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Translocation of Pesticides as Affected by Plant Nutrition

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The nutritional effects upon absorption and translocation of two organophosphate insecticides and two systemic fungicides into hydroponically grown bean plants are described. The insecticides included Guthion (*O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl] phosphorodithioate) and parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate). The fungicides included the major degradation product of benomyl (MBC) methyl 2-benzimidazole carbamate and thiophanate-methyl [dimethyl 4,4′-*o*-phenylenebis(3-thioallophanate)]. Translocation of the four compounds was related to the total root-absorbed activity in a complete nutrient solution.

The degree of penetration of a synthetic compound into plant roots and the extent of its subsequent translocation into other plant parts are both functions of the particular plant, soil type, and physicochemical properties of the compound, *e.g.*, water solubility, polarity, and/or its stability within the living cells (Lichtenstein *et al.*, 1970; Reynolds and Metcalf, 1962). Several investigators have reported to have observed nutritional influences relative to the penetration and translocation of pesticidal compounds within plants. Casida *et al.* (1952) reported a decreased schradan absorption by pea plants with a concomitant increase of available phosphorus, while Hacskaylo *et al.* (1961) observed a reduced dimethoate absorption by cotton plants grown in a phosphorus-deficient nutrient solution. More recently, Yu and Morrison (1969) discovered the alteration in uptake of mevinophos and phosphamidon by bean plants when the supply levels of phosphorus, potassium, magnesium, nitrogen, and calcium were varied. Finally, Talekar and Lichtenstein (1971) witnessed an increased penetration of lindane into the root system of pea plants grown in nitrogen-, sulfur-, or boron-deficient media. Actual translocation of lindane into the aerial parts of the pea plant, however, was reduced.

The present paper describes such nutritional effects upon absorption and translocation of four different systemic compounds. The test compounds used in this investigation included two organophosphate insecticides, Guthion (*O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] phosphorodithioate) and parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate), and two broad-spectrum fungicides, MBC (methyl 2-benzimidazole carbamate) and thiophanate-methyl [dimethyl 4,4′-*o*-phenylenebis(3-thioallophanate)].

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REAGENTS AND APPARATUS

Chemicals. Guthion (benzenoid-ring-U-^{14}C) (sp act. 1.0 μCi/mmol) was synthesized by White *et al.* (1972). The radiolabeled MBC-2-^{14}C (sp act. 2.83 μCi/mmol) was synthesized according to White and Kilgore (1972). Parathion (1,2-^{14}C ring labeled) (sp act. 1.52 μCi/mmol) was purchased from International Chemicals and Nuclear Corp., Irvine, Calif., and thiophanate-methyl (ring-U-^{14}C) (sp act. 2.9 μCi/mmol) was generously provided by the Biological Research Institute, Nippon Soda Co., Ltd., Japan. Analytical reagent grade chemicals and double-distilled solvents were used throughout this investigation.

Instruments. The Polytron, a high specific intensity ultrasonic generator (Type PT 3500, Brinkmann Instruments, Inc., Westbury, N. Y.) equipped with a saw tooth cutting head, was used to extract the labeled compounds from the plant tissues. Infrared spectra were obtained from potassium bromide disks, utilizing a Perkin-Elmer Model 337 spectrophotometer. The radioactivity (^{14}C) was measured in a Model 2425 Packard Tri-Carb liquid scintillation spectrometer. The scintillator fluid was composed of 15 g of 2,5-diphenyloxazole, 2 l. of toluene, and 1 l. of ethylene glycol monomethyl ether.

Thin-Layer Chromatograms. Precoated glass plates (silica gel UV-254, with fluorescent indicator) and precoated plastic sheets (polyamide II/UV-254, with fluorescent indicator) were purchased from Brinkmann Instruments, Inc., Westbury, N. Y.

PROCEDURE

Propagation of Plants. Bean seeds (*Phaseolus vulgaris* L. Tenderbest) were grown as described by Al-Adil *et al.* (1972).

