DISSEMINATION OF THE ANGULAR LEAFSPOT OF COTTON

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INTRODUCTION

A satisfactory explanation of the dissemination of many of the diseases of plants is lacking. The literature recognizes such agents as insects, wind, tools, laborers, drainage, spattering rains, etc., and yet in some cases one must doubt the importance attached to these by investigators. In other instances, particularly in the cases of bacterial diseases affecting the leaves, stems, flowers, and fruits, no satisfactory explanation has been offered. This was the status of affairs in the case of the angular leafspot of cotton (Gossypium spp.) when the author began the investigation of this disease at the South Carolina Experiment Station. It is the purpose of this paper to present the data obtained during the past summer and to offer the conclusion reached as to its dissemination under the conditions existing in western South Carolina, with a suggestion of the possible importance of these factors in the dissemination of other similar diseases.

HISTORICAL RÉSUMÉ

A brief résumé of the literature dealing with some of the most common bacterial diseases follows, being presented in order that the true situation may be understood and that the information found there may be used in this discussion.

Beach (1893)² and Halsted (1893) concluded that the blight of beans was a bacterial disease, because of the constant association of bacteria with the typical lesions. Halsted further stated that the disease was carried over in the seed. Sackett (1909, p. 21) states that—

Rain and dew are doubtless agents in spreading the germs from one part of the plant to another by washing them from old lesions onto unaffected parts, though no evidence of this fact is given. Edgerton and Moreland (1913) successfully inoculated plants without wounding, and concluded that infection can take place through the stomata.

Pierce (1901) successfully inoculated the fruit of the walnut with water suspensions of Bacterium juglandis. R. E. and C. O. Smith and

¹ The author is greatly indebted to Prof. R. A. Harper, of Columbia University, for the perusal and criticism of the manuscript, and to Mr. J. W. Sanders, assistant in this laboratory, for his most careful and untiring aid in the work.
² Bibliographic references in parenthesis refer to "Literature cited," pp. 473-475.
Ramsey (1912) agree that the uninjured host is susceptible, and conclude that water is apparently the principal agent in conveying the bacteria from the existing lesions to younger leaves and small nuts lower down on the tree. They state (p. 338) that—

During one of these [before mentioned] fogs the trees became saturated, water dripping from one portion of the tree to another which could easily carry the disease organisms to healthy tissue. Observations go to show that secondary infection in which large numbers of the small nuts become diseased is very likely to follow one of these foggy periods.

Arthur and Bolley (1896) suggest that wound-producing insects are probably an important factor in the spread of the carnation disease caused by *Bacterium dianthi*, though infections can also take place through the stomata. They recommend a method of watering the plants in which the foliage is kept dry, and experience has shown the efficacy of it. In their conclusion (p. 34) the authors state that—

As there must be moisture upon the leaves sufficient to enable the bacteria to move about and enter the stomata in order that they may gain access to the interior of the leaf, it is evident that keeping the foliage dry will prevent the disease.

It apparently does not occur to them that the water under pressure, being dashed from plant to plant, might serve as a means of dissemination.

Lewis (1914) describes a disease of *Erodium* spp. and *Pelargonium* spp. caused by *Bacterium erodii*, which, he says (p. 230),—

is more prevalent in crowded beds where the plants remain moist and light is not so dense.

Sprinkling is suggested as the most probable method of dissemination.

Sackett (1910) discusses a disease of the stems of alfalfa caused by *Pseudomonas medicaginis*. The organism is probably carried from place to place on wind-blown dust particles. O'Gara (1914) describes more fully the characteristic appearance of the affected parts, and adds that stomatal infections may occur, though by far the greatest infection takes place through openings produced by insect puncture and severe frost injury.

E. F. Smith, in the second volume of his work on Bacteria in relation to plant disease (1911), summarizes the data relating to water-pore inoculation of cruciferous plants with *Bacterium campestris*, and, in discussing Fischer's objections to this conclusion, writes (p. 308)—

** * * * (2) the hypothetical, dust-dry, wind-borne bacterium requiring a half day or more to moisten it, is probably not the one that usually enters the water-pores and induces the disease, but rather a fresh germ recently come from the interior of some affected leaf as an extrusion from some water-pore already diseased, or left in the vicinity of the water-pore by some wandering insect. * * * such a bacterium would be ready to grow as soon as it found lodgment in a moist place.

The disease progresses most in periods of frequent rains. Russell (1898, p. 31–32), writing on the same subject, states:

One direct agent by which the disease is spread is the wind. Whether the disease germ is present in the soil or in decaying plants, the dried bacilli can be carried through
the air *** Inasmuch as this [infection through the water pores] is by far the most common gate of entrance for the disease organism, it is highly probable that the disease is disseminated by means of the wind more than in any other way.

