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Breeding Strains of Cotton *Resistant to* Bacterial Blight

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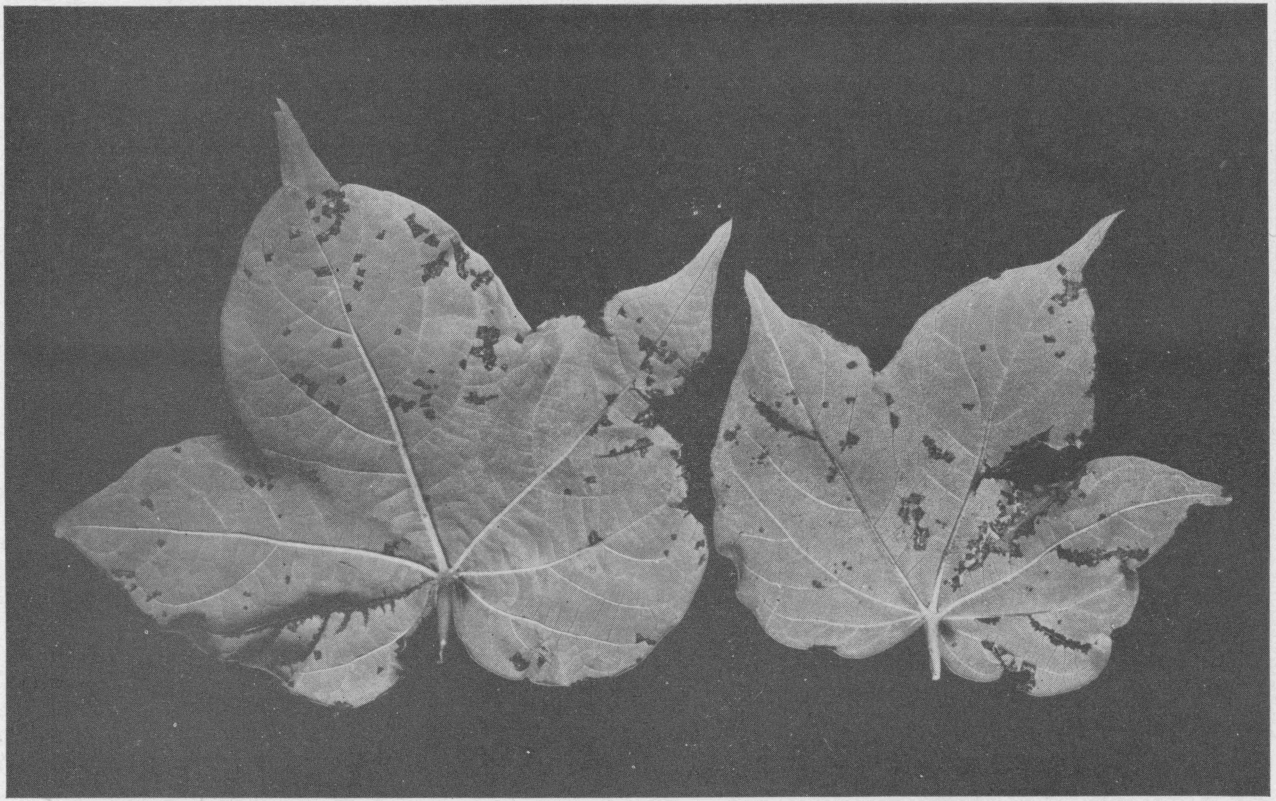

Figure 1. Bacterial blight infection of cotton leaves (angular leaf spot phase).

Digest

This bulletin describes methods used in Texas in developing strains of cotton resistant to bacterial blight, and reports studies related to the breeding program.

Stoneville 20, a strain highly resistant to bacterial blight, is being used as a source for transferring blight resistance to susceptible varieties of Upland cotton. The backcross method of breeding was used successfully for transferring resistance to commercial types. Results of tests conducted in 1950 indicate that the resistance of Stoneville 20 is being combined with the desirable agronomic qualities of several varieties. The varieties studied at College Station are Stoneville 2B, Deltapine and Empire.

Studies made of the variability in pathogenicity of isolates of the blight causal organism gave no evidence that races or strains existed. None of the isolates was capable of breaking down the resistance of Stoneville 20.

Methods developed for artifically inoculating cotton plants with the blight causal organism have proved effective to the extent that selections for resistance can be made with a high degree of certainty. Such methods (spraying inoculum through open stomata, on the lower side of leaves, into the intercellular spaces of the leaf) have made it possible for the breeding program to progress rapidly.

A method of grading the variation in blight infection is given. Results of grading the infection of individual plants of susceptible Deltapine, Stoneville 2B and Acala varieties indicated that Deltapine has a higher degree of tolerance than Stoneville 2B, which in turn has a higher tolerance than Acala.

Data obtained by grading infection of individual plants in F_2 progenies from crosses of Stoneville 20 with susceptible Deltapine and Acala supported the monohybrid hypothesis of the inheritance of Stoneville 20 resistance. The data strongly indicated that susceptibility was dominant and resistance was recessive.

The nature of the genetic data, along with the results of individual plant selections, indicated that minor genes greatly influence the degree of resistance given by the major genes.

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Breeding Strains of Cotton Resistant to Bacterial Blight

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BACTERIAL BLIGHT DISEASE of cotton is caused by *Xanthomonas malvacearum* (E. F. Sm.) Dows., and is capable of affecting all aboveground parts of the cotton plant. Bacterial blight occurs throughout the cotton producing areas of the world. In this country, it is one of the most widespread diseases of cotton and is particularly severe in the Southwestern cotton producing areas.

The disease shows up on the leaves as water-soaked angular lesions which turn brown when dry (Figure 1). On the bolls, it appears as water-soaked round lesions which are sunken and black when dry (Figure 2). On the stems and fruiting branches, it produces black elongated lesions. These characteristic symptoms cause the disease to be more commonly known as "angular leaf spot," "boll blight" and "blackarm." In the U. S. Cotton Belt, the blackarm phase does not occur as often on upland cottons as angular leaf spot and boll blight.

The bacteria are spread from old to newly-formed leaves and eventually to the bolls by wind-driven and splashing rain. Severe leaf infection may result in extreme defoliation which directly reduces the photosynthetic area of plants. This condition may result in slight to severe losses in the yield of seed cotton.

Leaf infection maintains the disease in the field during the entire growing season, thus providing a source of inoculum for boll infection. The boll-blight phase of the disease probably causes the greatest loss. In this phase, the causal organism penetrates the ovary wall by enzymatic action and enters the locules. The yellow bacterial slime stains the fibers, thus affecting the fiber grade. Also, blight lesions provide a port of entry for many of the secondary boll rotting fungi which normally do not infect healthy cotton bolls. Ray (17) stated that the key to control of boll rots in Oklahoma is control of bacterial blight.

