CHROM. 6279

Separation and determination of some systemic fungicides and their metabolites by thin-layer chromatography

The advantages and usefulness of systemic fungicides for the control of plant diseases have been recognized for a number of years[1,2].

A systemic fungicide is less subject to adverse weather conditions than a contact fungicide and will therefore offer protection from diseases for a longer period of time. The systemic properties of these fungicides allow protection of new plant growth, which otherwise would have to be treated after it develops. For these reasons systemic fungicides offer economical advantages in comparison with contact fungicides.

Benomyl, 1-(butylcarbamoyl)-2-benzimidazolecarbamic acid methyl ester is one fungicide which has received considerable attention during the last few years because of its ability to control a variety of plant diseases[3,4]. Thiophanate methyl, 1,2-bis(3-methoxycarbonyl-2-thioureido)benzene and the chemically closely related thiophanate, 1,2-bis(3-ethoxycarbonyl-2-thioureido)benzene are also of interest because of their broad spectrum of activity[5-7].

Several authors have shown that both benomyl and thiophanate methyl are degraded in aqueous solution to 2-benzimidazolecarbamic acid methyl ester (MBC)[8-10], which is also fungitoxic. It is reported to be the main component found in plants treated with benomyl or thiophanate methyl[8,9]. It is quite stable and persists in the plant for some time[8]. Further chemical degradation of MBC could produce chemical compounds such as 2-aminobenzimidazole and benzimidazole. The presence of these materials in the tissue of treated plants, however, has not been determined. Thin-layer chromatography (TLC) has been used by several workers to separate and determine mixtures of benomyl and MBC and of thiophanate methyl and MBC[8,9]. This paper describes the separation and determination of all three fungicides benomyl, thiophanate methyl and thiophanate with MBC, benzimidazole and 2-aminobenzimidazole. The two last compounds originate from MBC due to loss of the carbamic acid ester group. The separation and determination was achieved by two-dimensional TLC. A sensitive spray reagent for the detection of MBC was also found.

Experimental

Samples of analytical grade quality benomyl and MBC were supplied by the DuPont de Nemours Co. and samples of thiophanate methyl and thiophanate were obtained from Ciba-Geigy Canada Ltd. Benzimidazole and 2-aminobenzimidazole were purchased from chemical supply houses. Eastman Chromagram sheets coated with silica gel (100 μ) and containing a fluorescent indicator were used. The two-dimensional solvent systems used were: Solvent 1, benzene–methanol (9:1) and Solvent 2, ethyl acetate–chloroform (6:4). They were selected as the most efficient after experimenting with a large number of solvents and solvent mixtures. Compounds were detected under 254 nm UV light as dark spots. The chromogenic spray reagent used for the detection of MBC was a 0.5% solution of N-2,6-trichloro-*p*-benzoquinone-imine. This reagent has previously been used for the detection of carbamates[11].

The results of the two-dimensional TLC separation are shown in Fig. 1.

