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Note

Agricultural fungicides

I. Identification of systemic fungicides using thin-layer chromatography

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In the last few years a number of systemic fungicides have been developed and their use is now widespread. These compounds may be translocated throughout the plant and it is therefore desirable to be able to screen foodstuffs for residues before individual quantitative determination. Unlike the organochlorine[1] and organophosphorus[2] insecticides these compounds cannot be classified into a group of similar nature for residue analysis and they are not all amenable to direct gas–liquid chromatography. Von Stryk[3] has applied thin-layer chromatography (TLC) to the separation and determination of benomyl, thiophanate, thiophanate-methyl and their metabolites using a two-dimensional technique, but otherwise the TLC systems described apply only to single compounds[4–6].

A TLC method has been developed for the identification of eight systemic fungicides (see Table I) which are all widely used at present. Since benomyl is very rapidly broken down to methyl benzimidazol-2-ylcarbamate (MBC)[4,7], the latter compound has also been included. No single TLC system could be found that would separate all eight fungicides so it was decided to use a number of different systems together with a coding scheme not dependent on the measurement of R_F values. Silica gel and alumina were found to be the most useful adsorbents and all the fungicides except dodemorph and tridemorph could be distinguished using the four systems given in Table II. The fungicides are visualised under UV light or by spraying with potassium iodobismuthate solution[8] followed by exposure to bromine vapour. The detection limits are given in Table I.

EXPERIMENTAL

Materials and methods

Apparatus. The following apparatus was used: Chromatographic development tanks with internal liners of filter paper, 10-μl syringe, and a chromatographic spray.

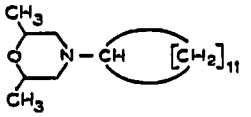
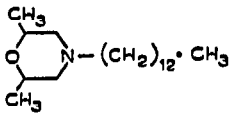
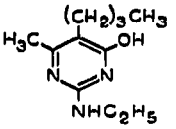
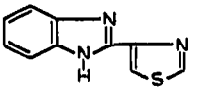
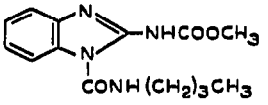
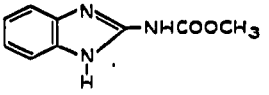
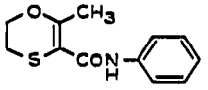
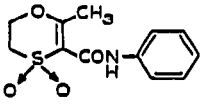
Reagents. The following TLC aluminium sheets were used: Aluminium Oxide F_{254} neutral (Type E), 20 × 20 cm, 0.20-mm layer thickness, Merck Catalogue No. 5550/0025*; Silica Gel 60 F_{254}, 20 × 20 cm, 0.20-mm layer thickness, Merck Catalogue No. 5554*.

* E. Merck, Darmstadt, G. F. R.

TABLE I

FUNGICIDES COVERED BY THE PROPOSED SCHEME

Method of detection: UV, using plates with fluorescent indicator and UV light (254 nm); colour, using potassium iodobismuthate solution followed by bromine vapour.

Fungicide	*Structure*	*Minimum detectable amount*	
		Method	*Amount (μg)*
Dodemorph		Colour	0.25
Tridemorph		Colour	0.25
Dimethirimol		Colour	0.6
Ethirimol		Colour	0.6
Thiabendazole		UV	1.0
Benomyl		UV	0.8
Methyl benzimidazol-2-ylcarbamate		UV	see text
Carboxin		UV	0.5
Oxycarboxin		UV	0.5

For chromatographic solvents used see Table II. All solvents were analytical-reagent grade and used as supplied. All solvent mixtures should be freshly prepared.

The potassium iodobismuthate solution[8] used was prepared as follows:

Solution A: Dissolve 1.7 g bismuth(III) nitrate and 20 g tartaric acid in 80 ml water. Solution B: Dissolve 16 g potassium iodide in 40 ml water-Stock solution: Mix equal parts of A and B. Spray solution: Dissolve 10 g tartaric acid in 50 ml water and add 10 ml of the stock solution. Prepare freshly each day.

