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**Effect of Agrochemicals and
Agricultural Practices on the Performance
and Fate of Selected Systemic and Non Systemic
Soil Pesticides**

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Title of Research Project: **EFFECT OF AGROCHEMICALS AND
AGRICULTURAL PRACTICES ON THE PERFORMANCE AND FATE
OF SELECTED SYSTEMIC AND NON SYSTEMIC SOIL PESTICIDES.**

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ABSTRACT

This study was intended to explore the effect of soil disinfestation, pesticides, fertilizers and other agricultural practices on the rate of degradation and fate of soil applied pesticides. Soil fumigation with methyl bromide or soil solarization significantly slowed down the rate of degradation of terbutryn, carbendazim (MBC) and other pesticides. The involvement and importance of soil microorganisms in the degradation was demonstrated. Results obtained by chemical analysis of treated soils incubated in biometer flasks were confirmed by bioassay in the greenhouse. Two fungi: Alternaria alternata and Bipolaris tetramera were capable to degrade carbendazim, thiabendazole and fuberidazol at same rate when grown on PDA media. The fungicides TMID and fentin acetate were effective in suppressing Alternaria. At 20 ppm in the soil they slowed down the degradation of terbutryn and carbendazim to similar extent as that found by disinfestation. The combined application of TMID and benomyl, as tested in the greenhouse and in the field, prolonged its persistence and its fungicidal activity. A simple and rapid bioassay for the determination of MBC in soil, without need for soil extraction, was developed. The effect of fertilizers, manure, captafol, and atrazine and the effect of soil application and soil incorporation (tillage) on the metabolic fate and translocation of [^{14}C] fonofos and [^{14}C] parathion from soils into oats and corn were studied within open and closed agricultural microcosms. After a 2-week plant growing period in open systems, more radiocarbon was recovered from fonofos-treated soils containing also cow manure, sewage sludge, or atrazine and less from oats or corn grown in these soils. The presence of these amendments in fonofos-treated soil also resulted in an increase of unextractable, bound ^{14}C residues, while ammonium sulfate and captafol had no effect. Soil amendments indirectly inhibited the production of $^{14}\text{CO}_2$ by directly affecting soil microorganisms, primarily soil fungi, responsible for the degradation of the insecticides. The effects of cow manure and sewage sludge on the fate of ^{14}C carbofuran applied to soil were also investigated in a run-off microcosm. Both manure and sludge reduced the uptake of ^{14}C carbofuran and its metabolites from soils into oat plant tissue. Movement of ^{14}C carbofuran-derived ^{14}C also occurred in the soil, because 22 to 25% of radiocarbon recovered from the soil was associated with the lower originally untreated soil layer. Run-off water and aquaria water were not toxic to Aedes aegypti L. mosquito larvae. Carbofuran was the major compound detected in terrestrial soils, whereas 3-hydroxycarbofuran was the major compound detected in leaves. Apparently, manure microorganisms were responsible for the enhanced ^{14}C carbofuran degradation in soil. In conclusion, this project emphasized the importance of the interactions, under laboratory and field conditions, between the pesticides and their surrounding environment in determining their bioactivity and fate.

Objectives of the research.

The objectives of this research proposal could be summerized as follow: (a) To study the nature of the interactions between agrochemicals and the persistence, metabolism and performance of soil applied pesticides. (b) To study in the laboratory and in the field the effect of agrochemicals on the bioactivity of soil microorganisms, especially as related to delayed or enhanced degradation, and to define specific mechanisms involved in these processes. (c) To study the relation between changes in microbial activity and the performance of these pesticides. (d) To use the information from the above studies for improving performance of soil applied pesticides.

3. DESCRIPTION OF THE RESEARCH.

I. Work carried out in Israel.

(a) EFFECT OF METHYL BROMIDE OR SOLAR HEATING TREATMENTS ON THE PERSISTENCE OF PESTICIDES IN THE SOIL

Abstract - The effect of soil disinfection treatments was investigated with respect to changes in the rate of degradation and fate of several pesticides in the soil. In one series of experiments the pesticides were added to sandy-loam soils collected from commercial fields which had been treated with methyl bromide or solar heated for the control of soilborne pathogens and weeds. Solar heating was accomplished by covering the wet soils with transparent polyethylene sheets during the hot season. The soils with the pesticides were adjusted to about 80% field capacity and kept at 27°C for 90 days. the rate of degradation of the fungicide carbendazim (MBC) and of some triazine herbicides was much slower in the disinfested soils. The degradation of the carbamate insecticide ethiofencarb (Croneton) was somewhat slower in the disinfested soils. The metabolite ethiofencarb sulfoxide accumulated to a larger extent in the solar-heated soil. In another series of experiments the pesticides were applied to the soil prior to the solar heating treatment and were thus exposed to the heating process. This treatment accelerated the rate of degradation of some pesticides. The fungicide thiabendazole (TBZ) disappeared faster in the solar heated soil.

Introduction

Microorganisms play an important role in the degradation and detoxification of pesticides in soil. Much work has been reported on the fate of soil applied pesticides (1-5). However, much less is known about interactions between agricultural chemicals and soil microflora and their effects on pesticide degradation under practical field conditions.

Processes that interfere with the activity of soil microorganisms might modify the performance and affect the fate of pesticides in the soil. Systemic insecticides have been shown to increase plant uptake of various herbicides (6) and herbicides were found to affect the persistence and metabolism of systemic insecticides (7). Other recent works have demonstrated the interaction of selected fungicides, herbicides and N-fertilizers with soil microorganisms and its effects on the degradation of parathion (8) and linuron (9). It has also been shown that the degradation of pesticides that are metabolized by dealkylation reactions was inhibited by sewage sludge and enhanced by manure (10).

Disinfection of soil for pest control is carried out by drastic means e.g. fumigation with methyl bromide. Recently a new method of solar heating of the soil, by means of polyethylene mulching was developed (11).

In this work two soil disinfection treatments were selected in order to demonstrate that some of the interactions which occur in the field may affect the performance as well as the persistence of soil-applied pesticides. It was assumed that any treatment that strongly affects soil microbial activity and the soil environment may interfere with the degradation of pesticides in the soil.

Materials and Methods

Soil treatment. the soil used in all the experiments was a sandy loam (hamra, pH 7.8, organic matter 0.9%, clay 8%, field capacity 11%).

Methyl bromide treatment. This fumigant was applied to the preirrigated soil, using a standard procedure, at a rate of 50 g/m² under a 0.1 mm thick polyethylene cover. After 48 h the polyethylene sheets were removed and the soil was left for aeration for additional 14 days.

Solar heating treatment. The soil was irrigated to a depth of 60-80 cm. Four days after irrigation, plots (2x15 m) were mulched with transparent polyethylene sheets (0.03-0.05mm thick) as described elsewhere (11). Mulching lasted for 5-9 weeks during July and August, after which the polyethylene sheets were removed.

Soil sampling. Samples were taken from the upper soil layer (0-10 cm) at five locations in each plot, these subsamples were mixed into one large composite sample. Control samples were taken from bare, unmulched, but similarly irrigated plots.

Pesticide application and soil incubation. For the first series of experiments, the pesticides carbendazim MBC - (2-benzimidazole carbamic acid methyl ester) and ethiofencarb - 2- (ethylthio) phenyl N-methylcarbamate were added to the soil in the laboratory. The pesticides, in acetone, were added dropwise to 50 g of air-dried soil. After solvent evaporation, the soil was mixed with 100 g of the same soil to provide a sample of 150 g. Each sample was placed in an incubating flask. The soil was watered to 10% moisture, the flask covered with thin polyethylene, and incubated in the dark at 27°C. A side arm of the flask, with water, maintained the moisture level of the soil. At different dates a subsample of 20 g was taken from the flask for chemical analysis. Each experiment was carried out in four replicates.

For the second series of experiments 50 g soil samples, containing the pesticide, were wrapped in a piece of nylon net and placed in the field at a depth of 10 or 30 cm prior to the solar-heating treatment. At different dates samples were removed from the soil, extracted and chemically analyzed for its pesticide residue content.

Soil extraction and analysis. The soil samples were stored at -15°C prior to the chemical analysis. Twenty grams of soil were extracted with the appropriate solvent; MBC and TBZ with aqueous NaOH and ethyl acetate, ethiofencarb with water + acetone and chloroform; and the herbicides with water + acetone and petroleum ether 40-60°. MBC and TBZ were cleaned up as described previously (12) and determined by high pressure liquid chromatography (HPLC, Tracor 985)

equipped with a Perkin Elmer 204 fluorescence spectrophotometer as the detector. The two benzimidazole derivatives were separated on a C 18 reversed phase column with a solvent of MeOH:H₂O 85:15 to which a few drops of conc. ammonia per liter solvent were added.

Ethiofencarb was analyzed by gas liquid chromatography (Tracor MT-220) equipped with a flame photometric detector (FPD) as previously described (10). The herbicides were determined by a Varian 3700 gas chromatograph equipped with a thermionic specific detector (TSD). Glass columns (180 cm x 0.2 cm i.d) were packed for the herbicides with 3% OV-17 on 80/100 mesh Gas Chrom. Q, and for the separation of ethiofencarb and its metabolites with 2.5% DC-200 and 3.5% QF-1 w/w) on 80/100 mesh Gas Chrom. Q.

Results and Discussion

Effect of soil disinfestation treatments on the degradation of carbendazim (MBC) and ethiofencarb. In the first series of experiments the degradation of pesticides was followed in soils that were previously treated with methyl bromide or were solar heated. The systemic fungicide MBC degraded much more slowly in the treated soils (Fig. 1). Three weeks after the incorporation of MBC into the soil, 20% of that applied was found in the non-treated soil, whereas in the methyl-bromide treated or solar-heated soils, more than 80% was found. The effect of methyl bromide and of solar heating on MBC degradation was very similar. After eight weeks of incubation the amount of MBC detected in the treated soil was still about 55% of that applied, which was much more than that of the non-treated soil.

Fig. 1. The effect of methyl bromide and solar heating treatments on the degradation of carbendazim (MBC). The fungicide was added (2.5 ug/g) to soil that was pretreated in the field with: methyl bromide, Δ - Δ ; solar heating, $\square$ - $\square$; and untreated control, $\circ$ - $\circ$.

Table 1. Degradation of the systemic insecticide ethiofencarb, added to a previously solar heated soil

DAYS AFTER TREATMENT	RESIDUES RECOVERED FROM							
	NONTREATED SOIL (ug/g)				SOLAR HEATED SOIL (ug/g)			
	Ethiofen- carb	Ethiofen- carb SO	Ethiofen- carb SO ₂	Total	Ethiofen- carb	Ethiofen- carb SO	Ethiofen- carb SO ₂	Total
0	7.10	-	-	7.10	7.00	-	-	7.00
11	0.30	1.70	1.40	3.40	0.30	3.20	1.30	4.80
25	-	0.10	0.30	0.40	-	0.50	0.40	0.90
45	-	0.07	0.03	0.10	-	0.30	0.06	0.36

The effect of soil disinfestation treatments on the degradation of the systemic carbamate ethiofencarb was not so profound as the effect on MBC. The results for ethiofencarb are presented in Table 1. The degradation of ethiofencarb in the solar heated soil was somewhat slower than in the non-treated soil. Moreover, it could be seen that the relative concentration of the biologically active metabolite - ethiofencarb sulfoxide - was higher in the treated soil.

Effect of solar heating on disappearance of pesticides present in the soil during the heating process. In the second series of experiments the pesticides were first incorporated into the soil and then exposed to the solar-heating treatment. Mulching of the wet soil with transparent polyethylene sheets during the hot season, increased the soil temperature to about 45-55°C in the upper layer of the soil (11).

Table 2 summarizes the results obtained for two benzimidazole fungicides and two herbicides. Solar heating in the early stages tends to accelerate the rate of disappearance of the systemic fungicides TBZ as demonstrated by chemical analysis. The faster disappearance of the pesticides in the heated wet soil could be the result of increased volatilization, thermal breakdown, or due to changes in microbial activity occurring during the solar heating process. The results of this investigation indicate that soil disinfestation treatments can alter the rate of degradation and persistence of pesticides in the soil. Certain pesticides are more persistent when applied to previously disinfested soil. The possible consequences are both pesticidal and environmental. This should be reflected in practice by lowering the dosage. Solar heating which accelerate the disappearance of certain pesticides from the soil may serve also as a decontamination tool. Both phenomena, of enhanced and reduced activity of pesticides, should be taken into consideration in establishing pest management practices.

Table 2. The effect of solar heating of the soil on the degradation of pesticides added to the soil at two depths prior to the solar heating.

PESTICIDE	DAYS AFTER ADDITION	RESIDUES RECOVERED FROM (ug/g)			
		NONTREATED SOIL		SOLAR HEATED SOIL	
		10 cm	30 cm	10 cm	30 cm
Thiabendazole	0	2.00	2.00	2.00	2.00
	30	1.50	1.70	0.60	1.00
Carbendazim	0	2.00	2.00	2.00	2.00
	14	1.10	0.90	1.00	0.90
	30	0.60	0.50	0.60	0.50
Atrazine	0	1.00	1.00	1.00	1.00
	30	0.40	0.40	0.30	0.30
	60	0.09	0.10	0.04	0.04
Trifluralin	0	2.50	2.50	2.50	2.50
	60	1.60	1.70	1.40	1.40

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(b) PERSISTENCE OF TERBUTRYN AND ATRAZINE IN SOIL AS AFFECTED BY SOIL DISINFESTATION AND FUNGICIDES.

Abstract.

The effect of soil disinfestation with methyl bromide (MB), or by soil solarization (solar heating) and the fungicides TMTD (tetramethylthiuram disulfide) and fentin acetate (triphenyltin acetate) on the degradation of terbutryn [2-(tert-butylamino)-4-(ethylamino)-6-(methylthio)-s-triazine], and atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] were investigated. The degradation of terbutryn appeared to follow first order kinetics with a "half-life" of about 2 weeks, and was much slower in MB-treated or solarized soils i.e. "half-life" of about 11 weeks. Suppression of terbutryn degradation in the MB-treated soil was still evident 8 months after soil fumigation, similar to that found in autoclaved soil. TMTD and fentin acetate at 20 ug/g soil, strongly inhibited degradation of terbutryn in soil. Degradation of atrazine was affected to a lesser extent by soil disinfestation. Results from the present study suggest that biocidal soil treatments may slow herbicide degradation. Thus, herbicide dosages in disinfested soil should be adjusted in order to avoid phytotoxicity. Moreover, lower dosages might be sufficient to attain weed control and combined disinfestation or fungicides with herbicide treatments might be intentionally used to extend herbicide activity.

Introduction

Performance of soil applied pesticides depends on maintaining a biologically effective concentration within the root zone or near the target organism for the desired period. Processes that cause rapid breakdown of the pesticide in the soil are important for nullifying residual toxic effects to the crop plant or to other non-target organisms. However, for many non-persistent, short-lived soil pesticides, a too rapid degradation may lower their effectiveness.

Many laboratory and field studies have shown that the degradation rate of certain pesticides has been affected by soil microorganisms. Soil disinfestation i.e. a drastic soil treatment which is regarded to have a broad spectrum biocidal effect, is carried out either by chemical means such as fumigation with MB, soil treatment with vapam or by physical means such as solarization, i.e. solar heating of the soil by covering (tarping) the soil with transparent polyethylene. The latter technique was recently introduced for practical uses for controlling soilborne diseases and weeds in Israel and USA (6). Soil disinfestation (6,12) as well as certain agrochemicals (4,5,11) may temporarily reduce or cause a shift in microbial activity and thus possibly prolong the persistence of certain pesticides (1,14,15). This work describes the effect of soil disinfestation and fungicides on the degradation of terbutryn and to a lesser extent, of atrazine in soil.

Materials and methods

Soil. Two soils were used; a sandy soil from Rehovot (6% clay, 1% organic matter 11% field capacity, and pH 7.5) and a sandy loam soil from Tira (15% clay, 0.7% organic matter, 14% field capacity and pH 7.2).

Pesticides. Terbutryn and atrazine (analytical grade 98-99%) were obtained from Agan Chemicals, Israel. TMTD and fentin acetate (Brestan) commercial formulations, were obtained from UCB Ltd., Belgium, and Makhteshim Ltd., Israel, respectively.

Soil treatment and sampling. Methyl bromide was applied to preirrigated soil, at 50 g/m² under a 0.1 mm thick polyethylene cover (12). After 48 h the polyethylene sheets were removed and the soil was left for aeration for 14 days. Solarization of the soil was as previously described (7). The soil was irrigated to a depth of 60-80 cm. One to two days later, plots (2x15 m) were covered with transparent polyethylene sheets (0.04 mm thick). Soil solarization lasted for 40 days during July and August, after which the polyethylene sheets were removed. Soil temperatures at depths of 10 and 30 cm were 38 and 31C, respectively in the nontreated soil. The corresponding figures for the solarized soils were 46 and 39C. Soil was sterilized by autoclaving (121C) for 1 hr on 2 successive days.

Samples were taken from the upper layer of the soil (2-10 cm) at five locations for each plot. These subsamples were combined and mixed into one large composite sample. Control samples were taken from nontreated but similarly irrigated plots. Soil samples were stored in polyethylene bags at 5C in the dark until used, up to 4 months after collection. A preliminary experiment showed that four months of cold storage did not affect the degradation of terbutryn in either treated or untreated soil.

Laboratory experiments. Acetone solutions of each herbicide were added dropwise to 40 g of air-dried soil. After solvent evaporation the soil was left uncovered in a ventilated hood for 24 h to remove traces of the solvent and then mixed with 110 g of the same soil to provide a sample of 150 g. The soil samples were placed in biometer flasks (3) and moistened to 80% of field capacity. The flasks were sealed with thin polyethylene and incubated in the dark at 26C. Twenty ml aliquots of water were put in the side arm of the flask to maintain a constant moisture level of the soil. Each experiment was prepared in four replicates. At different dates, subsamples of 20 g each were taken from the flasks and stored at -18 C until analyzed. When fungicides were used, TMTD and fentin acetate were added to the soil at 20 and 80 ug/g one day after terbutryn application and the soil was incubated in biometer flasks as described above. The experiments with terbutryn were repeated three times, at various periods, with similar results.

Herbicide extraction from soil and chemical analysis. Twenty g of soil were extracted in a 500 ml Erlenmeyer flask with a 150 ml mixture of water:acetone (1:1,v/v). The soil was separated and reextracted with 100 ml petroleum ether. The two extracts were combined in a separatory funnel. The lower aqueous phase

was reextracted twice with a mixture of 100 ml of petroleum ether + 30 ml acetone. The three upper organic extracts were combined, dried over anhydrous Na_2SO_4 and concentrated to 5 ml on a rotary evaporator. Terbutryn and atrazine levels in the concentrated extracts were determined using a Varian 3700 gas chromatograph equipped with a thermionic specific detector and a glass column (180 cm x 0.2 cm i.d.) packed with 3% OV-17 on 80/100 mesh Gas Chrom Q.

Field experiment and bioassay. Terbutryn was applied in the field to nontreated sandy soil or to previously MB-treated or solarized soils. The herbicide was applied to 2 x 4 m plots in a randomized block design with four replicates. Terbutryn (2 kg/ha as 50% WP formulation) was sprayed in a volume of 250 l/ha and mechanically incorporated by rototiller in the top 10 cm layer. The plots were irrigated (30 mm) immediately after treatment. Thirty-one days after treatment with terbutryn, four soil samples per replicate were taken from a depth of 2 to 10 cm. The samples from each replicate were combined, mixed and transferred to four pots (6 x 7 x 5 cm) in a greenhouse at 24±4°C. Ten seeds of foxtail millet [*Setaria italica* (L.) Beauv. #³ SETIT] were planted in each pot. The relative amount of the herbicide in the soil was determined by comparing the shoot fresh weight with that of plants grown in the herbicide-free soil. After cutting the shoots the same soil was bioassayed again by two additional successive plantings of the test plant.

Results

Effect of soil disinfestation on degradation of terbutryn and atrazine. Soil fumigation with MB or solarization significantly lowered the degradation of soil applied terbutryn and atrazine (Figure 1). The "half life" of terbutryn in the nontreated and the disinfested soils was about 15 and 77 days, respectively. After 110 days of incubation more than 97% of the terbutryn added to the soil was degraded, while in the disinfested soil about 25% of the initial amount was still present. Degradation of terbutryn in the nontreated soil appeared to follow first order kinetics, whereas the shape of the degradation curves of the disinfested soils was different. Atrazine was more persistent and was less affected than terbutryn by soil disinfestation (Figure 1).

The inhibitory effect of MB on terbutryn degradation was almost the same as that measured in autoclaved soil (Table 1), thus denoting the importance of microorganisms in terbutryn degradation. The inhibitory effect of MB was studied in two soils and at two concentrations; in all cases the trend was similar to that described in Figure 1. However, the degradation of terbutryn was somewhat slower when the initial concentration of the herbicide was higher.

Duration of the disinfestation inhibitory effect. Soil samples that were taken from the field at various periods after MB application, were treated with terbutryn in the laboratory and analyzed after various periods of incubation. Results show that the inhibitory effect on the degradation of terbutryn after 136 days was as strong as that found 14 days after soil fumigation (Figure 2).

Fig. 1. Degradation of terbutryn and atrazine in a sandy soil pretreated in the field with: methyl bromide ($\square$), solarization (Δ), or untreated control ($\circ$). Data prints with a common letter, at each incubation period, are not significantly different by Duncan's multiple range test ($p=0.05$).

Table 1. Effect of herbicide concentration, soil type, and pretreatment with methyl bromide (MB) or sterilization on terbutryn degradation in two soils.

Incubation (Days)	Terbutryn applied						
	5ug/g		5 ug/g			22 ug/g	
	Sandy loam soil (Tira) ^a		Sandy soil (Rehovot) ^a			Sandy soil (Rehovot) ^a	
	Untreated	MB	Untreated	MB	Autoclaved	Untreated	MB
	% Terbutryn						
0	100	100	100	100	100	100	100
15	71 b ^b	85 a	-	-	-	-	-
35	-	-	26 b	72 a	76 a	46 b	77 a
60	18 b	56 a	-	-	-	-	-
80	16 b	33 a	5 b	50 a	56 a	31 b	64 a

^aSampling location. ^bValues in rows for each soil experiment, followed by a different letter, are significantly different ($=0.05$) using Duncan's multiple range test.

