(1678 → 1673 cm^{-1}; 1620 → 1613 cm^{-1}), a covalent ring ν(NH) at 3220 cm^{-1}, and high frequency bands of 3400 and 3520 cm^{-1} (ring ν(NH)) of protonated-keto support the transition from keto to protonated-keto hydroxyatrazine at more acidic pH values.

The relationships reported herein between functional groups and infrared bands in the atrazine–hydroxyatrazine systems may be used to study the influence of colloids, cation saturation, temperature, pH, and moisture content on the hydrolysis of chloro-*s*-triazines. Infrared spectroscopy would also assist in adsorption–desorption–protonation experiments of chloro-*s*-triazines in aquatic and soil systems.

ACKNOWLEDGMENT

Appreciation is expressed to H. M. LeBaron and Ciba-Geigy Corporation for technical chemicals.

LITERATURE CITED

Armstrong, D. E., Chesters, G., Harris, R. F., *Soil Sci. Soc. Am. Proc.* **31**, 66 (1967).
Bellamy, L. J., "The Infrared Spectra of Complex Molecules", Wiley, New York, N.Y., 1958, 425 pp.
Chen, J. Y. T., *J. Assoc. Off. Anal. Chem.* **50**, 595 (1967).
Good, N. E., *Plant Physiol.* **36**, 788 (1961).
Harris, C. I., *J. Agric. Food Chem.* **15**, 157 (1967).
Herbicide Handbook of the Weed Science Society of America, 3rd ed, 1974, pp 29–35.
McCormick, L. L., Hiltbold, A. E., *Weeds* **14**, 77 (1966).
Pimemtel, G. C., McClellan, A. L., "The Hydrogen Bond", W. H. Freeman and Co., San Francisco, Calif., 1960, 475 pp.
Russell, J. D., Cruz, M., White, J. L., Bailey, G. W., Payne, W. R., Jr., Pope, J. D., Jr., Teasley, J. K., *Science* **160**, 1340 (1968).
Skipper, H. D., Gilmour, C. M., Furtick, W. R., *Soil Sci. Soc. Am. Proc.* **31**, 653 (1967).
Skipper, H. D., Volk, V. V., *Weed Sci.* **20**, 344 (1972).

Received for review May 27, 1975. Accepted October 10, 1975. Work supported by Public Health Service Grants 5 P01 00040 and 5T01 ES 00055, and the Oregon Agricultural Experiment Station. Technical Paper No. 3758.

Behavior and Fate of Ethylenethiourea in Plants

Robert E. Hoagland*[1] and D. Stuart Frear

When young seedlings and excised leaves of corn, lettuce, pepper, and tomato were pulse-treated with [4,5-^{14}C]ethylenethiourea (ETU), the labeled ETU was readily absorbed by either the roots or the petioles of excised leaves and was translocated primarily via the xylem. After 20 days, only 1 to 2% of the initial dose remained as [^{14}C]ETU, but several degradation and/or metabolic products were present in the methanol-soluble extracts, and part of the ^{14}C was found in the methanol-insoluble residue. Methanol-soluble and -insoluble degradation products were determined in the various tissue sections for a period of 20 days after treatment. Only minor amounts of $^{14}CO_2$ were obtained from treated plants or excised leaves, but a major degradation product was isolated from pepper plants and was tentatively identified by infrared and mass spectroscopy as ethyleneurea (EU), the oxygen analog of ETU.

Ethylenebis(dithiocarbamate) fungicides are used extensively on a number of food crops to control plant pathogenic fungi. These compounds are subject to decomposition, and they yield ethylenethiourea (ETU) as one of the degradation products. Recent reports indicate that ETU is carcinogenic and goitrogenic (Graham and Hansen, 1972; Graham et al., 1973; Innes et al., 1969; Ulland et al., 1972). Analysis of commercial formulations of these fungicides showed that a considerable quantity of ETU was present as a degradation product and an impurity (Bontoyan et al., 1972; Czegledi-Janko and Hollo, 1967; Fishbein and Fawkes, 1965; Lopatecki and Newton, 1952; Ludwig et al., 1954; Petrosini et al., 1963). However, information about the fate of ETU in plants is conflicting, and it has not been clearly established whether ETU is accumulated and persistent (Ross and Ludwig, 1957; Vonk and Sijpesteijn, 1970) or rapidly degraded (Yip et al., 1971).

