Effects of Sodium 2, 3-Dichloroisobutyrate, a Selective Gametocide, on Sugar Beets[1]

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Introduction

Some form of male sterility must be used in order to obtain crosses between self-fertile inbred lines of sugar beets or to insure a high percentage of crossing between heterogeneous strains. Cytoplasmic and Mendelian forms of male sterility have been reported (2, 3)[3], but much time and effort are required to incorporate them into desired lines or strains. Since sodium 2,3-dichloroisobutyrate (FW-450) has been shown to have a selective gametocidal effect on cotton (1), its effectiveness as a selective gametocide on sugar beets was tested. This paper reports results of preliminary studies dealing with this chemical. The objectives of this study were to determine whether sodium 2,3-dichloroisobutyrate has a selective gametocidal effect on sugar beets, to determine the optimum concentration and time of application of the chemical if a selective gametocidal action exists, and to obtain information on the nature and extent of undesirable side effects.

Materials and Methods

A greenhouse experiment and a field experiment were conducted in 1958 to determine the effects of FW-450 on sugar beets. A carrier rate of 100 gallons per acre was used in both experiments. The spray material was applied with a compressed-air sprayer consisting of an air storage tank into which air was introduced from a compressor, a regulator valve to control the pressure at which the material was applied, a reservoir for the liquid, and a spray nozzle. By calibrating the sprayer before making the applications, the dosage administered was controlled very accurately. Individual plants, with a shield around each to prevent drift of the spray material, were sprayed.

Field Experiment

The experimental design for the field experiment was a modified split-split plot with four replications. The whole plots were varieties, the sub plots times of application, and the sub-sub

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[3] Numbers in parentheses refer to literature cited.

plots concentrations of FW-450. The plant material consisted of 192 roots from each of three commercial varieties (A54-1, A54-6, and A54-7), a total of 576 roots. The roots were grown in 1957, overwintered in a root cellar, and planted on April 30, 1958. Each replication consisted of three blocks, each block representing a variety. Each variety block was divided into three groups of four rows. One group was given an initial application at emergence (May 19). A second group was given an initial application at early bolting (May 29), while the third group in each block was given an initial application at the pollen-mother-cell stage (June 6). One row within each group was untreated and served as a check and a pollen source, one received a treatment of 1/3 percent, one 1.0 percent, and one 3.0 percent FW-450 by weight. Each row consisted of four plants spaced 2 feet apart with 3 feet between rows.

Modifications of the split-split plot design were made to obtain information on the effect of repeated applications. Each plot treated at emergence was split in half, and one half, chosen at random, received repeat treatments at the early bolting and pollen-mother-cell stages while the other half received only the emergence spraying. The plots treated at early bolting also were divided and one-half received an additional treatment at the pollen-mother-cell stage.

Data were obtained for the following characters: period to first flowering, period from first flowering to first pollen shedding, seed yield, percentage of plants which shed pollen, and germination percentage. Period to first flowering was measured as the days from May 31 to the date when the first flower was observed to be open. Date of first pollen shedding was recorded as the first day on which any flower on the plant was observed to shed pollen. Period from first flowering to pollen shedding was the days from the date of first flowering to the date of first pollen shedding. The data listed were taken on an individual plant basis. Germination percentages were obtained for the following treatments: check, 1/3 and 1.0 percent applied at the pollen-mother-cell stage, 1/3 and 1.0 percent applied at early bolting, and 1/3 percent applied at both early bolting and the pollen-mother cell stage. Composite samples of two grams of seed from each of 24 plants from each treatment were obtained. Eight 100-seedball lots were taken from each such sample. The 48 lots thus obtained were arranged in a randomized complete-block design in the germinator. Germination percentage was computed as the percent of seedballs showing at least one radicle.

Since the three varieties were segregating for red and green hypocotyl color, each root in the field was classified for bud

color, and hypocotyl color counts were made on the open-pollinated progeny of the green-hypocotyl plants in the check plots, in the check plots in the pollen-mother-cell groups, in the plots to which the 1/3 percent treatment was applied at the pollen-mother-cell stage, and in the plots to which the 1.0 percent treatment was applied at the pollen-mother-cell stage. If an appreciable amount of selfing were occurring in the green-hypocotyl parents, then a comparison of the percentage of red-hypocotyl plants obtained in the progeny of green-hypocotyl plants in the check plots with the percentage of red-hypocotyl plants in the progeny of green-hypocotyl plants in the treated plots should give an indication of the effectiveness of the gametocide in promoting cross-fertilization.

