Mineralization of Parathion in the Rhizosphere of Rice and Pearl Millet

In a study on the ring cleavage of [*ring*-2,6-^{14}C]parathion [*O,O*-diethyl *O*-(*p*-nitrophenyl) phosphorothioate] applied to the root zone of rice plants grown under flooded conditions, evolution of $^{14}CO_2$ was more pronounced at the seedling stage than at the maximum tillering and panicle initiation stages. The degree of rhizosphere effect also depended on the variety used. The rhizosphere effect was related to the root oxidase activity in rice but not necessarily to the biomass of the plants. Pearl millet (*Pennisetum typhoides*), a C_4 plant, exerted a more pronounced rhizosphere effect on ring cleavage of parathion than rice, a C_3 plant.

Most of the studies on the metabolism of pesticides in the soil environment have been concerned with unplanted systems. But, a planted soil system with intense microbial activity and complex interactions in the immediate vicinity of the roots is more dynamic than an unplanted soil. In recent years, there are reports on the effect of plants on pesticide degradation in the soil. For instance, Hsu and Bartha (1979) reported that 17.9% of ^{14}C in labeled parathion [*O,O*-di[1-^{14}C]ethyl *O*-(*p*-nitrophenyl) phosphorothioate] was evolved as $^{14}CO_2$ from a nonflooded soil planted with bean seedlings as compared to 7.8% evolved from the unplanted soil during a 30-day incubation. Likewise, evolution of $^{14}CO_2$ from [*ring*-^{14}C]parathion [*O,O*-diethyl *O*-(*p*-nitrophenyl) phosphorothioate] was more pronounced in a soil planted with rice seedlings than in an unplanted soil under both flooded and nonflooded conditions (Reddy and Sethunathan, 1983); interestingly, the planted system evolved more $^{14}CO_2$ under flooded than under nonflooded conditions. The present study is concerned with ring cleavage of [*ring*-^{14}C]parathion in rice rhizosphere as related to plant age and variety. Also, we compared the ring cleavage of parathion in the rhizosphere of rice, a C_3 plant, and pearl millet, a C_4 plant.

MATERIALS AND METHODS

To study the rhizosphere effect of rice varieties on ring cleavage of parathion, four seedlings (25 day old) of rice varieties IR 8 and T 1242 were transplanted to 100-g alluvial soil (pH 6.2, organic matter 1.6%, total nitrogen 0.09%) samples contained in 100-mL beakers immediately after flooding to provide 5 cm of standing water. The planted and unplanted (control) systems were incubated in a greenhouse under natural light conditions. All beakers received 1 mL of modified Hoagland's solution (Yoshida et al., 1976) at 9 days after transplanting. The planted and unplanted systems were fortified with an aqueous solution of parathion (technical grade) at 20 μg/g of soil at 10 days after transplanting. At 20 days after transplanting, [*ring*-2,6-^{14}C]parathion (1.8×10^5 dpm/beaker) was introduced at three locations to the root zone (4 cm below the surface of the soil) of the rice plant in 0.05 mL of acetone with the help of a syringe. After 3 days of [^{14}C]parathion addition, four beakers for each variety were placed under a bell jar and $^{14}CO_2$ evolved during 3–6 and 6–9 days was trapped in 25 mL of N KOH as described earlier (Reddy and Sethunathan, 1983). This period of significant $^{14}CO_2$ evolution was chosen based on the earlier report (Reddy and Sethunathan, 1983). $^{14}CO_2$ trapped in KOH was analyzed by liquid scintillation. The soil samples after removal of $^{14}CO_2$ trap were extracted and analyzed for undegraded parathion and its metabolites, *p*-nitrophenol and aminoparathion (Reddy and Sethunathan, 1983). Unextractable residues (remaining after organic solvent extraction) in the soil were determined by combusting the soil (500 mg) in a Coleman carbon–hydrogen analyzer with excess of oxygen at 950 °C for 8 min. $^{14}CO_2$ evolved was trapped in 2 mL of hyamine hydroxide and the radioactivity determined.

The same procedure was followed to study the extent of evolution of $^{14}CO_2$ from [^{14}C]parathion in the rhizosphere of a C_3 plant, rice (Annapurna), and a C_4 plant, pearl millet (*Pennisetum typhoides*, variety IP 7973 from ICRISAT, Hyderabad). But in this experiment seedlings (one per beaker) were grown under nonflooded (60% water holding capacity) conditions and [^{14}C]parathion was applied at 40 days after sowing. $^{14}CO_2$ evolved during 3–9 days after [^{14}C]parathion was quantified as described in the first experiment.

