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# Comparative Degradation of Organophosphorus Insecticides in Soil: Specificity of Enhanced Microbial Degradation

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Laboratory experiments investigated the comparative degradation of six organophosphorus insecticides in soil as affected by enhanced microbial degradation. The degradation rates and product distributions of chlorpyrifos, fonofos, ethoprop, terbufos, and phorate were not dramatically altered in soils containing microbial populations adapted to rapidly degrade isofenphos. An *Arthrobacter sp.* isolated from soils with a history of isofenphos use rapidly metabolized isofenphos in pure culture but did not metabolize or cometabolize any of the other five organophosphorus insecticides. Likewise, only fonofos was rapidly degraded in soil with a long history of fonofos use. None of the organophosphorus insecticides were rapidly degraded in soil containing carbofuran-degrading microbial populations. Results indicate that the phenomenon of enhanced microbial degradation of soil insecticides may exhibit some degree of specificity.

Organophosphorus and carbamate insecticides are widely used in the Midwest to control such soil-dwelling pests of corn as larval corn rootworms (*Diabrotica sp.*) and cutworms (*Agrotis sp.*). Corn is now the crop receiving the most intensive use of soil-applied insecticides, and in Iowa alone, approximately $6 \times 10^6$ acres are treated annually with soil insecticides (Wintersteen and Hartzler, 1987). Although the degradation of individual insecticides in soil has been investigated, few comparative studies of their degradation have been conducted (Fuhremann and Lichtenstein, 1980).

Enhanced microbial degradation is an increasingly important phenomenon affecting the degradation of pesticides in soil (Tollefson, 1986). Enhanced degradation occurs when a population of soil microorganisms, which has adapted due to previous exposure to a pesticide, rapidly degrades a subsequent application of the pesticide. The result of this enhanced degradation is a failure of the pesticide to adequately control the target pests due to dramatically decreased persistence (Felsot et al., 1982). Enhanced degradation of several commonly used soil pesticides has been reported, including carbofuran (Felsot et al., 1981), EPTC (Wilson, 1984), and isofenphos (Racke and Coats, 1987).

Enhanced degradation is, at its core, a pesticide/microbe interaction. The decreased persistence of the pesticide involved results from rapid catabolism by populations of pesticide-adapted soil microorganisms (Fournier et al., 1981; Kaufman and Edwards, 1983; Racke and Coats, 1987). Pesticide catabolism by adapted soil microorganisms usually involves a hydrolysis, with further metabolism and utilization of one or more hydrolysis products as carbon or nutrient sources (Karns et al., 1986; Tam et al., 1987; Racke and Coats, 1987).

The current research was initiated to study the comparative degradation of six organophosphorus insecticides in soil as affected by enhanced microbial degradation. The study focused on determining the specificity of the enhanced degradative phenomenon, with special emphasis on the specificity of enhanced isofenphos degradation.

## MATERIALS AND METHODS

**Chemicals.** The following radiolabeled insecticides, along with model metabolites, were obtained from the respective manufacturers: [*U-ring*-14C]isofenphos, Mobay Chemical Corp., Kansas City, MO; [*2,6-phenyl*-14C]chlorpyrifos, Dow Chemical Co., Midland, MI; [*U-ring*-14C]fonofos, Stauffer Chemical Co., Mountain View, CA; [*1-ethyl*-14C]ethoprop, Rhone-Poulenc Inc., Monmouth Junction, NJ; [*methylene*-14C]terbufos and [*methylene*-

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**Table I. Structures and Selected Properties of Organophosphorus Insecticides Studied**