Because of concern about possible ETU residues in food crops, investigations were undertaken to obtain additional information about the behavior and fate of ETU in plants. Areas studied were ETU uptake and movement, ETU persistence, ETU degradation and/or metabolism, and the isolation and identification of major products of ETU in several mono- and dicotyledonous plants. The results are summarized in this report.

Metabolism and Radiation Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Fargo, North Dakota 58102.

[1]Present address: Southern Weed Science Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Stoneville, Mississippi 38776.

EXPERIMENTAL SECTION

Chemicals. [4,5-^{14}C]ETU (4.9 mCi/mmol) was purchased from Mallinckrodt (St. Louis, Mo.). Preparative TLC was used to remove minor radioactive impurities from the material before it was used for stock solutions. The nonradioactive ETU and EU used as reference compounds were obtained from Pfaltz and Bauer Chemical, Inc. (Flushing, N.Y.) and recrystallized before use. ETU was recrystallized twice from 95% ethanol (mp 202–203°C), and EU was recrystallized twice from chloroform (mp 130–131°C).

Plant Material. Seeds of bean (*Phaseolus vulgaris* L. var. Black Valentine), corn (*Zea mays* L. var. Northrup King, PX 448), lettuce (*Lactuca sativa* L. var. Great Lakes 659), pepper (*Capsicum frutescens* L. var. Early Calwonder), and tomato (*Lycopersicon esculentum* Mull. var. Sheyenne) were germinated in vermiculite saturated with one-third strength Hoagland's solution. After 15 to 21 days of growth, the seedlings were transplanted into individual containers filled with vermiculite and grown to maturity under greenhouse conditions. Mature plants were used as the source of excised leaves; the fruits of bean, pepper,

and tomato were used for direct injection of labeled ETU. Also, 3-week-old seedlings (grown as above) were used in root-absorption experiments with [^{14}C]ETU. Seedlings were pulse-treated for 3–4 hr with nonaerated distilled water solutions of labeled ETU. [For pulse treating, roots were immersed in [^{14}C]ETU solution for a short time period (as opposed to long term or continuous exposure to labeled compound) and then seedlings were transferred to nutrient solution.] The roots were rinsed with distilled water, and the seedlings were placed in jars with aerated one-third strength Hoagland's solution. These plants were grown in an environmental growth chamber with a relative humidity of 55%, a 12-hr photoperiod (26°C), and a 12-hr nyctoperiod (22°C).

Treatment of Plant Tissue with [^{14}C]ETU. Leaves of mature plants were excised by cutting the petioles under distilled water. The petiole of each leaf was then placed in a small test tube containing 0.5–1.0 ml of aqueous [^{14}C]ETU (17.7 μg) for pulse treatment. The tubes were wrapped with aluminum foil to prevent possible photodecomposition of [^{14}C]ETU during absorption, and distilled water was added as needed. After 3–4 hr, the petioles were removed from the tubes, rinsed with distilled water, and placed in tubes of distilled water in an environmental growth chamber. The ^{14}C absorbed through the petiole of each leaf was determined by measuring the difference between the ^{14}C in the treating solution and the ^{14}C remaining in solution after rinsing the petioles with distilled water.

Young seedlings were pulse-treated in small test tubes wrapped with foil and allowed to absorb [^{14}C]ETU through their roots. Additional distilled water was added to each tube after most of the original radioactive solution was absorbed. The treated seedlings were removed from the radioactive solution after 2–4 hr, and their roots were rinsed with distilled water. The plants were then placed in jars with aerated one-third strength Hoagland's solution.

Immature tomatoes, peppers, and bean pods were injected on the plants with 0.85 μCi of [^{14}C]ETU (173 nmol, 17.7 μg) prepared in distilled water. The fruits were allowed to remain on the plants for various intervals (up to 20 days after injection) before they were harvested and analyzed. Corn, lettuce, pepper, and tomato seedlings were also injected (center stem section) with 0.85 μCi of ETU in distilled water; these plants were likewise harvested at intervals up to 20 days and cut into sections (root, center, and upper) for methanol extraction of tissues.

