# Materials and Methods Used in Producing Commercial Male-Sterile Hybrids

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### Introduction

During the lifetime of the American Society of Sugar Beet Technologists, many reports have been made dealing with male sterility, self fertility, Mendelian male sterility and self sterility, as these principles are related to sugar beet breeding (3) (4) (6) (7) (9)<sup>2</sup>. It is not the purpose of this paper to discuss technical aspects of the principles mentioned above, but to indicate how the various "breeding tools" have been adapted to the commercial production of sugar beet hybrids.

Sugar beet hybrids, made by use of a cytoplasmic male-sterile and a self-fertile inbred, were first reported by Owen in 1946 (5). In the years to follow, many new inbred lines were produced by utilizing the true self-fertile character, and as lines of promise were developed, male-sterile equivalents of these lines were produced by introducing the cytoplasmic male-sterility factor and then backcrossing three or four times to the inbred line.

From this type of a program, the inbred CT9 and its male-sterile equivalent were developed. Top crosses and true hybrids made with CT9 demonstrated, that under controlled conditions where the male sterile could be rogued carefully, hybrids could be produced that would yield up to 125 percent of the mass selected varieties in common use by the sugar beet industry (1).

In 1952, Owen reported a second type of male sterility called by him, Mendelian male sterility, because its inheritance was controlled entirely by genes in the germ plasm or cell nucleus, in contrast to the major influence of the cytoplasm in cytoplasmic male sterility (8). While the  $\mathbf{F}_1$  generation of a cytoplasmic male-sterile and a type O pollinating line was completely male sterile, the  $\mathbf{F}_1$  generation of a Mendelian male-sterile and a pollinating line was completely male fertile. Owen pointed out the significance of Mendelian male sterility in producing vigorous  $\mathbf{F}_1$  pollinators for use in the commercial production of hybrid varieties (9) .

By 1952, the only apparent stumbling block in the way of producing cytoplasmic male-sterile hybrids on a commercial scale was the occurrence, in the CT9 male-sterile line, of an occasional pollen producer and more frequently, plants classed as semi-male steriles, which, under some conditions, might produce some pollen (7).

The seriousness of these semi-male-sterile plants was evaluated by both the Amalgamated and Utah-Idaho Sugar Company. Field hybrids were produced and the male sterile in one set of plots was carefully rogued, while in the other plot, no roguing was done. The hybrids from these two plots were then thoroughly tested in variety plots and found to be identical

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Numbers in parentheses refer to literature cited.

in performance (10). This demonstrated that the semi-male steriles in CT9 were not of commercial importance so long as Elite seed increases were rogued carefully to keep out any contaminants.

Hybrids were also produced to compare the value of keeping the pollinator and male sterile separate, as compared to blending in the pollinator as the commercial seed increase was made. Subsequent tests of these hybrids indicated, that where commercial top cross parents were used as pollinators, or where F<sub>1</sub> pollinators were used, there was no advantage in planting the male sterile and pollinator separate.

With this information as a background, the Utah-Idaho Sugar Company decided, in the Fall of 1958, to begin commercial production of multigerm hybrid sugar beet seed.

# Monogerm Hybrid Seed Development

From the references already cited, it is evident that many of the basic principles essential to the production of sugar beet hybrids were fairly well developed by 1948 and that it had already been demonstrated, with multigerm inbred lines, that hybrids of superior yielding ability could be produced.

In the Fall of 1948, discovery of the monogerm inbred lines SLC 101 and SLC 107 was made by V. F. and Halen Savitsky (41). During the next six years, monogerm lines of increased curly-top resistance and improved seed characteristics were developed (2) (14). Self fertility and male sterility were principal breeding tools used in the development of basic breeding stocks for the production of monogerm hybrids (12). By 1954, inbred lines with improved yielding ability, high sucrose content, and curly-top resistance, higher than U. S. 33, had been released to the sugar beet industry (13).

In 1954, additional monogerm lines, derived by V. F. Savitsky from backcrossing the monogerm lines SLC 600 and SLC 175 to such curly-top varieties as U. S. 22/4 and U. S. 35/2, were released by the U.S.D.A. to the sugar beet industry. The ground was now laid for the commercial production of monogerm hybrid seed. Just two years after commercial production of multigerm hybrids began, the Utah-Idaho Sugar Company shifted its entire commercial seed acreage to the production of monogerm male-sterile hybrid seed.

### Production of Elite Seed Stocks

Elite seed increases of cytoplasime male steriles are made on isolated plots of about .5 acres in size. The following planting pattern is quite generally followed: 2 rows pollinator; 2 rows blank; 12 rows male sterile; 2 rows blank; 2 rows pollinator, etc. This plot is rogued carefully to eliminate pollen producers and as many as possible of the semi-male steriles. In cases where monogerm elite stocks are being reproduced, the plot is also carefully rogued for multigerm plants. All roguing is done as early as possible to prevent pollination from undesirable plants. Roguing is facilitated if plants in the male-sterile strips are thinned to singles in the early spring.