| compound | chemical structure | water sol,[a] ppm | soil half-life, weeks | location | reference |
|---|---|---|---|---|---|
| isofenphos | (CH₃)₂CHN, C₂H₅O, P—O— with COOCH(CH₃)₂ on benzene ring, S | 22.1 | 12<br>5–8<br>>14 | field<br>field<br>lab | Chapman and Harris (1982)<br>Abou-Assaf et al. (1986)<br>Felsot (1984) |
| chlorpyrifos | C₂H₅O, C₂H₅O, P—O— pyridine ring with Cl, Cl, Cl, N; S | 0.7 | 2–8<br>2–7<br>4–12 | field<br>field<br>lab | Chapman and Harris (1980a)<br>Pike and Getzin (1981)<br>Getzin (1981) |
| fonofos | C₂H₅O, C₂H₅, P—S—benzene, S | 15.7 | 6<br>6<br>3–5 | field<br>field<br>lab | Kiigemagi and Terriere (1971)<br>Ahmad et al. (1979)<br>Miles et al. (1979) |
| ethoprop | C₃H₇S, C₃H₇S, P—OC₂H₅, O | 750 | 2–12<br>14 | field<br>lab | Smelt et al. (1977)<br>Jordan et al. (1986) |
| terbufos | C₂H₅O, C₂H₅O, P—S—CH₂—S—C(CH₃)₃, S | 5.5 | <2<br>2<br>1 | field<br>field<br>lab | Ahmad et al. (1979)<br>Szeto et al. (1986)<br>Chapman et al. (1982b) |
| phorate | C₂H₅O, C₂H₅O, P—S—CH₂—S—C₂H₅, S | 17.9 | <1<br><1<br><2 | field<br>lab<br>lab | Suett (1975)<br>Getzin and Chapman (1960)<br>Fuhremann and Lichtenstein (1980) |

[a] Sources: Bowman and Sans, 1979, 1983.

**Table II. Soils Used in the Comparative Insecticide Degradation Study**

| no. | soil series[a] | insecticide history[b] | pH | OM | texture sand | silt | clay | % H₂O (1/3 bar) |
|---|---|---|---|---|---|---|---|---|
| I | Tama | none | 7.3 | 3.9 | 8.2 | 58.6 | 33.2 | 32.9 |
| II | Tama | isofenphos | 6.7 | 3.2 | 5.8 | 63.8 | 30.4 | 28.7 |
| III | Canisteo | none | 7.4 | 4.8 | 30.2 | 34.0 | 35.8 | 31.5 |
| IV | Canisteo | isofenphos | 7.2 | 4.8 | 25.3 | 38.3 | 36.4 | 31.2 |
| V | Festina | fonofos | 7.3 | 2.5 | 17.1 | 60.1 | 22.9 | 26.6 |
| VI | Readlyn | mixed | 6.9 | 2.8 | 41.3 | 32.0 | 26.7 | 22.4 |

[a] Soils I and II (Tama) and soils III and IV (Canisteo) were companion soils taken from adjacent fields. [b] For isofenphos soils (II, IV), includes last 2 years; for fonofos soil (V), includes last 5 years; for insecticide-free soils (I, III), includes last 10 years; mixed soil (VI), carbofuran the previous year and mixed carbamate and organophosphorus insecticide exposure every other year for 10 years, with alternate years untreated.

[14C]phorate, American Cyanamid Corp., Princeton, NJ. All compounds were of greater than 98% radiopurity. Chemical structures and selected properties of these insecticides are shown in Table I.
Chemical structures and selected properties of these insecticides are shown in Table I.

**Soils.** Surface samples of Iowa soils were collected from cornfields at the end of the growing season, sieved to remove debris, and stored in a moist condition at 4 °C. Two soils (II and IV, isofenphos history) had histories of isofenphos use and performance failures and contained populations of isofenphos-degrading microorganisms (Racke and Coats, 1987). A companion soil for each isofenphos soil (I and III) was taken from an adjacent field with no recent insecticide exposure. A soil with 5 years of consecutive fonofos use was also used (V). A final soil (VI, mixed-insecticide history) had been exposed to a variety of organophosphorus and carbamate insecticides over the last 10 years and contained a microbial population that had adapted to rapidly degrade several carbamate insecticides, including carbofuran, carbaryl, bendiocarb, and cloethocarb (Racke and Coats, 1988). Properties of soils used in the comparative degradation study are listed in Table II.

**Extraction and Analyses.** At the end of the incubation period, [14C]insecticide residues in soil were extracted twice with acetone–methanol (1:1) and once with acetone–methanol–dichloromethane (1:1:1) and partitioned into dichloromethane as described by Fuhremann and Lichtenstein (1980). Aqueous samples of [14C]insecti-

cide-treated microbial cultures were acidified with concentrated HCl before partitioning.

