Influence of Mineral Nutrients on the Penetration, Translocation, and Metabolism of [14C]Dyfonate in Pea Plants

Narayan S. Talekar and E. Paul Lichtenstein*

The effects of mineral nutrient deficiencies on the penetration, translocation, and metabolism of [14C](ethoxy)Dyfonate in pea plants were investigated. With plants grown in nitrogen-deficient nutrient solutions, the penetration of radiocarbon into roots increased by 61%, while the translocation into greens was not affected. Deficiencies of potassium, calcium, and magnesium resulted in reduction of 14C into the roots by 48, 61, and 58%, respectively, while potassium deficiency caused an 80% increase in translocation. In roots, 81–91% of the recovered radiocarbon was associated with organic solvent phase, while in the greens these phases contained only 10–16%. Conversely, only 8 to 11% of the total 14C recovered from roots and 48 to 71% from greens was associated with the water phase. The amounts of unextractable radioactivity in the roots were relatively small (1 to 6% of the total recovered), while in the greens these figures were 19 to 47%. Nutrient deficiencies affected the production of water-soluble metabolites and the amounts of unextractable radioactivity. Concentrations of Dyfonate in control roots amounted to 80.2 ppm and of Dyfoxon was 0.87 ppm. In the greens these concentrations were considerably lower, but the amounts of Dyfoxon in the greens relative to the amounts of Dyfonate were much larger. A lack of nitrogen or sulfur resulted in the presence of higher concentrations of Dyfonate in the roots, while deficiencies in potassium, calcium, or magnesium caused significant decreases in concentrations of both Dyfonate and Dyfoxon in roots. Concentrations of Dyfoxon in greens were reduced due to deficiencies of all elements except nitrogen.

Pesticidal chemicals can penetrate into plant roots and be translocated and metabolized. The manner and rate of metabolic attacks on pesticidal chemicals within plants may depend on the extent of penetration and translocation of these chemicals within the plant. It has been shown that the extent of penetration and translocation of lindane in peas is affected by the availability of mineral nutrients (Talekar and Lichtenstein, 1971). Deficiencies of nitrogen, sulfur, or boron increased the penetration of lindane into the roots, but reduced its translocation into the greens. Magnesium deficiency resulted in reductions of both penetration and translocation of lindane, while a deficiency of potassium resulted in reduced lindane translocation. It is possible that nutrient deficiencies will also affect the metabolism of an insecticide both qualitatively and quantitatively, thus rendering the pesticide either more toxic or harmless to crop pests. Due to the replacement of organochlorine insecticides by organophosphorus compounds, it was felt that the effects of environmental conditions on the potential contamination of plants grown in insecticide-treated soils should be known. Experiments were conducted with Dyfonate and pea plants to obtain information on the effects of mineral plant nutrients on the penetration, translocation, and metabolism of this organophosphorus insecticide in pea plants.

METHODS AND PROCEDURES

Chemicals. [14C](Ethoxy)Dyfonate (*O*-ethyl *S*-phenyl ethylphosphonodithioate) (specific activity 5.75 mCi/mmol), Dyfoxon (*O*-ethyl *S*-phenyl ethylphosphonothiolate), ETP (*O*-ethylethanephosphonothioic acid), and EOP (*O*-ethylethanephosphonic acid) were obtained through the courtesy of the Stauffer Chemical Co. Solvents used were redistilled acetone and benzene and methanol.

Growing of Plants. Pea plants (*Pisum sativum*, variety Alaska Wilt Resistant) were grown for 15 days in insecticide-free Hoagland's nutrient solution or in solutions deficient in specific elements. Growth conditions and the preparation of nutrient solutions deficient in N, P, K, Ca, Mg, or S were previously described (Talekar and Lichtenstein, 1971). The pH of these solutions (pH 6.0 to 7.5) was determined every other day and, if necessary, adjusted to that of the control (complete nutrient solution) by adding either 0.1 *N* NaOH or 0.1 *N* HCl. All experiments were conducted with four replicates, each consisting of four pea plants.