To determine the effect of growth stage of rice plant on mineralization of [^{14}C]parathion in the rhizosphere, rice seedlings were grown in 500 g of alluvial soil contained in glass jars (7.7 × 13.7 cm) under flooded conditions. Staggered transplanting (four beakers with one seedling each for each growth stage) was done at 25-day intervals to synchronize the seedling (10 days after transplanting), maximum tillering (35 days after transplanting), and pa-

Table I. Mineralization of Parathion in Rhizosphere of Two Rice Varieties[a]

fractions	radioactivity, dpm × 10^3/100 g of soil[b]		
	unplanted	T 1242	IR 8
volatiles			
CO_2 (3–9 days)	3.2 ± 0.3	12.0 ± 1.2	19.5 ± 0.6
other than CO_2 (3–9 days)	0.3 ± 0.2	0.5 ± 0.2	0.8 ± 0.3
soil residue			
methanolic fraction	151.4 ± 9.4	139.2 ± 7.8	134.2 ± 6.9
parathion	62.8 ± 4.9	54.3 ± 3.4	44.6 ± 3.5
p-nitrophenol	65.5 ± 4.5	55.8 ± 2.3	53.1 ± 3.8
aminoparathion	9.2 ± 1.2	0	0
soil-bound residues	5.8 ± 0.6	3.8 ± 0.5	1.8 ± 0.3
total recovery[c]	160.7 ± 10.5	155.5 ± 9.7	156.3 ± 8.1

[a] Initial radioactivity added to each beaker: 1.8×10^5 dpm/100 g of soil. [b] Values are means of the data from two replicates ± standard deviations. [c] Sum of radioactivity in volatiles, methanolic fraction, and soil-bound residues.

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Table II. $^{14}CO_2$ Evolved from [*ring*-^{14}C]Parathion in a Flooded Soil Planted with Rice of Different Growth Stages[a]

growth stage	$^{14}CO_2$, dpm × 10^3/500 g of soil, evolved[b]	root oxidase activity,[c] μg of naphthylamine disappeared (g dry weight)$^{-1}$ h^{-1}	dry weight, g/plant[c]	
			root	shoot
seedling	26.5 ± 1.8	1633 ± 68	1.49 ± 0.21	2.82 ± 0.28
tillering	14.8 ± 0.6	962 ± 35	4.70 ± 0.34	6.45 ± 0.47
panicle initiation	10.2 ± 1.4	605 ± 83	5.81 ± 0.56	8.15 ± 0.60
unplanted	4.0 ± 0.3			

[a] Initial radioactivity added to each jar: 1.8 × 10^5 dpm/500 g of soil. [b] Values are means of the data from two experiments ± standard deviations. [c] Values are means of the data from three experiments ± standard deviations.

Table III. $^{14}CO_2$ Evolved from [*ring*-^{14}C]Parathion in a Nonflooded Soil Planted with Rice and Pearl Millet[a]

crop	$^{14}CO_2$, dpm × 10^3/500 g of soil, evolved[b]	dry weight, g/plant[c]	
		root	shoot
rice (Annapurna)	14.8 ± 1.5	1.39 ± 0.05	2.71 ± 0.12
pearl millet (IP 7973)	34.8 ± 2.5	4.87 ± 0.10	12.11 ± 0.17
unplanted	7.8 ± 0.8		

[a] Initial radioactivity added to each jar: 1.8 × 10^5 dpm/500 g of soil. [b] Values are means of the data from two experiments ± standard deviations. [c] Values are means of the data from three experiments ± standard deviations.

nicle initiation (60 days after transplanting) stages. The soil in all beakers was supplemented with 10 mL of modified Hoagland's solution at 20-day intervals. An aqueous solution of technical parathion was added to all beakers at 20 μg/g of soil at 10 days before [^{14}C]parathion addition. [^{14}C]Parathion (1.8 × 10^5 dpm/beaker) was applied to the root zone of rice plants of different growth stages (10, 35, and 60 days after transplanting). $^{14}CO_2$ evolved from four beakers for each growth stage was trapped in KOH during 3–9 days after [^{14}C]parathion addition as described earlier.

