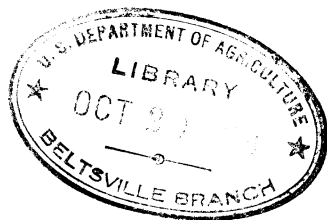# NATURAL

CROSS-POLLINATION

IN COTTON

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Agricultural Research Service

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Acknowledgments

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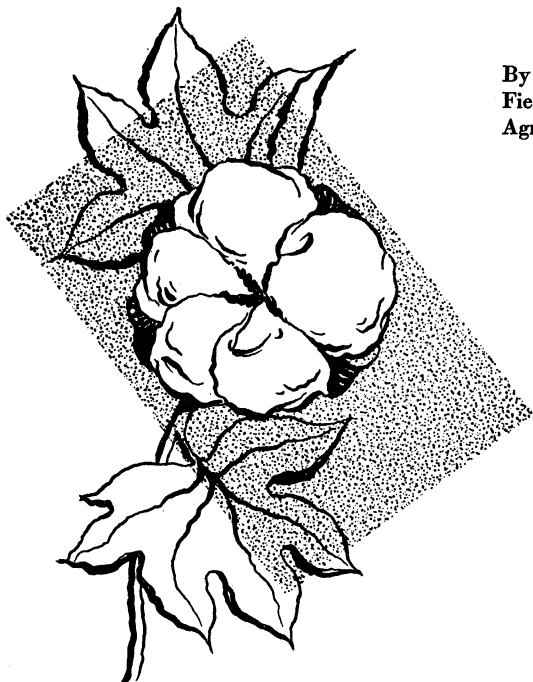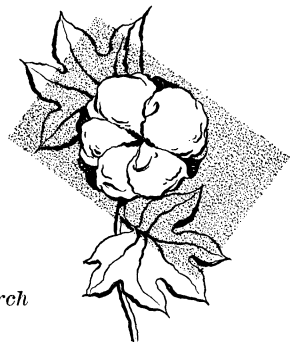
NATURAL CROSS-POLLINATION IN COTTON[1]

By D. M. SIMPSON, *agronomist, Field Crops Research Branch, Agricultural Research Service*

Natural cross-pollination has important implications in cotton breeding and cottonseed production. Control of the genetic complex that determines the potential value of a cotton plant for economic use is important, regardless of the breeding methods employed. In the past, natural crossing in cotton has received attention mainly as the cause of undesirable hybridization and mongrelization of seed stocks. Breeders have adopted artificial means of maintaining the genetic purity of their stocks, and allowable limits of segregation from other varieties have been set up for organizations engaged in the multiplication and distribution of cotton-planting seed. More recently, natural crossing in cotton has received increased attention because the phenomenon has possible use as a means of utilizing hybrid vigor and also for producing hybrid seed in commercial quantities. Formerly, studies were directed almost exclusively to reducing outcrossing; now, means of increasing natural crossing in cotton are of widespread interest.

REVIEW OF LITERATURE

Natural crossing in cotton has been investigated by numerous individuals during the past 50 years. The studies have served to emphasize the wide differences in amount of natural crossing in different localities and in different years. However, the materials used and the methods employed in these separate investigations were so varied and the results so contradictory that they furnished little in the way of uniform information.

Loden and Richmond (*3*),[2] in their excellent review of hybrid vigor in cotton, have summarized the pertinent data on natural crossing available up to 1950. The information in table 1 is taken from their article. There is no general pattern of behavior detectable from the data in table 1, but, in the discussions of their data, the investigators are in general agreement concerning the following factors that influence the amount of natural crossing: (1) Cotton pollen is relatively heavy, and wind is not an agent in pollen dispersal; (2) therefore, the amount of natural crossing in cotton is determined by the number of insect pollinators present in relation to the number of cotton flowers; and (3) intercrossing may be affected by the flower-

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[1] Submitted for publication March 15, 1954.
[2] Italic numbers in parentheses refer to Literature Cited, p. 17.

ing habits of the varieties grown, by the abundance of unlike pollen, by location of the fields in relation to insect habitats, by flowering periods of other plants attractive to insect pollinators, by distance between unlike varieties, by topography and barrier crops, and by other environmental, climatic, and biotic factors.

TABLE 1.—*Percentages of natural crossing reported in the literature, 1903–50* [1]

COTTON BELT OF THE UNITED STATES

Location	Investigators	Experimental design	Percent natural crossing	
			Max-imum	Mini-mum
South Carolina	Webber	Alternate rows	10	5
North Georgia	Allard	do	[2] 20	
	McLendon	do	[2] 2	
Tifton, Ga	Turner	do	34	11
	do	Male 90 percent	34	26
Alabama	do	Male 90 percent+	43	40
Knoxville, Tenn	Pope et al	Alternate rows	[2] 27	
	Simpson	Male 90 percent	53	44
Mississippi	Brown	Male 90 percent+	81	57
	Ricks and Brown	Alternate rows	11	5
	Brown	Adjacent plants	[2] 19	
Fayetteville, Ark	Ware	Alternate rows	[2] 41	
Scott, Ark	do	do	[2] 1	
College Station, Tex	Richmond et al	do	[2] 9	
	Stroman and Mahoney	Adjacent plants	3	2
Waco, Tex	Shoemaker	Excess of male	[2] 11	
Sacaton, Ariz	Kearney	Alternate rows	11	5
	do	Male 90 percent+	35	14

