Effect of Cysteine on the Stability of Ethylenethiourea and Ethylenebis(dithiocarbamate) in Crops during Storage and/or Analysis

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The stability of ethylenethiourea (ETU) in stored crops before analysis was studied. ETU stability in cucumber, Japanese pear, and potato was significantly less than in other crops examined, and ETU decreased with storage time. When L-cysteine monohydrochloride monohydrate (Cys-HCl) was used as an amendment, the degradation rate of ETU decreased. ETU degraded to 1.1% of initial concentration in Japanese pear stored for 100 days without Cys-HCl but degraded only to 81.5% when the samples were stored with Cys-HCl. Further, Cys-HCl also prevented degradation of ethylenebis(dithiocarbamate) (EBDC) to ETU during storage and analysis.

INTRODUCTION

It is desirable that residue analysis for pesticides in crops be conducted immediately after sampling. In most cases, however, samples must be stored at a low temperature (e.g., −20 °C) until analysis. The stability of pesticide residues during storage, therefore, becomes manifest. Photolysis may not be an important degradative reaction during storage since samples are usually stored in the dark at −20 °C. Pesticide degradation during storage results mainly from hydrolysis and oxidation (Egli, 1982). Oxidation, especially, is an important reaction for readily oxidizable thio compounds. Ethylenethiourea (ETU), which contains a thiocarbonyl group, is degraded from ethylenebis(dithiocarbamate) (EBDC) fungicides in crops (Rhodes, 1977; Ripley and Cox, 1978; Nash, 1976; Nash and Beall, 1980; Newsome et al., 1975), mice (Jordan and Neal, 1979), and aqueous media (Marshall, 1977) and by heat (Newsome, 1976; Lesage, 1980). ETU has teratogenic and tumorigenic properties in rats and mice (Khera, 1973; Teramoto et al., 1978) and may be toxic to man. Thus, development of a procedure to accurately measure the residue level of ETU at the time of sampling is desirable. During storage, ETU has been found to be unstable in certain crops (Uno et al., 1980) and tomato sauce and paste (Ankumah and Marshall, 1984). Our preliminary studies revealed also that a drastic decrease of ETU residue in cucumber and Japanese pear occurred during storage at −20 °C. In this study, the effect of an amendment, Cys-HCl, on the stability of ETU or EBDC in crops during storage and/or analysis was investigated.

MATERIALS AND METHODS

Chemicals. Ethylenethiourea (ETU) and ethyleneurea (EU) were obtained from Tokyo Kasei Kogyo Co., Ltd., and recrystallized from methanol. Mancozeb (an EBDC fungicide), L-cysteine monohydrochloride monohydrate (Cys-HCl), sodium ascorbate, and potassium fluoride (KF) were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). An Extrelut column (Art. 11738) was obtained from Merck (Darmstadt, FRG). Methanol and distilled water for HPLC were of HPLC grade, and other solvents were of analytical grade.

High-Performance Liquid Chromatography (HPLC). Analyses were carried out using a HPLC Shimadzu LC-6A equipped with a JASCO 870 UV–vis detector and Shimadzu C-R4A recording integrator. A Finepak SIL C_{18} column (250 × 4.6 mm i.d., 10 μm, JASCO), a flow rate of 1.0 mL/min, and mobile phases of water/methanol (95:5 v/v) for ETU and water/methanol (97:3 v/v) for EU were used. The detector was set at 240 nm for ETU and at 205 nm for EU.

Table I. Storage Stability of ETU in Cucumber and Japanese Pear at −20 °C

crop	cultivar	ETU fortifn, ppm	storage time, days	n[a]	ETU remaining, %
cucumber	Hokyoku	0.4	191	2	7.0 (8.1)[b]
	Megami	0.4	196	2	5.0 (5.8)
Japanese pear	Kousui	0.4	105	2	4.9 (5.3)
	Kousui	0.4	107	4	3.2 (3.4)

[a] n, number of samples analyzed. [b] Values in parentheses represent the percentage corrected for ETU recovery at zero time.

