Uptake and Translocation of Guthion by Beans and Barley

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The uptake and translocation of [^{14}C]benzenoid-ring-labeled Guthion {*O,O*-dimethyl *S*-[4-oxo-1,2,3-benzotriazin-3(4*H*)-yl methyl]phosphorodithioate} insecticide in bean and barley plants were investigated. The labeled compound was monitored throughout the plants by X-ray autoradiography and the systemic residues were determined quantitatively by ^{14}C analysis. Structural confirmation of Guthion, which represented 98% of the total systemic residue, was accomplished by utilizing appropriate spectroscopic techniques.

Guthion {*O,O*-dimethyl *S*-[4-oxo-1,2,3-benzotriazin-3(4*H*)-yl methyl]phosphorodithioate} is a broad spectrum organophosphate insecticide which is used extensively on a wide variety of food crops. The increasing acceptance of Guthion appears to be concomitant to the reduced application of the chlorinated hydrocarbons (Li and Fleck, 1972). Although the toxic organophosphates are undoubtedly less persistent than the highly stable organochlorine pesticides, the rates of decomposition under noncatalyzed conditions are still relatively slow (Ruzicka *et al.*, 1967). Guthion, which has a general toxicity range similar to the parathions, is reportedly one of the most persistent of the many "nonsystemic" organic phosphate insecticides (Chemagro, 1971).

The progressive use, toxicity, and general stability of Guthion has justified a broader investigation into the environmental fate of this compound. The present paper describes the heretofore unreported systemic behavior of Guthion in barley (monocotyledon) and bean (dicotyledon) plants.

REAGENTS AND APPARATUS

Chemicals. [^{14}C]Benzenoid-ring-labeled Guthion (specific activity 1.0 μCi/m*M*) was synthesized in this laboratory (White *et al.*, 1972). Reagent grade chemicals and double-distilled solvents were used throughout this investigation.

Instrumentation. The Polytron, a high specific intensity ultrasonic generator (Type PT3500, Brinkmann Instruments, Inc., Westbury, N. Y.) equipped with a sawtooth cutting head, was used to extract the labeled compounds from the plant samples. Infrared spectra were obtained from potassium bromide disks, utilizing a Perkin-Elmer Model 337 spectrophotometer. Mass spectra were acquired through the use of a Finnigan Model 3000 Gas Chromatograph/Peak Identifier. The radioactivity (^{14}C) on thin-layer plates was located by a Model LB 2722 Varian Radio Scanner and measured in a Model 2425 Packard Tri-Carb liquid scintillation spectrometer. The scintillator fluid was composed of 15 g of PPO, 2 l. of toluene, and 1 l. of ethyleneglycol monomethyl ether. A Varian gas chromatograph Model 1200 equipped with an alkali flame ionization detector (rubidium sulfate) and a 2 ft × 1/8 in. i.d. glass column packed with 5% Apiezon L on 80/100 mesh Chromosorb Q was used. Temperatures were 232° for the column, 205° for the injector, and 260° for the detector. Gas flow rates were 19 ml/min for the carrier gas nitrogen, 40 ml/min for hydrogen, and 230 ml/min for compressed air.

Thin-Layer Chromatograms. The precoated glass plates (Silica gel with fluorescent indicator F-254) used in this study were purchased from Brinkmann Instruments, Inc., Westbury, N. Y.

PROCEDURE

Propagation of Plants. Bean seeds (*Phaseolus vulgaris* L.) and barley seeds (*Hordeum vulgare* L.) were surface-sterilized by a 10-min immersion into a 0.5% sodium hypochlorite solution. The seeds were germinated and grown in vermiculite under 750 ft-candles of fluorescent light (Plant-Aid, KEN-RAD) providing a timed 14-hr photoperiod/24 hr at 27 ± 3° and 50 ± 5% relative humidity.