As pointed out by Ray (18), seed treatment is beneficial for controlling bacterial blight in the seedling stage. However, beyond the seedling stage *the use of resistant varieties offers the only known method of adequate control.*

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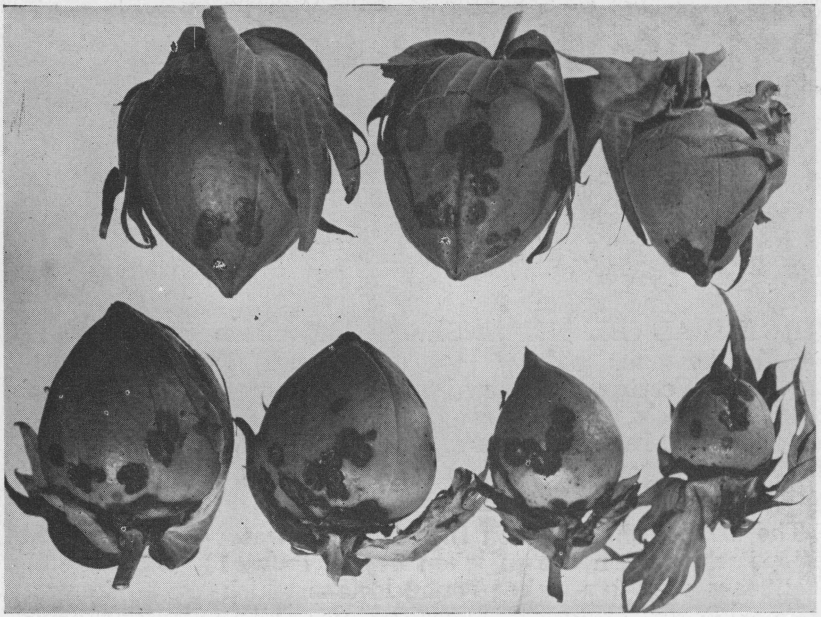

Figure 2. Bacterial blight infection of the cotton boll (boll blight phase). Upper row, young water-soaked lesions. Lower row, older lesions.

In the late 1930's, Simpson and Weindling (19) developed a strain of cotton known as Stoneville 20 that is highly resistant to bacterial blight. Stoneville 20 is now being used as a source of resistance for establishing blight resistance in commercial varieties of Upland cotton in this country. This bulletin describes methods used in developing blight-resistant strains of cotton in Texas, and reports studies related to the breeding program.

REVIEW OF LITERATURE

It has been estimated (15) that bacterial blight causes an annual reduction in yield of Upland cotton in the United States of 1 to 2 percent, and from 5 to 15 percent in severe outbreaks in Egyptian and Sea Island varieties. Leyendecker (13) reported that an epidemic of the disease affected 40,000 acres in the Pecos Valley of New Mexico in 1949. The reduction in yield was estimated at 35 to 50 percent. Leyendecker (14) again reported the disease in epiphytotic form in the Pecos Valley of New Mexico in 1950. Knight (8) estimated yield losses of 9 to 64 percent at various locations in the Egyptian Sudan.

Stoughton (21) showed that *X. malvacearum* was most active at temperatures of 25 to 28°C. with the relative humidity at about 80 to 90 percent. However, disease symptoms occur under any condition favorable to the development of the host plant. Weindling (23) observed that the susceptibility of cotton to bacterial blight was affected by both the varietal reaction and the stage of development and the condition of leaves and plants.

He also recognized that moisture conditions play an important part in the development of the disease and showed that high humidity levels favor its activity.

Knight (9) pointed out that no evidence of the occurrence of biological races of the blight organism has been obtained in the Egyptian Sudan. Balasubrahmayan and Raghavan (1) stated that biological races existed in India. They reported that Indian races broke down the resistance of Knight's B_2 B_3 genotypes unless they were strengthened by the addition of major or minor genes from the acclimatized Indian Upland cottons.

Weindling (22) found that the results of seedling tests conducted by soaking seed in suspensions of bacteria, in general, conformed to those obtained in field tests by spraying plants for varietal reactions to the disease. Knight and Clouston (5) and Weindling (23) recognized that varieties of American Upland cotton vary in their reaction to bacterial blight. Deltapine and Stoneville lines have tolerant reactions while Acala lines are highly susceptible.

Knight and Clouston (5) developed a method of inoculating cotton plants with *X. malvacearum.* Inoculum was prepared by soaking 10 pounds of infected leaves in 40 gallons of water for 2 hours. After filtering, the inoculum was applied by using knapsack sprayers. Simpson and Weindling (19) and Weindling (23) used essentially the same inoculating method as Knight and Clouston. In preparing the inoculum, they found that better results were obtained by growing the causal organism in petri plates and diluting the growth of one plate with 2½ gallons of water. Their inoculation was most effective when the spray was directed toward the lower surface of the leaves and when the inoculation was made from mid-morning to noon, when the majority of the stomata were open.

Knight and Clouston (5) showed that leaf and stem resistance are positively correlated. Bird (2) reported that leaf and stem resistance, and leaf and boll resistance are positively correlated. Knight and Clouston (5) conducted genetic studies of resistance to the blackarm disease of cotton. A disease grading system was established that ranged from "0" to "12" with "0" representing immunity and "12" full susceptibility. They reported that two factors for resistance to blackarm were found in *G. hirsutum,* variety Uganda B31. These factors were designated B_1 and B_2. B_2 was completely dominant and B_1 was partially dominant. B_2 gave grades of 6 and 7 resistance, while the weaker B_1 gave grade 10. The two factors were additive and when combined gave grades of 5 and 6. It was shown that B_1 and B_2 imparted greater resistance to Uganda B31 than they did when transferred to *G. barbadense,* variety Sakel. This difference was attributed to the presence of modifying factors in Uganda 31. Knight (7) found another factor, which was designated B_3, in some *G. hirsutum,* variety *punctatum,* cottons. Factor B_3 was shown to be weaker than B_2 but partially dominant and additive with B_2.

Factor B_4 was found by Knight (10) in *G. arboreum*, race *bengalense*. This gene, when transferred to *G. barbadense*, segregated independently of B_1, B_2 and B_3 and showed an additive effect in conjunction with B_2 and B_3. In *G. arboreum*, B_4 conferred immunity or near immunity to bacterial blight, but when transferred to *G. barbadense*, it conferred only a high degree of resistance. This difference was attributed to a complex of minor and modifying genes in *G. arboreum*. Another factor, B_5, was found by Knight (11) in *G. barbadense*, variety Grenadines White Pollen. B_5 appeared to be partially dominant and additive in conjunction with B_1, B_2, B_3 and B_4, respectively. Limited studies indicated that B_5 was probably independent of the other four genes.

Knight (9) suggested that factor B_2 was the standard factor controlling resistance in American Upland cottons, and that the highest degree of resistance was obtained with B_2 in conjunction with complexes of minor and modifying genes.

No extensive genetic studies of Stoneville 20 resistance have been made. However. as a result of his breeding work with this line, Simpson (20) suggested that resistance was transmitted as a major gene and that resistance was recessive, susceptibility being dominant. He also pointed out that results of crossing indicated that other genes or modifiers were necessary for the full expression of resistance. Results obtained by Blank (3) support Simpson's hypothesis. Knight (12) reported that F_1's of a cross between Stoneville 20 and Sakel gave grades of 7 to 9 on his grading scale.

Knight (8) used the backcrossing system to transfer major genes for resistance to fully susceptible strains. Blank (3) found that the backcross method was suitable for transferring Stoneville 20 resistance to susceptible commercial types used in his breeding program.