Table II. Effect of Amendments on ETU Recovery from ETU-Fortified Japanese Pear at Zero Time

amendment	amendment amount,[a] g	ETU recovery,[b] %
none		93.0 ± 3.7
Cys-HCl	1	94.2 ± 4.0[c]
Cys-HCl	2	95.7 ± 5.3[c]
sodium ascorbate	1	72.0 ± 14.3[c]
sodium ascorbate	2	74.3 ± 3.5[d]

[a] Amount of amendment to 50 g of Japanese pear fortified at an ETU level of 0.4 ppm. Concentrations of ETU in control Japanese pear: <0.01 ppm. [b] Mean ± SD of triplicates. [c] Not significantly different from values of samples without amendments. [d] Significantly different from values of samples without amendments and with Cys-HCl at $p < 0.01$.

Sample Preparation for Storage Stability Test. All samples were prepared for storage stability of ETU as follows: Vegetables and fruits were homogenized, and peanuts were milled. The samples (20–50 g) with and without 2 g of Cys-HCl were transferred into a 300-mL Erlenmeyer flask, fortified with ETU in methanol, and stored in the dark at −20 °C. After storage, the whole sample was analyzed to avoid nonuniform fortification.

Influence of pH on Stability of ETU. Since the pH of crops used in this study was in the range 3.4–6.8, pH for the stability test was kept at <7. Hydrochloric acid and sodium hydroxide were used to adjust solution pHs. pHs were adjusted to 2.0, 3.0, 6.0, and 7.0, respectively. Solutions were also amended with Cys-HCl aqueous solution (pH 0.74) and distilled water (pH 6.26). The several pH solutions were fortified with ETU and refluxed for 30 min at 120 °C. After refluxing, the fortified solutions were diluted with water to an appropriate volume and analyzed immediately.

Field Treatment by Mancozeb. Mancozeb (75 w/v %, WP) was diluted 400-fold with water and applied to Japanese summer orange and peanut in two and three replicate field plots,

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Table III. Recovery of ETU or EU from ETU- or EU-Fortified Crops with or without Cys-HCl as an Amendment at Zero Time

crop	fortifn, ppm ETU	fortifn, ppm EU	recovery of ETU or EU,[a] % without Cys-HCl	recovery of ETU or EU,[a] % with Cys-HCl[b]
cucumber	0.4		86.5 ± 9.6 (5)[c]	96.3 ± 6.5 (4)
		0.4	92.7 ± 8.4 (4)	93.0 ± 9.0 (4)
melon (flesh)	0.4		94.9 ± 2.6 (3)	95.4 ± 5.1 (3)
	2.0		96.0 ± 6.5 (3)	98.4 ± 3.3 (3)
potato	0.4		86.8 ± 7.8 (3)	93.0 ± 1.0 (3)
	2.0		101.7 ± 1.2 (3)	103.4 ± 0.6 (3)
		2.0	83.2 ± 5.7 (3)	85.9 ± 5.4 (3)
Japanese pear	0.4		93.0 ± 3.7 (3)	95.7 ± 5.3 (3)
		0.4	82.1 ± 4.5 (3)	78.5 ± 5.9 (4)
	2.0		97.0 ± 4.8 (3)	97.5 ± 8.3 (3)
peanut	0.4		93.4 (2)	99.2 (2)
Japanese summer orange (flesh)	0.4		86.8 ± 6.2 (3)	82.1 ± 4.5 (3)
Japanese summer orange (peel)	0.5		92.2 ± 6.1 (3)	91.6 ± 6.4 (3)
	1.0		100.7 ± 7.6 (3)	96.6 ± 2.0 (3)

[a] EU was determined using the ETU analytical method. Values are mean ± SD. [b] Amount of Cys-HCl: 2 g. [c] Values in parentheses represent the number of samples analyzed.

