Volatile Metabolites from Dimethyldithiocarbamate Fungicide Residues

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The decomposition of dithiocarbamic acids to amines and carbon disulfide in the presence of strong acid is well known and forms the basis for the analytical determination of dithiocarbamates (1). Evolution of gaseous products from dilute solutions of dithiocarbamate fungicides has also been reported (2, 3). Cox (4) and Sisler and Cox (5) showed that carbon disulfide was present in the air above dilute solutions of thiram and nabam in contact with cultures of Fusarium roseum. The

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fungicides were presumably decomposed by acids excreted into the medium by the microorganism. No similar study with higher plants has been reported, yet the vast majority of dithiocarbamate fungicides are applied to protect higher plants. During a study of the total fate of ziram residues on Carica papaya (papaya), it appeared conceivable that carbon disulfide and dimethylamine could be formed as volatile metabolites. Furthermore, it seemed likely that if dimethylamine were formed, it could be further metabolized to carbon dioxide. This paper presents the results of experiments designed to detect, identify and quantitate these volatile products.

Materials and Methods

Dithiocarbamates: Radioactive potassium dimethyldithiocarbamate (KDDC) labeled with ^{35}S was purchased from Volk Radiochemical Co., Chicago, Illinois. Prior to use it was exhaustively extracted with benzene to remove elemental ^{35}S which was the principal radiochemical impurity resulting from radiodecomposition. Radioactive KDDC labeled with ^{14}C in the methyl groups was synthesized from ^{14}C dimethylamine and carbon disulfide. Ziram [$Zn(DDC)_2$], either ^{35}S or methyl ^{14}C labeled, was prepared from the respective KDDC by addition of a stoichiometric amount of zinc chloride, dissolved in water, to an aqueous solution of the dithiocarbamate. The precipitated $Zn(DDC)_2$ was extracted into chloroform and isolated by removal of the solvent on a

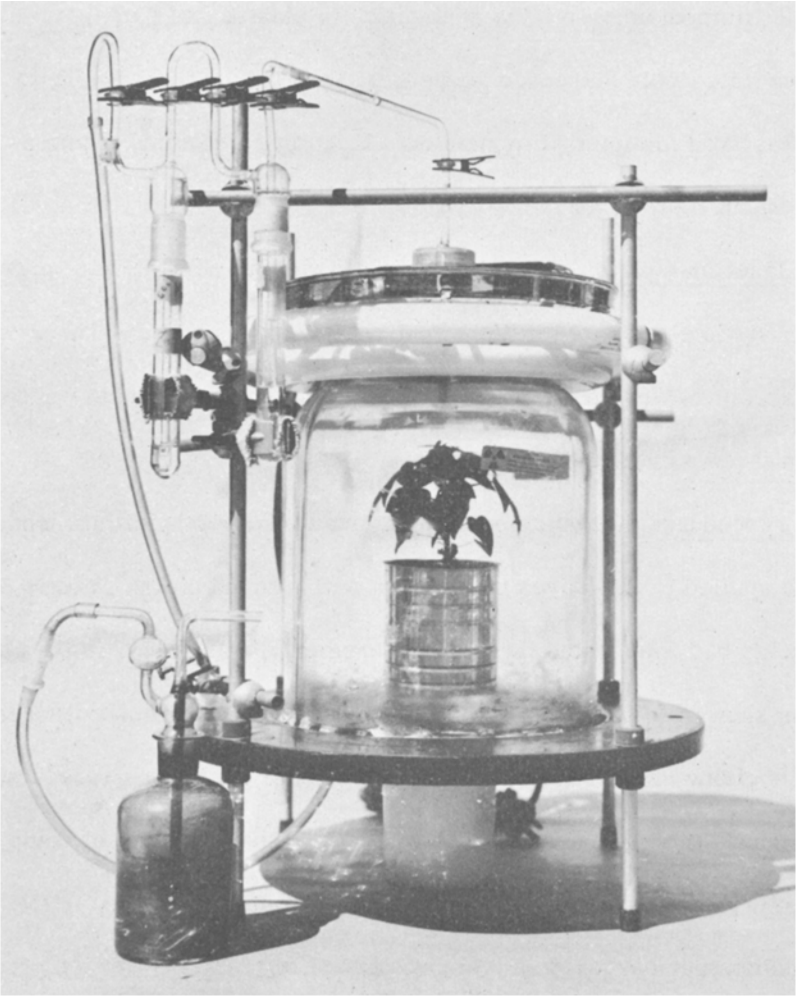


Figure 1. Apparatus used for the collection of volatile decomposition products.

rotating evaporator. Prior to use all dithiocarbamates were assayed for purity and radiopurity by chromatography (6) and radiochromatography. Water-soluble dithiocarbamates were dissolved in 0.02M potassium phosphate buffer, pH 7.4, containing 800 ppm Triton X-100 as a wetting agent, immediately prior to application to plants. Zn $(DDC)_2$ was applied either as a finely dispersed suspension in H_2O with 800 ppm Triton X-100, or dissolved in dimethylformamide. Dimethylformamide solutions did not produce any observable phytotoxicity.