MATERIALS AND METHODS

X. malvacearum was isolated from diseased material by the pour-plate and the streak methods of isolation. The authors found the streak method well adapted to the experiment and a higher percentage of blight isolates were obtained with it.

The organism grew vigorously on potato-carrot-dextrose agar. This medium was used for both culturing the organism and increasing it for preparing inoculum. Cultures of the organism remained viable at room temperature but had to be transferred to fresh medium every 20 to 25 days. Inoculum was prepared by repeatedly streaking the bacteria over the hardened medium in petri plates. After 5 to 7 days incubation at 24 to 28°C., the growth of one plate was diluted with $2\frac{1}{2}$ gallons of water for field inoculations and with one liter for greenhouse use.

The variability in pathogenicity of *X. malvacearum* was studied by using a seedling test similar to those described by Ray (16) and Weindling (22). Twenty isolates were compared

during 1949. These included two from Arizona, three from New Mexico, eight from Texas, three from Louisiana, and one each from Mississippi, Alabama, Georgia and South Carolina. Eleven isolates were compared during 1950. These included six from Texas, two from Arkansas, and one each from New Mexico, Mississippi and South Carolina. Four of these isolates were obtained from material collected in 1950. Separate seed lots of the variety of cotton used in a particular experiment were soaked for 4 hours in bacterial suspensions which were prepared from each isolate being studied. Necessary precautions were taken to prevent cross-contamination between the isolates.

The tests were conducted in the greenhouse using flats filled with coarse sand. The soaked seed lots for each isolate were planted in replicated, randomized, complete blocks at the rate of 25 seed per replication for each entry. For the first 3 days after planting, the sand flats were kept covered with heavy brown wrapping paper and after 3 days they were covered only during the night. The covering maintained a high relative humidity, thus favoring the development of the disease. At the end of 7 days, the cotyledons were graded for disease reaction, as follows: Grade 0, no infection; Grade 1, one or two small lesions less than one millimeter in diameter; Grade 2, more than two small lesions or one lesion larger than one millimeter in diameter; Grade 3, large lesions involving at least 50 percent of the cotyledon area; Grade 4, mass infection involving practically all the cotyledon area. A mean disease grade was obtained for each entry by dividing the sum of the disease grades by the number of seedlings in the entry. These data were studied by analysis of variance methods.

For studies of retention of pathogenicity by *X. malvacearum*, eight isolates that had been continuously cultured for 9 months were used. Each isolate had been transferred to fresh medium at intervals of approximately 25 days during the 9 months period. Seed lots were soaked in inoculum prepared from each isolate and planted in sand flats. When infection occurred on the young seedlings, isolations were made from each group. This gave a new isolate for each of the old ones. The pathogenicity of the new and old isolates was then compared, using the seedling testing technique previously described.

Spraying a bacterial suspension onto the lower surfaces of leaves was the most frequent method of inoculation used. With this method, the inoculum was forced through the open stomata into the substomatal cavity. Therefore, it was most effective when the majority of the stomata were open. For field inoculation a wheelbarrow sprayer (Figure 3), in which 125 to 150 pounds pressure was maintained, proved to be very effective. A hand sprayer was used in the greenhouse (Figure 4), in which carbon dioxide bulbs maintained approximately a pressure of 100 pounds. Best results were obtained with the wheelbarrow sprayer when the nozzle was held approximately 12 inches from the leaves, and the hand sprayer was most effective when the nozzle was held about 6 inches from the leaves.

Figure 3. Field inoculations being made with wheelbarrow sprayer. The bacterial suspension, under pressure, is directed against the under side of the leaves of young cotton plants.

An abrasion method of inoculation, developed by Brinkerhoff (4) was useful for greenhouse work. This method easily distinguished between resistant and susceptible types and could be used when the stomata were closed. It was most effective on cotyledons and true leaves. The inoculating bag was prepared by placing one teaspoon of coarse sand on a square piece of cheesecloth which had been doubled. The corners of the cheese cloth were then gathered and tied. The sandbag was dipped into a bacterial suspension and then rubbed over the surface of the plant part to be inoculated (Figure 5).

Several disease-grading methods were used. The grading system which seemed to be most adaptable to Texas conditions is illustrated in Figure 6. The grades range from 1 to 7, with 1 representing immunity and 7 full susceptibility. The disease grades were based entirely on leaf infection.

Simpson's Stoneville 20 strain of cotton, which is highly resistant to bacterial blight, was used as the source of resistance to be transferred to commercial varieties. The majority of Stoneville 20 plants had grade 1 resistance; however, an occasional grade 2 or 3 was observed.

The initial crosses were made in 1946 between Stoneville 20 and a number of adapted susceptible commercial varieties or advanced breeding strains. These included Stoneville 2B, Deltapine, Coker 100-7, Empire Wilt, CSS 3720, W 29-1, Stoneville 2B-85 and Stoneville 2B X Rogers Acala 4-128. All of these were carried through the third generation following the first backcross to the recurrent parent. At this stage, major emphasis was continued on the Stoneville, Empire and Deltapine lines, while the others received only minor attention or were discontinued.

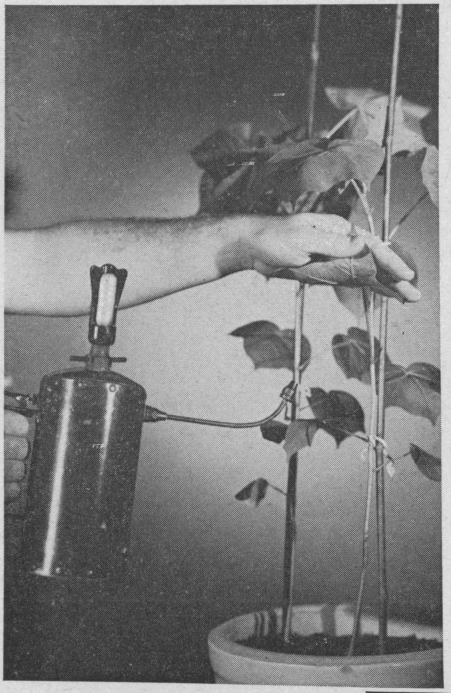

Figure 4

Hand sprayer being used to inoculate a greenhouse plant.

Figure 5
The abrasion method of inoculation being used on a greenhouse plant.

