1925

A new bacterial leaf spot (pseudomonas sp) on holcus sorghum L and Zea mays L.

James Blair Kendrick  
Iowa State College

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Agricultural Science Commons, Agriculture Commons, Agronomy and Crop Sciences Commons, and the Plant Pathology Commons

Recommended Citation
https://lib.dr.iastate.edu/rtd/14781

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.
NOTE TO USERS

This reproduction is the best copy available.

UMI*
A NEW BACTERIAL LEAF SPOT (PSEUDOMONAS SP.)
ON HOLCUS SORGHUM L. AND ZEA MAYS L.

BY

James Blair Kendrick

A Thesis submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major subject Plant Pathology

Approved

Signature was redacted for privacy.

In charge of Major work.

Signature was redacted for privacy.

Head of Major Department.

Signature was redacted for privacy.

Dean of Graduate College.

Iowa State College
1925.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>A Review of Earlier Literature on Bacterial Spot Diseases on Gramineous Hosts</td>
<td>2</td>
</tr>
<tr>
<td>Symptoms of the Disease on the Different Hosts</td>
<td>7</td>
</tr>
<tr>
<td><em>Holcus</em> <em>sorghum</em>, <em>H. sorghum</em> var. <em>sudanensis</em> and <em>H. halepensis</em></td>
<td>7</td>
</tr>
<tr>
<td><em>Zea</em> <em>mays</em></td>
<td>9</td>
</tr>
<tr>
<td><em>Pennisetum</em> <em>glaucum</em>, <em>Chaetochloa</em> <em>lutescens</em> and <em>C. italica</em></td>
<td>11</td>
</tr>
<tr>
<td>Studies of the Causal Organism</td>
<td>12</td>
</tr>
<tr>
<td>Isolations from <em>Zea</em> <em>mays</em></td>
<td>12</td>
</tr>
<tr>
<td>Isolations from <em>Holcus</em> species and <em>Chaetochloa</em> <em>lutescens</em></td>
<td>14</td>
</tr>
<tr>
<td>Morphology of the causal organism</td>
<td>19</td>
</tr>
<tr>
<td>Cultural characters</td>
<td>20</td>
</tr>
<tr>
<td>Temperature relations</td>
<td>33</td>
</tr>
<tr>
<td>Effect of freezing</td>
<td>34</td>
</tr>
<tr>
<td>Effect of sunlight</td>
<td>36</td>
</tr>
<tr>
<td>Resistance to desiccation</td>
<td>36</td>
</tr>
<tr>
<td>Taxonomy of the Causal Organism and its Host Range</td>
<td>38</td>
</tr>
<tr>
<td>Taxonomy</td>
<td>38</td>
</tr>
<tr>
<td>Host range of the <em>Holcus</em> bacterial spot</td>
<td>45</td>
</tr>
<tr>
<td>Relation of Parasite to Host Tissue</td>
<td>48</td>
</tr>
<tr>
<td>Pathogenicity studies</td>
<td>48</td>
</tr>
</tbody>
</table>
A NEW BACTERIAL LEAF SPOT (PSEUDOMONAS SP.)
ON HOLcus sorgHUM L. AND ZEA MAYS L.

Introduction
A bacterial leaf spot disease has been observed on
corn (Zea mays L.) throughout Iowa since 1916. In the
Fall of 1924, volunteer sorghum (Holcus sorghum L.) plants
in a Zea mays field near Ames, Iowa, were observed to
have a leaf spot of apparently bacterial origin. Further
examination revealed the presence of a similar leaf spot
on Holcus sorghum, sudan grass (H. sorghum var. sudanensis
(Fiper) Hitchc.), Johnson grass (H. halepenseis L.)
and pearl millet (Pennisetum glaucum (L.) R. Br.) on
the Farm Crops experimental plots. During the season of
1925, the disease again occurred generally on Zea mays,
Holcus sorghum, H. sorghum var. sudanensis, H. halepenseis
and Pennisetum glaucum and has been reported from widely
separated areas in the state on Holcus sorghum and H.
sorghum var. sudanensis. The disease was also found in
1925 to a limited extent on yellow foxtail (Chaetochloa
lutescens (Weigel) Stunts) and Japanese millet (Chaeto-
chloa italica (L.) Soribn.). It is primarily a leaf
spot disease. Often the necrotic areas became so numero-
us as to cause the death of the leaves and in many cases
impaired the vigor and production of the crops concerned.
The prevalence and injury of this disease led to a study of the relation of these spot diseases to one another, their relation to other previously described bacterial diseases on Gramineous hosts, and of the causal organism concerned. Repeated isolations and cross inoculations as well as morphological and cultural studies have shown the causal organisms associated with the above leaf spots to be alike.

A Review of Earlier Literature on Bacterial Spot Diseases on Gramineous Hosts.

As early as 1887 Burrill described a bacterial disease on sorghum and broom-corn (1, 2) and another on corn (3, 4) in 1889. Since that time, little has been added to our understanding of these diseases. Unfortunately Burrill's description of the symptoms and causal agents are so meagre that his contributions serve only to prove that spot diseases on these hosts are of long standing. The diseases as described by Burrill occurred on the roots, stems, leaves and leaf-sheath. From his descriptions of the disease and the organisms concerned, it is quite evident that he was not working with pure cultures and the condition which he describes
was at least partly due to saprophytic organisms and environmental factors. Kellerman and Swingle (14) in 1888 observed a spot disease of sorghum in Kansas and substantiated Burrill as to its bacterial nature. The disease herein described occurs only on the leaves, and the organism concerned differs widely from those described by Burrill.

In 1905 Smith and Hedges (25) briefly described a disease of broom-corn and sorghum as "Burrill's bacterial disease of broom-corn", but did not accept or reject the organism previously described by Burrill. In a later publication, Smith (26) briefly characterized the organism and named it *Bacterium andropogon*, apparently rejecting the name *Bacillus sorghi* previously suggested by Burrill (1, 2). The symptoms of the disease as described by Smith and Hedges somewhat resemble the disease under discussion here, but a comparison of the causal organisms show marked differences.

Two species of chromogenic bacteria were isolated from the red discoloration on the stalks and in the pith of sorghum plants in 1898 by Bruyning (5), which exhibited a symbiotic relationship and were considered by him to be the primary cause of the condition known as "Sorghum
A year later, Radais (19) isolated a small ovoid yeast from the red discolored stems of blighted sorghum plants and produced similar symptoms by hyperdermic injections of this organism, as well as with wine yeasts, into the stems of normal plants. He was also able to produce the same coloration by aseptically wounding the tissues of sorghum plants and concluded that the red discoloration was a host reaction and could be induced by yeasts or bacteria developing in the tissues. It is quite obvious that the above phenomenon is in no way related to the disease described herein.

In 1919 (21) and again in 1921 (22) Rosen described a bacterial root and stalk rot of field corn, but did not describe the causal organism further than to say it was a white rapidly growing bacterium. The disease as described by Rosen also occurred to a limited extent on the leaves. Since the disease described herein on *Zea mays* has been observed to occur on the leaves only and a comparison of the causal organisms is not possible, the two diseases are considered to be different. The purple leaf-sheath spot of corn described by Durrell (6) does not have a specific pathogen as the causal
agent, while the disease of sweet corn first described by Stewart (29) in 1897 is a vascular disease caused by a yellow bacterium which readily differentiates it from the spot disease herein described caused by a white bacterial organism.

The organism described by Rosen (23, 24) as causing a bacterial disease of foxtail (Chaetochloa lutescens) also under artificial greenhouse conditions produced spots on the leaves of wheat, oats, rye, barley, corn and sorghum. A comparison of the published description of the organism causing the above disease with the organism associated with the disease under discussion here shows marked differences; therefore the two diseases are not to be confused.

In 1924 (9) Elliott described a bacterial stripe disease of proso millet. The symptoms of this disease showed as watersoaked brown stripes on the leaves, sheaths and culms. Sorghum and other millets failed to become infected when inoculated artificially with the organism causing the stripe disease of proso millet. The organism differs materially from the one causing the spot disease described in this paper.

There are a number of other bacterial diseases on
closely related hosts that need be only briefly mention-
ed since the symptoms and causal organisms are obviously
different from the disease under discussion. Manns (16) in 1909 described a bacterial blade blight of oats as caused by a white and a yellow bacterial organism working symbiotically. Elliott (8) in a later study of the same disease has shown that it was caused by a white bacterium which he named *Bacterium acronofaciens*. This organism has been compared with the organism associated with the disease discussed herein and found to be markedly different. The yellow gum disease of western wheat grass (*Agropyron smithii*) described by O'Gara (17) as well as the disease of the stems and inflorescence of wheat described by Hutchinson (11) are caused by yellow bacterial organisms.

In 1916, Jones, Johnson, and Reddy (12) described a bacterial blight of barley and certain other cereals, caused by *Bacterium translucens*. Later Smith (27) and Smith, Jones and Reddy (28) described black chaff of wheat as due to *B. translucens var. undulosum*. Reddy, Godkin and Johnson (20) in 1921 described a bacterial blight of rye caused by still another variety of the same organism and named it *B. translucens var. secalis*. 
The above organisms are yellow which at once differentiates them from the white organism associated with the disease described in this paper. Brief mention should be made of the disease of wheat described by McCulloch (15) as basal glumerot, but which has no relation to the disease herein discussed.

