

Residues of Dikar and Ethylenethiourea in Treated Grapes and Commercial Grape Products

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Residues of Dikar, viz., EBDC (ethylenebisdithiocarbamate) as zineb equivalent and dinocap, and the EBDC degradation product ethylenethiourea (ETU) were monitored in Concord grapes following application of the fungicide in 1975 and 1976. Mean values of 6.8 ppm zineb, 0.38 ppm dinocap, and 0.03 ppm ETU were found immediately after application and these residues dissipated by 50% in the first 15–20 days; thereafter slight dilution due to growth was observed. Wine prepared from the harvested grapes contained a mean of 0.037 ppm ETU and no residues of EBDC. Heat treatment of the harvested grapes demonstrated an 18% conversion of EBDC to ETU. Commercial grape products were also analyzed and most showed <0.02 ppm ETU except concentrates which contained 0.06 ppm ETU; EBDC was found in only one of these samples.

Recent concern about the fate of ethylenebisdithiocarbamates (EBDC) and its metabolite ethylenethiourea (ETU; 2-imidazolidinethione) in the environment (Tweedy, 1973) and in the food supply (Pecka et al., 1975) have prompted renewed investigations into the residues of these compounds in various treated fruits and vegetables. Particular concern has been expressed for those crops where no alternative fungicides are available for prophylaxis.

Grapes are the second most important fruit crop grown in Ontario and represent about one-third of the total fruit hectarage. Dikar is a widely used fungicide for the control of downy mildew (*Plasmopara viticola*), powdery mildew (*Uncinula necator*), and black rot (*Guignardia bidwellii*) on grapes. The fungicide is usually applied several times over the growing season. This study was initiated to provide data on residues in Concord grapes after a normal spray program using Dikar, in wine processed at harvest from the treated grapes, and in commercial grape products.

EXPERIMENTAL SECTION

Grape Vineyard. The vineyard, occupying 14 ha, was located at the Grape Research Station, Horticultural Research Institute of Ontario (HRIO) in the Niagara Peninsula at Vineland Station, Ontario. The area was situated about 0.8 km below the Niagara Escarpment and about 2.5 km from Lake Ontario. The soil in this area is mainly clay.

Treatment with Dikar. The commercial pest control product Dikar (Rohm and Haas Co.) is a mixture of 72% coordination product of zinc ion and manganese ethylenebisdithiocarbamate, 4.4% dinocap [2-(1-methylheptyl)-4,6-dinitrophenyl crotonate], and 0.3% nitrooctyl phenols. Dikar was applied to the 14 ha of grapes using an airblast sprayer according to Ontario Ministry of Agriculture and Food (OMAF) recommendations (Publication 360). The study was conducted in 1975 and 1976; the spray program for both years is summarized in Table I.

Sampling. Sixty vines of grapes in one row were divided into three sections and eight bunches of grapes per 20 vines were sampled as replicates before and after the

last cover spray and thereafter at intervals up to 30 days. The samples were labelled and frozen at -29 °C until analysis. Weather conditions during the sampling period are presented in Table II.

Wine Preparation. Grapes were taken at harvest, 30 days after the last spray, and were processed unwashed into wine by the enologist at HRIO using standard methods.

Heat Treatment. Grape homogenate (20 g) was heated under reflux in a 125-mL flask in a 100 °C water bath for 15 min. After cooling, the samples were transferred with ethanol rinsings to a blender for extraction and ETU analysis.

Analytical Method. The EBDC was analyzed using the standard CS₂ evolution technique (Pease, 1957) with modifications (Keppel, 1969; Ripley and Simpson, 1977). Dinocap was analyzed following the method of Kilgore and Cheng (1963) based on the colorimetric measurement of dinocap in *N,N*-dimethylformamide at 444 nm after cleanup of the extracts by washing with sulfuric acid.

