translocated into the terminal buds and subtending internodes in amounts that inhibited terminal bud growth and induced internodal abscission. During the development of these responses, the lateral buds apparently did not absorb sufficient N-(3-nitro-1-naphthyl)phthalamic and N-(5-acenaphthenyl)phthalamic acids at the dosage levels used to inhibit their subsequent growth. It is probable that any residual amounts of these compounds became inactive during this period as far as growth inhibition was concerned, since there was no evidence of inhibitory or formative effects on axillary buds that developed subsequently.

Acknowledgment

The authors appreciate the cooperation of the Fundamental Research Co., Berkeley, Calif., which supplied the compounds.

Literature Cited

(1) Audus, L. J., "Plant Growth Substances," Leonard Hill (Books), Ltd., London, 1959.
(2) Chemical-Biological Coordination Center, National Research Council, Washington, D. C., "Plant Growth Regulators," Publ. **384** (1955).
(3) Mitchell, J. W., Livingston, G. A., Marth, P. C., U. S. Dept. Agr., *Agr. Handbook* **126** (1957).
(4) Roberts, A. N., Boller, C. A., Jr., "Handbook of Hollies," p. 164, Am. Soc. Hort. Sci. (spec. issue), 1957.
(5) Smale, B. C., Daly, E. J., J. Agr. Food Chem. **6,** 751 (1958).

Received for review October 12, 1964. Accepted January 29, 1965.

DEFOLIANT RESIDUES

Determination of Cyanamide Residues on Ginned Cottonseed

W. A. STELLER and I. B. FREDERICK
Agricultural Center, American Cyanamid Co., Princeton, N. J.
P. W. MORGAN
Department of Plant Physiology and Pathology, Texas Agricultural and Mechanical University, College Station, Tex.

A simple and sensitive colorimetric method for the determination of cyanamide residues on the surface of cottonseed has been developed. Residues are extracted from the seed with water and the extracts treated with activated charcoal to remove interfering substances. A red complex is formed by addition of sodium pentacyanoammine ferroate and measured at 530 mμ. Recoveries have averaged approximately 85% over the range from 0.03 to 0.20 p.p.m. The method is capable of detecting net cyanamide residues of 0.03 p.p.m. on cottonseed.

Calcium cyanamide and, more recently, hydrogen cyanamide have been used for the defoliation of cotton plants to facilitate harvesting of the bolls. Work with C^{14}-labeled cyanamide by Miller and Hall (*3*) has shown that under the conditions of their experiments cyanamide was rapidly metabolized by cotton plants and that the parent compound was undetectable in the plants as little as 8 hours after topical application to the leaves. Consequently, no cyanamide was found in the seeds of these plants. Therefore, the work reported here was directed toward the determination of surface residues which might result from late application or from accidental contamination during harvest.

Early attempts to apply the colorimetric procedure of Buyske and Downing (*2*) directly to aqueous extracts of cottonseed were unsuccessful because of the presence of sample extractives which caused high control values for untreated samples.

Attempts to remove the interfering extractives by partition against various organic solvents, ranging in polarity from 1-butanol to chloroform, were unsuccessful. Of a number of adsorbents evaluated for column cleanup of the extracts, only alumina and acid-washed alumina removed appreciable quantities of the interfering extractives. However, flow rates through these columns were very slow unless they were operated under pressure, in which case turbid effluents were obtained.

Treatment of the effluents with activated charcoal followed by filtration through a bed of diatomaceous earth proved satisfactory for the elimination of the turbidity. Further work with these adsorbents showed that the charcoal–diatomaceous earth treatment by itself produced solutions which were satisfactory for use with the colorimetric procedure.

The chromogenic reaction proved to be extremely sensitive to light in the presence of cottonseed extractives remaining after the charcoal treatment, and it was necessary to carry out the reaction in the dark in order to minimize color loss. Although the time for maximum color development in the absence of cottonseed extractives was about 40 minutes, in the presence of such materials the color tended to fade rapidly and the optimal color-development period was about 10 minutes. Under these conditions the color responses of known quantities of cyanamide added to processed control extracts just prior to the color development step were approximately 92% of those obtained for the standard curve prepared as described below. The standard curve obeyed Beer's law from 4 to 40 μg. of cyanamide per 21 ml. with a slope of 16.0 (absorbance *vs.* milligrams of cyanamide).

