Variability of Xanthomonas Malvacearum:

The Cotton Bacterial Blight Pathogen

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Variability of Xanthomonas Malvacearum, The Cotton Bacterial Blight Pathogen

By L. A. Brinkerhoff*

This bulletin reports results of a study to determine the prevalence of pathogenic races of *Xanthomonas malvacearum* (E.F.Sm.) Dowson and to learn more about their nature, origin and persistence.

Bacterial blight is particularly destructive to the long-stapled high-quality tetraploid cottons of *Gossypium barbadense L.* It also attacks the more widely grown tetraploid, *G. hirsutum L.* Commercial varieties of the latter differ in degree of resistance to the disease. The cultivated diploid Asiatic cottons, *G. herbaceaum L.* and *G. arboreum L.* are mostly resistant or immune.

The development of resistant varieties apparently offers the most economical means of controlling bacterial blight of cotton. In the Republic of Sudan where the disease is extremely destructive, British investigators have developed resistant varieties of *G. barbadense* that are now being grown commercially, and in the United States blight-resistant varieties of American upland (*G. hirsutum*) have been released to farmers in recent years in New Mexico, Arkansas, and Texas. Additional resistant varieties will soon be released in other parts of the United States and in other countries.

Once desirable agronomic characters are obtained, the success or failure of a resistant variety will depend upon its ability to withstand new races of the blight bacterium, *X. malvacearum.* The resistance that has been utilized thus far in cotton appears to be principally of the hypersensitive type comparable to the resistance in the cereal crops to the rust pathogens or in potatoes and flax to the late-blight and flax-rust fungi respectively. During the past quarter to half century a whole series of resistant varieties of cereals, flax and potatoes have been attacked by new races of the various disease organisms. It remains to be seen whether or not new races will pose an equal threat to the future cultivation of bacterial blight resistant cottons.

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REVIEW OF LITERATURE

Compared to the fungi, few critical investigations have been devoted to pathogenic variability within bacterial plant pathogens. The present status of the genetics of these bacteria is discussed in the text by Stakman and Harrar (63) and in a review of bacteria as plant pathogens by Starr (64).

Pathogenicity has been considered as a relatively stable character and has for many years served as a practical criterion for differentiating species, especially in the genera, Xanthomonas and Pseudomonas, which include a majority of the plant pathogens (10, 12, and 66). Starr (64), however, has emphasized that pathogenicity has been misused as a criterion for describing new species, and that in many instances it has not been investigated carefully enough.

The genus Xanthomonas has been described by Burkholder and Starr (12) as comprising plant pathogens that produce abundant slimy yellow growth on sugar-containing media; and as being aerobic, gram negative, non spore-forming rods with a single polar flagellum. They further characterized the genus as being able to produce certain specific biochemical reactions in different media; however, few of the species could be identified by biochemical reactions. Wernham (66), using three different methods of inoculation, concluded that the 17 species with which he worked were "remarkably specific" in their pathogenic reactions. *X. malvacearum* was included in his studies and attacked only cotton of 16 taxonomically distinct plants.

Variability in pathogenecity, however, has been recognized in several species of Xanthomonas. Smith et al. (61) in 1919, Reddy et al. (55) in 1924, Bamberg (4) in 1936, Hagborg (29) in 1942 and Wallin (65) in 1946 have shown that pathogenic races exist within *X. translucens* (L.R.J., A.G.J. and Reddy) Dows., a pathogen of cereals and bromegrass. The host range of the races are somewhat similar to those of *Puccinia graminis* Pers., the stem rust fungus and include wheat, barley, oats, rye and bromegrass. Wallin (65) distinguished six races by the differential reactions of different varieties of oats and bromegrass.

The destruction of a previously resistant variety of sugar cane in the Reunion Island (1) appears to be due to a new race of *X. vaculorum* (Cobb) Dows. Dye (24) reported that beans were attacked by a number of different species of Xanthomonas including *X. malvacearum*

after four successive passages through very young bean leaves. In a more recent investigation Logan (46) was unable to "adapt" *X. malvacearum* to bean or to break down resistance of a blight-resistant cotton, nor did *X. phaseoli* var. *fuscans* (Burkh.) Starr & Burkh. attack cotton.

That *X. malvacearum* is variable in pathogenicity has become increasingly evident as resistant strains and varieties of cotton have been developed and tested. When resistant strains of cotton, developed by Knight in the Sudan, were tested in Madras, India, between 1945 and 1947 Balasubrahmanyan and Raghaven (3) reported that resistance broke down and suggested that different races of the bacterium were responsible.

Manning (50), in a progress report for 1946-47 from Uganda, reported that one of Knight's resistant strains was severely attacked.

Hunter and Blank (32) described a new race of *X. malvacearum* in New Mexico in 1954. This race (now known as race 2) is widespread in New Mexico and Texas where resistant varieties have been grown (14).

Dark (18) and Rose (56) expressed concern over the breakdown of their blight resistant cottons in the Sudan in 1956-57. There seems to be a question as to whether unusual environmental conditions or a new race of the bacterium was responsible.

Simpson, working in Indonesia in 1958, distinguished isolates of *X. malvacearum* that differed from races 1 and 2 of the United States.[1]

More recently Schnathorst et al. (57) in California and Brodie and Cooper (11) in North Carolina reported only race 1 among isolates that were tested; however, in both instances only three upland differentials were used.

MATERIALS AND METHODS

Differential Lines

An attempt was made to use inbred lines of cotton with single disease-conditioning genes as advocated by Flor (25) for race studies with the flax rust fungus.

[1] Personal communication with J. T. Presley.

The source and the genetic make up of the various host differentials in so far as known are given in Table 1. Relationships of the blight-conditioning genes of upland and *G. barbadense* lines were not known, except for Knight's B_7, when these studies were initiated. The resistance of CR4 and 4-11 was not definitely known, but inheritance studies indicated a single major gene tentatively designated as B_L.[2] Inheritance studies (27) indicated that the upland line 8-3 possessed either the b_7 gene, or possibly an allele.

Seed of the differential lines were increased in isolation blocks and by selfing in nursery plots.

Isolation and Maintenance of Cultures

Nutrient agar prepared from 3 g beef extract, 5 g peptone, 17 g Difco-bacto agar per liter of distilled water and buffered to approximately pH_7 after the medium cooled proved to be an excellent medium for isolating single colonies of *X. malvacearum*. The bacterium was relatively short-lived on this medium and produced little slime.

Colonies failed to develop on dilution plates when the pH was much below seven, but mass transfers grew at pH 6 and somewhat below. The addition of dextrose greatly enhanced the production of slime; however, because sugar increased the growth of contaminants, it was omitted for isolations, while 2% dextrose, 5% K_2HPO_4 (of a 0.1 M solution) and 1% $CaCO_3$ were added for maintaining the cultures. Potato-dextrose agar buffered with $CaCO_3$ and $MgSO_4$ was also used as a medium for maintaining cultures. Cultures were transferred about every 4 to 8 weeks.

Cultures were also maintained by storing infested dry leaves at 50°F. Although the number of viable cells decreased, some usually remained viable for two or more years.

Single colonies of *X. malvacearum* were readily isolated as follows: Approximately 1/8-inch squares of diseased tissue from either fresh leaves or bolls were crushed in 10 ml of sterile tap water and placed in small 18 ml (perfume) bottles with plastic caps. Dilutions of 10^{-5} and 10^{-6} were then made with large bore 1-ml sterile pipettes by successive transfers. One-half ml of each of the final two dilutions was spread over the surface of nutrient agar in petri dishes with absorbent paper discs inside of either glass covers or special aluminum covers. The paper discs absorbed enough moisture to prevent condensation and

[2] Unpublished studies of J. M. Green and L. A. Brinkerhoff.

smearing of the surface, while storage of the plates inside of sterile plastic bags prevented excessive drying. Single colonies which developed from the dilute suspensions appeared to have originated from single cells. Microscopic examination of dilution plates prepared as outlined above from infected tissue and also from broth cultures of *X. malvacearum* showed the bacteria to be distributed singly rather than in clumps.

Single colonies were also isolated by the surface-flooding method from dried ooze and dried plant materials; lower dilutions were used, however. Fungal and bacterial contaminants which inhibited or overgrew *X. malvacearum* were more frequent from dry material and also from green tissue that had stood for several days. When contaminants were encountered, portions of the original samples were crushed in sterile mortars with a small amount of water and the bacterial suspension was used as inoculum for seedlings. Isolations were then made from fresh tissue as soon as lesions developed.

Single colonies were readily obtained from pure cultures by spreading concentrated inoculum over the surface of a plate of medium with a small wire loop, or with a tapered glass rod. The critical consideration was to eliminate most of the cells with the first two or three strokes. This technique was also used with bacterial suspensions from infected plant material, but was less reliable than dilutions from pipettes.

Preparation and Use of Inoculum

Cultures were grown in nutrient broth or nutrient broth supplemented with 2% dextrose on an Eberbach shaker for 24 to 48 hours at room temperature (about 80°F).

For field inoculum, the cultures were diluted 1:200 to 1:500 or to approximately 10^5 to 10^6 cells per ml. For seedling inoculations, cultures were used without diluting or with dilutions of not more than 1:100. Dilution tests with seedlings showed the time for disease expression and the amount of necrosis of resistant varieties to be dependent on the concentration of inoculum, and that concentrated inoculum produced more uniform infections (see Fig. 1). Innes and Last (34) have recently shown that lesion size in the field decreases as the inoculum is diluted. Inoculum for field tests was also prepared by thoroughly mincing fresh diseased leaves in a food blender with just enough water to cover the leaves. Bacteria were separated from the pulp by straining the leaf suspensions through several layers of cheesecloth, rinsing and squeezing the pulp dry. Dilutions of 1:100 of this inoculum produced infection about comparable to the 1:200 dilutions of shake cultures.

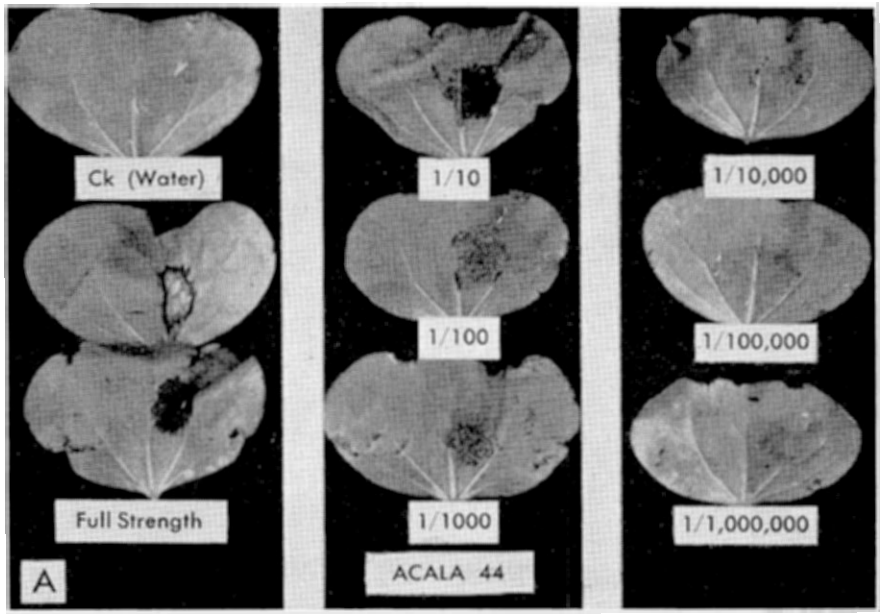
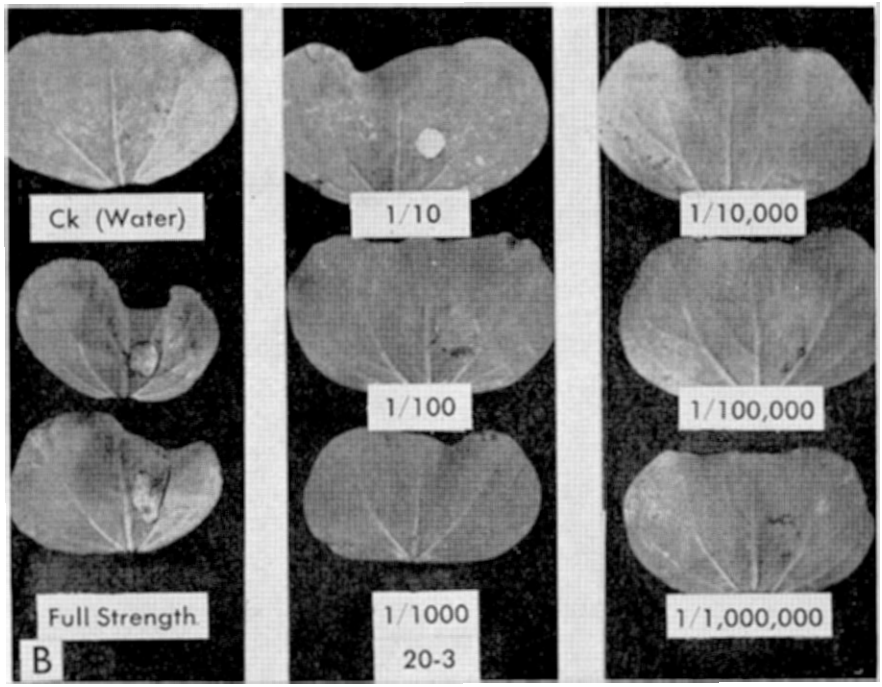


Fig. 1. Disease reactions after 14 days to different dilutions of a nutrient broth shake-culture of race 1. (A) Acala 44 is susceptible, and (B) 20-3 resistant. The central area of each cotyledon was watersoaked by injection with syringes with short rubber-tube tips.



Growing Plants for Inoculation

More uniform symptoms occurred on succulent, vigorous plants. Field plantings were inoculated when the plants had about four true leaves. Seedlings were inoculated immediately after the cotyledons had expanded, and bolls were inoculated when about ½ to ¾ grown.

Seedling tests were first made in the greenhouse at temperatures ranging from 70° to 95°F, or above. Later, results were more uniform in a temperature controlled room under fluorescent light at temperatures ranging from about 75° to 85°F. The light was left on continuously and measured from 300 to 400 ft-c illumination. In early tests 10 seedlings of each differential were grown in four-inch pots in steamed soil. Later the number was reduced to five seedlings per pot.

Inoculation Techniques

Plants in the field were inoculated by visibly water-soaking portions of the leaves with bacteria suspended in water. The inoculum was applied with single-nozzle guns from a power sprayer operated at about 400 psi (9). The spray stream was adjusted to provide visible water-soaking of the leaves without serious mechanical injury. The adjustment of the spray stream was more critical for successful water-soaking than was the pressure; a coarse spray water-soaked more readily when the stomata were open. Disease symptoms were usually visible within seven to 14 days, and appeared as single lesions, except on leaves which had received more than the usual amount of inoculum.

The following techniques were used to inoculate seedlings:

1) Small cheesecloth bags (half the size of tea bags) were filled with coarse sand, dipped in a concentrated bacterial suspension and rubbed on the lower surface of the cotyledon causing visible water-soaked scratches in the epidermis. The disease symptoms were visible five to 21 days after inoculation depending upon the initial concentration of the inoculum and the environment. Lesions appeared on susceptible cotyledons along the wounds and spread later to the tissue in between. The inoculated tissue of resistant varieties became necrotic but usually only along the wounds.

2) Five-ml-glass syringes were used to inject inoculum through the stomata or through wounds in cotyledons. The needles were replaced with short rubber tubes held in place by rubber bands. Small wounds permitted the cotyledons to water-soak readily when the stomata were not open. Symptoms were visible within four to 21 days depending upon the concentration of the inoculum and the environment.

3) Seedlings were submerged by placing plants in pots upside down over containers of inoculum in a vacuum chamber (8). The soil was held in the pot with paper towels placed under a wire frame. When a partial vacuum was released suddenly the cotyledons were completely water-soaked, provided the stomata were open. The inoculum was diluted, as concentrated inoculum caused necrosis and shedding of cotyledons. Single lesions (rather than general water-soaking) were obtained from bacterial suspensions from ½ g of dry leaves diluted in 500 ml of water. Resistant type reactions were not observed at this dilution. However, with broth cultures general necrosis and shedding of cotyledons occurred with dilutions of 1:500.

Classification of Disease Reactions

Disease reactions in the field were classified on the basis of grades devised in earlier studies in Oklahoma (9). Grades were based on five infection types: 0, 1, 2, 3, and 4, which were dependent upon the presence or absence, relative size, and appearance of individual lesions on the leaf blade (see Fig. 2A). As in previous studies (9) mixed or mesothetic infection types were encountered (see Fig. 2B), and were taken into consideration in classifying disease reactions. Descriptions of the various grades and infection types are given in Table 2.

Cotyledons, leaves, and bolls inoculated with concentrated inoculum by wounding or injection were classified on a somewhat similar scale which is summarized in Table 3. Lesion size is not a criterion for this scale as concentrated inoculum usually does not produce individual lesions.

Additional explanations of materials and methods are given with specific experiments.

EXPERIMENTAL RESULTS

Surveys for Different Races of X. Malvacearum

Two races of *X. malvacearum* were recognized when the present studies were started in the summer of 1955. Races 1 and 2 had been designated by a committee of the Cotton Disease Council (59) in February of 1955 on the basis of reactions of three upland differentials —Stoneville 2B (susceptible, i.e. no known major blight-conditioning genes), Stoneville 20 (with b_7 resistance) and Mebane B1 (resistant, but inheritance not determined). Race 1 attacked Stoneville 2B, and Race 2, Stoneville 2B and Stoneville 20; whereas, neither race attacked Mebane B1.

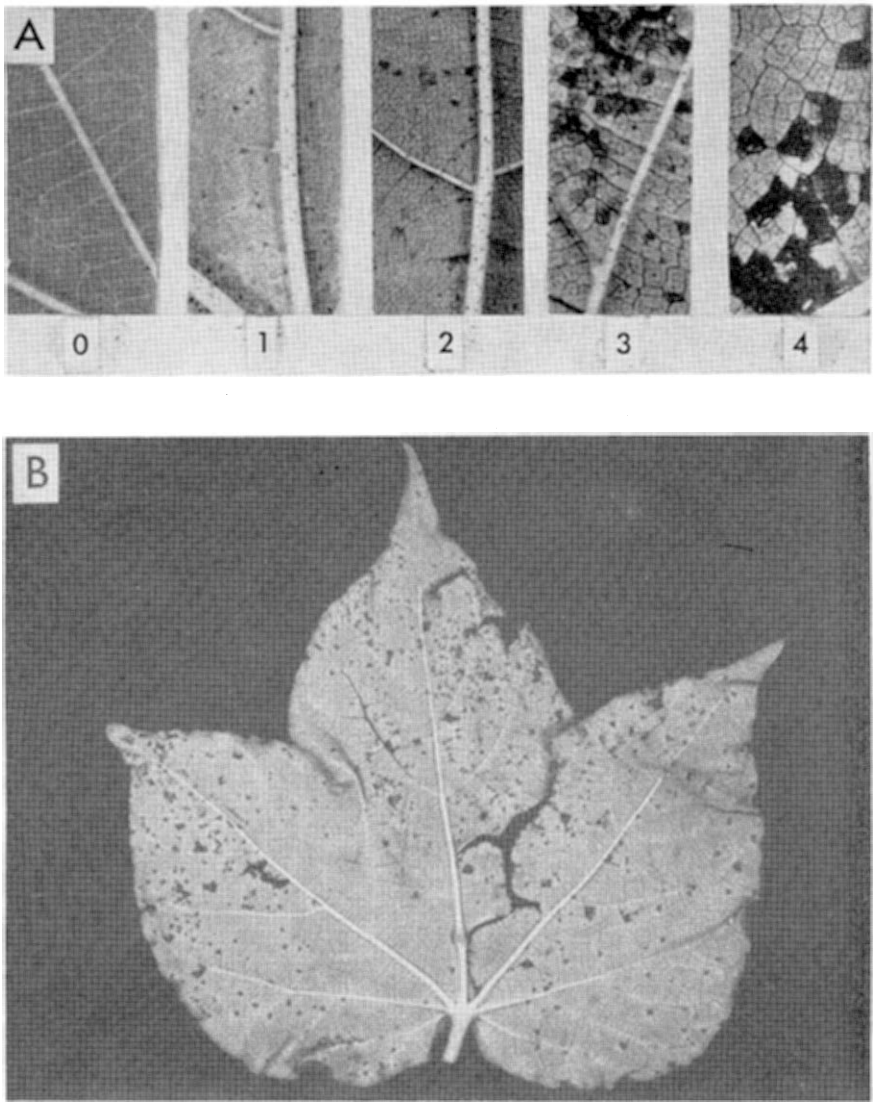


Fig. 2. Infection types representing field grades: (A) 0 = immune, 1 and 2 = resistant, 3 and 4 = susceptible. (B) Mesothetic or mixed reaction representing grade 2.3.

Isolates of *X. malvacearum* which reacted as race 2 had been described earlier by Hunter and Blank (32) in New Mexico, but no specific differentials or race designation had been proposed.

In the present study the Cotton-Disease-Council's differentials were included along with a large number of other upland lines with known blight-conditioning genes. The variety Acala 44 was soon substituted for Stoneville 2B as it proved to be somewhat more susceptible. Eventually, as the studies progressed Acala 44 (susceptible), 8-3 (b_7), CR4 ($B_{L?}$), 1-10B (B_{In}) and 20-3 (B_N) were adopted as differentials common to each test. The first three lines were comparable in reaction to the Cotton-Disease-Council's differentials, Acala 44 reacting as Stoneville 2B, 8-3 as Stoneville 20 and CR4 as Mebane B1.

Knight's *G. barbadense* lines were also included as differentials but were not used in all tests because of inadequate seed supplies.

Cultures from Susceptible Varieties from the United States.—During the seasons of 1955 through 1959 some 127 collections of blight-infested leaves and bolls were made from susceptible cotton varieties in Oklahoma and 40 collections were obtained from other cotton-producing states.[3] In all instances samples came from varieties without known major blight-conditioning genes. Resistant varieties, however, had been tested in some of the areas. The cultures were tested on seedling differentials usually soon after the samples were collected. The inoculum consisted of bacterial suspensions prepared by crushing the dried leaf samples in small amounts of water and was applied by the wounding technique.

Data from the seedling tests are summarized in Table 4, and arranged to show the relationship of each culture to races 1 and 2. Thirty-one percent of the cultures differed in pathogenicity from the previously known races. Of these, some attacked lines with the $B_{L?}$ gene (CR4 and Mebane B1), some attacked the B_N gene (20-3 and 20-8), and many others attacked the line with B_{In} (1-10B). Approximately 64% of the cultures reacted as race 1 and 5% as race 2.

Cultures from Previously Resistant Strains of Cotton.—A summary of data from seedling tests with cultures obtained from previously resistant plants during the period of 1956 to 1959 is shown in Table 5. Most of the cultures were derived as single-colonies and were isolated from plants in breeding nurseries, variety tests, increase plantings, or from seedlings that became infected with inoculum from susceptible varieties. The inoculum was usually grown as broth shake cultures and applied with hypodermic syringes.

The disease reactions (Table 5) show that 74% of these cultures

[3] Individuals who sent samples included: L. S. Bird, Texas; L. M. Blank, Arizona; N. Justus, Mississippi; B. B. Brodie, North Carolina; and W. P. Sappenfield, Missouri.

differed from races 1 and 2, and two groups differed from any of the cultures obtained from susceptible varieties (Table 4). One group of cultures attacked four of the five upland differential lines (all but the b_7 line) and the second attacked all five upland differentials.