To assay root oxidase activity, rice plants were pulled out of the soils with roots intact at different growth stages. Root oxidase was assayed following the method of Ota (1970). The roots were washed in water, cut into 1-cm pieces, and then squeezed gently with Whatman No. 1 filter paper to remove moisture. All operations were carried out with minimum exposure of the roots to the atmosphere. Root pieces (1 g) were incubated with 50 mL of a 20 ppm of naphthylamine solution in a 100-mL Erlenmeyer flask for 3 h on a shaker. One-milliliter aliquots of naphthylamine before and after incubation were treated with 1 mL each of a 100 ppm of $NaNO_2$ solution and 1% sulfanilic acid. The color developed was read at 500 nm after 30–60 min. Root pieces were subsequently dried at 60 °C for 6 h.

The KOH solution with trapped $^{14}CO_2$ was acidified with 1 N HCl and $^{14}CO_2$ evolved was absorbed in hyamine hydroxide as described earlier (Reddy and Sethunathan, 1983). The hyamine hydroxide (2 mL) was mixed with 5 mL of a scintillation cocktail [5 g of PPO (2,4-diphenyloxazole), 0.3 g of POPOP [1,4-bis[2-(5-phenyloxazolyl)]-benzene], and toluene to make 1 L] and the radiactivity determined by liquid scintillation.

RESULTS AND DISCUSSION

IR 8 exerted a more significant rhizosphere effect on ring cleavage of parathion than T 1242 (Table I). IR 8 is a new, short, high-tillering, and high-yielding variety while T 1242 is a traditional, tall, low-tillering, and low-yielding variety. The biomass of IR 8 (root and shoot, 2.15 ± 0.20 and 3.46 ± 0.14 g/four seedlings, respectively) was more than that of T 1242 (root and shoot, 1.53 ± 0.10 and 2.46 ± 0.81 g/four seedlings, respectively). Thus, the enhanced rhizosphere effect of IR 8 may be due to increased root exudates. Analysis of residues in the soil at 9 days showed that degradation of parathion proceeded essentially by hydrolysis and not by nitro group reduction in both planted and unplanted systems as indicated by the significant formation of *p*-nitrophenol. This is in agreement with the earlier report (Sudhakar-Barik et al., 1979) of significant hydrolysis of parathion after two or three applications to flooded soil.

The rhizosphere of 10-day-old rice seedlings effected more rapid evolution of $^{14}CO_2$ than did the rhizosphere of the 35-day-old (maximum tillering stage) and 60-day-old (panicle initiation stage) plant (Table II). The extent of $^{14}CO_2$ evolution followed the order seedling > maximum tillering > panicle initiation.

The evolution of $^{14}CO_2$ was not always related to the higher biomass of the rice plant. Thus, biomass of the rice plant increased from seedling to panicle initiation, but the amount of $^{14}CO_2$ evolved decreased. There is evidence that the root oxidizing capacity of a lowland rice plant decreases with an increase in the age of the rice plant (Yoshida, 1981). We also found that the root oxidase activity of rice plant under flooded conditions decreased in the order seedling > maximum tillering > panicle initiation. Although rice plants can transport oxygen from the foliage to the roots, the rhizosphere of the lowland rice need not always be aerobic. According to Kimura et al. (1979), the rhizosphere of the lowland rice may be aerobic in the initial stage of crop growth but becomes more reduced, in terms of the accumulation of ferrous iron and drop in redox potential, especially at or after panicle initiation. According to our study, the retardation of ring cleavage of parathion at later growth stage (panicle initiation) of lowland rice is possibly related to a decrease in the root oxidase activity and a depletion of oxygen in the root zone.

Mineralization of [^{14}C]parathion to $^{14}CO_2$ in the rhizosphere of rice (C_3 plant) and pearl millet (C_4 plant) was compared under nonflooded conditions. Interestingly, pearl millet exerted a more pronounced rhizosphere effect (>2-fold) than rice (Table III). C_4 plants differ from C_3 plants in anatomical, physiological, and biochemical parameters. More important, C_4 plants exhibit a higher photosynthetic efficiency than C_3 plants (Black, 1973). We found that pearl millet produced more biomass (root and shoot) than rice under the experimental conditions. Then, the enhanced rhizosphere effect in pearl millet through increased root exudates becomes difficult to dismiss.

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Registry No. Parathion, 56-38-2; oxidase, 9035-73-8; *p*-nitrophenol, 100-02-7; aminoparathion, 3735-01-1.

