Effects of Nutrient Deficiencies in Corn Plants on the in Vivo and in Vitro Metabolism of [^{14}C]Diazinon

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The effects of calcium, nitrogen, and magnesium deficiencies on the penetration, translocation, and the in vivo and in vitro metabolism of [^{14}C]diazinon in corn plants were investigated. On a per gram fresh weight basis, only roots from nitrogen-deficient solutions contained less ^{14}C, while tops from plants grown in calcium-deficient solutions contained nearly four times more radiocarbon than those from complete nutrient solutions. Due to calcium or nitrogen deficiencies a reduced degradation occurred in roots as indicated by the relatively higher recoveries of diazinon and the lower recoveries of an unidentified, more polar ^{14}C-ring compound. No differences in [^{14}C]diazinon degradation due to nutrient deficiencies were noticeable with corn tops. The metabolic activity of corn roots was due to a soluble enzyme. In studies with subcellular components from roots specific activities increased from the homogenate (4.09%/mg of protein) to the 105000*g* supernatant (7.77%/mg of protein). Subcellular components from calcium-deficient roots produced significantly less water-soluble radiocarbon (sp act., 0.60) than did control roots (sp act., 1.60), results similar to those observed with in vivo experiments. However, the 10000*g* supernatant from root material deficient in nitrogen produced significantly more water-soluble radiocarbon (sp act., 2.85) than subcellular fractions from control roots.

Agricultural soils contain pesticide residues which may penetrate the roots of various crop plants. After their translocation within the plant, they are often further metabolized. Among the many environmental factors which affect these processes, the availability of nutrients to the growing plant can be important. While pesticides can influence the nutrient content of plants (Adams and Espinoza, 1969; Voigt, 1955) nutrients can also affect the penetration into and translocation of pesticides within plants. Thus, Casida et al. (1952) showed a decreased absorption of schradan by pea plants with an increased phosphorus supply, while Hacskaylo et al. (1961) reported a reduced dimethoate absorption by cotton plants grown in phosphorus-deficient nutrient solutions. Rohrbaugh and Rice (1956) and Rice and Rohrbaugh (1958) showed that 2,4-D was not readily translocated within phosphorus or potassium-deficient tomato plants. With decreasing levels of nitrogen in nutrient solutions, a significant increase in schradan absorption into cotton plants was achieved (Hacskaylo and Ergle, 1955). Yu and Morrison (1969) demonstrated that the amount of mevinphos and phosphamidon that was translocated into leaves of bean plants was positively correlated with the external supply of nitrogen, potassium, phosphorus, and magnesium, while sulfur and calcium had little or no effect. Talekar and Lichtenstein (1971) showed that the penetration and translocation of lindane in peas is affected by the availability of mineral nutrients. 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 deficiency of potassium resulted in reduced lindane translocation. Talekar and Lichtenstein (1973) also reported that plant nutrients affected the penetration, translocation, and metabolism of [^{14}C]Dyfonate (fonofos) in pea plants. It is possible that nutrient deficiencies will also affect the metabolsim of an insecticide both qualitatively and quantitatively, thus rendering the pesticide either more or less toxic to crop pests.

In the present study we investigated the effects of some nutrient deficiencies on the penetration, translocation, and metabolism of [^{14}C]diazinon in corn plants.

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METHODS AND PROCEDURE

Chemicals. ^{14}C-ring-labeled diazinon [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl-2-^{14}C) phosphorothioate] (sp act., 3.6 μCi/mg), diazinon, diazoxon [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphate], 6-hydroxypyrimidine (2-isopropyl-4-methyl-6-hydroxypyrimidine), 6-mercaptopyrimidine (2-isopropyl-4-methyl-6-mercaptopyrimidine), 6-ethoxypyrimidine (2-isopropyl-4-methyl-6-ethoxypyrimidine), and hydroxypyrimidinol [2-(1′-hydroxy-1′-methyl)-ethyl-4-methyl-6-hydroxypyrimidine] were obtained through the courtesy of Ciba-Geigy Corp., Greensboro, NC.

