Residue Determination of Ethylenethiourea (2-Imidazolidinethione) from Tomato Foliage, Soil, and Water

Carlos H. Blazquez

A thin-layer chromatographic method was developed for the analysis of ethylenethiourea (ETU) (2-imidazolidinethione) from tomato foliage, soil, and water. High yields of ETU were obtained when heat was applied during the evaporation of dioxane–water suspensions of the fungicides ethylenethiuram monosulfide (ETM) and ethylenebisdithiocarbamate (Dithane M-45). Investigations indicated that no ETU was detected from Dithane M-45 applications on tomato foliage, soil surface, or ditch water. The tlc method had a sensitivity of 1 ppm, which is adequate in residue analysis experiments. ETU was detected as low as 1 ppm with the tlc method, a concentration adequate in field investigations of the degradation of ethylenebisdithiocarbamate fungicide residues and their biological activity. The tlc method coupled with a bioassay technique fit well into residue monitoring (RM) method of the EWDC system for more efficient timing of pesticide applications.

Concern with the continuous and frequent use of chemical pesticides has increased in recent years due to their predominance as pollutants in runoff waters from agricultural areas. In Florida, growers need to maintain a preventative cover of fungicides on their crops to arrest possible outbreak of disease which may lower the quality of the crop or completely destroy it. Present day recommended fungicide application schedules are based on trials carried out for a number of years under semi-controlled conditions at the various Agricultural Research Centers (ARC) of the University of Florida's Institute of Food and Agricultural Sciences.

At the ARC in Immokalee in southwest Florida, an early warning and disease control system (EWDC) has been developed for plant diseases disseminated by air. The system makes use of fungus spore traps for early disease detection, false color infrared photography for disease location, residue monitoring for more accurate timing of pesticide applications, and pesticide environmental degradation studies on cultivated and wild plants, soil, and water.

A thin-layer chromatography system (tlc) of fungicide residues and detection by a bioassay technique is part of the methodology of residue monitoring to determine concentration of foliar residues. Pesticide degradation studies on target and nontarget plants, soil, and water could reveal changes in the agricultural ecosystem that may necessitate modifications of agricultural practices. A number of specific analytical techniques have been used for detection of individual fungicides such as dyrene (2,3-dichloro-6-*O*-chloroanilino-*s*-triazine) and difolatan [*cis*-*N*-(1,1,2,2-tetrachloroethyl)thio-4-cyclohexene-1,2-dicarboximide] (Blazquez, 1971, 1972). The widespread use of maneb, maneb–zineb, and zineb fungicides in U.S. and Florida agriculture, which accounts for over 60% of all fungicides (Tweedy, 1972), necessitated the development of a rapid practical method for their detection and incorporation into the EWDC system. Tlc analysis for manganese and zinc salts of ethylenebisdithiocarbamic acid (Czegledi-Janko, 1967; Fishbein and Fawkes, 1965) was modified, and utilizing three solvent systems, it was possible to detect foliar residues as low as 10 ppm of maneb and 1 ppm of ethylenethiourea (ETU), a common breakdown product of the ethylenebisdithiocarbamate fungicides. The existence of ETU as a degradation product of nabam (disodium ethylenebisdithiocarbamate) under basic conditions has been known since 1954 (Ludwig *et al.*, 1954), and the possibilities of its homolog series have been tested for toxicant properties (Rich and Horsfall, 1954). It was not until 1969 that ETU was recognized (Innes *et al.*, 1969) as possibly having carcinogenic and tumorigenic properties.

