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Interactions between Agricultural Chemicals and Soil Microflora and Their Effects on the Degradation of [^{14}C]Parathion in a Cranberry Soil

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The interaction of selected fungicides, herbicides, and N-fertilizers with microorganisms in cranberry soils and their effects on the degradation of [*phenyl*-^{14}C]parathion were investigated. Soil microorganisms were responsible for the oxidative as well as reductive degradation of the insecticide. Incubation of soils with parathion or *p*-nitrophenol for 4 days followed by the addition of [^{14}C]parathion resulted after 24 h in an enhanced degradation of the insecticide to $^{14}CO_2$ (34–39% of the applied radiocarbon as opposed to 2% in controls) and also in an increased binding of ^{14}C to the soil. The fungicide captafol inhibited the degradation of soil-applied [^{14}C]parathion as evidenced by a reduction of both $^{14}CO_2$ evolution and ^{14}C-bound residues. Maneb and benomyl suppressed the degradation of [^{14}C]parathion to $^{14}CO_2$ but not the formation of bound residues. Pentachloronitrobenzene had no effect. Addition of (2,4-dichlorophenoxy)acetic acid to [^{14}C]parathion-treated soil also resulted in an increased persistence of the insecticide. Studies conducted with the insecticide and $(NH_4)_2SO_4$, NH_4NO_3, KNO_3, or urea showed that under all experimental conditions the total amounts of ^{14}C recovered were similar, yet the distribution of ^{14}C-labeled compounds into benzene-soluble, water-soluble, and bound residues was not. This possibly indicated a change in the pathway of [^{14}C]parathion degradation. The insecticide was most persistent in soils containing $(NH_4)_2SO_4$, as demonstrated by a recovery of 29% of the applied radiocarbon in the benzene-soluble form. Analyses by TLC of this benzene extraction phase revealed the presence of [^{14}C]parathion, *p*-amino[^{14}C]phenol, and amino[^{14}C]parathion. It appears that the form of the N-soil amendment and not the N amendment as such affected the degradation of [^{14}C]parathion. Results reported here stress the importance of investigating the environmental fate of a particular pesticide in relation to the presence of other agricultural chemicals.

Cranberries (*Vaccinium macrocarpon* Ait.) are an important fruit crop in the State of Wisconsin. A variety of pesticides and fertilizers are used each year to obtain high yields. Parathion is one of the major insecticides used, being applied at rates ranging from 0.5 to 5 lb/(acre/year). Since during part of the year cranberry bogs are flooded, concern has been expressed about the potential transport of parathion residues by water draining from cranberry bogs. Since fungicides, herbicides, and fertilizers are applied to cranberry soils, in addition to insecticides, we were interested in the potential interactions of some of these chemicals with the soil microflora and their effects on the degradation of parathion. Previous studies in our laboratory indicated that some herbicides synergized insecticides (Lichtenstein et al., 1973a; Liang and Lichtenstein, 1974) and that the herbicide Eptam affected the uptake and metabolism of [^{14}C]phorate in corn plants (Schulz et al., 1976). Gorder and Lichtenstein (1980) demonstrated parathion degradation in soil-free culture media inoculated with microorganisms obtained from cranberry soils. These microorganisms also grew in basal salt media utilizing parathion as the sole carbon source. Addition of 0.05% glucose to the basal salt media inhibited the degradation of parathion.

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This study was, therefore, conducted to investigate potential effects of selected fungicides, herbicides, and fertilizers on microorganism-mediated degradation of [*ring*-^{14}C]parathion in cranberry soils.

MATERIALS AND METHODS

Insecticides. [*ring*-^{14}C]Parathion (*O,O*-diethyl *O*-*p*-nitro[2,6-^{14}C]phenyl phosphorothionate) was purchased from International Chemicals and Nuclear Corp., Irvine, CA. Nonradioactive parathion and several of its potential metabolites were supplied by courtesy of Bayer Pflanzenschutz., Leverkusen, West Germany.

Fungicides. Captafol [*cis*-*N*-[(1,1,2,2-tetrachloroethyl)thio]-4-cyclohexene-1,2-dicarboximide] or a 40% AI flowable formulated material (Difolatan) was obtained from Ortho Chevron Chemical Co., Richmond, CA. Analytical grade benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] and Benlate, a wettable powder containing 50% AI benomyl, were obtained through the courtesy of du Pont de Nemours and Co., Wilmington, DE. Maneb [manganese ethylenebis(dithiocarbamate)] was used as Manzate, an 80% AI wettable powder manufactured by du Pont de Nemours and Co. PCNB (pentachloronitrobenzene) was used as Terraclor, a 75% AI wettable powder manufactured by Olin Corp., Little Rock, AR.

Herbicides. 2,4-D [(2,4-dichlorophenoxy)acetic acid] was obtained from Eastman Organic Chemicals, Rochester, NY. Atrazine was provided courtesy of Ciba-Geigy Corp., Greensboro, NC, and monolinuron by courtesy of du Pont de Nemours and Co., Wilmington, DE.

Fertilizers. Analytical grade $(NH_4)_2SO_4$, KNO_3, NH_4NO_3, and urea were utilized as nitrogen sources.

Antibiotics (Bactericides). Chloramphenicol [D(−)-*threo*-2,2-dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide] was purchased from Sigma Chemical Co., St. Louis, MO.

Tetracycline [4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide] hydrochloride was obtained courtesy of Upjohn Co., Kalamazoo, MI.

Solvents. Acetone, methanol, benzene, dioxane, Methyl Cellosolve, and hexane were redistilled before use. Diethyl ether, 95% ethanol, toluene, and ethanolamine were used without further purification. All other reagents used were analytical grade.

Cranberry Soil. A number of soil samples, 20-cm diameter and 10 cm deep, were collected from a representative area of a cranberry bog located in Juneau County, WI. The samples were mixed, and the surface debris was removed. The moist soil was then pressed through a no. 8 mesh stainless steel sieve. The sieved soil was stored in sealed plastic bags at 5 °C to prevent moisture loss and to minimize changes in the soil microflora. This cranberry soil consisted of sand (93%), silt (4%), clay (3%), and organic matter (3.5%) with a pH of 5.6. When used in the laboratory, the average moisture content was 23%.

Soil Treatments. Basically, the effects of fungicides, herbicides, and fertilizers on the fate of [*ring*-^{14}C]parathion in cranberry soils were investigated. To that effect, we mixed a specific nonradioactive chemical thoroughly with cranberry soil to yield a concentration of 100 ppm of active ingredient. Analytical grade captafol or atrazine was applied in acetone, while commercial formulations of PCNB (Terraclor), benomyl (Benlate), and maneb (Manzate) were applied in water. The herbicide 2,4-D, antibiotics chloramphenicol and tetracycline, glucose, and nitrogen fertilizers were also applied in water. Fifty-seven grams (dry weight equivalent) of uniformly treated soil was then placed into soil incubation jars (height 13 cm, diameter 5 cm) as depicted in Figure 1. An 8–8.5 μg/cm^2 amount of [*ring*-^{14}C]parathion in acetone was then applied to the surface of the cranberry soil, previously treated and mixed with one of the other chemicals. In experiments investigating the effects of nonradioactive parathion and *p*-nitrophenol on the fate of [*ring*-^{14}C]parathion, these materials were also applied only to soil surfaces (29 μg/cm^2) by utilizing acetone as the solvent.

