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FUNGICIDE RESIDUES

Colorimetric Method for the Determination of Dyrene Residues in Plant Material

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An analytical method for the microdetermination of the fungicide, 2,4-dichloro 6-(o-chloroanilino)-s-triazine (Dyrene) as a spray residue involves acid hydrolysis of the compound to form o-chloroaniline, which is diazotized and coupled with N-1-naphthylethylenediamine to produce a magenta color which is measured spectrophotometrically at 540 mμ. The method has been used for the determination of Dyrene residues in celery, onions, potatoes, and tomatoes.

THE IMPORTANCE OF DYRENE as a fungicide required the development of an accurate method for the determination of its residues on crops.

A colorimetric method developed by Burchfield and Storrs (*2*) at the Boyce Thompson Institute has been used to determine trace amounts of Dyrene and related compounds. The method depends upon the reaction of Dyrene with pyridine and measurement of the color developed when the solutions are made alkaline with aqueous sodium hydroxide. Quaternary pyridinium salts are first formed, which apparently undergo ring opening to form intensely colored Schiff bases. The color fades rapidly, but reproducible results can be obtained if the absorbance is read 2 minutes after addition of the alkali and compared to standards run under the same conditions.

An alternative method, reported in this paper, has been developed at the Pittsburgh Coke and Chemical, and Chemagro laboratories. It involves the acid hydrolysis of the Dyrene to form *o*-chloroaniline, which is diazotized and coupled with *N*-1-naphthylethylenediamine to yield an intensely colored azo dye. The color formed is essentially the same as in the Averell-Norris method for parathion (*1*).

In the development of a satisfactory method for the determination of Dyrene residues, a study was made to determine the optimum conditions for hydrolysis and color development. With pure solutions of hydrolyzed Dyrene, the color reaches a maximum in 10 minutes and is stable for at least another hour. However, the color develops more slowly in the presence of some plant extracts, taking up to 30 minutes to reach a maximum.

In view of this, all solutions should be measured for absorption one hour after addition of the coupling reagent. The color produced has an absorption maximum at 540 mμ (Figure 1). The absorption spectrum is identical with that obtained with *o*-chloroaniline, and Beer's law is obeyed over the range of 0 to 3.5 γ per ml. of final solution (Figure 2).

To determine the optimum conditions for the hydrolysis of Dyrene, hydrolyses were carried out in both acid and alkaline solutions and the efficiency of the hydrolysis reaction was measured by the colorimetric procedure. In these studies 215 γ of Dyrene in 10 ml. of isopropyl alcohol were refluxed on a steam bath for periods ranging from 0.5 to 5 hours with either 5 ml. of 5*N* hydrochloric acid or 7 ml. of 10*N* hydrochloric acid. An identical experiment was carried out using either 5 ml. of 5*N* potassium hydroxide or 10 ml. of 5*N* potassium hydroxide. In each case, an appropriate amount of acid or base was added to give an excess of 25 meq. of hydrochloric acid when the color was developed.

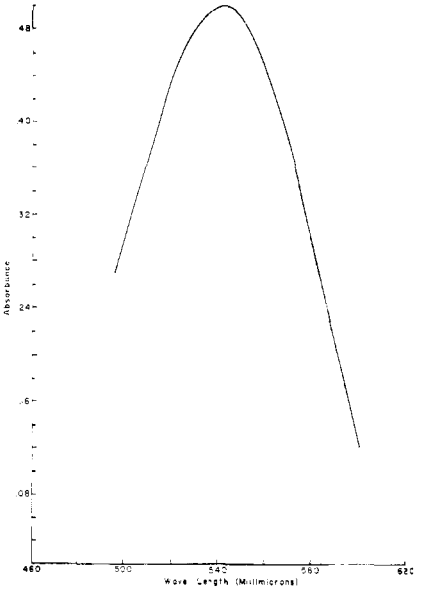

Figure 1. Absorption spectrum developed in determination of Dyrene

Complete hydrolysis of 215 γ of Dyrene should produce 100 γ of *o*-chloroaniline. Table I indicates that very little color develops when alkaline hydrolysis is used. The theoretical amount of color (based on *o*-chloroaniline) is obtained after 4 or 5 hours of refluxing with 5 ml. of 5*N* hydrochloric acid. At the higher

acid concentration, some Dyrene is apparently destroyed. A 5-hour hydrolysis period was adopted for routine work.

Experimental

Apparatus. Spectrophotometer, Beckman DU or equivalent.
Steam bath.
Waring Blendor.
Shaking machine.
Centrifuge, capable of holding 250-ml. bottles.

Reagents. All chemicals are reagent grade unless otherwise specified. All solvents were distilled in an all-glass apparatus.
Isopropyl alcohol.
Benzene.
Ethyl alcohol, 95%.
Acetone.
Hydrochloric acid 5*N*. Dilute 416 ml. of concentrated hydrochloric acid to 1 liter with distilled water.
Zinc metal, dust.
Filter aid, Hyflo Super-Cel (Johns-Manville).
Sodium nitrite reagent grade, 0.25% w./v. solution in water, prepared fresh daily.
Ammonium sulfamate, Fisher certified. 2.5% w./v. aqueous solution, prepared every 3 days.
Coupling reagent, *N*-1-naphthylethylene diamine dihydrochloride, 1% w./v. aqueous solution, stored in the refrigerator. Prepared fresh every 3 days.

