Postharvest Treatments for the Reduction of Mancozeb in Fresh Apples

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The objective of this study was to determine the effectiveness of chlorine, chlorine dioxide, ozone, and hydrogen peroxyacetic acid (HPA) treatments on the degradation of mancozeb and ethylenethiourea (ETU) in apples. This study was based on model experiments at neutral pH and temperature. Fresh apples were treated with two different levels of mancozeb (1 and 10 μg/mL). Several of the treatments were effective in reducing or removing mancozeb and ETU residues on spiked apples. Mancozeb residues decreased 56−99% with chlorine and 36−87% with chlorine dioxide treatments. ETU was completely degraded by 500 ppm of calcium hypochlorite and 10 ppm of chlorine dioxide at a 1 ppm spike level. However, at a 10 ppm spike level, the effectiveness of ETU degradation was lower than observed at 1 ppm level. Mancozeb residues decreased 56−97% with ozone treatment. At 1 and 3 ppm of ozone, no ETU residue was detected at 1 ppm of spiked mancozeb after both 3 and 30 min. HPA was also effective in degrading the mancozeb residues, with 44−99% reduction depending on treatment time and HPA concentrations. ETU was completely degraded at 500 ppm of HPA after 30 min of reaction time. These treatments indicated good potential for the removal of pesticide residues on fruit and in processed products.

Keywords: *Apple; calcium hypochlorite; chlorine dioxide; ozone; hydrogen peroxyacetic acid (HPA); mancozeb; ethylenethiourea (ETU); pesticide residue*

INTRODUCTION

Pesticide use in agriculture over the past several decades has proven to benefit food production by improving the yield of crops and the final quality. This has, in turn, lowered the cost of the household food budget. However, there is always the potential for trace amounts of pesticide residues to remain on commodities at the time of sale. Food safety has received increased attention in recent years as a major consumer concern, and reducing pesticides will increase consumer confidence (*1*, *2*).

The pesticide selected in this study was mancozeb (Dithane 75 DF), which is an ethylenebis(dithiocarbamate) (EBDC). EBDCs are fungicides that are frequently used for the control of fungal diseases in a wide range of fruits and vegetables (*3*). EBDCs are a class of highly effective fungicides that give very good disease control and have gradually replaced older products (*4*). In many cases, their use of multisite modes of action is essential in mixtures or program applications with more sophisticated products to control resistance (*4*). Recent concerns about the safety of mancozeb have been rebutted, and it remains on the market (*5*, *6*). EBDCs are subject to decomposition to ethylenethiourea (ETU) at elevated temperatures and humidity (*7*). ETU is also formed during the dissipation of the EBDC fungicides, and the conversion rate or degradation of ETU is greater than its formation rate (*8*). A major toxicological concern with EBDCs comes from ETU formation. Very little information is available regarding the health effects of ETU on humans. In animal studies, the acute oral LD_{50} for ETU was 1832 mg/kg in rats (*9*). ETU has caused cancer in experimental animals and has been classified by EPA as a group B2 probable human carcinogen on the basis of evidence from animal studies (*10*). Because of its carcinogenic (*11*), mutagenic (*12*), goitrogenic (*13*), and teratogenic (*14*) effects on laboratory animals, ETU has become a major human health concern among some consumer groups (*7*).

Chlorine, chlorine dioxide, ozone, and hydrogen peroxyacetic acid (HPA) have been employed historically for the oxidation of organic compounds at water treatment plants and have received a good deal of attention for their capacity to degrade organic pesticides. Chlorine and ozone treatments have shown to be effective in the reduction of azinphos-methyl, captan, formetanate hydrochloride, and propargite residues in apples and apple products (*15*, *16*).

The objective of this study was to determine if chlorine, chlorine dioxide, ozone, and HPA could reduce or eliminate mancozeb and ETU residues in spiked apples.

MATERIALS AND METHODS

Materials. Mature Golden Delicious apples were obtained from a commercial orchard in Onondaga, MI. These apples had not been sprayed with mancozeb or any other EBDCs at any time during the life of the orchard and had been isolated from

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Table 1. Recovery (n = 3) for Mancozeb on Apple Samples

	recovery, %		
	0.01 μg/mL spike	1 μg/mL spike	10 μg/mL spike
1	88.3	87.7	89.7
2	79.2	83.8	94.1
3	76.9	80.6	90.2
mean	81.5 ± 6.3[a]	84.0 ± 3.6	91.3 ± 2.4

[a] Mean ± standard deviation.