Incorporation of [^{14}C]Guthion. Forty 1-week-old and 80 1-week-old barley plants were selected for root absorption and translocation studies. Single bean plants were transplanted into foil-wrapped 125-ml Erlenmeyer flasks containing 100 ml of half-strength Hoagland solution (Hoagland and Arnon, 1950). Barley plants were transplanted similarly, except in pairs. Extra bean and barley plants were transplanted into nutrient solutions and utilized as controls for the treated plants; 8 ppm of [^{14}C]Guthion (0.4 μCi/40 ml of ethanol) was added to each flask marked for treatment. Five replicate samples of each plant were taken at 24-hr intervals throughout a time period of 8 days. The plants were removed from the treated media and the roots were placed in ultrasonic cups with 100 ml of chloroform to remove the surface residues. The wash was added to the medium to extract the unabsorbed radioactivity. One treated sample of each plant from each time interval was selected for autoradiography according to Crafts and Yamaguchi (1964). The remaining four samples of each time period were sectioned into root and shoot segments, weighed, and analyzed separately for ^{14}C content.

Absorption and translocation studies of topically applied [^{14}C]Guthion were conducted on bean plants which were grown in half-strength Hoagland solution for a period of 3–4 weeks. Fresh nutrient was added weekly. Labeled Guthion (0.3 μCi in 95% ethanol) was applied topically to the stem at the upper surface of the center leaflet of the first or second trifoliate leaf. The treated plants were allowed to grow in the nutrient solution for an additional time period of 7 days prior to the autoradiography. For the seed treatment, 0.2 μCi of [^{14}C]Guthion was injected under the seed coat of soaked beans, which were then planted in vermiculite. The seedlings were used for autoradiography 2, 4, and 12 days after emergence from soil.

Mites Bioassay. The systemic toxicity of root-treated plants was determined from the terminal leaflet of the first and second trifoliate leaf 7 days after the treatment using the adult two-spotted spider mite (*Tetranychus urticae* Koch). The toxicity tests were done according to Yu and Morrison (1969) and were carried out in the environmental chamber at 80 ± 2 F° and 50–60% relative humidity under fluorescent light. The mortality counts were made after 96 hr and have been corrected by Abbott's

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(1925) formula by using the same type of fresh nutrient solutions as control treatments.

Analytical Methods. Ultra-rapid extraction of all labeled compounds from the plant tissues was accomplished by utilizing the ultrasonic Polytron. Each sample was diced and placed separately into a 125-ml Erlenmeyer flask, together with 75 ml of chloroform. With the Polytron generator immersed in the sample, the plant segment was extracted for 30 sec at half maximum power. The mixture was filtered through anhydrous sodium sulfate and the resultant filtrate was evaporated to dryness on a rotary vacuum evaporator. The residue was redissolved in 5 ml of chloroform from which 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 in the media, plant extracts, and macerates was determined by combustion analysis (Krishna and Casida, 1966). The remaining plant extracts were combined and subjected to cleanup and analyses appropriate for structural elucidation, as described below.

A 25-μl aliquot of the concentrated extract was applied as a narrow band approximately 3 cm from the bottom of a commercially prepared silica gel thin-layer plate. A reference standard of [^{14}C]Guthion (10 μg) was spotted near the terminal end of the applied band, and the chromatogram was developed in a solvent system containing a 5:1 mixture of chloroform–acetone (Schulz *et al.*, 1970). Major constituents were located under ultraviolet light (2537 A). Labeled areas were mapped by using a scanning radioisotope counter or by exposing the developed thin-layer plate to a sheet of X-ray film (Kodak No. screen) for a period of 2 weeks. The band having the same R_f as the Guthion standard was extracted from the plate with a vacuum-assisted spot collector (Brinkmann Instruments, Inc., Westbury, N. Y.) and the compound was eluted from the support with chloroform. The eluted compound was then subjected to gas chromatography, infrared, and gc/ms analyses.