Thin-layer chromatographic and autoradiographic systems were used to characterize isofenphos (Racke and Coats, 1987), chlorpyrifos (Getzin, 1981), fonofos (Fuhremann and Lichtenstein, 1980), ethoprop (Menzer et al., 1971), terbufos (Laveglia and Dahm, 1975), phorate (Fuhremann and Lichtenstein, 1980), and their respective metabolites. Qualitative confirmation of the identity of parent insecticides was by gas–liquid chromatography, using a Varian 3700 chromatograph equipped with a 10% DC-200/2% OV-225 on 80/100-mesh Chromosorb column (2 mm × 90 cm) and a TSD (N–P) detector. Unextractable, soil-bound [14C]insecticide residues were recovered by combustion to 14CO₂ in a Packard sample oxidizer. Quantification of 14C, including that present in vapor traps, on TLC plates, and in alkaline CO₂ traps, was by liquid scintillation counting.

EXPERIMENTAL PROCEDURES

**Comparative Degradation of Organophosphorus Insecticides in Soil.** The six soils utilized in the study (Table II) were separately treated with each of the six [14C]insecticides at 5 ppm (0.5 or 1.0 µCi) in acetone (Fuhremann and Lichtenstein, 1980) in duplicate 100-g samples and added to 250-mL glass jars (5-cm diameter × 11-cm height). The effect of laboratory induction of enhanced degradation was also investigated by pretreating an insecticide-free soil (soil I) with 10 ppm of isofenphos at 2-week intervals for a total of four pretreatments. Two

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weeks after the last pretreatment, this soil was then treated with [$^{14}$C]insecticides in the same manner as the other six soils. Soils were moistened to field capacity ($^1/_3$-bar soil moisture tension) with distilled water and incubated at 25 °C in the dark by using a flow-through incubation system (Ferris and Lichtenstein, 1980). Air was periodically purged from the tightly sealed soil jars through both vapor and $CO_2$ traps, and this allowed maintenance of aerobic conditions and monitoring of [$^{14}$C]insecticide degradation to $^{14}CO_2$. Distilled water was added as necessary to replace that lost due to evaporation. After 4 weeks, the soil in each jar was analyzed for the presence of the respective [$^{14}$C]-insecticide and its metabolites as previously described.

**Comparative Metabolism of Organophosphorus Insecticides by an Isofenphos-Degrading Microorganism.** To isolate microorganisms involved in the enhanced degradation of isofenphos, aqueous suspensions of soils II and IV were incubated on basal salts agar plates containing formulated isofenphos insecticide (Amaze 20G) as the sole carbon source. Colonies appearing on the plates were tested for their ability to mineralize [$^{14}$C]isofenphos to $^{14}CO_2$ (Racke and Coats, 1987) and were characterized by using standard microbial taxonomic methodology (Gerhardt, 1981).

The comparative metabolism of the six organophosphorus insecticides was determined in a pure culture of the most active degrader, an *Arthrobacter sp.* Late exponential phase cultures of this *Arthrobacter sp.*, grown on basal salts medium with salicylate as the sole carbon source, were harvested by centrifugation (500$g$ × 30 min) and washed twice by suspension of the pelleted cells in basal salts medium. One-millimeter aliquots of 1.0 Au (Beckman DB spectrophotometer, 540 nm) bacterial suspension were added to 25-mL Erlenmeyer flasks containing 4 mL of basal salts medium, following which one set was sterilized by autoclaving. Glass fiber disks impregnated with 250 $\mu$g of [$^{14}$C]insecticide (0.1 $\mu$Ci) were then added separately to the cultures, with half of the cultures receiving both [$^{14}$C]insecticide and 250 $\mu$g of isofenphos to test for cometabolism. The flasks were incubated on a throw shaker at 120 rpm for 12 h at 25 °C, after which the cultures were analyzed for the presence of [$^{14}$C]insecticides and metabolites as described earlier.

## RESULTS

**Comparative Degradation of Organophosphorus Insecticides in Soil.** Results of the comparative degradation study (Table III) will be summarized for each insecticide.

*Isofenphos* and its oxon metabolite were most rapidly degraded in soils with previous exposure to isofenphos (II and IV). This rapid degradation resulted in the production of significantly more $CO_2$ and soil-bound residues than in companion soils (I and III). The overall degradation of isofenphos was not significantly altered in soil with fonofos history (V) or mixed-insecticide history (VI). A relatively constant 62–76% of the applied isofenphos persisted after the 4-week incubation in soils with a variety of textural properties and no prior isofenphos exposure. Isofenphos degradation was extremely rapid in the isofenphos-pretreated soil (10 ppm × four pretreatments), with more than 50% of the applied insecticide being mineralized to $CO_2$ within 2 days (results not shown in Table III).

