SURVIVAL OF XANTHOMONAS MALVACEARUM (E. F. S.) DOWSON IN COTTON PLANT DEBRIS AND SOIL

SURVIVAL OF XANTHOMONAS MALVACEARUM (E. F. S.) DOWSON IN COTTON PLANT DEBRIS AND SOIL

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Bachelor of Science

Oklahoma Agricultural and Mechanical College

Stillwater, Oklahoma

1946

Submitted to the Department of Botany and Plant Pathology

Oklahoma Agricultural and Mechanical College

In partial fulfillment of requirements for

the degree of

MASTER OF SCIENCE

1950

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AGRICULTURAL & MECHANICAL COLLEGE
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PREFACE

The writer wishes to express his appreciation to Mr. Lloyd A. Brinkerhoff, Associate Plant Pathologist, U. S. Department of Agriculture and Oklahoma Agricultural and Mechanical College, for suggesting the problem, and for his advice and many helpful suggestions; to Dr. Walter W. Hansen, Head of the Department of Botany and Plant Pathology, Oklahoma Agricultural and Mechanical College, for permitting the generous use of departmental equipment and greenhouse.

The writer is indebted to Dr. John E. Thomas, Associate Plant Pathologist, Department of Botany and Plant Pathology, Oklahoma Agricultural and Mechanical College for advice on presentation of the material.

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INTRODUCTION

Bacterial blight of cotton, caused by Xanthomonas malvacearum (E.F.S.) Dowson, is one of the most important cotton diseases of Oklahoma. Atkinson (2) first described the disease in Alabama in 1891. The first extensive studies were made by Rolfs (15) and Faulwetter (6) in 1915 and 1917 respectively. The disease is destructive from the Carolinas to the irrigated valleys of the southwest, except for California; however, it is more severe in the southwestern part of the United States. Planting infested seed has long been considered (1, 3, 12, 14, 16, 19) to be a major factor in transmitting the organism from one crop of cotton to another. Primary infection occurs on the cotyledons soon after the cotton seedlings emerge. Wind-driven rains (7), (15) and irrigation water (9) have been proven to be important agents in disseminating the organism throughout the growing season. Seed treatments with either acid or chemical disinfestant dusts will greatly reduce bacterial blight in the seedling stage (14), (15), under Oklahoma conditions. However, seed treatments do not give adequate control throughout the entire season.[1] It would appear that factors other than planting infested seed may be of importance in perpetuating the disease.

Information at the present time is lacking on just how long the organism will survive in decomposing plant debris and soil under Oklahoma conditions.

The present study was an attempt to determine how long the organism would survive: (1) in infested cotton debris when placed under conditions that favor decomposition, and (2) as free living bacteria in the soil.

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[1] Final project report (Adams 416.1) Oklahoma Agricultural Experiment Station, March 15, 1949.

LITERATURE REVIEW

Early investigators, Faulwetter (6) and Rolfs (15), attributed little importance to the survival of the bacterial blight organism in either the soil or infested cotton debris left in the field. In Egypt, Massey (13) was unable to isolate the bacterium from the soil except when it contained undecomposed cotton debris. Infested powdered plant debris as found along roadways was innocuous after three months providing it was kept dry. Dry woody tissues retained the organism in a viable condition for a long period. Weindling (20), working in South Carolina, collected leaf material from the ground, from a field of cotton plants infested with bacterial blight the previous season, at intervals throughout the winter. The leaves collected were of two sample parts, 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 indications of containing a larger number of pathogenic bacteria than the moist part. However both of the leaf portions yielded viable bacteria as late as March of the following spring.

Hare and King (9), demonstrated that the organism was able to survive the winter on infested seed cotton left in the field in Arizona, and suggested that the bacterium may be transmitted to the planted crop from the volunteer seedlings by irrigation water and wind-driven rains.

Archibald (1) was unable to isolate the bacterium from the soil, irrigation water or from water standing between the cotton rows. However, King and Brinkerhoff (11) found the bacterium to be disseminated by irrigation water in Arizona.

