CORRELATION BETWEEN GREENHOUSE AND FIELD REACTION OF SOME VARIETIES AND STRAINS OF COTTON TO XANTHOMONAS MALVACEARUM (E.F.S.) Dowson

CORRELATION BETWEEN GREENHOUSE AND FIELD REACTION OF SOME VARIETIES AND STRAINS OF COTTON TO XANTHOMONAS MALVACEARUM (E.F.S.) Dowson

By

R. A. KILPATRICK

Bachelor of Science

Oklahoma Agricultural and Mechanical College

Stillwater, Oklahoma

1948

Submitted to the Department of Botany and Plant Pathology

Oklahoma Agricultural and Mechanical College

In Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

1949

APPROVED BY:

Lloyd A. Brinkerhoff
Chairman, Thesis Committee

L. D. Murphy
Member of the Thesis Committee

Walter W. Hauser
Acting Head of the Department

W. C. McTurlo
Dean of the Graduate School

ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Dr. K. Starr Chester, former Head of the Botany and Plant Pathology Department, Oklahoma A. and M. College, for suggesting the problem, and permitting the use of departmental equipment; to Mr. Lloyd A. Brinkerhoff, Associate Pathologist, U. S. Dept. Agri. and Oklahoma A. and M. College, Stillwater, Oklahoma, for his many helpful suggestions.

The author is indebted to Dr. W. W. Ray, Head of the Botany Department, University of Nebraska, Lincoln, Nebraska, for advice by correspondence; to Dr. L. M. Blank, Pathologist, U. S. Dept. Agri., College Station, Texas, for cultures of the bacterial blight organism; to Mr. Henry Dunlavy, Agronomist, U. S. Dept. Agri. and Oklahoma A. and M. College, Stillwater, Oklahoma; to Mr. I. M. Parrot, Agronomist, U. S. Dept. Agri. and Oklahoma A. and M. College Sub-station, Chickasha, Oklahoma; to Mr. Dow M. Porter, Agronomist, Greenville, Texas; and to Mr. D. M. Simpson, Agronomist, U. S. Dept. Agri., Knoxville, Tennessee, for supplying the seed used in this study.

R. A. Kilpatrick

TABLE OF CONTENTS

INTRODUCTION

The following study with bacterial blight of cotton, caused by Xanthomonas malvacearum (E.F.S.) Dowson, was made for the purpose of determining whether cotyledonary infection in the greenhouse would serve as an index of varietal reaction of mature plants in the field. If so, the greenhouse method would have a number of obvious advantages over field testing. Among these would be the saving of space and time, and an opportunity to test breeding material during the winter.

For this investigation one-hundred different varieties and strains of cotton were grown in the greenhouse and rated as seedlings and the same varieties and strains were also grown in the field where readings were made on the leaves, bolls, and bracts.

LITERATURE REVIEW

As early as 1907, Orton (5) reported that Sea Island cotton, Gossypium barbadense L., was severely damaged by bacterial blight in the southeastern United States. Both Atkinson (1, 2) and Smith (10, 11) had worked with the angular leaf spot disease prior to 1907, but neither of these workers mention differences in varietal susceptibility.

In 1915, Rolfs (8) observed and reported varietal susceptibility to the disease in a cotton breeding nursery in South Carolina. He reported Egyptian cotton, G. barbadense L., to be the most severely affected, whereas Willet Red Leaf, an upland variety, G. hirsutum L. showed considerable resistance.

The development of varieties resistant to bacterial blight of cotton, was first begun by two British workers, Knight and Clouston (4), in 1934. Crosses were made between a resistant American upland variety, Nye's Uganda B-31, G. hirsutum L., and two Sakel strains, G. barbadense L.

Nye's Uganda B-31 was developed in the Uganda and, although it originated from an American upland cotton, it apparently was never used by American workers. As far as the literature indicated, the primary use made of this variety was for the transmission of its resistant factor to G. barbadense L.

One of the outstanding features of the work of Knight and Clouston (4) was the development of an inoculation technique by which they obtained uniform infection in susceptible field grown cotton seedlings. They prepared inoculum from 10 pounds of air-dried, bacterial-blight-infested cotton leaves, which were crushed and stirred into 40 gallons of water and allowed to soak for 1 hour. The leaf particles were then separated

and the water containing the causal bacterium was sprayed on 5-weeks old plants. Thirteen grades were used to designate the susceptibility of the leaves. In crosses made between Uganda B-31 and Sakel strains, resistance was found to be governed by two factors which were called B_1 and B_2.

Weindling (15) developed a technique for testing cotton seedlings by which he soaked seeds for different intervals, and grew the seedlings in glass tumblers. At the same time he also modified Knight and Clouston's field inoculation method. The inoculum was grown in pure culture rather than obtaining it from naturally infested leaves, and the suspension was applied in a coarse spray probably with more pressure than Knight and Clouston used. Weindling (16) grew the causal bacterium, Xanthomonas malvacearum (E.F.S.) Dowson, on potato-dextrose agar for 5 to 7 days and used the bacterium at a concentration of 1 plate per 100 cc. of water. By adding this suspension at the rate of 10 cc. to 1,000 cc. of water, a spray suspension was prepared for field inoculation.

