Colorimetric Microdetermination of Vitavax (5,6-Dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) Residues in Crops

John R. Lane

Vitavax (carboxin) is a systemic fungicide. This method determines 0.2 ppm (negligible residue) to 20 ppm in green forage crops including barley, cotton, oat, peanut, sorghum, and wheat plants; also potatoes and sugar beets. From 67 fortifications the average recovery is 89%; the range 74 to 110%. Crop interference is 0.05 to 0.10 ppm. Aniline is liberated from Vitavax in NaOH solution and distilled. Hexane extraction removes interference and the pH of the distillate is adjusted to 6.5 with H_3PO_4. The distillate is extracted with benzene. The aniline is extracted from the benzene with 20% acetic acid. Two ml of Ehrlich's reagent are added. In 30 min the pH is adjusted to 3.5 with 50% NaOH solution. After 10 min the absorbance is measured at 440 and 490 mμ in 50-mm silica cells. Crop interference forms a red color; aniline from Vitavax forms a yellow color.

Vitavax (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) Figure 1, a new systemic fungicide discovered in the laboratories of Uniroyal (von Schmeling *et al.*, 1966; Kulka *et al.*, 1968), is useful as a seed treatment on grain and forage crops (Uniroyal, 1969; von Schmeling, 1969). Vitavax treated seed at germination absorb the fungicide to kill seed-borne pathogens. Initial concentrations of 20 ppm of Vitavax in 1 in. seedlings diminish to 0.2 ppm (negligible residue) in about 8 weeks (Table I). To provide a means of determining as little as 0.2 ppm in various crops (Table III) the following method was developed.

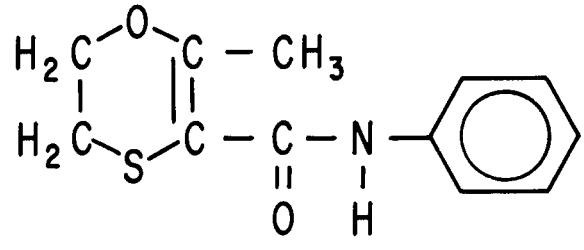

Figure 1. Chemical structure of Vitavax

EXPERIMENTAL

Apparatus. ALAR STEAM DISTILLATION HEAD. Described by Edgerton *et al.* (1967). A slightly adapted head, using a Graham type coil condensing tube of about five loops to replace the original 45° angle condensing tube, was employed (Macalaster Bicknell Co., 181 Henry Street, New Haven, Conn.). The spectrophotometer was equipped with 50-mm silica cells.

REAGENTS

Antifoam A, paste form, Dow Corning Corp., 401 Sylvan Ave., Englewood Cliffs, N.J.

DAB reagent (Ehrlich's), 2 g *p*-dimethylaminobenzaldehyde, dissolved in 100 ml of 4% HCl solution.

A normal cell blank (see Color Measurement) is very light yellow, almost colorless at pH 3.5. Aging reagent causes the cell blank to become bluish at pH 3.5 and crop analyses bluish or greenish. When this condition develops, prepare fresh reagent.

Vitavax standard solution: 100 μg/ml acetone.

Titanium(ous) trichloride, 20% aqueous (Fisher SO-T-43).

Zinc Granules, 10 mesh.

PROCEDURE

Add the weight of macerated crop sample and the volume of NaOH solution required to a suitable flask (Table II). To the flask also add 5 ml of titanium trichloride solution, 2 ml of granular zinc, and 1 ml of Antifoam A. Potatoes and sugar beets normally require 5 cc of paraffin wax also as a defoamer.

Grease the flask joint and connect it to the distillation head. Seat the flask in a heating mantle and heat with a powerstat setting of 110/140 units. Distill 150 ml into a graduated 250-

Table I. Typical Residues in Cotton Seedlings Emerging from Vitavax Treated Seed

Weeks after emergence	Net ppm Vitavax 8 oz trmt/cwt	Net ppm Vitavax 4 oz trmt/cwt
1	22	15
2	17	10
3	8	6
4	4	2
5	1	0.5
6	0.5	0.2
7	0.2	not detectable (<0.1)
8	not detectable (<0.1)	

Uniroyal Chemical, Naugatuck, Conn. 06770

Table II. Distillation Conditions for the Various Crops

Crop	Anticipated Residue (ppm)	Sample (grams)	Flask	NaOH Solution
Green plants	3 to 20	10	1-liter	500 ml of 35%
Green plants	0.5 to 2.0	50	1-liter	500 ml of 35%
Green plants	0.2 to 0.4	100	2-liter	500 ml of 35%
Sugar beets	0.2	100	2-liter	400 ml of 40%
Potatoes	0.2	100	2-liter	400 ml of 40%

ml suction flask attached to a slight vacuum source to remove obnoxious odors. (This is *NOT* vacuum distillation.)