Exposure of Plants to Dyfonate and Plant Harvesting. After 15 days of growth, the plants were transferred to freshly prepared complete (control) or nutrient-deficient solutions which had been treated with 4 ppm (1.0 μCi) of [14C]Dyfonate in ethanol. After 8 additional days of growth, during which the insecticide could penetrate into the root system, translocate into the greens, and also be metabolized, the plants were removed from the growth jars and roots were washed with running tap water and then dried with blotting paper. This procedure did not differentiate between the residues within the root system and those adhering to the outer side of the root epidermis. Analytical data referring to the residues "in roots" are to be interpreted in that way. Roots and greens from each replicate were then separated and their fresh weight was determined. They were then placed into jars and covered with 25 ml of redistilled methanol–acetone (1:1). The nutrient solutions in which the plants had grown, as well as the plant material, were stored at −15° for future extraction and analysis.

A preliminary experiment was set up in duplicate to quantitate the overall recovery of radiocarbon from the system and to determine the distribution of [14C]Dyfonate and its metabolites between the nutrient media and the pea plants over the 8-day exposure period. The final experiments were then conducted with four replicates to determine the effects of nutrient deficiencies on the uptake and metabolism of [14C]Dyfonate by the plants.

Extraction Procedure. *Plants.* The roots or greens and the methanol–acetone mixture in the storage jars were quantitatively transferred into a Waring blender and the plant material was extracted with two 50-ml portions of methanol–acetone. A third extraction was made with 50 ml of a 1:1:1 mixture of methanol–acetone–benzene. The extracts were filtered under vacuum, resulting in a "dry residue" of extracted plant material and a filtrate. The

Department of Entomology, University of Wisconsin, Madison, Wisconsin 53706.

filtrate was concentrated to near dryness in a flash evaporator at 35°, adjusted to 100 ml with water, and extracted three times with 75-ml portions of ethyl acetate. Both the ethyl acetate and water phases were adjusted to volume for analysis.

Nutrient Solutions. After defrosting the stale nutrient solutions, suspended root particles were removed by filtration and the volume of each solution was determined. Each solution was extracted three times with 100-ml portions of ethyl acetate. The pH of the water phase was adjusted to pH 1–1.5 with concentrated HCl and extracted with three 75-ml portions of diethyl ether. The ether and ethyl acetate extracts were combined, concentrated in a flash evaporator, and adjusted to volume with ethyl acetate for analysis. Determination of the radiocarbon content of freshly treated nutrient solutions was done as described for the nutrient solutions in which plants had grown for 8 days.

Analytical Procedures. *Liquid Scintillation Counting (lsc).* The radiocarbon content in organic solvent extracts obtained from plants or nutrient solutions was determined by adding 2-ml aliquots to 14 ml of a scintillation mixture containing 6 g of PPO and 0.25 g of dimethyl POPOP in 1000 ml of toluene. The radiocarbon content of the water phases was determined by adding 2 ml of water to 14 ml of a scintillation mixture consisting of 4 g of PPO, 0.05 g of dimethyl POPOP, 120 g of naphthalene, and 880 ml of 1,4-dioxane. These mixtures were then counted in a Model 3320 Packard Tri-Carb scintillation spectrometer. Data were corrected for background, counter efficiency, and dilution. The unextractable radiocarbon in the "dry residue" of the plant material was determined by drying the samples overnight at 60°, and then combusting subsamples in an oxygen-filled 1000-ml Schöninger flask according to the method of Kelly *et al.* (1961). The liberated $^{14}CO_2$ was adsorbed in 3 ml of a 1:2 phenethylamine–methanol mixture which was then dissolved in 14 ml of the toluene-based scintillation mixture and analyzed by lsc. Data obtained from the combustion of extracted plant material is referred to as "bound" radioactivity.

Gas–Liquid Chromatography (glc). The organic solvent extraction phases were analyzed by glc using a Tracor Model 550 gas chromatograph (Tracor Inc., Austin, Tex.) equipped with Melpar flame photometric detector and 526 or 394 mμ interference filters for the detection of phosphorus- or sulfur-containing compounds. A 183-cm Pyrex glass column (4-mm i.d.) containing 10% DC-200 on 80/100 Gas Chrom Q was conditioned at 230° before use. Gas flow rates in ml/min were: nitrogen (carrier), 120; oxygen, 12; and hydrogen, 150. The column oven temperatures were 180° for Dyfonate and 160° for Dyfoxon. The injector was held at 240° and the detector at 205°.

