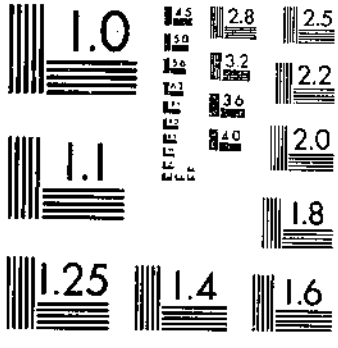
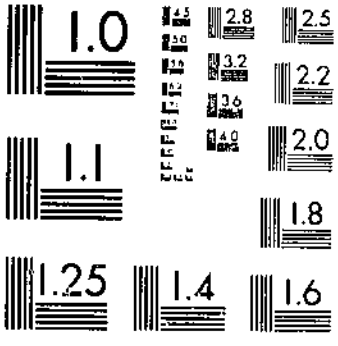
START



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

Technical Bulletin No. 956 • May 1948

UNITED STATES
DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

Bacterial Blight of Cotton Under Conditions of Artificial Inoculation[1]

By RICHARD WEINDLING, formerly *associate pathologist, Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration*

CONTENTS

BACTERIAL blight, caused by *Xanthomonas malvacearum* (E. F. Sm.) Dawson, is capable of affecting all above-ground organs of the cotton plant *Gossypium*. Damage from the disease involves mainly

[1] Submitted for publication July 15, 1947. Grateful acknowledgment is made to H. D. Barker and Paul Marsh, of this Bureau, for their generous assistance in the preparation of the bulletin.

775908°—48——1

the leaves, stems, and bolls; hence, according to the organ affected, it is known as angular leaf spot, black arm, or bacterial boll rot. Although the disease is present in all cotton-producing countries, crop losses vary widely with region, varieties grown, and seasonal conditions.

Varieties of Egyptian and sea-island (*G. barbadense* L.) are more susceptible to the disease than varieties of upland cotton (*G. hirsutum* L.). In the western part of the Cotton Belt bacterial blight is considered second in importance only to cotton root rot. Losses due to the disease are difficult to estimate because its effects are cumulative. Plants are rarely killed, and destructive epidemics are infrequent, but when these do occur, the affected leaves and bolls tend to shed rapidly. Thus the set-back to the crop is not readily recognized as brought about by disease except when stands of seedling plants are destroyed by black arm.

Two methods of controlling bacterial blight of cotton are known to be of practical value: (1) Treating seed with certain germicidal dusts; (2) using resistant varieties. Seed treatment reduces primary infection on seed leaves but cannot be expected to prevent the disease from building up later to serious proportions if conditions are favorable. On the other hand, under conditions of natural infection it is difficult to select and breed varieties resistant to bacterial blight, because of the erratic behavior of the disease. Under these conditions plants that escape the disease are likely to be considered resistant. A systematic program of breeding for resistance to such a disease requires methods of artificial inoculation that give uniformly severe infection.

The work reported here was concerned in part with developing suitable methods of artificial inoculation and of grading varieties with respect to relative resistance. In our first trials with the method Knight (*12*)[2] used in breeding work in the Anglo-Egyptian Sudan, infection was erratic. Thus a need arose to determine the factors and conditions essential for producing epidemics artificially. Previous investigations in the epidemiology of the disease have been concerned primarily with survival, dissemination, and spread of the causal organism (*7, 8, 9, 14, 16*) and with the effects of environmental conditions upon disease development (*20*). The present study emphasizes the host-parasite relationship itself. Special consideration was given to various methods of inoculation and to the factors affecting invasion and disease expression. The results of the study may be summarized as follows.

SUMMARY

The investigations conducted were concerned with bacterial blight of cotton, with emphasis on (1) the host-parasite relationships fundamental in epidemics of the disease, involving intensive studies on infection of individual leaves; and (2) methods for producing epidemics by artificial inoculation, including greenhouse and field experiments on the epidemiology of the disease.

Severe infection of expanded leaves was found to depend upon methods of inoculation that provide rapid penetration of large bacterial masses into susceptible tissues. Placing bacterial suspensions upon

[2] Italic numbers in parentheses refer to Literature Cited, p. 33.

mature leaves did not result in bacterial invasion, because the bacteria apparently require a liquid passage into the intercellular spaces.

Rapid stomatal invasion depends upon the degree of stomatal opening and upon the extent of water congestion induced in the intercellular spaces by water forced against the leaf surface. The term "water congestion" designates an abnormally high water content of the intercellular spaces. If leaf tisses are visibly water-congested at the time of inoculation they later become entirely diseased; if water congestion is not visible the disease develops as scattered spots.

The larger the numbers of bacteria introduced into the tissue, the shorter was the period of incubation and the more severe the disease. These relationships suggest a hypothesis of mass action effective in abundant infection of cotton leaves. Hydrosis, the first symptom, becomes visible when the masses of bacteria have built up to the point that they, together with the water or cell sap withdrawn from the surrounding cells, practically fill the intercellular spaces. The larger the initial number of bacteria introduced per unit volume of intercellular space, the shorter the period necessary for incubation and the greater the chances for further spread through the tissues.

Thus, incubation is subject to a biological mass effect. Invasion apparently is dependent upon physical mass effects, such as the number of liquid passages and the forces with which bacteria are drawn into the infection courts. These factors appear to depend upon the extent of temporary water congestion and in turn upon the water pressure effective at the leaf surface.

Susceptibility or resistance appears to be connected with the degree of compatibility of host tissue and parasite. In susceptible tissues the pathogenic bacteria spread from a given infection court into surrounding leaf areas. In resistant tissues infection is restricted to small spots or may not even result in visible lesions. It is well known that varieties differ in susceptibility. Moreover, leaves of plants that are in active growing condition are more susceptible than those of plants not actively growing; young succulent leaves are generally more susceptible than older leaves of the same plant. Under epidemic conditions, factors of susceptibility may be obscured by physical mass effects, e. g., leaves on plants of tolerant varieties may become as severely diseased as comparable leaves of susceptible varieties. Obviously so many factors affect the disease that the importance of any single factor will vary from one set of conditions to another.

Epidemics have been induced in the field by spraying plants with bacterial suspensions, using a knapsack or wheelbarrow sprayer. This method has consistently given uniformly severe disease on mature leaves if the previously stated requirements for successful inoculation were satisfied, as follows:

1. Spray-inoculate the open stomata in midmorning.
2. Apply pressure by holding the nozzle close to the leaves for producing water congestion.
3. Use sufficient concentration of inoculum by employing about 1 million bacteria per milliliter.
4. Provide susceptible plant tissue by using plants in good growing condition when 5 to 8 weeks old.

Leaf spot served as the principal indicator of relative resistance in grading inoculated field plants. Varieties were tentatively grouped in three classes: Susceptible, tolerant, and resistant, depending on the

predominant reaction of the leaves. Tolerant reaction was more readily distinguished from resistant than from susceptible reaction, which latter was recognized by larger slower drying spots and by greater secondary infection. Degrees of susceptibility within the groups were often more clearly in evidence on stems, on bolls, and on leaves in the bud stage at the time of inoculation. On these organs stomatal invasion seemed to be of minor importance.

There is general agreement with regard to relative varietal resistance in the several phases of the disease—leaf spot, black arm, boll rot, and seedling blight. The extent of the agreement, however, is not known with certainty. Deviations have been noted. Thus, stem and boll reaction should also be considered in breeding programs.

Field experiments were conducted on the effects of various factors, which had been studied first on greenhouse plants. Mild forms of the disease resulted (1) when plants were sprayed late in the evening; (2) when the spray was applied as a fine mist; (3) when plants were old or in poor growing condition. Experiments in the greenhouse were conducted in parallel with field tests; secondary infection, common in the field, was excluded in the greenhouse. On susceptible varieties it was demonstrated that leaves that were in the bud stage at the time of inoculation became rather severely infected, even when the inoculum was not applied forcefully.

Methods of artificial inoculation imitated natural inoculation during rains. Applying inoculum in the form of a mist corresponds to the effect of applying it in gentle rain; fine spray when applied as described above, to a driving rain; and coarse spray, to a rain storm. The coarse spray frequently injured tender organs. On leaves it was not much more effective than the fine spray, but on bolls and stems much heavier infection was produced by coarse than by fine spray.

Evidence was obtained showing that mild infections, especially those of bud leaves and seedlings, may be seriously aggravated by treatments imitating dew and frequent gentle rains, such as exposure to high humidity or frequent sprinkling following inoculation. These procedures deserve consideration for use in conjunction with the more severe methods recommended for inoculation of breeding plots. The severe methods have the advantage of excluding escapes from disease, as they produce symptoms in the most resistant upland varieties known. Comparative studies of inoculation methods imitating various natural conditions may also be useful in further investigations on the epidemiology of the disease. More information is particularly needed in regard to the boll rot and black arm phases.

INFECTION AND DISEASE REACTION OF INDIVIDUAL LEAVES

Recent investigations on bacterial pathogens of tobacco have demonstrated the importance of water congestion for infection of the host plant and for the development of epidemics (*3, 5, 11*). The term "water congestion," proposed by Johnson (*11*), is used here for designating an abnormally high moisture content of the intercellular spaces of leaf tissue. The present investigations are concerned primarily with water congestion produced by external means. When noticeable to the unaided eye, water congestion will be called "visible," in contrast to "nonvisible" water congestion. This is a crude differ-

entiation, considering the complex nature of the phenomena, for obviously there are various kinds and degrees of visible and nonvisible water congestion that cannot be defined properly at our present state of knowledge.

The investigations on bacterial blight of cotton were conducted from 1942 to 1945. The experiments discussed in this section were concerned with certain factors affecting the host-parasite relationship. Nearly every one of these experiments involved several factors. In order to facilitate the presentation these will be treated separately and then attempts will be made in the discussion to correlate and to interpret the interrelations. In tables 1 to 7 the results of representative experiments are given in a condensed form because it is impractical to present here all the experiments and all details of the reaction of individual leaves. To illustrate the detailed observations on which the tables are based, results from a single plant are shown in table 7, omitting notes on disease severity earlier and later than the 18th day after inoculation. The data obtained by inoculating individual leaves were confirmed also by experimental results on inoculation of whole plants, as presented in a subsequent section.

Materials and Methods

Plants were grown in the greenhouse in 2-gallon glazed pots with sandy loam soil to which 5–5–10 fertilizer had been added at the rate of 1½ ounces per pot. To reduce soil packing a porous 2-inch clay pot was placed in the center for watering. The plants were kept in a thrifty condition by adding calcium nitrate solution about once a week at the rate of 300 ml. of a ⅓ molar solution. At first, when the importance of using thrifty plants was not fully realized, 4 to 6 plants were grown in each pot and inoculated when 8 to 14 weeks old. Later, 6 to 8 plants were grown in each pot and inoculated at the age of 6 weeks or earlier.

The varieties of cotton used most frequently in these experiments were, in order from highly susceptible to resistant: S × P Egyptian, Shafter Acala, Acala 11, Trice 2A, Stoneville 37, Stoneville 4–8, and Stoneville 20. Acala 11 was used in many of the basic experiments.[3]

Bacterial suspensions of *Xanthomonas malvacearum* were prepared as follows. The bacteria were streaked thickly over hard potato dextrose (or sucrose) agar in petri dishes. After 4 to 6 days' incubation at 26° C. the slimy masses of bacteria, which covered the agar, were removed with a pot label onto a moist piece of cheesecloth spread over a funnel. Water was added to give the desired dilution, and the suspension was repeatedly filtered through the cheesecloth until uniform. The number of bacteria in suspension was estimated for several tests by dilution cultures and by direct count. When relative concentration was the factor to be considered, it was found satisfactory to base calculations on the following estimate:

The average number of viable bacteria obtained from one petri dish by the outlined procedure is about 10 billions. Suspending these in 1,000 milliliters gives 10 million per milliliter, which was the concentration frequently employed in preliminary greenhouse tests. In field inoculations, suspensions containing 1 million bacteria per milliliter

[3] The writer is indebted to the following workers for furnishing the seed lots: C. J. King, G. Harrison, D. R. Hooton, and D. M. Simpson.

were used generally. One isolate of *Xanthomonas malvacearum* was used throughout this work, including the field work, and its pathogenicity kept constant by frequent reisolation from infected tissue.

Procedures of water congesting and inoculating cotton leaves were developed and standardized so as to furnish results permitting valid comparisons within any given test. Two types of water congestion are distinguished—"visible" and "nonvisible." For producing visible water congestion the method of Diachun and others (*5*) was used. A No. 20 needle of a 10-milliliter B–D Luer-type hypodermic syringe was cut off to give a circular opening. A stream of water was forced from the syringe against the leaf surface. The syringe was moved rapidly, inducing a water-congested streak that remained visible to the naked eye for 10 to 20 minutes. Nonvisible water congestion was produced by means of a 1-quart sprayer, and more often by means of a No. 16 DeVilbiss atomizer.

An attempt was made to attain uniform pressure at the leaf surface for each of the described methods. The leaf was backed by holding it appressed to a rubber pad while the water was forced against its surface from a distance of 1 to 1½ inches. Atomizing or spraying was done from the same distance, and the number of strokes adjusted so as to apply about the same quantity of liquid per unit of leaf surface. For instance, leaves of 5-inch diameter would receive six strokes, and leaves of 3½-inch only three strokes from the atomizer. Water congestion was induced at the lower surface of the leaf, unless otherwise stated.

The degree of visible water congestion was used as an indicator of the degree of stomatal opening (footnote 1, table 2), as Diachun (*4*) has shown that satisfactory results are obtained by this method. It is realized that terms such as degree of water congestion are ill-defined. They are used here as temporary expedients, pending a clearer understanding of the complex phenomena.

Inoculation was carried out by various procedures. An attempt was made to apply uniform quantities of bacterial suspension to corresponding leaf surfaces when using any one procedure. In some experiments the inoculum was poured over the leaf surface or leaves were dipped into a jar containing the bacterial suspension. More often inoculation was carried out by atomizing or by brushing with a 2-inch paint brush. Two methods of brushing were used—"gentle brushing" and "brushing with moderate pressure." In gentle brushing the brush was moistened with bacterial suspension and held parallel to the leaf surface; the latter was touched at several places, taking care to avoid rubbing. In brushing with moderate pressure, the moistened brush was drawn over the leaf as in painting, but held at an acute angle to the surface. Care had to be taken to avoid permanent injury, which results if excess pressure is applied to any one point of the leaf by brushing or by forcing water against it.

In the course of work conducted over several years it was not feasible to avoid modifications of techniques. Data of one experiment were not strictly comparable with those of another. Moreover, the greenhouse work was done under conditions that did not permit rigid control of temperature and soil moisture. Temperatures were generally in the range of 23° to 35° C., and the pots were kept well watered. Despite considerable variations from one experiment to another, the results within any given experiment were generally consistent.

In the first experiment each test was repeated from 2 to 4 times, using at least 10 leaves for a given treatment. Whenever possible, parallel treatments were compared on individual leaves by applying each treatment to half of the leaf.

As the studies progressed and as the factors affecting infection and susceptibility were defined, procedures were adopted that made it possible to obtain comparable numerical data. The leaves were tagged and the degree of water congestion noted at the time of inoculation. Relative development of expanding leaves was indicated by measuring their diameter at the time of inoculation (table 6, footnote 1). When the incubation periods were to be determined, daily notes had to be taken. Arbitrary scales were established, showing the degree of water congestion at the time of inoculation and the relative severity of disease 3 weeks afterward (table 2, footnotes 1 and 3).

Factors Affecting Infection of Leaves

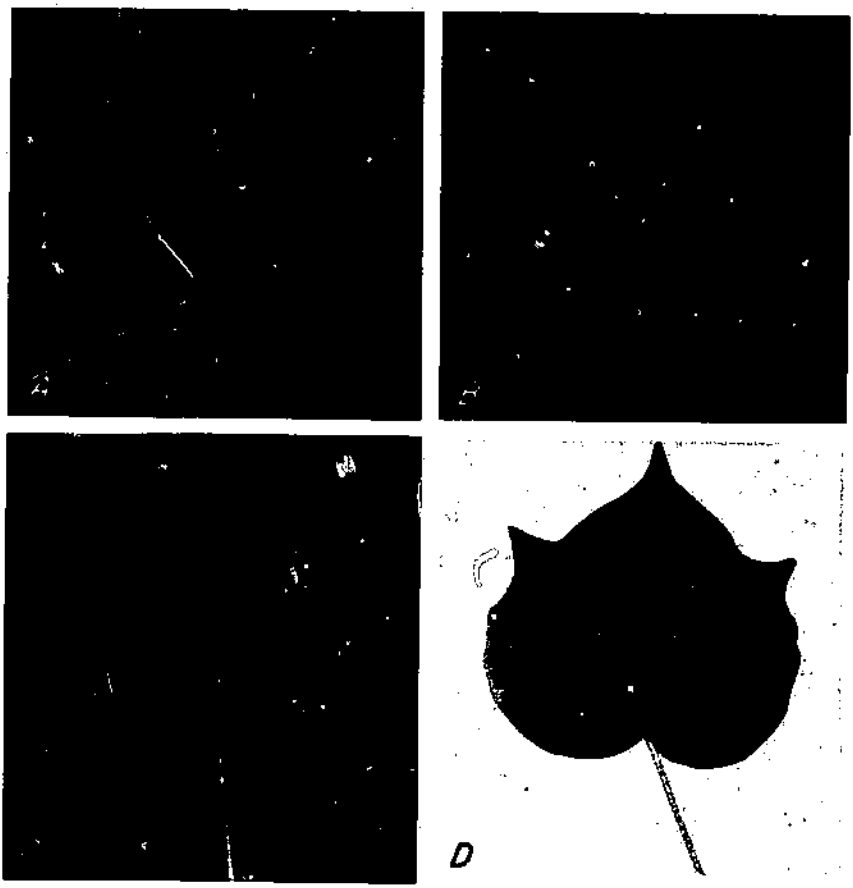
STOMATAL OPENING

Stomata of cotton leaves of greenhouse and field plants showed wide variation in stomatal reaction when tested by Dinchun's method (*5*). The pattern of stomatal behavior agreed with data reported in the literature (*15*) as follows: Stomata of mature cotton leaves are practically closed during the night. They open in the morning, rapidly in sunlight and slowly on cloudy days. Closing of stomata is less regular than opening, particularly with stomata of the upper surface. The period of maximum opening may be shortened by various factors, such as drought, shading, and low relative humidity. Stomata of bud leaves are undeveloped, those of expanding leaves tend to be open for short periods only, and those of very old leaves become permanently closed.