Solvents used were redistilled toluene, benzene, chloroform, methylene chloride, acetone, dioxane, ethyl acetate, and 99% isopropyl alcohol, 95% ethanol, and methanol.

Growing of Plants. Corn seeds (Funk Hybrid G4444, blight resistant) were obtained through the courtesy of Funk Seed International, Bloomington, IL. Plants were grown in complete or nutrient deficient solutions as described by Talekar and Lichtenstein (1973), except that the iron concentration had to be increased to 9 ppm to provide an adequate supply for corn plants. In most experiments, corn seedlings were grown for 12 days in complete nutrient solutions or in solutions deficient in calcium, nitrogen, or magnesium. Freshly prepared Hoaglands nutrient solution has a pH of 5.0. This pH was maintained by daily adjustment. The effects of plant nutrient deficiencies on the penetration, translocation, and metabolism of [^{14}C]diazinon were studied by adding the insecticide to the respective solutions in which the plants had grown for 6 days. Plants were then exposed to [^{14}C]diazinon during the last 6 days of their growing period. For in vitro tests, plants were grown in complete or deficient nutrient solutions, but without the insecticide. After that, subcellular fractions of roots were prepared as described by Lichtenstein and Corbett (1969), and the resulting components were incubated with [^{14}C]diazinon in phosphate buffer (pH 6.3).

Extraction Procedure. Roots and tops were extracted three times in a Waring blender with a mixture of benzene–methanol–acetone (1:1:1). The extracts were filtered under a vacuum, resulting in a filtrate and the dry extracted plant pulp. A 20-mL aliquot of the filtrate was concentrated to near dryness in a flash evaporator at 35 °C, adjusted to 20 mL with water, and partitioned with three 25-mL portions of benzene.

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Table I. Effects of Nutrient Deficiencies on Growth of Corn Plants[a]

plants grown in solutions deficient in	roots				tops			
	length[b]		fresh weight[b]		length[b]		fresh weight[b]	
	cm	% Ck[c]	grams	% Ck	cm	% Ck	grams	% Ck
none	37.0 ± 5.3	100	2.23 ± 0.6	100	34.4 ± 6.9	100	2.81 ± 1.0	100
calcium	18.3 ± 2.6[d]	49	0.73 ± 0.2[e]	33	20.5 ± 2.9[d]	60	1.02 ± 0.1[f]	36
nitrogen	32.7 ± 4.8	88	1.05 ± 0.2[f]	47	21.7 ± 4.1[f]	63	1.04 ± 0.2[f]	37
magnesium	39.0 ± 2.3	105	1.82 ± 0.4	82	32.1 ± 4.1	93	2.18 ± 0.5	78

[a] Corn was grown for 6 days in complete nutrient solutions, followed by 8 days growing in complete or nutrient-deficient solutions. [b] Length and fresh weight per one plant. Averages of three separate tests with ten plants each. [c] % Ck, in percent of control (complete nutrient solution). [d-f] Results were significantly different from controls (complete nutrient solutions) at the 0.1% (*d*), 1.0% (*e*), and 5% (*f*) levels.

Nutrient solutions were filtered to remove suspended root particles. Each solution was then extracted with three 100-mL portions of benzene.

Incubation mixtures from in vitro tests were extracted with three 10-mL portions of benzene.

Analytical Procedures. Liquid scintillation counting (LSC) was used to determine the radiocarbon content in organic solvent extracts or in water extraction phases from plants or nutrient solutions as described by Fuhremann and Lichtenstein (1978).