Even 8 months after disinfestation with MB, the degradation of terbutryn was still significantly slower than that in the nontreated soil.

The similarity between the degradation curves obtained in the nontreated soil taken from the field at different dates (Figure 2) indicates that the procedure followed in the present study is reproducible and might be useful for qualitative and quantitative assesment of pesticide interactions under practical field conditions.

Fig.2. The effect of soil fumigation with methyl bromide on the degradation of terbutryn. The herbicide was added to the soil at 14 (□), 136 (△) and 252 (○) days after methyl bromide treatment. Data prints with a common letter, at each incubation period, are not significantly different by Duncan's multiple range test ($p=0.05$). (△,□,○) symbols, methyl bromide treated; (◻,◼,◉) symbols, untreated controls.

Bioassay studies. The results described in the previous section were obtained from soils that were disinfested in the field but the degradation study of the herbicide was carried out in biometer flasks, in the laboratory. In another series of experiments both treatments, disinfestation and terbutryn application, were carried out in the field and the degradation of terbutryn was followed in the greenhouse using foxtail millet seedlings as a test organism for the presence of the herbicide in the soil. The results (Table 2) basically confirmed those obtained from the biometer flask experiments, namely, both solarization as well as MB treatments slowed down terbutryn degradation.

Effect of fungicides. The effect of two fungicides (at 20 ug/g soil) on terbutryn degradation was studied. Results (Figure 3) showed that the inhibitory effect of TMTD and fentin acetate was as pronounced as that of MB treatment. Eighty days after incubation the amount of terbutryn (as a percentage of that applied initially) was 6, 57, 56 and 43 for the nontreated, TMTD, fentin acetate and MB-treated soils, respectively. A similar inhibitory effect was obtained when the fungicides were applied at 80 ug/g soil.

Table 2. The effect of soil disinfestation with methyl bromide (MB) or solarization on the persistence of terbutryn applied in the field. Herbicide activity was determined by a bioassay using foxtail millet seedlings.

Days after terbutryn application	Shoot fresh weight (% of herbicide - free control)					
	MB			Solarization		
	MB	Terbutryn	⁺ Terbutryn	Terbutryn	⁺ Terbutryn	
31	89 ^a	a	81 ab	52 bc	83 ab	68 ab
43	99	a	80 a	57 b	83 a	79 a
56	112	a	97 ab	72 bc	91 abc	75 bc

^aFigures in each row (on each sampling date) with a common letter are not significantly different by Duncan's multiple range test (p=0.05).

Fig.3. The effect of TMTD (O), fentin acetate (Δ), and methyl bromide (□) on terbutryn degradation in sandy soil compared to soil treated only with terbutryn (●). Data prints with a common letter, at each incubation period, are not significantly different by Duncan's multiple range test (p=0.05).

Discussion

The degradation of terbutryn or atrazine in disinfested soils or of terbutryn in fungicide-treated soils was slower from that in the non-treated ones. This might be attributed to the suppressive effect of disinfestation or fungicides on microbial activity (8,9,13). In the present study MB suppressed degradation of terbutryn to about the same extent as sterilization (Table 1). Moreover, the inhibitory effect of MB lasted for several months (Figure 2) which indicates that the recovery of the populations of the microbial degraders in the

MB-treated soil is slow. TMTD, which suppressed terbutryn degradation, also strongly reduces soil fungal populations (10,13) but not the bacterial ones (13). This indicates that fungi may play an important role in terbutryn degradation. TMTD slowed the degradation of carbendazim⁴ [2-(Methoxycarbonylamino)-benzimidazole] which indicates that its suppressive effect is not restricted to terbutryn. Other fungicides or biocides with antimicrobial activity which are routinely used in agriculture may also affect the persistence of terbutryn or other pesticides. For example, the fungicides captafol ['cis-N-((1,1,2,2-tetrachloroethyl)thio)-4-cyclohexene-1,2-dicarb-oximide], maneb [manganese ethylene bis dithiocarbamate] and benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate] inhibited degradation of parathion [o,o-diethyl o-p-nitrophenyl phosphorothioate] in soil (5). Captafol also affected the fate and translocation of fonofos [o-ethyl-s-phenylethyl-phosphonodithioate] in a soil-plant microcosm (11). Soil disinfestation may also affect pesticide degradation in a qualitative way, as was shown with the carbamate, ethiofencarb [2-ethyl-mercaptomethyl-phenyl-N-methylcarbamate] (1).

Degradation of herbicides in solarized soils, which were incorporated into the soil prior to tarping with polyethylene and therefore exposed to heating were affected differently depending on the herbicide (15). Thus, the fate of any pesticide applied to a crop might be also affected by the type and dosage of the other biocide used as well as by other agricultural practices.

The process of degradation of terbutryn in the soil is mainly microbial (Table 1), while the first step of atrazine degradation is mainly chemical (2). Thus, the stronger inhibition of terbutryn degradation as compared to atrazine, indicates that the main effect of soil disinfestation is an inhibition of microbial activity. Our results (Figure 1 and Table 1) suggest that soil disinfestation can be utilized as a tool for determining the role of soil microorganisms in pesticide degradation, under practical field conditions. The close agreement between results obtained when terbutryn was applied to the soil in the laboratory (Figures 1 and 2; Table 1) and those obtained when the herbicide was applied in the field (Table 2), indicates that our systems are reliable and closely resemble the field situation. There was a clear decline in the concentration of terbutryn in the treated and nontreated soils during the first two weeks after application. This may be attributed to physical processes such as adsorption or binding, or to residual enzymatic activity which was not completely destroyed during the disinfestation treatment.

It is not economically feasible to use soil disinfestation only for the purpose of controlling pesticide fate in soils. However, since this practice is followed with certain crops, extended effectiveness of the pesticide can be regarded as an additional benefit. TMTD and fentin acetate were highly effective at relatively low dosages (Figure 3). Therefore, their potential use as "extenders" (4) might be economical under the right circumstances.

Results from this present study suggest that dosages of herbicides should be adjusted if the soil was previously disinfested or if certain fungicides are present, in order to avoid possible phytotoxicity. When necessary, disinfestation or fungicides might also be used as a tool for extending herbicide effectiveness or for reducing herbicide dosage. Thus, recommendations for herbicide dosages should take into consideration the presence of other pesticides or the use of soil treatments which affect the soil biophase.

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(c) A RAPID BIOASSAY FOR THE DETERMINATION OF CARBENDAZIM RESIDUES IN SOIL*

Abstract. A simple and rapid bioassay for the direct determination of carbendazim residues in soil was developed. Pellets composed of mixtures of soil (200-500 mg) and agar were placed on an agar medium pre-inoculated with the test organism Pencillium digitatum. After incubation, the size of the inhibition zone was determined. The lowest detectable concentrations of carbendazim and thiabendazole in a sandy soil were 0.25 ug/g and 1.0 ug/g, respectively and were higher in heavy soils. Chemical analysis and the pellet bioassay technique yielded similar results. This technique requires only small quantities of soil, without need for soil extraction.

Introduction

Studies of the persistence of methyl benzimidazole-2-ylcarbamate (carbendazim, known also as MBC) and related compounds in soil have been reported by a number of investigators (1,4,12). However, continuous efforts are made to simplify methods for the determination of pesticide residues in soil. Methods for analysis of residues in soil, are usually dependent on extraction procedures prior to residue determination, and require soil samples of 10-20g (10). A bioassay using sensitive fungi for the quantitative determination of benzimidazole compounds has been reported, however, it also has the drawback of being dependent on soil extraction (5,7,10,13). We developed a reliable bioassay technique for the direct determination of carbendazim in soil without need for soil extraction, and utilizing small soil quantities.

Materials and Methods

Chemicals, fungus and soil. Carbendazim (99% purity) was obtained from Agan Chemical Co., 2(thiazol-4-yl) benzimidazole (thiabendazole, known also as TBZ) was obtained from Asia-Rizel co. (Israel) as Pitrizet (30% active ingredient). Soils used for the experiments were collected from various sites in Israel and consisted of a sandy loam soil from Rehovot, a light clay from Hulda and two clay loam soils from Mt. Reyhan and from Kisalon. All experiments were performed with Rehovot sandy loam soil, excluding the comparison of bioassay efficiency in various soils. The fungus Penicillium digitatum sensitive to carbendazim and thiabendazole, was used. Carbendazim dissolved in acetone

(10 ml) was added to Rehovot air-dried soil (40 g). After solvents had evaporated, this subsample was mixed with air dried soil (110 g), bringing the final concentration to 10 ug/g soil. The soil samples (in three replicates) were adjusted to 9% water content (field capacity) and incubated in Bartha Pramer biometer flasks in the dark at 27°C (3). The side arm of the flask was filled with distilled water (30 ml) and the flask openings were covered with polyethylene film to prevent evaporation, in order to maintain constant soil moisture.

The soil-agar pellet bioassay. The bioassay was based on the ability of carbendazim to diffuse in agar medium (6,14). Three replicates of soil (200 mg) from every sample were each put into glass rings (13 mm diameter, 13 mm height) placed on a glass plate. Aliquots of 0.3 ml melted agar (3%) cooled to 50°C were mixed with the soil in each ring, using a botanical needle. After cooling and solidification the rings were removed and the pellets were ready for use. Each pellet was placed in the centre of a Petri dish containing 10 ml potato dextrose agar with 2.5 mg chloramphenicol and subsequently inoculated by spreading 0.1 ml of a conidial suspension in sterile water (2×10^6 conidia/ml). Inoculated dishes with the pellets were stored in the dark for 24 hours at 4°C and then transferred to a dark incubator (27°C). After 48 hours a clear zone of fungus growth inhibition was evident (Fig. 1). Standard curves were prepared by using soil samples containing known quantities of carbendazim and thiabendazole. Fungus inhibition was determined by measuring the distance between the pellet edge and the margin of the inhibition zone. The square value of this distance was used for comparing the inhibitory effect of the diffused fungicide (square width of fungus growth inhibition).

Fig 1. Growth inhibition of Penicillium digitatum caused by carbendazim diffused from a soil-agar pellet which was placed in the center of a 90 mm Petri dish containing 10 ml of potato dextrose agar. Numbers indicate carbendazim concentration in soil (ug/g)

Results

Bioassay efficiency. In order to develop a sensitive and reproducible bioassay for the direct determination of carbendazim residues in soil, it was necessary to prepare a compact and firm soil sample with maximal surface contact with the agar medium. Soil samples, when placed on the agar, tend to collapse and spread unevenly on the agar medium and therefore the diffusion of the compound from the soil to the medium was rather limited and not uniform. These difficulties were overcome by using a soil-agar pellet. Various soil-agar mixtures were tested, and the most suitable soil-agar composition was obtained by mixing 0.3 ml agar with 200 mg soil. For the detection of lower levels of carbendazim the pellet size could be increased to 0.5 ml agar mixed with 500 mg soil. For every soil-agar pellet composition a standard concentration-response curve should be prepared. Examples of such standard curves of carbendazim and thiabendazole are given in Figures 2-3. The minimum detectable concentrations of carbendazim and thiabendazole using the various soil-agar pellet compositions are summarized in Table 1. The linear range of the bioassay response to carbendazim residues (calculations based on the standard curve of the 0.3 ml/200 mg pellet) lies between 0.25 ug and 2.5 ug/g soil. In this range, the correlation coefficient (r) was found to be 0.997. For carbendazim concentrations above 2.5 ug/g, the soil samples were diluted with fungicide free soil prior to the assay.

Table 1. Minimum detectable concentrations (ug/g soil) of carbendazim and thiabendazole, using the soil-agar pellet bioassay with Penicillium digitatum.

Pellet composition (ml agar/mg soil)	Concentration in soil (ug/g)	
	Carbendazim	Thiabendazole
0.3/200	0.25	2
0.35/300	0.25	2
0.4/400	0.25	1
0.5/500	0.25	1

The thickness of the medium is of importance. A thinner layer increases the sensitivity of the method. However, applying less than 10 ml agar medium (approx. 2 mm thick in a standard 90 mm Petri dish) may cause variations in the reproducibility of the method due to partial drying of the agar.

It is necessary to maintain the pre-inoculated Petri dishes with the soil-agar pellet at 4C for 24 hours, prior to incubation at 27C, in order to obtain clearly-identifiable inhibition zones. This step enables diffusion of the fungicide from the pellet to the medium in the Petri dish while fungus growth is temporarily inhibited due to the low temperatures. If this stage is omitted, the bioassay fungus may grow in the pellet vicinity before the fungicide has

diffused to the agar. It is obvious that, except for experiments involving sterile soil, the soil in the pellets contains a variety of microorganisms that may multiply and grow during the incubation period. Although such organisms do appear, they usually do not affect the ability to define the inhibition zone in the inoculated Petri dishes.

Fig. 2. EFFECT OF CARBENDAZIM CONC. IN SOIL ON GROWTH INHIBITION OF *PENICILLIUM DIGITATUM* USING THE SOIL-AGAR PELLET TECHNIQUE. GROWTH INHIBITION IS EXPRESSED AS THE SQUARE WIDTH OF THE INHIBITION ZONE. PELLET COMPOSITION (ML AGAR/MG SOIL):
 △ — △ 0.5/500, □ — □ 0.4/400
 ○ — ○ 0.35/300, ● — ● 0.3/200.

Fig. 3. EFFECT OF THIABENDAZOLE CONC. IN SOIL ON GROWTH INHIBITION OF *PENICILLIUM DIGITATUM* USING THE SOIL-AGAR PELLET TECHNIQUE. GROWTH INHIBITION IS EXPRESSED AS THE SQUARE WIDTH OF THE INHIBITION ZONE. PELLET COMPOSITION (ML AGAR/MG SOIL):
 △ — △ 0.5/500, □ — □ 0.4/400
 ○ — ○ 0.35/300, ● — ● 0.3/200.

Soils used for the bioassay may contain substances that can affect the test organism. Appropriate control samples should be therefore taken to show whether such compounds are present and to determine their possible interference with the bioassay results. This is particularly true in soil with previous treatment or residues of fungicides which can affect the assay. As with many bioassays, this technique does not enable distinction between the parent compound and its toxic metabolites.

Bioassay of carbendazim in various soils. Carbendazim (0.1-10 $\mu\text{g/g}$) was added to soils of different types and soil-agar pellets (500 mg/0.5 ml) were prepared. The bioassay results are summarized in standard curves for the different soils (Figure 4). In all tested soils, increasing carbendazim concentrations resulted in increases in the width of the fungus inhibition zone. However, with the heavier soils (which contain higher amounts of organic matter and clay minerals) the slope of the curve was less steep and the minimum detectable concentration of the fungicide was higher. When using the 500 mg/0.5 ml soil-agar pellets, the minimum detectable concentration of carbendazim in soil was 0.25 carbendazim/g soil in the sandy loam and light clay soils. In the Mt. Reyhan and Kisalon clay loam soils the lowest concentration detected was 0.5 and 2.0 μg carbendazim/g soil, respectively. This reduction in the sensitivity of the bioassay can be

related to higher adsorption of carbendazim in the heavier soils, as shown by Aharonson & Kafkafi (1975). The calculated adsorption coefficients of carbendazim (g/ml) in the sandy loam, light clay and Mt. Reyhan clay loam soils are 1.5, 4.0 and 5.8, respectively. The adsorption coefficients of thiabendazole for the same soils were calculated to be 6.0, 16.1 and 23.1, respectively.

Comparison of the soil-agar pellet bioassay with chemical analysis of carbendazim-treated soil. Carbendazim was added to the sandy loam soil (10 ug/g) and determined after various incubation periods by chemical analysis and by the soil-agar pellet bioassay. chemical and bioassay techniques show similar results (Fig. 5). Data obtained by the two methods were analysed statistically and no significant difference between the methods was found. These results indicate the reliability of the soil-agar pellet technique for detecting carbendazim residues. As expected, carbendazim level decreases with time, apparently due to microbial degradation, as shown in various studies (2,8,9,11)

Figure 4. Effect of carbendazim concentration in soil on growth inhibition of Penicillium digitatum using the soil-agar pellet technique. Growth inhibition is expressed as the square width of the inhibition zone. Soil types: ○-○ Rehovot sandy loam, △-△ Hulda light clay, □-□ Mt. Reyhan clay loam, ◇-◇ Kisalon clay loam. Pellet composition was 0.5/500 (ml agar/mg soil).

Discussion

The proposed bioassay has been shown to be a simple, rapid and inexpensive method for determining carbendazim residues in soil, without the need for soil extraction. The possible applicability of this test to thiabendazole has also been shown. The amount of soil required for residue determination has been

reduced to 200 mg, thus providing an option for low-volume soil experiments and soil microsites investigations. In spite of the relative low sensitivity of this technique when compared to other procedures, we feel that the method has advantages and can be very helpful in many cases, for example when conducting screening tests and other experiments requiring multiple sampling and quick results. A comparison of the soil-agar pellet bioassay with the chemical extraction and analysis from a viewpoint of work efficiency clearly shows the advantage of the bioassay in terms of throughput. We were able to prepare over 100 soil samples per day for the bioassay compared with eight for chemical extraction. Another advantage of the bioassay is a substantial reduction in consumption of expendable supplies and the need of only basic laboratory equipment as compared with the requirements for chemical extraction and analysis. The proposed bioassay could prove useful for analysing pesticides incorporated into the soil or applied by a drench treatment. The treatments would in many cases provide residual concentrations in soil (usually 1-5 ug/g) that are within the bioassay detection limits. Pesticides in soil, originating from foliar spray application (runoff, drift) could probably not be detected with the described method.

The soil-agar pellet bioassay may be adopted for the determination of other pesticides in soil, provided they are diffusible and non-volatile and that an appropriate test organism is available.

Figure 5. Persistence of carbendazim in soil, as determined by ○ - ○ chemical analysis, and by Δ - Δ soil-agar pellet bioassay.

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(d) DELAYED AND ENHANCED DEGRADATION OF BENOMYL AND CARBENDAZIM IN DISINFESTED AND FUNGICIDE-TREATED SOILS

Abstract

Factors affecting biodegradation of benomyl and its fungitoxic hydrolysis product, carbendazim, in soil were studied. Soil disinfestation by fumigation or solarization prior to fungicide incorporation inhibited degradation and extended the persistence of carbendazim in soil. Carbendazim was present for a significantly longer time and at higher concentrations in leaves of pepper grown in a field soil disinfested and treated with benomyl than with plants grown in nondisinfested soil. The fungicides TMTD (thiram) and fentin acetate strongly inhibited the degradation of carbendazim, when added to the soil together with carbendazim. A drastic reduction in general enzyme activity in soil amended with TMTD was observed. Alternaria alternata and Bipolaris tetramera, capable of degrading carbendazim, were isolated from soil. The two fungi were sensitive to low concentrations of TMTD and fentin acetate. The combined application of TMTD with benomyl in the field, intended to prolong the biological activity of benomyl, further reduced the number of apothecia of Sclerotinia sclerotiorum when compared with benomyl treatment alone. In soil with a history of benomyl treatment, carbendazim was degraded more rapidly than in a soil with no such history.

Introduction

The increased use of pesticides in modern agriculture has posed challenging problems for research. On the one hand there is the need to extend the effectiveness of the pesticides in the field from both a pest control and economic point of view, but on the other hand there is the need to shorten their persistence due to environmental concerns and possible side-effects on nontarget organisms. Microbial degradation is usually a very significant process which affects the persistence of pesticides (12). Although many studies have dealt with biodegradation of pesticides in culture and field soil, only few have studied the fate of pesticides in disinfested soils. Soil disinfestation by fumigation or solarization affects microbial activity in soil, and may affect the fate and behavior of pesticides (2,11,15,17,20). Rapid degradation of pesticides and a concomitant loss of pest control have been reported where pesticides are applied repeatedly to soil. This might be due to a build-up of populations of microorganisms which degrade the pesticides. These reports have focused mainly on degradation of herbicides and insecticides (13,14). The possibility that the effectiveness of benomyl in mushroom casing is related to enhanced degradation of the fungicide has also been raised (6). The fate and microbial degradation of the fungicide benomyl and its fungitoxic hydrolysis product, carbendazim, have been studied under laboratory and field conditions. These compounds are exposed to physical and microbial transformations in soil.

They are adsorbed to organic matter and clay minerals (1,10), and are degraded by soil-borne microorganisms to nonfungitoxic compounds in periods ranging from weeks to months, depending on soil type, humidity and temperature conditions (4,7-10).

In the present study we investigated the persistence of benomyl and carbendazim in disinfested soils and their interaction in soil with other fungicides in an attempt to extend their effectiveness. Slowing down the degradation process of benomyl and carbendazim may extend their persistence and consequently prolong their effectiveness in disease control. The possibility of enhanced degradation in soil with previous benomyl application was also studied.

Materials and methods

Fungicides. The following fungicides were used: carbendazim (technical grade, Agan Chemical Co., Israel), Benlate (benomyl 50WP, E.I. du Pont de Nemours and Co.), thiram (tetramethylthiuram disulfide or TMTD) (Tirpa 80 WP, Machteshim Inc., Israel), and fentin acetate (Bdilan 60WP Machteshim Inc., Israel).

Medium: Potato dextrose agar (PDA) was used for isolation and growth of fungi. Fungicide suspensions were prepared in acetone and appropriate dilutions were added to PDA (50C) prior to pouring into petri dishes (10 ml per dish). After pouring, the dishes were left open in a sterile hood to allow acetone residues to evaporate.

Cellophane discs. Clear celophane was cut to fit petri-dish diameter. After rinsing (5 min tap water followed by 5 min distilled water), discs were placed in boiling distilled water for 2 hr. After an additional rinse (5 min distilled water), discs, along with paper dividers between them, were placed 10 per petri dish and autoclaved for 20 min.