Hewison (10) reported that Archibald obtained infection by scattering dust, collected from beneath apparently healthy cotton plants, upon the leaves

of healthy plants providing the leaves were kept moist. Archibald also claimed to have recovered the organism from the dust washed from leaves collected in the field from apparently healthy plants.[2] Hewison (10) found that secondary infection was greatest where the rainfall was greatest, and was of the opinion that secondary infection was due to the activation of the causal bacterium, which he believed to be disseminated in an inactive condition over an area long before any symptoms of the disease became observable, rather than from infested plants.[3] The infection was more severe where cotton was grown the previous season. Brown (5) reported that wind-blown dust following a severe hail was an agent in transmitting bacterial blight.

As previously stated infested seed is accepted as one of the major factors in transmitting the organism, but there is disagreement as to the importance of internal infection. Faulwetter (3), Hansford (4), and Stroughton (13) all failed to obtain evidence of internal infection. However, Archibald (1), Bain (2), and Rolfs (12) have reported small percentages of internal infection. Rolfs (11), found the organism was able to over-winter in or on the seed. Tennyson (14), said, "The seedling infection resulting from internal contamination may reach as high as 20 per cent." Brinkerhoff[4] found that seedlings grown from seeds obtained from infected bolls of American Egyptian cotton, _Gossypium barbadense_ L., averaged 60.2 per cent infection for un-

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[2] The writer was unable to find whether Archibald's studies referred to by Hewison were ever published. They could not be found in the library.

[3] So far as the writer was able to determine the work of neither Archibald nor Hewison has been substantiated.

[4] Unpublished data.

treated seed compared to 20.1 per cent for surface sterilized seed. Indirect evidence of internal infection was presented by Leding and Brinkerhoff (8) who found that soaking infested seed for 24 and 48 hours greatly increased primary infection.

Ray (14) found that dusting contaminated cotton seed with New Improved Ceresan reduced the amount of infection in one test from 17.0 per cent to 0 per cent, and from 16.8 per cent to 0.7 per cent in a second test.

GREENHOUSE STUDIES

This experiment was an attempt to determine whether different temperature conditions would effect the length of time X. malvacearum remained viable in infested cotton debris buried in moist soil.

Methods and Materials

Leaves, stems, and carpels were obtained from bacterial blight infested cotton plants from the field during the fall of 1949. Eight samples of each plant part plus a noninfested check sample were buried in individual four-inch clay pots December 17, 1949 in three locations representing different temperature conditions. The locations were as follows: (1) outside and against the north wall of the greenhouse, (2) in a cool section of the greenhouse maintained at approximately 70°F., (3) in a warm section of the greenhouse maintained at approximately 80°F. Table I. lists the weekly mean maximum and minimum air temperature outside the greenhouse. Table II. lists the same data for the warm section of the greenhouse beginning with the week ending January 22, 1950 and continuing for the duration of the experiment. The material in the three locations was kept moist throughout the experiment. The pots outside were buried about six inches deep, while in the greenhouse they were buried in sand in wooden flats. All pots were washed and filled about one third full with sand, after which the infested cotton debris was buried in a layer of unsterilized soil two thirds of an inch in depth. The remaining space was filled with sand.

Seedlings used for testing the viability of the bacterium contained in the decomposing debris were grown from disease-free seed. Seedlings were inoculated shortly after the cotyledons expanded which was usually about ten to twelve days after planting. New wood veneer plant bands were filled with

Table I. Air temperature outside the greenhouse for winter 1949-50. Weekly means recorded in degrees Fahrenheit.

Week ending	Maximum	Minimum
Dec. 23, 1949	52	30
Dec. 30, 1949	53	27
Jan. 6, 1950	45	26
Jan. 13, 1950	50	30
Jan. 20, 1950	50	21
Jan. 27, 1950	49	28
Feb. 3, 1950	39	14
Feb. 10, 1950	56	32
Feb. 17, 1950	51	29
Feb. 24, 1950	60	27
Mar. 3, 1950	59	29
Mar. 8, 1950	65	37

Table II. Air temperature in the warm greenhouse for winter 1949-50. Weekly means recorded in degrees Fahrenheit.

Week ending	Maximum	Minimum
Jan. 22, 1950	85	71
Jan. 29, 1950	89	69
Feb. 5, 1950	93	78
Feb. 12, 1950	95	79
Feb. 19, 1950	101	86
Feb. 26, 1950	94	80
Mar. 5, 1950	94	80
Mar. 8, 1950	93	75

uncropped soil for each planting. There was ample space between each plant to prevent contamination by contact. The plants were watered with a 125 ml. flask being especially careful to avoid splashing.