#### Stock Seed Increases

Stock seed increases are made on fields of from 5 to 10 acres in size. The general planting scheme, as used with Elite seed plots, is followed.

Stock seed fields are too large to make detailed roguing practical. Some roguing in monogerm stock seed increases may be done to eliminate stray multigerm plants. Contamination with occasional multigerm seed will possibly not be eliminated until multigerm seed is no longer being handled in any of the same equipment that is being used for monogerm seed.

As stock seed increases are being made, it is important that the male sterile, being produced for planting commercial seed acreage, has good vigor. This can, of course, be accomplished by producing an F<sub>1</sub> male sterile for commercial seed production. This can be illustrated by an actual example. In 1952, 610 MSmm x 610 mm was increased at Saint George. In 1953, 610 MS x 91 mm was increased in Saint George. In 1954 (610 MS x 91 mm) x 91 mm was increased at both Saint George and in Oregon. In the Fall of 1955, the monogerm stock seed increases were (610 MS x 91 mm x 91 mm) x 108 mm. The Elite seed plot in Saint George, planted in the Fall of 1955, was (610 MS x 91 mm x 91 mm) x 117 mm and, in the Fall of 1956, the stock seed plot will be planted using this MS and 108 mm as the pollinator. All of these monogerm inbreds, except SLC 117 mm, were developed by V. F. Savitsky, working in cooperation with the Beet Sugar Development Foundation and the Sugar Crops Section of the United States Department of Agriculture. The SLC 117 mm inbred line was developed by Owen.

Use of these respective lines so as to keep the male-sterile seed in the  ${\rm F_1}$  generation insures a vigourous male sterile for both stock seed and commercial seed acreage.

# Pollinators for Hybrid Seed

In producing CT9 hybrids, two types of pollinators have been used: Commercial varieties as top-cross parents and F<sub>1</sub> pollinators produced by using the SLC 824 aa Mendelian Male sterile.

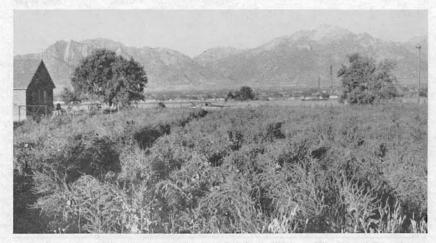


Figure 1.—Elite seed plot for production of  $F_1$  pollinator. The 824 aa Mendelian male sterile pollinator strips on each side were 12-rows wide. The pollinator variety was a sugar selection of SLC 202. Approximately 60 percent of the plants were rogued from the 824 aa strip.

In producing monogerm hybrids we have used almost exclusively  $F_1$  pollinators. Variety test results indicate that the  $F_1$  824 as x CT9 is one of the best we have used to date.

The production of  $F_1$  pollinators is a rather tedious procedure because all the Mendelian male-sterile plants must be inspected for sterility while still in the bud stage and approximately 50 percent of the plants rogued out. However, we have found it practical to produce 500 to 600 pounds of  $F_1$  pollinator in any one year (Figure 1).

In producing hybrid seed of either multigerm or monogerm, we have dyed the pollinator and then mixed it with the male sterile in the ratio of one pound of pollinator to twenty pounds of male sterile.

### Practical Results of Hybrid Seed Program

Detailed data obtained from extensive field trials are included in another paper and will not be repeated here. However, a summary of the data pertaining to four of the CT9 hybrids is shown in Table 1.

The figures shown in Table 1 are averages of four CT9 hybrids as compared to 5 and 6 of what might be listed as leading commercial varieties. In each case several of the commercial varieties were used as top-cross pollinators in making the CT9 hybrids that were averaged for comparison. It will be noted that the CT9 hybrids gave an encouraging performance at all locations.

It was also interesting to note the performance of top-cross hybrids as compared to the commercial top-cross pollinator parent. These comparisons are given in Table 2.

Table 1.—Comparison of the Average	Performance	o£	Commercial	Varieties	With	the
Performance of CT9 Male-Sterile Hybrids.						