OTHER COTTON-PRODUCING AREAS

India	Kottur	Alternate rows	6	
	Afzal and Khan	do	3	1
	do	do	[2] 2	
	do	Adjacent plants	2	2
	do	do	[2] 2	
	Gammie	Alternate rows	0	
China	Yu and Hsieh	do	[2] 8	
U.S.S.R.	Anonymous	do	9	4
Egypt	Balls	do	15	5
	do	do	35	5

[1] After Loden and Richmond (*3*), tables 2 and 3.
[2] Average figures.

Several reliable methods of artificial self-pollination are available to cotton breeders, and the genetic purity of small stocks may be readily maintained by such methods. However, the use of barrier crops (preferably cotton) and of adequate isolation from unlike geno-

types are the only practical methods suggested for reducing intervarietal crossing in field-size plantings (*2, 5*). As natural crossing in cotton heretofore has generally been considered a handicap in breeding programs and a hazard to be avoided, it is not surprising that the literature contains little data on methods or means of increasing the amount of crossing. From consideration of the factors that have been suggested as influencing the amount of natural crossing, it is apparent that interstrain crossing can be increased by intermingling the unlike genotypes and by increasing the population of insect pollinators. Meade (*4*) reported that honey bees (*Apis mellifera* L.) are effective cotton pollinators and suggested beekeeping as an·aid to cotton growing. Bumble bees (*Bombus* spp.) are most frequently mentioned as pollen carriers. From other suggestions, we may infer that insect activity might be highest in small fields adjacent to uncultivated areas, such as timberlands, pastures, meadows, or wasteland.

Other investigations (*6, 9*) have indicated that outcrossing occurs more frequently in some varieties than in others when exposed to cross-fertilization under similar conditions. Adequate studies of varietal interactions have not been reported, nor have the morphological and physiological bases for such interactions been thoroughly explored. Use of a male-sterile or semi-male-sterile stock as the female parent (*3*) has been suggested as a means of obtaining maximum crossing. A number of workers have studied this problem, but as yet there have been no practical results from this line of approach.

REGIONAL NATURAL-CROSSING TEST

Plan of Experiment

Tentative plans for a regional natural-crossing test were formulated at the First Cotton Improvement Conference held at Baton Rouge, La., in February 1949. Under the direction of a committee appointed at that conference, a uniform planting plan was prepared and seed stocks were obtained for distribution to all cooperators.

The general plan of the experiment provided for planting a small block (20 rows wide by 100 feet long) of upland red-leaf cotton in a location semi-isolated from other sorts. A small proportion of upland green-leaf cotton (approximately 10 percent of the total plant population) was interplanted systematically at 10-foot intervals, staggered in alternate rows, in this red-leaf block. The seed cotton from the green-leaf plants was retained separately, and the seed was subsequently planted for determination of the percentage of red (♂) × green (♀) hybrids in the population.

The determinations of the outcrossing percentages were made by each cooperator for his own location. Various methods of growing out the plants were used to determine the proportion of hybrids. These included sprouting the seeds in the germinator, greenhouse plantings, and field plantings. Red and green seedlings are easily recognized in greenhouse plantings or under continuous illumination in germinators. Checks of the different methods indicate that all are about equally accurate if the counts are made on populations of equal size.

Since the test plots were seeded with the ratio of 9 reds to 1 green, the estimates obtained were actually 90 percent of the total outcrossing, assuming the same frequency of outcrossing between green plants as that between red and green. The red-leaf variety used was DeRidder, and the green-leaf variety was Empire. F_1s of this cross are easily recognized by the intermediate-red pigmentation in the seedlings or in the mature plants.

The results obtained by the method used here in measuring natural crossing are strictly applicable only to the two varieties employed. As pointed out by Stephens and Finkner (8), the results could be biased by marked differences in flower production, noncoincidence of flowering period, or other physiological or morphological differences in the varieties. Actually, initial flowering was somewhat later in DeRidder than in Empire, so that in some cases a few very early Empire flowers may have had no opportunity for cross-pollination with DeRidder. Available information indicates that in other respects the two varieties are near average among upland cottons for potential outcrossing and that reciprocal natural crossing between them is about equal. The results, therefore, are considered fairly representative for the locations under the specific environmental conditions in the season of test.