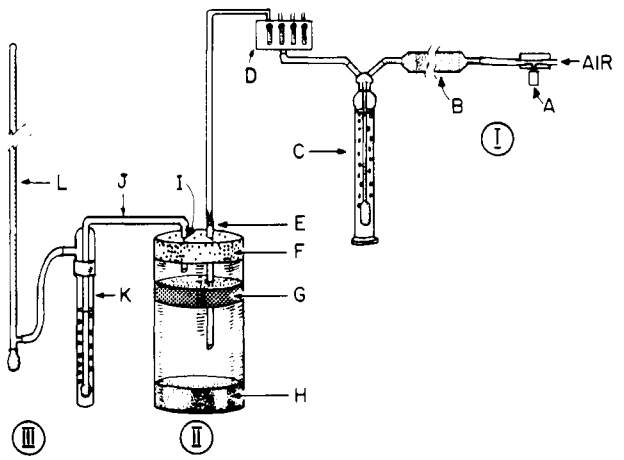

Figure 1. Diagram of the closed system used to study the fate of ^{14}C-labeled insecticides in soil [based on Kearny and Kontson (1976)]. (I) Regulation and treatment of air: (A) needle valve; (B) carbon dioxide filter; (C) air humidifier; (D) manifold to split the airstream; (E) syringe. (II) Soil incubation jar: (F) No. 10 rubber stopper; (G) polyurethane trap; (H) 250-mL jar (13 cm height, 5 cm diameter) containing ^{14}C-labeled insecticide-treated soil; (I) 5-mm i.d. Pyrex exit tube; (J) 7-mm i.d. Tygon tube with a pinch clamp. (III) $^{14}CO_2$ trap: (K) gas dispersion tube with 15 mL of 0.1 M KOH; (L) bubble flow meter.

Soil Incubation and Trapping of $^{14}CO_2$. With the exception of the experiment conducted with soil-free cultures of microorganisms, all studies were conducted in a closed system as shown in Figure 1, a modification of the one described by Kearney and Kontson (1976). The apparatus facilitates the trapping of volatile substances, such as lipid-soluble materials and $^{14}CO_2$, derived from [*ring*-^{14}C]parathion. Soils were incubated for 3 weeks at room temperature (23 ± 2 °C) in the dark. Volatile ^{14}C-labeled compounds were purged from the system, for 2 h with 15 mL/min moist CO_2-free air, each day for the first week and biweekly thereafter. The volatile ^{14}C-labeled compounds trapped by the KOH were quantified by liquid scintillation counting as described below. A procedure was used as described by Gorder and Lichtenstein (1980) to determine whether the radiocarbon which was trapped in KOH was actually associated with CO_2.

Extraction. Soils were extracted twice with a mixture of acetone–methanol (1:1) at a ratio of 2 mL per g of soil. This was followed by a third extraction with acetone–methanol–benzene (1:1:1) as described by Lichtenstein et al. (1973b). Use of this method resulted finally in a benzene and a water extraction phase. After the soils had been extracted, they were combusted to determine the amount of unextractable, bound ^{14}C-labeled residues. Volatile lipid-soluble compounds retained by the polyurethane trap (Figure 1, G) were extracted twice with 75 mL of hexane.

Analyses. *Liquid scintillation counting* (LSC) of organic and water extraction phases was performed as described by Fuhremann and Lichtenstein (1978). Extracted soil samples were pelleted and combusted in an automatic oxidizer to determine the amounts of unextractable, bound

^{14}C-labeled residues as described by Fuhremann and Lichtenstein (1978). For determination of the amounts of radiocarbon in the KOH traps, aliquots of KOH were counted in a dioxane-based scintillation solvent containing 3.5% Cab-O-Sil to prevent precipitation.

Thin-Layer Chromatography (TLC). The benzene extracts of soils and of soil-free incubation mixtures were analyzed by TLC to determine the identity of ^{14}C-labeled compounds. Aliquots of the benzene extract were spotted on precoated 0.25-mm silica gel 60 plates (E. Merck, Darmstadt, West Germany). The plates were developed first in CCl_4, to separate interfering lipid materials from [^{14}C]parathion and its metabolites, and then in diethyl ether. Parathion and its metabolites were visualized by spraying the plates with 0.5 g of $PdCl_2$ in 2 mL of concentrated HCl and 98 mL of water. Additional spraying was then performed with 5 N NaOH (Lichtenstein and Schulz, 1964). Detection of radioactive compounds in thin-layer chromatograms was performed by autoradiography, using Kodak No-Screen X-ray films.

Gas–liquid chromatography (GLC) was used for the separation and detection of parathion, paraoxon, and aminoparathion as described by Fuhremann and Lichtenstein (1980).

EXPERIMENTAL SECTION

Role of Microorganisms on the Fate of [*ring*-^{14}C]-Parathion in Cranberry Soils. Previous studies conducted in our laboratory showed that parathion was degraded in soil-free cultures which had been inoculated with microorganisms derived from cranberry soils (Gorder and Lichtenstein, 1980). For further investigation of the role of microorganisms in the degradation of insecticides, experiments were conducted with parathion in actual cranberry soil. To this effect, we used five different ways to reduce or change the soil microflora or fauna. Soils were autoclaved 3 times for 1 h at 120 °C to eliminate microbial activity or they were aerated for 30 min with 60 °C steam to selectively remove Pseudomonads and Phycomycetes (Broadbent et al., 1971). Bacteriostatic agents (chloramphenicol or tetracycline) were mixed with soils at 100 ppm to eliminate or reduce the population of soil bacteria. Finally, glucose was mixed with soils at 2000 ppm to stimulate microbial activity as shown by Anderson and Domsch (1974).

Triplicate aliquots (58 g dry weight, each) of nontreated soils (controls) or those treated as described were then placed into the soil incubation jars as shown in Figure 1. After that, 0.3 μCi of [*ring*-^{14}C]parathion was applied at a rate of 8 $\mu g/cm^2$ to the soil surface in each of the 18 jars (controls and five treatments).

Each unit (Figure 1) was assembled in a transfer chamber purged with sterile air to prevent inadvertent microbial contamination of the soil. Soils were then incubated at room temperature (23 ± 2 °C) for 3 weeks. Volatile ^{14}C-labeled compounds were purged with air from the system for 2 h each day during the first week but only biweekly thereafter. $^{14}CO_2$ evolved from the incubation jars was trapped in KOH and quantitated by LSC as described. After incubation, all units were dismantled and the soils were sampled to determine the soil dry weight and populations of aerobic bacteria and fungi. Bacterial and fungal counts were performed by dilution plate techniques as described by Johnson and Curl (1972). The remaining soil and the vapor traps were then extracted and analyzed by LSC and GLC.