Thin-Layer Chromatography (tlc). Organic Phase. Root extract replicates were combined and analyzed by tlc on 20 × 20 cm silica gel precoated plates (Brinkmann Instruments, Inc., N. Y.). Chromatograms were developed in chloroform–ethyl acetate (1:1) (McBain *et al.*, 1970). Reference compound spots on the developed plates were visualized as described by Lichtenstein *et al.* (1972). Radioactive metabolites were visualized by autoradiography of the developed tlc plates with Kodak No-Screen X-ray films. Areas on plates, corresponding to the spots appearing on the X-ray films, were scraped off, extracted with methanol, and analyzed by lsc and glc. Tlc of the ethyl acetate phases of the greens could not be performed due to interfering substances. When these extracts were subjected to the cleanup procedures described by Storherr and Watts (1965), Watts *et al.* (1969), Mills *et al.* (1972), or the procedure recommended in the Pesticide Analytical Manual (1968), 48 to 71% of the radioactivity was lost.

Water Phase. After the radiocarbon content in each water phase had been determined, the replicates were combined and concentrated to 5–10 ml at 35°. Proteins were then precipitated by adding 150 ml of cold acetone. After centrifugation at 2000 × *g* for 15 min, the acetone–water was filtered through Whatman no. 40 filter paper, concentrated to approximately 0.5 ml, and spotted on precoated silica gel plates along with reference compounds, and developed in 1-propanol–1-butanol–0.17 *N* ammonium hydroxide (2:1:1) (McBain *et al.*, 1970). Water-soluble metabolites were identified as previously described for the organic phases. The protein precipitates in the centrifuge tubes and on the filter papers were dissolved in water and the radiocarbon bound to proteins was determined by lsc.

RESULTS AND DISCUSSION

The term *penetration* refers to the radioactive compounds found within, or adhering to, the pea roots, while the radiocarbon recovered from the greens is referred to as *translocation* from the roots to the greens. The term *uptake* refers to the total ^{14}C in an entire plant.

Preliminary experiments showed that an 8-day exposure of pea plant roots to [^{14}C]Dyfonate in both complete and mineral-deficient nutrient solutions resulted in the uptake of the insecticide by the plants. Between 44 and 61% of the [^{14}C]Dyfonate applied to the nutrient solution was recovered from the entire system of plants plus nutrient solutions. The radiocarbon recovered from plants alone ranged from 9 to 36% of the applied dosage and from 22 to 48% of the applied dosage remaining in the nutrient solutions. The loss of 39 to 56% of the applied radioactivity from the total system could be due to the fact that the nutrient solutions were continuously aerated for the entire 8-day exposure period, thereby enhancing volatilization. Lichtenstein and Schulz (1970) found that 68% of [^{14}C]Dyfonate was lost from water kept at 30° in a shaker over an 8-day period. It is also possible that small amounts of [^{14}C]Dyfonate taken up by the plants were metabolized and $^{14}CO_2$ or other radioactive compounds were lost from the plant surfaces.

I. Effects of Mineral Nutrition on the Uptake of [^{14}C]Dyfonate in Pea Plants. When these tests were repeated with four replicates, the radiocarbon recovered from the plants similarly ranged between 4 and 33% of the applied dosage. Considerable differences due to deficiencies of various essential elements were observed in the uptake by plants and in the distribution of the radioactivity between the roots and greens. However, these were also differences in plant growth due to the lack of a particular nutrient element, therefore the uptake of radiocarbon per gram of plant material was calculated. Data were statistically evaluated by the analysis of variance (F test) to compare the mean amounts of ^{14}C uptake due to a deficiency of a particular element with that of control plants. Results (Table I) indicated that the absence of N, K, Ca, and Mg significantly affected the penetration and/or translocation of radiocarbon into pea plants. In the absence of sufficient amounts of N, the penetration of radiocarbon into roots increased by 61%, while the translocation was not affected. Talekar and Lichtenstein (1971) observed that a nitrogen deficiency resulted in an increased penetration of lindane into roots, but also in a decreased translocation of this insecticide. They further demonstrated (Talekar and Lichtenstein, 1972) that the selective transport of lindane into roots was dependent on the energy supply systems of the root cells and that, in the presence of metabolic inhibitors, an increased penetration of lindane into roots occurred. A decreased nitrogen supply to the plant roots could have resulted in an adverse effect on the energy supply system of the roots, thus permitting an increased penetration of foreign compound into the root system.