Abundant infection of mature cotton leaves by *Xanthomonas malvacearum* was found to take place only if stomata were open (table 1, experiments 1 and 2). Stomatal invasion took place regardless of whether bacterial suspensions were applied to the lower or upper leaf surface, using any of the following procedures: (1) Forcing the suspensions against the leaf by atomizer, sprayer, or hypodermic syringe; (2) forcing water against the leaf by atomizer, sprayer, or syringe immediately following or prior to pouring the suspension or to brushing it gently over the leaf; and (3) inoculating as in (2), but from the leaf surface opposite the one treated (tables 1 and 2).

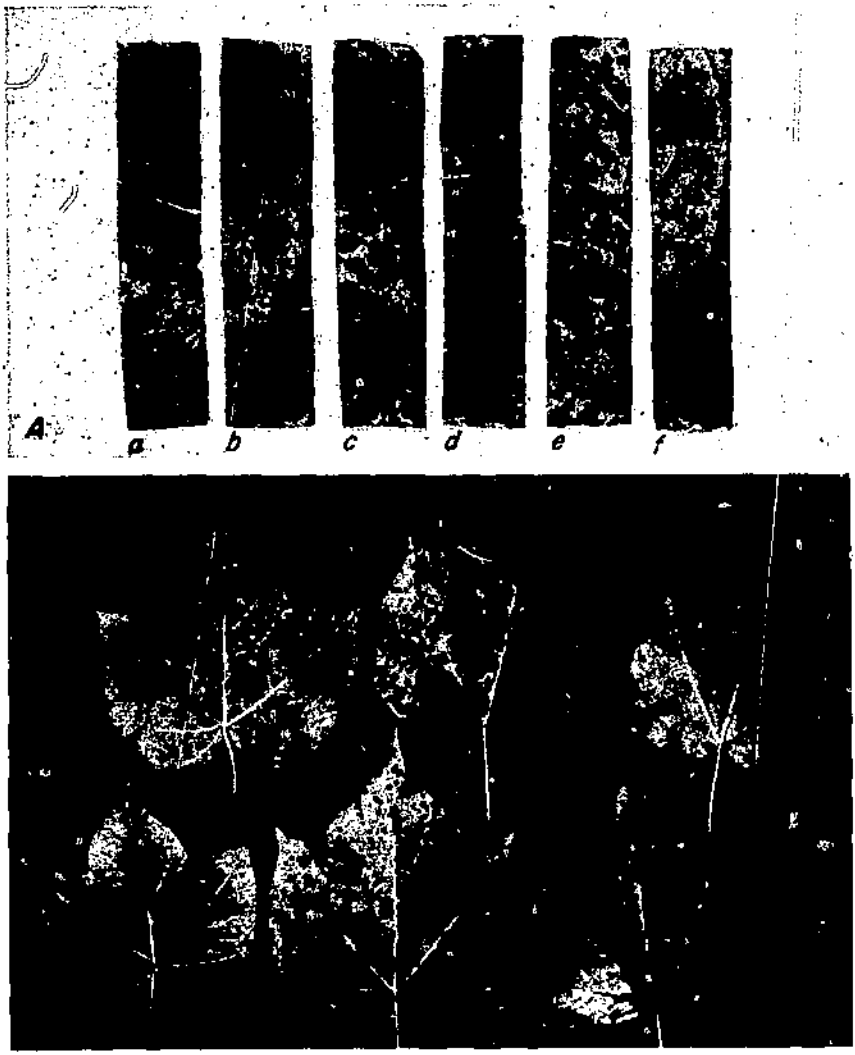
WATER CONGESTION

Proof was obtained in the same experiments that in mature cotton leaves abundant invasion through stomata depends upon the presence of water in intercellular spaces normally filled with air. This is demonstrated most strikingly by pouring the bacterial suspension over the lower surface of the whole leaf after inducing visible water congestion in the form of streaks by means of a hypodermic syringe as described above. The leaf parts thus congested at the time of inoculation become solidly infected later on, while in the rest of the leaf the disease either does not develop at all or occasionally manifests itself in the appearance of a few small spots (pl. 1, *A*). Moreover, abundant infection does not occur if the bacterial suspension



Disease symptoms produced by *Xanthomonas malvacearum* on cotton leaves, illustrating the differences in symptoms resulting from two different water-congestion procedures (*A* and *B*) and from differences in inherent resistance to the disease (*C* and *D*): *A*, Leaf of a susceptible variety, 3 weeks after inoculation, water congestion by a stream of water from a hypodermic syringe directed against lower surface of left half of leaf, followed immediately by pouring the bacterial suspension over entire lower leaf surface (table 1, expt. 3); *B*, same as *A*, except water congestion by spraying with water; *C*, leaf of a susceptible variety (Acala 11) 3½ weeks after inoculation, visible water congestion on right half of leaf by stream of water from a hypodermic syringe, immediately prior to inoculation by atomizing the whole leaf with the bacterial suspension; *D*, same as *C*, except that leaf is of a resistant variety (Stoneville 20).

775908°—48 2



Relative susceptibility of leaves as affected by stage of leaf development at time of inoculation. Plants 4 weeks old. Inoculation by spraying with a knapsack sprayer. Stomata of mature leaves open. Photographed 2½ weeks after inoculation. *A*, Sections from leaves of a Shafter Acala plant: *a* and *b*, From expanding leaves; *c* and *d*, from barely mature leaves; *e* and *f*, from fully mature leaves. × 1½. *B*, Leaves of an S×P plant: *a*, Bud leaf; *b*, expanding leaf; *c* and *d*, barely mature leaves; *e* and *f*, fully mature leaves. × ½.

the incubation period and therefore the development of the disease. This hypothesis is favored by all the results obtained in the investigation. Other conditions being the same, the concentration of the inoculum suspension determines the duration of the period of incubation and subsequently affects the severity of disease expression. The incubation period may be reduced to 2 days by applying a highly concentrated suspension of bacteria to visibly water-congested parts of leaves (tables 5 and 7), or it may be extended to 2 or 3 weeks if the leaves are atomized with dilute suspensions (table 3). Furthermore, atomizing or brushing leaves with a concentrated suspension (table 7) may induce disease symptoms earlier than applying a very dilute suspension to completely water-congested parts of the leaf (table 5).

Factors Affecting Susceptibility

Susceptibility of cotton to bacterial blight is affected not only by varietal reaction but also by the stage of development and the condition of leaves and plants. E. F. Smith (*19*), in his pioneer work on bacterial diseases of plants, recognized that "juicy" or succulent tissues favor disease development, and this fact has since been confirmed by all workers dealing with this or similar diseases.

STAGE OF LEAF DEVELOPMENT

In the course of the present investigation the need arose for a more precise definition of factors affecting susceptibility, especially with respect to individual leaves. Frequently, because of variations between leaves, averages of numerical data did not adequately express differences in disease severity resulting from various treatments, although large differences were readily seen on examining the leaves.

In the early experiments this difficulty was partly overcome by using mature leaves only and by giving the contrasting treatments to the opposite halves. It was noted, however, that barely mature leaves tended to become more severely diseased than older ones, even though stomata were uniformly wide open, as shown by the visible water congestion at the time of inoculation. In these barely mature leaves the diseased streaks appeared earlier and the lesions extended farther into the neighboring tissues than in older leaves. When atomized with bacterial suspensions, old leaves developed smaller lesions than younger leaves and sometimes the old leaves did not become diseased at all.

The brush inoculation method permitted extension of these tests to expanding and bud leaves. Stomata are generally closed in bud leaves and in leaves at early stages of expansion, while at later stages stomatal opening varies much more than in mature leaves. When inoculated by brushing with moderate pressure, leaves in the expanding stage became more severely diseased than mature leaves, whereas the latter were generally the more severely infected by methods involving stomatal invasion (table 7). Bud leaves tended to have few lesions, which in highly susceptible varieties became very large at a later stage, often resulting in distortion of the leaf.

The high susceptibility of bud leaves is often not reflected in the data on disease severity because the latter is an estimate of diseased leaf area, depending on both number and size of lesions. However,

when methods of inoculation that cause very mild infection (dipping leaves into bacterial suspension or gentle brushing) are used, leaves of all stages have few spots; the disease in bud leaves is then the more severe, particularly in highly susceptible varieties (table 6). Vein blight was prevalent in leaves inoculated at the early expanding and bud stages (pl. 2, *B*). The vein blight consisted usually of bands of infected tissues adjoining the veins and rarely affected the vein tissues themselves.

The size of individual lesions tended to decrease gradually from bud leaves to those fully mature. This effect was most clearly shown (pl. 2, *A*) when whole plants with four to six fully expanded leaves were sprayed uniformly with bacterial suspension, using the coarse spray of a knapsack sprayer as later described. In presenting such data on plants with more than three expanded leaves, the following groups were distinguished: "Bud;" "top" (expanding leaves with diameters ranging from 10 to 70 mm. at the time of inoculation); "mid" (barely mature); and "low" (fully mature) leaves (tables 6 and 7).

Among leaves at various stages of development, the incubation period becomes generally longer the older the leaves (tables 6 and 7). The period of incubation, however, is usually longer in leaves of the early expanding and bud stages than in those of the late expanding and early mature stages. Intercellular spaces are small at first, increasing gradually during expansion, particularly through development of spongy parenchyma. Thus, room for large masses of bacteria is provided only after some days, accounting for the delay in symptom expression.

AGE AND CONDITION OF PLANTS

It was commonly observed that leaves were fully susceptible to the disease only when the plants were in vigorous growing condition before and during the development of lesions. In this field of investigation studies are needed to define more clearly the following factors and their interrelations: (1) Temperature and water relationships, particularly with respect to soil moisture; (2) nutritional conditions; (3) general growing condition and age of plants. The few critical data obtained in the present work will be briefly discussed, together with incidental observations.

Data on the effect of soil moisture were obtained in one of the earlier experiments, using mature leaves on 3-month-old plants. The plants of three pots had been kept rather dry, while those in three others were watered normally. At the time of inoculation water congestion of leaves was equally good in both series. On the leaves of plants growing in moist soil the disease was more severe and the average period of incubation shorter. The results were consistent under two conditions of inoculation and at two concentrations of inoculum (table 3). In a later test similar results were obtained with younger plants (7 weeks old when inoculated), but the low leaves were more severely diseased in the "dry" plants, apparently because they remained longer in a "juicy" susceptible condition, whereas bud and expanding leaves were retarded in growth and therefore less susceptible.

Considering data obtained in the early tests with old plants in rather poor growing condition, in contrast to later data on young plants in good growing condition, water congestion was obtained much more readily in leaves of the young plants than in those of the old.

Moreover, disease symptoms tended to be more severe in thrifty plants regardless of conditions affecting water congestion.

VARIETAL REACTION

Under the conditions of these greenhouse experiments, outstanding resistance of mature leaves was evident in some strains of the Stoneville variety only. When comparing highly susceptible, susceptible, and tolerant varieties, striking differences were sometimes noted with respect to the reaction of bud leaves. When mature leaves of thrifty plants of tolerant varieties, however, were inoculated under conditions of water congestion, they became as severely diseased as those of susceptible varieties (table 5). The lesions of the former tended to be only slightly smaller and tended to dry a little faster than lesions on the more susceptible plants. When such differences were present they were not reflected in the duration of the period of incubation but seemed to involve the subsequent spread of bacteria through the leaf tissue.

The high resistance of Stoneville 20 was manifested either in absence of lesions or in very small lesions, which were brown and dry from the beginning. Complete water congestion in leaves of this strain was as readily obtained as in susceptible varieties. When, subsequent to such water congestion, leaves were inoculated with a highly concentrated suspension of bacteria (100 to 300 million per ml.) a light-brown streak was obtained (pl. 1, *D*) on the following day; that is, a day earlier than hydrosis would have appeared under the same conditions in leaves of susceptible varieties.

This extremely rapid reaction suggested a phenomenon similar to the hypersensitivity reported in rusts, but this idea is contradicted by such evidence as is now available. Neither host tissues nor bacteria seem to be killed. It appears rather that the discoloration of the tissue is connected with a resistant reaction of the host cells that restricts multiplication and spread of the bacteria. No reaction ensued when bacterial suspensions of rather low concentrations were applied to water-congested leaves of Stoneville 20; pale-yellowish streaks resulted on some leaves when suspensions of intermediate concentration were used, usually becoming apparent shortly after typical symptoms of hydrosis were noted on leaves of susceptible varieties correspondingly inoculated. Furthermore, in some instances, a few typical lesions were seen on leaves; under epidemic conditions in the field, greenish-yellow halos appeared around necrotic spots at the time when abundant secondary spread was evident around diseased lesions on leaves of susceptible plants.

Discussion

HYPOTHESIS OF INFECTION

When bacterial suspensions are merely placed on the surface of mature cotton leaves, infection does not result. Apparently the bacterial masses require a liquid passage leading from the surface into the intercellular spaces. Such invasion passages are provided by methods that produce water congestion by external means.

When India ink is placed on the surface of cotton leaves while parts of their tissues are completely or partially water-congested,

some of the ink is drawn immediately into the water-congested parts. This effect has been demonstrated by Diachun and others (*6*), and supports the view that bacteria are drawn into the intercellular spaces passively, apparently by capillary forces. This work has been extended here to include the aforementioned brushing method. When India ink was introduced into the intercellular spaces of cotton leaves by brushing with moderate pressure, it could not be removed by washing the leaf with water. Apparently the small wounds caused by brushing furnish the passages, while cell sap from broken cells provides water congestion.

By coupling a hypothesis of incubation with that of invasion, infection of water-congested leaf tissue may be visualized as follows: The first symptom of the disease, hydrosis, is outwardly similar to the visible (temporary) water congestion produced by a hypodermic syringe. Apparently hydrosis sets in when the zoogloeal masses of bacteria have built up to the point at which they are able to withdraw water or cell sap from the surrounding cells to such an extent that the intercellular spaces become permanently congested. The period necessary for building up the bacterial masses to the critical point is the period of incubation.

Evidence cited above indicates that the larger the initial number of bacteria introduced into the unit volume of intercellular spaces the shorter the incubation period becomes. This initial number depends upon (1) the concentration of the bacterial suspension used as inoculum and (2) the degree of water congestion. This latter, in turn, involves two factors—the number of liquid passages and the force with which the bacterial suspension is drawn through them. Thus, in addition to the biological mass effect that dominates incubation, physical mass effects may be postulated as governing invasion.

It is realized that the effects of moisture and other factors in infection are of a very complex nature and that there is need of detailed basic investigation. The hypothesis discussed in the preceding paragraph should be considered therefore as an outline, oversimplified for the purpose of presenting a coherent picture of infection.

SUSCEPTIBILITY OR RESISTANCE

The terms "susceptibility" and "resistance" are applied here to the reaction of invaded tissues. The severity of individual lesions was taken as a criterion, regardless of whether relative reaction was compared within any of the kinds of susceptibility; that is, among leaves at various stages of development, among plants grown under various conditions, or among varieties. When used in this sense, susceptibility and resistance were expressions of the compatibility of host and parasite and were shown to be independent of invasion phenomena such as stomatal opening and of water congestion produced by external means at the time of inoculation. In other words, if the tissues surrounding a given infection court are susceptible, the pathogenic bacteria will spread quickly and continuously; in less susceptible tissues, multiplication and spread are retarded; in resistant tissues, spread is restricted so much that the lesions are very small, or no visible symptoms appear.

The nature of the susceptibility or resistance of cotton to bacterial blight is understood as little as are basic factors of most other plant

diseases. Obviously, host-parasite relations are complex. There may be factors of susceptibility involving moisture and nutritional relationships; and there may be factors of resistance, such as substances produced by the host that inhibit the pathogen. (The existence of three genetical factors of resistance has been indicated by Knight (*12*, *13*).)

The lack of definite information with respect to the basic factors involved makes it difficult to propose an adequate theory. Evidence and observations suggest that susceptibility of cotton to the various forms of bacterial blight is associated with immature tissues and with a slow rate of maturing. It is not known, however, whether the various kinds of susceptibility are fundamentally the same. The effects are similar, but the causes need not be the same. Obviously mass phenomena are involved, as has been discussed in relation to incubation. Mass development of bacteria in susceptible tissues, however, is a consequence and not a cause of susceptibility.

INTERRELATIONS

It remains to examine how susceptibility and resistance affect disease expression when the various kinds of susceptibility are considered in conjunction with one another and with factors affecting infection.

Susceptibility and resistance are relative reactions. Varieties may be arranged in the order of increasing resistance as determined under a given set of conditions. A change in these conditions does not affect the relative resistance rating of the different varieties, even though the general level of disease in all varieties may be altered. Likewise, on a given plant with leaves at various stages of development, susceptibility tends to increase from the oldest to the youngest leaf; this relative reaction is independent of concentration of inoculum and of variety. When highly resistant varieties show any disease symptoms at all, they appear in the expanding and barely mature leaves.

The element of time is important in considering the combined action of the various factors that affect the disease. This time factor has been discussed already in connection with effects of mass action upon the duration of the incubation period. When inoculated with dilute bacterial suspensions, fully mature leaves that water-congested well at the time of inoculation often failed to become diseased, while expanding leaves that water-congested poorly developed severe symptoms. By the time these symptoms appeared, that is, from 10 to 20 days after inoculation, the older leaves had lost their susceptibility. Thus the kinds of tissue susceptibility that are connected with leaf development and plant condition are dynamic, that is, they may change while the host-parasite relationship is in progress. Obviously this is more likely to occur when incubation and symptom development extend over a long period.