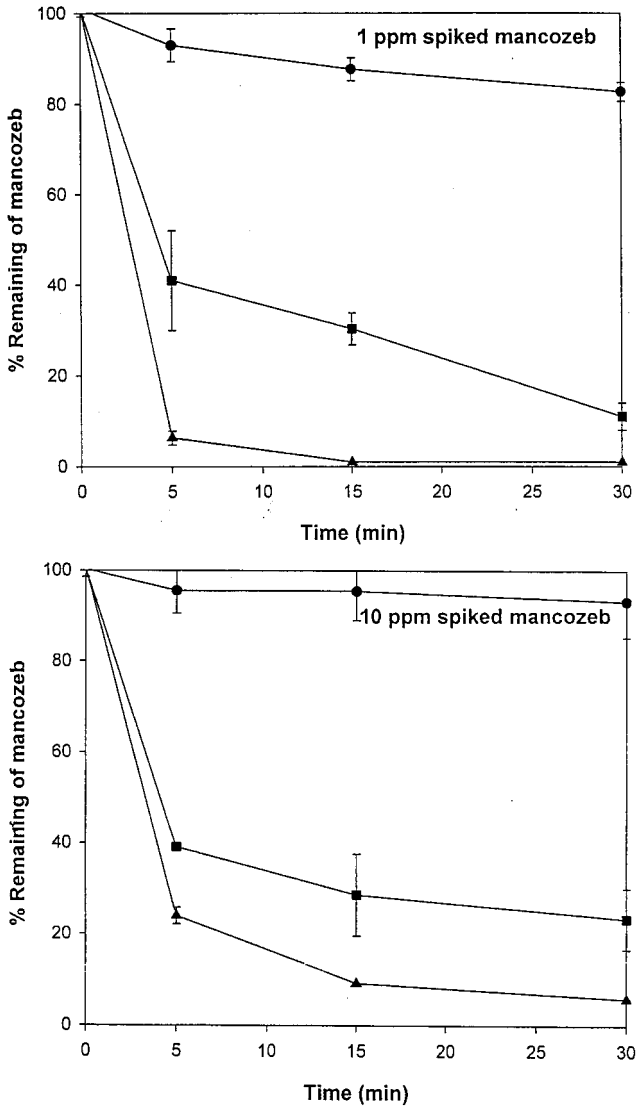

Figure 1. Effect of calcium hypochlorite on the degradation of mancozeb in spiked apples: (●) control; (■) 50 ppm of $Ca(OCl)_2$; (▲) 500 ppm of $Ca(OCl)_2$.

other orchards so there was no possibility of contamination. The fruits were hand picked randomly from various regions of the trees, thoroughly mixed, and stored at 4 °C until they were prepared for residue analysis. All organic solvents used for the preparation of stock solutions, extraction, gas chromatography (GC), and high-performance liquid chromatography (HPLC) were of distilled-in-glass residue grade or better. Acetone and methylene chloride were obtained from J. T. Baker Co. (Phillipsburg, NJ). Mancozeb standard was obtained from Rohm & Haas (Philadelphia, PA). ETU standard was obtained from Aldrich Chemical Co. (Milwaukee, WI). The stock solutions of mancozeb and ETU were prepared in distilled water at a concentration of 100 μg/100 mL. The standards were protected from light and stored under refrigeration at 4 °C. Chlorine solutions were prepared from calcium

