A Comparative Study of Water Transpiration and the Uptake and Metabolism of [^{14}C]Phorate by C_3 and C_4 Plants

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Physiological and anatomical differences existing between C_3 (*Atriplex patula*, oats, peas, barley, and wheat) and C_4 (*Atriplex rosea*, corn, sorghum, and millet) plants were utilized to investigate the mechanism of uptake, translocation, and metabolism of soil-derived [^{14}C]phorate residues by plants. C_3 plants transpired 2.5 times more water and took up twice as much [^{14}C]phorate residues than did C_4 plants, indicating that a direct correlation existed between the water transpired and the uptake of radiocarbon by all plants. Ranked in decreasing order of water transpired, their order was barley, oats, wheat, peas, *A. patula*, sorghum, *A. rosea*, corn, and millet. A similar pattern was noted relative to radiocarbon accumulation. Metabolism studies indicated that relatively greater amounts of water-soluble and smaller amounts of benzene-soluble radiocarbon were recovered from C_3 plants (*A. patula* and oats) than from C_4 plants (*A. rosea* and corn). Phorate sulfoxide and phorate sulfone were apparently further oxidized in plant tops into phoratoxon sulfoxide and phoratoxon sulfone, more so in oat leaves than in corn leaves.

Numerous studies have been conducted on the movement and metabolism of insecticides in plants, yet relatively few have addressed themselves to the question of the mechanisms of insecticide uptake and translocation in plant tissues. In this study, differences existing in water transpiration between C_3 and C_4 plants were utilized to further investigate the uptake, translocation, and metabolism of [^{14}C]phorate residues by plants. So-called C_4 plants (Table I) are able to grow faster than C_3 plants—particularly in hot, dry climates (Black, 1973)—and transpire considerably less water. In addition, they have the capacity to photosynthesize more efficiently at higher temperatures and light intensities and release little or no previously fixed CO_2 by photorespiration.

Means of insecticide transport in plants have been attributed to the transpiration stream. Nearly 25 years ago, girdling experiments conducted with willows indicated that the upward movement of demeton in plants was in the xylem (Tietz, 1954), and Hacskaylo et al. (1961a,b) reported that cotton plants grown in sand treated with [^{32}P]dimethoate or [^{14}C]phorate accumulated more of either insecticide in the leaves under environmental conditions which favored transpiration. Crisp (1972) stated that the systemic insecticides are apparently transported by the xylem and accumulate at the margins and evaporative surfaces of plant leaves. In view of the fact that C_3 and C_4 plants have different transpiration rates and that plants probably transport insecticides in the transpiration stream, this study was conducted to investigate and compare the water transpiration and the uptake of [^{14}C]phorate and its metabolism by selected C_3 and C_4 plants.

MATERIALS AND METHODS

Chemicals. [*methylene*-^{14}C]Phorate (sp act. 9.7 mCi/mmol) was obtained through the courtesy of American Cyanamid Co. The insecticide was diluted with nonradioactive phorate before its addition to soils. These insecticides were determined to be at least 97% pure by thin-layer chromatography (TLC) and autoradiography. Nonradioactive phorate sulfoxide, phorate sulfone, phoratoxon, phoratoxon sulfoxide, and phoratoxon sulfone were also obtained from the American Cyanamid Co. Solvents used were redistilled acetone, benzene, and hexane as well as analytical-grade methanol, toluene, nitromethane, and acetonitrile.

Plant Material and Germination Procedures. Differences in insecticide uptake and metabolism between C_3 and C_4 plants could possibly be attributed to differences in plant genera rather than those existing between C_3 and C plants. Use of *Atriplex patula* (C_3) and *Atriplex rosea* (C_4), however, eliminates this possibility since these plants are two species within the same genus. In addition, four C_3 plants (oats, peas, barley, and wheat) and three C_4 plants (corn, sorghum, and millet) were used in these studies. Corn seeds (hybrid variety Funk-G4444-0900, wilt resistant) were obtained through the courtesy of Funk Seeds International, Bloomington, IL, while barley (Trophy), wheat (Kenosha winter wheat), millet (Red Proso), and sorghum (Waconia orange sorghum cane) seeds were obtained through the courtesy of Dr. Gerald E. Edwards, Department of Horticulture, University of Wisconsin, Madison, WI. *A. rosea* L. seeds were obtained through the courtesy of the Carnegie Institution of Washington, Stanford, CA, and *A. patula* variety hastata L. Gray seeds were obtained through the courtesy of Dr. G. Fred Somers, University of Delaware, Newark, DE. Oats (Lodi) and peas (Alaska wilt resistant) were purchased from a local seed dealer. None of the seeds mentioned above had been treated with pesticides.