Workers investigating the degradation of ETU (Allen *et al.*, 1955) found that the addition of hydrochloric acid and the application of heat converted the inner salt of the dithiocarbamic acid to the thiourea. ETU was found in sap from cucumber plants after treatment with labeled zineb (Sato and Tamizawa, 1960). Additional interest was created in 1971 by a description of the translocation of ETU in cucumber seedlings after 48 hr in a nabam solution (Vonk and Sijpesteijn, 1970). The translocation of ETU was confirmed in a later report (Vonk, 1971). It was found, however, that in an acid soil nabam does not form ETU but instead degrades into ethylenediamine (EDA) and carbon disulfide. A combination of tlc and gas chromatographic methods has been used to determine ETU in food samples at a recovery level of 0.02 to 10 ppm (Onley and Yip, 1971) and in field-sprayed lettuce and kale (Yip *et al.*, 1971). The purpose of this report is to describe a tlc method for the detection of ETU from foliar, soil, and water residues of maneb–zineb fungicides and to report that the tlc method coupled with a bioassay technique was successfully used as a part of a residue monitoring system for better timing of fungicide applications.

EXPERIMENTAL SECTION

Ethylenethiourea (ETU), ethylenethiuram monosulfide (ETM), ethylenediamine (EDA), the zinc and manganese salts of ethylenebisdithiocarbamic acid (Dithanes Z-78 and M-22, respectively), their combination product (Dithane M-22 Special), and their coordination product (Dithane M-45) were separated by thin-layer chromatography with three solvent systems and visualized with two different detection reagents. Dioxane (1,4-ethylene dioxide) was used both in extraction and chromatographic separations.

Solvent Systems. Solvent systems used were dioxane–formalin–acetic acid–water (3:1:1.5:1) (v/v) (4X), methylene chloride (MC), and chloroform–*n*-butyl alcohol–methanol–water (100:5:1:0.5) (CBMW).

Detection Reagents. Detection reagents used were iodine–starch, potassium ferricyanide–ferric chloride (1:1), and 1% aqueous solutions for both.

Preparation of Chromatoplates. A standard method of chromatoplate and microscope slide preparation was used

University of Florida, Agricultural Research Center, Immokalee, Florida 33934.

(Morley and Chiba, 1964). Silica Gel G was applied on glass plates (20 cm × 20 cm) and slides (2.5 cm × 7.6 cm) to a thickness of 280 μ. The plates and slides were not activated after air drying. Channels were cut into the silica layer following a channel technique (Purdy and Truter, 1962).

Development. A semiquantitative analysis of the spots developed by the ascending method was made by visualizing with both detection reagents (in duplicate plates) and compared visually to standards spotted on the same plate. In later experiments only the iodine fume–starch solution detection method was used. A measure of the quantitative accuracy of this method indicated an error of ±10% in the detection of ETU. The percent error for Dithane M-45 and ETM determinations was ±25 to 35%, while that for EDA was ±40 to 50%.

Foliage and Soil Applications. ETU, ETM, EDA, Dithane M-45, and Dithane M-45 plus 1 pt of Nu-Film 17 (B-pinene polymer) were applied to replicated plots (three replicates) of Homestead 24 tomato plants and on the surface of prepared soil beds at the rate of 0.91 kg per 378.5 l. of water (2 lb per 100 gal of water). Weekly applications were made to the tomato plants, while a single application was made to the soil beds and ditches. Leaf samples were harvested before application, 3 hr after application, and every 24 hr thereafter for 7 days. Water and soil samples were collected before application, 3 hr after application, and every 7 days for 28 days. All samples were immediately frozen until analyzed. Leaf and soil samples were washed with dioxane–water (1:1) for 1 hr in a horizontal shaker before filtering and evaporation with heat (water bath at 100° for 10 min) to less than 1 ml. Each sample was made up to 1 ml by rinsing the evaporating dish. Each 1-ml vial was then kept frozen until used. Water samples were not shaken, but were otherwise treated as the leaf and soil samples.

Water Suspensions. *Laboratory.* Three replicates of ETU, ETM, EDA, and Dithane M-45 were mixed in 1000 ml of deionized water in beakers and allowed to stand in the laboratory at an average temperature of 26° and 55% relative humidity.