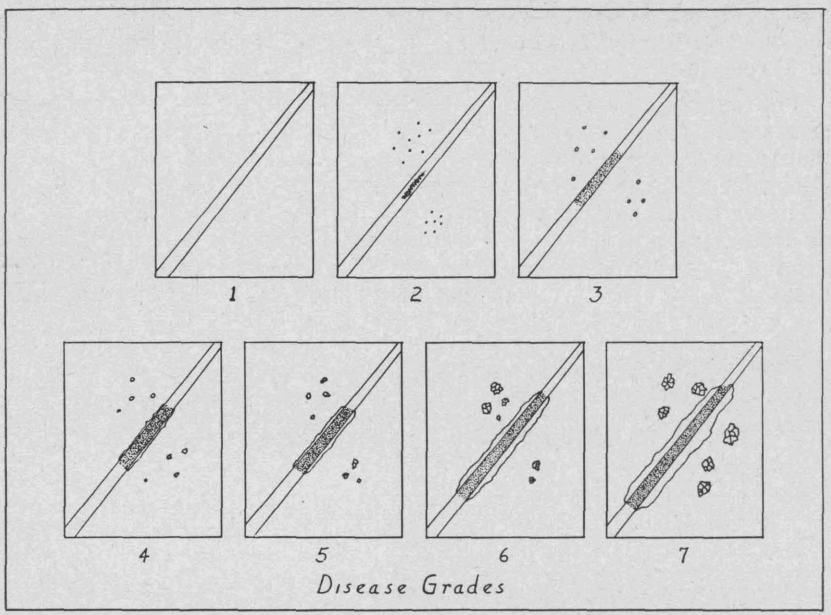

Figure 6. Stylized drawings of portion of leaf blades illustrating the varying degrees of symptom expression among plants in an F_2 population. Grading system. Grade (1) no infection; (2) lesions less than ½ mm, brown, rounded and raised, never angular, wet or coalesced. Brownish red marks on veins; (3) lesions about ¾ mm, rounded and wet, never angular or coalesced, sunken when dry. Veins occasionally show brown lesions but the infection does not spread to blade tissue; (4) lesions about 1 mm, rounded to slightly angular and wet, never coalesced. Veins have brownish black lesions which have spread about ½ mm into blade tissue; (5) lesions about 1½ mm, angular with occasional coalescing and wet, never rounded. Veins have black lesions which have spread about 1 mm into blade tissue; (6) lesions 2-3 mm, angular with frequent coalescing. Veins have black lesions which have spread about 2 mm into blade tissue; (7) lesions 3-5 mm, angular and always coalesced. Veins have black lesions which have spread about 3 mm into blade tissue.

A field test was conducted to ascertain the relationship in degree of tolerance to bacterial blight of three commercial varieties of cotton. Acala, Stoneville 2B and Deltapine were the commercial types used and Stoneville 20 was the known resistant entry. The design was randomized complete blocks replicated five times. Twenty plants of each entry were grown per replication, giving a total of 100 plants for each strain. The plants were inoculated three times during the first half of the growing season. Each plant in the test was given a disease grade 4 weeks after the last inoculation. These data were studied with the usual analysis of variance methods.

In another test, individual plants from 10 Deltapine and 9 Acala F_2 progenies were given disease grades. The F_2 progenies were from crosses of fully susceptible plants to resistant (with Stoneville 20 resistance) Deltapine and Acala plants (as shown in step 6, Figure 7). These data were used to study the distri-

bution of the disease grades within F_2 progenies and for fitting the observed genetic ratios to the expected ratio of 3 susceptible to 1 resistant.

The degree of tolerance found in commercial types was used as a guide for differentiating between resistant and susceptible plants in F_2 progenies from a cross with Stoneville 20. Plants with disease grade 1 were selected from crosses of Stoneville 20 with varieties that have an appreciable degree of tolerance, such as Deltapine and Stoneville. However, some plants with disease grades 2 and 3 were selected for future work. Varieties with little or no tolerance, such as Acala, had very few plants with

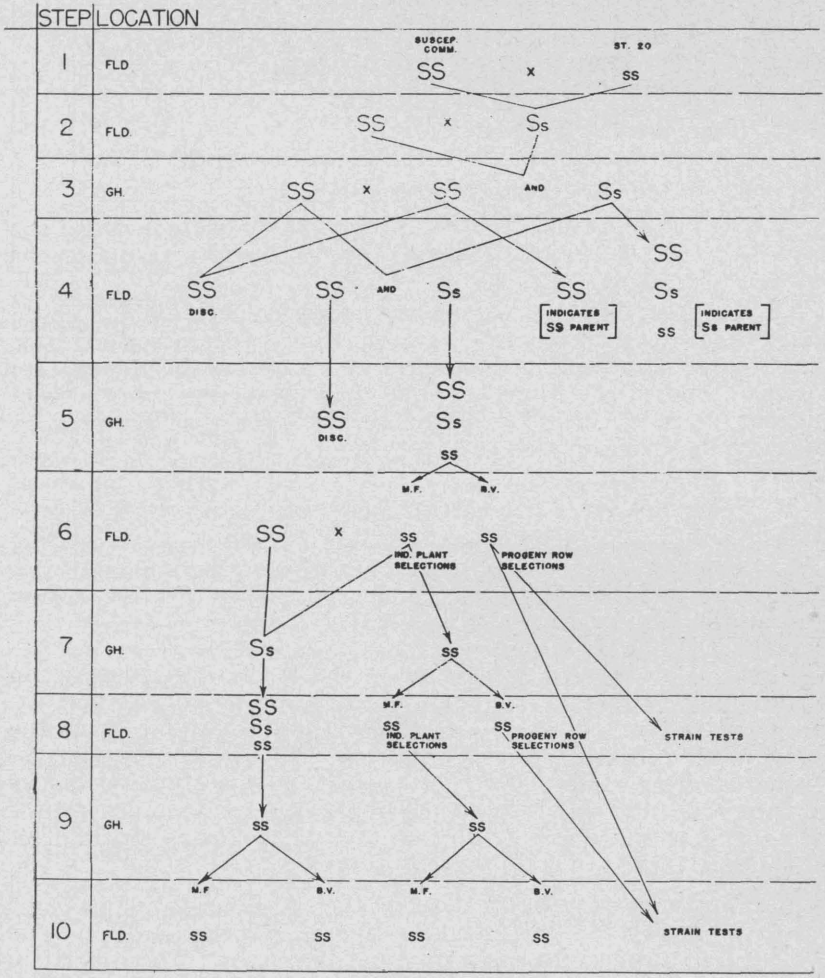

Figure 7. Diagram of successive steps in the breeding procedures. SS, susceptible commercial types or homozygous susceptible; ss, resistant types; Ss, heterozygous plants; M.F., Main Station Farm; B.V., Brazos River Valley Laboratory; Gh., greenhouse planting; Fld., field planting. For explanation, see text.

disease grade 1 in F_2 progenies. In such cases, plants with disease grades 2, 3 and even 4, were selected. As a rule, with highly tolerant types, F_2 plants with disease grades 1 to 3 were homozygous for the major resistant genes. With less tolerant types, it was found that F_2 plants with disease grades 1 to 4 were homozygous resistant.

The breeding methods used for transferring Stoneville 20 blight resistance to commercial types were based on the hypothesis that resistance was recessive and was transmitted as a single gene. For convenience, the symbols "SS" were used to designate homozygous-susceptible types and "ss" homozygous-resistant types. A diagram giving the various steps in the breeding program is shown in Figure 7. The plants were inoculated when it was necessary to distinguish between susceptible and resistant types. The various breeding steps were as follows:

1. Commercial types were crossed to Stoneville 20.
2. F_1's were backcrossed to commercials.
3. All plants of the backcross generation were again backcrossed to commercials. Plants of the first backcross generation were also selfed.
4. The second backcross generations were planted and, at the same time, selfed progenies from the first backcross generation plants were planted. The progenies which segregated for disease reaction indicated which of the first backcross plants were heterozygous. On this basis, the second backcross generations, which were from crosses to heterozygous plants, were kept and the rest were eliminated.
5. Progenies from the second backcross plants were planted and resistant plants of the segregation progenies were selected.
6. Progeny rows from each of the resistant plants were planted at both the Main Station Farm, College Station, Texas, and at the nearby Brazos River Valley Laboratory (conditions for about 90 to 95% selfing). The Brazos Valley planting was used to make progeny selections on the basis of resistance and agronomic characters. The Main Station Farm planting was used for individual plant selections and for making the third cross to the recurrent commercial types. Selections and the third backcross were made only in progenies which were outstanding, as shown by the sister progenies in the Brazos Valley planting.
7. F_1's of the third cross to the recurrent parents and plants from each selection were grown.
8. Plantings of progenies from the greenhouse plants were made at both the Main Station Farm and the Brazos River Valley Laboratory. Progeny row and individual plant selections were made by using the procedure outlined in step 6. F_2 progenies of the third cross to the recurrent parents were planted and resistant plant selections were made. A limited number of fourth crosses to the recurrent commercial types (not shown in the diagram) were made.