Location and Comparison	Tons Per Acre	Sucrose Percentage	Gross Suga Per Acre	
Washington	· · · · · · · · · · · · · · · · · · ·		**************************************	
Average Commercial Variety <sup>†</sup>	19.46	15.56	6060	
Average Male-Sterile Hybrid	20.72	15.69	6502	
Idaho				
Average Commercial Variety	20.93	18.43	7714	
Average Male-Sterile Hybrid	22.19	18.31	8126	
Garland				
Average Commercial Variety	21.44	14.75	6324	
Average Male-Sterile Hybrid	22.58	14.72	6648	
So. Dakota				
Average Commercial Variety	17.86	15.05	5376	
Average Male-Sterile Hybrid	19.80	15.02	5948	
Nebraska				
Average Commercial Variety	17.64	15.00	5292	
Average Malc-Sterile Hybrid	18.28	15.06	5482	
Combined Averages	······································			
Commercial varieties	19.47	15.76	6153	
Male-Sterile Hybrids	20.71	15.76	6541	

<sup>&</sup>lt;sup>4</sup> The six Commercial Varieties compared in these tests were:

In Idaho, Nebraska, South Dakota—U. S. 35/2; U. S. 22/3; SLC 202; G. W. 359; Klein E, and American No. 2.

In Utah and Washington -- U. S. 35/2, U. S. 22/3, SLC 202, and Sugar Selection SLC 202.

Table 2.	—Comparison	of CT9	MS T	op Crosses	with	the	Pollinator	Parent.	Average	of
Results from	Idaho, Nebras	ska, and	South	Dakota V	ariety	Test	s (Total, f	32 Replic	ations).	

Top Cross Pollinators and CT9 MS Hybrids	Tons Beets Per Acre	Sucrose Percentage	Grøss Sugar Per Acre
G.W. 359	19.52	16,14	6302
CT9 MS x G.W. 359	20.44	15.92	6508
GT9 MS x F <sub>1</sub> (824 aa x G.W. 359)	20.13	16.19	6518
American No. 2	17.74	16.44	5832
CT9 M8 x American No. 2	20.14	16.16	6510
U. S. 35/2	17.50	16.52	5782
CT9 MS x U. S. 35/2	19.14	16.37	6266
Klein E	21.79	15.65	6820
CT9 MS x Klein E	21.93	15.63	6856
CT9 MS x F <sub>1</sub> (824 aa x Klein E)	19.14	16.00	6124
Average of Top Cross Pollinator	19.14	16.19	6184
Average of CT9 Hybrids	20.41	16.02	6540

Table 3.—Comparison of 610 mm M8 Top Crosses with the Pollinator Parent. Results from Shelley, Idaho, Test, 1955 (8 Replications).

Top Cross Pollinators and 610 Hybrids	Tons Beets Per Acre	Sucrose Percentage	Gross Sugar
G.W. 359	21.15	17.26	7302
610 MS x G.W. 359	21.50	17.20	7396
610 MS x Fr (824 aa x G.W. 359)	19.82	17.79	7052
American No. 2	19.03	17.19	6542
610 MS x American No. 2	22.01	17.05	7506
U. S. 35/2 (824)	18.53	17.36	6434
610 MS x U. S. 35/2	20.62	17.29	7130
Klein E	23.10	16.90	7808
610 MS x Klein E	22.68	17.26	7830
610 MS x F <sub>1</sub> (824 aa x Klein E)	18.32	17.33	6350
Average to Top Cross Pollinators	20.45	17.18	7021
Average of 610 mm MS Hybrids	21.70	17.20	7466

It will be noted that in every case the hybrids were higher than the top-cross pollinators in yield, although the hybrid with Klein E was not significantly higher than the Klein E parent. The increase in tonnage was strikingly higher when high sugar top-cross parents were used as compared to when tonnage type varieties were used as the top-cross parent.

These same relationships were obtained when the monogerm 610 MS was used in hybrids with these same top-cross parents. These results are shown in Table 3. It should be noted that the hybrids with the monogerm 610 MS gave the same percentage increase over the top-cross pollinator parents as did the multigerm hybrids with CT9.

In both Table 2 and Table 3, data are listed for a three-way hybrid where SLC 824 as Mendelian male sterile was used to produce an  $\mathbf{F}_1$  pollinator for the main purpose of increasing the sucrose percentage of the hybrid. A further summary of the effect of introducing a high sugar variety into the pollinator is shown in Table 4. It is evident from these data, that the use of SLC 824 as to produce an  $\mathbf{F}_1$  pollinator did increase the

sucrose percentage, but it also decreased tonnage sufficiently to result in less gross sugar per acre. This does not mean that some combinations cannot be made where results would be more favorable, or that a different Mendelian male sterile might not react differently.

Table 4.—Comparison	of	Two-Way and	Three-Way	Hybrids	Based	on	Results	with
G.W. 359 and Klein E Hyb	rids	Planted at For	r Locations.					