Field. ETU, ETM, EDA, and Dithane M-45 were mixed in four separate ditches containing approximately 946 l. (250 gal) of water. Each compound was applied at the rate of 500 g except for EDA, which was applied at the rate of 500 ml in each respective ditch and thoroughly mixed.

Bioassays. Leaf disks from Homestead 24 tomato leaves were tested by a cellophane technique (Neely, 1968) with spore suspensions of the early blight fungus (*Alternaria solani* Weber) for the percent germination to indicate fungicidal activity of the sprayed ETU, ETM, and Dithane M-45 compounds.

RESULTS

***In Vitro* Experiments.** All the manebs, zinebs, and combinations moved from the origin in the 4X solvent. The undegraded compounds and ETM appeared as streaks at the higher concentrations (800 ppm), while ETU appeared as a distinct spot on the solvent front. EDA was the only exception, as it appeared as a spot at R_f 0.75. The range of detectability for the maneb and maneb–zineb parent compounds varied from 80 to 50 ppm. In the CBMW solvent system, EDA and the maneb–zineb undegraded compounds remained at the origin, while ETU moved to R_f 0.4 and ETM moved to R_f 0.8. In the MC solvent system, ETU moved slightly from the origin and ETM had an R_f of 0.25, whereas EDA and the undegraded maneb–zineb parent compounds remained at the origin.

High concentrations (800 ppm) of the maneb and zineb were needed on leaves for successful recoveries without the application of heat during the evaporation and concentration processes. In preliminary experiments it was found that the application of heat (water bath at 100° for 10 min) would degrade the maneb and zineb parent compounds largely to ETU and some ETM (depending on the compound) so that it was possible to consistently detect concentrations as low as 1 ppm of ETU on leaf washings from sprayed plants. Dithane M-45 yielded only ETU upon boiling, while zineb yielded ETU and ETM. A greater number of breakdown products were detected in the heat-treated M-45 than in the zineb.

***In Vivo* Experiments.** High concentrations (800 ppm) of ETU, ETM, and M-45 can be readily washed off leaves with either water, dioxane, or a water–dioxane combination (1:1). At lower concentrations (100 ppm and below) the water–dioxane combination gave the best recovery of the three compounds. In contrast to ETU and M-45, ETM was more easily detected in leaf washings concentrated without heat, although in general the recovery of ETM was not as good as that of the heat-concentrated ETU. No ETU was ever recovered from leaf washings from either ETM or Dithane M-45 that had not been subjected to heat treatment. ETU sprayed on tomato plants (at 0.91 kg per 378.5 l. or 2 lb per 100 gal) was easily recovered 3 hr after application (0 day); however, 2 days later the concentration had decreased considerably and none was detected 6 days after application (Table I). Although M-45 was detected at a higher concentration than ETM 3 hr after application, both compounds degraded at approximately the same rate and could still be detected 8 days after spraying. The concentrations of Dithane M-45 detected by the tlc method were in agreement with those obtained by the CS_2 evolution method (Cullen, 1964) of the Miller Chemical & Fertilizer Corporation laboratories in Hanover, Pa. (Table I). The addition of Nu-Film 17 (NF), a new type of sticker-extender, increased the initial deposition of Dithane M-45 residues and extended their duration past the average breakdown of the compound on the leaves (Table I).

ETU sprayed on the soil surface (Immokalee fine sand soil type) was easily detected by cold extraction 3 hr after application and did not degrade as rapidly as Dithane M-45 or ETM. It could still be detected at 68 ppm 13 days after application, but only a trace could be found after 27 days. M-45 was detected from 0 to 6 days after application, while a trace of ETM was detectable after 13 days. EDA was incompletely recovered shortly after application and could still be recovered after 13 days (Table II). No ETU was detected in either the ETM or M-45 soil extracts.