Weindling (16) found spraying with a Knap-sack sprayer to give satisfactory results when applied during the hours of mid-morning until noon - the period corresponding to maximum stomatal opening. He also found infection occurred best when the spray was applied at a close range and directed toward the lower side of the leaf. One spray tank containing $2\frac{1}{2}$ gallons of suspension was sufficient for a 300 foot row of plants 5 to 7 weeks old.

Ray (6) also developed a greenhouse seedling inoculation technique. A suspension of 100 cc. per plate of bacteria was used by Ray instead of 600 cc. per plate as used by Weindling. Seedlings were grown in the greenhouse in sand rather than in glass tumblers and under such conditions Ray found seedlings could be graded after 1 week. Weindling grew his seedlings for 3 weeks before grading.

The methods of inoculation used by Weindling proved valuable in the development of a bacterial-blight-resistant strain of cotton by workers in this country. This strain, which has been designated as Stoneville 20, originated from a naturally infected field plot of Stoneville 2-A at Knoxville, Tennessee in 1939. (Simpson and Weindling 9). Later it was thoroughly tested by artificial infection. By subjecting crosses of Stoneville 20 and susceptible varieties to bacterial blight infection, resistance was found to be inherited as a single recessive factor. The strain has recently been distributed to cotton breeders for use in bacterial-blight-resistant breeding programs.

Weindling (15, 17) and Ray (6) have observed some correlation to exist between seedling reaction in the greenhouse and field reaction, but no extensive study has been reported.

MATERIALS AND METHODS

Description of Material

One-hundred strains of upland cotton, Gossypium hirsutum L., representing 21 different varieties, were used. Seed of all varieties were obtained from cotton breeders in Oklahoma, Texas, and Tennessee and a majority of them originated in the states from which seed was procured. Among the chief varieties were: Stoneville, Acala, Mebane, Mucala, Acala Okra Leaf, Deltapine, Half and Half, Coker's 100, Northern Star, and Oklahoma Triumph.

Method of Delinting

Seeds were acid delinted for both greenhouse and field plantings. The procedure was carried out as follows: One pound of fuzzy seed of each variety was acid delinted by adding approximately 30 cc. of concentrated sulfuric acid. To decrease the period of delinting, 10 cc. of water was added to the seed. Seeds were removed when a black gummy mass was obtained and after all the lint was removed. Washing the acid from the seed was accomplished by running water over the seed and quickly pouring off to prevent the overheating of the seed. This process of washing was repeated approximately 10 times or more until the water was no longer colored after having covered the surface of all of the seeds. The fraction of seed that floated in water, "floaters", was removed at the time of washing. The heavy seed, "sinkers", were dried on paper toweling and constituted the portion used in the plantings. The shriveled and immature seeds are always removed with the "floaters". Some fully viable seeds may also be removed with this fraction.

Planting Procedure

Field planting.- The field planting was on a sandy loam soil located 1½ miles west of Stillwater, Oklahoma, on one of the Oklahoma Agriculture and Mechanical College Experimental Farms.

Seeds were hand planted at the rate of 5 per hill and spaced 1 foot apart. Two replications of all varieties were planted in a series of 4 blocks, each consisting of 25 rows. A resistant and susceptible check was planted on both sides of each block.

The first replication was located on a slight slope and had good drainage. Replication 2 was located in a low area and as a result water stood 2 inches deep upon 2 different occasions for as long as 3 days during the growing season.

Greenhouse planting.- For greenhouse plantings the writer used a bench that was 24 feet long and 3 feet wide. The bench was filled with sand and then sterilized with steam for 14 hours. A sloping frame was built over the bench and covered with canvas. The cover extended to the top of the bench on 3 sides and could be raised or lowered as desired. The frame was sloped sufficiently to prevent water from standing on the canvas. A fabric hose, sold under the trade name of "Soil Soaker", was placed on top of the canvas and a small stream of water was kept constantly running for the duration of each test.

Two plantings were made in the greenhouse, the first on August 31, 1948, and the second, September 11, 1948. Both plantings were made as follows: Seeds were planted in moist sand in rows 22 inches long, 2 inches apart, and 1 inch in depth. To insure uniformity in depth in planting a yard stick was pressed firmly in the sand to a depth of 1 inch. Seeds

were placed at the bottom of the rows and covered with sand. After planting, the sand was thoroughly soaked with water and covered with a wet newspaper to maintain high humidity. The newspaper was removed the third day for the first replication and the fifth day for the second replication. For the first replication the canvas sides were raised on the fourth day and for the second replication on the sixth day. Lower temperatures which prolonged the germination of the seed in the second replication accounted for the above differences. Before making the second planting the sand was sifted through a ¼ inch wire mesh to a depth of 1½ inches; this removed the old seed hulls and ungerminated seed.