Symptoms of the Disease on the Different Hosts:

*Holcus sorghum, H. sorghum var. sudanensis, H. halepensis.*

The symptoms of the disease on these three hosts are very similar. Different varieties of *Holcus* may have varying degrees of red color associated with the lesions, while the variety known as Shallu has a dark brown border instead of a red. The spots are usually round or oblong, sometimes linear to irregular, of variable size with a light straw colored parchmentlike center and a deep red border (Pl. I. B. & C.). Under field conditions the spots first appear on the lower leaves and gradually spread to the upper leaves as the plants approach maturity. Often marginal infection occurs, causing rather long necrotic areas along the
edge of the leaves (Pl. I, A. & C.). The lesions are usually limited by the veins, but often the coloration extends across the veins and where several spots are close together they converge to form rather large necrotic areas on the leaves (Pl. I, A.).

The lesions occur at any place on the leaf and vary in size from one to eight millimeters in diameter. The spots are at first dark green water-soaked, and in a few hours after infection is visible the red color appears, even before they lose their water-soaked appearance. The lesions soon become dry and assume a parchmentlike appearance with a narrow to broad red border (brown in the case of Shallu, Pl. I, A.). Often the smaller lesions are entirely red with a very small somewhat sunken center.

The intensity of the red color varies somewhat with the different varieties of Holcus. The varieties orange cane, Kansas orange, ribbon cane, and broom-corn have a light red border while feterita, white kafir, higeria, red amber, black amber, and Sudan grass have a darker red border. H. halepensis also has a dark red border.
The lesions occur primarily on the leaves, although occasionally leaf-sheath lesions occur late in the season. Often red spots occur on the glumes covering the seed, sometimes with a small light colored center, but more often as red spots or blotches.

Similar leaf lesions have been repeatedly secured by artificial inoculations in the greenhouse except they were usually smaller. The only difference between spots produced artificially in the greenhouse and those occurring naturally in the field is in the size, which was probably due to the difference in the vigor and environment of the plants. The lesions often became so numerous on the leaves of *H. sorghum* var. *sudanensis* as to cause the death of the leaf.

Lesions have been noted on the first leaf of seedling *H. sorghum* plants growing in sterile soil and in the field. These spots are small and occur usually in the center (Pl. III) or along the margin near the tip of the first leaf, and are similar to the lesions occurring on the plants in the field.

*Zea mays*.

The disease has been observed only on the leaves of these hosts. The spots occur on the lower leaves and
usually more numerous on the tip. Incipient lesions are round, oblong or irregular, dark green water-soaked, varying in size from two to ten millimeters in diameter (Pl. II). The spots lose their water-soaked appearance and become brown centered with a somewhat darker brown to reddish brown narrow border. A narrow yellowish halo when viewed by transmitted light is usually visible, especially around the larger lesions (Pl. II, A). The darker spots in Plate II, B, are incipient lesions among the numerous older lesions on the leaf.

When conditions are especially favorable for the spread of the organisms and infection, occasionally marginal infection develops along the entire edge of some leaves and causes a dark brown necrotic condition of the entire margin. This condition is only brought about during periods of rainy weather on the lowest leaves.

Infection was first noted in the field on the lower leaves on June 25, 1925, following a period of rainy weather. Incipient lesions were noted following rains, but none during periods of dry weather. By July 20, lesions were found on leaves up to the seventh and eight node.
The bacterial lesions should not be confused with round, oblong to irregular necrotic areas, common on leaves of *Zea mays* in practically all fields, which have a white papery appearance and no definite border. Repeated isolations and examinations have failed to reveal any organism associated with such lesions and they probably result from some mechanical injury.

The symptoms on *Zea mays* in the greenhouse, resulting from artificial inoculations are somewhat different from those observed in the field. They differ in being smaller and thin light colored papery necrotic areas with a narrow light brown border when they lose their water-soaked appearance.

*Pennisetum glaucum, Chaetochloa lutescens* and *C. italica*.

The disease on *Pennisetum glaucum* does not differ materially from that on the other hosts except in the color of the spots. They are usually a dark brown color with a light greenish halo. Marginal infection is more common on this than any of the other hosts, causing elongated necrotic areas on the edge of the leaves. The lesions on *Chaetochloa lutescens* and *C. italica* are very similar to the ones on *Pennisetum glaucum*, except they
are smaller and these hosts have shown only slight susceptibility.

**Studies of the Causal Organism**

**Isolations from Zea Mays.**

On September 24, 1924, a large number of isolations were made from conspicuous light centered, dark bordered spots on leaves and leaf-sheaths of *Zea Mays* collected in the Agronomy experimental plots at Ames, Iowa. These isolations were made by surface sterilizing the tissues in mercuric chloride (1:1000), and then washing in sterile water. The tissues were crushed in a drop of sterile water on a flamed slide and dilution plates made in potato dextrose agar. In 48 hours, a number of the plates from the leaf lesions showed an abundance of bacterial colonies, some yellow and others white. Transfers were made of single isolated colonies of the white and yellow organism. Subsequent inoculations on *Zea mays*, *Holcus sorghum* and *H. sorghum* var. *sudanensis*, in the greenhouse with the two organisms, showed that the white organism only was pathogenic. A majority of the tissue plantings in the poured plates were sterile at the end of two days,
but from a number, a white organism grew out which in transfers resembled in every way the white organism secured in the dilution plates. Many different organisms were secured from the corn leaf-sheath spots which is in accord with Durrell's (6) findings.

Again, on October 20, 1924, dilution plates were made from *Zea mays* leaf spots similar to those mentioned above. In these plates white bacteria of a uniform type developed, which resembled the organism secured from previous isolations and which also proved pathogenic to *Zea mays* and *Holcus sorghum* in the greenhouse.

On June 25, 1925, following a period of rainy weather, spots were found on the lower leaves of *Zea mays* var. *saccharata* (variety Country Gentleman) in a field near Ames, which were apparently bacterial in nature and resembled the spots noted in the fields of *Zea mays* in the Fall of 1924. Other *Zea mays* fields were examined near Ames at this time and similar leaf lesions noted in four fields. A large number of isolations were made from these lesions by the method previously described and a white fluorescent bacterial organism was consistently secured that resembled the
organism previously isolated from *Zea mays* and *Holcus sorghum*. The organisms secured proved pathogenic to *Zea mays* and *Holcus sorghum* by subsequent inoculation tests in the greenhouse.

**Isolations from Holcus species and Chaetochloa lutescens.**

While examining *Zea mays* in a field near Ames in September 1924, a round to oblong red or light centered and red bordered spot was noted on leaves of volunteer *Holcus sorghum* plants. Leaves showing these spots were brought in and examined under the microscope by cutting the lesions laying in a drop of water on a slide. Bacteria oozed from the tissues from many of the necrotic areas. Isolations were made using the method previously described, and in two days white bacterial colonies of a uniform type appeared in the plates which resembled the previous isolations from spots on *Zea mays*. Transfers were made to agar slants and the pathogenicity of the organism proved.

An examination of the Forage Crops experimental plots on October 24, 1924, showed the presence of a similar leaf spot on *Holcus sorghum* and *H. sorghum* var. *sudanensis*. A round, oblong to irregular spot of
apparently bacterial nature was also noted on Pennisetum glaucum growing in the same plot. A white bacterial organism similar to the one discussed above was isolated from the above named plants and the pathogenicity of the organism determined. A killing frost occurred throughout Iowa about this time and prevented further work on field material until the Spring of 1925.

A few Holcus sorghum heads were collected in the field of Zea mays previously mentioned near Ames. Some of the seed from these heads were planted in pots in the greenhouse to furnish plants for inoculation. When the plants were very small, a light centered, red bordered, round to oblong spot was noted on the margin or near the center of several of the first leaves. These spots resembled some of the spots noted on the Holcus sorghum leaves in the field. The spots were surface sterilized with mercuric chloride, rinsed in sterile water and cut in a drop of sterile water on a flamed slide. Bacteria oozed from the tissues. Isolations were made and a white organism secured which appeared to be the same as the organism previously isolated from Zea mays and Holcus sorghum.
Large *Holcus sorghum* plants growing in a greenhouse bench were sprayed early in May, 1925 with a suspension of bacteria isolated from a *Holcus sorghum* lesion. In a few days, typical leaf lesions occurred and ten days after inoculation, small red spots occurred on the sheath enclosing the head of one of the plants. When the head emerged from the sheath, the glumes that were immediately under the infected area on the sheath appeared to be infected. They were dark red on the tip and the color soon involved the entire glume, while the normal glumes were still green. This color change should not be confused with the natural reddening of the glumes in the process of maturity of the seed, which starts at the base and extends towards the tip. Some of the diseased glumes were removed, surface sterilized in mercuric chloride, and then washed in sterile water and successful reisolations secured. Transfers were made to agar slants and the pathogenicity of the reisolated bacterium proven on *Holcus sorghum*.

On June 26, 1925, a brown, round to irregular spot was noted on a few leaves of *Chaetochloa lutescens* growing near infected *Zea mays* var. *saccharata* plants. Exam-
ination under the microscope revealed bacteria in the tissues, and isolations and subsequent inoculations showed the organism to be the same as previously isolated from Zea mays and Holcus sorghum.