Smith discusses the wilt of cucurbits in the same volume and presents evidence of the dissemination of that disease by insects, especially one *Diabrotica vittata*. Concerning Cobb’s disease of sugar cane, this author in his third volume (1914) writes (p. 48):

We can well imagine, however, that under ordinary field conditions, with an abundance of dew or rainfall, and plenty of insect depredators, diseased plants might readily infect neighboring healthy ones, especially when young.

In the chapter on Stewart’s disease of sweet corn, which is also in the third volume, the author states (p. 124), in the discussion of an experiment relating to seed infection:

If the disease was actually derived from the seed-corn there probably would have been some cases during the seedling stage, and fragments of these soft plants full of the bacteria would have been blown upon neighboring plants, or dragged by cultivators, or carried on the feet of men and horses, or bitten into by insects, or washed about by rains and dews. There are ways enough to account for the dissemination of the bacteria in the infection of a few plants when the distance is only a matter of a few feet.

In an address before the Massachusetts Horticultural Society (1897) Smith discussed the subject under the headings: (1) Spread by insects; (2) spread by snails and slugs; (3) spread through manure pile; (4) spread by way of the soil; (5) spread by way of seeds, seedlings, buds, tubers, cuttings, and nursery stock.

Macchiati (1891) and Boyer and Lambert (1893) describe a leaf and twig disease of mulberry caused by bacteria. Both claim to have isolated the organism, and the latter authors report successful inoculations with a bacterium named by them "*Bacterium mori*." E. F. Smith (1910) uses this name for an organism which he determined to be the cause of the same disease in Georgia, though it differed from that of Boyer and Lambert. In no case, however, is an explanation of the method of dissemination offered.

Manns (1909) concluded that the bladeblight of oats was caused by two bacteria to which he assigned the names "*Pseudomonas avenae*" and "*Bacillus avenae*," and that these bacteria were present in the soil reaching the host through "spattering rains." Manns and Taubenhaus (1913) report their studies of the streak disease of sweet peas and clovers which they found caused by a bacterium named by them "*Bacillus lathyri*." Later, Manns (1915) discusses this disease and considers more fully the subject of dissemination, saying (p. 12) that the disease attacks the plants about the beginning of the blooming period—having its origin usually near the ground, indicating distribution by spattering rain and infections through the stomata.

Neither of them states by what means the upper parts of the plants become infected.

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1 According to Migula's system of classification this name would be "*Pseudomonas mori*."
Griffin (1911), Barss (1913, 1915), and Rees (1915), writing separately of the gummosis of cherry trees, agree that the disease is caused by a motile bacterium, and suggest that sucking insects are the probable agent in its dissemination. Barss (1915, p. 239) says:

The exact manner in which the disease is disseminated is not known, but indications seem to point to the possibility that sucking insects may be largely responsible for infections.

No data are available in the literature as to the susceptibility of any part of the plant without previous injury, all inoculations having been made by needle puncture.

Stewart and Leonard (1913) show clearly that certain sucking insects, among them the tarnished plant bug, are capable of inoculating tender shoots of pears when *Bacillus amylovorus* is present at the immediate point of puncture or upon the mouth parts of the insect. These authors (1915) conclude that certain flies are not active agents of inoculation, but, because of their abundant presence in the orchards and among nursery stock, they are probably a factor in spreading the bacteria from place to place. Attention is attracted by Stewart (1913, 1915) to the increased disease following rainy weather, due to both the increased susceptibility of the host because of the more succulent growth and the increased activity of the bacteria because of more favorable environment. Heald (1915) is of the opinion that leaf infection of pears by fireblight is possible through water pores, while Hotson (1915, 1916) offers further evidence in support of this.

Rolfs (1915), writing on the disease of stone fruits caused by *Bacterium pruni*, states that infection takes place through stomata and that a film of moisture is necessary to successful inoculation. He says (p. 416, 421):

Rain and dew are not only important factors for inoculation, but they also carry the bacteria to the healthy leaves, twigs, and fruits, and thus frequently serve as agents of transportation. * * * The warm, slow, continued rains of the summer furnish the best conditions for the rapid spread of the disease. Heavy, driving rains of short duration followed by sunshine and winds are not favorable to its spread, since many of the bacteria are washed to the ground, and the leaves are quickly dried off and the few bacteria that may be spread will be quickly dried and killed.

Rolfs (1915), in his discussion of the angular leafspot of cotton, states (p. 17-18):

Wet weather, of course, materially aids in the dissemination of the organism. Even if the weather is excessively dry, the dew at night will often furnish sufficient moisture for inoculation. * * * If for any reason the first leaves fail to become inoculated in this way [by contact in presence of dew] the movement of the leaves in the wind especially during a storm, will soon bring them in contact with some of the virus on diseased leaves. * * * The rain carries large numbers of the organism to the new tissue and to the ground under the plants. * * * The soil under the infected plants may thus become an important means of inoculating many of the lower leaves.

