

- L., *Proc. Am. Soc. Hort. Sci.*, **54**, 435-7 (1949).
- (72) Vaughn, J. R., and Klomparens, W., *Phytopathology*, **42**, 22 (1952).
- (73) Wain, R. L., *Ann. Appl. Biol.*, **39**, 429-32 (1952).
- (74) Wallen, V. R., and Skolko, A. J., *Can. J. Botany*, **29**, 316-23 (1951).
- (75) Wallen, V. R., and Skolko, A. J., *Can. J. Research, Sect. C*, **28**, 632-6 (1950).
- (76) Wallen, V. R., Sutton, M. D., and Skolko, A. J., *Phytopathology*, **40**, 156-60 (1950).
- (77) Weindling, R., Katznelson, H., and Beale, H. P., *Ann. Rev. Microbiol.*, **4**, 247-60 (1950).
- (78) Wellman, R. H., *Contrib. Boyce Thompson Inst.*, **16**, 295-8 (1951).
- (79) Yarwood, C. E., *Proc. 2nd Intern. Congr. Crop Protection, 1951*, 1-22.
- (80) Yoshi, H., *Ann. Phytopathol. Soc. Japan*, **13**, 37-40 (1949); *Rev. Appl. Mycol.*, **29**, 475 (1950).
- (81) Yoshi, H., *Ann. Phytopathol. Soc. Japan*, **14**, 9-10 (1950); *Rev. Appl. Mycol.*, **30**, 190 (1951).
- (82) Yoshi, H., *Ann. Phytopathol. Soc. Japan*, **15**, 13-16 (1950); *Rev. Appl. Mycol.*, **31**, 82 (1952).
- (83) Young, W. J., and Fulton, R. H., *Plant Disease Repr.*, **35**, 540-1 (1951).
- (84) Zaumeyer, W. J., Thomas, H. R., Mitchell, J. W., and Fisher, H. H., *Phytopathology*, **43**, 407 (1953).

Received for review September 14, 1953. Accepted January 22, 1954. Presented before the Division of Agricultural and Food Chemistry, Fermentation Subdivision, Symposium on Non-therapeutic Uses for Antibiotics, at the 124th Meeting of the AMERICAN CHEMICAL SOCIETY, Chicago, Ill. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

## SIDE EFFECTS OF CONTROL AGENTS

# Effects of 2,4-D on Quality of Cotton As Determined by Alkali Centrifuge Value

A. DAVID BASKIN

Fungicide & Herbicide Unit, Pesticide Regulation Section, Plant Pest Control Branch, U. S. Department of Agriculture, Beltsville, Md.

Information was needed as to the extent of damage to cotton fiber caused by direct contact of 2,4-D with field-grown plants. Field-grown Coker 100 Wilt cotton sprayed with the sodium salt of monohydrated 2,4-dichlorophenoxyacetic acid in concentrations of 5 through 500 p.p.m. acid equivalent had shown reduced yield of cotton lint. This cotton fiber when tested by the alkali centrifuge value method was found to have been damaged. Statistical analysis of the data showed the cotton fiber absorbed the alkali, essentially, in direct proportion to concentrations of 2,4-D from 5 through 50 p.p.m. regardless of the time of application, whether applied at time of first true leaves, first flowers, or first true leaves and again at time of first flowers. Relatively small amounts of 2,4-D may impair cotton quality severely, depending upon the stage of growth when field-grown plants come in contact with this herbicide. Therefore, 2,4-D should not be used near cotton.

SMALL AMOUNTS OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) applied to cotton grown in the field have been found to cause severe formative effects (7). It was considered important, therefore, to determine further the extent of damage to cotton fiber which may be caused by 2,4-D, so as to afford a better working knowledge of the probable extent of injury to cotton in contact with 2,4-D by direct application or by drift.

Field tests using Coker 100 Wilt cotton sprayed to runoff at two stages of growth with known concentrations of the sodium monohydrate salt of 2,4-D, were made in 1952 with the cooperation of the Virginia Tidewater Experiment Station, Holland, Va. The fiber harvested from this test was examined for

quality by the alkali centrifuge value test.

It was found (7) that plants sprayed only at the time of first two true leaves (L stage) showed less severe response relative to number of flowers and squares, bolls set, and yield, than did plants sprayed twice (LF stage) or only at time of flowering (F stage). Formative responses to treatments appeared to be in direct proportion to concentration of the acid applied at any stage. The severity of response for plants treated in the first true leaf stage decreased in time and appeared only to delay the maturing of the plants. Plants twice sprayed or treated at the flowering stage showed more pronounced formative effects which were not mitigated in time. Yields responded to the major effects of concentration applied as well as the stage of

plant growth when the plants were treated. Lint yields were drastically reduced by even 5 p.p.m. of 2,4-D acid, which caused a 60.2, 84.4, and 65.2% decrease compared with the untreated checks in the L, LF, and F stages, respectively. As the concentration of the acid was increased, lint yields were further reduced but at a slower rate. These decreases were found by analysis of variance of the data to be significant at the 1% level. Furthermore, plants treated twice (LF stages) produced no cotton when from 100 through 500 p.p.m. of 2,4-D acid were applied.

It was believed that this 2,4-D effect on lint yield could possibly be correlated with differences in fiber quality—length of fiber and thickness of wall, for example.

