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# Percent of Blend of Male Sterile and Male Fertile Corn Seed for Optimum Fertilization in Hybrid Corn at two Locations in South Dakota

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# PERCENT OF BLEND OF MALE STERILE AND MALE FERTILE CORN SEED FOR OPTIMUM FERTILIZATION IN HYBRID CORN AT TWO LOCATIONS IN SOUTH DAKOTA

By

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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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## INTRODUCTION

Cytoplasmic male sterility has become a factor in corn seed production during the past number of years. The advantage of male sterility is that the producer is saved the expense and trouble of detasseling in the single-cross and double-cross seed fields. It also could be an advantage in certified seed production in that it would eliminate the human error factor in the detasseling process. The disadvantage is that, at present, male sterile and male fertile seed must be mixed or blended to insure a kernel set when grown by the farmer. It is highly desirable that no seed go out to the consuming farmer with any question or possibility that there be a shortage of viable pollen during the time of fertilization. Therefore, the percentage of blend has become a problem of concern in hybrid corn seed production and certification work.

Most federal, state and commercial corn breeders are working with male sterility. They are finding it to be a rather unstable type of tool with which to work because male sterile plants may have tassels that do not shed viable pollen in one location while in another location some viable pollen may be shed. No one has a sure way of knowing what value male sterility will eventually have in the overall picture of corn production.

A desirable use of male sterile would be to have it in the female single-cross in producing double-cross seed and also to have male fertile restorer genes in the pollen parent of the double-cross seed production field so the consumer would have

pollen in his fields. Since restorer genes are not yet ready for use, producers must still blend their seed when they use male sterility in their seed production program. Since environment affects the ability of plants to shed pollen, this study was set up to try to determine for South Dakota conditions what mixtures or blends it would be desirable to have to insure kernel set in the farmers fields and to aid in establishing certification standards for hybrid corn seed production.

## REVIEW OF LITERATURE

The literature on male sterility in corn in general is rather limited while that dealing specifically with blends of seed is confined to seed certification standards (3, 5, 6, 10, 11, 12, 17, 18).* The necessity of proper blends has been indicated by Ingersoll (4). He also states that in determining the use of cytoplasmic male sterility in seed production programs, one should consider the hazards involved, the benefits that may be derived, and the production procedure necessary for success. Every hybrid seed corn producer has a grave responsibility to his farmer customers. Hybrid corn seed, when sold to a farmer, must produce fields of corn with an abundance of viable pollen. Ingersoll further emphasizes that the incorporation of the male sterile factor into the proper inbred line and conserving the fine performance of the line is a task for no amateur corn breeder.

During the past few years steps have been taken to get some standards established for seed blending. Certification standards and regulations on seed blending in South Dakota and neighboring