Air temperatures were recorded with a thermograph; soil temperatures were taken intermittantly with a thermometer.

The seedlings were watered twice a day to maintain high humidity and at the same time the canvas was given a thorough soaking.

Preparation of Agar, Methods of Isolating and Culturing the Organism

A potato-dextrose agar was found suitable for Petri dish cultures when made up as follows: 200 grams of potatoes, 30 grams of agar, 10 grams of dextrose, and 1,000 cc. of tap water.

The technique of isolating the organism was not mastered until late in the summer of 1948. The organism was then isolated from fresh infected leaf tissue by a modification of a standard dilution plate method (Riker and Riker 7). The modification consisted of macerating infected leaf tissue in sterile tap water in a Wearing Blender for 10 minutes.

Xanthomonas malvacearum (E.F.S.) Dowson, the bacterial blight organism, is very similar to a non-pathogenic bacterium which has been described

by earlier workers (Edgerton 3, Smith 12). These two bacteria are commonly associated when cultured on agar from bacterial blight infected tissue. The non-pathogenic form usually is a darker yellow and develops more rapidly, at least for the first 4 or 5 days.

For both greenhouse and field innoculations, transfer of culture from tube to Petri dish was accomplished by the use of a small flattened needle, 1/8 inch in width. Three to four heavy smears from a pure culture of X. malvacearum were thoroughly streaked over the agar. Four to six day old cultures were used in making the bacterial suspensions.

Preparation of the Inoculum and Methods of Inoculations

Sterile water was poured over the cultures and the bacteria were loosened from the agar by the use of a glass spatula. The resulting suspension was then placed in a Wearing Blender for 5 seconds. The mixer thoroughly broke up the bacterial masses to give a uniform suspension. The concentrated suspension from each plate was then diluted with 100 cc. of distilled water.

Greenhouse inoculations.-The method employed was essentially the seed soaking method devised by Ray (6). Twenty-five cc. of the above diluted bacterial suspension was poured over 65 seeds. Each lot of seed was agitated every 20 minutes for a period of 3 hours and then placed on paper toweling to dry.

Field inoculations.-Because of an abundance of natural infection that developed in the early part of the season, field inoculations were delayed until early September when the following procedures were attempted: (1) rubbing cheese cloth covered with carborundom powder soaked in a bacterial

suspension, 1 plate per 1,000 cc. of tap water, over young bolls and bracts from different sides; (2), rubbing cheese cloth soaked in a bacterial suspension, the same as above but without the carborundum; and (3), spraying the entire plant from the top downward with a knap-sack sprayer that was maintained at its maximum pressure. The spray dilution was 1 plate per 2½ gallons of water. Spraying was accomplished during and after a light rain and during cloudy weather.

Description of Index Numbers

Five different grades were used to designate differences in disease reaction. These were: 0, 1, 2, 3, and 4. Photographs of the latter four grades are shown for the cotyledons, mature leaves, bolls, and bracts (Fig. 1, 2, 3, and 4). A disease index was determined by taking the number of individuals in each grade and multiplying by the class number; this number was then divided by 5 times the number of individuals, and multiplied by 100. This always resulted in an index number that fell within the range of 0 to 100 inclusive. The greater the susceptibility the higher the index number.

The grades for the different plant organs were based on the size and the number of lesions. In the greenhouse the disease rating was based on cotyledonary infection. All of the seedlings were graded.

In the field each plant was observed and the leaf showing the most severe infection was graded. The same method applied to the bolls and bracts except that only 10 plants for the bolls and 15 plants for the bracts were selected from each row. These were plants in which the bolls and the bracts showed the most severe infection.

Fig. 1. Cotyledons of cotton illustrating the four different grades of bacterial blight infection used in grading seedlings in the greenhouse. Grades read from the top, left to right.