Plantings of this test were made at 12 locations in 1949, 15 in 1950, 15 in 1951, and 6 in 1952. All cooperators were requested to conform as nearly as possible to the general plan of the experiment so that data from the different areas would be comparable. This was done in most cases. A different procedure, however, was used at Sacaton, Ariz., in all 3 years of tests. Homozygous red-leaf plants of Acala were randomly spaced in a plot of green-leaf cotton, and the percentage of green (♂) × red (♀) hybrids was determined from a plant population grown from the female (red) parent. At Raleigh, N. C., in 1950, the ratio of red-leaf plants to other types in the crossing block was 9 : 11 instead of 9 : 1 as in the general plan. The 1950 data for Raleigh were corrected for the different proportion of red stocks used.

Results and Discussion

The Cotton-Belt-wide scope of the experiment is illustrated in the map (fig. 1) which shows the geographic locations of the tests. At least one test was conducted in every cotton-growing State except California.

The data for the 4 years of test are summarized (table 2). For the purposes of this experiment, the data have been divided into regional groups. This grouping involves certain overlapping of and divergence from the generally accepted geographic subdivisions of the Cotton Belt. This was necessitated by ecological relationships of these subdivisions, as discussed on ensuing pages of this bulletin. The percentages of natural crossing shown within the regional groups indicate a definite pattern of behavior dependent on the climatic and biotic environment generally imposed by geographic location. The wide difference in percentage of outcrossing between and within regions shows clearly why this has been a highly controversial subject in the past.

The southeastern region of the Cotton Belt (not including the Mississippi Valley) contains more than one-third the total acreage
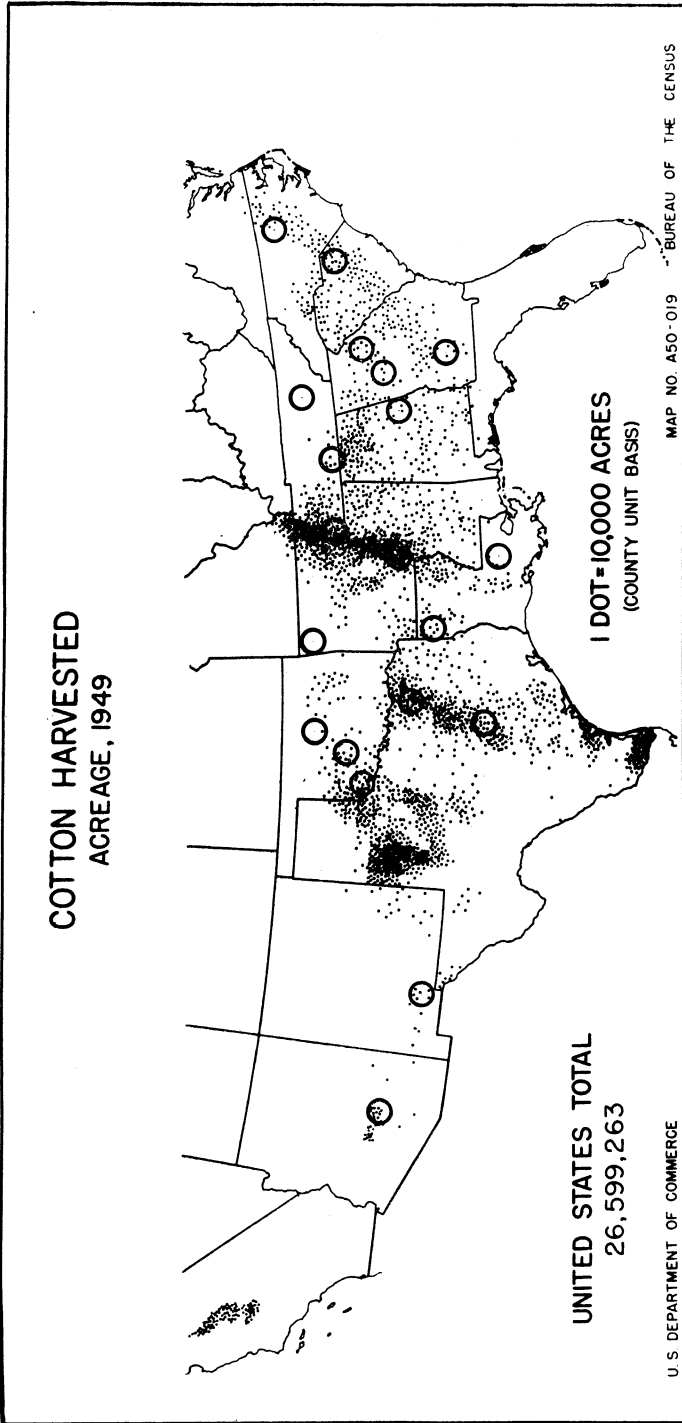
FIGURE 1.—The distribution of cotton acreage in the United States, 1949. The circles indicate the locations at which one or more of the natural-crossing tests were conducted.