Deficiencies of K, Ca, and Mg resulted in reductions of ^{14}C penetration into the roots by 48, 61, and 58%, respec-

Table I. Effects of Nutrient Deficiencies on the Penetration and Translocation of [14C](Ethoxy)Dyfonate into Pea Plants after Exposure to the Insecticide During the Last 8 Days of the Growing Period. Results Are Averages of Four Replicates (Four Plants Each)

Nutrient solution	Plant parts	Fresh weights, g	14C recovered in % of applied[a]		
			Total	Per g of plant material	% CK[b]
Complete	Roots (R)	2.50 ± 0.00	21.77 ± 0.32	8.71 ± 0.13	100
(CK)	Greens (G)	5.23 ± 0.15	9.16 ± 1.77	1.75 ± 0.29	100
	R + G	7.73 ± 0.15	30.93 ± 2.08	4.00 ± 2.00	100
Deficient in					
N	Roots (R)	2.20 ± 0.18	30.79 ± 3.76	13.99 ± 2.35	161[c]
	Greens (G)	1.13 ± 0.27	1.82 ± 0.46	1.61 ± 0.24	92
	R + G	3.33 ± 0.41	32.61 ± 4.17	9.86 ± 1.51	247[c]
P	Roots (R)	2.54 ± 0.28	18.55 ± 1.29	7.30 ± 0.82	84
	Greens (G)	2.38 ± 0.12	4.12 ± 0.50	1.73 ± 0.16	99
	R + G	4.92 ± 0.39	22.67 ± 1.25	4.61 ± 0.31	115
K	Roots (R)	1.82 ± 0.18	8.19 ± 1.42	4.50 ± 0.70	52[c]
	Greens (G)	1.67 ± 0.21	5.26 ± 0.93	3.15 ± 0.17	180[c]
	R + G	3.49 ± 0.38	13.45 ± 1.77	3.85 ± 0.33	96
Ca	Roots (R)	1.03 ± 0.16	3.52 ± 0.49	3.42 ± 1.06	39[c]
	Greens (G)	0.58 ± 0.14	1.03 ± 0.38	1.78 ± 0.42	102
	R + G	1.61 ± 0.28	4.55 ± 0.42	2.83 ± 0.50	71
Mg	Roots (R)	1.80 ± 0.19	6.56 ± 2.30	3.64 ± 1.10	42[c]
	Greens (G)	1.56 ± 0.19	2.33 ± 0.37	1.49 ± 0.11	85
	R + G	3.36 ± 0.36	8.89 ± 2.55	2.65 ± 0.54	66[d]
S	Roots (R)	2.60 ± 0.23	25.51 ± 2.07	9.81 ± 0.94	113
	Greens (G)	4.08 ± 0.60	7.67 ± 0.83	1.88 ± 0.13	107
	R + G	6.67 ± 0.81	33.18 ± 2.89	4.97 ± 0.41	124

[a] Applied 1.0 μCi (4 ppm) of [14C]Dyfonate to each nutrient solution. [b] % CK = in % of control (complete nutrient solution). [c,d] Differences between 14C in plant parts from nutrient deficient solutions and those from controls were significant at [c] 1% or [d] 5% level.

tively, while K deficiency caused an increase in translocation of 14C by 80%. It is possible that K deficiency increased the movement of [14C]Dyfonate or its metabolites from the roots to the greens, thus accounting for smaller amounts in the roots. No effects on these processes as measured by uptake of radiocarbon were observed when plants were grown in P- or S-deficient nutrient solutions. In previous work (Talekar and Lichtenstein, 1971) it was observed that deficiencies of K, Mg, and S affected the penetration and translocation of lindane in pea plants, whereas the deficiencies of P and Ca affected neither penetration nor translocation of the insecticide when pea roots were exposed to lindane for 24 hr.