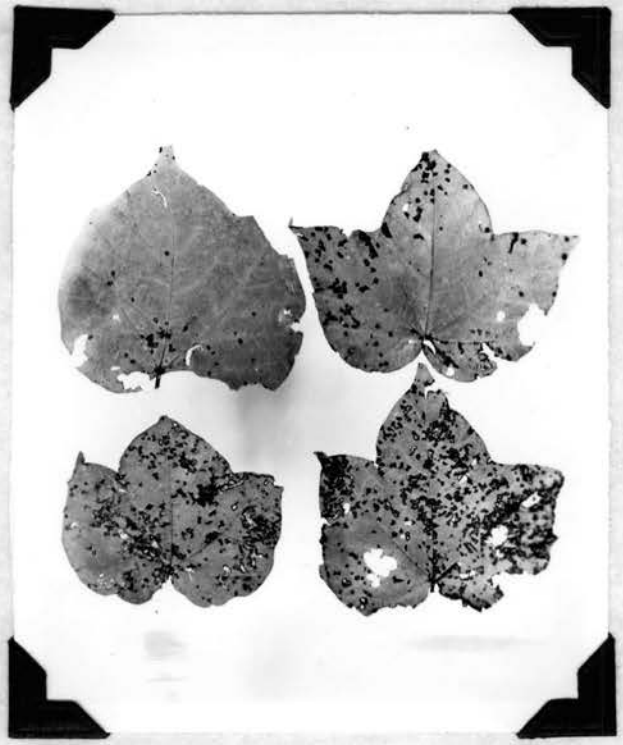

Fig. 2. Leaves of cotton illustrating the four different grades of bacterial blight infection used in grading seedlings in the field. Grades read from the top, left to right.

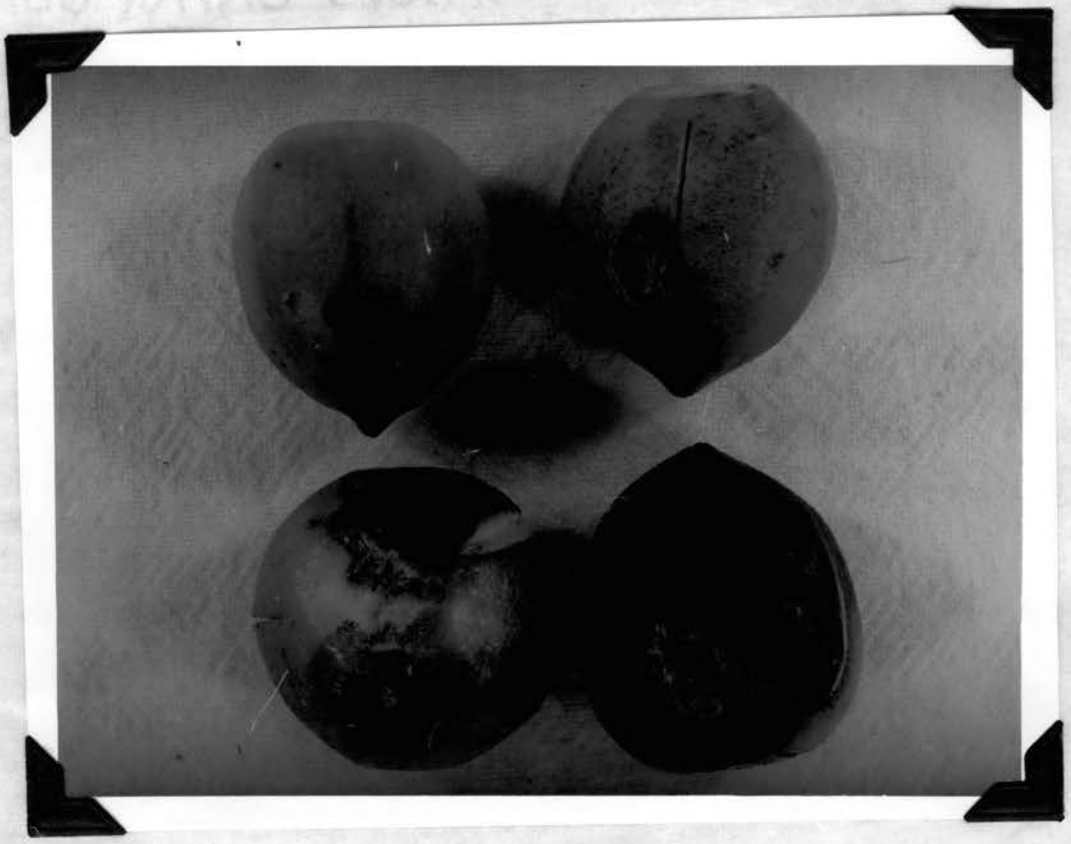

Fig. 3. Bolls of cotton illustrating the four different grades of bacterial blight infection used in grading seedlings in the field. Grades read from the top, left to right.

Fig. 4. Bracts of cotton illustrating the four different grades of bacterial blight infection used in grading bracts in the field. Grades read from the top, left to right.

OBSERVATIONS AND RESULTS

In the Field

Readings of bacterial blight infection were taken on the leaves when the plants were 7 weeks old and at that time natural infection was uniform throughout the first replication. Growth of plants in the second replication was retarded by excessive soil moisture. Thus, readings were delayed until the latter part of July when the infestation of bacterial blight became severe throughout this replication.

Stoneville 20, the highly resistant variety, showed no lesions at the time leaf infection was graded; however, small lesions were noted in some instances toward the end of the season.

During early August small halo leaf spots were noticed which later enlarged. These brown, angular leaf-spots were surrounded by a greenish-yellow margin which later formed a circular, brown lesion. Weindling (17) describes this condition when severe epiphytotics of bacterial blight occur.