Dyrene. Technical material of known purity (Chemagro Corp., Kansas City, Mo.).

Preparation of Sample for Analysis. Grind 100 grams of the chopped material with 100 ml. of isopropyl alcohol in a Waring Blendor, operating at high speed. Blend for 6 minutes after the sample has been added. In the case of onions, blend for 15 minutes. Transfer the contents of the Blendor to a quart Mason jar and rinse with successive portions of distilled water. Add a total of 400 ml. of distilled water to the sample. Add 200 ml. of benzene to the jar and shake vigorously for 30 minutes. Pour a portion of the contents of the jar into a 250-ml. centrifuge bottle and centrifuge for 15 minutes to separate the phases. Pipet off a 40-ml. aliquot of the supernatant benzene and filter through a Whatman No. 41 filter paper into a 150-ml. beaker. Wash the paper with additional benzene. Evaporate on a steam bath in a current of air to a volume of about 2 ml.

Colorimetric Procedure. Rinse the residue into a 100-ml. volumetric flask with 10 ml. of 95% ethyl alcohol. For onion extracts, rinse with an additional 5 ml. of acetone to remove the waxy residue completely. Add 5 ml. of 5*N* hydrochloric acid. Invert a 10-ml. beaker over the volumetric flask and reflux for 5 hours on a steam bath. Then add 0.2 gram of zinc dust and reflux for an additional 5 minutes or until the solution is decolorized. Cool, and dilute to volume with distilled water. Pour the contents of the flask into a clean, dry beaker. Add 1 teaspoonful of filter aid, mix, let stand for 10 minutes, and then filter through a Whatman No. 12 filter paper. Place 40-ml. aliquots of the clear filtrate in each of two 50-ml. stoppered graduates. Add 1.0 ml. of sodium nitrite solution and mix. After 10 minutes add 1.0 ml. of ammonium sulfamate reagent, mix, and let stand for 10 minutes. Add 2 ml. of coupling reagent to each of the two cylinders. Dilute all solutions to volume with distilled water and mix thoroughly. After 60 minutes, determine the absorbance at 540 mμ in 1.0- or 10.0-cm. cells against a solvent blank prepared in the same way as the samples.

Preparation of Standard Curve. Pipet suitable quantities of a 95% ethyl alcohol solution containing from 50 to 500 γ of Dyrene into a series of 100-ml. volumetric flasks. Add 95% ethyl al-

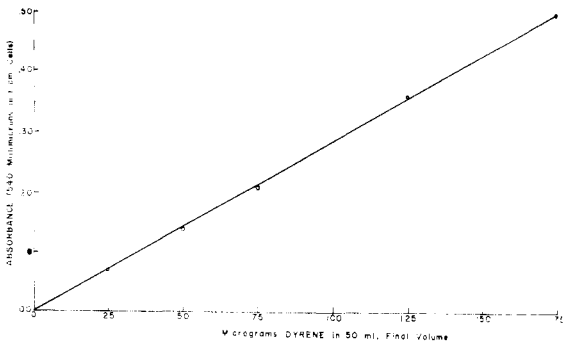

Figure 2. Dyrene standard curve

Table I. Hydrolysis of Dyrene[a]

Reflux Time, Hours	Acid Hydrolysis		Alkaline Hydrolysis	
	HCl, 5N[b]	HCl, 10N[c]	KOH, 5N[b]	KOH, 10N[b]
0.0	0	0	0	0
0.5	15	16	2	2
1.0	...	26	3	2
2.0	76	75	3	3
3.0	89	62	6	3
4.0	100	62	6	4
5.0	100	89	5	4

[a] All results reported as micrograms of *o*-chloroaniline.
[b] 5 ml.
[c] 7 ml.

Table II. Precision of Dyrene Method for Tomatoes

Dyrene Added, P.P.M.	Dyrene Found,[a] P.P.M.	Dyrene Recovered, P.P.M.	Recovery, %
0.00	0.25 ± 0.00 (2)	...	
0.50	0.75 ± 0.07 (3)	0.50	100
1.00	1.30 ± 0.03 (3)	1.05	105
2.50	2.88 ± 0.16 (3)	2.63	105

[a] Values are followed by average deviation from the mean and, in parentheses, by number of determinations.

Table III. Recovery of Dyrene Added to Crops

Crop	Dyrene Added, γ	Dyrene Found,[a] γ	Mean Recovery, %
Celery	100	86.4 ± 2.0 (2)	86.4
	150	146.1 ± 18.0 (2)	97.4
	250	212.0 ± 5.0 (2)	84.2
Onions	50	41.7 (1)	83.3
	100	88.9 ± 2.1 (4)	88.9
	250	229.2 ± 8.9 (6)	91.6
Potatoes	50	41.7 (1)	83.3
	100	89.5 ± 2.1 (4)	89.5
	150	155.0 (1)	103.0
	250	236.5 (1)	94.7
Tomatoes	100	99.0 (1)	99.0
	150	147.0 (1)	98.0
	250	242.5 (1)	97.0

[a] Values are followed by average deviations and number of determinations.

cohol to each flask to give a volume of 10 ml. Add 5 ml. of 5*N* hydrochloric acid to each flask and proceed with the hydrolysis and color development as described above.

