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Effects of Sodium 2,3-Dichloroisobutyrate on Six Characteristics of American Upland Cotton[1]

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EATON'S[3] announcement that cotton plants sprayed with certain concentrations of a solution of sodium 2,3-dichloroisobutyrate produced flowers with little or no functional pollen, but produced fertile seeds when pollinated by pollen from untreated plants, caused considerable excitement among workers interested in methods of producing hybrid cotton. A field experiment, conducted on four varieties of American Upland cotton at College Station, Texas, in 1958, was designed to provide additional information on the gametocidial properties of sodium 2,3-dichloroisobutyrate (which in this report will be referred to by the manufacturer's designation, FW-450) with special reference to dosage and varietal effects when natural crossing is relatively high.

EXPERIMENTAL PROCEDURES

To provide genetically different plant material for testing varietal response to the gametocide dosage and for the estimation of the amount of natural cross-pollination in the various plots, four varieties (Arizona 44, Empire WR, Deltapine 15, and Brazos) were grown on the Agronomy Farm at College Station, Texas in unreplicated blocks with alternate rows of a "marker" stock which carried a simple dominant genetic factor for red leaves and stems.

Three gametocide dosages were used: *(1)* check (0.00% FW-450), *(2)* 0.25% FW-450, and *(3)* 0.40% FW-450.[4] A gametocide-dosage plot consisted of a single row 30 feet long. Each plot contained approximately 20 plants and was replicated 3 times in each varietal block. Each gametocide-dosage plot was divided into 2 equal subplots to provide for a comparison of flowering and fruiting behavior under open-pollination and controlled self-pollination. Seeds of the experimental varieties and the marker stock were planted May 5. Seedlings were thinned to a spacing of 18 inches. The plants were given routine cultural treatment throughout the growing season.

The FW-450 concentrate was dissolved in water to dilutions previously mentioned. Plants were sprayed initially on June 17 when the first "squares" or floral buds appeared. The same plants were sprayed again on July 1 and July 21. Sufficient quantities of spray were applied to wet the leaves to the point of run-off. A shield was used to prevent the spray from getting on the plants in the check plots and marker rows.

All flowers born on plants in the self-pollinated subplots were sealed by hand on the day prior to anthesis with a cellulose acetate compound which prevents the unfurling of the corolla and thus excludes bees and other pollinating insects, but allows self-pollination to occur on the day of normal anthesis. Flowers on plants in the open-pollinated subplots were allowed to bloom normally and were thus exposed to visitation by bees and other pollen carriers. Flowers were tagged daily for 6 weeks beginning on June 26. Data were recorded by weekly intervals, but the statistical analysis was based on the data for the total 6-week period.

The following characteristics were analyzed: *(1)* percentage of bolls set, *(2)* numbers of flowers, *(3)* lint production, *(4)* seed production, *(5)* germination percent, and *(6)* outcrossing percent. Estimates of male (pollen) sterility were obtained from the number of bolls set in the self- and open-pollinated subplots, the number of seed, and the percentage of outcrossing. The other measurements or deteminations povided valuable agronomic information on the effect of the gametocide.

The summary of data for each of the six characteristics investigated is given in Table 1. The presentation of experimental results and the discussion to follow are based largely on data given in this table.

EXPERIMENTAL RESULTS

Percentage of Bolls Set

In this experiment, the percentage of bolls set refers to the number of bolls that grew to maturity expressed as a percentage of the total number of flowers that bloomed. Thus the percentage of bolls set is the anthesis of shedding percentage.

The four experimental varieties differed significantly in percentages of bolls set. However, the gametocide dosages had no differential effect on the varieties as evidenced by the nonsignificant variety × gametocide-dosage interaction. In other words the varieties responded similarly at the different levels of gametocide treatment.

Irrespective of variety, the gametocide dosages resulted in significant differences in bolls set. In general there was a decrease in percentage of bolls set as the concentration of the gametocide material increased. The check plots were significantly higher in bolls set than the treated plots and the 0.25% FW-450 plots were significantly higher than the 0.40% FW-450 plots.