Hasse's (1915) conclusion that *Pseudomonas citri* is the cause of Citrus canker has been confirmed by Wolf (1916). The latter states (p. 94), regarding its dissemination:
Definite experimental data are wanting on the agencies by which Citrus canker is spread. It is evident that rain and dew are important factors in carrying the disease to unaffected leaves, twigs, and fruits of trees in which the disease is already present.

Even though Stevens (1914) thought the disease to be due to a fungus, he writes (p. 41):

The disease seems to develop and spread rapidly during rainy weather, but it is more or less retarded during periods of drought or in a dry season.

Brown and Jamieson (1913) present their work on a disease of nasturtium and sugar-beet leaves caused by the same organism, which they named "Bacterium aptatum." While the authors state they had no opportunity to study this disease under field conditions, their experiments would suggest that infection takes place only in bruised or wounded tissue caused by insects or mechanical injury.

A number of preliminary reports of work on bacterial diseases have been made in recent years; and, since in most of these the subject of dissemination is not mentioned, the present author takes it that this phase will be discussed later and, therefore, he will not review this literature.

The facts in the dissemination of certain types of fungus diseases have a close bearing on the subject; yet this literature is so voluminous as to be impossible of review here. However, because of its relation to one of the most serious cotton diseases, anthracnose, Whetzel's (1906) paper on bean diseases is mentioned. Of anthracnose, the author writes (p. 205):

The spores may be scattered by the cultivator, the pickers, by animals, or by the wind in damp or rainy weather.

EXPERIMENTAL INVESTIGATIONS

Infection of the leaves of cotton by Bacterium malvacearum can easily be brought about by superficial inoculation in the presence of sufficient moisture. This has been done by applying small amounts of agar-slab cultures of the organism with glass rods and spreading with rubber-gloved fingers when the dew was on the leaves, by applying water suspensions of the bacteria with cotton swabs at all times of the day or evening, spraying such suspensions with an atomizer, or by painting them upon the leaves with camel's-hair brushes. The first signs of infection are minute, dark-green, angular (triangular or quadrilateral) spots on the underside, whether the inoculation was made upon the upper or lower surface of the leaf, usually in 7 to 10 days, though often not earlier than 15 days. In a day or two following the first appearance on the underside of the leaf the same dark-green water-soaked spot will appear on the upper side, though less conspicuously angular. When held up to the light, such spots show a translucency as contrasted with the light, impervious normal leaf and the irregular transparency of some insect injuries. The spot usually increases in size simultaneously on both sides of the leaf, but never crosses the veins. Single infections seldom increase to a size larger than 3 to 4 mm. in the longest dimension. The larger spots, so con-
spicuous on the leaves, are usually caused by the coalescence of two or more separate infections which often occur in clusters when in the mesophyll regions and in a linear direction when along the veins, seemingly where the water gathers and is last to evaporate. The top side of the spot thus appearing becomes a reddish brown color over a circular area at the center and shrinks slightly in thickness. The colored area increases in size until it conforms to the angular shape of the affected part, the red advancing to the margin and the center shading off to gray. On the underside the spot becomes a brown color, though the dark green, characteristic of the young spot, remains as a narrow band about the margin until the latest stages of development, a place where the activity of the disease continues longest. When the green band finally disappears, the brown replaces it, giving a sharp angular margin to the spot. These color changes may develop quickly, so that the entire spot will be brown eight or nine days after inoculation; but usually they proceed more slowly, leaving the spot dark green for five or six days.

**SEED INFECTION**

The source of bacteria for the first infections of each season is as yet undetermined, because of conflicting experimental results obtained during the past summer; however, such a problem can be solved satisfactorily only when great numbers are used under the most favorable circumstances. One experiment, or series of experiments, can not be expected to settle the matter.

Over 2,500 seedlings from seed of various sources grown during the winter in all variations of temperature and moisture in the greenhouse and laboratory failed entirely to show the disease. Two instances of cotyledonary infection occurred in the late spring upon seedlings in the greenhouse. An acre field planted on April 20 was entirely free from the disease until August, except in such portion as had been used for inoculation experiments. Another field near by, planted partly with these same seeds and partly with others on May 15, developed considerable disease in both lots of seedlings, though the season was rainy and the chopping was delayed; and no data as to the amount of disease due to the seed and that due to local dissemination could be secured. It was true that, of the diseased seedlings examined, equally as many had cotyledons free from the disease (84) as had them spotted (78).

In order to decide more carefully the probability of seed infection, an experiment was arranged in which samples of various lots of seeds were planted in plots, seven rows wide, the rows being 50 feet long and 3 feet apart. Because of the triangular shape of the land available, these dimensions could not be strictly adhered to; yet each lot of seed was represented by one such plot at least. Most seeds used were samples obtained from several growers in various parts of the State, who had fields badly infected early in the summer, such as would likely be due to
a high percentage of seed infection. These are labeled "Lots II-V," Lot I being seed of the same lot planted on April 20 and remaining free from seedling infection. The other lots (VI-X) are Mississippi Cook seed grown on the Experiment Station farm under differing conditions the previous summer.