Plant materials: Papaya plants (Solo variety, line 5) were grown from seeds germinated in Vermiculite in the greenhouse. Three-week-old seedlings were transplanted to sterile soil and the plants were used when three months old.

Procedure: Known amounts of radioactive dimethyldithiocarbamates were applied to the leaves of the plant with a micropipet. When the solvents had evaporated the well-watered plant was placed in the apparatus shown in Figure 1. It was illuminated by two circular fluorescent lamps giving an average of 250-foot candles at the leaf surface. Air was drawn through the system by a small mechanical vacuum pump. Incoming air first passed through a trap containing acidified 0.1M potassium permanganate to remove impurities which otherwise produced an epinastic response in the plant within 4 hours. The effluent air stream passed through two traps in series. In the case of ^{35}S-labeled materials, the first of these traps contained 20% zinc acetate to retain H_2S, and the second contained Viles reagent (7) for CS_2. With ^{14}C-labeled substances, the first trap contained 1.0N HCl, to retain dimethylamine, and the second contained 15% KOH to trap CO_2. The trapping solutions were changed at suitable intervals.

Radioactivity determinations: Hydrogen sulfide was removed from the trapping solution by addition of 7.8 mg of carrier sodium sulfide. The resulting precipitate was collected on a glass fiber disc, washed, dried and weighed. Aliquots of the carbon disulfide and dimethylamine trapping solutions were transferred to planchets, dried and weighed. The radioactivity in these samples was determined with a Nuclear Chicago thin window gas flow counter with an efficiency of 26% for ^{14}C. Radioactive carbon dioxide was precipitated as barium carbonate by addition of 10% barium chloride to the trapping solution. The precipitate was collected on a sintered glass filter, washed with water and 95% ethanol, and dried. A portion of the precipitate was ground to a fine powder, transferred to a tared 20 ml liquid scintillation counting vial and dried to constant weight. The vial was filled with Cab-O-Sil thixotropic agent and 20 ml of scintillator solution (8). These samples were counted in a Packard Tri-Carb Liquid Scintillation Spectrometer which had an efficiency of 55% for ^{14}C. The results of radioactivity determinations were corrected for background, decay, and self absorption.

TABLE 1

Radioactive Carbon Disulfide Released by Plants Treated with Radioactive Dimethyldithiocarbamates (Average of 3 Replications)

Days after application	Compound applied ^{35}S-KDDC cpm	 ^{35}S-Zn(DDC)$_2$ cpm
1	105,600	1,290
2+3	218,800	2,790
4+5+6	270,800	4,110
7+8+9	36,300	3,980
10+11+12+13	6,270	5,230
Total	637,770	17,400
Counts applied	1.65×10^7	1.36×10^7

TABLE 2

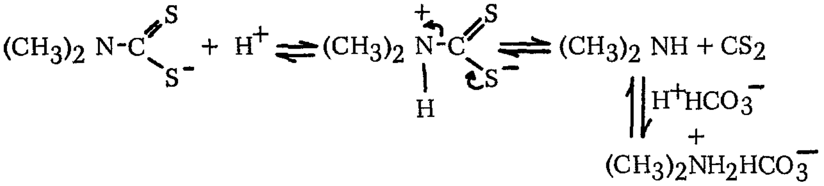
Radioactive Carbon Dioxide Released by Plants Treated with Radioactive Dimethyldithiocarbamates (Average of 3 Replications)

Days after application	Compound applied $^{14}CH_3$-KDDC cpm	 $^{14}CH_3$-Zn(DDC)$_2$ cpm
1-3	2,001	547
4-6	2,295	582
7-9	2,351	568
10-12	2,227	543
Total	8,874	2,240
Counts applied	2.9×10^5	2.04×10^6

Results and Discussion

The possibility that volatile products could be formed from residues of dithiocarbamates on higher plants seems to have been overlooked. Our data, presented in Tables 1 and 2, demonstrate that such products are formed by higher plants, and that significant amounts of carbon disulfide are released from residues of both KDDC and $Zn(DDC)_2$. The rate of release (Fig. 2) is much greater with KDDC shortly after application, but by the ninth day the rates of carbon disulfide evolution are essentially the same for both compounds. The much slower evolution of carbon disulfide from $Zn(DDC)_2$ residues is presumably due to the low-water solubility of this substance when compared with that of the potassium salt. The decrease in carbon disulfide production observed with the latter may also be due to solubility, since it has been shown that KDDC residues are gradually oxidized to thiram on leaf surfaces (9).