On July 27, 1925, a total of 30 isolations were made from Zea mays, Holcus sorghum, H. sorghum var. sudanensis, H. halepensis and Pennisetum glaucum leaf lesions collected in the field. The lesions were surfaced sterilized and the isolations made in the usual way. All plates showed an even and abundant seeding of apparently the same white florescent bacterial organism as secured in previous isolations. Transfers were made and their pathogenicity proven.

Early in the course of the study of this disease it became quite apparent that the organism never occurred in as great abundance in the lesions on the leaves of Holcus sorghum, H. sorghum var. sudanensis and H. halepensis as in lesions on Zea mays and Pennisetum glaucum leaves. Often isolations from older lesions on the first named hosts resulted in only a few typical bacterial colonies, or sterile plates. In every case where incipient lesions were cut in a drop of water on a slide, and placed under
low power of a microscope, bacteria oozed from the tissues in the center of the lesion. No trouble was encountered in securing cultures from such lesions. Similar lesions taken after the tissues had dried out and treated the same way often failed to reveal any bacteria oozing from the tissues and isolations resulted in relatively few bacterial colonies or sterile plates. The above phenomenon was repeatedly demonstrated from artificial inoculations by pouring plates from incipient lesions and from lesions on the same plants after the infected tissues had dried out. A large number of lesions from field material were examined under the microscope by cutting the lesions in a water drop on a slide and watching for the bacteria to ooze out. It was observed that in practically every case where a large lesion with a rather large light area in the center was cut in a drop of water, bacteria oozed from the tissues, but in the case of smaller lesions with a very small light center or lesions entirely red, bacteria rarely oozed from the tissues. Cultures of the organism were easily secured from the lesions in which bacteria oozed out from the tissues. Lesions of all sizes and ages on *Pennisetum glaucum* leaves showed bacteria
oozing from the tissues in great abundance.

It seems that the bacteria are rather quickly arrested in their development in the case of the Holcus species possibly by some toxic substance, and never become as abundant in the lesions as in the case of Zea mays and Pennisetum glaucum. Bacteria were not observed to ooze from the red discolored tissues bordering the lesions. This red color is apparently a substance produced when the tissues are wounded by bacterial invasion and diffuses through the healthy tissue immediately surrounding the wound. No evidence is presented as to why the bacteria are apparently arrested in their development or die in the tissues in these hosts.

Morphology of the causal organism.

The organism is a short rod with rounded ends, usually solitary, but sometimes occurring joined together in pairs. The bacteria stain readily with Ziehl's carbol fuchsin or anilin gentian violet. The size of the organism from 24 hour to four day old cultures shows little variation. When stained with Ziehl's carbol fuchsin and anilin gentian violet, the cells measured from 0.6 to 1.μ in width and 1.5 to 2.9μ in length with an average
The organism is motile by means of polor flagella (1-4) at one pole only. The usual number being one or two. (Plate IV). For flagella staining, a two millimeter loop of growth from a 20 to 24 hour agar slant was removed and placed in a ten cubic centimeter water blank which was permitted to stand several hours to allow the motile bacteria to diffuse throughout the water. A two millimeter loop was then removed and carefully smeared on a clean cover glass and stained by Plimmer's method.

Endospores or involution forms have not been observed and the presence of capsules have not been demonstrated. The organism is gram-negative.

Cultural characters.

The organism grows readily on the ordinary laboratory culture media but grows markedly better when one per cent dextrose is added to the basal medium. For general laboratory use neutral potato agar and neutral beef-peptone agar containing one per cent dextrose were found to be most satisfactory. The reaction of all culture media was adjusted to PH 7.0 with normal sodium
hydroxide or normal hydrochloric acid.

The special media were prepared and allowed to incubate at room temperature for several days to insure sterility. Inoculations were made from heavy water suspensions made by removing the growth from a 24 to 36 hour old agar slant to a ten cubic centimeter water blank. Unless otherwise noted, all cultures were incubated at room temperature. Strains of the organism isolated from Holcus sorghum, H. sorghum var. sudanensis, Zea mays and Pennisetum glaucum were carried in parallel series. These cultures were purified by poured plates and their pathogenicity tested before being used. A culture of Bacterium coli was carried in parallel series in most of the tests.

Agar poured plates. On poured plates of beef-peptone agar small, circular, white fluorescent colonies appeared in 24 hours. In 36 to 40 hours, the surface colonies were round, convex, one to two millimeters in diameter. Surface colonies reached their maximum growth in four to five days, and were two to four millimeters in diameter, margin entire, surface smooth, finely amorphous, viscid, grayish white in reflected light and slightly greenish-yellow fluorescent in transmitted light.
Submerged colonies were lens-shaped, white and very small. The agar was unchanged in color.

On poured plates of potato agar containing one per cent dextrose, growth was similar to that on beef-peptone agar except more vigorous. Colonies appeared in 24 hours and by 36 to 40 hours were one to three millimeters in diameter. The medium was unchanged in color and there was no odor present.

**Agar stabs.** Stab cultures on beef-peptone agar showed best growth at the top. There was slight growth along the line of puncture near the surface only. In four days, the growth had spread over the entire surface with a raised area around the point of puncture. A slight greenish-yellow pigment was produced which diffused through the medium to a depth of three centimeters from the surface.

**Agar slants.** Slant cultures on beef-peptone agar showed moderate growth in 24 hours, filiform, slightly raised along the edges, smooth surface, grayish white color in reflected light and greenish fluorescent in transmitted light, especially along the edges. The cultures reached their maximum growth in three days and never entirely covered the surface of the slant. There
was slight evidence of a greenish-yellow pigment which diffused through the medium directly under the slant. The medium was otherwise unchanged.

Cultures on slants of potato dextrose agar showed more abundant growth than on beef-peptone agar, but otherwise appeared the same.

On slants of beef-peptone agar containing one per cent dextrose, growth was more abundant than on the other slanted medias. In three days the entire surface of the slant was covered with a slightly raised growth of much the same appearance as on the other medias.

Cultures on veal infusion agar slants, made only moderate growth. There was slightly more evidence of the greenish-yellow pigment produced on this medium than on the other agar slants.

**Gelatin plates.** Small circular colonies appeared on poured plates of plain nutrient gelatin incubated at 20°C. in 24 to 36 hours. Liquefaction was evident in 36 hours by the round colonies being present in small saucer-shaped cavities. In 48 hours the entire medium was liquefied.

**Gelatin stabs.** Stab cultures on plain nutrient
gelatin were held at 20°C. Growth in 24 hours was best on the surface and extended down the line of puncture to the bottom of the tube. Liquefaction was evident by a slight crateriform liquid area on the surface. Liquefaction progressed rather rapidly, later becoming striatiform, and at the end of seven days liquefaction was infundibuliform to striatiform with a white flocculent precipitate at the bottom of the liquid portion of the medium. The medium was practically half liquefied at the end of ten days.

**Potato cylinders.** Growth on steamed potato cylinders was moderate, grayish white, smooth, glistening, and spread rapidly over the surface of the medium. In four days, the growth was a dull white color, and the medium was slightly grayed.

**Milk.** There was no apparent change in cultures of plain milk until the third day when there was evidence of slight clearing in the top layer of the medium. No coagulation occurred at any time and in 15 days, the top one-half of the medium was cleared and had a slight greenish tinge. The consistency of the semitransparent liquid was unchanged.
Azolitmin milk. Lavender colored azolitmin milk showed a pale blue layer on the surface in three days. In 15 days, one-half the medium had been cleared without coagulation. The pale blue zone still remained on the surface, but the remainder of the cleared liquid was colorless. The undigested portion showed no color change. No pink color occurred; therefore no acid was produced.

Methylene blue in milk. In this medium, digestion occurred as in the other milks, but no color change was noted until the eight day when the color had disappeared in the cleared portion of the medium. The undigested portion remained the same color as the control.

Brom cresol purple in milk. Brom cresol purple in a concentration of .0016 per cent, produces a light bluish color in milk. If the acidity is increased, the color turns yellow and if the medium becomes more alkaline a purple color results. Cultures in this medium showed the usual digestion without coagulation and a reddish purple color occurred in the cleared liquid. This further substantiates the fact that no acid is produced from milk.
Nitrate reduction. A one per cent potassium nitrate bouillon in fermentation tubes showed moderate growth in the open arm, but no growth in the closed arm and no gas produced. A further test was made by growing the organism in a two per cent peptone water containing 0.2 per cent potassium nitrate. The cultures were tested at the end of seven days for nitrites, using the sulphanilic acid and naphthyl-amine-acetate test. Strong positive tests were secured for nitrites.

Carbon Metabolism. To test for gas production from different carbon compounds, one per cent solutions of dextrose, saccharose, maltose, lactose, mannit, and glycerol were made up in a one per cent difco peptone water. Instead of using the ordinary U tubes, a simpler and more convenient type of fermentation tube was used, consisting of a smaller test tube inverted within a larger one.