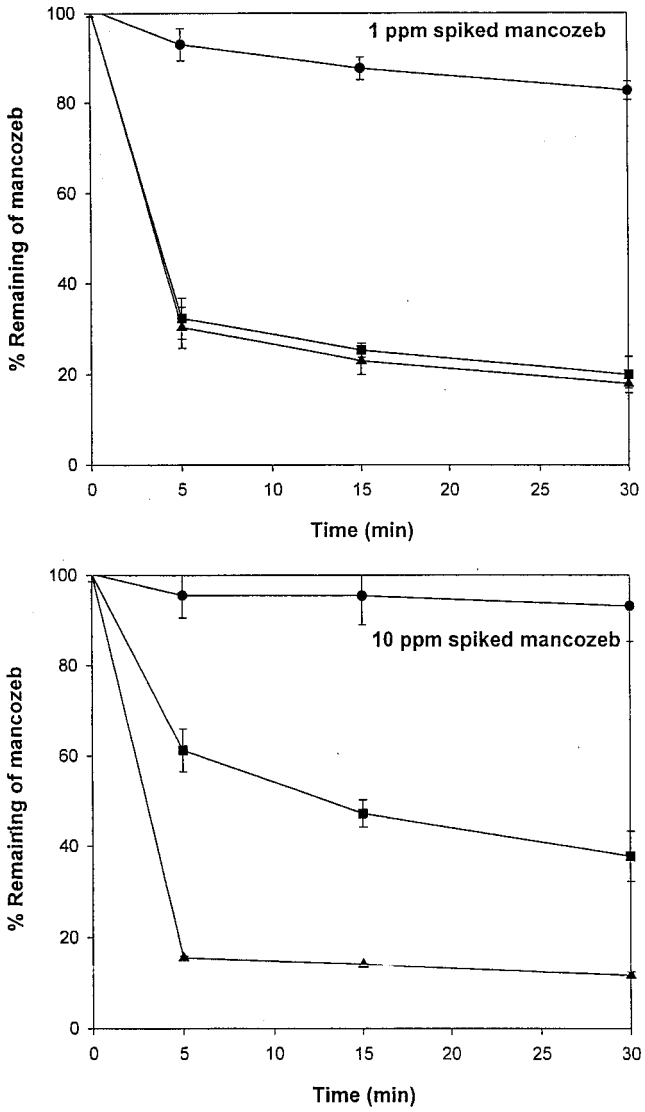

Figure 2. Effect of chlorine dioxide on the degradation of mancozeb in spiked apples: (●) control; (■) 5 ppm of ClO_2; (▲) 10 ppm of ClO_2.

hypochlorite (Aldrich). Sodium thiosulfate, sodium sulfate, potassium iodide, and potassium indigo trisulfonate were all of reagent grade.

Methods. (i) To degrade pesticides, calcium hypochlorite at two concentrations (50 and 500 ppm), chlorine dioxide at two concentrations (5 and 10 ppm), ozone at two concentrations (1 and 3 ppm), and HPA at two concentrations (50 and 500 ppm) were prepared. (ii) A pH of 6.7 (distilled water) and (iii) ambient temperature (21 °C) were used. Degradation of mancozeb was studied over a 30 min period because the typical water contact time in a commercial processing operation is ~10−15 min, and under normal conditions would rarely exceed 30 min. There were three replications per treatment. Solution was taken at appropriate intervals for analysis of mancozeb and ETU residues. Calcium hypochlorite stock solution (5000 ppm) and HPA stock solution were used as chlorine and peroxyacetic acid sources. Chlorine dioxide was generated in the laboratory using the manufacturer's (S. C. Johnson Professional) instructions as follows: 100 mL of the stock 2% Oxine FP solution was added to a 200 mL French square screw-capped bottle; 25 mL of 75% w/w food grade phosphoric acid was added, the bottle was sealed, and the mixture was allowed to generate chlorine dioxide for 5 min with a magnetic stirrer to ensure thorough mixing. The final concentration of chlorine dioxide was determined using the HACH chlorine colorimeter (model CN-66, catalog no. 2231-01, HACH Co., Loveland, CO)