The site of decomposition may be either the leaf surface or within the outer layers of cells. The plants were in an atmosphere at or near 100% relative humidity, and under these conditions a film of moisture on the leaf surface could be sufficiently acidic, due to dissolved carbon dioxide, to catalyze a reaction similar to that proposed by Zuman and Zahradnik (10) for the acid decomposition of dithiocarbamates



$$(CH_3)_2N\text{-}C\overset{S}{\underset{S^-}{\lessgtr}} + H^+ \rightleftharpoons (CH_3)_2\overset{+}{\underset{H}{N}}\text{-}C\overset{S}{\underset{S^-}{\lessgtr}} \rightleftharpoons (CH_3)_2NH + CS_2 \xrightleftharpoons{H^+HCO_3^-} (CH_3)_2NH_2HCO_3^-$$

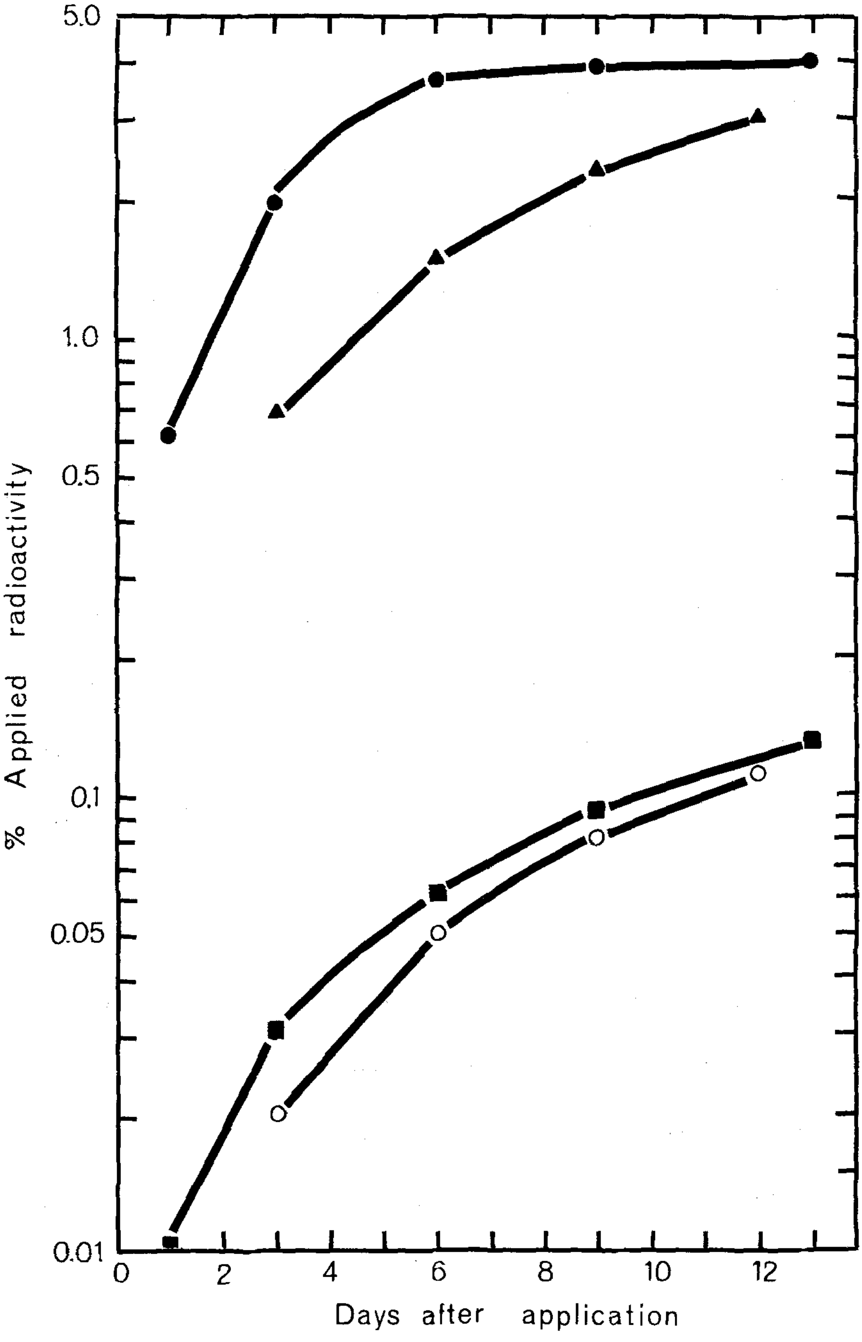


Figure 2. Evolution of volatile products from plants treated with radioactive dimethyldithiocarbamates. CS_2 from ^{35}S-KDDC ●; CS_2 from ^{35}S-Zn(DDC)$_2$ ■ ; CO_2 from $^{14}CH_3$-KDDC ▲; CO_2 from $^{14}CH_3$-Zn(DDC)$_2$ ○ .

