Determination of Residues of Vitavax and Its Sulfoxide in Seeds

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Residues of Vitavax (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide), a systemic fungicide and its sulfoxide, are determined in wheat, barley, oats, peanuts, sorghum, flax, and cotton seeds and extracted seed oils. The Vitavax is extracted from the seeds or oils and the extract is cleaned up. Vitavax is hydrolyzed to liberate aniline in a caustic reducing medium. The aniline is distilled and concentrated. An aliquot of the concentrate is injected into a gas chromatograph equipped with a microcoulometric nitrogen detector. The method is designed to minimize the interference from natural components of seed such as tryptophan and its analogs which may also produce aniline. The method is sensitive to less than 0.2 ppm. No residues have been detected in seed harvested from plants grown from treated seed.

Vitavax (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) is a systemic fungicide useful as a seed treatment prior to planting of wheat, barley, oats, peanuts, sorghum, flax, and cotton. An analytical method sensitive to 0.2 ppm has been developed to measure the residues of Vitavax and its sulfoxide metabolite in seed harvested from plants grown from treated seed.

The previously described colorimetric test (Lane, 1970) for determining Vitavax residues in crops was unable to determine 0.2 ppm of Vitavax in grains and other seeds because natural interferences were too great. The need for a specific means of determining 0.2 ppm of Vitavax and/or its sulfoxide residue in seed crops led to the development of the following test.

MATERIALS AND EQUIPMENT

The water used in the method for extractions and distillations was distilled from a commercial laboratory still and then redistilled from alkaline permanganate. When this water was charged into the steam generator for steam distillation, it was acidified with sulfuric acid and rendered purple with potassium permanganate.

The water used in the cell of the microcoulometric detector was laboratory distilled, then distilled from ferrous sulfate–sulfuric acid, and finally distilled from alkaline permanganate and stored in glass bottles.

The hexane and diethyl ether were extracted with 10 *N* sulfuric acid before use. The titanium trichloride (Fisher) and sodium hydroxide, 50% (J. T. Baker) were used as received, as were the following (Baker and Adamson's) chemicals: methanol, toluene, benzene, and acetone. The rotary evaporator had a Teflon drive shaft (Model 5001, California Laboratory Equipment Co., Berkeley, Calif.). The steam distillation head is described by Edgerton *et al.* (1967) and Lane (1970).

GAS CHROMATOGRAPH, DETECTOR, AND RECORDER

The gas chromatograph was a Perkin-Elmer 881 chromatograph with the flame detector bypassed.

The detector was a Dohrmann C-200-A microcoulometer with a T-400 titration cell. The cell was enclosed in a box made of plywood to insulate it thermally, and the plywood was lined with thin galvanized iron sheets, all grounded, to insulate it from magnetic or electric pickup. The microcoulometer was operated with a Range Ohms setting of 400–700, depending on background noise.

The recorder was a Bristol Dynamaster Recorder, Model 66A-1PH553, 1 mV span.

Constant voltage transformers were used for the magnetic stirrer, microcoulometer, and recorder. A noisy recorder signal was traced to distortion of the output sine wave from the constant voltage transformer between the microcoulometer and the mains. This transformer must give an undistorted sine wave output, as determined with an oscilloscope.

COLUMN AND OPERATING CONDITIONS

The column was 18 ft long, $^1/_8$ in. o.d., $^1/_{10}$ in. i.d., type 304 stainless steel, filled with 70/80 mesh Anakrom SD, coated with 4% Carbowax 20 M, 1% Igepal CO-880 and 1% potassium hydroxide. The injection port temperature was 150° C and the column temperature was programmed from 100° C to 200° C at 6°/min.

The carrier gas was ultrapure hydrogen moistened by passing it through damp silica gel. The moisture in the hydrogen prolonged the life of the nickel catalyst in the reduction tube. The damp silica gel was contained in a JU-9 gas drier (Analabs, North Haven, Conn.). The silica had been removed from the drier and divided into two approximately equal parts. One part had been thoroughly wet with water and then recombined with the dry portion. The blend was then replaced in the drier and installed downstream from the regulating gauge on the hydrogen tank.