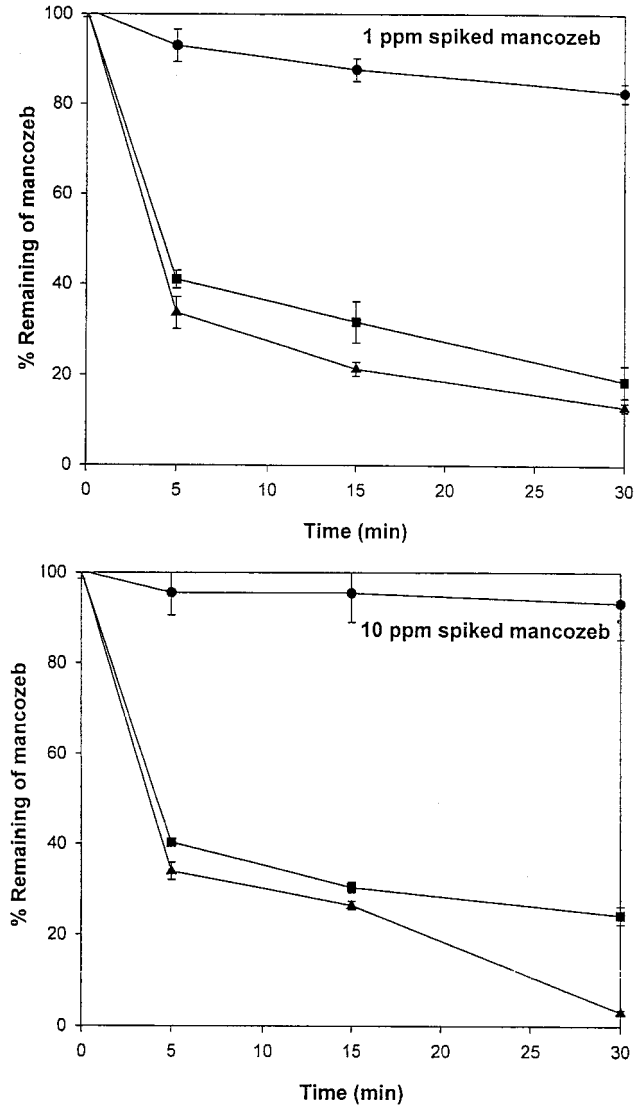

Figure 3. Effect of ozone on the degradation of mancozeb in spiked apples: (●) control; (■) 1 ppm of O_3; (▲) 3 ppm of O_3.

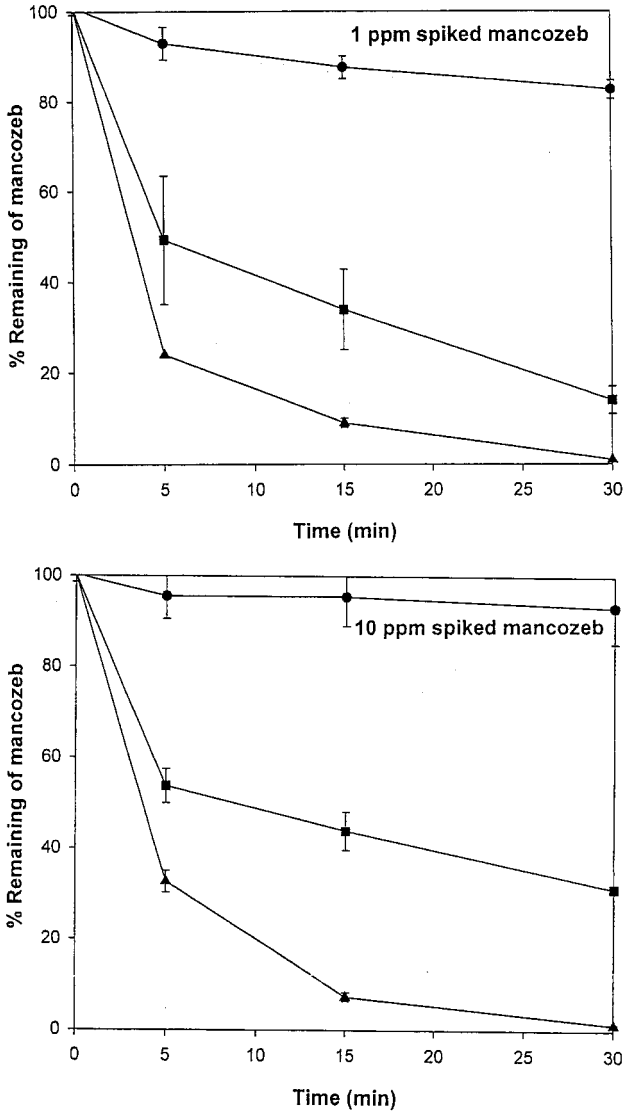

Figure 4. Effect of hydrogen peroxyacetic acid on the degradation of mancozeb in spiked apples: (●) control; (■) 50 ppm of HPA; (▲) 500 ppm of HPA.

before and after each sampling run. A 1:2000 dilution of unactivated Oxine FP solution was used as a control blank. Ozone was bubbled through a glass sparger (i.e., bubbles of ~10 mm i.d.) into 990 mL of distilled water under 25 psi at 15 SCFH of oxygen until the desired ozone concentration was attained. Ozone detection and monitoring were performed using the indigo colorimetric method as described in *Standard Methods for the Examination of Water and Wastewater* (*17*). All reagents were prepared just prior to use. The ozone concentration was monitored before and after each sampling run. The ozonated water was collected into a 100 mL volumetric flask containing 10 mL of the indigo reagent to minimize loss of ozone. A separate volumetric flask was filled with distilled water containing 10 mL of indigo reagent to serve as a blank. The solutions were mixed thoroughly, and the absorbance of each solution was immediately measured at 600 nm in a 1 cm cell.