	Ton	s Beets	Sucrose Percentage		Sucrose Percentage		Gros	s Sugar
Location of Test	Two-Way Hybrids	Three-Way Hybrids	Two-Way Hybrids	Three-Way Hybrids	Two-Way Hybrids	Three-Way Hybrids		
Shelley, Idaho	- Andrewson - Andrewson to the AND	MARKETT METEROTOTICS STATES STATES AND ADDRESS AND ADD			N. A. CHARLES OF	7 90 98		
8 Replications	21.93	22.69	17.77	18.41	7794	8354		
8 Replications	21.50	19.82	17.20	17.79	7396	7052		
8 Replications	23.58	20.26	17.81	18,31	8400	7420		
8 Replications	22.68	18.32	17.33	17.26	7860	6324		
Nebraska								
12 Replications	17.75	18.52	15.01	15.08	5328	5586		
12 Replications	20.31	18.79	14.54	14.86	5906	5584		
South Dakota								
12 Replications	21.64	19.19	14.98	15.08	6484	5788		
12 Replications	21.90	18.37	14.53	14.85	6364	5456		
Garland, Utah								
15 Replications	24.01	22.25	14.14	14.51	6790	6456		
Combined Average	VALUE OF THE PARTY							
of 95 Plots	21.70	19.80	15.92	16.24	6910	6432		

### Commercial Seed Acreage Planted in 1955

In the Fall of 1955, 588 acres of sugar beet seed were planted for the purpose of producing commercial monogerm hybrids. Based on monogerm seed yields obtained in 1954, this acreage should produce well in excess of 1,000,000 pounds of monogerm seed. This seed will be harvested in 1956 and will be put to commercial use in 1957 and 1958. No data are available on the performance of the exact hybrids being produced. However, we have already seen in comparing data in Tables 2 and 3 that, when the same pollinators were used, hybrids with CT9 MS and 610 MS were very similar. Data are available on the pollinators used for the 1955 seed plantings. The Monogerm male sterile used in the seed plantings was 91 MS rather than the 610 MS, for which data are available. However, from available data (1954 Government Report to Beet Sugar Development Foundation) the 94 MS averaged 103 percent of 610 MS in both yield and sucrose percentage. We can expect, therefore, that hybrids produced using 91 MS will, in general, be three percent better than hybrids produced using 610 MS.

Actual performance of the pollinators used in 1955 seed plantings of 610 MS hybrids and their calculated performance with 91 MS are shown in Table 5. These data indicate that monogerm hybrids, now planted for seed production, will exceed the present commercial variety in both tonnage and sucrose percentage and that their performance will be equal to the best CT9 hybrids which we have produced.

Table 5.—Comparison of U. S. 22H Commercial with Monogerm Hybrids Using 610 MS and Multigerm Pollinators Used in Commercial Seed Plantings in Fall of 1955 (Shelley Test, 8 Replications).

Variety and Hybrid	Tons Bects Per Acre	Sucrose Percentage	Gross Sugar Per Acre
U. S. 2211 Commercial	19.09	17.48	6658
610 M8 x F <sub>1</sub> (824 aa x CT9)	22.57	17.34	7817
610 MS x Sug. Sel. 202	21.77	17.23	7482
610 MS x U-I 114	21.50	17.20	7394
610 MS x Fi (824 aa x U I 114)	19.82	17.79	7018

Calculated Performance of Actual Monogerm Hybrids Planted for Seed Production in Fall of 1955 Based on Use of 91 MS Rather Than 610 MS<sup>1</sup>

Hybrid	Tons Beets Per Acre	Sucrose Percentage	Gross Sugar Per Acre
U. S. 22H Commercial	19.09	17.43	6658
91 MS x F <sub>1</sub> (824 aa x CT9)	23.25	17.86	8286
91 MS F <sub>1</sub> (824 aa x S.S. 202)	22.42	17.75	7931
91 MS x U-I 114	22.15	17.72	7838
91 MS x F <sub>1</sub> (824 aa x U-I 114)	20.41	18.32	7439

<sup>&</sup>lt;sup>3</sup> Calculations shown above are based on the improved performance of 91 MS over 610 MS as shown by Government tests in 1954. These comparisons are shown below.

Comparison of 610 MS and 91 MS in Hybrid Combinations When 610 mm and 91 mm Lines Were Used to Pollinate a Common Multigerm Male Sterile—1954 Gov. Report.

		$610 \; \mathrm{mm}$				
Location	Tons Beets	Sucrose	Sugar	Beets	Sucrose	Sugar
Taylorsville, Utah	29,70	14,60	8672	32.90	14.93	9824
Twin Falls, Idaho	31.40	17.77	11160	30.90	18.18	11236
Brawley, California	24.90	14.33	7136	24.40	15.02	7330
Average	28.67	15.57	8928	29,40	16.04	9432
Percent Increase				103%	103%	106%

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