In all cases there was abundant growth in the open arm but no growth in the closed arm, or inner inverted tube. No gas was produced. Parallel cultures of *Bacterium coli* showed heavy clouding in the closed arm and gas produced from all six carbon compounds.
Acid production from carbon compounds. To determine accurately if acid was produced from carbon compounds the three sulphonephthalein indicators, brom cresol purple, brom thymol blue, and phenol red were used. These three indicators serve for a PH range from 5.2 to 8.4. Since the neutrality point is at PH 7.0 it is obvious that a series of media containing these indicators will serve to detect considerable acid or alkali production by the organism growing in culture.

A series of liquid media were prepared containing one per cent difico peptone and one per cent of the following carbon compounds, dextrose, saccharose, lactose, maltose, mannit, and glycerol. These media were prepared in triplicate. To one series brom cresol purple was added, in another brom thymol blue, and to the other one, phenol red. The indicators were added at the rate of eight cubic centimeters of a .2 per cent alcoholic solution per liter of medium, giving a concentration of the indicator of 0.0016 per cent. These media when prepared had a PH value of 6.8 to 7.0, as evidenced by the grass-green color of the medium containing brom thymol blue.
The series containing brom cresol purple were near the full alkaline color, while the phenol red series were a very faint pink. In 24 hours after inoculation, there was slight acid production from dextrose indicated by a slight fading of the green color in the brom thymol blue series, and in four days, there was strong acid production, shown by a yellow color with all three indicators. In the case of saccharose, acid production was not evident until two days after inoculation and in five days the brom thymol blue series were entirely yellow, but the brom cresol purple series still had a slight purple color. There was not enough acid produced from saccharose to give the full acid color to brom cresol purple, while with dextrose the purple color entirely disappeared. In the case of lactose and maltose there was considerable alkali produced, indicated by the change to the alkaline color, while with mannit and glycerol there was very little color change from the controls. The above tests demonstrated that acid is produced from dextrose and saccharose, but not from lactose, maltose, mannit, and glycerol.

A series of solid media were prepared using beef-
peptone agar and one per cent of the following sugars, dextrose, saccharose, maltose, and lactose. The same three indicators enumerated above were used in the same concentration. Slant cultures were made. The color changes were the same as in the liquid media containing the same sugars. This further demonstrated that acid is produced from dextrose and saccharose, but not from maltose and lactose.

An additional series of carbon compounds were used for acid production in both liquid and solid media, using only the indicator brom thymol blue. The following carbon compounds were used in a one per cent concentration; arabinose, galactose, mannose, melezitose, raffinose, rhamnose, salicin, trehalose, and xylose. The results were the same in both the liquid and solid media. Slight acid was produced from arabinose, galactose, mannose, and xylose, indicated by the change from green to yellow color, while in the case of melezitose, rhamnose, raffinose, salicin and trehalose, the color became slightly more alkaline than the controls. A summary of acid production from the above named compounds is presented in table 1.
Table 1. Acid production from carbon compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Acid production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose</td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td></td>
</tr>
<tr>
<td>Maltose</td>
<td></td>
</tr>
<tr>
<td>Mannit</td>
<td></td>
</tr>
<tr>
<td>Mannose</td>
<td></td>
</tr>
<tr>
<td>Melezitose</td>
<td></td>
</tr>
<tr>
<td>Raffinose</td>
<td></td>
</tr>
<tr>
<td>Rhamnose</td>
<td></td>
</tr>
<tr>
<td>Saccharose</td>
<td></td>
</tr>
<tr>
<td>Salicin</td>
<td></td>
</tr>
<tr>
<td>Trehalose</td>
<td></td>
</tr>
<tr>
<td>Xylose</td>
<td></td>
</tr>
</tbody>
</table>

Azolitmin sugar agars. A series of beef-peptone agars containing azolitmin and one per cent of the following carbon compounds, dextrose, saccharose, maltose, and lactose were prepared and the organism grown on them in slant cultures. In 24 hours the medium containing dextrose was pink along the slant and in 15 days, the color had entirely disappeared from the unslanted portion of the medium, but the slant was the same color as the control. The saccharose medium showed slight pink along the slant in two days and in 15 days was similar to the dextrose series. There was no change to pink color in the maltose or lactose cultures, but the color turned...
dark blue throughout in 15 days.

**Action on starch.** Cultures of the organism growing on plates of beef-peptone agar to which starch had been added were flooded with a saturated solution of iodine in 50 per cent alcohol. The entire medium turned a dark blue color indicating that there had been no dia-static action on the starch.

**Tests for indol and ammonia.** Cultures were made in beef-peptone bouillon containing two per cent difico peptone to test for indol production. After seven days, there was no indol present when tested with potassium nitrite and sulphuric acid.

Cultures of the same medium were tested at the end of seven days for ammonia with Nessler's reagent and no positive test secured.

**Cohn's solution.** No growth occurred in Cohn's solution.

**Uschinsky's solution.** Slight growth occurred in 24 hours and in seven days, growth was good with a floc-culent surface pellicle which settled on being disturbed. A greenish-yellow pigment was produced, first in the upper layer of the medium and later throughout the liquid.

**Fermi's solution.** Growth was slightly more vigor-
ous in this medium at first than in Uschinsky's. The flocculent surface pellicle and the greenish-yellow pigment were produced as in Uschinsky's solution.

**Toleration of sodium chloride.** The organism produced slight clouding in tubes of neutral (PH 7) beef-peptone bouillon containing as high as six per cent sodium chloride. The growth of the organism was inhibited by 7 per cent sodium chloride in the same medium.

**Hydrogen-ion toleration.** A series of beef-peptone bouillons were adjusted with normal hydrochloric acid to the following PH values, 6.0, 5.5, 5.0, 4.5 and 4.0. The PH values were determined colorometrically by comparison with color standards. Tube cultures were made in each medium. The results are presented in table 2.

**Table 2. Acidity tolerance as indicated by growth in media at various hydrogen-ion concentrations.**

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>PH 6.0</th>
<th>PH 5.5</th>
<th>PH 5.0</th>
<th>PH 4.5</th>
<th>PH 4.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>good</td>
<td>good</td>
<td>light</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>growth</td>
<td>growth</td>
<td>growth</td>
<td>growth</td>
<td></td>
</tr>
<tr>
<td>2 days</td>
<td>heavy</td>
<td>heavy</td>
<td>moderate no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td></td>
<td>growth</td>
<td>growth</td>
<td>growth</td>
<td>growth</td>
<td></td>
</tr>
<tr>
<td>5 days</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>
From the above table it will be seen that the organism tolerates hydrogen-ion concentration of PH 5.0 and that the limit of tolerance is between PH 4.5 and PH 5.0.

Temperature relations.

The organism grows in a wide range of temperatures. A series of slant and plate cultures were made on potato dextrose agar and incubated at the following temperatures: 0° C, 5°-6° C, 11°-12° C, room temperature, (22°-23°), 25° C, 27.5° C, 30° C and 35° C. The organism grew slowly at 0° C, moderately at 5°-6° C and 11°-12° C, while at room temperature, 25° C, 27.5° C and 30° C, good growth occurred. At 35° C, growth was very meagre. The maximum growth occurred at 25° to 30° C.

In determining the thermal death point water suspensions of the organism in small thin walled test tubes were subjected to a ten minute exposure to a series of temperatures in an electrically controlled water bath. After the exposure, the tubes were cooled at once and tests made by loop transfers to agar slants and pouring dilution plates. A ten minute exposure at 45° and 46° C, killed a large percentage of the organisms, 47° and 48° C, left few viable organisms and 49°, 50°, and 51° C.
all were killed; therefore the thermal death point may be considered to be 49°C.

**Effect of Freezing.**

Water suspensions of the organism were made and transferred aseptically to sterile, thin-walled, test tubes, which were placed in a mixture of ice and water in a compartment of a frigidaire ice box. Plates were poured when the suspensions were placed in ice and water mixture. The suspensions did not freeze for 14 hours, but the mixture of ice and water was held at from 1°-2°C. At the end of 14 hours, the tubes froze solid and remained frozen throughout the duration of the experiment except the two cases where noted. From time to time tubes were removed, thawed out and plates poured. The approximate number of bacteria per cubic centimeter as indicated by plate counts were determined and the results are presented in table 3.
Table 3. Effect of freezing of the bacteria in water.

<table>
<thead>
<tr>
<th>Length of time frozen</th>
<th>Approximate number of bacteria per c.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (original suspension)</td>
<td>8,382,000</td>
</tr>
<tr>
<td>14 hrs. at 1°-2°C. Frozen 75 min.</td>
<td>69,375,000</td>
</tr>
<tr>
<td>10 hrs.</td>
<td>7,400,000</td>
</tr>
<tr>
<td>9 days</td>
<td>3,908,000</td>
</tr>
<tr>
<td>11 days</td>
<td>624,000</td>
</tr>
<tr>
<td>21 days</td>
<td>7,800</td>
</tr>
<tr>
<td>23 days</td>
<td>5,300</td>
</tr>
<tr>
<td>34 days</td>
<td>4,200</td>
</tr>
<tr>
<td>50 days</td>
<td>700</td>
</tr>
</tbody>
</table>

*Thawed for a few hours due to regulator sticking on the ice box control.

The above table shows that there were 700 viable bacteria per cubic centimeter after 50 days in ice, indicating that some of the organisms are very resistant to freezing. The fact that the organism grows at 0°C explains why there was a much larger number per cubic centimeter present when the second test was made than were in the original suspension. At the end of 50 days, the last of the suspensions were used and no further tests could be made.
Effect of sunlight.