On two sides of this triangular plot were cotton variety tests of the Station, and the disease was present upon these plants. To guard against insect activity in spreading the disease to the young seedlings and thus confusing the results, cages of cheesecloth were erected over 12 feet of a row of each lot of seed, giving an opportunity for the disease to appear under such protection if the bacteria were present upon or in the seed. The field was planted on August 12. The seed germinated quickly, and the seedlings began to appear above the ground by the 16th and 17th. On the 22d a careful examination was made of all the plants, but no disease was found. On the 28th 11 cases of cotyledonary infection were found, and on September 1, 20 days after planting and 15 days after the cotyledons were spread, affording ample time for the appearance of the disease in view of our earlier work, the final counts were made. Of 34 diseased plants found, only one was beneath a cage; yet that one lends weight to the conclusion that seed infection does occur. The tabulated data by plots of each lot of seed follows in Table I.

**Table I.—Results of the infection of cottonseed by angular leafspot**

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Plot No.</th>
<th>Number of seedlings.</th>
<th>Number diseased in—</th>
<th>Total number of seedlings</th>
<th>Total number diseased</th>
<th>Percentage diseased.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>11 days.</td>
<td>20 days.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>820</td>
<td>0</td>
<td>0</td>
<td>820</td>
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</tr>
<tr>
<td></td>
<td>2</td>
<td>4,096</td>
<td>0</td>
<td>0</td>
<td>4,096</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1,743</td>
<td>0</td>
<td>0</td>
<td>1,743</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>468</td>
<td>0</td>
<td>0</td>
<td>468</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cage</td>
<td>207</td>
<td>0</td>
<td>0</td>
<td></td>
<td>8,750</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>3,523</td>
<td>4</td>
<td>2</td>
<td>3,523</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>5,543</td>
<td>0</td>
<td>7</td>
<td>5,543</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cage</td>
<td>179</td>
<td>1</td>
<td>1</td>
<td>179</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2,622</td>
<td>4</td>
<td>1</td>
<td>2,622</td>
<td>4</td>
</tr>
<tr>
<td>III</td>
<td>9</td>
<td>700</td>
<td>0</td>
<td>0</td>
<td>700</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cage</td>
<td>86</td>
<td>0</td>
<td>0</td>
<td></td>
<td>3,408</td>
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<tr>
<td></td>
<td>10</td>
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<td>0</td>
<td>6</td>
<td>2,884</td>
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<tr>
<td></td>
<td>11</td>
<td>1,242</td>
<td>0</td>
<td>4</td>
<td>1,242</td>
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</tr>
<tr>
<td></td>
<td>12</td>
<td>1,402</td>
<td>0</td>
<td>8</td>
<td>1,402</td>
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<tr>
<td></td>
<td>Cage</td>
<td>117</td>
<td>0</td>
<td>0</td>
<td>117</td>
<td>5</td>
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<td>IV</td>
<td>13</td>
<td>142</td>
<td>0</td>
<td>0</td>
<td>142</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2,030</td>
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<tr>
<td></td>
<td>15</td>
<td>1,150</td>
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</tr>
<tr>
<td></td>
<td>16</td>
<td>195</td>
<td>0</td>
<td>0</td>
<td>195</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cage</td>
<td>145</td>
<td>0</td>
<td>0</td>
<td>145</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>2,002</td>
<td>0</td>
<td>0</td>
<td>2,002</td>
<td>0</td>
</tr>
<tr>
<td>VIII</td>
<td>18</td>
<td>1,247</td>
<td>0</td>
<td>0</td>
<td>1,247</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>3,678</td>
<td>0</td>
<td>0</td>
<td>3,678</td>
<td>0</td>
</tr>
<tr>
<td>IX</td>
<td>20</td>
<td>1,415</td>
<td>0</td>
<td>0</td>
<td>1,415</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cage</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Grand total</td>
<td></td>
<td>36,568</td>
<td>45</td>
<td>0</td>
<td></td>
<td>36,568</td>
</tr>
</tbody>
</table>
In this experiment the seedlings of Lot I were slightly diseased (0.011 per cent), the results being comparable with those obtained from the field planted on April 20. It is improbable that the diseased plants in the field planted with these seed on May 15, which varied from 2.7 to 8.1 per cent of the plants of single rows, were due to seed infection, but rather to the spread of the disease from other infected plants at an extremely favorable time.

The other results obtained, 0.2, 0.14, and 0.31 per cent, do not account for the amount of disease observed in fields planted with these seed. The field planted with seed of Lot V was the most badly diseased of any seen on a trip about the State in June; and, while the author obtained only 142 seedlings, none of them were diseased.