Before planting in insecticide-treated soils, oat, corn, and pea seeds were pregerminated between moist paper towels for 72–96 h at 22 °C. Barley, wheat, sorghum, and millet seeds were first soaked for 2 h in tap water and then incubated in a moist condition at 28 °C for 40 h. Since the germination of *Atriplex* seeds is inhibited by the high concentration of chloride ions in the protective bracteoles surrounding the seeds (Beadle, 1952; Twitchell, 1955), they had to be removed by hand, prior to soaking the seeds in distilled water for 18 h. These seeds were then rinsed several times with distilled water and germinated on moist filter paper at 28 °C for 24–48 h. It turned out that *A. patula* and *A. rosea* were extremely slow growing. For this reason they were first grown in insecticide-free quartz sand at 30 °C for 30 days, before they were transplanted into insecticide-treated sandy soil.

Soil Treatment. A Plainfield sand (0.6% organic matter, 93.4% sand, 3.6% silt, and 3.0% clay and a pH of 5.6), free of insecticidal residues, was collected in Adams

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Co., WI, and stored at 24 ± 2 °C in a moist condition prior to use. The sandy soil was treated as described by Lichtenstein and Schulz (1959) with acetone solutions of [^{14}C]phorate to yield a dry weight concentration of 1 ppm (4.3 μCi) for the experiment with *Atriplex*, oats, peas, and corn and at 0.5 ppm (2.15 μCi) for the experiments with wheat, barley, sorghum, and millet. After removal of the acetone and a thorough mixing of the insecticide-treated soil, aliquots were extracted for analysis to determine the actual dose of radiocarbon applied. In experiments with corn, oats, and peas, portions of the treated soil were combusted to $^{14}CO_2$, to determine the total dose of radiocarbon applied. Data resulting from all experiments were finally expressed in percent of these initially recovered doses.

Plant Growth Procedures. The inside of paper cartons (9 × 8.5 cm diameter) was lined with plastic bags and then filled with 700 g of moist insecticide-treated sandy soil each. One-hundred milliliters of modified Hoagland's nutrient solution (Hoagland and Arnon, 1950) was then added to the soil in each carton. After these cartons were placed into a second plastic bag, they were sealed. Eight (corn, peas, *A. patula*, and *A. rosea*) or twenty-five (oats, barley, wheat, sorghum, and millet) seedlings were planted through perforations in the plastic cover, and the "initial weight" of each of the assembled cartons was recorded. Unless especially mentioned, all plants were then grown for 5 days at room temperature to allow them to grow tall enough so that cotton plugs could be inserted around the base of each plant growing through the perforations in the plastic cover. In this way, it was assumed that water loss would occur primarily by transpiration through the leaves and could thus be quantitated by weighing the container periodically (Noggle and Fritz, 1976). Water lost was replaced on a daily basis with distilled water added through a temporarily unplugged perforation in the plastic cover. Every third day, the cartons were watered with Hoagland's nutrient solution.

Experiments were also conducted to determine potential water losses from soils in which no plants were grown by closing the 25 holes in the plastic covers with cotton plugs.

Extraction and Analyses. At harvest, the tops of the various C_3 and C_4 plants were cut 1 cm above the soil surface, weighed, rinsed with cold tap water, cut into 2-cm pieces, and immersed in acetone–methanol (1:1). Roots were separated from the soils with a forceps, washed with a forceful stream of cold tap water, dried with paper towels, and weighed. They were then cut into 1-cm pieces and immersed in acetone–methanol (1:1). Soil was thoroughly mixed to ensure uniformity, and weighed aliquots were removed for moisture determination and for extraction. The soil to be extracted was immersed in acetone–methanol (1:1). Extraction and analyses of the plant greens, roots, and soil were performed as described by Lichtenstein et al. (1973). Benzene and water extraction phases, as well as $^{14}CO_2$ resulting from the oxidation of unextractable (bound) residues, were analyzed by liquid scintillation counting (LSC) as described by Fuhremann and Lichtenstein (1978). Qualitative and quantitative analyses of ^{14}C-labeled residues in the organic solvent extracts of plant greens and soils were performed by TLC, autoradiography, and LSC (Lichtenstein et al., 1978).