*Literature cited

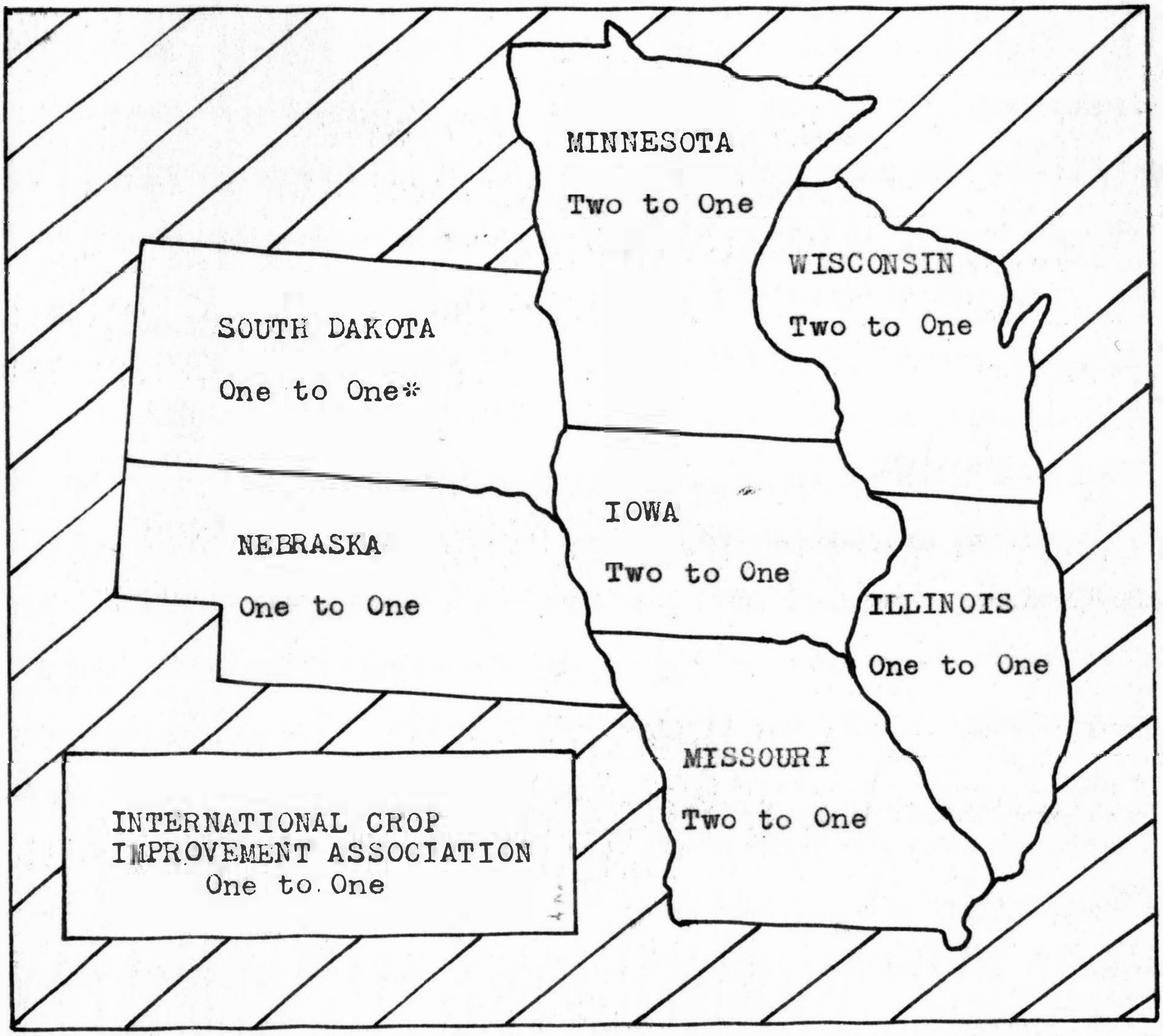



Figure 1. Seed Certification regulations on blends of male sterile and male fertile corn seed.

* Ratio male sterile to male fertile

states are indicated in Figure 1.

South Dakota Seed Certification Standards (17) contain the following statement: "A male sterile ear parent can be used to produce certified commercial hybrid corn seed. Seed of the normal fertile ear parent must be mixed with seed of the male sterile ear parent either by blending in the field at harvest or by grade at processing time. The ratio of male sterile ear parent seed to normal ear parent seed should not exceed one to one." The neighboring state of Nebraska (12) gives its regulation in the following manner: "We do not make any specific requirements in regard to blending male sterile and male fertile corn seed since it is almost impossible to absolutely control and check; however, we do suggest that there not be more than 50 percent sterile seed in any given lot of seed corn." The Minnesota (10) plan of corn seed certification is as follows: "A male sterile ear parent can be used to produce certified commercial hybrid corn seed. Seed of the normal fertile ear parent must be mixed with seed of the male sterile ear parent either by blending in the field at harvest or in processing. The ratio of male sterile ear parent seed to normal fertile ear parent seed should not exceed two to one. The producer is solely responsible for proper blending of the fertile and sterile ear parent. The Minnesota Crop Improvement Association assumes no risk in this procedure."

Beadle (2) states that in his work, due to the number of genes which affect the process of pollen development, sterility must depend on easily disturbed physiological conditions. As pointed out

by Rhoades (13), the inheritance of the male sterile condition does not conform to Mendelian behavior. He also points out that cytological investigation shows the meiotic divisions in microsporgenesis to be normal. The degeneration of the pollen occurs usually after the first vegetative division.

Rhoades (13) reports that the genetic constitution of the male parent crossed with a male sterile individual has no demonstrable affect on the degree of sterility. However, Bauman (1) reports that some workers have isolated genes which counteract the action of sterile cytoplasm so that fertility is restored. If such genes could restore complete fertility and were present in the male single-cross used in double-cross seed production, then blending of seed would not be necessary. At the present time there is some question on how completely fertility is restored.

It was pointed out by Bauman (1) that there are four different sources of cytoplasmic male sterility which are: (a) Texas male sterile; (b) Connecticut male sterile; (c) 33-16 male sterile, and (d) Kys male sterile. The Connecticut source, also called "S", was used in this study.

As stated by Rogers and Edwardson (15), the information obtained so far on the behavior of the male sterile character definitely indicates that male-sterile inbreds may be utilized to obviate detasseling in the production of corn hybrids.

## MATERIALS AND METHODS

In this study three different blends of male sterile and male

fertile seed were grown in isolated fields in each of two counties for a period of two years.

The seed of two single-crosses, (WF9 ms x M14) and (WF9 x M14), were used. This seed was Iowa certified and was produced in 1952 by Clyde Black and Son of Ames, Iowa. The male sterility factor came from the "S" source.

The study was carried on during 1953 and 1954. The experiments were conducted at two locations which were Lincoln County in the eastern part of the state, and Charles Mix County in the south central part of South Dakota. Lincoln County was chosen because it is in the center of the corn producing area. Charles Mix County was chosen because it is west of the first location and therefore is in the more marginal corn area and has less annual rainfall in the amount of three to four inches along with higher summer temperatures. There was about an 80-mile east-west distance between the two locations (Figure 2).

At both Lincoln County and Charles Mix County locations there were three isolations on different farms in the area. A different blend of fertile to sterile seed corn was planted in each isolation in each county. These were as follows: (1) 5% male fertile to 95% male sterile; (2) 15% male fertile to 85% male sterile, and (3) 45% male fertile to 55% male sterile. The male fertile percentages of 5, 15, and 45 were chosen with the probability that 45% would give adequate pollen while the other two might be at the critical level or even inadequate.