Without doubt, 0.46 per cent of diseased seedlings at the beginning of a season, especially if rainy weather prevails before chopping, would be sufficient to start a general field infection, and this could be called the primary infection. It is interesting to note in this connection, however, that 1,218 seedlings grown in the greenhouse from seeds planted on August 5 and taken from the same bag as those in Lot VII were entirely free from the disease as late as August 29. It is improbable that the conditions in the greenhouse were unfavorable to the development of the disease, since successful artificial inoculations have been made here besides the two cases of natural cotyledonary infection already mentioned; and, if we accept this as the case, adding these seedlings to those observed in the field, making a total of 7 diseased seedlings in 2,708, the 0.46 per cent is reduced to 0.25 per cent.

Whatever the true situation in this regard may be, and only further carefully checked observations can decide, it is a fact that the disease appears sooner in some parts and later in others, yet almost inevitably in every cotton field. In any method of primary infection it is improbable that every plant will be attacked, so that the spread of this disease from leaf to leaf and plant to plant becomes a subject of considerable interest and importance.

INSECT DISSEMINATION

In view of all the work—much of it recent—done on the subject of the spread of plant diseases by insects, an effort was made to determine carefully the extent of their activities in the dissemination of the angular leafspot. Leaf-eating beetles (flea beetles and cucumber beetles) were abundant in some fields of seedling cotton, but in only one case has the author observed the disease developing about the margin of eaten areas. The most common insects upon the older plants were the jassids; and, since they were more active than any other except the ants (the latter, however, being active only after the dew had disappeared), especial attention was given them. Five large plants were
caged in the greenhouse, with cheesecloth stretched over wooden frames. Into one of these cages were placed 50 jassids caught in the early morning while the dew was on the plants; into another, 75 jassids caught about 9 a.m., after the dew had evaporated. Two cages were used as un inoculated controls, while the plant in the fifth cage was sprayed with a water suspension of a 6-day-old 1 per cent saccharose agar slant culture of *Bacterium malvacearum*. After the insects had been upon the plants for 18 days in one instance and 14 days in the other and after the inoculated control had developed for 11 days the cages were opened and observations made. The results are given in Table II.

**Table II.**—Results of a greenhouse experiment to determine the agency of insects in the dissemination of angular leaf spot

<table>
<thead>
<tr>
<th>Test</th>
<th>Number of leaves not infected</th>
<th>Number of leaves infected.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jassids caught in dew (18 days)</td>
<td>58</td>
<td>1 (1 spot?)</td>
</tr>
<tr>
<td>Control</td>
<td>58</td>
<td>0</td>
</tr>
<tr>
<td>Jassids caught dry (14 days)</td>
<td>132</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>124</td>
<td>0</td>
</tr>
<tr>
<td>Inoculated control</td>
<td>99</td>
<td>22 (871 spots)</td>
</tr>
</tbody>
</table>

While the data are not conclusive as to the activities of these insects the conclusion that they have a very slight effect, if any at all, is supported by the later developments in the same patch used for seed-infection studies and described above.

Shortly after September 1 this field was chopped to a close stand and the plants allowed to develop. There was considerable angular leaf-spot present upon the plants in the adjoining fields, and an excellent opportunity was presented for the insects to demonstrate their influence upon the spread of the disease, since the growing plants were at a favorably susceptible age. A careful examination was made of this field on October 2, particularly of those plots lying next to the older cotton. Table III shows the amount of disease present, the data being presented in rows per plot. Those of the first five plots were parallel to the rows of the adjacent cotton field, the seventh row being slightly over 21 feet away, while the remaining plots were bordering the cotton on another side of the triangle, with the rows running toward the other field, so that no idea of distance can be had. The numbers of the plots are identical with those in Table II, which facilitates comparison.
TABLE III.—Results of a field experiment to determine the agency of insects in the dissemination of angular leaf spot

<table>
<thead>
<tr>
<th>Row</th>
<th>Plot 1</th>
<th>Plot 6</th>
<th>Plot 14</th>
<th>Plot 8</th>
<th>Plot 2</th>
<th>Plot 10</th>
<th>Plot 9</th>
<th>Plot 4</th>
<th>Plot 5</th>
<th>Plot 12</th>
<th>Plot 18</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plants</td>
<td>Plants</td>
<td>Plants</td>
<td>Plants</td>
<td>Plants</td>
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<tr>
<td>1</td>
<td>41</td>
<td>1</td>
<td>49</td>
<td>0</td>
<td>35</td>
<td>0</td>
<td>46</td>
<td>1</td>
<td>80</td>
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Considering the irregular distribution of the diseased plants in plots 1, 6, 14, 8, and 2, little evidence of insect activity is noted; and this fact, together with the higher percentage of diseased plants in plot 9, which was credited with high seedling infection (Table I), might lead one to believe this disease present to be more likely due to seed infection or spread from such plants.