Cultures from Mexico and Africa.—[4] Cultures were obtained from a winter-increase planting for cotton breeders in the United States from Iguala, Mexico and from Uganda, Nigeria, Republic of Sudan and Portuguese East Africa. The cultures from Mexico were single-colony isolates tested in 1956 and 1957 in both the greenhouse and field in Oklahoma, while the African cultures were tested as bacterial suspensions from leaves on seedlings in the greenhouse in Minnesota in 1958 and 1959. A group of single-colony cultures isolated from one of the leaf samples from Uganda were tested in the field in Minnesota during the summer of 1958.

Disease reactions of seedling differentials inoculated with Mexican and African cultures are shown in Table 6. The predominant reaction of the Mexican isolates was that of race 1. An occasional seedling, however, of 8-3, CR4 and 1-10B was also attacked. When the original single colony isolates were tested later in the field the disease reactions were typical for race 1.

The culture from Uganda, Africa, designated XL 57/21, attacked all of the upland differentials that were tested and also all of Knight's *G. barbadense* lines except the one with the genes B_2B_{6m}.[5] Disease reactions of adult plants in the field were obtained for a composite culture made up of single-colony cultures isolated from each of the five upland differentials and also from each of Knight's *G. barbadense* lines with single blight genes. Again all of the single-gene lines previously tested were attacked as were the lines, Mebane B1 ($B_{L?}$), 4-11 ($B_{L?}$), 6-77 (B_S), B251 (genes?), CR4 x 20-3 ($B_{L?}$ & B_N), 4-11 x Ac250 ($B_{L?}$ & $b_{7?}$) and Knight's B_2B_3.

Most of the other African cultures differed from races 1 and 2 but did not have as wide a range of pathogenicity as Uganda XL 57/21. Some of the African cultures were probably mixtures of races as successive tests with leaf inoculum differed considerably in disease reactions. Single colony-isolates were not tested except for the Uganda XL 57/21 culture.

[4] Cultures were obtained by Dr. J. T. Presley from research stations in the various countries.

[5] This culture represented inoculum used by Dr. G. M. Wickens, Normbye, Uganda, in his breeding nursery.

If the various cultures from Africa are representative, their wide range of pathogenicity may well account for the difficulty expressed by Hutchinson (33) in following the inheritance of Knight's blight-conditioning genes. Hutchinson attributed the problem to variable disease reactions under environmental conditions less favorable for disease development than the Sudan.

Designation of 10 New Races.—Disease reactions of 117 cultures from the United States and one from Africa are summarized in Table 7. All of the cultures were tested on seedlings of both upland and *G. barbadense* lines and most had been reisolated as single colonies from the previous seedling tests summarized in Tables 4 and 5. Disease reactions of only one of the African cultures, Uganda XL 57/21 are included in Table 7 as the other cultures were only tested as suspensions from leaves and may have been composed of mixtures. Reactions of a number of isolates from B_2 and B_4 are not included, as these cultures were not tested on the upland differentials.

Ten races in addition to races 1 and 2 are designated in Table 7. The disease reactions of a number of these races are illustrated in Figs. 3 to 6 inclusive. The 12 races can be identified by the reactions of eight of the 11 differentials, as Knight's B_3 and B_5 were susceptible and B_1 and 8-3 reacted alike.

Hence, for the present, eight lines are proposed as race differentials. Knight's B_1, B_3 and B_5 are omitted as they are not needed to differentiate the 12 races and more specific information is needed on their relationship to the blight-conditioning genes in the upland lines. Mebane B_1 is suggested in place of CR4 as it reacted as CR4 to the proposed races and was originally designated by the Cotton Disease Council (59). Acala 1517 BR-1 is suggested in place of Stoneville 20, or 8-3 as the b_7 differential and 20-3X4 Ac44 is suggested in place of 20-3 or 20-8 as the B_N differential. Acala 44 (susceptible) would be used in place of the Cotton Disease Council's Stoneville 2B which has a complex of minor blight-conditioning genes (6). The other Acala lines are suggested because they appear to have fewer minor genes and have produced less variable disease reactions. The proposed differentials and their reactions to the 12 races (of Table 7) are shown in Table 8.

Eventually, a more complete set of differentials with single major-blight-resistance genes, each in an Acala background, appears very desirable for studying races as well as for physiological studies on the nature of blight resistance. Flor (25) pointed out that flax differential lines with single rust-conditioning genes usually show less variation in

infection type than do lines with two or more genes. Also if resistance in the host is dominant, differentials with two or more rust-conditioning genes do not differentiate between races that attack only one of the genes. Thus, some races may escape detection.

Considering the races shown in Tables 7 and 8, races 1 and 2 are the same as designated by the Cotton Disease Council (59). The former is the prevalent race of the United States and attacks only Acala

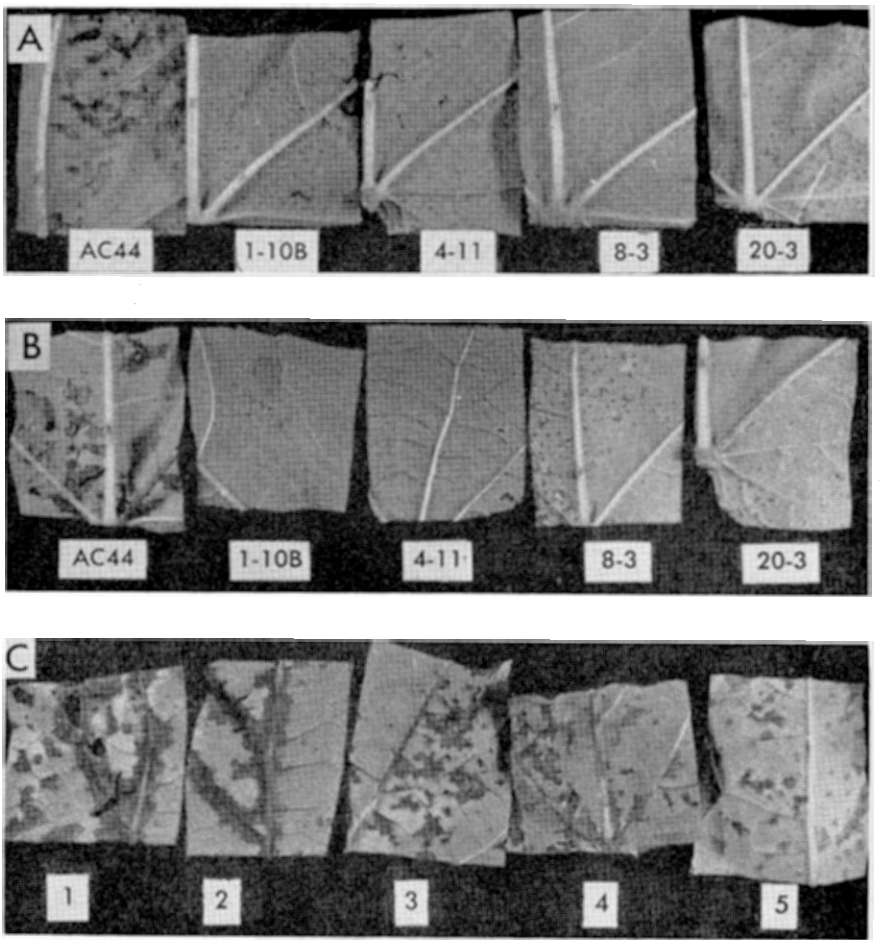


Fig. 3. Disease reactions of upland differentials in the field to: (A) race 1; (B) and (C) race 2. The 8-3 line is usually more susceptible to race 2. Lines shown in (C) are: (1) Acala 1517C, (2) Acala 1517 BR-1, (3) Austin, (4) Rex, (5) (Sto 20 x 6 Sto. 62)F_8. Acala 1517C is susceptible to all known races and the latter have Stoneville 20, or b_7 resistance.

44 of the differential lines. Race 2 occurs primarily in New Mexico and Texas where Acala varieties with b_7 resistance have been grown (14). The U. S. cultures of race 2 consistently attacked Knight's B_1 as well as b_7. Race 3 attacks the B_{In} differential and is fairly common on

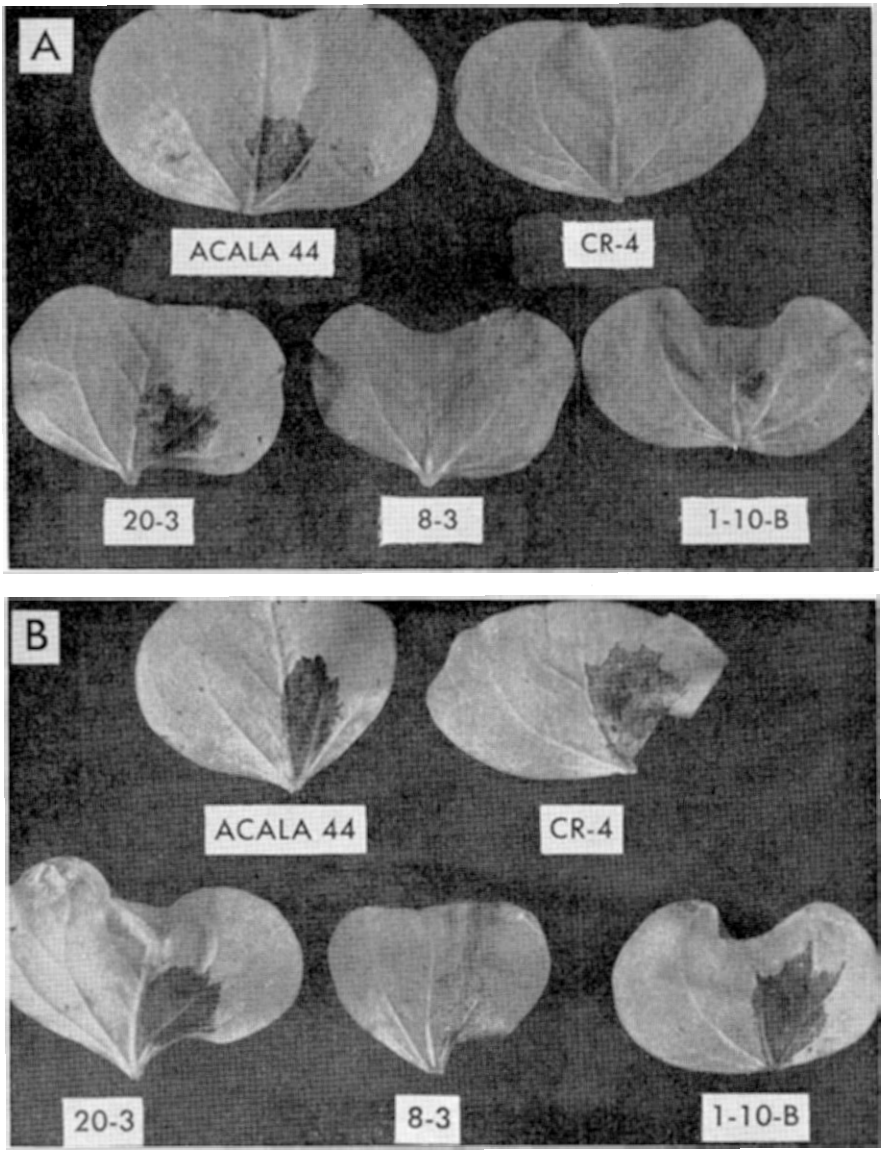


Fig. 4. Bacterial blight reactions on cotyledons incited by: (A) race 4 and (B) race 7. Inoculation was by the syringe method.

susceptible varieties in Oklahoma and probably also in Texas. It was also obtained from North Carolina and Missouri. Races 4 to 12 inclusive were obtained principally from resistant lines grown in breeding nurseries, variety tests and increase plantings. Race 12 was also obtained from Uganda, Africa. The latter culture was more virulent than the culture of race 12 obtained from resistant varieties in the United States.

Nature of Variability in X. Malvacearum

Much has been learned in recent years about variation in the bacteria. Although most studies have dealt with non-plant pathogens, the findings will no doubt prove to be valid for most species. Recent con-

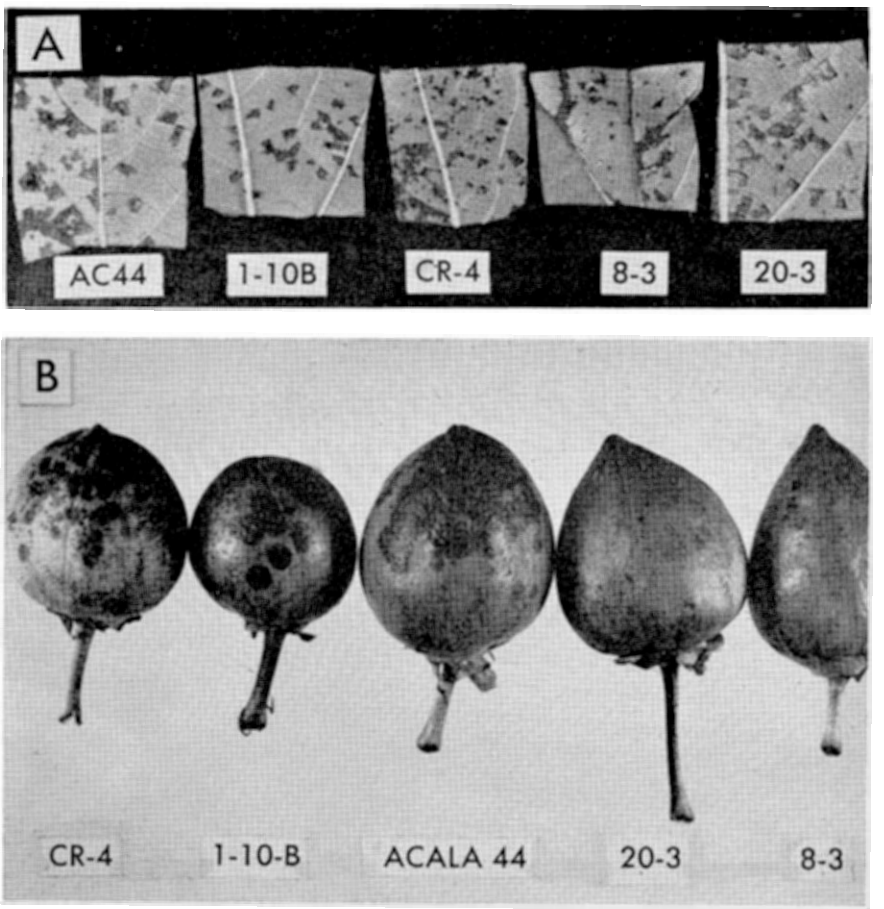


Fig. 5. Bacterial blight reactions in the field to a mixture of: (A) race 4, 7 and 10, and (B) to race 6.

cepts pertinent to the present study are: 1) A nucleus that divides with the cell has been readily demonstrated in all recently studied species (52). 2) Spontaneous and undirected mutations for such characters as resistance to bacteriophages (49, 53), resistance to antibiotics (13) and dependence on specific nutrients (44) have been demonstrated in a number of different species. 3) Complex loci have been demonstrated

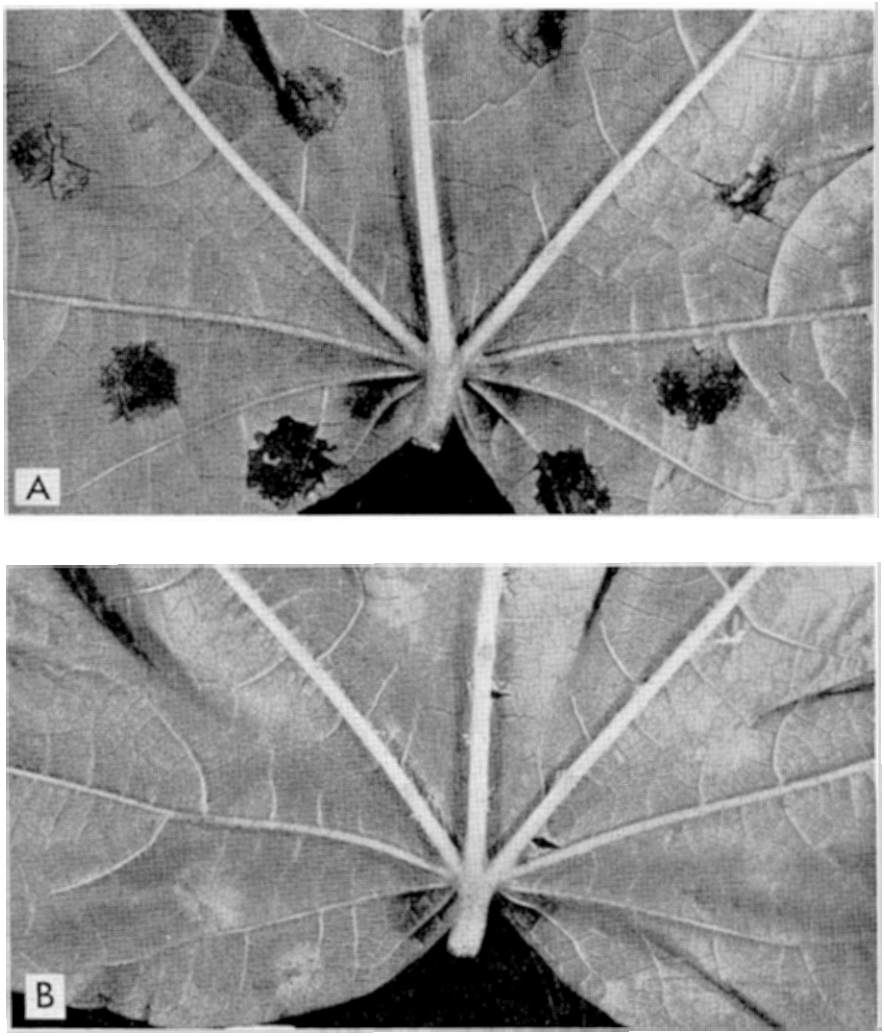


Fig. 6. Disease reactions incited by 8 cultures of *X. malvacearum* on leaves of 2 lines of Knight's *G. barbadense*. (A) Line B_5 and (B) line B_2B_6. The cultures clockwise from the 7 o'clock position are: races 1, 6, 1, 2, 6, 7, 1 and 4.

for bacteria as for other organisms, and recombinations within these may give rise to different but related types (21, 23).

Mechanisms that produce additional genetic variability include: 1) conjugation (5, 45, 68); 2) transformation (2, 28, 62); and 3) transduction (69). How much these latter processes contribute to genetic variability outside the laboratory is not known; nor is it known how generally they occur among the species of bacteria. Nevertheless, Corey and Starr (16, 17) transformed *X. phaseoli* (E.F.Sm.) Dows. for different colony types and for streptomycin resistance, and Klein and Klein (35, 36) transformed *Agrobacterium tumefaciens* (E.F.Sm. & Town.) Conn. for pathogenicity. Both species are plant pathogens and the former is a close relative of *X. malvacearum*.

The race-identification studies with *X. malvacearum* have shown this pathogen much more variable than previously believed. Many of the cultures tested as leaf suspensions comprised two or more races, but usually race 1 predominated in the cultures from susceptible varieties. The other races usually were present only in very minute fractions of the total population of cells.

Mutation is the most likely mechanism producing the differences in pathogenicity in *X. malvacearum*; however, recombination might produce similar changes. Cultures derived from single colonies, readily identified by one or more specific markers, appeared to offer a feasible means of identifying pathogenic variants in a given culture and also provide information on the nature and frequency of such changes. If contaminations from insects (47) or other sources are important factors in bringing about race mixtures, these could be distinguished from the marked cultures.

Tests for Genetic Markers.—A number of different characters were considered as possible markers. These included differences in colony morphology and resistance to different chemicals.

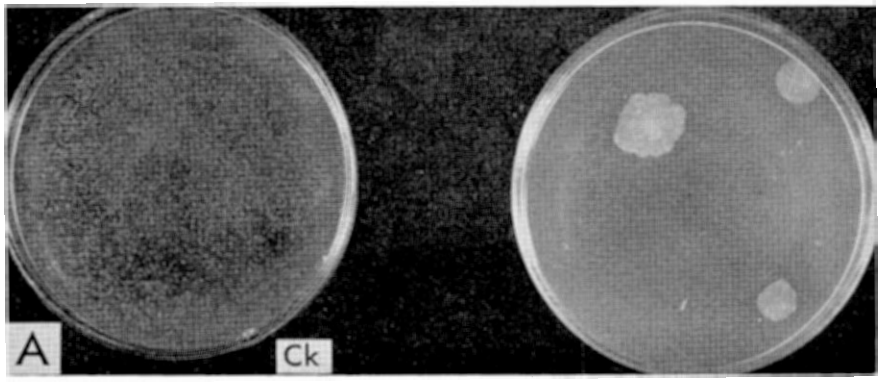
Morphological variants.—Cultures were selected from colonies of *X. malvacearum* grown on nutrient agar which differed in type of growth (watery vs. viscid), pigmentation (mottled vs. normal), type of margins (irregular vs. smooth), and size and shape (large, appressed vs. normal, raised). Progeny tests showed that each of these except the large-appressed colony type reverted to the normal type. Further tests showed the appressed colony type to be fairly stable but when the colonies were crowded they could not be distinguished from the normal type.

Tetrazolium variants.—Tests were made with cultures from colonies showing different staining patterns when grown on nutrient agar with .005% tetrazolium.[6] None of the variant types proved to be genetically stable.

Antibiotic-resistant variants.—Cultures resistant to streptomycin sulfate were readily isolated from normal cultures of *X. malvacearum* provided sufficiently large numbers of cells were screened. The antibiotic was added as a dry powder to hot, sterilized nutrient agar immediately after the medium was removed from the autoclave. Usually 0.5 to 1.0 ml of a non-diluted 24 to 48 hr nutrient-broth-shake culture, grown at about 80°F, provided sufficient cells so that some resistant colonies developed on each plate (see Fig. 7). In one test with 0.5 ml of undiluted inoculum on nutrient agar with 10 ppm or 200 ppm streptomycin sulfate the average number of colonies (18 plates) was 10.9 and 5.0 per plate after four days incubation. The parent culture contained approximately 10^9 cells per ml, and had been grown for 24 hrs on a shaker in nutrient broth with 2% dextrose.

Streptomycin-resistant colonies were readily obtained from all cultures tested including those from Africa. These mutations apparently are of general occurrence, but do not become the predominant type unless screened on a medium with the drug.

Resistant colonies developed on media with the antibiotics Penicillin G., or the Charles Pfizer and Co's Terramycin, P496 and GS-1 in much the same manner as for streptomycin sulfate. None of the cultures from these colonies retained resistance for the given antibiotic after successive transfers on media without the antibiotic or after pas-



[6] 2, 3, 5 triphenyl tetrazolium chloride.

sage through the host; whereas, the majority of the streptomycin-resistant cultures retained their resistance. Nevertheless, stable resistant mutants might have been obtained had more extensive tests been made with the other antibiotics.

Increases in resistance to Terramycin, Penicillin G., and in some instances to streptomycin sulfate were obtained by stepwise mutations.