General Methods. Plant tissues were harvested, cut into small pieces, and homogenized at 0–10°C in an electric blender with 8–10 vol of methanol. Homogenates were filtered through a 10-μ Millipore Teflon filter. The plant residue on the filter was homogenized again with methanol and filtered. The methanol extracts were combined and concentrated with a rotary vacuum evaporator at 35°C. [^{14}C]ETU and its ^{14}C-labeled methanol-soluble degradation and/or metabolic products were quantitatively determined by liquid scintillation counting after extraction or separation by TLC. The methanol-insoluble residue was dried in a vacuum oven at 35°C, and the ^{14}C content was converted to $^{14}CO_2$ by an oxygen-flask combustion method (Oliverio et al., 1962). The $^{14}CO_2$ was quantitatively determined in a scintillation counter. The $^{14}CO_2$ evolved from treated plants or treated excised leaves was collected in a series of traps containing methyl Cellosolve–monoethanolamine (7:1, v/v) and quantitatively measured by liquid scintillation counting (Jeffay and Alvarez, 1961).

Thin-Layer Chromatography. Thin-layer chromatograms were developed to 15 cm on glass plates coated with a 500-μ layer of silica gel HF. Solvent systems routinely used were 1-propanol–water (85:15, v/v) and chloroform–1-butanol–methanol–water (100:5:1:0.5, v/v/v/v). Radioactive zones were detected by autoradiography or with a radiochromatogram scanner.

Isolation and Purification of ETU Metabolite. Two pepper plants were grown for 6 weeks in nutrient culture under greenhouse conditions until each plant had 6–8 leaves and a well-developed root system. The roots of these plants were washed with distilled water and then immersed in a small beaker containing 40 μCi of [^{14}C]ETU in 15 ml of distilled water. The beaker was wrapped with foil to prevent possible photodecomposition. After the ^{14}C-labeled solution was absorbed by the plants (~2–3 hr), the plants were transferred back to nutrient culture for 7 days. Only excised leaf and stem tissue was used for methanol extraction. The methanol was then removed under vacuum, and the remaining aqueous fraction was concentrated to a small volume and partitioned with chloroform and ether. The aqueous phase was concentrated to a small volume and chromatographed by TLC in benzene–95% ethanol (2:1, v/v). The radioactive zone, which cochromatographed with EU, was removed from the plate and eluted from the silica gel with methanol. The radioactive compound was further purified by TLC in chloroform–methanol–water (65:25:4, v/v/v). The radioactive zone was again removed from the silica gel and chromatographed at 22°C on a 1.2 × 25 cm DEAE column that had been previously equilibrated with 2 *M* Tris-HCl buffer (pH 8.0) and exhaustively washed with distilled water. The column was eluted with water. This step was effective in removing yellowish pigmentation. The ^{14}C peak from the DEAE column was concentrated and purified further by TLC in chloroform–ethanol–acetic acid (90:5:5, v/v/v). The separated ^{14}C zone was eluted, concentrated, and chromatographed on a 1.2 × 25 cm Sephadex G-10 column eluted with water. This purified [^{14}C]ETU product was analyzed by infrared and mass spectroscopy. Mass spectra were obtained with a Varian M-66 spectrometer equipped with a V-5500 console. The samples were introduced with a solid sample probe. Micro KBr pellets (1.5 mm) were prepared, and infrared spectra were recorded with an infrared spectrophotometer equipped with a beam condenser.