RESULTS AND DISCUSSION

The systemic property of Guthion is clearly illustrated in the autoradiographs in Figure 1. Figure 1 shows specie representatives of hydroponically grown plants in which each plant was subjected to a Guthion-fortified nutrient solution (0.4 μCi of Guthion/100 ml of nutrient) for a period of 7 days. The distribution of radioactivity in the plant extracts is shown in Figure 2. The assimilation of Guthion by the roots and the translocation of the labeled compound into the aerial parts of both plant species were most rapid during the first 24 hr of exposure. After 1 day, however, the general increasing rate of Guthion accumulation slackened markedly, with both sets of shoots reaching an unexplainable low fluctuation of 11 cpm/mg after 5 days. The incorporation of radioactivity (cpm/mg) into the roots of both plant species during this experiment always exceeded the accumulation of radioactivity (cpm/mg) in the plant shoots. However, precise interpretation of root data is made complex by the fact that adsorption contributes, as well as absorption, to the total root activity. The degree to which Guthion was translocated, relative to the total amount of Guthion adsorbed and absorbed by the roots of both species, is illustrated in Figure 3.

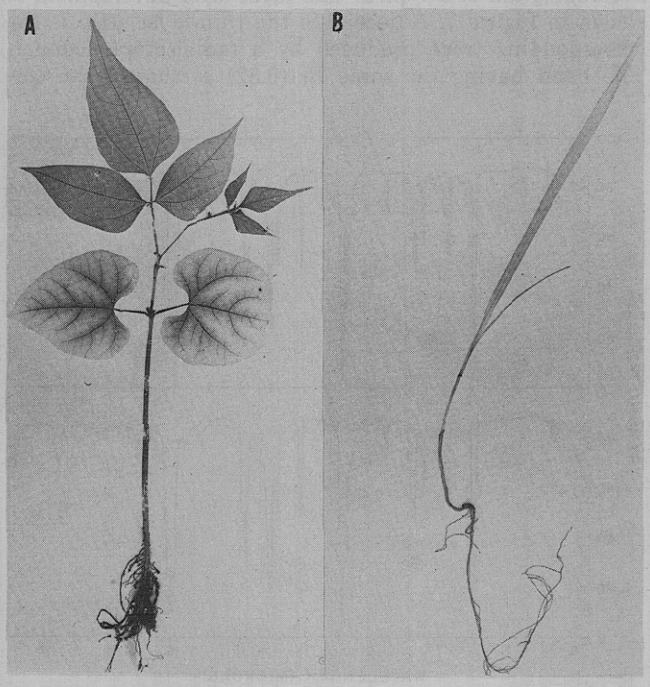

Figure 1. Autoradiograms of bean plant (A) and barley plant (B) grown for 7 days in [^{14}C]Guthion containing nutrient solution.

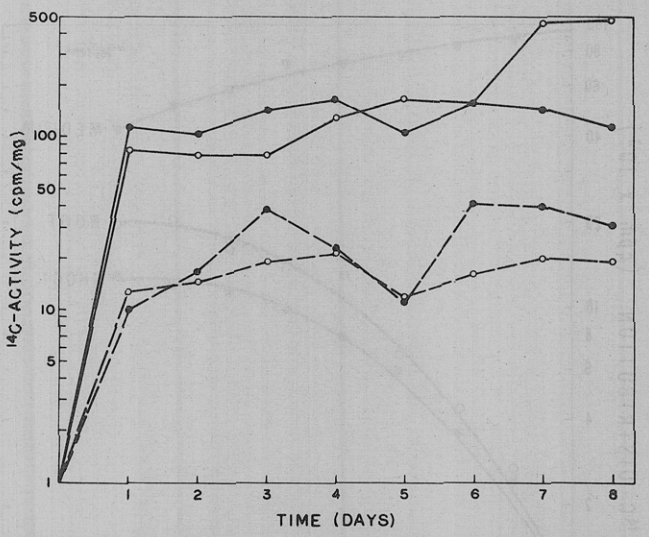

Figure 2. Uptake of ^{14}C from nutrient solution containing [^{14}C]Guthion by beans (●——●, root; O- - - -O, shoot) and barley (O——O, root, ●- - - -●, shoot).