TABLE 2.—*Summary of results of regional natural-crossing tests with cotton, 1949–52*

Location	Total plants examined (in thousands)				Natural-crossing					Standard deviation of the mean
	1949	1950	1951	1952	1949	1950	1951	1952	Mean	
Southeastern (except Mississippi Valley):					*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Raleigh, N. C.	12	—	—	—	55	—	—	—	49	6
Knoxville, Tenn.	25	8	14	14	46	42	42	52	46	2
Loretto, Tenn.	—	—	8	5	—	42	29	39	34	5
Experiment, Ga.	4	—	6	—	25	—	42	—	34	8
Athens, Ga.	—	19	27	3	—	27	48	50	42	7
Tifton, Ga.	3	3	—	—	27	33	—	—	30	3
Auburn, Ala.	2	3	5	—	29	24	45	—	33	3
Hartsville, S. C.	.2	—	—	—	12	—	—	—	[1]12	—
Mean	—	—	—	—	—	—	—	—	39	2
Mississippi Valley:										
Munford, Tenn.	10	12	10	4	12	11	34	26	30	4
Stoneville, Miss.	—	—	5	—	—	—	31	—	18	6
Baton Rouge, La.	1	3	2	—	51	31	30	—	37	7
Mean	—	—	—	—	—	—	—	—	28	5
South Central:										
Bossier City, La.	—	18	10	—	—	10	14	—	14	—
Fayetteville, Ark.	—	7	14	15	—	34	9	42	20	11
Stillwater, Okla.	—	4	6	—	—	26	15	—	34	—
Chickasha, Okla.	—	7	—	—	—	24	—	—	20	6
Tipton, Okla.	—	7	—	—	—	24	—	—	24	—
Mean	—	—	—	—	—	—	—	—	22	4

Central Texas:										
Greenville, Tex	19	13	4	--	8	19	17	--	15	3
College Station, Tex	15	6	2	4	3	10	11	3	7	2
Mean									10	2
Western (irrigated):										
Sacaton, Ariz	10	7	6	--	29	25	46	--	33	6
State College, N. M	25	3	3	--	12	12	5	--	10	3
Mean									22	6

[1] Based on less than 1,000 plants; not included in regional mean.

planted to cotton in the United States and produces more than one-third the total cotton yield. This estimate is based on the 10-year average for the period 1939–48, as given in Agricultural Statistics, 1951. The region is characterized by rolling to hilly topography, relatively small fields of cotton, and relatively large areas of woodland, pasture, and wasteland. With abundant natural cover in proximity, insect pollinators occur in great numbers in the cotton-fields. Natural crossing in this region approximated 39 percent under the conditions of this experiment. This relatively high percentage of natural crossing undoubtedly reflects the general physiographic ecology of the region.

The cotton region bordering the Mississippi River is a well-defined geographic area, but it is less well-defined in terms of ecological relationships. On the alluvial soils in the flood plain of the Mississippi River, a high proportion of the land is planted in cotton, and this region is one of the most productive cotton areas in the United States. Along the margins of the flood plain and in places where the plains are narrow, the river bottoms are adjacent to considerable areas of rolling, eroded loess soils, much of which is wasteland. In such areas, the insect-plant association is much like that in the southeastern region. Munford, Tenn., and Baton Rouge, La., with natural-crossing percentages of 30 and 37, respectively, are representative of such locations. However, where flood plains widen, as in parts of eastern Arkansas, northwest Mississippi, and northeast Louisiana, the topography is flat or undulating, and the land is intensely cultivated, predominantly in cotton; and there are few areas of waste or uncultivated land suitable as a natural habitat for insect pollinators. Stoneville, Miss., is situated in an area of this type and natural crossing (18 percent) was generally lower there than at Munford, Tenn., and Baton Rouge, La.

There is a wide area west of the Mississippi Delta, including parts of Arkansas, Louisiana, east Texas, and Oklahoma, that lies within the humid region. In soil type and topography, it is not unlike the Coastal Plain that extends through the South Atlantic and Gulf coast areas of the Cotton Belt. No data on natural crossing are available from this general area, but it may be assumed that the percentage of crossing would be somewhat intermediate to that obtained in the southeastern and south central regions.

The great cotton-producing region of the Blackland Prairies and contiguous areas of central Texas, and possibly similar climatic areas of Oklahoma and west Texas, are regions of relatively low natural crossing (tables 1 and 2). A large proportion of the land in this region is under cultivation, with large acreages in cotton and few bordering areas of wasteland suitable for pollinator-insect habitats. Low summer rainfall and scarcity of wild flowering plants that would provide year-round food supplies for pollinator insects are probably contributing causes of low insect population and low natural crossing.

The data from the western irrigated areas, with 33 percent natural crossing at Sacaton, Ariz., and 10 percent at State College, N. Mex., are indicative of local conditions at the places of the tests. In the broad irrigated valleys in areas of intensive cotton production, such as exist in parts of the Salt River Valley in Arizona and the San Joaquin Valley in California, natural crossing would be generally low. In areas not predominantly planted to cotton and with more

favorable biotic conditions, as at Sacaton, Ariz., a high percentage of natural crossing is not unexpected.