Preparation of Nutrient-Deficient Solutions. Basically, a modified Hoagland nutrient solution (Johnson *et al.*, 1957) containing the following elements was used: nitrogen, 140 ppm (as KNO_3 or $Ca(NO_3)_2 \cdot 4H_2O$); potassium, 130 ppm (as KNO_3 or KCl); calcium, 200 ppm (as $Ca(NO_3)_2 \cdot 4H_2O$ or $CaCl_2 \cdot 2H_2O$); phosphorus, 31 ppm (as $NaH_2PO_4 \cdot H_2O$ or $NH_4H_2PO_4$); sulfur, 64 ppm (as $MgSO_4 \cdot 7H_2O$ or Na_2SO_4); magnesium, 49 ppm (as $MgSO_4 \cdot 7H_2O$ or $MgCl_2 \cdot 6H_2O$); boron, 0.5 ppm (as H_3BO_3); molybdenum, 0.01 ppm (as $H_2MoO_4 \cdot H_2O$); manganese, 0.5 ppm (as $MnSO_4 \cdot H_2O$ or $MnCl_2 \cdot 4H_2O$); copper, 0.5 ppm (as $CuSO_4 \cdot 5H_2O$ or $CuCl_2 \cdot H_2O$); zinc, 0.05 ppm (as $ZnSO_4 \cdot 7H_2O$ or $ZnCl_2$, 95%); and iron, 2.24 ppm (as Na-FeHEDTA (monosodium ferric hydroxyethylenediaminetriacetate)). A deficiency of a particular element was created by eliminating this element selectively from the complete nutrient solution. Whenever a particular anion or cation was removed from the complete nutrient solution, the simultaneous removal of another important cation or anion was restored by adding an appropriate substitute salt. Nitrogen or calcium was removed by eliminating $Ca(NO_3)_2 \cdot 4H_2O$, while the calcium was replaced by $CaCl_2 \cdot 4H_2O$ and nitrogen was replaced by KNO_3 or $NaNO_3$. Potassium was removed by eliminating KNO_3 while nitrogen was replaced by $Ca(NO_3)_2 \cdot 4H_2O$. Magnesium or sulfur was removed by eliminating $MgSO_4 \cdot 7H_2O$, while sulfur was replaced by Na_2SO_4 and magnesium was replaced by $MgCl_2 \cdot 6H_2O$. Micronutrient elements were selectively eliminated by withholding the appropriate salt. The pH of the nutrient solutions (pH 6.75–6.85) was checked and adjusted to that of the control (complete nutrient solution).

Exposure of Plants to the Four Different Pesticides. One-week-old bean plants were selected for translocation studies. Single bean plants were transplanted into foil-wrapped 125-ml erlenmeyer flasks containing 100 ml of freshly prepared complete (control) or deficient nutrient solution. The cotyledons were removed 1 day after plants were exposed to nutrient solution so that any elemental deficiency would appear more rapidly (Casida *et al.*, 1952). Plants were allowed to grow for 15 days and the nutrient solutions were renewed after 1 week. Following 15 days of growth the different nutrient solutions were again renewed and appropriate amounts of ^{14}C-radiolabeled pesticides (0.4 μCi) were added. The plants were then allowed to grow for an additional 48 hr, during which time the pesticide could penetrate into the roots and ultimately translocate to the shoots. The plants were removed from the treated media and the roots were washed with an appropriate solvent to remove surface residues. Each plant was divided into root and shoot segments, weighed, and analyzed separately for ^{14}C content. All tests with complete or deficient nutrient solutions involved seven replicates.

Analytical Methods. Ultrarapid extraction of all labeled compounds from the plant tissues was accomplished by utilizing the ultrasonic Polytron. Each sample was separately diced and placed into a 125-ml erlenmeyer flask together with 75 ml of solvent [parathion–acetonitrile; Guthion–chloroform; MBC–ethyl acetate; and thiophanate-methyl–chloroform–methanol, 1:1]. The plant samples were extracted for 30 sec at half-maximum power of the Polytron. The mixtures were filtered through anhydrous sodium sulfate, and the resultant filtrates were evaporated to dryness on a rotary-vacuum evaporator. The residues were redissolved in 5 ml of solvent (parathion–acetone; Guthion and thiophanate-methyl–chloroform; and MBC–tetrahydrofuran). A 1-ml aliquot was removed and evaporated to dryness on an ashless Whatman No. 42 sample wrapper (Arthur H. Thomas Co., Philadelphia, Pa.). Radiocarbon content was determined by combustion analysis (Krishna and Casida, 1966). The remaining plant extracts were combined and subjected to clean-up and analyses for identification purposes as described below.