An intentionally produced deficiency of one element also affects the concentrations of other elements in plant tissues (Talekar and Lichtenstein, 1971). Therefore, a combination of factors could have been responsible for the differences observed in the uptake of radioactivity by the pea plants.

Since all these data indicate quantitative differences based on total radiocarbon content in roots or greens, some qualitative information was obtained by determining the distribution of the radiocarbon in organic and water-soluble phases.

II. Effects of Plant Mineral Nutrition on the Metabolism of [14C]Dyfonate. *Production of Water-Soluble and Nonextractable Substances from [14C]Dyfonate.* Since both Dyfonate and Dyfoxon partition into the organic solvent phase with the described extraction procedure, the amount of radioactivity recovered from the water phase serves as an indicator of the metabolism into water-soluble substances. Analytical data presented in Table II show that in all root samples 81 to 91% of the recovered radioactivity was associated with the organic solvent phase, while in the greens these phases contained only 10–16%. Conversely, only 8 to 11% of the total radiocarbon recovered from the roots and 48 to 71% from the greens was associated with the water phase, indicating that the greens contained most of the water-soluble metabolites. These compounds could have been produced in the roots and then translocated into the greens, or possibly formed in the greens themselves. The amounts of unextractable radioactivity ("Bound," Table II) in the roots were relatively small (1 to 6% of the total recovered), while in the greens these figures were 19 to 47%. The increase in unex-

Table II. Effects of Nutrient Deficiencies on the Production of Water-Soluble Metabolites and Unextractable 14C after Exposure of Pea Roots to [14C](Ethoxy)Dyfonate (4 ppm) During the Last 8 Days of Growing Period. Results Are Averages of Four Replicates (Four Plants Each)

Nutrient solution	In % of total radioactivity recovered		
	Plant extract		
	Ethyl acetate	Water	Bound[a]
	Roots		
Complete (CK)	86.87 ± 0.65	10.95 ± 0.57	2.18 ± 0.16
Deficient in			
N	91.09 ± 0.28[b]	7.57 ± 0.36[b]	1.34 ± 0.10
P	85.73 ± 1.19	10.78 ± 1.01	3.49 ± 0.32[b]
K	84.04 ± 1.00	10.24 ± 0.74	5.72 ± 0.39[c]
Ca	85.28 ± 2.71	9.82 ± 2.96	3.90 ± 1.02[c]
Mg	81.08 ± 4.20[c]	14.83 ± 3.07[c]	4.09 ± 1.27[c]
S	89.96 ± 1.58	8.14 ± 1.32	1.90 ± 0.28
	Greens		
Complete (CK)	10.94 ± 1.04	48.09 ± 4.12	40.97 ± 3.74
Deficient in			
N	16.13 ± 0.69[c]	54.78 ± 1.70[c]	29.09 ± 1.42[c]
P	13.30 ± 0.82[b]	40.15 ± 1.43[c]	46.55 ± 2.65[c]
K	13.44 ± 1.55[b]	46.65 ± 0.63	39.91 ± 2.05
Ca	10.35 ± 2.89	70.90 ± 2.12[c]	18.75 ± 0.81[c]
Mg	10.69 ± 0.93	62.97 ± 2.31[c]	26.34 ± 1.87[c]
S	11.97 ± 0.60	50.75 ± 1.21	37.28 ± 1.36[c]

[a] Determined after combustion of previously extracted plant material. [b,c] Differences between 14C in plant parts from nutrient-deficient solutions and those from controls were significant at the [b] 5% or [c] 1% level.