Plates of beef-peptone agar were poured from a suspension of the organism and exposed to the direct rays of the sun at 1 P. M. The plates were placed on cracked ice and one-half of each plate covered with black paper. At intervals of 15 minutes plates were removed and incubated at room temperature. Plates exposed 15 minutes had a normal development of bacteria in them; while 30 minutes reduced the number of colonies developing in the unshaded portion of the plate one-half. The plates exposed 45 and 60 minutes had no bacteria developing in the unshaded portion while the shaded portion of the plates had a normal development of bacterial colonies.

Resistance to desiccation.

Water suspensions of young vigorous growing agar slant cultures were made and a two millimeter loop of this suspension was smeared on a series of sterile cover glasses in a petri dish and allowed to dry. Tests were made from time to time for viable bacteria
by dropping the cover glasses in a tube of beef-peptone bouillon and by dropping slides on an agar slant so that the smear came in contact with the surface of the medium. Tests were made just as the smears were drying, after drying 15, 23 and 60 hours respectively. From these tests, it was found that the organism survived 60 hours drying on sterile glass.

To determine the resistance to drying on seed, *Holcus sorghum* seed were placed in petri dishes, moistened and sterilized in the autoclave. Water suspensions of the organism were poured on the sterilized seed and allowed to stand until the seed were thoroughly wet. The excess suspension was then poured off and the dishes placed in a large sterilized glass culture chamber to dry. From time to time, seeds were removed from these dishes and planted in agar poured plates. Tests thus far have shown the organism to be alive after three months drying on the seed. Furthermore it has been found that the organism lives over winter on or in *Holcus sorghum* seed, showing that it is highly resistant to drying on or in the seed.
Taxonomy of the Causal Organism and its Host Range

Taxonomy.

The organism causing the disease under consideration apparently differs from any previously described. The earlier work of Bruyning (5) and Radais (19) are hardly to be considered in the taxonomy of the organism under discussion. Bruyning claimed to have isolated two chromogenic bacteria from blighted sorghum which he named Micrococcus aurantiacus sorghi, a yellow pigment producer, and Bacillus ruber ovatus producing a red pigment. He claimed that the two organisms working symbiotically produced the disease known as sorghum blight. Since the symptoms of the disease as described elsewhere are quite different and the organism concerned is in no way related to the organisms which he described, the two diseases should not be confused. Radais (19) working with apparently the same condition found no specific organism associated with the disease, but attributed it to the action of yeasts.

The organism studied is obviously different from
the spore producing organism, *Bacillus sorghi*, described by Burrill (1, 2) as causing a blight of sorghum. This is also true of the bacillus which he described later (3, 4), as causing a disease of field corn. His descriptions are rather brief and lacking in detail, making it impossible to now identify the organisms with which he was concerned.

The organism causing Holous bacterial spot, in some respects, rather closely resembles *Bacterium andropogoni* described by Smith and Hedges (25) and Smith (26) as causing "Burrill's bacterial disease of broom corn". However a careful comparison shows several important differential characters. *Bacterium andropogoni* does not liquefy gelatin, reduce nitrates or produce a green pigment; while the organism associated with Holous spot liquefies gelatin readily, reduces nitrates and produces a green water-soluble pigment.

Rosen (21, 22) described a bacterial root and stalk rot of field corn but did not describe the organism other than to say that it was a white rapidly growing bacterium. In the case of Stewart's disease of sweet corn (29) the causal organism is a yellow bacterium and a vascular parasite only. Durrell (6) found no specific parasite
associated with the purple leaf sheath spot disease which he described on corn, but found that a number of more or less saprophytic organisms might produce the disease under favorable conditions.

A bacterial blight of barley and certain other cereals caused by *Bacterium translucens* was described by Jones, Johnson and Reddy (12, 13). Later Smith, Jones and Reddy (28) described a black chaff disease of wheat caused by a variety of the above named organism (*B. translucens* var. *undulatum*). In 1924 Reddy, Godkin, and Johnson (20) described a bacterial disease of rye caused by still another variety of the same organism which they named *B. translucens* var. *secalis*. The above named organisms are all yellow bacteria and differ only in pathogenicity.

*Aplanobacter agropyri* described by O’Gara (17, 18) as causing a disease of Western wheat grass is obviously different, as well as *Pseudomonas tritici* described on wheat by Hutchinson (11). This yellow color at once differentiates them without taking up the other differences. The white bacterial organism described by McCulloch (15) as causing basal glumerot of wheat differs
materially in cultural and morphological characters from the organism associated with Holcus bacterial spot.

Manns (16) in 1909 isolated two bacterial organisms from a blade blight of oats which he claimed had a symbiotic relationship and named them *Bacillus avenae*, a white organism and *Pseudomonas avenae*, a yellow organism. Elliott (8) in 1920, has shown that Manns was probably working with mixed cultures, and she describes a white organism, *Bacterium coronofaciens*, as causing "Halo Blight" of oats, which is in all probability the same disease that Manns was working with. Since *B. coronofaciens* has failed to infect *Holcus sorghum* and *Zea mays* when inoculated in the greenhouse and the organism causing Holcus bacterial spot does not attack *Avena sativa* it is quite apparent that they are two distinct organisms. The two organisms were compared culturally and found to be different.

In 1924, Elliott (9) described a Bacterial stripe disease of proso millet caused by a white bacterium, *Bacterium panici*, which somewhat resembles the organism under discussion here. However she was not able to
produce infection on *Holcus sorghum*; pathogenicity being limited to proso millet. Culturally the organism differs from the organism causing *Holcus* bacterial spot in that it grows in Cohn's solution, non-fluorescent in Fermi's or Uschinsky's solution, and did not produce acid with either dextrose or saccharose.

Rosen (24) described a disease of foxtail (*Chaetochloa lutescens*) caused by a white bacterium which he named *Pseudomonas alboprecipitans*. He was able to artificially infect *Zea mays*, *Holcus sorghum*, *Triticum aestivum*, *Avena sativa*, *Hordeum vulgare*, and *Secale cereale* with this organism. The organism described herein has not produced infection on *Triticum aestivum*, *Avena sativa* or *Chaetochloa lutescens*. It should be explained here that the *Chaetochloa lutescens* seed were obtained from the Agronomy office, U. S. Department of Agriculture, Washington, D. C. According to Hitchcock (10) *Setaria glauca* occurring in Iowa is synonymous with *Chaetochloa lutescens*. The seed from Washington produced plants that appeared to be slightly different from the *Setaria glauca* plants occurring in Iowa. This difference was probably due to different environmental conditions. Very sparing infection was
secured on Chaetochloa lutescens of Iowa, and infection is uncommon in the field. The two organisms differ materially in cultural characters. The organism described by Rosen as *Pseudomonas alboprecipitans* differs from the organism described here in producing a colorless zone around the colonies on agar plates, no gelatin liquefaction, no pigment production in Ushinsky's or Fermi's solution or other mediums, produced diastatic action on starch, and produced no acid in the presence of carbohydrates.

This review of the cultural and morphological characters of the plant pathogens concerned in the diseases of gramineous hosts indicates clearly that the causal agent of Holcus bacterial spot is an undescribed organism.

**Technical description**

*Bacterium holci* ¹, n. sp.

Cylindrical rods with rounded ends, single or in

pairs; average measurement of individual rods 7.3 μ by
2.13 μ; motile by polar flagella (1-4); aerobic; non-
spore forming; no capsules; involution forms not ob-
served. Stains readily with Ziehl's carbol fuchsin
and gentian violet; gram-negative.

Surface colonies on agar plates are round, smooth,
glistening, raised or pulvinate, margin entire, internal
structure finely amorphous; grayish white by reflected
light; greenish fluorescent by transmitted light; pro-
duces greenish pigment in Fermi's and Uschinsky's solu-
tion, less pronounced on beef-peptone, and veal infusion
agars; no growth in Cohn's solution.

Gelatin liquefied; milk cleared in three weeks with
no coagulation nor acid production; nitrates reduced; no
indol; no gas with dextrose, saccharose, lactose, maltose,
mannit or glycerol; acid produced from arabinose, dex-
trose, galactose, mannose, saccharose and xylose; no
diastatic action on starch. No growth in PH 4.5 beef-
peptone broth, slight growth in PH 5.0 beef-peptone
broth; growth inhibited by seven per cent sodium chlo-
ride in PH 7.0 beef-peptone broth.

Grows 0°-35°C. optimum 25°-30°C.; thermal death
point $49^\circ$C.; resistant to freezing in water; slight growth after 60 hours drying on cover glasses; resistant to desiccation on *Holcus* *sorghum* seed; succumbs to 45 minutes exposure to direct sunlight.

Group number according to later chart adopted by the Society of American Bacteriologists is 5222-31121-2232. Group number according to the old bacteriological chart is 211.2323133.


**Host range of the Holcus bacterial spot.**

The hosts for this disease as determined by field observations are as follows: sorghum (*Holcus* *sorghum* L.), sudan grass (*Holcus* *sorghum* var. *sudanensis* (Piper) Hitchc.).