Laboratory studies of carbendazim degradation in soil. An acetone solution of carbendazim (100 ug/g) was added dropwise to 40 g of air-dried soil. After solvent had evaporated, the soil was left uncovered in a ventilated hood for 24 hr to remove traces of the solvent and then mixed with 110 g of the same soil to provide a 150 g replicate. The soil samples were placed in biometer flasks (3) and moistened to the approximate equivalent of 80% field capacity by adding 12 ml water to each 150 g replicate. The flasks were sealed with thin polyethylene and incubated in the dark at 27°C. Twenty ml of water was put in the side arm of the flask to maintain a constant moisture level in the soil. Soil treatments were replicated three times in each experiment. At various time intervals, subsamples of 20 g each were taken from the flasks and stored at -18C until analyzed.

Uptake of carbendazim by pepper. A randomized block design field trial was used to assess carbendazim uptake by pepper in untreated and disinfested soil. The trial was located on the experiment farm of the Faculty of Agriculture in Rehovot (red-brown sandy soil; 0.6% organic matter, 3.8% clay, pH 7.9, field capacity 9%). Disinfestation treatments consisted of fumigation with methyl

bromide at two rates, 500 and 1000 kg/ha. Another disinfection treatment applied was soil solarization, by tarping the moist soil with transparent polyethylene for one month prior to planting (11). Benlate was incorporated into the soil of the untreated and the disinfested plots at the rate of 10 kg/ha. One-month-old pepper (Capsicum annum L. 'Maor') seedlings were planted after fungicide incorporation. The plants were uprooted at various time intervals for determination of carbendazim in leaves.

Bioassay for carbendazim in leaves of pepper plants. The bioassay used was similar to that described by Solel and Edgington (19). Discs (13 mm diameter) were cut out of the edge of the youngest leaf exceeding 40 mm in length of plants freshly uprooted from the field. Discs were submerged for 10 seconds in liquid nitrogen in order to disrupt cell membranes. Immediately following, the leaf discs were placed in the center of a petri dish (10 ml PDA). The petri dishes were preinoculated with 0.1 ml of a conidial suspension in sterile water (5×10^6 conidia per ml water) of an isolate of Penicillium digitatum Sacc. that was sensitive to carbendazim and was obtained from the Dept. of Plant Pathology and Microbiology, Faculty of Agriculture, Rehovot. Inoculated dishes with the discs were stored in the dark for 24 hr at 4C and then transferred to a dark incubator (27C). After 48 hr a clear zone of fungus growth inhibition was evident in cases where carbendazim had diffused from the leaf discs. A comparison of fungus inhibition area, caused by diffusion of carbendazim from the leaf discs of the various treatments, indicated the relative uptake of carbendazim by plants.

Isolation of carbendazim-degrading microorganisms. Soil dilutions on PDA containing 250 ug/ml chloramphenicol were made in order to isolate a variety of soilborne fungi. Addition of carbendazim at 10 ug/ml to the medium served as a selective factor. The capacity of each tested fungus to degrade carbendazim was determined by the turnover method described here. Pure cultures of the tested fungi were placed in the center of petri dishes containing PDA amended with 1 ug/ml carbendazim. After cultures had grown to cover approximately one half of the medium surface, the agar (with the culture) was removed in one piece, overturned, and placed back in the same petri dish. A conidial suspension of P. digitatum (5×10^5 conidia per 0.1 ml sterile water), sensitive to carbendazim, was spread over the newly exposed agar surface and incubated 48 hr at 27C. If conidia on carbendazim-amended media germinated, indicating the possibility that carbendazim had disappeared, carbendazim degradation by the tested fungi was investigated further.

Actual carbendazim degradation by these select fungi was determined as follows: the fungi were reisolated from the preliminary test dishes and maintained on PDA (27C) for 10 days. Discs (13 mm diameter) of the newly grown cultures were placed in the center of a large petri dish (15 cm diameter) containing 50 ml PDA amended with carbendazim (10 ug/ml). This test was carried out in 3 replicates, placing one disc in the center of every large dish. After the fungus had grown to cover approximately one half of the medium surface,

round discs (13 mm diameter) were cut out along the diameter of the petri dish, and removed for bioassay of carbendazim. The bioassay consisted of placing the discs on PDA in petri dishes preinoculated with the sensitive strain of P. digitatum. The dishes (with discs) were placed in the dark for 24 hr at 4C and then transferred to a dark incubator (27C). After 48 hr a fungus inhibition zone was evident. The area of germination inhibition zone was presumed to be related to the amount of carbendazim in the tested disc; the higher the concentration of carbendazim in the test disc - the larger the inhibition zone. this was verified by comparing the size of the inhibition zone to that obtained in tests with discs from medium containing various concentrations of carbendazim. A reduction in inhibition zone size in experiments with the tested fungi was considered as an indication of carbendazim degradation capacity.

Another test was conducted to determine whether loss of carbendazim activity was due to degradation or rather to adsorption of the compound to the mycelium of the tested fungus, thus preventing it from affecting the bioassay organism. In similar dishes containing the same amended medium, a sterile cellophane paper disk was placed on the medium (prior to placing the tested culture on it), serving as a physical barrier between the organism and the medium. After the growth period, the cellophane paper was removed, and along with the fungal mat attached, placed in acetone and shaken 15 min for carbendazim extraction. The extract, after concentration, was tested for carbendazim residues.

Determination of general enzyme activity in soil. The hydrolysis of fluorescein diacetate (FDA) (Sigma Chemical Co., St. Louis, MO) was used as an assay for the determination of general microbial activity in soil as indicated by enzyme activity levels. This test is an indicator of general microbial activity (18). Samples (5g) of soil were preincubated with 50 ml 0.06 M sodium phosphate buffer (pH 7.6) for 30 min. at 24C on a shaker. After preincubation, 250 ul of FDA solution (2 mg/ml acetone) were added to the suspension. After 180 min of shaking at 24C, 4 ml of the suspension were removed and transferred to a test tube with 4 ml acetone (reaction termination). The suspension was centrifuged in a clinical centrifuge (HEC HN-SII) for 10 min at 1500 rpm. Optical density (O.D.) of the supernatant was determined at 490 nm (Kontron Uvikon 810 spectrophotometer). All phases of the assay were carried out at minimal lighting conditions. The O.D. is proportional to enzyme activity.

Effect of benlate and TMTD on the number of apothecia of Sclerotinia sclerotiorum (de Bary). A field trial was carried out at Gilat Experimental station (silty loam loessial sierozem type soil, 0.5% organic matter, 20% clay, pH 8.4 field capacity 20%) in a lettuce field naturally infested with sclerotia of S. sclerotiorum. A motoric sprayer (spraying boom of 1.2m and 10 nozzles) was used to spray the soil surface with benlate (WP 50% a.i.) at the rate of 5kg/ha. Other treatments included spraying with TMTD (WP 80% a.i.) at the rate of 5kg/ha and a combination of both fungicides. The experiment consisted of

four replicates in a randomized block design. All fungicide suspensions were sprayed at the equivalent of 2000 L/ha. Fungicide application took place during December 1983, 36 days after planting. the number of apothecia was recorded at various time intervals following fungicide treatment during February and March 1984.

Results

Effect of disinfestation and sterilization on persistence of carbendazim in soil and on its uptake by pepper plants. Carbendazim residues were determined after various incubation periods in biometer flasks containing autoclaved or disinfested and untreated soil. The most pronounced degradation occurred in the untreated soil; about 80% of the amount applied was degraded within 30 days (Fig. 1). Autoclaving resulted in a pronounced inhibition of degradation throughout the experiment. Both methods of soil disinfestation strongly slowed down carbendazim degradation. Thus, half life of carbendazim in the soil was extended from about 20 days in the untreated soil to 40-55 days in the disinfested soils.

Fig. 1. Effect of soil sterilization by autoclaving, disinfestation with methyl bromide at 500 kg/ha, or solarization on persistence of carbendazim subsequently added to soils at 10 ug/g. Points are the mean of 3 replicates. Carbendazim was determined by fluorescence spectrophotometry following extraction and HPLC.

Disinfestation of soil in the field, prior to incorporation of benlate, resulted in an increase of carbendazim uptake by pepper plants grown in this soil as compared to plants grown in the nondisinfested plots. The increase observed was both greater and longer at the higher dosage of methyl bromide (Table 1). Fourteen days after soil treatment, carbendazim could be detected only in plants grown in disinfested soil. Twenty-two days after soil treatment, only plants grown in a soil treated with 1000 kg/ha methyl bromide contained carbendazim at

TABLE 1. Effect of fumigation with methyl bromide and soil solarization on relative uptake of carbendazim from soil by pepper plants as determined by a bioassay with Penicillium digitatum

Soil treatment	Relative amount of Carbendazim in pepper leaves at indicated days after application to soil ^Y			
	6	14	22	29
No disinfestation	90 a ^Z	2 a	0 a	0 a
Methyl bromide (500 kg/ha)	92 a	19 b	13 ab	0 a
Methyl bromide (1000 kg/ha)	100 a	28 b	19 b	5 a
Soil solarization	96 a	32 b	3 a	1 a

^YAll bioassay results (inhibition zones) were compared with that of Methyl bromide (1000 kg/ha) after 6 days which was assigned a value of 100.

^ZWithin columns, numbers followed by a common letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

significantly higher rates than plants in the untreatad plots.

Isolation of carbendazim degrading fungi from soil. While conducting the turnover assay for detection of carbendazim degrading fungi, following the incubation period, germination of P. digitatum was very clear on carbendazim-free PDA dishes, and it was completly inhibited on carbendazim-amended dishes free of a tested culture. Germination inhibition occurred in most cases in which a test culture had grown. However, in a few cases, P. digitatum germination was evident over the medium above an overturned fungus colony grown on carbendazim-amended PDA. The medium below these germination spots was suspected to not containing carbendazim, possibly due to degradation of the fungicide by the fungus previously grown on the opposite side of the same medium. Out of 20 fungi isolated from soil on carbendazim-amended dilution plates, 2 were determined capable of degrading this fungicide in culture. They were identified as Alternaria alternata (Fr.) Keissler and Bipolaris tetramera (McKinney) Shoemaker. Their degradation capacity was further verified by growing them on PDA supplemented with carbendazim at 10 ug/ml. After two weeks of fungal growth on the plates, bioassays of agar discs taken from the center of the inoculated plates showed a 50% reduction in the amount of the fungicide.

The apparaent disappearance of carbendazim from the inoculated plates could be attributed either to degradation by the fungi or to inactivation by adsorption to mycelia. In order to examine these two possibilities, cellophane discs were used as mechanical barriers between the fungi and the medium, with no evidence of the compound in the fungal mat. Thus, fungicide disappearance could not be attributed to its adsorption to the mycelium. It was also found that

Fig. 2. Concentration of the fungicides; carbendazim, thiabendazole, and fuberidazole (% of applied) in petri dishes two weeks after beginning of growth of the fungi Alternaria alternata and Bipolaris tetramera.

both fungi are tolerant to carbendazim since ED_{50} of growth on PDA amended with carbendazim was approximately 500 ug/ml.

Microbial degradation of structurally related compounds

Two soil-borne fungi, capable of degrading MBC were isolated from soil. They were identified as Alternaria alternata and Bipolaris tetramera. Their capability to degrade two other structurally related benzimidazole fungicides in vitro was tested. Fungus mycelium discs (13 mm diameter) were placed in the center of a 15 cm diameter petri dish containing 50 ml potato dextrose agar (with 25 ug/ml chloramphenicol) amended with MBC, TBZ and fuberidazole at 10 ug/ml. Two weeks after fungus growth (27°C), agar discs (13 mm diameter) from along the dish diameter were removed and placed on new petri dishes (10 ml PDA), pre-inoculated with conidia of Penicillium digitatum sensitive to the fungicides. After 24 h at 4°C and 48 h at 27°C test fungus germination occurred. Diffused fungicides from the discs inhibited germination, forming a measurable inhibition zone that gave an indication of the amount of fungicide in each disc. Both fungi degraded all three fungicides. Tests for possible adsorption of the fungicides to the fungal mycelium were conducted, yielding no detectable residues of the compounds in the fungal extracts.

B. tetramera seems to have higher degrading capabilities of all fungicides tested. The fact that both fungi degrade the fungicides in a similar pattern may indicate that the degrading mechanism is based on the structure relationship between the fungicides. TBZ, known to persist longer in soil than MBC, was degraded in vitro as readily as MBC. The absence of soil related factors known to effect availability of pesticides to the soil solution (clay minerals and organic matter), consequently resulted in degradation of TBZ present in the medium. The findings emphasize that although structurally related in fungitoxic activity, characteristics derived from differences in the molecular structure may influence other important factors such as microbial degradation in soil. Typical results are presented in Fig. 2.

Inhibition of carbendazim degradation in soil by fungicides. The previous results indicated that treatments which potentially affect soil microbial activity inhibit carbendazim degradation. Thus, the effect of fungicides which were found in preliminary experiments to be inhibitory towards the two carbendazim-degrading fungi was examined. Two fungicides, TMTD and fentin acetate, which strongly reduced the growth of the two fungi at 20 ug/g in culture, were examined for effects on carbendazim degradation in soil in biometer flasks. Both fungicides had a pronounced inhibitory effect on the degradation of carbendazim in soil, which was even more lasting than that of soil fumigation (Table 2). Soil microbial activity, as reflected by enzyme activity, was still low after 45 days of incubation in TMTD-treated soils, in which enzyme activity was inhibited by about 50%. Enzymatic activity in the methyl bromide treated soil, after 45 days incubation was similar to that of the control, while soil sterilization almost completely inhibited it (Table 3).

TABLE 2. Effect of fungicide treatments or soil fumigation with methyl bromide on persistence of carbendazim added to soil^Y

Soil treatment	% of carbendazim remaining	
	31 days	45 days
Untreated	0 b	0 b
Carbendazim	7 b	2 b
Methyl bromide + carbendazim	83 a	9 b
Carbendazim + tetramethylthiuram disulfide (20 ug/g)	85 a	78 a
Carbendazim + tetramethylthiuram disulfide (100 ug/g)	93 a	94 a
Carbendazim + fentin acetate (20 ug/g)	94 a	79 a
Carbendazim + fentin acetate (100 ug/g)	95 a	98 a

^YMethyl bromide was applied in the field at 500 kg/ha. Carbendazim was added to soil at 10 ug/g and analysed by fluorescence spectrophotometry following extraction and HPLC.

^ZWithin columns, numbers followed by a common letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 3. Effect of soil treatments on general enzymatic activity in soil and its relationship to carbendazim persistence in soil^X

Soil treatment	Optical density (490 nm)	Carbendazim in soil (% initial) after 45 days incubation ^Y
Untreated	0.35 a ^Z	2 b
Methyl bromide	0.30 a	9 b
Tetramethylthiuram disulfide (20 ug/g)	0.18 b	78 a
Tetramethylthiuram disulfide (100 ug/g)	0.13 b	94 a
Autoclaved	0.01 c	95 a

^XEnzymatic activity was determined by fluorescein diacetate hydrolysis as detected in soil, 45 days after adding carbendazim (10 ug/g). Optical density values represent relative enzymatic activity.

^YData for this column (with the exception of the autoclaved treatment) were taken from results described in Table 2 for comparison.

^ZWithin columns, numbers followed by a common letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Effect of soil treatment with benlate and TMTD on apothecia of *S. sclerotiorum* in a lettuce field. Soil treatment with benlate significantly reduced the number of apothecia in treated plots. An average of 11 apothecia per m² was recorded throughout the growing season in untreated plots whereas 1.6 were found on the surface of benlate-treated soil. The combined treatment of benlate and TMTD further reduced the number of apothecia to an average of 0.4 per m². Soil treatment with TMTD alone did not significantly affect the number of apothecia when compared to the untreated control (7.5 per m²).

Enhanced degradation of carbendazim in field soil pretreated with benlate. Soil was taken from benlate-treated plots in the lettuce field described above, three and a half months after benlate incorporation. This soil proved to have the capability of more rapidly degrading carbendazim, as tested in biometer flasks, when compared to a soil from the same field, which had no carbendazim history (Fig. 3). This enhanced biodegradation resulted in a substantial shortening of the half life of the fungicide from 11 to about 4 days in this soil system. It is clearly evident that the major change in the degradation process 'acquired' by the carbendazim-history soil is the drastic diminishing of the lag period before actual breakdown occurs.

Fig. 3. Degradation of carbendazim in soils with or without a history of prior carbendazim treatment. Points are the mean of three replicates. Carbendazim was added to soils at 10 ug/g and determined by fluorescence spectrophotometry following extraction and HPLC. Initial treatment of carbendazim history soil was at 10 ug/g, 3 months prior to the experiment.

Discussion

The manipulation of microbial processes related to pesticide persistence in soil may prove to be of great value both when attempting to prolong or shorten the biological activity of these compounds. This study has shown that inhibiting

the breakdown of benomyl or carbendazim in soil by general biocidal (soil disinfection) or relatively selective means (fungicides) extends its effectiveness under laboratory and field conditions. The implications of these observations could be used to reassess the use of carbendazim where the benefits of soil incorporation of this compound are considered marginal. It might well be that prolonging the persistence and consequently the effectiveness of benomyl or carbendazim as soil applied fungicides, may enable their use in a wider range of cases. Furthermore, other relatively short life compounds should be tested for improved performance by means of inhibiting the breakdown process. Degradation of parathion was inhibited by captafol (5) and of linuron by the fumigant vorlex (17). TMTD has special advantages in this respect since it also controls certain soilborne pathogens and even renders the soil suppressive to Pythium (16).

Degradation of benomyl and carbendazim is mainly microbial though a certain proportion might be immobilized by a nonbiological process (10). The determination of microorganisms involved in the process may prove an important step in the better control of the degradation process, especially when enhanced degradation occurs. The turnover method may be a useful tool in screening for such organisms. The excretion of antibiotic substances by the organism tested may inhibit the pesticide test organism. This would perhaps mask the ability to identify pesticide breakdown in cases where both degradation and antibiotic excretion occur simultaneously. We believe such a case would be rare, yet must be taken into account while using the method. A similar problem could arise should the breakdown product be toxic to the test organism.

TMTD and methyl bromide suppress fungal populations in soil but not bacteria; they may even increase bacteria in certain cases (15,16). This indicates that fungi may play an important role in benomyl and carbendazim degradation, though this has yet to be established.

Attention should also be drawn to the expanding phenomenon of soils in which pesticides are rapidly degraded ("problem soils"). The enhanced degradation of pesticides can result from either frequent application of the same compound ("history soils") or from acquired degradation capability due to contact with other structurally related pesticides. This phenomenon may be analogous to cross-resistance as found in the case of pathogen resistance to structurally related pesticides. In the present study, enhanced degradation of benomyl in soil under field conditions was still evident two months after crop harvest. Some reports on loss of effectiveness of benomyl may be related to such a phenomenon, as already has been shown with mushroom casing (6). The use of soil disinfection or fungicidal amendments may serve not only to extend and increase effectiveness of soil fungicides, but also to prevent enhanced degradation or even to correct "problem soils" which already rapidly degrade the fungicide. It is not economically feasible to disinfect soils solely to inhibit pesticide degradation. However, this potential benefit should be taken into account when disinfection is considered for pest control. Moreover, under

the right circumstances, combining soil disinfection with a fungicide may not only improve the performance of the fungicide but might also extend effectiveness of disinfection by delaying reinfestation of the soil.

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II. Research carried out in the USA.

(e) EFFECTS OF FERTILIZERS, CAPTAFOL AND ATRAZINE ON THE FATE AND TRANSLOCATION OF [^{14}C] FONOFOS AND [^{14}C] PARATHION IN A SOIL-PLANT MICROCOSM

Abstract

The effect of fertilizers, captafol, and atrazine on the metabolic fate and translocation of [^{14}C] fonofos and [^{14}C] parathion from soils into oats and corn were studied within open and closed agricultural microcosms. After a 2-week plant growing period in open systems, more radiocarbon was recovered from fonofos-treated soils containing also cow manure, sewage sludge, or atrazine and less from oats or corn grown in these soils. The presence of these amendments in fonofos-treated soil also resulted in an increase of unextractable, bound ^{14}C residues, while ammonium sulfate and captafol had no effect. In parathion-treated soils manure and sludge increased the formation of bound residues, while ammonia sulfate, atrazine, and in particular captafol inhibited their formation. Oat greens from fonofos-treated soils contained primarily insecticidal metabolites. Oats from parathion-treated soil contained less ^{14}C residues, most of them in the form of parathion. Production of $^{14}\text{CO}_2$ from the ^{14}C -labeled insecticide treated soils was significantly inhibited by all amendments, in particular by captafol and manure. Results indicate that soil amendments indirectly inhibited the production of $^{14}\text{CO}_2$ by directly affecting soil microorganisms, primarily soil fungi, responsible for the degradation of the insecticides. Microbiological degradation of [^{14}C] glucose in soils to $^{14}\text{CO}_2$ was inhibited by manure and sludge, was increased in the presence of captafol and atrazine, and was not affected by ammonium sulfate. Results indicate that the fate of pesticide chemicals in the agricultural environment should be studied by taking various ecological factors into consideration.

Introduction

The environmental behavior of pesticides has in the past been investigated primarily with one specific chemical, usually after its application to soils, water, or plants. Under normal agricultural practices, however, a number of chemicals are often used, resulting in a variety of mixed pesticide residues in soils, water, or crop plants. In addition to pesticide chemicals, organic and inorganic fertilizers are applied that could affect the fate of soil pesticides, possibly via an effect on the soil microflora. Thus Rajaram et al. (1978) reported that a single application of ammonium sulfate or potassium nitrate inhibited the hydrolysis of parathion in flooded soil that had been inoculated with a parathion-hydrolyzing enrichment culture. Doyle et al. (1978), utilizing a closed flow-through incubation unit, reported that "altered rates of pesticide degradation were observed in sludge and/or manure amended soils for a number of structurally unrelated pesticides. ^{14}C -product distribution varied with soil amendments". The pesticides investigated in Doyle's study were primarily herbicides. Studies conducted in our laboratory with [^{14}C] parathion (Ferris and Lichtenstein, 1980) showed that selected fungicides and inorganic nitrogen fertilizers affected the degradation of [^{14}C]parathion in Wisconsin cranberry soils.

For further investigation of interaction phenomena, open and closed agricultural microcosms or systems were used (Figure 1) with or without plants that were grown in insecticide-treated loam soil. With this setup the potential effects of fertilizers, captafol, and atrazine on the fate and translocation of soil-applied insecticides in soils and crop plants were studied.