Effects of Parathion Residues in Soil on the Fate of [*ring*-^{14}C]Parathion. Thirty years ago, Newman and Thomas (1950) reported that "the persistence of 2,4-D in soil was decreased by pretreatment of the soil with 2,4-D and certain other compounds having similar constituent groups". The authors also stated that the "increased rate of breakdown must be due to the development of a population capable of decomposing the pretreatment compound and also 2,4-D". In recent studies conducted in India (Barik et al., 1979) it was shown that parathion or *p*-nitrophenol treatment of a flooded soil increased the population of soil microorganisms and accelerated the degradation of parathion applied subsequently. However, both of the above studies employed unlabeled compounds, making it impossible to determine whether the remaining parent compound was derived from the treatment or pretreatment compound. Furthermore, unextractable (bound) residues were not determined by Barik et al. (1979) even though they may contain a major proportion of the aminoparathion formed from the metabolism of parathion (Katan and Lichtenstein, 1977).

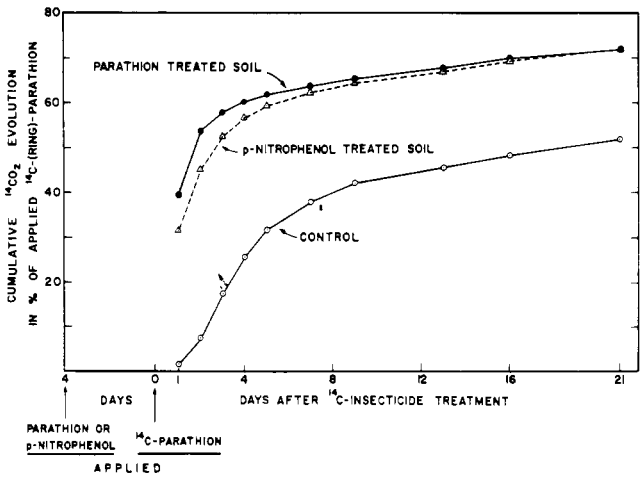

Figure 2. Effect of parathion or *p*-nitrophenol in soil on the evolution of $^{14}CO_2$ from soil-applied [*ring*-^{14}C]parathion. Results are means of duplicate tests and represent accumulated $^{14}CO_2$ evolution.

Since parathion is used for the control of insects in cranberry soils, it was felt that both parathion and its hydrolysis product *p*-nitrophenol might foster the development of a parathion-degrading microflora, thus inducing a more rapid dissipation of a subsequently applied insecticide. Experiments were therefore conducted in which cranberry soils were pretreated with either parathion or *p*-nitrophenol, followed later by the addition of [*ring*-^{14}C]parathion. To that effect, we placed portions of cranberry soil (58 g dry weight each) into each of 21 soil incubation jars. Soils in seven jars (group 1) were then surface treated with 0.5 mL of acetone each (controls), while group 2 (seven replicates) was surface treated with 0.5 mL of acetone containing 0.58 mg of parathion per jar (group 2). Soils in the seven remaining jars (group 3) were each surface treated with 0.5 mL of acetone containing 0.58 mg of *p*-nitrophenol. After 4 days of incubation at room temperature, three replicates from each group were sampled to determine the number of aerobic bacteria in the soil. The remaining four replicates of each of the three groups were then surface treated with [*ring*-^{14}C]parathion (0.32 μCi or 8 $\mu g/cm^2$), sealed, and purged continuously for 1 day to trap $^{14}CO_2$. At that time, two replicates from each group were extracted and analyzed as described. The remaining replicates were incubated for 20 additional days. During this time $^{14}CO_2$ was trapped by purging the system as described on the days indicated in Figure 2. After a total of 21 days of incubation each remaining system was dismantled and analyzed as described.

Table I. Effects of Microorganisms on the Fate of [*ring*-^{14}C]Parathion in Cranberry Soils[a]

	cranberry soils plus:					
	none (control)	autoclaved[b]	aerated steam[b]	chloramphenicol[c]	tetracycline[c]	glucose[c]
	^{14}C Recovered in % of Applied [*ring*-^{14}C]Parathion[d]					
soil						
extraction phase						
benzene						
^{14}C	8.41 ± 1.39	96.2 ± 7.2[i]	89.8 ± 0.25[i]	84.2 ± 1.7[i]	8.45 ± 0.53	29.3 ± 2.2[i]
parathion[e]	3.30 ± 0.73	92.8 ± 7.5	68.7 ± 2.0	65.2 ± 2.4	3.95 ± 0.32	7.15 ± 2.8
water	0.71 ± 0.29	0.97 ± 0.28	0.40 ± 0.13	0.53 ± 0.05	0.35 ± 0.13	0.43 ± 0.07
bound[f]	39.7 ± 2.8	1.39 ± 0.19[i]	4.54 ± 0.70[i]	6.66 ± 0.38[i]	30.3 ± 1.4[j]	57.8 ± 9.2
vapor traps						
polyurethane	1.43 ± 0.28	2.47 ± 0.33	2.43 ± 0.21[j]	2.61 ± 0.32[j]	1.65 ± 0.13	1.82 ± 0.76
KOH	45.0 ± 3.5	0.11 ± 0.02[i]	1.04 ± 0.10[i]	2.18 ± 0.13[i]	49.5 ± 1.28	0.62 ± 0.38[i]
total	95.2 ± 3.4	101.0 ± 6.3	98.2 ± 0.58	96.1 ± 1.68	90.2 ± 0.83	89.9 ± 6.7
	Microorganisms/g Dry Wt of Soil, Million					
aerobic bacteria[g]	10.7 ± 7.9	ND[h]	5.6 ± 2.6	0.57 ± 0.47	7.3 ± 3.7	NM[h]
fungi[g]	0.029 ± 0.020	ND	0.26 ± 0.14	0.24 ± 0.02[i]	0.027 ± 0.006	NM

[a] Results obtained after 3 weeks of incubation are means ± SD of triplicate tests. [b] Soil autoclaved 3 times or treated for 30 min with aerated steamat 60 °C. [c] Soil treated by mixing with chloramphenicol or tetracycline at 100 ppm or with glucose at 2000 ppm on a dry weight basis. [d] Surface-applied [*ring*-^{14}C]parathion (0.3 μCi) at 8 μg/cm^2. [e] Determined by GLC. [f] Unextractable, bound ^{14}C. [g] Determined by dilution plate technique. [h] ND = not detected; NM = not measured. [i,j] Data are significantly different from the respective controls (none) at the 0.1%[i] or 1%[j] level (Student's *t* test).