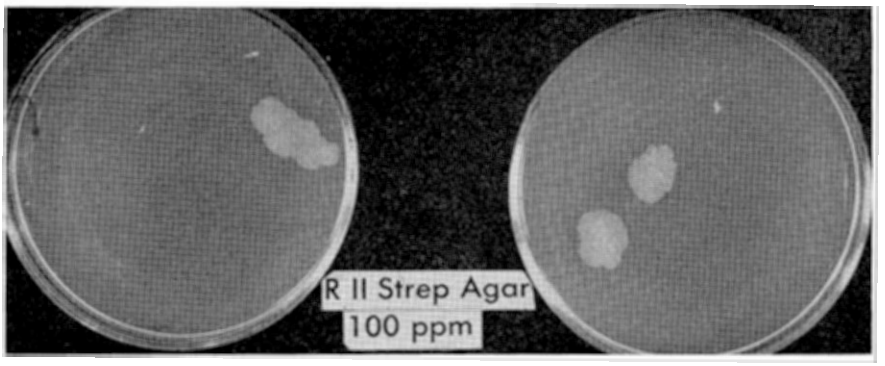
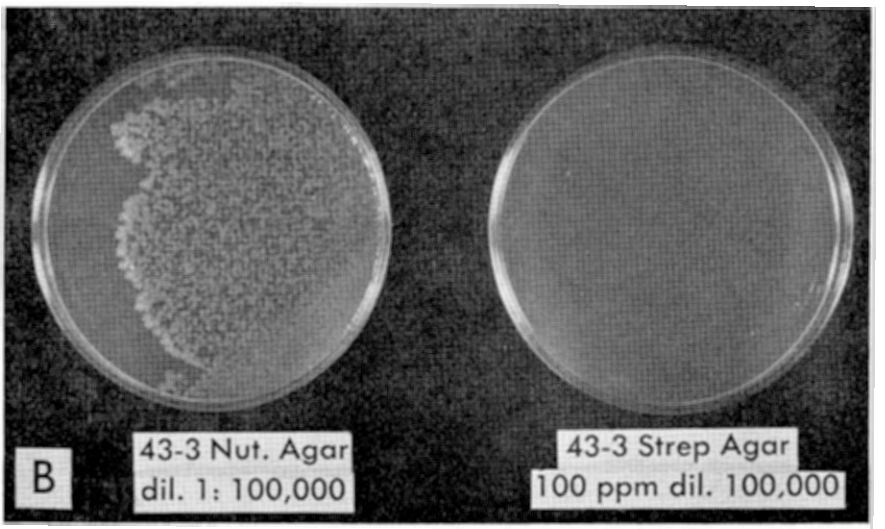


Fig. 7. (A) Streptomycin resistant colonies of race 2 screened from 1 ml of nondiluted 16-hour nutrient-broth shake-culture of *X. malvacearum*. The check (opposite page) shows colonies obtained from 1 ml of a 10^{-5} dilution of the same culture on nutrient agar without streptomycin sulfate. (B) Colonies were obtained from 1 ml of a 10^{-5} dilution of race 7 (43-3) on nutrient agar without and with 100 ppm streptomycin sulfate. Note that at this dilution no resistant colonies were obtained; resistant colonies, however, were readily screened from larger populations of this same culture.



Resistant colonies were first isolated at relatively low concentrations of the antibiotic; later, after successive selections were made at intermediate levels, cultures thrived on media with five, 20 and 100 times the original concentrations, respectively, of the three antibiotics. These cultures, however, lost resistance after being maintained for three months on media without the antibiotic.

A few cultures were found to be dependent upon streptomycin sulfate for growth. Others varied in pigmentation at the higher concentrations of the antibiotic. Some resistant cultures that were almost white on streptomycin sulfate or Terramycin were the normal yellow when cultured on media with little or no antibiotic.

These studies indicated that a number of different mutations for resistance to antibiotics occurred in relatively low frequencies in normal populations of *X. malvacearum*. However, the mutants were readily screened on a selective medium and immediately became the predominant type. It seems probable that mutants which could attack various lines of the host possessing different genes for blight resistance might also occur in normal populations of *X. malvacearum,* and might be similarly screened by the resistant lines.

Stability of Streptomycin-Resistant Cultures.—Of the characters studied, single-step mutants for resistance to streptomycin sulfate was the only one that appeared to meet the requirements of a good genetic marker. The appressed-colony type was included in additional tests, but no selective method was devised for screening it from normal populations. A streptomycin-resistant clone, however, was readily obtained from the one appressed-colony type culture (race 7) found as were single-step streptomycin-resistant clones also readily selected from normal cultures of races 1, 2, 4, 6 and 7. These were derived from single colonies on nutrient agar with 200 ppm streptomycin sulfate. Studies were then undertaken to determine the genetic stability of the selected clones.

An attempt was made to grow 20 successive shake cultures of each clone for 48-hr periods in 6-oz prescription bottles containing 100 ml of nutrient broth or nutrient broth with 1.6% dextrose. All cultures were started from mass transfers with a small wire loop. A comparison of relative numbers of cells susceptible or resistant to the antibiotic was made with equal amounts of the diluted cultures by flooding the surface of nutrient agar with and without streptomycin sulfate. Pathogenicity tests were made with the same culture on seedling differentials grown under fluorescent light. Difficulty was encountered with a white bac-

terial contaminant which inhibited the growth of *X. malvacearum*. When a culture became contaminated, it was discarded and the series started over by mass transfer from the preceeding series.

Tests that were made with mass cultures of the 1st, 3rd, 6th, 9th, 12th, 15th, 17th, and 20th culture of each of the five races in the different media showed no loss of resistance to streptomycin sulfate, nor any loss in pathogenicity except in race 2. Similar losses in pathogenicity occurred in the streptomycin-susceptible parent of race 2. The disease reactions were typical of the different races and the cultures appeared to be as virulent as the non-streptomycin resistant parents. The relative numbers of colonies reisolated from infected seedling differentials were usually greater on media with streptomycin sulfate. This was probably due to inhibition by the antibiotic of contaminants present in the infected tissue.

Single-colony cultures obtained from streptomycin plates of the 17th and 20th transfer series were tested on two differentials. Disease reactions for 30 different cultures are shown in Table 9. Several cultures appeared to have lost virulence (S- or R reactions) but gave the same reaction on both differentials. One culture of the normal clone of race 7 isolates (No. 3) appeared to have lost pathogenicity for 20-3, or the B_N gene, but retained full virulence for Acala 44.

Two of 100 race 7 single-colony cultures isolated on nutrient agar from the 12th serial transfer, were not pathogenic on either Acala 44 or 20-3. When reisolations were made from 100 inoculated 20-3 seedlings, colonies of *X. malvacearum* developed from 97 of these seedlings on a medium with streptomycin sulfate, while all of the seedlings produced colonies on the medium without the antibiotic.

These studies indicated that back mutations to susceptiblity to streptomycin had occurred. The susceptible biotypes, however, had not increased and became the predominant type in either the synthetic media or host tissue.

New Pathogenic Races from Streptomycin-Resistant Cultures.—The studies with antibiotics indicated that resistance to streptomycin sulfate should be a good genetic marker since the single-step mutants were relatively stable, did not usually differ in pathogenicity from the parental cultures, and could be readily obtained from normal populations simply by plating large numbers of cells on agar with the antibiotic. Thus, tests were initiated in 1958 with a streptomycin-marked culture to determine whether pathogenic mutants might be derived from it.

Resistant lines of the host were used as a selective medium, in somewhat the same way agar with streptomycin sulfate was used to screen for streptomycin resistant mutants.

A clone of race 3 was obtained from a single colony selected on nutrient agar with 800 ppm. of streptomycin sulfate in July of 1958.[7] A seedling test with an undiluted broth culture showed the resistant clone to be the same race and of virulence comparable to the parent culture.

Four differential lines in three 100-ft plots were inoculated on August 20, 1958, at St. Paul, Minnesota, with a diluted (1:200) nutrient-broth-dextrose shake culture when the plants had four to six true leaves. The disease reactions were evident after 10 to 14 days and showed Acala 44 to be uniformly susceptible (field grade 4), and all plants of the other lines to be resistant but varying in reactions from field grades 0.1 to 1.2. Four weeks after inoculating, a few plants of lines 8-3 and 20-3 had an occasional water-soaked lesion on leaves which previously had only resistant reactions. The majority of these water-soaked lesions were grade 3 or somewhat less than the fully susceptible grade 4 reaction. During the remainder of the season the numbers of susceptible lesions increased but none of the plants became uniformly infected. Water-soaked lesions were not observed on plants of Mebane B1. Shortly before frost, leaves with lesions of the various infection types were removed and air dried.

Greenhouse tests with seedling differentials were made to determine the pathogenicity of the cultures from leaves with different reactions. All cultures were prepared as ground-leaf suspensions, but in each instance the inoculum was increased first on Acala 44 as a preliminary test showed relatively few viable cells present in the resistant-type lesions. No attempt was made to separate resistant- and susceptible-type lesions where they occurred on the same leaf.

The disease reactions in Table 10 show that race 3 was recovered from the fully susceptible leaves of Acala 44 (grade 4) and also from the resistant leaves (grade 1.2) of Mebane B1 and 8-3. Race 5 (pathogenic to 20-3) was recovered from leaves of 20-3 with the mesothetic

[7] The parent culture was obtained from Dr. L. S. Bird of Texas A.&M. College in the spring of 1958 and had been designated as race 1. However, seedling tests made by the author when the culture was received showed it to be race 3 rather than race 1. That the streptomycin-resistant clone used in this study was descended from a single resistant cell would appear to be almost certain on the basis of probability, as it was derived from a series of single colonies each screened from the minute resistant fraction constituting probably less than a millionth part of each of four successive populations by plating first on a medium with 50 ppm, and then 100 ppm and 200 ppm Penicillin G, and finally on streptomycin sulfate. Resistance to penicillin as discussed in the section on antibiotics proved not to be stable in the absence of the drug.

reactions 1.3 and 1.4 and also from the 8-3 line with 1.3 reactions.

A test on differential media (nutrient agar with and without streptomycin sulfate) with leaf suspensions from the final greenhouse test showed that the cultures derived from the resistant clone were still resistant to streptomycin sulfate, and therefore were not contaminants (see Table 11).

In 1959, more extensive tests were undertaken with single-colony streptomycin-resistant clones of races 2, 6, 7, 9 and 10. It was intended to include race 1 among the cultures, but the clone selected from it proved to be race 6, and since there was not time to reselect, race 1 was omitted. The clone from race 10 was only weakly pathogenic to the 8-3 differentials while the parent culture had been fully pathogenic the previous season. Also the resistant clone from race 2 was not fully pathogenic to 8-3, although the parent had been the previous season.[8]

The experiments were made at Stillwater, Oklahoma in single 25-ft row plots comprising four upland differentials interspaced between spreader rows of susceptible Acala 44. Blocks of differentials were isolated from each other by 20-ft barriers of a tall variety forage sorghum. The single-colony clones were isolated on nutrient agar with 200 ppm streptomycin sulfate on July 16 and tested on seedlings between July 21 and August 3. Field inoculations were made with 1:200 dilution of shake cultures on August 5. After three or four weeks mesothetic reactions had developed on differentials that were at first resistant. There was, however, considerable variation among individual plants of the different lines.

Bacterial suspensions were obtained directly from single fresh susceptible-type lesions from leaves with mesothetic reactions and also from leaves with uniformly susceptible lesions. Each suspension was divided and tested on seedling differentials and on nutrient agar with and without streptomycin.

The disease reactions to the resistant clones in the field and to the cultures derived from different host lines are shown in Table 12. Subsequent tests (Table 13) were made with bacterial suspensions from leaf tissue of the seedling tests and also with single-colony cultures obtained on nutrient agar with streptomycin. The reactions obtained with the original clones and cultures derived from each of the differential lines are illustrated in Figs. 8 to 11 inclusive.

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[8] The parent cultures of races 10 and 2 were selected in 1958 from the lines 20-8 and Stoneville 20, respectively, by Mr. R. E. Hunter and had been stored for approximately one year under mineral oil at 50°F.

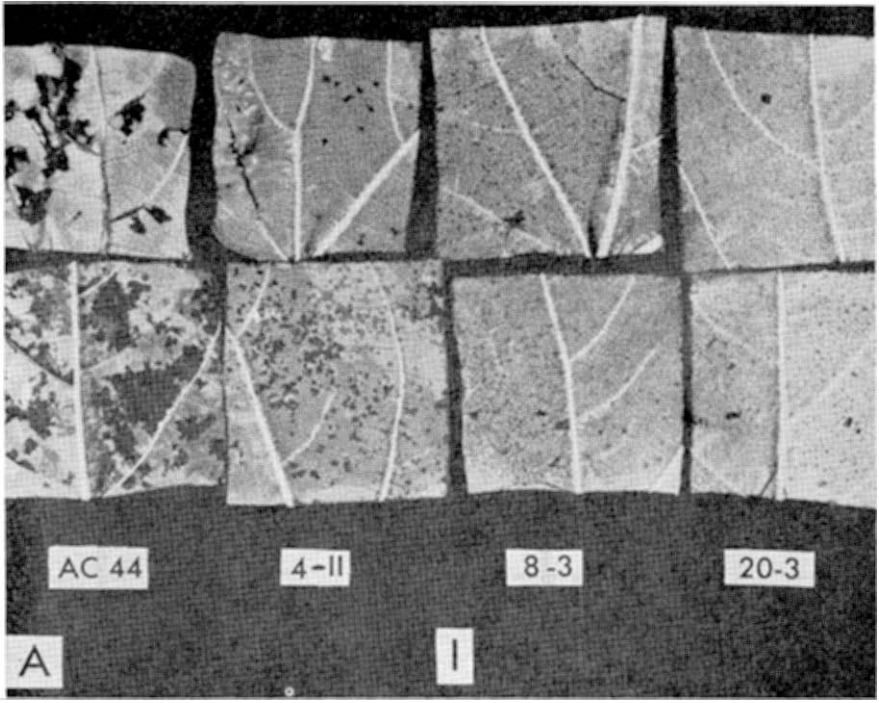
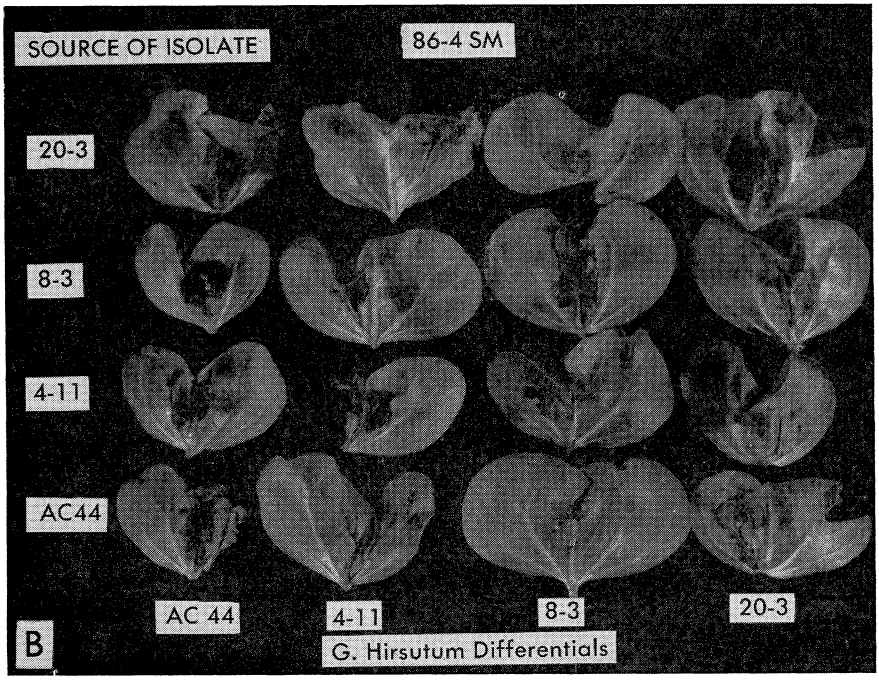


Fig. 8. (A) Infected leaf sections showing the range of blight reactions on upland differentials in the field three weeks after inoculation with a single-colony streptomycin-resistant clone of race 6. Note "mutant"-type lesions on 8-3 and 20-3. The leaf surface was wet to enhance contrast. (B) Reactions obtained on cotyledons indoors to cultures derived from susceptible-type lesions of the four differentials. Starting with the isolate from 20-3 at the top the cultures represent races 4, 8, 6 and 1.



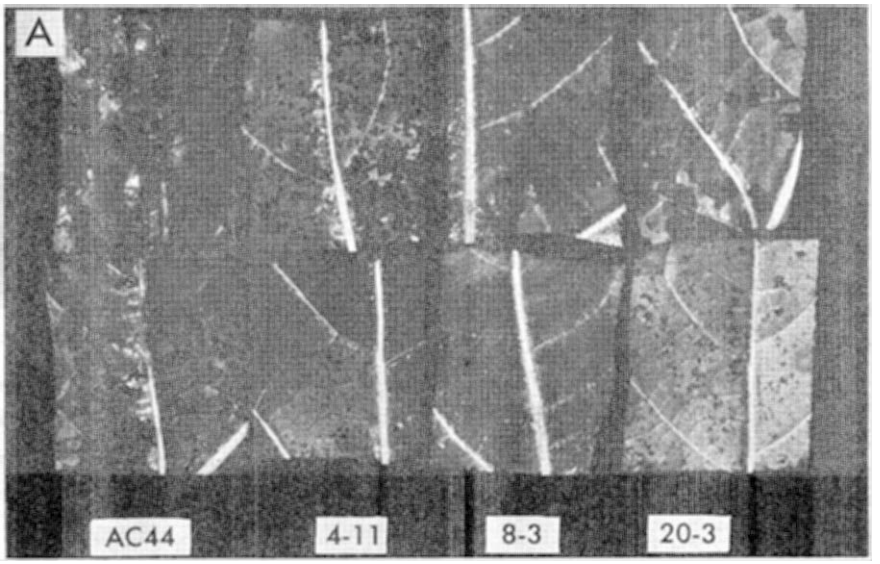
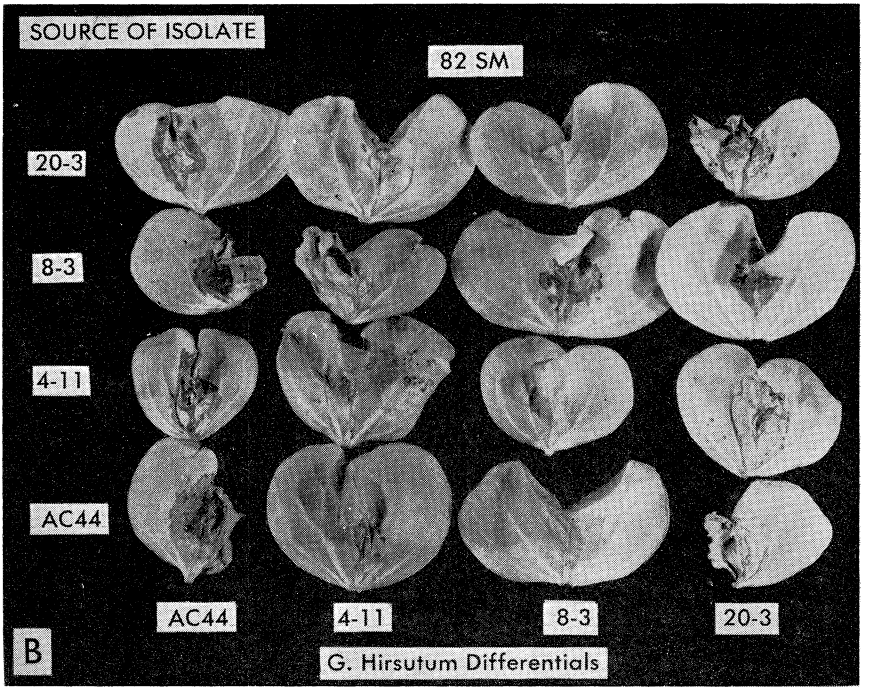


Fig. 9. (A) Infected leaf sections showing the range of blight reactions on upland differentials in the field to a single-colony streptomycin-resistant clone of race 7. Note "mutant"-type lesions on 8-3. The leaf surface was wet to enhance contrast. (B) Reactions obtained on cotyledons indoors to cultures derived from susceptible-type lesions of the four differentials. Starting at the top the isolates represent races 5, 10, 5 and 7.



As in 1958, clones with increased pathogenicity were isolated from the susceptible-type lesions of mesothetic reactions of the 20-3 and 8-3 lines, but not from the $B_{L?}$ line. Full pathogenicity was restored to races 2 and 10 by selecting clones from lesions of the 8-3 line, and a race not previously encountered was selected from a "mutant" lesion produced by race 2 on 20-3. Also race 12 was obtained from race 10 by selecting from the 8-3 differential. This culture differed somewhat from the race 12 culture from Uganda, Africa in that it was not as virulent on Knight's B_4, but was more virulent on Knight's B_3 line.

The more complex races (7, 9 and 10) appeared to be somewhat less stable than race 2 and 6 (see Table 13).

The results in 1959 confirmed those of the previous season and showed that new races originated as mutants in clones derived from single colonies which presumably originated from single cells. Thus, mutations for pathogenicity appear to be present in normal populations just as are mutations for antibiotics and other characters.

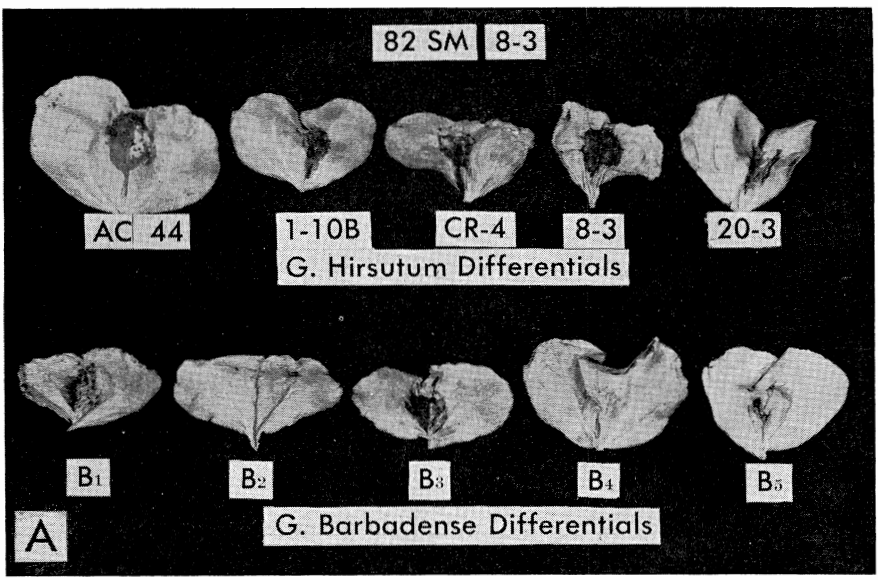
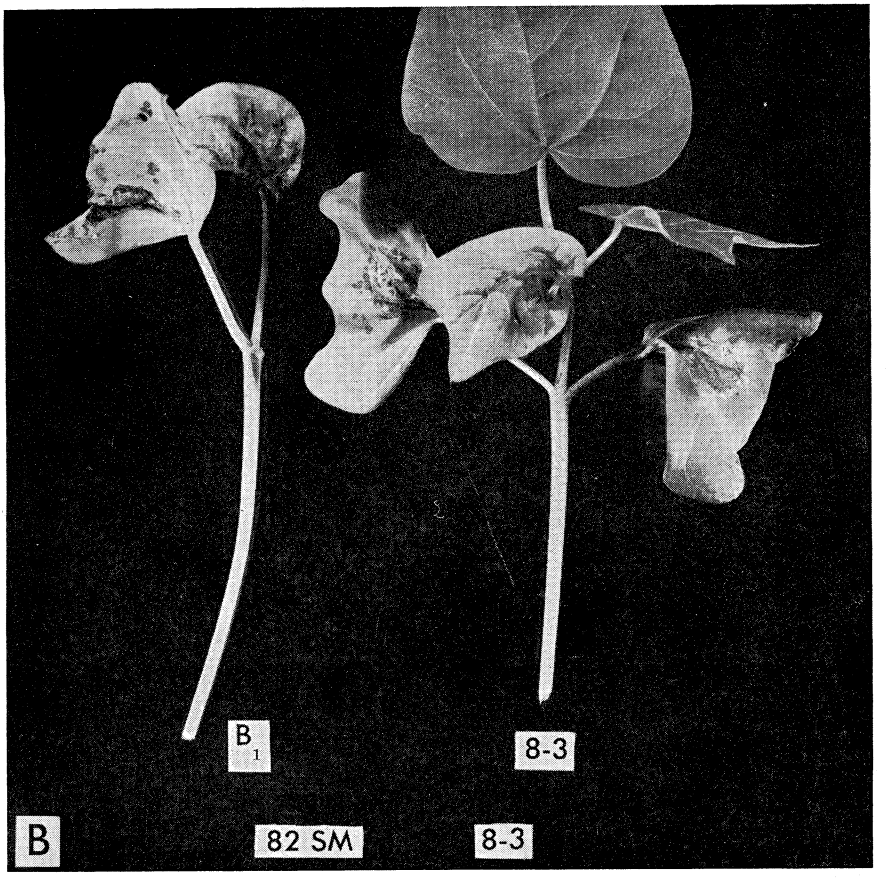


Fig. 10. Additional bacterial blight reactions to the clone of the culture 82Sm obtained from a "mutant" lesion of 8-3 (see Fig. 9A): (A) cotyledons of *G. hirsutum* and *G. barbadense* differentials and (B) (Opp. page) young plants of lines B_1 and 8-3 showing systemic infection from cotyledon inoculation.