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Nontransmission of Deoxynivalenol (Vomitoxin) to Eggs and Meat in Chickens Fed Deoxynivalenol-Contaminated Diets

Deoxynivalenol (DON, vomitoxin) present in the rations of Leghorn chicks and laying hens and broiler chickens at dietary levels of 4–5 μg/g for periods of between 28 and 190 days was not detected in eggs or tissues (drumstick, breast, liver, and gizzard). The detection limit of the method used was 10 μg of DON/kg of tissue. It was also determined that DON was stable in feed (kept at room temperature, ≥10 °C), eggs (23 °C), and chicken tissue (4 °C).

Considerable evidence has now accumulated on the natural occurrence of vomitoxin [3α, 7α, 15-trihydroxy-12,13-epoxytrichothec-9-en-8-one; deoxynivalenol (DON)] in grains grown in many parts of the world (Scott et al., 1981; Trenholm et al., 1981; Ueno, 1980; Vesonder and Hesseltine, 1981). DON is a metabolite of *Fusarium graminearum* (perfect stage *Gibberella zeae*) that has been found to be an emetic agent in swine, dogs, and ducklings (Ueno, 1980), to cause feed refusal by swine and rats (Forsyth et al., 1977; Yoshizawa et al., 1978), and to be teratogenic in mice (Khera et al., 1982). Poultry, however, apear to be relatively insensitive to DON (Hamilton et al., 1981a,b; Huff et al., 1981; Hulan and Proudfoot, 1982; Moran et al., 1982), which raises the possibility that DON-contaminated feed grains may be incorporated into rations of laying hens and broiler chickens without affecting performance. Therefore, it is important from the human health viewpoint to establish whether DON residues occur in eggs and tissues when poultry are given rations that contain DON. This study was undertaken to answer this question when DON naturally present in wheat was fed to White Leghorn chicks and laying hens and broiler chickens at levels about 4–5 μg/g of feed over continuous periods of between 28 and 190 days (Hamilton et al., 1983). No previous residue studies on DON in any animal species have been published. However, the transmission of other polar mycotoxins (ochratoxin A and aflatoxins) to eggs and tissues of chickens fed contaminated feed has been reported by several investigators (Harwig et al., 1983; Juszkiewicz et al., 1982; Rodricks and Stoloff, 1977).

EXPERIMENTAL SECTION

Feed Samples. Spring wheat was the major component of the control and DON-containing rations that were otherwise nutritionally balanced for laying hens, Leghorn chicks, and broiler chickens. These rations, whose major source of protein was a 48% protein-containing soybean meal, were formulated to be isocaloric as well as isonitrogenous because the protein content of the DON-contaminated wheat was much higher than that of the noncontaminated wheat (13.6 vs. 9.8%, N × 5.8, respectively). Therefore, the contaminated and noncontaminated rations contained 51–66% and 57–74% wheat, respectively, depending on the type of ratio prepared. Chemical analyses (Laboratory Services Division, Agriculture Canada) indicated that the noncontaminated wheat contained <0.05 μg of DON/g while the contaminated wheat contained 7.6 μg of DON/g. Sufficient quantities of the Leghorn chick and broiler chicken rations were mixed at one time to last throughout the experimental period, while approximately a 3-month supply of the laying hens rations were mixed at one time. Calculated DON concentrations in the contaminated diets were about 5 μg/g for the laying hens rations and Leghorn chick and broiler chicken starter rations and about 5.5 μg/g for the broiler chicken finisher rations. Samples of all rations were collected at the time of mixing for chemical analyses and were subsequently stored at −20 °C. The remainder of the rations were stored at room temperature (≥10 °C) until they were fed to the birds in the form of a mash.

Laying Hens: Eggs and Tissues. Two sets of eggs were collected from Leghorn hens that had been fed ad libitum contaminated or control laying rations for 146 and 160 days, respectively. Each set contained equal numbers of eggs from the hens given each of the two diets. After having been fed the aforementioned diets for 168 or 190 days, randomly selected hens were killed by carbon dioxide inhalation between 8.00 and 10.00 a.m. and samples of breast muscle, drumstick, liver and gizzard were collected and immediately frozen. The hens were 361 days of age at the beginning of the experimental period.

Chicks: Tissues. White Leghorn chicks and broiler chickens were killed as described previously, and breast

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