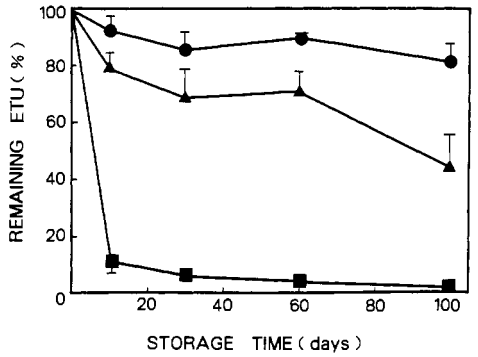

Figure 1. Effect of 2-g amendment on storage stability of ETU in 50 g of Japanese pear. Values are mean ± SD of triplicates and represent the percent ETU remaining with time. Error bars indicate standard deviations. ETU fortification: 0.4 ppm. (●) Cys-HCl; (▲) sodium ascorbate; (■) none.

respectively, at 4000 L ha^{-1}. Samples were collected 60 and 75 days for Japanese summer orange and 21 and 30 days for peanut, respectively, after the last application and then frozen immediately and stored at −20 °C.

Mancozeb Conversion to ETU during Analysis. A 0.2 or 2.0 ppm mancozeb solution (100 mL) was slowly evaporated in vacuo at 40 °C for 1 h. The residue was dissolved in water/methanol (95:5 v/v) for determination by HPLC.

A 20 ppm mancozeb solution (50 mL) was refluxed for 30 or 60 min at 120 °C. After heating, the solution was diluted to a suitable volume with water for HPLC analysis.

Analytical Methods. The ETU and EU extractions and assays were performed according to a modification of the method published by Kobayashi et al. (1986). Crop samples (20–50 g) were extracted by adding 15 g of KF and 150 mL of methanol/water (3:1 v/v). The mixture was shaken for 30 min at room temperature and filtered. The filtrate was concentrated to approximately 10 mL in vacuo at 40 °C. The concentrate was added to 100 mL of water and washed with 50 mL of hexane. The aqueous phase was reconcentrated. After basification (pH 8) of the concentrate with ammonium hydroxide, the extract was placed on an Extrelut column and ETU was eluted with 100 mL of dichloromethane. The eluate was evaporated to dryness on a rotary evaporator (water bath 40 °C), dissolved in 4 mL of water/methanol (95:5 v/v), and injected into the HPLC. By this method, the retention times of ETU and EU were 5.4 and 6.2 min, respectively, when the mobile phase was water/methanol (97:3 v/v). The limit of detection for ETU and EU was 5 ng.

Mancozeb was determined according to the conventional carbon disulfide (CS_2) evolution method (Goto and Kato, 1981).

Statistical Analyses. The data are presented as mean ± SD and analyzed either by Student's or Aspin Welch's *t*-test or by Mann Whiteney *U*-test (one-sided) for the effect of Cys-HCl on the storage stability of ETU and mancozeb.

RESULTS AND DISCUSSION

Analytical Procedure. ETU recovery from cucumber was only 40–50% by both methods previously described (Nitz et al., 1982; Kobayashi et al., 1986). This low-recovery problem was solved by the addition of KF to the sample before extraction with methanol/water (3:1 v/v). The recoveries of ETU from 0.4 ppm of ETU-fortified cucumber along with 10 and 15 g of KF were 79.8 ± 11.8% ($n = 4$) and 86.5 ± 9.6% ($n = 5$), respectively. Therefore, 15 g of KF was used in this study.

Storage Stability of ETU in Cucumber and Japanese Pear. As listed in Table I, ETU decreased to 3–8% of the initial concentration when ETU-fortified cucumber and Japanese pear were stored for 105–196 days at −20 °C. Webster and Reimer (1976) reported that several pesticides degraded during sample storage even at −20 °C. Stability of ETU in cucumber and Japanese pear did not differ between the crops or among their respective cultivars. These results indicated that ETU stability in crops during storage may be influenced by plant components.