In a shaken deionized water solution, ETU gradually decomposed so that 21 days after it was dissolved in water, only 50% could be detected. ETM degraded at a faster rate, although it could still be detected at 5 ppm after 21 days. EDA quickly decomposed 1 day after appli-

Table I. Percent Gray Leaf Spot (*Stemphylium solani*) Disease and Residual Concentration of Fungicides in ppm (μg/g of Leaves) Remaining on Leaf Surface of Homestead Tomato Plants 8 Days after Spraying

Fungicide applied	Days after application					% disease +8 days
	0	1	3	6	8	
ETU	680	510	185	0	0	44 ab[a]
ETM	460	400	350	220	55	4 b
M-45[b]	540	450	195	50	30	10 b
M-45 + NF[b]	764	563	234	60	43	3 b
Check	0	0	0	0	0	45 a

[a] Numbers within a given column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at 1% level. [b] Analyzed by Miller Chemical Co. (CS_2 evolution).

Table II. Residual Concentration of Fungicides in ppm (μg/g of Soil) Remaining on the Soil Surface of a Plant Bed up to 27 Days after Spraying

Fungicide applied	Days after spraying				
	0	1	6	13	27
ETU	220	116	86	68	TR
ETM	80	60	40	TR	0
EDA	40	22	15	20	0
M-45	360	230	125	0	0

cation, remained fairly constant in concentration at 14 days, but could only be found as a trace at 21 days (Table III). M-45 degraded slowly in solution and was detected at 60% of the original concentration after 21 days. ETU was found 7 days after application in both the ETM and M-45 suspensions at the ratio of 5:1. None of the following were detected in suspension after 34 days: ETU, ETM, and Dithane M-45. In contrast to the above, when all four chemicals were applied to water in field ditches, most degraded rapidly within 1 day. Both ETU and EDA were not easily recovered, although low concentrations of ETU were found after 7 and 14 days. With the exception of ETU, none of the other three chemicals could be found 7 days after application (Table III). Traces of ETU were found on the EDA samples 1 and 7 days after application.

DISCUSSION

The results obtained with the tlc analytical method for the maneb, zineb, and their combination or coordination products were generally in agreement with those obtained by other workers working with standards (Czegledi-Janko, 1967; Fishbein and Fawkes, 1965).

The use of the dioxane–water (1:1) solvent followed by heat extraction greatly facilitated the analysis of leaf washings from tomato plants that had been sprayed with the test compounds ETU, ETM, and M-45.

The breakdown of Dithane M-45 by heat extraction (boiling in water bath at 100° for 10 min) had not been reported in the literature, although the application of heat to dithiocarbamic acid converted the inner salt to ETU, reported previously (Allen *et al.*, 1955). However, the reported reaction required the addition of hydrochloric acid for dithiocarbamic acid breakdown, while the Dithane M-45 breakdown was produced by heat alone.

ETU's lack of fungicidal activity, both in the field and in a bioassay test, confirmed findings by other workers (Rich and Horsfall, 1954; Sato and Tamizawa, 1960; Vonk, 1971). The low concentration of ETU on tomato foliage could possibly be accounted by its reported ability to be translocated from lower to upper leaves (Sato and Tamizawa, 1960; Vonk, 1971; Vonk, and Sijpesteijn, 1970).

In contrast, both Dithane M-45 and ETM degraded at a slower rate and indicated high fungicidal activities.

While some ETU was found in the *in vitro* suspension of ETM and Dithane M-45, none was found with the described system in foliar residues, soil samples, or water suspensions from field ditches as a degradation product of Dithane M-45 or ETM.

The absence of ETU from foliar residues suggests that either it may be produced in small amounts and be rapidly taken up by the plant, or that there may be a fungicide–plant tissue interaction bypassing ETU formation. It may be that the acidic nature of plant tissue can not support the breakdown reaction, which requires an alkaline environment as reported by other workers (Ludwig *et al.*, 1954; Vonk, 1971).

Both Dithane M-45 and ETM appeared to have degraded on the soil surface without forming ETU as a bioproduct, a previously reported phenomenon for nabam (Vonk, 1971).