Even though the system of factors affecting infection and susceptibility is complex, the data presented in tables 1 to 7 show that single factors and their effects can be clearly recognized. It is necessary, of course, to choose a range at which reactions to a given factor are well differentiated. Moreover, the aforementioned interrelations of fac-

tors have to be taken into consideration. In table 7, disease reaction of leaf No. 7 is shown to be definitely less severe than for leaf No. 8, taking into account the stomatal condition at the time of inoculation as expressed by the degree of water congestion. For both leaves the ratio of water congestion to severity is about 0.6.

Generalizations have been made here on the basis of such evidence as is now available. They are submitted as a possible basis for work toward a more complete understanding of the disease, a goal that can be reached only by further research on the factors studied here as well as on other factors. In these experiments no concerted attack has been made on the role of moisture conditions as they affect the host-parasite relationship. The complex nature of this problem has become obvious through many observations. Thus, "juicy" leaf tissues have been found more susceptible than "dry" tissues, but often juicy leaves water-congest easier than dry ones, other factors being equal. Extensive investigations on other bacterial diseases have demonstrated the importance of internal humidity (*17*) and internal water congestion (*11*). Indicative data along this line will be given in the last part of this bulletin. Emphasis has been placed here on methods of inoculation that involve water congestion by external means. These methods have proved practical for producing epiphytotics by artificial means.

ARTIFICIALLY INDUCED EPIDEMICS IN RELATION TO VARIETAL RESISTANCE AND EPIDEMIOLOGY

METHODS

DEVELOPMENT OF FIELD TECHNIQUES

The data presented in the first part of this bulletin provide an explanation for the inconsistent results obtained in previous field trials of the inoculation technique described by Knight (*13*). It had not been recognized that this technique was fully effective only when the plants are in good growing condition and when stomata are open at the time of inoculation.[4] The methods subsequently developed are modifications of those used by Knight for producing artificial epidemics and for determining relative varietal resistance.

In order to be useful to the plant breeder such methods should be as simple as possible and standardized so as to give consistent results. Methods should be simple enough to permit easy and rapid preparation of inoculum, inoculation of large numbers of field plants, and grading of individual plants. Standardization should minimize the effects of variable natural conditions and provide uniformly severe disease through (1) uniform coverage with inoculum, preventing any plant from escaping disease; (2) uniform procedures of inoculation; and (3) a comprehensive system of grading.

In developing satisfactory field techniques the outlined requirements were kept in mind, together with the understanding of disease factors gained by studies on individual leaves. Some work in inocu-

[4] These points are not clearly brought out in Knight's original description. A recent communication from him indicates that they have been considered in practice.

lating individual leaves was done on field plants, using procedures that involved visible or nonvisible water congestion. The results were similar to those reported for greenhouse plants. Once, after a heavy rain, leaves of young field plants were found to be so "juicy" that atomizing produced visible water congestion.

Inoculation with the knapsack sprayer was first tested in the greenhouse. The effect of the water congestion induced was intermediate between that of hypodermic syringe and atomizer. Usually water congestion was not visible; but visible water congestion resulted when a leaf was backed with a rubber pad while the spray was applied from a distance of 5 to 6 inches. Such visible water congestion did not appear if stomata were closed; for instance, in leaves of plants shaded for 2 hours prior to inoculation.

In developing the method, it was found that greenhouse conditions have the advantage of being semicontrolled and permit continuous close observation of the development of the disease. Furthermore, the direct effects of the inoculation could be recognized independent of the secondary infection that is frequently responsible for variations in the field. On the other hand, greenhouse tests had to be restricted to a small number of plants. Moreover, greenhouse plants growing in pots with well-fertilized soil generally differ from field plants. They are more tender and succulent at first but tend to get more rapidly into poor growing condition. With a limited quantity of soil available, the roots of the plants become pot-bound and growth is retarded despite frequent watering and heavy fertilization.

INOCULATING PLANTS IN BREEDING PLOTS

The method of inoculating field plants consisted in applying bacterial suspensions with a sprayer. A knapsack sprayer was satisfactory for small plots; a wheelbarrow sprayer equipped with a pressure tank was preferable for larger plots. When spraying with the wheelbarrow type, it was found convenient to hold the pressure at 125 pounds. This method was first tried in 1944, applied on a larger scale in 1945, and briefly described in a recent article (*18*).

Field plants were inoculated when in vigorous growing condition, usually when 5 to 7 weeks old. Growing condition is dependent upon soil moisture and other environmental factors that cannot be controlled. Growing condition, therefore, is the feature least susceptible to standardization.

Preparation of inoculum was standardized, using the procedure described for experiments on individual leaves. Concentrated bacterial suspensions containing the yields of 10 petri dishes in 1,000 ml. were taken to the field. By adding this suspension at the rate of 10 ml. to 1,000 ml. of water, a spray suspension was prepared that contained about 1 million bacteria per milliliter. One gallon of this suspension was sufficient for spraying a 100-foot row of plants 5 to 7 weeks of age.

Stomatal opening was tested in the first trials, using as a criterion the degree of water congestion produced by a stream of water from a hypodermic syringe, as described above. It was found later that this test is not necessary when plants in good growing condition are sprayed in midmorning of clear days, because at that time the stomata on the under side of most of the mature leaves are wide open.

775908°—48—— 2

Spraying aimed at producing a high degree of nonvisible water congestion in a large percentage of leaves was effected by (1) directing the spray toward the under side of the leaves, where stomata are more uniformly open than on the upper side; (2) holding the nozzle as close to the leaves as possible; and (3) adjusting it so that it would deliver a semicoarse spray. Thus, the procedure resembles that of applying pesticides to obtain uniform coverage, but differs from that method in that the spray is made to strike the leaves forcefully, like a driving rain. When carefully applied under favorable conditions, this method gave uniformly severe disease, even though the spraying was done only once. Repeating the spray on the following day from the opposite direction, and with order of rows reversed, insures against the possibility that any of the plants might escape infection.

GRADING FOR RELATIVE RESISTANCE

When plants are sprayed according to the method just described, severe disease symptoms appear on mature leaves within 5 to 7 days. Except on highly resistant plants these lesions coalesce frequently. Severely affected leaves start shedding from 2 to 3 weeks after inoculation. In 1944, attempts were made to judge relative resistance of varieties by severity of disease, as expressed by the number of diseased leaves and by the diseased areas of individual leaves. This procedure was laborious and not satisfactory unless secondary infection also was considered.

A practical scheme of grading varieties with respect to resistance was developed in 1944 and extensively used in 1945. Essentially it is a simplified version of Knight's method (*13*) and has been briefly described previously (*18*). Grading is based primarily upon the size and appearance of disease spots (outside of coalescent areas) about 3 weeks after inoculation. In addition to spot size, the following characteristics indicate the degree of compatibility between host and parasite: Rapidity of drying of lesions; color when dry; bacterial exudate; secondary infection; and vein blight (pl. 3).

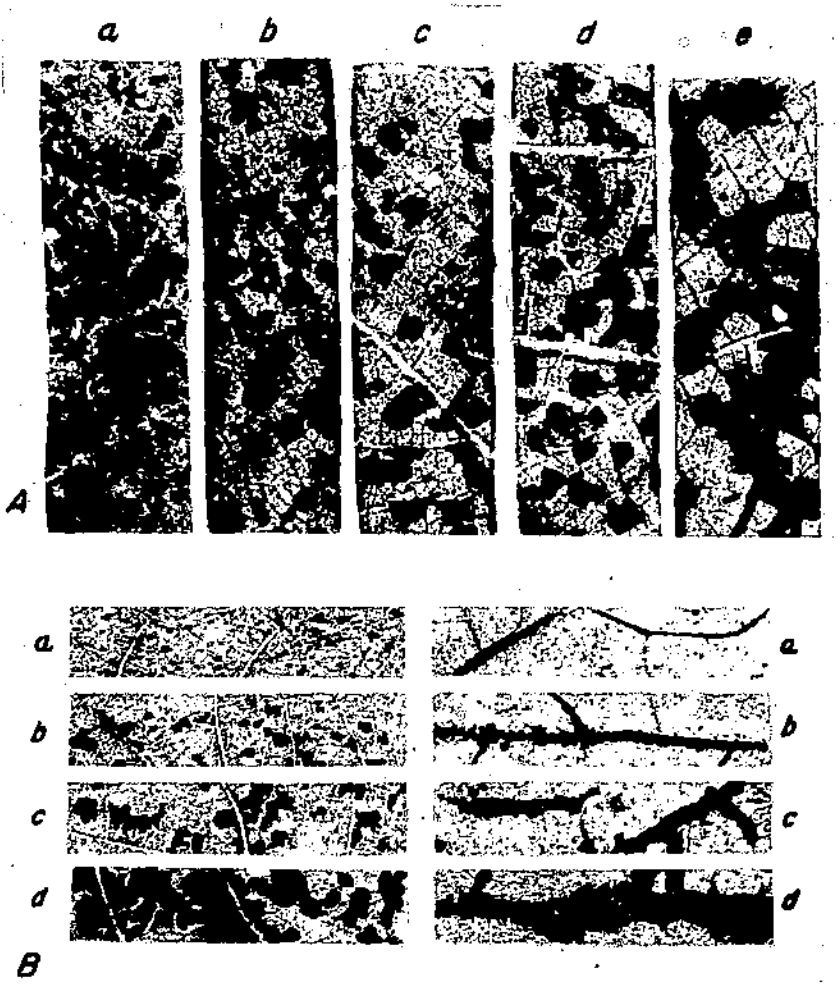
Three groups have been established as a tentative basis for classifying plants or varieties with respect to resistance to bacterial blight. These groups are defined by their prevailing leaf reaction, three types of which are differentiated (table 8).

(1) A resistant reaction is easily recognized by nontypical small roundish spots, which appear some days after typical lesions and are dry and brown from the beginning; coalescent areas and vein blight are very rare.

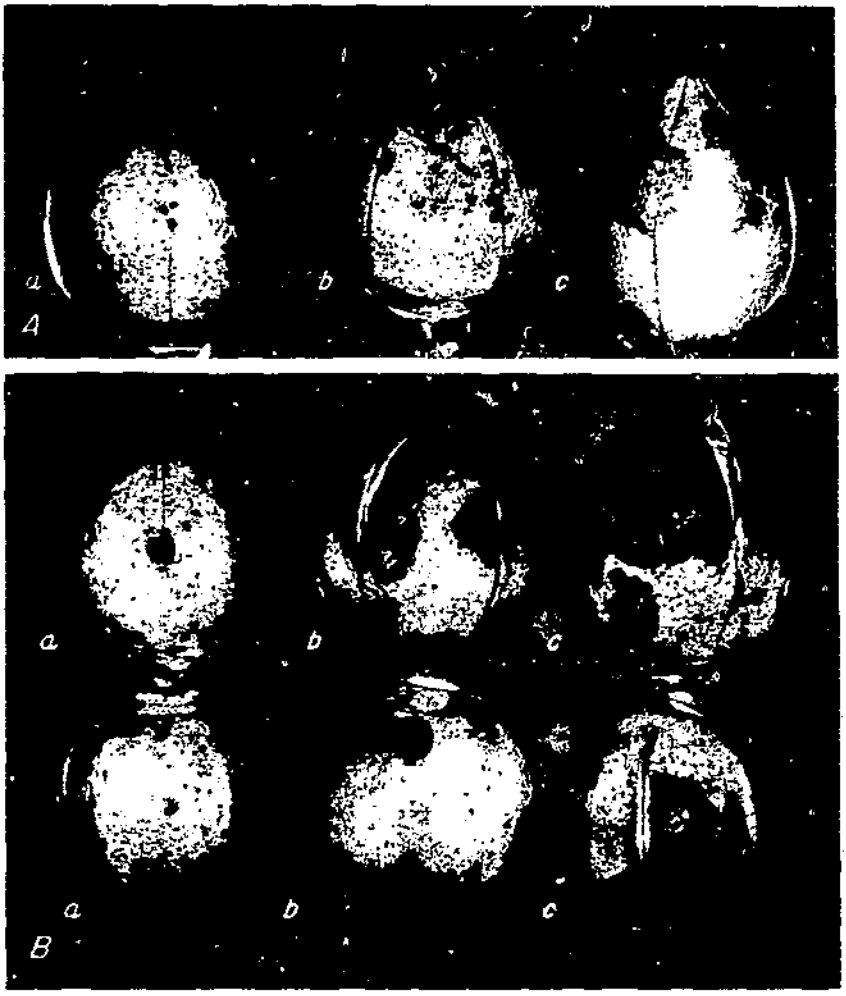
(2) A tolerant reaction is identified by typical green angular spots of 1 to 2 mm. in diameter, which become brown when dry and often coalesce; secondary spread and vein blight are rather limited.

(3) A susceptible reaction is characterized by large green angular spots, which tend to dry slowly, becoming dark brown or black and are frequently covered by dry grayish films of bacterial exudate; secondary spread and vein blight are abundant. Tolerant and susceptible reactions are not so readily distinguished from one another as are tolerant and resistant reaction.

On the basis of this classification, a numerical system of grading upland varieties was devised in 1945. The following grades were used: Grade 1, resistant reactions; grades 2 and 3, tolerant; and grade 4,



Relative varietal resistance in the leaf spot phase (table 8). *A*, Sections cut from representative leaves of plants in field plots that had been sprayed with bacterial suspension, using a wheelbarrow sprayer: *a*, Stoneville 20, *b*, Stoneville 4; *c*, Stoneville 37; *d*, Trice 2A; and *e*, Shafter Acala. Leaves collected in September 1944, 4 weeks after inoculation. × 2. *B*, Leaves collected in August 1945, 3 weeks after inoculation. × 1½.



Relative varietal resistance in the boll rot phase. Full-sized bolls inoculated by rubbing with cheesecloth wetted in a concentrated suspension of *Xanthomonas malvacearum*. *A*, Bolls of greenhouse plants photographed 2 weeks after inoculation when most lesions were in the green water-soaked stage: *a*, Stoneville 20; *b*, Stoneville 4-15; *c*, Stoneville 37. About ½. *B*, Bolls of field plants photographed 5 weeks after inoculation when green water-soaked areas were present only at the margin of spreading lesions; two bolls of each of the following strains: *a*, Stoneville 20; *b*, selection made by D. M. Simpson from a backcross of Trice 2A to a hybrid of Stoneville 20 and Trice 2A; *c*, Trice 2A. About ½.

susceptible (table 9, pl. 3). Intermediate reactions are indicated by decimals. This tentative scheme may serve as a basis for a more comprehensive system, possibly including varietal reaction in other phases of the disease.

DETERMINING RELATIVE RESISTANCE IN THE BLACK ARM AND BOLL ROT PHASES

It would be desirable to have one method of inoculation for all phases of the disease. This, however, does not seem to be feasible. Special methods have been developed, therefore, for inoculating and grading in the black arm and boll rot phases. These methods induce invasion of bacteria primarily by mechanical injury. It appears that these methods are not so dependent upon stomatal opening as the leaf inoculation method. It is not known to what extent stomatal behavior of leaves agrees with that of bolls and stems.

Field tests in 1944 indicated that in upland varieties black arm was serious only when a very coarse spray was applied to young plants. Otherwise the method of inoculation did not differ from that previously described. Under field conditions stem lesions appeared within 2 to 5 weeks after inoculation, mainly on the internodes of the stems. This indicated that most of the black arm originated from direct infection and that little was secondary, that is, working down from the leaves through the petioles.

Grading for resistance in the black arm phase was based on (1) percentage of diseased plants, and (2) severity of individual lesions. Severity was estimated by a scale ranging from 1, very slight, to 5, very severe (table 10, footnote 3), depending upon size and depth of the cankers.

Preliminary tests in 1944 suggested that severe bacterial boll rot may be obtained in the field by two methods, both of which were used extensively in 1945: (1) Rubbing individual bolls with a tooth brush or with a piece of cheesecloth wetted with a bacterial suspension and (2) spraying the bolls as described above, but using a coarse spray. The rubbing method was very effective but slow. Rubbing produces many small abrasions through which the bacteria enter. Boll infection was not induced without such wounds; however, severe wounds made by a scalpel resulted in rapid drying of the induced lesions. Coarse spraying required large quantities of spray, at least 3 gallons to each 100-foot row; semicoarse spray was ineffective. Boll lesions appeared within 6 to 14 days after inoculation.

Grading for resistance in the boll rot phase was based on two criteria: (1) Percentage of diseased bolls; (2) severity of individual lesions. Severity has been defined by an arbitrary scale ranging from 1, resistant, to 10, highly susceptible, as indicated by size of lesion, color, rapidity of drying, depth, and tendency to spread. The extremes of disease reactions on bolls are recognized as easily as those on leaves. The highly susceptible reaction is characterized at first by green water-soaked raised lesions. They tend to spread rapidly and to dry rather slowly in the center, forming craterlike depressions. Frequently the whole boll becomes involved, the lint is discolored, and the rot is aggravated by fungus infection. The resistant reaction is manifested at first by small pale-green flat spots. They dry rather quickly, resulting in brown scabby lesions that usually do not result in serious injury

to the boll. Intermediate reactions cover the range between these two extremes (pl. 4). At the time of taking data on severity, the predominant reaction (mode) and the range of reaction were determined for each variety.

In view of the practical importance of boll rot, methods of inoculation need further investigation. In developing satisfactory procedures the following factors may need consideration: (1) Bolls lack uniformity in development on the same plant and on the different varieties; (2) shedding of young bolls interferes because it is difficult to determine whether it is due to physiological causes, boll weevil punctures, or bacterial blight.

EPIDEMIOLOGICAL STUDIES

The described method of field inoculation was adopted after comparing various procedures in greenhouse and field tests. These experiments also gave interesting information with respect to the epidemiology of the disease. Factors affecting the disease under conditions of artificial inoculation are likely to operate under conditions of natural inoculation. Similar tests were conducted therefore in 1945 in order to check on the results obtained in 1944.