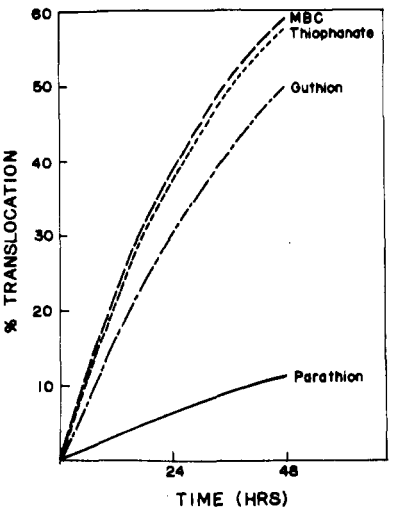

Figure 1. Translocation percentages of absorbed parathion, Guthion, thiophanate, and MBC in bean plants grown in a complete nutrient solution fortified with ^{14}C-radiolabeled pesticides.

Separate aliquots (25–50 μl) of the concentrated extracts were applied as narrow bands to commercially prepared thin-layer plates. Polyamide-11 sheets were used for MBC and silica gel plates were used for the other pesticides. A reference standard of each ^{14}C-radiolabeled compound was spotted near the terminal end of the applied band. The plates were developed in an appropriate solvent system [parathion; ethyl ether–benzene (5:95) and Guthion; chloroform–acetone (5:1) and thiophanate-methyl; chloroform–methanol (9:1) and MBC; chloroform–ethyl acetate–acetic acid (190:10:4)]. Major constituents were located under ultraviolet light (2537 Å). Labeled areas were mapped by exposing the developed thin-layer plates to X-ray film (Kodak no-screen) for a period of 2 weeks. The bands having the same R_f values as those of the standards were extracted from the plates with a vacuum-assisted spot collector (Brinkmann Instruments, Inc., Westbury, N. Y.). The compounds were eluted from the support medium and subjected to a number of spectroscopic techniques appropriate to structure elucidation. Radioactivity was determined by combustion analysis as described earlier above.

RESULTS

The degree (per cent) to which the four absorbed pesticides were translocated after 48 hr [(translocated ^{14}C activity in shoots/total ^{14}C activity in the whole plant) (100)] is shown in Figure 1. Thiophanate-methyl and MBC were translocated to the extent of 58 and 59%, respectively. Similarly, 50.9% of the absorbed Guthion and 11.7% of the absorbed parathion were translocated. Metabolites comprised a small percentage of the total ^{14}C activity extracted from all plants. For example, the recovery of MBC represented 100% of the total radioactivity with no detectable metabolite formation attributed to the use of this compound, intact parathion and unaltered Guthion each comprised 98% of the total radioactivity recovered from the use of these two compounds, and finally, thiophanate-methyl was recovered to the extent of 73% with MBC representing the major observed metabolite.

Effects of Nutrient Deficiencies on the Translocation of the Four Pesticides. The varied effects of elemental deficiencies on the translocation of parathion are shown in Figure 2. Bean plants grown in parathion-treated nutrient solutions deficient in nitrogen–sulfur, iron, boron, or potassium resulted in greater shoot residues than plants similarly grown in a complete nutrient solution. Only a marginal decrease in shoot residues was observed in bean plants grown in a phosphorus-deficient nutrient solution.

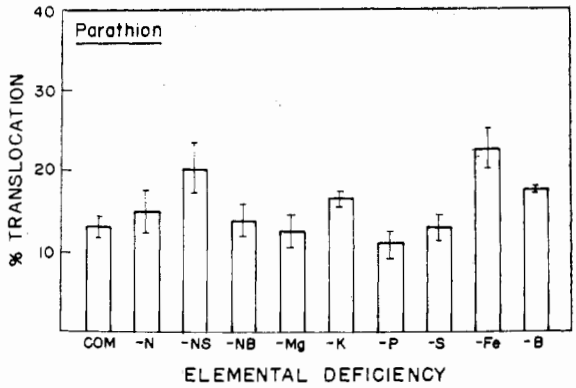

Figure 2. Translocation percentages of absorbed parathion in bean plants grown in different element deficient nutrient solutions. Abbreviations used in this and following figures are: COM, complete nutrient media; N, nitrogen deficient media; NS, nitrogen–sulfur deficient media; NB, nitrogen–boron deficient media; Mg, magnesium deficient media; K, potassium deficient media; P, phosphorus deficient media; S, sulfur deficient media; Fe, iron deficient media; B, boron deficient media.