Materials and methods

Chemicals. [U-phenyl- ^{14}C]Fonofos (Dyfonate) (sp act. = 17.1 mCi/mM) was obtained through the courtesy of Stauffer Chemical Co., Mountain View, CA, and [2,6-phenyl- ^{14}C]parathion (sp act. = 2.2 mCi/mM) was purchased from the ICN Corp., Irvine, CA. Specific activities indicated in the different experiments were obtained by dilution with nonradioactive fonofos or parathion. These insecticides were determined to be at least 97% pure by thin-layer chromatography. Nonradioactive fonofos or parathion and some of their potential metabolites were obtained from Stauffer Chemical Co. and from Farbenfabriken Bayer, West Germany, respectively. Analytical-grade captafol (Difolatan) was obtained from Ortho Chevron Chemical Co., Richmond, CA and atrazine was provided courtesy of Ciba-Geigy Corp., Greensboro, NC. D-[6- ^{14}C]Glucose (sp act. = 51.2 mCi/mM) was purchased from New England Nuclear Corp. Boston, MA.

Fertilizers. Cow manure was obtained from the University of Wisconsin dairy barn and sewage sludge from the Madison Metropolitan Sewage District. The sludge was in a liquid, digested form (pH 7.0), as sold to Wisconsin farmers. Both manure and sludge were air-dried at room temperature, ground to a fine powder, and stored at 5°C. Granular analytical-grade ammonium sulfate was obtained from Mallinkrodt, St. Louis, MO.

Figure 1. Soils treated with [^{14}C]fonofos or [^{14}C]parathion to which also cow manure (CM), sewage sludge (SL), $(\text{NH}_4)_2\text{SO}_4$ (NH), Difolatan (captafol) (DI), or atrazine (AT) had been added. (A) depicts the open system with oats or corn and (B) the closed system.

Solvents. Acetone, benzene, chloroform, ethyl acetate, dioxane, methyl-Cellosolve, and hexane were redistilled before use. Methanol, toluene, and ethanolamine were of analytical grade.

Soil. A Plano silt loam soil (4.7% organic matter, 5% sand, 71% silt, and 24% clay with a pH of 6.0), free of insecticide residues, was collected at the University of Wisconsin Experimental Farm near Madison and stored in a moist condition at $22 \pm 2^\circ\text{C}$ prior to use.

Soil treatments. Studies described here deal with the potential effects of cow manure, sewage sludge, ammonium sulfate, captafol, and atrazine on the fate and metabolism of soil-applied [^{14}C]fonofos and [^{14}C]parathion in soils and their translocation into crop plants. Loam soils were first treated at 4 ppm with an acetone solution of one of the insecticides and then thoroughly mixed, while the acetone was removed with a gentle stream of air (Lichtenstein and Schulz, 1959). Immediately following treatment, two 50-g aliquots of each treated soil were removed for extraction and analyses to determine the actual treatment levels. All data are reported as percentages of the initially determined doses. Soil amendments were added to these soils as described later under Experimental Procedures.

So that the effect of these soil amendments on glucose-degrading microorganisms could be studied, soils were also treated with [^{14}C]glucose followed by the addition of one of the soil amendments.

Extraction. Soils. All soils were extracted with a mixture of acetone-methanol-benzene (1:1:1), followed by solvent evaporation and partitioning of the extracted residues between benzene and water as described by Lichtenstein et al (1973).

Plants. Oats or corn grown in all ^{14}C -labeled insecticide treated soils were extracted with acetone-methanol-benzene as described above. Cleanup of organic

extraction phases of oat or corn tops was necessary prior to analysis by thin-layer chromatography. For this purpose benzene extraction phases containing [^{14}C]parathion were purified with charcoal as described by Lichtenstein et al. (1973).

Analyses. Analyses by liquid scintillation counting (LSC), thin-layer chromatography (TLC), and autoradiography as well as gas-liquid chromatography (GLC) were performed as described by Fuhremann and Lichtenstein (1980). Results were expressed in percent of the insecticidal dose as determined by initial soil analyses following soil treatment.

Experimental procedures

Effects of Soil Amendments on the Fate of ^{14}C -Labeled Insecticides in an Open Soil-Plant Microcosm. Oats or corn were grown in insecticide-treated soils, to which fertilizers, captafol, or atrazine had also been added. Initially two 5000 g (dry weight) each of soil were treated at 4 ppm as described (Lichtenstein and Schulz, 1959) with 125 mL of acetone containing 20 mg of [^{14}C]fonofos (30.2 uCi) or 20 mg of [^{14}C]parathion (53.3 uCi). Each of 14 cartons (9x8.5 cm diameter) was then filled with 300 g (dry weight) of [^{14}C]fonofos-treated soil and each of 14 additional cartons with 300 g of [^{14}C]parathion-treated soil. All tests were conducted in duplicate: two cartons containing either [^{14}C]fonofos- or [^{14}C]parathion-treated soil served as controls. Additional soil treatments consisted of mixing 13.5 g of dried cow manure or sewage sludge with insecticide-treated soil, resulting in a dose equivalent to 100 metric tons of manure or sludge per hectare of soil. On the basis of Walsh et al (1976), the amounts of sludge used in these experiments appear to be on the high side of practical use rates, although Doyle et al. (1978) reported that the rate of 100 metric tons/ha "are in accordance with those rates which have been considered for possible use in Maryland agricultural soils". Ferris and Lichtenstein (1980) showed that in the presence of ammonium sulfate applied at 100 ppm of N equivalent to Wisconsin cranberry soils, parathion was more persistent and amounts of $^{14}\text{CO}_2$ produced from [^{14}C]parathion were reduced by 83%. In the experiments reported here, ammonium sulfate was added to the insecticide-treated soils at 106 ppm of N equivalent. Captafol or atrazine was mixed with soils at 100 ppm. These later concentrations, although higher than those used normally, were selected to demonstrate the potential effects of captafol and atrazine (Ferris and Lichtenstein, 1980). Percich and Lockwood (1978) used atrazine at 10, 30 and 100 ppm to study the interaction of atrazine with soil microorganisms.

Planting and Growing Oats and Corn. Oats were used as test plants in these experiments with the exception of those where the effects of atrazine were investigated. In these latter tests corn plants were utilized.

Seeds of oats and corn were germinated until the roots reached a length of ca. 1 cm. After soil treatment as described, 25 oat seedlings were planted in the soil of each of 20 cartons (10 with [^{14}C]fonofos-treated soil and 10 with

[^{14}C]parathion-treated soil). In addition, eight corn seedlings were planted in each of the soils in eight cartons (four with [^{14}C]fonofos-treated soil and four with [^{14}C]-parathion-treated soil) designated for the study of the effects of atrazine. The soil in each carton was then watered with 40 mL of distilled water and weighed. This initial weight was maintained by addition of water as necessary. The plants were grown for 14 days in a growth chamber at a temperature of 28°C and on a 14-h light, 10-h dark cycle.

Harvesting Plants and Soils. Tops of plants were cut 1 cm above the soil surface, weighed, rinsed with cold tap water, cut into 1-cm pieces, and immersed in appropriate solvents before extraction as described above. Results obtained

Table I. Effects of Fertilizers^a and Captafol^b on the Fate and Translocation of [^{14}C]Fonofos in Loam Soils and Oat Plants Incubated for 14 Days in an Open System at $28\pm 1^{\circ}\text{C}$

^{14}C recovered, % of applied [<u>ring</u> - ^{14}C]fonofos ^d in soil plus					
	none (control)	cow manure	sewage sludge	ammonium sulfate	captafol (Difolatan)
soil					
benzene: ^e ^{14}C	35.6 $\pm$ 0.9	26.8 $\pm$ 1.3 ⁱ	40.5 $\pm$ 2.2	38.6 $\pm$ 1.0	31.5 $\pm$ 4.1
FO ^f	36.3 $\pm$ 1.0	20.5 $\pm$ 0.6 ^h	33.8 $\pm$ 0.8	38.2 $\pm$ 0.4	34.2 $\pm$ 5.8
water ^e	1.0 $\pm$ 0.1	2.1 $\pm$ 0.1 ⁱ	0.7 $\pm$ 0.2	0.9 $\pm$ 0.1	10.2 $\pm$ 0.3 ^g
bound ^e	28.3 $\pm$ 1.4	43.4 $\pm$ 0.8 ^h	36.3 $\pm$ 0.9 ⁱ	26.0 $\pm$ 0.4	29.0 $\pm$ 0.1
total (S)	64.9 $\pm$ 2.4	72.3 $\pm$ 0.3 ⁱ	77.4 $\pm$ 1.1 ⁱ	65.6 $\pm$ 1.4	70.7 $\pm$ 3.9
oat greens					
benzene: ^{14}C ⁱ	1.0 $\pm$ 0.1	1.1 $\pm$ 0.0	1.0 $\pm$ 0.0	1.0 $\pm$ 0.1	0.2 $\pm$ 0.0 ^h
water	7.4 $\pm$ 0.5	0.7 $\pm$ 0.1 ^h	0.5 $\pm$ 0.1 ^h	15.0 $\pm$ 1.0 ^h	3.7 $\pm$ 0.5 ⁱ
bound	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0 ⁱ	0.1 $\pm$ 0.0 ⁱ	0.4 $\pm$ 0.0 ^h	0.1 $\pm$ 0.0 ⁱ
total (O)	8.6 $\pm$ 0.5	1.9 $\pm$ 0.0 ^h	1.6 $\pm$ 0.1 ^h	16.4 $\pm$ 0.1 ^h	4.0 $\pm$ 0.5 ⁱ
total/g of green	2.4	0.3	0.2	3.1	1.3
total (S+O)	73.5 $\pm$ 1.9	74.2 $\pm$ 0.3	79.1 $\pm$ 1.0	81.9 $\pm$ 0.4 ⁱ	74.7 $\pm$ 3.4

^aCow manure and sewage sludge were mixed with soil (13.5 g of dry powder/300 g of dry soil) and $(\text{NH}_4)_2\text{SO}_4$ at 106 ppm of nitrogen equivalent. ^bCaptafol was mixed with soil at 100 ppm (10 mg/100g of dry soil). ^cResults are average of duplicated tests. ^d[ring- ^{14}C]Fonofos (1.81 uCi) was mixed with 300 g of soil (dry weight) at 4 ppm. ^eBenzene and water extraction phases of soil. Bound = unextractable ^{14}C . ^fFO = fonofos; the amount was determined by GLC. ^{g-i}Data are significantly different from controls (none) at the 0.1% (g), 1% (h), and 5% (i) level (Student's t test). ⁱOver 90% of benzene-soluble ^{14}C compounds were associated with methyl phenyl sulfone (see Figure 2).

after analyses of plant greens were reported as radiocarbon recovered from the leaves in percent of the amount previously applied to the soil and were also calculated on a per gram fresh weight basis (Tables I-III).

After the soils had dried for 2 days, roots were removed but were not analyzed. The soils were sieved through a 2-mm screen and thoroughly mixed. A 100-g aliquot was removed for moisture determination while a second 100-g aliquot was immersed in 200 mL of extraction solvents for analyses.

Table II. Effect of Fertilizers^a and Captafol^b on the Fate and Translocation of [¹⁴C]Parathion in Loam Soils and Oat Plants Incubated for 14 Days in an Open System at 28±1°C^c

	¹⁴ C recovered, % of applied [ring- ¹⁴ C]parathion ^d in soil plus				
	none (control)	cow manure	sewage sludge	ammonium sulfate	captafol (Difolatan)
soil					
benzene: ^e ¹⁴ C	42.6±3.7	10.7±2.4 ^h	29.5±2.9 ⁱ	28.6±0.2 ⁱ	44.9±0.4
PA ^f	26.4±0.1	7.0±2.1 ^h	10.6±1.3 ^h	20.7±0.0 ^g	39.0±1.7 ^h
water ^e	11.3±3.8	24.0±1.4 ⁱ	2.5±0.7	28.0±0.7 ⁱ	41.7±0.5 ^h
bound ^e	31.7±0.0	51.0±2.9 ^h	60.9±2.7 ^h	27.1±0.4 ^h	7.3±0.6 ^g
total (S)	85.6±0.1	85.7±2.0	92.9±0.6 ^h	83.7±0.2 ^h	93.9±0.6 ^h
oat greens					
benzene: ¹⁴ C ⁱ	0.1±0.0	0.1±0.0	trace ⁱ	0.1±0.0	trace ⁱ
water	0.3±0.0	0.2±0.0 ^h	0.1±0.0 ^h	0.3±0.0	0.1±0.0 ^h
bound	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0 ⁱ	0.1±0.0
total (O)	0.5±0.0	0.4±0.0 ^h	0.2±0.0 ^g	0.6±0.0	0.2±0.0 ^h
total/g of greens	0.08	0.07	0.05	0.09	0.05
total (S+O)	86.5±0.1	86.1±2.0	93.1±0.5 ^h	84.3±0.2 ^h	94.1±0.6 ^h

a,b,e,g-i Footnotes a,b,e, and g-i are the same as in Table I. ^cResults are average of duplicated texts. ^d[ring ¹⁴C]parathion (3.2 uCi) was mixed with 300 g soil (dry weight) at 4 ppm. ^fPA = parathion; the amounts in soils were determined by GLC. Amounts in greens could not be determined because of impurities. ⁱ0.02 or less.

Effects of Soil Amendments on the Fate and Metabolism of ¹⁴C-Labeled Insecticides in a Closed Soil Microcosm. Studies conducted previously in our laboratory with plants grown under bell jars had been designed to obtain a total balance of the soil-applied radiocarbon. Results obtained, however, were quite different from similar studies with open systems (Anderegg and Lichtenstin, 1981). This was due to the different growing conditions and in particular due

Table III. Effects of Atrazine^a on the Fate and Translocation of ¹⁴C-Labeled Insecticides in Loam Soil and Corn Plants Incubated for 14 Days in an Open System at 28±1°C^b

¹⁴ C recovered, % of applied ^c to soil				
	[¹⁴ C]fonofos plus		[¹⁴ C]parathion plus	
	none	atrazine	none	atrazine
soil				
benzene: ^d ¹⁴ C	36.6±0.1	36.0±1.6	30.0±6.3	40.4±1.6
IN ^e	30.2±0.1	32.2±0.8 ^g	24.9±6.3	33.1±0.8 ^h
water ^d	1.2±0.1	2.1±0.1 ^h	18.9±3.7	29.9±0.6 ^h
bound ^d	23.7±0.4	33.0±1.0 ^g	32.0±0.6	20.3±1.0 ^g
total (S)	61.5±0.3	71.1±0.7 ^g	80.9±2.0	90.6±0.1 ^h
oat greens				
benzene: ¹⁴ C ^e	1.0±0.0	0.2±0.0 ^f	0.2±0.0	0.2±0.0 ⁰
water	14.1±0.4	10.8±0.0 ^g	0.2±0.0	0.6±0.1 ^h
bound	0.2±0.0	0.1±0.0	0.2±0.0	0.3±0.0
total (C)	15.3±0.4	11.1±0.0 ^g	0.6±0.0	1.1±0.1 ^h
total/g of greens	1.14	0.87	0.04	0.08
total (S+C)	76.8±0.1	82.2±0.7 ^g	81.5±2.0	91.7±0.0 ^h

^aAtrazine was mixed with soil at 100 ppm (10mg/100g of dry soil). ^b Results are average of duplicate tests. ^c Same as footnote d in Tables I and II. ^d Benzene and water extraction phases of soil. Bound = unextractable ¹⁴C. ^e IN=insecticide (fonofos or parathion, respectively) determined by GLC. Data are not available for greens. ^{f-h} Data are significantly different from controls (none) at the 0.1% (f), 1% (g), and 5% (h) level (Student's t test).

to reduced rates of leaf transpiration in the closed system. It was for these reasons that the investigations with soil amendments as described above were conducted with plants under the more realistic environmental conditions of open systems. Since the production of ¹⁴CO₂ from ¹⁴C-labeled insecticides is a good indicator of the potential effects of soil amendments on the breakdown of ¹⁴C-labeled insecticides, additional experiments were performed in duplicate with soils kept in closed systems (Figure 1B) as described by Ferris and Lichtenstein (1980). One hundred grams (dry weight) of [¹⁴C]fonofos-treated soil (4 ppm) was placed into each of 12 incubation jars (13 cm x 6 cm diameter). Of these, two served as controls, while cow manure or sewage sludge was applied at 4.5 g (dry weight)/100g of soil, ammonium sulfate at 106 ppm of N equivalent, and captafol or atrazine at 100 ppm. A second experiment identical with the one described was set up, except that [¹⁴C]parathion was used as the insecticide.

Soils were incubated for 4 weeks at room temperature ($23 \pm 2^{\circ}\text{C}$) and in the dark, while $^{14}\text{CO}_2$ was collected in KOH traps at intervals and quantified by LSC as described by Ferris and Lichtenstein (1980).

At the end of the incubation period, soils from each jar were extracted and analyzed, resulting in data for organic and water-soluble as well as unextractable, soil bound radiocarbon. Volatile lipid-soluble compounds retained in the polyurethane trap were extracted twice with 75 ml of hexane.

Effects of Captafol and Atrazine on Populations of Microorganisms in Parathion-Treated Soil. Results obtained in experiments with captafol or atrazine suggested that both the fungicide and the herbicide inhibited soil microorganisms which in turn could have altered the degradation of the insecticides in the soil. For determination of the effects of captafol and atrazine on fungal and bacterial populations in those soils, an experiment complementary to the one described with the closed systems was conducted in duplicate with soils treated with parathion at 4 ppm and with captafol or atrazine at 100 ppm. Soils treated only with parathion served as controls. After a 21-day incubation period, soils were thoroughly mixed, and 15-g samples were removed from each incubation jar and shaken for 20 min with 150 mL of sterilized distilled water to produce a soil inoculum. After that, fungal selective agar media (Martin, 1950) were inoculated with a 1:10 dilution prepared from control or captafol- or atrazine-treated soils. Bacteria selective agar media (Ridge and Rovira, 1971) were inoculated with a 1:1000 dilution prepared from the respective soils samples. After a 3-day incubation period of the inoculated plates at room temperature, they were photographed.

Effects of Soil Amendments on the Activity of [^{14}C]Glucose-Degrading Soil Microorganisms. Studies conducted previously in our laboratory had shown that soil microorganisms in basal salt media utilized parathion as the sole carbon source. Addition of glucose, however, drastically reduced the degradation of the insecticide (Gorder and Lichtenstein, 1980). This phenomenon was also demonstrated with parathion in cranberry soils (Ferris and Lichtenstein, 1980). To test the hypothesis that soil microorganisms are affected by the soil amendments used in the experiments described above, we used [^{14}C]glucose as the substrate instead of ^{14}C -labeled insecticides.

In the first study, the potential degradation of [^{14}C]glucose by soil microorganisms was investigated. Two jars (10 cm x 5 cm diameter) (Figure 1B) were each filled with 25 g (dry weight) of loam soil that was then sterilized by irradiation (45000 rad for 70 h). The sterility of these soil was confirmed by incubating samples in yeast extract-dextrose medium. Two additional jars were filled with 25 g (dry weight) of nonsterilized soil (controls). Four milliliters of sterilized water containing 50 mg of [^{14}C]glucose (0.97 uCi) was then pipetted onto each soil surface in the four jars. The soils were then incubated for 28 days at $24 \pm 1^{\circ}\text{C}$ and $^{14}\text{CO}_2$ produced from [^{14}C]glucose was trapped at intervals during the 4-week period. Sterility tests conducted at the end of

the incubation period with the original irradiated soils indicated that these soils were no longer sterile.

The second study was conducted with [^{14}C]glucose-treated soil to which the five amendments had been added as described above. After 100 g (dry weight) of soil in each of two incubation jars had been mixed with either cow manure, sewage sludge, ammonium sulfate, captafol, or atrazine as described, 2 mL of water containing 200 mg of [^{14}C]glucose (1.0 uCi) as added to each of the soils containing amendments and to two control soils. To facilitate a better penetration of [^{14}C]glucose throughout these soils, 10 mL of additional water was added to each soil in the 12 incubation jars. Soils were then incubated as described for 31 days while the released $^{14}\text{CO}_2$ was trapped at intervals and quantitated by LSC.

Results and discussion

Effects of soil Amendments on the Fate of ^{14}C -Labeled Insecticides in an Open Soil-Plant Microcosm. The effects of fertilizers and captafol on the fate of soil-applied [^{14}C]fonofos were conducted in open systems as shown in Figure 1A. Due to the presence of the soil amendments, significant changes in the distribution of radiocarbon within the soil-plant system were noticed after the 2-week incubation period (Table I). While the total recoveries of ^{14}C -labeled compounds from soils plus oats (Table I, total, S + O) were similar under all conditions (except for ammonium sulfate), significant differences were noticed when results from soils or oats were compared with those of controls. Thus, more total radiocarbon was recovered from soils containing cow manure or sewage sludge and considerably less from oats that had grown in these soils.

Effects of soil amendments on the distribution of radiocarbon in the extraction phases of [^{14}C]fonofos-treated soils were primarily noticed with cow manure. In comparison to controls, manure-treated soils contained less benzene soluble radiocarbon and less fonofos (21% of applied as opposed to 36% in controls) but showed a significant increase in bound ^{14}C residues (43% of applied as opposed to 28% in controls). In the presence of sewage sludge, an increase in bound soils residues had occurred, while in the presence of captafol a 10-fold increase in water-soluble radiocarbon was noticed. The pH of control soils and of those containing also cow manure, sewage sludge, or ammonium sulfate were initially 5.9, 6.4, 6.5, and 5.9, respectively. Fourteen days later, these figures were 4.9 (controls), 6.0, 6.5, and 5.0, respectively. Determinations of the pH in captafol-treated soils are not available.

Differences observed with oats due to manure or sludge were reflected by a 90% reduction in the formation of water-soluble radiocarbon amounting to only 0.7% or 0.5% of applied ^{14}C , respectively, as compared to 7.4% in controls. It appears that in the presence of manure and sludge where an increase in binding of radiocarbon to soil had occurred less water-soluble ^{14}C had been translocated from soils into the leaves or the metabolism within leaves of benzene-soluble ^{14}C into water-soluble radiocarbon had been drastically reduced. The presence of

ammonium sulfate in soils, however, resulted in an increase in water-soluble ^{14}C within oat greens (15% of applied as opposed to 7.4% in controls).