Four procedures of spraying were used—mist, fine, semicoarse, and coarse spray. The semicoarse spray has been described in detail. The fine spray follows the same procedure, except that the adjustable nozzle is closed to produce the finest spray possible. The usefulness of the coarse spray has been pointed out in describing methods of stem and boll inoculation. This spray differs from semicoarse in that the nozzle is opened to the point at which it still produces a cone of spray rather than a solid stream. When applied from a distance of 1 foot, coarse spray will sway plants and occasionally tear tender leaves. For the mist spray the nozzle is adjusted as for fine spray. When the nozzle is held at a distance of 1 to 2 feet to the side of the plant, a mist is made to drift onto the leaves, settling mostly on the upper surface. The four types of spray may be likened to three types of rain: Mist, to drizzle; fine and semicoarse sprays, to driving rain; and coarse spray, to a rainstorm.

In addition to spray type, four factors were investigated and are here listed, indicating the specific comparisons made in the tests: (1) Replication of sprays—spray applied on 1 vs. 2 and 3 days; (2) stomatal opening—spraying in midmorning vs. late evening; (3) age of plant—4 vs. 6 weeks; (4) concentration of inoculum—suspensions having 1/10 vs. 1 and 10 million bacteria per milliliter.

In the field tests treatments were applied to 50-foot rows arranged in randomized blocks, allowing at least two replicates for each treatment. In most experiments more than one variety was used.

Data on results in the black arm and boll rot phases were obtained as outlined for those on varietal resistance. In order to determine the effects of the various treatments in the leaf spot phase other procedures had to be devised. Two kinds of data were taken. (1) The immediate effects of inoculation, apparent within 2 to 3 weeks, were determined by rapid estimates of number of diseased leaves and relative severity of disease, as indicated by leaf area affected. (2) Long range effects were investigated in an attempt to differentiate leaves mainly affected by primary infection (originating from the artificial inoculation) from those affected by secondary infection (due to natural spread through rain).

In each 50-foot replicate row 10 plants were chosen at random. A tag was attached to the leaf node that was judged to separate leaves affected mainly by each of the above 2 types of infection. In 1944 this was done 3 weeks after inoculation, when the first symptoms due to secondary infection appeared. In 1945 it was done 1 week after inoculation. The leaf, which was at that time in the late expanding stage, was marked, as this was known to be usually the highest leaf subject to primary infection. Data were recorded 5 to 6 weeks after inoculation. Counts were made of leaves shed and of diseased leaves, and the relative severity of disease was estimated on individual leaves. Only the leaves attached to the main stem were considered.

Spots on bracts, obviously due to secondary infection, were common in 1944. Counts were made of the number of squares, blooms, or young bolls thus affected. In 1945 bract infection was too scarce to be considered.

GREENHOUSE AND FIELD PRACTICES

For the greenhouse experiments plants were grown as described in the first part of this bulletin, page 5.

The field plots were located near Clemson, S. C., in a field of about 1 acre on Cecil sandy loam. The land was fairly uniform, considering the sloping soils of the Piedmont section, and had not been under cultivation for several years but was plowed in the spring of 1944.

Acid-delinted seed was planted between May 9 and 11, in 1944 and 1945. A hand-type planter was used by drilling small lots in the variety plots. Otherwise, seed were planted with a horse-drawn planter in hills 13 inches apart. Rows were 3 feet apart. Three weeks after planting the seedlings were thinned, generally to three plants per hill.

Cultivation followed practices commonly used in the Piedmont section of South Carolina. Before bedding, a 5-10-5 fertilizer was disked into the soil, at the rate of 400 pounds per acre. Side dressings of nitrate of soda were applied shortly after thinning and 3 weeks later, using a total of 250 pounds per acre in 1944 and 500 pounds in 1945.

Early plant growth was good in 1944, but in 1945 it was retarded by cool moist weather during May and June. (This is the reason for the heavier nitrate application given in 1945.) In May and June, average monthly mean temperatures were from 3° to 4° F. lower in 1945 than in 1944, while total rainfall for the 2 months in 1945 was more than twice that of 1944 (table 11). The rest of the 1944 season was rather dry, and the growing condition of the cotton plants was much poorer during that time than in 1945, when frequent rains continuing late into the season induced rank growth. Thus, taking into consideration the first 2 months and the remaining months of the growing season apart from one another, growing conditions for cotton plants in 1945 were exactly the reverse of those for 1944. Boll weevil damage was slight in 1944, but heavy in 1945.

In both years the available field space was divided about equally between plots for (1) testing varieties and strains with respect to relative resistance and (2) investigating epidemiological aspects of the disease. In 1944 this separation was not strict, as experiments were made on the effectiveness of various methods of spraying in

conjunction with testing relative varietal resistance. In 1945 the standardized methods described above were applied to two variety plots of one-fourth acre each, planted to the same 54 strains. One of the plots was a newly plowed section at a little distance from the rest of the field. The strains were planted to allow one 50-foot row for each strain in each plot. Every sixth row was a checkrow of either Acala 11 or Coker 100 Wilt, varieties whose reaction had been established previously as "susceptible" and "tolerant." These varieties were chosen because large quantities of seed were available. Using interspersed checkrows instead of replicates was satisfactory with respect to excluding the effects of variable soil conditions. This procedure would seem to be particularly suitable for breeding plots when one wishes to determine the relative resistance of single plants or of selections consisting of a few plants.

Epidemiological experiments were conducted in 1945 on two quarter-acre plots consisting of alternate 100-foot rows of Acala 11 and Coker 100 Wilt. The plots were arranged in randomized blocks providing two or three 50-foot rows of each variety for every one of the treatments. In the epidemiological tests of 1944 plot arrangements were similar but not so well standardized.

Table 9 gives information relating to the strains used in the variety plots; no useful purpose would be served by enumerating here all the strains tested. When selecting the strains for tabulation, emphasis was placed on those developed by D. M. Simpson, because they are representative of a wide range of reaction.

The experiments on the effect of seed and seedling inoculation were conducted in 1945 on a small plot of 16 100-foot rows, using 4 susceptible varieties.

Greenhouse Experiments

RESULTS

Uniformly severe infection was obtained on susceptible varieties in early experiments testing the use of a knapsack sprayer. In the first trial made, leaves of all plants with the exception of Stoneville 20 became so severely diseased that at first glance there seemed to be little difference among them. As may be seen from the data of table 12, however, closer observation revealed varietal differences with regard to disease susceptibility, particularly on the older plants and on bud leaves. In another experiment, the results of which are not here recorded, it was found that variations in inoculation spray type, such as doubling the quantity of spray, repeating the spray on three consecutive days, or using fine or coarse spray, had little influence on the disease reaction. In this experiment, carried out on 5½-week-old plants, it was interesting to note, however, that Stoneville 20 showed a slight disease reaction, with very small atypical lesions on the leaves and without hydrosis. As might have been expected from earlier experiments on isolated leaves, leaf blight was greatly reduced by the use of such distinctly less severe inoculation techniques as applying the inoculum when the stomata were closed or applying it as a mist when the stomata were open (table 13). The mildness of these inoculation procedures was particularly evident in the case of midleaves and least evident on bud leaves.

Influence of concentration of inoculum on severity of bacterial blight. Greenhouse seedling plants of Acala 11 inoculated by use of a coarse spray from a knapsack sprayer (table 10). Appearance of plants 3½ weeks after inoculation with three concentrations of bacterial suspension: *A*, Low; *B*, intermediate; *C*, high. × ¼.

775808 48 4

Blight-infected stem sections of plants of varieties differing in resistance to *Xanthomonas malvacearum* in the black arm phase: *A*, Stoneville 20; *B*, Stoneville 1-15; *C*, Stoneville 37; *D*, Trice 2A. Inoculation by use of a concentrated suspension of the bacteria applied in a coarse inoculation spray from a knapsack sprayer. Plants 4 weeks old. Photograph of representative sections of infected stems taken 4 weeks after inoculation.

Results of an experiment designed to indicate the influence of age of plant, variety of plant, and concentration of inoculum on infection of leaves and stems of cotton plants with *Xanthomonas malvacearum* are shown in table 10. The influence of age of plant appeared most strikingly in the case of the black arm phase. The influence of concentration of inoculum was apparent in both leaf spot and black arm phases in all cases. It is worthy of note that in this experiment all plants were in excellent growing condition and that the succulent condition of leaves and stems helped to make the coarse spray very effective in producing extremely severe disease, especially at the highest concentration of inoculum. Several plants of S × P were completely killed back by stem blight, as were also plants of Acala 11, that had been inoculated in the seedling stage (pl. 5). For the first time stem lesions were noted on Stoneville 20 (pls. 6 and 7) and leaves of the same variety were found with typical angular spots, although such leaf spots were few and dried very rapidly.

The high susceptibility of S × P and the outstanding resistance of Stoneville 20, when compared with varieties of the intermediate range, are illustrated by the data of table 10. Among the varieties of this range, differentiation was not consistent; apparently the number of plants used was too small for accurate evaluation of susceptibility.

DISCUSSION

The greenhouse experiments on testing varietal resistance led to the adoption of a field method of inoculation consisting essentially of application of a vigorous spray of bacterial suspension to plants in good growing condition. This method seemed preferable to others because it provided good differentiation among plants falling in the range of resistant reactions so important to the plant breeder. Although other procedures, such as spraying older plants forcefully or using less vigorous sprays, may give better differentiation among varieties outside the resistant range, these procedures are not suitable for the resistant range, in which they frequently do not produce any symptoms at all.

The data obtained in greenhouse experiments on epidemiological aspects of the disease generally confirmed results discussed in connection with inoculation of individual leaves. When whole plants were inoculated (tables 10, 12, 13), the disease reactions of leaves were affected by the following factors in the directions previously pointed out: (1) Stomatal opening; (2) sprays inducing water congestion; (3) concentration of inoculum; (4) relative development of leaves; (5) growing condition and age of plants. Leaves at various stages of development commonly reacted according to the pattern indicated by plate 2, *B*, the youngest leaves having the vein blight. Sometimes, however, in young leaves the disease manifested itself mainly in the appearance of spots, such as are illustrated by the leaf sections of plate 2, *A*. Plate 2, *A*, also serves to illustrate the point that leaves of comparable stages of development must be considered when using size of leaf spot as a criterion for relative varietal resistance (pl. 3).

High severity of blight in the black arm phase was dependent upon the application of a concentrated bacterial suspension to young plants in good growing condition. When considering plants well beyond the seedling stage the susceptibility to black arm of the

S × P (Egyptian) variety was outstanding. This high susceptibility is probably connected, at least in part, with the nondelimited character of growth of this variety.

Bud leaves exhibited considerable similarity to seedlings in their reaction to various factors. On S × P plants, bud leaves became more severely diseased than in any of the upland varieties and the number of diseased bud leaves tended to be larger. Furthermore, mild inoculations, e. g., those involving mist spray or a low concentration of inoculum, were capable of producing rather severe disease in bud leaves of S × P but not in other varieties. This finding suggests that in bud leaves of S × P the threshold concentration of inoculum required is lower than in other varieties.

The disease reaction of bud leaves was found to be characteristically different from that of other leaves in two respects: (1) Mist spray may produce a considerable degree of disease in bud leaves; (2) forceful spray is effective on bud leaves independent of conditions that influence stomatal opening on mature leaves. These findings are perhaps not unexpected, since stomata of bud leaves are thought to be nonfunctional. It is also not surprising that more variation in disease reaction was observed among bud leaves than among mature leaves, since the bud leaves considered here, that is, the first or the first two leaves above the expanding leaf, do not expand either at the same time or at the same rate.

Field Experiments

The field experiments carried out in these studies fall into two groups, according to whether they were concerned primarily (1) with varietal reaction to the bacterial blight organism or (2) with epidemiological aspects of the disease.

VARIETAL REACTION TO XANTHOMONAS MALVACEARUM

Data summarizing the results obtained with the methods of inoculating and grading described in detail on pages 18 and 19 of this bulletin are presented in table 9 and tables 14 to 18.

In the experiments of 1944 the high susceptibility of S × P and the resistance of Stoneville 20 were easily recognizable in the field and were outstanding with regard to black arm, secondary leaf spot symptoms, and bract spot (table 14). Differences were not so clear-cut in the intermediate range of varietal reaction, although the susceptible and tolerant groups were fairly well defined. Leaf blight seemed about equally severe in all varieties except Stoneville 20, so that leaf data were taken only with respect to secondary infection.

A satisfactory leaf grading method was developed in the late season of 1944 (pl. 3, *A*) and was used exclusively in 1945 (pl. 3, table 8). Leaf grading data are summarized in table 9 for 10 representative strains covering the range of susceptibility reactions of the 54 strains tested. The most favorable time for grading leaves was found to be 2 to 3 weeks after inoculation. Later grading, however, gave similar results and permitted observations on secondary infection and vein blight. Data not given in table 9 indicated high resistance for other selections made by D. M. Simpson from hybrids between Stoneville 20 and Stoneville 4 and from their backcrosses.

In general there was remarkable uniformity with respect to varietal reaction to the disease in plants of the selections made from the above crosses or plants of selfed lines of other varieties and strains. In the disease reaction resulting from the July 24 inoculation in plot 1 (table 9), it was noted that the plants of Simpson's Stoneville 4–3 segregated into susceptible and resistant individuals. The latter corresponded in reaction to Stoneville 4–2, which had definite resistance in the leaf spot phase, even though this variety was not quite so resistant as Stoneville 20. Such segregation was not found in any other strain used in the experiments, the results of which are recorded in table 9, nor did any of these strains approach the resistance of Stoneville 2.

Despite the wide differences in growing conditions between 1944 and 1945, susceptibility ratings on leaves and bolls showed approximately the same relative order of ranking of the different varieties in the 2 years. In 1944 growing conditions for cotton were favorable early in the season and inoculation of plants 5 weeks old resulted in more severe disease than occurred as a result of inoculation on older plants. At the time of the first inoculation of 1945, plants 5 weeks old had only two to four leaves. They were in their thriftiest state at the time of the third inoculation when 11 weeks old, furnishing excellent leaf material for uniform severe infection. In contrast with the situation observed in 1944, black arm infection from spray inoculation was negligible in the experiments in 1945 except on seedlings. This was apparently due to the fact that in 1945 the plants were in poor growing condition at the early age at which upland varieties are susceptible to this phase of the disease.

Data on boll rot reaction are summarized in table 15. The trend of varietal reaction to the boll rot phase was similar to that observed with the leaf spot phase (table 9). The agreement was particularly interesting with respect to the resistant and susceptible segregates of Stoneville 4–3. The results were also rather consistent from one test to another. Within the individual strains, however, there was much variation in reaction, as may be seen in the range of grades shown in table 15. With the exception of Stoneville 20, all strains showed at least some severe boll lesions, even including strains having leaf reaction similar to that of Stoneville 20. The percentage of diseased bolls was generally in line with the grade, but there were some notable deviations. Further studies on this point are needed, as only one of the tests was made on sizable numbers of bolls per strain.

Varietal susceptibility in the early and late seedling stages appears in general to parallel the reactions observed in other phases of the disease. This is indicated by the data of table 16 concerning infection of cotyledons subsequent to seed inoculation, and by the data of table 17 concerning bacterial blight of seedling plants inoculated with a knapsack sprayer. In the latter experiment the findings on Acala 11 were out of line, apparently as a result of natural infection of the seed with *Xanthomonas malvacearum* (see footnote 4, table 16).

RELATIVE VARIETAL RESISTANCE IN THE VARIOUS PHASES OF BACTERIAL BLIGHT

The relative ranking of the different varieties for resistance to bacterial blight has been remarkably similar in the different phases

of the disease. In order to facilitate critical examination of experimental results bearing on this point, representative data were assembled in table 18, including results of leaf grading in September 1944 and greenhouse results obtained by the seed inoculation method (*21*) in 1943. The general consistency of the data is evident, particularly when strains are considered in the four groups of prevailing reactions: (1) Highly susceptible (S × P); (2) susceptible (Shafter Acala, Acala 11, and Trice 2A); (3) tolerant (Coker 100 Wilt, Stoneville 37, and Stoneville 4–15); (4) resistant (Stoneville 20 and hybrid backcross). Heterozygosity with respect to bacterial blight resistance has been found recently in a line of Stoneville 4 (table 15). Thus the position of Stoneville 4–15 in table 18 is tentative.

Available evidence indicates that some Stoneville 4 lines, such as Stoneville 4–2, are intermediate in reaction between other varieties of the tolerant group and Stoneville 20. Likewise Shafter Acala is intermediate between other members of the susceptible group and S × P. Although there is, as shown in table 18, a general similarity in relative rankings of susceptibility of the different varieties and strains when judged according to the different phases of the disease, it is equally obvious that large differences between two groups of strains may occur in one phase of the disease, while in another phase there is little or no difference between the same groups. For instance, S × P is much more susceptible than any of the upland varieties in the black arm phase; leaf symptoms of S × P, as indicated by leaf grading, are on the other hand no more severe than those of Shafter Acala. Similarly, some selections derived from hybrids of Stoneville 20 with other varieties and previously found to be as resistant as Stoneville 20 in the leaf spot phase are decidedly more susceptible than Stoneville 20 in the boll rot phase.

Such divergent reactions in the various phases of bacterial blight will have to be considered in working toward a comprehensive system of classifying varieties and strains with respect to their relative resistance to the disease.

EPIDEMIOLOGICAL ASPECTS OF BACTERIAL BLIGHT

Data from experiments dealing with epidemiological aspects of bacterial blight are presented in tables 19 to 22, the methods employed in these experiments having been discussed on pages 18 and 19 of this bulletin.

The data of table 19 emphasize differences in effectiveness of different inoculation spray techniques. Disease symptoms produced by coarse and fine sprays were consistently more abundant and more severe than disease symptoms following mist spray inoculation, other conditions being the same. Age of plant at time of inoculation and concentration of inoculum were also considered in the experiment that provided the data for the second part of table 19. There is an obvious tendency toward increase in disease when using younger plants and higher concentrations of inoculum. Wide generalizations, however, cannot be drawn with respect to the effect of any single factor on all phases of the disease.

Thus, although young plants of Acala 11 developed much more extensive black arm than older plants, in the case of bract infection the situation was reversed, the younger plants being the less severely

affected. This situation apparently resulted from the fact that squares on younger plants developed later than those on older plants and had less chance to become infected.