9. Plantings were made from resistant plant selections.

10. Plantings of progenies from greenhouse plants were again made at both the Main Station Farm and the Brazos River Valley Laboratory. Progeny row selections and individual plant selections were made again, as outlined in step 6.

Strain testing of the blight-resistant lines, as shown in step 8 of the breeding diagram (Figure 7), was initiated in 1950. The testing was conducted as an aid to selection and as a check on the progress being made in developing blight-resistant lines with agronomic characteristics equal to the parental types. The 1950 tests were conducted at the Brazos River Valley Laboratory near College Station, and at the U. S. Cotton Field Station at Greenville. The material included in the test represented the blight-resistant lines which had shown evidence of possessing the desirable characters of the recurrent parent. Appropriate commercial varieties or breeding strains were used for comparison. The strains were planted in single row, 50-foot plots. They were replicated three times in the Brazos Valley test and six times in the Greenville test. All plants in both tests received one spray inoculation during the early part of the growing season. The plants were observed during the remainder of the season for evidence of disease symptoms. A disease rating was obtained for each strain by determining the average number of infected plants. Boll size, lint percent and fiber properties for each entry in the two tests were based on 50-boll samples collected at the first picking.

RESULTS

The analysis of variance for the 1949 and 1950 tests pertaining to the variability in pathogenicity of isolates of *X. malvacearum* showed that differences between the mean disease grades for the isolates were not significant. The 1950 test also indicated that the isolates which were obtained from diseased material collected in 1949 and 1950 had the same degree of pathogenicity. There was no evidence of loss in virulence in cultures which had been cultured for 9 months, as compared with new reisolates of the same cultures.

Results showed that the inoculation methods were effective. The wheelbarrow sprayer proved to be very efficient in that two men operating one sprayer could inoculate one to two acres of cotton in 4 hours. The hand sprayer also proved to be a convenient method of inoculating large numbers of greenhouse plants. Results in the field showed that successful inoculation of all plants was obtained with spray inoculation if certain inoculating procedures were followed. These were: (1) inoculate only on clear sunny days; (2) inoculate only between 9:00 a.m. and 2:30 p.m.; and (3) direct the spray to the lower surface of the leaves. The results with spray inoculation were very similar to those resulting from natural infection. Therefore, the plants could be graded very accurately as to their disease reaction. Infection which resulted from spray inoculation of field plants is shown in Figure 8.

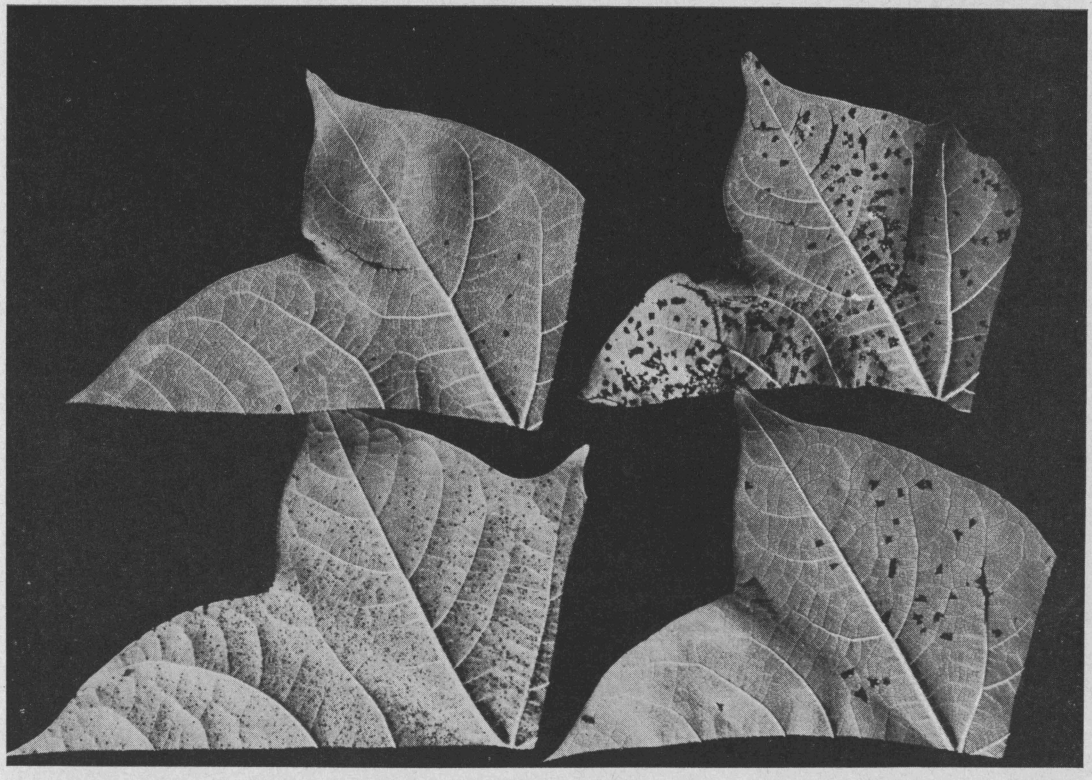

Figure 8. Infection which resulted from the spray method of inoculation. Lower left, infection corresponding to disease grade 2 as illustrated in Figure 6. Upper left, infection of disease grade 4. Lower right, infection of disease grade 5. Upper right, infection of disease grade 6.

The abrasion method of inoculation was found to be effective for distinguishing between resistant and susceptible plants. The abrasion method appeared to be more effective than spray inoculation of cotyledons of young seedlings. However, it was difficult to assign disease grades accurately to infection resulting from the abrasion method. Infection which resulted from the abrasion method of inoculating true leaves is shown in Figure 9.

In the grading of plants as to their disease reaction, it was observed that environmental factors, such as temperature and humidity, greatly influenced the expression of symptoms, as has been noted by other workers. If the disease grading was made too soon, a large number of grades were too low, suggesting a greater degree of tolerance than actually existed. In general, the proper time for grading was considered to be about 10 days after full symptoms appeared on known susceptible checks. In all cases, the disease grade was determined for a plant by the leaf which had the greatest degree of infection.

The means of the disease grades and the distribution of the grades for the varieties in the blight-tolerance comparison test are given in Table 1. The analysis of variance showed a highly significant difference in blight tolerance among entries. As expected, the mean disease grade for Stoneville 20, the known resistant entry, was very low. Three different levels of tolerance were present in the three susceptible varieties. The highest level was represented by Deltapine, the intermediate level by Stoneville 2B and the lowest level by Acala.