For identification of organic-soluble compounds, benzene extraction phases of both soils and oats were also analyzed by TLC, autoradiography, and GLC (soils only). Results are shown in Figure 2, where the right-hand column presents in figures the total amounts of benzene-soluble radiocarbon recovered from soils and oats (expressed in percent of soil-applied ^{14}C). The horizontal bar graphs, however, depict the percent distribution of the various compounds in these benzene extraction phases. It is evident, that fonofos was the major compound recovered from soils, amounting from 49% to 72% of all benzene-soluble compounds. In oat greens, however, most of the ^{14}C recovered (1% or less of the soil-applied radiocarbon) was associated with the fonofos breakdown product methyl phenyl sulfone (MPSO₂). Small amounts of the oxygen analogue of fonofos (F=oxon in Figure 2) were found in both soils and oats. Compounds recovered in trace quantities ("Others" in Figure 2) were thiophenol, diphenyl disulfide, and 2-, 3-, or 4-hydroxymethyl phenyl sulfone. As demonstrated by Liang and Liang and Lichtenstein (1980) with houseflies, only fonofos (0.1 ug/fly) and its oxygen analogue (0.1 ug/fly) are insecticidal and "no mortalities were observed after flies had been treated with 1 ug of methyl phenyl sulfone, thiophenol, diphenyldisulfide, or one of the hydroxy methyl phenyl sulfone compounds". It is

Figure 2. Metabolism of [ring- ^{14}C]fonofos in soils and plants growing in an open system. Data are based on TLC of the organic extraction phase, followed by autoradiography, LSC, and GLC (soils). MPSO₂ = methyl phenyl sulfone; F=oxon = oxygen analogue of fonofos; others = thiophenol, diphenyl disulfide, and 2-, 3-, or 4-hydroxymethyl phenyl sulfone.

Figure 3. Metabolism of [ring- ^{14}C]parathion in soils and plants grown in an open system. Data are based on TLC of the organic extraction phase, followed by autoradiography, LSC, and GLC (soils). PS = parathion; PO = paraoxon; PO-NH₂ = aminoparaoxon; others = aminoparaoxon and p aminophenol.

evident, therefore, that after 2 weeks of incubation, soils still contained 20% to 38% of the applied fonofos (Table I), while nearly all of the radiocarbon recovered from oat greens was associated with detoxified, noninsecticidal metabolites of fonofos.

As shown in Figure 2, relatively more MPSO_2 was recovered from soils amended with cow manure and less from soils amended with ammonium sulfate. The addition of captafol to soils, however, suppressed the production of MPSO_2 , which amounted to only 5% of all benzene-soluble ^{14}C in the soil.

Experiments identical with those described for [^{14}C]fonofos in open systems were also conducted with [^{14}C]parathion. As shown in Table II, the effects of soil amendments on the fate of [^{14}C]parathion were quite pronounced, although they were different in nature from those observed with [^{14}C]fonofos. The overall recoveries (total, S + O in Table II) of [^{14}C]parathion-derived radiocarbon were larger in the presence of sewage sludge and captafol, due to larger recoveries from soils. In the presence of ammonium sulfate, however, the total recovery of radiocarbon was slightly small than in controls. Due to the presence of manure, recoveries of benzene-soluble ^{14}C and of parathion (determined by GLC) from soils were drastically reduced and to a lesser extent in the presence of sludge and ammonium sulfate. Conversely, the presence of captafol resulted in an increased persistence of parathion as determined by GLC.

Most noticeable were the effects of these soil amendments on the production of unextractable, bound ^{14}C residues in soils. While in control soils 31.7% of the applied radiocarbon was associated with bound soil residues, the presence of manure and sludge increased these bound residues to 51% and 61% of applied, respectively, while ammonium sulfate and captafol drastically decreased the formation of bound residues. It appears that in particular captafol inhibited soil microorganisms responsible for the reduction of parathion to amino compounds, which in turn are bound to soils (Katan and Lichtenstein, 1977). This might explain one of the reasons for the increased persistence of the originally applied parathion and the decreased binding of [^{14}C]parathion derivatives in captafol-treated soils. Results relative to the binding of [^{14}C]parathion derived radiocarbon are different from those observed with [^{14}C] fonofos. This was not surprising, since previous investigations with [^{14}C]fonofos showed that the mechanism of binding to soil is different than that observed with [^{14}C]parathion (Lichtenstein et al., 1977).

The amounts of ^{14}C water-soluble compounds produced in [^{14}C]parathion treated soils increased from 11% of applied in controls to 24%, 28%, and 42% of applied in the presence of manure, ammonium sulfate, and captafol, respectively, and decreased to 2.5% of applied when sewage sludge had been added.

Oat leaves grown in these soils contained small amounts (0.2-0.5% of applied or 0.05-0.09%/g of fresh greens) of radiocarbon. On the basis of TLC, autoradiography, and GLC (soils only), parathion was the major compound recovered from the benzene extraction phases of both soils and oat greens (Figure 3), although in greens the actual amounts were minimal. Paraoxon,

aminoparaoxon, and p-nitrophenol were the major metabolites detected. All data, therefore, indicate that the effects of soil amendments on the fate of [^{14}C]parathion in these soil-plant systems were primarily noticeable with soils, where they especially affected the production of bound and water-soluble [^{14}C]parathion derivatives. In plants, however, effects were not noticeable.

Table IV. Effects of Fertilizers^a Difolatan, and Atrazine^b on the Fate of Insecticides in a Loam Soil Incubated for 28 Days in a Closed System at $24\pm 2^\circ\text{C}$

soil plus	^{14}C recovered from soil treated ^d with			
	[^{14}C]fonofos		[^{14}C]parathion	
	$^{14}\text{CO}_2^e$	bound ^f	$^{14}\text{CO}_2$	bound
Percent of Applied ^d				
none (control)	16.7 $\pm$ 0.5	30.8 $\pm$ 0.4	23.2 $\pm$ 1.1	48.5 $\pm$ 0.1
Percent of control (=100)				
cow manure	7.8 ^g	99.4	4.3 ^h	178.6 ^g
Sewage sludge	21.6 ^g	78.3 ^h	4.3 ^h	172.8 ^g
ammonium sulfate	61.7 ^h	97.1	85.3 ⁱ	84.1 ^g
captafol	2.4 ^g	89.0 ⁱ	4.3 ^h	46.0 ^g
atrazine	26.4 ^h	115.9 ⁱ	26.7 ^h	79.4 ^h

^aAs in Table 1, except that 4.5g dry powder of manure or sludge per 100 g of soil was added. ^bDifolatan or atrazine was mixed with soil at 100 ppm (10 mg/100g of dry soil). ^cResults are averages of duplicated tests. ^d[ring- ^{14}C] Fonofos (0.69 uCi) or [ring- ^{14}C]parathion (1.1 uCi) was mixed with 100 g of soil (dry weight) at 4 ppm. ^eCumulative $^{14}\text{CO}_2$ trapped over a 28-day incubation period in KOH within the closed system. ^fBound = unextractable ^{14}C soil residues. ^{g-i}Data are significantly different from controls (none) at the 0.1% (g), 1% (h), and 5% (i) level (Student's t test).

In order that potential insecticidal activity of oat greens grown in fonofos or parathion-treated soils could be tested organic-soluble compounds extracted from leaves were also bioassayed with fruit flies (*Drosophila melanogaster* Meig.) as described by Lichtenstein (1960). In a separate experiment, two containers as shown in Figure 1A were each filled with soil treated with fonofos at 4 ppm, two with parathion treated at 4 ppm, and two with untreated soil (control). Oat greens were harvested after a 2-week growing time in these soils and were extracted as described. Duplicate organic solvent extracts, representing 5.8, 5.6, and 5.7 g (fresh weight) of oat greens from fonofos-treated soils, from parathion-treated soils, and from control soils, respectively, were transferred into bioassay jars. As an additional control, only solvents were pipetted into

two bioassay jars. After evaporation of these solvents, fruit flies were exposed to the dry residues in the eight jars and held for 48 h. At the end of the exposure period to solvent controls, oat extracts from nontreated soils, fonofos treated soils, and parathion-treated soils insect mortalities of 4%, 6%, 4%, and 33%, respectively, were observed. Data, therefore, indicate, that only oat greens from parathion-treated soils exhibited some toxicity to fruit flies.

Effects of Soil-Applied Atrazine on the Fate of Insecticides in a Soil-Corn System. Since corn plants were used in experiments with atrazine, results are reported separately in Table III. On the basis of analyses by GLC of the benzene extraction phase of soils (IN in Table III), both fonofos and parathion were more persistent in the presence of atrazine. Binding of radiocarbon to soils was increased with [^{14}C]fonofos but decreased with [^{14}C]parathion. In addition, more water-soluble ^{14}C compounds had been produced from both insecticides in the presence of the herbicide.

Corn greens grown in [^{14}C]fonofos-treated soils (controls) contained a total of 15% of the applied radiocarbon but only 11% when atrazine was also present. As shown in Table III and Figure 2, respectively, most of these residues in corn were in the form of water-soluble fonofos metabolites and 81% of the benzene-soluble radiocarbon was associated with the noninsecticidal methyl phenyl sulfone.

Corn greens grown in [^{14}C]parathion or [^{14}C]parathion plus atrazine treated soils contained only 0.6% and 1.1% respectively, of the originally soil-applied radiocarbon. Of these residues, one-third were water-soluble metabolites (Table III) and most of the benzene-soluble radio-carbon was in the form of parathion (Figure 3).

Effects of Soil Amendments on the Fate of ^{14}C -Labeled Insecticides in a Closed Soil Microcosm. Since these experiments were conducted in closed systems (Figure 1B) without plants, results obtained cannot be strictly compared with those obtained with open systems. Furthermore, soils in closed systems were incubated for 28 days as opposed to 14 days with open systems. However, soil amendments did alter the fate of both [^{14}C]fonofos and [^{14}C]parathion in soils incubated in closed systems. Although analytical data for benzene-soluble and water-soluble radiocarbon are available, the most significant effects of the soil amendments were noticed in the production of $^{14}\text{CO}_2$ and of bound ^{14}C residues and are, therefore, reported here. Data presented in Table IV show the recoveries of $^{14}\text{CO}_2$ and of bound ^{14}C compounds for controls in percent of the amounts of ^{14}C -labeled insecticides applied to soil. However, data pertaining to the effects of the various soil amendments are expressed relative to controls and were calculated as ^{14}C recoveries in percent of those determined for controls (=100%). Production of $^{14}\text{CO}_2$ from the ^{14}C -labeled insecticide treated soils was significantly inhibited by all amendments. With [^{14}C]fonofos this inhibition was most pronounced in the presence of captafol (2.4% of control or 97.6% inhibition) and cow manure (7.8% of control or 92.2% inhibition). With [^{14}C]parathion, 95.7% of inhibition was noticed with cow manure, sewage sludge,

or captafol. Since microorganisms affect the persistence of insecticides in soils (Lichtenstein and Schulz, 1964) and also the formation of unextractable soil-bound insecticide derivatives (Katan and Lichtenstein, 1977), it appears that the soil amendments indirectly inhibited the production of CO_2 by directly affecting soil microorganisms. No lipid-soluble volatile ^{14}C was found in the polyurethane traps.

The production of soil-bound ^{14}C residues was also affected by the amendments (Table IV). With [^{14}C]fonofos, less binding occurred in the presence of sewage sludge and captafol and more with atrazine. With [^{14}C]parathion, however, increased binding was noticed with cow manure and sewage sludge and decreased binding with the remaining amendments.

Data, therefore, indicate that the two insecticides were affected in different ways. This makes it difficult or impossible to draw general conclusions from results obtained with one particular chemical.

Effects of Captafol and Atrazine on Populations of Microorganisms in [^{14}C] Parathion-Treated Soil. Populations of fungi or bacteria in parathion-treated soils (controls) and in soils containing also captafol or atrazine were estimated by using selective agar media. After a 3-day incubation period, no fungal growth had occurred on plates inoculated with microorganisms from soils that had also been treated with captafol or atrazine, but bacterial colonies had appeared (Figure 4). The fact that atrazine also inhibited fungal growth was somewhat surprising, although this phenomenon has been reported. As stated by Kaiser et al. (1970) "triazines have highly variable effects on the soil microflora. They have no action at times (four authors), they stimulate the microflora of certain species (six authors), or inhibit fungi (eight authors)" Results seem to indicate that soil fungi were involved in the effects exhibited by captafol and atrazine on the fate of parathion in the soils. No attempt was made to identify fungal or bacterial species.

Effects of Soil Amendments on the Activity of [^{14}C]Glucose-Degrading Soil Microorganisms. [^{14}C]-Glucose was rapidly degraded to $^{14}\text{CO}_2$ in nonsterilized soils but not in irradiated soils (Figure 5). Two days after soil treatment $16.12 \pm 0.3\%$ of the radiocarbon applied to control soils was released as $^{14}\text{CO}_2$ but only $0.48 \pm 0.1\%$ from irradiated soils. Four days later (day 6), these figures were $32.4 \pm 1.2\%$ and $3.0 \pm 0.03\%$, respectively. After that, a rapid increase in $^{14}\text{CO}_2$ production took place in the irradiated soils, similar to the end of a lag phase and the beginning of a logarithmic growth phase of soil microorganisms. After 18 and 28 days of incubation the amounts of $^{14}\text{CO}_2$ released from control soils were $45.4 \pm 0.9\%$ and $49.6 \pm 1.3\%$ of the applied [^{14}C]glucose and from irradiated soils $32.8 \pm 0.1\%$ and $39.8 \pm 0.1\%$, respectively. Tests conducted at that time with the irradiated soils showed that they were no longer sterile. The production of $^{14}\text{CO}_2$ in these irradiated soils indicates that microorganisms developed and grew along lines of a typical microorganism population growth curve. It appears, therefore, that the degradation of [^{14}C]glucose to $^{14}\text{CO}_2$ was related to the activity of soil microorganisms.

Figure 4. Fungal (A and B) and bacterial (C and D) growth on nutrient media inoculated with microorganisms from soils treated with parathion (controls) or parathion plus Difolatan (captafol) or atrazine.

Figure 5. Evolution of $^{14}\text{CO}_2$ from soil applied ^{14}C glucose in soils sterilized by γ irradiation and in nonirradiated soils (controls).

Figure 6. Effects of fertilizers (CM = cow manure; SL = sewage sludge; NH = ammonium sulfate), Difolatan (captafol) (DI), and atrazine (AT) on the evolution of $^{14}\text{CO}_2$ from soil applied ^{14}C glucose during a 31-day incubation period. None = controls (soil treated only with ^{14}C glucose). Differences observed from controls are significant at the 0.02% level (Student's t test) except for ammonium sulfate.

The effect of soil amendments on the degradation of ^{14}C glucose in soils was studied with closed systems (Figure 1B), and the production of $^{14}\text{CO}_2$ was used as the criterion to appraise these effects. Results (Figure 6) indicated that cow manure and sewage sludge significantly (at the 0.1% level) decreased the production of $^{14}\text{CO}_2$ from $43.4 \pm 0.6\%$ in controls to $30.5 \pm 1.4\%$ and $36.1\% \pm$

0.5% of the applied radiocarbon, respectively. Conversely, captafol and atrazine significantly increased the production of $^{14}\text{CO}_2$ to $56.9 \pm 0.4\%$ and $57.3 \pm 3.3\%$, respectively. Ammonium sulfate in [^{14}C]glucose-treated soils, however, had no effect. Soil amendmens apparently affected certain soil microorganisms that were responsible for the breakdown of [^{14}C]glucose to $^{14}\text{CO}_2$. It is conceivable, therefore, that the effects of soil amendments on the fate of fonofos or parathion in soils also took place via an effect on soil microorganisms, although different groups might have been involved.

Results obtained with the insecticides under the different environmental conditions do show that soil amendments affect the persistence, metabolism, and translocation of both fonofos and parathion in soil and plants but the nature of these effects was different for each insecticide and no general conclusions can be draw. It appears that the effects of some amendments occurred via a direct effect on soil microorganism populations that in turn are responsible for the degradation of the insecticides. In addition, organic fertilizers such as cow manure and sewage sludge are not of a specific, fixed chemical and microbiological composition. Their qualitative and quantitative makeup could be dependent on the source of the fertilizer and possibly on the food of the producing organism at a particular time. The major fact, however, is that the soil amendments did in one way or another change the fate and metabolism of the insecticides under the experimental conditions employed. It is for these reasons that the behavior and fate of pesticide chemicals in the agricultural environment should be studied by taking various ecological factors into consideration.

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(f) EFFECTS OF ORGANIC FERTILIZERS ON THE FATE OF ^{14}C CARBOFURAN IN AN AGRO-MICROCOSM UNDER SOIL RUN-OFF CONDITIONS

Abstract.

The effects of cow manure and sewage sludge on the fate of ^{14}C carbofuran applied to soil were investigated in a run-off microcosm. Both manure and sludge reduced the uptake of ^{14}C carbofuran and its metabolites from soils into oat plant tissue. Movement of ^{14}C carbofuran-derived ^{14}C also occurred in the soil, because 22 to 25% of radiocarbon recovered from the soil was associated with the lower originally untreated soil layer. After two applications of "rain" 6.4 to 7.0% of soil-applied ^{14}C had been carried off with the run-off soil and water, but only 3.3 to 3.7% of soil-applied ^{14}C were associated with the aquatic component at the end of the experiment. Of these ^{14}C residues, more than half were recovered from the bottom soil-mud sediments. Run-off water and aquaria water were not toxic to *Aedes aegypti* L. mosquito larvae. Carbofuran was the major compound detected in terrestrial soils, whereas 3-hydroxycarbofuran was the major compound detected in oat leaves. In all experiments with manure-amended soil, an increased degradation of ^{14}C carbofuran was noticed as evidenced by recoveries of reduced amounts of organic-soluble ^{14}C , by an increased evolution of $^{14}\text{CO}_2$ (144% of control) and by an increased production of bound ^{14}C residues. Apparently, manure microorganisms were responsible for the enhanced ^{14}C carbofuran degradation in soil, based on increased carbofuran recoveries and formation of reduced amounts of bound ^{14}C residues and $^{14}\text{CO}_2$ in soils previously mixed with sterilized manure.

Introduction.

Since the introduction of synthetic pesticides more than 35 years ago, movement of pesticides from their place of original application has presented an important environmental problem. The movement of pesticides occurs to a large extent with water, but it also occurs in vapor form, depending on the water solubility and vapor pressure of the particular pesticide. In addition, many pesticides or their usually noninsecticidal degradation products translocate into plants grown in pesticide-treated soils. The movement of pesticides in or through soil can occur during heavy rainfalls in the form of soil run-off or in solution, depending on the water-solubility of a particular compound, thus resulting in percolation of pesticides through soils. To investigate phenomena of pesticide movement from insecticide-contaminated soils, microcosms were developed in our laboratory for the study of soil run-off (Lichtenstein et al. 1978) and of water contamination via percolation of insecticides through soils

(Lichtenstein et al. 1974, Koeppe and Lichtenstein 1982).

Previous investigations with fonofos and parathion (Lichtenstein et al. 1982) were designed to determine the effects of cow manure and sewage sludge on the fate of insecticides in soil-plant systems. The present study represents a continuation of our efforts and was conducted to investigate the effects of these organic fertilizers on the fate of soil-applied ^{14}C carbofuran under run-off conditions in a soil-plant-water microcosm.

Materials and methods.

Chemicals. ^{14}C carbofuran (benzene- $^{14}\text{C}(\text{U})$ 2,3 dihydro 2,2 dimethyl 7 benzo furanyl methylcarbamate) (sp. act. 26.73 mCi/mM), carbofuran, 3-hydroxy carbofuran, 3-ketocarbofuran, 3-hydroxycarbofuran phenol, 3-ketocarbofuran phenol, and carbofuran phenol were obtained through the courtesy of FMC Corp. (Middleport, N.Y.). ^{14}C -Ring-carbofuran was diluted with nonradioactive insecticide to 0.41 mCi/mM before its addition to soil. Solvents used were redistilled acetone, benzene, dichloromethane, methyl cellosolve, dioxane, toluene, ethyl ether and methanol.

Soils. A Plano silt loam (4.7% organic matter/5% sand/71% silt/24% clay, pH 6.), free of insecticide residues, was collected at the University of Wisconsin Experimental Farm near Madison, Wis., and stored at $22 \pm 2^\circ\text{C}$ in a moist condition before use. Lake mud (12.5% organic matter/37% sand/57% silt/6% clay, pH 7.4) collected from Lake Mendota, Madison, Wisc., at a depth of 9 m, was drained of excess water and stored under refrigeration.

Fig. 1. Flow sheet depicting experimental procedures.

Organic Fertilizers. Fresh cow manure was obtained from dairy cows kept at the Dairy Science Department, University of Wisconsin, Madison. Anaerobically digested sewage sludge was obtained from the Madison, Wis., Metropolitan Sewage Treatment Plant. The manure and sludge were air-dried, ground to a fine powder with a Wiley Mill and stored at 4°C. All experiments were conducted with the same batch of manure or sludge.

Soil Treatment. Studies described here deal with the potential effects of cow manure and sewage sludge on the movement and metabolism of soil-applied ^{14}C carbofuran in soil, plant, and water systems. Loam soils were treated with an acetone solution of ^{14}C carbofuran, as described below under experimental procedures. After the initial insecticide concentration in the soil had been determined, data collected during the study were reported as percentages of the initially determined dose and were statistically analyzed by Student's *t* test. Cow manure or sewage sludge were mixed with the insecticide-treated soils at a ratio of 4.4 g (dry powder) per 100 g (dry wt) soil, which is equivalent to 100 metric ton/ha (Doyle et al. 1978).

Oat Plants. Oat seeds (*Avena sativa*), purchased from a local seed dealer, were pregerminated for 72 h before they were planted in insecticide-treated soils.