The direct effects of differences in type of spray inoculation on extent of leaf spot infection are indicated most adequately by the data on shed leaves in table 19. In the experiment recorded in this table, coarse and fine sprays generally produced abundant and severe lesions within 5 to 8 days after inoculation, while mist spray resulted in irregularly scattered spots that developed slowly over 2 to 3 weeks. Data on leaf spot were taken more than 3 weeks after inoculation. At that time severely affected leaves had shed prematurely. On the remaining leaves secondary lesions obscured the contrasts observed in the early stages of disease development.

The influence on severity of infection of degree of stomatal opening, as determined by the time of day when inoculations were made, was the main factor under investigation in another experiment (table 20). Severity and frequency of black arm lesions were consistently greater for morning than for evening spray inoculation. Black arm seemed to have originated therefore at least in part from stomatal invasion of stem tissues, as most of the lesions appeared on internodal parts of stems and were not connected with infection passing down from the leaves. Data on secondary leaf infection presented in table 20 give only a slight indication of the immediate effects of the spray treatments observed 2 weeks after inoculation. At that time leaf spot was abundant and severe only on plants that had been inoculated with coarse or fine spray in the morning.

The data of table 21 represent results from a 1945 field experiment relating to three of the factors considered in 1944, namely, stomatal opening, spray inoculation type, and concentration of inoculum. The results in 1945 were in general agreement with those obtained in 1944.

The importance of degree of stomatal opening in inoculations for producing black arm were clearly shown, the incidence of black arm being about the same for morning inoculations involving 0.1 million bacteria per milliliter as was obtained by evening inoculations with 10 times as high a bacterial concentration. In the same experiment it was found that within 2 weeks after inoculation epiphytotic forms of leaf spot occurred as a result of morning applications of inoculum in either coarse or fine spray, more than 95 percent of the leaves becoming affected, 50 to 90 percent severely. All evening spray applications, however, induced milder forms of the disease, affecting 33 to 63 percent of the leaves, with less than 15 percent of them severely diseased. As shown by the data of table 21, however, 4 to 5 weeks after inoculation the effects of coarse and fine spray applied in the evening became more severe.

Apparently, pressure at the boll surface is the decisive factor in producing boll infection by spraying. This conclusion had been suggested by experiments conducted in 1944 and was confirmed by later experiments carried out in 1945 (table 22). Holding the nozzle as close as possible to the bolls increased the incidence of bacterial spots on bolls as compared with holding the nozzle at some distance. Semicoarse spray gave better results than fine one, but was not so effective as coarse spray.

Further evidence along this line was obtained in connection with the boll inoculation of 1945 in variety plot 2. Two applications of

semicoarse spray were ineffective, only a few bolls becoming diseased within 2 to 3 weeks. The third inoculation, however, using coarse spray, produced an epidemic of bacterial boll spots within 8 to 12 days. Included in table 22 are data suggesting an increase of bacterial boll rot as a consequence of cutting back the tops of rank plants after inoculation. The effect may be caused by nutritional or temperature factors. On sunny days maximum air temperatures around bolls of cut plants ranged from 8° to 11° C. higher than those around bolls of other plants.

MISCELLANEOUS STUDIES

In earlier parts of this bulletin there have been presented results of detailed experiments relating to a few of the factors that determine the incidence and severity of bacterial blight of cotton under conditions of artificial inoculation. In addition to these experiments, preliminary tests were carried out in relation to other factors concerning the disease and its development. While the experimental evidence on these additional points in some cases is not extensive, such data as are available reveal a number of highly interesting new facts and suggest promising lines of attack toward further knowledge of the disease.

Moisture Relations in Infection by *Xanthomonas malvacearum*

It has been claimed by E. F. Smith (*19*), and later by other plant pathologists, that greenhouse plants are more readily infected by bacterial pathogens if they are placed in a moist chamber for 1 or 2 days after inoculation. Diachun, Valleau, and Johnson (*5*) have demonstrated that when inoculation procedures involving water congestion are used, infection of tobacco leaves by bacterial pathogens takes place without moist-chamber exposure. The fact that severe infection of cotton leaves by *Xanthomonas malvacearum* may take place after inoculation procedures involving water congestion but without recourse to moist-chamber exposures is amply demonstrated by experimental results reported in earlier sections of this bulletin. Further experiments of a preliminary nature, however, revealed that when mild forms of the disease were induced in vigorously growing plants of highly susceptible varieties by such inoculation methods, for example, as the application of mist spray, the moist-chamber treatment increased the severity of the bacterial blight on bud leaves and on leaves in the early expanding stage.

In view of the intensifying effect of post-inoculation moist-chamber treatment on blight production in bud and young expanding leaves, it seemed probable that a similar effect might occur with seedling plants because of the vigorous growing condition of their leaves. An experiment with seedlings did in fact confirm this idea (table 23). Moist-chamber treatment resulted generally in a greater disease incidence and more severe disease. The seedlings for the experiment reported in table 23 were grown in 4 wooden flats with well-fertilized soil, 60 seedlings to a flat, 10 seedlings of each of the 6 varieties used. The plants were kept in vigorous growing condition throughout the experiment and became crowded and rank. At the time of inoculation they were 12 days old, with bud leaves just beginning to expand, the largest having a diameter of about 15 mm. The inoculum con-

tained about 60 million bacteria per milliliter and was applied at the rate of 700 ml. per flat. About a fifth of the inoculum was atomized onto the seedlings, an attempt being made to wet the bud leaves. The rest was sprinkled over the plants with a small can. After inoculation, two of the flats were placed in a moist chamber for 2 days; the other two were kept in a greenhouse and were shaded until the plants were dry. When watering the flats, an attempt was made to avoid wetting the leaves.

The seedling experiment reported in table 23 indicates that relative varietal susceptibility is similar to that observed in previous experiments on grown plants. Incidental to the findings made in connection with this experiment (table 23) observations on other disease symptoms were made. A wilt of the leaves exhibiting symptoms very similar to fusarium wilt was frequently noticed, and when the stems of the plants were cut, black streaks were often seen. Data on these wilt symptoms are included in table 23 and will be discussed in a subsequent section.

The increase in infection of seedlings brought about by the post-inoculation moist-chamber treatment described above suggested that similar effects might be induced by frequent light sprinkling of plants following inoculation. The results of an experiment to test this hypothesis (table 24) indicated that such was indeed the case. On seedlings grown from inoculated seed, the most striking effect of sprinkling was on the true leaves. Very few of these became infected unless seedlings were moistened after emergence. When the seedlings were sprinkled with water once a day for 8 days after emergence, bacterial spots appeared on the true leaves of nearly all seedlings that had diseased cotyledons. Much of the black arm that occurred resulted from invasion of the stems through the petioles of the cotyledons and developed without sprinkling; sprinkling, however, did cause some increase in black arm.

In another part of the experiment reported in table 24, seedlings were inoculated by sprinkling with bacterial suspension shortly after emergence (8 days), at a stage when the bud leaves were still rudimentary. Sprinkling with water twice a day for 4 days following inoculation tripled the number of plants of Shafter Acala on which the leaves became diseased, and while it did not change infection of leaves on Stoneville 37, this strain showed a large increase in number and severity of lesions on cotyledons as a consequence of water sprinkling. On seedlings of both these varieties inoculated 16 days after planting, repeated sprinkling with water subsequent to inoculation resulted in slightly more leaf spot than on control plants. (Plants of the two groups inoculated at the seedling stage became so crowded that they could not be grown long enough to develop black arm.)

In experiments to determine the relative effectiveness of seedling inoculation by sprinkling as compared with inoculation by forceful spray from a knapsack sprayer, the results obtained were not entirely consistent from one experiment to the next, apparently because the condition of seedlings changes rapidly during growth and is greatly influenced by environmental factors. The results of one such experiment are reported in table 25. In this experiment the inoculated plants were in good growing condition and some of the leaves had a diameter as great as 5 cm., stomata being wide open at the time of inoculation. Sprinkling inoculation produced spots on the leaves of

all plants, but the disease was more widespread and severe on leaves of plants inoculated with a knapsack sprayer.

It is clear from the experimental evidence presented that infection of bud leaves of mature or seedling plants differs in several respects from infection of older leaves. It seems probable that the epidemiology of bacterial blight might become better understood through studies on infection of bud leaves, especially those of seedlings. Infection of bud leaves is not dependent on forceful spraying. Bud leaves, in addition, are generally more susceptible than others. A mature leaf does not become severely diseased when only a few lesions are present, whereas bud leaves frequently do. During expansion of a bud leaf the disease may spread from a single infection court over much of the leaf area as vein blight, or as "wilt," which is discussed in a subsequent section.

Abundant infection of mature leaves involves instantaneous invasion, which occurs most readily when the stomata are open and the tissues water-congested. It is not known, however, how invasion proceeds in bud leaves. The bacteria may enter through stomata if a few of them are functional, or possibly through openings in the cuticle. The cuticle is very thin at this stage and therefore more subject to injury; leaves that are expanding while plants are in a moist chamber have been noted to be more tender than corresponding leaves on plants outside the chamber.

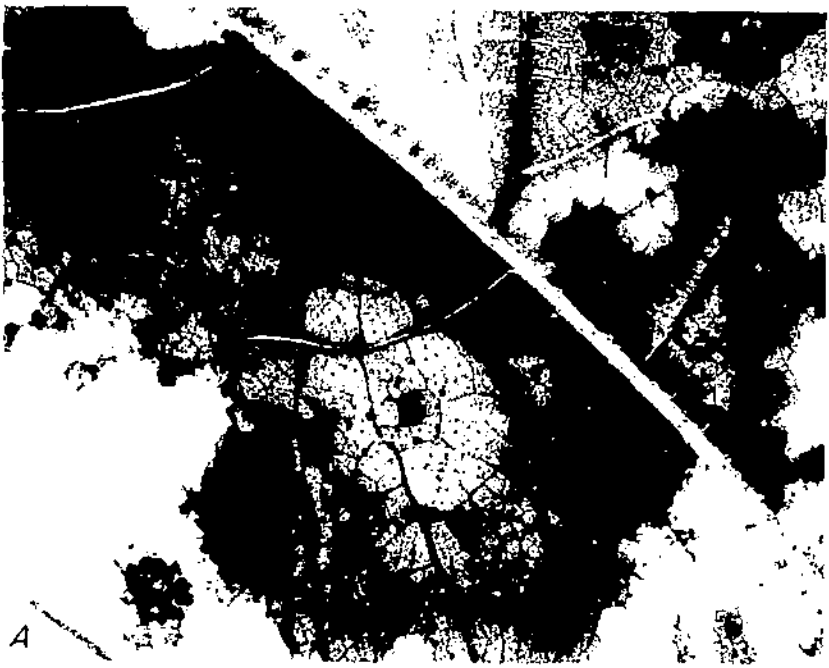
Data given here indicate that infection of bud leaves on seedlings is increased by repeated wetting of leaves as well as by moist-chamber treatment after inoculation; that is, frequent presence of free moisture on the leaf surface appears to be as effective as its continuous presence. Under natural conditions, therefore, frequent dews or light rains may facilitate invasion of bud leaves and of leaves in the early expanding stage. In highly susceptible varieties this may result in a gradual build-up of the disease to epidemic proportions.

Vascular Infection

Bryan (*1*) described as "atypical" lesions on cotton leaves "a gradual and progressive fading out and dying of the tissues," apparently the result of vascular infection by *Xanthomonas malvacearum*. She found the bacteria to "pour out in great numbers from the cut ends of the veins but not from the intervening tissue"—a condition opposite to the usual situation. In the present work this form of the disease was frequently noted on inoculated plants in the greenhouse and, although not so frequently, in the field. The condition occurred on seedlings and on bud leaves and expanding leaves of older plants of susceptible varieties. Thus, this phase of the disease appears to occur primarily on highly susceptible leaves. The vascular nature of this type of infection was confirmed by reisolating the pathogenic bacteria from discolored streaks that extended from the veins through petioles into the stem. Such dark streaks were followed in stems of seedling plants for distances of 5 to 8 cm., the most pronounced discoloration appearing in the primary xylem (pl. 8, *B*). On greenhouse plants, leaf symptoms were similar to those of fusarium wilt.

Data were taken in connection with the seedling experiments represented by tables 23 to 25 on this bacterial wilt and on internal discoloration of stems. These data were in line with those on other

Early stage of black arm lesion on stem, produced by bacteria invading from diseased leaf. Stem infection derived from vein blight of leaf by progression of bacteria through petiole. Variety, Trice 2A.



Symptoms of "wilt," or vascular infection, on cotton by *Xanthomonas malvacearum*. A, Leaf taken from a greenhouse plant 5 weeks after inoculation by knapsack sprayer. Leaf in the expanding stage at the time of inoculation. × 2. B, Section through the stem of a seedling plant of Acala 44, showing vascular infection as black streaks. Stem infection detected by leaf spot progression through petiole. Plant photographed 7 weeks after inoculation by spraying with a concentrated suspension of *X. malvacearum* on June 21. ½

symptoms of the disease, giving similar responses for factors under tests, such as varietal reaction and moist-chamber and sprinkling treatments.

An interesting late stage of the wilt phase was observed on older greenhouse plants of susceptible varieties. In the center of the wilt lesions was the original typical angular spot, which was dry and dark brown at this time and surrounded by a light-brown area of dry tissue. Then a faded-green zone followed, which retained the green color even when the healthy parts of the leaf turned yellow. At the margin of this zone were typical angular spots or minute spots along veinlets, giving the appearance of a dark staining in the network of veins (pl. 8, *A*).

The following hypothesis is offered as a possible approach toward explaining why bacterial blight, which is typically a disease of parenchymatic tissues, may adopt a behavior characteristic of vascular wilts. On hot days, leaves in the expanding stage are the first to show temporary physiological wilting in both field and greenhouse. The water content of expanding leaves is known to be subject to larger diurnal fluctuations than that of other leaves (*15*). Bacteria require abundant moisture for their development in the leaf tissues. During expansion of leaves, the pathogenic bacteria are most likely to find favorable moisture conditions close to veins, as is indicated by the common occurrence of vein blight on expanding leaves in the parenchymatic tissues adjoining the veins. Conditions of water stress, however, may induce the bacteria to invade the water-conducting tissues themselves, resulting in "wilt," as described above.

Survival of *Xanthomonas malvacearum* in Plant Tissue

The life cycle of *Xanthomonas malvacearum* may be divided into active and quiescent stages. In the active stages moisture is essential for the spread of the bacterium and for infection. In quiescent stages dry tissues provide the most favorable condition for survival of the pathogen. Many plant pathogenic bacteria, including *Xanthomonas malvacearum*, may persist in dry plant tissues for years (*14*, *16*). Rapid drying of the bacterial slime seems to be important, since the bacteria tend to disappear from rotting tissues (*10*).

Proof of the presence of *Xanthomonas malvacearum* in quiescent condition in old diseased tissue was not difficult to obtain. Trap methods were found to yield the pathogenic bacteria easily, even when attempts at isolation failed in dilution cultures as a result of the predominance of secondary organisms. Suspensions made from dry diseased tissues by crushing them in distilled water were poured over leaves of susceptible plants after inducing visible water congestion in the leaves by means of a hypodermic syringe or after gently rubbing expanding leaves with a moist cheesecloth. As an alternative method seed of S × P were soaked in the suspension for 2 to 3 hours, then germinated between paper towels. In some of these tests estimates of relative abundance of bacteria in leaf tissue were obtained by making suspensions of comparable samples of dry material in 10-ml. water blanks. Severity of lesions and duration of the incubation period served as indicators of the relative numbers of bacteria present; the incubation period of different samples differed by as much as 2 weeks.

In order to investigate the possibility of survival of *Xanthomonas malvacearum* in diseased tissues through the winter, samples of leaves and of diseased parts of stems were collected during the winter of 1944–45 from field sections in which there had been blight late in the season of 1944. Leaves still attached to the plants were collected and also leaves from the ground. In the case of leaf collections from the ground, two sample parts were taken, one from the upper dry layer of leaves and the other from the moist leaf layer directly on the ground. The dry-leaf part of the sample consistently gave indication of containing a larger number of pathogenic bacteria than the moist part. The bacterial blight pathogen was recovered from all types of samples collected up until and including the last collection date, March 9, 1945; that is, up until a few weeks prior to the time of planting cotton.

Preliminary experiments were also conducted in the laboratory on factors affecting the survival of *Xanthomonas malvacearum* in leaf tissue. In one experiment diseased leaves were removed from greenhouse plants, were then dried and broken into small pieces, subdivided, and stored in two screw-top bottles, one bottle being kept in the laboratory at room temperature, the other at 8° C. Three years later *X. malvacearum* was recovered from the tissue in each of the bottles. The material kept at 8° C. contained more bacteria. Other tests were made by cutting disks of 8-mm. diameter with a cork borer from lesions on the leaves of field plants, mounting such disks on pins stuck into corks, and attempting to recover the blight bacterium after different periods and conditions of storage.

Five groups of samples were stored for 9 months. One group was stored in the open in the laboratory. Three groups were stored at different relative humidities in desiccators, one at 25 percent, one at 55 percent, and one at 85 percent relative humidity. A fifth group was held in a moist chamber for 8 weeks, at the end of which period the samples had become covered with mold growth and were removed to the open laboratory for exposure in parallel with the first group. *Xanthomonas malvacearum* was recovered by trapping methods from samples in each of the five groups after as much as 9 months. Some of the disks exposed to the moist chamber, however, did not yield the bacteria. Moreover, a smaller number of bacteria was present in disks kept for more than 2 months at 85 percent relative humidity than in disks kept open in the laboratory at 25 and 55 percent.

In view of the preliminary evidence obtained from the experiments described above it seems quite possible that the angular leaf spot bacterium may frequently overwinter in the field and that such overwintered bacteria may be responsible for some seedling infection in spring. Observations made in connection with field experiments in 1945, however, confirmed the prevalent idea that infected seed is the most important source of primary inoculum. In 1945 the disease appeared on cotyledons of many seedlings, but it was first noted on those from infected Texas-grown seed, spreading later to other strains whose seed were not infested.