For the reference fungicide solutions a concentration of 2 mg/ml in acetone is used. Prepare freshly each day and keep in stoppered vials.

Procedure

Apply 1–2 μl of the solutions containing the unknown fungicides onto each of four TLC sheets (3 silica gel and 1 aluminium oxide) at a distance of at least 15 mm from the bottom and 25 mm from the sides of the sheet. Dry the sheets in air for 5 min. Develop the sheets in solvents 1, 2, 3 and 4 as shown in Table II for a length of run of 120 mm at room temperature. Remove the sheets from the tanks and allow them to air dry. Rerun the sheet from system 3 in the same developing solvent. When the sheets are dry, rule lines across them at R_F values of 0.25, 0.50 and 0.75 so as to

TABLE II

CHROMATOGRAPHIC SYSTEMS USED IN THE SEPARATION OF THE FUNGICIDES

System No.	*Adsorbent*	*Mobile phase*
1	Silica gel	Diethyl ether–glacial acetic acid–methanol (100:5:2)
2	Silica gel	Acetone
3*	Silica gel	Light petroleum (60–80°)–acetone (3:1)
4	Aluminium oxide	Diethyl ether–methanol (40:1)

* The sheets were run twice in this solvent.

TABLE III

CODES FOR FUNGICIDES CHROMATOGRAPHED IN SYSTEMS 1, 2, 3 AND 4

Code	*Fungicide*
BBAA	Ethirimol
BBAB	Thiabendazole
BCAA	Oxycarboxin
BCCD	Dodemorph
	Tridemorph
CBAA	Methyl benzimidazol-2-ylcarbamate
CBBA	Dimethirimol
DCBC	Carboxin
DCCC	Benomyl

divide the sheets horizontally into the following sections: code A: spots located in the section bounded by R_F 0.00 to 0.25; code B: spots located in the section bounded by R_F 0.25 to 0.50; code C: spots located in the section bounded by R_F 0.50 to 0.75; code D: spots located in the section bounded by R_F 0.75 to 1.00. Visualise the position of the compounds first by observation under UV light of wavelength 254 nm and then by spraying with potassium iodobismuthate solution followed by exposure to bromine vapour.

Record the letters in which the spots appear on each sheet as a composite code of four letters in the sequence corresponding to chromatographic system number (Table II). Compare the codes with the list given in Table III to obtain a preliminary identification of the sample. The identification of the sample is then confirmed by chromatography on a sheet or sheets with standard spots of the suspected fungicides. Spots of the sample solution are also overspotted with spots of the suspected compounds. The unknown fungicide is identified by giving a single spot with the correct standard while all the other standards give rise to double spots.

DISCUSSION

A method for the separation and identification of eight commonly used systemic fungicides has been presented. The method should be capable of being extended by the use of further TLC adsorbents and solvent systems to cover new systemic fungicides.

Dodemorph and tridemorph could not be separated in any of the systems tried and both showed signs of decomposition with tailing and double spots on silica gel sheets.

The presence of thiabendazole may be confirmed by running on Merck silica gel sheets not containing the fluorescent indicator. This increases the R_F of this compound without affecting the other fungicides. The formation of transition metal complexes of thiabendazole has been reported[9] and a complex between this compound and the fluorescent indicator may be formed in this instance.

No limit of detection has been stated for methyl benzimidazol-2-ylcarbamate owing to the difficulty of detection of this compound. Von Stryk[3] has reported a detection limit of 25 ng on a silica gel sheet by spraying with a 0.5% solution of N-2,6-trichloro-*p*-benzoquinoneimine followed by heating for 10 min at 100 ° to give a blue colour, but we were unable to reproduce this result.

During the identification of these fungicides extracted from foodstuffs, co-extractives may interfere and affect the separation of the fungicides on the TLC sheets and colouring matter may affect the identification. Publication of the results of work on these aspects will follow and quantitative estimation of fungicides using bioassay is in progress.

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