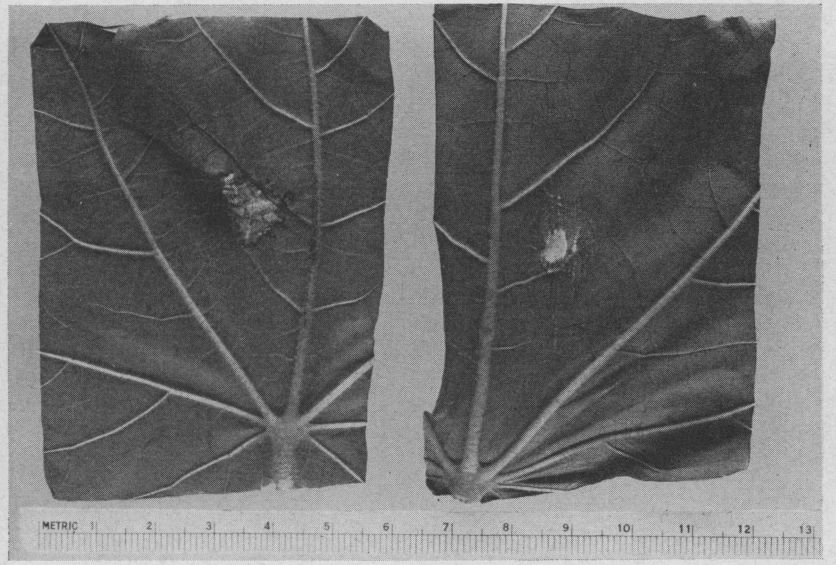

Figure 9. Infection which resulted from the abrasion method of inoculating true leaves. Left, fully susceptible reaction; right, highly resistant reaction.

Table 1. Variety means for the blight-tolerance comparison test and the distribution of the disease grades of individual plants in each variety

Variety	Disease grades							N	Mean of disease grades
	1	2	3	4	5	6	7		
Stoneville 20	94	2	4	..	..	..	..	100	1.11 ± 0.0447
Deltapine	..	..	..	15	56	26	3	100	5.17 ± 0.0711
Stoneville 2B	..	..	..	2	22	58	18	100	5.91 ± 0.1003
Acala	..	..	..	..	..	29	71	100	6.71 ± 0.0456

Additional information is presented in Table 2 on the distribution of the disease grades of individual plants and the means for the Deltapine and Acala F_2 progenies segregating for resistance. The averages showed that the Deltapine and Acala F_2 progenies differed in the average level of resistance. The mode of the disease grades for the Deltapine F_2's was grade 4 while for the Acala F_2's it was grade 6.

Table 2. Distribution and means of the disease grades of individual plants in Deltapine and Acala F_2 progenies

Strain	Disease grades							N	Mean of disease grades
	1	2	3	4	5	6	7		
Deltapine F_2's	36	13	35	122	103	74	11	394	4.292 ± 0.0751
Acala F_2's	18	6	6	54	55	126	93	358	5.436 ± 0.0814

Table 1 shows that the commercial Deltapine variety had the disease grade range of 4 to 7. On this basis, the F_2 progenies from crosses involving Deltapine were divided into susceptible (grades 4 to 7) and resistant (grades 1 to 3) groups, and the 10 F_2 progenies were tested by fitting the observed groups to the theoretical ratio of 3 susceptible to 1 resistant. The test is shown in Table 3. By the same assumption, the Acala F_2 progenies were divided into susceptible (grades 6 and 7) and resistant (grades 1 to 5) groups. However, when divided into these observed groups, the chi-square value for fitting the expected 3 to 1 ratio indicated a poor fit. The Acala F_2 progenies were then divided into susceptible (grades 5 to 7) and resistant (grades 1 to 4) groups, with the results presented in Table 4. The data in Tables 3 and 4 pertaining to Deltapine and Acala F_2 progenies, support the monohybrid hypothesis of the inheritance of Stoneville 20 blight resistance. The results strongly

Table 3. Total, pooled and heterogeneity chi-square values for testing the 10 Deltapine F_2 progenies to the expected ratio of 3 susceptible (grades 4 to 7) to 1 resistant (grades 1 to 3)

	D:F	X^2	P
Total	10	11.9997	0.2 — 0.3
Pooled[1]	1	2.4670	0.1 — 0.2
Heterogeneity	9	9.5327	0.3 — 0.5

[1]310 susceptible to 84 resistant plants.

Table 4. Total, pooled and heterogeneity chi-square values for testing the 9 Acala F_2 progenies to the expected ratio of 3 susceptible (grades 5 to 7) to 1 resistant (grades 1 to 4)

	D:F	X^2	P
Total	9	5.9477	0.7 — 0.8
Pooled[1]	1	0.4507	0.5 — 0.7
Heterogeneity	8	5.4970	0.7 — 0.8

[1]274 susceptible to 84 resistant plants.

indicate that resistance is recessive, susceptibility being dominant. The data from the F_2 distributions give curves which are continuous. This fact possibly indicates that minor gene action greatly influences the degree of resistance produced by the major genes.

The general procedure of the breeding program has been discussed in preceding pages and presented diagrammatically in figure 7. During the earliest stages of the study, major emphasis was placed on the selection of plants showing the desired degree of resistance to bacterial blight, with somewhat less emphasis being given to agronomic characteristics. It was evident that a satisfactory degree of blight resistance was expressed following one or two backcrosses to the recurrent parent. The field plantings that were made of progenies arising from selfing resistant F_2 plants, following the second backcross to the recurrent parents, were checked carefully for blight reaction and for agronomic characters. Fiber analysis was obtained on the progenies which appeared to fulfill the requirements as regards blight reaction and apparent yielding ability. Based on these several criteria, a number of the lines were chosen for inclusion in strain tests in 1950. These lines represented selections from material of which Stoneville, Empire and Deltapine were recurrent parents in the backcross program.

The lines selected for strain tests in 1950 are listed in Tables 5 and 6. Although all plants in the two tests were subjected to spray inoculation, only a mild to medium development of blight symptoms occurred. Apparently environmental conditions in 1950 were not favorable to secondary spread of the disease and most of the disease expression was limited to the angular leaf spot phase. Only a small amount of boll blight was observed, and blackarm, if present at all, was inconsequential in its effects. Disease ratings for the entries in the two strain tests are given in Tables 5 and 6. The disease ratings show that the inoculations were effective in initiating blight infection. However, as previously pointed out the infection failed to develop to any extent.

The blight-resistant lines were similar to the susceptible parental checks for plant type, uniformity and earliness. Yield data, boll size, lint percent and fiber data for the two tests are given in Tables 5 and 6. These tables show that several of the blight-resistant Stoneville lines are equal to the susceptible Stoneville checks for all agronomic characters considered. The

Empire lines are equal to the commercial Empire and, with the possible exception of lint percent and fiber strength, they are also equal to the susceptible Empire breeding lines 8-0-8 and 2-1-2. Several of the resistant Deltapine lines, with the exception of yielding ability, are equal to the susceptible Deltapine checks. As pointed out, only mild blight infection developed in the test areas during 1950. Therefore, it is possible that, under conditions of severe blight infection, the performance of the resistant lines would have been more outstanding, especially in regard to yield. The performance of these blight-resistant lines will be checked in similar tests in 1951.