New Pathogenic Races from Normal Cultures.—Normal as used in the following studies refers to cultures of *X. malvacearum* that had not been selected for resistance to streptomycin sulfate. Most of the studies with normal cultures were made prior to those with the streptomycin-resistant cultures, but are presented in this sequence because the results can be interpreted better in light of the preceeding studies.

Isolations from mesothetic reactions.—During the summer of 1957, single-colony cultures were obtained from plants in breeding nurseries at Stillwater and Chickasha, Oklahoma. The plants had been inoculated with bacterial suspensions from leaves of naturally-infected susceptible plants. At both locations the leaf inoculum originally incited race 1 reactions, but many plants later developed mesothetic reactions. Knight's line with B_2B_{6m} resistance was the only differential that did not eventually have some water-soaked lesions. Two or more single-colony

cultures were obtained from individual susceptible-type lesions from lines with mesothetic reactions and from lines that were fully susceptible. Races were identified on seedlings growing indoors under artificial light. Inoculation was by the syringe method with full-strength shake cultures.

The disease reactions incited by isolates from various differentials are shown in Table 14. As with the streptomycin-resistant clones, new races were obtained from the susceptible-type lesions of plants with predominantly resistant reactions. Relatively fewer cultures attacked the host line than was true of the marked cultures, however. Race 6, which is pathogenic to lines with the $B_{L?}$ gene, was obtained from $B_{L?}$ lines and from other lines as well. Races which attacked the b_7 line (8-3) were not obtained although other races were isolated from the susceptible-type lesions. A number of the cultures from lines with blight-conditioning

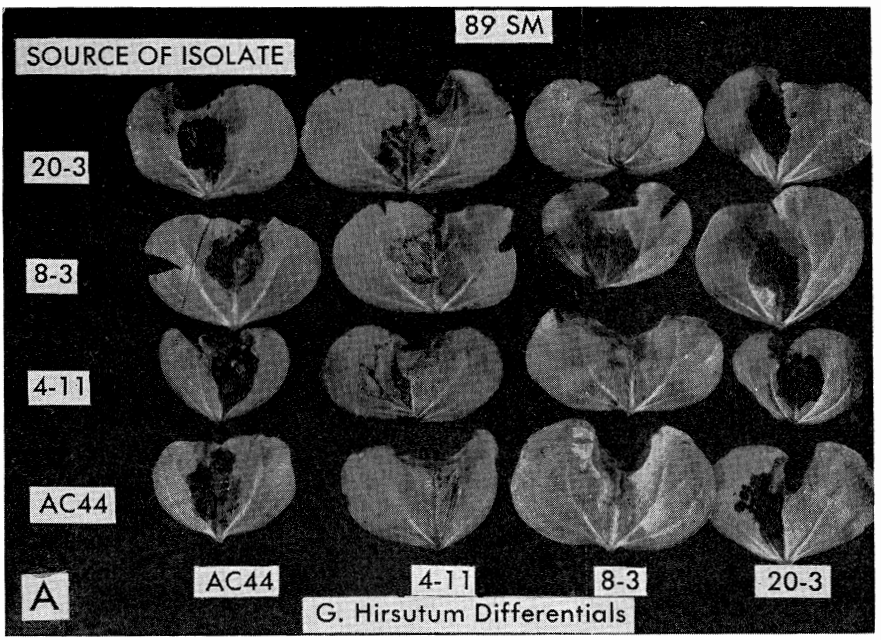
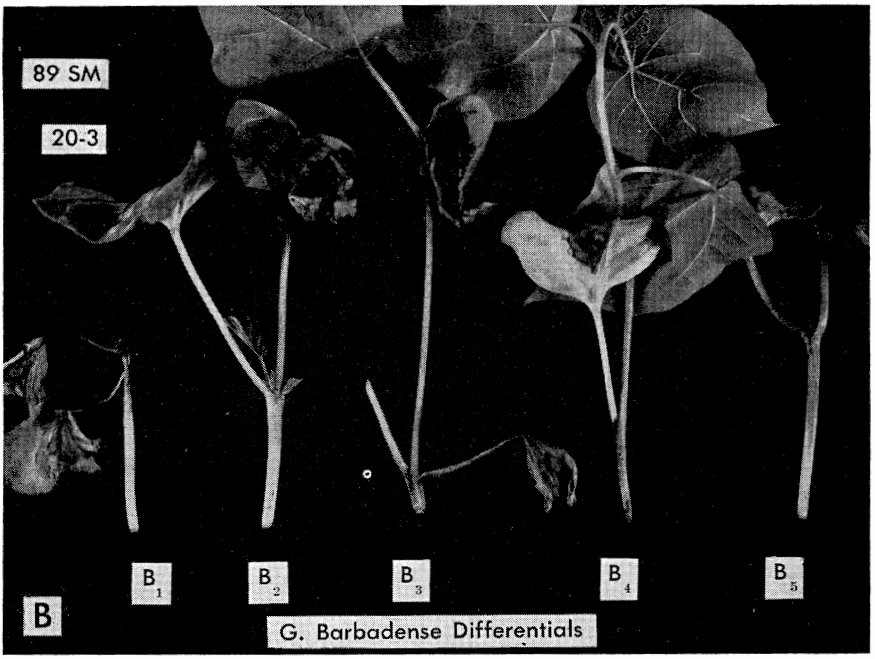


Fig. 11. (A) Cotyledon reactions to clones of *X. malvacearum* selected from susceptible-type lesions of upland differentials that were inoculated with race 10. The parent streptomycin-resistant culture had lost pathogenicity to the 8-3 line except for a few "mutant"-type lesions. The reactions incited by the 8-3 clone shows that full pathogenicity to 8-3 had been regained. (B) (Opp. page) Systemic infection of lines B_1, B_2, B_3 and B_5 and more restricted infection of B_4 with the 8-3 culture. The reactions in (A) and (B) and Table 12 show the 8-3 clone to be race 12.



genes proved to be race 1. Several of the cultures reacted as mixtures of races 3 and 6.

The origin of some of the variant races in Table 14 is not as evident as was the origin of most of the variants from the marked clones. On the other hand, the inoculum was probably more heterogeneous. The race 1 cultures from the susceptible lesions of the mesothetic reactions may have stemmed from the original inoculum; however, it seems more likely that they were back mutations from unstable mutants. Instability of many of the original mutants might account for the failure to isolate race 2 and for the occurrence of races not pathogenic to the various parent lines. The isolation of races 3 and 5 from lesions of fully susceptible varieties may have resulted from secondary infection (from mutants screened by resistant lines), or variant cells of these races may have been present in considerable numbers in the original inoculum. The numbers of susceptible-type lesions of race 3 on 1-10B indicated the latter hypothesis; however, 20-3 which differentiates race 5 had relatively few susceptible-type lesions and these must have had some other origin.

Tests with pairs of clones from the same lesions show either more than one race was present in the same lesion or that additional muta-

tions or segregation of some type had occurred after the original isolations were made (see Table 15).

Although many of the cultures obtained from the mesothetic reactions behaved as new races, no general build up of new races occurred on differentials in the field. The weather for 1957, however, was not especially favorable for disease development during the summer and fall period.

In 1959 the reactions of single-colony cultures from resistant- and susceptible-type lesions of the 20-3 line were compared. The parent planting was an isolated seed-increase block that had been inoculated with race 1. Three weeks after inoculating, the ½-acre planting was carefully inspected for infected plants. None was found, but about one plant of each 10,000 had one or more leaves with mesothetic reactions with very small water-soaked lesions. Although all of the plants with mesothetic reactions were removed, by six weeks after inoculation about 50% of the plants had relatively large water-soaked lesions. The disease had nearly disappeared, however, by the end of the season.

The disease reactions incited by cultures isolated from different types of lesions from the 20-3 planting at three different dates are shown in Table 16. These results agree with those obtained in 1958 with the streptomycin-resistant clone of race 3. Race 1 was recovered from the resistant-type lesions and a race that attacked the parent line from the susceptible-type lesions.[9]

From naturally infected plants of Cr4 ($B_{L?}$).—During the 1956 season, which was especially favorable for bacterial blight, CR4 became generally diseased at Tipton, Oklahoma. The resistant line had been included among susceptible varieties in a large randomized and replicated field planting.[10] The susceptible varieties became generally infected early in the season from inoculum carried in the seed and debris from a previous crop of cotton. At the beginning of the season CR4 was resistant but became generally infected as the season advanced. The lesions never became as numerous nor quite as severe as on the susceptible varieties. The resistant-type lesions usually associated with mesothetic reactions were either absent or much smaller than on leaves with artificial inoculation.[11]

Single-colony cultures were isolated from individual leaf lesions of

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[9] Dilutions of only 10^{-2} or 10^{-3} were needed for isolating from resistant type lesions rather than 10^{-5} or 10^{-6} that were used for susceptible-type lesions.

[10] This was a cold-tolerance experiment conducted by Dr. C. L. Leinweber.

[11] Less concentrated inoculum is probably responsible for the differences under natural conditions (34).

CR4 and cultures were also obtained as bacterial suspensions from dried infected bolls. The inoculum for test seedlings was applied by scratching the cotyledons with small bags of sand rather than by water-soaking with hypodermic syringes. The former method which leaves patches of uninjured cells between wounds, now appears to be more favorable for the development of pathogenic mutants in resistant cotyledons.

The results of two greenhouse tests are shown in Table 17. Neither group of cultures consistently attacked CR4, although some cultures produced S- reactions on part of the seedlings. Of the 18 cultures obtained by crushing dried bolls (Table 17) one was pathogenic to two of 10 seedlings of the 8-3 line (b_7 resistance) and two other cultures attacked one seedling each of 10 seedlings of 20-8 (B_N resistance).

The results of seedling tests with single colonies isolated from diseased CR4 seedlings (that were originally inoculated with single-colony clones, Table 17) are shown in Table 18. Again, most of the CR4 seedlings were resistant but a few were attacked by each culture, and the seedlings of the lines 8-3 and 1-10B were also variable in some of the tests.

The results of inoculations with 39 single-colony clones derived from one of the 20-8 seedlings originally infected by inoculum from CR4 bolls are shown in Table 19. Thirty-six of these cultures attacked the B_N differential (line 20-3), while three were not pathogenic to any of the lines. Races 5 and 7 predominated but races 4 and 10 were also obtained. Again, certain cultures attacked only part of the seedlings of CR4 and 8-3. It seems probable that these isolates stemmed from a single mutant screened from the field population of cells pathogenic to CR4. If this were true the original mutation must not have been stable since four different races were obtained which attacked the B_N differential. On the other hand, each of these races could have stemmed from separate mutations in the original population. But it does not seem likely that all would have been present in the original lesion on 20-8.

The failure of single-colony cultures derived from susceptible CR4 and especially of cultures obtained from infected bolls to attack CR4 seedlings uniformly is puzzling because 100% of the original plants in the field had been attacked. The same cultures failed to attack CR4 uniformly in the field during the next season, while a culture from a different source was fully pathogenic. Hence, the loss of pathogenicity appeared to have a genetic basis, and may in some way be comparable to the loss of resistance of the multiple-step mutants for penicillin and

certain of the other antibiotics encountered in *X. malvacearum*.

The variable reactions of the $B_{L?}$ lines as well as other uplands with single major blight-conditioning genes definitely indicate that resistance is influenced by modifying or so-called minor genes and that the lines are not homozygous. This is not only indicated by variable seedling reactions to single-colony clones but also by the fact that mesothetic reactions usually do not develop on all of the adult plants of a given line in the field. Furthermore, progeny derived from plants without mesothetic reactions are more resistant to the development of mesothetic reactions. This confirms results of Bird and Hadley (6) and others (27, 37, 42) who have suggested that modifying genes contribute to resistance.

Isolations from cultures maintained on synthetic media.—Before streptomycin-marked clones were available, usually there was no way to be certain that the occasional infection of a resistant-variety seedling had not resulted from contamination from one source or another. One exception in which there was no question about contamination was the sudden appearance in a test with single-colony clones of a variant which attacked the B_N lines (20-8 and 20-3). These lines had never been attacked in previous seedling tests.

Results of a series of seedling tests in the greenhouse with clones of races 1, 2 and 3 are shown in Table 20. Cultures were maintained continuously on PDA and seedlings were inoculated with concentrated inoculum by the wounding method. Leaf-suspension cultures from infected CR4 and 1-10B of the first test (see Table 20) attacked 1-10B, but not CR4. Leaf inoculum, however, from the single diseased seedling of 20-8 (B_N) in the third test with race 3 (see Table 20) was pathogenic to each of 50 seedlings of 20-3 (B_N). Single-colony cultures were isolated from a single lesion of one of the infected 20-3 seedlings. Of 32 clones that were tested (see Table 21), 27 reacted as race 4, three as mixtures of races 4 and 7, one as a mixture of races 4 and 5, and one as race 1.

The particular test in which the 20-8 seedling became infected (Test 3 of Table 20) was made during a period of frequent rains and high humidity which was exceptionally favorable for disease development. Many subsequent tests have indicated that to produce visible symptoms pathogenic mutants require succulent tissue and an environment that definitely favors blight development.

Whether the variant cells originated before or after entry into the host is not known for this or the other tests, and until some means is known of selecting pathogenic variants other than by screening in the

resistant host tissue, this question may not be answered. However, the classic studies of Luria and Delbruck (49) and of Newcombe (53) on the origin of resistance in *Escherichia coli* (Migula) Castellani and Chalmers to phage TI in the absence of the phage would indicate that contact with the host is not necessary.

Relative Stability of Races of X. Malvacearum.—The data in Table 20 (which have been discussed in relation to the origin of new races) also indicate that there was a shift to less pathogenic biotypes in each of the three single-colony clones by the end of the test period. This has been encountered repeatedly with cultures of race 2, and also with races that attack more than two lines. Results of a series of tests with races 4, 6 and 7 are shown in Table 22. Again the cultures were single-colony clones, but instead of PDA the medium was nutrient agar with 2% dextrose. One of the race 6 cultures lost pathogenicity for the B_{L_2} differential after three months, and race 7 was only weakly pathogenic to any of the differentials after 15 months. Race 4, however, had gained pathogenicity for 1-10B and reacted as race 5 at the end of the test period.

Preliminary tests indicated that some races may lose or shift in pathogenicity after storage in dried leaves, but pathogenicity has been regained by reisolation from the host differentials which apparently have selected more virulent types.

Variability and Pathogenicity of Small-Colony Variants.—Some very small colonies were observed in a culture of race 7 on plates of nutrient agar containing streptomycin sulfate after incubation for three weeks. These resembled the colony variants of *X. phaseoli* reported by Corey and Starr (15) and were also similar to some of the variants of pneumococcus (28, 2).

In a second test, plates of nutrient agar with and without streptomycin sulfate were examined under 10X magnification six and 19 days after inoculation with race 1 (see Table 23).[12] Numerous small colonies were observed after 19 days in the plates with 200 ppm streptomycin sulfate and small variants were also found, but in fewer numbers, in the plates containing 1000 ppm of the antibiotic and in plates with only nutrient agar.

The results of a test to determine the relative stability of the two colony types are shown in Table 24. Diluted bacterial suspensions were tested by flooding nutrient agar and nutrient agar with streptomycin sulfate. Two cultures from wild-type non-streptomycin-resistant colonies,

[12]This culture (designated 59-59-1) was isolated from a resistant-type lesion of the 20-3 differential and was identified as race 1.

[NA-5L and Sm (3) -13-1L] produced some small colonies as did one culture from a large streptomycin-resistant colony [Sm (1000) -40-2L]. Small colonies of the latter were observed only at the high concentration of the antibiotic. Cultures from the small types produced variable progeny in most instances, that is, some were large, some irregular and some small.

Table 25 shows results of an additional progeny test on nutrient agar with bacterial suspensions derived from single colonies of the test shown in Table 24. Some of the cultures were stable for large colonies and some for small colonies; others produced variable types. Some intermediate colonies with irregular margins were also observed. Figure 12 shows illustrations of some of the variable colonies.

A test to determine the pathogenicity of some of the variant colony types was made with seedlings of Acala 44 with full strength nutrient broth-shake cultures. The results, included as part of Table 26, show most of the small- and intermediate-colony cultures produced disease reactions after 26 days different from those at seven days. The host tissue at first reacted as either immune, resistant or only slightly susceptible, but later the same tissue developed water-soaked spots or became diseased over all of the inoculated area. Two cultures, however, produced only a slight chlorosis (see disease reactions for cultures NA3-5L-4s and Sm (3) 13-1L-1s in Table 26).

Sixteen days after inoculation, isolations were made from host tissue inoculated with eight of the 40 cultures (see Table 26). Colonies derived from plants inoculated with a normal wild-type colony (cultures NA-5L-2L) were all large. Those derived from plants inoculated with small-rough or intermediate colonies were of mixed colony types. Usually the small types were recovered, but large wild-type colonies were also present. Isolations from plants inoculated with culture NA3-5L-4s (which produced only a slight chlorosis on Acala 44) yielded colonies predominantly small and rough, but a very few intermediate and large colonies were also obtained (see Fig. 12). Cultures derived from these large colonies were fully pathogenic. The inoculated cotyledons that became only slightly chlorotic contained fewer viable bacteria, as colonies were obtained only from dilutions of 10^{-2}, but not from 10^{-4} or 10^{-6}. Fully diseased host tissue produced a comparable number of colonies at dilutions of 10^{-6}.

Two progeny tests were made with cultures derived from single colonies obtained from cotyledons which showed different reactions in the previous test. On nutrient agar, 14 of 18 viable cultures produced

either large or small colonies (see Table 27). These included cultures from four large wild-type colonies that stemmed from variants of small colonies (refer to cultures 60-13 . . . #1L and #2L; and 60-16 . . . #18L and 19L in Table 27. On nutrient agar with dextrose, however, quite

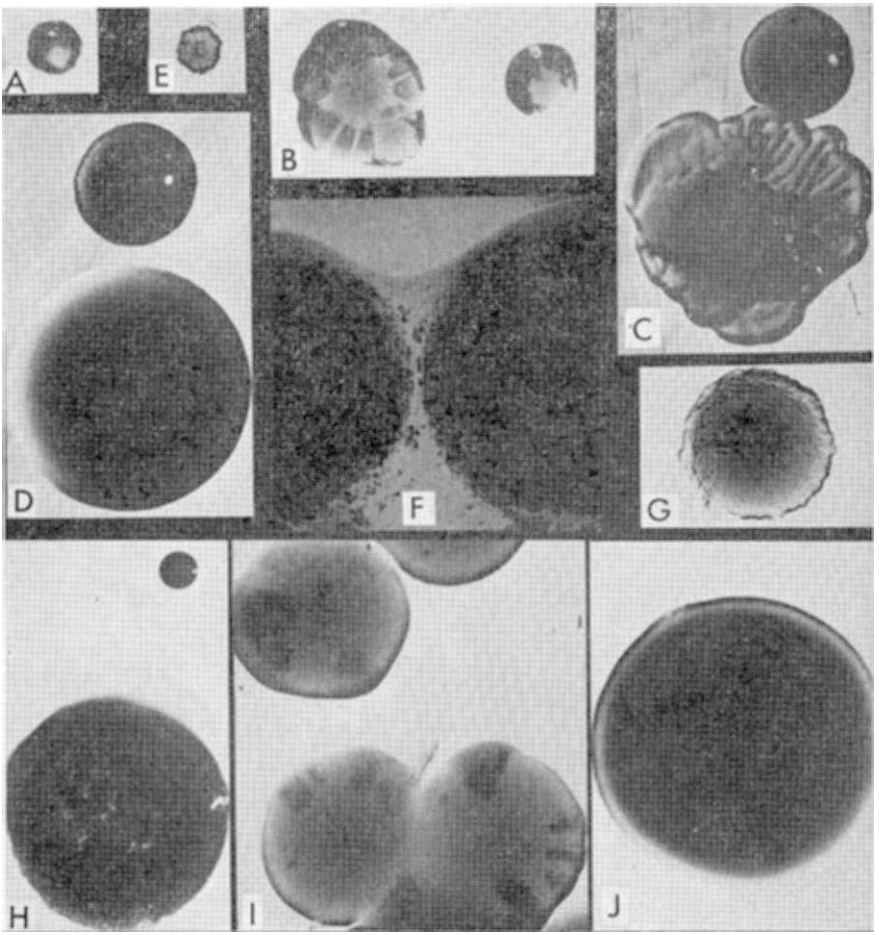


Fig. 12. Some of the variant-colony types of *X. malvacearum* on nutrient agar and on nutrient agar with dextrose. (A) to (D) inclusive are variant colonies on nutrient agar. (E) to (J) inclusive were from single colonies isolated from a seedling of Acala 44 inoculated with a small-colony variant that was nonpathogenic (Culture NA3-5 L-4s in Table 26). (E) and (F) are transfers from the same small rough colony grown without dextrose (E) and with dextrose (F), and (G) and (H) represent transfers from a second small rough colony grown without dextrose (G) and with dextrose (H). Only transfers from (F) were fully pathogenic. The sectored and large wrinkled types were least stable. Photographed after 10 days growth; X8.

different results were obtained (Table 28). Some cultures which produced small or intermediate colonies on nutrient agar gave rise to large colonies that produced abundant slime on nutrient agar with sugar. For some of these cultures the pigmentation was extremely granluar; for others it was normal in appearance, while others had mosaic patterns or sectors. Other cultures produced large, or small colonies on both media. Two cultures (60-14 . . 1 & 2, Table 28) gave rise to small colonies on nutrient agar and different colony types on the same medium with dextrose while others were stable (see Figure 13).