Sample Treatment and Extraction. Apples were coated by dipping fruit in 200 mL of water containing 1 or 10 μg/mL of mancozeb. The water was allowed to evaporate, and then the apples (five at a time) were placed in 500 mL of distilled water, at room temperature with the desired treatment solutions of calcium hypochlorite (50 and 500 ppm), chlorine dioxide (5 and 10 ppm), ozone (1 and 3 ppm), or HPA (50 and 500 ppm). At the predetermined reaction time (0, 3, 15, and 30 min) the apples were removed and the surface was extracted with 20 mL of water and analyzed for mancozeb residues by GC.

Pesticide Residue Analyses. Mancozeb residues were analyzed as carbon disulfide (CS_2) by gas−liquid chromatographic headspace analysis (*18*). Twenty milliliters of sample was transferred into sample bottles, and 0.1 M sodium thiosulfate solution was added to the samples at the appropriate time to quench the reaction. Forty milliliters of 1.5% stannous chloride in 5 M HCl was added, and bottles were immediately sealed with a crimped septum. Fifty microliters of a 1 mg/mL thiophene solution was injected into each bottle and incubated at 70−80 °C in a water bath for 15 min. Bottles were removed and agitated for 2 min by hand. Bottles were replaced in the water bath with repeated shaking for 1 h. A 100 μL gas sample was removed with a gastight syringe from the bottle headspace and injected into the GC.

ETU residues were determined using a modification of the HPLC method published by Ahmad et al. (*18*): 20 g of sample was weighed into an Erlenmeyer flask, and then 8 g of potassium fluoride and 0.6 g of ammonium chloride were added. This mixture was extracted with 50 mL of methylene chloride two times. The methylene chloride layer was passed through a bed of 25 g of anhydrous sodium sulfate, collected in a Zymark Turbovap tube, and evaporated to dryness on an automated Zymark Turbovap evaporator (Zymark Inc., Hopkin, MA) at 40 °C. The residue was dissolved in 3 mL of distilled water, and 75 μL was injected into an HPLC column.