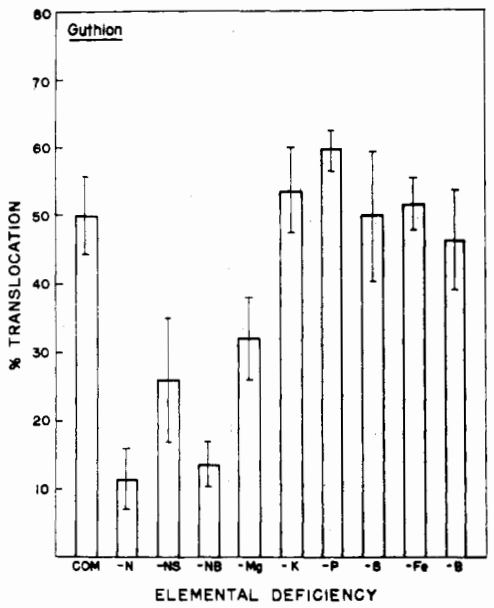

Figure 3. Translocation percentages of absorbed Guthion in bean plants grown in different element deficient nutrient solutions. For abbreviations, see caption to Figure 2.

In contrast, the varied effects of elemental deficiencies on the translocation of Guthion are more dramatic (Figure 3). Shoot residues of bean plants grown in Guthion-treated nutrient solutions deficient in nitrogen, nitrogen–sulfur, nitrogen–boron, and magnesium were statistically lower than shoot residues of plants similarly grown in a complete nutrient solution fortified with Guthion. The only statistically significant increase in Guthion translocation was attributed to the bean plants grown in a phosphorus-deficient nutrient solution.

Marked differences (lower shoot residues) were also noted in bean plants grown in nitrogen, nitrogen–sulfur, and nitrogen–boron deficient nutrient solutions fortified with thiophanate-methyl and MBC (Figures 4 and 5). Comparatively lower thiophanate-methyl residues were also observed in nutrient solutions deficient in magnesium, phosphorus, and sulfur, whereas similar nutrient deficient solutions fortified with MBC resulted in translocated residues similar to the complete MBC fortified nutrient solution. Statistically significant increases in MBC shoot residues resulted from nutrient solutions deficient in potassium, iron, and boron.

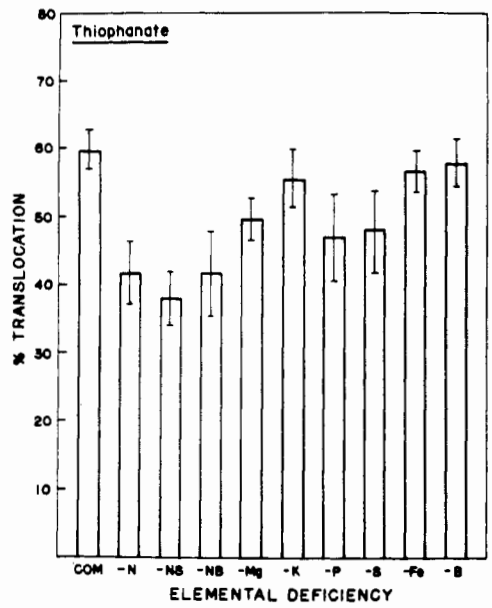

Figure 4. Translocation percentages of absorbed thiophanate-methyl in bean plants grown in different element deficient nutrient solutions. For abbreviations, see caption to Figure 2.

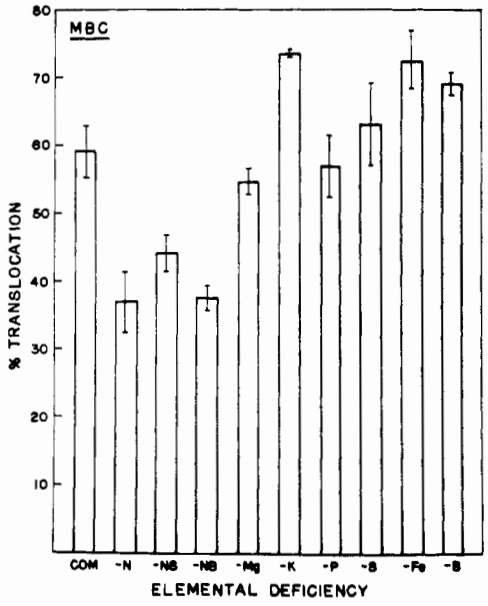

Figure 5. Translocation percentages of absorbed MBC in bean plants grown in different element deficient nutrient solutions. For abbreviations, see caption to Figure 2.

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