---

broom-corn (*H. sorghum* var. *technicus* Bailey), Johnson
grass (*H. halepensis* L.), pearl millet (*Pennisetum
glaucum* (L.) R. Br.), dent corn (*Zea mays* var. *indentata*
Bailey), sweet corn (*Z. mays* var. *saccharata* Bailey) and
foxtail (*Chaetochloa luteecens* (Weigel) Stuntz).

Greenhouse inoculations have failed to reveal any
other hosts for the disease. A large number of varieties
of *Holcus sorghum* and *Zea mays* have been infected artificial­
ly in the greenhouse.

The varieties of *Holcus sorghum* that have been found
to be susceptible either in the field or by greenhouse
inoculations are as follows: orange cane, white kafir,
red amber, black amber, mile, white durre, standard black-
hull kafir, shallu, shrock, sunrise, kaferita, dwarf he-
gari, dwarf kafir, dwarf sumac, red kafir, sumac, Kansas
orange black glume, pink kafir, jap sugar cane, higeria,
feterita, and ribbon cane, a total of 22 varieties. All
varieties tested have proved susceptible.

*Holcus sorghum* var. *sudanensis*, *H. sorghum* var.
technicus and *H. halepensis* have proved to be hosts for
the disease in the field and greenhouse. The disease
has been observed in the field on *Pennisetum glaucum* and
the same host has repeatedly responded to artificial
inoculations in the greenhouse. A few spots were observed on Chaetochloa lutescens in the field and slight infection has been secured in the greenhouse, but indications are that it is highly resistant.

The disease has been observed in the field on Zea mays var. indentata and Z. mays var. saccharata. The following varieties of Z. mays var. indentata (dent corn) have been artificially infected in the greenhouse: Reid's yellow dent, Wimple's yellow dent, St. Charles white, champion white pearl, Minnesota number 13, Pickett's yellow dent, Boone county white, lancaster sure crop, golden orange, silver king, and calico. Two varieties of Z. mays var. indurata (flint corn): rainbow flint, and Rhode Island white flint have also responded to artificial greenhouse inoculations. The disease has been observed in the field on the following varieties of Z. mays var. saccharata (sweet corn): country gentleman, howling mob and early crosby. In addition the varieties black mexican and narrow grained were artificially infected in the greenhouse. Zea mays var. everta has been artificially infected under greenhouse conditions.
The following grasses have failed to become infected under repeated greenhouse tests and the disease has not been observed on them in the field where field observations have been possible: brome grass, (Bromus inermis Leyss), English rye grass, (Lolium perenne L.), phalaris grass (Phalaris L., species undetermined), meadow fescue (Festuca elatior L.), orchard grass (Dactylis glomerata L.), tall meadow oat grass (Arrhenatherum elatius L. Mort and Koch.), timothy (Phleum pratense L.), red top (Agrostis palustris Huds.), wheat and (Triticum aestivum L.), oats (Avena sativa L.).

The following millet (Chaetochloa italicca (L.) Scribn.) varieties have failed to become infected artificially in the greenhouse: Japanese, Siberian, common, Hungarian, and brome-corn.

Relation of Parasite to Host Tissue

Pathogenicity studies.

During the past year a large number of inoculation experiments have been made under greenhouse conditions upon various species of the family Gramineae. A large number of strains of the organism have been used, but
three strains were used consistently throughout the course of these tests. One strain was isolated from Zea mays var. indentata in the Fall of 1924, one from a seedling Holcus sorghum plant, and one a reisolation from H. sorghum var. sudanensis which had been previously inoculated with the strain from Zea mays. Reisolations have been made repeatedly and the identity of the causal organism determined. These three strains as well as others have been used in the study of the cultural characters of the organism. During the past season, a large number of fresh isolations have been extensively used in greenhouse inoculations and compared culturally with former strains of the organism.

The method used in making inoculations was to rub the leaves of the plants between wet fingers and then with an atomizer, spray a water suspension of the organism on the plants. The best results were obtained by using cultures 24 to 30 hours old. As soon as the plants were sprayed, they were placed in a large glass chamber having a layer of wet sphagnum moss in the bottom. The incubation period was found to be from two to three days. After being in the inoculation chamber two days, the plants were removed to a greenhouse bench. All plants
were grown in pots to facilitate moving from one place to another.

Inoculation tests on Zea mays have usually been made on young plants, and successful results have not always been obtained. When older plants were available for inoculation trials, more consistent results were obtained. Greenhouse studies and field observations indicate that Z. mays is more susceptible in its later stages of development, but doubtless environmental factors play an important role in the severity of the disease on this host. Holcus sorghum, H. sorghum var. sudanensis and Pennisetum glaucum are very susceptible in all stages, and markedly so in the seedling stage.

On October 15, 1924, 30 young Zea mays var. indentata plants were thoroughly sprayed with a suspension made from a culture of the organism isolated from Z. mays. The plants were incubated in a large glass cage. Three days after they were inoculated, 21 of the 30 plants showed abundant evidence of bacterial infection in the form of round, oblong or irregular water-soaked areas on the leaves. The 28 plants sprayed with sterile water as controls remained healthy.

Again on Oct. 22, 30 young Zea mays var. indentata
plants were sprayed with a suspension of the same strain and only three showed evidence of infection, while 19 young plants inoculated six days later showed no infection. On Nov. 5, three series of inoculations were made on young plants of Zea mays, Holcus sorghum, H. sorghum var. sudanensis, Bromus inermis, Triticum aestivum and Avena sativa using the organism mentioned above, one from Holcus sorghum and a reisolation from Zea mays. In two days, all Zea mays plants showed typical water-soaked lesions on the leaves as well as the Holcus sorghum and H. sorghum var. sudanensis. The lesions appeared on the last two named hosts as round, oblong to water-soaked areas, with a reddish green color which soon turned to red, or light centered, red bordered lesions. All three organisms produced similar lesions and the organism was recovered from all hosts. Triticum aestivum, Avena sativa and Bromus inermis showed no evidence of infection.

Another series of inoculations were made on Dec. 31, including Holcus sorghum, H. sorghum var. sudanensis, H. halepensis, Arrhenatherum elatius, Dactylis glomerata, Lolium perenne, Phalaris species, Agrostis palustris, Triticum aestivum, Phleum pratense, Festuca elatior and
and Bromus inermis. Two days later, typical lesions were evident on the leaves of Holcus sorghum, *H. sorghum* var. *sudanensis*, and *H. halepensis*, but no indication of infection on the other grasses. The lesions on *H. halepensis* were the same as those on *H. sorghum* var. *sudanensis*. During the period from Jan. 29, to Feb. 9, the same series of plants were inoculated at four different times, fresh Holcus sorghum, *H. sorghum* var. *sudanensis* and *H. halepensis* plants being substituted in the series each time. Two strains of the organism were used. In each case, two days after inoculation, reddish-green water-soaked, round oblong to irregular lesions appeared abundantly on the leaves of the Holcus species but no evidence of infection on the other grasses appeared in any cases.

Numerous inoculations were made during the past year on Holcus sorghum and *H. sorghum* var. *sudanensis* to test out the pathogenicity of reisolations. In practically every case, typical bacterial lesions resulted.

A series of plants including Holcus sorghum, *H. sorghum* var. *sudanensis*, Pennisetum glaucum, Chaetochloa lutescens, and the following varieties of *C. italic*: 
Japanese, Siberian, Hungarian and common were repeatedly inoculated with the different strains of the organism. In every case, typical infection occurred on the Holcus species and Pennisetum glaucum but none on the varieties of Chaetochloa italica. The lesions appeared on the leaves of Pennisetum glaucum as round, oblong, to linear irregular dark green water-soaked areas, which later became dark brown with a slight light greenish halo. There was no evidence of red color and the lesions were typical of those noted on plants growing in the field. This host was even more susceptible under greenhouse conditions then the Holcus species.

In order to test out the susceptibility of the different varieties of Zea mays, a series of inoculations were made on Mar. 23, including the following varieties: (Z. mays var. indentata) blue flower, Minnesota number 13, Io Jap striped, Boone county white, lancaster sure crop, silver king, golden orange, northwestern dent, Wimple’s yellow dent, St. Charles white, Hackett’s yellow dent, champion white pearl, and calico; (Z. mays var. indurata) rainbow flint and Rhode Island white flint; (Z. mays var. saccharata) howling mob, golden bantam and black mexican;
(Z. mays var. everta) yellow pearl and jap hulless. Holcus sorghum and H. sorghum var. technicus were included in this series to test the viability of the cultures used. All plants were in the seedling stage, and were divided into parallel series, using two strains of the organism. After three days incubation, there was abundant infection on Holcus and slight infection on the following varieties of Z. mays var. indentata: Boone county white, lancaster sure crop and golden orange. No evidence of infection occurred on the other varieties of Zea mays. The same plants were inoculated in parallel series again in 15 days, after the plants were 12 to 18 inches high. In addition Pennisetum glaucum and the following varieties of Chaetochloa italic: common, Hungarian, Siberian, Japanese and broom-corn were included. After the usual incubation period, good infection showed on the Holcus species and Pennisetum glaucum. Slight infection occurred on the following varieties of Zea mays var. indentata: Wimple's yellow dent, champion white pearl, St. Charles white, Minnesota number 13 and Pickett's yellow dent; Z. mays var. indurata, rainbow flint and Rhode Island white flint; Z. mays var. saccharata, golden bantam.
Other pots of the varieties of *Zea mays* mentioned above were allowed to grow until they were in the tasseling stage. The plants never reached normal development due to growing in pots and were short and somewhat stunted. On May 7, a series of these plants including the varieties named in the previous series were inoculated and after two days incubation, abundant evidence of infection showed on all varieties.