Since it appeared to be clear that beans can absorb and translocate Guthion, attempts were made to determine the approximate amount of Guthion absorbed by bean plants grown in a solution containing 0.4 μCi of [^{14}C]Guthion. Figure 4 shows how rapidly Guthion was absorbed by bean roots and subsequently translocated into the shoots. Loss of Guthion in the medium was undoubtedly due to absorption by the plants. The overall average re-

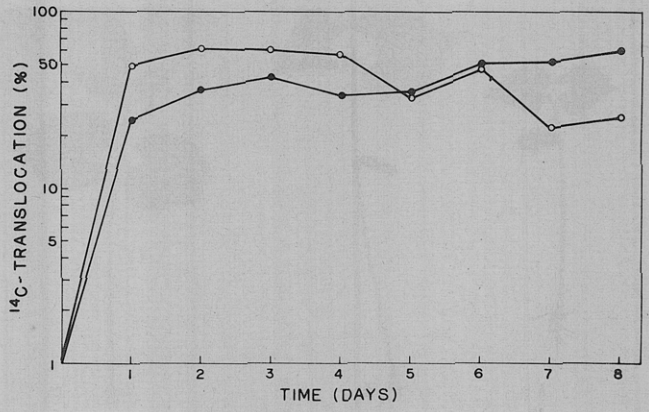

Figure 3. Percentage ^{14}C translocation with time by beans (O——O) and barley (●——●) grown in nutrient solution containing [^{14}C]Guthion.

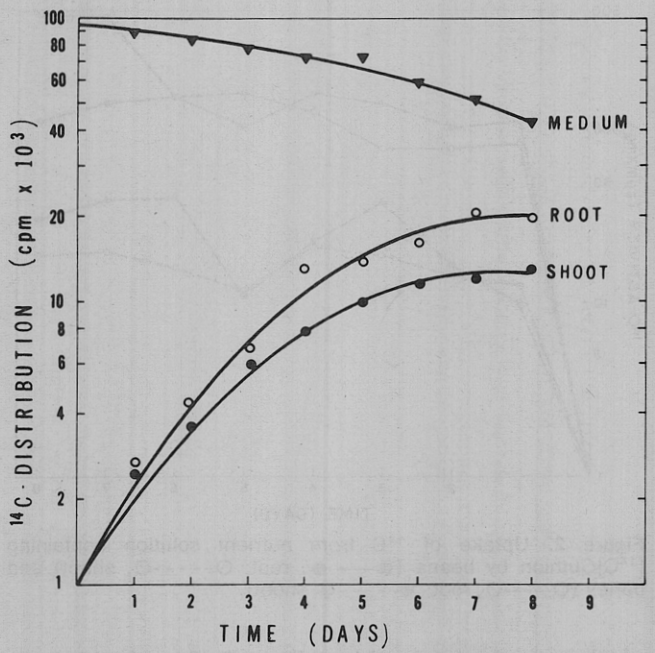

Figure 4. Absorption of [^{14}C]Guthion by 1-week-old bean plants. The medium contained approximately 8 ppm (0.4 μCi) of [^{14}C]Guthion when plants were transplanted.

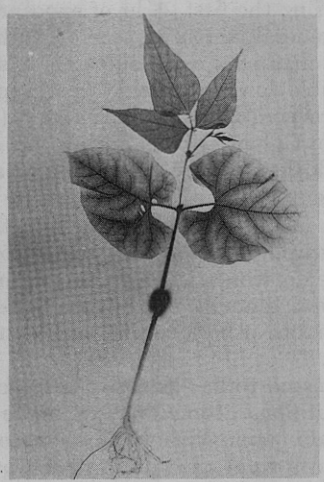

Figure 5. Autoradiogram of bean plant treated topically (stem) with [^{14}C]Guthion and grown for 7 days in nutrient solution.