Figure 2 shows a typical calibration curve.

Discussion

The zinc reduction step after the hydrolysis greatly reduces the amount of extraneous color present in some extracts. A 5-minute reflux is sufficient for most samples, but onions require a 15-minute reflux period.

The filtrate obtained after zinc reduction, when diluted with distilled water, develops a turbidity due to precipitation of lipide materials. The addition of Celite and filtration remove them completely.

The determination from which the coupling reagent is omitted allows for correction due to extraneous color in the solution.

The reproducibility of the method was tested by macerating a large number of tomatoes in isopropyl alcohol as previously described (Table II).

The puree was thoroughly mixed and divided into 12 equal portions by weight. Each portion contained the equivalent of 100 grams of tomatoes and 100 ml. of isopropyl alcohol. Two samples were analyzed without the addition of Dyrene and the other nine were divided into three groups. Each group was treated with various amounts of Dyrene.

In spite of the low absorbance values read, the average deviation in the concentration range of 0.5 to 2.5 p.p.m. is about 5%.

To test the efficiency of the method, Dyrene in isopropyl alcohol was added to plant tissue in the blending operation and carried through the entire procedure (Table III).

The values for untreated control material show considerable variation. In the samples analyzed, untreated controls gave values which were equivalent to 1.0 p.p.m. or less. The reproducibility of values from any particular location was good. In using the method for residue determination, the values for the untreated controls are always subtracted before reporting the results. An initial concentration of 1 p.p.m. gives an absorbance in the final solution of 0.02 using the dilutions and aliquots described in the method. Readings of untreated controls ranged up to values equivalent to 1 p.p.m. of Dyrene.

The recovery data (Tables II and III) justify using 1.0 p.p.m. as the limit of sensitivity of the method. In view of the low toxicity of Dyrene, this sensitivity is considered satisfactory.

For concentrations of Dyrene of less than 5 p.p.m., the final readings should be made in 10-cm. quartz cells. By reading in this way, the sensitivity of the method can be extended to 1 p.p.m.

Acknowledgment

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FUNGICIDE RESIDUES

Determination of Dyrene in Apples by Application of the Zincke Reaction

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A method is presented for the determination of small amounts of the fungicide, Dyrene, in apples. Apple tissue is extracted with acetone and the acetone filtrate is extracted with chloroform. The evaporated extract is taken up in benzene and chromatographed on alumina to remove interferences. The Zincke reaction with pyridine and alkali is used to determine Dyrene. Recovery of Dyrene, added to apples, averaged 97 ± 10%. About 0.05 p.p.m. of Dyrene can be detected, if the entire chloroform extract from a 100-gram sample of apple is analyzed. Analysis of apples treated with Dyrene for scab control shows residues of about 0.1 p.p.m.

DYRENE, 2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine, controls certain foliage diseases of higher plants. Its effectiveness against apple scab and rust prompted the development of a rapid analytical method for its determination in apple fruit.

Meagher *et al.* (*3*) have developed a colorimetric method for the determination of Dyrene residues in plant material. It is based on the acid hydrolysis of Dyrene to *o*-chloroaniline followed by diazotization and coupling with *N*-1-naphthylethylenediamine.

Burchfield and Schuldt (*1*) have shown that the colorimetric reaction of pesticides containing active halogen with pyridine followed by alkali (Zincke reaction) can be used for their quantitative determination. Burchfield and Storrs (*2*) used the Zincke reaction to determine 2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine in solvent extracts of fungus spores. In the work reported Dyrene is extracted from apple tissue by the method of Meagher and MacDougall (*4*) for Guthion, using acetone and chloroform. The evaporated extract is taken up in benzene and the solution is passed through an alumina column to remove interfering substances. The color reaction, essentially that of Burchfield and Storrs (*2*), is then used to determine Dyrene.

Reagents

DYRENE STOCK SOLUTION. Dissolve 0.1000 gram of analytical standard Dyrene (Chemagro Corp., P. O. Box 4913, Kansas City, Mo.) in C.P. acetone. Transfer to a 500-ml. volumetric flask, make up to volume with acetone, and mix. One milliliter of this solution contains 200 γ of Dyrene.

DYRENE STANDARD SOLUTION. Dilute 5 ml. of the stock solution to 100 ml. with acetone in a volumetric flask. One milliliter of this solution contains 10 γ of Dyrene.

Procedure

Preparation of Standard Curve (0 to 100 γ of Dyrene). Pipet aliquots of the Dyrene standard solution corresponding to from 0 to 100 γ of Dyrene into a series of 20 × 150 mm. test tubes. Evaporate the solution to dryness on a