Table 5. Agronomic data on bacterial blight-resistant strains of cotton grown at the U. S. Cotton Field Station, Greenville, 1950[1]

Strains[2]	Test rank	Yield lint, lbs. per acre[3]	Disease rating[4]	Boll size[5]	Lint, percent	Length U.H.M. (inches)	Fiber strength[6]	Fineness mm^2/mm^3
226-4-4	1	620	1.5	54	40.5	1.11	6.33	412
Empire 2-1-2	2	587	80.0	59	41.3	1.10	7.46	391
226-4-5	3	585	1.0	53	38.5	1.11	7.05	389
226-4-2	4	581	6.5	63	39.3	1.06	6.79	440
226-6-5	5	562	2.0	62	40.5	1.08	7.08	424
Empire 8-0-8	6	559	82.0	63	41.2	1.08	6.71	391
226-6-6	7	558	6.5	61	39.7	1.10	6.86	420
131-1-5	8	551	2.5	68	36.7	1.13	6.20	434
212-1-1	9	524	3.0	57	39.7	1.12	6.82	391
131-3-2	10	513	2.0	67	37.5	1.12	6.06	416
Deltapine (Miss.)	11	496	82.5	76	43.3	1.14	6.59	380
76-2-3	12	496	18.0	61	39.4	1.10	6.90	399
76-5-3	13	493	6.0	63	37.5	1.15	6.94	439
B-23	14	466	0.0	76	38.3	1.18	7.29	410
Deltapine (TPSA)	15	459	79.5	71	41.7	1.15	6.62	379
131-2-1	16	443	0.5	65	39.3	1.07	6.51	407
76-2-2	17	438	3.0	67	39.5	1.06	6.72	408
79-6-6	18	437	5.0	71	38.8	1.12	6.85	414
131-1-4	19	434	5.5	63	38.3	1.02	6.95	403
M-22y	20	422	6.5	62	38.5	1.10	7.14	492
342-3-4	21	414	2.5	74	41.4	1.19	6.90	367
M-12y	23	395	0.0	66	37.3	1.06	6.97	400
B-3	24	394	1.5	76	37.8	1.09	6.69	444
Stoneville 2B (Miss.)	25	374	78.5	62	39.1	1.11	6.51	432
M-20y	26	359	3.0	63	36.8	1.10	6.90	423
Stoneville 2B 39	27	356	77.5	71	38.9	1.16	6.71	369
342-5-2	28	350	2.5	74	40.7	1.15	6.74	359
Texas BR St. No. 1	29	341	8.0	62	37.3	1.08	6.80	383
M-18y	30	341	4.0	64	39.4	1.10	6.87	417
342-3-2	31	330	0.5	73	43.9	1.12	6.47	350
342-5-4	32	326	2.0	78	41.4	1.13	6.56	372
342-5-5	33	318	1.5	78	41.4	1.11	6.73	380
M-17y	35	300	0.0	64	37.0	1.13	6.71	397
342-5-3	36	281	2.0	81	40.4	1.15	6.61	439

[1]There were 36 entries in the test; however, these data are only for blight-resistant strains that were developed at College Station, plus appropriate checks. The test was conducted jointly with the U. S. Cotton Field Station, Greenville, and the Cotton Genetics Section, College Station.

[2]Strain designations:

Blight-resistant types;
Stoneville—B—, M—, 76—, 79—, 131—, and Tex. BR St. No. 1.
Empire—212— and 226—.
Deltapine—342—.

Susceptible checks;
Stoneville—Stoneville 2B (Miss.) and Stoneville 2B 39.
Empire—Empire 8-0-8 and Empire 2-1-2.
Deltapine—Deltapine (Miss.) and Deltapine (TPSA).

[3]Differences required for significance: odds 19 to 1, 137 pounds; odds 99 to 1, 180 pounds.

[4]Expressed as the mean number of infected plants.

[5]Expressed as the number of bolls necessary to produce one pound of seed cotton.

[6]Pressley index.

Table 6. Agronomic data for bacterial blight-resistant strains of cotton grown at the Brazos River Valley Laboratory, 1950[1]

Strains[2]	Test rank	Yield lint, lbs. per acre[3]	Disease rating[4]	Boll size[5]	Lint, percent	Length U.H.M. (inches)	Fiber strength[6]	Fineness mm^2/mm^3
Deltapine (Miss.)	1	935	66.0	78	42.9	1.15	6.42	388
226-4-4	2	876	7.0	63	38.4	1.11	6.87	441
M-12y	3	843	4.7	67	34.6	1.09	7.04	432
342-3-2	4	819	2.0	75	43.8	1.14	6.54	367
131-2-1	5	805	2.7	61	37.4	1.12	7.43	430
226-4-2	6	796	5.7	58	35.8	1.21	7.25	463
M-22y	7	795	0.7	65	36.0	1.15	7.14	441
226-4-5	9	777	7.3	63	38.7	1.18	7.02	575
226-6-6	10	775	1.7	60	37.7	1.12	7.19	446
76-5-3	11	772	7.0	77	37.4	1.08	6.97	442
Texas BR St. No. 1	12	756	2.0	67	36.3	1.04	7.37	430
Empire (Comm.)	13	756	64.3	60	37.7	1.12	7.19	446
B-23	14	755	3.0	70	35.7	1.13	7.32	448
Stoneville 2B (Miss.)	16	743	65.0	68	37.6	1.08	7.53	463
79-6-6	17	728	9.0	77	35.3	1.10	7.07	465
342-3-4	18	726	1.7	73	41.2	1.16	6.82	384
212-1-1	19	721	5.0	67	37.4	1.11	7.29	405
342-5-5	20	720	0.0	77	39.6	1.07	7.10	363
131-1-4	21	717	4.0	73	36.5	1.06	7.08	429
M-20y	22	704	3.7	77	37.8	1.11	7.33	442
342-5-2	23	686	2.3	73	37.5	1.11	6.46	456
131-1-5	24	683	2.0	69	36.5	1.11	6.55	413
B-3	26	661	1.7	79	37.6	1.15	6.44	429
342-5-3	27	660	1.3	78	39.3	1.11	6.75	406
131-3-2	29	648	3.0	73	36.5	1.10	6.63	434
M-18y	30	646	4.0	73	37.6	1.10	6.85	432
M-17y	32	642	1.0	63	35.2	1.11	7.15	407
342-5-4	33	628	2.7	69	39.7	1.14	6.76	411
76-2-3	35	610	16.0	66	36.5	1.12	7.39	423
Stoneville 2B 39	36	590	63.3	77	34.9	1.11	6.76	442

[1]There were 36 entries in the test; however, these data are only for blight-resistant strains that were developed at College Station, plus appropriate checks.
[2]Strain designations:
Blight-resistant types;
Stoneville— B—, M—, 76—, 79—, 131— and Tex. BR St. No. 1.
Empire— 212— and 226—.
Deltapine— 342—.
Susceptible checks;
Stoneville—Stoneville 2B (Miss.) and Stoneville 2B 39.
Empire—Empire (Comm.). Empire 8-0-8 and Empire 2-1-2.
Deltapine—Deltapine (Miss.)
[3]The test was composed of three replications. Differences were not significant.
[4]Expressed as the mean number of infected plants.
[5]Expressed as the number of bolls necessary to produce one pound of seed cotton.
[6]Pressley index.