As pointed out by Jones and Brooks (7) in their work on

SOUTH DAKOTA

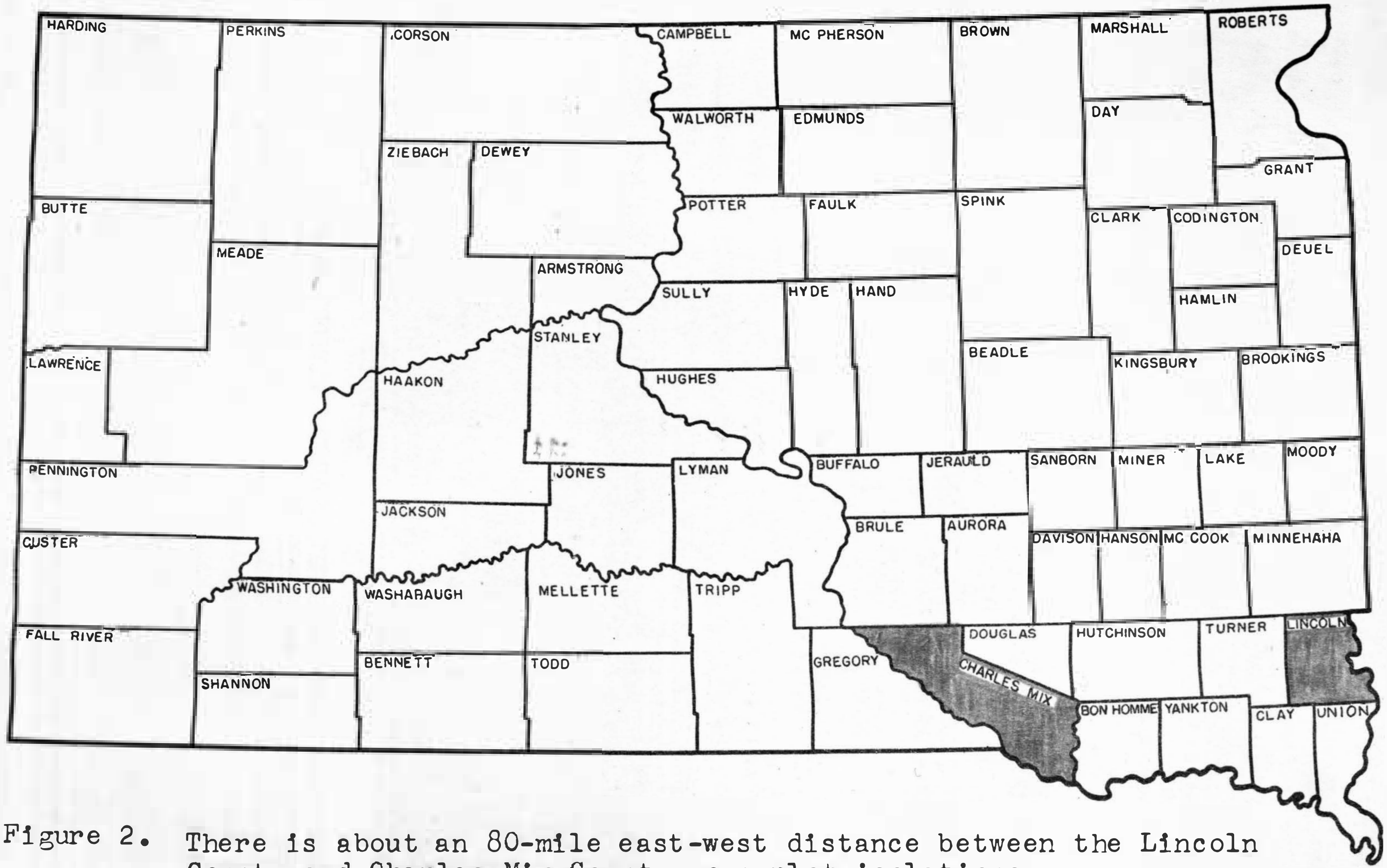



Figure 2. There is about an 80-mile east-west distance between the Lincoln County and Charles Mix County corn plot isolations.

isolation distances, it was advisable to have forty rods isolation to reduce contamination. This study would have had little or no value if there was foreign pollen getting to the silks, thus giving an improved seed set in the lower levels of 5 and 15 percent blends. Therefore, each isolation was located 40 rods or more distance from any other corn (Figure 3). Each isolation was about one-fourth acre in size, being 28 corn rows wide and 112 feet long.

Figure 3. Isolations were 40 rods or more. (Lincoln County, 1953)

In putting up seed, it was calculated that for forty-inch rows with kernels spaced fourteen inches apart, 2882 viable kernels would be needed per isolation. Extra allowances were made to adjust to the 2882 viable kernels desired because there was only 95% germination in the male fertile seed and 90% germination

in the male sterile seed. The seed was treated with Arasan to control soil borne diseases.

The seed bed preparation, planting, and cultivation were done by the farmers with their regular farm equipment (Figures 4 and 5).

Figure 4. Planting in Lincoln County was done with a four-row corn planter.

No commercial fertilizer was applied in either of the years. The previous crop in Lincoln County for 1952 was corn, therefore corn followed corn on spring plowing. There were no volunteer corn pollen problems in the grain fields around the isolations in Lincoln County in 1953. In Charles Mix County the previous crop was winter wheat. The 1954 isolations in Lincoln County were all located on small grain stubble which was fall plowed. The Charles Mix County isolations were seeded to small grain in the spring

Figure 5. Planting in Charles Mix County was done with a two-row lister.

before the listing of the corn at planting time.

Counts of male sterile and fertile tassels were made only once during the pollen shedding period.

