Fungicide Residues

Part I. The Detection, Identification and Determination of Residues of Quintozene in Tomatoes, Lettuces and Bananas by Gas Chromatography

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A simple method for determining residues of quintozene in tomatoes, lettuces and bananas is presented. After extraction with hexane, quintozene is separated from interfering co-extractives by a partition process with dimethylformamide followed by chromatography on an alumina column, and is quantitatively determined by electron-capture gas - liquid chromatography. A confirmatory chemical test for quintozene is also described.

Quintozene (pentachloronitrobenzene) is used mainly as a soil fungicide or seed or transplant dressing for the control of many root-rotting and damping-off diseases; it is also applied directly to foliage for botrytis control, especially on lettuce grown under glasshouse conditions. It is a persistent compound, retaining its fungicidal properties for a considerable time,[1] but in moist soil it is slowly reduced to pentachloroaniline,[2] which is also stable and shows lower fungicidal activity. Quintozene is generally regarded as being non-systemic in action.

Methods of analysis for residues of quintozene have been reviewed by Zweig.[3] The three principal methods described are colorimetric,[4,5] polarographic[5] and gas chromatographic with either microcoulometric[5,6] or electron-capture detectors.[7,8] This last procedure is widely used for the determination of residues of organochlorine insecticides and consequently is suitable for the detection and determination of residues of quintozene on a routine basis.

The report of the F.A.O./W.H.O. Joint Meeting in 1969 expressed a desire for further work on the development of analytical methods with greater sensitivity and their evaluation for regulatory purposes. The procedure described in this paper has therefore been devised to provide suitable extraction, clean-up and gas-chromatographic conditions for the detection, identification and determination of residues of quintozene and its degradation product pentachloroaniline in tomatoes, lettuces and bananas, these crops being taken as representatives of the types of product on which quintozene may be used.

Experimental

The samples used in this work were obtained from retail sources and therefore had unknown histories of treatment except for some control lettuces, obtained by courtesy of the Glasshouse Crops Research Institute, Littlehampton, which were known not to have been treated with quintozene. The procedure of a partition process with dimethylformamide followed by clean-up on an alumina column has been used successfully in this laboratory for many years for the determination of residues of organochlorine insecticides in a wide variety of sample substrates.[9] A similar procedure was therefore applied to samples to which quintozene had been added in various concentrations, and gas-chromatographic determinations of the cleaned-up extract were carried out on several stationary phases. Of the columns tested, that containing 5 per cent. of EGSS-X on Chromosorb G (100 to 120 mesh) was found to be the most suitable for quantitative determinations. For confirmatory purposes, the SE-52 and Apiezon L columns used for organochlorine compounds[10] were found to be suitable. Table I gives the relative retention times of quintozene and pentachloroaniline under the operational conditions.

Both quintozene and pentachloroaniline have very short retention times on the columns (Table I) that are normally used in the determination of organochlorine pesticides, and to avoid interferences from co-extracted materials that are generally also eluted rapidly from the column, all the reagents used in the method must be carefully purified before use (see under Reagents). Many interfering peaks were initially obtained from the batches of sodium sulphate and alumina used owing to inadequate heat treatments. In any chromatographic analytical procedure, in which a chemical species is "identified" by measuring its retention

TABLE I

RETENTION TIMES OF QUINTOZENE AND PENTACHLOROANILINE RELATIVE TO DIELDRIN (= 1·00)

Column	Column temperature/°C	Relative retention time	
		Quintozene	Pentachloroaniline
EGSS-X	200	0·24	0·53
	170	0·19	0·48
SE-52..	200	0·25	0·37
Apiezon L	196	0·26	0·37