The summer for 1948 was unusually wet during the early part of the growing season as compared with other years. During the months of June, July, and August, approximately 12 inches of rain were recorded. As might be expected, the temperatures were rather low during the rainy period, which extended through the middle of August. The remainder of the growing season was dry and temperatures somewhat above normal. (See table 1).

At the time bolls were first beginning to form, the relative humidity was still high and precipitation was frequent, boll and bract inoculations were considered unnecessary due to the heavy natural infestation of bacterial blight throughout the field. However, due to the high temperatures

and low humidity which followed the rainy period the bolls and bracts did not become uniformly infected. Artificial inoculations were then attempted.

Table 1. Field temperatures and precipitation for the 1948 growing season, West Agronomy Farm, Stillwater, Oklahoma.

Month	Temperatures in degrees Fahrenheit Mean maximum	Mean minimum	Total precipitation in inches
May	77	62	3.41
June	87	69	6.72
July	87	71	3.74
August	89	70	3.20
September	85	61	0.7

Spraying the plants late in the season gave good leaf infection although lesions remained small and soon dried out. Spraying did not produce uniform boll and bract infection.

Inoculations of bolls and bracts using carborundom powder as an abrasive caused rapid drying of the tissues and resulted in premature shedding of the young bolls. Artificial inoculations without carborundom powder resulted in lesions appearing on approximately 50 per cent of the bolls and bracts inoculated. It should be emphasized that all artificial inoculations were made during the dry period of the summer when temperatures were high and soil moisture was deficient.

Bract lesions were found to be most abundant on bolls that had basal infection although this did not apply in all cases. No lesions were found on very young bolls or bracts.

Readings on bolls and bracts were based on both natural and artificial infection but many plants showed no boll or bract infection.

In the Greenhouse

Experiments were conducted late in the season, August 31 to September

23. Minimum temperatures, especially for the second planting, were somewhat lower than some workers consider desirable for the optimum development of primary infection (Table 2). Stoughton (14) reported that temperatures ranging from 77° to 80.6° F. were necessary in order to obtain maximum primary infection. The percentage of diseased plants was 59.8 for an over-all average of the two replications. Readings were based on cotyledonary infection.

Table 2. Greenhouse temperatures in degrees Fahrenheit.

Date	Maximum	Minimum
August 31	89	70
September 1	88	68
2	99	65
3	82	70
4	83	67
5	79	63
6	78	55
7	82	70
8	77	64
9	68	57
10	72	58
11	71	58
12	85	59
13	72	58
14	68	62
15	71	62
16	73	67
17	77	67
18	84	67
19	80	70
20	83	70
21	81	69
22	83	68
23	77	62

At the time of the planting of the first replication, the air temperature was 84°F., while the soil temperature was 75°F. The percentage of germination was higher and infection was more uniform throughout the first

replication. Temperatures during the period of growth of the first replication ranged from a high of 99°F. to a low of 55°F. Both maximum and minimum temperatures were somewhat lower for the second replication. The percentage of infection for the first replication was 70 percent while in the second replication the percentage of infection was only 41 per cent.

Some damping-off occurred during both plantings although only a small percentage of seedlings were affected.

Comparison of Bacterial Blight Reaction in the Field and Greenhouse

Disease reaction of the susceptible varieties and strains in the greenhouse and field.- Index numbers representing bacterial blight reactions for the varieties and strains that were found to be susceptible are shown in table 3. These are shown for both field and greenhouse grown plants. All zero readings are omitted from the calculation in table 3 because they are believed to represent escape plants.

Table 3. Bacterial blight infection as indicated by index numbers for 99 susceptible varieties and strains of cotton, ranked according to degree of susceptibility in the greenhouse.

No.	Variety	Index numbers			
		Greenhouse	Leaves	Bolls	Bracts
1	A-5-6574-20-7-1-4-2-6-2-2	20.0	44.6	43.0	29.3
2	Stoneville 462	20.4	32.6	38.8	28.0
3	Stoneville 62-1-14	22.1	42.3	32.6	25.3
4	Stoneville 62-1-2	22.9	39.5	31.1	30.0
5	Stoneville 62-1-2-2	23.3	38.9	37.9	28.0
6	Stoneville 62-1-2-3	23.4	41.1	35.5	24.0
7	Stoneville 62-1-26	23.4	41.8	35.0	29.3
8	Stoneville 62-1-0-0-84	23.4	46.0	39.0	26.7
9	Acala 892-17898-3-4-2-5	23.7	42.1	36.0	27.3
10	Acala 892-17898-3-4-3-8	23.9	32.7	39.0	36.7

Table 3. (Continued)