Greenhouse Experiment

The greenhouse planting consisted of 40 quarter roots of each of two inbred lines; 52-407, which has red roots, and 54-565, which has yellow roots. The quarters of these lines were alternated. Spray applications consisted of a 1/2 percent solution of FW-450. Alternate pairs of plants, one of each inbred, were sprayed; thus one-half of the plants of each line were treated while the other half were untreated and served as pollen parents and as a check on the effectiveness of the spray applications. Of the pairs of sprayed plants, one-half received two applications—one at the emergence stage (April 28) and one at the early bolting stage (May 12). The other pairs of sprayed plants received an additional spraying at the pollen-mother-cell stage (May 26).

Hypocotyl color counts on progeny of 54-565 were made on composite samples of seed from the untreated check plants, the plants sprayed twice, and the plants sprayed three times. Since 54-565 is self-fertile, the percentage of red hypocotyl plants in progeny of check plants as compared with the percentage obtained in progeny of the treated plants provides a measure of the effectiveness of the gametocide. Seed yield was measured as grams per plant. Composite samples of seed from each of the three treatments from each inbred were used to obtain germination percentage by the method described for the field experiment.

Statistical Methods

The days to first flowering and seed yield data from the field experiment were subjected to analysis of variance. Since a single orthogonal analysis of the design used was not possible, separate analyses of variance were made for time of single application

and for number of applications. Chi-square analysis was used for the data on percent of plants shedding pollen, germination percentage, and hypocotyl color.

Results

The results of the field and greenhouse experiments will be discussed separately.

Field Experiment

Homogeneity chi-square tests reveal that, for the single applications, the emergence and early-bolting applications are different in percent of plants shedding pollen with odds between 19:1 and 49:1 (see Table 1). Further analysis of Table 1 reveals that the average percent of plants shedding pollen is less for the single applications at the pollen-mother-cell stage than for single applications at either the emergence or early-bolting stage with odds greater than 99:1. Within the pollen-mother-cell stage, the 1.0- and 3.0-percent applications have significantly lower percentages of plants shedding pollen than the ⅓-percent application or the check. The double and triple applications reduced the percent of plants shedding pollen significantly more than any of the single applications. The repeated applications of the 1.0 and 3.0 percent solutions caused the greatest reduction in percent of plants shedding pollen.

The means for the characters number of days from May 31 to first flowering, number of days from first flowering to pollen shedding, and seed yield in grams for the various concentration and time of application combinations are shown in Table 2. The 1.0-percent treatment applied three times and the 3.0-percent applied twice delayed flowering about 5 days, the 3.0 percent applied three times delayed flowering about 8 days. Both delays were significant.

The means for days from first flowering to pollen shedding were computed from only those plants which actually shed pollen. Pollen shedding on the check plots occurred, on the average, one to two days after the first flowers were observed to be open. Of the single applications, those made at the early bolting and the pollen-mother-cell stages with concentrations of 1.0 and 3.0 percent delayed pollen shedding 10 to 12 days, while a single application of ⅓ percent at the pollen-mother-cell stage or of 3.0 percent at emergence delayed pollen shedding about 6.5 days. All the repeat applications delayed pollen shedding an average of at least 5 days.

The analyses of variance for seed yield revealed highly significant differences between concentrations, number of appli-

Table 1.—Total Number of Plants in Three Varieties of Sugar Beets, and Percent of Plants Shedding Pollen for Time of Application and Concentration Combinations in Field Gametocide Test.

Stage of Growth at Application and Concentration	Total Plants (Number)	Plants Shedding (Percent)
Emergence		
Check	46	100.0
1/3%	24	95.8
1.0%	22	81.9
3.0%	24	79.2
Total[1]	70	85.7
Early Bolting		
Check	46	97.8
1/3%	23	82.6
1.0%	24	66.7
3.0%	23	65.2
Total[1]	70	71.4
Pollen Mother Cell		
Check	46	97.8
1/3%	47	87.2
1.0%	47	38.3
3.0%	46	26.1
Total[1]	140	50.7
Early Bolting and Pollen Mother Cell		
Check[2]	46	97.8
1/3%	24	70.8
1.0%	24	29.2
3.0%	24	0.0
Total[1]	72	33.3
Emergence, Early Bolting, and Pollen Mother Cell		
Check[3]	46	100.0
1/3%	24	79.2
1.0%	23	34.8
3.0%	23	4.3
Total[1]	70	40.0