The hydrogen gas flow was 55 ml/min when the gc column was 100° C and 40 ml/min, when the column was 200° C. The flow rates were measured at the vent tube of the T-400 cell.

REDUCING TUBE AND FURNACE

The gc column delivered the hydrogen carrier gas into a 370 mm long, 9.5 ± 0.5 mm o.d., 4.5 ± 0.5 mm i.d. quartz reducing tube. The reducing tube (Figure 1) had a 105 mm empty lead-in from the chromatograph, followed successively by 25 mm of 0.6% rhodium on silica gel, 70 mm of 20/30 mesh nickel shot, 30 mm of 30/50 mesh calcium oxide, 75 mm of Lithasorb, and 50 mm of lead-out to the microcoulometric titration cell. The different column fillings were separated by about 3 mm quartz wool plugs. The reduction tube was connected to the titration cell by a piece of Teflon tubing and a 12/5 ball joint.

The furnace (Figure 2) consisted of two independently controlled heated zones. The high temperature zone was 5.5 in. long, and the low temperature zone was 4.5 in. long.

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The furnace was constructed of two heavy-walled stainless steel tubes reamed out to fit loosely on the quartz reducing tube. Both ends of each tube were turned down to permit insertion to a depth of 1/4 in. into 0.5-in. thick marinite walls in the furnace housing. The tubes were wound with asbestos-covered, No. 23 gauge nichrome wire, one layer, tightly wound. The nichrome winding was wrapped with several layers of asbestos tape until the diameter of the winding was 2 in. The nichrome wire was connected to asbestos-covered copper wire lead-outs with short crimped-on couplings of 1/8 in. o.d. copper tubing. The two furnace sections were installed in a 10.5-in. piece of 4-in. diameter pipe using marinite supports. The space between the asbestos-wrapped heaters and pipe was filled with blown mica. The sections were independently heated, the 5.5-in. zone at 750° C, and the other at 300° C by means of variable transformers, and were left on continuously.

PROCEDURE

To 30 g of whole seed was added 100 ml of methanol and the mixture was ground in a Waring Blendor for 2 min. The solid matter was allowed to settle and the methanol was decanted through a 150-mm powder funnel extended with a piece of glass tubing to deliver into a 43 × 123 mm Soxhlet thimble in an extractor attached to a 300-ml round-bottomed flask. A No. 16 standard taper glass stopper was under the Soxhlet thimble to promote efficient drainage. The seed in the Blendor was treated with an additional 75 ml of methanol and blended for 2 more min. Both seed and methanol were transferred to the Soxhlet thimble. The Blendor was rinsed with 100 ml of methanol and the rinsings were added to the thimble. Using a heating mantle and a variable transformer setting of 100 V, the seed meal was Soxhlet extracted for 3 hr. While the extraction was in progress, the warm parts of the extraction apparatus were insulated with corrugated paper to accelerate the cycle. Excessive heating should be avoided because it promotes scorching on the inside flask walls, giving erratic blanks due to the thermal degradation of natural components of the extract.

The methanol extract was cooled and transferred to a 1-l. separatory funnel. The flask was rinsed with two 25-ml portions of water. The rinsings were added to the separatory funnel, followed by 2 ml of saturated NaCl solution. The methanol–water mixture was extracted twice with 50 ml portions of *n*-hexane, which were discarded. Then 70 ml of water was added, and the contents of the funnel were extracted with 100 ml of chloroform, followed by four successive extractions with 30-ml portions of chloroform. The chloroform extracts were combined in a 250-ml separatory funnel with 15 g of anhydrous Na_2SO_4 powder, and the separatory funnel was shaken. After 1 min the chloroform was decanted from the separatory funnel through Whatman No. 4 filter paper into a 500-ml round-bottomed flask. The Na_2SO_4 in the separatory funnel was rinsed with two 15-ml portions of chloroform which were filtered through the filter paper into the flask. The flask, connected to a vacuum rotary evaporator, was half submerged in warm water and the chloroform solution concentrated to about 2 ml. Then 30 ml of toluene was added to the flask and the solution concentrated again until about 3 ml of liquid remained.