No.	Variety	Index numbers Greenhouse	Leaves	Bolls	Bracts
11	A. O. L. 16-5	23.9	27.7	31.2	25.3
12	Delfos 9169	24.4	44.6	35.4	24.7
13	Stoneville 62-1-8	24.4	45.4	43.5	28.0
14	Stoneville 62-1-2-4	24.5	40.4	31.0	24.0
15	Lankart	24.6	34.1	30.0	22.7
16	Stoneville 62-1-1-6	24.8	39.5	35.0	32.0
17	Mebane 140-Sta.	24.8	38.9	27.8	25.3
18	A-5-6566-18-8-2-1-2-2-3-6	25.2	52.4	40.0	32.7
19	A-5-6566-18-8-2-1-2-2-2-6	25.4	47.4	47.0	37.3
20	A-5-6566-18-8-2-1-2-2-1-3	25.6	49.6	47.0	35.3
21	Meb. 140-6802-2-3-1-3-1-5	25.6	40.2	24.4	26.7
22	Watson New Rowden	25.7	45.8	41.3	30.7
23	Watson	25.8	46.7	36.0	24.7
24	A-5-6574-20-2-4-3-4-2-6-2	25.8	49.8	39.0	27.3
25	Stoneville 62-1-6-8	25.9	37.3	35.5	26.7
26	Oklahoma Triumph 92-1-1	26.0	45.5	43.0	30.7
27	D and P L-15	26.1	41.4	34.7	29.3
28	Half and Half	26.1	46.3	38.0	38.0
29	Meb. 140-6801-2-5-1-1-1-3	26.3	31.9	28.7	24.7
30	Meb. 140-6801-2-1	26.5	49.7	35.4	27.3
31	Meb. 140-28192	26.7	36.6	35.3	20.7
32	Mucala 7480-2-2-3-3-3-12	26.7	35.7	32.3	27.3
33	Acala 892-17898-3-4-3-5	26.8	42.2	37.6	32.0
34	A-5-6583-21-2-2-1-4-5-6-4	26.9	39.8	43.3	34.7
35	Meb. 140-6819-2-2-3-4-3-2	26.9	33.9	35.8	35.3
36	Meb. 140-6801-2-5-2-2-3-4	26.9	36.3	32.3	28.7
37	Acala 892-17898-3-2-3-5	26.9	40.0	41.0	30.0
38	A-5-6566-18-8-2-1-2-2-1-2	27.0	47.4	43.7	35.3
39	A-5-6574-20-7-1-4-2-6-2-10	27.0	45.7	44.0	43.3
40	Acala 892-17898-3-4-6-10	27.1	44.7	42.0	26.0
41	Meb. 140-6801-2-5-2-1-2-2	27.3	34.0	33.8	22.0
42	Acala 892-17898-3-4-1-3	27.4	41.2	33.7	28.0
43	Acala 892-17863-1-1-9-10	27.4	40.5	33.7	28.0
44	Acala 892-12194-8A.-2-1-9-5	27.5	42.4	37.6	26.7
45	Acala 892-17898-3-4-2-8	27.6	42.0	35.0	31.3
46	D and P L-041	27.7	42.2	42.0	26.0
47	Lockett 140	27.7	49.4	36.8	38.0
48	Meb. 140-28209	27.7	29.1	27.1	28.7
49	A-5-6583-21-2-1-3-1-5-10-4	27.8	49.5	38.0	36.0
50	Stoneville 62-1-21	28.0	38.8	37.1	26.0
51	Stoneville 62-1-0-0-108	28.0	38.8	37.1	26.0
52	Meb. 140-6819-2-4-2-5-1-2	28.0	36.5	35.0	24.7
53	Stoneville 62-1-25	28.1	36.2	37.0	31.3
54	Stoneville 62-1-2-1	28.2	39.1	31.6	30.0
55	Acala 892-17898-3-1-8-3	28.3	37.7	37.0	26.0
56	Acala 892-17997-1-4-9-3	28.4	41.5	37.6	25.5
57	Meb. 140-6801-2-5-1-1-15	28.6	40.0	24.0	23.3
58	Coker's 100	28.6	40.7	33.3	28.7

Table 3. (Contimued)