Method

Reagents. Water, extraction, pH 5. Adjust CO_2-free distilled water to pH 5 with 0.5N hydrochloric acid.

Decolorizing charcoal, Darco S-51, Atlas Powder Co.

Diatomaceous earth, Hyflo Super-Cel, Johns-Manville.

Buffer solution, pH 10.4. Mix 3 parts of 0.1N sodium carbonate solution with 1 part of 0.1N sodium bicarbonate solution.

Cyanamide Standard Solution. Dilute 60% hydrogen cyanamide solution (American Cyanamid Co., P. O. Box 400, Princeton, N. J.) with distilled CO_2-free water to contain 4 μg. of cyanamide per ml. Adjust to pH 5 with 0.5N hydrochloric acid. Store in a refrigerator; prepare fresh solution every 2 days.

Color Reagent. Prepare a 4% solution of trisodium pentacyanoammine ferroate, $Na_3[Fe(CN)_5NH_3]$ (K and K Laboratories, Inc., Jamaica 33, N. Y.), in distilled water. Filter the solution through a folded Whatman No. 12 filter

paper and store in a closed amber bottle in a refrigerator. Prepare fresh solution daily.

Apparatus. Spectrophotometer, Beckman Model DU, with 5-cm. cells (approximately 17-ml. capacity).

Glassware, 1000-ml. round-bottomed flasks; 250-ml. acetylation flasks; 1-quart wide-mouthed screw-cap bottles; and a jar-mill roller.

Concentration apparatus. Rotating film evaporator connected to a large capacity dry ice trap and evacuated by a mechanical vacuum pump.

Procedure

Extraction of Cottonseed. Extract a representative 200-gram sample of cottonseed with 400 ml. of distilled water (pH 5.0) by tumbling in a 1-quart wide-mouthed jar for 20 minutes. Filter the extract through No. 42 Whatman filter paper on a Büchner funnel by applying vacuum. Transfer the cottonseed to the Büchner funnel and pack down in order to recover as much as possible of the aqueous extract. Measure the volume of the filtered extract and record the percentage of the extract recovered. Adjust the pH to 5.0 and transfer the solution to a 1-liter round-bottomed flask. Concentrate the solution on the described concentration apparatus to approximately 50 ml. while holding the temperature of the water bath below 40° C. Transfer the concentrate and washings to a 125-ml. Erlenmeyer flask (total volume should not exceed 75 ml.). Adjust the pH to 5.0 and if necessary store the solution overnight in a refrigerator.

For cleanup of the extract, add 0.75 gram of decolorizing charcoal to the Erlenmeyer flask and stir with a magnetic stirrer for 15 minutes. Prepare a filter by pouring a water slurry containing 1 gram of diatomaceous earth onto a 4.25-cm. Whatman No. 1 filter paper in a Büchner funnel and applying a gentle vacuum to pack the bed uniformly; place a second piece of Whatman No. 1 paper on top of the bed. Allow the charcoal to settle out of the sample solution for 5 minutes and pass the solution through the diatomaceous earth filter. Wash the charcoal and filter bed with two 10-ml. portions of water, combining the filtrate and washes and adjusting the pH to 5.0. Transfer the solution to a 250-ml. acetylation flask and concentrate to 5 ml. on the described concentration apparatus while holding the temperature of the water bath below 40° C.

Development of Color. Transfer the solution quantitatively to a 25-ml. volumetric flask, being careful to keep the total volume of extract plus washings below 15 ml. Add 10 ml. of pH 10.4 buffer, dilute to the mark, and mix well. Allow to stand 5 minutes and gravity-filter through No. 12 Whatman folded filter paper into a clean, dry, 50-ml. Erlenmeyer flask. Transfer a 20-ml. aliquot measured with a pipet into a clean, dry, 25-ml. volumetric flask. Add 1 ml. of the color reagent, mix well (do not dilute to the mark), and place the flask in the dark for 10 minutes. Then measure the absorbance of the solution in a 5-cm. light path cell at 530 mμ, using a similarly prepared reagent blank as a reference solution. Correct the absorbance of each sample for that of an untreated control sample carried through the entire procedure and read the micrograms of cyanamide present from the calibration curve.