In experiments in which plants were grown in closed systems under bell jars or in open systems (controls), plant tops, roots, and soils were extracted as above, except that the resulting organic solvent extracts were not partitioned into benzene and water extraction phases. Aliquots of these extracts were pipetted onto 7-cm disks of filter paper. After the solvents had evaporated at 22 °C, the paper disks were pelleted and combusted to $^{14}CO_2$. Unextractable, ^{14}C-labeled residues, bound to plants or soils, were determined as described above. Polyurethane traps for lipid-soluble volatiles were extracted with hexane. $^{14}CO_2$ evolving from the plant–soil system was trapped in a NaOH trap and analyzed as described by Walter-Echols and Lichtenstein (1978).

EXPERIMENTAL PROCEDURES

Comparative Study of the Uptake of Water and [^{14}C]Phorate Residues from Soils by Selected C_3 and C_4 Plants. Since C_3 plants have higher transpiration rates than C_4 plants, we were interested to find out if C_3 plants also translocate and metabolize soil-applied [^{14}C]phorate differently than C_4 plants. Therefore, the uptake of water and translocation of [^{14}C]phorate from soils by selected C_3 and C_4 plants were determined in three separate experiments (I, II, and III in Table I). All plants in these experiments were grown in soil for 5 days before transfer to one of the following conditions. (I) Triplicate cartons with soil containing *A. patula* or *A. rosea* plants were incubated for 10 days at 32 °C, 50% relative humidity, and 1600 ft-c of mixed incandescent and fluorescent light on a 12-h photoperiod. The uptake of water and [^{14}C]phorate residues by the two *Atriplex* species were of particular interest, since they belong to the same genus. (II) Triplicate cartons with soil containing oats (C_3), peas (C_3), or corn (C_4) were incubated at 28 °C, 35% relative humidity, and 3000 ft-c of light on a 15-h light, 9-h dark cycle. While the corn and oats were grown in these conditions for 10 days, the slower growing peas were grown for 12 days. (III) Duplicate cartons with soil each containing barley (C_3), wheat (C_3), sorghum (C_4), or millet (C_4) were incubated for 13 days at 28 °C, 30% relative humidity, and 1400 ft-c of light on a 12-h photoperiod. In all experiments the uptake of water from the soil was determined by the gravimetric transpiration determinations as described.

Plant tops were extracted and partitioned into benzene-soluble, water-soluble, and unextractable residues for the determination of their radiocarbon content. In experiments with oat (C_3) and corn (C_4) plants, roots and the soil in which the plants had grown were also extracted and partitioned as above. In addition, radioactive compounds in the benzene extracts of oat and corn tops and in soils were identified and quantified by TLC, autoradiography, and LSC.

Uptake of Water and [^{14}C]Phorate from Soils by Oats (C_3) and Corn (C_4) under Reduced Transpiration Conditions. The correlation between water transpiration and [^{14}C]phorate uptake by plants was further investigated by artificially reducing water loss from plants. This was achieved by covering plants with bell jars, which resulted in a closed system, that also facilitated the measurement of ^{14}C-labeled compounds lost through volatilization, in particular $^{14}CO_2$. Noncovered soils with plants (open system) served as controls. Cartons containing [^{14}C]phorate-treated soil plus 25 oat seedlings or 8 corn seedlings were prepared as described so that water losses via transpiration could be quantitated. Duplicate containers each with corn or oats were grown in a closed system by placing them under a glass bell jar (28 × 10 cm diameter) immediately after planting of the seedlings. The lower, open end of each bell jar was closed with a no. 15 rubber stopper. Clean, dry air flowed continuously through each bell jar at an approximate rate of 100 mL/min during the 15-day growing period. This closed system was, in principle, similar to that described by Ferris and Lichtenstein (1980) and facilitated the measurement of volatile lipid-soluble

Table I. Influence of Water Transpiration on the Uptake of Radiocarbon from [^{14}C]Phorate-Treated Soil by C_3 and C_4 Plants[a]

	mL of water transpired/g fresh weight of greens				
expt	C_3 plants		C_4 plants		C_3/C_4[b]
I	*A. patula*	75.4 ± 3.1	*A. rosea*	55.3 ± 3.8	
II	oats	127.0 ± 12.2[c]	corn	52.2 ± 4.4	
	peas	118.0 ± 11.2[c]			
III	barley	134.4 ± 1.1	sorghum	57.0 ± 2.0	
	wheat	118.5 ± 4.8	millet	39.5 ± 2.6	
	av	114.7		51.0	2.25
	radiocarbon recovered/g of plant tops, % of [^{14}C]phorate applied to soil				
expt	C_3 plants		C_4 plants		C_3/C_4[b]
I	*A. patula*	4.77 ± 0.35	*A. rosea*	3.90 ± 0.06	
II	oats	7.59 ± 0.19[d]	corn	3.14 ± 0.29	
	peas	6.53 ± 1.12[d]			
III	barley	10.00 ± 1.20	sorghum	4.70 ± 0.06[e]	
	wheat	7.80 ± 0.62	millet	2.93 ± 0.21[e]	
	av	7.34		3.67	2.00