[1] Excluding the check.
[2] Check is the same as that for the Early Bolting block.
[3] Check is the same as that for the Emergence block.

cations, and times of application. From Table 2 it can be seen that all treatments except the 1/3 percent applied at emergence reduced seed yield to some extent. Of the other treatments, the 1.0 percent application at emergence reduced seed yield the least. The single 1/3 percent applications at early bolting and the pollen-mother-cell stage, and the double application of 1/3 percent caused about the same reduction in seed yield, to an average of 103.3 grams per plant compared with 131.7 grams per plant for the average of the checks. The rest of the treat-

ments range from an average of 90.1 grams for 1.0 percent applied at early bolting to 5.9 grams for 3.0 percent applied three times.

The number of red- and green-hypocotyl plants of each variety in the field experiment is shown in Table 3. The red-

Table 2.—Period to First Flowering, Period from First Flowering to Pollen Shedding and Seed Yields for Treatments Applied to Three Varieties of Sugar Beets in the Field Gametocide Test.

Stage of Growth at Application and Concentration (percent)	Days from May 31 to First Flowering (Number)	Days from First Flowering to Pollen Shedding (Number)	Seed Yield (Grams)
Emergence			
Check	19.2	1.9	131.2
1/3%	18.9	3.4	128.9
1.0%	18.6	4.9	118.0
3.0%	21.0	8.2	77.1
Early Bolting			
Check	19.4	1.0	137.2
1/3%	20.5	5.7	100.4
1.0%	19.5	10.8	90.1
3.0%	20.3	10.9	63.1
Pollen Mother Cell			
Check	20.6	1.1	126.6
1/3%	19.7	7.7	104.6
1.0%	19.9	12.7	72.7
3.0%	20.5	11.9	35.9
Early Bolting and Pollen Mother Cell			
Check	19.4	1.0	137.2
1/3%	20.9	9.8	105.0
1.0%	20.9	12.3	38.9
3.0%	24.6	—	10.0
Emergence, Early Bolting, and Pollen Mother Cell			
Check	19.2	1.9	131.2
1/3%	21.0	10.2	69.1
1.0%	24.6	13.4	27.5
3.0%	27.8	7.0	5.9

Table 3.—Number of Sugar Beet Plants with Red- and Green-Hypocotyls, q^2, and q Values for Field Experiment.

Variety	Hypocotyl color		q^2	q
	Red	Green		
A54-1	162	28	0.1474	0.3839
A54-6	130	50	0.2778	0.5271
A54-7	149	39	0.2074	0.4554

and green-hypocotyl plants were distributed at random throughout the field. Assuming that each variety represents a panmictic population, q, the frequency of the r gene, can be estimated as the square root of q^2, the proportion of green-hypocotyl plants in each variety. Weighting the values of q thus obtained by the number of plants in each population values of q = 0.4543 and p = 0.5457, where p = 1-q, are obtained.

The number of red- and green-hypocotyl plants obtained by germinating open-pollinated seed from green-hypocotyl plants in plots of certain treatments are shown in Table 4. The percent of red-hypocotyl plants in these is a direct estimate of p since all the red-hypocotyl plants had to result from cross-fertilization. Chi-square values for the fit of the obtained numbers of red-hypocotyl plants to the number expected on the basis of random mating are shown in Table 5. From this table it can be seen that while none of the individual chi-squares exceeded the 5

Table 4.—Number of Red- and Green-Hypocotyl Plants in the Progeny of Green-Hypocotyl Sugar Beet Plants for Certain Treatments in the Field Experiment.

Treatment	Hypocotyl Color			Percent Red	
	Red	Green	Total	Obtained	Expected[2]
Check	357	333	690	51.74	54.57
PMC[1] Check	217	192	409	53.06	54.57
PMC 1/3%	64	59	123	52.03	54.57
PMC 1.0%	57	65	122	46.72	54.57

[1] PMC designates pollen-mother-cell.
[2] On the basis of random mating.

Table 5.—Chi-Square and P Values for Fit of Obtained Numbers of Red-and Green-Hypocotyl Sugar Beet Seedlings to the Numbers Expected on the Basis of Random Mating.