Gas-liquid chromatography (GLC) was used for the analyses of the benzene-extraction phases containing diazinon and/or diazoxon with a Tracor Model 560 gas chromatograph (Tracor, Inc., Austin, TX). Separation of these two compounds was obtained utilizing a 122 cm × 2 mm i.d. Pyrex column packed with 3% DEGS on 80–100 mesh chromosorb W (AW-DMCS) at a temperature of 170 °C. Detection of the compounds was accomplished with a flame photometric detector equipped with a 526-nm interference filter specific for the detection of phosphorus-containing compounds. Gas flow rates were: hydrogen, 60 mL/min; air, 110 mL/min; and nitrogen carrier, 60 mL/min. Temperatures of the injector and detector were 225 and 205 °C, respectively. Retention times and peak heights for chromatograms produced by injection of sample extracts were compared to those produced by known amounts of analytical-grade diazinon and diazoxon.

High-pressure liquid chromatography (LC) was used to confirm the presence of 6-hydroxypyrimidine in plant extracts. LC could also be used for the detection of diazinon and diazoxon, but thin-layer chromatography (TLC) and GLC methods were more sensitive. Analyses by LC were performed with a Varian Model 8500 liquid chromatograph equipped with a variable wavelength UV-scanning spectrophotometer. Analytical separation of 6-hydroxypyrimidine from plant extracts was accomplished using a 25 cm × 2 mm Micropak CN-10 column (Varian Instruments). The solvent system used was isooctane–methylene chloride–isopropyl alcohol (70:27:3) and was pumped at a flow rate of 1 cm^3/min. The absorption maximum for 6-hydroxypyrimidine is 265 nm but the effluent was monitored at 270 nm to avoid interference due to solvent absorption. In addition to retention time, identification was aided by stopped-flow UV scans of observed peaks which were compared to authentic reference compounds.

Thin-layer chromatography (TLC) of organic solvent extraction phases from corn roots and tops were performed using 20 × 20 cm silica gel 60 precoated plates treated with a fluorescent indicator (E. Merck, Darmstadt, Germany). Chromatograms were developed with a mixture of benzene–chloroform–acetone (1:1:1) and were visualized by observing them under UV light at 254 nm. The following R_f values were observed: 0.62 for diazinon, 0.51 for diazoxon, 0.22 for 6-hydroxypyrimidine, and 0.08 for hydroxypyrimidinol. Separation of authentic 6-mercaptopyrimidine, 6-ethoxypyrimidine, diazinon, and diazoxon was achieved with a mixture of chloroform–toluene–ethyl acetate (5:4:1). However, 6-mercaptopyrimidine and 6-ethoxypyrimidine could not be detected in any sample. Radioactive metabolites were visualized by autoradiography of the developed TLC plates with Kodak No-Screen X-ray films. Areas on the plates corresponding to the spots observed on the X-ray films were scraped off and sonicated in 1 mL of 0.1 N KOH. This mixture was then added to 14 mL of scintillation solvent, which contained 3.5% (w/v) thixotropic gel powder (CAB-O-SIL).

RESULTS AND DISCUSSION

Effect of Nutrient Deficiencies on Growth of Corn Plants. To determine the effects of nutrient deficiencies on plant growth, corn seedlings were grown for 6 days in complete nutrient solutions. After that, triplicate groups of ten plants were grown for 8 days in complete nutrient solutions (controls), or in solutions deficient in calcium, nitrogen, or magnesium. The length and fresh weight of roots and tops were then determined. Results (Table I) indicated that lack of calcium resulted in a 40–50% reduction in length and in a 65% reduction in the fresh weight of roots and tops. Deficiencies in nitrogen resulted in similar reductions, except that root length was not affected.

Exploratory tests conducted in triplicate with three plants per container indicated that corn grown in nutrient-deficient solutions had a reduced water content in both roots and tops, thus increasing the dry weight from 8% (controls) to 11% (no magnesium), 12% (no nitrogen), and 15% (no calcium) of the recorded fresh weight.