Four methods were used to measure pollen shedding. The first method was pollen counting which was done in late July when the tassels were shedding. Weather vane stands (Figure 6) were made to hold a greased microscope slide. These stands were placed at random in each plot in the forenoon when the shedding of pollen was high. The greased slides were kept carefully covered before and after being on the vane for twenty minutes. After exposure the slides were examined under the microscope and pollen counts were made.

Figure 6. Weather vane standard to hold greased microscope slide to catch corn pollen.

The second, third and fourth methods of measurement were made on the corn ears after harvest. Five hundred ears were picked in each isolation. Five 100-ear samples were harvested as shown in Figure 7. Four border rows on each side and approximately ten feet on each end of each isolation were left from which no sample ears were picked because some of this border area would be at a disadvantage in receiving pollen. Twenty-five ears were picked from each of four rows in each of the five sample locations. The corn was harvested following the first frost in late September or early October and brought into the crop dryer for about two days. The dryer was operated at a temperature of 110°F. Then the samples were ready for the second method of measuring which was score rating.

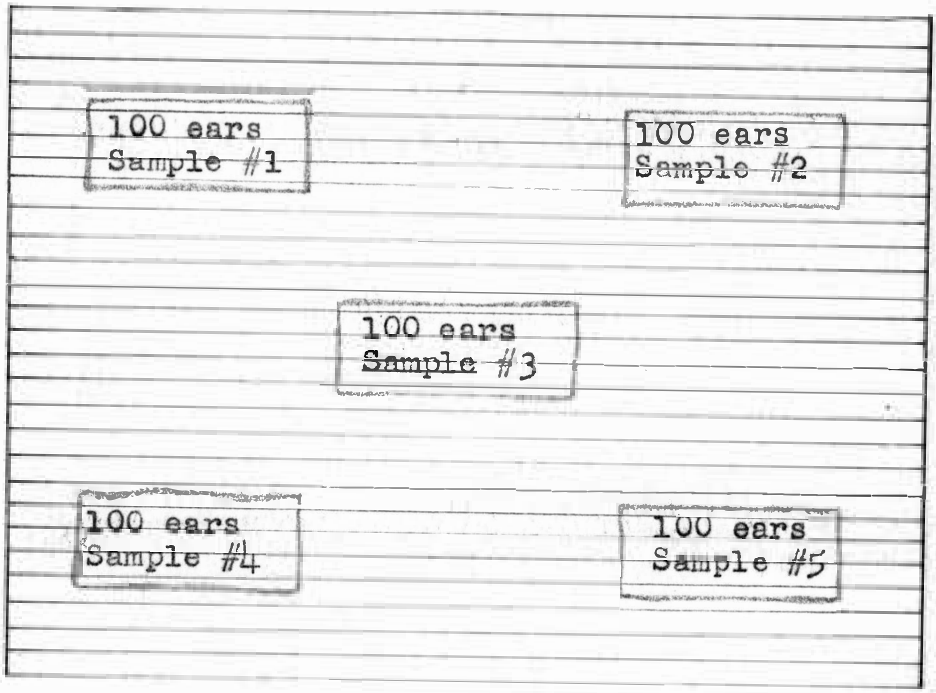



Figure 7. Five 100 ear samples were picked from each isolation.

The score rating method was to score each of 3000 ears on three different days by using a rating of one to ten. Number one was given to the poorest set ears while ten was high and given to an ear with full set. These 3000 ears were spread on the floor in six groups of five hundred, one hundred ears to a row. There were three groups for each county.

The third method of measuring was shelling percentage. To get shelling percentage of the ears, every fifth ear from each row of one hundred ears was weighed on a gram scale, shelled, then the shelled corn weighed. To calculate the shelling percentage, the ear weight was divided into shelled corn weight. This is a good way to measure the pollen distribution.

The fourth method of measurement was kernel count. The shelled corn from the shelling percentage determinations was bulked and weighed and total kernel counts made by using a counting board.

## EXPERIMENTAL RESULTS

TASSEL COUNTS An inspection and count was made each year of the shedding tassels. If two inches or more of the main spike or side branches of the tassel had anthers extended from the glumes, it was counted as shedding pollen. The results of these counts are given in Table 1.

Table 1. Percentage of tassels that were shedding pollen when various combinations of fertile and sterile corn seed were used.

| Ratio of Fertile to Sterile | | Lincoln 1953 | Lincoln 1954 | Charles Mix 1953 | Charles Mix 1954 |
|---|---|---|---|---|---|
| 5 | 95 | 7.0 | 7.4 | 5.6 | 6.0 |
| 15 | 85 | 17.4 | 14.4 | 14.2 | 15.4 |
| 45 | 55 | 46.0 | 44.0 | 50.2 | 49.2 |

The percentage of shedding tassels followed very closely the percentage of male fertile seed used. Figure 8 gives a good idea of how a random sample of the ears looked from Charles Mix County in 1953 for each of the three levels of seed blends.