The method of pollination (open or self) had a significant effect on the percentage of bolls set in the various gametocide-dosage plots and furthermore, there was a significant pollination-method × gametocide-dosage interaction. Contrary to impressions gained at this station from other experiments involving natural (open) pollination and hand (self) pollination, the self-pollinated check (0.00% FW-450) plots set a higher percentage of bolls than the open-pollinated check plots. This relationship was definitely reversed in the 0.40% FW-450 plots. Boll setting in the 0.25% FW-450 plots approached that of the check plots and the differences between the check and the 0.25% FW-450 plots were smaller in the open-pollinated

Table 1—Effects of sodium 2,3-dichloroisobutyrate on 6 characteristics of 4 varieties of cotton.

Gametocide dosage	Variable						
	Boll set, %		Flowers per plot	Lint, g. per plant	Seed, g. per plant	% germination	% outcrossing
	OP*	SP					
			Arizona 44				
0.00%	40	49	812	25	40	56	31
0.25%	37	42	862	22	32	45	36
0.40%	28	21	894	12	19	33	39
			Empire WR				
0.00%	29	38	1176	22	42	63	24
0.25%	30	30	989	16	31	56	40
0.40%	26	11	935	12	25	44	47
			Deltapine 15				
0.00%	33	44	1072	20	30	51	29
0.25%	32	42	1141	17	26	47	37
0.40%	24	21	984	10	14	32	53
			Brazos				
0.00%	36	50	1067	20	36	63	40
0.25%	36	40	1116	20	33	47	46
0.40%	23	15	973	8	14	29	53

* OP and SP = open-pollination and self-pollination.

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[3] Eaton, Frank M. Selective gametocide opens way to hybrid cotton. Science 126:1174–1175. 1957.

[4] Material used was supplied by Rohm and Haas Company, Philadelphia, Pa

subplots than in the self-pollinated subplots. The extent to which bolls were set in the treated plots of the self-pollinated subplots represents a measure of the failure of FW-450 to produce male sterility; on the other hand, the slight reduction in percentage of bolls set in the 0.25% FW-450 plots and the considerable reduction in the 0.40% plots can be attributed to the gametocidal effect of FW-450. Obviously it was impossible to distinguish between pollen and ovule sterility in the self-pollinated plots. Assuming an adequate supply of viable pollen for a full set of bolls, estimates of these effects can be obtained from a comparison of the treated open-pollinated plots with corresponding self-pollinated plots. As already noted, there were practically no differences between the check and the 0.25% FW-450 plots under open-pollination, but the 0.40% treatment plot set a significantly lower percentage of bolls than were set in the check or the 0.25% FW-450 plots. However, the percentages of bolls set in the open-pollinated 0.40% treatments were consistently and in most cases considerably higher than those set in the self-pollinated 0.40% plots. From these comparisons it is apparent that ovule damage occurred at the 0.40% rate and that the pollen was damaged slightly at the 0.25% rate and severely at 0.40%.

Number of Flowers

Flowering in this experiment followed the usual sigmoid curve; relatively few flowers were produced the first and second weeks, peak flowering occurred during the fifth week, and flowering dropped off sharply in the sixth week. On the average, the open-pollinated plots produced more flowers than the self-pollinated, but this difference apparently was not due to the gametocide dosages. Data from the open-pollinated plots were used for the statistical analysis of the number of flowers produced. No significant differences were found to be associated with varieties or with gametocide dosages; in all varieties, except Arizona 44, the 0.40% FW-450 plots tended to produce fewer flowers than the other two treatment plots.

Lint and Seed

The varieties did not differ significantly in production of lint and seed although the differences among the varieties in these respects appeared to be more variable in seed production than in lint production. Within varieties, the correlation between lint and seed production was significant ($r = 0.93$ for 10 degrees of freedom).

As was expected from the observed reduction in bolls set in the open-pollinated gametocide-treatment plots (particularly the 0.40% FW-450 plots), there was a corresponding highly significant reduction in the amounts of lint and seed produced. The magnitude and direction of the differences in lint and seed production that may be ascribed to different concentrations of the gametocide followed the pattern of the data on the percentage of bolls set. Lint and seed determinations were not obtained from the self-pollinated plots. However, in view of the highly significant dosage × pollination-method interaction in respect to percentage of bolls set reported earlier, production of lint and seed in these plots also must have been proportional to the number of bolls set and at a generally lower level.

