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# Mechanical Separation of Hybrid and Self-Pollinated Seed as a Means of Increasing Percentage Hybrids in Upland Cotton[1]

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HYBRID vigor has been utilized extensively in many crops, but a method is not currently available for large-scale production of cottonseed consisting of a high percentage of $F_1$ hybrids. Cotton has a perfect flower which makes production of large quantities of $F_1$ hybrid cottonseed difficult. Also, cotton pollen is not wind-borne and must be carried from flower to flower by insects. Simpson (9) reported that the percentage of natural crossing at various locations in the Cotton Belt ranged from 7 to 49% for 1948–52. Loden and Richmond (4) reviewed reports of hybrid vigor in cotton and indicated that many agronomically desirable characters of cotton, including yield of seedcotton, exhibit heterosis.

Cotton geneticists have tried to develop a workable method of utilizing male-sterility to produce hybrid cotton, but no successful method has been reported in the literature. Kohel and Richmond (3) reported that lint yield reduction on male-sterile plants in a natural-crossing block would make the production of $F_1$ hybrid seed very costly even in areas where natural-crossing is high.

Since a workable method of producing large quantities of $F_1$ hybrids is not available, methods of producing partial hybrids are being investigated. Ware (10) reported that seeds produced on $F_1$ cotton plants were larger than seeds produced on their parent plants. Pressley (7), and Ganesan (1) reported that $F_1$ cottonseeds are often heavier than those of either parent. The most striking differences were reported by Ganesan, who found that the $F_1$ seeds from crosses of strains of *Gossypium arboreum* L. were 21 to 41% heavier than those of the self-pollinated parents.

These findings are in accordance with those reported by corn researchers. Whaley (11) reviewed the literature pertaining to hybrid vigor in seed and indicated increased $F_1$ seed weight is common in corn. Whaley (12) indicated that $F_1$ corn kernels from selected crosses exceeded the weight of their larger parent, but that most crosses produced kernels that were intermediate between those of their parents.

If heavier cottonseeds are also larger, it might be possible to remove hybrid seeds from mixed populations with sieves. Hand crosses made in 1958 and 1959 indicate that some parental combinations produced $F_1$ seeds that were larger than self-pollinated seeds from the respective female parent. Additional studies were initiated in 1960 and 1961 to determine whether these results could be duplicated under natural-crossing conditions.

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## MATERIALS AND METHODS

Since the percentage of natural crossing on the Delta Branch Station at Stoneville is very low, natural-crossing blocks were planted at two locations in Mississippi where a higher percentage of natural-crossing was known to occur in cotton. The first natural-crossing block was planted on the North Mississippi Branch Experiment Station at Holly Springs in 1960. In this experiment, lines used as female and male were planted in alternate rows. Female rows were M8 marked with the recessive glandless factor $gl_1$ $gl_1$. Part of the female rows were also homozygous for *ms*-3, a partial male-sterility factor described by Justus et al. (2). Other rows were segregating for this factor. An attempt was made to rogue the fertile segregates, but at the end of the season a few fertile plants remained. The percentage of partially male-sterile plants served only to increase the total number of hybrids. Male rows were M11, a strain with larger seeds than M8 and carrying the dominant factor for glanded. At the end of the season, cotton from the female rows was harvested and ginned, and the seeds were acid-delinted. The seeds were then run through a series of revolving sieves with the appropriate perforation widths to separate them into the classes described in Table 1, except the 12.5 (D) sieve was not available in 1960. The seeds were then germinated and the hybrids identified and counted as described by McMichael (5).

A second natural-crossing block consisting of alternate male and female rows was grown at the Pontotoc Ridge–Flatwoods Experiment Station, Pontotoc, Mississippi, in 1961. The male rows were planted to M11. The female rows were planted to 5 small-seeded lines and varieties (M8 $gl_1$ $gl_1$, $D_2$ 716, 'Coker 100A', 'Deltapine Smooth Leaf', and 'Stoneville 3202'). M8 is a doubled haploid from Deltapine 14 (6). M8 $gl_1$ $gl_1$ was produced by back-crossing $gl_1$ $gl_1$ to M8. $D_2$ 716 is essentially M8 with $D_2$ Smoothness. Coker 100A, Deltapine Smooth Leaf, and Stoneville 3202 are commercial varieties. The strains were grown in a randomized complete-block design replicated four times. The 1-row plots were 100 feet long. The field was rectangular; replications 1 and 3 and 2 and 4 were grown side by side. Thus, each replication had 2 sides bordered by cotton and 2 not bordered.