RESULTS AND DISCUSSION

Exposure of ETU on silica gel to near-ultraviolet irradiation (Cruickshank and Jarrow, 1973) showed that photodecomposition is an important factor in ETU degradation. Indeed, our preliminary studies showed that ETU was degraded despite very mild conditions. For example, a significant loss of [^{14}C]ETU resulted if TLC plates spotted with [^{14}C]ETU were exposed to normal laboratory temperature, light (natural and artificial), and humidity. When TLC plates spotted with [^{14}C]ETU were allowed to remain on the laboratory bench for 0, 4, 12, and 24 hr before they were developed in chloroform–1-butanol–methanol–water (100:5:1:0.5, v/v/v/v), the rate of ETU degradation was constant, and only 80% of the original [^{14}C]ETU remained after 24 hr. Unknown radioactive degradation products were detected at or near the sample origin. Total recovery of ^{14}C was 98–100%. Therefore, we minimized the [^{14}C]ETU decomposition by drying all TLC plates rapidly with a stream of cool air after spotting and developing them immediately. After development, a stream of cool air was again used to dry the plates rapidly.

Other precautions were also observed in an attempt to avoid the production of ETU decomposition products. For

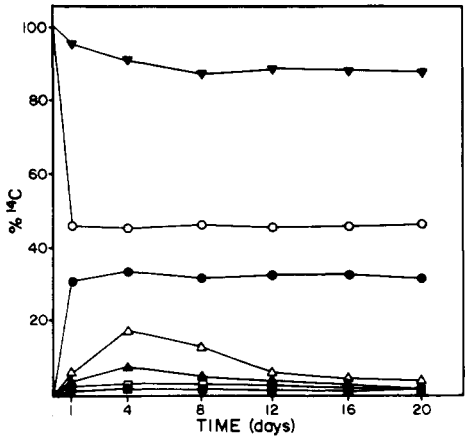

Figure 1. Distribution of ^{14}C in tomato seedlings injected with [^{14}C]ETU: total ^{14}C recovery (▼); center-soluble fraction (○); upper-soluble fraction (△); root-soluble fraction (□); center-insoluble fraction (●); upper-insoluble fraction (▲); root-insoluble fraction (■). The data represent the average of three replications. Variability of the values shown did not exceed ±6%.

example, all procedures of extraction, concentration, and chromatography were carried out at temperatures below 35°C. Also, plant tissues were treated with [^{14}C]ETU as rapidly as possible. This was achieved either by injection or by pulse treatment, instead of long-term or continuous treatment with aerated nutrient cultures or application of spray to the leaf surface. In addition, caution was exercised to eliminate possible ETU photodecomposition. Thus, all [^{14}C]ETU stock solutions were prepared in distilled water and stored at 4°C in containers wrapped with aluminum foil (solutions were stable for several months). When these precautions were taken, minimum decomposition of [^{14}C]ETU was observed.

ETU has been reported to be degraded when incubated with expressed sap from cucumber or tomato plants (Vonk and Sijpesteijn, 1970). In our injection studies with corn, lettuce, pepper, and tomato seedlings, [^{14}C]ETU was degraded, but was still present (20–30%) in these tissues after about 12 days. Movement of ^{14}C from the injection site was relatively low. Figure 1 shows the distribution of ^{14}C in the methanol-soluble and insoluble fractions from tomato tissue at various intervals after stem injection. No significant change is apparent in the total ^{14}C-labeled methanol-soluble content in the center stem section through the 20 days, which indicates that acropetal and basipetal movement of [^{14}C]ETU and ^{14}C-labeled products was very limited. After 12 days, about 28% of the methanol-soluble radioactivity in the center stem section was [^{14}C]ETU, and 30% of the total ^{14}C remained in the methanol-insoluble center stem section. Total ^{14}C recovery over the 20 days decreased slightly (92–85%). Similar results were obtained with injected corn, lettuce, and pepper seedlings.

The stability or persistence of [^{14}C]ETU injected into the fruits was also examined. Figure 2A shows the distribution of methanol-soluble ^{14}C in immature tomato, pepper, and bean fruits after injection. All three curves show a similar decrease in ^{14}C-labeled methanol-soluble material with time. Total recovery of ^{14}C (methanol soluble and methanol insoluble) was initially high, and it decreased only slightly in each tissue, over the 20-day period (96–92%). This indicates that [^{14}C]ETU and its labeled degradation products did not move from the injected fruits into other plant tissues. Figure 2B shows the rate of degradation of [^{14}C]ETU in these fruits. The rate was highest in bean pod tissue, but it was significant in