A second pathogenicity test with reisolations from host tissue was made with cultures derived from single colonies from the test represented in Table 28. The results given in Table 29 are fairly comparable to those obtained in the first pathogenicity test (see Table 26). The host reaction to cultures from small or intermediate colonies eventually became slightly or fully susceptible and reisolations indicated that reversion to wild type had taken place. On the other hand, two of the wild-type cultures (50-13 . . . #1L-1L-1L and #1s-1s-1L) which stemmed from a small-rough culture that was non-pathogenic, were fully pathogenic; but, reisolations from infected tissue in each instance showed that there had been some reversion to the small colony types.

The pathogenicity of the small-colony variants of *X. malvacearum* resembled that of the colony variants of *X. phaseoli* studied by Corey and Starr (15). The ability of a culture to produce slime or polysaccharide definitely appeared to be associated with pathogenicity. The small-rough colonies were non-pathogenic, or nearly so, while the intermediate types were intermediate. There was, however, a rapid reversion of most of the variants to the normal mucoid-colony type when placed in susceptible host tissue. The normal types appeared to have a very definite selective advantage over the aberrant types. Corey and Starr (15) on the other hand, found that the numbers of cells were similar for variant and normal type cultures of *X. phaseoli* after 6 days in bean-leaf tissue. In the present study, isolations after 16 days indicated that normal types had produced up to 10^4 times as many cells as the small-rough variants.

Segregation of unstable heterocaryotic cells of *E. coli* was shown by Witkin (67) to be responsible for new genotypes in irradiated cultures. She concluded that colonies showing sectors for non-lactose fermentation were derived largely from multinucleate heterocaryotic cells while non-sectored colonies originated from uninucleate cells. However, a secondary mechanism for variability was demonstrated and was postulated to be due to delayed mutation, to genetic recombination of some kind,

or to segregation of extranuclear particles. Whatever the nature of this secondary sector-producing event, it occurred during the first two divisions following irradiation. Presumably, the unstable heterocaryotic cells resulted from mutations which in some manner at first interfered with normal cell division but not with the division of the nuclei.

Although no attempt was made to identify multinucleate cells in *X. malvacearum,* the behavior of both the small-colony variants and

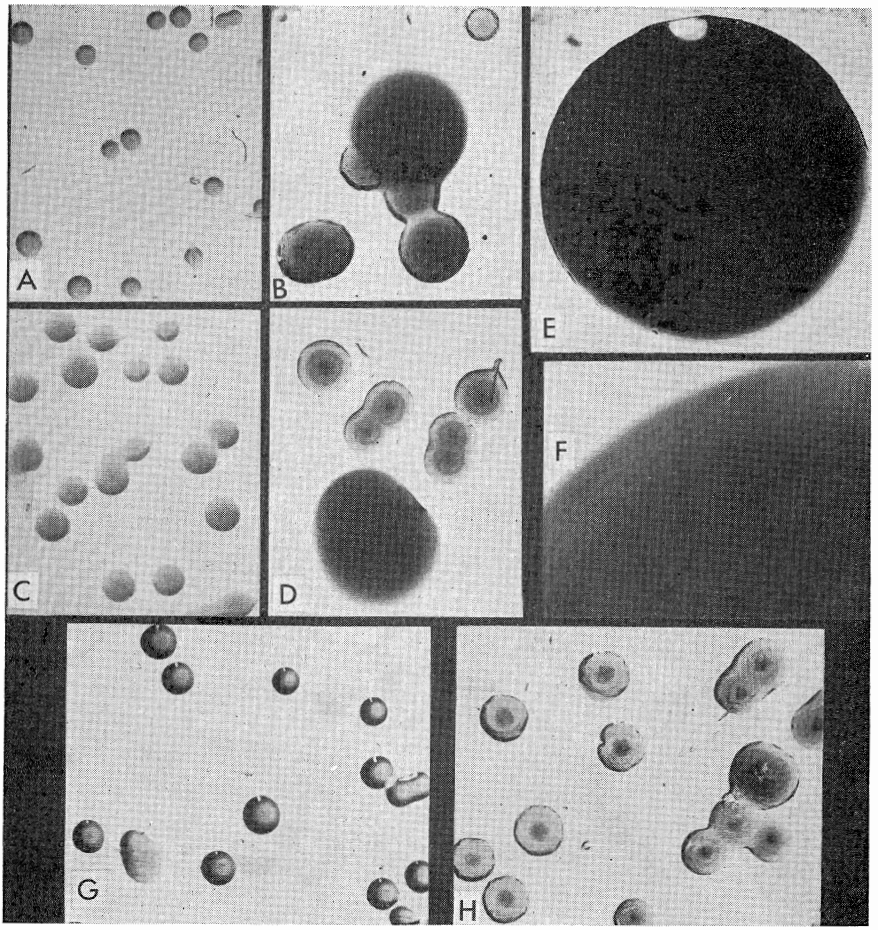


Fig. 13. Ten-day-old colonies of *X. malvacearum* from 3 different small-colony-variant cultures on nutrient agar (A), (C), and (G) and a normal culture on nutrient agar (E). Colonies from the same small-colony cultures on nutrient agar with sugar (B), (D) and (H) and from the normal culture (F). Note segregation for slime producing colonies in (B) and (D) on nutrient agar with sugar. X8.

also of the pathogenic mutants suggests that mechanisms for variability similar to those described by Witkin (67) may be present. This is indicated by the delayed development and subsequent instability of the original small-colony variants as contrasted to relatively normal development and stability in later generations, and also by the delayed development of the mutants that produced susceptible-type lesions in previously resistant leaf tissue. Also many of the pathogenic mutants which were unstable at first, were more stable later, and these also incited lesions more rapidly in later generations.

Influence of Environmental Factors on Disease Expression

Pathogenicity tests in both the field and greenhouse have indicated that the upland differentials, especially, tend to become more resistant at high temperatures and low relative humidities. The leaves of the susceptible variety Acala 892 were almost uniformly resistant when inoculated with *X. malvacearum* during a hot dry period in the fall of 1957. Young bolls were susceptible, but older ones tended to be resistant. The plants did not lack moisture since water had been supplied by irrigation. Upland lines with known blight-conditioning genes in the same planting also tended to be resistant to races that later attacked seedlings and adult plants in other plantings.

Table 30 shows the bacterial blight reactions of adult cotton plants grown in the greenhouse at high temperatures and of plants grown at more moderate temperatures in the field. Greenhouse plants were growing in soil beds in which moisture and fertility levels favored rapid growth. The upland differentials were blooming, while the *G. barbadense* lines had flowered very sparsely, but had made very rank growth. Fully expanded leaves on the upper part of the plant were inoculated with broth cultures of 6 races by the syringe method with concentrated inoculum (about 5 x 10^6 cells per ml). All of the upland differentials developed necrotic reactions including those which were normally susceptible. Later some water-soaking appeared around the necrotic areas on some of the susceptible lines (see Fig. 14). The *G. barbadense* lines developed typical reactions for the different races, except that race 7 attacked B_2, and race 1 was not pathogenic. Five of the seven cultures were tested later in the field at temperatures considerably below those in the greenhouse. The differentials that reacted as R- or S- in the greenhouse were fully susceptible except for the reaction of 8-3 to race 2, which was S-.

A greenhouse test with seedlings of the upland differentials was made at relatively high temperatures (about 85°F to 90°F) and at

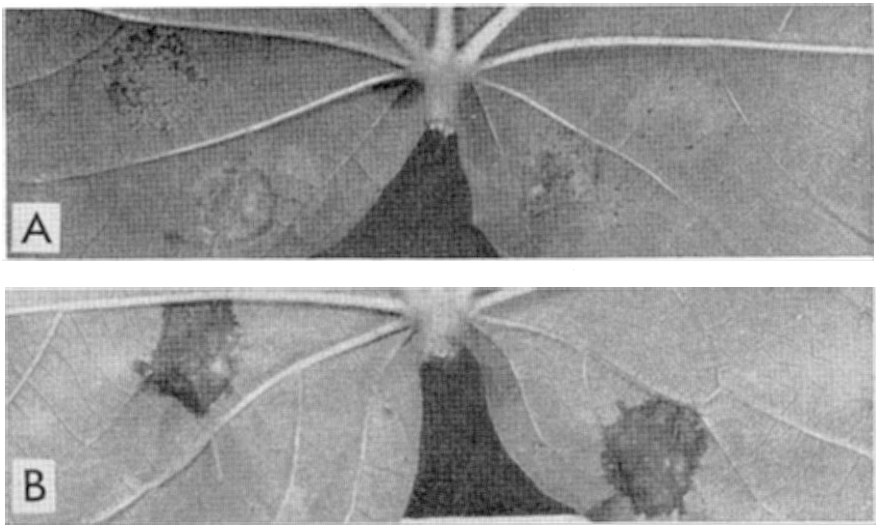


Fig. 14. (A) Partially resistant reactions on an Acala 44 plant grown at high temperatures. (B) Fully susceptible reactions for comparison.

relatively low temperatures (about 65°F to 70°F). Cultures of six races were used, four of which had been maintained as separate but duplicate cultures. The inoculum was grown in broth shake-cultures and used at a concentration of about 10^6 cells per ml. The seedlings were all started at the warmer temperatures.

Disease reactions are shown in Table 31. At the lower temperatures 8-3 (b_7) was attacked by three races (3, 4 and 6) which normally caused resistant reactions. The cultures of races 2, 7 and 12 had lost pathogenicity for some differentials and tended to react about the same at both temperatures. The lesions dried up faster at the higher temperatures, and there was more necrosis associated with all of the cultures, which indicated that the tissues were somewhat more resistant. Although seedlings were used in this experiment, the results seemed to confirm the previous field and greenhouse observations that high temperatures and relatively low humidity tend to produce tissues which are more resistant to *X. malvacearum*.

In a third greenhouse test with a culture of race 6, cotton seedlings were incubated at high temperatures (about 70°F to 100°F), but at two relative humidities (about 20% to 50%) and (60% to 90%). Replicated flats of 50 seedlings each of Acala 44 (susceptible) and CR4 ($B_{L?}$ gene) were inoculated by the wounding method as soon as the cotyledons were expanded. After a month, the CR4 seedlings at the

lower humidity range graded S- to R while at the higher humidities they graded S to S-. The Acala 44 was fully susceptible at the higher humidity range but graded S to S- at the lower.

These studies substantiate many observations that high relative humidity is conducive to the development of bacterial blight.

Persistence and Buildup of Races in the Field

The previous studies have clearly shown that *X. malvacearum* is a variable organism and that new races are constantly being produced in the field as well as when the bacterium is cultured in the laboratory. Just how much of a threat these variants might be to the maintenance of resistant varieties was, of course, not known. Race 2, however, rapidly became a problem in New Mexico when resistant Acala was introduced (14). Field tests designed to study the persistence and spread of new races were started in 1957 and continued through 1960.

Studies in 1957.—Eight upland and seven of Knight's differentials were planted between spreader rows of Acala 44 in 14 blocks isolated from each other by barriers of the resistant strain, 8-3. Similar plantings were made at Chickasha and Stillwater, except that Knight's lines were omitted and the upland lines Mebane B1 and NMB9 were added at Chickasha.

At Stillwater, plants were inoculated with seven cultures comprising five races. These included: race 1 (from Texas); race 2 (from Texas); race 4; three cultures of race 6 (one of high virulence, one of intermediate virulence, and one of low virulence); and race 7. The same cultures were used at Chickasha except that race 3 was substituted for the race 6 culture of low virulence.

Both the spreader rows and the differential rows were inoculated when the plants had four to six true leaves. Cultures were grown in nutrient broth for approximately 27 hrs, diluted in water 1 to 200, and applied as a coarse spray which partially water-soaked the leaves.

The different races attacked the differential strains for which they were designated, but usually the plants of the susceptible spreader rows of Acala 44 were more severely infected than the differentials with blight-conditioning genes. However, race 7 was not fully pathogenic on Acala 44; the lesions were classed as grade 3.

The weather was relatively dry and hot through July, August, and part of September, so the plots were sprinkle-irrigated at three to four week intervals at Chickasha, and furrow-irrigated at Stillwater. At

Chickasha, the infected leaves were shed during August and the disease completely disappeared from all but the spreader rows in all of the plots. By the end of the season at Stillwater only the spreader rows and Knight's B_3 and B_5 lines were generally infected. A small amount of secondary infection was found on 8-3 and Knight's B_1 in the race 2 block, and on CR4 and 1-10B in one of the race 6 blocks .

A second inoculation made at Chickasha on September 20 caused leaf and boll infection, but was generally mild. Many of the leaves had mesothetic reactions, and appeared to have acquired considerable tolerance as compared to the reactions to the first inoculation.

Tests were made on seedling differentials with leaf-inoculum cultures obtained at Stillwater from Acala 44 at the end of the season. Inoculum from blocks representing each of the five races in each instance reacted as race 1. A second test was then made with leaf-inoculum cultures prepared from the infected cotyledons of Acala 44 of the first test. A few lesions developed on 20-3 from inoculum stemming from race 7. Further tests established that race 7 had been recovered.

Studies in 1958.—Race 3 (from Texas) and race 12 (from Uganda, Africa) were observed in isolation blocks on different strains of cotton throughout the summer of 1958 at St. Paul, Minnesota. With relatively frequent showers and mild temperatures both of these races maintained their pathogenicity for the differential strains. Race 12 was somewhat less severe on Knight's B_3 and B_2B_3 strains at the end of the season. Although race 3 caused mesothetic-type lesions on two resistant differentials, the disease did not build up on these differentials in the field.

Studies in 1959.—These studies have been partially outlined under the section on the nature of variability. Five streptomycin-resistant clones of races 2, 6, 7, 9 and 10 were used individually to inoculate isolated blocks of five upland differentials. Also an adjacent planting was inoculated with a mixture of race 1 cultures not resistant to streptomycin.

The weather during the summer of 1959 was especially favorable for bacterial blight development at Stillwater, Oklahoma, where these plantings were located. Over 30 inches of rain was recorded for July, late September and early October and most of the rains were accompanied by strong winds. A severe hail and rainstorm occurred at the end of August which favored disease development and in addition the plots were sprinkle-irrigated during dry periods occurring in August.

The five races maintained their identity (as well as resistance to

streptomycin) and each was recovered at the end of the season. However, race 10 lost in virulence for the 8-3 differential, but both races 7 and 10 remained virulent for 20-3. Severe boll damage occurred on the latter differential at the end of the season (see Fig. 15A). Although new races were recovered from "mutant" lesions and from infected bolls from previously resistant differentials, the buildup of new races was generally mild on upland cotton. However, this was not true for the *G. barbadense* differentials. Variants originated from race 1 and attacked Knight's B_1, B_2 and B_4 lines which were at first resistant. B_2B_3 was affected only very slightly, while B_2B_{6m} remained immune (see Fig. 15B).

Studies in 1960.—Two different types of studies were made in this season. At Stillwater, tests were patterned after those of the three previous seasons but were expanded both in numbers and kinds of differential strains. Four well-isolated blocks were planted with spreader rows

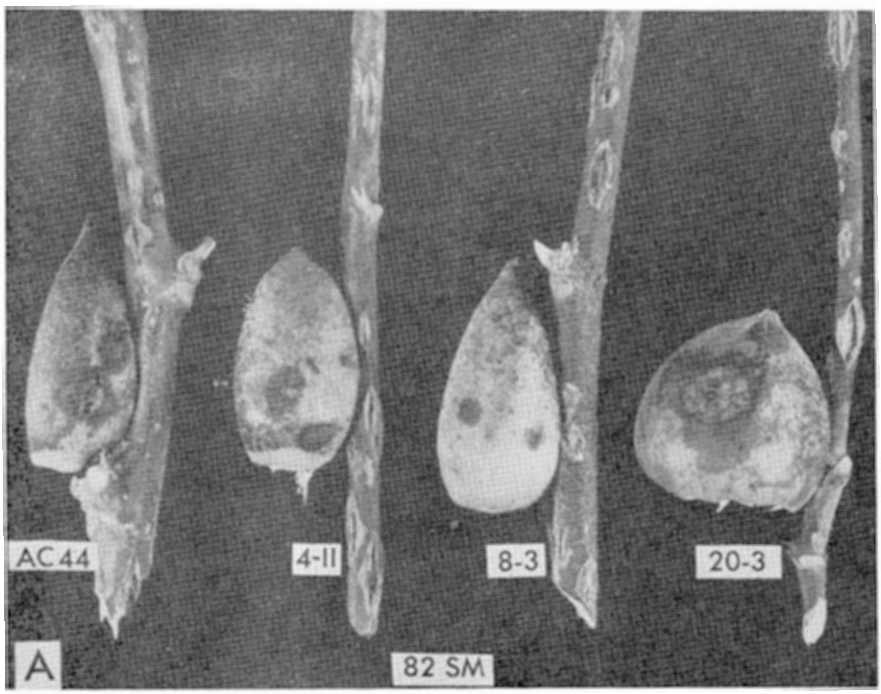
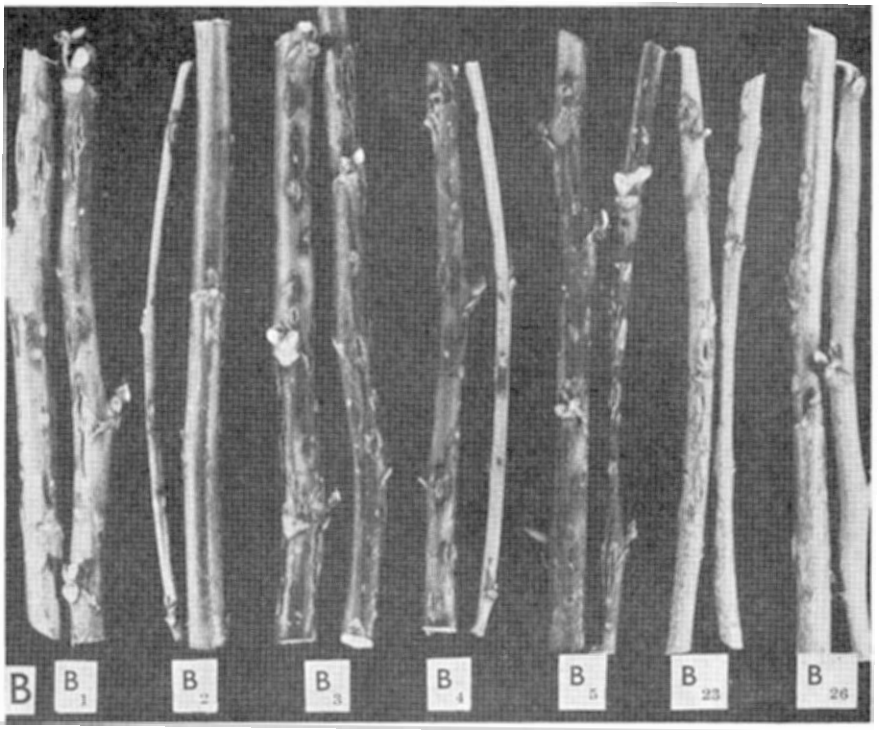


Fig. 15. (A) Boll and stem injury at the end of the season from plants in a plot inoculated with race 7. Note that 8-3 which was resistant at the beginning of the season shows some injury. Although the stems were damaged by hail only Acala 44 showed extensive stem lesions. (B) (Opp. page) Stem lesions on Knight's *G. barbadense* lines in a plot originally inoculated with race 1. B_2B_3 showed only mild injury and B_2B_{6m} remained resistant.



as previously, and each inoculated with a different race or mixture of races when the plants had four to six true leaves. Races 1 and 2 were each used to inoculate a separate block as was a mixture of races 6 and 9 and a mixture of races 4, 7 and 10.[13] A composite of nine different cultures was used for races 6 and 9, and 15 difference cultures for races 4, 7 and 10.

Rains were accompanied by less wind and were more evenly spaced than in the three previous summers. Irrigation water was applied by sprinkling during one dry period in August. September and the first half of October were relatively dry and somewhat less favorable for disease development than the earlier part of the season. Although a favorable season for disease development, 1960 was not as favorable as 1959 at Stillwater.

Disease readings were taken two to three weeks after inoculation and at the end of the season. As the plants approached maturity and

[13]The cultures of races 1 and 2 were obtained in the spring of 1960 from Dr. T. E. Smith of New Mexico State University.

drier weather prevailed the upland differentials including the susceptible Acalas became more tolerant to bacterial blight as was also true of Knight's B_3 and B_2B_3 lines.

Sharply defined resistant reactions were incited by race 1 at the start of the season (see Table 32 and Fig. 3) on 17 of 23 upland lines. Mixed or mesothetic infection types (classed here as intermediate) were produced on 10 of 24 plants of 20-3 and on all except two plants, which were fully susceptible, of the 20-3 backcross of Acala 44. Plants of B_2, B_3 and B_4 of Knight's lines were at first mostly intermediate. By the end of the season the Acala backcross was considered susceptible as was also Knight's B_1, B_3, and B_4. However, the Acala backcross had fewer lesions than Acala 44. Race 4 was obtained from lesions of the backcross and race 2 from Knight's B_1. Isolates from other differentials were not identified as to race.

Race 2 incited resistant infection types on 1-10B, and on crosses possessing $B_{L?}$ and B_N as was expected (see Table 33). Acala 1517BR1 with b_7 resistance and Knight's B_1 were typically susceptible. Other uplands including Rex, and Austin with the b_7 gene were susceptible while young (see Fig. 3) but became intermediate when older. Plants of the differential 8-3 were mostly resistant at the end of the season, as was the strain 18-37.

The mixed culture composed of races 6 and 9 (Table 34) was more virulent than races 1 or 2 or the other mixed cultures as judged by the size and nature of the lesions produced on Acala 44. However, this difference was less pronounced at the end of the season. The plants of the differentials believed to possess $B_{L?}$ resistance (CR4, 4-11, 17-3, 8373, 2B4, and Mebane B1) were mostly intermediate after inoculation and then became resistant as the season progressed. The backcross (20-3 x 4 Ac44) F_4 with B_N resistance appeared to be segregating as were also CR4, Mebane B1 and (20-3 x CR4) F_4 7-11.

The mixed culture composed of races 4, 7 and 10 incited susceptible or mesothetic reactions on all of the Oklahoma lines including those presumed to have two major genes for resistance (see Table 35 and Fig. 5A). Two single plants, one of strain 6-77 and the other of the cross (20-3 x 17-3) F_4 7-5 were the only resistant plants at the start of the season in this entire group. Nevertheless, by the end of the season many of the susceptible plants had become intermediate and the intermediate plants resistant.

Two of the nine upland breeding lines obtained from Dr. L. S. Bird of Texas A & M College were resistant, except for two plants in

one of the lines. The line LCBV59 resulted from a complex cross and possessed the genes B_2, B_3 and b_7 according to Dr. Bird.[14] All of the Texas lines acquired greater tolerance as the season progressed.

A single line of *G. herbaceum* was immune until late fall when some small necrotic vein lesions appeared.[15] Isolation did not yield *X. malvacearum,* nor did leaf-inoculum cultures incite infection on Acala 44.

Two of five lines of *G. arboreum* were classified as resistant, two as intermediate, and one as segregating to the race mixture; but, by the end of the season all five lines were classed as resistant.

The parent races 4, 7 and 10, were recovered at the end of the season from Acala 1517C which was completely removed from differentials with B_N resistance indicating that these races had survived on this susceptible variety. However, of 23 single-colony cultures from Acala 1517C, eight were identified as races other than those of the original mixture. Seven of these were race 5 and one was race 1.