After concentration, 2 ml of 20 mesh zinc granules, 5 ml of titanium trichloride, and 100 ml of 50% NaOH were added to the flask which was placed in a heating mantle for insulation, connected to a steam generator, and steam distilled until 100 ml of distillate had been collected.

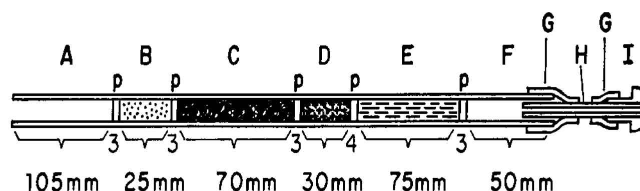

Figure 1. Quartz reduction tube. Total length: 370 mm (o.d. 9.5 ± 0.5, i.d. 4.5 mm). A and F—empty; B—0.6% rhodium on silica gel; C—20/30 mesh nickel shot; D—30/50 mesh calcium oxide; E—Lithasorb; p—quartz wool plug; G—rubber tubing; H—Teflon tubing; I—12/5 ball joint

The distillate was transferred to a separatory funnel, brought to a pH of 1 with 10 *N* H_2SO_4 (using 1 to 11 range pH paper), and extracted with 20 ml of benzene which was discarded. The aqueous phase was then rendered alkaline to pH 11 with 50% NaOH solution and was extracted with 40 ml of diethyl ether, followed by three extractions with 20-ml portions of diethyl ether. The 40-ml extract was drained into a 50-ml beaker containing 6 g of anhydrous Na_2SO_4 powder and stirred to remove most of the water present. The ether was decanted into a 30-ml beaker and 30 μl of 3 *N* H_2SO_4 were added. The beaker was placed in a dish of warm water (not greater than 30° C) and the ether evaporated under a fine jet of nitrogen gas. The 20-ml ether extracts were successively dried with the same Na_2SO_4 in the first beaker, and were added to the ether under the jet. When the ether was about 5 ml in volume, it was transferred portionwise to a tared conical tube and the evaporation continued. The beaker was rinsed with 1 ml of acetone, the rinsing added to the conical tube, and evaporated to 0.4–0.7 ml (ether free). Forty microliters of 3 *N* NaOH were added and the tube reweighed. Since the sample solution is mostly water, the weight in milligrams was assumed to be equal to the volume in microliters. The tube was stoppered and set aside for microcoulometric gas chromatographic (mcgc) analysis.

Ten microliters from the tube were injected into the gas chromatograph to determine any aniline present in the sample. The aniline peak from Vitavax and its sulfoxide appeared in about 15 min, but other peaks continued to emerge for 30 min more. Typical chromatograms of wheat seed, untreated, and untreated plus 0.2 ppm Vitavax, are shown in Figure 3.

The response of the mcgc was tested several times a day by injecting 60 ng of aniline in aqueous solution into the instrument.

CALCULATION

The peaks due to aniline were traced on tracing paper, cut out, and weighed. From the weights the areas of the peaks

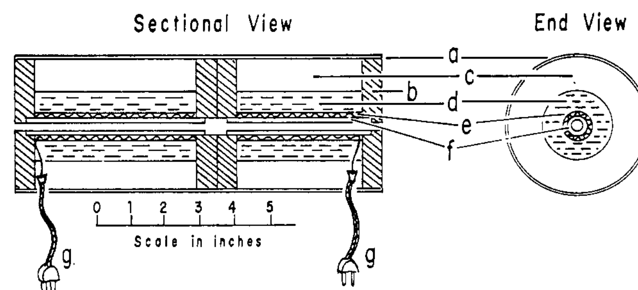

Figure 2. Reduction furnace. a. 10$^1/_2$ in. × 4 in. o.d. pipe; b. marinite; c. blown mica; d. asbestos tape; e. nichrome wire; f. stainless steel tubes; g. wires to variable transformers