**Dissemination by wind during rainfall**

An extensive inoculation experiment was arranged in one of the college cotton fields to demonstrate beyond all doubt the pathogenicity of the organism which preliminary inoculations indicated was *Bacterium malvacearum*. This field was located on a plateau overlooking a valley 2 miles wide sloping from east to west, with no higher hills near by. The row chosen for the experiment was parallel to a roadway and about 20 rows from it (80 feet). The inoculations were made on May 26, one person applying the bacteria from agar-slant cultures to the underside of two or three leaves of each plant by means of a sterile glass rod. One tube culture and one rod were used as far as the culture served, when another culture and rod were used. The bacteria were gently spread over the underside of the leaf with the fingers by a second person, using rubber gloves to facilitate sterilization after each culture. The success of the experiment was noted on June 3 (eight days later), though a complete record was not taken until June 5, when an examination showed that none of the control plants were diseased and that all those inoculated were diseased except two. These also showed infection shortly afterwards.

A record was taken on June 5 of the number of leaves infected and uninfected. Comparison with a similar record taken on June 15 showed an increase from 2 to 10 leaves per plant, usually 3 to 6, and a similar
increase of diseased leaves. At the time two control plants standing next to inoculated plants were also diseased. Thus, there is without doubt a rapid increase of the number of the spots on infected plants and those adjacent, probably due, as Rolfs (1915, a) points out, to the spread of the bacteria by contact with uninfected leaves and plants in the presence of sufficient moisture.

During the observations made on June 26, to note the spread of the disease in this field, it was found that the row on each side of that inoculated contained infected plants, though the disease appeared to be spread farther toward the east than the west. That part of the field was carefully diagramed, an accurate record made of the disease on each plant, and the data then charted for closer study. The chart is reproduced here to show the true situation in the field at that time (fig. 1, B.)

All the plants are represented by cross marks, those originally inoculated with *Bacterium malvacearum* are divided into series, each series being given the number of our culture used. The numbers near the plants of the neighboring rows and of the checks designate the number of angular leafspots found on them on June 27 and 28. The inoculated plants were so badly diseased as to make such data useless.

Two facts stand out upon examination of the chart: (1) There had been very little spread of the disease in a westerly direction as contrasted with that toward the east, and (2) the spread toward the east was strikingly opposite the inoculated plants as compared to the control plants. Upon closer study of these points it will be noticed that one had to proceed 14 rows to the east to find a row comparable in amount of disease with the second one west of the inoculated row. Further, a decrease in disease is noticed west of wide skips in the inoculated row almost as pronounced as that west of the control plants.

The significance of these data was much of a surprise to the author; therefore, he immediately set about to duplicate the original conditions, hoping at least to obtain some partial repetition of these results. A row in the same field, 35 rows west of the first inoculated row, was chosen for the second experiment. For this inoculation a bacterial suspension was made by grinding a large number of badly spotted leaves in a food chopper and diluting the macerated tissue with water. The rapid spread of the disease after the first inoculation led the author to believe that the bacteria in culture had been more or less attenuated, since it was eight days after inoculation before the disease appeared on the leaves. Unless a great amount of this spread had taken place at one time, it would have been difficult to understand how the disease could progress so far in one direction if each spot took 8 to 10 days to appear.

In the row used, a number of plants serving as controls were not inoculated, a number were inoculated, and others again left as controls. A shallow dish of the fresh suspension of bacteria was held in one hand while each of the leaves of the plants to be inoculated was immersed;
in this way inoculations were made with bacteria which had never been in culture. A record made of the amount of disease in this row at the time of inoculation is shown in figure 1, c. There were some infected plants, but not enough to interfere with the work as long as their location was known. Frequent examinations of the plants were made; and any idea of attenuation of the virility of the organism by artificial culturing proved unfounded, since few of the spots appeared in less than eight days and most of them later. A record taken on July 15 (fig. 1, c) shows how slight the inoculation was, probably because of the high dilution of the bacterial suspension. No count was made, and no idea is now had of the number of bacteria per cubic centimeter of that suspension.

It was fortunate, from the author’s viewpoint, that this experiment was arranged before the prolonged rainy period of July, in which rain fell on July 7 and almost every day afterwards until the 25th. During this time the effect of the two tropical hurricanes, which did considerable damage in the Gulf States, was felt, the wind blowing during all the time from the southeast.