Germination

Germination tests were made on seeds from plants in the open-pollinated plots. On the average, germination decreased significantly as the concentration of the gametocide solution increased. The viability of seeds from all plots was low by commercial standards; the average germination percentages were 60, 49, and 34 for the check, 0.25% and 0.40% FW-450 dosage plots, respectively.

Natural Crossing

As explained under experimental procedures, the green-leaf plants of the varieties in the gametocide-dosage plots were grown between rows of red-leaf plants and in the open-pollinated subplots these green-leaf plants were allowed to pollinate under natural (open) conditions. Seedlings grown from a random sample of seeds from each open-pollinated plot had either green or red leaves. The number of red-leaf seedlings expressed as the percentage of the total number of seedlings in the sample was considered a measure of the percentage of natural crossing. Such percentages are, no doubt, minimum values, since hybrids between green-leaf plants could not be detected by the method employed. Therefore, the "true" percentage of natural crossing that occurred in the area where the plots of this experiment were located would be represented by a considerably higher value.

The percentage of outcrossing in the check plots ranged from 24% in Empire to 40% in Brazos. However, the over-all difference in outcrossing among the varieties was not statistically significant.

There were significant differences in the percentages of outcrossing among the gametocide-dosage plots and the data show a positive relationship between the concentration of FW-450 applied and the percentage of outcrossing obtained. As an average of the four varieties, the percentage of outcrossing was 31%, 40%, and 48% for the check (0.00% FW-450), 0.25% FW-450, and 0.40% FW-450 dosage plots, respectively. In view of these findings there can be little doubt that the chemical (FW-450) induced a significant amount of male sterility and that significant increases in the percentage of outcrossing occurred as a result.

The percentage of outcrossing in the 0.25% FW-450 plots was approximately halfway between the values for the check and the 0.40% FW-450 plots. This is in striking contrast to the results obtained for the other characteristics measured in which the values at the 0.25% treatment level, though intermediate in position, lay very near those for the check plots.

Also noteworthy is the strong negative within-variety association between outcrossing percent and germination percent, $r = -.98$ for 10 d.f.

DISCUSSION

A relatively high rate of natural crossing was one of the prime requirements of this experiment. Therefore the Agronomy Farm adjoining the Main Campus at College Station was chosen as the experimental site. At this location natural crossing in excess of 25% has been recorded on several different occasions. Unfortunately the soils at the Agronomy Farm are not well suited to cotton performance trials in which reliable estimates of yielding potential are sought.

The four varieties used were chosen to represent a range of different genotypes within the American Upland group of cultivated cottons. Their pedigrees, as well as the fact that they had shown significant differences in yield in formal yield trials, were major considerations in their selection. That these varieties did not exhibit significant

differences in any of the six characteristics measured except percentage of bolls set may be attributed to the fact that the fertility and water-holding capacity of the soils of the Agronomy Farm are so low that varieties or strains seldom are able to manifest their inherent capacity in terms of production or other economic characteristics. This behavior also may have contributed to the lack of association between variety and gametocide dosage found in every case. However, even under the low "performance ceiling" of the experiment there is no reason to believe that strong variety × gametocide-dosage interactions would not have been detected statistically if they had been present. At any rate the role of the varieties in this experiment was insignificant and so far as the analyses of the effects of the gametocidal treatments are concerned all of the plants may as well have been of a single stock.