*Chlorpyrifos* was as persistent or more persistent in the isofenphos history soils (II and IV) than in the companion soils (I and III). However, a significantly different distribution of degradation products was evident in comparing the Tama soils (I and II) with the Canisteo soils (III and IV). Chlorpyrifos was more persistent, yet gave rise to

greater $CO_2$ in the Tama soils, whereas greater production of soil-bound residues and 3,5,6-trichloropyridinol occurred in the Canisteo soils. The degradation of chlorpyrifos was not distinctly greater in either the fonofos history soil (V) or the mixed-insecticide history soil (VI). Chlorpyrifos was most persistent in the soils with pH <7 (II and VI).

The degradation of *fonofos* was not dramatically affected by soil isofenphos history. A decreased persistence of fonofos and methyl phenyl sulfone and an increased production of $CO_2$ were evident in Tama soils (I and II) as compared with Canisteo soils (III and IV). The degradation of fonofos was most rapid in the fonofos history soil (V), with less than 8% of the applied fonofos remaining in the soil after 4 weeks. This increased degradation resulted in much greater production of $^{14}CO_2$, with 41% of the applied [$^{14}$C]fonofos cumulatively mineralized to $^{14}CO_2$ during the first 11 days of incubation. Fonofos was most persistent in the soil with mixed-insecticide history.

*Ethoprop* was equally or more persistent in the isofenphos history soils (II and IV) than in the companion soils (III and V). As was the case with fonofos, greater degradation of ethoprop, especially to $CO_2$, was noted in the Tama soils (I and II) compared with the Canisteo soils (III and IV). The persistence of ethoprop was not reduced in the fonofos history soil (V), and it was the greatest in the mixed-insecticide history soil (VI). Significant amounts (>0.5%) of extractable ethoprop metabolites were not detected.

Neither *terbufos* nor *phorate* was very persistent in any of the six soils, and both were nearly completely metabolized after 4 weeks to their primary and secondary oxidative products, sulfoxides and sulfones. In all soils, greater quantities of terbufos sulfoxide were present than terbufos sulfone, whereas phorate sulfone was the major phorate metabolite detected. The persistence of these oxidative metabolites was as great or greater in the isofenphos history soils (II and IV) than in the companion soils (I and III). Differences also were not evident in the fonofos soil (V) nor in the mixed-insecticide history soil (VI). Greater quantities of terbufos sulfone, in relation to quantities of terbufos sulfoxide, were present in the Tama soils (I and II) than in the Canisteo soils (III and IV).

Insecticide degradation results for the isofenphos-pretreated soil (soil I: 10 ppm × four pretreatments) are not shown because, with the exception of isofenphos, results were not significantly different from those obtained in soil I with no pretreatments.

**Comparative Metabolism of Organophosphorus Insecticides by an Isofenphos-Degrading Microorganism.** Several bacterial isolates from isofenphos history soils (II and IV) were capable of degrading [$^{14}$C]isofenphos to $^{14}CO_2$. One bacterium isolated from soil II was identified as a *Pseudomonas sp.* and was capable of utilizing isofenphos as a sole carbon source (Racke and Coats, 1987). However, this isofenphos-degrading trait was unstable and was lost after successive culturings on a rich medium, perhaps indicating plasmid involvement. In contrast, an *Arthrobacter sp.* isolated from soils II and IV exhibited a very stable isofenphos-degrading trait, and this isolate proved to be the most efficient isofenphos degrader. A pure culture of this bacterium completely degraded 10, 50, or 100 ppm of [$^{14}$C]isofenphos in less than 6 h, with the production of nearly equal quantities of $^{14}CO_2$ and [$^{14}$C]-polar, water-soluble products. Although preliminary evidence suggests that this *Arthrobacter* may be able to utilize isofenphos as a sole carbon source, growth on isofenphos in pure culture was not conclusively quantified.