Elodea and Mosquito Larvae. *Elodea nuttallii* (Planch.) St. John, obtained from the Dept of Botany, University of Wisconsin, Madison, were grown in a 38 liter aquarium containing tap water and a 2-cm bottom deposit of insecticide-free loam soil. Mosquito eggs (*Aedes aegypti* L.) were obtained from Raltech Scientific Services, Inc., Madison, and the larvae were reared in our laboratory as needed.

Description of Microcosm. We used the soil-plant-water microcosm described by Lichtenstein et al (1978). Its terrestrial component consisted of two stainless steel containers, each 10 by 22 by 10 cm. Each container was filled with 200 g (dry wt) ^{14}C carbofuran-treated soil (4.18 ppm, 1.56 uCi) and placed on top of 800 g (dry wt) insecticide-free soil, because under agricultural conditions pesticide residues are primarily located in the upper soil layers. The aquatic component of the microcosm consisted of two glass aquaria (each 16 cm deep by 10 cm wide by 14 cm long and 2-liter capacity) into which a bottom layer of 500 g moist lake mud was placed before water was added.

Extraction and Analyses. Soils, plant material, and water were extracted and analyzed as described by Koeppel and Lichtenstein (1982). Presence of $^{14}\text{CO}_2$ in KOH traps was determined by liquid scintillation counting (LSC). The run-off soil from each container was air-dried, weighed and ca. 20% were combusted to $^{14}\text{CO}_2$ to determine the radiocarbon content. The run-off water was mixed well and a 122 ml aliquot was removed for analyses by LSC (2 by 1 ml) and extraction with dichloromethane (100 ml). Twenty milliliter samples of run-off water and water from the aquaria were also bioassayed with *Aedes aegypti* mosquito larvae as described by Lichtenstein et al. (1974).

Experimental Procedures. Effects of Cow Manure or Sewage Sludge on Fate of ^{14}C Carbofuran in a "Run-off" Soil-plant-water Microcosm. In a recent study with a

"percolation microcosm," Koeppe and Lichtenstein (1982) demonstrated that carbofuran was metabolized and transported through soils. The present investigation, however, was designed to study the movement and metabolism of this insecticide under soil run-off conditions.

Procedures used in our experiments are outlined in Fig. 1. A total of 1,600 g (dry wt) of Plano silt loam was treated as described by Lichtenstein and Schulz (1959) in two, 800-g (dry wt) portions, each with 32 ml of an acetone solution containing 3.35 mg (6.25 uCi) of ^{14}C carbofuran. This resulted in an insecticide concentration of 4.18 ppm on a dry weight soil basis. As indicated in fig. 1, all experiments were conducted in duplicate; soils in two of these containers served as controls (containers I and II). The upper 200 g of insecticide-treated soil in containers III and IV, however, were mixed with 8.8 g (dry powder) of cow manure; those in containers V and VI were mixed with 8.8 g (dry powder) of sewage sludge. These six containers were then held for 7 days in a growth chamber at 28°C during the day (16h) and 21°C at night. Soil moisture was kept constant by determining daily the loss of water and then replacing it. Because the ^{14}C carbofuran-treated soils were aged for 1 week, differences in the amounts of soil-bound ^{14}C residues produced under the three experimental conditions might be reflected in the amounts of radiocarbon taken up by the plants. Seven days after soil treatment, ca. 400 oat seedlings were planted in the soil surface of each of the six containers. The containers were again placed in the growth chamber for an additional 15 days (days 7-22). Soils were kept moist by alternately adding water or Hoagland's nutrient solution (Hoagland and Arnon 1938) on a daily basis.

On day 14 (Fig. 1), artificial rain was applied as described by Lichtenstein et al. (1978) to soils in all six containers. A total of 1,875 ml of water (0.85 in. or 2.1 cm of rainfall) covered, in cone form, two soil containers which were held at a slope of 5° . This resulted in a run-off of 610, 610, and 630 ml of water from control, manure, or sludge-treated soils, respectively. Although the amounts of run-off water were similar under all conditions, the amounts of soil carried with the water varied, amounting to 27.4 ± 2.1 , 8.3 ± 2.5 , and 8.8 ± 0.9 g from control, manure, or sludge-treated soils, respectively. Run-off water and soil were separated by vacuum filtration through Whatman No. 1 filter paper. A 120 ml aliquot of the water was removed for analyses while the remainder from each container was carefully poured into the appropriate 2-liter aquaria (containers I-VI) into which 500 g (fresh wt) lake mud had previously been deposited. After 1 or 2 h, when the lake mud had settled, the remaining run-off soil, from which 20% had been removed for analysis, was carefully added to the appropriate aquaria.

On day 21,, a second rain was applied to the soils in each container. Run-off water and soil were collected and placed into the appropriate aquaria as described previously. A total of 2,500 ml of water (1.11 in or 2.84 cm of rainfall) in cone form covered two soil containers, which resulted in a run-off of ca. 900 ml from each container. The amounts of soil which were carried with

the water were 8.8 ± 1.1 , 2.6 ± 1.4 , and 7.5 ± 0.6 g from control, manure, and sludge-treated soil, respectively. On day 23, after soil from the second run-off had settled in the aquaria, sixty 3.5 cm pieces of Elodea plants were suspended with thread in the water of each aquarium ca. 4-5 cm above the lake mud, thus preventing the roots from touching the bottom layer of sediments. Tap water was added as necessary to replace evaporated water. The aquaria were then further incubated for 19 days (to day 44, Fig. 1) under a bank of Gro-Lux lamps on a photoperiod of LD 16:16 at room temperature.

Twenty-two days after soil treatment, the terrestrial component of the microcosm was dismantled and separated into soil, roots, and leaves as described by Koeppe and Lichtenstein (1982). The soils were cut horizontally into two equal portions, and 150-g aliquots from each were analyzed separately. Twenty-five grams (fresh wt) of oat greens and the total portion of roots were extracted and analyzed as described above. On day 44, the aquatic component of the microcosm was also dismantled and separated into soil-lake mud sediments, water and Elodea plants. Water (500 ml), 150 g of sediments and all Elodea plants were extracted and analyzed as described above.

Effects of Manure on the Fate of ^{14}C -Ring-carbofuran in a Closed Soil-plant System. At the end of the experiments described above, 38% of the soil-applied radiocarbon could not be accounted for. To obtain a total ^{14}C balance, a closed system with soils and oats was set up under bell jars as described by Anderegg and Lichtenstein (1981). A total of 450 g (dry wt) loam soil was treated with 25 ml of an acetone solution containing 1.86 mg (3.48 uCi) of ^{14}C carbofuran. This resulted in an insecticide concentration of 4.14 ppm on a dry weight soil basis. Eighty-five grams (dry wt) of the ^{14}C carbofuran-treated soil (4.14 ppm, 0.66 uCi) were then placed on top of 250 g (dry wt) untreated soil in each of four cartons (9 by 8.6 cm ID). Soils in two of these cartons served as controls, while 3.8 g (dry powder) of cow manure were mixed with the 85 g of insecticide-treated soil in each of the remaining two cartons. All cartons were placed under bell jars and $^{14}\text{CO}_2$ was collected as described by Anderegg and Lichtenstein (1981). The soils were then incubated under a bank of Gro-Lux lamps on photoperiod of LD 16:16 at $23 \pm 1^\circ\text{C}$. Twenty-five oat seedlings were planted in the soil surface of each of the four cartons after 7 days of incubation. Distilled water was added to each soil as necessary, and all systems were incubated under bell jars for an additional 15 days. On day 22, the soil-plant systems were dismantled and separated into soil, oat roots, and leaves as described by Anderegg and Lichtenstein (1981). All roots were air-dried, weighed and combusted to $^{14}\text{CO}_2$ as described above. Two oat leaves were removed for moisture determination while the remainder were extracted and analyzed. All 335 g of soil from each carton were mixed well and a 100-g aliquot was removed for moisture determination while a second 100-g aliquot was extracted and analyzed.

Effects of Microorganisms in Cow Manure on ^{14}C Carbofuran Persistence in Soil. Because the presence of cow manure in ^{14}C carbofuran-treated soil did result in

a more rapid degradation of the insecticide, an experiment was conducted to determine whether microorganisms in manure might play a role. Sterilized manure was mixed with ^{14}C carbofuran-treated loam soil to determine whether the absence of these microorganisms might alter the fate of the insecticide in soil.

Table 1. Effects of cow manure and sewage sludge on the fate of ^{14}C -ring carbofuran in a soil-plant-water microcosm (Results are averages of duplicated tests).

	Terrestrial soil treated with ^{14}C -ring-carbofuran at 4.2 ppm ^a plus:					
	None		Manure ^b		Sludge ^b	
	Total sample	Per g wt ^c	Total sample	Per g wt	Total sample	Per g wt
^{14}C Recovered in % of applied to upper soil layer ^{ae}						
Terrestrial part						
Oats (O)						
Greens	18.9 ± 1.5	0.41	13.4 ± 2.1	0.26*	19.5 ± 1.5	0.30*
Roots	4.6 ± 0.4	0.14	4.0 ± 0.5	0.12	4.9 ± 0.5	0.13
Total (O)	23.5 ± 1.4		17.4 ± 2.0		24.4 ± 0.5	
Soil (S)						
Upper (U) ^a	26.4 ± 1.4	0.06	32.0 ± 2.7	0.07	28.0 ± 0.6	0.06
Lower (L) ^a	9.1 ± 0.9	0.02	8.9 ± 2.0	0.01	8.1 ± 0.3	0.01
Total U + L (S)	35.5 ± 0.5		40.9 ± 0.7**		36.1 ± 0.2	
Total (O + S)	59.0 ± 0.9		58.3 ± 0.7		60.5 ± 0.7	
Run-off 1						
Water (W)	3.6 ± 0.0	0.006	5.2 ± 0.5*	0.009	4.8 ± 0.9	0.008
Soil (S)	2.3 ± 0.2	0.083	0.5 ± 0.2**	0.064	0.6 ± 0.1**	0.071
Total W + S	5.9 ± 0.2		5.7 ± 0.5		5.4 ± 1.1	
Run-off 2						
Water (W)	0.6 ± 0.0	0.001	0.9 ± 0.2	0.000	0.5 ± 0.1	0.001
Soil (S)	0.5 ± 0.1	0.057	0.2 ± 0.2	0.086	0.5 ± 0.0	0.064
Total W + S	1.1 ± 0.1		1.1 ± 0.1		1.0 ± 0.1	
Total Run-off (1 + 2)						
Water (W)	4.2 ± 0.0		6.1 ± 0.3**		5.3 ± 0.8	
Soil (S)	2.8 ± 0.1		0.7 ± 0.1***		1.1 ± 0.1**	
Total W + S	7.0 ± 0.1		6.8 ± 0.3		6.4 ± 0.7	
Aquatic part						
Elodea (E)	0.6 ± 0.0	0.113	0.6 ± 0.0	0.100	0.5 ± 0.0	0.093
Water (W)	0.6 ± 0.1	0.000	0.9 ± 0.1	0.001	0.9 ± 0.2	0.001
Soil + Lake Mud (S)	2.1 ± 0.2	0.005	2.2 ± 0.4	0.005	1.9 ± 0.1	0.005
Total (E + W + S)	3.3 ± 0.2		3.7 ± 0.5		3.3 ± 0.3	
Total						
Terrestrial (T)	59.0 ± 0.9		58.3 ± 0.7		60.5 ± 0.7	
Aquatic (A)	3.3 ± 0.2		3.7 ± 0.5		3.3 ± 0.3	
T + A	62.3 ± 0.7		62.0 ± 1.8		63.8 ± 0.4	

^a ^{14}C -Ring-carbofuran applied to upper 200 g (dry wt) of soil and placed on top of 800 g (dry wt) of untreated soil. Recovery data, however, refer to the upper ½ (U) and the lower ½ (L) layer of soil.

^b 8.8 g (dry powder) of cow manure or sewage sludge was mixed with the upper 200 g (dry wt) of soil.

^c Per g fresh weight for plants, dry weight for terrestrial soils, moist weight for lake mud, or per milliliter of water.

^d Data based on extraction analysis except run-off soils were analyzed by combustion and LSC and run-off water 2 by direct LSC analysis.

^e Results are significantly different from controls at the 5% (*), 1% (**), or 0.1% (***) level (Student's *t* test).

Microorganisms were eliminated from manure by γ irradiation (45,000 rads for 70 h) as described for soil by Katan et al. (1976). The sterility of the manure was confirmed by incubating samples for 3 days in glucose-yeast extract medium. The experiment was conducted in such a way that a total of 100 g (dry wt) soil was treated with ^{14}C carbofuran at 4.5 ppm. Ten grams of ^{14}C carbofuran-treated soil (0.08 uCi) were placed into each of 6 glass vials (78 by 23 mm ID). Soils in two vials served as controls, while cow manure (0.45 g dry powder) was mixed with each soil in two additional vials. Soils in the remaining two vials were mixed with 0.45 g (dry powder) of sterilized cow manure. Each of the six vials was closed with a rubber stopper with holes for air inlet and outlet. Soils were incubated in the dark at $23 \pm 1^\circ\text{C}$ for 10 days. Evolved $^{14}\text{CO}_2$ was collected by passing air through the vials and then through 0.1 N KOH ($^{14}\text{CO}_2$ trap) for 2 h (at 15 ml per min) 1, 2, 4, 7, 9 and 10 days after soil treatment. On day 10, all soils were extracted and analyzed as previously described.

Results and discussion.

Effects of Cow Manure or Sewage Sludge on the Fate of ^{14}C Carbofuran in a "Run-off" Soil-plant-water Microcosm. Results obtained after analyses of the different components of the run-off microcosm show that, under all experimental conditions, considerable amounts of carbofuran-derived radiocarbon were taken up by oat plants (Table 1). Approximately 77 to 80% of the ^{14}C detected in oats was associated with leaves. Total ^{14}C residues recovered from oat greens were similar under the three experimental conditions. Expressed on a per gram fresh weight basis, however, the presence of fertilizers in these soils resulted in a significant reduction of ^{14}C uptake by the oats. An appreciable movement of carbofuran-derived ^{14}C from the upper insecticide-treated soil layer into the lower, untreated soil layer had also occurred because, under all experimental conditions, 22 to 25% of the total radiocarbon recovered from the soil was associated with the lower soil layer. This mobility was probably related to the relatively high water solubility (320 ppm) of carbofuran. Carbofuran-derived ^{14}C compounds were more persistent in soil amended with cow manure, as indicated by recoveries of 41 and 36% of all the soil-applied ^{14}C from manure-amended and control soils, respectively.

After two applications of "rain", 6.4 to 7.0% of all soil-applied ^{14}C had been carried with the combined run-off soil plus water under the three experimental conditions. However, run-off soil from manure- or sludge-treated soils contained significantly less ^{14}C than soil from control experiments. Soil amended with cow manure or sludge appeared to adhere or "stick together" and thus less soil had been carried with the run-off water under these conditions (Table 1). In experiments conducted under percolating or leaching conditions (Koeppel and Lichtenstein 1982), considerably more ^{14}C compounds (49% of the soil-applied radiocarbon) had been removed from the soil with water than had occurred under the above described soil run-off conditions.

At the end of the experiment, the aquatic components contained 3.3 to 3.7%

of the soil-applied ^{14}C , although 6.4 to 7.0% had initially been placed into aquaria (Table 1). These losses of radiocarbon were most likely due to the evolution of $^{14}\text{CO}_2$ or other volatile ^{14}C compounds. More than half of the radiocarbon recovered from the aquatic system was associated with the soil-lake mud sediments while the distribution of the remainder was similar between Elodea plants and the water. Addition of manure or sludge to insecticide-treated soil had no effect on the amounts and distribution of ^{14}C within the aquatic system.

Further analyses of benzene and water extraction phases as well as

Table 2. ^{14}C Carbofuran and metabolites recovered from the organic-soluble extraction phases^a of terrestrial soils^b and oat greens

Soils treated with ^{14}C carbofuran plus:							
	None		Manure		Sludge		
Organic-soluble ^{14}C recovered from oats and soil, % of soil-applied $^{14}\text{C}^c$							
	Oat greens	Soil ^b	Oat greens	Soil	Oat greens	Soil	
Rf	7.9±0.3	14.5±0.2	6.6±0.4	9.8±0.1 ^d	10.0±0.5 ^e	16.2±0.2 ^d	
(Compounds isolated, ^f in % of soil-applied $^{14}\text{C}^c$)							
Carbofuran	0.46	0.2	6.9	0.2	5.3	0.4	8.6
3-Keto CF	0.39	1.9	4.1	0.7	2.0	1.3	4.8
3-OH CF	0.05	4.3	0.3	3.9	0.4	6.0	0.3
3-Keto CFP	0.55	0.5	2.8	0.7	1.8	0.9	2.0
Others		1.0	0.4	1.1	0.3	1.5	0.5

^a Organic, total of benzene plus dichloromethane extraction phases. ^b Results obtained from soils are the total ^{14}C recovered from the upper + lower layers of soils. ^c ^{14}C carbofuran was applied to the upper 200 g (dry wt) of soil at 4.2 ppm (1.56 uCi) on top of 800 g (dry wt) of untreated soil. ^d Results are significantly different from controls at the 1% level (Student's t test). ^e Results are significantly different at the 5% level (Student's t test).

^f Compounds identified were CF, carbofuran; 3-keto CF, 3-ketocarbofuran; 3-OH CF, 3-hydroxycarbofuran; 3-keto CFP, 3-ketocarbofuran phenol; others, carbofuran phenol, 3-hydroxycarbofuran phenol, and unknowns I, II, and III.

quantification of unextractable, bound ^{14}C residues showed that, in manure amended soils, bound ^{14}C residues amounted to $26.9 \pm 0.4\%$ of the originally applied radiocarbon but only to $17.1 \pm 0.7\%$ in control soils. Sludge amended soils, however, contained bound ^{14}C residues similar to those determined with

control soils. Conversely, organic-soluble ^{14}C in manure-amended soils amounted to only $9.8 \pm 0.1\%$ of the applied radiocarbon, compared with $14.5 \pm 0.2\%$ in control soils and to $16.2 \pm 0.2\%$ in sludge-amended soils. Therefore, these data indicate that, in the presence of cow manure, reduced amounts of organic-soluble radiocarbon and increased amounts of bound ^{14}C residues were recovered. The amounts of organic-soluble, water-soluble and bound ^{14}C residues in oat leaves were not affected by the addition of manure to soil. In the presence of sludge, however, more organic-soluble ^{14}C ($10.0 \pm 0.5\%$ of soil-applied) was recovered

Table 3. Fate of ^{14}C -ring-carbofuran in a closed soil-plant system as affected by cow manure soil amendments (Results are averages of duplicated tests).

^{14}C Carbofuran-treated soil plus:		
	None	Manure ^a
	(^{14}C Recovered, % of applied to soil ^b)	
Soil(s)		
Organic ^c	57.5 ± 0.9	$29.2 \pm 0.2^{***d}$
Water	3.3 ± 0.2	$5.9 \pm 0.4^*$
Bound ^c	14.9 ± 0.1	$31.4 \pm 0.4^{***}$
Total	75.7 ± 1.0	$66.5 \pm 0.7^{**}$
Oat greens		
Organic	2.9 ± 0.2	2.1 ± 0.2
Water	1.7 ± 0.0	1.8 ± 0.2
Bound	1.5 ± 0.0	$1.9 \pm 0.1^*$
Total	6.1 ± 0.2	5.8 ± 0.4
Oat roots	2.7 ± 0.1	3.1 ± 0.2
Total oats (O)	8.8 ± 0.1	8.9 ± 0.6
Volatiles (V)		
$^{14}\text{CO}_2$	8.8 ± 0.3	$13.2 \pm 0.6^{**}$
Lipid solubles ^e	0.0 ± 0.0	0.0 ± 0.0
Total (S + O + V)	93.4 ± 0.8	$88.7 \pm 0.7^*$

^a Cow manure (3.8 g, dry wt) was mixed with the upper 85 g of soil.

^b Soil (85 g, dry wt), treated with ^{14}C carbofuran at 4.14 ppm (0.66 uCi), was placed on top of 250 g untreated soil and incubated for 22 days. Results obtained are reported as recovery from total soil ^c Organic, total of benzene + Dichloromethane extraction phase. Bound, unextractable ^{14}C residues. ^d Results are significantly different from control (none) at 5% (*), 1% (**), or 0.1% (***) level (student's t test). ^e ^{14}C trapped in polyurethane.

from oat leaves than from those which had grown in control soils ($7.9 \pm 0.3\%$ of soil-applied). This finding was probably related to the recovery of larger amounts of organic-soluble ^{14}C in sludge-treated soils compared to control soils. Analyses of the aquatic components of the microcosm showed that treatment of terrestrial soils with manure or sludge resulted in a reduction of organic-soluble ^{14}C in the soil-lake mud sediments.

To identify potential ^{14}C carbofuran metabolites in terrestrial soils and oats, organic-soluble ^{14}C compounds were analyzed by thin-layer chromatography (TLC), autoradiography, and LSC. Because aquatic components contained very little organic-soluble radiocarbon, no further analyses were performed. As shown in Table 2, carbofuran was the major compound detected in unamended control, manure and sludge-amended soils (upper + lower layers), amounting to 6.9, 5.3, and 8.6% of the soil-applied ^{14}C , respectively. Metabolites of carbofuran in these soils included 3-ketocarbofuran, 3-ketocarbofuran phenol, and 3-hydroxy carbofuran. Although only small amounts of 3-hydroxycarbofuran were detected in soils, this compound was the primary constituent in oat leaves which were grown in these soils. Conversely, only small amounts of carbofuran were recovered from these leaves. Other metabolites of carbofuran in the oat leaves included 3-ketocarbofuran and 3-ketocarbofuran phenol. Under all experimental conditions, no major differences in the metabolism of carbofuran in oat leaves could be observed. Oat roots did not contain enough organic-soluble radiocarbon for TLC analyses, and therefore no data are available. Data for run-off water are not presented in Table 2. However, 2.7 to 4.0% of soil-applied ^{14}C present in the first runoff water was recovered as carbofuran.