Effect of Amendments on Recovery of ETU at Time Zero. Cys-HCl and sodium ascorbate were tested as amendments. ETU recovery was satisfactory (94–96%) when Japanese pear was amended with Cys-HCl (Table II). Sodium ascorbate reduced ETU recovery to <75%. Extensive extraction studies were conducted on several crops to determine ETU recovery after sample amendment with Cys-HCl. Both ETU and EU were fully recovered in the presence of Cys-HCl (Table III). On the basis of these results, 2 g of Cys-HCl/20–50 g of sample was used for the storage stability test.

Influence of pH on Stability of ETU. The pH values of cucumber, melon (flesh), potato, Japanese pear, peanut, Japanese summer orange (flesh), and Japanese summer orange (peel) used in this study were 6.23, 5.88, 6.05, 6.18, 6.70, 3.45, and 4.62, respectively. ETU storage stability was greatly reduced in Japanese pear and potato without Cys-HCl at 30 days compared to other samples, and likewise for cucumber and peanut stored for 200 and 204 days (Table IV). It was possibly caused by their higher

Table IV. Cys-HCl Effect on Storage Stability of ETU in Crops

crop/cultivar[a]	ETU fortifn, ppm	storage time, days	remaining ETU, % without[c,e]	remaining ETU, % with[d,e]	concn of EU,[b] ppm without	concn of EU,[b] ppm with
cucumber						
Megami	0.4	100	47.5 ± 6.7	90.3 ± 0.9[f]	0.023 ± 0.013 (6.8)	<0.005
Megami	0.4	200	15.4 ± 3.4	89.0 ± 1.1[g]	0.033[h] (9.8)	<0.005
melon (flesh)						
Andes	0.4	102	54.8 ± 2.3	95.4 ± 5.5[g]		
Andes	0.4	250	35.3 ± 5.3	81.1 ± 4.1[g]		
Andes	2.0	248	65.3 ± 3.3	88.8 ± 5.9[f]		
Amuse	2.0	275	68.0 ± 2.9	92.3 ± 3.0[g]		
potato						
Danshaku	0.4	102	6.9 ± 1.3	100.9 ± 2.8[g]		
Dejima	0.4	260	1.6 ± 0.5	98.8 ± 3.9[g]		
Danshaku	2.0	30	13.8 ± 0.5	94.9 ± 3.8[g]	0.010 ± 0.000 (0.6)	<0.005
Danshaku	2.0	60	5.7 ± 0.3		0.079 ± 0.013 (4.7)	
Danshaku	2.0	238	3.5 ± 1.0	95.4 ± 2.9[g]		
Dejima	2.0	252	5.2 ± 1.3	92.7 ± 3.8[g]		
Japanese pear						
Kousui	2.0	10	37.3 ± 2.2	98.9 ± 8.5[g]	0.126 ± 0.027 (7.5)	<0.005
Kousui	2.0	30	22.4 ± 3.0	96.2 ± 0.9[g]	0.135 ± 0.013 (8.0)	<0.005
peanut						
Nakateyutaka	0.4	204	42.0 ± 7.0	80.0 ± 1.2[g]		
Japanese summer orange (flesh)						
Natsudaidai	0.4	222	92.8 ± 3.3	96.7 ± 9.5		
Amanatsu	0.4	214	88.6 ± 2.7	97.9 ± 3.5[i]		
Japanese summer orange (peel)						
Natsudaidai	1.0	60	49.7 ± 4.2	86.9 ± 1.7[g,j]		
				56.3 ± 3.6[k,l]		
Natsudaidai	1.0	100	42.0 ± 5.6	81.9 ± 0.8[f,j]		
				50.5 ± 2.8[k,l]		
Natsudaidai	1.0	222	37.5 ± 2.7	89.4 ± 1.7[g]		
Amanatsu	1.0	214	30.7 ± 3.8	88.2 ± 2.3[g]		