Observations made toward the end of July, allowing a time factor as long as we had in our first experiment, showed that the disease had spread to the northwest of the inoculated row with very little disease on the row to the east (fig. 1, c). Upon further examination of the plants in the first experiment the disease was found to have spread over the plants in the rows west of that inoculated (fig. 1, A), the direction of spread having been reversed. Many of these plants were recorded as being free from disease on June 28, while on July 30 it was so abundant as to make careful counting needless. Consequently “1,000” is used to denote relatively a great amount of disease, more than 1,000 spots. The only obvious factor which is capable of such far-reaching action is the wind, and this only when sufficient moisture is present over the leaves to enable the bacteria to become detached from the colony within the diseased area, either through the stomata or from the surface of the spots. Even heavy dews afford sufficient moisture for this purpose, as has been demonstrated by experiment.

Absorbent cotton, sterilized in a plugged Erlenmeyer flask, was carried to the field on two occasions in the early morning while the dew was present. Small bits of this were pulled off with forceps, and each bit placed upon the top side of the diseased leaves, not especially over lesions, but promiscuously upon the leaf. After these were placed, each was taken up and placed into a tube containing sterile water (a sterile water “blank”). These were taken to the laboratory, shaken thoroughly, and samples of the water plated in agar. In the first preliminary experiment 5 out of 30 plates showed Bacillus malvacearum. In the second case, 12 out of 84 plates showed the organism. It would seem that the close percentages (16 and 14) of these two experiments is merely coincidental;
yet in another experiment in which cold, poured agar plates were taken to the field and with covers removed, dew dashed onto the agar surface, about the same percentage gave growths of *Bact. malvacearum*.

It is reasonable to expect that the bacteria would become as free in a film of water due to rains as to dew. When bacteria have thus escaped from the interior of diseased areas through stomata (possibly in other ways) and have been suspended in this water film, any agency carrying this water from plant to plant becomes a means of dissemination. Wind during rainfall is the most probable agency which has been active in this disease of cotton. Infection by contact of plants is precluded by the distance between rows (4 feet) and the height of the plants at the time of the first experiment—8 to 10 inches at its beginning and not over 18 inches high at its close.

**METEOROLOGICAL CONDITIONS**

Little can be said concerning the wind during the rainfall which spread the disease in these experiments. Future studies are planned in which these factors will be more closely observed, and it is hoped to learn what variations and what minimum amounts of wind and rain, separately and together, will have this effect. It is a fact of record that the wind blows during the rainfall of thunderstorms of this region, and it is common knowledge that the wind blows violently during the rainfall of West Indian hurricanes. As types of the thunderstorms most important in this regard, three graphs are presented (fig. 2), records of storms observed at the office of the United States Weather Bureau, Little Rock, Ark., and Vicksburg, Miss. The high wind preceding the rain, or at its beginning, should be noted. It is improbable that this wind at the beginning is instrumental in spreading bacterial diseases, but it will be noted that later the wind reaches a velocity of 25, 29, and 35 miles per hour during periods in the storms when rain is falling heavily and after the foliage has been wet for some time. It is extremely probable that winds of these velocities blowing during heavy rainfall will serve to disseminate such diseases as the angular leafspot of cotton.

The records of storms in the extreme western portion of the cotton belt are used here so that it will be noted that these meteorological conditions are not peculiar to this locality but that such conditions are prevalent throughout the cotton-growing States and probably elsewhere as well.¹

**DISCUSSION OF AGENCY**

Suitable conditions of wind and sufficient moisture occur during the summer rains of this region. The method of action of this combination has been and will be the subject of close attention. During June, 1916,

¹ Thanks are due to Messrs. H. S. Cole and William E. Barron, officers in charge of the Weather Bureau offices at Little Rock and Vicksburg, respectively, for their kind assistance and suggestions in this phase of the subject.
there occurred one or two typical thunderstorms. During July a rainy period continued through the greater part of the month, though from figure 1 it can be seen that the inoculated plants in the second experiment were only slightly diseased on July 15, most of the spread probably occurring after that time. During this time, however, the effects of two West Indian hurricanes were felt, and the wind blew during most of the rain.

Just how far a falling raindrop could splash water from the surface film of a leaf is hard to conjecture, but this must be a factor in the problem. It is probably this that previous authors had in mind in discussing disease dissemination; yet the distance which the wind blowing at the time of the splash will carry this ascending water is a problem for determination. The possibilities of this chain of action during a driving rain are considerable if one includes the distance bacteria may be carried from the original lesion, then splashed up again and carried farther, and so on, until a dilution too great for infection is obtained.

Rolfs concluded that rains followed by sunshine and wind were not favorable to the spread of the bac-
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terial disease of peaches, etc., since the desiccation resulting killed the bacteria which had been spread. The physiology involved in our subject has not as yet been determined—whether negative heliotropism, positive chemotropism, or mere chance determines the time within which the bacteria enter the stomata—but it is true that inﬂection resulted almost uniformly when the inoculation was made in direct sunshine on the upper or lower surface of the leaves with a temporary unprotected layer of water over the leaf surface. It may have been that a small percentage of these organisms withstood the unfavorable environment until night and that penetration of the leaf occurred then. The result of the author’s experiments on the effect of sunlight upon Bacterium malvacearum agree with those of Rolfs (1915, a) and Edgerton (1912), in that the present writer found that 15 minutes’ exposure kills some bacteria, 30 minutes a considerable number, and in an hour almost total destruction of the bacteria in the exposed parts of poured agar plates occurs. Some growth always appeared at the edge of the exposed area, and this was thought to be due to spreading from the margin of the shaded portion. More evidence on this point is needed. The conditions following rains seem to exert little inﬂuence on the spread of the angular leaﬂspot of cotton.