The second study in 1960 consisted of naturally infested plantings at three locations in southwestern Oklahoma in fields where blight had been severe in previous seasons. Both upland and *G. barbadense* differentials were included and each resistant line was interspaced with two spreader rows of a fully susceptible variety. Also in each instance the planting was adjacent to or surrounded by a fully susceptible variety of cotton, and each planting was irrigated as was the surrounding cotton. The irrigations and several severe summer storms at each location favored general blight infection.

The disease reactions of the differentials were characteristic of race 1 during the early part of the growing season. However, before the season was over it was apparent that additional races had developed. The percentages of infected plants on October 31 and November 1 are shown in Table 36. Two upland lines 8-3 (b_7) and 20-3 (B_N) remained resistant at the three locations as did Knight's B_2B_3 and B_2B_{6m}. The upland backcrosses on Acala with b_7 or B_N resistance, nevertheless, became infected. The pattern of blight development and the percentages of plants infected in the different tests indicated that new races were responsible. Extensive isolations and pathogenicity tests were not undertaken. Race 2, however, was identified from Acala 1517BR-1 (b_7)

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[14]Personal communication.

[15]The seed of *G. herbaceum* and *G. arboreum* were obtained from Dr. J. R. Meyer of the U. S. Cotton Field Station, Stoneville, Mississippi.

and Knight's B_1, and races 4 and 7 from (20-3 x 4 Ac44) F_4. Other races were probably also present.

These field studies covering four seasons show that new races can maintain themselves under favorable conditions as in 1958 at St. Paul, Minnesota or as in 1959 and 1960 at Stillwater, Oklahoma. However, in a less favorable season like 1957 at Stillwater and Chickasha, Oklahoma, the new races may not survive to any appreciable extent and may revert to race 1.

On the other hand, studies with race 1, under conditions that favored disease development, showed that new races readily build up presumably from mutants screened on certain of the differentials. The buildup of new races was particularly striking on Knight's *G. barbadense* lines with single genes and on upland lines with major blight-conditioning genes in an Acala background.

These studies also indicate that Knight's B_{6m} gene in combination with B_2 is the least apt to be attacked by new races of the breeding lines thus far tested. Also that most of the upland lines possess genes which confer a certain amount of tolerance to all of the races of bacterial blight especially after the seedling stage.

DISCUSSION

The results show that new pathogenic races of *X. malvacearum* probably will be a serious problem in the maintenance of bacterial blight-resistant varieties of cotton. The problem has, in fact, already arisen with race 2 in New Mexico and western Texas (14). Varieties which have resistance conferred only by one major-blight-conditioning gene appear to be especially susceptible to the buildup of new races. The Acala and *G. barbadense* genotypes definitely favored new races in the persent studies. Nevertheless, other genotypes which do not have a complex of modifying genes probably also will be readily attacked.

The designation of 10 new races in addition to races 1 and 2 indicates the pathogenic diversity of *X. malvacearum*. Further studies will undoubtedly show this bacterium capable of producing numerous races in addition to those described in these studies.

Considerable hope of avoiding the buildup of new races, however, appears to lie in the use of Knight's modifying gene, B_{6m}, in combination with major genes. Also the modifying or minor genes found in a number of uplands definitely appear to help inhibit the buidlup of

new races when used in combination with the major genes. Perhaps of particular significance is the fact that Knight's B_2B_{6m} line was not attacked.

Mutation would appear to account for much of the genetic variability encountered in *X. malvacearum,* as it has now been demonstrated to account for variation in many types of haploid and somatic cells (13). Other genetic mechanisms such as heterocaryosis and recombination might also be involved, but these were not proven. Nor, can the possibility of adaptive enzymes (54) be ruled out as a mechanism to account for some of the variability observed in *X. malvacearum.*

Mutations for colony variation and other specific characters have been studied extensively in haploid cells of the fungi (63), and during recent years mutations for resistance to antibiotics have received a great deal of attention in the bacteria. Recent studies have shown that mutations for cell morphology and other characters occur in single-cell clones of populations of somatic animal-tissue cultures (30). Also cancerous cells of animals have been shown to produce mutants that resist chemotheroputants (31). Although very specific types of mutations are apparently being produced continuously by many if not all different kinds of dividing cells, their frequencies are so low that usually they go undetected (13). However, with populations of single cells a mutant or a group of related mutants may immediately become the predominant or only type when screened or isolated in a selective manner.

In the present study with *X. malvacearum,* this sudden change in the makeup of a population was very striking for streptomycin resistant mutants which were screened on agar with the antibiotic. The same type of phenomenon was demonstrated for the origin of new pathogenic races from genetically-marked single-colony clones of *X. malvacearum.* The previously resistant lines of cotton served as the selective media in the latter instance.

Of interest in relation to mutations for new races in *X. malvacearum* are the studies of Graham et al. (26) with populations of haploid sporangia derived from single-zoospore cultures of *Phytopthora infestans* (Mont.) de Bary. New fully pathogenic races were derived by alternate passages through resistant and susceptible varieties on excised leaves of potatoes or tomatoes. At first the new mutants were able to sporulate only to a limited degree on juvenile or senescent leaf tissue of resistant varieties. The authors suggest that such tissues were more susceptible and permitted the fungus to grow and mutate, and the "residual" resistance had a selective effect upon the heterocaryon created by mutation.

New races were not obtained from parallel series which were maintained an equal or greater number of generations on susceptible hosts.

Whether the mutations occurred before or after entry into the resistant host does not appear to have been demonstrated, as the susceptible hosts would not be expected to screen or select the new mutants. Thus mutants for increased pathogenicity may have been present in low numbers but not detected. Nor, have the present studies with *X. malvacearum* demonstrated whether the mutations for increased pathogenicity occurred before or after entry into the host, although the relative frequencies would indicate that the mutations occurred before entry. It has been known for a number of years, however, that mutations for resistance to phages in bacteria occur before contact (49, 53).

The development of new mutants in resistant leaves of cotton appears comparable to the development of the new poorly sporulating mutants of *P. infestans* in the juvenile or senescent tissue of resistant potatoes or tomatoes (26). With *X. malvacearum* the "mutant" lesions usually developed more slowly and only in succulent tissues under favorable environmental conditions. This suggests that pathogenicity of the mutant cells was not fully expressed until considerable time after entry into the host. If this should hold true for all mutations for pathogenicity of *X. malvacearum* it might constitute a potential weakness in the pathogen and help explain why races did not build up on some lines of cotton. A somewhat similar situation was observed for newly transformed cells of *X. phaseoli* by Corey and Starr (17). No streptomycin-resistant colonies developed unless the cultures were permitted to grow for several hours in the absence of streptomycin. Replica-plating tests indicated that the newly transformed cells were genotypically resistant but phenotypically susceptible.

Delayed expression of induced mutations for resistance to phage and to streptomycin, and certain nutritional deficiencies has been observed (19, 20, 22, 43). As many as 12 cell divisions were required before all of the mutations were expressed. The slow emergence of the original small-varient-type colonies of *X. malvacearum* and of part of the streptomycin-resistant colonies appears to be the same type of a phenomenon.

Single-colony isolates from the "mutant" lesions produced by *X. malvacearum* frequently represented several different races. If the parent cells are unstable heterocaryons following mutation as suggested by Witkin (67) for lactose minus mutants in *E. coli* this might account for the different races and possibly also for the reduced pathogenicity

that was first evident. Also the unstable colony-types of *X. malvacearum* which segregated for both colony morphology and for pathogenicity might have originated as heterocaryons.

The added resistance produced by Knight's B_{6m} and other modifiers appears to be of a general type resistance since all races reacted alike. The upland differentials such as 20-3, 8-3, CR4 and 4-11 each appeared to have a minor gene complex which conferred a low level of field resistance to adult plants, but the "mutant" lesions were not completely inhibited. However, when $B_{L?}$ and B_N were combined, or B_2, B_3 and b_7 as in some of Bird's lines, some individual plants were resistant to a mixture of races that attacked lines possessing each of the major genes individually. It appeared that favorable combinations with modifying genes were responsible for the differences. Bird has consistantly subjected his lines to a mixture of races 1 and 2 and has emphasized the importance of minor genes.[16]

Bird and Hadley (6) showed that two varieties from the Mississippi Delta, Stoneville 2B and a variety of Deltapine, possessed modifying genes while a variety of Acala did not. Both of the Mississippi varieties have a certain amount of field resistance. The fact that bacterial blight is not more of a problem in the older parts of the U. S. Cotton Belt may be due at least in part to the level of field resistance possessed by many of these varieties which have been derived through numerous generations of selection under disease conditions.

It remains to be seen how much influence the environment will have on the expression of the resistance due to both major and modifying genes like Knight's B_2B_{6m}. Last (48) has recently shown that the natural spread of *X. malvacearum* was more limited for varieties with the B_2B_{6m} genes than for varieties with B_2 or B_2B_3. The present studies showed that low relative humidity and high temperatures enhanced resistance, while lower temperatures and high humidity favored the pathogen. However, adequate tests were not made with lines possessing combined resistance like B_2B_{6m}. The use of race mixtures which attack all of the major genes as suggested by Graham et al. (26) as a means of selecting for field resistance to *P. infestans* in potatoes may be very useful in screening for increased resistance to *X. malvacearum*.

Combined resistance resulting from major and modifying genes should prove of real value in inhibiting the buildup of new races.

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[16]Personal communications.

SUMMARY

Ten new races of *X. malvacearum* were described on the basis of their disease reactions on eight differential lines of cotton. These are in addition to the previously known races 1 and 2 which were retained, and are also distinguished by the eight differentials.

Clones resistant to streptomycin, penicillin, or several other antibiotics were readily screened from relatively large populations of cells of *X. malvacearum* on solid media containing the antibiotic. Likewise, colony variants were isolated from populations of *X. malvacearum* on solid media after an incubation period of 10 to 20 days. Similarly, new pathogenic races were isolated from genetically marked single-colony clones by screening populations of cells on previously resistant varieties of cotton. Mutation and segregation from heterocaryotic cells are suggested as probable mechanisms to account for the genetic variability encountered.

"Mutant" lesions usually developed more slowly and only in succulent tissues under favorable environmental conditions. Mutations for increased pathogenicity, colony-type variation and in some instances for resistance to antibiotics did not appear to be fully expressed until after several cell divisions. Nuclear segregation is suggested as a mechanism to account for this delay.

Many of the mutations for pathogenicity tended to be unstable and in this respect appeared comparable to multiple-step mutations for resistance to antibiotics like penicillin and terramycin. Under unfavorable environmental conditions new pathogenic races reverted to race 1 presumably by back mutations and natural selection.

New races of *X. malvacearum* built up readily on the Acala and *G. barbadense* genotypes possessing major-blight-conditioning genes, but without modifiers or a minor gene complex.

The *G. barbadense* line, B_2B_{6m}, developed by Knight, was not attacked by new mutants. This combination of genes and probably other combinations of major and modifying gene should prove useful in breeding varieties that resist new races.

Minor genes possessed by a number of upland lines confer a low level of field resistance to adult plants and enhance the resistance of major genes.

Screening with mixtures of races which attack the major genes is suggested as a means of identifying and maintaining gene combinations which confer resistance to new mutants.

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Table 1. Source and major blight-conditioning genes of differential lines of cotton

Designation of line	Major blight gene and source	Inheritance determined by
	Knight's G. barbadense lines	
B_1	B_1; transferred from upland acclimatized in Uganda	Knight and Clouston (42)
B_2	B_2; same source as B_1	(42)
B_3	B_3; transferred from *G. hirsutum L.* var. *punctatum* (Schum.) Hutchinson et al.	Knight (37)
B_4	B_4; transferred from *G. arboreum* by chromosome doubling	Knight (38)
B_5	B_5; transferred from a perennial *G. barbadense,* Grenadines white pollen	Knight (39)
B_2B_3	B_2 and B_3 combined by Knight	
B_2B_{6m}	B_2 as above; and B_{6m} an intensifying gene tranferred from *G. arboreum* and not expressed when alone	Knight (40)
	Upland (G. hirsutum) lines	
Acala 44	Susceptible; developed in Arizona by E. H. Pressley	
1-10B	B_{In}; selected from an unidentified susceptible upland variety; originally designated B_I, but this was changed because of confusion with Knight's B_1	Green and Brinkerhoff (27)
CR4	$B_{L?}$; selected from a susceptible upland breeding line, CA122 (9)	Not published (tentative)
8-3	b_7 or an allele selected from a susceptible upland variety, Half and Half (9)	Green and Brinkerhoff (27)
20-3	B_N; selected from Northern Star, a susceptible upland variety (9)	Brinkerhoff (not published)
20-8	B_N; a sister line of 20-3 (9)	Green and Brinkerhoff (27)
4-11	$B_{L?}$; a sister line of CR4; it has usually reacted as CR4 (9)	Not published (tentative)
Mebane B1	$B_{L?}$; selected by Hooton (58) from Mebane, a susceptible upland; it has usually reacted as CR4	Not published (tentative)

Table 1. Continued

Designation of line	Major blight gene and source	Inheritance determined by
Stoneville 20	b_7, or B_7; selected by Simpson (60) from Stoneville 2A, a susceptible upland. In *G. barbadense* background reacts as a partially dominant gene; in upland reacts as a recessive or nearly so	Knight (41) Blank (7) Green and Brinkerhoff (27) Bird and Hadley (6)
BBR-3	Probably b_7; selected by Moosberg from Stoneville 2B	Moosberg (51)
6-77	B_S; selected from Stormproof No. 1, a susceptible upland	Green and Brinkerhoff (27)

Table 2. A summary of leaf grades used in classifying blight infection in the field

Grade	Host reaction	Description of infection type or types
0	Immune	No visible lesions
1	Resistant	Dry pin-point to small round lesions
2	Resistant	Dry small angular lesions between veins; sometimes dry vein lesions
3	Mildly susceptible	Small to intermediate, angular, wet lesions between veins; intermediate to water-soaked vein lesions
4	Susceptible	Large, water-soaked, angular lesions that turn black on drying; large water-soaked vein lesions
Mesothetic reactions		
0.1, 0.2 & 0.3	Resistant	Predominantly immune; with a few lesions of infection types 1, 2, 3; or, more than one type may be present
1.2 & 1.3	Resistant	Predominantly type 1; with type 2, or types 2 and 3 lesions present
2.3	Intermediate	Predominantly type 2, but with type 3 lesions also present; type 1 lesions may also be present especially if the environment does not favor disease expression

Table 3. Bacterial blight reaction of cotyledons, leaves and bolls that were inoculated with concentrated bacteria suspensions

Grade	Host reaction	Description of infection type
O	Immune	No visible reaction
R	Resistant	Chlorosis, or necrosis of inoculated tissue; necrotic tissue usually dry and brown
R-	Resistant	Necrosis of inoculated tissue; brown but remains somewhat water-soaked especially along veins
S-	Mildly susceptible	Partial necrosis of inoculated tissue with some water-soaking usually at margin of necrotic tissue; or no necrotic tissue with little water-soaking
S	Susceptible	Water-soaking of inoculated tissue; often spreading beyond inoculated area especially along veins; may become systemic above inoculated leaf

Table 4. Disease reactions of upland seedling differentials to 167 cultures obtained from blight-susceptible varieties in the United States[a]

Number of cultures	Lines which differentiate races 1 and 2[b]			Additional differentials		
	Ac44 (Sus.)	8-3 (b_7)	CR4 ($B_{L?}$)	1-10B (B_{In})	20-3 (B_N)	Race[c]
			Oklahoma			
78	S	R	R	R	R	1
7	S	S	R	R	R	2
38	S	R	R	S	R	Other
3	S	R	S	S	R	Other
1	S	R	S	S	S	Other
			Texas			
7	S	R	R	R	R	1
1	S	S	R	R	R	2
5	S	R	R	S	R	Other
			Arkansas			
4	S	R	R	R	R	1
			Mississippi			
5	S	R	R	R	R	1
			North Carolina			
4	S	R	R	R	R	1
3	S	R	R	S	R	Other
			Missouri			
8	S	R	R	R	R	1
1	S	R	R	S	S	Other
			Arizona			
1	S	R	R	R	R	1
1	S	R	R	R	S	Other
Number pathogenic	167	8	4	51	20	

[a] Data are from tests made over the period of 1955-1960.
[b] These differentials give blight reactions comparable to the Cotton Disease Council's differentials: Acala 44 reacts as Stoneville 2B, 8-3 as Stoneville 20, CR4 as Mebane B1.
[c] "Other" indicates that the cultures react differently than races 1 and 2.

Table 5. Bacterial blight reaction of 5 upland seedling differentials to 202 cultures obtained from previously resistant plants[a]

Number of cultures	Lines which differentiate races 1 and 2		Additional differentials			
	Ac44 (Sus.)	8-3 (b_7)	CR4 ($B_{L?}$)	1-10B (B_{In})	20-3 (B_N)	Race[b]
40	S	R	R	R	R	1
18	S	S	R	R	R	2
45	S	R	S	S	R	Other
3	S	S	S	S	R	Other
21	S	R	R	S	R	Other
40	S	R	R	R	S	Other
14	S	R	R	S	S	Other
12	S	R	S	S	S	Other
9	S	S	S	S	S	Other
Number pathogenic	202	30	69	110	75	

[a] Summary of seedling tests made in the greenhouse or in a temperature-control room between 1956 and 1960.

[b] "Other" as used here means different from races 1 and 2.

Table 6. Bacterial blight reactions of upland and G. barbadense seedling differentials to 2 cultures obtained from Mexico and 8 cultures from Africa

Designation of culture	Lines which differentiate races 1 and 2			Additional differentials								Race[a]
				Upland		G. barbadense						
	Ac44 (Sus.)	8-3 b_7	CR4 $B_{L?}$	1-10B B_{In}	20-3 B_N	B_1	B_2	B_3	B_4	B_5	B_2B_{6m}	
						Iguala, Mexico						
56-96	S	R,S[b]	R,S	R,S	R							1, other
56-97	S	R	R	R,S	R							1, other
						Normbye, Uganda, Africa						
XL57/21	S	S	S	S	S	S	S	S	S	S	R	other
Albar 51	S	R	S	S	R	R	R	R	R	S	R	other
						Samaru, Zaria, Northern Nigeria, Africa						
	S	S	R	S	R	R	R	R	R	R	R	other
						Shambat, Republic of Sudan, Africa						
BAR14/22#1	S	R	R	S	R	R	R	S	R	S	R	other
BAR14/22#2	S	R	R	R	R	R	S	S	R	S	R	other
BAR14/22#3	S	R	R	S	R	R	R	R	R	S	R	1[c]
						Portuguese E. Africa						
G.b. #1	S	R	R	R	R	R	S	S	S	S	R	1[c]
G.b. #2	S	R	R	R	R	R	R	R	R	S	R	1
G.h. #1	S	R	R	R	R	R	R	S	R	S	R	1[c]

[a] "Other" means different from races 1 and 2.
[b] Two reactions for the same differential mean that part of the seedlings reacted as resistant and part as susceptible.
[c] These are race 1 on the basis of the Cotton-Disease-Council's upland differentials, but different from race 1 on Knight's lines.

Table 7. Disease reactions of upland and G. barbadense seedlings and race designations for 118 cultures that represent pathogenic differences encountered in X. malvacearum

Numbers of cultures tested	Lines which differentiate races 1 and 2			Additional differentials								Race
				Upland		G. barbadense						
	Ac44 (Sus.)	8-3 (b_7)	CR4 ($B_{L'}$)	1-10B (B_{In})	20-3 (B_N)	B_1	B_2	B_3	B_4	B_5	B_2B_{6m}	
39	S	R	R	R	R	R	R	S	R	S	R	1
10	S	S	R	R	R	S	R	S	R	S	R	2
22	S	R	R	S	R	R	R	S	R	S	R	3
10	S	R	R	R	S	R	R	S	R	S	R	4
5	S	R	R	S	S	R	R	S	R	S	R	5
8	S	R	S	S	R	R	R	S	R	S	R	6
11	S	R	S	S	S	R	R	S	R	S	R	7
3	S	S	S	S	R	S	R	S	R	S	R	8
5	S	R	S	S	R	R	S	S	R	S	R	9
2	S	S	S	S	S	S	R	S	R	S	R	10
1	S	S	S	S	S	S	S	S	R	S	R	11
2	S	S	S	S	S	S	S	S	S	S	R	12

Table 8. The disease reactions of 8 cotton lines that are proposed as differentials to distinguish 12 races of X. malvacearum

Race designation	Differential lines							
		G. hirsutum				G. barbadense		
	Ac44 (Sus.)	1-10B (B_{In})	Meb B1 ($B_{L'}$)	Ac 1517 BR-1 (b_7)	20-3 x 4 Ac44 (B_N)	B_2	B_4	B_2B_{6m}
1	+	—	—	—	—	—	—	—
2	+	—	—	+	—	—	—	—
3	+	+	—	—	—	—	—	—
4	+	—	—	—	+	—	—	—
5	+	+	—	—	+	—	—	—
6	+	+	+	—	—	—	—	—
7	+	+	+	—	+	—	—	—
8	+	+	+	+	—	—	—	—
9	+	+	+	—	—	+	—	—
10	+	+	+	+	+	—	—	—
11	+	+	+	+	+	+	—	—
12	+	+	+	+	+	+	+	—

Table 9. Bacterial blight reactions to 30 single-colony isolates obtained from dilution plates with streptomycin sulfate after 17 or 20 serial transfers in nutrient broth

Culture designation		Transfer series	Differential strains Ac44 (Sus.)	20-3 (B_N)
Race 4	#1	17th	S —	S —
	2	17th	S —	S —
	3	17th	S	S
	4	17th	S	S
	5	17th	S	S
	6	17th	S	S
	7	17th	S	S
	8	17th	S —	S —
	9	17th	S	S
	10	17th	S —	S —
Race 7	#1	20th	S	S
	2	20th	S	S
	3	20th	S	R, S—[a]
	4	20th	S	S
	5	20th	S	S
	6	20th	S —	S —
	7	20th	R	R
	8	20th	S	S
	9	20th	S	S
	10	20th	S	S
Race 7 (appressed)	#1	17th	S	S
	2	17th	S	S
	3	17th	S	S
	4	17th	S	S
	5	17th	S	S
	6	17th	S	S
	7	17th	S	S
	8	17th	S	S
	9	17th	S	S
	10	17th	S	S

[a] Some of the seedlings of this differential reacted as resistant, while some reacted as S—.

Table 10. Disease reactions to a streptomycin-resistant clone of race 3 and to leaf-suspension cultures derived from differentials infected by this clone

Source of culture			Reaction of differentials				
Host	Lesion types	Ac44 (Sus.)	1-10B (B_{In})	Meb B1 ($B_{L?}$)	8-3 (b_7)	20-3 (B_N)	Race recovered
		Disease grade of adult plants in the field[b]					
Sm clone[a]		4		1.2	1.2,1.3	1.3,1.4	
		Seedling reactions with cultures from field test					
				4-11			
Ac44	4	S	S—	R	R	R	3
Meb B1	1.2	S	S—	R	R	R	3
8-3	1.2	S	S—	R	R	R	3
8-3	1.3	S	S—	R	R—	S—	5
20-3	1.3	S	S—	R	R	S	5
20-3	1.4	S	S—	R	R	S	5

[a] Sm = streptomycin resistant.
[b] Two different infection types occurred on the same plants of 8-3 and 20-3.