Effects of Selected Fungicides on the Fate of [*ring*-^{14}C]Parathion in Cranberry Soils. Since most agricultural soils contain a mixture of man-made chemicals, environmentally significant interactions must be considered. Of special concern are those synthetic compounds which affect the soil microflora, in particular those microorganisms which in turn affect the fate of biodegradable substances such as parathion. It was for this reason that we investigated the potential effects of four fungicides, of which two (captafol and maneb) are registered for use in the production of cranberries, on the fate of [^{14}C]parathion in cranberry soils. The other two fungicides were PCNB (Terraclor) and benomyl (Benlate). Soils were uniformly treated at 100 ppm as described with one of the fungicides, and triplicate aliquots were placed into soil incubation jars. [^{14}C]Parathion was then applied to the soil surfaces. Incubation jars were sealed, and the systems for the trapping of volatile substances were set up. Soils were incubated for 21 days. Each unit was purged for 2 h at the times indicated in Figure 3 to trap $^{14}CO_2$. At the end of the incubation period, all systems were dismantled and analyzed as previously described. Fungal and bacterial counts were conducted with control and captafol-treated soils.

Additional experiments as described were conducted with captafol applied at 1, 5, 25, or 100 ppm to test for a dose–response relationship.

In another series of experiments, we were interested to determine whether the effect of a particular fungicide on [^{14}C]parathion degradation would be the same in soil-free media inoculated with soil microorganisms as that observed with soils. These experiments were similar to those described by Gorder and Lichtenstein (1980). [^{14}C]Parathion was added at 10 ppm (0.15 μCi) to 7.5 mL of basal salt media in six 25-mL Erlenmeyer flasks. Benomyl was added to three of the flasks to yield a concentration equivalent to 100 ppm. After that, all six media were inoculated with microorganisms derived from cranberry soils as described (Gorder and Lichtenstein, 1980) and incubated in the dark at 25 ± 2 °C on an Eberbach shaker. Four days later, the incubation mixtures were centrifuged and the supernatant and pellet were analyzed as described (Gorder and Lichtenstein, 1980).

Effects of Selected Herbicides on the Fate of [*ring*-^{14}C]Parathion in Cranberry Soils. Experiments similar to those conducted with fungicides were repeated with 2,4-D. In addition, nonreplicated preliminary tests were performed with atrazine and monolinuron.

Effects of Fertilizers on the Fate of [*ring*-^{14}C]-Parathion in Cranberry Soils. Nitrogen fertilizers, principally $(NH_4)_2SO_4$, are used in the production of cranberries. Previous studies in our laboratory (Anderson and Lichtenstein, 1971) showed that the biodegradation of DDT by *Mucor alternans* was reduced by 54% in cultures treated with $(NH_4)_2SO_4$ as compared with those treated with identical amounts of nitrogen in the form of NH_4NO_3 or urea. The degradation of the organophosphorus insecticide fonofos into water-soluble compounds by soil fungi in culture was also a function of the available nitrogen source (Flashinski and Lichtenstein, 1974).

For an investigation of the potential effects of nitrogen fertilizers on the fate of [^{14}C]parathion, experiments were conducted in which cranberry soils were mixed with $(NH_4)_2SO_4$, NH_4NO_3, KNO_3, or urea at 100 ppm of actual nitrogen. Untreated soils served as controls. After initial pH measurements had been performed, each of the five soils was placed in triplicate into incubation jars. [^{14}C]-Parathion (0.81 μCi) was then applied to all soil surfaces (8 μg/cm^2), and each soil was placed into the closed system as described. They were then incubated in the dark at 23 ± 2 °C for 3 weeks. Volatile ^{14}C-labeled compounds were purged from the system for 2 h each day for the first 7 days but only biweekly thereafter. $^{14}CO_2$ evolved on each sampling day was trapped in KOH and quantitated by LSC. After 3 weeks of incubation, the units were dismantled and the soils were sampled to determine the final soil pH. Vapor traps and soils from two replicates in each group were extracted and analyzed as described.

RESULTS AND DISCUSSION

Effects of Microorganisms on the Fate of [*ring*-^{14}C]Parathion in Cranberry Soils. As shown in Table I, treatment of soils by autoclaving, steam aeration, chloramphenicol, or glucose drastically changed the degradation of parathion in cranberry soils. Although in all cases the total radiocarbon recovered was similar, the fate of the insecticide differed. In control soils only 3% of the applied insecticide could be accounted for by GLC and 40% of the applied radiocarbon was soil bound. However, autoclaving of the soil stopped degradation of the insecticide to $^{14}CO_2$

Table II. Effect of Soil Pretreatment with Parathion or *p*-Nitrophenol on the Fate of [*ring*-^{14}C]Parathion in Cranberry Soils[a]

	cranberry soils plus:		
	none (control)	parathion[b]	*p*-nitrophenol[b]
	4 Days Incubation		
aerobic bacteria[c] per g dry wt of soil, million	2.3 ± 0.2	3.1 ± 0.9	3.9 ± 0.5
	plus [*ring*-^{14}C]parathion[d]		
	^{14}C recovered in % of applied [*ring*-^{14}C]parathion, incubated for:		
	1 Day		
soil			
extraction phase			
benzene	83.1 ± 0.45	13.6 ± 0.91	24.7 ± 3.4
water	0.83 ± 0.44	5.04 ± 0.49	3.31 ± 0.36
bound[e]	7.75 ± 0.06	39.8 ± 1.4	34.3 ± 3.4
vapor traps			
polyurethane	0.56 ± 0.20	0.25 ± 0.02	0.37 ± 0.06
KOH	2.17 ± 0.42	39.2 ± 1.2	34.5 ± 0.5
total	94.4 ± 0.6	98.0 ± 1.1	97.2 ± 0.9
	21 Days		
soil			
extraction phase			
benzene	8.92 ± 0.26	2.70 ± 0.05	4.88 ± 0.24
water	0.21 ± 0.05	0.10 ± 0.06	0.16 ± 0.07
bound	44.0 ± 4.1	36.2 ± 3.1	32.8 ± 0.35
vapor traps			
polyurethane	1.65 ± 0.13	0.46 ± 0.02	0.72 ± 0.04
KOH	51.3 ± 3.9	71.5 ± 1.2	72.2 ± 1.5
total	106 ± 0	111 ± 1.4	111 ± 1.4

[a] Results are averages of duplicate tests. [b] Parathion or *p*-nitrophenol was surface applied at 29 μg/cm² to 75 g of moist cranberry soil before [^{14}C]parathion was applied to these soil surfaces 4 days later. [c] Mean ± SD of triplicate tests determined by dilution plate technique. Data from *p*-nitrophenol pretreated soils were significantly different (at the 1% level, Student's *t* test) from the control. [d] Applied [*ring*-^{14}C]parathion (0.32 μCi) at 8 μg/cm². [e] Unextractable, bound ^{14}C.

as well as formation of bound residues. By the end of the incubation period 93% of the applied parathion was still recovered by GLC. Neither aerobic bacteria nor fungi could be detected in autoclaved soils. Similar results were obtained with soils that had been aerated with steam at 60 °C for 30 min. Although 60 °C aerated steam selectively kills vegetative cells, many spore-forming microorganisms survive this treatment (Baker, 1970). This suggests that parathion is degraded by 60 °C heat-sensitive microorganisms, i.e., by non-spore-forming bacteria or certain fungi. This is supported by the decline in the bacterial population (Table I) of principally Gram-negative rods and the concomitant proliferation of spore-forming fungi of which most appeared to belong to Penicillium and Fusarium species.