**Table III. Effect of Insecticide History[a] on the Fate and Degradation of [$^{14}$C]Insecticides in Soil, during a 4-Week Incubation[b] (Results Expressed as $^{14}$C Recovered in Percent of Applied [$^{14}$C]Insecticide)**

| fraction | none (I) | isofenphos (II) | none (III) | isofenphos (IV) | fonofos (V) | mixed (VI) |
|---|---|---|---|---|---|---|
| | | | Isofenphos | | | |
| isofenphos | 62.8[d] | 12.9[e] | 75.2[f] | 24.9[g] | 76.3[f] | 74.9[f] |
| isofenphos oxon | 15.2[d] | 2.8[e] | 8.1[f] | 4.1[g] | 12.1[h] | 11.6[h] |
| soil bound | 9.2[d] | 23.6[e] | 7.9[f] | 25.8[g] | 8.0[f] | 7.3[f] |
| $^{14}$CO$_2$ | 10.0[d] | 52.4[e] | 5.2[f] | 34.3[g] | 4.7[f] | 4.5[f] |
| other[c] | 0.9[d] | 0.8[d] | 0.8[d] | 0.7[d] | 1.4[d] | 0.7[d] |
| total | 98.2[d] | 92.8[e] | 97.1[d] | 89.6[e] | 102.4[f] | 99.1[df] |
| | | | Chlorpyrifos | | | |
| chlorpyrifos | 23.6[d] | 50.3[e] | 11.9[f] | 14.1[f] | 33.9[g] | 55.8[h] |
| 3,5,6-trichloropyridinol | 16.3[d] | 5.6[e] | 34.4[f] | 36.0[f] | 28.7[g] | 11.8[h] |
| chlorpyrifos oxon | 1.9[d] | 0.6[e] | 0.9[e] | 1.0[e] | 1.8[d] | 0.6[e] |
| soil bound | 20.8[d] | 21.5[d] | 39.1[e] | 35.2[f] | 18.6[g] | 15.4[h] |
| $^{14}$CO$_2$ | 25.0[d] | 16.4[e] | 6.5[f] | 7.6[f] | 9.1[g] | 10.9[h] |
| other[c] | 3.6[d] | 2.2[e] | 2.5[e] | 3.7[d] | 2.1[e] | 1.0[f] |
| total | 91.1[d] | 96.6[d] | 95.2[d] | 97.4[d] | 94.1[d] | 95.5[d] |
| | | | Fonofos | | | |
| fonofos | 34.5[d] | 35.3[d] | 51.2[e] | 55.2[e] | 7.8[f] | 60.4[g] |
| methyl phenyl sulfone | 1.1[d] | 1.4[d] | 6.2[e] | 5.3[f] | 0.2[g] | 0.4[h] |
| fonfos oxon | 0.4[de] | 0.7[d] | 0.5[de] | 0.2[e] | 0.4[de] | 0.6[de] |
| soil bound | 28.9[d] | 37.3[e] | 27.7[f] | 21.3[g] | 33.1[e] | 20.8[g] |
| $^{14}$CO$_2$ | 29.6[d] | 25.6[e] | 15.5[f] | 12.6[g] | 53.7[h] | 15.1[f] |
| other[c] | 1.4[d] | 0.9[ef] | 0.9[ef] | 1.0[f] | 2.1[g] | 0.7[e] |
| total | 96.0[d] | 101.2[e] | 101.8[d] | 95.6[d] | 97.3[d] | 97.9[d] |
| | | | Ethoprop | | | |
| ethoprop | 6.9[d] | 14.6[e] | 24.4[f] | 25.3[f] | 24.1[f] | 56.8[g] |
| soil bound | 31.7[de] | 29.9[e] | 33.7[d] | 31.8[de] | 25.3[f] | 19.2[g] |
| $^{14}$CO$_2$ | 50.9[d] | 50.6[d] | 39.4[e] | 38.6[e] | 45.7[f] | 23.4[g] |
| other[c] | 0.2[d] | 0.2[de] | 0.1[de] | 0.0[e] | 0.1[de] | 0.1[de] |
| total | 89.8[d] | 95.3[e] | 97.7[e] | 95.7[e] | 95.3[e] | 99.5[e] |
| | | | Terbufos | | | |
| terbufos | 1.4[de] | 1.0[de] | 2.4[ef] | 3.5[f] | 0.3[d] | 3.8[f] |
| terbufos sulfoxide | 31.3[d] | 37.0[e] | 53.4[f] | 52.3[f] | 42.6[g] | 59.1[h] |
| terbufos sulfone | 22.9[d] | 20.6[d] | 11.7[e] | 9.5[e] | 17.6[f] | 11.6[e] |
| soil bound | 17.6[d] | 22.9[e] | 17.0[f] | 16.3[f] | 15.1[g] | 11.2[h] |
| $^{14}$CO$_2$ | 18.5[d] | 16.3[e] | 13.4[f] | 13.1[f] | 14.5[g] | 12.2[h] |
| other[c] | 9.2[d] | 6.6[e] | 4.0[f] | 5.9[e] | 8.1[d] | 4.9[ef] |
| total | 100.8[df] | 104.4[e] | 101.8[ef] | 100.6[df] | 98.2[d] | 102.8[ef] |
| | | | Phorate | | | |
| phorate | 0.4[d] | 0.4[d] | 0.2[d] | 1.1[e] | 0.5[d] | 0.2[d] |
| phorate sulfoxide | 3.8[d] | 2.7[e] | 8.2[f] | 8.9[f] | 8.1[f] | 18.2[g] |
| phorate sulfone | 35.0[d] | 46.0[e] | 46.8[e] | 49.5[f] | 49.6[f] | 57.7[g] |
| soil bound | 22.7[de] | 23.6[e] | 20.1[f] | 19.5[f] | 21.6[d] | 13.8[g] |
| $^{14}$CO$_2$ | 19.3[d] | 26.9[e] | 12.0[f] | 11.3[g] | 16.4[h] | 10.8[g] |
| other[c] | 18.3[d] | 1.0[e] | 13.6[f] | 11.8[g] | 9.4[h] | 2.8[i] |
| total | 99.5[d] | 100.5[d] | 100.8[d] | 102.2[de] | 105.7[f] | 103.5[d] |