The mortar and pestle used to grind the material was sterilized in the autoclave at 15 pounds of pressure for thirty minutes. After the plant material was placed in the mortar, a small amount of fine unsterilized sand was used as an abrasive and a few drops of tap water were added to make a suspension. Three plants were inoculated with each sample. As a precaution against contamination by handling the plants, the investigator's hands were washed with warm water and soap, rinsed in 70 per cent alcohol and dried with individual paper towels between each inoculation.

The technique used to test the viability of the organism in the decomposing debris was similar to one previously developed at this station.[5] A small bag was made by wrapping approximately two grams of fine sand in two thicknesses of cheese cloth. Individual sterile bags were used for each sample of decomposing plant debris. The method of applying the inoculum was as follows: the leaf was held with one hand and the lower epidermis well scratched with the small sterile sand bag that was first wet in the suspension, Fig. 1. The scratches were made by moving the sand bag at right angles to the veination of the leaf. The bag was then dipped again in the suspension and daubed several times on the wound.

Readings were made from nine to sixteen days after inoculation. At first an attempt was made to grade the plants as to the degree of infection as indicated by the size of the lesions on the cotyledons. Later all attempts at

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[5] Op. cit.

Fig. 1. Method of inoculating cotton cotyledons with X. malvacearum by the use of a small sand bag dipped in a bacterial suspension.

grading the amount of infection was abandoned and the infection simply considered either as positive or negative. If there was any indication of a lesion as evidenced by water soaking, the plant was considered to be infected.

Results

Until it was almost thoroughly decomposed, the infested plant debris always produced infection when used as inoculum. At first the symptoms on the cotyledons were very easily read. However as the plant debris became more decomposed the lesions developed more slowly and became progressively smaller until eventually it was very difficult to determine whether or not the test plants were infected. The leaves decomposed most rapidly and lost their infectivity first, with the carpels apparently retaining the organism in a viable condition longest. Figs. 2 and 3 show the variation in degree of infection encountered during the experiment. Table III. lists the results of the experiments in the different locations. X. malvacearum, as indicated by the ability of infested plant debris to produce infection in disease-free seedlings, remained viable longest outside the greenhouse where the average mean air temperature was about 40° F. The bacterium appeared to lose its viability with approximately the same incubation period in the warm and cooler sections of the greenhouse where the average mean air temperature was approximately 85° F. and 70° F. respectively. However data was obtained that would raise some question as to the accuracy of the method used in the inoculation technique as a means of determining viability of the bacterium. When cotyledons of two questionable test plants that occurred in the third series were used as inoculum, the disease was produced in both instances. Later as readings became more difficult, tests were made with cotyledons that were classed as negative as well as questionable. In all instances test cotyledons that

Fig. 2. Different degrees of bacterial blight infection obtained with inoculum from decomposing infested cotton debris.