SUMMARIZED DATA The summarized data for both locations in each of the two years on score rating, shelling percentage, and kernel count are presented in Table 2. Score rating increased with an increase in percentage of male fertile plants irrespective of location or year grown. This was true whether the percentage of

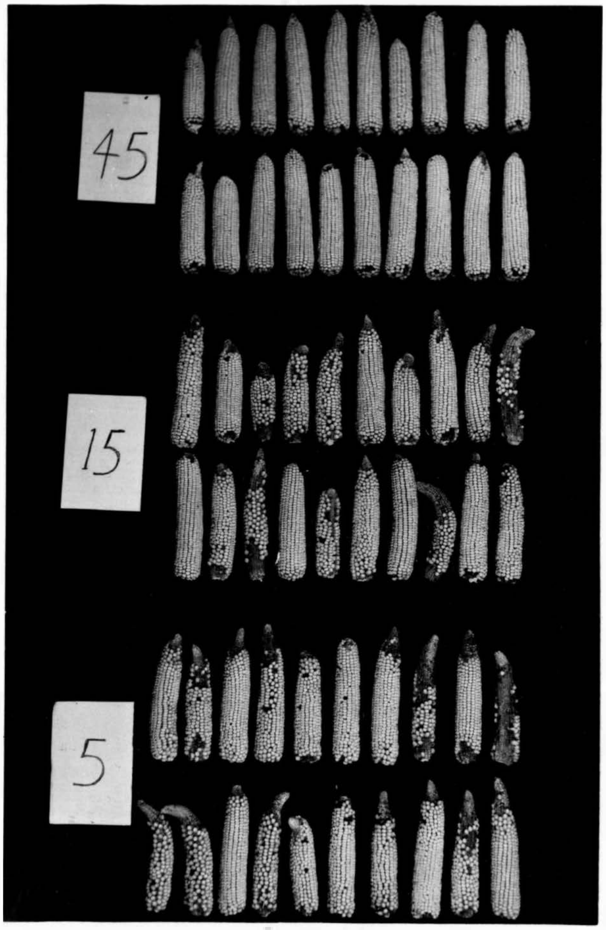



Figure 8. Random sample of 20 ears for each of the three seed blends from Charles Mix County, 1953

male fertile plants was raised from five to fifteen percent or from fifteen to forty-five percent. Similar results were obtained when the adequacy of pollen was studied for the various blends by determining the shelling percentage and the kernel count of 100 ears. In only one case was there no sizeable

Table 2. Summary of results for score rating, shelling percentage and kernel count obtained on 100 ears harvested from blends of male fertile and male sterile seed corn.

| Blends of Seed | | Lincoln County | | | | | | Charles Mix County | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1953 | | | 1954 | | | 1953 | | | 1954 | | |
| M.F. | M.S.* | Score | Shell-ing % | Kernel Count | Score | Shell-ing % | Kernel Count | Score | Shell-ing % | Kernel Count | Score | Shell-ing % | Kernel Count |
| 5% | 95% | 662 | 73.4 | 35200 | 644 | 78.8 | 47300 | 722 | 72.9 | 49500 | 754 | 70.1 | 59700 |
| 15% | 85% | 692 | 77.9 | 40600 | 777 | 79.9 | 63900 | 796 | 76.2 | 61700 | 811 | 82.2 | 68900 |
| 45% | 55% | 830 | 80.5 | 57200 | 825 | 82.8 | 65500 | 915 | 80.4 | 70900 | 854 | 84.2 | 71400 |

* M.F. - male fertile  M.S. - male sterile

increased kernel set with larger percentages of male fertile plants in the field. That was for shelling percentage in Lincoln County in 1954. There the increase in shelling percentage from the five percent male fertile to fifteen percent male fertile was only 1.1 percent. At this level the shelling percentage was not statistically significant.

ANALYSES OF VARIANCE The seed set differences among levels of seed blends were highly significant statistically for score, kernel count and shelling percent except Charles Mix County in 1953. At this location the shelling percentage was significant at the five percent level. Analysis of variance for these three methods of measuring are given in Table 3.

The greased microscope slide data was not used because it was felt that the technique needs to be improved by setting out the greased slides about eight times instead of only once during the pollinating period.

CURVE COMPARISONS In the three methods of measuring kernel set there is a sharp break just before reaching the fifteen percent level. By using the mathematical formula two log (600 X + 1)*, which fitted the data best of the formulas examined, it is possible to draw a curve for the data of this study. Figures 9 and 10 show the comparison of the curves. The graph which results from these mathematics will show how far one should go in blending male fertile and male sterile seed corn for optimum production of corn for the two locations used in this study in South Dakota.