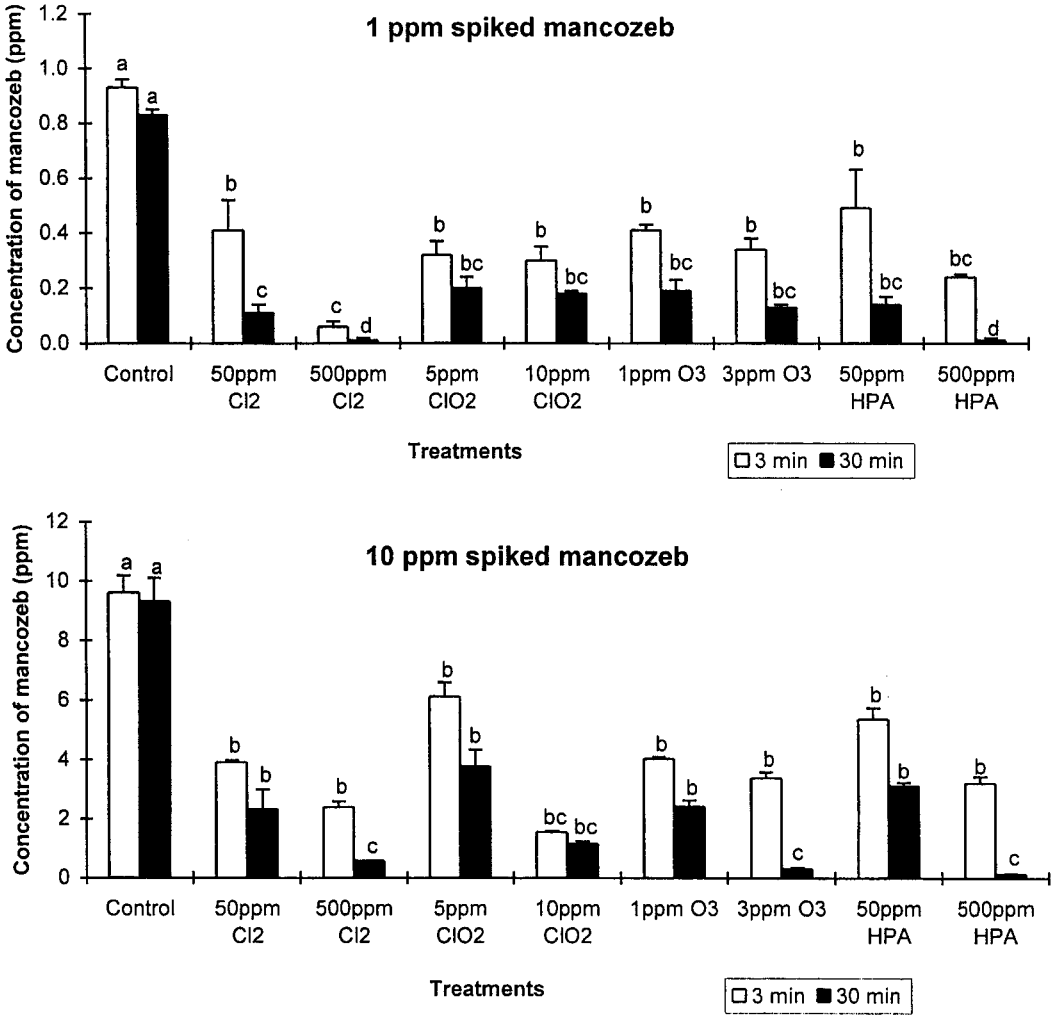

Figure 5. Comparison of various oxidizing agents on the degradation of mancozeb.

Chromatographic Analyses. Mancozeb residues were detected and quantified using a Hewlett-Packard series II 5890 gas chromatograph equipped with a flame photometric detector (FPD) in the sulfur mode. Hydrogen and air were used for the FPD. The GC was equipped with a Supel-Q-Plot fused silica capillary column (30 m long × 0.53 mm i.d.) with a film thickness of 0.25 μm (Supelco Inc.). The oven temperature was 80 °C, and the injector and detector temperatures were 230 and 300 °C, respectively. Helium and nitrogen were used as the GC carrier gas and makeup gas, respectively. Carrier gas flow through the column was 20 mL/min. Integration was carried out with HP Chemstation software interfaced to the GC.

ETU residues were detected and quantified using a 2487 Waters liquid chromatograph with a Hypersil BDS C_{18} column (250 mm × 4.6 mm, 5 μm particles), a Hypersil BDS C_{18} guard column (10 mm × 4.6 mm, 5 μm particles), and a UV detector (Waters Associates, Inc., Milford, MA) set at 240 nm. The mobile phase was 0.72% butylamine in distilled water at pH 3.0−3.2. An M-45 Waters HPLC pump (Waters Associates, Inc.) was used for solvent delivery at a flow rate of 0.5 mL/min. After the system was stabilized (~1 h from initial warmup), 75 μL of extract sample was injected via a Rheodyne syringe loop injector (50 μL loop) for analysis. Integration was carried out using a 3390 A Hewlett-Packard integrator.

Calculation of Pesticide Residue Concentration. Mancozeb and ETU residue concentrations in solution were calculated on the basis of the area of the integrated peaks of the samples compared with known concentrations of analytical standard of the respective pesticides. Standard curvess of the mancozeb and ETU were plotted, and least-squares linear regression was performed using Microsoft Excel (Microsoft Corp., Redmond, WA) software.

Statistical Analyses. All determinations were replicated three times. Mean standard deviations, mean square errors, two-factor ANOVA, correlation, and interaction of main effects were calculated using Sigmastat computer software 1.0 (Jandel Corp., San Rafael, CA). Appropriate comparisons were made using the Student−Newman−Keuls method for multiple comparisons. A $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Recovery Study. On the basis of model system studies, whole fruit studies were conducted. To determine the extraction efficiency of mancozeb, five apples (~700 g) were treated with mancozeb at three concentration levels (0.01, 1, and 10 μg/mL). Table 1 gives the percent recoveries obtained from these treated apples. On the basis of the regression equation, average recoveries of mancozeb were 84.0% at 1 μg/mL spike level and 91.3% at 10 μg/mL spike level. The method detection limit (MDL) for mancozeb was determined to be 0.01 μg/mL. The MDL did not relate to the actual sample weight of the apples and did not conduct for ETU. Relatively high recoveries were obtained for all three spike levels. Recoveries appeared to decline when apples were spiked at a lower level. The lower recoveries may be a result of matrix effects on extraction efficiency. Samples that contain low levels initially are more likely to show these discrepancies (*19*).