It is increasingly apparent from the data on this subject that natural crossing is lowest in areas of intensive cotton cultivation where fields are large and contiguous to other fields of cotton or other cultivated lands. Under such conditions insect populations are small in relation to the total cotton acreage, and visitations to flowers are too infrequent or too long delayed to be greatly effective in cross-fertilization. The distribution of cotton acreage in the United States (fig. 1) indicates roughly the areas of high and low natural crossings, but these areas cannot be clearly defined on the basis of data currently available.

POPULATION COUNTS NECESSARY FOR ACCURACY OF DATA

At the beginning of this study, no information was available as to the size of sample necessary to determine with accuracy the percentage of natural crossing. Population counts in 1949 at the various locations ranged from approximately 200 plants at Hartsville, S. C., to 25,000 at Knoxville, Tenn., and at State College, N. Mex. At Raleigh, N. C., Knoxville, Tenn., and State College, N. Mex., populations counts were made by units of planting that made it possible to calculate the percentage of hybrids on one or more units and judge the accuracy of results from populations of different sizes (table 3). It is apparent that reasonably accurate determinations of the percentage of hybrid plants can be obtained by examining a population of 1,000 plants. Counts of larger populations reduced the standard deviation of the mean but did not materially change the mean. The natural-crossing percentages shown in table 2, with one exception, are based on counts of 1,000 or more plants. The data are therefore considered highly reliable. The arithmetic averages of table 2 are justified since the percentage data from all locations furnish approximately equal information.

TABLE 3.—*Variation in natural-crossing percentages at three locations when based on variously sized populations, 1949*

[The populations used are cumulative]

Approximate total population	Hybrids at the locations shown		
	Raleigh, N. C.	Knoxville, Tenn.	State College, N. Mex.
	Percent	*Percent*	*Percent*
500	55. 0	41. 2	
1,000	52. 4 ± 2. 9	45. 3 ± 4. 1	9. 5
1,500	52. 2 ± 1. 7	43. 9 ± 2. 1	9. 3 ± 0. 2
2,500	57. 0 ± 3. 1	44. 1 ± 1. 5	10. 0 ± 0. 7
4,500	53. 5 ± 2. 7	44. 5 ± 1. 0	10. 2 ± 0. 9
6,500	53. 7 ± 1. 9	44. 9 ± 0. 8	10. 1 ± 0. 7
9,500	54. 2 ± 1. 5	44. 3 ± 0. 6	10. 5 ± 0. 6
12,500	54. 9 ± 1. 2	44. 5 ± 0. 5	10. 8 ± 0. 4
25,000		45. 5 ± 0. 3	11. 6 ± 0. 3

The data (table 3) from Knoxville, Tenn., and from State College, N. Mex., show tendency of the mean percentage of hybrids to increase progressively with the larger population counts. At Knoxville the counts by units consumed considerable time and the work was spread over a period of about 2 weeks. It was observed that the hybrid plants in the population were apparently less susceptible to seedling diseases than the plants of the maternal green-leaf variety. On June 2, a re-check of the counts originally scored on May 16 on 10-row units indi-cated that 6 percent of the original stand had been lost from seedling diseases or other causes. The loss, however, was 8 percent among green-leaf plants and only 3 percent among the intermediate reds. The percentage of hybrid plants in the original count was 44.2, whereas the percentage from the counts made on June 2 on the same row units was 45.4. Thus, under such conditions, the longer the count was de-layed, the higher the apparent percentage of natural crossing.

Several of the cooperators in the regional natural-crossing test have raised the point that hybrid plants are more vigorous and that possibly a larger percentage of the hybrid seeds produce seedlings than do self-pollinated seeds in the same lot. The vigorous growth of the DeRidder × Empire hybrids was noted during the course of the re-gional tests. At Knoxville in 1950, hybrid seedlings were 14 percent heavier, based on green weight, than those of the green-leaf parent variety. These observations indicate the possibility that the greater vigor of the hybrids may result in a higher percentage of emergence and survival of seedlings from hybrid seed than from selfed seed.

ROW AND PLANT VARIATION

The general plan for the regional natural-crossing tests specified a field planting of about 20 rows approximately 100 feet long in a loca-tion at least semi-isolated from other cotton. This general plan was adhered to in most of the tests. Several of the cooperators maintained the identity of the seed from their crossing plots by row and plant number until the percentage of natural crossing was determined. The very complete data obtained at Raleigh, N. C., Athens, Ga., and Fayetteville, Ark., are illustrative of row and plant variations in this type of test.

The row variations (table 4) shown by the data from Raleigh, N. C., and Athens, Ga., appear to be a random distribution of values around the mean percentage of natural crossing obtained for the respective locations. Natural crossing in these tests does not appear to have been influenced by row position. At Fayetteville, Ark., however, the values do not appear to be a random distribution around the location mean but show a progressive increase in percentage of natural crossing from row 1 to row 20. It is presumed that in this field location, the two sides of the plot were unlike in relation to topography, adjacent plantings, or other environmental factors, although no information is available as to the nature of such possible differences. The percentages of hybrids in the Fayetteville samples were determined from seed sprouted in germinators, and the results could not have been due to differential seedling mortality as described for delayed counts at Knoxville (see table 3).