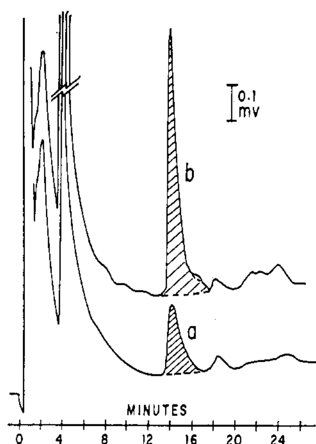

Figure 3. Chromatograms of untreated, and Vitavax fortified wheat seed. Chromatogram b has been displaced upward for clarity. a. Chromatogram of untreated wheat seed. 10 μl from 525 μl injected. b. Chromatogram of untreated wheat seed fortified with 0.20 ppm Vitavax. 10 μl from 513 μl injected

were calculated. The residue was calculated as Vitavax from

$$\text{ppm} = \frac{\text{area of unknown peak}}{\text{area of aniline std. peak}} \times 0.060 \times 2.53 \times \frac{\text{total volume}}{\text{volume injected}} \times \frac{1}{\text{sample wt.}} \quad (1)$$

which, for a 30-g sample, and 10 μl injection, reduced to

$$\text{ppm} = 5.06 \times 10^{-4} \times \frac{\text{area of unknown peak} \times \text{total volume}}{\text{area of aniline std. peak}} \quad (2)$$

In eq 1 the 0.060 arises from a 0.060 μg aniline injection, and the 2.53 is the factor for conversion of aniline to Vitavax. To calculate the result as ppm sulfoxide a factor of 2.70 is used, and the factor in eq 2 is 5.40×10^{-4}.

The area of the aniline standard peak was the average area of the standards for the day.

Periodically the efficiency of the system was calculated by the method recommended in the manufacturer's manual. The efficiencies were from 80 to 105%.

MODIFICATION OF METHOD FOR COTTON SEED

Two modifications of the foregoing method were necessary to analyze cotton seed because the lint on the ginned seed soaked up much of the solvent, making extraction difficult, and abnormally high interference from natural components was encountered.

In the modified procedure for cotton seed, 30 g of ginned seed was ground in a Waring Blendor with 150 ml of methanol, and the methanol decanted to a Soxhlet thimble. The cotton seed was blended again with 100 ml of methanol, and the seed and methanol were transferred to the Soxhlet thimble. The Blendor jar was rinsed with 50 ml of methanol and the rinsings added to the Soxhlet thimble. The sample was extracted for 3 hr.

The methanol extract was cooled, 1 ml of 30% hydrogen peroxide was added, and the mixture was swirled and allowed to stand 15 min. The mixture was transferred to a 1-l. separatory funnel. The hexane and water treatment and the chloroform extraction were as described in the general procedure. The chloroform extracts were combined in a 500-ml separatory funnel. To the combined extracts were added 45 ml of water, 2 drops of 50% NaOH solution, and 2 ml of saturated NaCl solution. The mixture was shaken, the phases allowed to separate, and the lower chloroform layer drawn off and filtered through Whatman No. 4 filter paper into a 500-ml round-bottomed flask. To the aqueous layer in the separatory funnel was added 2 ml more of NaCl solution and 30 ml of chloroform. After shaking and separating, the 30-ml of chloroform extract was filtered and added to the 500-ml flask. The flask was attached to the vacuum rotary evaporator and the analysis completed as described in the general procedure.