Treatment	D.F.[1]	Chi-Square	P
Check	1	2.2229	0.20-0.10
PMC[2] Check	1	0.3791	0.70-0.50
PMC 1/3%	1	0.3151	0.70-0.50
PMC 1.0%	1	3.0473	0.10-0.05
Sum	4	5.9644	0.30-0.20
Total	1	4.4255	0.05-0.02
Interaction	3	1.5389	0.70-0.50

[1] D.F. designates degrees of freedom.
[2] PMC designates pollen-mother-cell.

percent probability level, the chi-square for the totals fell between the 5 and 2 percent points. The sum of the individual chi-squares with four degrees of freedom fell between the 0.30 and 0.20 probability levels while the interaction chi-square with three degrees of freedom fell between the 0.70 and 0.50 levels. Study of Table 4 reveals that fewer red-hypocotyl plants were obtained than were expected in all of the populations. The difference is so small that for all practical purposes the populations can be considered as random mating.

The treatments fall into three distinct groups as regards percentage of germination (see Table 6). Germination percentage was reduced the least by the 1/3 percent treatment at early bolting. Reduction in germination percentage was intermediate for the 1/3 percent treatment at the pollen-mother-cell stage and greatest for 1.0 percent at the pollen-mother-cell stage, 1.0 percent at early boting, and 1/3 percent applied at both early bolting and pollen-mother-cell stages.

Table 6.—Total Number of Seedballs of Three Varieties of Sugar Beets, and the Percentage of Germination for Certain Treatments from the Field Gametocide Test.

Treatment	Seedballs Tested (Number)	Seedballs Germinating (Percent)
Check	800	49.1
1/3% PMC[1]	800	35.9
1.0% PMC	800	23.1
1/3% EB[2]	800	43.6
1/3% EB & PMC	800	26.0
1.0% EB	800	29.0

[1] PMC designates pollen-mother-cell.
[2] EB designates early bolting.

Greenhouse Experiment

Since 54-565 is rr and 52-407 is RR any red-hypocotyl plants in the progeny of 54-565 must have resulted from crosses with 52-407. From Table 7 it can be seen that the percentage of red-hypocotyl plants in a composite sample of seed from the untreated check plants of 54-565 is only 3.51. This indicates that inbred 54-565 is mostly self-fertile. The progeny of plants of 54-565 which were sprayed twice contained 13.89 percent red-hypocotyl plants while progeny of those which were sprayed three times contained 40.27 percent red-hypocotyl plants. Chi-square tests reveal that the proportion of red-hypocotyl plants, and thus the percentage of crossing, is significantly higher for the plants sprayed three times than for either the check plants or those sprayed twice.

Table 7.—Number of Red- and Yellow-Hypocotyl Seedlings in Progeny of Sugar Beet Inbred 54-565.

Number of Sprayings	Total Plants (Number)	Plants with Red-Hypocotyl (Percent)
Check (0)	1940	3.51
Two	1497	13.89
Three	956	10.27

Study of Table 8 reveals that the seed yield of 54-565 was not reduced by two applications of gametocide and that three applications reduced seed yield only 10 grams per plant. For 52-407 two sprayings reduced seed yield 18.61 grams while the third spraying reduced seed yield an additional 15.49 grams.

Table 8.—Mean Seed Yield in Grams per Plant for Two Inbred Lines of Sugar Beets Sprayed with FW-450 0, 2 and 3 Times in the Greenhouse Test.

Number of Sprayings	Inbred		Mean
	54-565	52-407	
Check (0)	132.11	126.21	129.16
Two	133.80	107.60	120.70
Three	122.11	92.11	107.11
Mean	130.18	113.24	121.71

The numbers and percentages of seedballs germinated from the greenhouse seed are shown in Table 9. Chi-square analysis of these data reveals that, on the average, two treatments significantly increased the germination percentage while three sprayings caused a significant decrease in germination percentage.

Table 9.—Germination Percentage for Two Inbred Lines of Sugar Beets Sprayed with FW-450 0, 2 and 3 Times in the Greenhouse Test.

Inbred and Number of Sprays	Total Seedballs (Number)	Germinated Seedballs (Percent)
54-565		
Check	800	87.1
Two	800	91.2
Three	800	76.1
52-407		
Check	800	86.8
Two	800	89.1
Three	800	58.2

The decrease obtained from 52-407 sprayed three times is larger than the decrease obtained from 54-565 sprayed three times with odds greater than 99:1.

Discussion

Eaton (1) reported induction of male sterility in cotton by use of FW-450. The results reported in this paper provide preliminary data on the effects of this chemical on sugar beets.