To determine potential effects of nutrient deficiencies on the chemical composition of corn roots, a 10000*g* supernatant was prepared as described (Lichtenstein and Corbett, 1969) from corn which had been grown in complete or nutrient-deficient solutions. This supernatant, which we found to be relatively active in degrading [^{14}C]diazinon, was analyzed for its protein content by the modified method of Lowry (Geiger and Bessman, 1972). Analyses for amino acids were performed with an amino acid analyzer as described by Kemp and Sutton (1971). No differences due to nutrient deficiencies were found in protein (1.2 ± 0.04 mg/0.25 g of root material) or amino acid content and their composition in the 10000*g* supernatant from corn roots.

In Vivo Effects of Nutrient Deficiencies on the Penetration, Translocation, and Metabolism of [^{14}C]Diazinon in Corn. Since visible effects due to nutrient deficiencies were primarily noticed with plants grown in the absence of calcium or nitrogen, further experiments were conducted to study the effects of these nutrient deficiencies on the penetration, translocation, and metabolism of [^{14}C]diazinon in corn. Three groups of six

Table II. Effect of Calcium- and Nitrogen-Deficiencies on the Uptake of Radiocarbon by Corn Plants Grown in [^{14}C]Diazinon-Treated Nutrient Solutions[a]

radiocarbon recov.[b] from	^{14}C, in % of applied[a]		
	plants grown in nutrients solutions deficient in		
	none (Ck)	calcium	nitrogen
A. nutrient solutions	34.4 ± 7.8	54.5 ± 1.7[d]	73.5 ± 8.8[d]
B. plants	23.3 ± 0.9	11.9 ± 2.1[c]	6.3 ± 1.0[c]
roots[f]	14.3 ± 0.03	6.2 ± 0.64[c]	3.03 ± 0.48[c]
roots/gram[g]	1.04 ± 0.05	1.06 ± 0.10	0.53 ± 0.07[c]
tops[f]	9.0 ± 0.85	5.68 ± 1.53[e]	3.27 ± 0.89[d]
tops/gram[g]	0.36 ± 0.05	1.65 ± 0.07[c]	0.60 ± 0.15
total (A + B)	57.7 ± 8.7	66.4 ± 2.2	79.8 ± 9.4[e]

[a] Plants were grown for 12 days in complete or nutrient-deficient solutions. ^{14}C-diazinon was applied after 6 days of growth at the rate of 5ppm (0.31 μCi/500 mL). [b] Recovered from nutrient solution or extracts of plants plus their bound residues. Results are the average of three replicate tests with six plants each. [c-e] Results were significantly different from controls at the 0.1% (*c*), 1.0% (*d*), or 5% (*e*) levels. [f] Roots or tops from six plants. [g] Per gram fresh weight.

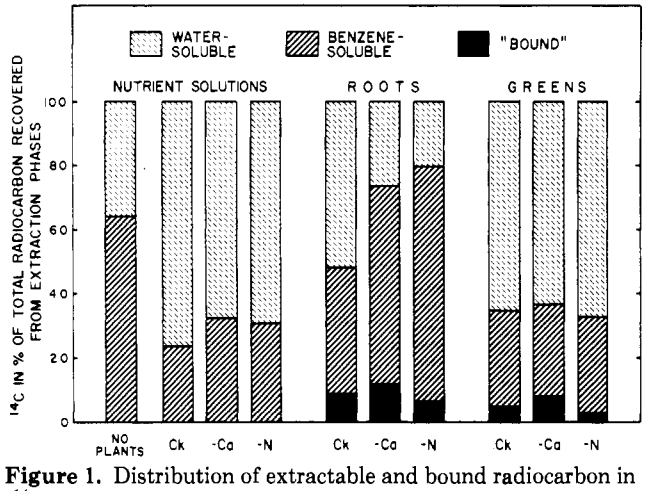

Figure 1. Distribution of extractable and bound radiocarbon in [^{14}C]diazinon treated nutrient solutions and in corn plants, as affected by calcium (-Ca) and nitrogen (-N) nutrient deficiencies. "No Plants" = complete nutrient solutions in which no plants had grown. Ck = complete nutrient solutions in which plants had grown.