* X = percent of male fertile in the seed blend

Table 3. Analyses of variance of score, shelling percentage and kernel count

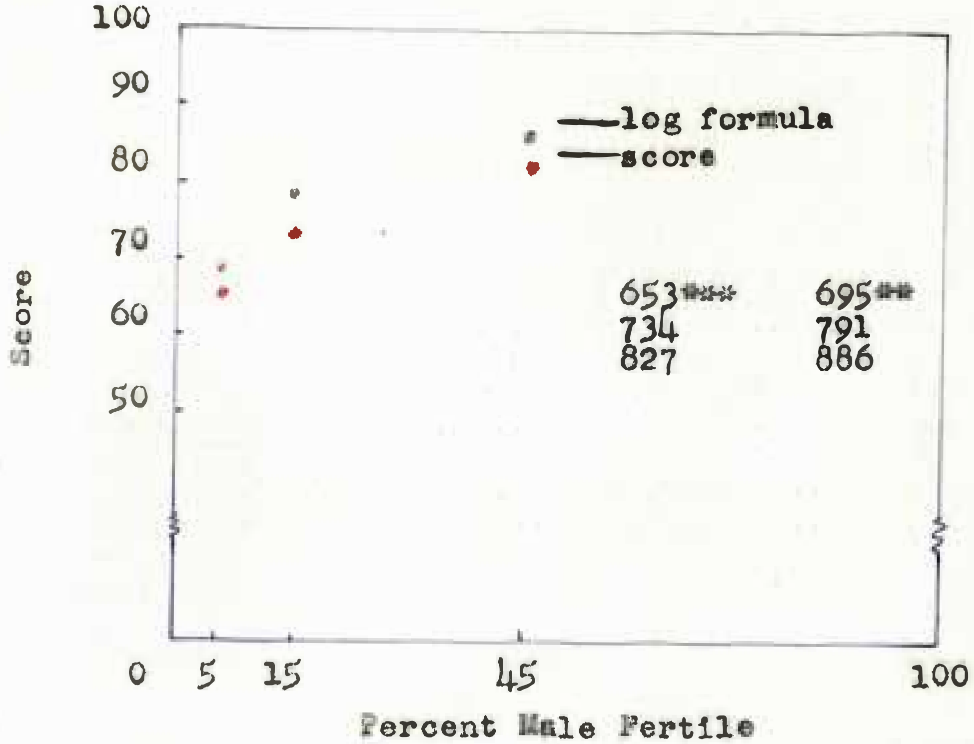
Lincoln County

| Source of Variation | df | 1953 MS | 1954 MS |
|---|---|---|---|
| **Score** | | | |
| Levels of Seed Blend | 2 | 40483** | 43933** |
| Within Isolations | 12 | 1742 | 1486 |
| **Shelling Percentage** | | | |
| Levels of Seed Blend | 2 | 64.33** | 20.91** |
| Within Isolations | 12 | 4.42 | 2.43 |
| **Kernel Count** | | | |
| Levels of Seed Blend | 2 | 263490** | 202489** |
| Within Isolations | 12 | 20873 | 9779 |

Charles Mix County

| Source of Variation | df | 1953 MS | 1954 MS |
|---|---|---|---|
| **Score** | | | |
| Levels of Seed Blend | 2 | 47316** | 12792** |
| Within Isolations | 12 | 3271 | 1103 |
| **Shelling Percentage** | | | |
| Levels of Seed Blend | 2 | 71.01* | 48.08** |
| Within Isolations | 12 | 15.81 | 1.89 |
| **Kernel Count** | | | |
| Levels of Seed Blend | 2 | 232011** | 76345** |
| Within Isolations | 12 | 26090 | 7558 |