[a] Samples were stored at −20 °C until analysis. [b] EU formed during storage. Values in parentheses represent the percentage of conversion of ETU to EU. [c] Samples were stored without Cys-HCl. [d] Cys-HCl (2 g) was added to the samples (20–50 g) immediately before storage. [e] Mean ± SD of triplicates. ETU values were corrected for recovery. [f,g,j] Significantly different from values of samples without Cys-HCl at $p < 0.01$, $p < 0.001$, and $p < 0.05$, respectively. [h] Mean of duplicates. ETU values were corrected for recovery. [i] Significantly different from values (k) of samples treated with Cys-HCl before analysis at $p < 0.001$. [k] Cys-HCl (2 g) was added to stored samples (20 g) immediately before analysis. [l] Not significantly different from values of samples without Cys-HCl.

pH values (>5.8). However, a few exceptions were found. ETU in stored Japanese summer orange (flesh) at pH 3.45 was stable, while ETU in the peel at pH 4.62 was fairly unstable. When Cys-HCl was added to the peel, the peel pH decreased to 1.89 and ETU was quantitatively recovered. These results demonstrated that ETU was stable in stored crops at low pH values.

To further elucidate the effect of pH on ETU in stored crops, the stability of ETU was studied in various pH solutions. These solutions containing 2 ppm ETU were refluxed for 30 min at 120 °C. The mean recoveries (n = 2) of ETU in solutions of pH 2, 3, 6, and 7, reagent blank (water; pH 6.26), and 10% Cys-HCl aqueous solution (pH 0.74) were 102, 106, 103, 103, 106, and 101%, respectively. Thus, ETU stability was independent of pH within the range 0.74–7.0. Ankumah and Marshall (1984) also reported that ETU was appreciably more stable in both phosphate and acetate buffers (pH 4.18) than in tomato products such as tomato sauce and paste; therefore, the pH alone was not a major factor in ETU decomposition.

Behavior of ETU in Japanese Pear Stored with or without Amendment. The behavior of ETU in Japanese pear during storage was examined with or without Cys-HCl and sodium ascorbate amendment. The results are presented in Figure 1. ETU decreased in Japanese pear drastically (1.1%) after 100 days of storage when no amendment was added, while 81.5 and 44.3% of ETU remained in samples containing Cys-HCl and sodium ascorbate, respectively. Thus, Cys-HCl was found to be more effective than sodium ascorbate for the storage stability of ETU in Japanese pear.

Cys-HCl Effect on ETU Storage Stability in Crops. Cys-HCl effect on ETU stability was also verified in various crops as listed in Table IV. All samples except Japanese summer orange (flesh) demonstrated that Cys-HCl increased the ETU stability. ETU in stored potato (13.8% after 30 days), cucumber (47.5% after 100 days), and Japanese pear (22.4% after 30 days) decreased without Cys-HCl amendment and formed very small amounts of EU. However, 90.3–96.2% of ETU was recovered when stored for 30 or 100 days after amendment with Cys-HCl, and EU was not detected.

Cys-HCl effect on ETU storage and analytical stability depended upon the stage Cys-HCl was added to process. When Cys-HCl was added to the stored Japanese summer orange (peel) immediately before analysis, ETU recovery was only 6–8% higher than that without Cys-HCl. For example, ETU recovery at 100 days of Japanese summer orange (peel) storage was 42.0% in the absence of Cys-HCl and 50.5% when Cys-HCl was added immediately before analysis. However, ETU recovery from samples stored for 100 days in the presence of Cys-HCl was 81.9% for Japanese summer orange (peel). Thus, ETU was significantly ($p < 0.01$–0.001) more stable in various crops during the entire period of storage when Cys-HCl was added to the samples immediately before storage.

ETU stability was different among plant species, but not among cultivars.