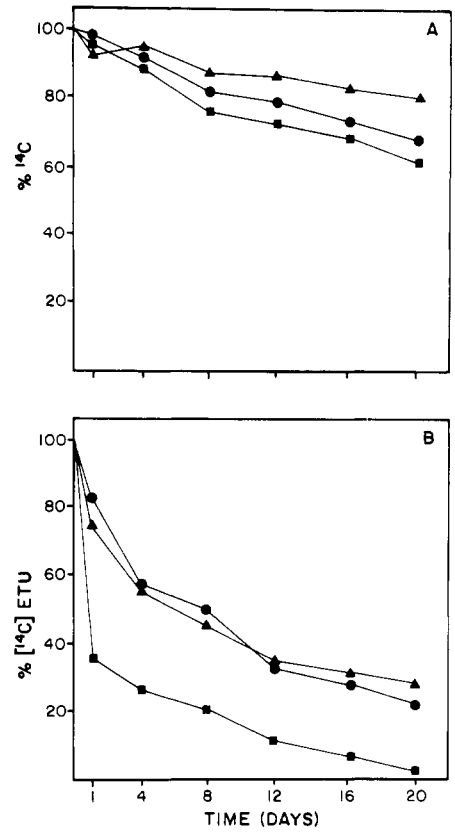

Figure 2. (A) Methanol-soluble ^{14}C in fruits injected with [^{14}C]ETU: tomato (●); bean (■); and pepper (▲); (B) [^{14}C]ETU degradation in injected fruits; [^{14}C]ETU is given as percentage of the initial dose: tomato (●); pepper (▲); and bean (■). The data represent the average of three replications. Variability of the values shown did not exceed ±4%.

all fruit. After 20 days, [^{14}C]ETU was present in a range of 3–28% of the initial dose. Although injection is not a normal route of entry of a chemical into plant tissue, these experiments are useful for comparative purposes and for giving some indication of the persistence of a chemical under in vivo conditions.

Since root absorption was considered a more normal route of ETU entry into plants under field conditions, the roots of young corn, lettuce, tomato, and pepper seedlings were pulse-treated with [^{14}C]ETU. [^{14}C]ETU was rapidly absorbed by the roots and translocated to the foliar tissues. Similar absorption and translocation of ETU in plants were reported by other workers (Ross and Ludwig, 1957; Sato and Tamizawa, 1960; Vonk, 1971; Vonk and Sijpesteijn, 1970). Figure 3A shows the distribution of ^{14}C in tomato seedlings for 20 days after pulse treatment: the total recovery decreased from 90 to 85% over the 20-day period; the methanol-soluble ^{14}C content in the leaf tissue (including stem) increased with time; and the leaf residue, root residue, and root soluble ^{14}C fractions increased only slightly or remained almost constant. Figure 3B shows the distribution in pepper seedlings: it was about the same as in tomato except that the methanol-soluble ^{14}C in the pepper leaves decreased considerably with time. Lettuce seedlings (Figure 3C) gave similar results, except that the methanol-soluble ^{14}C in the leaves decreased only slightly. Corn seedlings (Figure 3D) had a much higher content of ^{14}C in the root and a lower methanol-soluble ^{14}C content in the leaves compared with the other species. These differences among seedlings of these four species may indicate differences in metabolism, translocation, and extractability of [^{14}C]ETU and its ^{14}C-labeled degradation

Table I. $^{14}CO_2$ Evolution by [^{14}C]ETU-Treated Plants and Excised Leaves

Plant or leaf	Treatment	Initial dose, dpm	Trapping time, days	% of dose evolved
Lettuce seedling, 5 weeks old	Root	1194900	5	2.5
Pepper seedling, 4 weeks old	Root	1376100	5	0.9
Tomato seedling, 3 weeks old	Root	592900	5	1.1
Corn seedling, 3 weeks old	Root	805630	5	2.1
Tomato seedling, 3 weeks old	Injection	1909900	5	0.2
Tomato seedling, 3 weeks old	Injection	1909900	16	0.7
Tomato leaf, fr. mature plant	Petiole	1875200	3	0.1
Pepper leaf, fr. mature plant	Petiole	1899500	3	0.2