** Significant at the one percent level
* Significant at the five percent level



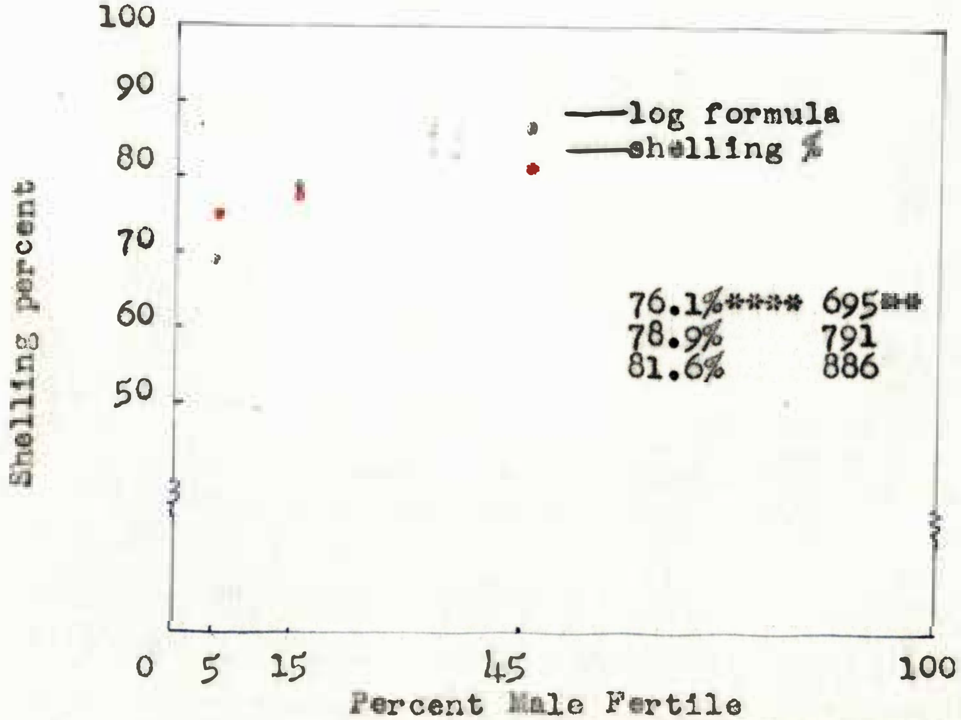



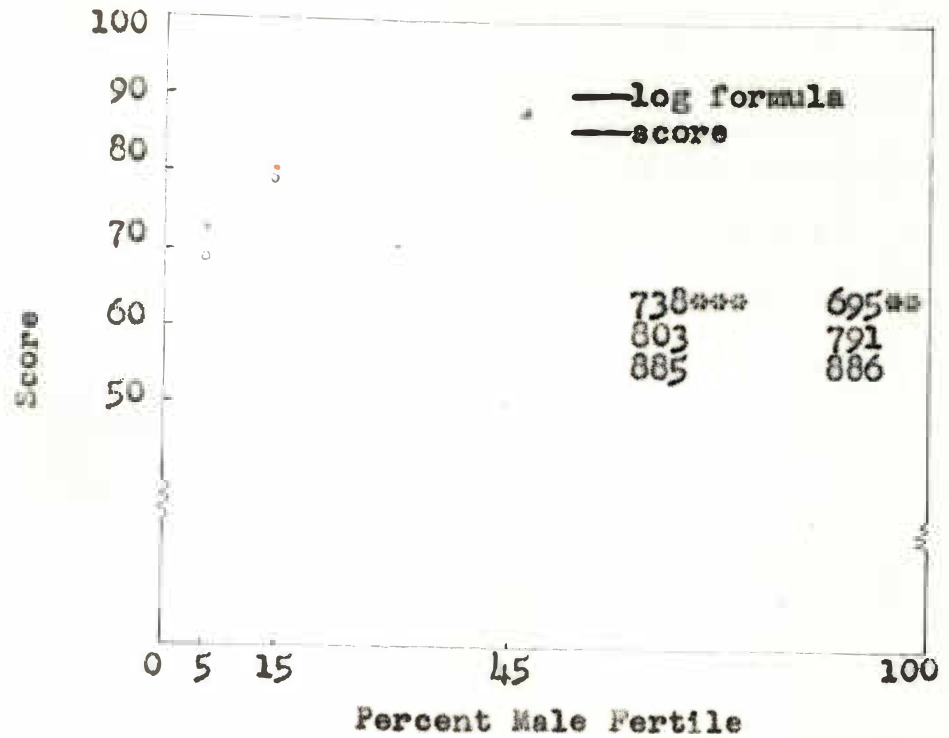

Figure 9. Average score and shelling percentage Lincoln County compared with formula 2 log (600 X + 1)*

* X = percent of male fertile

** Results from formula 2 log (600 X + 1)

*** Score for Lincoln County

**** Shelling percent for Lincoln County



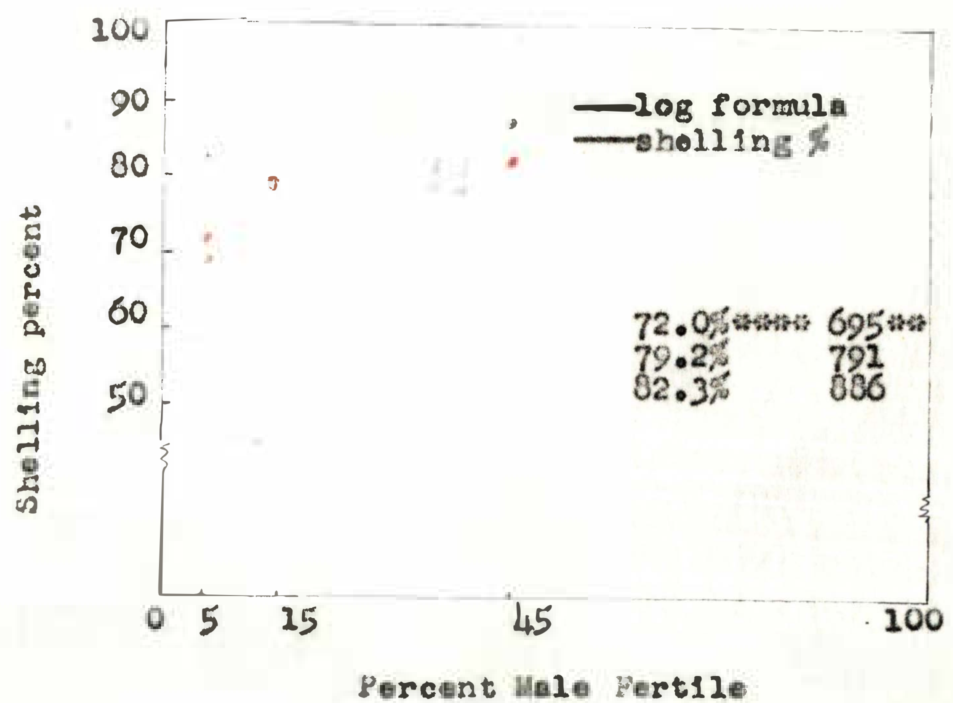




Figure 10. Average score and shelling percentage Charles Mix County compared with formula 2 log (600 X + 1)*

* X = percent of male fertile

** Results from formula 2 log (600 X + 1)

*** Score for Charles Mix County

**** Shelling percent for Charles Mix County

## DISCUSSION

The use of cytoplasmic male sterility in the production of hybrid seed corn probably has some advantages, particularly from the standpoint of the producer. The detasseling job is sometimes difficult due to adverse weather conditions and personnel problems which can and do occur at critical times. Male sterility would eliminate this job of detasseling. Certification services probably would also favor the use of male sterility because it would cut down on selfing and sibbing resulting from seed parent tassels that were shedding pollen because they were missed when the detasseling crew went through the field. The disadvantage of male sterility is that it does not always remain stable under a wide range of environmental conditions.

Hybrid seed corn when sold to a farmer must produce fields of corn with an abundance of pollen. Kiesselbach (9) states that the average fertile tassel will shed 18 million pollen grains. With a figure this large there certainly is an abundance of pollen. In the case of male sterility it is the responsibility of the seed producers to see that there is plenty of pollen and not a shortage due to poor blending of seed. When a blending process is used, each bushel of seed must be the same. There should be no possibility of error.