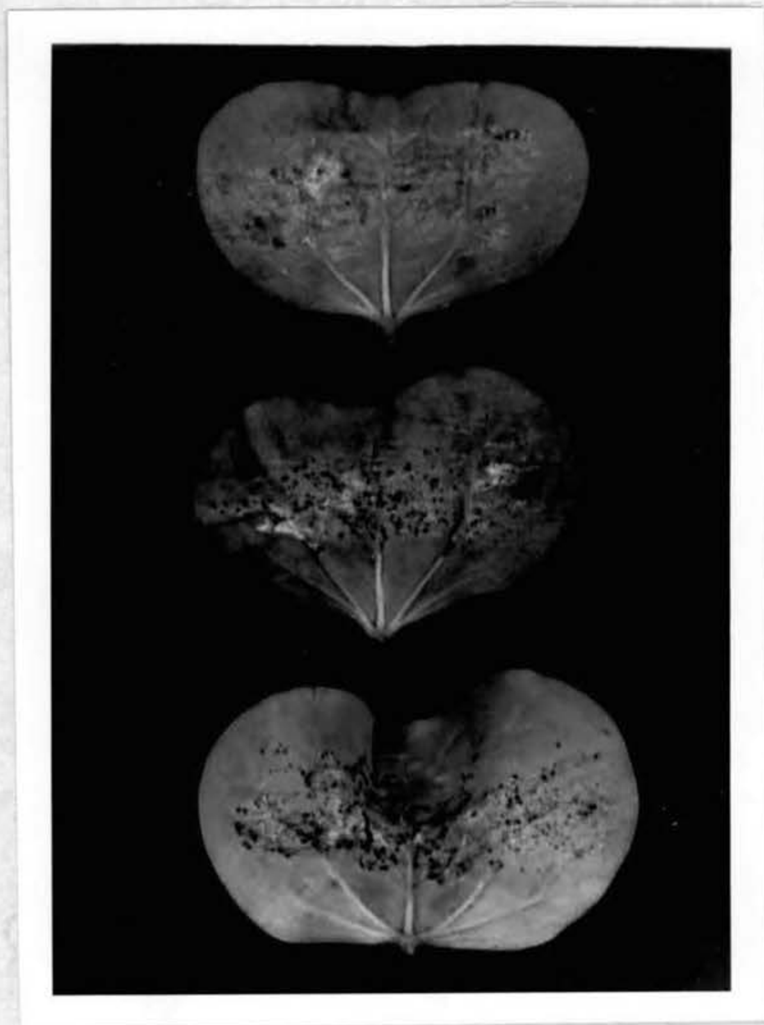

Fig. 3. Different degrees of bacterial blight infection obtained with inoculum from decomposing infested cotton debris.

Table III. Development of bacterial blight in cotton seedlings inoculated with decomposing infested plant debris.

Date of inoculation	Days of incubation	Outside				Cool greenhouse				Warm greenhouse			
		Check	Leaves	Carpels	Stems	Check	Leaves	Carpels	Stems	Check	Leaves	Carpels	Stems
12-27-49	10	- - -	+ + +	+ + +	+ + +	- - -	+ + +	+ + +	+ + +	- - -	+ + +	+ + +	+ + +
1- 4-50	18	- - -	+ + +	+ + +	+ + +	- - -	+ + +	+ + +	+ + +	- - -	+ - ?	+ + +	+ + +
1-17-50	31	- - -	+ + +	+ + +	+ + +	- - -	?[1] ? ?	- - ?[1]	? ? ?	- - -	- + +	- - -	+ + +
1-26-50	40	- - -	+ + +	+ + +	+ + +	- - +	- + -	+ + +	- - -	- - -	- - -	+ + +	- - -
2- 7-50	52	- - -	- - -	+ + +	+ + +	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
2-17-50	62	- - -	? ?[1] ?[1]	+ + +	? + ?	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
2-25-50	70	- - -	-[1] -[1] -[1]	?[1] -[1] -[1]	?[1] -[2] -[1]	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
3- 8-50	81	- - -	-[1] -[1] -[1]	?[1] ?[1] +[1]	?[1] ?[1] ?[1]	- - -	-[2] -[2] -[2]	-[2] -[2] -[2]	-[2] -[2] -[2]	- - -	- - -	- - -	- - -

+ Positive
- Negative
1 Reinoculated and found to be positive.
2 Reinoculated and found to be negative.

were considered questionable produced infection. Of ten test cotyledons that were inoculated with debris from outside and previously classed negative, nine produced infection when used as inoculum. On the other hand nine test cotyledons that were inoculated with debris from the cool section of the greenhouse and previously classed negative did not produce the disease.

FIELD STUDIES

The following study was made to determine what effect burying infested cotton carpels and leaves at different depths in the soil would have on the viability of the bacteria.

Methods and Materials

Infested cotton carpels and leaves were buried April 12, 1950 in three locations, namely, Paradise, Perkins, and Stillwater, Oklahoma. The carpels were buried at two depths, while the leaves were buried at four different depths. The plots and treatments were as follows:

1. Carpels, 6 inches deep
2. Carpels, top of soil
3. Leaves, 6 inches deep
4. Leaves, 4 inches deep
5. Leaves, 2 inches deep
6. Leaves, top of soil.

The material on top of the soil was slightly covered to prevent it from being blown away.