Natural Spread of Bacterial Blight

Observations made during the course of the 1945 experiments indicated that most of the natural spread of bacterial blight in the

seedling stage in the field was brought about by movement of inoculum in drainage water at the time of washing rains late in May. These observations were in accord with reports of Hansford (*8*) on the influence of sloping land on such spread. In varietal plot 1, for example, a comparison of strains of known susceptibility showed that the incidence of seedling blight was much higher at the top of the slope, where the rows joined those of the infected Acala, than at the bottom. Varietal plot 2, also bordering the infected Acala rows, had less slope than plot 1 and also less seedling infection.

Observations were made on the natural spread of blight on older plants in plots where spraying had produced epiphytotic disease conditions. In 1944, spread of the disease by secondary infection occurred mainly on inoculated plants and to only a very slight or negligible extent on plants adjoining them. In 1945 more natural spread was noted. This spread seemed to originate mostly, however, from early seedling infection and was never so uniform and severe as that produced by artificial inoculation with a vigorous spray. There was likewise very little natural spread of bacterial boll rot, even on plants whose leaves had become severely diseased prior to the development of bolls.

GENERAL DISCUSSION

Bacterial blight of cotton presents baffling problems to the investigator who is in search of a comprehensive picture of the disease. This situation has been aptly expressed by Butler (*2, p. 195*) in comparing the forms in which the disease occurs in India and Africa:

> If one were working with a different type of cotton, grown under entirely different conditions, everything in the behaviour of the disease might be different in the two areas, so that for practical purposes it would seem as if one really had to do with two distinct diseases.

The present investigation may be considered as an experimental approach toward exploring the bacterial blight complex with regard to epidemiology and varietal reaction. Many of the basic factors and phenomena of the disease have been recognized by previous investigators (*7, 14, 16, 19, 20*). Fundamentally the present work is in agreement with the ideas of these workers, but some of the issues have been more clearly defined.

In approaching epidemiological problems of bacterial blight, various methods of artificial inoculation have been employed in our experiments to define (1) basic factors affecting the host-parasite relationship under semicontrolled conditions; and (2) the operation of these factors under field conditions. It is realized that in generalizing from the results of such experiments one needs to consider the data critically, correlating them with observations on the behavior of the disease under various natural conditions. Caution in interpretations needs to be exercised, particularly with regard to (1) intermediate effects under natural conditions in contrast to relatively sharp lines drawn in the experimental work; and (2) time factors in relation to gradual development of the disease.

A good example falling under the first of these categories concerns the matter of stomatal opening and invasion. Under conditions of natural invasion on a rainy day, stomata of leaves are likely to be partly open, the degree of opening depending on the time of day and

on the extent of cloudiness. The second group of effects, that of relation of time factors to development of the disease, is most obvious when the disease occurs in mild forms, building up gradually through the season. It would seem difficult to imitate adequately the building-up through a succession of stages: (1) Primary infection originating from infected planted seed; (2) inoculum from primary lesions on seedlings spreading by runoff water or splashing raindrops or by wind carrying dry leaf debris; and (3) secondary infection cycles repeating the processes of infection and of spread of inoculum with the aid of rains or heavy dews.

Two forms of the disease have been distinguished in our experimental work—mild and epiphytotic. Under natural conditions, mild forms are most common, proceeding as described. Apparently, epidemic forms occur only when at some stage of the building-up process conditions for the disease become exceptionally favorable. In 1944, such outbreaks were seen in two fields in the sand hill section of South Carolina. The plants involved were about 6 weeks old and the disease appeared in all respects similar to the epiphytotic form produced artificially at Clemson, S. C., each individual plant being severely affected. A heavy rainstorm was connected with the natural outbreak.

Since the western parts of the Cotton Belt have in general a drier climate than the eastern parts and since plant diseases are commonly favored by moist rather than dry weather, one is led to wonder why bacterial blight of cotton seems to occur more frequently in epidemic form in the western rather than in the eastern regions. The work reported here, together with other points discussed, suggests the following reasons for this apparent contradiction: (1) In Texas and Oklahoma, summer rains consist commonly of brief hard downpours. Experimental evidence indicates that such driving rains provide better conditions for epiphytotics than the frequent light rains common in the eastern cotton States. (2) Plants growing under dry conditions tend to have open foliage so that the bolls are more readily accessible to infection when driving rains occur. (3) Dry weather provides better conditions for survival of the pathogen in diseased tissues of leaves and bolls. In the eastern section of the belt, bacterial boll infection is followed by extensive fungus rot more commonly than in the western section, and seed from Texas has been found to carry more infection by *Xanthomonas malvacearum* than seed from the eastern sections. (4) Several varieties grown in Texas, such as Acala, are more susceptible to the disease than those grown in the East.

In the present work the influence of environmental and other factors on the development of the disease has been shown to vary according to the phase of the disease, the method of inoculation, and the type of infection. The factor of stomatal opening, for example, is most important for infection of mature leaves inoculated by forceful spraying; less important for stem and boll infection by the same methods; and least important for infection of bud leaves of susceptible varieties, using gentle methods of inoculation. On the other hand, with bud leaves of susceptible varieties the severity of the disease may be increased by the frequent or continuous presence on the plant of free moisture or by high atmospheric moisture subsequent to inoculation; such moisture relations, however, seem to be of little importance when

invasion is instantaneous, involving rapid entrance of bacteria through stomata or wounds.

After carrying out a series of investigations on the relation of environmental factors to bacterial blight of cotton under controlled conditions, Stoughton (*20*) concluded that the severity of the disease is increased when temperatures are high throughout the period of its development, and when humidity is high during the first 2 days after inoculation. Stoughton's work was apparently carried out under the following conditions: (1) Young plants of a highly susceptible variety (Egyptian) were used; (2) inoculation was accomplished by atomizing, a rather gentle procedure unless carried out from very close range; and (3) inoculation was made under rather weak artificial light, the results suggesting that the stomatal opening was slight and irregular. As may be seen by reference to the preceding discussion, external moisture relations have been shown to be an important factor under conditions and with plant materials similar to those used by Stoughton (*20*), but not under some other conditions or with other plant materials. These considerations demonstrate the danger of generalizing with regard to the effect of environmental factors on bacterial blight, even if such generalizations are based on very careful experimentation.

It is perhaps desirable to consider effects of individual factors as trends subject to modification by other factors. Such trends were noted in tables 19 to 21 with respect to the effects of concentration of inoculum, age of plant, stomatal opening, and method of inoculation. The complex nature of the disease is obvious when the tabulated data are viewed as a whole, considering all the factors involved and their relation to the various phases of the disease.

The leaf spot phase has been emphasized in fundamental investigations discussed in the first part of this bulletin. Similar studies of the other phases may lead to a better understanding of the entire disease complex.

The need for including phases other than leaf spot in the further development of methods of artificial inoculation and of grading plants for varietal resistance in breeding plots has been pointed out. Consideration may have to be given to those phases and factors that are of special importance under the local or sectional conditions for which the breeding work is done. For general purposes the methods of artificial inoculation that induce severe epidemics are thought to be most practical because they exclude escapes and provide assurance that plants resistant under those conditions will survive milder forms of the disease.

LITERATURE CITED

(1) BRYAN, M. K.
1932. AN ATYPICAL LESION ON COTTON LEAVES CAUSED BY BACTERIUM MALVACEARUM. Phytopathology (Note) 22: 263–264, illus.

(2) BUTLER, E. J.
1934. [DISCUSSION ON BLACK ARM DISEASE IN UGANDA.] *In* Report and Summary of Proceedings, Second Conference on Cotton Growing Problems, July 1934. Empire Cotton Growing Corp., 340 pp., illus. London.

(3) CLAYTON, E. E.
1936. WATER SOAKING OF LEAVES IN RELATION TO DEVELOPMENT OF THE WILDFIRE DISEASE OF TOBACCO. Jour. Agr. Res. 52: 239–269, illus.

(4) DIACHUN, S., and VALLEAU, W. D.
1939. RELATION OF STOMATAL OPENING TO WATER SOAKING OF TOBACCO LEAVES. Amer. Jour. Bot. 26: 347–351, illus.
(5) ——— VALLEAU, W. D., and JOHNSON, E. M.
1942. RELATION OF MOISTURE TO INVASION OF TOBACCO LEAVES BY BACTERIUM TABACUM AND BACTERIUM ANGULATUM. Phytopathology 32: 379–387, illus.
(6) ——— VALLEAU, W. D., and JOHNSON, E. M.
1944. INVASION OF WATER-SOAKED TOBACCO LEAVES BY BACTERIA, SOLUTIONS, AND TOBACCO-MOSAIC VIRUS. Phytopathology 34: 250–253.
(7) FAULWETTER, R. C.
1917. WIND-BLOWN RAIN, A FACTOR IN DISEASE DISSEMINATION. Jour. Agr. Res. 10: 639–648, illus.
(8) HANSFORD, C. G., HOSKING, H. R., STOUGHTON, R. H., and YATES, F.
1933. AN EXPERIMENT ON THE INCIDENCE AND SPREAD OF ANGULAR LEAF-SPOT DISEASE OF COTTON IN UGANDA. Ann. Appl. Biol. 20: 404–420, illus.
(9) HARE, J. F., and KING, C. J.
1940. THE WINTER CARRY-OVER OF ANGULAR LEAF SPOT INFECTION IN ARIZONA COTTON FIELDS. Phytopathology 30: 679–684, illus.
(10) HILDEBRAND, E. M.
1939. STUDIES ON FIRE-BLIGHT OOZE. Phytopathology 29: 142–156, illus.
(11) JOHNSON, J.
1945. INFECTION EXPERIMENTS WITH DETACHED WATER-CONGESTED LEAVES. Phytopathology 35: 1017–1028, illus.
(12) KNIGHT, R. L.
1944. THE GENETICS OF BLACKARM RESISTANCE. IV. GOSSYPIUM FUNCTATUM (SCH. & THON.) CROSSES. Jour. Genet. 46: 1–27, illus.
(13) ——— and CLOUSTON, T. W.
1939. THE GENETICS OF BLACKARM RESISTANCE. I. FACTORS B_1 AND B_2. Jour. Genet. 38: 133–159, illus.
(14) MASSEY, R. E.
1930. STUDIES ON BLACKARM DISEASE OF COTTON. Empire Cotton Growing Rev. 7: 185–195, illus.
(15) MAXIMOV, N. A.
1929. THE PLANT IN RELATION TO WATER. 451 pp., illus. London.
(16) ROLFS, F. M.
1935. DISSEMINATION OF THE BACTERIAL LEAF SPOT ORGANISM. (Abstract) Phytopathology 25: 971.
(17) SHAW, L.
1935. INTERCELLULAR HUMIDITY IN RELATION TO FIRE-BLIGHT SUSCEPTIBILITY IN APPLE AND PEAR. N. Y. (Cornell) Agr. Expt. Sta. Mem. 181, 40 pp., illus.
(18) SIMPSON, D. M., and WEINDLING, R.
1946. BACTERIAL BLIGHT RESISTANCE IN A STRAIN OF STONEVILLE COTTON. Amer. Soc. Agron. Jour. 38: 630–635, illus.
(19) SMITH, E. F.
1920. AN INTRODUCTION TO BACTERIAL DISEASES OF PLANTS. 688 pp., illus. Philadelphia.
(20) STOUGHTON, R. H.
1933. THE INFLUENCE OF ENVIRONMENTAL CONDITION ON THE DEVELOPMENT OF THE ANGULAR LEAF-SPOT DISEASE OF COTTON. V. THE INFLUENCE OF ALTERNATING AND VARYING CONDITIONS OF INFECTION. Ann. Appl. Biol. 20: 590–611.
(21) WEINDLING, R.
1944. A TECHNIQUE FOR TESTING RESISTANCE OF COTTON SEEDLINGS TO THE ANGULAR LEAF SPOT BACTERIUM. Phytopathology 34: 235–239, illus.

APPENDIX (TABLES 1 TO 25)

TABLE 1.—*Effect of preinoculation treatments on invasion of mature cotton leaves by Xanthomonas malvacearum, experiments of August–September 1942*

[Plants of susceptible varieties, 8 to 9 weeks old]

Experiment No.[1]	Number of leaves	Leaf surface[2]	Preinoculation treatment[3]	Method of inoculation[4]	Leaves with lesions	
					Numerous	Very few
					Percent	*Percent*
1	30	Lower	Shading for 2 hours	Spraying[5]	0	23.6
2	38	do	No shading	do	100	0
3a	33	do	Visible water congesting[6]	Drenching	100	0
3b	33	do	No water congesting	do	0	45.5
4a	48	Upper	Visible water congesting	do	100	0
4b	48	do	No water congesting	do	0	27.1
5a	11	Lower	Spraying with water[7]	do	91	0
5b	11	do	No spraying	do	0	9.0
6a	12	Upper	Spraying with water	do	75	0
6b	12	do	No spraying	do	0	8.3
7	18	Lower	Visible water congesting[6]	do	100	0
8	18	do	Visible water congesting 20 minutes prior to inoculation.	do	0	23.6

[1] In experiments Nos. 3 to 6, the preinoculation treatment was applied to (a) one-half of the leaf, but not to (b), the other half of the same leaf. Subsequent disease symptoms reflecting the preinoculation treatments are shown in fig. 1.

[2] Leaf surface to which preinoculation treatment and inoculation were applied.

[3] Treatment immediately prior to inoculation, except in experiment No. 8 in which one-half of the leaf was treated 20 minutes before inoculation.

[4] Using suspensions containing 5 to 10 million bacteria per milliliter.

[5] Spraying with bacterial suspension using a 1-quart sprayer.

[6] Induced by means of a hypodermic syringe.

[7] Spraying with a 1-quart sprayer when stomata were open.

TABLE 2.—*Effectiveness of water congestion induced on the leaf surface opposite the one to which the bacteria were subsequently applied in allowing invasion of cotton leaves by Xanthomonas malvacearum, experiment of July 1943*

[Plants of variety Acala 11, 12 weeks old]

Number of leaves	Preinoculation treatment			Leaf surface inoculated[2]	Incubation period		Disease symptoms	
	Leaf surface	Water congestion			Average	Minimum	Leaves affected	Average severity
		Method of production	Degree[1]					
			Value		*Days*	*Days*	*Percent*	*Value*
27	Upper	Hypodermic syringe.	2. 7	Lower	8.	4	100	[3] 1. 9
33	Lower	do	2. 1	Upper	9	5	100	[3] 1. 5
27	Upper	Spraying		Lower	15	8	100	[4] 1. 3
33	Lower	do		Upper	16	10	58	1. 0

[1] The degree of visible water congestion produced by a stream of water from a hypodermic syringe at the time of inoculation is a measure of the degree of stomatal opening. The recorded figures are averages of estimates on individual leaves. Estimates of degree of water congestion are made according to the following scale:

Appearance of streak:	*Value*
Solid heavy	3. 0
Solid faint	2. 0
Irregular	1. 0
Intermediate reactions expressed by decimals from	0. 2–3. 0

[2] Inoculation done by gently applying with a paint brush a suspension containing 10 million bacteria per milliliter.

[3] Disease severity in lesions appearing along the streaks that had been visibly water congested at the time of inoculation, expressed as an average of estimates on diseased leaves. Estimates of severity were made 18 to 21 days after inoculation, using the following scale:

Appearance of lesions:	*Value*
Spreading much to both sides of the streak	4. 0
Spreading little to both sides of the streak	3. 0
Confined to the streak, forming a continuous band	2. 0
Along the streak, not continuous	0. 2–1. 8
Intermediate reactions expressed by decimals from	0. 2–4. 0

[4] Disease severity in leaf areas which had nonvisible water congestion or no water congestion at the time of inoculation expressed as an average of estimates on diseased leaves. Estimates made 18 to 21 days after inoculation, using the following scale:

Fraction of inoculated area diseased (percent):	*Value*
100	5. 0
80	4. 0
60	3. 0
40	2. 0
20	1. 0
Intermediate reactions expressed by decimals from	0.2–5. 0

TABLE 3.—*Effect of soil moisture, inoculum concentration, and degree of water congestion at the time of inoculation on infection of cotton leaves by Xanthomonas malvacearum, inoculation done by atomizing the under side of leaves with a bacterial suspension immediately after inducing water congestion, experiment of July 1943*

[Plants of variety Acala 11, 3 months old]

Number of leaves	Factors tested for influence on infection				Incubation period		Disease symptoms	
	Soil condition	Bacterial concentration in inoculum	Water congestion					
			Method of production	Degree [1]	Average	Minimum	Leaves infected	Average severity
		Millions per cubic centimeter		*Value*	*Days*	*Days*	*Percent*	*Value*
25	Moist	35.0	Hypodermic syringe.	2.5	5	4	100	[2] 2.5
28	do	.7	do	2.4	9	8	100	2.1
25	Dry	35.0	do	2.3	6	4	100	1.9
21	do	.7	do	2.1	14	8	100	.7
25	Moist	35.0	Atomizing		8	6	100	[3] 2.4
28	do	.7	do		13	10	100	.4
25	Dry	35.0	do		13	9	100	1.3
21	do	.7	do		19	16	76	.4

[1] See footnote 1, table 2. [2] See footnote 3, table 2. [3] See footnote 4, table 2.

TABLE 4.—*Effect of intensity of water pressure at the leaf surface on infection of cotton leaves by Xanthomonas malvacearum. One water-congested streak was produced on each half leaf by means of a hypodermic syringe.*[1] *Inoculum, containing 50 million bacteria per cc., applied by atomizing. Left under side of leaf atomized from 1 inch distance, right under side from 5 to 6 inches. A rubber pad was used when leaves were "backed" during inoculation; when not "backed" leaves were held at petiole and allowed to sway when atomized; experiment of May 1944*

[Plants of variety Acala 11, 8 weeks old; 16 leaves per treatment]

Stage of leaf development	Inoculation conditions		Incubation period		Disease symptoms	
	Atomizing distance	Leaf backing	Average	Minimum	Leaves infected	Average severity[2]
	Inches		*Days*	*Days*	*Percent*	*Value*
Barely mature	1	+	5.0	4	100	1.9
Do	1	−	10.2	6	100	.7
Do	5	+	8.2	7	100	.5
Do	5	−	14.1	12	100	.3
Fully mature	1	+	12.0	9	100	.8
Do	1	−	14.3	10	57	.4
Do	5	+	14.0	12	100	.4
Do	5	−	17.0	15	57	.2

[1] The average degree of water congestion was 2.2 for the barely mature leaves and 2.5 for those fully mature.