There were 31 plots in each replication. Genetically marked M8 plants were arranged in 16 rows per replication to determine the percentage of crossing. The seed-sizing data obtained from the plots containing genetically marked M8 were secondary to obtaining seeds for a combining-ability study.

Five individual genetically marked M8 plants were harvested from each of the 16 plots containing this marker stock in each replication. The seeds from each plant were separated into 5 width classes (C, D, E, F, and G). All seeds passed through the 14.0/64-inch and 13.5/64-inch (A and B) width sieves. Also, small quantities of seeds from each width class were separated into length classes by hand. The seeds were germinated and the percentage of outcrossing was thus determined.

## RESULTS AND DISCUSSION

The percentage of hybrids, the cumulative percentage of hybrids, the cumulative percent of the total lot, and increase of percentage of hybrids over the mean of the population are presented in Table 2 for the 1960 seed lot. Six percent of the seeds were in the largest width class and 72% were hybrid as compared with 48% in the original lot. This was a 50% increase in percentage hybrids over the mean of the population. These data indicate that as width decreased the percentage of hybrid seed decreased at an almost linear rate. Also, the percentage of the population retained increased as the width classes decreased.

An analyses of variance was calculated on the percentage of hybrids in 5 width classes of cottonseed from the M8 $gl_1$ $gl_1$ plants grown in 1961.[3] Duncan's Multiple Range Test (Table 3) indicated that width class C had a significantly higher percentage of hybrids than the smaller width classes. The D, E, and F size classes and the F and G width classes were not different. The cumulative percent hybrids in width class C was significantly greater than the smaller width classes at the .05 level. The D and E size classes and the E, F, and G size classes did not differ. The percentage of hybrids decreased at an almost linear rate even though the size classes were not always significantly different. These data indicate that the percentage of hybrids in a mixed seed lot can be increased by seed separation into size classes. The practicality of the method depends on the economics involved. That is, the boost in yield and other desirable characteristics caused by a shift in percentage hybrids would have to be enough to pay for the cost of separating the seeds and discarding a portion of them.

The principal advantage of this method of producing partial-hybrids is that there is no reduction in yield as Kohel and Richmond (3) indicated there would be with male-steriles. The principal disadvantage of this method is that a portion of the seeds would not be saved for planting.

For commercial production the seed loss could be partially overcome by growing the large seeds from the first-year natural-crossing block and producing an advanced generation as described by Simpson (8). Seeds in the next largest classes could be saved and blended with the advanced-generation material. Seeds in the smallest width classes could be used to plant the primary block each year because it consists mostly of $S_1$ seeds.

Several methods of seed separation have been investigated and width separation has been the best single method with the materials tested. The data in Tables 4 and 5 indicate that a combination of width and length separation would be more effective than width separation alone. The width separations were done with sieves and the length

Table 1. Code letters used for cottonseed-width classes obtained from cylinder sieves with perforations having length of 1¼ inches and widths as indicated.

| Class code | Seed width, 64th inch | |
|---|---|---|
| | Less than | Greater than |
| A | - | 14.0 |
| B | 14.0 | 13.5 |
| C | 13.5 | 13.0 |
| D | 13.0 | 12.5 |
| E | 12.5 | 12.0 |
| F | 12.0 | 11.0 |
| G | 11.0 | - |

Table 2. Effects of separating mixed lot of hybrid and self-pollinated cottonseeds into width classes on percentage hybrids in Upland cotton in 1960.

| Seed-width class code | Seed | | | Hybrids | | |
|---|---|---|---|---|---|---|
| | No. | % of total | Cumulative % | % | Cumulative %* | % increase† |
| A | 112 | 6 | 6 | 72 | 72 | 50 |
| B | 297 | 17 | 23 | 60 | 63 | 31 |
| C | 379 | 20 | 43 | 54 | 59 | 22 |
| DE | 402 | 23 | 66 | 44 | 54 | 12 |
| F | 423 | 23 | 89 | 40 | 50 | 4 |
| G | 201 | 11 | 100 | 33 | 48 | - |
| Total | 1814 | | | | | |

* Percentage hybrids in this class and all larger classes combined.
† Percentage increase in hybrids over mean of population.