Table 11. Relative numbers of colonies on differential media of 10^{-5} dilutions from infected leaves of seedlings inoculated with streptomycin-resistant clones that originated from race 3

Race	Source of culture[a]	Selective media[b]	
		Nutrient agar	Nutrient agar + 1000 ppm strep. sul.
5	20-3	+++	+++
5	20-3	+++	+++
3	Ac44	+++	+++
Check			
3	Parent culture (non-selected)	+++	—

[a] The cultures with the exception of the parent are from the seedling test shown in Table 10.
[b] The scale for relative numbers of colonies is: — = none, +++ = between 101 and 1000 per plate.

Table 12. Disease reactions to streptomycin-resistant clones of different races in the field and to leaf-inoculum cultures from susceptible-type lesions of different host lines

Original race	Source Host Lesion	Reaction of differentials: Ac44(Sus.)	1-10B(B_{In})	4-11($B_{L?}$)	8-3(b_7)	20-3(B_N)	Relative no. of colonies on media with:[a] No strep. sul.	200 ppm strep. sul.	Races recovered
	Disease grades of adult plants in field								
2		4		1.3	2.3	1.3			2?
	Seedling reactions with cultures from single lesions								
	Ac44	4	S[b]	R—	R	S—	R	++	++	2
	4-11	3	S—	R—	R[b]	S—	R	+	—	2
	8-3	3	S	R	R	S[b]	R	++	+	2
	20-3	3	S[b]	S	R	S	R	—	—	?[c]
	Disease grades of adult plants in field								
6		4		2.3	1.3	1.3			6?
	Seedling reactions with cultures from single lesions								
	Ac44	4	S[b]	S	S—	R	R	++++	+++	6
	4-11	3	S	S	S—[b]	R	R	++++	+++	6
	8-3	3	S	S	S—	S+[b]	R	++	++	8
	20-3	3	S	S	S—	R	S[b]	++	++	7
	Disease grades of adult plants in field								
7		4		3—	1.3	4			7
	Seedling reactions with cultures from single lesions								
	Ac44	4	S[b]	S	S—	R	S	++	+	7
	4-11	3—	S	S	S—[b]	R	S	+	—	7
	8-3	3	S	S	S—	S+[b]	S+	+	+	10
	20-3	4	S	S	R	R	S[b]	+	++	5
	Disease grades of adult plants in field								
9		4		3	1.3	1.3			9
	Seedling reactions with cultures from single lesions								
	Ac44	4	S[b]	S	R	R	R	++++	+	3
	4-11	3	S	S	S—[b]	R	R	++	++	9
	8-3	3	S	S	S—	S[b]	R	++++	+	8
	20-3	3	—	—	—	—	—	—	—	—

Table 12. Continued

Original race	Source Host	Source Lesion	Reaction of differentials: Ac44(Sus.)	1-10B(B_{In})	4-11($B_{L?}$)	8-3(b_7)	20-3(B_N)	Relative no. of colonies on media with:[a] sul. No.	strep.	200 ppm strep. sul.	Races recovered
			Disease grades of adult plants in field								
10			4		2.3	1.3	4				10?
			Seedling reactions with cultures from single lesions								
	Ac44	4	S[b]	S	S—	R	S	++		—	7
	4-11	3	S	S	S—[b]	S— —	S	+		—	10
	8-3	3	S	S	S—	S+[b]	S	+		+	10
	20-3	4	S	S	S—	S— —	S[b]	+		—	10

[a] The scale for relative numbers of colonies is: — = none, + = 1 to 10, ++ = 11 to 100, +++ = 101 to 1000, ++++ = >1000.

[b] Dilutions from infected tissues of these seedlings on nutrient agar with and without streptomycin sulfate showed no loss in resistance to the antibiotic.

[c] This race has not been numbered.

Table 13. Relative stability of parent and mutant races derived from single-colony streptomycin resistant clones

Original race (sm clone)	Source (host in field test)	Races recovered: Test 3[a] Inoculum — Leaf suspensions	Races recovered: Test 3[a] Inoculum — Single colony cultures	Races recovered: Test 4[b] Inoculum — Leaf suspensions
2	Ac44	2	2	
2	4-11	2	2	
2	8-3	2	2	2
6	Ac44	6	6	6
6	4-11	6	6	
6	8-3	8	8	8
6	20-3	7	7	7
7	Ac44	5	5	
7	4-11	5	5	
7	8-3	10	10	10
7	20-3	7	5	7
9	Ac44	3,6	6	
9	4-11	6	6	6
9	8-3	8	8	8
10	Ac44	5	5	
10	4-11	5	7	3,5,7
10	8-3	10	12	12
10	20-3	7	7	10

[a] Cultures derived from the seedling test shown in Table 12. Test made between September 17 and 29, 1959.

[b] Cultures derived from Test 3 from infected cotyledons that had been inoculated with single-colony cultures. Test made between October 6 and 22, 1959. All cultures retained streptomycin resistance.

Table 14. Disease reactions of test seedlings to single-colony clones from susceptible-type lesions on susceptible lines and lines with mesothetic reactions[a]

Source (Var. or line)	Blight resistance gene	Reaction of differentials					Races isolated[b]
		Ac44 (Sus.)	1-10B (B_{In})	CR4 ($B_{L?}$)	8-3 (b_7)	20-3 (B_N)	
Ac44	Sus.	S	R—	R	R	R	1
Ac44	Sus.	S	S	R	R	R	3
Parrott	Sus.	S	S	R	R	S—	5
Ac4-42	Sus.	S	R—	R	R	R	1
1-10B	B_{In}	S	R—	R	R	R	1
1-10B	B_{In}	S	S	R,S[c]	R	R	3 & 6
CR4	$B_{L?}$	S	S	S	R	R	6
CR4	$B_{L?}$	S	R	R	R	R	1
CR4	$B_{L?}$	S	S	R,S[c]	R	R	3 & 6
Ac892 x P1	$B_{L?}$	S	S	S—	R	R	6
Ac8373	$B_{L?}$	S	S	R,S—[c]	R	R	3 & 6
8-3	b_7	S	R—	R	R—	R	1
8-3	b_7	S	S	S	R,R—	S—	7
8-3	b_7	S	R—	R	R	R	1
8-3	b_7	S	S	S	R	R	6
8-3	b_7	S	R	R	R	R	1
C100W2	$b_{7?}$	S	S—	R,S—[c]	R	R	3 & 6
D2-6	b_7	S	S	R	R	R	3
20-3	B_N	S	R—	R	R	R	1
20-3	B_N	S	S	R	R	S	7
20-3 x 2Ac	B_N	S	S	S	R	R	6
G.b.	B_3	S	R—	R	R	R	1
G.b.	B_5	S	R—	R	R	R	1

[a] Th original inoculum was race 1 from infected leaves.

[b] Identification was based on upland differentials only.

[c] Some of the seedlings were resistant and others susceptible.

Table 15. Disease reactions of test seedlings to pairs of single-colony clones isolated from the same lesion[a]

Source (var. or line)	Blight resistance gene	Reaction of differentials[b] Ac44 (Sus.)	1-10B (B_{In})	4-11 ($B_{L?}$)	8-3 (b_7)	20-3 (B_N)	Races isolated[c]
Ac44	Sus.	S	R	R	R,R—	R	1
Ac44	Sus.	S	R	R	R	R	1
1-10B	B_{In}	S	R—	R	R	R	1
1-10B	B_{In}	S	S	R	R,S	R	3 & ?
8-3	b_7	S	R—	R	R	R	1
8-3	b_7	S	R—	R	R	R	1
8-3	b_7	S	S	R	R	R,S	3 & 5
8-3	b_7	S	R	R	R	R	1
20-3	B_N	S	S—	R	R	S	5
20-3	B_N	S	S—	R	R	S	5
Check cultures							
Race 1		S	R—	R	R	R	1
Race 2		S—	R—	R	S—	R	2

[a] The original inoculum was race 1 from infected leaves.
[b] Two reactions indicate that different test seedlings reacted differently.
[c] Races identified on upland differentials only.

Table 16. Bacterial blight reactions to cultures obtained from both resistant and susceptible-type lesions of leaves with mesothetic reactions of adult plants of 20-3 inoculated with race 1

Culture designation	Host lesion type	Differential seedlings Ac44 (Sus.)	CR4 ($B_{L?}$)	8-3 (b_7)	20-3 (B_N)	Race recovered
Single colonies			**Test 8/2/59 to 9/8/59**			
59-90-1	1	S	R	R	R	1
59-91-1	1	S	R	R	R	1
59-92-1	2	S	R	R	R	1
95-93-3	3	S	R	R	S	4
Single colonies			**Test 9/11/59 to 9/30/59**			
59-106-1	3	S	R—	R	S	4
59-106-2	3	S	R—	R	S	4
59-93-3-1	3	S	R	R	S	4
Leaf inoculum			**Test 9/30/59 to 11/2/59**			
59-94	1	S	R	R	R	1
59-95	3	S	R	R	R	1

Table 17. Disease reactions of test seedlings to cultures obtained from leaves and bolls of uniformly infected CR4

Number of cultures reacting	Reaction of differentials[a]			
	Ac44 (Sus.)	CR4 ($B_{L?}$)	Sto20 (b_7)	20-8 (B_N)
		Single-colony cultures from leaves		
8	S	R	R	
3	S	R,S—	R	
		Bacterial suspensions from bolls		
			8-3 (b_7)	
9	S	R	R	R
1	S	R,R—	R	R
1	S	R,S—	R	R
3	S	R,S—	R,R—	R
1	S	R—	R,S—	R
1	S	R,R—	R	R,R—
2	S	R	R	R,S—
		Check cultures		
Race 1 (Okla.)	S	R	R	R
Race 1 (Texas)	S	R	R	R
Race 2 (Texas)	S	R	S—	R

[a] Two reactions indicate that individual seedlings reacted differently.

Table 18. Bacterial blight reactions to cultures reisolated as single colonies from infected seedlings of CR4[a]

Culture designation	Ac44 (Sus.)	Upland differentials[b] 1-10B (B_{In})	CR4 ($B_{L?}$)	8-3 (b_7)	20-8 (B_N)
			1st test		
56-67	S		R—,S—	R—,S—	R
56-68	S		R—,S—	R,R—	R
56-69	S		R,S—	R,R—	R
					20-3 (B_N)
			2nd test		
56-67	S	R—	R—	R	R
56-68	S	R—	R—	R	R
56-69	S	R—	R—	R—	R
			3rd test		
56-67	S	R—,S—	R,S—	R	R
56-68	S	R,R—	R,R—	R	R
56-69	S	R—	R,R—	R	R
			4th test		
56-67	S	R—,S—	R—,S—	R,R—	R
56-69	S	R—,S—	R—,S—	R,R—	R

[a] The cultures were isolated from seedlings inoculated with single colonies shown in Table 17.

[b] Two reactions indicate that individual seedlings reacted differently.

Table 19. Disease reactions of test seedlings to single-colony clones isolated from a single lesion of 20-3[a]

Number of cultures reacting	Reaction of differentials[b]					Race[c]
	Ac44 (Sus.)	1-10B (B_{In})	CR4 ($B_{L?}$)	8-3 (b_7)	20-3 (B_N)	
3	R	R	R	R	R	—
1	S	R—	R,S	R	S	4 & ?
1	S—	S—	R—	R,S—	S—	5 & ?
1	S—	S—	R	R—	S—	5
1	S	S	R—	R,R—	S	5
1	S	S	R—	R	S	5
8	S	S	R—	R—	S	5
1	S+	S	R—	R—,R—	S	5
1	S+	S	R—	R—,S	S	5 & ?
2	S	S	R—,S	R	S	5 & 7
3	S	S	R—,S	R,R—	S	5 & 7
5	S	S	R—,S	R—	S	5 & 7
1	S+	S	S	R,R—	S	7
1	S+	S	S	R—	S	7
6	S	S	S	R—	S	7
2	S+	S	S	R—,S—	S	7 & 10
1	S+	S	S	S	S	10

[a] The 20-3 seedling was inoculated with leaf inoculum from the 20-8 seedling infected by a bacterial suspension from a CR4 boll.

[b] Two reactions indicate that individual seedlings reacted differently.

[c] Identified on the upland differentials only.

Table 20. Disease reactions of differential seedlings to 3 single-colony clones maintained continuously on PDA

Race and source	Date of test	Reaction of differentials[a]				
		Ac892 or Ac44 (Sus.)	1-10B (B_{In})	CR4 ($B_{L?}$)	8-3 (b_7)	20-8 or 20-3 (B_N)
3	2/14/56	S	R,S	R,S	R	R
(Okla.)	8/19/56	S		R	R	R
	9/21/56	S	R,S	R—,S—	R—,S—	R,S
	10/18/56	S	S—	R	R—	R
	11/15/56	S	R—	R	R	R
	1/17/57	S	R,R—	R	R	R
1	2/14/56	S	R,S	R,S—	R	R
(Texas)	8/19/56	S		R	R	R
	9/21/56	S	R,S	R—,S—	R—,S—	R
	10/18/56	S	S	R	R—	R
	11/15/56	S—	R—	R	R	R
	1/17/57	S	R,R—	R	R	R
	2/15/57	R,S—	R	R	R	R
2	8/14/56	S	R	R	S	R
(Texas)	8/19/56	S		R	S—	R
	9/21/56	S	R	R—,S	S	R
	10/18/56	S	R	R	S	R
	11/15/56	S	R—	R—	S	R
	1/17/57	S	R,R—	R,R—	R—	R
	2/15/57	R,S—	R	R	R	R

[a] Two reactions indicate that individual seedlings reacted differently. Usually only 1 of 10 seedlings was susceptible in these tests.

Table 21. Disease reactions to single-colony cultures derived from the single diseased seedling of 20-8 in the 9/21/56 test with race 3[a] (see Table 20)

Number of cultures reacting	Reaction of differentials[b]					Race[c]
	Ac44 (Sus.)	1-10B (B_{In})	CR4 ($B_{L?}$)	8-3 (b_7)	20-3 (B_N)	
1	S	R	R	R	R—	1
1	S	R	R	R	S	4
1	S	R—	R	R	S	4
9	S	R,R—	R	R	S	4
1	S	R,R—	R,R—	R	S	4
1	S	R—	R,R—	R	S	4
1	S	R—,S—	R,S	R	S	4 & 7
2	S	R—,S—	R,S	R,R—	S	4 & 7
9	S	R,R—	R	R,R—	S	4
4	S	R—	R	R,R—	S	4
1	S	R—,S	R	R,R—	S	4 & 5
1	S	R	R	R,R—	S	4

[a] Leaf inoculum from the 20-8 seedling was used to inoculate seedlings of 20-3. The single colonies were isolated from a single lesion of a 20-3 seedling.

[b] Two reactions for a given differential indicate that individual seedlings reacted differently.

[c] Races were identified on upland differentials only.

Table 22. Successive tests with cultures of races 4, 6 and 7 showing losses in pathogenicity after cultivation on nutrient agar with dextrose

Original race and designation		Reaction of differentials[a]					Race(s) recovered[b]
		Ac44 (Sus.)	1-10B (B_{In})	CR4 ($B_{L?}$)	8-3 (b_7)	20-3 (B_N)	
			Cotyledon test 8/6/57 to 9/10/57				
4	48-2	S	R—	R	R	S	4
6	47-3	S	S	S	R	R	6
6	42-2	S	S	S,R	R	R	3,6
7	43-3	S	S	S	R	S	7
			Boll test 9/24/57 to 10/14/57				
				4-11			
3	48-2	S		R		S	4
6	47-3	S		S		R	6
6	42-2	S		S		R	6
7	43-3	S		S		S	7
			Leaf test 11/15/57 to 11/25/57				
				CR4			
4	48-2	S	R,R—	R	R,R—	S	4
6	47-3	S	R—,S	R	R	R	3,6
6	42-2	S	S	S—	R,R—	R	6
7	43-3	S	S	S—	R	S	7
			Cotyledon test 11/18/57 to 12/6/57				
				4-11			
4	48-2	S	R—	R	R,R—	S	4
6	47-3	S	R—	R	R	R	1
6	42-2	S	S	S—	R	R	6
7	43-3	S	S	S—	R	S	7
			Cotyledon test 1/9/58 to 1/28/58				
				CR4			
4	48-2	S	R	R	R	S	4
6	42-2	S	S	S—	R	R	6
7	43-3	S	S	S—	R	S	7
			Cotyledon test 11/5/58 to 11/26/58				
4	48-2	S	S —	R	R—	S	5
6	42-2	S	S	S—	R	R	6
7	43-3	S— —	S— —	R—	R	S— —	7

[a] Two reactions indicate that seedlings reacted differently.

[b] Races identified on upland differentials only.

Table 23. Number of normal and small variant colonies of X. malvacearum from 1 ml of a 48-hour shake culture on nutrient agar with and without streptomycin sulfate

	Concentration of streptomycin sulfate[a]											
	None				200 ppm				1000 ppm			
Rep.	Normal		Small		Normal		Small		Normal		Small	
no.	6 days	19 days	6 days	19 days	6 days	19 days	6 days	19 days	6 days	19 days	6 days	19 days
	Culture diluted 10^{-7}						Culture not diluted					
1	37	38	0	4	3	6	0	>50	1	3	0	19
2	41	41	0	3	2	3	0	>50	1	1	0	1
3	25	26	0	2	1	1	0	>50	0	0	0	0
4	23	23	0	0	3	4	0	>50	2	2	0	21
5	41	41	0	0	5	5	0	>50	3	3	0	0
6	28	28	0	3	2	3	0	>50	4	4	0	1
7	44	44	0	1	3	4	0	>50	1	1	0	0
8	21	23	0	3	3	3	0	>50	1	1	0	0
9	34	34	0	1	2	4	0	>50	1	3	0	13
10	34	36	0	2	2	2	0	>50	3	5	0	10
11	35	36	0	1	2	3	0	>50	1	2	0	18
12					4	4	0	>50	0	0	0	3

[a] On media with 3 ppm of the antibiotic a film of growth developed over the surface of the plate. Later, individual large colonies grew on top of the film.

Table 24. Relative numbers of colonies on nutrient agar with or without streptomycin sulfate of cultures from large and small colonies[a]

	Concentration of streptomycin sulfate											
	None				200 ppm				1000 ppm			
	Large	Int.	Small		Large	Int.	Small		Large	Int.	Small	
Culture designation[b]	Smooth	Irreg.	Smooth	Rough	Smooth	Irreg.	Smooth	Rough	Smooth	Irreg.	Smooth	Rough
Originally from large colonies												
NA3-5L	+++	+	—	+	—	—	—	—	—	—	—	—
Sm(3)13-1L	+++	—	—	+	—	—	—	—	—	—	—	—
Sm(200)26-2L	+++	—	—	—	++	+	—	—	+	++	—	—
Sm(200)28-4L	+++	—	—	—	+++	—	—	—	+	++	—	—
Sm(200)29-3L	+++	—	—	—	+++	—	—	—	+	++	—	—
Sm(1000)37-1L	+++	—	—	—	+++	—	—	—	—	—	—	—
Sm(1000)40-2L	+++	—	—	—	+++[c]	—	—	—	—	—	—	—
Originally from small colonies												
NA7-1s	—	++	—	—	—	—	—	—	—	—	—	—
NA8-6s	++	+	—	—	—	+[d]	—	—	—	—	—	—
NA2-5s	+	++	—	+	—	—	—	—	—	—	—	—
Sm(3)13-1s	—	—	—	+++	—	—	—	—	—	—	—	—
Sm(200)30-5s	—	+++	—	—	—	—	+	+	—	—	+	+
Sm(200)35-1s	+	+	+	—	—	+	+	+	+	+	+	—
Sm(200)26-2s	—	—	—	—	+	++	—	+	—	—	—	—
Sm(200)28-4s	++	+	+	—	+	+	+	—	+	+[e]	—	—
Sm(1000)37-5s	—	+++	—	—	—	—	—	+++[f]	—	—	++[g]	—
Sm(1000)40-4s	—	+++	—	—	—	—	+[g]	++	—	—	—	—
Sm(1000)37-1s	++	+	—	—	—[h]	—[h]	—[h]	—[h]	++	+	+	—
Sm(1000)40-7s	—	+++	—	—	—	++	+	+	—	++	+	+

[a] The relative scale for numbers of colonies is: — = none, + = 1 to 10, ++ = 11 to 100, +++ = 101 to 1000, ++++ = more than 1000.

[b] "NA" represents nutrient agar; "SM—(3), (200) and (1000)" represent cultures selected from media with 3, 200, or 1000 ppm, respectively, of streptomycin sulfate; "L" stands for large and "s" for small colonies.

[c] All colonies deep yellow.

[d] One colony only.

[e] Pigmentation occurs as mosaic patterns in colonies.

[f] Some colonies with raised centers, others with depressed centers.

[g] Almost white in color.

[h] Plates covered with a white bacterial contaminant.

Table 25. Relative numbers of colonies on nutrient agar with or without streptomycin sulfate from single colonies shown in Table 24[a]

Culture designation[b]	Concentration of streptomycin sulfate[c] None				200 ppm			
	Large	Small			Large	Small		
	Smooth	Int. Irr.	Smooth	Rough	Smooth	Int. Irr.	Smooth	Rough
Originally from large colonies								
NA3-82L	+++	—	—	—	—	—	—	—
Sm(3)13-1L-1L	+++	—	—	—	—	—	—	—
Sm(3)13-1L-2L	+++	—	—	—	—	—	—	—
Sm(3)13-1L-3L	+++	—	—	—	—	—	—	—
Sm(3)13-1L-4L	+++	—	—	—	—	—	—	—
Sm(3)13-1L-1s	—	—	—	+++	—	—	—	—
Sm(3)13-1L-2s	—	—	—	+++	—	—	—	—
Sm(3)13-1L-3s	—	—	—	+++	—	—	—	—
Sm(3)13-1L-4s	—	—	—	+++	—	—	—	—
Sm(3)13-1L-5s	—	—	—	+++	—	—	—	—
Sm(1000)37-1L	+++	—	—	—	+++	—	—	—
Originally from small colonies								
NA7-1s	+	+	—	+	—	—	—	—
Sm(200)25-13s	—	+	+	+	—	—	—	—
Sm(200)25-16s	+++	—	—	—	+++	—	—	—
Sm(200)28-9s	+	+	—	—	—	+	+	—
Sm(200)31-8s	—	—	—	+++	—	—	+	+++
Sm(200)35-1s	—	—	+	++	—	+	—	++
Sm(1000)37-1s	+++	—	—	—	+	+	—	+
Sm(1000)37-5s	—	+	—	++	—	—	+	+
Sm(1000)44-8s	—	—	+++	—	—	+	—	++

[a] See Table 24 for the scale indicating relative numbers of colonies.

[b] See Table 24 for explanation of culture designations.

[c] "Int." is intermediate size colonies; "irreg." is irregular margins and surface of colonies.