Table I. Analytical Results from Untreated and Fortified Seed

Untreated Seeds	No. of Samples	ppm Apparent Vitavax[a] Low	High	Avg.	Recovery Avg.
Wheat	6	0.00	0.09	0.04	...
Oats	1	...	...	0.11	...
Barley	6	−0.01	0.12	0.05	...
Peanuts	5	0.01	0.10	0.05	...
Peanut oil	2	0.01	0.14	0.07	...
Peanut meal[b]	2	0.01	0.03	0.02	...
Sorghum	4	−0.04	0.01	−0.01	...
Cotton	13	−0.01	0.10	0.07	...
Cotton seed oil	2	0.10	0.14	0.12	...
Cotton seed meal[b]	2	0.03	0.06	0.04	...
Above Seeds, Fortified (0.20 ppm)[c]					
Wheat	4	0.15	0.20	0.18	90%
Wheat[d]	4	0.16	0.22	0.18	90%
Oats	1	...	...	0.22	110%
Barley	4	0.15	0.25	0.20	103%
Barley[d]	4	0.14	0.21	0.17	85%
Peanuts	4	0.17	0.20	0.18	91%
Peanuts[d]	2	0.20	0.24	0.22	110%
Peanut oil	2	0.16	0.20	0.18	90%
Peanut meal[b]	2	0.19	0.21	0.20	100%
Sorghum	3	0.14	0.16	0.15	73%
Sorghum[d]	2	0.14	0.17	0.16	78%
Cotton	10	0.13	0.21	0.17	84%
Cotton seed oil	2	0.19	0.21	0.20	100%
Cotton seed meal[b]	2	0.18	0.19	0.18	92%

[a] All of the data in the "ppm Apparent Vitavax" column have been corrected for reagent blanks and the data for the samples fortified with 0.20 ppm Vitavax have been corrected for the interference in the untreated seeds. [b] Petroleum ether extracted. [c] Fortified with Vitavax unless otherwise indicated. [d] Fortified with 0.10 ppm Vitavax and 0.10 ppm sulfoxide.

MODIFICATION OF METHOD FOR PEANUT AND COTTON SEED OILS

Two hundred grams of peanuts or 400 g of cotton seed were ground in a Waring Blendor. For peanuts the sample was divided into two approximately equal portions, each blended in a leak-tight grounded Waring Blendor with 200 ml of petroleum ether (CAUTION), and combined in a 60 $\times$ 180 mm thimble in a Soxhlet extractor attached to a 1000-ml round-bottomed flask. Sufficient petroleum ether was added for Soxhlet extraction, and the sample extracted for 4 hr. Due to the bulky nature of the cotton seed and the larger sample used, four extractors were used, each containing 100 g.

The petroleum ether was rotary evaporated under vacuum until only peanut or cotton seed oil remained. To a 1-l. separatory funnel were added 30 g of the oil, 200 ml of methanol, 50 ml of water, and 2 ml of saturated NaCl solution and the mixture was extracted twice with 50-ml portions of hexane which were discarded. Seventy milliliters of water was added and the analysis completed, as described in the general procedure.

RESULTS

The analytical results were corrected for reagent blanks from the reagents in use at the time. The reagent blanks were reproducible to ± 0.02 ppm equivalent Vitavax. Over a period of weeks the reagent blanks varied from 0.04 to 0.10 ppm equivalent Vitavax throughout work consuming several gallons of many of the reagents.

Results of analyses of seed from untreated plots and of untreated seed fortified with 0.20 ppm Vitavax are summarized in Table I. Analysis of about 100 samples of seed of these crops, harvested from plants grown from seed treated at recommended and exaggerated rates, has shown no evidence of residues. The data on such seed showed no detectable difference from untreated control samples.

SPECIFICITY OF METHOD

When 80 pesticides were combined at their tolerance levels (NAC News, February 1969) and added to their respective crops (seeds of wheat, barley, peanuts, sorghum, or cotton), there was no detectable increase in background interference and no apparent effect on recoveries of 0.2 ppm Vitavax fortifications.

ACKNOWLEDGMENT

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