Cys-HCl Effect on Mancozeb Conversion to ETU. For the determination of ETU in mancozeb-treated crops, it is important to determine whether mancozeb residue in the crops is or is not converted to ETU during analysis. To clarify this problem, mancozeb with or without Cys-HCl was reacted under conditions (evaporation at 50 °C under vacuum) similar to those of ETU analysis. In addition, mancozeb was reacted at 120 °C at atmospheric

Table V. Cys-HCl Effect on Mancozeb Conversion to ETU

mancozeb, ppm	conditions	ETU, ppm without Cys-HCl[a]	ETU, ppm with Cys-HCl[a,b]
water			
0.2	50 °C, 1 h under vacuum	0.004 ± 0.001 (5.3)[c]	<0.002[d] (0)
2.0	50 °C, 1 h under vacuum	0.079 ± 0.010 (10.4)	0.007 ± 0.001[d] (0.9)
2.0	120 °C, 1 h reflux	0.613 ± 0.023 (80.7)	<0.002[d] (0)
20.0	120 °C, 30 min reflux	5.46 ± 0.25 (72.0)	<0.02[d] (0)
20.0	120 °C, 1 h reflux	5.58 ± 0.32 (73.6)	<0.02[d] (0)
20.0	120 °C, 2 h reflux	5.91 ± 0.34 (77.9)	<0.02[d] (0)
peanut			
2.0	analytical method of ETU	0.066 ± 0.016 (8.7)	0.005 ± 0.000[d] (0.7)
Japanese summer orange (peel)			
2.0	analytical method of ETU	0.030 ± 0.002 (4.0)	0.009 ± 0.001[d] (1.2)

[a] Mean ± SD of triplicates. [b] Amount of Cys-HCl: 2 g. [c] Values in parentheses represent the percentage of mancozeb conversion to ETU. [d] Significantly different from value of sample without Cys-HCl at $p < 0.05$ by Mann Whiteney U-test (one-sided).

Table VI. Cys-HCl Influence on Mancozeb Recovery from Mancozeb-Fortified or -Treated Sample

	concn of mancozeb, ppm without Cys-HCl[a]	concn of mancozeb, ppm with Cys-HCl[a,b]
water		
0.1 ppm of mancozeb added	0.085 (85.0)[c]	0.082 (82.0)
2.0 ppm of mancozeb added	1.84 ± 0.11 (92.0)	1.76 ± 0.10 (88.0)
Japanese summer orange (peel)		
blank	<0.05	<0.05
1.0 ppm of mancozeb added	0.83 ± 0.05 (83.0)	0.82 ■ 0.06 (82.0)
field treated[d]	12.95	12.85

[a] Mean ± SD of duplicates except for values for 1.0 and 2.0 ppm of mancozeb which are of triplicates. [b] Amount of Cys-HCl: 2 g. [c] Values in parentheses represent the percent recovery. [d] Number of field treatment × days after last treatment: 2 × 60. The samples were analyzed immediately after sampling.

Table VII. ETU Residues in Mancozeb-Treated Crops during Storage

number of field treatment × days after last treatment	storage time, days	concn of ETU, ppm no Cys-HCl	A[a]	B[b]
Japanese summer orange (peel)				
2 × 60	195	0.334 ± 0.075[c]	0.170 ± 0.010[c,f]	0.143 ± 0.021[d,f]
2 × 75	195	0.203 ± 0.038[d]	0.123 ± 0.012[c,g]	0.097 ± 0.006[c,g,h]
peanut				
3 × 21	195	0.01[e]		<0.01[e]
3 × 30	195	0.02[e]		<0.01[e]

[a] Two grams of Cys-HCl was added to 20 g of stored sample immediately before analysis. [b] Twenty grams of Cys-HCl was added to 200 g of sample immediately before being stored at −20 °C. An aliquot (20 g equivalent) of sample was analyzed. [c,d,e] Values are mean ± SD of triplicates, quadruplicates, and duplicates (mean only), respectively. [f,g] Significantly different from value of sample without Cys-HCl at $p < 0.01$ and $p < 0.05$, respectively. [h] Significantly different from value in column A at $p < 0.05$.

pressure to avoid drying that occurs during evaporation under vacuum; 50 °C under vacuum (20 mmHg) corresponds to approximately 120 °C at atmospheric pressure.