Hallaway (11) has shown that the half life of diethyldithiocarbamate in acid solution ranges from 2 min at pH 4.0 to 860 min at pH 6.6. At pH 5.7, the approximate pH of moist leaf surfaces, the half life was 107 min. Although these results are for the diethyl derivative, it is reasonable to expect that the dimethyl compound would behave in a similar fashion. The KDDC compounds were applied in pH 7.4 phosphate buffer in an attempt to reduce the effects of dissolved CO_2. The other product of acidic decomposition, trimethylammonium bicarbonate, would be unstable under these conditions, and presumably give rise to appreciable quantities of trimethylamine. Only trace amounts (0.013% of applied ^{14}C-KDDC and 0.016% of applied ^{14}C-$Zn(DDC)_2$) of this substance were detected over a 14-day period, indicating that either decomposition at the leaf surface is a minor phenomenon, or that the liberated trimethylamine or trimethylammonium bicarbonate is rapidly and selectively absorbed by the plant tissues.

The alternative to surface decomposition is degradation of the dithiocarbamates in the outer cell layers of the leaf. Ziram and thiram, or at least DDC ions derived therefrom, are known to be absorbed by intact leaves (9, 12). Direct decomposition of these compounds by acidic cellular fluids would follow the reaction already discussed. Released carbon disulfide would diffuse from the cells into the atmosphere while the trimethylamine would be oxidatively metabolized.

It would appear that DDC ion is not demethylated prior to release of CS_2 since this would give rise to methylisothiocyanate by rearrangement of the monomethyldithiocarbamate, and this substance was not detected. Neither was another potential volatile decomposition product, hydrogen sulfide, detected in these experiments.

DDC ions are conjugated in higher plants to form derivatives of glucose and alanine (13, 14). These substances are in equilibrium with each other and with a small amount of DDC ion (15). It is probable that part or all of the carbon disulfide produced later than seven days after application of KDDC is derived from decomposition of DDC ions present in this equilibrium.

A more complete analysis of the mechanisms underlying the release of volatile decomposition products of dithiocarbamate fungicide residues on higher plants is not possible at this time. The salient point illustrated by our results is that such volatile products are formed and constitute one possible route for the dissipation of these pesticides.

References

1. T. CALLAN and N. J. STRAFFORD, J. Soc. Chem. Ind. 43, 1T (1924).
2. R. M. WEED, S. E. A. MCCALLAN, and L. P. MILLER, Contribs. Boyce Thompson Inst. 17, 299 (1953).
3. S. E. A. MCCALLAN, L. P. MILLER, and R. M. WEED, Contribs. Boyce Thompson Inst. 18, 39 (1954).
4. C. E. COX, H. D. SISLER, and R. A. SPURR, Science 114, 643 (1951).
5. H. D. SISLER and C. E. COX, Phytopathology 41, 565 (1961).
6. J. W. HYLIN, Bull. Environ. Contamination Toxicol. 1, 76 (1966).
7. H. L. PEASE, J. Ass. Offic. Agr. Chem. 40, 1113 (1957).
8. C. F. GORDON and A. L. WOLFE, Anal. Chem. 32, 574 (1960).
9. J. W. HYLIN, Abstracts, 145th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept. 1963, p 17a.
10. P. ZUMAN and R. ZAHRADNIK, Z. Phys. Chem. (Leipzig) 208, 135 (1957).
11. M. HALLAWAY, Biochim. Biophys. Acta 36, 538 (1959).
12. F. MASSAUX, Mededel. landbHogesch. Gent 28, 590 (1963).
13. J. KASLANDER, A. KAARS SIJPESTEYN, and G. J. M. VAN DER KERK, Biochim. Biophys. Acta 52, 396 (1961).
14. J. KASLANDER, A. KAARS SIJPESTEYN, and G. J. M. VAN DER KERK, Biochim. Biophys. Acta 60, 417 (1962).
15. H. M. DEKHUIJZEN, Neth. J. Plant Path. 70, Suppl. 1, 75 pp (1964).