TABLE 4.—*Variation in percentage of natural crossing as affected by row position*

Row No.	Hybrid plants by rows at—			
	Raleigh, 1949	Athens, 1950	Athens, 1951	Fayette-ville, 1950
	Percent	*Percent*	*Percent*	*Percent*
1	53. 9	20. 5	37. 9	8. 7
2	43. 2	25. 0	42. 2	7. 2
3	54. 1	26. 7	44. 4	9. 1
4	52. 0	33. 3	41. 9	8. 7
5	64. 7	30. 9	46. 0	6. 4
Average, rows 1–5	53. 6	27. 3	42. 5	8. 0
6	55. 4	28. 7	50. 3	9. 3
7	43. 7	33. 0	46. 7	9. 7
8	54. 7	36. 1	51. 0	12. 7
9	55. 3	32. 7	52. 7	10. 2
10	54. 5	23. 4	50. 3	10. 4
Average, rows 6–10	52. 7	30. 8	50. 2	10. 5
11	53. 4	32. 5	52. 5	10. 3
12	51. 7	31. 7	49. 3	11. 8
13	61. 7	24. 0	47. 1	9. 5
14	52. 1	23. 4	47. 6	11. 9
15	55. 2	27. 6	45. 1	12. 5
Average, rows 11–15	54. 8	27. 8	48. 3	11. 2
16	54. 0	29. 8	45. 8	17. 1
17	63. 2	34. 0	49. 2	17. 4
18	59. 8	24. 0	47. 8	11. 7
19	51. 6	26. 7	45. 5	10. 6
20	55. 8	24. 4	42. 1	15. 2
Average, rows 16–20	56. 9	27. 8	46. 1	14. 4
Location mean [1]	54. 9	27. 4	47. 8	10. 3

[1] Obtained from total count.

Data on natural crossing by individual plants were obtained at Raleigh, N. C., Athens, Ga., and Fayetteville, Ark. The class frequencies derived from these data (table 5) are from relatively small populations and therefore show many irregularities, but they appear to be near-normal distributions around the location means. This is an expected result, as both parental varieties are fairly homozygous line-bred types.

VARIETAL RESPONSE TO NATURAL CROSSING

In general, DeRidder and Empire have been used as parental varieties for the regional natural-crossing tests, with Empire as the female parent. Some different result might have been obtained if

other varieties had been used. It is possible, even highly probable, that the extent of outcrossing is affected by varietal differences in mechanical and physiological characters, such as time of flower opening, time of anther dehiscense, style length, abundance of pollen, and rate of pollen-tube growth. Varietal response to natural crossing has received some attention, and preliminary evidence indicates that varietal differences may be quite large. Turner (9) reported 30 percent crossed seed from an Acala strain as compared with 13 percent from Florida Greenseed when similarly exposed to cross-pollination. At Raleigh, N. C., 79 percent natural crossing was recorded when a virescent yellow stock was used as a female parent as compared with 42 percent from Empire (green-leaf) and 45 percent from DeRidder (red-leaf).

TABLE 5.—*Frequency distribution of natural crossing by individual plants*

Class interval (percent hybrids)	Number of plants in respective class intervals at—			
	Raleigh, N. C. 1949 [1]	Athens, Ga. 1950 [1]	Athens, Ga. 1951 [1]	Fayette-ville, Ark. 1952
0.0–5.0	0	5	0	0
5.1–10.0	0	18	0	0
10.1–15.0	1	19	1	0
15.1–20.0	0	41	1	3
20.1–25.0	2	41	0	3
25.1–30.0	3	33	3	5
30.1–35.0	6	31	6	20
35.1–40.0	10	19	12	34
40.1–45.0	16	20	34	39
45.1–50.0	22	14	51	33
50.1–55.0	24	11	21	21
55.1–60.0	21	11	11	13
60.1–65.0	20	4	4	4
65.1–70.0	21	0	1	5
70.1–75.0	11	2	0	0
75.1–80.0	5	2	0	0
80.1–85.0	2	2	0	0
85.1–90.0	1	0	0	0
90.1–95.0	1	1	0	0
95.1–100.0	0	0	0	0
Total plants	166	274	145	180
Location mean (percent hybrids)	54. 9	27. 4	47. 8	42. 2

[1] Plants with 10 or less seeds omitted.

At Knoxville, Tennessee, in 1951, 2 replications of 79 varieties or strains of green-leaf cotton were planted in alternate rows with De-Ridder red-leaf, to study response to natural crossing (6). The average natural crossing between red-leaf and green-leaf was 41 percent. The range among the 79 strains was from a low of 29 percent to a high of 60 percent. A statistical analysis of the data indicated that a

difference of 13 percent between strains was highly significant. Further tests will be necessary to determine whether genetic differences that can be used to increase or decrease the normal percentage of natural crossing exist in these strains.