time (relative to a standard pure compound) on at least three columns with widely differing polarities, it is preferable that at some stage during the analysis the species is unambiguously characterised. This is usually achieved by either (*a*) isolating the chromatographic fraction corresponding to the "identifying" retention time and then characterising the species chemically or by using instrumental methods of analysis (*e.g.*, infrared or mass spectrometry), or (*b*) chemically pre-treating the injected solution to give a suitable derivative that is chromatographically distinguishable from the parent material under the same conditions. The latter approach has been used to provide a chemical confirmatory test for quintozene, namely, reduction with lithium aluminium hydride in diethyl ether to form pentachloroaniline in high yield. Formation of pentachloroaniline can be confirmed by its removal by shaking with sulphuric acid. However, this confirmatory test is complicated by the fact that pentachloroaniline is also a natural metabolite of quintozene[4,6] and consequently vegetable samples that contain quintozene may also contain pentachloroaniline. Therefore, in applying this chemical test for quintozene, all traces of pentachloroaniline present in the untreated sample must first be removed by treatment with sulphuric acid. In all the vegetable samples examined, no co-extracted materials gave rise to peaks that interfered with the gas-chromatographic determination of quintozene. In both retail and control lettuce samples, a peak having a retention time similar to that of pentachloroaniline was observed, but as this peak disappeared after reduction of the sample with lithium aluminium hydride no interference occurred in the identification and determination of pentachloroaniline formed during the confirmatory test for quintozene. Lettuce samples, however, which were known to have been treated with thiram and zineb (*i.e.*, control lettuces) showed several additional peaks in the vicinity of that of pentachloroaniline. These peaks were probably due to sulphur-containing degradation products derived from the added fungicides and were easily removed from the organic extract by elution through a silver nitrate - alumina column prior to carrying out the confirmatory test. By using carefully purified reagents, and following the extraction and clean-up procedures described above to remove interfering co-extractives, this method allows the chemical identification and quantitative determination of quintozene in vegetable materials at the required residue level.

REAGENTS—

Analytical-reagent grade materials should be used whenever possible.

Hexane—Distil hexane from sodium hydroxide solution and, to check its suitability, concentrate a 50-ml volume to 1 ml and examine the product by gas - liquid chromatography.

Sodium sulphate—Heat anhydrous sodium sulphate at 500 °C for 36 hours and allow it to cool in a desiccator.

Alumina—Heat alkaline aluminium oxide (100 to 240 mesh) for 4 hours at 500 °C. Allow it to cool in a desiccator, add 5 per cent. w/w of water dropwise and shake the mixture for 2 hours; store the product in a tightly stoppered bottle.

Dimethylformamide—Laboratory-reagent grade material is used.

Quintozene—Recrystallise technical quintozene from ethanol and dry it in a desiccator (m.p. 142 °C; literature value,[2] 143·6 °C).

Pentachloroaniline—Add 0·3 g of lithium aluminium hydride to 1 g of recrystallised quintozene in 30 ml of dry diethyl ether. Destroy the excess of reagent by adding water dropwise, separate the ethereal layer, dry it over anhydrous sodium sulphate, filter it and evaporate it to dryness. Recrystallise the product from ethanol and purify it by sublimation (m.p. 228 °C; literature value,[11] 232 °C). The infrared spectrum of the product in a Nujol mull was identical with the published spectrum of pentachloroaniline.[4]

APPARATUS—

Gas chromatograph—The detector was of the tritium-foil electron-capture type; the columns, used isothermally, were as follows.

1. 1·6 m × 4 mm i.d. glass column containing 5 per cent. of EGSS-X on Chromosorb W (100 to 120 mesh), operated at 170 or 200 °C.
2. 1·8 m × 3 mm i.d. glass column containing 1·3 per cent. of SE-52 and 0·15 per cent. of Epikote 1001 on silanised Chromosorb G (60 to 80 mesh), operated at 200 °C.
3. 1·2 m × 3 mm i.d. glass column containing 1 per cent. of Apiezon L and 0·15 per cent. of Epikote 1001 on silanised Chromosorb G (60 to 80 mesh), operated at 196 °C.

For all columns, oxygen-free nitrogen was used as the carrier gas at a flow-rate of about 100 ml min^{-1}.

Homogeniser.

Evaporator—A Kuderna-Danish instrument, of 500-ml capacity, was used.