Lichtenstein et al. (1979) reported that LD_{50} 's for *Aedes aegypti* larvae were 0.22, 2.5, 15, 30, 75, and 200 ppm with carbofuran, 3-hydroxycarbofuran, 3-ketocarbofuran, carbofuran phenol, 3-hydroxycarbofuran phenol, and 3-ketocarbofuran phenol, respectively. *A. aegypti* bioassays, conducted in our experiments with run-off water collected after application of "rain" on days 14 and 21, and with water from the aquaria, did not result in insect mortality after 24- and 48-h exposure periods. Thus, the concentration of carbofuran and each of its metabolites in these waters was most likely below the LD_{50} 's listed above.

Effects of Manure on the Fate of ^{14}C Carbofuran in a Closed Soil-plant System.

Because a total of only 62 to 64% of the applied ^{14}C could be accounted for in all experiments (T + A, bottom of Table 1) we assumed that part of the originally applied ^{14}C carbofuran had been degraded to $^{14}\text{CO}_2$. To verify this assumption, experiments were conducted with soil-plant systems held under bell jars. However, data obtained from these closed systems cannot be directly compared to data obtained with open systems because of different plant growing conditions and, in particular, due to reduced rates of leaf transpiration in closed systems (Anderegg and Lichtenstein 1981). Table 3 summarizes results obtained after analyses of soils, oats and vapor traps. Total amounts of radiocarbon recovered after the 22-day incubation period were 93 and 89% of the

soil-applied ^{14}C from control and manure-amended soil-plant systems, respectively, of which 9 and 13% had been released in the form of $^{14}\text{CO}_2$. Polyurethane traps did not contain detectable amounts of radiocarbon. In addition, an increased degradation of ^{14}C carbofuran was noticed in manure-amended soils, as evidenced by increased amounts of water-soluble ^{14}C compounds (181% of control), increased evolution of $^{14}\text{CO}_2$ (144% of control), and an increased production of bound ^{14}C residues (210% of control). Lichtenstein et al. (1982) also reported an increased formation of water-soluble and bound ^{14}C residues in ^{14}C fonofos-treated soils that had been amended with cow manure.

Table 4. Effects of manure microorganisms on the fate of ^{14}C carbofuran in soil within a closed system (Results are averages of duplicated tests).

Soil treated with ^{14}C -ring-carbofuran plus:			
	sterile manure ^b (^{14}C recovered, % of soil-applied radiocarbon) ^d	Manure ^c	None
Soil (S)			
Benzene: ^{14}C	85.2 $\pm$ 1.2*	80.2 $\pm$ 0.6	87.2 $\pm$ 1.2
CF ^e	73.5	63.7	80.9
CH ₂ Cl ₂	9.8 $\pm$ 2.2	8.7 $\pm$ 1.6	6.4 $\pm$ 0.7
Water	1.1 $\pm$ 0.3	1.5 $\pm$ 0.3	0.5 $\pm$ 0.1
Bound ^f	2.9 $\pm$ 0.2**	6.1 $\pm$ 0.5	1.9 $\pm$ 0.0
Total	99.9 $\pm$ 1.2	96.5 $\pm$ 0.3	96.0 $\pm$ 0.5
Volatiles (V)			
$^{14}\text{CO}_2$	0.8 $\pm$ 0.1**	1.8 $\pm$ 0.1	1.0 $\pm$ 0.1
Lipid soluble ^{14}C	0.03 $\pm$ 0.0	0.02 $\pm$ 0.0	0.05 $\pm$ 0.0
Total (S + V)	99.8 $\pm$ 1.0	98.2 $\pm$ 0.4	97.0 $\pm$ 0.4

^a 10 g (dry wt) soil, treated with ^{14}C carbofuran at 4.5 ppm (0.08 uCi), was incubated for 10 days. ^b Manure was sterilized by irradiation (45,000 rads for 70 h). ^c 0.45 g (dry wt) cow manure was mixed with 10 g (dry wt) soil. ^d Results from irradiated manure-amended soils are significantly different from manure-amended soil at the 5% (*) or 1% (**) level (Student's t test). ^e CF, Carbofuran, as determined by TLC. ^f Bound, unextractable ^{14}C residues.

Effects of Manure Microorganisms on ^{14}C Carbofuran Persistence in Soil. As shown in Table 3, the presence of cow manure in ^{14}C carbofuran-treated soil increased the degradation of the insecticide. Results obtained from experiments conducted

with insecticide-treated soil to which sterilized or nonsterilized cow manure had been added and with insecticide-treated soil without amendments are summarized in Table 4. Data indicate that the elimination of manure microorganisms by γ irradiation resulted in a reduction of the degradation of ^{14}C carbofuran in soils as evidenced by a higher recovery of organic-soluble ^{14}C compounds and a reduced production of bound ^{14}C residues as well as of $^{14}\text{CO}_2$. Separation, identification and quantitation of benzene-soluble ^{14}C by TLC, autoradiography, and LSC showed that carbofuran amounted to 74% of all the radiocarbon in soils mixed with sterilized manure, but only to 64% in soils mixed with nonsterilized manure.

Determination of the production of $^{14}\text{CO}_2$ during the 10-day incubation period also suggests that manure microorganisms were involved in the degradation of the insecticide to CO_2 (Fig. 2). In soils containing sterilized manure, the rate of $^{14}\text{CO}_2$ evolution was initially inhibited in comparison to controls (no manure), but started to increase one week later, finally resulting in $^{14}\text{CO}_2$ recoveries which were similar to controls. In soils to which nonsterilized manure had been added, however, $^{14}\text{CO}_2$ evolution during the first 6 days of incubation was similar to controls, but after that time increased considerably. At the end of the 10-day incubation period, the amounts of $^{14}\text{CO}_2$ released from nonsterilized manure-treated soils were double of that observed with controls.

Results of these experiments indicate the importance of rainfall and the presence of organic fertilizers in soil on the movement and metabolic fate of ^{14}C carbofuran in a soil-plant environment.

Fig. 2. Degradation of ^{14}C carbofuran to $^{14}\text{CO}_2$ as affected by the sterilization of cow manure.

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(g) EFFECTS OF SOIL MIXING AND FLOODING ON THE FATE AND METABOLISM OF ^{14}C -FONOFOS AND ^{14}C -PARATHION IN OPEN AND CLOSED AGRICULTURAL MICROCOSMS¹

Abstract.

The effects of soil surface application and soil incorporation ("tillage") of ^{14}C -fonofos and ^{14}C -parathion on the metabolic fate of the insecticides in soils and their translocation into oat leaves were investigated in the laboratory, using both open and closed microcosms. The effects of flooding of ^{14}C insecticide treated soils were also studied. The insecticides were least persistent after their application to the soil surface, whereas mixing the insecticides with the soil increased the persistence of fonofos and parathion by factors of 2 and 5.3, respectively. Most of the organic solvent-soluble radiocarbon in ^{14}C insecticide-treated soils was recovered as the undegraded parent compound, more so with fonofos than with parathion. In oat leaves from fonofos-treated soils, most of the organic soluble ^{14}C recovered (1% or less of the soil-applied radiocarbon) was associated with the non-insecticidal fonofos metabolite methyl phenyl sulfone; in leaves from parathion-treated soil, > 90% of the organic soluble ^{14}C (0.1% of the soil-applied dose) was associated with parathion. In experiments with closed systems, the amounts of $^{14}\text{CO}_2$ produced from soil-applied ^{14}C -parathion were 30% of the insecticide applied to the soil surface, 23% of that mixed with the soil, and only 0.9% of that mixed with the soil and kept under flooded conditions. With fonofos-treated soils, less $^{14}\text{CO}_2$ was produced, but the general trend was the same as that observed with parathion. Binding of ^{14}C compounds to soils increased after the insecticides had been mixed with the soil and, in particular, after soil flooding when 72% of the soil-applied ^{14}C -parathion was unextractable.

Introduction

With decreased agricultural tillage practices, the effects of various environmental conditions on the fate of pesticide chemicals in soils should be reconsidered. Although in this respect a number of studies were conducted by several research groups, for reasons of brevity only a few pertinent data from our own studies are presented to illustrate the significance of soil cultivation and mode of insecticide application on the fate of pesticide chemicals in soils. Studies conducted in 1961 showed that, under field conditions, daily disking of a loam soil treated with aldrin or DDT at 4 lb/5-in. acre caused a reduction of 38% of the aldrin residues and 25% of the DDT residues during a 3-month period (Lichtenstein and Schulz 1961). Four months after a soil surface application of aldrin and heptachlor, residues ranged from 2.7 to 5.3% of the applied dosages, whereas incorporation of these insecticides into the upper soil layers increased the persistence of the residues by a factor of 10 (Lichtenstein et al. 1962). Field experiments conducted over an 11-year period indicated that soil cultivation resulted in a 76 to 82% reduction of aldrin and heptachlor residues in comparison with noncultivated soils (Lichtenstein et al. 1971). Phorate and

its metabolites were least persistent in field soils after the insecticide had been applied to the soil surface (half-life = 6 days), whereas treatment of the upper 10- to 12-cm soil layer increased the persistence by a factor of 5 (Lichtenstein et al. 1973).

Since field experiments had been conducted with nonlabeled insecticides, and in view of the importance of no-tillage practices, we conducted laboratory studies with ^{14}C -labeled fonofos and ^{14}C -labeled parathion applied to soil in both open soil-plant microcosms and closed systems as described (Lichtenstein et al. 1982). In this way, we were able to further investigate the effects of surface application or soil incorporation of the insecticides on the metabolic fate of the radiolabeled chemicals in these systems. The effects of flooding of ^{14}C insecticide-treated soil were also investigated.

Materials and Methods.

Chemicals. - [U-phenyl- ^{14}C]fonofos (Dyfonate) (specific activity, 17.1 mCi/mM) was obtained through the courtesy of Stauffer Chemical Co., Mountain View, Calif., and [2,6-phenyl- ^{14}C]parathion (specific activity, 2.2 mCi/mM) was purchased from the ICN Corp., Irvine, Calif. Specific activities indicated in the different experiments were obtained by dilution with nonradioactive fonofos or parathion. These insecticides were determined to be at least 97% pure by thin-layer chromatography (TLC). Nonradioactive fonofos or parathion and some of their potential metabolites were obtained from Stauffer Chemical Co. and from Farbenfabriken Bayer, W. Germany, respectively.

Solvents. - Acetone, benzene, chloroform, ethyl acetate, dioxane, methyl cellosolve, and hexane were redistilled before use. Methanol, toluene, and ethanolamine were of analytical grade.

Soil. - A Plano silt loam soil (4.7% organic matter, 5% sand, 71% silt, and 24% clay, with a pH of 6.0), free of insecticide residues, was collected at the University of Wisconsin Experimental Farm near Madison and stored in a moist condition at $22 \pm 2^\circ\text{C}$ before use.

Soil Treatments. - For surface applications, specific amounts of ^{14}C -fonofos or of ^{14}C -parathion were pipetted in hexane onto untreated moist loam soil, representing a weight of 300 g of dry soil in open systems, or onto 100 g (dry-weight basis) of soil in closed systems as described below.

To mix the insecticide with the soils ("tillage," Tables 1 and 2), an acetone solution of one of the insecticides was pipetted onto the soil, followed by thorough mixing with the soil while the acetone was removed with a gentle stream of air (Lichtenstein and Schulz 1959). Immediately after treatment, two 50-g aliquots of each insecticide-treated soil were removed for extraction and analysed to determine the actual treatment levels. All data are reported as percentages of the initially determined doses.

Experimental Design:

Effects of Surface Application and Soil Incorporation of Insecticides on the Fate of ^{14}C fonofos and ^{14}C -Parathion in an Open Soil-Plant Microcosm.

Insecticide Application. - Oats were grown in soils treated with insecticides as described by Lichtenstein et al. 1982. For surface applications of the chemicals, duplicate cartons (9 by 8.5 cm in diameter) were each filled with 300 g (dry-weight basis) of soil; 2.4 ml of hexane containing 1,200 ug of ^{14}C -fonofos (1.81 uCi) were then pipetted onto each soil surface (56.8 cm²). Three hundred grams (dry-weight basis) of soil in each of two additional cartons was treated the same way, except that the hexane contained 1,200 ug of ^{14}C -parathion (3.2 uCi).

To study the effects of insecticide incorporation into the soil, four additional cartons were each filled with 300 g (dry-weight basis) of soil, of which two had previously been mixed at 4 ppm with ^{14}C -fonofos (1.81 uCi) and 2 at 4 ppm with ^{14}C -parathion (3.2 uCi).

Planting and Growing of Oats. - Seeds of oats were germinated until the roots reached a length of ca. 1 cm. After insecticide application, 25 oat seedlings were planted in the soil of each of eight cartons (four with ^{14}C -fonofos and four with ^{14}C -parathion-treated soil). A 40-ml amount of distilled water was then added to the soil in each carton, followed by weighting of each carton. This initial weight was maintained by addition of water as necessary. The plants were grown for 14 days in a growth chamber at a temperature of 28°C and a photoperiod of LD 14:10.

Harvesting Plants and Soils. - Tops of plants were cut 1 cm above the soil surface, weighed, rinsed with cold tap water, cut into 1-cm pieces, and immersed in appropriate extraction solvents. Results obtained after analyses of plant leaves were reported as radiocarbon recovered from the leaves in percentage of the amount initially applied to the soil, and were also calculated on a per-g (fresh weight) basis (Table 1).

After the soils had dried for 2 days to a 10 to 15% moisture content, roots were removed but were not analyzed. The soils were sieved through a 2-mm screen and thoroughly mixed. A 100-g aliquot was removed for moisture determination, whereas a second 100-g aliquot was immersed in 200 ml of extraction solvents for analyses.

Effects of Surface Application, Soil Incorporation of Insecticides, and Soil Flooding on the Fate and metabolism of ^{14}C -Insecticides in a Closed Soil Microcosm.

Studies conducted previously in our laboratory with plants in closed systems under bell jars had been designed to obtain a total balance of the soil-applied radiocarbon. Results obtained, however, were quite different from similar studies with open systems (Anderegg and Lichtenstein 1981), due to the different growing conditions and, in particular, to reduced rates of leaf transpiration in the closed system. For these reasons, the investigations as described above were not repeated with oats grown under bell jars but were conducted only with insecticide-treated soils incubated in closed systems as described in detail by Ferris and Lichtenstein (1980). This procedure facilitated the measurements of $^{14}\text{CO}_2$ produced from ^{14}C -insecticides, which is a good indicator of the potential

effects of the different environmental conditions (insecticide application and soil flooding) on the breakdown of ^{14}C -insecticides (Table 2).

For surface applications, 100 g (dry wt) of soil was placed into each of four glass incubation jars (13 cm tall by 6 cm in diameter). After that, 0.4 ml each of hexane containing 400 ug of ^{14}C -fonofos (0.69 uCi) was pipetted onto the soil surface (20 cm²) in two containers, and 0.4 ml each of hexane containing 400 ug of ^{14}C -parathion (1.1 uCi) was pipetted onto the soil in the two remaining incubation jars.

For soil incorporation of the insecticides, eight additional jars (13 cm tall by 6 cm in diameter) were prepared; four were each filled with 100 g (dry-weight basis) of soil mixed previously with ^{14}C -fonofos (0.69 uCi) at 4 ppm, and four were filled with 100 g of soil each which had previously been mixed with ^{14}C -parathion (1.1 uCi) at 4 ppm. Two of the soils mixed with ^{14}C -fonofos and two mixed with ^{14}C -parathion were then flooded by carefully adding 70 ml of water to each soil. This resulted in water standing above the soil surface at a height of ca. 1 cm. All flasks were closed with a no. 10 rubber stopper containing inlets and outlets for air passage and an attached trap for $^{14}\text{CO}_2$. Soils in all 12 incubation jars (6 with ^{14}C -fonofos [Table 2A] and 6 with ^{14}C -parathion [Table 2B] were then incubated for 4 weeks in the dark at room temperature ($23 \pm 2^\circ\text{C}$), whereas $^{14}\text{CO}_2$ was collected in KOH traps at intervals and quantified by LSC as described by Ferris and Lichtenstein (1980).

At the end of the incubation period, soils or soils and water (flooded treatment) from each jar were extracted and analyzed as described below. This resulted in data for organic and water-soluble, as well as unextractable, soil-bound radiocarbon. Volatile lipid-soluble compounds retained in the polyurethane trap were extracted twice with 75 ml of hexane.

Soil Extraction. - All soils were extracted three times in a Lourdes homogenizer with a mixture of acetone-methanol-benzene (1:1:1), followed by solvent evaporation and partitioning of the extracted residues between benzene and water as described by Lichtenstein et al. (1973). Flooded soils were extracted the same way.

Plant Extraction. - Oats grown in all ^{14}C -insecticide treated soils were extracted with acetone-methanol-benzene (1:1:1), as described above for soils. Cleanup of organic extraction phases of oat tops was necessary before analyses by TLC. For this purpose, benzene extraction phases containing ^{14}C -fonofos or ^{14}C -parathion were purified with charcoal as described by Lichtenstein et al. (1973).

Analysis. - Analyses by liquid scintillation counting (LSC), TLC, and autoradiography, as by gas liquid chromatography (GLC), were performed as described by Fuhremann and Lichtenstein (1980). Results were expressed in percentage of insecticidal dose pipetted initially onto the soil surface or in percentage of dose determined by initial soil analyses following soil mixing with the insecticide.

Results and discussion

Effects of surface application and soil incorporation of insecticides on the fate of ^{14}C -fonofos and ^{14}C -parathion in an open soil-plant microcosm.

Results obtained by LSC are summarized in Table 1. As indicated by the total recoveries of radiocarbon from soils and oats (Table 1, total: S + O), mixing of soil with the insecticides ("tillage") increased their persistence and that of their derivatives by factors of 1.56 with fonofos (73.5% of applied recovered)

Table 1. - Effects of soil "tillage"^a on the fate and translocation of [^{14}C]-fonofos and [^{14}C]parathion in loam soils and oat plants incubated for 14 days in an open system at $28 \pm 1^\circ\text{C}$

	^{14}C Recovered (% applied to soil) with the following insecticides applied ^b to soil:			
	^{14}C -fonofos ^b		^{14}C -parathion ^b	
	Surface ^c	Mixed ^a	Surface	Mixed
Soil (S)				
Benzene ^d - ^{14}C	20.1 $\pm$ 1.3	35.6 $\pm$ 0.9 ^g	7.2 $\pm$ 1.7	42.6 $\pm$ 3.7 ^g
Benzene-INS ^e	18.1 $\pm$ 1.3	36.3 $\pm$ 1.0 ^g	5.0 $\pm$ 0.8	26.4 $\pm$ 0.1 ^f
Water ^d	1.5 $\pm$ 0.2	1.0 $\pm$ 0.1	5.4 $\pm$ 0.9	11.3 $\pm$ 3.8
Bound ^d	20.0 $\pm$ 0.2	28.3 $\pm$ 1.4 ^h	36.8 $\pm$ 0.4	31.7 $\pm$ 0.0 ^g
Total: S	41.6 $\pm$ 1.2	64.9 $\pm$ 2.4 ^g	49.4 $\pm$ 0.3	85.6 $\pm$ 0.1 ^f
Oat leaves (O)				
Benzene	0.4 $\pm$ 0.1	1.0 $\pm$ 0.1 ^h	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0
Water	4.9 $\pm$ 0.2	7.4 $\pm$ 0.5 ^h	0.4 $\pm$ 0.0	0.3 $\pm$ 0.0 ^h
Bound	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.5 $\pm$ 0.1	0.1 $\pm$ 0.0 ^h
Total: O	5.5 $\pm$ 0.3	8.6 $\pm$ 0.5 ^h	1.0 $\pm$ 0.1	0.5 $\pm$ 0.0 ^h
Total per g of leaves	1.4	2.4	0.16	0.08
Total: S + O	47.1 $\pm$ 1.5	73.5 $\pm$ 1.9 ^g	50.4 $\pm$ 0.3	86.1 $\pm$ 0.1 ^f

^a Insecticides were pipetted onto soil surface followed by immediate mixing with the total soil volume ("tillage," mixed). Results are averages of duplicated tests. ^b [^{14}C -ring]fonofos (1.81 uCi) or [^{14}C -ring]parathion (3.2 uCi) were applied to 300g of soil(dry weight) at 4 ppm. ^c Insecticides were pipetted -onto soil surface only. ^d Benzene and water extraction phases of soil. Bound=unextractable ^{14}C . ^e INS, Insecticide (fonofos or parathion, respectively) determined by GLC. Data are not available for leaves. ^{f.g.h} Data are significantly different from surface applied at the 0.1%, f 1%, g and 5%h level (Student's t test).

and 1.7 with parathion (86.1% of applied recovered) in comparison with results obtained with soil surface applications. This increase was primarily due to increased recoveries of ^{14}C compounds from the soils (Table 1, total: S), amounting to 64.9% of the applied fonofos and to 85.6% of the applied parathion in soils mixed with the insecticides, as opposed to 41.6 and 49.4% of applied in surface treated soils, respectively. Based on GLC analyses of the benzene-extraction phase (Table 1, INS), most of the benzene-soluble radiocarbon in fonofos-treated soils was associated with fonofos, and to a lesser extent with parathion in parathion-treated soils. Conversely, parathion-treated soils contained more water-soluble radiocarbon and also more bound ^{14}C residues than did fonofos-treated soils. We noted that ^{14}C -fonofos-derived bound residues were larger in soils mixed with the insecticide (28.3% of applied) than in surface-treated soils (20% of applied), whereas ^{14}C -parathion-derived bound residues were smaller in soils mixed with the insecticide (31.7% of applied) than in surface-treated soils (36.8% of applied).

Oat leaves grown in fonofos-treated soils contained considerably more radiocarbon than those grown in parathion-treated soils (Table 1, total: O). However, > 86% of ^{14}C -fonofos-derived residues were associated with water-soluble fonofos breakdown products (4.9 and 7.4% of applied ^{14}C in surface-treated and "mixed soils," respectively), whereas the remainder were benzene-soluble or were bound to the plant tissue.