INSECT ACTIVITY IN COTTON FLOWERS

Cross-pollination is not readily detected in cotton unless distinctive marker characters are present in the parental lines. For this reason, the extent of natural crossing in open-pollinated fields has been consistently underestimated by some cotton breeders. Some idea of the potential for crossing may be gained from a study of insect activity and visitations to cotton flowers.

Eckert (1) found that when colonies of honey bees were separated from a given nectar-producing area by badlands, with no other source of food intervening, they would fly a distance of at least 8.5 miles. Experiments on the distribution of bees from apiaries located within a nectar-producing area showed that bees would fly for 2.75 to 4.6 miles in one direction and confine their efforts to working in that direction rather than in nearer fields of seemingly equal attractiveness. The results indicate that bees establish feeding habits and tend to return to the same portion of a field, or to the same small field, on successive days for nectar or pollen, even though other areas of the same forage plant are nearer. Bee experts state that when bees are brought into fields to improve pollination and increase seed-set, best results are obtained if the colonies are moved in *after* the particular plants are in flower, so that flight lanes to some other food source will not be established.

A unique method for studying insect activity has been used by S. G. Stephens at Raleigh, N. C. The method and results as described by Stephens are as follows: [3]

If you grind fairly finely a dye like methylene blue and dry it thoroughly before use it works as a good marker of the movements of the bees. We put a light sprinkling of the methylene blue powder over the anther column early in the morning when the anthers burst. The next day we open the stale flowers from the previous day and examine for blue stain. It works very well. There is enough moisture accumulated when the flower fades and closes to spread even one tiny grain of the dye and make an easily recognizable blue spot. Furthermore you can see whether the bee has been collecting pollen or visiting nectaries or both by the position of the stain. In our plots we placed the dye in four flowers at one end of the field. Next day we found blue stained flowers for a distance of 30 yards as far as a broad alleyway. Apparently the bees did not cross the alleyway or if they did the blue stain had become exhausted by the time they reached it. In the vicinity of the "dye-dusted" flowers *all* flowers examined were blue pigmented. Apparently very few flowers are missed by bees under our conditions which accords with the high percent of outcrossing observed [in Kime's experiment]. The methylene blue apparently does not deter the bees from visiting flowers, neither does it cause the flowers to drop without setting. So it appears to be quite a useful technique in finding out how much visitation goes on, how far the bees range and also one could probably determine what effect a neighboring field of, say, alfalfa would have on the activity of the bees in the cotton. I think one could improve the technique by grinding the dye really fine and possibly diluting with talc.

This method appears useful in studying insect visitations, including how far the bees range, and the percentage coverage of flowers in a

given area. It should be of particular interest to breeders who have hesitated to conduct conventional natural-crossing studies for fear of introducing red-leaf or other distinctive marker characters into their seed stocks.

HETEROZYGOSITY IN SUCCESSIVE HYBRID GENERATIONS

Hybrid vigor is associated with heterozygosity, and the maximum expression of heterosis is obtained in the F_1 generation. Cotton is a partially cross-pollinated plant; thus, some degree of heterozygosity is maintained indefinitely when open-pollinated seed from an original F_1 population is continued on through F_2, F_3, and subsequent generations. The relative proportions of selfing and outcrossing determine the amount of hybrid vigor retained in later generations. Stephens (7) has given the following formula for calculating the proportion of heterozygotes in the current generation when the proportion of heterozygotes in the preceding generation and the proportion of outcrossing is known:

$$h = \frac{1}{2} \left[(1-k) h' + k \right]$$

in which h is the proportion of heterozygotes in the current generation, h' is the proportion of heterozygotes in the preceding generation, and k is the proportion of outcrossing. The formula provides an estimate of the average heterozygosity of any number of gene pairs expressed as a percentage of the F_1 heterozygosity.

A practical test of heterozygosity, as measured by the frequency of 1 gene pair in successive hybrid generations, has been conducted at Knoxville, Tenn. In 1948, mass crosses were made between pilose-leaf (T-611) and a smooth-leaf cotton. The pilose character in T-611 is controlled by a single gene. All F_1 plants of this cross were intermediate with incomplete dominance of pilosity. The F_2 generation showed 3 leaf-class phenotypes readily recognizable by field examination of mature plants.

The F_1 generation was grown, open-pollinated, in an isolated plot on the station in 1949. The seed cotton was harvested in bulk from this planting and ginned; the seed was thoroughly mixed, and part of it was used for a similar planting in 1950. The same procedure was followed in obtaining seed for 1951 and 1952 plantings.

A count was made each year (1949 to 1952, inclusive) of the number of plants in each leaf-class phenotype in the total population. The amount of natural crossing at Knoxville during this period averaged about 45 percent. The actual proportions of plants heterozygous for the pilose character is compared with the expected proportions calculated on the basis of 45 percent natural crossing (table 6).