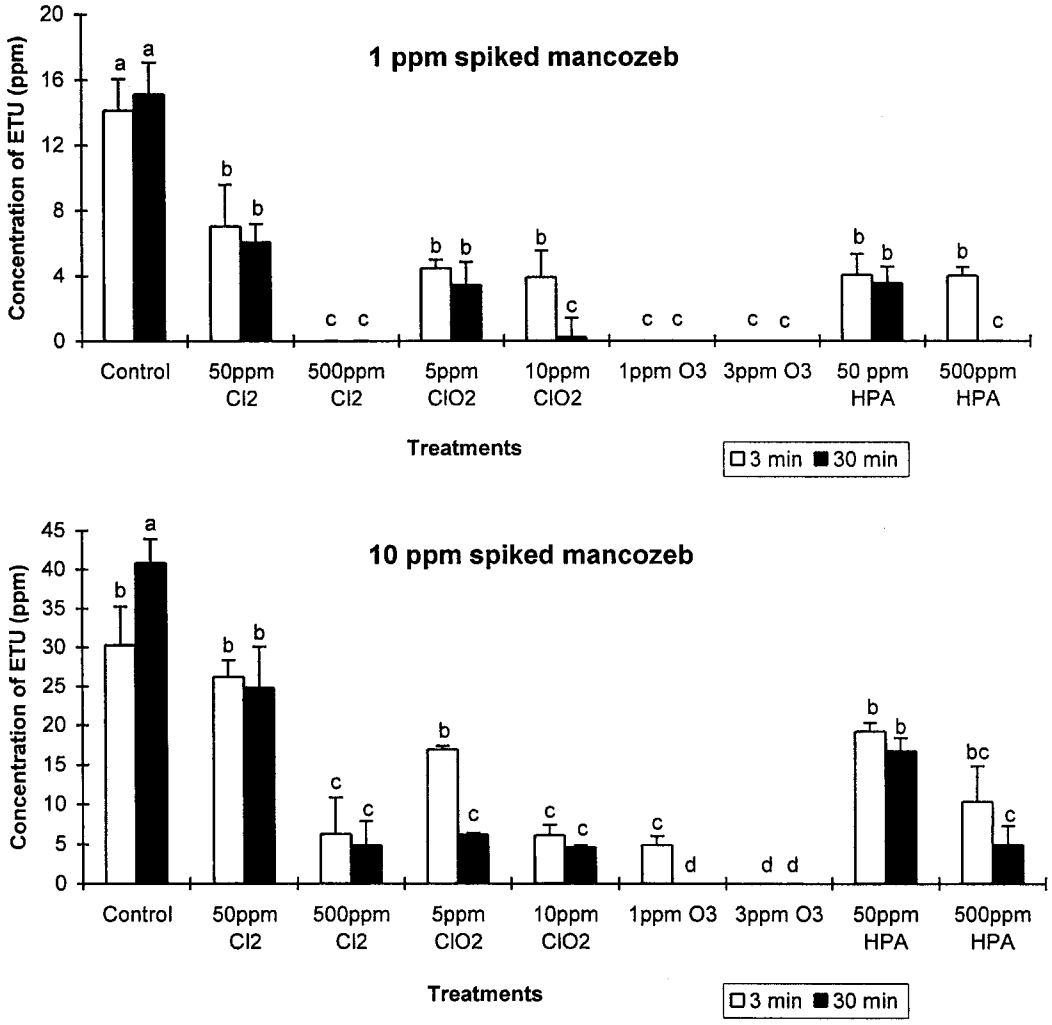

Figure 6. Comparison of various oxidizing agents on the conversion of mancozeb to ETU.

Removal of Mancozeb in Spiked Apples. On the basis of model system studies, ambient temperature (21 °C) and pH 6.7 were used in this study. This experiment utilized five apples (~700 g of apples) coated with 1 or 10 ppm of mancozeb. The whole fruit spiked with mancozeb gave results similar to those found in the model system studies. Control studies conducted with mancozeb-coated apples under the exact conditions as the treated samples but exposed only to distilled water with no other treatments showed only slight dissipation of mancozeb residues (Figure 1). This indicates that mancozeb was relatively stable in distilled water for at least 30 min. Figure 1 shows the rates of decline for mancozeb on apples. At zero reaction time, the spiked mancozeb concentration was ~1 ppm. This decreased gradually to about 0.11 and 0.01 ppm at 50 and 500 ppm of calcium hypochlorite, respectively, after 30 min of reaction time. In the 50 ppm of calcium hypochlorite treatment, approximately 94 and 75% of the initial amounts of mancozeb were eliminated after 30 min at 1 and 10 ppm spike levels, respectively. Chlorine at 500 ppm significantly ($p < 0.05$) increased the rate of degradation of mancozeb. Only about 0.01 and 0.04% of mancozeb remained at 1 and 10 ppm spike levels after 30 min of reaction time.