The following varieties of *Holcus sorghum* were tested in the greenhouse as to their relative susceptibility: orange cane, white kafir, red amber, black amber, milo, white durra, standard black-hull kafir, shallu, shrock, sunrise, kaferita, dwarf hegeria, dwarf kafir, dwarf sumac, red kafir, sumac, Kansas orange, pink kafir and the two varieties of *H. sorghum* var. technicus, evergreen and acme. All proved susceptible. Under greenhouse conditions the orange cane, Kansas orange, shallu and the two varieties of broom-corn were perhaps slightly more susceptible than the others.

Fresh isolations were made from *Zea mays* var. seccharata and *Chaetochloa lutescens* on June 25, 1925. These cultures proved pathogenic to *Holcus sorghum* and *Zea mays* and slight infection was produced on *Chaetochloa lutescens*. 
Numerous fresh isolations from *Holcus sorghum*, *H. sorghum* var. *sudanensis*, *H. halepensis*, *Pennisetum glaucum* and *Zea mays* made during July yielded a similar white bacterial organism which appeared to be identical to the organisms isolated previously, and their pathogenicity proven by greenhouse inoculations.

On July 16, the lower leaves of plants of *Zea mays* var. *saccharata*, golden bantam, in a home garden and several selections of *Z. mays* var. *indica* in an experimental plot were sprayed with two strains of the organism. The weather was dry and very unfavorable for infection at this time. However, in four to five days, typical lesions were produced on a few of the lower older leaves in all cases.

A general summary of the pathogenicity studies shows that a transmissible bacterial leaf spot disease occurs on *Holcus sorghum*, *H. sorghum* var. *sudanensis*, *H. halepensis*, *Pennisetum glaucum*, *Zea mays* and *Chaetochloa lutescens*. Cultures of the organism from these different hosts have been repeatedly crossed from one host to the other. Consistent infection has been secured on the *Holcus* species and *Pennisetum glaucum*, but *Zea mays* has not responded as readily under greenhouse conditions.
as the first named hosts. Field observations have shown abundant infection on the lower leaves of *Zea mays* plants after June 25. Indications are that the age of the *Z. mays* plants and the environmental factors play an important part in the susceptibility of this host. *Chaetochloa lutescens* has shown only slight susceptibility in the field and greenhouse.

**Infection**

Since infection occurs when suspensions of the organism are sprayed on the leaves without wounding the tissues, it seems likely that infection takes place through the stomata. Apparently the bacteria cause a necrosis of the tissues within a few hours after entering the plant. An examination of the lower and upper epidermis of the leaves showed an abundance of stomata on both surfaces. The bacteria are at first apparently intercellular but soon cause a collapse of the tissues and become intracellular.

When young lesions were cut in drops of water on a slide and examined under the microscope, bacteria in abundance oozed from the intracellular spaces as a wavy, slow moving, cloud-like mass. Such sections showed that
in the case of the Holcus species the bacteria were usually limited to a rather small central portion of the necrotic area. The red coloration associated with the lesions on Holcus species is a host reaction, apparently responsive to the wound produced by bacterial invasion. This color usually extends through the tissues immediately surrounding the wound. The lesions appeared in cross-sections as saucer-shaped sunken areas.

The presence of considerable moisture is apparently necessary for infection. Plants inoculated in a dry atmosphere resulted in only meagre infection. Natural infection in the field also occurs rarely during periods of dry weather.

Seed transmission.

While examining Holcus sorghum plants in the field in the Fall of 1924, many glumes were noted with red spots on them which resembled those on the leaves. This suggested the possibility that the causal organism might be carried over from year to year in this way. Several heads were cut from infected plants in this field and brought into the laboratory.
Seed from the above named heads were planted in pots of soil in the greenhouse. A red spot was noted on the first leaves of a few of the seedling plants (Pl. III). These spots resembled the smaller spots noted on the leaves in the field. Isolations were made from these lesions in the usual way and a white bacterial organism appeared in the plates that was apparently identical to the ones formerly isolated from the leaf lesions collected in the field. The organism secured later proved to be pathogenic when sprayed on species of Holcus in the greenhouse.

On January 27, 1925 a few seed from the same source were planted in a pot of soil which had been sterilized in an autoclave at 25 pounds pressure for 30 minutes on three consecutive days. Among the 17 seedlings grown in this soil, one showed a typical lesion on the first leaf identical to the lesions previously noted on seedlings in unsterilized soil.

A small package of Holcus sorghum seed of the white milo variety were secured from Dr. L. W. Durrell, of the Colorado Agricultural Experiment Station early in January 1925. Many of these seeds showed red lesions on the seed coat. An attempt was made to select diseased and healthy
seed from this packet. The two selections were planted on January 27, 1925 in pots of soil that had been sterilized in the autoclave as previously noted. A third planting was made from general seed from the same packet. Fifty-eight seeds were selected that had red lesions and 60 that were apparently free from such lesions. From the 58 seeds, 54 seedling plants were produced and one plant showed a definite bacterial lesion on the first leaf. The 60 seeds produced 56 plants and two had similar lesions on the first leaf. From the plantings of general seed, 156 seedlings resulted, and nine had lesions on the first leaf similar to those on the other plants. A total of 266 plants were grown from this seed and 12, or 4.5 per cent showed first leaf infection. This primary infection consists of small round or oblong, red, or red bordered, light centered spots. They may be located in the middle near the center or on the margin near the center of the first leaf.

On May 13, 1925 a similar seed test was made with *Holcus sorghum* of the black and red amber varieties with seed secured from the Iowa State College Seed Laboratory. The age and source of the seed could not be determined. Of the 188 seedlings from the black amber variety, eight
showed typical first leaf lesions, while four of the 219 red amber seedlings showed similar lesions.

Similar tests in pots of sterile soil were made on May 19, and June 1, 1925, using a large number of varieties. The seed were from different sources. The results are presented in table 4.
Table 4. *Holcus sorghum* seed test of May 19, 1925.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Source of Seed</th>
<th>No. :Infecting:</th>
<th>Per. :Infecting:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black amber</td>
<td>I.S.C. Seed Laboratory</td>
<td>169</td>
<td>0</td>
</tr>
<tr>
<td>Red amber</td>
<td>&quot;</td>
<td>159</td>
<td>1</td>
</tr>
<tr>
<td>Orange cane</td>
<td>&quot;</td>
<td>193</td>
<td>0</td>
</tr>
<tr>
<td>White durra</td>
<td>&quot;</td>
<td>124</td>
<td>0</td>
</tr>
<tr>
<td>Dakota red</td>
<td>Kansas</td>
<td>197</td>
<td>0</td>
</tr>
<tr>
<td>Black amber</td>
<td>&quot;</td>
<td>152</td>
<td>0</td>
</tr>
<tr>
<td>Red amber</td>
<td>&quot;</td>
<td>145</td>
<td>1</td>
</tr>
<tr>
<td>Kansas orange</td>
<td>&quot;</td>
<td>159</td>
<td>0</td>
</tr>
<tr>
<td>White milo</td>
<td>Colorado</td>
<td>189</td>
<td>10</td>
</tr>
</tbody>
</table>

*Holcus sorghum* seed test of June 1, 1925.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Source of Seed</th>
<th>No.</th>
<th>Per.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dwarf hegari</td>
<td>Kansas</td>
<td>84</td>
<td>2</td>
</tr>
<tr>
<td>Kansas orange</td>
<td>&quot;</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>Shrock</td>
<td>&quot;</td>
<td>134</td>
<td>0</td>
</tr>
<tr>
<td>White Africa</td>
<td>&quot;</td>
<td>125</td>
<td>0</td>
</tr>
<tr>
<td>Dorso</td>
<td>&quot;</td>
<td>133</td>
<td>0</td>
</tr>
<tr>
<td>Sumac</td>
<td>&quot;</td>
<td>145</td>
<td>0</td>
</tr>
<tr>
<td>Dwarf kafir</td>
<td>&quot;</td>
<td>93</td>
<td>0</td>
</tr>
<tr>
<td>Reed kafir</td>
<td>&quot;</td>
<td>89</td>
<td>9</td>
</tr>
<tr>
<td>Dwarf sumac</td>
<td>&quot;</td>
<td>133</td>
<td>0</td>
</tr>
<tr>
<td>Sunrise</td>
<td>&quot;</td>
<td>117</td>
<td>0</td>
</tr>
<tr>
<td>Black hull kafir</td>
<td>&quot;</td>
<td>91</td>
<td>0</td>
</tr>
<tr>
<td>X sourless</td>
<td>&quot;</td>
<td>174</td>
<td>5</td>
</tr>
<tr>
<td>Kaferita</td>
<td>&quot;</td>
<td>169</td>
<td>5</td>
</tr>
<tr>
<td>Pink kafir</td>
<td>&quot;</td>
<td>134</td>
<td>7</td>
</tr>
<tr>
<td>Red kafir</td>
<td>&quot;</td>
<td>248</td>
<td>4</td>
</tr>
<tr>
<td>Kansas orange</td>
<td>&quot;</td>
<td>152</td>
<td>1</td>
</tr>
</tbody>
</table>

From the above tests, which were made well beyond the time when *Holcus sorghum* seed are planted, it is very evident that the organism may survive from one
season to the next in or on the seed. A total of 11 out of 25 tests resulted in diseased seedlings, varying from 0.6 to 10.1 per cent. The white milo seed tested on May 19, were from the same packet that were tested on Jan. 27, 1925. The early test showed 4.5 per cent of infected seedlings while the latter test showed 5.3 per cent. In all cases where the seedlings were held for some time after the initial infection, secondary infection occurred on the upper leaves.