TABLE 6.—*Frequency of pilose, intermediate, and smooth-leaf plants in F_1 and later open-pollinated generations of the hybrid, and proportions of plants heterozygous for pilosity, at Knoxville, Tenn.*

Year	Generation	Plants in leaf-class phenotypes				Proportion of plants heterozygous for pilosity	
		Pilose	Inter-mediate	Smooth	Total	Actual	Ex-pected [1]
		Number	*Number*	*Number*	*Number*	*Percent*	*Percent*
1949_____	F_1____	0	(²)	0	_____	100	100
1950_____	F_2____	151	384	144	679	57	50
1951_____	F_3____	257	361	250	868	42	36
1952_____	F_4____	206	319	150	675	47	32

[1] Based on 45 percent natural crossing.
[2] All plants.

The theoretical proportion of heterozygotes suggested by Stephen's formula is based on equal viability and equal productivity of all types in the mixture. With types which show heterosis, the formula is likely to underestimate the proportion of heterozygotes actually obtained. For instance, if the more heterozygous plants in a given population had higher than average yield, the succeeding generation would contain a larger proportion of seed from such plants than would be indicated by the actual number of plants of that class in the current generation. Disproportionate frequencies could occur if differences in seedling vigor, disease and insect resistance, or other factors caused a larger proportion of one class of plants than another to be left in the stand of mature plants. It is likely in this material that natural selective processes have operated to favor the heterozygous phenotype, although the alternative possibility that the amount of natural crossing was greater than 45 percent cannot at present be disregarded.

ROLE OF NATURAL CROSSING IN CURRENT BREEDING

The fundamental but perhaps unsuspected role of natural crossing in current breeding techniques has been pointed out by Stephens and Finkner (*8*) :

In developing new varieties of cotton the breeder attempts to extract superior lines from his breeding stocks. Following systematic testing the best lines are commonly bulked and multiplied for two or three years in increase blocks to provide seed for commercial planting. It will be evident that the composition of the "variety" finally released to the farmer will be dependent on the initial heterozygosity of the breeder's selections and the amount of natural crossing which has taken place during multiplication. It could vary between the following limits: (a) a mechanical mixture of different but more or less homozygous biotypes, (b) a hybrid swarm in which the identities of the original selections have been lost. In the latter case the combining ability of the lines included in the multiplication mixture and the amount of hybrid vigor retained during multiplication might be important, though perhaps unconsidered, factors in the success of the variety.

Since medium to high percentages of cross-pollination occur in extensive areas of the Cotton Belt, including highly important seed-producing areas, the phenomenon must have considerable practical effect on the genetic complex of varieties produced in such areas under open-pollinated conditions. If hybrid vigor, retained through natural crossing among component strains, is a contributing factor to the productivity, quality, and adaptability of some of our present-day commercial cotton varieties, recognition of this fact may suggest modifications of present breeding techniques. In regions of moderate to high natural crossing, the component strains might better be selected on the basis of their combining ability rather than extreme uniformity as represented by closely related homozygous lines. On the contrary, in regions of low natural crossing, the combining ability of the component strains would have little effect on the composition of a variety. Varieties grown in such regions would tend to become or remain mechanical mixtures of relatively homozygous biotypes.

SUMMARY

The amount of natural crossing in cotton, determined from 48 separate tests in 12 States over a period of 4 years, ranged from less than 10 percent in parts of central Texas to approximately 50 percent in parts of the southeastern region of the Cotton Belt. The extent of natural crossing apparently is closely related to the frequency and timeliness of the visits of insect pollinators to the cotton flowers. The data from the several areas suggest a regional pattern for natural crossing somewhat coincident with the cotton-acreage distribution. In areas of intensive cotton cultivation, the ratio of insect pollinators to cotton flowers is low and cross-fertilization is infrequent. In areas of small fields and "patch" cotton, as in most of the southeastern region, the ratio of insect pollinators to cotton flowers is high, and cross-fertilization is correspondingly high. Thus, the phenomenon reflects the general physiographic ecology of the region.

Reasonably accurate determinations of the percentage of hybrid plants can be obtained by examining a population of 1,000 plants. Equally accurate results were obtained by growing out the populations in germinators, greenhouse benches, or in the field. In general, data on row and plant variation in natural crossing indicate a random distribution of values around the mean percentage obtained for the respective locations.

From related studies it appears that cotton varieties differ in the amount of cross-fertilization that occurs when they are similarly exposed to foreign pollen. This fact may be of considerable significance with regard to development of new breeding methods utilizing hybrid vigor.

Bumble bees and honey bees are most frequently mentioned as insect carriers of cotton pollen. The activity of these insects in the cotton flowers is a good index of the potential for crossing. The spread of methylene blue from a few "dye-dusted" flowers to other flowers in the vicinity has been used to study such activity.

A study of heterozygosity in advanced generations of an original F_1 population made at Knoxville, Tenn., indicated a higher percentage of heterozygous plants in the F_2, F_3, and F_4 generations than the

theoretical expectation. The disparity may have been caused by natural selective processes operating to favor the heterozygous phenotype.

Natural crossings may play a fundamental but perhaps unsuspected role in current breeding techniques. Recognition of this role may suggest modifications of breeding methods to employ natural crossing as a useful tool for cotton improvement.

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