[a] Soil insecticide history includes last 2 years for II and IV, last 10 years for I and III, and last 5 years for V. Soil VI had carbofuran last year and mixed carbamate and organophosphorus insecticide exposure previously. [b] [$^{14}$C]Insecticides were each uniformly applied to duplicate 100-g portions of soil at 5 ppm (0.5 $\mu$Ci for isofenphos, 1.0 $\mu$Ci for other insecticides). [c] Includes volatile products other than CO$_2$ as well as polar, water-soluble products. [d-i] Means followed by the same letter in each horizontal row are not significantly different at the 5% level (Student–Newman–Keuls Test).

The strain exhibited typical *Arthrobacter* characteristics, including snapping division and a rod to coccus, gram-negative to gram-positive life cycle (Figure 1). This isolate utilized a variety of simple organic substrates as sole carbon sources (e.g., benzoate, salicylate, phenyl acetate), tolerated up to 4.5% NaCl, and was highly aerobic and nonmotile, sensitive to penicillin, carbenicillin, and bacitracin, and insensitive to colistin.

A pure culture of the isofenphos-degrading *Arthrobacter* sp was tested for the ability to metabolize or cometabolize chlorpyrifos, fonofos, ethoprop, terbufos, or phorate. After a 12-h exposure to each of these insecticides individually, or in combination with isofenphos, no evidence of metabolism or cometabolism of any of the insecticides other than isofenphos was noted (Figure 2). In the case of isofenphos, 45% of the applied [$^{14}$C]isofenphos was re-

covered as polar, water-soluble products after 12 h. When this incubation was repeated in a flow-through system (Racke and Coats, 1987), 50% of the applied [$^{14}$C]isofenphos was mineralized to $^{14}$CO$_2$.