To identify organic-soluble compounds, benzene extraction phases of both soils and oats were also analyzed by TLC, by autoradiography (Fig. 1), and by GLC of soil extracts (Table 1, INS). Results obtained by TLC are summarized in Fig. 1, where the right-hand column presents the total amounts of benzene-soluble radiocarbon recovered from soils or oats. These amounts are expressed in percentage of soil-applied ^{14}C . The horizontal bar graphs, however, depict the percent distribution of the various compounds in these benzene extraction phases. Although the amount of benzene-soluble radiocarbon recovered from soils were considerably larger than those found in oats when expressed in percentage of the applied insecticide, the percent distribution of the relative amounts of fonofos and its metabolites expressed in percentage of the total recovered were not affected by the two forms of insecticide application. As shown in Fig. 1A, fonofos was the major compound recovered from soils, amounting to 66 and 69% of all benzene-soluble compounds. In oat greens, however, most of the benzene soluble ^{14}C recovered (1% or less of the soil-applied radiocarbon) was associated with the fonofos metabolite methyl phenyl sulfone (MPSO₂). Small amounts of the oxygen analogue of fonofos (F = oxon in Fig. 1A) were found in both soils and oats. Compounds recovered in trace quantities ("others" in Fig. 1A) were thiophenol, diphenyldisulfide, and 2-, 3-, or 4-hydroxymethyl phenyl sulfone. As demonstrated by Liang and Lichtenstein (1980) with house flies, only fonofos (0.1 ug per fly) and its oxygen analogue (0.1 ug per fly) are insecticidal, and no mortalities were observed after flies had been treated with 1 ug of methyl phenyl sulfone, thiophenol, diphenyl-disulfide, or one of the

hydroxymethyl phenyl sulfone compounds. It is evident, therefore, that after 2 weeks of incubation, soils still contained 18 to 36% of the applied fonofos (Table 1), whereas nearly all of the radiocarbon recovered from oat greens was associated with detoxified, non-insecticidal metabolites of fonofos.

FIG. 1. - Effects of surface application and soil mixing of insecticides on the metabolism of [ring- ¹⁴C]fonofos and of [ring- ¹⁴C]parathion in soils and plants grown in an open system. Data are based on TLC of the organic extraction phase, followed by autoradiography. LSC, and GLC (soils). (A) MPSO₂: methyl phenyl sulfone; F = OXON: oxygen analogue of fonofos. Others: thiophenol, diphenyldisulfide and 2-, 3-, or 4-hydroxymethyl phenyl sulfone. (B) p-NO₂: p-nitrophenol. Others: amino parathion and aminoparaoxon.

As with fonofos, mixing of parathion with the soil also increased its persistence. Based on TLC and autoradiography, parathion was the major compound recovered from the benzene extraction phases of both soils and oat leaves (Fig. 1B), although in leaves the actual amounts were minimal (0.1% of the soil-applied radiocarbon). Paraoxon, aminoparaoxon, aminoparathion and p-nitrophenol were the major metabolites detected.

Based on GLC analyses of soil-benzene extraction phases (Table 1, INS), the insecticides were 2.0 and 5.3 times more persistent after soil incorporation of fonofos and parathion, respectively.

Effects of Surface Application, Soil Incorporation of Insecticides, and Soil Flooding on the Fate and Metabolism of ¹⁴C-insecticides in a Closed Soil Microcosm. Since these experiments were conducted in closed systems without plants, results obtained cannot be compared with those obtained with soils and

oats in open systems. Furthermore, soils in closed systems were incubated for 28 days, as opposed to 14 days in experiments with open systems. Results (Table 2) show the amounts of radiocarbon recovered from soils and vapor traps. Data are expressed in percentage of soil-applied ^{14}C -fonofos (Table 2A) or ^{14}C -parathion (Table 2B).

Since the closed microcosm facilitated the measurement of the amounts of $^{14}\text{CO}_2$ produced from the originally applied ^{14}C -insecticides, a good indicator was available to study the effects of the different conditions on the breakdown of the ^{14}C -insecticides to $^{14}\text{CO}_2$. Thus, the amounts of $^{14}\text{CO}_2$ produced from ^{14}C -fonofos and retained in the KOH vapor trap were 19.3% of the insecticide applied to the soil surface, 16.7% of that mixed with the soil, but only 0.5% of that mixed with the soil and kept under flooded conditions. Oxidative conditions affecting the formation of CO_2 were obviously most prevalent on the soil surface and nearly absent when the soil had been flooded. In addition, a total of 66% of the applied radiocarbon was recovered (or 44% were lost) from the surfacetreated soil, whereas 75% (25% loss) and 88% (12% loss) were recovered from soils previously mixed with the insecticide or mixed and flooded, respectively (Table 2A: Total S). Also, the production of unextractable, soil-bound ^{14}C -fonofos residues had significantly increased under flooded conditions.

Results obtained with parathion-treated soils (Table 2B), also show differences in the insecticide persistence and metabolism under the three experimental conditions. In comparison to ^{14}C -fonofos, the effects on $^{14}\text{CO}_2$ production and production of soil-bound residues were more pronounced with parathion. Thus, more $^{14}\text{CO}_2$ (30% of the applied ^{14}C -parathion, as opposed to 19% with ^{14}C -fonofos) was produced from ^{14}C -parathion surface treated soil. Mixing the insecticide with the soil reduced this amount to 23% of applied radiocarbon, whereas soil flooding practically stopped the degradation of ^{14}C -parathion to $^{14}\text{CO}_2$.

Data also show that 44% of the ^{14}C -parathion-derived radiocarbon had become soil bound after an insecticide surface application as opposed to 29% with ^{14}C -fonofos. Mixing ^{14}C -parathion with the soil increased the amounts of bound residues to 49% and additional flooding to 72% of the originally applied ^{14}C -parathion. These findings agree with earlier reports (Katan and Lichtenstein 1977) demonstrating that the production of parathion-derived bound residues is a result of reductive conditions which favor the metabolism of parathion to amino-derivatives. Because of the increased ^{14}C -parathion-derived bound residues in these soils, the overall recoveries of ^{14}C -parathion residues were larger than those observed with ^{14}C -fonofos under all experimental conditions.

The effects of various agricultural chemicals on the fate of ^{14}C -parathion in Wisconsin nonflooded cranberry soils were investigated by Ferris and Lichtenstein (1980). In these experiments, different non-radioactive fungicides and inorganic fertilizers were mixed with cranberry soils, whereas ^{14}C -parathion was only applied to the soil surface. Results obtained in these experiments with

Table 2. - Effects of soil "tillage"^a and soil flooding^b on the fate of ¹⁴C-insecticides in a loam soil incubated for 28 days in a closed system at 24±2°C

Insecticides applied ^c to soil:			
	Surface ^d	Mixed ^a	
		None	Flooded ^b
¹⁴ C Recovered (% of ¹⁴ C fonofos applied) ^e			
A Soil (S)			
Benzene ^e	33.0 ± 0.2	35.4 ± 1.3	39.6 ± 1.1 ^h
Water ^e	3.8 ± 0.2	9.0 ± 0.7 ^g	12.0 ± 0.2 ^f
Bound ^e	29.3 ± 0.4	30.8 ± 0.4	36.4 ± 0.3 ^g
Total: S	66.1 ± 0.8	75.2 ± 1.0 ^g	88.0 ± 1.0 ^g
Vapor traps (V)			
Polyurethane	0.3	<0.04	<0.04
KOH (¹⁴ CO ₂)	19.3 ± 0.7	16.7 ± 0.5 ^h	0.5 ± 0.1 ^f
Total: S + V	85.7 ± 0.7	91.9 ± 1.5 ^h	88.5 ± 1.1
¹⁴ C Recovered (% of ¹⁴ C-parathion applied) ^c			
B. Soil (S)			
Benzene	12.2 ± 0.7	20.7 ± 0.1 ^g	16.7 ± 0.6 ^h
Water	6.8 ± 0.1	6.0 ± 0.7	8.0 ± 0.8
Bound	43.8 ± 1.6	48.5 ± 0.1 ^h	72.1 ± 0.9 ^g
Total: S	62.8 ± 0.7	75.2 ± 0.8 ^g	96.8 ± 2.3 ^g
Vapor traps (V)			
Polyurethane	0.02	<0.02	0.02
KOH (¹⁴ CO ₂)	30.0 ± 0.2	23.2 ± 1.1 ^h	0.9 ± 0.01 ^f
Total: S + V	92.8 ± 0.5	98.4 ± 0.3 ^g	97.7 ± 2.3

^a Insecticides were pipetted onto soil surface followed by immediate mixing with the total soil volume ("tillage," mixed). Results are averages of duplicated tests. ^b Flooding: after insecticides had been mixed with the soil, water was added until it stood to about 1 cm above soil surface. ^c [¹⁴C-ring] fonofos (0.69 uCi) or [¹⁴C-ring]parathion (1.1 uCi) were applied to 100 g of soil (dry weight) at 4 ppm. ^d Insecticides were pipetted onto soil surface only. ^e Benzene and water extraction phases of soil. Bound = unextractable ¹⁴C. ^{f,g,h} See respective footnotes to Table 1.

control soils treated only with ¹⁴C-parathion showed that the overall recoveries of radiocarbon were very similar to those observed with loam soils reported here (Table 2). However, the amounts of ¹⁴CO₂ produced from ¹⁴C-parathion in

cranberry soils were considerably larger (44 to 46% of applied) than those produced in loam soils (30% in Table 2). Conversely, the amounts of bound ^{14}C -residues were smaller in cranberry soils (34 to 39%) than in loam soils (44% in Table 2). Results, therefore, seem to indicate that the nonflooded sandy cranberry soil was oxidatively more and reductively less active than the loam soil used in the present study, probably due to different physical properties of the soil and different populations of soil microorganisms.

Data discussed here show that both fonofos and parathion were least persistent after their application to the soil surface. Evidently, the availability of oxygen in the soil environment presents a major factor which affects the metabolism and degradation of the insecticides in soils.

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(h) PENETRATION OF VOLATILE [^{14}C] RESIDUES INTO THE AERIAL PARTS OF PLANTS GROWN ADJACENT TO OR IN [^{14}C] FONOFOS OF [^{14}C] CARBOFURAN TREATED SOILS

The potential vapor absorption by plant greens of volatile [^{14}C] compounds from soils treated with [^{14}C]fonofos or [^{14}C] carbofuran was investigated. Oats or peas were grown in insecticide-free soil adjacent to insecticide-treated soil or

Table 1. [^{14}C]fonofos and metabolites recovered from plant greens grown in insecticide-free soil, but surrounded" by [^{14}C]fonofos-treated soil, or grown directly in insecticide-treated soil ("[^{14}C]fonofos").

	Peas grown in soil					Oats grown in soil				
	surrounded	[^{14}C]fonofos				surrounded	[^{14}C]fonofos			
Compound ^a R _f	C ^b Open	C ^c D.Closed	D ^d E.Open	F ^e F.Closed	C ^e C.Open	D ^e D.Closed	E ^e E.Open	F ^e F.Closed		
¹⁴ C recovered from plant greens, % of all benzene-soluble ^e radiocarbon										
Fonofos	0.69	0.0	10.0	0.6	2.3	0.0	19.1	1.8	12.6	
F = Oxon	0.30	1.8	1.9	7.9	6.4	0.0	12.0	6.0	9.6	
MPSO ₂	0.40	0.0	5.5	45.3	18.7	0.0	0.0	42.7	28.9	
PS, TP	0.78	0.0	0.0	31.1	52.5	0.5	0.0	11.7	5.9	
Others	0.18	0.0	0.0	0.3	0.1	0.0	0.0	0.4	2.2	
Unknown ¹	0.60	0.7	0.6	0.8	3.4	0.1	0.3	3.8	4.0	
2	0.50	0.0	0.1	0.1	0.9	0.1	0.0	0.9	1.3	
3	0.00	97.5	81.9	13.9	15.7	99.3	68.6	32.7	35.5	

^aDetermined by TLC separation and LSC: F = oxon: fonofos-oxon; MPSO₂: methyl phenyl sulfone; PS: diiphenyldisulfide; TP: thiophenol; Others: trace amounts of 2-, and/or 4-(hydroxyphenyl) methyl sulfone. ^bCapital letters C through F refer to Figure 2. ^{c,d}Plants grown (c) in open systems or (d) in soils under bell jars ("closed"). ^eData for all benzene-soluble ¹⁴C in peas are presented in Table I. Comparable data obtained with oats expressed in % of soil-applied ¹⁴C, are 0.01% ("surrounded-open"), 0.22% ("surrounded-closed"), 0.34% ("[^{14}C]fonofos-open") and 0.30% ("[^{14}C]fonofos-closed").

directly in the insecticide-treated soil. Some experiments were conducted in such a way that volatile [^{14}C] compounds released from the insecticide-treated soil could be removed, thus preventing their contact with plant greens. Both [^{14}C]fonofos and [^{14}C]carbofuran in soils were to some extent broken down to ¹⁴CO₂. After its release into the air, some of this ¹⁴CO₂ was absorbed by the aerial parts of plants which grew in untreated soil adjacent to insecticide-treated soil or directly within insecticide-treated soil. Since the major portion of radiocarbon in plant greens was water-soluble, it appears that the

Table 2. Pick up of ^{14}C by peas and oats grown in insecticide-free soil but surrounded by [^{14}C]fonofos-treated soil^a.

	Peas or oats grown ^b in untreated soil			
	Peas		Oats	
	I ^c Open	J ^c Covered	I. Open	J. Covered
^{14}C recovered, % of applied to soil ^a				
$^{14}\text{CO}_2$ from soil ^d	---	1.2±0.0	---	1.0±0.0
PLANTS				
Greens				
benzene ^e	0.01± .00	---	0.01± .00	---
water ^e	0.04± .01	---	0.07± .01	---
bound ^e	0.01± .00	---	0.00± .00	---
total	0.06± .00	0.0	0.08± .00	0.0
Total/gram	0.03± .01	0.0	0.04± .00	0.0
Roots	0	0	0	0
SOILS				
Treated U ^c	85.4±3.3	83.4±2.1	83.0±0.6	83.8±2.3
I ^c	7.3±1.7	7.7±0.9	5.2±0.2	6.6±0.6
Total	92.7±1.4	91.1±1.3	88.2±0.8	90.4±3.0
Untreated ^f	TR ^g	0	TR	0
Misc ^h	---	0.13± .01	---	0.12± .01
TOTAL	92.8±1.3	92.4±1.2	88.3±0.8	91.5±3.0

^a [ring- ^{14}C]fonofos (1.33 uCi) was mixed with 300 g soil (dry wt) at 4 ppm and placed on top of 300 g (dry wt) of untreated soil, both surrounding a glass vial containing 300 g (dry wt) of insecticide-free soil. Results are averages of duplicated tests. ^bOats and peas were grown for 11 and 14 days, respectively

^cFigure 3: I=Outer ring consisting of an upper layer (U) of treated soil and a lower layer (L) of untreated soil, was not covered. J=same as I, but treated soil was covered. ^dSame as c in Table III ^eSame as d in Table III

^fUntreated: the inner core of soil in which the plants were grown, had not been treated with the insecticide. ^gTR = Trace ^hSame as footnote f in Table III

$^{14}\text{CO}_2$ picked up from the air was photosynthesized into water-soluble products. Only the smallest fraction of radiocarbon recovered from plants grown adjacent to or in [^{14}C]fonofos-treated soil was benzene-soluble, and represented less than 0.5% of the total soil-applied ^{14}C . This radiocarbon was primarily associated with non-insecticidal metabolites. The addition of cow manure to [^{14}C]carbofuran-treated soil resulted in an increased production of $^{14}\text{CO}_2$ and in

an increased presence of water soluble radiocarbon within the aerial plant parts. Typical results are presented in tables 1 and 2.

(i) MICROBIAL ASPECTS OF PESTICIDE METABOLISM:

A. EFFECTS OF SOIL MICROORGANISMS ON THE RELEASE OF [^{14}C] RESIDUES FROM SOILS, PREVIOUSLY TREATED WITH [^{14}C] PARATHION

Experiments were conducted to investigate the importance and significance of soil microorganisms relative to the release of unextractable, bound [^{14}C] residues from soils, previously treated with [^{14}C] parathion. Results obtained indicate that soil-bound [^{14}C] residues can be released, metabolized and picked up by oat plants grown in these soils. It became evident that with an increase in soil microorganism populations a concomitant increase in the release of soil-bound [^{14}C] residues had occurred. Addition of biologically active substances (glucose, parathion, chloramphenicol and captafol), known to increase or decrease microbiological activities, to soil-bound [^{14}C] residues also resulted in an increase or decrease of the release of soil-bound radiocarbon and in particular in the evolution and release of $^{14}\text{CO}_2$. Addition of either cow manure or autoclaved soil to soil-bound [^{14}C] residues, also resulted in most cases in larger populations of soil microorganisms, in a decrease of initially soil-bound ^{14}C -residues, a decrease in the amounts of total [^{14}C] soil-residues remaining, a dramatic increase in the evolution of $^{14}\text{CO}_2$ and, in comparison to controls, in quantitatively different pick up patterns of [^{14}C] residues by oats which were grown in these soils. It is obvious, therefore, that soil-bound residues should not be regarded as unavailable for further degradation.

B. EFFECT OF PLANT PATHOGENS ON THE FATE OF SOIL-APPLIED ^{14}C -CARBOFURAN IN CORN PLANTS

The effects of common root rot and leaf rust diseases of corn plants on the translocation and metabolism of soil-applied (5 ppm) [^{14}C]carbofuran in corn were investigated. The corn diseases studied were caused by the fungi Giberella zeae (Schw.) Petch or Puccinia sorghi Schw., respectively. Both root rot and leaf rust affected the translocation of [^{14}C]carbofuran from soils into the plant tissue. Soil-applied radiocarbon accumulated in the diseased organs of the plants, i.e. in roots of corn with root rot and in leaves of plants with rust, which contained 1.4 and 1.7 times more radiocarbon than roots or leaves from control plants, respectively. The amounts of ^{14}C translocated into leaves were directly related to the transpiration stream, which was increased in both root rot and leaf rust-infected plants. Carbofuran was the major compound recovered from control leaves, amounting to 42% of all organic-soluble radiocarbon present. Similar amounts were isolated as 3-hydroxycarbofuran. Leaves from plants with root rot, however, contained primarily 3-hydroxycarbofuran (50% of organic-soluble ^{14}C) and considerably less carbofuran (24% of organic-soluble ^{14}C). Conversely, leaves with rust contained more carbofuran (47% of organic soluble ^{14}C) and slightly less

3-hydroxycarbofuran that did control leaves. based on analyses by gas-liquid chromatography, concentrations of carbofuran in leaves from control plants and from plants with root rot or rust amounted to 8.8, 3.7 and 16.7 ppm, respectively.

4. DESCRIPTION OF COOPERATION.

The work in Israel emphasized interactions occurring in the laboratory as well as under practical field conditions, employing commercial soil disinfection treatments and fungicides. The work in the USA was devoted to the effect of various agrochemicals, irrigation, light intensity, temperatures, soil mixing, etc., which was studied under controlled laboratory conditions employing a soil plant microcosm and taking advantage of the excellent facilities available at the University of Wisconsin, and the model systems developed there. continuous contacts, mutual exchange of data and visits enabled a coordinated complimentary work in both countries.

5. MAIN ACHIEVEMENTS OF THE RESEARCH.

In this research we studied the nature of the interactions between agrochemicals and agricultural practices and the persistence and metabolism of soil applied pesticides. Attempts were made to define specific mechanisms involved in these processes in order to improve the performance of these pesticides.

The results from this work clearly demonstrate that persistence and efficacy of various soil applied fungicides, herbicides, insecticides and nematicides may be drastically altered by agrochemicals and/or agricultural practices including soil disinfection by methyl bromide (MB) or solarization. We have tried to examine the scope and the extent of the problem which seems to include a series of agrochemicals interacting with the persistence of a relative large group of soil applied pesticides. The fate of soil applied pesticides such as terbutryn or atrazine in disinfested soils, or of terbutryn and MBC in fungicide-treated soils, was different from that in the nontreated ones i.e the degradation was much slowed down. This was attributed to the strong effect of disinfection or of the fungicides on microbial activity. Microorganisms play an important role in degrading many pesticides. In the present study MB suppressed degradation of several pesticides to about the same extent as sterilization. Moreover, the inhibitory effect of MB lasted for several months, which indicates that the recovery of the populations of the microbial degraders in the MB-treated soil is slow.

TMTD, which suppressed terbutryn and MBC degradation, also strongly reduced soil fungal populations but not the bacterial ones. This indicates that fungi may play an important role in the degradation of MBC and terbutryn. Other fungicides or biocides with antimicrobial activity which are routinely used in agriculture may also affect the persistence of soil applied pesticides. For example, the fungicides captafol, maneb and benomyl inhibited degradation of parathion in soil. Soil disinfection may also affect pesticide degradation in

a qualitative way, as shown with the carbamate ethiofencarb. Degradation of herbicides in solarized soils, which were incorporated into the soil prior to tarping with polyethylene, and therefore were exposed to the heating process, were affected in different ways - depending on the herbicide. Thus, the fate of any pesticide applied to a crop might be affected also by the type and dosage of the other pesticides used.

There was a clear decline in the concentration of the herbicides in the treated and nontreated soils during the first days after application. This may be attributed to physical processes such as adsorption and binding, or to residual enzymatic activity which was not completely destroyed during the disinfestation treatment.

The results suggest that soil disinfestation can be utilized as a tool for determining the role of soil microorganisms in pesticide degradation, under practical field conditions. The good agreement between results obtained when terbutryn was applied to the soil in the laboratory and those obtained when the herbicide was applied in the field denotes the effectiveness and reliability of the systems used in this study.

It is not economically feasible to use soil disinfestation only for the purpose of controlling the fate of pesticides applied to soil. However, since this practice is followed with certain crops for pest control, extended effectiveness of the pesticide can be regarded as an additional side benefit. TMTD and fentin acetate were highly effective at relatively low dosages. Therefore, their potential use as "extenders" might be economic under the right circumstances.

Results from the present study suggest that dosages of pesticides, especially herbicides should be adjusted if the soil was previously disinfested or when certain fungicides are present, in order to avoid possible phytotoxicity. When necessary, disinfestation or fungicides might also be used as a tool for extending the effectiveness of herbicides or for reducing herbicide dosage. Thus, recommendations for pesticides dosages should take into consideration the presence of other, or the use of soil treatments which affect the soil biophase.

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