Table 3. Effects of separating mixed lot of hybrid and self-pollinated cottonseeds into width classes on percentage hybrids in Upland cotton in 1961.

| Seed-width class code | Seed | | | Hybrids | | |
|---|---|---|---|---|---|---|
| | No. | % of total | Cumulative % | % | Cumulative %* | % increase† |
| AB | 0 | - | - | - | - | - |
| C | 667 | 2 | 2 | 39a‡ | 39a | 77 |
| D | 2,104 | 6 | 8 | 31 b | 33 b | 48 |
| E | 3,867 | 11 | 19 | 27 b | 29 bc | 32 |
| F | 16,698 | 48 | 67 | 22 bc | 24 c | 9 |
| G | 11,520 | 33 | 100 | 18 c | 22 c | - |
| Total | 34,856 | | | | | |

* Percentage hybrids in this class and all larger classes combined. † Percentage increase in hybrids over mean of population. ‡ Means not followed by the same letter are significantly different at the .05 level of probability.

[3] Calculation of analyses by Biometrical Services, ARS, USDA, Plant Industry Station, Beltsville, Maryland, is gratefully acknowledged.

Table 4. Data on percentage of hybrids in 5 width classes and 4 length classes of cottonseed grown in a natural-crossing block at Pontotoc, Miss., in 1961.

| Seed length and width* | Treat-ment No. | Seeds | Hybrids % | Seed length and width* | Treat-ment | Seeds | Hybrids % |
|---|---|---|---|---|---|---|---|
| 10: C | 1 | 1 | 100 | 9.0: C | 11 | 3 | 100 |
| D | 2 | 14 | 71 | D | 12 | 13 | 23 |
| E | 3 | 2 | 100 | E | 13 | 26 | 11 |
| F | 4 | 68 | 41 | F | 14 | 58 | 14 |
| G | 5 | 78 | 23 | G | 15 | 21 | 10 |
| Total | | 163 | $\bar{x}$ 36 | Total | | 121 | $\bar{x}$ 16 |
| 9.5: C | 6 | 11 | 46 | < 9.0: C | 16 | 1 | 0 |
| D | 7 | 25 | 32 | D | 17 | 3 | 0 |
| E | 8 | 27 | 26 | E | 18 | 19 | 5 |
| F | 9 | 79 | 19 | F | 19 | 35 | 17 |
| G | 10 | 56 | 5 | G | 20 | 1 | 0 |
| Total | | 198 | $\bar{x}$ 19 | Total | | 59 | $\bar{x}$ 10 |
| | | | | Grand total | | 541 | $\bar{x}$ 23 |

* Length in mm.; width code from Table 1.

separations were done by hand. To date no machine that will separate cottonseeds into finite length classes has been found.

The use of male and female rows in a natural-crossing block increases production costs because the male rows are not used for seed production and the chance of mixing the seeds from male and female rows at harvest time is great. The ideal situation would be to find lines with small seeds that produce a hetorotic effect for seed size. The mechanical mixtures could be made and the entire block could be harvested for seed purposes and thus eliminate the expense of growing the male rows.

## SUMMARY

The percentage of $F_1$ hybrids in different width classes of cottonseeds from two populations were determined. The percentage of hybrids was always greatest in the largest width class and decreased progressively as the width decreased. The percentage of the seed lot retained was inversely proportional to the percentage of $F_1$ hybrid seeds retained. Width and length separation used together produce classes with a higher percentage of $F_1$ hybrid cottonseeds than either used alone.

Table 5. Increasing percent of cotton hybrids picked out by combining treatments in Table 4.

| Treatment combination | Additional treatments | % hybrids | % of population | % increase over mean of population |
|---|---|---|---|---|
| 1+2+3+4+5+6+7+11 | | 46 | 23 | 103 |
| | +5 | 37 | 37 | 63 |
| | +5+8 | 36 | 42 | 58 |
| | +5+8+9 | 32 | 57 | 39 |
| | +5+8+9+12 | 31 | 59 | 37 |

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