Dissolve 5 g of NaCl in the distillate and extract with 50 ml of hexane in a 250-ml separator. Retain the aqueous phase, transfer it to a 250-ml beaker and, by dropwise addition of 85% H_3PO_4, adjust the pH to 6.5 on a pH meter. (Alternatively make the dropwise addition of H_3PO_4 to the distillate in a 250-ml separator, mix well, and test the wet glass stopper with 4.5 to 7.5 range Hydrion pH paper until the pH of 6.5 is obtained.) Extract the distillate two times with 30-ml portions of benzene. Combine the recovered benzene extracts and rinse two times with 50 ml of H_2O in a separator. Retain the benzene layer and extract it with 12 ml of 20% acetic acid solution. Ensure a pH of 3.0 or lower by testing the wet stopper of the separator with 3.0 to 5.5 range Hydrion pH paper. If the pH is above 3.0, bring it down to 3.0 by dropwise addition of glacial acetic acid. Allow 2 min for any benzene droplets or fog to clear from the lower aqueous phase and transfer the aqueous phase to a 25-ml mixing cylinder. (Be sure no benzene is drawn off with the aqueous phase to cause a foggy solution.) Add 2 ml of DAB reagent to the mixing cylinder and mix well. In 30 min add 1 ml of glacial acetic acid and adjust the pH to 3.5 by dropwise addition of 50% NaOH solution (about five drops) by testing the wet cylinder stopper with 3.0 to 5.5 range Hydrion pH paper. Mix well. (If the pH rises above 3.5 at this point, the DAB precipitates as yellowish, creamy solids. To make the solution crystal clear again, add 1 ml of glacial acetic acid, mix well, and readjust the pH to 3.5 by more cautious dropwise addition of 50% NaOH solution.) Adjust the volume to 16 ml with distilled H_2O, if necessary. This is sufficient volume to fill the 50-mm silica cell. Record all volumes of color solutions for use in the calculation. The color solutions must be crystal clear for measurement. If a color solution is foggy at this point, filter it through Whatman No. 1 paper.

COLOR MEASUREMENT

Prepare a cell blank: To a 25-ml mixing cylinder add 12 ml of 20% acetic acid solution, 2 ml of DAB reagent, and 1 ml of glacial acetic acid. Mix well. Adjust the pH to 3.5 and the volume to 16 ml as given in the procedure. Measure the absorbance of crop analyses or standard analyses against the cell blank as the reference in 50-mm silica cells at 440 and 490 mμ on a spectrophotometer. If the absorbance measured at 440 mμ is greater than 1.5 units, remeasure the solutions in 1-cm silica cells and multiply by 5 to fit the calculation.

CALCULATION

$$\text{net absorbance} = (\text{Abs.}_{440\text{m}\mu} - \text{Abs.}_{490\text{m}\mu}) \times \text{(50-mm cells)} \times \frac{\text{total ml of color solution}}{16 \text{ (the standard color volume and capacity of the 50-mm cell)}} \quad (1)$$

$$\text{ppm} = \frac{\text{net absorbance} \times K}{\text{sample wt}} \quad (2)$$

where

$$K_{\text{av.}} = \frac{\mu\text{g}}{\text{net absorbance}} = \text{about 60 (from the average standard curve)} \quad (3)$$

STANDARD CURVE

To a 1-liter flask add 500 ml of 35% NaOH solution, 2 ml of zinc granules, 5 ml of 20% titanium trichloride solution, 5 ml of conc. NH_4OH (to simulate the amines and NH_4OH in a crop analysis), and finally 0, 20, 40, 60, or 80 μg of Vitavax A.R. as the standard solution. Distill 150 ml and complete the analysis as described above in the procedure. Measure the absorbance in 50-mm silica cells. Plot the net absorbance *vs.* μg of Vitavax. From the average straight line through the points, determine K for the calculation. $K_{\text{av.}}$ is about 60.

