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Note

Agricultural fungicides

II. Detection and identification of systemic benzimidazole fungicides on citrus fruit

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Systemic fungicides are increasingly being used for the post-harvest protection of citrus fruit. A limit of 6 mg/kg (based on the weight of the whole fruit) for residues of thiabendazole has been recommended[1–3] and tolerances for benomyl, methyl benzimidazol-2-ylcarbamate and thiophanate-methyl are under consideration[4]. Consequently, there is a need for methods for the detection and determination of residues of these fungicides in citrus fruit. When the treatment of the fruit is unknown, much time may be wasted in discovering which (if any) fungicide is present. A simple method for the detection and identification of thiabendazole, benomyl, methyl benzimidazol-2-ylcarbamate (MBC) and thiophanate-methyl on citrus fruit skin has been developed.

It has been reported that, by a careful choice of mobile phase and adsorbent, the separation of nine systemic fungicides can be achieved[5]. These included thiabendazole, benomyl and MBC; thiophanate-methyl may also be separated using the same system. However, it was found that neither of the detection systems used in that method (viewing under ultraviolet light of wavelength 254 nm or spraying with potassium iodobismuthate) was capable of detecting small amounts of fungicide in the presence of large amounts of coloured co-extractives such as are obtained from citrus fruit skins. By combining this thin-layer separation of the fungicides with the bioassay technique of Homans and Fuchs[6], it is possible to detect and identify the four systemic benzimidazole fungicides on citrus fruit skin at the levels shown in Table I. This enables the analyst to identify the fungicide present and carry out the relevant quantitative residue method if necessary.

MATERIALS AND METHODS

Apparatus and reagents

Chromatographic tanks, solvents, sprayguns and pre-coated TLC sheets have all been described in Part I[5].

50-μl syringe.

Kuderna–Danish evaporator of 500 ml capacity for large volumes of solvents and a micro-Snyder column for small volumes of solvents.

Automatic shaker.

Ethyl acetate, AR grade.

Reference fungicide solutions: 100 mg/100 ml in acetone (except MBC, dissolved in 1,4-dioxane) and diluted as required.

Fungal spore suspension

The fungus (*Cladosporium cladosporioides*, Herb. IMI 45534) was obtained from the Commonwealth Mycological Institute, Ferry Lane, Kew, Great Britain, grown on dextrose agar and suspended in a medium containing glucose and mineral salts as described by Homans and Fuchs[6]. Spraying was carried out with a chromatographic spraygun.

PROCEDURE

Weigh the whole fruit and then carefully peel the outside layer of skin from the fruit, chop finely and mix well. Weigh the separated peel. Weigh 10 g of the peelings into a 50-ml conical flask and add 30 ml of ethyl acetate. Shake the flask for 15 min and then decant the solvent through a small filter plugged with cotton wool into a Kuderna–Danish evaporator fitted with a 10-ml graduated test tube. Wash the cotton wool with 10 ml of ethyl acetate. Shake the peelings with a further 30 ml of ethyl acetate for 15 min, decant the solvent into the evaporator and wash the cotton wool as before. Reduce the volume of the solvent to approximately 4 ml on a steam-bath and finally to 1 ml using the micro-Snyder column. Make this volume up to 2 ml with acetone. Apply 20 μl of this extract (corresponding to 0.1 g of fruit) to four TLC sheets[5] by repeated spotting from a 50-μl syringe to give a spot of 5 mm diameter. To each plate also apply a small amount (approximately 100 ng) of each fungicide for comparison of R_F values. Develop the sheets as previously described[5]. After air drying, the sheet from solvent system 1 must be lightly sprayed with saturated sodium bicarbonate solution to neutralise the last traces of acetic acid. Spray each sheet evenly but lightly with the spore suspension of *Cladosporium cladosporioides* and incubate them in a humid atmosphere at 25° for 2 days. Zones of inhibition of growth indicate the positions of the fungicides. The identity of any fungicide in the sample is obtained by comparing the R_F values of the inhibition zones of the unknown fungitoxic substances with those of the standard fungicides.

RESULTS

Recovery experiments were performed with samples of orange, lemon and grapefruit skin to which various amounts of these fungicides had been added. The minimum detectable amount of the fungicides was determined for each fruit (Table I). These minimum levels may be further reduced if the sample is applied to the TLC plate as a streak by means of a semi-automatic applicator.

Thiophanate-methyl was not included in the original method[5], but no modification of the technique has been necessary in order to include this compound. In this method each fungicide was given a code depending on the R_F value obtained in the four different TLC systems. The code for thiophanate-methyl is DCAB.

One sample of grapefruit of unknown origin was found to contain approximately 3 mg/kg of thiabendazole in the peel.

TABLE I

MINIMUM AMOUNTS OF FUNCIGIDES DETECTABLE IN CITRUS FRUIT SKIN (mg/kg)

Fungicide	*Orange*	*Lemon*	*Grapefruit*
Thiabendazole	2.0	2.0	2.0
Benomyl*	1.0	1.0	1.0
MBC	0.5	0.5	0.5
Thiophanate-methyl**	1.0	1.0	1.0

* Detected as MBC.
** Detected in part as MBC.

DISCUSSION

A method for the extraction, detection and identification of thiabendazole, benomyl, MBC and thiophanate-methyl on citrus fruit has been presented. The method should be capable of extension to other systemic fungicides provided suitable fungi can be found for the bioassay stage. A number of different fungi has been studied in order to produce a suitable test for all systemic fungicides simultaneously but none was suitable. *Cladosporium cladosporioides* was chosen for this method because of its high sensitivity towards benzimidazole fungicides and low sensitivity towards other systemic fungicides. Table II shows the minimum amount of fungicide needed to inhibit growth (applied as a spot from a standard solution).

TABLE II

MINIMUM AMOUNTS OF FUNGICIDES DETECTABLE AS STANDARDS

Fungicide	*Minimum amount (μg)*
Thiabendazole	0.05
Benomyl*	0.05
MBC	0.01
Thiophanate-methyl	0.02
Dodemorph	10
Tridemorph	10
Dimethirimol	10
Ethirimol	10
Carboxin	0.5
Oxycarboxin	10

* As benomyl, 0.02 μg as MBC.

This method is considerably more sensitive and specific than those described in the previous paper[5]. Benomyl decomposes to MBC even on a TLC sheet and extracts from fruit were totally decomposed to MBC. Thiophanate-methyl also breaks down to MBC, though apparently at a slower rate than benomyl. A close examination of the method proposed by Von Stryk[7] for the detection of small amounts of MBC (0.025 μg) using N,2,6-trichlorobenzoquinoneimine showed that the coloration appeared to be due to traces of peroxide in the solvent (1,4-dioxane), not to MBC. Further tests on other peroxide-containing solvents (tetrahydrofuran, di-*n*-butyl ether) and on benzoyl peroxide showed this to be the case.

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