## DISCUSSION

Each of the organophosphorus insecticides exhibited different persistence and a different product distribution in soil. On average, the persistence of parent insecticide after 4 weeks, in order of decreasing persistence, was isofenphos > fonofos > chlorpyrifos > ethoprop ≫ terbufos > phorate. This is consistent with earlier reports of the persistent nature of isofenphos (Chapman and Harris, 1982; Felsot, 1984) and of the relatively greater persistence of fonofos than of both chlorpyrifos (Miles et al., 1979) and phorate (Ahmad et al., 1979; Fuhremann and Lichtenstein,
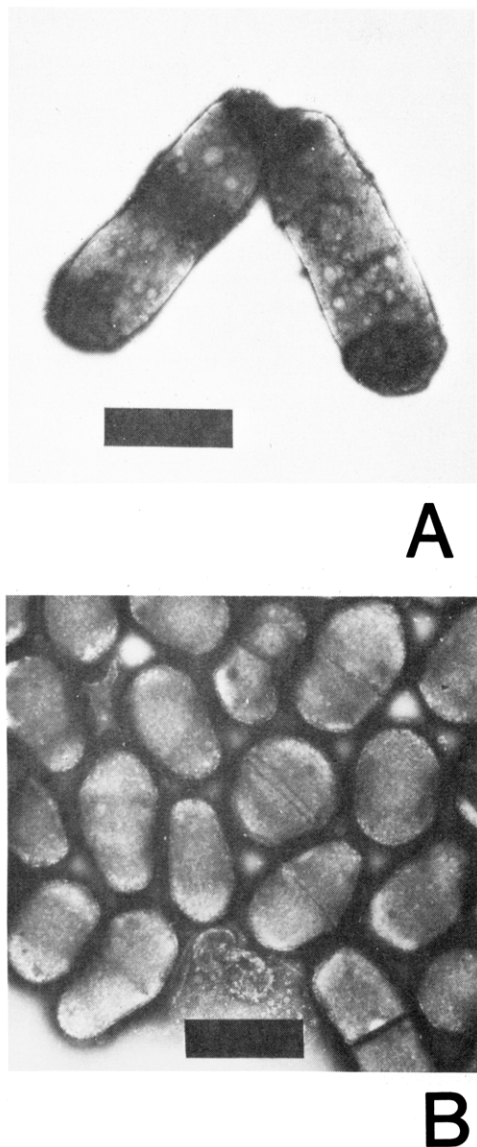
**Figure 1.** Isofenphos-degrading *Arthrobacter sp.* isolated from isofenphos history soils: A, 12-h-old culture; B, 72-h-old culture. Negatively stained with phosphotungstate, 1 bar = 1 $\mu$M.
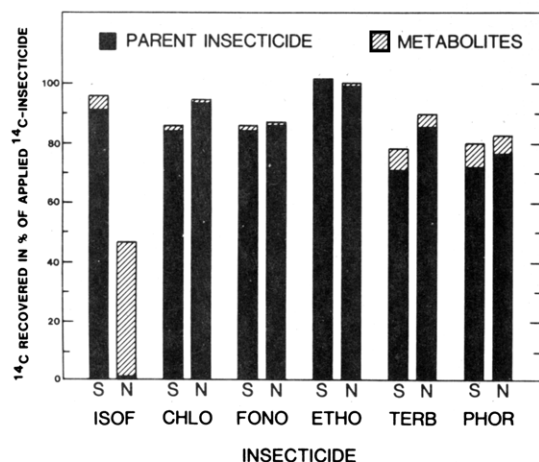


**Figure 2.** Metabolism of organophosphorus insecticides in a pure culture of *Arthrobacter sp.* during a 12-h incubation: S = sterile; N = nonsterile. Results are means of triplicate tests.

1980). The ephemeral nature of terbufos and phorate in soil has been well documented (Getzin and Chapman, 1960; Suett, 1975; Sellers, et al., 1976; Chapman et al., 1982a,b), and it is terbufos sulfoxide and phorate sulfone that are

most persistent in soil and may contribute to target pest toxicity (Laveglia and Dahm, 1975; Chapman and Harris, 1980b; Harris and Chapman, 1980).

The importance of soil properties in modulating the degradation of insecticides has long been recognized (Edwards, 1966). In the current study, all the organophosphorus insecticides were most persistent in the Readlyn soil, which had the least organic matter content, greatest sand content, and a near-neutral pH. Isofenphos, fonofos, ethoprop, terbufos, and phorate were all more persistent in the higher organic matter Canisteo soils than in the Tama soils. Increased organic matter in soil is often associated with increased adsorption of pesticides (Stevenson, 1972), which can render pesticides less susceptible to microbial degradation (Ogram et al., 1985). In the present study, only chlorpyrifos was less persistent in the Canisteo soils than in the Tama soils, being represented by greater quantities of 3,5,6-trichloropyridinol in the former soils. Chemical degradation is an important factor modulating chlorpyrifos persistence in soil (Miles et al., 1979), and increased production of its hydrolysis product in soil has been correlated with increased soil organic matter content (Chapman and Harris, 1980a; Getzin, 1981).

Clearly, the degradation of isofenphos was profoundly affected by its prior application to soil. Decreased persistence of isofenphos in the field after several years of use has been documented (Chapman et al., 1986; Abou-Assaf et al., 1986). Racke and Coats (1987) subsequently demonstrated that isofenphos had undergone enhanced degradation by inducible populations of soil microorganisms.