COMMENTS ON THE METHOD

Zinc granules are added to the distillation flask to produce nascent hydrogen, which aids substantially in the distillation of micro-amounts of amines from NaOH solution by maintaining a reducing effect (Lane *et al.*, 1958; Lane, 1964a,b). Titanium trichloride is a more powerful reducing agent which is needed, together with zinc, to quantitatively distill microamounts of aniline and various hydrazines from NaOH solution (Lane, 1967). Analytical grade pellet, flake, or 50% liquid NaOH may be used to prepare the NaOH solutions for analysis.

When analyzing 10-gm crop samples containing 5 to 20 ppm of Vitavax, the raw distillate plus NaCl may be extracted directly with benzene, omitting the intermediate cleanup steps because the interference is, relatively speaking, very low.

From limited data the DAB color solutions from crop analyses appear to maintain the yellow aniline color with <5% deviation for 4 to 6 hr. Although the red interference color does diminish slightly, the net effect on the ppm of Vitavax present is minimal. For convenience the acetic acid extracts of a number of analyses may be collected and, together with a cell blank, colored and measured. Usually an untreated sample, a Vitavax fortified sample, and two or four field treated samples are analyzed as a group.

FORTIFICATIONS

To analyze, for example, cotton plants fortified with 0.2 ppm of Vitavax, select the analytical conditions listed in Table II. Thus, to a 2-liter flask, charge 100 gm of untreated macerated cotton plants. Add 20 μg of Vitavax (0.2 ml of the 100 μg/ml standard solution), then 500 ml of 35% NaOH solution, and the remaining materials listed in the procedure. Complete the analysis as directed.

$$\%\text{ recovery} = \frac{\text{ppm found (fortified analysis-untreated analysis)} \times 100}{\text{ppm fortification}} \quad (4)$$

All recovery data in Table III were obtained in this manner.

RECOVERIES

Table III lists the % recovery data obtained from untreated crops fortified with Vitavax and analyzed as directed under FORTIFICATIONS. Where possible, the % recovery data for each crop is divided into three groups. The first group shows

Table III. Recoveries of Vitavax from Fortified Crops

Crop	Added ppm	No. of Detns.	% Recovery Average	% Recovery Range	Interference from Untreated Crop (ppm)
Barley plants	0.125[a]	3	87	76–110	
Barley plants	0.2	3	92	85–105	0.05–0.10
Barley plants	1–20	7	94	78–105	
Cotton plants	0.125[a]	1	...	120	
Cotton plants	0.2	5	91	80–105	0.05–0.10
Cotton plants	1–20	5	99	88–110	
Oat plants	0.125[a]	2	62	56, 57	
Oat plants	0.2	2	81	87, 75	0.05–0.10
Oat plants	10	1	...	91	
Peanut plants	0.2	5	86	76–92	0.05–0.10
Peanut plants	1–20	7	85	78–92	
Sorghum plants	0.2	6	90	74–106	0.05–0.10
Sorghum plants	5	2	82	82, 82	
Wheat plants	0.125[a]	6	84	69–100	
Wheat plants	0.2	12	95	80–105	0.05–0.10
Wheat plants	1–20	6	88	76–101	
Potatoes	0.125[a]	3	65	54–81	
Potatoes	0.2	3	85	79–89	0.00–0.06
Sugar beets	0.125[a]	1	...	107	
Sugar beets	0.2	3	91	82–102	0.00–0.06

[a] Detectability.

recoveries of 0.2 ppm. This area determines whether the "negligible residue" of 0.2 ppm is or is not exceeded for policing purposes. The second group shows recoveries of 1 to 20 ppm. This area provides degradation curve data such as is listed in Table I. The third group shows recoveries in the 0.125 ppm (minimum detectable) area of the method. The 0.125 ppm fortifications require a 200-g sample, but in other respects are analyzed as normal 100 g samples (see FORTIFICATIONS).

Of the 67 recovery determinations in Table III, 39 are 0.2 ppm fortifications. They average 89% recovery and range from 74 to 106%. The remaining 28 determinations are 1 to 20 ppm fortifications. They average 89% recovery and range from 76 to 110%. The 0.125 ppm fortifications consistently detect 50% or better.