ETU was determined by gas-liquid chromatography of the *N*-trifluoroacetyl-*S*-(*m*-trifluoromethylbenzyl) derivative (King, 1977; Ripley and Simpson, 1977) using a flame photometric detector in the sulfur mode and a 1 m × 4.0 mm i.d. glass column packed with 3% (w/w) OV-225 on Chromosorb W, H.P. Temperatures (°C) were: column, 175; detector, 185; and injector, 225. Gas flow (mL/min) were: carrier (N₂), 80; H₂, 120; air, 40; and O₂, 20. These GLC conditions were optimized for a retention time of 3 min and linearity in the range 1–10 ng of the derivative. Substrates were fortified with ETU (0.01–0.25 μg) prior to blending and analyzed concurrently with samples. Quantitation was based on a calibration curve and results varied by ±0.01 ppm ETU.

RESULTS AND DISCUSSION

Mean residue levels of the two main active ingredients of Dikar, the EBDC (expressed as zineb) and dinocap, in the grapes for the 2 years are tabulated in Table III. Reported values represent the mean ± the standard deviation of the three replicates in each of the years. The error between duplicates was less than the experimental error associated with the replicates.

The initial residues present at day 0, prespray, represent those residues remaining from the previous four or five applications. The difference in residue concentrations between the years is probably due to these previous sprays since the values for the last cover spray for both years are similar after correcting for the prespray residues. After application of the last cover spray, the mean residue

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Table I. Summary of Dikar Spray Schedule to Concord Grapes in 1975 and 1976^a

Spray time	1975 date	Rate AI/ha	1976 date	Rate AI/ha
Prebloom	June 7	2.58 kg/560 L	June 15	2.58 kg/560 L
Postbloom	June 21	3.85 kg/739 L	June 29	3.85 kg/739 L
First cover	July 10	3.85 kg/739 L	July 12	3.85 kg/739 L
Second cover	July 30	4.30 kg/930 L	July 28	4.30 kg/930 L
Third cover	Aug. 26	4.30 kg/930 L	Aug. 10	4.30 kg/930 L
Fourth cover	Not applied		Aug. 24	4.30 kg/930 L

^a All sprays were concentrate 3× aqueous suspensions of Dikar (72% coordination product of Zn-Mn EBDC, 4.4% dinocap, and 0.3% nitrooctyl phenols, Rohm and Haas, Co.).

Table II. Meteorological Data for the Sampling Period (Last Spray to Harvest in 1975 and 1976)

	1975		1976	
	Mean	Range	Mean	Range
Temperature, high (°C)	20.1	11.7-26.1	22.9	13.3-29.4
Temperature, low (°C)	11.7	4.4-18.9	13.3	4.4-23.3
Rainfall, (mm)	5.2	Tr-17.8	4.7	Tr-28.2
Number of days		20		14

concentrations of zineb and dinocap increased by about 3.8 and 0.3 ppm to about 6.8 and 0.38 ppm, respectively. In the first 6 days there was a rapid decrease in residue and this was followed by a slight dissipation in the next 24 days. The residues at harvest, 30 days following the last spray, are below the current Canadian tolerance of 7 ppm zineb equivalent EBDC and are essentially negligible (0.1 ppm) for dinocap.

On a total deposit basis (product of residue concentration and weight of grapes), it appeared that about 50% of the pesticide was lost in the first 7 days after application; thereafter, the total residue appeared to decrease mainly as the result of dilution due to growth of the fruit.

Initially, problems were encountered in the analysis of ETU in the grapes. It was determined that the immature grapes required more base for neutralization prior to extracting the *S*-(*m*-trifluoromethylbenzyl)ethylenethiourea; there was a pronounced color change in the extract when the correct pH was reached. Also, due to reagent blank and substrate background, electron-capture detection GLC could not be used and the second derivative, *N*-trifluoroacetyl-*S*-(*m*-trifluoromethylbenzyl)ethylenethiourea, was prepared and quantitation accomplished with the flame photometric detector in the S-mode. On the 3% OV-275 column (King, 1977) a background peak, probably due to a natural product or flavor constituent in the grape, was found at the same retention time as the ETU de-