PROCEDURE—

Quantitative procedure—To 10 g of sample (taken from a 100 to 120-g batch of well mixed sample) add 50 ml of hexane and 30 g of anhydrous sodium sulphate and blend the mixture in the homogeniser for 2 minutes. Filter the solution through a short column of anhydrous sodium sulphate into the Kuderna-Danish evaporator and repeat the extraction three times with 25 ml of hexane. Reduce the volume of the combined hexane extracts to approximately 5 ml on a steam-bath. Make the volume up to 20 ml with hexane in a 100-ml separating funnel and extract the solution with 10 ml of dimethylformamide saturated with hexane. Transfer the lower dimethylformamide extract into a second 100-ml separating funnel and extract the remaining hexane phase twice with 10 ml of dimethylformamide saturated with hexane. Wash the combined extracts with 10 ml of hexane saturated with dimethylformamide and transfer the dimethylformamide extract into a 500-ml separating funnel. Wash the residual hexane solution with 10 ml of dimethylformamide saturated with hexane and add the mixture to the first extract. To the combined extracts, add 300 ml of a 2 per cent. w/v sodium sulphate solution, shake the mixture well and allow the hexane layer to separate. Fill a chromatographic column (30 cm × 13 mm) with a slurry of 10 g of alumina in hexane, allow it to settle and drain off the hexane until the solvent level reaches the top of the alumina. Discard the aqueous layer from the separating funnel and slowly run the hexane solution on to the column. Wash the separating funnel twice with 2 ml of hexane and add the washings to the column. Elute the column with hexane and collect 100 ml of the eluate. Examine a 5-μl volume of the eluate by gas - liquid chromatography and then adjust the volume to give a quintozene concentration of approximately 0·1 μg ml^{-1}. Calculate the concentration of the quintozene present by comparing the peak height with that obtained from a 5-μl injection of a standard solution (0·1 μg ml^{-1}) of quintozene in hexane. A calibration graph, prepared by injecting 5-μl volumes of standard solutions and plotting the resultant peak height against the weight of quintozene taken, showed that the detector response was linear over the range 0·1 to 1·0 ng. With a signal-to-noise ratio of 3:1 the limit of detection for quintozene was found to be 5 pg on the 10-g sample taken.

Reduction of quintozene—To 1 ml of a solution of pure, recrystallised quintozene in dry diethyl ether (1 μg ml^{-1}) in a 10-ml calibrated test-tube, add a small amount of lithium aluminium hydride and shake for 0·5 minute. Add 5 ml of distilled water, dropwise, with shaking, and centrifuge the mixture if necessary. Add diethyl ether to give a total volume of the ether of 1 ml and examine a 5-μl volume of the ethereal extract by gas - liquid chromatography on each of the columns specified above. Calculate the concentration of pentachloroaniline present by comparison with a standard solution of pure pentachloroaniline in diethyl ether (1 μg ml^{-1}). The detector response for pentachloroaniline was shown to be linear over the range 1 to 5 ng injected, and the limit of detection (with a signal-to-noise ratio of 3:1) was 50 pg on the 10-g sample taken. It should be noted that if the reduction stage is allowed to continue for longer than the stated time, the yield of pentachloroaniline obtained is decreased owing to competing side reactions. By using the above procedure, three separate experiments gave yields of 105, 87 and 83 per cent. of pentachloroaniline.

Confirmatory test for quintozene in vegetable samples—After the extraction and clean-up procedures have been carried out on the vegetable sample, the quintozene content of the hexane extract is determined gas chromatographically and the quintozene is confirmed chemically by the following procedure.

Dissolve 0·75 g of recrystallised silver nitrate in a mixture of 0·7 ml of water *plus* 3 ml of acetone. Add the solution obtained to 10 g of alumina, shake the mixture well and warm it in a current of air to remove the acetone. Fill a chromatographic column (4 cm × 6 mm) with a slurry of 1 g of the prepared alumina in hexane and drain off the hexane until the solvent level reaches the top of the alumina. Run 1 ml of the hexane extract of the vegetable (0·1 μg ml^{-1}) on to the column, elute it with hexane and collect 20 ml of the eluate. Concentrate the eluate to 2 ml, add 0·5 ml of concentrated sulphuric acid and shake the mixture well to remove any pentachloroaniline that is already present. Evaporate 1 ml of the hexane phase to dryness in a gentle stream of air. Add 1 ml of diethyl ether to the residue and reduce the quintozene with lithium aluminium hydride as described above. Determine the concentration of pentachloroaniline in the reduced extract by gas - liquid chromatography. A final test can be made by shaking the reduced ethereal extract with a further 0·5 ml of concentrated sulphuric acid (to remove the pentachloroaniline formed from the reduction of quintozene) and examining the organic phase by gas - liquid chromatography. A completely clean trace should be obtained.