Chloramphenicol, a bacteriostatic agent which inhibits protein synthesis, also reduced the degradation of [^{14}C]-parathion. As with steam-treated soils, relatively large amounts of radiocarbon were recovered from the benzene extraction phases, but less parathion remained in comparison to autoclaved soils. This indicated that in steam- or chloramphenicol-treated soils benzene-soluble compounds had been produced from [*ring*-^{14}C]parathion, apparently by the remaining microflora which survived the treatment. The inhibition of parathion metabolism by chloramphenicol was correlated with a 95% reduction in the population of aerobic bacteria. It was also observed that chloramphenicol drastically reduced the diversity of cranberry soil microorganisms. Only *Bacillus cereus* va. mycoides, the fungus *Trichoderma* sp., and an unidentified bacterium were detected in chloramphenicol-treated cranberry soil. Tetracycline, another bactericide which inhibits protein synthesis, had little effect on parathion degradation since results obtained were similar to those obtained with controls. This apparently resulted from its inactivation in soil since microbial populations were not affected by its addition to soil (Table I).

The addition of glucose to soils increased the persistence of the insecticide and the appearance of other benzene-soluble ^{14}C-labeled compounds, which were identified by TLC as amino[^{14}C]parathion and *p*-amino[^{14}C]phenol. This appearance of amino metabolites was probably related to the increase in unextractable, bound ^{14}C-labeled residues (Katan and Lichtenstein, 1977), since over one-half of the radiocarbon originally applied to the soils was unextractable. Glucose added to cranberry soil also inhibited the formation of $^{14}CO_2$, which indicates that reductive conditions prevailed. It is likely that glucose is a better carbon source than parathion. These experiments suggest that certain groups of soil microorganisms are primarily responsible for the degradation and dissipation of parathion residues from cranberry soils.

Effects of Parathion Residues in Soil on the Fate of [^{14}C]Parathion. Pretreatment of cranberry soils with parathion or *p*-nitrophenol considerably increased the degradation of [*ring*-^{14}C]parathion. This is indicated by the increase in the evolution of $^{14}CO_2$, as shown in Figure 2. The effects of soil pretreatment were in particular evident 1 day after soil treatment with [^{14}C]parathion. While in controls only 2% of the applied radiocarbon had been released as $^{14}CO_2$, this figure amounted to 34 and 39% with soils pretreated with *p*-nitrophenol or parathion, respectively. Counts of aerobic bacteria in soils before treatment with radiocarbon indicated a significant increase in bacteria in *p*-nitrophenol-pretreated soils (Table II), in comparison to controls. It appears that the presence of *p*-nitrophenol applied directly or derived from parathion resulted in an increase of parathion-degrading microorganisms. Data in Table II also show that in parathion- or *p*-nitrophenol-pretreated soils, [^{14}C]parathion was degraded rapidly within 1 day after its application. At the same time, a remarkable increase in unextractable bound

Table III. Effect of Fungicides[a] on the Fate of [*ring*-^{14}C]Parathion in Cranberry Soil[b]

	^{14}C recovered in % of applied [*ring*-^{14}C]parathion[c] in cranberry soil plus:				
	none (control)	Difolatan (captafol)	Manzate (maneb)	Benlate (benomyl)	Terraclor (PCNB)
soil					
extraction phases					
benzene					
^{14}C	7.30 ± 2.03	74.8 ± 10.2[f]	22.9 ± 2.1[f]	16.7 ± 1.78[g]	4.93 ± 0.25
parathion[d]	2.83 ± 1.01	65.6 ± 14.0	8.00 ± 3.6	4.12 ± 0.88	1.40 ± 0.17
water	0.69 ± 0.55	1.02 ± 0.02	0.67 ± 0.19	0.47 ± 0.05	0.56 ± 0.04
bound[e]	37.5 ± 2.1	6.63 ± 2.59[f]	65.6 ± 5.7[g]	56.0 ± 5.9[g]	39.1 ± 1.04
vapor traps					
polyurethane	1.45 ± 0.22	2.83 ± 0.46[g]	2.78 ± 0.20[g]	1.76 ± 0.20[g]	1.61 ± 0.14
KOH					
^{14}C	44.5 ± 3.0	7.72 ± 6.78[g]	2.87 ± 1.58[f]	18.4 ± 4.4[g]	44.0 ± 1.7
% of control	100	17	6.4	41	99
total	91.4 ± 2.5	93.0 ± 3.5	94.9 ± 2.4	93.3 ± 1.8	90.2 ± 0.5

[a] Captafol (analytical grade) and commerical formulations of Manzate, Benlate, or Terraclor were mixed with soil at 100 ppm of AI on a dry weight basis. [b] Results obtained after 3 weeks of incubation are means ± SD of triplicate tests. [c] Applied [*ring*-^{14}C]parathion (0.34 μCi) at 8.5 μg/cm^2. [d] Determined by GLC. [e] Unextractable, bound ^{14}C. [f,g] Data are significantly different from respective controls (none) at the 0.1%[f] and 1%[g] level (Student's *t* test).

^{14}C-labeled soil residues had occurred, amounting to 4–5 times more than in control soils. After 20 additional days of incubation (day 21, Table II), however, these differences had largely disappeared. At that time, [^{14}C]parathion had also been degraded in controls and relatively large amounts of bound ^{14}C-labeled residues had been produced. Still, considerably more $^{14}CO_2$ had evolved from the pretreated soils, indicating that aerobic microorganisms had been more active.

Effects of Selected Fungicides on the Fate of [*ring*-^{14}C]Parathion in Cranberry Soils. The evolution of $^{14}CO_2$ from [^{14}C]parathion is one method to obtain information about the rate of degradation of the insecticide. As shown in Figure 3, some of the fungicides, indeed, inhibited the degradation of [^{14}C]parathion in cranberry soils. While with control soils 45% of the applied radiocarbon had been released in the form of $^{14}CO_2$ over a 3-week period, only 3 or 8% was released from soils treated with maneb or captafol, respectively. As shown in Table III, the degradation of [^{14}C]parathion was considerably inhibited by captafol, since 66% of the applied insecticide was still present in these soils as opposed to only 3% in controls. The decreased metabolism of the insecticide is also indicated by a significant reduction in bound radiocarbon. Captafol apparently inhibited those soil microorganisms which are usually responsible for the degradation of the insecticide. The effects of captafol on the biodegradation of [^{14}C]parathion were similar to those observed with autoclaved soils (Table I). On the basis of visual, nonmicroscopic observations, captafol totally suppressed the population of soil fungi, but, contrary to the effect of soil autoclaving, the total population of aerobic bacteria, determined by plate dilution techniques, increased 5.9-fold. These results are similar to those reported by Corden and Young (1965). Only one colony type of bacteria predominated on 1:10^5 soil/dilution plates prepared from captafol-treated soils. This suggests that captafol reduced bacterial diversity.