No.	Variety	Index numbers Greenhouse	Leaves	Bolls	Bracts
59	A-5-6583-21-2-2-1-4-5-6-7	28.9	53.2	48.7	30.7
60	Meb. 140-6801-2-5-1-1-11	28.9	39.5	24.0	28.7
61	Acala 892-17991-1-1-2-10	28.9	47.0	41.0	31.3
62	Acala 892-17898-3-4-5-4	28.9	41.3	37.8	31.3
63	Mucala 7480-2-2-3-3-3-3	29.0	44.4	36.0	25.3
64	Meb. 140-6801-2-5-1-3-3-6	29.0	36.2	35.2	34.7
65	Meb. 140-6801-2-5-1-1-7	29.0	32.5	22.8	23.3
66	A. O. L. 33-6-54	29.0	29.7	33.3	25.3
67	Acala 892-18024	29.2	38.4	35.5	23.3
68	Meb. 140-6801-2-5-1-3-3-3	29.3	40.4	31.8	37.3
69	A. O. L. 31-5-1	29.3	31.1	22.2	20.7
70	A-5-6566-18-8-2-1-2-2-1-1	29.4	51.8	37.0	34.7
71	Acala 892-17898-3-4-6-1	29.4	49.4	31.2	28.0
72	Acala 892-17871-1-3-6-4	29.6	44.3	32.9	26.0
73	Bob Shaw	29.7	52.7	40.0	31.3
74	A-5-6583-21-2-2-1-4-5-6-2	29.7	46.9	52.0	35.3
75	Hi Bred	29.8	50.2	48.0	34.7
76	A-5-6566-18-8-2-1-2-2-1-5	30.0	50.4	40.0	36.7
77	Meb. 140-6801-2-5-1-3-3-4	30.2	35.6	28.2	24.0
78	Meb. 140-13731-1-3-2-1	30.3	44.2	31.6	31.3
79	Mucala 7480-2-2-3-3-3-9	30.6	34.4	20.0	29.7
80	Meb. 140-6801-2-5-1-1-6	30.8	40.9	24.4	22.1
81	A-5-6574-20-7-1-4-2-6-2-5	30.9	41.8	41.0	32.0
82	Acala 892-17898-3-2-8-6	30.9	43.0	29.0	30.7
83	Stoneville 62-1-2-6	31.0	36.1	32.0	30.7
84	A-5-6583-21-2-2-1-4-5-6-3	31.2	51.3	41.0	33.3
85	A-5-6595-2-4-3-1-1-3-5-8-4	31.5	48.7	48.0	41.3
86	A-5-6595-24-3-1-1-3-5-8-6	31.5	41.7	40.0	36.0
87	Acala 892-17837-3-4-7-2	31.5	39.8	36.7	28.7
88	A-5-6574-20-2-1-6-2-2-3-2	31.7	50.3	45.5	37.3
89	Meb. 140-6801-2-5-1-1-18	31.0	39.8	23.3	23.4
90	Northern Star	32.1	35.4	31.0	28.0
91	Meb. 140-6801-2-5-1-2-7	32.3	33.2	35.4	26.0
92	Acala 892-17837-2-3-6-4	32.9	41.3	35.0	26.7
93	Acala 892-17898-3-4-4-5	33.1	45.7	35.0	26.0
94	Meb. 140-6801-2-5-2-2-2-2	33.5	43.3	32.9	33.3
95	Acala 108-2-19	33.7	45.0	38.0	34.7
96	Meb. 140-6801-2-5-1-1-1-1	35.6	38.9	25.4	27.3
97	Acala 892-17920	35.7	48.1	38.9	25.3
98	Stoneville 2-B	36.4	36.6	37.1	25.3
99	Acala 6566-18	37.3	51.5	43.0	38.7

In the field where conditions were favorable for leaf infection 99.7 per cent of all of the plants of the susceptible varieties became infected. On the other hand, in the greenhouse apparently under less favorable

conditions only 59.8 per cent of the seedlings grown from seed of the same varieties became infected. The range of index numbers for field grown plants with the escape plants omitted were: leaves 27.7 to 53.2 with an average of 41.8; bolls 20.0 to 52.0, averaging 35.8; and bracts 20.7 to 43.3, for an average of 29.4. Index numbers for cotyledons of plants grown in the greenhouse with the escape plants omitted ranged from 20.0 to 37.3, for an average of 28.1.

Table 4. Correlation coefficients (r) of the bacterial-blight reaction of 99 varieties and strains of cotton grown in the greenhouse and in the field.

Pairs of Characters	Correlation coefficient (r) Excluding zero readings	Including zero readings
Greenhouse cotyledons Field grown leaves	0.105	0.086
Greenhouse cotyledons Field grown bolls	0.073	0.064
Greenhouse cotyledons Field grown bracts	0.125	0.112

At the 5% level 0.198 is required for significance.

The correlation coefficient (r) is shown in table 4 for: (1) greenhouse cotyledons and field grown leaves, (2) greenhouse cotyledons and field grown bolls, and (3) greenhouse cotyledons and field grown bracts. Correlations are shown for indexes in which the zero readings (escape plants) are omitted and also with the zero readings included. When the zero readings are included the index numbers are somewhat lower. The data show no significant correlation to exist between the disease reactions of greenhouse grown susceptible plants and field grown susceptible plants.

The degree of bract infection was found to be more variable than for

either the leaves, bolls, or greenhouse cotyledons (Table 3 and Table 5).

Disease reaction of the resistant variety in the greenhouse and field.- In the greenhouse Stoneville 20 showed 40 out of 502 plants with bacterial blight infected cotyledons, or 8.0 per cent. See table 5. In the field 12.0 per cent of the bolls became infected, whereas only 1.2 per cent of the leaves showed infection at the time readings were made. Later in the season the percentage of infected leaves increased somewhat. Field grown plants showed 27.7 per cent bract infection.

Table 5. Bacterial blight reaction and percentage of infection for Stoneville 20 in the field and in the greenhouse.