[a] Results are means ± SD of triplicate tests (I and II) or averages of duplicate tests (III). Grown in a Plainfield sand treated with [^{14}C]phorate at 1 ppm (4.3 μCi, for experiments I and II) or at 0.5 ppm (2.2 μCi, for experiment III). Growing conditions in experiment I were 32 °C, 50% relative humidity, in experiment II, 28 °C, 35% relative humidity, and in experiment III, 28 °C, 30% relative humidity. [b] C_3/C_4 = ratio between average milliliters of water transpired or the average ^{14}C recovered from C_3 and C_4 plants, respectively. [c-e] Within each block (I, II, and III), data without a letter in common are significantly different (5% level, Duncan's new multiple range test).

compounds and $^{14}CO_2$ as well as the determination of radiocarbon compounds in insecticide-treated soils and in plants. All plants were grown for 15 days at 28 °C and 1300 ft-c of light on a 12-h photoperiod. The relative humidity in the closed system was close to 100% as evidenced by water condensing on the walls of the bell jar. In control experiments, duplicate soil containers with corn or oats were grown in an open system as described previously. The relative humidity around plants in the open system, however, amounted to only 40%. The water lost by transpiration from the soil–plant system was then measured for the remaining 10 days. During the total 15-day period, corn and oats grown in a closed system were watered 3 and 4 times, respectively, while all plants grown in the open system had to be watered daily.

After 15 days of incubation, the systems were dismantled, and the soil, plants, and polyurethane traps were extracted. Both soluble and unextractable ^{14}C-labeled residues were then analyzed by LSC as described. The trap for $^{14}CO_2$ was analyzed for radiocarbon by LSC on a daily basis. For verification that the radioactivity found in the NaOH was actually $^{14}CO_2$, the procedure described by Ferris and Lichtenstein (1980) was utilized. Of the radiocarbon trapped in the NaOH, an average of 96% was identified as $^{14}CO_2$. Two sets of duplicate cartons containing [^{14}C]phorate-treated soil either with or without 25 oat plants were placed under bell jars as described to test whether the $^{14}CO_2$ evolved in the closed system was of plant or soil origin. All four containers were incubated for 10 days at 28 °C and 600 ft-c of light on a 15-h photoperiod. The $^{14}CO_2$ generated in the two systems was quantitated as described above.

RESULTS AND DISCUSSION

Comparative Study of the Uptake of Water and [^{14}C]Phorate Residues from Soils by Groups of Selected C_3 and C_4 Plants. The amounts of water transpired by plants during the growing time and the ^{14}C content in plant tops were determined after the plants had grown in [^{14}C]phorate-treated soils. Results (Table I, experiment I) show that over a 10-day period *A. patula* (C_3) did in fact transpire 1.4 times more water than *A. rosea* (C_4), a difference significant at the 5% level (Duncan's new multiple range test). A similar pattern was seen in the uptake of [^{14}C]phorate residues by the two *Atriplex* species since over 1.2 times more [^{14}C]phorate residues were taken up by *A. patula* than by *A. rosea*. This difference was also significant at the 5% level (Duncan's new multiple range test). The data, therefore, indicate that there was a significant correlation between the water transpiration and the accumulation of [^{14}C]phorate residues in these two closely related plants.

Additional results obtained with the seven other C_3 and C_4 plants (Table I) showed that on an average C_3 plants transpired significantly more water (C_3/C_4 = 2.25) and took up significantly greater quantities of [^{14}C]phorate residues (C_3/C_4 = 2.00) than did C_4 plants, thus establishing a direct correlation between the water transpired and [^{14}C]phorate-derived radiocarbon translocated into the plant tops. These results, therefore, add much strength to the theory that the transpiration stream is the vehicle of [^{14}C]phorate residue transport in plants.