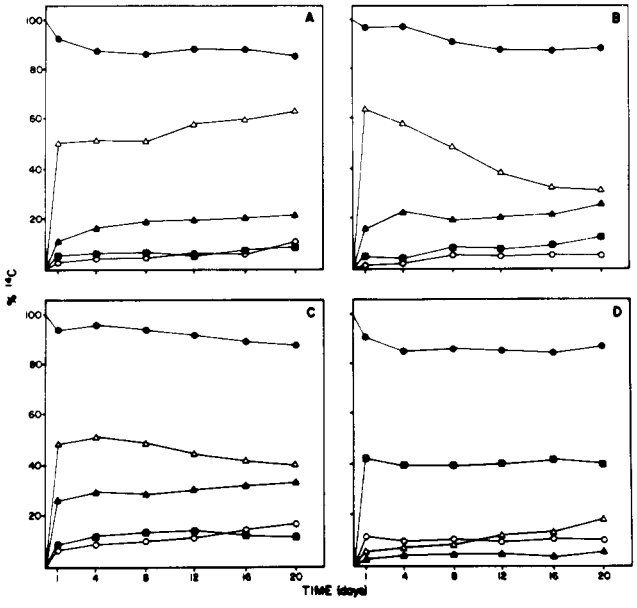

Figure 3. Distribution of ^{14}C in seedlings treated with [^{14}C]ETU: (A) tomato; (B) pepper; (C) lettuce; (D) corn; total recovery (•); leaf insoluble fraction (▲); root insoluble fraction (■); root soluble fraction (○); leaf soluble fraction (△). The data represent the average of three replications. Variability of the values shown did not exceed ±6%.

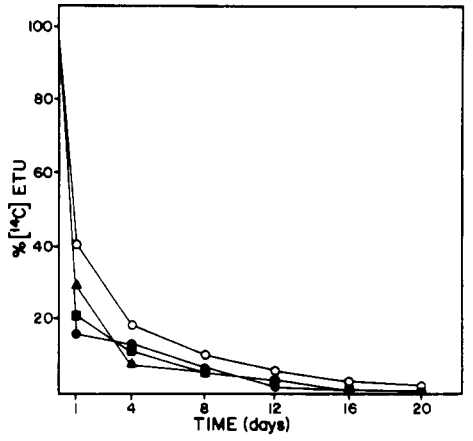

Figure 4. [^{14}C]ETU degradation in excised leaves. [^{14}C]-ETU is given as percentage of the total methanol-soluble ^{14}C: tomato (•); pepper (○); lettuce (▲); and corn (■). The data represent the average of three replications. Variability of the values shown did not exceed ±5%.

products. Certainly, the results appear quite complex, and interpretation is made more difficult because [^{14}C]ETU is transformed into several ^{14}C-labeled compounds by each of the plant species.

The relative amounts of ETU remaining in the tissues of excised leaves after pulse treatment of the petioles with [^{14}C]ETU are shown in Figure 4. ETU degraded very rapidly, and essentially none was detected after 20 days. Apparently, ETU degrades significantly faster in pulse-treated plants than in injected plants, perhaps because it is translocated more rapidly to active sites of degradation or metabolism when it is absorbed by the roots or through the cut surfaces of the petioles.

Autoradiography also indicated that absorption and translocation of [^{14}C]ETU occurred rapidly. For example, [^{14}C]ETU was absorbed through the cut surface of tomato petioles and translocated throughout the leaf within 4 hr after pulse treatment, and a movement of ^{14}C toward the leaf margins was visible in autoradiograms of excised tomato leaves 72 hr after treatment. Excised pepper leaves also absorbed and translocated ^{14}C throughout the leaf tissue 4 hr after treatment, and the concentration of ^{14}C increased near the leaf margin after 72 hr. A similar distribution of ^{14}C was observed when excised corn and lettuce leaves were pulse-treated with [^{14}C]ETU. Likewise, autoradiograms of root-treated pepper seedlings showed a considerable amount of ^{14}C present in the roots, stem, and leaves 8 hr after treatment. Leaves that developed up to 2 weeks after pulse treatment, however, contained only a small amount of ^{14}C. Therefore, translocation of [^{14}C]ETU and its degradation products and/or metabolic products was apparently limited to the xylem, which is consistent with the evidence that the ^{14}C was not readily translocated to newly developing tissues after pulse treatment.