Table III. Soluble and Protein Bound ^{14}C in the Water Extraction Phase from Pea Plants after Exposure of Pea Roots to [^{14}C](Ethoxy)Dyfonate During the Last 8 Days of the Growing Period

	In % of total (organic and water-soluble) radioactivity recovered from roots or greens				
		Protein bound[b]		Soluble[c]	
Nutrient solution	Water[a]		% water[d]		% water
		Roots			
Complete (CK)	10.95	3.92	36	7.03	64
Deficient in					
N	7.57	2.57	34	5.00	66
P	10.78	6.36	59	4.42	41
K	10.24	4.04	40	6.18	60
Ca	9.82	2.38	24	7.44	76
Mg	14.83	6.29	42	8.54	58
S	8.14	3.07	38	5.07	62
		Greens			
Complete (CK)	48.09	27.55	57	20.54	43
Deficient in					
N	54.78	6.50	12	48.28	88
P	40.15	15.66	39	24.49	61
K	46.65	23.75	51	22.90	49
Ca	70.90	33.09	47	37.81	53
Mg	62.97	29.20	46	33.77	54
S	50.75	21.21	42	29.54	58

[a] ^{14}C in the water phase before protein precipitation. [b] ^{14}C bound to the precipitated proteins. [c] Calculated: "water" minus "protein bound." [d] Protein bound ^{14}C in % of radiocarbon content of total water extraction phase.

tractable radiocarbon in greens could be due to the formation of stable conjugates or complexes with certain plant constituents which are not extractable with the organic solvents used.

Nutrient deficiencies affected the production of water-soluble metabolites and the amounts of unextractable radioactivity. In nitrogen-deficient plants, the metabolism of Dyfonate in the roots was reduced, as determined by decreases in water-soluble radioactivity and increases in organic soluble radioactivity. Greens from N-deficient plants contained more organic and water-soluble radioactivity than did control plants, but contained considerably less unextractable ^{14}C compounds. It appears that a deficiency in nitrogenous nutrients resulted in increased penetration of [^{14}C]Dyfonate into the roots but in decreased metabolism of the penetrated insecticide into water-soluble compounds. A decrease in metabolism in the greens is indicated by the lower amounts of unextractable radioactivity, which appear to be associated with metabolic processes.

Based on the amounts of radioactivity recovered from the water phases, magnesium deficiency resulted in increased metabolism of [^{14}C]Dyfonate in both the roots and the greens, while deficiencies of P and Ca only affected the metabolism in the greens; there were significantly less water-soluble metabolites in greens from plants grown in P-deficient nutrient solution and more in those grown in Ca-deficient nutrient solutions.

Attempts were made to characterize the water-soluble metabolites produced. Analysis of the precipitated proteins (Table III) showed that 36 and 57% of the radioactivity in the water phases of the control roots and greens, respectively, were associated with the proteins. As shown in Table III, these figures were different in the nutrient-deficient plants, especially with the roots from P- and Ca-deficient plants and with the greens from P- and N-deficient plants. Since these estimates were made with pooled samples, no statistical analyses were performed. The observed differences may be related to the amounts of proteins present in the water.

Analyses by tlc and autoradiography of the deproteinized water phase ("Soluble," Table III) were inconclusive because of streaking. However, none of the spots visualized by autoradiography had the same R_f value as Dyfonate (R_f 0.9), Dyfoxon (R_f 0.9), and ETP (R_f 0.68). A prominent elongated spot, between R_f 0.14 and 0.57, was observed with all samples. The presence of EOP (R_f 0.43) was indicated but not confirmed.

Effects of Nutrient Deficiencies on the Penetration, Translocation of Dyfonate, and Its Conversion into Dyfoxon. Analyses of the organic solvent phases by tlc resulted in the detection of Dyfonate (R_f 0.72) and Dyfoxon (R_f 0.47) and a minor spot at the origin. Analysis of these same extracts by glc showed the presence of Dyfonate and Dyfoxon (Table IV). In the control roots, concentrations of 80.22 ppm of Dyfonate and 0.87 ppm of Dyfoxon were determined, while in the greens these concentrations were considerably lower, but the amounts of Dyfoxon in the greens relative to the amounts of Dyfonate were much larger.