Preparation of Standard Curve. Because the chromogenic reagent is added to a 20-ml. aliquot of the cottonseed samples after the filtration step, it is convenient to prepare the calibration curve as follows:

Add 0-, 1.0-, 2.0-, 4.0-, 8.0-, and 10.0-ml. aliquots of standard cyanamide solution to 25-ml. volumetric flasks, add 10 ml. of buffer, dilute to the mark, and mix well. Remove exactly 5.0 ml. from each volumetric flask, add 1 ml. of color reagent to the flask, and mix well. Exactly 10 minutes later, measure the absorbance at 530 mμ, using the reagent blank as a reference solution. Allow an appropriate time interval between the addition of the color reagent to each solution, in order that each sample may be read exactly 10 minutes after the addition of the color reagent. Do not allow the dilute solutions of cyanamide to remain in contact with the pH 10.4 buffer for more than 30 minutes before addition of the color reagent. The color reaction itself is not light-sensitive; hence, it is not necessary to place the standard curve solution in the dark during the 10-minute color-development period.

Results and Discussion

The recovery of cyanamide through the procedure was determined by adding known quantities of hydrogen cyanamide to mixtures of untreated cottonseed and water (pH 5.0), tumbling, and processing as described for field samples. Recovery values determined in this manner averaged 85% over the range from 0.03 to 0.20 p.p.m. (Table I).

Table I. Recovery Values for Cyanamide on Cottonseed

Cyanamide Added		Cyanamide Recovered	
μg.	P.p.m.	μg.	%
5	0.025	2.5	50
	0.025	4.0	80
9	0.045	7.7	86
	0.045	7.4	82
10	0.05	8.9	89
	0.05	10.2	102
	0.05	10.0	100
20	0.10	15.0	75
	0.10	14.5	73
40	0.20	29.5	74
	Average recovery		85%
	Standard deviation		15%

Eleven samples of untreated cottonseed yielded control values (Table II) which averaged 0.015 p.p.m. with a standard deviation of 0.014 p.p.m. The lowest apparent value which can be considered different from the control at a 95% confidence level is 0.043 p.p.m. Therefore, this figure was taken as the limit of detectability on the basis of apparent residue. Subtraction of the average control yields 0.028 p.p.m. as the limit of detectability on a net residue basis.

Table II. Apparent Cyanamide on Untreated Cottonseed

Absorbance	Apparent Cyanamide, P.P.M.
0.074	0.021
0.043	0.013
0.037	0.011
0.029	0.008
0.125	0.037
0.125	0.037
0.000	0
0.070	0.021
0.000	0
0.023	0.007
0.007	0.002
Average control	0.015 p.p.m.
Standard deviation	0.014 p.p.m.

Analysis of 11 field samples harvested 6 to 16 days after treatments with 25 to 60 pounds of actual calcium cyanamide per acre showed complete absence of detectable residues in all cases. Similarly, residues could not be detected in any of ten field samples harvested 9 to 15 days following treatment with 3 to 10 pounds of hydrogen cyanamide per acre.

Although none of the cottonseed samples analyzed in the course of this study contained residues of cyanamide, it was of interest to determine whether any cyanamide which might be present on cottonseed would be destroyed by the steam cooking step of the oil-extraction process (*1*). Cottonseed samples, fortified at levels of 0.092, 0.92, and 9.2 p.p.m. calcium cyanamide, were exposed for 15 minutes to steam under 4 pounds' pressure at a temperature of 223° F. Residues could not be detected in any of the samples following this treatment.

The limited residue data obtained during the development of this method suggest that surface residues of cyanamide on cottonseed probably would not be present at harvest when this material is used according to current recommendations.

Literature Cited

(1) Bailey, A. E., "Industrial Oil and Fat Products," 2nd ed., pp. 561–3, Interscience, New York, 1951.
(2) Buyske, D. A., Downing, V., *Anal. Chem.* **32,** 1798 (1960).
(3) Miller, C. S., Hall, W. C., J. AGR. FOOD CHEM. **11,** 222 (1963).

Received for review October 26, 1964. Accepted April 27, 1965. Division of Agricultural and Food Chemistry, 148th Meeting, ACS, Chicago, Ill., September 1964.