Overwintering.

From the above discussion, it is apparent that the causal organism may overwinter in or on *Holcus sorghum* seed. In testing the effect of freezing on the causal organism, it was found that it survived 50 days freezing in water. In the course of the 50 days, the suspensions were thawed only twice for a very short time. The organisms that survived the 50 days freezing were equally as vigorous on culture media as those that were carried under normal laboratory conditions. Laboratory tests also showed that the bacteria grew at 0°C. These two facts indicate that the organism might easily survive the winter in the field in old infected plants or on
decaying organic matter. It is entirely possible that during a period of warmer weather, the organism continues growth on the dead and decaying host plants in the soil.

Field observations during the spring of 1925 at Ames, Iowa, indicate that the organism does live over in the soil. Late in the Fall of 1924, an examination was made of the forage crop experimental plots at Ames. Holcus bacterial spot was found abundantly on Holcus sorghum, H. sorghum var. sudanensis and Pennisetum glaucum. These same plots were again used for forage crops in 1925 and observations were made on the plants in the seedling stage before much if any secondary infection could have taken place. The majority of the Holcus varieties were planted in hills, 18 to 24 inches apart in two foot rows. There were three to eight plants per hill. On June 13, 1925, the plots were first examined for bacterial infection. The plants were six to ten inches high and still had the first leaves attached to the plants. Typical round, oblong to irregular red lesions were noted on the first leaves of a considerable number of plants. In nearly every case where infection occurred in a hill, a spot could be found on the first leaf of one or more plants. The infected hills
in each variety were counted and the data presented in table 5.

Table 5. Seedling infection in *Holcus sorghum*
plots, June 13, 1925.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Total</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jap sugar cane</td>
<td>55</td>
<td>37</td>
<td>67.3</td>
</tr>
<tr>
<td>Evergreen broom-corn</td>
<td>46</td>
<td>12</td>
<td>26.1</td>
</tr>
<tr>
<td>Paterita</td>
<td>36</td>
<td>9</td>
<td>25.0</td>
</tr>
<tr>
<td>White kafir</td>
<td>43</td>
<td>14</td>
<td>30.2</td>
</tr>
<tr>
<td>Nigeria</td>
<td>53</td>
<td>15</td>
<td>28.3</td>
</tr>
</tbody>
</table>

Besides the above there were several other varieties planted in the drill in adjacent rows and the plants were not counted. *Holcus sorghum*, varieties ribbon cane and orange cane, *H. sorghum var. sudanensis* and *Pennisetum glaucum* were all in rows in such large numbers that counts were not attempted, but examination showed approximately as heavy seedling infection as in case of the above counted varieties. Some of this infection very likely came from infected seed, but since in the numerous seed tests in sterile soil, never more than ten and usually only one to two per cent were found infected, it is quite evident that a relative large per cent of the seedling infection in these plots came from the soil.
No evidence of the organism being harbored on seed of *Zea mays* has been obtained, since seedling infection has not been noted. On June 25, 1925, bacterial lesions were observed on the lower leaves of *Z. mays* var. *saccharata* plants in an experimental field at Ames, Iowa. The plants were about ready to tassel. On further examination of *Z. mays* var. *indentata* fields near Ames the following week, several were found where the plants had similar lesions in abundance on the lower leaves. Since there is no evidence that the *Z. mays* seed carry the causal organism, and considering the way the first infection occurred, the primary lesions very likely came from the soil.

**Control.**

As a possible control measure, it is suggested that a crop rotation be used for *Holcus sorghum*, *H. sorghum* var. *sudanensis* and *Pennisetum glaucum*, and all volunteer *Holcus* plants be destroyed in *Zea mays* fields.
Summary

The Holcus bacterial spot herein described is a leaf spot disease of Holcus sorghum (sorghum), H. sorghum var. sudanensis (sudan grass), H. halepensis (Johnson grass), Zea mays (corn), Pennisetum glaucum (pearl millet) and Chaetochloa lutescens (foxtail).

The disease has been known to occur in Iowa every year since 1916.

The spots vary in size and shape. They are round, oblong to irregular, often converging to cause large necrotic areas or the death of the entire leaf. Lesions on Holcus sorghum, H. sorghum var. sudanensis and H. halepensis usually have a light brown center with a wide red border. Sometimes the spots are red throughout. On H. sorghum variety shalu, the lesions have a light brown center with a dark brown border, while on Pennisetum glaucum they are a dark brown with a narrow light green halo.

The lesions on Zea mays have light to darker brown centers with a narrow reddish brown border. Marginal infection often results in necrotic areas along the edge of the leaf.
The causal organism is an undescribed white, fluorescent, cylindrical rod, polar flagellate bacterium for which the name *Bacterium holo* n. sp. is suggested. The organism grows from 0°C to 35°C, optimum 25°C to 30°C; resistant to freezing; susceptible to drying on glass, but resistant to desiccation on *Holcus sorghum* seed.

Field observations and greenhouse inoculations have revealed the following as hosts for the disease: 22 varieties of *Holcus sorghum* and *H. sorghum* var. *technicus*, *H. sorghum* var. *sudanensis*, *H. halopensis*, *Pennisetum glaucum*; 11 varieties of *Zea mays* var. *indentata*; two varieties of *Z. mays* var. *indurata*; six varieties of *Z. mays* var. *saccharata*; *Z. mays* var. *everta* and *Chaetochloa lutescens*.

Infection is apparently stomatal. The organism is at first intercellular, but soon cause a collapse of the tissues and becomes intracellular. The red color produced in the case of the *Holcus* species is a host reaction resulting from the wound produced by the invading bacteria.

A total of 26 tests of *Holcus sorghum* seed made in sterile soil, including 23 varieties and selections,
resulted in seedling infection of from 0.6 to 10.1 per cent in 11 of the 26 tests. The causal organism may be carried over winter in or on Holcus sorghum seed. Evidence is presented which indicates that the organism also overwinters in the soil.
Literature Cited

(1) Burrill, T. J.


(5) Bruyning, F. F. Jr.

(6) Durrell, L. W.

(7) Elliott, Charlotte


(10) Hitchcock, A. S.
(11) Hutchinson, C. H.


(14) Kellerman, W. A. and W. T. Swingle

(15) McCulloch, Lucia

(16) Manna, T. F.

(17) O'Gara, F. G.


(19) Radais, Maxine

(20) Reddy, C. S., J. Godkin, and A. G. Johnson
(21) Rosen, H. H.
(22) 1921. Further observations on a bacterial root and stalk rot of field corn. Phytopath. 11: 74-79.
(28) L. R. Jones and C. S. Reddy.
1919. The black chaff of wheat. Sci. n. s. 50: 48.
(29) Stewart, F. C.
Plate I.

Bacterial lesions on Holcus species and Pennisetum glaucum resulting from natural infection in the field.

A. Leaf of Holcus sorghum (variety shallu) showing the light centered, dark brown bordered lesions and the rather large necrotic areas resulting from the converging of the spots. Marginal lesions are also shown.

B. H. sorghum (variety orange cane) leaf showing the various types of lesions. Some are small red sunken dots, while others are larger, round, oblong to irregular with parchmentlike center and light red border.

C. H. sorghum var. sudanensis leaf showing lesions similar to B.

D. Leaf of Pennisetum glaucum showing dark brown round to oblong lesions of various size.
Plate II.

Tips of lower *Zea mays* leaves showing the lesions resulting from natural infection in the field. Photographed by transmitted light.

A. Leaf showing small and large lesions as well as necrotic marginal areas. The brown centers with the darker brown border and the narrow halo are evident in the larger lesions. The smaller light colored lesions resemble somewhat the lesions produced artificially in the greenhouse.

B. Old and incipient lesions of various size on the same leaf. The darker areas are incipient lesions, and are dark green water-soaked with a narrow light colored halo.
Plate III.

Seedling *Holcus sorghum* plant grown in sterile soil showing primary lesion in the center of the first leaf. The lesion has a very small light center and a wide red border.
Plate III.
Plate IV.

Causal organism stained by Flimmer's method to show polar flagella. The light outer area surrounding the dark center is the result of the focus and not a capsule as it appears. Photomicrograph x 1500.
Plate IV.
Plate V.

Four day old plates of the causal organism on beef-peptone dextrose agar and two day old transfer to the same medium. These are plates from dilution two and three made by surface sterilizing a Zea mays lesion in mercuric chloride and crushing in sterile water.