1982). Spectrophotometric readings for each enzyme were adjusted according to controls receiving no culture filtrates.

Replicate aliquots of each culture filtrate were also assayed for protein content (Lowry et al. 1951). Specific enzyme activities are reported as micromoles of product produced per milligram of protein per hour at 25°C.

RESULTS AND DISCUSSION

The response of soil microorganisms to pesticides in pure culture was variable with no consistent relationship evident between the source of the microorganisms (treatment plot) and the observed response. Therefore, results from bioassays on microorganisms representative of the dominant isolates occurring at both field sites are presented (Table 1). The greatest number of isolates inhibited by the pesticides occurred among the bacterial group while the least inhibited were among the fungi. The number of isolates able to grow on pesticide substrates alone was highest also for the bacteria. These observations demonstrate the diversity of metabolic pathways available to bacteria for metabolism of the pesticides. Representative actinomycete isolates grew on all pesticide compounds except the chlorinated pesticides alachlor and cloethocarb which markedly inhibited

growth of many of the isolates. Most of the fungal isolates were not inhibited by any of the pesticides. Overall, carbofuran stimulated growth of the greatest number of microorganisms. These results support previous reports implicating the involvement of individual soil microorganisms in accelerated degradation of several of the pesticides under study (Kazano et al. 1972; Lee 1984; Ou et al. 1982; Williams et al. 1976). Although the pesticides were assayed at 100 ppm, a concentration higher than field rates, this concentration is likely surpassed in the soil solution within "microsites" near the soil surface (Kaufman 1984 personal communication) and soil microorganisms may thereby respond as observed in the pure culture study.

Table 1. Effect of pesticides on growth of various soil microorganisms in pure culture[a]

Pesticide	Bacteria			Actinomycetes			Fungi		
	+	-	N	+	-	N	+	-	N
	number of isolates								
Alachlor	3	6	11	0	4	10	0	1	8
Butylate	1	3	13	1	0	9	1	0	7
EPTC	2	3	12	2	0	8	2	1	6
Carbofuran	3	4	10	3	1	7	4	1	4
Cloethocarb	3	1	11	0	4	10	0	1	8
Isophenfos	2	2	12	1	0	9	0	0	8
Terbufos	1	3	13	3	2	7	1	0	7
Total	15	22	82	10	11	60	8	4	48

[a]Growth ratings: +, Stimulated growth; -, Inhibited growth (inhibition zone formed); N, No effect (no zone formed).

Microbial isolates exhibiting growth on pesticides during the sensitivity assays were examined for specific enzymatic activities (Table 2). The activities varied among the isolates and, in some instances, were significantly greater for "field" isolates than for reference strains indicating a possible selection for increased enzymatic activity in the presence of applied pesticides. Several individual isolates capable of growing on the carbamate insecticide, carbofuran, and the thiocarbamate herbicides, butylate and EPTC, also exhibited high rhodanese activity suggesting that this enzyme may be involved in the metabolism of these pesticides. These results also suggest that soil microorganisms developing high rhodanese activity in response to applications of thiocarbamate herbicides (ie. butylate) may also be involved in the metabolism of the chemically similar carbamate insecticides (ie. carbofuran) without prior exposure to the insecticides. Previous reports have indicated that such cross-adaptations by

Table 2. Enzyme activities of microorganisms from soils exhibiting accelerated pesticide degradation.

Organisms	Pesticide Substrate(s)[a]	Rhodanese	Alkaline Phosphatase	Phosphodi-esterase
		ug product/mg protein/h at 25° C		
Bacteria				
Alealigenes sp.	IS	8.0 a*	1.8 a	12.6 a
Micrococcus sp.	None	3.2 b	1.8 a	11.6 a
Pseudomonas sp. 3	CB, BU	9.1 a	1.8 a	5.2 b
Pseudomonas sp. 6	CB, IS, AL	4.3 b	1.8 a	12.1 a
Serratia sp.	CB	8.5 a	1.7 a	4.3 b
Pseudomonas (Ref)[b]	None	4.5 b	1.8 a	11.0 a
Actinomycetes				
Nocardia sp	None	0 b	12.0 a	30.4 b
Streptomyces sp. 13	TB, AL, BU	5.5 a	11.6 a	41.2 a
Streptomyces sp. 15	None	0 b	10.3 b	20.6 b
Streptomyces sp. 25	All	5.5 a	11.8 a	61.0 a
Streptomyces (Ref)[b]	None	0 b	12.7 a	30.1 b
Fungi				
Aspergillus sp.	EP, CL	6.4 b	11.8 a	26.8 b
Fusarium sp. 25	EP, BU	8.2 a	12.1 a	18.8 b
Fusarium sp. 210	IS	0 b	12.5 a	45.5 a
Penicillium sp.	None	4.3 b	12.0 a	27.7 b
Penicillium (Ref)[b]	None	0 b	11.3 a	21.2 b

[a]Pesticides metabolized by the microbial isolate: AL=Alachlor; BU=Butylate; EP=EPTC; CB=Carbofuran; CL=Cloethocarb; IS=Isophenfos; TB=Terbufos.

[b]Reference strains from microbial culture collection of R. J. Kremer.

*Means within a column followed by a common letter do not differ significantly ($P<.05$).

soil microorganisms may occur for herbicides within the thiocarbamate class (Obrigawitch et al. 1983; Skipper et al. 1986). A relationship between growth on organophosphate insecticides (isophenfos and terbufos) and high alkaline phosphatase and phosphodiesterase activities was observed for only two actinomycete isolates (Streptomyces spp. 13 and 25) and one fungal species (Fusarium sp. 210).

The apparent relationship between pesticide substrate utilization and increased activities of specific enzymes by individual microorganisms presumably involved in degradation agrees with Senior et al. (1976) who reported that individuals of a microbial community may acquire the ability to grow on a pesticide through evolution of specific enzymes. However, recent reports have also shown that more than one microbial species may be involved in the degradation of pesticides in natural environments (Lappin et al. 1985; Senior et al. 1976) and this may be the case with the organophosphates. It is likely, therefore, that several microorganisms coexisting as a soil community possess a synergistic capacity to metabolize one or more of the pesticides examined in this study. This situation has yet to be investigated for pesticides subject to accelerated degradation.

The present study indicates that assays for activities of enzymes presumed to be involved in accelerated degradation of pesticides may be useful in characterizing the responsible soil microorganisms. Also, identification of specific enzymes involved in accelerated degradation may result in the development of more specific microbial inhibitors or extenders than those used currently to decrease the rate of degradation of certain pesticides (Obrigawitch et al. 1983; Rahman and James 1983; Skipper et al. 1986). Assays for specific enzymatic activities of microorganisms shown to metabolize certain pesticide substrates indicate that cross adaptation of the microorganisms for degradation of chemically similar pesticides in the soil environment may exist.

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