Maneb also inhibited $^{14}CO_2$ evolution from [^{14}C]parathion but not the total degradation of the insecticide. As shown in Table III, only 8% of the applied insecticide was recovered; yet considerably more benzene-soluble radiocarbon (23%) was still present. TLC of the benzene extraction phase revealed the presence of *p*-amino[^{14}C]phenol in addition to [^{14}C]parathion. As demonstrated by Katan and Lichtenstein (1977), binding of parathion residues to soils occurs after the insecticide has been reduced to amino

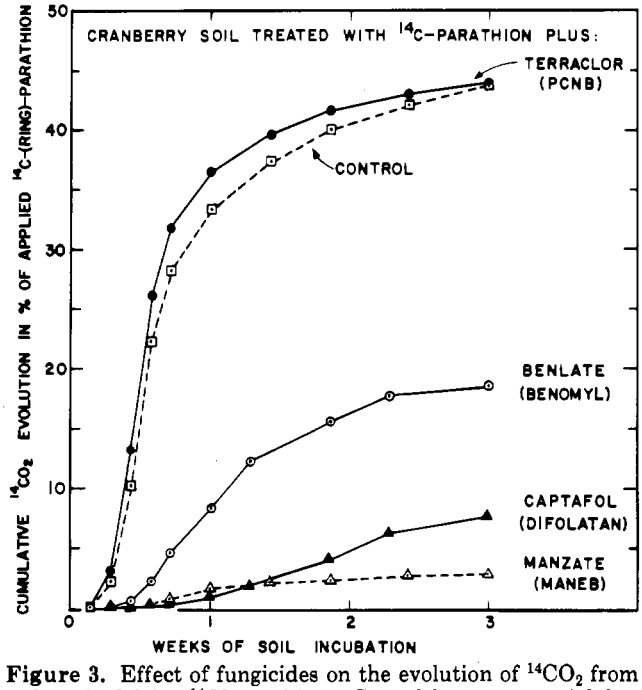

Figure 3. Effect of fungicides on the evolution of $^{14}CO_2$ from soil-applied [*ring*-^{14}C]parathion. Captafol or commercial formulations of Manzate, Benlate, and Terraclor were mixed with cranberry soil at 100 ppm of AI before a surface application of [*ring*-^{14}C]parathion (0.34 μCi) at 8.5 μg/cm^2. Results are means of triplicate tests and represent accumulative $^{14}CO_2$ evolution.

compounds. Thus, in the presence of maneb, 66% of the soil-applied radiocarbon was unextractable as opposed to 38% with control soils. It appears, therefore, that maneb did not affect soil microorganisms which are responsible for the reduction of parathion in cranberry soils.

Benomyl affected [^{14}C]parathion degradation, although to a lesser extent than did captafol and maneb. Thus, in benomyl-treated soils the amount of $^{14}CO_2$ evolved was only 18% of the applied radiocarbon. As with maneb, the presence of benomyl in the soil resulted in recoveries of larger amounts of benzene-soluble radiocarbon and in increased binding of ^{14}C-labeled compounds.

PCNB did not affect the fate of [^{14}C]parathion in cranberry soils. This fungicide is not harmful to bacteria, in some cases increases their number in soils, and only affects streptomycetes and certain fungi in nutrient-amended soils (Farley and Lockwood, 1969).

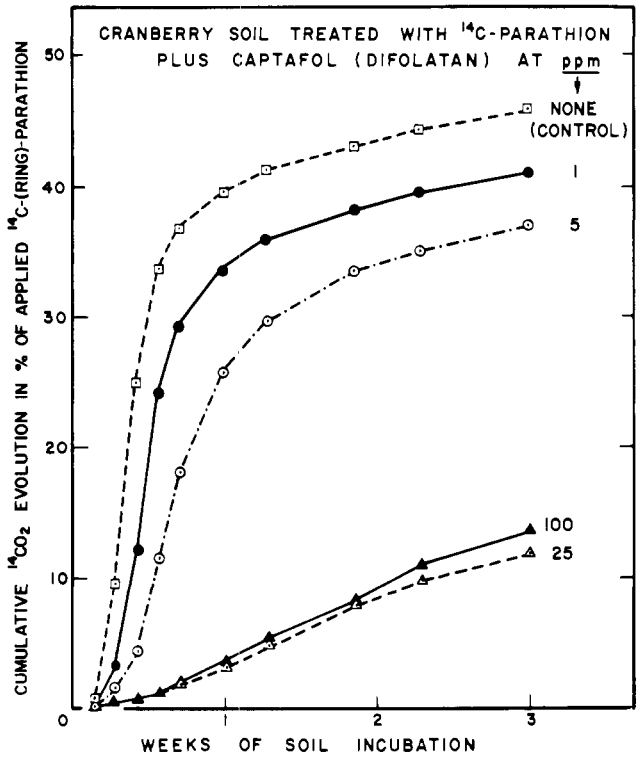

Figure 4. Effect of increasing concentrations of captafol (Difolatan) on the evolution of $^{14}CO_2$ from soil-applied [*ring*-^{14}C]-parathion. Captafol was applied and mixed with soil before application of [*ring*-^{14}C]parathion (0.78 μCi) at 8 $\mu g/cm^2$. Results are means of triplicate tests.

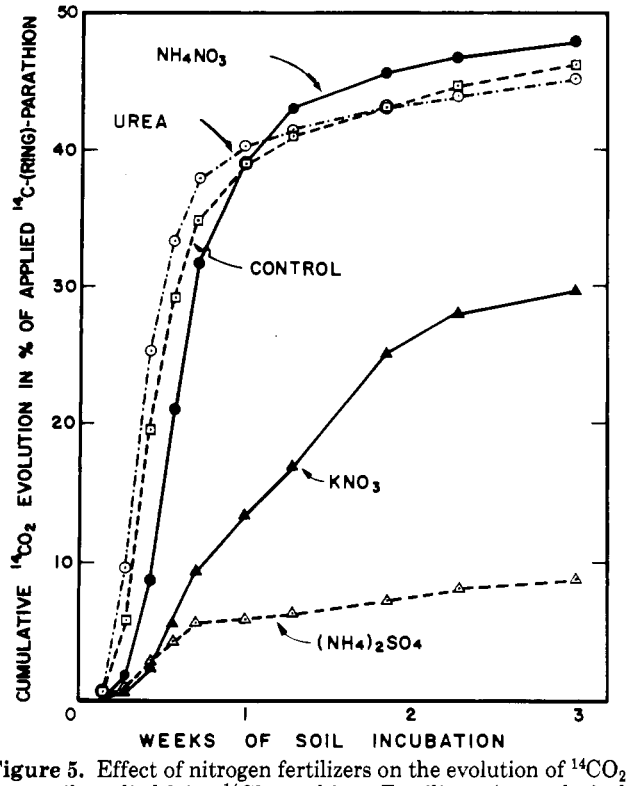

Figure 5. Effect of nitrogen fertilizers on the evolution of $^{14}CO_2$ from soil-applied [*ring*-^{14}C]parathion. Fertilizers (as analytical compounds) were mixed with cranberry soil at 100 ppm of nitrogen equivalent prior to the application of [*ring*-^{14}C]parathion (0.81 μCi) at 8 $\mu g/cm^2$. Results are means of triplicate tests.