Differences in the metabolism of [^{14}C]phorate in C_3 and C_4 plants were also evident when the amounts of benzene-soluble, water-soluble, and unextractable (bound) radiocarbon determined in various plant tops were compared (Figure 1). While insecticidal phorate metabolites are associated with the benzene phase, detoxified metabolites are usually water soluble. The appearance of relatively large amounts of water-soluble radiocarbon could, therefore, serve as an indicator of increased metabolic activity. The nature of bound residues, often thought to be the product of metabolic activity, is unknown. As shown in Figure 1, significantly greater quantities of water-soluble radiocarbon compounds were recovered from *A. patula* (C_3) than from *A. rosea* (C_4). Thus water-soluble residues accounted for 75% of the total radiocarbon recovered from the tops of *A. patula* (AP, Figure 1) while those in *A. rosea* (AR, Figure 1) comprised only 57%. These results seemed to indicate that *A. patula* metabolized [^{14}C]phorate to a greater extent than *A. rosea*. On the other hand, significantly greater quantities of bound residues were recovered from *A. rosea* (28% of total ^{14}C) than from *A. patula* (13% of total ^{14}C). The quantities of benzene-soluble radiocarbon, however, were similar to both *A. patula* and *A. rosea*.

Table II. Movement, Fate, and Metabolism of [^{14}C]Phorate in Oat (C_3) and Corn (C_4) Plants Grown in a Sandy Soil[a]

	radiocarbon recovered, % of [^{14}C]phorate applied to soil			
	oats (C_3)		corn (C_4)	
extraction phase	total sample (% T[d])	per g[b]	total sample (% T)	per g
tops (Gr)				
benzene	15.45 ± 0.79 (38%)	2.92 ± 0.05	18.84 ± 3.42 (48%)	1.52 ± 0.32[f]
water	20.29 ± 1.50 (51%)	3.83 ± 0.08	15.84 ± 1.47[g] (41%)	1.27 ± 0.07[e]
bound[c]	4.46 ± 1.19 (11%)	0.84 ± 0.18	4.32 ± 0.42 (11%)	0.35 ± 0.04[g]
total	40.20 ± 3.40 (100%)	7.59 ± 0.19	39.00 ± 4.79 (100%)	3.14 ± 0.29[e]
roots (R)				
benzene	0.34 ± 0.04 (8%)	0.09 ± 0.01	1.13 ± 0.05[e] (17%)	0.06 ± 0.01[g]
water	0.47 ± 0.07 (10%)	0.12 ± 0.02	2.14 ± 0.07[e] (32%)	0.11 ± 0.01
bound	3.72 ± 0.22 (82%)	0.95 ± 0.04	3.34 ± 0.22 (51%)	0.18 ± 0.02[e]
total	4.52 ± 0.30 (100%)	1.16 ± 0.06	6.61 ± 0.17[e] (100%)	0.35 ± 0.03[e]
soil (S)				
benzene	7.74 ± 0.92 (51%)		6.48 ± 0.87 (58%)	
water	0.53 ± 0.27 (4%)		0.39 ± 0.14 (3%)	
bound	6.81 ± 1.42 (45%)		4.38 ± 0.82 (39%)	
total	15.08 ± 2.58 (100%)		11.25 ± 0.75 (100%)	
total (Gr + R + S)	59.81 ± 4.17		56.86 ± 4.06	

[a] Results are means ± SD of triplicate tests. Grown for 10 days at 28 °C, 3000 ft-c. [^{14}C]Phorate was applied at 1 ppm (4.3 μCi) to a Plainfield sand. [b] Per gram of fresh greens or roots. [c] Unextractable ^{14}C residues determined by combustion to $^{14}CO_2$. [d] % T = ^{14}C in percent of total radiocarbon recovered. [e-g] "Total sample" or "per gram" corn data are significantly different from the respective oat data at the 0.1% (*e*), 1% (*f*), or 5% (*g*) level (Student's *t* test).