The involvement of an *Arthrobacter* in the enhanced degradation of isofenphos is not surprising inasmuch as *Arthrobacter spp.* are metabolically diverse degraders in soil (Hagedorn and Holt, 1975) and have been implicated in the enhanced degradation of both 2,4-D and EPTC (Tiedje et al., 1969; Tam et al., 1987). Several soil bacteria have been isolated that could rapidly metabolize isofenphos, including a *Corynebacterium sp.* (Murphy and Cohick, 1985), a *Pseudomonas sp.* (Racke and Coats, 1987), and a *Streptomyces sp.* (Gauger et al., 1986). Thus, it is possible that the enhanced degradation of isofenphos in soil may be due to the activities of more than one bacterial strain. Indeed, several genera of bacteria have been implicated in the enhanced degradation of carbofuran (Felsot, et al., 1981; Karns et al., 1986) and diazinon (Siddaramappa et al., 1973; Forrest et al., 1981).

Although fonofos was rapidly degraded in the soil with fonofos history, more research is needed to determine whether it too has undergone enhanced degradation. Unlike the rapid degradation of isofenphos and carbofuran, preliminary results in several soils indicate that the ability of a soil to rapidly degrade fonofos does not persist through the winter. It may be important to distinguish between temporary soil adaptation to rapidly degrade a pesticide and the persistence of that adaptive trait to affect a subsequent field application of the chemical.

None of the other organophosphorus insecticides were rapidly degraded in soil in which enhanced degradation of isofenphos was evident. Likewise, only fonofos was rapidly degraded in the fonofos history soil. It was in the soil with mixed-insecticide history that the organophosphorus insecticides were most persistent, although several carbamates had undergone enhanced degradation in this soil (Racke and Coats, 1988).

In several instances, the enhanced degradation of a specific pesticide in a given soil has evidently involved a rather broad adaptation, with other members of the same pesticide class also subject to rapid degradation. This has

been true to some extent with the carbamate insecticides (Harris et al., 1984; Felsot, 1986), thiocarbamate herbicides (Wilson, 1984; Harvey et al., 1986), and phenoxyacetic acid herbicides (Kirkland and Fryer, 1972). However, the enhanced degradation of organophosphorus insecticides in soil has usually been associated with a lesser degree of cross adaptation. Thus, the enhanced degradation of diazinon has displayed some degree of specificity within the organophosphorus class (Sethunathan and Pathak, 1972; Forrest et al., 1981). Likewise, the enhanced degradation of chlorfenvinphos is specific, and the degradation of other organophosphorus insecticides is not accelerated in soil capable of rapidly degrading chlorfenvinphos (Read, 1987). Horng and Kaufman (1987) did find some evidence that cross adaptations may be associated with the enhanced degradation of some organophosphorus insecticides in soil. Cross adaptations involving members of different classes of pesticides have not generally been noted (Forrest et al., 1981; Harris et al., 1984).

The isofenphos-degrading *Arthrobacter sp.* isolated in the current investigation was able to rapidly metabolize this compound yet did not metabolize or cometabolize the other organophosphorus insecticides tested. Because hydrolysis has been shown to be an important step in the enhanced microbial degradation of isofenphos (Racke and Coats, 1987), it is possible that the enzymes involved in isofenphos hydrolysis may be somewhat specific. Of the six organophosphorus insecticides studied, isofenphos is unique in possessing a phosphoramide bond (Table I), and this may confer properties requiring the action of a different type of hydrolytic enzyme. Although the hydrolase isolated from diazinon-metabolizing *Flavobacterium* and *Pseudomonas* strains is able to hydrolyze some of the other organophosphorus insecticides (Kearney et al., 1986), only the hydrolysis product of diazinon is utilized as a carbon source by these bacteria. Consequently, only diazinon is rapidly degraded by these bacteria in soil (Sethunathan and Pathak, 1972; Forrest et al., 1981). It is possible that the metabolic specificity exhibited by organophosphorus-degrading bacteria may relate to their ability to beneficially metabolize the hydrolysis products formed.

In conclusion, results indicate that the enhanced degradative phenomenon may exhibit a high degree of specificity in soil and at the microbial level. Further research is needed to shed light on the importance of this phenomenon in soil and provide a basic understanding of the microbial ecology and genetics of its occurrence.

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