Results from experiments conducted with soils treated with captafol at 1, 5, 25, or 100 ppm and with [^{14}C]parathion showed that fungicide concentrations of 25 and 100 ppm equally inhibited the evolution of $^{14}CO_2$ (Figure 4). Soil residue levels of captafol more closely approximating those resulting from normal agricultural practice, 1 and 5 ppm, reduced total $^{14}CO_2$ evolution by 11 and 19%, respectively. These reductions were significant at the 5 and 1% levels (Student's *t* test), respectively, suggesting that in these experiments the "no effect" concentration of captafol was approximately 1 ppm.

Data presented in Table III indicated that in the presence of benomyl, relatively large amounts of [^{14}C]parathion-derived radiocarbon were bound to soil. Experiments were therefore conducted with [^{14}C]parathion and benomyl, but without soil. Results showed that in culture media containing microorganisms from cranberry soils, the degradation of the insecticide was totally inhibited by the fungicide. Thus, in the presence of benomyl, 100 ± 2.9% of the applied radiocarbon was recovered from the media. Of this radiocarbon, 92.1 ± 3.7% was still associated with the benzene extraction phase and only 4.02 ± 0.11% was water soluble. The pellet, obtained after centrifuging the incubation mixture, contained 3.95 ± 0.9% of the applied radiocarbon. Conversely, in benomyl-free incubation mixtures (controls), a total of only 37.1 ± 3.6% of the applied radiocarbon was recovered, of which 7.51 ± 0.47% was benzene soluble, 11.6 ± 1.6% was water soluble, and 17.9 ± 2.9% was associated with the microbial pellet. The overall low recovery of radiocarbon in the absence of benomyl (controls) was probably due to the loss of $^{14}CO_2$, which, as shown by Gorder and Lichtenstein (1980), had been produced by the microorganisms from [^{14}C]parathion. Benomyl, therefore, affected the persistence of [^{14}C]-parathion by eliminating or inhibiting those soil microorganisms which degrade the insecticide. It should be kept in mind, however, that the effects of benomyl at a concentration of 100 ppm in culture media would be different from the same concentration in soil, where binding, adsorption, and other phenomena are of importance.

Effects of Selected Herbicides on the Fate of [^{14}C]Parathion in Cranberry Soils. Results obtained in experiments with 2,4-D and [^{14}C]parathion showed that the persistence of parathion in cranberry soils was increased in the presence of the herbicide. Thus, after 3 weeks of incubation, only 2.09 ± 0.31% of the applied [^{14}C]parathion could be recovered from control soils, but 12.2 ± 2.3% of the applied [^{14}C]parathion was recovered from 2,4-D-treated soils. In addition, the benzene soil extraction phases contained no reduced ^{14}C-labeled amino compounds, while in control soils *p*-amino[^{14}C]phenol was detected by TLC. This suggests that 2,4-D may have inhibited the reduction of [^{14}C]parathion by affecting the activity of nitroreductase-producing microorganisms. The undetectability of [^{14}C]parathion-derived amino metabolites in 2,4-D-treated soils was also reflected in the reduced recoveries of soil-bound radiocarbon, which amounted to only 18.1 ± 1.9% of applied in 2,4-D-treated soils but to 37.6 ± 3.1% of applied in control soils.

Preliminary, unreplicated tests with atrazine or monolinuron at 100 ppm gave results similar to those observed with 2,4-D.

Effects of Fertilizers on the Fate of [^{14}C]Parathion in Cranberry Soils. Previous studies in our laboratory with cultures of soil fungi indicated that the degradation of both DDT (Anderson and Lichtenstein, 1971) and fonofos (Flashinski and Lichtenstein, 1975) was a function of available nitrogen sources. Studies reported here confirmed that some nitrogen fertilizers can indeed inhibit the degradation of [^{14}C]parathion in cranberry soils. Thus, application of $(NH_4)_2SO_4$ to [^{14}C]parathion-treated cranberry soils inhibited the degradation of the insecticide to

Table IV. Effects of Nitrogen Fertilizers[a] on the Fate of [*ring*-^{14}C]Parathion in Cranberry Soils[b]

	^{14}C recovered in % of applied [*ring*-^{14}C]parathion[c] in cranberry soils plus:				
	none (control)	$(NH_4)_2SO_4$	KNO_3	NH_4NO_3	urea
soil					
extraction phases					
benzene	5.47 ± 0.39	28.7 ± 9.4	12.1 ± 1.7	5.86 ± 0.55	5.26 ± 0.11
water	0.37 ± 0.09	0.70 ± 0.28	0.65 ± 0.11	0.57 ± 0.05	0.50 ± 0.36
bound[d]	33.7 ± 0.6	48.6 ± 10.4	42.4 ± 4.8	31.5 ± 0.7	36.7 ± 1.3
vapor traps					
polyurethane	2.21 ± 0.44	3.90 ± 1.0	3.55 ± 0.39	1.88 ± 0.89	2.10 ± 0.56
KOH	45.8 ± 1.0	7.94 ± 0.83	29.5 ± 5.1	48.7 ± 1.1	45.4 ± 0.07
total	87.5 ± 0.4	89.8 ± 0.6	88.2 ± 1.7	88.6 ± 0.6	89.9 ± 1.6
soil pH after:					
no incubn	5.97 ± 0.02	5.34 ± 0.04	5.24 ± 0.03	5.37 ± 0.03	6.06 ± 0.01
3-week incubn	5.64 ± 0.06	5.14 ± 0.15	6.69 ± 0.04	6.06 ± 0.07	6.40 ± 0.06

[a] Mixed with soils at 100 ppm of nitrogen equivalent. [b] Results obtained after 3 weeks of soil incubation are averages of duplicate tests. [c] [*ring*-^{14}C]Parathion (0.81 μCi) was applied to the soil surface at 8 μg/cm^2. [d] Unextractable, bound ^{14}C.

$^{14}CO_2$, since only 8% of the applied radiocarbon evolved as $^{14}CO_2$ during the 3-week incubation period (Figure 5). In controls, however, this figure amounted to 46%. KNO_3 also reduced the formation of $^{14}CO_2$ but not to the extent observed with $(NH_4)_2SO_4$. Addition of NH_4NO_3 or urea to [^{14}C]parathion-treated soils had no apparent effect.