The results of the field experiment indicate that complete elimination of pollen shedding by application of the chemical at the stages studied is possible only with extremely heavy applications. Pollen shedding could be delayed as much as a week or 10 days by use of much smaller applications. The greatest degree of control with the least reduction in viable seed produced was achieved with the 1/3-percent application at the pollen-mother-cell stage. Plants given this treatment had a germination percentage of 35.9 percent, compared to 49.1 percent for the check; seed yield of 104.6 grams per plant, compared to 126.6 for the check; 87.2 percent of the plants shedding pollen; and period to pollen shedding, for plants which did shed pollen, of 7.7 days.

Significant delays in date of first flowering were found only where extremely heavy applications were made. In these cases the delay was probably caused by severe burning of the main flowering stalk so that first flowering had to await the development of secondary flowering branches. No significant delay in flowering was found when potentially useful concentrations of the chemical were used.

Hypocotyl color counts made on the progeny of green-hypocotyl plants indicated that the varieties used in the field experiment are for all practical purposes completely cross-pollinated. Thus it should be possible to obtain synthetic varieties, made up of individuals selected from such populations, which are essentially completely cross-pollinated.

When a self-fertile inbred line was treated with three applications of a 1/2-percent solution in the greenhouse the percent of hybrids was increased from 3.51 to 40.27 with only a slight reduction in seed yield and germination percentage. The figure 40.27 percent represents only the measureable crossing which occurred. Since 50 percent of the untreated plants were yellow-rooted, the amount of actual cross-pollination caused by the gametocide is probably somewhat greater than 40.27 percent. Thus chemical control of pollen formation offers an effective method of forcing certain self-fertile inbred lines to hybridize.

For the plant breeder interested in testing combining ability of self-fertile lines or in obtaining crosses between populations which, while not self-fertile, are intra-population fertile, chemically induced male sterility may be an extremely useful tool.

Another aspect of the problem apparent from the greenhouse experiment is the different responses of the two inbred lines to the chemical. Inbred 52-407 showed a greater reduction in both seed yield and germination percentage than 54-565 when three applications of the chemical were made. This indicates that some lines are more resistant to the effects of FW-450. Similar results have been reported for cotton (1).

Many problems connected with the use of selective gametocides on sugar beets remain to be solved. From the practical viewpoint, more work needs to be done in determining the exact combination of concentrations, carrier rates, time of application, and number of applications which will give maximum selective gametocidal action with minimum undesirable effects. A large number of strains, inbred lines, hybrids, and commercial varieties should be treated to determine the range of response to the chemical. From a theoretical standpoint, it would be of interest to study the cytological and histological effects of the chemical. At what stage in the process of anther and pollen formation does it have its effects? Another problem which should be studied is that of possible production of mutations in the megasporocytes of treated plants.

Although many problems remain unsolved, it can be concluded from the results of these preliminary investigations that some lines of sugar beets can be made effectively male sterile while retaining the major part of their seed-producing ability; the optimum concentration of FW-450, when applied at a carrier rate of 100 gallons per acre, is probably between 1/3 and 1/2 percent; and inbred lines differ in their response to the chemical.

Summary

(a) A field and a greenhouse experiment were conducted to determine the effectiveness of sodium 2,3-dichloroisobutyrate (FW-450) as a selective gametocide on sugar beets.

(b) Concentrations used in the field experiment were 1/3%, 1.0%, and 3.0% applied at a carrier rate of 100 gallons per acre. Stages of growth at time of application were emergence; early bolting; pollen-mother-cell stage; early bolting, and the pollen-mother-cell stage; and emergence, early bolting, and the pollen-mother-cell stage. All possible combinations of times of application and concentrations were applied to three varieties in four replications of a modified split-split plot design.

(c) A concentration of ½ percent at a carrier rate of 100 gallons per acre was used in the greenhouse experiment. Two different treatments (applications at emergence and early bolting and applications at emergence, early bolting, and the pollen-mother-cell stage) were used. Forty quarter roots of each of two inbred lines were used.

(d) Of the treatments used in the field experiment, applications of ⅓ percent at the pollen-mother-cell stage gave a maximum of delay in pollen shedding with a minimum of adverse effect.

(e) Hypocotyl color counts on seedlings obtained from green-hypocotyl plants in the field experiment revealed that the three varieties used are essentially completely cross-pollinated.

(f) Hypocotyl color counts on progeny of the yellow-rooted inbred used in the greenhouse study revealed that the percent of hybrids had been increased from 3.51 for the check to 40.27 for plants treated three times.

(g) Different responses to the effects of the gametocide, as measured by seed yield and germination percentage, were found for the two inbreds studied.

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