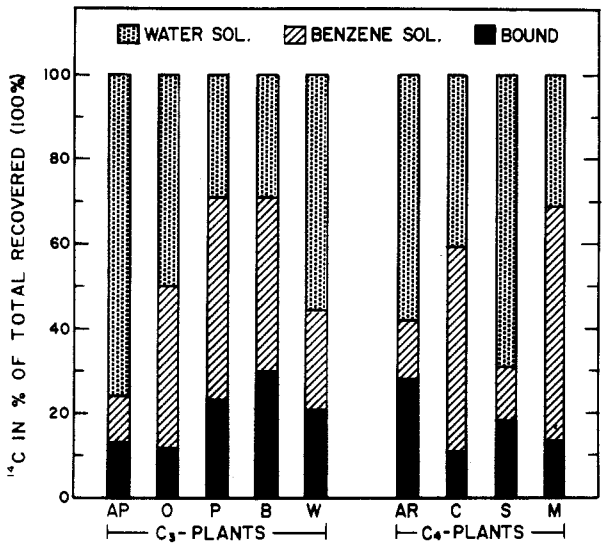

Figure 1. Distribution of radiocarbon between water and benzene extraction phases as well as unextractable (bound) residues from plant tops. Data show the metabolism of [^{14}C]phorate by selected C_3 (AP = *A. patula*, O = oats, P = peas, B = barley, and W = wheat) and C_4 (AR = *A. rosea*, C = corn, S = sorghum, and M = millet) plants grown in [^{14}C]phorate-treated soil. Results are averages of triplicate (oats, peas, *Atriplex* sp., and corn) or duplicate (barley, wheat, sorghum, and millet) tests.

In addition to the determinations of the total radiocarbon content in plant tops (Table I and Figure 1), further analyses were conducted with oats and corn. In this case, the amounts of [^{14}C]phorate-derived radiocarbon were also determined in plant roots and the soils they had grown in (Table II). In addition, the benzene extraction phases of plant tops and soils were quantitatively and qualitatively analyzed by TLC, autoradiography, and LSC (Table III). Table II presents the total radiocarbon content of each component (tops, roots, and soil) analyzed; for comparative purposes, this total radiocarbon was also expressed as ^{14}C recovered per gram of plant tops or roots. Both corn and oat plants had translocated large quantities of ^{14}C into their tops, and only small amounts remained in roots and soils. In addition, larger quantities of [^{14}C]phorate were taken up by oat greens than by corn greens when expressed on a per gram fresh weight basis. Radiocarbon recovered from the benzene- and water-soluble extraction phases, plus the portion of unextractable, bound radiocarbon in oat tops, was compared with that recovered from corn tops (Figure 1 and Table II). Relatively larger amounts (% T = % of total radiocarbon recovered) of water-soluble ^{14}C and concomitantly smaller amounts of benzene-soluble ^{14}C were recovered from oat tops than from corn tops. Such results indicated that in oat tops the insecticide was more rapidly metabolized or had more rapidly been translocated, thus making more time available for metabolic processes.

Smaller quantities of [^{14}C]phorate-derived radiocarbon were recovered from plant roots than from plant tops or soils. The significance of root-associated ^{14}C is difficult to interpret since ^{14}C inside the root cannot be distinguished from that adhering to root surfaces. On a per gram basis (Table II, larger quantities of [^{14}C]phorate residues were found in oat roots than in corn roots. This is probably related to the higher per gram uptake of [^{14}C]phorate residues by oat tops than by corn tops.

No significant analytical differences were evident in the data obtained from soils in which either oats or corn had grown (Table II). Benzene-soluble soil residues comprised 7–8% and bound residues 4–7% of the radiocarbon originally applied to the soils. Only small amounts of water-soluble residues were recovered from soils in which plants had grown. Similar results were reported by Walter-Echols and Lichtenstein (1978), who found that water-soluble residues accounted for only 1% of the total radiocarbon recovered from a [^{14}C]phorate-treated loam soil incubated for 2 weeks without any plants. Thus it appears that the metabolism of phorate in soils mainly involved the production of benzene-soluble metabolites and bound residues. However, the possibility that water-soluble metabolites were taken up by plants immediately upon production cannot be ruled out.

The total amounts of ^{14}C recovered from systems with oats or corn were similar, amounting to 60 and 57% of soil-applied [^{14}C]phorate. Of these amounts, 40–39% were associated with oat or corn tops, 4–7% with oat or corn

Table III. [^{14}C]Phorate and Metabolites Recovered from the Benzene Extraction Phases of Oats (C_3), Corn (C_4), and Sandy Soil[a] by Thin-Layer Separation, Autoradiography, and LSC

benzene-soluble metabolites	total benzene-soluble ^{14}C, % of applied[b]	recovered, % of all benzene-soluble radiocarbon[c]				
		phorate (R_f 0.94)	phorate sulfoxide (R_f 0.46)	phorate sulfone (R_f 0.86)	phoratoxon sulfoxide (R_f 0.11)	phoratoxon sulfone (R_f 0.56)
tops						
oats (C_3)	2.92 ± 0.05	ND[d]	40.1 ± 0.9	27.9 ± 0.8	12.1 ± 0.4	19.8 ± 1.1
corn (C_4)	1.52 ± 0.32[f]	ND	40.3 ± 2.6	33.8 ± 4.7	10.2 ± 0.8[g]	15.7 ± 1.4[g]
soil						
oats (C_3)	7.74 ± 0.92	24.6 ± 0.8	42.3 ± 1.8	31.2 ± 2.6	0.8 ± 0.1	1.1 ± 0.2
corn (C_4)	6.48 ± 0.87	32.4 ± 2.1[f]	41.5 ± 1.7	24.3 ± 3.4[g]	0.6 ± 0.1	1.2 ± 0.2