Mancozeb without Cys-HCl rapidly decomposed to ETU (Table V). The conversion percentages of 0.2 and 2.0 ppm of mancozeb were 5.3 and 10.4%, respectively, when Cys-HCl was not added to the mancozeb solution but less than 1% in the presence of Cys-HCl. The mancozeb standard solution (1000 ppm) used in this study contained 1.33 ± 0.14 ppm ($n = 4$) ETU as the contaminant; hence, ETU in a 2 ppm mancozeb solution is theoretically present at 0.0027 ppm. This indicated, therefore, that ETU was formed during reaction of mancozeb without Cys-HCl.

When an aqueous solution of mancozeb (20 ppm) was refluxed at 120 °C, 72–78% of mancozeb was converted to ETU in the absence of Cys-HCl. On the other hand, decomposition of mancozeb to ETU was <1% in the presence of Cys-HCl. Similar results were obtained in mancozeb-fortified peanut and Japanese summer orange, also. Therefore, in the absence of Cys-HCl, ETU was formed during analysis. These findings suggest that Cys-HCl drastically reduced the thermal degradation of mancozeb to ETU.

These results suggest that analysis of samples containing mancozeb and ETU residues should be amended with Cys-HCl before storage. In addition, even if the samples are analyzed immediately after sampling, the addition of Cys-HCl is necessary to prevent the degradation of mancozeb and ETU during analysis. Lesage (1980) reported that the formation of ETU by the thermal degradation of EBDC and the evolution of CS_2 in aqueous media were reduced by the addition of copper salts. These results demonstrate that Cys-HCl reduced both the degradation of ETU and the formation of ETU from mancozeb during analysis and storage.

Cys-HCl Influence on Mancozeb Recovery from Mancozeb-Fortified or -Treated Samples. Since Cys-HCl was an effective amendment to prevent the thermal degradation of mancozeb, the effect of Cys-HCl on mancozeb residue analysis was examined. When Cys-HCl-treated stored samples were analyzed for residues, it was confirmed that Cys-HCl had no influence on stabilizing mancozeb (Table VI).

ETU Residues in Mancozeb-Treated Crops during Storage. Degradation of mancozeb in crops can be estimated by the level of ETU formed. As shown in Table VII, ETU in Japanese summer orange at 60 days after mancozeb application plus 195 days storage at −20 °C resulted in 0.334 ppm of ETU. When Cys-HCl was added immediately before analysis of stored samples, ETU was

present at 0.17 ppm. When Cys-HCl was added before storage, ETU was detected at only 0.143 ppm. Thus, the ETU levels were significantly ($p < 0.01$) lower in field samples when Cys-HCl was added both before storage and before analysis, compared to samples without Cys-HCl. This indicated that ETU in the mancozeb-treated crops is formed mainly during analysis rather than during storage. Indeed, it has been shown that ETU in EBDC-treated crops increases as a result of heating (Watts et al., 1974; Newsome, 1976; Onley et al., 1977; Lesage, 1980). In conclusion, the addition of Cys-HCl retards residue decomposition during analysis of mancozeb-treated crops and permits a true estimate of ETU residue in the crops.

Apparent Role of Cys-HCl in Stabilizing ETU. ETU loss may be attributed to conversion to known compounds. Indeed, ETU was degraded to EU and other metabolites in plants and animals (Hoagland and Frear, 1976; Nash, 1976; Engst, 1977) and decomposed to EU in tomato sauce and paste stored at room temperature after industrial processing (Ankumah and Marshall, 1984). As given in Table IV, 0.6–9.8% of the ETU in cucumber, Japanese pear, and potato degraded to EU during storage. Thus, oxidation is one decomposition factor of ETU during storage. In spite of drastic ETU decomposition, however, increased amounts of EU were not detected. This apparently demonstrates that ETU converted to compounds other than EU by hydrolysis and plant components or that EU was rapidly degraded in the plant tissues examined. Cys-HCl inhibits such phenomena.

Conclusions. ETU in cucumber, Japanese pear, and potato was stabilized by the use of Cys-HCl during storage. Cys-HCl also prevented EBDC degradation to ETU during storage and analysis. Addition of Cys-HCl proved to be a useful way to prevent unnecessary degradation or formation of ETU in EBDC-treated crops during storage and/or analysis.

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