Table III. Residual Concentration of Fungicides in ppm Remaining in Deionized and Ditch Water 21 Days after Application

Fungicide applied	Days after application				
	0	1	7	14	21
	Ionized water				
ETU	100	95	80	80	50
ETM	100	60	40[a]	40[a]	5[a]
EDA	100	45	40	40	TR
M-45	100	90	80[a]	70[a]	60[a]
	Ditches				
ETU	40	20	10	5	0
ETM	250	100	20	0	0
EDA	40	10[a]	TR[a]	0	0
M-45	250	140	51	0	0

[a] Ethylenethiourea present.

Traces of ETU found in the EDA ditch water could not be accounted for either by repeated sampling or possible contamination from ditches sprayed with ETU.

The tlc method used for ETU detection was satisfactory for field analysis of Dithane M-45 fungicide residues since the range of fungicidal activity for Dithane M-45 was found to be above the necessary 400-ppm concentration and well within the detection range of the tlc system. These results are in agreement with bioassay experiments with Dithane M-45 and other maneb–zineb compounds (Neely, 1968).

Under the conditions of these investigations, no ETU was formed on plants, soil surface, or ditch water from the application of Dithane M-45 or ETM sprays. The application of heat to extracts containing Dithane M-45 and ETM quickly degraded them mostly to ETU. The presence of Dithane M-45 residues on foliage could be best detected by heat extraction. The combination of the tlc method and the bioassay technique was successfully used to more accurately determine Dithane M-45 application schedules, indicating that residue monitoring can be a functional part of the early warning and disease control system.

LITERATURE CITED

Allen, C. F., Edens, C. W., Van Allan, J., *Org. Syn. Collect.* **8,** 394 (1955).
Blazquez, C. H., *Phytopathology* **61,** 885 (1971).
Blazquez, C. H., *Phytopathology* **62,** 11 (1972).
Cullen, T. E., *Anal. Chem.* **36,** 221 (1964).
Czegledi-Janko, G., *J. Chromatogr.* **31,** 89 (1967).
Fishbein, L., Fawkes, J., *J. Chromatogr.* **19,** 364 (1965).
Innes, J. R. M., Ulland, B. M., Valerio, M. G., Petrucelli, L., Fishbein, L., Hart, E. R., Pallotta, A. J., *J. Nat. Cancer Res. Inst.* **41,** 1101 (1969).
Ludwig, R. A., Thorn, G. D., Miller, D. M., *Can. J. Bot.* **32,** 48 (1954).
Morley, H. V., Chiba, M., *J. Ass. Offic. Anal. Chem.* **47,** 306 (1964).
Neely, D., *Phytopathology* **58,** 1061 (1968).
Onley, J., Yip, G., *J. Ass. Offic. Anal. Chem.* **54,** 165 (1971).
Purdy, S. J., Truter, E. V., *Chem. Ind.* **17,** 506 (1962).
Rich, S., Horsfall, J. G., *Science* **120,** 122 (1954).
Sato, T., Tamizawa, C., *Bull. Nat. Inst. Agr. Sci. Ser. C* **12,** 181 (1960).
Tweedy, B. G., Department of Plant Pathology, University of Missouri, Columbia, Mo., personal communication, 1972.
Vonk, J., *Ned. Fak. Land. Wetten. Gent.* **36** 109 (1971).
Vonk, J. W., Kaars Sijpesteijn, A., *Ann. Appl. Biol.* **65,** 489 (1970).
Yip, G., Onley, J., Howard, C., *J. Ass. Offic. Anat. Chem.* **54,** 1373 (1971).

Received for review October 16, 1972. Accepted January 29, 1973. Florida Agricultural Experiment Station Journal Series.
Presented at symposium on Origin and Fate of Ethylenethiourea Fungicides, 164th National Meeting of the American Chemical Society, New York, N. Y., August 1972.