Degradation of mancozeb residues by chlorine dioxide is shown in Figure 2. At the 1 ppm of mancozeb spike level, there was no significant difference between 5 and 10 ppm of chlorine dioxide treatments and the effects were lower than those observed with calcium hypochlorite. In this case, between 34 and 32% of mancozeb remained after 5 min at both 5 and 10 ppm of chlorine dioxide treatments, respectively. After 15 min, degradation of mancozeb increased, with 24 and 22% remaining; however, there was no significant difference with reaction time. At the 10 ppm of mancozeb spike level, 64 and 16% of mancozeb remained after 5 min and 41 and 13% of mancozeb remained after 30 min at 5 and 10 ppm of chlorine dioxide treatments, respectively (Figure 2). It is anticipated that residue levels would be reduced considerably by the chlorine dioxide treatment if the concentration of chlorine dioxide were increased above the 10 ppm that was used in this study.

Ozonation at 1 and 3 ppm significantly ($p < 0.05$) influenced the rate of degradation of mancozeb at the 10 ppm of mancozeb spike level (Figure 3). At the 3 ppm of ozone concentration, 3% of the mancozeb residue remained after 30 min at the 10 ppm spike level, with 16% of the mancozeb residue remaining from the 1 ppm spike level. Ozone has shown to be reasonably stable at the neutral pH range of distilled water, so it is possible that this treatment could be applied in commercial plants.

Degradation of mancozeb by HPA was significantly increased at higher HPA concentration. In 50 ppm of HPA treatment, almost 83 and 66% of the initial amounts of mancozeb were degraded after 30 min. HPA treatments at 500 ppm showed greater effects than 50

ppm of HPA at 1 and 10 ppm of mancozeb spike levels after 30 min, with 99 and 98% degradation of mancozeb, respectively (Figure 4).

Comparison of the Effects of Various Oxidizing Agents on the Degradation of Mancozeb Residues. The effects of various oxidizing agents on the degradation of mancozeb are shown in Figure 5. Mancozeb residues in all of the samples were significantly reduced compared to control by exposure to various oxidizing agents. In 1 ppm of mancozeb, there were no significant differences among various treatments except chlorine at 500 ppm at both 3 and 30 min of treatment time. At the 10 ppm of mancozeb spike level, 10 ppm of chlorine dioxide treatment showed the best effect at 3 min of reaction time. With longer reaction times of 30 min, chlorine at 500 ppm, chlorine dioxide at 10 ppm, ozone at 3 ppm, and HPA at 500 ppm showed greater effects than other treatments. Treatments with 500 ppm of calcium hypochlorite and 500 ppm of HPA showed the greatest effects with both 1 and 10 ppm of mancozeb after 30 min.

Degradation of Mancozeb to ETU in Spiked Apples. The second phase of this work was the determination of conversion of mancozeb to ETU in spiked apples. Figure 6 shows the ETU residues formed from the 1 and 10 ppm of mancozeb spiked apples at 3 and 30 min reaction times. Mancozeb produced significant quantities of ETU. At 1 ppm of mancozeb, ETU after 3 min was 14.13 ppb and slowly increased to 15.12 ppb after 30 min of reaction time for the control, which was treated with only distilled water. Various oxidizing agents significantly reduced ETU residue levels compared to the control. For the 1 ppm of mancozeb experiments, 500 ppm of calcium hypochlorite treatment and 1 and 3 ppm of ozone treatments completely inhibited the conversion of mancozeb to ETU. At 3 min, chlorine dioxide and HPA were very effective in reducing ETU levels compared to the control; however, there was no statistical ($p < 0.05$) difference between 5 and 10 ppm of chlorine dioxide and 50 and 500 ppm of HPA. After 30 min, all ETU residues were degraded at high concentrations of the oxidizing agents, and small amounts of ETU were still determined at lower concentrations of oxidizing agents.

At 10 ppm of mancozeb, the conversion rate of mancozeb to ETU was higher and the oxidizing agent treatments showed less effect than at the 1 ppm level. In this case, increased reaction time and higher concentration of oxidizing agents played an important role in the reduction of ETU residues. Ozone at 3 ppm was still highly effective in reducing ETU levels. Ozone was also very effective in the degradation of mancozeb as compared to other oxidants at low concentration. This is probably due to the high oxidation potential of ozone (2.07 V).

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