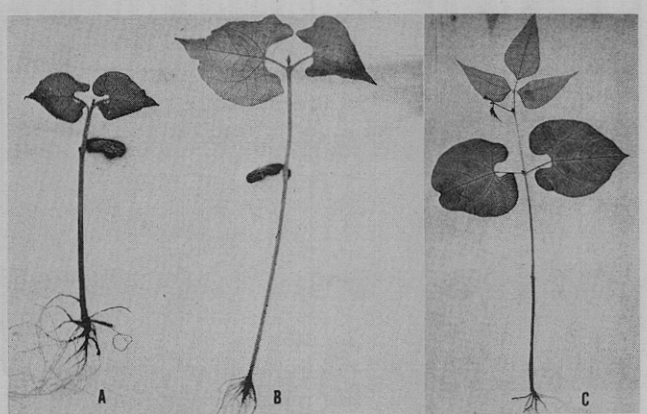

Figure 6. Autoradiogram of bean seedlings grown from [^{14}C]Guthion treated seeds. The plants were taken for autoradiography 2 (A), 4 (B), and 12 (C) days after soil emergence.

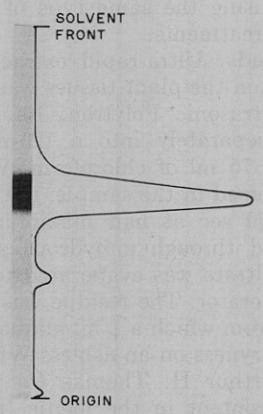

Figure 7. Autoradiogram of a thin-layer plate and the corresponding trace produced by a radioisotope scanner of chromatographed shoot extract.

covery of all applied radioactivity during this course of investigation was 99 ± 4%, and the average extraction efficiency of grown-in ^{14}C from bean plants was 90.9 ± 0.9% (9.1% remains in the macerate).

The systemic nature of Guthion is illustrated further in Figure 5. This autoradiogram clearly shows both an upward and downward movement of the topically applied (stem) Guthion in a bean plant. Obviously, the downward movement of the insecticide might be used to advantage in protecting the roots of the host plant against certain soil insects. Furthermore, the rapidity of Guthion absorption and mobility in bean plants was evidenced by injecting the ^{14}C-labeled pesticide under the seed coat. The autoradiogram in Figure 6 shows heavy labeling of all plant parts 2, 4, and 12 days after plant emergence from soil. The mites bioassay revealed that the mortalities of adult mites fed on the 1st and 2nd trifoliates were 28 ± 7 and 69 ± 11%, respectively. This finding was supported by the fact that the autoradiograms of the treated plants showed higher intensity of the tracer at the new growth.

Tentative identification of the major systemic compound Guthion was accomplished after cleanup of the plant shoot extract by thin-layer chromatography. The autoradiogram of a typical thin layer after development is shown in Figure 7. Adjacent to the thin-layer strip is the corresponding trace produced by a radioisotope scanner. The band having the same R_f (0.52) as that of the Gu-

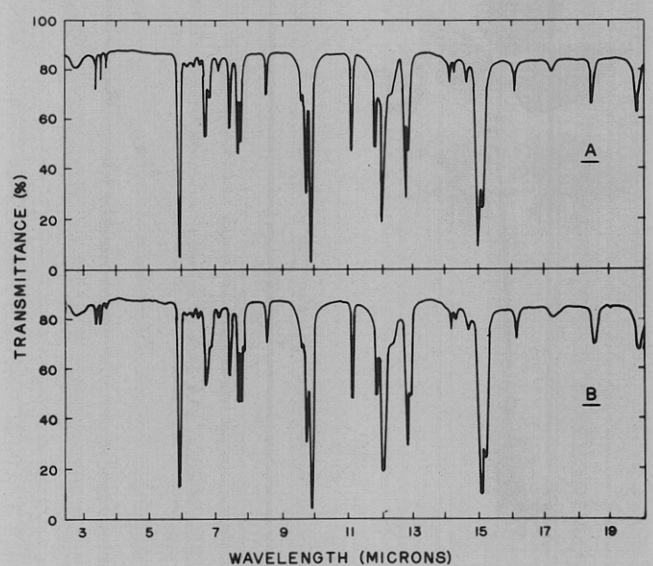

Figure 8. Infrared spectra of (A) [^{14}C]Guthion standard and (B) the major tlc band.

thion standard represents 97.7% of the total shoot radioactivity. The remaining two unidentified bands (R_f's 0.05 and 0.31) represent 0.7 and 1.6% of the total shoot radioactivity.