The gametocide induced significant effects in 5 of the 6 characteristics studied. The data on percentage of bolls set in open- and self-pollinated subplots and the percentage of outcrossing, determined from the relative number of red-leaf plants in open-pollinated subplots, led to the conclusion that FW-450 was capable of inducing a certain amount of sterility and that a somewhat higher percentage of male than female sterility was induced. The different concentrations produced noticeably different results. In all cases except the percentage of outcrossing, the plants treated with 0.25% FW-450 resembled those in the check (0.00% FW-450) rather than those in the plots that received the higher concentration of the material. This was true of the appearance of the plants and the condition of the anthers as well as of the six characteristics measured. Plants in the 0.25% FW-450 plots showed only a trace of leaf burning, were barely taller than the plants in the check rows, and showed no striking differences in anther dehiscence or in the amount of "normal" pollen. On the other hand, by the end of the test period the plants in the 0.40% FW-450 plots were taller than those in the check plots and showed definite symptoms of leaf burning. Differences in height no doubt were due to differences in number of bolls set (see Table 1). However, the anthers and the pollen of the plants in the 0.40% FW-450 plants were not strikingly abnormal. Apparently the sterility or partial sterility induced by the FW-450 material was not completely associated with the appearance or the behavior of the plants, particularly so in the case of the lower concentration. A comparison of the percentage of bolls set in the open-pollinated subplots treated with 0.25% FW-450 and the check shows no appreciable difference, but there was a 30% increase in outcrossing in the 0.40% FW-450 plots.

Highest percentages of hybrid seed were obtained from the plots treated with 0.40% FW-450 and if other economic considerations such as production of lint and seeds per plant are ignored, the higher concentration of the gametocidal material would be recommended for hybrid seed production. However, it must be pointed out that outcrossing was only 50% in the 0.40% FW-450 plots as compared with the check plots which themselves showed an average of 31% outcrossing. Furthermore, this increase in outcrossing by use of a 0.40% concentration of FW-450, was accompanied by significant and serious reductions in percentage of bolls set, amount of lint and seed produced, and germination percentage. It should be emphasized that not only were the amounts of lint and seed reduced when concentrations of FW-450 as high as 0.40% were used but effective production of hybrid seed was further reduced by lower germination. Pate and Duncan[5] reported similar results but their data show that FW-450 had even less selective effect on the male gametes than was found in the present experiment.

It might be argued that higher concentrations (and a more critical timing of the applications) would result in complete male sterility. If further experimentation should prove this to be the case, the assurance of complete hybridity in all of the seed produced from treated plants might compensate partly for the reductions in yields of lint and seed and in viability of the seed produced from gametocide-treated cotton plants. Viewed from the standpoint of practical production of hybrid seed, there was no evidence from the present experiment that would encourage the expectation that complete male sterility, accompanied by a sufficient level of female fertility, yield and seed viability, to produce hybrid seed in economical quantity can be achieved by treatment with higher concentrations of FW-450.

Since a considerable proportion of the seeds from plants treated with FW-450 will be inbred or nonhybrid, hybrid-seed production schemes involving the treatment of the seed-production rows with FW-450 must be designed to cope with both hybrid and nonhybrid plants in production fields in essentially the same manner as would be required in hybrid-seed production systems in which the plants in the seed-production rows were normal in respect to male sterility, i.e. not treated with a gametocide. If one were convinced of the feasibility of using FW-450 as a gametocidal material, the practical question to be answered is whether it would be more economical to use a concentration that will give a higher percentage of hybrids than to use one that will give a lower percentage of hybrids but a relatively higher total quantity of viable hybrids and a higher over-all yield of lint and viable seed.

SUMMARY

The effects of concentrations of 0.00%, 0.25%, and 0.40% of FW-450 (2,3-dichloroisobutyrate) on 6 characteristics of each of 4 cotton varieties were investigated and the following conclusions drawn:

1. Regardless of the concentration of FW-450 used, the varieties did not differ significantly in any of the characters measured except percentage of bolls set nor was there a significant variety × gametocide-dosage interaction.

2. Significant effects were induced in 5 of the 6 characters.

3. A measurable amount of sterility was induced and there was a somewhat higher percentage of male than female sterility.

4. Plants treated with 0.40% FW-450 were markedly different from the untreated plants in appearance, but such differences were not observed in the comparisons of the 0.25% FW-450 and the check plots.

5. The highest percentage of outcrossing occurred in the 0.40% FW-450 plots, but plants in these plots were significantly lower than the plants in the check plots in percentage of bolls set, amounts of lint and seed produced, and germination percentage of the seed.

6. The practicability of using FW-450 as a gametocide in hybrid-seed production schemes was questioned.

[5] Pate, J. B., and Duncan, E. N. Evaluation of sodium 2,3-dichloroisobutyrate as a selective male gametocide in cotton. Agron. J. 52:506–508. 1960.