Each test was a two foot square plot with the plots being 15 feet apart in a row. The soil was stirred to a depth of six inches in each square before burying the material, and the loose soil removed to the desired depth, viz., 2, 4, and 6 inches. The plant debris was then evenly distributed in the respective plots and the loose soil replaced. Approximately forty disease-free cotton seed were planted in each plot May 18, 1950.

The soil at Paradise and Perkins was a sandy loam while at Stillwater it was a black clay type.

Table IV. lists the weekly mean maximum and minimum air temperature and precipitation in inches at Stillwater; table V. lists the same data recorded at the weather station at the Horticulture Farm at Perkins. No

Table IV. Air temperature and precipitation at Stillwater, Oklahoma for spring 1950 recorded as weekly means in degrees Fahrenheit. Precipitation recorded in inches.

Week ending	Maximum	Minimum	Precipitation
April 18, 1950	67	43	.06
April 25, 1950	77	51	.00
May 2, 1950	74	47	.04
May 9, 1950	85	62	.84
May 16, 1950	75	52	1.97
May 23, 1950	83	59	.56
May 30, 1950	79	59	1.53
June 6, 1950	78	55	1.21
June 13, 1950	87	67	.21
June 16, 1950	93	72	.00

Table V. Air temperature and precipitation at Horticulture weather station, Perkins, Oklahoma for spring 1950 recorded as weekly means in degrees Fahrenheit. Precipitation recorded in inches.

Week ending	Maximum	Minimum	Precipitation
April 18, 1950	63	45	.19
April 25, 1950	72	48	.13
May 2, 1950	70	46	.03
May 9, 1950	78	61	.14
May 16, 1950	73	53	1.93
May 23, 1950	83	62	.86
May 30, 1950	79	62	2.38
June 6, 1950	75	59	1.32
June 13, 1950	84	66	.30
June 16, 1950	90	72	.00

data were taken at Paradise. The seedlings at Stillwater were thoroughly sprinkled with a garden hose during dry periods.

The first disease readings were made May 8, 1950 and plants were classed as either positive or negative, as previously described. Three readings were made between the above date and May 15, 1950, when the experiment was terminated. When a plant was classed as positive it was removed from the plot which eliminated this source of inoculum.

Results

The only plants that developed disease symptoms on the cotyledons were those planted in the plots where the blight infested carpels and leaves were placed on top of the soil. The results of this part of the experiment for the three locations are listed in table VI. Where the infested leaves were placed on top of the soil, none of the test plants developed bacterial blight in the plot at Paradise, 56 per cent developed blight at Perkins and 38 per cent at Stillwater. Where the infested carpels were placed on top of the soil, 18 per cent of the plants developed bacterial blight in the plot at Paradise, 9 per cent developed blight at Perkins and 3 per cent at Stillwater. At the time the seeds were planted, the leaves at the surface of the soil appeared to be decomposed except for the veins. The carpels showed very little decomposition.

Table VI. Number of diseased seedlings that developed from diseased leaves and carpels placed on top of the soil at Paradise, Perkins, and Stillwater, Oklahoma.

Location	Kind of material	Number healthy	Number diseased	Percent diseased
Paradise	Leaves	27	0	0
Paradise	Carpels	14	3	18.0
Perkins	Leaves	8	10	56.0
Perkins	Carpels	21	2	9.0
Stillwater	Leaves	23	14	38.0
Stillwater	Carpels	35	1	3.0

LABORATORY STUDIES

The following study was made to determine how long *X. malvacearum*, when free from plant debris, would remain viable in soil maintained under different moisture conditions.

Methods and Materials

Preparation of inoculum.-- A pure culture suspension was made by adding a small quantity of tap water to one test tube culture of *X. malvacearum*, and shaking until the bacteria came free from the agar. The contents of the tube was then emptied into a small quantity of water and strained through several thicknesses of cheese cloth, and then diluted with tap water to make one liter.

A second suspension of *X. malvacearum* was made by submerging 30.4 grams of dry infested cotton leaves in one liter of water for one hour. The leaf debris was then removed by straining through several thicknesses of cheese cloth.

Incubation in moist soil.-- Two four-inch pots were filled with screened, uncropped soil. The pure culture suspension was poured over one of the pots, and the leaf suspension over the other. In both cases the suspensions were poured into the pots until an excess ran out at the bottom. The pots were kept at room temperature throughout the experiment, and watered with tap water each time the soil began to dry on top.