[a] Same as footnote *a* in Table II. [b] Total benzene-soluble ^{14}C recovered in percent of soil-applied [^{14}C]phorate per gram of fresh greens (tops) or in percent of soil-applied [^{14}C]phorate (soil). [c] No phoratoxon was recovered from plants or soils. [d] ND = nondetectable. [e-g] Oat greens or soil data are significantly different from respective corn data at the 0.1% (*e*), 1% (*f*), or 5% (*g*) level (Student's *t* test).

Table IV. Water Transpiration and [^{14}C]Phorate Uptake in Oats (C_3) and Corn (C_4) Grown in Sandy Soil in an Open or a Closed System[a]

	mL of water transpired/g fresh weight of tops			
	oats (C_3)		corn (C_4)	
	open system	closed system	open system	closed system
	104.5 ± 7.2	24.4 ± 1.5[d]	34.6 ± 1.1[d]	11.9 ± 0.2
	^{14}C recovered from tops, % of applied/g fresh weight[a,b]			
	oats (C_3)		corn (C_4)	
	open system	closed system	open system	closed system
	3.08 ± 0.07	1.33 ± 0.03	1.06 ± 0.02	0.92 ± 0.01
	^{14}C recovered, % of applied to soil[a,b]			
	oats (C_3)		corn (C_4)	
	open system	closed system	open system	closed system
tops (T)	13.59 ± 0.31	4.12 ± 0.18	12.22 ± 0.20	6.22 ± 0.52
roots (R)	3.04 ± 0.23[f]	3.36 ± 0.30[f]	6.00 ± 0.63[e]	4.74 ± 1.04[e,f]
soil (S)	38.92 ± 1.35	57.44 ± 0.77	25.16 ± 0.47	51.99 ± 0.52
volatiles (V)				
CO_2 evolved	NM[i]	10.70 ± 0.67[g]	NM	9.38 ± 0.03[g]
lipid soluble[c]	NM	0.00 ± 0.00[h]	NM	0.00 ± 0.00[h]
total (T + R + S + V)	55.55 ± 1.43	75.64 ± 0.59	43.38 ± 0.90	72.32 ± 1.07

[a] Results are averages of duplicate tests. [^{14}C]Phorate was applied at 1 ppm (4.3 μCi) to a Plainfield sand. Plants were grown for 10 days at 28 °C, 1300 ft-c, in an open (controls) or a closed system. [b] Data are totals of extractable and unextractable residues from plant tops, roots, and soils. [c] ^{14}C trapped in polyurethane. [d-h] For each line, data followed by the same letter are not significantly different (5% level, Duncan's new multiple range test). [i] NM = not measured in open systems.

roots, and 15–11% with soils in which oats or corn had grown, respectively.

Results obtained by quantitative and qualitative analyses of plant tops and soils are summarized in Table III. Because of the presence of only small amounts of [^{14}C]-phorate-derived radiocarbon in roots, they were not analyzed by TLC. As indicated, phorate sulfoxide was the major phorate metabolite in both soils and plant tops, amounting to 40–42% of the total benzene-soluble radiocarbon recovered. Phorate sulfone accounted for 24–34% of the total benzene-soluble ^{14}C in soils and plant tops. While there was no detectable phorate in plant tissues, soils still contained 25–32% of the benzene-soluble ^{14}C as phorate. Similar results were reported by Lichtenstein et al. (1974), who concluded that corn plants either absorbed phorate from the soil and metabolized it to its sulfoxide and sulfone or absorbed these metabolites directly from the soil. Since the water solubilities of phorate sulfoxide and phorate sulfone are >8000 and 860 ppm, respectively (Bowman and Sans, 1979), their penetration into root tissue would occur to a greater extent than that of phorate, which has a water solubility of 18 ppm. The relationships between relatively higher water solubilities of six different insecticides relative to their translocation in oats has been demonstrated (Fuhremann and Lichtenstein, 1980).