This paper presents an analysis of the

**Table I. Average Alkali Centrifuge Values for Coker 100 Wilt Raw Cotton Fiber Treated with 2,4-D Acid Applied as Contact Spray**

Concn. of 2,4-D Acid, P.P.M.	Av. Alkali Centrifuge Values of Cotton Fiber Treated at Different Stages of Growth			
	L	LF	F	Av. for concn.
Untreated check	194	202	204	200
5	213	210	235	219
10	221	246	246	238
25	248	195	244	229
50	235	244	255	245
L.S.D. <sup>a</sup>				
5% level				20
1% level				27
100	213	...	286	
200	218 <sup>c</sup>	...	278	
500	201 <sup>d</sup>	...	315	

<sup>a</sup> Least significant difference.

<sup>b</sup> No cotton bolls matured following these treatments.

<sup>c</sup> Average of 4 determinations from 1 replication (no yield obtained in 2 replications).

<sup>d</sup> Average of 8 determinations from 2 replications (no yield obtained in 1 replication).

Data for treatments with 100 through 500 p.p.m. 2,4-D acid not included in statistical analysis.

quality of this cotton fiber (roller ginned) using the alkali centrifuge value developed by Marsh (2) as an index of the damaging effects of 2,4-D on cotton. This method was used by Marsh to determine the extent of microorganic and extracellular enzymatic deterioration of cotton fiber, and its usefulness as a measure of the response of cotton fiber to such injurious agents as acids, hypochlorite, heat, and weathering was suggested.

### Method

The treatment of a weighed sample of raw cotton fiber with sodium hydroxide (2) to determine the per cent increase in weight, called the alkali centrifuge value, was the method used to assay the effects of 2,4-D on cotton fiber quality. Determination of this value reflects the effect of some damaging agent on the thickness of the fiber wall and the diameter of the fiber lumen. As the wall thickness is reduced, the amount of alkali that can be absorbed increases, and hence the alkali centrifuge value increases. All tests were made in a room maintained at 70° ± 2° F. and 65 ± 5% relative humidity. Four determinations were made for each of three replications for every treatment; thus a total of 12 assays was obtained for each field treatment wherever possible.

### Results

The factor of concentration was found by analysis of variance to be a significant cause (Table I) of the differences in alkali centrifuge values determined for cottons treated with 2,4-D sprays containing from 5 through 50 p.p.m. of acid. The average values for 10 through 50 p.p.m. are significantly different at the 1% level from the untreated check.

The average value obtained for the 5 p.p.m. treatment approaches a significant difference at the 5% level compared to the untreated check and the treatment with 10 p.p.m. of 2,4-D acid, and is significantly different at the 1% level compared to the 50 p.p.m. acid treatment.

Treatments with 2,4-D acid at concentrations higher than 50 p.p.m. were not included in the statistical analysis because plants sprayed with these higher dosages produced no cotton fiber at the LF stage. It is also evident from the data summarized in Table I that 100 through 500 p.p.m. of 2,4-D acid applied during the flowering stage obviously caused more severe damage to cotton than any other treatment. The statistical analysis was therefore applied to the 0 through 50 p.p.m. concentrations, for with these treatments equal numbers of determinations could be compared.

Neither time of application (L, or LF, or F) nor replication in time of application was a cause of variance, as far as could be determined by the alkali centrifuge values obtained. Analysis of field error, as it may be reflected in fiber quality, showed this factor to be significant, indicating that some of the differences obtained might be ascribed to variation in the field—soil, drainage conditions, and local variations in cultural practices, for example.

Treatment of Coker 100 Wilt cotton at the first leaf stage with higher than 50 p.p.m. of 2,4-D acid caused relatively slight impairment of quality. It is not known why the alkali centrifuge values determined for the three highest concentrations here are not so great as those determined for cotton treated with from 10 through 50 p.p.m. Cotton treated at the flowering stage was more severely injured by the three highest concentrations applied than by any other treatment.

### Discussion

Analysis of variance for 36 determinations made on untreated checks showed no significant differences between the alkali centrifuge values obtained. A coefficient of variation for these untreated samples was calculated to be 7.33%. The standard deviation for untreated checks was found to be 200 ± 15. The coefficient of variation for all the determinations made (a total of 240) was 7.42%, indicating that laboratory error was essentially the same for all determinations. These coefficient values for a bioassay method are considered fair and might be reduced by improving the method used for selecting the weighed raw cotton aliquots to be assayed. The coefficient of variation for the field test performed was calculated to be 17.7%. This value is within the range normally expected for field tests, but a lower value would have been desirable. As it stands, however, the coefficient for the field test is believed to reflect the significant field error as computed.

### Conclusion

The alkali centrifuge value is believed to afford a good index of gross injurious effect of 2,4-D on cotton fiber.

Cotton fiber quality was impaired, essentially, in direct proportion to the average for concentration from 5 to 50 p.p.m., regardless of the time of application.

Statistical analysis for variance in time of application of 5 to 50 p.p.m. (comparing L, LF, and F with one another) was found to be nonsignificant.

Significant differences for the alkali centrifuge value were found for cotton treated in the field with contact sprays containing up to 50 p.p.m. of 2,4-D acid, indicating that relatively small amounts of 2,4-D may cause considerable impairment of fiber quality and yields, as previously determined.

Concentrations higher than 50 p.p.m. in the flowering stage produced the most severe impairment of cotton quality, as indicated by the alkali centrifuge values determined.

Determinations made for quality of cotton produced by plants treated in the first leaf stage showed more severe damage as the dosage was increased up to 50 p.p.m. The reason for less severe injury of cotton in the first leaf stage treated with 100 through 500 p.p.m. is not known.

### Literature Cited

- (1) Baskin, A. D., *Proc. 7th Ann. Meeting N.E. Weed Control Conf.*, 23-31 (1953).
- (2) Marsh, P. B., *Plant Disease Repr.*, 37 (2), 71-6 (1953).

Received for review December 3, 1953. Accepted February 6, 1954.