[2] See footnote 4, table 2. Lesions along the streaks which had been visibly water-congested at the time of inoculation were disregarded.

TABLE 5.—*Effect of cotton variety and of inoculum concentration on infection of cotton leaves by Xanthomonas malvacearum, experiment of November 1943*

[Plants were 4 weeks old; 10 leaves inoculated on 4 plants of each variety per treatment]

Variety	Method of inoculation	Average incubation period; inoculum (expressed in millions of bacteria per milliliter) at 3 concentrations			Average severity [1] of disease; inoculum (expressed in millions of bacteria per milliliter) at 3 concentrations		
		300	10	0.3	300	10	0.3
		Days	*Days*	*Days*	*Value*	*Value*	*Value*
S × P	Gentle brushing [2] subsequent to water congesting. [3]	3.7	6.8	10.2	2.9	2.1	1.3
Acala 11	do	3.9	7.0	9.5	2.6	1.9	1.2
Stoneville 37	do	3.9	7.8	11.7	2.4	1.3	.8
Stoneville 4–8	do	4.3	9.3	..	[4] 1.3	[4] 1.2	([5])
Stoneville 20	do	2.3	16.0	..	[6] .7	[6] .3	0
S × P	Brushing [2] with moderate pressure, no previous water congesting.	5.8	7.3	10.3	[7] 1.5	1.2	.8
Acala 11	do	6.2	8.2	10.1	1.5	1.2	.8
Stoneville 37	do	6.5	8.2	11.1	1.4	1.2	.7
Stoneville 4–8	do	9.0	..	..	.3	0	0
Stoneville 20	do	..	..	..	([5])	0	0

[1] See footnote 3, table 2.
[2] Using a paint brush with bacterial suspension. One-half of each leaf received the preinoculation treatment and was brushed gently; the other half was brushed with moderate pressure.
[3] Using a hypodermic syringe.
[4] Symptoms on some plants atypical.
[5] Trace.
[6] Symptoms on all plants atypical.
[7] See footnote 4, table 2.

TABLE 6.—*Influence of stage of leaf development and method of inoculation on infection of cotton leaves by Xanthomonas malvacearum; experiment of March 1944*

[Plants of variety S × P Egyptian, 5½ weeks old, inoculated 23 to 27 leaves per treatment]

Number of leaves	Stage of development of leaves (position on plant)[1]	Preinoculation treatment		Inoculation procedure[2]	Incubation period		Disease symptoms	
		Type	Degree[2]		Average	Minimum	Leaves diseased	Average severity
					Days	*Days*	*Percent*	*Value*
10	Top	Hypodermic syringe.	0.3	Gentle brushing.[4]	4.0	4	30	[5] 2.8
20	Mid	do	1.5	do	4.5	3	100	2.4
20	Low	do	.7	do	9.5	6	55	.6
10	Bud	None		Brushing with moderate pressure.	5.8	5	100	[6] 1.6
10	Top	do		do	4.6	4	100	2.6
20	Mid	do		do	6.4	4	100	2.0
20	Low	do		do	8.4	6	85	1.8
10	Bud	do		Dipping	7.9	5	100	[6] 1.6
10	Top	do		do	5.2	5	100	1.2
20	Mid	do		do	10.2	5	100	.9
20	Low	do		do	14.1	12	60	.4

[1] Stage of development at the time of inoculation:
Bud=bud leaves.
Top=expanding leaves, up to 70 mm. in diameter.
Mid=barely mature leaves.
Low=fully mature leaves.
Bud leaves are not susceptible to water congestion with a hypodermic syringe.

[2] See footnote 1, table 2.

[3] Using 50 million bacteria per milliliter.

[4] See footnote 2, table 5.

[5] See footnote 3, table 2.

[6] See footnote 4, table 2.

TABLE 7.—*Influence of stage of development of leaves on a single cotton plant and of method of inoculation on infection by Xanthomonas malvacearum; experiment of December 1948*

[Plant of variety S×P Egyptian, 7 weeks old]

Stage of leaf development			Preinoculation treatment		Inoculation procedure [4]	Incubation period	Average disease severity
Number (rank in order of age) [1]	Position on plant [2]	Diameter	Type	Degree [2]			
		Millimeter		*Value*		*Days*	*Value*
2	Top	30	Water congestion by hypodermic syringe.	0	Gentle brushing [5]		[6] 0
3	do	70	do	1.0	do	2	2.8
4	Mid	110	do	2.5	do	2	3.0
5	do		do	3.0	do	2	3.3
6	do		do	2.8	do	3	3.0
7	Low		do	1.5	do	5	.9
8	do		do	2.5	do	4	1.4
2	Top	30	Atomizing		do		[7] 0
3	do	70	do		do	4	2.0
4	Mid	110	do		do	4	2.6
5	do		do		do	4	2.8
6	do		do		do	4	.8
7	Low		do		do	9	.3
8	do		do		do	12	.2
1	Bud	8	None		Brushing with moderate pressure. [5]	9	[7] .5
2	Top	30	do		do	4	2.5
3	do	70	do		do	4	2.7
4	Mid	110	do		do	4	1.8
5	do		do		do	5	.8
6	do		do		do	5	.6
7	Low		do		do	7	.4
8	do		do		do	14	.2

[1] The leaves are numbered in order from tip to base of stem; a bud leaf is not large enough to be subjected to more than 1 treatment.
[2] See footnote 1, table 6.
[3] See footnote 1, table 2; water congestion by atomizing is not visible.
[4] Using 300 million bacteria per milliliter.
[5] See footnote 2, table 5.
[6] See footnote 3, table 2.
[7] See footnote 4, table 2.

TABLE 8.—*Basis of method for classifying cotton varieties for relative resistance to Xanthomonas malvacearum, according to characteristic disease symptoms on leaves*

Reaction class	Character of leaf spots				Extent of vein blight	Bacterial exudate and extent of secondary spread
	Shape	Diameter	Color	Time and rate of drying		
			Early stage	Late stage		
		Millimeter				
Resistant	Roundish	1	Light brown	Reddish brown	Dry from beginning.	Very little, limited to vein.	Negligible.
Tolerant	Angular	1–2	Green	Brown	Rather rapid	Considerable, along veins.	Very little.
Susceptible	do	1–3	do	Dark brown or black.	Slow	Extensive, spreading.	Abundant.

TABLE 9.—*Differences in susceptibility of several cotton varieties to Xanthomonas malvacearum, as measured by leaf grading in two previously inoculated field plots; experiment of 1945*

[Plants were 1, 1½, and 2½ months old at time of inoculation]

Strain [1]	Date of inoculation				
	June 12, plot 1, graded June 27	June 26, plot 2		July 24, plot 1	
		Graded July 14	Graded July 24	Graded August 9	Graded August 13
	Grade [2]	*Grade*	*Grade*	*Grade*	*Grade*
Acala 11	4	3.5	4	4	4
Trice 2A	4	4	3.5	3.5	4
Coker 100 Wilt	3.5	3	3.5	3	3.5
Stoneville 37–13	3	3	3	3	3
Empire	3	2.5	3	3	3
Stoneville 62	2.5	2.5	3	2.5	3
Stoneville 4–3	[3] 2.5	[3] 3	[3] 2.5	[4] 1.5–3.5	1.5–3.5
Stoneville 4–2	1.5	1	1	1.5	1.5
Hybrid, backcross	1	1	1	1	1.5
Stoneville 20–1	1	1	1	1	1

[1] The seed lots of these strains were obtained from the following sources: Acala 11 from D. R. Hooton, Greenville, Tex.; Coker 100 Wilt from C. H. Rogers, Hartsville, S. C.; Empire from W.W. Ballard and A. L. Smith, Experiment, Ga.; Stoneville 62 from W. W. Ray, Stillwater, Okla.; all remaining strains from D. M. Simpson, Knoxville, Tenn. "Hybrid, backcross" refers to a backcross to Trice 2A of a Stoneville 20 × Trice 2A hybrid made by D. M. Simpson.

[2] Grades: 1=resistant; 2 and 3=tolerant; 4=susceptible.

[3] Results variable.

[4] In plot 1 plants of Stoneville 4–3 tagged to differentiate resistant (R) in the leaf spot phase from susceptible (S); 24 plants resistant, grade 1.5; 58 plants susceptible, grade 3.5.

TABLE 10.—*Influence of age and variety of plant and of concentration of inoculum on infection of leaves and stems of cotton plants spray-inoculated with Xanthomonas malvacearum, experiment of December 1944*

Variety	Age of plant	Plants inoculated [1]	Bacterial concentration in inoculum	Leaf spot [2]						Black arm	
				Incubation period			Severity			Plants affected	Severity [3]
				Bud	Top	Mid	Bud	Top	Mid		
	Days	*Number*	*Millions per milliliter*	*Days*	*Days*	*Days*	*Value*	*Value*	*Value*	*Percent*	*Value*
Acala 11	12	5	50	4.3	3.0	-------	[4] 2.8	[4] 2.8	-------	100	4.5
Do	12	5	1	10.0	4.4	-------	1.5	2.8	-------	100	4.0
Do	12	5	.05	10.5	5.8	-------	1.6	1.8	-------	100	.9
Do	28	5	50	9.3	3.7	4.4	.8	1.9	1.9	100	1.5
Do	28	5	1	9.5	4.2	8.1	.5	1.9	1.4	60	.7
Do	28	5	.05	-------	6.8	10.6	-------	.6	.9	40	.3
Stoneville 20	28	5	50	7.0	5.3	7.5	[4] .1	[5] .4	[5] .2	100	.3
Do	28	5	1	-------	6.3	-------	0	.2	0	60	.1
Do	28	5	.05	-------	13.3	-------	0	.1	0	([6])	([6])
S × P	28	5	50	4.5	3.3	5.4	[4] 3.4	[4] 3.7	[4] 3.2	100	4.0
Do	28	5	1	8.0	5.0	9.0	2.9	2.8	2.0	100	2.8
Do	28	5	.05	6.0	5.0	11.0	1.0	1.3	.6	60	1.2
5 varieties [7]	28	25	50	6.5	4.0	5.3	.9	2.0	1.6	100	2.0
Do	28	25	1	7.2	5.1	8.5	.8	1.6	1.1	76	.7
Do	28	25	.05	9.4	8.2	11.2	.5	.7	.5	24	.4

[1] Inoculation by applying a coarse spray with a knapsack sprayer.

[2] See footnote 1, table 6.

[3] Severity scale for black arm lesions:

	Value
Slight lesions	1.0
Moderate lesions	2.0
Severe lesions	3.0
Very severe lesions	4.0
Stem killed	5.0

[4] See footnote 4, table 2.

[5] A few angular spots showing hydrosis at first, but drying very rapidly. One of the few cases in which typical angular spots were produced on Stoneville 20.

[6] Trace.

[7] Shafter Acala, Acala 11, Trice, Stoneville 37, Stoneville 4–15; they cover the range of disease reaction intermediate between S×P and Stoneville 20

TABLE 11.—*Monthly average temperature and total rainfall at Clemson, S. C., during the growing seasons of 1944 and 1945*

Weather conditions	April	May	June	July	August	September
Temperatures, 1944:		°F.	°F.	°F.	°F.	°F.
Minimum	-------	55. 5	65. 8	66. 0	66. 0	63. 5
Maximum	-------	84. 5	93. 4	89. 2	86. 8	84. 0
Mean	-------	70. 0	79. 6	77. 6	76. 4	73. 7
Temperatures, 1945:						
Minimum	-------	53. 4	64. 2	68. 3	66. 0	65. 1
Maximum	-------	79. 2	89. 5	87. 9	87. 9	85. 9
Mean	-------	66. 3	76. 8	78. 1	76. 9	75. 5
Rainfall:		*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
1944	5. 3	1. 4	2. 3	3. 9	3. 4	4. 4
1945	5. 2	4. 5	5. 0	4. 8	3. 7	4. 8

TABLE 12.—*Degree of differentiation of cotton varieties according to susceptibility to Xanthomonas malvacearum when tested by knapsack sprayer inoculation technique; experiment of May 1944*

[Plants 7½ weeks old]

Variety	Number of plants inoculated [1]	Stage of leaf development [2]			
		Bud	Top	Mid	Low
		Number of leaves infected per plant			
S × P	18	0. 7	0. 9	3. 1	2. 1
Acala 11	18	. 3	. 9	3. 0	2. 2
Stoneville 37	18	. 1	. 5	3. 0	1. 9
Stoneville 20	12	0	0	0	0
		Average severity of disease (value)			
S × P	18	[3] 1. 0	2. 2	2. 4	1. 1
Acala 11	18	. 5	1. 5	2. 2	. 5
Stoneville 37	18	. 5	. 9	1. 9	. 4
Stoneville 20	12	0	0	0	0

[1] A suspension of 8 million bacteria per milliliter applied at the rate of 600 ml. per pot of 6 plants with a semicoarse spray. Stomata of mature leaves fairly well open.

[2] See footnote 1, table 6.

[3] See footnote 4, table 2.

TABLE 13.—*Influence of inoculation spray type, stage of leaf development, and stomatal opening on infection of leaves of cotton plants by Xanthomonas malvacearum*

[Plants 4½ to 5 weeks old]

EXPERIMENT OF JULY 28, 1944

Variety	Inoculation spray type	Stomatal condition on matured leaves	Disease symptoms [1]									
			Leaves infected per plant				Severity					
							19 days after inoculation				33 days after inoculation	
			Bud	Top	Mid	Low	Bud	Top	Mid	Low	Bud	Top
			Number	*Number*	*Number*	*Number*	*Value*	*Value*	*Value*	*Value*	*Value*	*Value*
S × P	Semicoarse [2]	Open	1.2	1.0	2.8	0.8	[3] 0.9	1.8	2.4	0.6	2.8	3.5
Do	do	Closed	1.5	.8	1.7	1.0	.6	.6	.5	.4	2.3	2.7
Do	Mist [4]	Open	.8	1.0	2.8	.7	.8	.4	.3	.3	1.6	.7
Acala 11	Semicoarse	do	.5	1.0	3.0	1.2	.5	1.2	2.3	.5	.6	1.6
Do	do	Closed	.8	1.0	2.5	.7	.5	.7	.4	.3	.7	1.1
Do	Mist	Open	.7	.7	1.8	.3	.4	.3	.3	.2	.6	.4
Stoneville 37	Semicoarse	do	1.0	.8	3.0	1.0	.5	1.1	3.0	1.4	.5	1.5
Do	Mist	do	.2	.8	1.2	0	.4	.3	.3	.2	.6	.4
Stoneville 20	Semicoarse	do	.2	.8	.5	0	.2	.4	.3	0	.2	.4
Do	Mist	do	0	0	0	0						

EXPERIMENT OF AUGUST 28, 1944

[illegible] P	Semicoarse [2]	Open	1.3	1.0	3.0	2.3	1.3	1.4	2.4	1.3	
Do	do	Closed	1.5	.8	2.0	1.5	1.0	.8	.8	.5	
Do	Mist [4]	Open	1.4	.8	1.8	1.2	.4	.5	.4	.3	
Acala 11	Semicoarse	do	.8	1.0	3.0	2.0	.5	1.5	1.8	.5	
Do	do	Closed	.8	1.0	2.4	1.2	.5	.8	.3	.2	
Do	Mist	Open	.8	.8	2.4	1.0	.3	.4	.3	.2	
Stoneville 37	Semicoarse	do	.8	1.0	3.0	1.8	.6	2.0	1.0	.4	
Do	do	Closed	.8	.8	2.6	1.6	.4	.9	.5	.2	
Do	do	Open	.2	.8	1.2	0	.2	.3	.2		

[1] See footnote 1, table 6.

[2] Semicoarse spray applied at the rate of 600 to 750 ml. per pot with 5 plants, holding the nozzle close to leaves; July 28 experiment, 1 million bacteria per milliliter; August 28 experiment, ½ million per milliliter.

[3] See footnote 4, table 2.

[4] Mist spray containing 1 million bacteria per milliliter applied at the rate of 1,500 ml. per pot with 5 plants, holding the nozzle 2 to 3 feet from the plants.

TABLE 14.—*Varietal differences in reaction of cotton to Xanthomonas malvacearum applied by spray inoculation in two field plots; experiment of June 16 to 18, 1944*

[Plants 5 weeks old]

DATA FROM PLOT A [1]

Variety	Secondary leaf spot				Bract spot		Black arm		
	Time after inoculation	Diseased leaves on 24 plants		Area infected on diseased leaves	Time after inoculation	Diseased bracts [2]	Time after inoculation	Diseased plants [3]	Average severity [4]
		Total	Shed						
	Days	*Number*	*Number*	*Percent*	*Days*	*Percent*	*Days*	*Percent*	*Value*
S × P	62	120	75	8.9	40	99	53	91.0	3.1
Shafter Acala	62	99	37	9.2	40	67	53	25.0	1.2
Acala 11	62	79	15	6.8	40	49	53	23.0	1.3
Trice 2A	62	85	17	9.8	40	48	53	13.0	1.3
Coker 100	62	87	6	6.7	40	41	53	4.0	1.0
Stoneville 37	62	83	12	6.1	40	49	53	9.3	1.0
Stoneville 4–15	62	67	13	3.6	40	31	53	1.3	1.0
Stoneville 20–1	62	8	0	1.0	40	5	53	0	0

DATA FROM PLOT B [5]

S × P	55	134	64	6.6	54	100	46	98.0	3.8
Shafter Acala	55	84	19	6.8	54	87	46	52.0	1.5
Acala 11	55	99	26	5.3	54	71	46	22.0	1.3
Trice 2A	55	97	31	6.7	54	73	46	9.3	1.2
Coker 100	55	82	11	4.4	54	65	46	3.5	1.0
Stoneville 37	55	78	10	4.1	54	60	46	11.0	1.0

[1] Plot A was inoculated by semicoarse spray.