corn seedings were grown for 12 days in either complete (control), calcium, or nitrogen-deficient solutions. After 6 days of growing, [^{14}C]diazinon was added at 5 ppm to all solutions. After six additional days, analyses of nutrient solutions and plant parts were conducted to determine the total radiocarbon in the system (Table II), the distribution of ^{14}C between the organic solvent and water extraction phases as well as the amounts of unextractable, bound ^{14}C residues (Figure 1) and finally the identities and quantities of organic soluble ^{14}C compounds (Table III). In the following discussions, the term penetration refers to the radioactive compounds found within, or adhering to, the corn roots, while the radiocarbon recovered from plant tops is referred to as having been translocated from the roots.

The total amounts of radiocarbon recovered from nutrient solutions and plants—including unextractable, bound ^{14}C—are presented in Table II. Only 57.7% of the radiocarbon applied to complete nutrient solutions were recovered from the whole system (solutions plus plants), indicating a loss of 42%. In the absence of calcium or nitrogen, however, more ^{14}C was recovered, with a loss of only 34 or 20% from plants plus solutions, which were

Table III. Radiocarbon, Diazinon, and Metabolites Recovered from the Organic Solvent Extraction Phase of Corn Plants Grown in [^{14}C]Diazinon-Treated Nutrient Solutions[a]

plants grown in sol. defic. in	roots	tops
	A. Organic Solvent-Soluble ^{14}C[b] in % of [^{14}C]Diazinon Applied	
none (Ck)	13.18 ± 0.14	8.59 ± 0.83
Ca	5.56 ± 0.51[c]	5.30 ± 1.42[d]
N	2.86 ± 0.47[c]	3.19 ± 0.87[c]

plants grown in sol. defic. in	roots				tops			
	I	II	III	IV	I	II	III	IV
	B. Distribution on TLC in % of Organic Solvent-Soluble ^{14}C[e]							
none	22	2	22	54	5	2	78	15
Ca	57	2	20	21	6	1	79	14
N	44	2	44	10	5	1	85	9

[a] As in Table II, footnote *a*. [b] Benzene-methanol-acetone soluble ^{14}C. Results are averages of three replicate tests with six plants each. [c,d] Significantly different from controls at the 0.1% (*c*) and 5% (*d*) level. [e] I, diazinon; II, diazoxon; III, 6-hydroxypyrimidine; IV, unknown ^{14}C-ring-labeled metabolites.

deficient in calcium or nitrogen, respectively. Assuming that loss from the water surfaces due to volatilization was similar under all three conditions, losses via the plants could account for these differences. Corn grown in nutrient-deficient solutions contained significantly less radiocarbon than did control plants, which metabolized the insecticide more extensively, as indicated in Figure 1 and Table III for corn roots.

Since plants grown in calcium or nitrogen-deficient solutions had reduced fresh weights, the amounts of radiocarbon which penetrated into roots and was translocated into corn tops were also calculated on a per gram fresh weight basis (Table II). Expressed this way, only roots from nitrogen-deficient solutions contained less ^{14}C, while tops from plants grown in calcium-deficient solutions contained nearly four times more radiocarbon than those from complete nutrient solutions.

To study the metabolism of [^{14}C]diazinon in corn plants, the benzene–methanol–acetone extract of both roots and tops were analyzed by TLC as described. Table IIIA presents data for the organic solvent-soluble radiocarbon (excluding bound ^{14}C) recovered from roots and tops. Lack of calcium or nitrogen in the [^{14}C]diazinon-treated nutrient solution, significantly reduced the organic-soluble radiocarbon which had penetrated the roots and was translocated into the tops. Resolution of the ^{14}C compounds by TLC (Table IIIB) indicated that a reduced degradation occurred in roots grown in nutrient-deficient solutions as indicated by the relatively higher recoveries of diazinon and the lower recoveries of a more polar compound which remained at the origin of the TLC plate. No differences in [^{14}C]diazinon degradation due to nutrient deficiencies were noticeable with corn tops. The presence of 6-hydroxypyrimidine was confirmed by LC.