Injection of a sample of the major tlc band into the gas chromatograph described earlier produced a single peak having the same retention time (3 min) as that of a Guthion standard injected under the same conditions. Further chromatographic analyses involving the use of a gc/ms produced a mass spectrum of unit resolution similar to that reported by Damico (1966) for Guthion. Final proof of structure was apparent after comparing the infrared spectrum of the major labeled tlc band to the infrared spectrum of [^{14}C]Guthion standard (Figure 8). Guthion, therefore, may be classified as a systemic since it is readily absorbed through roots and foliage of the plants and is translocated intact without harm throughout the plant system, thus rendering untreated areas insecticidal.

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Metabolism and Residues of Temik Aldicarb Pesticide in Cotton Foliage and Seed Under Field Conditions

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The fate of *S*-methyl-^{14}*C*-aldicarb pesticide [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime] was studied in field-grown cotton. Aldicarb sulfoxide [2-methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)-oxime], aldicarb sulfone [2-methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)-oxime] and water-soluble noncarbamate metabolites constituted the major portion of the residual ^{14}C materials in the foliage and seed. Pesticide residue analysis of samples from commercially treated fields qualitatively agreed with the carbamate residues found in the metabolism studies with [^{14}C]aldicarb. Residue methods are described for characterizing the aldicarb carbamate residues in cotton foliage and for determining the total toxic residue in cottonseed.

The metabolism of Temik aldicarb pesticide [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime] in the cotton plant is well documented. In a series of greenhouse experiments, the pathways of biotransformation of aldicarb in cotton plants were studied following uptake by excised leaves (Coppedge *et al.*, 1967; Metcalf *et al.*, 1966), injection into the petiole of intact leaves (Coppedge *et al.*, 1967), root uptake into young seedlings (Bartley *et al.*, 1970), stem application to large plants (Metcalf *et al.*, 1966; Ridgway *et al.*, 1968), and soil application (Bartley *et al.*, 1970; Coppedge *et al.*, 1967; Ridgway *et al.*, 1968). Under field conditions, the metabolic behavior was studied by treating individual leaves and stems of cotton plants (Bull, 1968). These reports have dealt with the individual aspects of the fate of aldicarb in cotton plants. The present study provides detailed analyses of the metabolites and residues resulting from soil application of the formulated compound under field conditions.

METHODS AND MATERIALS

Chemicals and Apparatus. Radiolabeled aldicarb (*S*-methyl-^{14}*C*, specific activity 5.85 mCi/mmol) as well as nonlabeled standards of aldicarb degradation products were prepared and authenticated according to previously described procedures (Bartley *et al.*, 1966; Durden *et al.*, 1970). The radiochemical purity of the [^{14}C]aldicarb sample was in excess of 98.5%, as determined by thin-layer chromatography, radioautography, and liquid scintillation counting. The impurities consisted largely of ^{14}C material(s) remaining at the origin of the thin-layer chromatogram. Nonlabeled standards and the abbreviations used in the present study are as follows: 2-methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)oxime (aldicarb sulfoxide), 2-methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)oxime (aldicarb sulfone), 2-methyl-2-(methylsulfinyl)propionaldehyde oxime (oxime sulfoxide), 2-methyl-2-(methylsulfonyl)propionaldehyde oxime (oxime sulfone), 2-methyl-2-(methylsulfinyl)propionitrile (nitrile sulfoxide), 2-methyl-2-(methylsulfonyl)-propionitrile (nitrile sulfone), 2-methyl-2-(methylsulfinyl)propanol (alcohol sulfoxide), 2-methyl-2-(methylsulfonyl)propanol (alcohol sulfone), 2-methyl-2-(methylsulfinyl)propionamide (amide sulfoxide), 2-methyl-2-(methylsulfinyl)propionic acid (acid sulfoxide), and 2-methyl-2-(methylsulfonyl)propionic acid (acid sulfone).

Radioactivity was determined with a Beckman LS-150 liquid scintillation spectrometer with its carbon-14 channel adjusted to count a nonquenched [^{14}C]hexadecane standard (Beckman Instruments, Fullerton, Calif.) at 90%

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