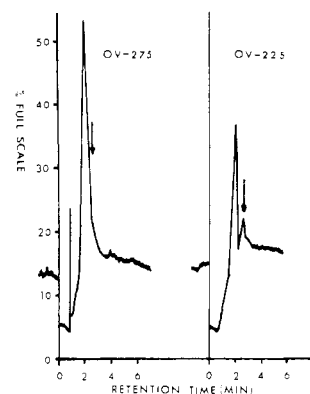


Figure 1. Chromatogram of mature grape (30 days after last application) on 3% OV-275 and 3% OV-225; conditions as in text; 1.024×10^{-8} amps full scale; 80 mg of grape extract.

rivative; a clear window was found with a 3% OV-225 column (Figure 1). Typical chromatograms of the derivatized standard, day 3 and day 30 treated grapes, fortified grape, and wine sample are shown in Figure 2. ETU results varied by ± 0.01 ppm.

Mean concentrations of ETU determined in the grapes for the 2 years are shown in Figure 3. After application of Dikar, the ETU level rose about 0.016 to 0.03 ppm and declined to about 0.015 ppm in 15–20 days and remained at this concentration until harvest.

Meteorological data for the sampling periods are shown in Table II. No correlation between rainfall or temperature and the removal of residue deposits could be found. Throughout the sampling period, in both years, the ratio of dinocap-ETU-zineb concentration remained fairly constant at about 4.5:0.44:100, respectively.

Wine was prepared in triplicate, using standard methods, from the 1975 unwashed grapes obtained at harvest (30 days). No zineb residue (<0.1 ppm) was found in the wine; no analysis for dinocap was performed. ETU

Table III. Residues of EBDC and Dinocap in Concord Grapes after the Last Cover Spray with 5.6 kg/ha Dikar^a in 1975 and 1976

Days after application	Residues ^b in grapes, ppm					
	1975			1976		
	Mean wt, g/grape	Zineb equivalent	Dinocap	Mean wt, g/grape	Zineb equivalent	Dinocap
0 prespray ^c	2.25 ± 0.16	1.6 ± 0.3	0.06 ± 0.01	2.08 ± 0.19	4.4 ± 1.1	0.13 ± 0.02
0 postspray	2.31 ± 0.03	5.2 ± 0.6	0.25 ± 0.13	2.31 ± 0.16	8.3 ± 2.1	0.50 ± 0.27
3	2.38 ± 0.21	3.8 ± 0.5	0.19 ± 0.05	2.34 ± 0.22	6.6 ± 3.8	0.27 ± 0.04
6	2.41 ± 0.13	2.6 ± 0.2	0.21 ± 0.09	2.21 ± 0.03	5.3 ± 1.4	0.20 ± 0.06
9	2.41 ± 0.09	3.6 ± 0.1	0.23 ± 0.04	2.31 ± 0.06	6.0 ± 1.5	0.24 ± 0.12
12	2.40 ± 0.25	3.2 ± 0.8	0.13 ± 0.07	2.39 ± 0.22	5.9 ± 0.2	0.15 ± 0.08
15	2.49 ± 0.26	2.8 ± 0.3	0.15 ± 0.07	NA	NA	NA
16	NA	NA	NA	2.46 ± 0.16	5.4 ± 0.9	0.15 ± 0.05
20	2.59 ± 0.40	3.3 ± 0.4	0.14 ± 0.06	2.64 ± 0.17	4.4 ± 1.3	0.13 ± 0.06
25	2.63 ± 0.38	2.0 ± 0.2	0.16 ± 0.06	3.04 ± 0.30	3.4 ± 0.7	0.10 ± 0.05
30	2.56 ± 0.27	2.8 ± 0.9	0.16 ± 0.02	3.11 ± 0.37	3.5 ± 1.0	0.11 ± 0.04

^a See Table I. ^b EBDC concentration expressed as zineb equivalent. Mean ± standard deviation of the three replicates. ^cResidues remaining from previous cover spray (Table I).