Benzene–methanol–acetone extracts were further partitioned as described, resulting in water and benzene extraction phases. This analytical step was important since water-soluble ^{14}C compounds represent degradation products of [^{14}C]diazinon. Amounts of radiocarbon found in these extraction phases and the unextractable amounts determined by combustion are presented in Figure 1 and

Table IV. Production of Water-Soluble Radiocarbon from [^{14}C]Diazinon by Subcellular Fractions of Corn Roots, Grown for 14 Days in Complete Nutrient Solutions

incubation mixtures[a]	^{14}C recov. in % of applied[b]	sp act.[c]
buffer	2.3 ± 0.1	
homogenate	39.1 ± 1.1	4.09 ± 0.12
1000*g* P	4.9 ± 0.1	0.33 ± 0.01
1000*g* S	46.2 ± 0.8	5.15 ± 0.09
10000*g* P	4.2 ± 0.1	0.33 ± 0.02
10000*g* S	51.5 ± 0.3	6.01 ± 0.04
105000*g* P	4.4 ± 0.2	0.47 ± 0.04
105000*g* S	54.5 ± 0.5	7.77 ± 0.07
105000*g* S boiled	3.3 ± 0.1	0.15 ± 0.01

[a] P, pellet; S, supernatant. [b] Water-soluble radiocarbon in percent of [^{14}C]diazinon applied to incubation mixtures (25 μg/3 mL). Average of triplicate incubation mixtures. [c] Water-soluble ^{14}C recovered per milligram of protein, in percent of applied [^{14}C]diazinon.

are expressed in percent of the total ^{14}C recovered. Complete nutrient solutions in which plants had grown contained 34.4% of the applied radiocarbon (Table II), but 74.5% was recovered from nutrient solutions in which no plants had grown. Thus radiocarbon must have been lost via the plants. Complete nutrient solutions in which no plants had grown contained two-thirds of the recovered radiocarbon in benzene-soluble form. In the presence of plants (Ck, Figure 1), however, only one-fourth of the applied ^{14}C partitioned into benzene, while the remainder was present as water-soluble [^{14}C]diazinon degradation products. With plants growing in nutrient solutions, differences in the partition behavior of the extracted radiocarbon due to nutrient deficiencies were only observed with roots. Due to lack of calcium or nitrogen, roots contained increased amounts of benzene-soluble and decreased amounts of water-soluble radiocarbon, indicating a reduced metabolism [^{14}C]diazinon as was also indicated by TLC (Table IIIB).

Effects of Nutrient Deficiencies on the in Vitro Metabolism of [^{14}C]Diazinon. Experiments were conducted initially to determine which subcellular fractions would be the most active in degrading [^{14}C]diazinon. For this purpose, corn plants were grown for 14 days in quartz sand supplied with a complete nutrient solution. Since previous tests had shown that nutrient deficiencies only affected the metabolism of the insecticide in roots, they were used for subcellular studies. After the preparation of subcellular components as described, triplicate 3-mL incubation mixtures, each containing the equivalent of 1.2 g of root material, were treated with 25 μg (0.08 μCi) of [^{14}C]diazinon in 25 μL of ethanol. After incubation for 8 h at 25 °C, the reaction was stopped by adding 5 mL of acetone. Reaction mixtures were extracted with three 10-mL portions of benzene, resulting in a benzene and water extraction phase. After adjustment to volume, analyses by LSC were performed as described. Since water-soluble radiocarbon produced from [^{14}C]diazinon represents degradation products of the insecticide, these data were used to indicate the relative activity of each fraction. Results (Table IV) show that the metabolic activity was due to a soluble enzyme. The specific activity increased from the homogenate (4.09%/mg of protein) to the 105000*g* supernatant (7.77%/mg of protein). Incubation of [^{14}C]diazinon with buffer only or with 105000*g* supernatant after boiling did not produce significant amounts of water-soluble radiocarbon, indicating the enzymatic nature of the degradation process.