DISCUSSION AND CONCLUSIONS

In any program of breeding for resistance to a plant pathogen, the possibility of the existence of races or strains of the parasite must be considered, and in many instances the apparent success of a program is threatened by the appearance of new strains of the causal organism of the particular disease. Therefore, a number of isolates of *X. malvacearum* were examined to determine the extent of variability in pathogenicity. It was found that the isolates had the same degree of pathogenicity and were not capable of breaking down Stoneville 20 resistance.

It has been reported that strains of the blight organism in India were capable of breaking down resistant lines developed in the Egyptian Sudan, and that such breeding material was strengthened as regards resistance by the addition of major or minor genes from the acclimatized Indian Upland cottons. In the present study, resistance to bacterial blight was transferred from Stoneville 20 to varieties of cotton which were

adapted to Texas conditions, and it has been demonstrated that these adapted varieties have various levels of minor genes for blight tolerance in their genetic makeup. This fact may forestall or prevent any breakdown of Stoneville 20 resistance.

The fact that the pathogenicity of *X. malvacearum* did not decrease when cultured on potato-carrot-dextrose agar for 9 months had one important aspect to the breeding program. It meant that isolates of the causal organism, which were obtained from diseased material collected over the Cotton Belt and cultured during the winter, could be used for preparing inoculum the following spring. By following this practice each year, the breeding material was exposed to pathogenic isolates of the bacteria from over the Cotton Belt.

The effective inoculations methods (19) made it possible to select for resistance with a high degree of certainty and contributed largely to the rapid progress of the breeding program. The efficiency of the wheelbarrow sprayer for field inoculations made it possible to work with large numbers of plants.

It was demonstrated that commercial Deltapine, Stoneville 2B and Acala varieties had different levels of tolerance. Knight (9, 12) and Simpson (20) have assumed that the observed differences in blight tolerance in commercial varieties were due to minor genes. It is concluded that Deltapine had the highest level of minor genes, Stoneville 2B had about half the Deltapine level and Acala had a very low level.

The grading of Deltapine and of Acala F_2 progenies showed that the total level of resistance in the Deltapine progenies was significantly higher than the level in the Acala progenies despite the fact that they had, proportionately, the same complement of major genes for blight resistance. It follows that the difference in the level of resistance was probably due to minor genes inasmuch as the difference corresponds to the different levels of minor genes in the commercial types.

The experimental results indicated that one pair of major genes transmits Stoneville 20 blight resistance. The grouping of the resistant and susceptible disease grades for the Deltapine progenies was justifiable on the basis of the grading of the commercial type. The grouping of the Acala progenies was questionable, except for the fact that, as a result of the cross to Stoneville 20, both minor and major genes for blight resistance were transferred. The presence of the minor genes would have caused the susceptible group to extend to grade 5. The Acala F_2's, with a low level of minor genes, would have given a more accurate indication of the segregation of the major gene for resistance.

The fact that the larger group of Deltapine F_2 plants tended to be intermediate suggested that the heterozygous plants had an intermediate disease grade. Since the difference in the Deltapine and Acala F_2's was due to minor genes, the action of a major gene with the minor genes must have caused the heterozygous Deltapine plants to be intermediate. The results obtained

did not indicate whether the gene action between the major and minor genes was additive or an interaction. Bird (2) showed that heterozygous plants of a F_2 progeny from a cross of Stoneville 2B with Stoneville 20 tended to be intermediate on a disease grading scale and that the heterozygous plants could be selected with a high degree of accuracy. Knight (12) reported that heterozygous plants from a cross of Stoneville 20 X Sakel gave grades 7 to 9 with his grading method. It follows that, with the Acala F_2 distribution shown in Table 2, a large number of the plants with grade 6 were probably heterozygous for the major genes for resistance.

If the minor genes caused heterozygous plants to have higher tolerance, then high levels of minor genes in homozygous resistant plants would produce a higher degree of resistance than homozygous plants with low levels of minor genes. This action would have caused the range in the degree of resistance within the groups that were homozygous for the major genes.

The ability to distinguish between heterozygous and fully susceptible plants suggests a modification of the breeding methods shown in Figure 7 which would be of value for future work. In step 3 the fully susceptible plants would be removed from the first backcross generation and then the second backcross made onto the remaining plants which would be largely heterozygous. This would reduce the number of backcrosses to be made and at the same time eliminate the necessity of planting progenies to determine the heterozygous plants, as shown in step 4.

Deltapine, Stoneville 2B and Empire Wilt lines with a high degree of blight resistance have been obtained after two backcrosses to the recurrent commercial parents. Acala lines with blight resistance were obtained, although it was not as high as that obtained in the Deltapine, Stoneville 2B and Empire Wilt lines.

Theoretically, each time resistant Acala plants were backcrossed to the commercial parent, the minor gene component from Stoneville 20 was reduced by one-half. This fact, along with the results obtained, possibly suggests the necessity of continued selection, within lines homozygous for the major resistant genes to obtain lines highly resistant to bacterial blight.

The two strain tests of 1950 indicated that considerable progress has been made in developing blight-resistant strains of cotton which are equal to the susceptible variety types for yielding ability, boll size, lint percent and fiber qualities. The blight-resistant strains that were tested in 1950 were developed after two backcrosses to the susceptible parents. As shown in the breeding diagram (Figure 7), the third backcross to the recurrent parents was made in 1949. With the progress that was made after two backcrosses, it is highly possible that the third backcross material will give highly resistant lines which are

equal to the susceptible types for all agronomic characters. Such varieties should go far towards eliminating the losses resulting from bacterial blight.

SUMMARY

Isolates of *X. malvacearum* from diseased material collected from various locations over the Cotton Belt showed no differences in pathogenicity.

Cultures which had been maintained on potato-carrot-dextrose agar for 9 months were similar in pathogenicity to newly isolated cultures.

Blight tolerance in commercial varieties is influenced by different levels of minor genes for resistance within these varieties. Deltapine has a high level of minor genes, Stoneville 2B somewhat less and Acala a very low level.

The monohybrid ratios may be obscured by the segregation of minor genes. The data strongly indicate that resistance is recessive, with susceptibility being dominant.

The genetic data indicated that heterozygous plants could be distinguished from fully susceptible ones. The data also suggest that minor genes greatly influence the degree of resistance produced by major genes. Whether the action is an additive effect or an interaction between major and minor genes, was not determined.

A modified backcrossing method for transferring Stoneville 20 blight resistance to commercial varieties of cotton is presented.

It was suggested that, by distinguishing between heterozygous and fully susceptible plants, the straight backcross method of breeding could be used instead of the modified method that was employed.

To develop highly resistant strains of cotton it appears to be necessary to combine major genes with high levels of minor genes.

Tests of blight-resistant strains derived after two backcrosses to the recurrent parents showed that the strains were comparable with commercial varieties for many agronomic characters. No association has been observed between blight resistance and either deleterious yield factors or poor fiber quality.

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