[2] Counts made on 150 plants per variety (squares, blooms, and young bolls). Specimens recorded as diseased when they had 1 or more typical green spots.

[3] Counts made on 75 plants per variety in plot A and 150 plants in plot B.

[4] See footnote 3, table 10.

[5] Average of data from 3 subplots inoculated by fine, semicoarse, and coarse spray.

TABLE 15.—*Differences in susceptibility of several cotton varieties to boll rot as measured after artificial inoculation with Xanthomonas malvacearum; experiment of 1945*

Strain	Plot 1 [1]		Plot 2 [2]		
	Bolls diseased	Severity of disease symptoms, predominant grade [3]	Bolls diseased	Severity of disease symptoms	
				Predominant grade [3]	Range in grade [3]
	Percent		*Percent*	*Value*	*Value*
Acala 11 [4]	100	9	61	9	6–10
Trice 2A	90	8	67	9	6–10
Coker 100 Wilt [4]	90	7	39	7	6– 8
Stoneville 37–13	90	5	33	6	4– 7
Empire [4]	80	5	16	6	4– 9
Stoneville 62 [4]	80	5	42	6	5– 7
Stoneville 4–3S [5]	80	6	26	6	3– 8
Stoneville 4–3R [5]	50	4			
Stoneville 4–2	100	4	20	3	1– 5
Hybrid backeross [4]	50	3	30	4	2– 6
Stoneville 20	20	1	20	1	.5– 2

[1] Bolls inoculated by rubbing tagged specimens with a piece of cheesecloth wet with bacterial suspension, Aug. 31. Data taken 3 weeks later, 10 bolls per variety.

[2] Bolls inoculated by a heavy application of coarse spray, Aug. 27. Data taken 3 weeks later, 80 to 170 bolls per variety.

[3] Bolls graded by a scale ranging from 1=very slight, to 10=very severe.

[4] See footnote 1, table 9.

[5] See footnote 4, table 9.

TABLE 16.—*Differences in susceptibility in the seedling stage of four cotton varieties grown in field plots from seed inoculated with Xanthomonas malvacearum; experiment of May 1945*

Variety	Seedlings grown [1]	Seedlings diseased [2]		Disease index [3]
		Total	Severely diseased cotyledons	
	Number	*Percent*	*Percent*	
S × P	254	70	11	24.2
Shafter Acala	386	67	5.6	21.0
Acala 11 [4]	635	56	.8	10.7
Coker 100	647	33	.1	3.1

[1] 2 100-foot rows per variety.

[2] Data taken May 29–30 on seed planted May 9.

[3] Disease index=percentage of seedlings diseased × relative severity, considering disease severity of destroyed seedlings as 1.0.

[4] In checkrows planted with seed that had not been inoculated a count of 960 seedlings of Acala 11 gave 24.2 percent diseased. Practically no seedling infection was noted at this time in other varieties. This indicated that the Texas-grown Acala 11 was naturally infected.

TABLE 17.—*Severity of leaf spot and black arm symptoms on cotton varieties inoculated in the seedling stage by spraying with suspension of Xanthomonas malvacearum; experiment of May–June, 1945*

Variety	Inoculation spray type	Leaf spot [1]		Black arm [1]	
		Leaves diseased per plant	Average severity [2]	Plants diseased	Average severity [3]
		Number	*Value*	*Percent*	*Value*
Shafter Acala	Coarse spray	2. 4	17. 5	92	2. 3
Acala 11 [4]	do	2. 0	17. 5	87	2. 0
Coker 100	do	2. 0	12. 0	65	. 9
Shafter Acala	Fine spray	1. 5	7. 5	30	. 9
Acala 11 [4]	do	1. 8	10. 0	65	1. 3
Coker 100	do	. 7	4. 0	7	. 3

[1] Disease counts made June 25 on 40 thinned plants of each variety per treatment.
[2] Expressed as estimated percentage of diseased leaf areas.
[3] See footnote 3, table 10.
[4] See footnote 4, table 16.

TABLE 18.—*Summary of relative susceptibility of cotton varieties to various phases of bacterial blight caused by Xanthomonas malvacearum*

Variety	Cotyledon spot,[1] 1943	Leaf spot [2]		Bract spot,[3] June 1944	Black arm [4] (plants diseased)		Boll rot,[5] September 1945	
		September 1944	June to August 1945		1944	1945	Plot 1	Plot 2
	Disease index	*Grade*	*Grade*	*Percent*	*Percent*	*Percent*	*Grade*	*Grade*
S × P (Egyptian)	91	5		99	91			
Shafter Acala	56	5		67	25	92		
Acala 11	47	4	4	49	23	87	9	9
Trice 2A	35	3. 5	4	48	13		8	9
Coker 100 Wilt		3	3. 5	41	4	65	7	7
Stoneville 37	22	3	3	49	9		5	6
Stoneville 4–15	5	2		31	1			
Hybrid backcross [6]			1				3	4
Stoneville 20	3	1	1	5	0		1	1

[1] Seed inoculated and cotyledons graded for disease reaction, as described in a previous paper (*21*).
[2] Leaf grades range from 1=resistant to 5=highly susceptible; 1945 data from table 9.
[3] Data from plot A, table 14.
[4] Age of plants at time of inoculation: 5 weeks in 1944, 3 weeks in 1945. The 1944 data are from plot A, table 14, the 1945 data from table 17.
[5] Boll grades range from 1=resistant, to 10=highly susceptible; data from table 15.
[6] See footnote 1, table 9.

TABLE 19.—*Influence of inoculation spray type, age of plants, and concentration of inoculum on infection of cotton plants in field plots with Xanthomonas malvacearum; experiments of June 1944*

DATA OF PLOT C, INOCULATED JUNE 22 TO 24, 1944

Variety	Age of plants at time of inoculation	Concentration of inoculum	Inoculation spray type	Leaf spot					Bract spot [3]	Black arm	
				Primary infection,[1] diseased leaves on 10 plants			Secondary infection [1]				
				Total	Shed	Average severity [2]	Diseased leaves	Average severity [2]		Diseased plants [4]	Average severity [5]
	Days	*Million bacteria per milliliter*		*Number*	*Number*		*Percent*		*Percent*	*Percent*	
S × P	43	1	Coarse	97	74	92	100	17	96	98	4.5
Do	43	1	Fine	93	64	89	94	13	96	92	3.5
Do	43	1	Mist	83	58	84	79	4	92	26	2.0
Shafter Acala	43	1	Coarse	82	52	89	71	6	92	8	1.0
Do	43	1	Fine	70	46	89	67	6	84	4	1.0
Do	43	1	Mist	63	22	80	51	3	24	2	.7
Coker 100	43	1	Coarse	78	55	89	54	3	76	6	.7
Do	43	1	Fine	77	47	86	55	3	48	2	.7
Do	43	1	Mist	69	43	85	49	2	16	0	----

DATA OF PLOT D, INOCULATED JUNE 21 TO 23, 1944

Acala 11	28	10.0	Coarse	63	39	89	77	8	44	90	
Do	28	1.0	do	65	39	85	73	6	28	72	
Do	28	.1	do	57	35	75	67	4	28	60	
Do	28	10.0	Mist	59	31	69	70	5	20	16	
Do	28	1.0	do	53	26	59	67	3	12	6	
Do	28	.1	do	33	0	22	43	2	8	0	
Do	42	10.0	Coarse	77	58	92	97	8	88	32	
Do	42	1.0	do	60	42	87	90	6	64	8	
Do	42	.1	do	57	27	82	87	6	48	2	
Do	42	10.0	Mist	52	23	63	77	5	28	0	
Do	42	1.0	do	48	11	38	80	4	28	0	
Do	42	.1	do	31	7	23	80	3	16	0	

[1] Counts made on 10 plants per treatment 25 days after inoculation.
[2] Expressed as a percentage of infected leaf surface, considering shed leaves as 100 percent infected.
[3] Counts made 47 days after inoculation in plot C; 38 days after inoculation in plot D.
[4] Counts made on 50 plants per treatment 47 days after inoculation in plot C; 41 days after inoculation in plot D.
[5] See footnote 3, table 10.

TABLE 20.—*Effect of time of day and inoculation spray type on infection of cotton plants in field plots with Xanthomonas malvacearum; experiment of June 1944*

[Plants 5 weeks old]

Variety[1]	Inoculation		Secondary leaf spot[2]			Black arm[4]	
	Time of day[1]	Spray type	Diseased leaves on 10 plants		Area infected on diseased leaves[3]	Diseased plants[3]	Average severity[5]
			Total	Shed			
			Number	*Number*	*Percent*	*Percent*	*Value*
S × P	Late morning	Coarse	52	27	5	98	4.0
Do	do	Fine	46	27	7	86	3.3
Do	do	Mist	33	20	3	15	1.8
Do	Late evening	Coarse	36	11	6	46	2.5
Do	do	Fine	36	16	4	26	2.0
Do	do	Mist	15	3	3	5	1.2
Shafter Acala	Late morning	Coarse	28	6	11	20	1.0
Do	do	Fine	28	5	8	7	.7
Do	do	Mist	29	2	3	1	.7
Do	Late evening	Coarse	24	4	6	8	.7
Do	do	Fine	21	2	3	3	.7
Do	do	Mist	14	0	3	0	----

[1] During the morning inoculation the stomata of most leaves were wide open; during the evening inoculation they were closed or nearly closed.
[2] Observations made 50 days after inoculation.
[3] Estimated percentages, excluding shed leaves.
[4] Counts on 50 plants per treatment.
[5] See footnote 3, table 10.

TABLE 21.—*Effect of inoculation spray type, concentrations of inoculum, and time of day on infection of cotton plants in field plots with Xanthomonas malvacearum; experiment of June 1945*

[Plants of varieties Acala 11 and Coker 100, 6 weeks old]

Inoculation			Leaf spot [1]				Black arm [2]	
			Primary infection		Secondary infection			
Time of day	Concentration	Spray type	Acala 11	Coker 100	Acala 11	Coker 100	Acala 11	Coker 100
	Million bacteria per milliliter		*Disease index* [3]	*Disease index* [3]	*Disease index* [3]	*Disease index* [3]	*Disease index* [4]	*Disease index* [4]
Late morning	10.0	Coarse	74	70	11	10	82	23
Do	10.0	Fine	73	62	13	9	41	14
Do	10.0	Mist	20	32	4	4	9	0
Do	1.0	Coarse	58	60	8	7	29	2
Do	1.0	Fine	53	53	6	6	20	1
Do	1.0	Mist	13	16	3	2	7	0
Do	.1	Coarse	31	32	3	4	14	0
Do	.1	Fine	24	25	2	4	7	0
Do	.1	Mist	12	18	2	3	4	0
Late evening	1.0	Coarse	31	32	3	4	18	0
Do	1.0	Fine	24	26	2	4	9	0
Do	1.0	Mist	8	9	1	2	5	0

[1] Counts made on 20 plants per treatment, 35 days after inoculation.
[2] Counts made on 100 plants per treatment, 36 days after inoculation.
[3] Calculation of leaf disease index: (Number of shed leaves × 100) + (number of diseased leaves × severity) ÷ 100.
[4] Calculation of black arm index: Percentage of diseased plants × severity. All black arm lesions were rather slight.

TABLE 22.—*Effect of inoculation spray type on boll rot in field plots of cotton plants inoculated with Xanthomonas malvacearum; experiment of August 1945*

Inoculation			Total number of bolls inoculated		Bolls with bacterial spots [1]	
Date	Spray type	Method of spray application	Acala 11	Coker 100	Acala 11	Coker 100
			Number	*Number*	*Percent*	*Percent*
Aug. 20	Fine	Low [2]	61	197	8.2	3.5
Do	do	High	76	211	1.3	2.8
Do	Semicoarse	Low	67	231	16	7.8
Do	do	High	61	202	10	1.5
Aug. 28	Coarse	Low [3]	111	232	40	29
Do	do	do. [3]	88	287	29	26
	Uninoculated		39	118	0	3.3

[1] Four weeks after inoculation.
[2] Low = nozzle held close to bolls. High = nozzle 1 to 2 feet farther away from bolls.
[3] Tops of these plants cut above the boll-bearing laterals immediately after inoculation.
[4] Plants not cut.

TABLE 23.- *Effect of moist-chamber treatment on bacterial blight of seedling cotton plants; experiment of March 1945*

[Plants 12 days old]

Variety	Moist-chamber treatment [1]	Leaf spot [2]			Stem infection [5]				Leaf wilt [5]
					Black arm			Vascular	
		Diseased leaves per plant	Severity [3]	Disease index [4]	Lesions per plant	Severity [6]	Disease index [7]	Lesions per plant	Disease index [4]
		Number	*Value*		*Number*	*Value*		*Number*	
S × P	−	1.7	1.1	18.7	1.4	2.4	33.6	0.9	33
Do	+	2.4	1.7	40.8	1.9	2.6	49.4	1.4	38
Shafter Acala	−	1.3	.8	10.4	1.3	1.3	16.9	.4	7
Do	+	1.8	1.0	18.0	1.5	2.0	30.0	.7	18
Trice 2A	−	1.3	.6	7.1	.9	.8	7.2	.1	3
Do	+	1.7	1.1	18.7	1.1	1.6	17.6	.5	6
Stoneville 37	−	1.0	.6	6.0	.7	.7	4.9		2
Do	+	1.2	1.1	13.2	1.2	1.1	13.2	.4	4
Stoneville 4-15	−	.6	.5	3.0	.4	.5	2.0	.1	1
Do	+	1.6	.9	14.4	.7	.7	4.9	.1	3
Stoneville 20	−	.3	.2	.6	0	---	0	0	0
Do	+	.1	.1	.1	0	---	0	0	0

[1] One group of seedlings, placed in a moist chamber for 48 hours following inoculation; other group kept outside the moist chamber. Inoculation done by applying a suspension of 60 million bacteria per milliliter, partly with a quart sprayer and partly with a small sprinkling can.

[2] Data taken 15 days after inoculation.

[3] See footnote 4, table 2.

[4] Disease index = 10 × number of diseased leaves per plant × severity.

[5] Data taken 33 days after inoculation.

[6] See footnote 3, table 10.

[7] Disease index = 10 × number of lesions per plant × severity.

TABLE 24.—*Effect of sprinkling seedling cotton plants with water several days after inoculation upon subsequent infection by Xanthomonas malvacearum; experiment of April 1945*

[Seedling plants, 25 to 30 of each variety per treatment, grown in ½-gallon pots; soil kept very moist by watering from beneath]

Growth stage at time of inoculation [1]	Variety	Sprinkling [2]	Lesions on cotyledons [3]		Leaf spot [5]		Infection [6]		
			Plants diseased	Disease index [4]	Plants diseased	Disease index [4]	Black arm	Vascular symptoms	Total [7]
			Percent		*Percent*		*Percent*	*Percent*	*Percent*
Seed	Shafter Acala	−	55.2	20.0	3.4	0.3	24.1	20.7	24.1
Do	do	+	58.8	20.9	52.9	5.6	44.1	26.5	44.1
Do	Stoneville 37	−	17.2	4.5	0	0	6.9	0	6.9
Do	do	+	25.9	7.2	29.6	1.5	14.8	11.1	14.8
8-day-old seedling	Shafter Acala	−	61.5	9.1	11.5	.6			
Do	do	+	74.1	13.0	37.0	1.2			
Do	Stoneville 37	−	28.0	3.0	8.0	.3			
Do	do	+	76.0	11.0	8.0	.4			
16-day-old seedling	Shafter Acala	−			84.0	4.8			
Do	do	+			100.0	5.7			
Do	Stoneville 37	−			77.8	2.7			
Do	do	+			87.5	4.2			

[1] Seed inoculation carried out by soaking seed for 2 hours in a suspension containing 60 million bacteria per milliliter; seedlings inoculated by sprinkling with bacterial suspension on two successive days.

[2] − = Plants not sprinkled and not wetted when watering; + = plants sprinkled with water from a small sprinkling can: (*a*) Seedlings from inoculated seed sprinkled early in the morning for 8 days, starting with the day of emergence; (*b*) other seedlings sprinkled early in the morning and late in the evening of the 4 days following inoculation.

[3] Data taken 13 days after inoculation.

[4] See footnote 4, table 23.

[5] Data of seed inoculation taken 28 days after planting those of other seedlings, 13 days after inoculation.

[6] Data taken 40 days after planting.

[7] Total number of stems with black arm, vascular symptoms, or both.

TABLE 25.—*Effect of method of inoculation on bacterial blight of seedling cotton plants; experiment of April 1945*

[Plants of variety Shafter Acala, 12 days old at time of inoculation, 27 plants per treatment]

Method of inoculation [1]	Diseased cotyledons [2]	Leaf spot			Stem infection [5]			Leaf wilt [7]	
		Diseased leaves per plant	Spots per leaf [3]	Severity [4]	Black arm	Vascular symptoms	Total [6]	Leaves per plant	Severity [3]
	Number	*Number*	*Number*	*Value*	*Percent*	*Percent*	*Percent*	*Number*	*Value*
Sprinkling	0. 1	1. 3	2. 2	0. 4	33. 3	14. 9	30. 4	0. 5	2. 0
Semicoarse spray	. 4	2. 1	25. 6	1. 0	40. 7	22. 2	47. 7	1. 6	3. 3

[1] Inoculated in the morning of 2 successive days using, for each 2-gallon pot with 9 plants, 600 ml. of a suspension containing 10 million bacteria per milliliters. Sprinkling done with a small sprinkling can. Semicoarse spray applied with a knapsack sprayer.

[2] Data taken 13 days after inoculation.

[3] See footnote 4, table 2.

[4] Average number of spots per diseased leaf.

[5] Data taken 40 days after inoculation.

[6] Total number of stems with black arm, vascular symptoms, or both.

[7] Data taken 30 days after inoculation.

U. S. GOVERNMENT PRINTING OFFICE: 1948

For sale by the Superintendent of Documents, U. S. Government Printing Office
Washington 25, D. C. - Price 20 cents

END