Possible degradation of [^{14}C]ETU to $^{14}CO_2$ in the plants was also investigated by monitoring intact seedlings and excised leaves (from plants of various ages) for $^{14}CO_2$ after pulse treatment of the roots or petioles, or after stem injection with [^{14}C]ETU (Table I). Root-treated lettuce seedlings produced the largest amount of $^{14}CO_2$; however, the lettuce seedlings were the oldest seedlings tested and had a much larger leaf surface area than the other plants. Stem-injected tomato seedlings produced less $^{14}CO_2$ than root-treated tomato seedlings, probably because of the reduced translocation and degradation of [^{14}C]ETU in stem-injected plants. The amount of $^{14}CO_2$ evolved every 24 hr by [^{14}C]ETU root-treated lettuce and corn seedlings appeared to plateau at a relatively constant rate after 48 hr and remained at that level through the 5 days of the test. Tomato and pepper seedlings, however, showed a steady increase in production of $^{14}CO_2$ with time. The degradation of [^{14}C]ETU and its ^{14}C-labeled products to $^{14}CO_2$ is apparently a very slow and minor catabolic route in plants.

Autoradiograms of TLC plates indicated that several methanol-soluble [^{14}C]ETU products were present in all the plant tissues, and that at least four radioactive zones could be distinguished. The radiochromatogram and autoradiogram of a TLC plate in Figure 5 show the ^{14}C products in bean pod tissue 1 day after [^{14}C]ETU injection: [^{14}C]ETU was still a major component of the methanol-soluble extract. [^{14}C]ETU concentration then decreased with time up to 20 days after injection when the con-

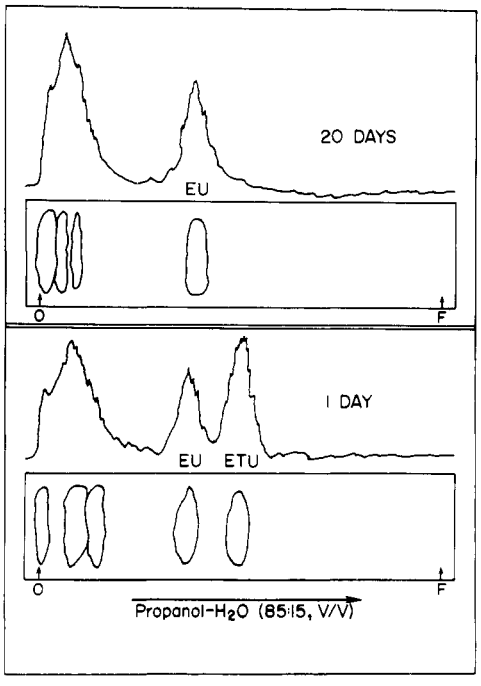

Figure 5. Autoradiograms and radiochromatogram scans of methanol-soluble ^{14}C-labeled products in bean pod tissue injected with [^{14}C]ETU. Thin-layer plates were spotted with approximately equal amounts of ^{14}C from methanol extracts. The results are typical of several tests.

centration was insignificant compared with other ^{14}C-labeled products.

A major [^{14}C]ETU degradation product found in all plant tissues cochromatographed with ethyleneurea (EU), and this product was isolated and purified from [^{14}C]-ETU-treated pepper plants, as previously stated. Mass spectra (70 eV) of the radioactive metabolite and of standard EU were characterized by a simple ion fragmentation pattern and a strong molecular ion, M = 86. The infrared spectra of the metabolite and the standard EU were very similar. However, two weak bands appeared at 1012 and 650 cm^{-1} in the spectrum of the metabolite that were absent in the standard EU spectrum. These extraneous absorption bands are probably due to substances eluted from the silica gel or to traces of solvent used in the isolation and purification procedure. EU has been identified as a photodecomposition product of ETU (Ross and Crosby, 1973), and soils have also been shown to convert ETU to EU (Kaufman and Fletcher, 1973). Another product of ETU degradation found in plants has been tentatively identified as 2-imidazoline (Vonk and Sijpesteijn, 1970).