Our data indicate (Table III) that in plant tops phorate sulfoxide and phorate sulfone were apparently further oxidized into the oxygen analogues, phoratoxon sulfoxide and phoratoxon sulfone. Larger quantities of these two oxygen analogues were recovered from oat tops than from corn tops. These differences were particularly apparent with phoratoxon sulfone, which accounted for 19.8% of the total benzene-soluble ^{14}C in oat tops as opposed to 15.7% in corn tops (differences significant at the 5% level, Student's *t* test). Thus not only were relatively larger amounts of water-soluble ^{14}C and smaller amounts of benzene-soluble ^{14}C recovered from oat tops than from corn tops (% *T* in Table II) but also oats contained larger quantities of both phoratoxon sulfoxide and sulfone than did corn (Table III). Oat tops seemed to have metabolized [^{14}C]phorate to a greater extent than corn tops. Neither phorate nor phoratoxon were found in the plant tops.

In soils in which oats had grown, less phorate (24.6% of benzene-soluble ^{14}C) but similar amounts of phorate sulfoxide (42%) and more phorate sulfone (31%) were present than in those in which corn had been grown (Table

III). This may indicate a difference between oat and corn plants relative to their influence on the metabolism of phorate in the soil. Alternatively, the various phorate metabolites may have been selectively translocated by the two plants. No phoratoxon and only traces of the oxygen analogue metabolites were recovered in either of the soils. This is consistent with findings that phoratoxon derivatives were recovered only in small quantities or not at all from soils (Schulz et al., 1973; Lichtenstein et al., 1974).

Uptake of Water and [^{14}C]Phorate from Soils by Oats (C_3) and Corn (C_4) under Reduced Transpiration Conditions. The transpiration rate was artificially reduced by placing plants in a water-saturated environment under bell jars as described to test the importance of the transpiration stream relative to the translocation of soil-applied [^{14}C]phorate. Results showed (Table IV) that both corn and oat greens grown in closed systems transpired considerably less water and took up significantly smaller amounts of [^{14}C]phorate residues than plants grown in open systems (controls), indicating that the insecticide was translocated via the transpiration stream. Oat tops (C_3) grown under both open or closed conditions transpired more water and took up more [^{14}C]phorate residues than did respective corn tops (C_4).

Water loss from soils was also measured in the absence of plants. Results showed that the amounts of water lost by evaporation through the cotton plugs were negligible in comparison to that lost due to plant transpiration. Therefore, no corrections for water loss in the absence of plants was deemed necessary.

Data presented in Table IV indicate that the reduced air movement and the high relative humidity within the bell jars resulted in low [^{14}C]phorate residue uptake by plants. A total of 72–76% of the soil-applied ^{14}C was recovered from the closed systems as compared to a total of only 43–55% recovered from the open systems. The fact that in the closed systems only 72–76% of the soil-applied ^{14}C could be accounted for was unexpected since the [^{14}C]phorate-treated soils were placed under bell jars soon after soil treatment and planting of the seedlings. It is possible that some of the volatile lipid-soluble metabolites which were trapped in the polyurethane trap at the beginning of the experiment were lost after exposure to a continuous air flow for 15 days. Probably, due to the reduced transpiration in the closed systems, less radiocarbon had been translocated into the plant tops than under the conditions favoring transpiration (open system). Conversely, more radiocarbon remained in soils, being kept with the plants under these conditions.

For determination of the source of $^{14}CO_2$ (soils or plants), additional experiments as described were conducted under closed conditions with [^{14}C]phorate-treated soils only and with soils in which oats were grown. Results showed that in the absence of oat plants, 84.6 ± 2.0% of the soil-applied radiocarbon was still recovered from the soil and 9.5 ± 0.1% in the form of $^{14}CO_2$, thus representing a total recovery of 94%. With oat plants, however, only 70.4 ± 0.2% of the soil-applied radiocarbon was associated with the soil, 23.7 ± 1.8% with the oats (roots and tops), and 5.7 ± 0.1% as $^{14}CO_2$, representing a total recovery of 99.8% of the soil-applied radiocarbon. No explanation is available as to why in these experiments a good balance of radiocarbon was obtained and not in those summarized in Table IV. The amounts of $^{14}CO_2$ evolved in these closed systems in the absence of plants were nearly double of that determined in systems with oats. This seems to indicate that the $^{14}CO_2$ evolved primarily from the soil rather than from the plants. Also, a fixation and assimilation of $^{14}CO_2$ into photosynthetic products could have occurred in oat plants.

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