Results and discussion

Quintozene was added to tomato samples to give concentrations from 0·005 to 0·1 mg kg^{-1}. The recoveries obtained are listed in Table II. All blank determinations carried out on untreated samples, prior to recovery experiments, were in the range 0·0006 to 0·001 mg kg^{-1} and are substantially lower than the recommended tolerance level[12] (0·1 mg kg^{-1}) set for quintozene in tomatoes.

Similarly, Table III lists the recoveries obtained from the skins and fruits of bananas spiked separately with quintozene to give concentrations from 0·01 to 5·0 mg kg^{-1}. Blank

Table II

Recovery of quintozene added to tomatoes

Quintozene added/mg kg^{-1}	Quintozene recovered	
	Range, per cent.	Mean, per cent.*
0·1	87–106	94
0·05	79–92	83
0·025	88–92	90
0·01	81–93	87
0·005	85–102	91

* Average of three determinations on each sample.

Table III

Recovery of quintozene added to bananas

Sample	Quintozene added/mg kg^{-1}	Quintozene recovered	
		Range, per cent.	Mean, per cent.*
Skin	5·0	75–79	77
Fruit	5·0	75–90	82
Skin	1·0	79–87	83
Fruit	1·0	77–84	80
Skin	0·5	75–76	75
Fruit	0·5	75–76	75
Skin	0·1	74–80	77
Fruit	0·1	75–86	81
Skin	0·05	76–81	79
Fruit	0·05	75–79	77
Skin	0·01	85–99	92
Fruit	0·01	95–110	103

* Average of three determinations on each sample.

TABLE IV

RECOVERY OF QUINTOZENE ADDED TO CONTROL LETTUCES

Quintozene added/mg kg^{-1}	Quintozene recovered	
	Range, per cent.	Mean, per cent.*
5·0	98–101	99
1·0	96–107	100
0·5	97–101	99
0·1	110–113	111
0·05	86–94	90
0·01	95–125	108

* Average of three determinations on each sample.

determinations were carried out on all banana skins and fruits used and the amounts of quintozene residues found were in the range 0·006 to 0·01 mg kg^{-1} for banana fruits and 0·008 to 0·01 mg kg^{-1} for banana skins. The majority of the banana samples examined contained quintozene residues that were well below the recommended tolerance levels[12] set for quintozene, *i.e.*, 0·01 mg kg^{-1} for banana fruits and 1·0 mg kg^{-1} for whole bananas.

Recovery experiments were carried out on control lettuces, which were then treated with quintozene to give concentrations from 0·01 to 5·0 mg kg^{-1}. The results obtained are shown in Table IV. Blank determinations were in the range 0·012 to 0·018 mg kg^{-1}. The distribution of quintozene residues in retail lettuces is shown in Table V and, as expected, most of the applied fungicide was found to reside on the outermost leaves of the plant. Residue levels were found to be in the range 0·78 to 2·6 mg kg^{-1} for retail lettuces of Dutch origin (Table V) and 0·70 to 1·2 mg kg^{-1} for four English retail lettuces. All the retail lettuce samples examined contained quintozene residues that were above the recommended tolerance level[12] of 0·3 mg kg^{-1} set for lettuces; even the hearts of three out of four samples contained amounts of quintozene that exceeded this level.

TABLE V

DISTRIBUTION OF QUINTOZENE RESIDUES IN RETAIL LETTUCES*

Outer leaves†/ mg kg^{-1}	Inner leaves†/ mg kg^{-1}	Heart†/ mg kg^{-1}	Weighted average per lettuce/mg kg^{-1}
2·92	1·52	1·00	1·99
1·00	0·80	0·21	0·78
1·62	0·98	0·37	1·10
4·85	1·27	0·40	2·60

* Imported lettuces of Dutch origin.
† For a 10-g sample.

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