To study the effects of nutrient deficiencies on the subcellular degradation of [^{14}C]diazinon, all corn plants were first grown for 6 days in a complete nutrient solution, followed by 8 additional days of growth in solutions deficient in calcium, nitrogen, or magnesium or in complete nutrient solutions (controls). The 10000*g* supernatant obtained from the corn roots was then used for the study of the in vitro degradation of the insecticide. [^{14}C]Diazinon (200 μg, 0.15 μCi) was added in 50 μL of ethanol to triplicate 4-mL incubation mixtures. Each mixture contained an equivalent of 0.9 g of root material prepared from plants grown in complete or nutrient-deficient solutions. After a 2-h incubation at 25 °C, the reaction was stopped by adding 5 mL of acetone. Amounts of water-soluble ^{14}C compounds were determined as described and served as an indicator for the activity of the root material. Incubation mixtures from calcium-deficient roots produced significantly less water-soluble radiocarbon than those from control roots (Table V). Similar results were observed with the in vivo experiments (Figure 1). However, contrary to results observed with in vivo tests, the 10000*g* supernatant from root material deficient in nitrogen produced significantly more water-soluble radiocarbon (sp act., 2.84) than did subcellular fractions from control roots (sp act., 1.60).

Table V. Effects of Nutrient Deficiencies in Corn Roots on the in Vitro Degradation of [^{14}C]Diazinon[a]

corn grown in nutrient solutions	water-soluble ^{14}C recov.[b] in % of applied[c]	% Ck[d]	sp act.[e]
complete (Ck)	8.93 ± 0.23	100	1.60 ± 0.15
deficient in			
calcium	3.67 ± 1.23[f]	41	0.60 ± 0.17[f]
nitrogen	14.70 ± 1.12[g]	165	2.84 ± 0.09[g]
magnesium	9.52 ± 0.36	107	1.85 ± 0.22

[a] As in Table I. [b] Recovered from the 10000*g* supernatant of corn roots after a 2-h incubation. Results are average of three replicate tests. [c] Applied [^{14}C]diazinon (200 μg/0.15 μCi) to each 4 mL of incubation mixture, containing an equivalent of 0.9 g of root material. [d] In percent of controls (complete nutrient solutions). [e] Water-soluble ^{14}C recovered in percent of applied per milligram of protein. [f,g] Significantly different from controls at the 1% (*f*) and 0.1% (*g*) level.

Results observed with in vivo experiments did not necessarily agree with in vitro observations. Perhaps differences in the degradation of diazinon by the 10000*g* supernatants from control and nutrient-deficient roots were due to differences in actual amounts of enzymes present or due to other factors. Since no differences could be found in the amounts of protein in preparations from control or nutrient-deficient roots, it is possible that different amounts of cofactors or inhibitors might have been present. When subcellular fractions are prepared, cellular contents become disturbed and redistributed and may, therefore, not reflect the activity of an intact plant tissue. With in vivo experiments, penetration phenomena from the nutrient solution into the corn roots could have played a role in addition to the metabolism of the insecticide within the root tissue.