Figure 11 shows that with the fertile tassel, on the right, there should be normal pollen shedding, fertilization and seed set, but with only sterile tassels like the one on the left it would result in a field having only cobs without any corn on them.

Figure 11. Sterile tassel on left and normal on right.

It was expected that the environmental effects of the more marginal location used in this study might give some reduction in seed set due to environment, mainly by high temperatures killing pollen.

Sonneborn (16) calls attention to the fact that one role of the genes is to function in yet unknown ways with genetic components of the cytoplasm in the control of particular hereditary traits. Determination of these traits cannot be attributed to the action of either genes alone or cytoplasmic genetic factors alone, but only to their interactions (16). It is felt that the environment also has its effects on these interactions which cause tassels to shed pollen or not to shed pollen.

The United States grew nearly 70 million acres of hybrid corn in 1951 according to Jugenheimer (8). His estimation is that on a peak day of the detasseling season some 125,000 persons are engaged in removing tassels from corn plants in seed production fields. By using male sterile corn seed in a recommended blend, much of this hand labor will be reduced and a superior quality of seed will be produced.

The commercial use and application of cytoplasmic male sterility is an opportunity for the nation's corn breeders and seed producers because it is an improvement in the business of seed corn production. Since the United States grows about seventy million acres of corn, it is estimated that 500,000 acres are used to produce seed (8). If we can accept male sterility and thereby eliminate detasseling, which costs about $15.00 per acre, there would be a saving of seven and one-half million dollars in detasseling expenses per year. It is doubtful if all of this can be saved, but if we can blend our seed one to one, there is still a huge saving. Male sterility would also cut down on some of the labor problems at detasseling time. It is believed by Jugenheimer (8) and the author and probably others that male sterility might do the job better than hand detasseling. The usual certification requirements require that detasseling shall be carefully done that not more than one percent for any one inspection or a total of two percent for all inspections of tassels of ear parent shall have shed pollen. If more than two percent of the tassels have shed pollen, the field is disqualified for seed production.

The saving to the farmer would be very small. He pays two dollars or less per acre for seed corn now. It is believed that through the use of male sterility he would receive higher quality seed even though it were not certified seed. It is known that some seed corn producers sell corn seed that was detasseled very poorly. If male sterile seed was used in the double-cross production, it probably would be an improvement in the seed sold.

Two large single-cross producers in Iowa have seed lists which show 14 male sterile singles out of 150 for one, and 12 male sterile singles out of 140 for the other. The two organizations referred to are probably the largest of their kind in the seed corn business. These comparisons show that there is still a long way to go before we get male sterility into all of our double-cross hybrids even if that was desirable.

The weather conditions of the marginal corn area could probably cause severe effects on male sterility. There appear to be variations in male sterility due to genetic and environmental conditions.

From the evidence presented and using the formula 2 log (600 X + 1), it can be concluded that the minimum level of seed blending of male fertile to male sterile for the two years of this study in South Dakota would probably be 33% fertile to 66% sterile. This two to one ratio would give an 86 value on the curve in Figures 9 and 10 where 100% gives a 95 value and the one to one ratio gives a 90 value on the curve in Figures 9 and 10, according to the formula 2 log (600 X + 1).

A study of temperature and precipitation was made over a 17-day period each year starting about a week before the peak of pollen shedding and continuing about a week following. July 25 was the starting and August 10 was the closing date. The average temperature and precipitation for July 25 to August 10, inclusive, for Lincoln County with Canton and Centerville as the two U. S. weather reporting stations, and for Charles Mix County with Wagner, Armour and Pickstown as the three U. S. weather reporting stations is given in Table 4.

Table 4. Average temperature and rainfall during pollen shedding.

| | Lincoln | | Charles Mix | |
|---|---|---|---|---|
| | Temperature | Rainfall | Temperature | Rainfall |
| 1953 | 76°F | 3.62 in. | 76.1°F | 3.87 in. |
| 1954 | 72.6°F | 1.03 in. | 73°F | 1.45 in. |

Since this period involved two good corn years for 1953 and 1954, the years of this study, there was no reason to suspect any seed set differences due to environmental effects on cytoplasm. The data of this study did not bear out the expectation that Charles Mix County would not have as good a seed set as it did. The fertilization was higher in Charles Mix County than in Lincoln County.

## SUMMARY

Male sterility is becoming more widely used in the production of hybrid seed corn each year. This study was designed to compare

the ability of various blends of male sterile and male fertile seed corn to produce a full kernel set when they were grown under two different sets of environmental conditions in South Dakota.

To do this study, three levels of male sterile and male fertile seed corn were used. The experiment ran for 1953 and 1954 with one isolation for each level of seed blend in each of the counties of Lincoln and Charles Mix, which are about 80 miles apart in an east to west direction. The reason for selecting the western location was to possibly find an environment which would be more difficult for corn fertilization.

During the two years of 1953 and 1954, the weather was favorable to the growing of corn in nearly all places in South Dakota. Therefore, the differences which were expected, and which we know sometimes come about due to adverse weather, did not occur. The comparisons of seed blends did give differences which when plotted on a curve by using the formula 2 log (600 X + 1) show the percent of seed blending that the Certification Service can recommend.

The conclusion resulting from this study is that a seed blend of one part male fertile to two parts male sterile are adequate for the years of good corn production, but for the years of adverse weather, the blend of one part male sterile to one part male fertile is a safer recommendation.

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