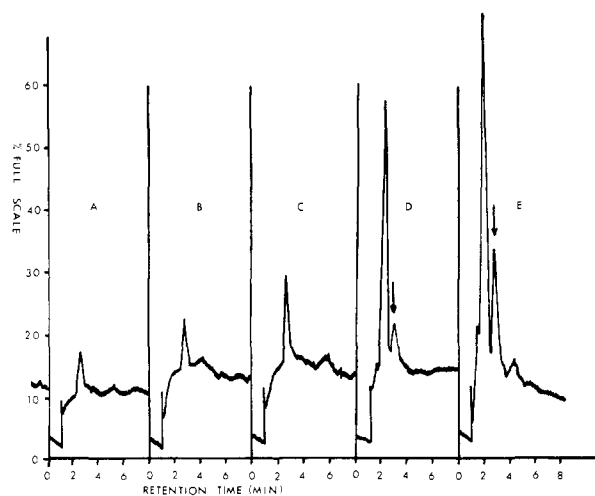


Figure 2. Chromatograms of *N*-trifluoroacetyl-*S*-(*m*-trifluoromethylbenzyl)ethylenethiourea. Conditions as in text; 1.024×10^{-8} amps full scale; final grape extract, 4 g/mL. (A) 10 μ L, standard 0.25 μ g/mL; (B) 20 μ L, grapes day 3; (C) 20 μ L, grapes day 3 fortified with 0.03 ppm ETU; (D) 20 μ L, grapes day 30; (E) 20 μ L, wine prepared from grapes day 30.

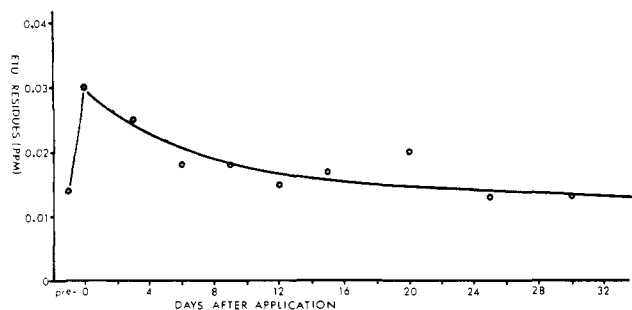


Figure 3. ETU residues in grapes at intervals after last application of Dikar. Mean of six results, three from each of 1975 and 1976 study.

was determined at 0.037 ± 0.012 ppm.

ETU is known to be formed when EBDC-treated crops are heated (Newsome and Laver, 1973; Watts et al., 1974). The samples of grapes taken at harvest for both years were also boiled to determine the degree of conversion. After boiling 15 min, ETU was found at 0.10–1.09 ppm which represents an $18.6 \pm 15.6\%$ conversion. These results underline the necessity of analyzing not only for the parent compound but also for toxic metabolites and degradation products.

Due to the potential of elevated ETU residues being present in heated products prepared from EBDC-treated crops, several commercial grape products were analyzed (Table IV). Although there was no evidence that the

Table IV. ETU Found in Commercial Grape Products^a

Product	No. of samples	No. positive	Concentration of ETU (ppm) range
Jelly	3	2	ND(<0.01)–0.02
Jam	1	1	0.01
Concentrate	2	2	0.06
Drink	3	2	ND(<0.01)–0.01
Wine	2	1	ND(<0.01)–Tr(0.01)

^a Purchased locally; amount of EBDC treatment unknown. Zineb concentration in all samples was ND(<0.2 ppm) except one wine (0.25 ppm).

grapes had been sprayed with any EBDC prior to processing, samples of commercial Concord grape wine, jam, jelly, concentrated juice, and drink were purchased locally for examination. The two grape juice concentrates contained the highest concentration of ETU (0.06 ppm) whereas six of the nine other products had detectable ETU levels of 0.02 ppm or less. In only one wine sample was zineb detected (0.25 ppm); all the other products contained no detectable zineb (<0.2 ppm). Assuming the grapes, prior to processing, had been treated in accordance with OMAF recommendations and were harvested 30 days after the last application of EBDC, these data would indicate that there is little conversion of EBDC to ETU during commercial heat treatment or that the commodities are prepared from both treated and nontreated grapes. Elevated ETU concentrations would be expected in the products that are concentrated such as the juice.

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