	Number of plants showing infection:			
Grade	Leaves (field)	Bolls (field)	Bracts (field)	Greenhouse (cotyledons)
0	408	88	107	462
1	5	11	35	36
2	0	1	6	4
3	0	0	0	0
4	0	0	0	0
Percent diseased	1.2	12.0	27.7	8.0

DISCUSSION AND CONCLUSIONS

This study shows a susceptible variety producing a high reading in the greenhouse inoculated by the seed soaking method will not always produce high readings in the field, instead, varying degrees of susceptibility may be recorded. If genetic differences exist between the susceptible varieties and strains used, apparently they are not great. The lack of significant positive correlations between greenhouse and field disease reaction may have been due in part to the fact that there were many escape plants (with the exception of leaf infection in the field), or that optimum conditions for development of the disease did not occur when bolls and bracts were forming. Another factor that might have been involved was the method of determining grade scales. The grades were not thoroughly studied with special reference to such factors as the production of bacterial ooze or drying of the lesions. If these were also considered along with size and number of lesions the degree of susceptibility of the different varieties and strains might have been more accurately obtained.

Stoneville 20 reacted about the same in the greenhouse as in the field. Most of the cotyledons developed no lesions at all in the greenhouse while in the field this was also true for the leaves of older plants. The bolls and especially the bracts, on the other hand, appeared to show less resistance than the greenhouse reaction would have indicated. This study might suggest that inoculation of bracts in the field would be a highly sensitive test for resistance.

It would appear that the particular lot of Stoneville 20 seed that was used for this study was not pure for blight resistance.

The study would indicate that greenhouse tests might be employed to obtain high resistance.

SUMMARY

Ninety-nine susceptible varieties and strains and one highly resistant variety of upland cotton were studied. Acid-delinted seeds were grown in the greenhouse in sand under high humidity and in the field under conditions favorable for the development of the disease during the early part of the season. Seeds were inoculated for the greenhouse tests by soaking in bacterial suspensions for three hours. The disease developed uniformly on the leaves from natural infection; artificial inoculation on the bolls and bracts was employed with only partial success late in the growing season. Disease reactions were based on cotyledonary infection in the greenhouse while leaf, boll, and bract infection was recorded in the field.

No significant correlation was found to exist between the greenhouse reaction and the field reaction of the 99 susceptible varieties and strains.

The resistant variety, Stoneville 20, gave about the same disease reaction in the greenhouse as in the field.

BIBLIOGRAPHY

1. Atkinson, G. F. (1895). Angular spot of cotton. Ala. Agr. Exp. Bull. 41: 54-55.

2. _____ (1896). Diseases of cotton. U. S. Dept. Agr. off. Exp. Sta. Bull. 33: 286-287.

3. Edgerton, C. W. (1912). The rots of the cotton boll. La. Agr. Exp. Sta. Bull. 137: P. 10.

4. Knight, R. L. and Clouston, T. W. (1939). The genetics of black arm disease. Jour. Gen. 38: 133-159.

5. Orton, W. A. (1907). Bacterial blight in Sea Island cotton. U. S. Dept. Agr. Farm. Bull. 302: 41-42.

6. Ray, W. W. (1946). Varietal reaction of cotton to bacterial blight. Phytopath. 36: P. 409.

7. Riker, A. J. and Riker, R. S. (1936). Introduction to research on plant diseases. John S. Swift Co. Inc., Chicago, Ill.

8. Rolfs, F. M. (1915). Angular leaf spot of cotton. S. C. Agr. Exp. Sta. Bull. 184: 1-28.

9. Simpson, D. M. and Weindling, Richard (1946). Bacterial blight resistance in a strain of Stoneville cotton. Jour. Amer. Soc. Agron. 38: 630-635.

10. Smith, Erwin F. (1901). U. S. Dept. Agr. Div. Veg. Phys. and Path. Bull. 28: P. 153.

11. _____ (1905). Bacteria in relation to plant diseases. Carnegie Institute, Washington, D. C. Vol. 1: P. 95.

12. _____ (1920). Bacterial Diseases of Plants. W. B. Saunders Co., Philadelphia, Pa. Pp. 314-339.

13. Snedecor, George W. (1946). Statistical Methods. Iowa State College Press, Ames, Iowa.

14. Stoughton, R. H. (1930). The influence of environmental conditions on the development of the angular leaf spot disease of cotton. Ann. Appl. Biol. 17: 493-503.

15. Weindling, Richard (1944a). A technique for testing resistance of cotton seedlings to the angular leaf spot bacterium. Phytopath. 34: 235-239.

16. Weindling, Richard (1944b). Field experiments on bacterial blight of cotton. Clemson Annual Report. (Unpublished)

17. _____ (1948). Bacterial blight of cotton under conditions of artificial inoculation. U. S. Dept. Agr. Tech. Bull. 956.

Typist: Mary Wallace Spohn