Table 26. Disease reactions incited on Acala 44 by nutrient broth-shake cultures derived from large or small colonies, and colony types reisolated

Culture designation[a]	Disease reaction on Ac44 after: 7 days	26 days	Colony types recovered on nutrient agar from host tissue 16 days after inoculation:
Originally from large colonies on nutrient agar			
NA3-5L-1L	S	S	
NA3-5L-2L	S	S	Large, smooth
NA3-5L-3L	S	S	
NA3-5L-4L	S—	S	
NA3-5L-5L	S	S	
NA2-3L-1s	S— —	S—	
NA3-5L-2s	S—	S	Large & inter.; smooth
NA3-5L-3s	O	S— —	
NA3-5L-4s	O	O[b]	Small, rough; very few inter. & large
NA3-5L-5s	O	S— —	
Originally from large colonies on NA + 3 ppm. strep. sulfate			
SM(3)13-1L-1L	S	S	
SM(3)13-1L-2L	S	S	
SM(3)13-1L-3L	S	S	
SM(3)13-1L-4L	S	S	
SM(3)13-1L-5L	S	S	
SM(3)13-1L-1s	O	O[b]	
SM(3)13-1L-2s	O	S—	
SM(3)13-1L-3s	O	S—	
SM(3)13-1L-4s	S— —	S	
SM(3)13-1L-5s	S— —	S	
Originally from small colonies on NA + 200 ppm strep. sulfate			
SM(200)30-5s-1I	S— —	S	
SM(200)30-5s-2I	S— —	S	
SM(200)30-5s-3I	S— —	S	
SM(200)30-5s-4I	S— —	S	Inter. & small, smooth
SM(200)30-5s-5I	S— —	S	
SM(200)30-5s-1s	R—	R—,S—	Small rough from R—; small smooth & rough from S—
SM(200)30-5s-2s	R—	R—,S—	Small rough with sunken centers
SM(200)30-5s-3s	R—	R—,S—	
SM(200)30-5s-4s	R—	S	
SM(200)30-5s-5s	R—	S—	All types, mostly small

Table 26. Continued.

Culture designation	Disease reaction on Ac44 after: 7 days	26 days	Colony types recovered on nutrient agar from host tissue 16 days after inoculation:
Orignally from small colonies on NA + 1000 ppm strep. sul.			
SM(1000)37-1s-1L	S	S	
SM(1000)37-1s-2L	S	S	
SM(1000)37-1s-3L	S	S	
SM(1000)37-1s-4L	S	S	
SM(1000)37-1s-5L	S	S	
SM(1000)37-1s-1s[c]	S—	S	
SM(1000)37-1s-2s[c]	S—	S	
SM(1000)37-1s-3s[c]	S—	S	
SM(1000)37-1s-4s[c]	S— —	S—	
SM(1000)37-1s-5s[c]	S— —	S—	Small to inter., smooth & translucent

[a] "I" represents cultures from an intermediate-size colony.

[b] These seedlings showed an immune reaction except for a slight amount of chlorosis.

[c] The parent colonies were small and smooth.

Table 27. Types of colonies on nutrient agar from cultures isolated from Acala 44 as indicated in Table 26

Culture designation	Kind of progeny
60-13 NA3-5L-4s-Ac44 #1s	Small, rough
60-13 NA3-5L-4s-Ac44 #3s	Small, rough
60-13 NA3-5L-4s-Ac44 #1I	Intermed., smooth or rough
60-13 NA3-5L-4s-Ac44 #2I	Intermed., smooth or rough
60-13 NA3-5L-4s-Ac44 #1L	Large, smooth
60-13 NA3-5L-4s-Ac44 #2L	Large, smooth
60-16 NA3-5L-2s-Ac44 #18L	Large, smooth
60-16 NA3-5L-2s-Ac44 #19L	Large, smooth
60-16 NA3-5L-2s-Ac44 #20L	No colonies
60-16 NA3-5L-2s-Ac44 #21L fl[a]	Large, flat
60-18 Sm(200)30-5s-1s-Ac44 #18s	No colonies
60-18 Sm(200)30-5s-1s-Ac44 #9s	No colonies
60-18 Sm(200)30-5s-1s-Ac44 #10L fl[a]	Large, flat
60-18 Sm(200)30-5s-1s-Ac44 #10L fl[a]	Large, flat
60-14 Sm(200)30-5s-Ac44 #1s[b]	Small, rough
60-14 Sm(200)30-5s-Ac44 #2I	Small, rough; and a few large, smooth
60-11 Sm(200)30-5s-4I-Ac44 #1I	Intermediate, smooth
60-11 Sm(200)30-5s-4I-Ac44 #2I	Intermediate, smooth
60-17 Sm(200)30-5s-2s-Ac44 #1s[c]	No colonies
60-17 Sm(200)30-5s-2s-Ac44 #2s	Small, rough
60-17 Sm(200)30-5s-2s-Ac44 #3s	Small, rough
60-10 Sm(1000)37-1s-6s[b]-Ac44 #14s[b]	Intermed., irreg., or rough

[a] "fl" stands for flat. These colonies were probably not from *X. malvacearum,* as in later tests they produced gas on media with sugar and were not pathogenic.
[b] This culture came from a small, smooth colony.
[c] This culture came from a small, rough colony with a sunken center.

Table 28. Types of colonies on nutrient agar with or without sugar, from cultures isolated on nutrient agar of the test shown in Table 27

Culture designation[a]	On nutrient agar	On NA + 2% dextrose
60-13 #1L-1L	Large	Large, smooth[b]
60-13 #1L-2L	Large	Large, smooth[c]
60-13 #1s-1s	Contaminated	Large, granular[d]
60-13 #3s-1s	Contaminated	Large, smooth
60-13 #3s-2s	Small	Large, granular[d]
60-13 #1I-1I	Intermed.	Large, smooth
60-13 #2I-1I	Large	Large, smooth
60-16 #18L-1Lf1	Large, flat	Large, flat, smooth
60-16 #19L-1Lf1	Large, flat	Large, flat, smooth
60-14 #1s[e]-1s	Small[e]	Small,[f] a few large
60-14 #2I-1s	Small[e]	Small, a few large
60-14 #2I-2L	Large	Large, smooth
60-11 #1I-1I	Intermed.	No colonies
60-10 #14-1I	Intermed.	No colonies
60-17 #1s-1s	Small	Small, rough[g]
59-90-1 (Check)	Large	Large, smooth

[a] The designations are abbreviations of those in Table 27.
[b] All large, smooth colonies produced abundant slime on this medium.
[c] This culture came from a large colony with a mosaic pattern of pigmentation. The progeny in this test were all large and of normal pigmentation.
[d] Granular refers to relatively large particles that developed within colonies that also produced abundant slime.
[e] Colony type small, smooth.
[f] Colonies small, rough with a raised center.
[g] Colonies small, rough with a sunken center.

Table 29. Disease reactions and types of colonies produced from transfers of selected colonies from the test represented in Table 28

Culture designation[a]	Disease reaction on Ac44 after 45 days	Colony types recovered from host tissue after 45 days
60-13 #1L-1L-1L (smooth on NA+D)	S	Large & small (1:1)[b]
#1s-1s-1L (granular on NA+D)	S	4 large: 13 small[c]
#1s-1s-2L (granular on NA+D)	S	
#1s-1s-3L (granular on NA+D)	S	
#3s-2s-5s (small on NA)	S— —	
#2I-1I	S	95 large; 91 have sectors or clear zones
60-16 #18L fl-1L flat (large, flat on NAD)	O	All large flat[d]
60-10 #14s-1I (intermed. on NA)	S—	
60-11 #1I-1I-7I (intermed. on NA & light color)	S— —	
60-53 Sm(200)30s-#4s-Ac44 #1s	S— —	7 large; 37 small[e]

[a] See Tables 28 and 27 for full designation, except for culture 60-53 which is complete.

[b] Dilutions from a large colony gave rise to all large colonies; dilutions from a small colony produced small and large in a ratio of about 1:1.

[c] Dilutions from a large colony and from a small colony each gave rise to a mixture of types.

[d] When tested on nutrient agar with dextrose these cultures produced gas. It was concluded that these probably originated from contaminants and were not variants of *X. malvacearum*.

[e] These isolations were on nutrient agar with 200 ppm streptomycin sulfate. Eight of the 37 small colonies were then tested individually on NA and NA+ streptomycin sulfate. Each of the 8 produced only small colonies on both media. A single large colony was similarly tested and produced all large colonies.

Table 30. Disease reactions of adult cotton plants incited by different races of X. malvacearum at high temperatures in the greenhouse and at moderate temperatures in the field

Race	Location of test	Reaction of differentials										
		G. hirsutum				*G. barbadense*						
		Ac44 (Sus.)	CR4 (B_{L_2})	8-3 (b_7)	20-3 (B_N)	B_1	B_2	B_3	B_4	B_5	B_2B_3	B_2B_{6m}
1	G.h.[a]	R	R	R	R	R	R	R	R	R	R	R
	Field[b]	S	R	R	R	R	R	S	R	S	R	R
2	G.h.	S—	R	R—	R	S	R	S—	R	S—	R	R
	Field	S	R	S—	R	S	R	S	R	S	R	R
4	G.h.	S—	R—	R	R—	R	R	S—	R	S—	R	R
	Field	S	R	R	S	R	R	S	R	S	R	R
6	G.h.	S—	R—	R	R	R	R	S—	R	S	R	R
	Field	S	S	R	R	R	R	S	R	S	R	R
7	G.h.	S—	R—	R	R—	R	S—	S—	R	S	R	R
	Field	S	S	R	S	R	R	S	R	S	R	R
8	G.h.	S—	R	R	R	R	R	S	R	S—	R	R
9	G.h.	S—	R—	R	R	R	S—	S	R	S	R	R

[a] Greenhouse test in which average minimum and maximum temperatures were 82°F and 102°F.

[b] Field test in which average minimum and maximum temperatures were 64°F and 97°F.

Table 31. Bacterial blight reactions at low and high temperatures in the greenhouse at St. Paul, Minnesota

Race		Differentials and temperatures (F) of tests									
		Ac44(Sus.)		1-10B(B_{III})		4-11($B_{L?}$)		8-3(b_7)		20-3(B_N)	
		Low 65° to 70°	High 85° to 90°	Low 65° to 70°	High 85° to 90°	Low 65° to 70°	High 85° to 90°	Low 65° to 70°	High 85° to 90°	Low 65° to 70°	High 85° to 90°
2	A	S	S	R—	R—	R	R	S—	S—	R	R
2	B	R—	S—	R	R	R	R	R—	R—	R	R—
2	C	S	S	R	R	R	R	R	R—	R	R
3	A	S	S	S	S—	R	R	S—	R	R	R
4	A	S	S	R	R	R	R	S—	R	S	S
4	B	S	S	R	R	R	R	S—	R	S	S
9	A	S	S	S	S	R—	S	S	R	R	R
9	B	S	S	S	S	R—	S	S	R	R	R
7	A	S—	R—	R—	R—	R	R	R	R	R—	R
7	B	R—	R—	R	R—	R	R	R	R	R	R
12		S—	S—	S—	S—	S—	R—	S—	S—	S—	S—

Table 32. Disease reactions 2 weeks and 11 weeks after inoculation with race 1[a]

Differential line	Major gene(s) for resistance	Number of plants grading:[b] S	I	R	S	I	R
G. hirsutum			July 26			October 3	
1-10B	B_{In}	0	0	18	0	0	18
4-11	$B_{L?}$	0	0	15	0	0	15
8-3	b_7	0	0	17	0	0	17
20-3	B_N	0	10	14	1	9	14
(20-3 × 4Ac44)F_4	B_N	2	22	0	24[c]	0	0
B_2B_{6m} × 4 Empire	B_2B_{6m}	0	4	0	0	0	4
(20-3 × 17-3)F_5 7-5-1	$B_NB_{L?}$	0	0	2	0	0	2
(20-3 × 17-3)F_5 7-5-3	$B_NB_{L?}$	0	0	3	0	0	3
(20-3 × 17-3)F_5 7-5-4	$B_NB_{L?}$	0	0	2	0	0	2
(20-3 × B251)F_4 7-8-1	$B_NB_?$	0	0	1	1	0	0
(20-3 × B251)F_4 7-8-6	$B_NB_?$	0	0	2	0	2	0
(20-3 × B251)F_4 7-8-9	$B_NB_?$	0	2	0	0	0	2
(20-3 × CR4)F_4 7-9-1	$B_NB_{L?}$	0	0	2	0	0	2
(20-3 × CR4)F_4 7-9-4	$B_NB_{L?}$	0	3	0	0	0	3
(20-3 × CR4)F_4 7-10	$B_NB_{L?}$	0	3	10	0	0	13
(20-3 × CR4)F_4 7-11	$B_NB_{L?}$	0	1	17	0	0	17
(4-11 × 250-9)F_4 7-12	B_Lb_7	0	0	15	0	0	15
(4-11 × 250-9)F_4 7-13	B_Lb_7	0	0	15	0	0	15
(4-11 × B251)F_4 7-15	$B_{L?}$	0	0	15	0	0	15
(4-11 × B251)F_4 7-16	$B_{L?}$	0	0	15	0	0	15
(4-11 × 20-3)F_4 7-18	B_LB_N	0	0	8	0	0	8
(4-11 × 20-3)F_4 7-19	B_LB_N	0	0	8	0	0	8
296-5		0	0	9	0	0	9
Ac44	Sus.	300	0	0	300[c]	0	0
G. barbadense							
Knight's B_1	B_1	0	1	11	11[c]	0	1
Knight's B_2	B_2	0	16	0	1	0	15
Knight's B_3	B_3	0	8	0	8	0	0
Knight's B_4	B_4	2	19	0	21	0	0
Knight's B_5	B_5	6	0	0	6	0	0
Knight's B_2B_{6m}	B_2B_{6m}	0	0	16	0	0	16

[a] Inoculated July 12, 1960.
[b] S includes field grades 3 and 4; I includes field grades 1.3 and 2.3; R includes field grades 0, 1, and 2 and combinations of these.
[c] Not fully susceptible reactions; fewer lesions and some intermediate types.

Table 33. Disease reactions 2 weeks and 12 weeks after inoculation with race 2 NM[a]

Differential line	Major gene(s) for resistance	Number of plants grading:[b] S	I	R	S	I	R
G. hirsutum			July 15			October 4	
1-10B	B_{In}	0	0	24	1	0	23
8-3	b_7	0	27	2	0	3	26
Ac 1517 BR-1	b_7	22	0	0	22[c]	0	0
Austin	b_7	21	6	0	1	26	0
Rex	b_7	0	27	0	0	27	0
(Sto.20 × 6 Sto.62)F_8	b_7	0	28	0	0	28	0
18-37	b_7	0	23	0	0	0	23
20-3	B_N	0	0	29	0	0	29
B251	?	0	23	3	2	21	3
(20-3 × B251)F_4 7-8	B_NB?	0	0	8	0	0	8
B_2B_{6m} × 4 Empire	B_2B_{6m}	0	3	0	0	0	3
(4-11 × 250-9)F_4 7-12	B_L?b_7	0	0	30	0	0	30
(4-11 × B251)F_4 7-15	B_L?	0	0	20	0	0	20
Ac 1517C	Sus.	300	0	0	300[c]	0	0
Ac44	Sus.	49	0	0	49[c]	0	0
G. barbadense							
Knight's B_1	B_1	24	0	0	24[d]	0	0
Knight's B_2	B_2	0	20	0	5	15	0
Knight's B_3	B_3	0	3	0	0	3	0
Knight's B_4	B_4	1	0	16	17	0	0
Knight's B_5	B_5	2	0	0	2	0	0
Knight's B_2B_3	B_2B_3	0	0	12	0	0	12
Knight's B_2B_{6m}	B_2B_{6m}	0	0	13	0	0	13

[a] Inoculum applied on July 1, 1960.
[b] See Table 32 for relationship to field-grade scale.
[c] Not fully susceptible reactions; fewer lesions and some intermediate types.
[d] Severely attacked.

Table 34. Disease reactions 3 weeks and 13 weeks after inoculating with a mixture of races 6 and 9[a]

Differential line	Major gene(s) for resistance	Number of plants grading:[b] S	I	R	S	I	R
G. hirsutum			July 21			October 6	
1-10B	B_{In}	36	0	0	0	36	0
CR4	$B_{L?}$	1	19	0	1	2	16
4-11	$B_{L?}$	0	14	0	0	0	14
17-3	$B_{L?}$	0	19	0	0	0	19
8373	$B_{L?}$	0	17	0	0	0	17
2B4	$B_{L?}$	0	26	0	0	0	26
Mebane B1	$B_{L?}$	2	18	3	2	0	21
8-3	b_7	0	34	0	0	0	34
(B_2B_{6m} × 4 Empire)	B_2B_{6m}	0	2	0	0	0	2
20-3	B_N	0	27	0	0	0	27
(20-3 × 4 Ac44)F_4	B_N	1	5	1	3	3	1
(20-3 × 17-3)F_5 7-5	$B_NB_{L?}$	0	0	9	0	0	9
(20-3 × 17-3)F_5 7-10	$B_NB_{L?}$	0	0	15	0	0	15
(20-3 × CR4)F_4 7-9	$B_NB_{L?}$	0	0	14	0	0	14
(20-3 × CR4)F_4 7-11	$B_NB_{L?}$	0	0	10	1	0	9
(4-11 × 250-9)F_4 7-12	B_Nb_7	0	0	31	0	0	31
(4-11 × 250-3)F_4 7-13	B_Nb_7	0	0	13	0	0	13
(4-11 × B251)F_4 7-15	$B_NB_?$	0	0	12	0	0	12
(4-11 × 20-3)F_4 7-19	$B_{L?}B_N$	0	0	1	0	0	1
Ac44	Sus.	300[d]	0	0	300[c]	0	0
G. barbadense							
Knight's B_1	B_1	0	2	0	2[d]	0	0
Knight's B_2	B_2	2[d]	0	0	0	2	0
Knight's B_3	B_3	2	0	0	2	0	0
Knight's B_4	B_4	2	3	0	5	0	0
Knight's B_5	B_5	2	0	0	2	0	0
Knight's B_2B_3	B_2B_3	0	3	0	0	0	3
Knight's B_2B_{6m}	B_2B_{6m}	0	12	0	0	0	12

[a] Inoculum applied on July 1, 1960.

[b] See Table 32 for relationship to field-grade scale.

[c] Not a fully susceptible reaction.

[d] Severely attacked.

Table 35. Disease reactions 3 weeks and 13 weeks after inoculating with a mixture of races 4, 7 and 10[a]

Differential line	Major gene(s) for resistance	Number of plants grading:[b] S	I	R	S	I	R
G. hirsutum (Okla. lines)			July 21			October 6	
1-10B	B_{In}	12	0	0	0	12	0
CR4	$B_{L?}$	0	8	0	1	0	7
4-11	$B_{L?}$	0	27	0	0	0	27
8-3	b_7	0	30	0	0	0	30
20-8	B_N	27	0	0	0	27	0
20-3	B_N	28	0	0	0	28	0
(20-3 × Ac44)F_2	B_N	22	0	0	22	0	0
(20-3 × 3Ac44)F_2	B_N	24	0	0	24	0	0
(20-3 × 4Ac44)F_4	B_N	13	0	0	13	0	0
6-77	$B_{S?}$	27	0	1	0	0	27
(20-3 × CR4)F_4 7-9	$B_NB_{L?}$	1	13	0	1	0	13
(20-3 × CR4)F_4 7-11	$B_NB_{L?}$	5	14	0	5	0	14
(20-3 × 17-3)F_4 7-5	$B_NB_{L?}$	0	7	1	0	0	8
(20-3 × 17-3)F_4 7-10	$B_NB_{L?}$	4	10	0	4	0	10
(20-3 × B251)F_4 7-8	$B_NB_?$	5	0	0	0	5	0
(4-11 × 250-9)F_4 7-12	$B_{L?}b_7$	0	4	0	0	0	4
(4-11 × 20-3)F_4	$B_{L?}B_N$	5	0	0	0	5	0
CR4-45 Blk FWN(op)	$B_{L?}$	1	23	0	0	1	23
CR4-52-2 Blk FWN(op)	$B_{L?}$	6	26	0	6	26	0
Cluster 3-18-6B FWN(op)	$B_{L?}$	5	25	0	0	5	25
Cluster 3-10-1B FWN(op)	$B_{L?}$	7	21	0	0	7	21
Sto. 62	Sus.	30	0	0	0	30	0
Ac 1517C	Sus.	1000	0	0	1000[c]	0	0
G. hirsutum (Texas lines from L. S. Bird)							
B_2B_{6m} × 4 Empire (op)	B_2B_{6m}	0	4	0	0	0	4
L C BV 59 (op)	?	0	20	0	0	0	20
19B-UF 59 (op)	?	25	0	0	0	25	0
8M-BV 59 (op)	?	20	0	0	2	18	0
7M-BV 59 (op)	?	1	2	19	1	2	19
6M-BV 59 (op)	?	2	17	0	1	1	17
8A-BV 59 (op)	?	0	14	0	0	0	14
51-Lok 59 (op)	?	0	6	18	0	0	24
45-Lok 59 (op)	?	1	21	0	1	21	0
G. barbadense							
Knight's B_1	B_1	0	20	0	20	0	0
Knight's B_2	B_2	0	25	0	2	23	0
Knight's B_3	B_3	2	0	0	0	2	0
Knight's B_4	B_4	4	0	19	23	0	0
Knight's B_5	B_5	2	0	0	2	0	0
Knight's B_2B_{6m}	B_2B_{6m}	0	0	5	0	0	5

Table 35. Continued.

Differential line	Major gene(s) for resistance	Number of plants grading:[b] S	I	R	S	I	R
G. herbaceum (from J. R. Meyer, Stoneville, Miss.)							
59-3-32	?	0	0	18[d]	0	0	18
G. arboreum (from J. R. Meyer, Stoneville, Miss.)							
59-3064 Okinawa	?	0	0	35	0	0	35
59-311 Current leaf	?	0	25	0	0	0	25
59-3165 Garo Hill	?	0	8	13	0	0	21
59-3170 Brown lint	?	0	0	28	0	0	28
59-3187 Green gland	?	0	36	0	0	0	36

[a] Inoculated July 1, 1960.
[b] See Table 32 for relationship to field-grade scale.
[c] Not a fully susceptible reaction.
[d] Immune.

Table 36. Percentages of plants of previously resistant differential lines that were attacked by new races that built up under natural infection at three locations in Oklahoma in 1960[a]

Differential lines	Kind of resistance	Percentage of plant grading:[b] S	R	S	R	S	R
G. hirsutum		Chickasha		Tipton		Hollis	
1-10B	B_{In}	17	83	20	80	5	95
4-11	$B_{L?}$	0	100	5	95	2	98
8-3	b_7	0	100	0	100	0	100
Ac 1517 BR-1	b_7	20	80	0	100	3	97
20-3	B_N	0	100	0	100	0	100
(20-3 × 4 Ac44)F_4	B_N	34	66	2	98	0	100
Checks							
Ac44	Sus.	100	0	100	0	100	0
Ac 1517C	Sus.	100	0	100	0	100	0
Auburn 56	Sus.					100	0
G. barbadense							
Knight's B_1	B_1	100	0	39	61	42	58
Knight's B_2	B_2	0	100	0	100	6	94
Knight's B_3	B_3	100	0	100	0	100	0
Knight's B_4	B_4	54	46	100	0	15	85
Knight's B_5	B_5	100	0	100	0	100	0
Knight's B_2B_3	B_2B_3	0	100	0	100	0	100
Knight's B_2B_{6m}	B_2B_{6m}	0	100	0	100	0	100

[a] Readings taken 10/31/60 and 11/1/60.
[b] S includes plants with mixed reactions. In most instances infected plants were adjacent to each other in the row, however, in some rows there was more than one center of infection.

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Agriculture is Oklahoma's number one industry. It has more capital invested and employs more people than any other industry in the state. Farms and ranches alone represent a capital investment of four billion dollars—three billion in land and buildings, one-half billion in machinery and one-half billion in livestock.

Farm income currently amounts to more than $700,000,000 annually. The value added by manufacture of farm products adds another $130,000,000 annually.

Some 175,000 Oklahomans manage and operate its nearly 100,000 farms and ranches. Another 14,000 workers are required to keep farmers supplied with production items. Approximately 300,000 full-time employees are engaged by the firms that market and process Oklahoma farm products.

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