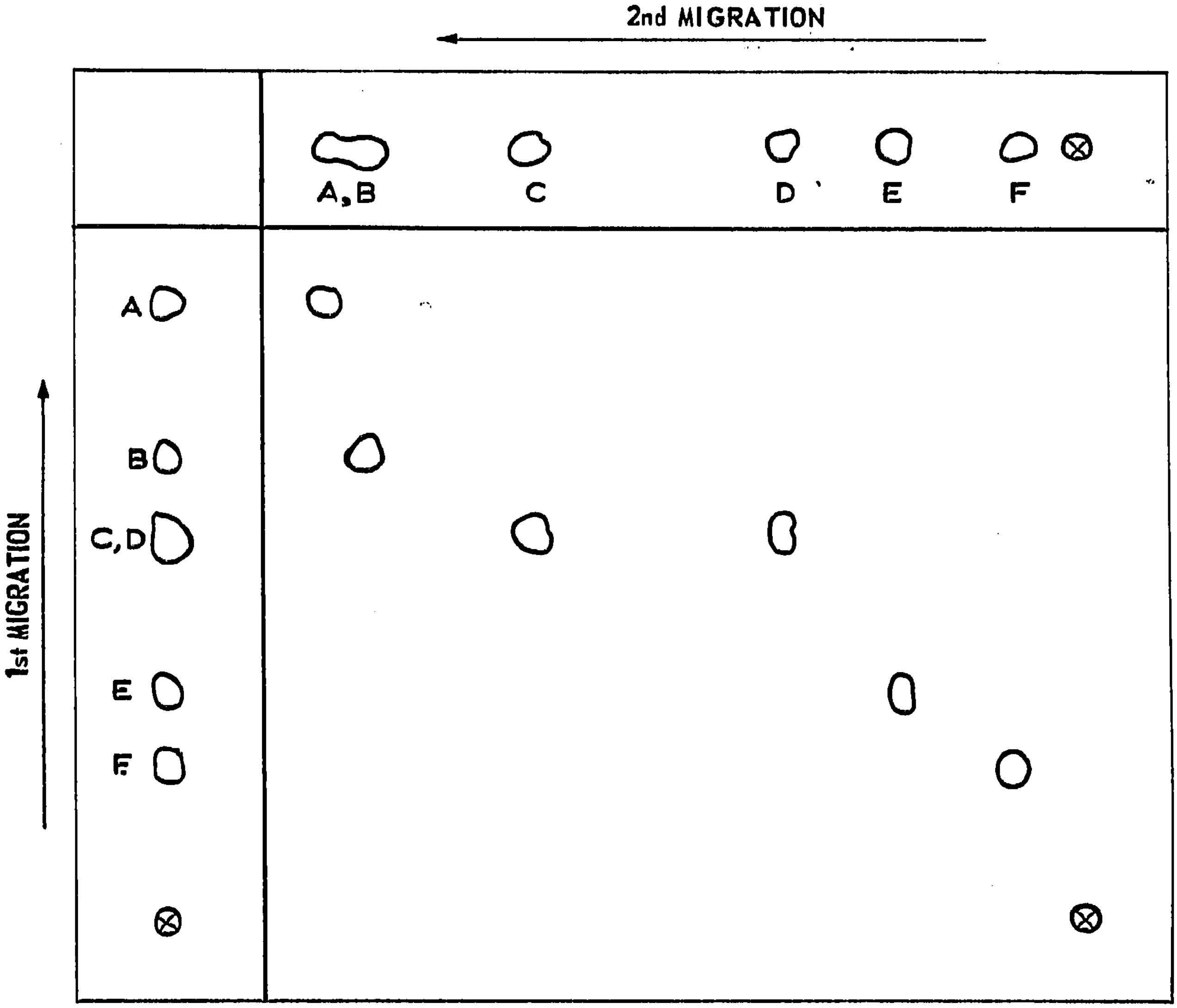

Fig. 1. Two-dimensional thin-layer chromatogram of benomyl (A), thiophanate (B), thiophanate methyl (C), MBC (D), benzimidazole (E), and 2-aminobenzimidazole (F). Solvent systems: (1) benzene–methanol (9:1); (2) ethyl acetate–chloroform (6:4).

Results and discussion

The R_F values of the TLC separation are presented in Table I.

After development of the chromatogram in the two solvent systems, the compounds were visible under short-wave (254 nm) UV light as distinct dark spots on a pink fluorescent background.

It was also found that MBC gave a very sensitive colour reaction when sprayed with a 0.5% solution of N-2,6-trichloro-*p*-benzoquinoneimine in cyclohexane, a reagent which has been used previously for the detection of carbamates[11]. After the spray application the chromatogram was developed at 100° for 10 min to form an intensely blue coloured spot. The colour is not persistent and will fade after a period of several minutes. The other compounds did not give this colour reaction. Amounts as low as 25 ng of MBC can be detected by this method.

TABLE I

APPROXIMATE R_F VALUES

Compounds	*Solvent 1*	*Solvent 2*
Benomyl	0.88	0.91
Thiophanate	0.66	0.87
Thiophanate methyl	0.56	0.68
MBC	0.56	0.37
Benzimidazole	0.34	0.23
2-Aminobenzimidazole	0.25	0.07

It was found that the two-dimensional technique offers excellent accuracy and better separation than the development in one direction only.

Efforts are under way to apply this method to extracts of plant material and soil samples containing these fungicides.

The assistance of G. F. ZAJACZ, technical assistant, is gratefully acknowledged. The author thanks the DuPont de Nemours Co. and Ciba-Geigy Canada Ltd. for the analytical grade samples of benomyl, MBC, thiophanate and thiophanate methyl.

Canada Department of Agriculture, Research Station,
Harrow, Ontario (Canada)
F. G. VON STRYK

1 D. C. ERWIN, *FAO Plant Prot. Bull.*, 18 (1970) 73.
2 E. EVANS, *Pestic. Sci.*, 2 (1971) 192.
3 H. M. LOUX, *US Pat.*, 3,010,068 (1961).
4 A. FUCHS, A. L. HOMANS AND F. W. DE VRIES, *Phytopathol. Z.*, 69 (1970) 4.
5 D. C. ERWIN, H. MEE AND J. J. SIMS, *Phytopathology*, 58 (1968) 528.
6 *Ger. Pat.*, Offen. 1,930,540 (1970) to Nippon Soda Co.
7 R. T. MERCER, *Pestic. Sci.*, 2 (1971) 214.
8 W. W. KILGORE AND E. R. WHITE, *Bull. Environ. Contam. Toxicol.*, 1 (1970) 67.
9 J. W. VONK AND A. KAARS SIJPESTEIJN, *Pestic. Sci.*, 2 (1971) 160.
10 H. A. SELLING, J. W. VONK AND A. KAARS SIJPESTEIJN, *Chem. Ind. (London)*, (1970) 1625.
11 R. L. METCALF, T. R. FUKUTO, C. COLLINS, K. BORCK, H. T. REYNOLDS AND M. F. OSMAN, *J. Agr. Food Chem.*, 14 (1966) 580.

Received April 28th, 1972

J. Chromatogr., 72 (1972) 410–412