Results obtained after extraction and analyses of fertilizer-treated soils and vapor traps are summarized in Table IV. Although under all experimental conditions the total amounts of ^{14}C recovered were similar, the distribution of ^{14}C-labeled compounds into benzene-soluble, water-soluble, and bound residues was not, thus possibly indicating a drift in the pathway of [^{14}C]parathion degradation. The insecticide was most persistent in soils containing $(NH_4)_2SO_4$. This is demonstrated by a recovery of 29% of the applied radiocarbon in the benzene-soluble form. Analyses by TLC and autoradiography of this benzene extraction phase revealed the presence of [^{14}C]-parathion, *p*-amino[^{14}C]phenol, and amino[^{14}C]parathion. Compared to controls, some increase in bound radiocarbon was also noticeable in the presence of $(NH_4)_2SO_4$. Inhibitory effects of KNO_3 on parathion metabolism as shown in Figure 5 were also evident after extraction and analyses of soils and vapor traps (Table IV). All analytical results obtained with fertilizers in the form of NH_4NO_3 or urea were similar to those observed with controls (Table IV).

The pH of the treated soils was slightly different; yet not enough data are available to draw any definite conclusions.

The preceding data, therefore, indicate that the form of the N-soil amendment and not the N amendment as such affected the degradation of [^{14}C]parathion. One explanation for the inhibition of the metabolism of [^{14}C]-parathion to $^{14}CO_2$ by $(NH_4)_2SO_4$ could be the lower pH level induced (Table IV), since bacterial activity is known to be suppressed by low pH. Soil microorganisms differ in their ability to utilize various forms of nitrogen. Thus, microbial composition in soil is expected to be affected by the source of nitrogen and this in turn could affect the metabolism of pesticides. In urea-treated soils, nitrogen amendments resulted in a marked proliferation of fungal and bacterial colonies. Eck (1971) reported that cranberry soils contain large quantities of plant debris low in total nitrogen. This in turn may limit the metabolism and breakdown of organic matter. If nitrogen amendments resulted in a more favorable C/N ratio, a shift in the population of soil microorganisms and an increase in microbial activity may result. Whether fungi or bacteria will predominate will depend on many factors (Stotzky, 1974), but the form of the nitrogen amendment may apply selection pressure that results in conditions more or less favorable for parathion-metabolizing organisms.

Nitrogen fertilizer as $(NH_4)_2SO_4$ is used in many Wisconsin cranberry bogs, and the amounts applied per year average 45 lb of actual nitrogen per acre. By the assumption that a 3-in. deep acre has an approximate weight of 1 million pounds, the concentration of nitrogen equivalent in this soil would amount to 45 ppm. Since this concentration is below that used with all fertilizers in the experiments described above, similar experiments were conducted with $(NH_4)_2SO_4$ at concentrations of 55 and 110 ppm of nitrogen equivalent. Test were also conducted with NH_4NO_3 at 91 and 182 ppm of nitrogen equivalent, since no effects on the degradation of [^{14}C]parathion had been noticed with NH_4NO_3 concentrations of 100 ppm (Figure 5 and Table IV). After the amount of $^{14}CO_2$ evolved from the fertilizer-treated soils over a 3-week period was measured, it was found that $(NH_4)_2SO_4$ at 55 ppm of nitrogen equivalent inhibited $^{14}CO_2$ evolution to the same extent as did a concentration of 110 ppm. Therefore, these data do not show the potential threshold concentration for $(NH_4)_2SO_4$ relative to the inhibition of [^{14}C]parathion degradation. With NH_4NO_3 concentrations at 91 ppm of nitrogen equivalent, results similar to control and to those obtained with concentrations of 100 ppm (Figure 5) were obtained. However, an increase of the NH_4NO_3 concentration to 182 ppm of nitrogen equivalent did result in a 52% inhibition of $^{14}CO_2$ evolution from [^{14}C]parathion. This suggests that above a threshold concentration of 100 ppm of nitrogen equivalent NH_4NO_3 suppression of $^{14}CO_2$ evolution is dose related. Flashinski and Lichtenstein (1975) reported that the metabolism of fonofos by the fungus *Rhizopus arrhizus* was also inhibited by NH_4NO_3 at high concentrations, which is consistent with these findings.

Results reported in this study show that a reduction in the population of soil microorganisms resulted in a decrease in one or more of the degradation processes of [^{14}C]parathion. It appears that not only the number but also the composition of the microflora affect the fate of the insecticide. Thus, degradation of [^{14}C]parathion was similar in both steam-aerated and chloramphenicol-treated soils, yet the number of aerobic soil bacteria was different (Table I). Apparently, degradation of [^{14}C]parathion to $^{14}CO_2$ and production of bound residues are carried out by different groups of microorganisms, since in certain cases only one of the two processes could be stopped as with glucose, maneb, or $(NH_4)_2SO_4$.

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Comparison of Liquid and Gas Chromatography for the Determination of Bromoxynil Octanoate and Benzoylprop Ethyl in Wheat Products

James F. Lawrence,* Luz G. Panopio, and Harry A. McLeod

Wheat products, including whole grain wheat, shredded wheat cereal, whole wheat flour and bread, and refined white flour and bread were blended with methanol to extract bromoxynil octanoate [[(3,5-dibromo-4-octanoyl)oxy]benzonitrile] and benzoylprop ethyl [ethyl *N*-benzoyl-*N*-(3,4-dichlorophenyl)-2-aminopropionate] from the samples. An aliquot of the extract was partitioned between methylene chloride and water. The organic extract was reduced to a small volume and passed through a 3% deactivated Florisil column. The fraction containing the herbicides was analyzed by both gas (GC) and liquid (LC) chromatography. Detection limits in the samples were about 0.05 ppm by LC and about 0.005 ppm by GC. Recoveries were generally higher than 80% by both LC and GC at 0.1 ppm or greater.

Statistics released by the Canadian government in 1977 (Statistics Canada) indicated that wild oat herbicides [including bromoxynil octanoate [[(3,5-dibromo-4-octanoyl)oxy]benzonitrile], benzoylprop ethyl [ethyl *N*-benzoyl-*N*-(3,4-dichlorophenyl)-2-aminopropionate], barban, difenzoquat, and asulam] accounted for about 50% by weight of all pesticides sold in Canada in 1976. Essentially all (99.9%) were used in western Canada. As a result of this great usage, a need has arisen to monitor cereal grains, particularly wheat, for residues of these herbicides. At the present time no routine method exists which can adequately screen for them. Of the five herbicides, only bromoxynil octanoate (Helfant, 1979) and benzoylprop ethyl (Wright and Mathews, 1976) pass through a gas chromatograph (GC) without derivatization. Little or no work has been carried out on the analysis of the remaining three by GC after derivatization. Barban has been analyzed by GC after conversion to 3-chloro-2,4,6-tribromoaniline (Harris and Whiteoak, 1972). Recently, a GC method involving hydrolysis and derivatization has been reported for asulam (Bardalaye et al., 1979). No GC method has been reported for difenzoquat although it is possible that the sodium borohydride method for diquat reported by King (1978) might be suitable.

An alternative approach to GC is LC where most organic compounds can be separated by some means without recourse to derivatization. In fact, derivatization in LC is usually employed to improve detectability and not chromatographic behavior (Lawrence, 1979; Jupille, 1979). The wild oat herbicides mentioned above have adequate UV absorbance so that such derivatization becomes unnecessary. Thus, it should be possible to analyze all five herbicides directly by LC at levels of 0.1 ppm or greater. This

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