Incubation in dry soil.-- A small quantity of screened, uncropped soil was placed in each of ten Petri dishes. The soil in five of the dishes was well soaked with the pure culture suspension and the other

five with the leaf suspension. The Petri dishes were then placed in an oven at 105° F. and the door was left open throughout the experiment to increase the rate of evaporation. The soil in the Petri dishes was dry and hard after 24 hours in the oven, but measurements of the soil moisture content were not made at any time during the experiment.

To test the viability of the organism, disease-free seedlings were inoculated with samples from the pots and from the Petri dishes at 0, 24, 48, 96, and 192 hours after treating the soil.

The samples were taken two inches below the soil surface in the moist pots. The dry samples were removed from the oven and sufficient tap water then added to each Petri dish to make a suspension. The inoculations were made with sterile sand bags as previously described. One plant was inoculated from each soil sample. A check plant was inoculated with tap water.

Results

Table VII. lists the results of the tests on the survival of X. *malvacearum* in the soil. The disease-free seedlings developed water soaked spots when inoculated with X. *malvacearum* that was incubated 48 hours or less in the soil. When disease-free seedlings were inoculated with bacteria that were incubated 96 hours in the soil the readings were questionable, but when the cotyledons from the questionable plants were used to inoculate disease-free seedlings the water soaked spots developed from all the samples except the infested leaf suspension in moist soil. The organism produced negative results when incubated 196 hours in the soil, and remained negative when disease-free seedlings were inoculated with the leaves from these plants.

Table VII. Recovery of X. *malvacearum* from dry and moist soil.

Inoculum	Condition of soil during incubation	Incubation period in hours				
		0	24	48	96	192
Pure culture suspension	Dry [1]	+	+	+	? [3]	- [4]
Pure culture suspension	Moist [2]	+	+	+	? [3]	- [4]
Check (Tap water)		-	-	-	-	-
Infested leaf suspension	Dry [1]	+	+	+	? [3]	- [4]
Infested leaf suspension	Moist [2]	+	+	+	? [4]	- [4]
Check (Tap water)		-	-	-	-	-

[1] Soil dried in Petri dishes in an oven at 105° F. after being wet with bacterial suspensions. The soil appeared to be dry after 24 hours.

[2] Moisture was maintained by adding tap water. Incubation was at room temperature.

[3] Reinoculated and found to be positive.

[4] Reinoculated and found to be negative.

RECOVERY OF *X. MALVACEARUM* FROM OVER-WINTERED INFESTED COTTON DEBRIS AND FROM DRIED MATERIAL STORED IN THE LABORATORY

Methods and Materials

Diseased carpels were collected from the ground May 10, 1950, in a field of cotton at Altus, Oklahoma, that had been infested with bacterial blight the previous year. They were used to inoculate disease-free cotton plants.

Diseased carpels and leaves, collected from bacterial blight infested fields during the summer and fall of 1949, were stored in the laboratory and used to inoculate disease-free seedlings in the spring.

The method of planting and spacing the seedlings were the same as previously described. Three plants were inoculated April 20, 1950, with material from each location. A check plant was inoculated with tap water. The inoculations were with the sand bag, and upon reading were classed as positive or negative as previously described.

The material was collected from the following locations:

1. Paradise, Oklahoma; the leaves collected October 15, 1949.
2. Indiahoma, Oklahoma; the carpels collected September 15, 1949.
3. Tipton, Oklahoma; carpels collected September 14, 1949.
4. Chickasha, Oklahoma; carpels collected September 13, 1949, near west Bitter Creek, northeast of town.
5. Tucumcari, New Mexico; leaves collected August 23, 1949, from Acala cotton.
6. Altus, Oklahoma; carpels collected October 30, 1949.
7. Chilocothe, Texas; carpels collected October 4, 1949.
8. Greenville, Texas; carpels collected August 20, 1949.
9. Hobart, Oklahoma; leaves collected July 7, 1949.