Stewart and Leonard (1915, 1916) show conclusively that sucking insects are capable of inoculating young pear trees with Bacillus amylovorus when the bacteria are present upon the twigs at the immediate point of puncture or upon the mouth parts of the insects. Flies, etc., also have been determined to be a probable means of spread of the bacteria. To what extent these agents work together, in what other ways the bacteria may be spread from place to place to be inoculated by sucking insects, or to what extent inoculation may follow after insect punctures (since insect activity is lessened by rains, and the spread of the disease, according to these authors, is most noticeable after rainy periods) have not been determined. These authors state (1915, p. 121) that—

On the other hand, it is to be noted that sucking bugs may be present in great numbers without the occurrence of much blight,

then describe two situations differing only in the amount of rainfall, and conclude that the resistant condition of the trees in the case where the drought prevailed lessened the blight as compared to a severe outbreak in the case of an abundant rainfall.

The work on Citrus canker is of particular interest and importance in this connection because the southeastern Citrus region is subjected to the tropical hurricanes, storms in which the wind blows highest and longest during rainfall and which above all others would serve for this purpose. Hasse determined the pathogenicity of her organism without wounds or injuries of the host plant, and Wolf concludes that rain and dew must serve as agents of local dissemination. If the action of a hurricane be combined with the motility of such a bacterium, in view of
the writer's results, this disease would undoubtedly be spread rapidly, as
is noted by Stevens (1914).

The application of these conclusions to other diseases of such plants
as walnut, cabbage, curcubits, oats, clover, sugar cane, and sweet corn,
caused by motile bacteria which infect the aerial portions of the host,
depends solely upon the prevalence of these meteorological conditions—
that is, sufficient wind during rain to blow the water from plant to
plant. It need not be from host to host, because other plants may serve
as "stepping stones," as it were, provided the bacteria reached their
host before the suspension became too dilute for infection. Their appli-
cation is not at all limited to bacterial diseases, since there are many
fungi, parasitic upon plants, having spores in sori, such as the Glomerellas
and rusts, others having spores in pycnidia and in asci, where water is a
necessary factor in their dissemination, and others having aerial spores,
such as the Monilias and downy-mildews, where water is not necessary,
yet in all probability would greatly augment infection by spreading the
spores in the most favorable environment.

It is this factor which places all of these diseases in one theoretical
class as regards control. Fungicides have not been generally accepted
as a logical means of preventing bacterial diseases, though their effi-
ciency should be the same as in the cases of potato-mildew, apple-scab,
etc. A protective compound applied to the susceptible parts, active in
the presence of water and of sufficient toxicity to kill the several bacteria
quickly, would be expected to have the same result as in cases where
sprays are recommended. In fact, Rolfs (1915, a) reports successful results
with Bordeaux mixture in the prevention of this disease. Stewart (1913, a)
mentions certain unpublished observations by Reddick in which a spray
on the blossoms of pear lessened fireblight. Wolf and Massey (1914,
p. 100), referring to Citrus canker, state:

Very encouraging indications of successful control have been obtained by the use
of each of these fungicides [Bordeaux mixture, ammoniacal copper carbonate, and
soluble sulphur].

Sprays have been tried by some one or another in the case of almost
every bacterial disease, and in the majority of instances negative results
have been reported. This has developed the more or less current opinion
that the theory of such methods is incorrect; whereas from these data
one might conclude that the theory is correct, improper fungicides or
ill-timed applications having been the cause of failures.

CONCLUSIONS

In conclusion and by way of summary the following points may be
reiterated as of greatest importance:

(1) The methods of dissemination of many plant diseases, especially
those caused by bacteria, are not satisfactorily described in the literature.
(2) In the case of the angular leafspot of cotton there is evidence of but little seed dissemination, though this is a probable factor in primary infection.

(3) Insects play a very unimportant part in the spread of this disease.

(4) Data have been obtained which point to the conclusion that wind-blown rain is an important factor.

(5) The records of the United States Weather Bureau show that such an agency is possible.

(6) Nothing in the literature precludes the assumption that such an agency may be effective in the dissemination of other similar diseases, while certain facts are on record which render it probable that this factor is of importance.

(7) The control measures used in the case of diseases disseminated by the wind which require water for infection may be expected to serve satisfactorily for this type of disease.

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Dissemination of Angular Leafspot of Cotton

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