As a "byproduct" of this study, the effects of the amounts of root material, length of incubation time, pH, and plant age on the subcellular metabolism of the insecticide were investigated. To that effect, 200 μg (0.15 μCi) of [^{14}C]diazinon was added to 4 mL of incubation mixtures derived from the 10000*g* supernatant of corn roots grown in complete nutrient solutions. Results showed that up to 0.9 g of root material (Figure 2,I) and up to 4 h of incubation time (Figure 2, II) yielded a linear response which resulted in the production of increasing

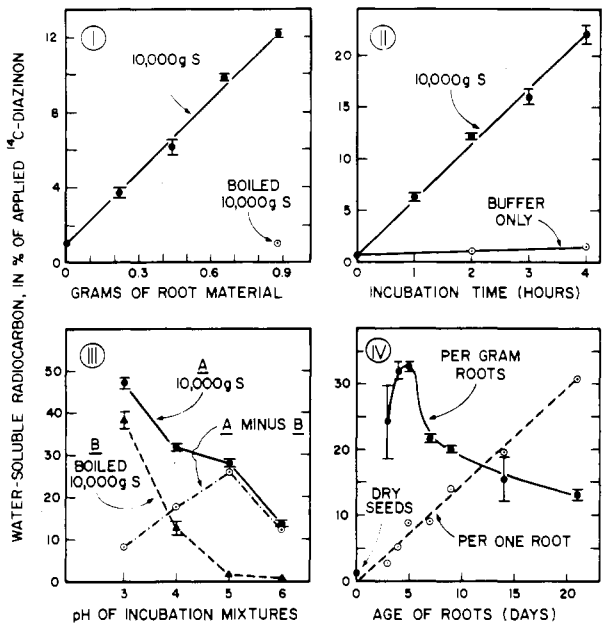

Figure 2. Effects of: (I) amounts of root materials, (II) incubation time, (III) pH, and (IV) root age on the production of water-soluble radiocarbon from [^{14}C]diazinon by the 10000*g* supernatant prepared from corn roots. Plants were grown in complete nutrient solutions.

amounts of water-soluble radiocarbon. An optimum activity was observed at pH 5 (Figure 2,III) after subtraction of the amounts of water-soluble radiocarbon produced by boiled plant material (controls) from that produced by the nonboiled incubation mixtures. Incubation mixtures prepared from 5-day-old roots were more active than those prepared from younger ones (Figure 2,IV).

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Effect of Nonionic Surfactants on the Photochemistry of 3-(4-Chlorophenyl)-1,1-dimethylurea in Aqueous Solution

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Solutions of 3-(4-chlorophenyl)-1,1-dimethylurea (monuron) at approximately 200-ppm concentration were photolyzed in aqueous media containing nonionic surface-active agents. The Triton X and the Tergitol TMN series of nonionic surfactants were employed to observe the difference in effects provided by aryl- and alkyl-substituted polyoxyethylene glycols. X-100, X-405, TMN-6, and TMN-10 were used at 0.2, 0.4, 1, and 2% concentrations. All surfactant solutions were at concentrations in excess of the critical micelle concentration. Samples were examined under oxygenated and nonoxygenated conditions. The identified photoproducts were obtained from demethylation, coupling, and reductive dechlorination reactions. Surfactants increase the rate of monuron degradation, eliminate ring hydroxylation reactions, and enhance the reductive dechlorination reaction. The results indicate that the photochemical reactions occurred in the organic phase of the micelles rather than in the aqueous phase of the solvent.

The photolysis of 3-(4-chlorophenyl)-1,1-dimethylurea (monuron, I) has been studied in aqueous solution (Crosby and Tang, 1969; Rosen et al., 1969; Tanaka et al., 1977) and in methanolic solution (Mazzocchi and Rao, 1972). In these studies, pure materials were placed into solution and photolyzed. Under normal environmental conditions, however, pesticides are generally applied as formulations which include surface-active agents. The amount of surfactant applied with a given pesticide depends on the formulation. In some instances, approximately as much surfactant (on a weight-to-weight basis) is applied as active ingredient. Therefore, there appears to be a need to determine the effect of surface-active agents on the photolytic degradation of pesticides.

Although there are three main classes of surfactants available (cationic, anionic, and nonionic), this investigation was conducted with the nonionic surfactants because of the widespread usage of these materials in pesticidal formulations. The nonionic surfactants usually contain the polyoxyethylene glycol moiety and are gen-

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