10. Stillwater, Oklahoma; leaves collected at West Agronomy Farm, August 15, 1949.

11. Chickasha, Oklahoma; carpels collected July 28, 1949.

Results

Cotyledons inoculated with bacterial blight infested carpels that over-wintered in the field were classed as positive. Cotyledons inoculated with diseased carpels and leaves that were stored in the laboratory for several months were classed as positive for all the test samples, except diseased leaves collected at Stillwater, Oklahoma, August 15, 1949. The organism was isolated in pure culture and its pathogenicity established.

Table VIII. lists the results of the recovery of _X. malvacearum_ from diseased carpels that over-wintered in the field, and diseased carpels and leaves kept under dry conditions.

Table VIII. Recovery of *X. malvacearum* from diseased carpels that over-wintered in the field, and diseased carpels and leaves kept under dry conditions. Plants inoculated April 20, 1950, readings made May 1, 1950.

Location of Material	Kind of Material	Date Collected	Readings
Hobart, Okla.	Leaves	July 7, 1949	+ + +
Chickasha, Okla.	Leaves	July 28, 1949	+ + +
Stillwater, Okla.	Leaves	August 15, 1949	- - -
Greenville, Tex.	Carpels	August 20, 1949	+ + +
Tucumcari, N. Mex.	Leaves	August 23, 1949	+ + +
Chickasha, Okla.	Carpels	September 13, 1949	+ + +
Tipton, Okla.	Carpels	September 14, 1949	+ + +
Indiahoma, Okla.	Carpels	September 15, 1949	+ + +
Chilocothe, Tex.	Carpels	October 4, 1949	+ + +
Paradise, Okla.	Leaves	October 15, 1949	+ + +
Altus, Okla.	Carpels	October 30, 1949	+ + +
*Altus, Okla.	Carpels	May 10, 1950	+ + +

* Over-wintered in the field

+ Positive

- Negative

DISCUSSION

The results of the foregoing experiments, although not conclusive, indicate that X. malvacearum can remain viable for a much longer period in infested cotton plant debris buried in soil and maintained at near freezing temperatures than it can at higher temperatures, providing the moisture content is approximately the same under both conditions. The more suitable the conditions are for decomposition, viz., high temperature and adequate moisture, the less chance the organism has of survival. This is in agreement with Weindlings (24) findings that the leaves collected directly on the moist surface of the soil consistently contained a fewer number of viable bacteria than the dryer leaves.

In this study the organism maintained its viability as long as ten months in infested plant debris kept under dry conditions. This material was tested only once; therefore, the findings were inconclusive as to how long the organism would live under dry conditions.

The fact that the bacterium was found to over-winter in Oklahoma supports the hypothesis that infested plant debris left in the field may be a source of infection for the planted crop in the spring, (9), (24).

Disease-free seedlings did not develop bacterial blight when planted where the diseased debris was buried for 36 days before planting time. The organism, however, was viable when placed on top of the soil. The buried debris was not recovered and used as inoculum to test the viability of the organism.

Rolfs (16) found the organism to live approximately 60 hours in distilled water, while Tennyson (23) found the organism to live from 46

to 56 hours in run off water. Massey (13) stated that the bacterium disappeared after 72 hours in river water, but might live a month in rain or distilled water. In these studies it was demonstrated that the organism may live in the soil in a free condition somewhere between 96 and 192 hours.

SUMMARY

Experiments were conducted in different locations to test the viability of _X. malvacearum_ under different temperature conditions.

X. malvacearum in cotton debris was still viable outside the greenhouse after 81 days of incubation in the soil where the average mean air temperature was approximately 40° F. However, in the cool and warm sections inside the greenhouse, where the average mean air temperatures were approximately 70° F. and 85° F., respectively, the organism apparently died out after 40 days of incubation in the soil.

In a field study diseased carpels and leaves were buried April 12, 1950, at different depths in the soil at three locations. The only positive results occurred where the diseased material was placed on top of the soil. The diseased leaves produced from 0 to 56 per cent infection for the three locations, while diseased carpels produced from 3 to 18 per cent infection.

The laboratory studies demonstrated that the organism may live in the soil in a free condition somewhere between 96 and 192 hours. The bacterium over-wintered on diseased carpels collected at Altus, Oklahoma. _X. malvacearum_ was recovered from diseased carpels and leaves kept in a dry condition for as long as ten months.

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