THE COLOR REACTION

Ten micrograms of aniline react with DAB reagent to form a weak yellow color at pH 1. After color development at pH 1, adjustment of the pH to 3.5 increases the intensity of the aniline color twofold. This is adequate sensitivity for determining 0.2 ppm of Vitavax in crops. Other amines showing a twofold increase in intensity of the yellow color at pH 3.5 were hydrazine, μ-toluidine, and the chloroanilines. The nitroanilines and the phenylenediamines maintain essentially the same color intensity at pH 1 or 3.5.

CROP INTERFERENCE IN THE COLOR REACTION

In any crop analysis, aniline from Vitavax and some crop interference remain in the final acetic acid extract. Coloration with DAB reagent (pH 1) produces a weak red color with the crop interference and a yellow color with aniline. Adjusting the pH to 3.5 strengthens the aniline yellow color twofold but does not affect the weak red color. This dual color development permits the base-line technique type of calculation to sensitively select and determine the yellow aniline color (peak—440 mμ; base—490 mμ) as Vitavax, yet equate the weak red interference color to a near zero value. For this reason the normal crop interference from 100-g samples is as low as 0.05 to 0.10 ppm (Table III). Previously similar types of baseline technique were used to determine maleic hydrazide residues with DAB reagent and N-1-naphthylphthalamic acid residues with diazotized sulfanilic acid reagent (Lane *et al.*, 1958); also succinic acid 2,2-dimethylhydrazide residues with trisodium pentacyanoamino Ferrate reagent (Lane, 1967). Analysis of untreated, mature seed of each plant variety listed in Table III produced yellow interference color with DAB reagent similar to the color produced with aniline and equivalent to 1 ppm of Vitavax. Therefore, analysis of the seed by this test was not possible. Mature safflower seed, however, was an exception, showing interference of <0.2 ppm.

INTERFERENCE FROM TOLERANCE PESTICIDES

When all of the pesticides granted a tolerance (Dec. 31, 1968) on cotton plants, peanut plants, or sugar beets are combined and present on the respective crop at the tolerance allowed, they cause no objectionable background and do not intefere in the normal recovery of 0.2 ppm of Vitavax from these crops.

When all the pesticides granted a tolerance (Dec. 31, 1968) on barley, oat, sorghum, and wheat plants and potatoes are present on the respective crop at the tolerance allowed, only Diuron and Linuron interfere in the Vitavax residue method. In analyses of these crops, any Diuron or Linuron present liberates dichloroaniline, which is distilled and carried through the analytical steps in exactly the same manner as any aniline from Vitavax. Both dichloroaniline and aniline react with DAB reagent to produce indistinguishable yellow colors. The 2 ppm Diuron and 1 ppm Linuron tolerance allowed on sorghum plants when present cause an apparent net Vitavax residue of 1.0 ppm. To identify quantitatively any net residue greater than the "negligible residue" of 0.2 ppm as either dichloroaniline or aniline, inject concentrated benzene extract of the distillate into a MCGC equipped with a halogen detection system. Any dichloroaniline present is determined by the peak at its characteristic retention time, whereas aniline from Vitavax will give no response.

The Tolerance Pesticides used in this study were compiled by the National Agricultural Chemicals Association, Washington, D.C., 20005, from the official tolerance orders published in the Federal Register and re-published as complete lists in *NAC News*, Jan./Feb. 1969.

OTHER AMINE REAGENTS

The naphthylethylenediamine (NEDA) reagent of Averell and Norris (1948) was examined first to determine Vitavax residues in crops. But the crop interference found was about four times greater (as ppm) than the interference subsequently obtained using DAB reagent. Since NEDA forms a magenta dye with both the crop interference and the aniline from Vitavax, a base-line technique calculation is ineffective in reducing the interference value. But DAB reagent with its dual-color formation is well adapted to the baseline approach.

Other diazotization amine reagents tried were nonspecific, like NEDA, and/or produced colors too weak for the sensitivity of 0.2 ppm required. Other aniline reagents also examined were 9-chloroacridine (Stewart *et al.*, 1969); $KBrO_3$ oxidation (Legradi, Laszlo, 1969); and 4-dimethylaminocinnamaldehyde (Tulus and Aydogan, 1967).

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