It is not known whether the formation of [^{14}C]EU and other ^{14}C-labeled products is the result of enzymatic or chemical reactions in plants treated with [^{14}C]ETU. However, several chemical photosensitizers have been reported to catalyze the photolysis of ETU (Ross and Crosby, 1973; Cruickshank and Jarrow, 1973) and plants may also contain photosensitizer compounds that aid in this process. Various cyclic thioureas have also been reported to be oxidized by nonphotochemical reactions (Ware, 1950).

ETU is therefore readily absorbed and translocated by root-treated seedlings. It is also metabolized and/or degraded to at least four methanol-soluble components and incorporated into a methanol-insoluble residue fraction in several plant species. However, complete ETU degradation to CO_2 appears to be a slow and minor catabolic pathway in higher plants. The plant species tested can readily degrade ETU once it is absorbed and translocated in the plant.

ACKNOWLEDGMENT

The authors wish to thank H. R. Swanson for his advice and technical assistance during a portion of this work.

LITERATURE CITED

Bontoyan, W. R., Looker, J. D., Kaiser, T. E., Giang, P., Olive, B. M., *J. Assoc. Off. Anal. Chem.* **55**, 923 (1972).
Cruickshank, P. A., Jarrow, H. C., *J. Agric. Food Chem.* **21**, 333 (1973).
Czegledi-Janko, G., Hollo, J., *J. Chromatogr.* **31**, 89 (1967).
Fishbein, L., Fawkes, J., *J. Chromatogr.* **19**, 364 (1965).
Graham, S. L., Hansen, W. H., *Bull. Environ. Contam. Toxicol.* **7**, 19 (1972).
Graham, S. L., Hansen, W. H., Davis, K. J., Perry, C. H., *J. Agric. Food Chem.* **21**, 324 (1973).
Innes, J. R. M., Ulland, B. M., Valerio, M. G., Petrucelli, L., Hart, E. R., Pallotta, A. J., Bates, R. R., Falk, H. L., Gart, J. J., Klein, M., Mitchell, I., Peters, J., *J. Natl. Cancer Inst.* **42**, 1101 (1969).
Jeffay, H., Alvarez, J., *Anal. Chem.* **33**, 612 (1961).
Kaufman, D. D., Fletcher, C. L., presented at the 165th National Meeting of the American Chemical Society, Dallas, Tex., April 1973, PEST-1.
Lopatecki, L. E., Newton, W., *Can. J. Bot.* **30**, 131 (1952).
Ludwig, R. A., Thorn, G. D., Miller, D. M., *Can. J. Bot.* **32**, 48 (1954).
Oliverio, V. T., Denham, C., Davidson, J. D., *Anal. Chem.* **4**, 188 (1962).
Petrosini, G., Tafuri, F., Businelli, M., *Not. Mal. Piante* **65**, 9 (1963); *Chem. Abstr.* **59**, 8066 (1963).
Ross, R. D., Crosby, D. G., *J. Agric. Food Chem.* **21**, 335 (1973).
Ross, R. G., Ludwig, R. A., *Can. J. Bot.* **35**, 65 (1957).
Sato, T., Tamizawa, C., *Nogyo Gijutsu Kenkyusho Hokoku C* **12**, 181 (1960).
Ulland, B. M., Weisburger, J. H., Weisburger, E. K., Rice, J. M., Cypher, R., *J. Natl. Cancer Inst.* **49**, 583 (1972).
Vonk, J. W., *Meded. Fac. Landbouww., Rijksuniv Gent* **36**, 109 (1971).
Vonk, J. W., Sijpesteijn, K., *Ann. Appl. Biol.* **65**, 489 (1970).
Ware, E., *Chem. Rev.* **46**, 403 (1950).
Yip, G., Onley, J. H., Howard, S. F., *J. Assoc. Off. Anal. Chem.* **54**, 1373 (1971).

Received for review December 27, 1974. Accepted October 3, 1975. Presented in part at the 166th National Meeting of the American Chemical Society, Division of Pesticide Chemistry, Chicago, Ill., Aug 1973. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.