Deficiencies in certain mineral nutrients had various effects, as indicated by data presented in Table IV. As mentioned above, a lack of nitrogen resulted in the increased penetration of radioactive compounds into the roots (Table I). This was also confirmed by the presence of higher concentrations of both Dyfonate and Dyfoxon in the roots (Table IV). Significant increases in Dyfonate

Table IV. Effects of Nutrient Deficiencies on the Penetration, Translocation, and Metabolism of [^{14}C](Ethoxy)Dyfonate in Pea Plants after Growing for 8 Days in a [^{14}C](Ethoxy)Dyfonate-Treated (4 ppm) Nutrient Solution. Results Are Averages of Four Replicates (Four Plants Each)

	Recovered from plant parts[a]							
	Dyfonate				Dyfoxon			
	Roots		Greens		Roots		Greens	
Nutrient solution	ppm	% CK[b]	ppm	% CK[b]	ppm	% CK[b]	ppm	% CK[b]
Complete (CK)	80.22 ± 1.26	100	0.48 ± 0.21	100	0.87 ± 0.11	100	0.25 ± 0.03	100
Deficient in								
N	135.40 ± 22.76	169[c]	0.25 ± 0.12	52	1.29 ± 0.32	148[c]	0.47 ± 0.34	188
P	64.82 ± 9.43	81	0.34 ± 0.25	71	0.57 ± 0.22	66	0.16 ± 0.03	64[d]
K	38.41 ± 7.94	48[c]	1.63 ± 0.41	340[c]	0.44 ± 0.05	51[d]	0.16 ± 0.04	64[d]
Ca	35.29 ± 10.33	44[c]	0.90 ± 0.73	187	0.30 ± 0.07	35[c]	0.13 ± 0.06	52[d]
Mg	35.36 ± 11.57	44[c]	0.46 ± 0.11	96	0.46 ± 0.18	53[d]	0.04 ± 0.01	16[c]
S	107.10 ± 11.04	134[d]	0.60 ± 0.19	125	0.69 ± 0.12	79	0.10 ± 0.02	40[d]

[a] Recovered by glc from roots and greens. Root data were confirmed by tlc. Analysis of the greens by tlc was inconclusive due to interfering substances. [b] % CK = in % of control (complete nutrient solutions). [c,d] Differences in Dyfonate and Dyfoxon concentrations between plant parts from nutrient-deficient solutions and those from controls were significant at the [c] 1% or [d] 5% level.

concentrations were also observed in the roots of plants grown in S-deficient solutions and in the greens of plants from K-deficient solutions.

Significant decreases in the concentrations of both Dyfonate and Dyfoxon in the roots were observed with plants grown in solutions deficient in K, Ca, or Mg. The concentrations of Dyfoxon were significantly decreased by deficiencies of all elements except nitrogen.

Analysis of the nutrient solutions after 8 days of plant growth showed that 2.7 to 13.3% of the recovered radioactivity was water soluble, compared to 2.1% immediately after treatment with [^{14}C]Dyfonate. This indicates that either water-soluble ^{14}C metabolites are released from the plant roots into the nutrient solutions or that exoenzymes metabolized the Dyfonate in the nutrient solutions.

LITERATURE CITED

Kelly, R. G., Peets, E. A., Gordon, S., Buyske, D. A., *Anal. Biochem.* **2**, 267 (1961).
Lichtenstein, E. P., Schulz, K. R., *J. Agr. Food Chem.* **18**, 814 (1970).
Lichtenstein, E. P., Schulz, K. R., Fuhremann, T. W., *J. Agr. Food Chem.* **20**, 831 (1972).
McBain, J. B., Hoffman, L. J., Menn, J. J., *J. Agr. Food Chem.* **18**, 1139 (1970).
Mills, P. A., Bong, B. A., Kamps, L. R., Burke, J. A., *J. Ass. Offic. Anal. Chem.* **55**, 39 (1972).
Pesticide Analytical Manual, Vol. II, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, 1968.
Storherr, R. W., Watts, R. R., *J. Ass. Offic. Agr. Chem.* **48**, 1154 (1965).
Talekar, N. S., Lichtenstein, E. P., *J. Agr. Food Chem.* **19**, 846 (1971).
Talekar, N. S., Lichtenstein, E. P., *J. Agr. Food Chem.* **20**, 1260 (1972).
Watts, R. R., Storherr, R. W., Pardue, J. R., Osgood, T., *J. Ass. Offic. Anal. Chem.* **52**, 522 (1969).

Received for review April 4, 1973. Accepted July 13, 1973. Research supported in part by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wis. (Hatch Project No. 1387), and by a grant from the NSF (GB-35021). Contribution from the Wisconsin Agricultural Experiment Station as a collaborator under North Central Cooperative Research Project 96, "Environmental Implications of Pesticide Usage."

Fate of Aldrin-^{14}C in Sugar Beets and Soil under Outdoor Conditions

Jagmohan Kohli, Sarban Zarif, Irene Weisgerber, Werner Klein,* and Friedhelm Korte

Aldrin-^{14}C has been applied to soils under outdoor conditions in Germany (2.9 kg/ha) and England (3.2 kg/ha) and sugar beets have been grown. Upon harvest, more than 95% of the total radioactivity recovered from the plants and more than 40% of the radioactivity recovered from the soils was due to metabolites, mainly dieldrin and a group of hydrophilic metabolic products. The main hydrophilic compound was identified as dihydrochlordene-^{14}C dicarboxylic acid (1,2,3,4,8,-8-hexachloro-1,4,4a,6,7,7a-hexahydro-1,4-endomethylene-indene-5,7-dicarboxylic acid); additionally, photodieldrin and two minor acidic compounds were detected. Between 52 and 58% of the total residue in the surface soils was aldrin, but the percentage of aldrin decreased with increasing depth. Only very low residues were detected in the deeper soil layers in England, whereas more radioactivity was found in the soil samples in Germany.

In a previous paper (Klein *et al.*, 1973) we reported upon outdoor soil application studies of aldrin-^{14}C in potatoes carried out in 1969 at two locations in Germany and England. These outdoor studies have been set up to obtain an approach to field conditions, since radioisotope studies can not be carried out under real conditions. The simulated field conditions, however, give information which is not easily obtainable with small-scale indoor experiments. For potatoes, it was shown that more than 60% of the total radioactivity which was recovered in plants and soil after the harvest was due to metabolites, mainly dieldrin and a group of hydrophilic metabolic products, the main compound of which was identified as dihydrochlordene-^{14}C dicarboxylic acid (Figure 1). The same compound was detected in the leaching water draining from the experimental box at a depth of 60 cm (Klein *et al.*, 1973; Moza *et al.*, 1972).

In the same year, experiments with other crops were conducted in Birlinghoven, Germany, and Sittingbourne, U. K., under the same conditions. The full results obtained from experiments with sugar beets are described in this paper.

Institut für ökologische Chemie, Schloss Birlinghoven, der Gesellschaft für Strahlen- und Umweltforschung mbH, Munich, Germany.

APPARATUS AND REAGENTS

The apparatus for radioactive counting, glc, and mass spectrometry, as well as the reagents, was the same as described in the previous paper (Klein *et al.*, 1973).

PROCEDURE

The sugar beets were grown in Birlinghoven, Germany, and Sittingbourne, U. K., in the open air in boxes 60 × 60 × 60 cm, constructed from water-resistant plywood. The base of the boxes contained holes to permit the drainage of excess water. The boxes were sunk into a pit such that the upper surface of the soil was level with the surrounding ground. The soils in the boxes were typical for sugar beet growing in the two locations. Fertilizers were applied as in agricultural practice. The aldrin-^{14}C was applied as a diluted 30% emulsifiable concentrate using commercial surfactants; application rate and specific activity of aldrin-^{14}C are given in Table I. The concentrate was diluted 100 times with water before application. The insecticide was incorporated in the soil to a 10-cm depth; then 40 sugar beet seeds were sown. The plants were thinned out to 20 when they were 5-cm high, and to 4 at the six-leaf stage. Air temperature, humidity, and pressure, as well as rainfall, were recorded during the vegetation period. A summary of the climatic conditions, the analyses of both soils, and further cultural details are included in Table I.