BACTERIAL LEAF BLIGHT AND STALK ROT OF CORN[1]

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INTRODUCTION

A bacterial disease of dent corn (*Zea mays* L.) different from any previously described has occurred sporadically in several Southern and Central States in the past 20 years. It was reported first from Alabama (*5*).[2] In 1928, specimens of the diseased corn were received from S. H. Gibbons, county agent of Baldwin County, Ala., and the disease was found in Virginia shortly thereafter in the same year. In later years it was found in Georgia, Texas, Kansas, and Nebraska (*6*, *11*, *19*, *20*, *21*). Fortunately, the disease has never assumed major importance, although in a few cases it has been rather severe in localized areas. A very similar disease has been reported from Australia by Ludbrook (*10*).

Most varieties of dent corn seem to be rather resistant, whereas some lines of popcorn (*Zea mays* var. *everta* (Sturtev.) Bailey) have been reported as especially susceptible.[3] Sweet corn (*Z. mays* var. *saccharata* (Sturtev.) Bailey) also is susceptible. The purpose of the present paper is to describe the disease more fully and to report the identification of the causal organism.

DESCRIPTION OF THE DISEASE

The disease occurs usually in localized areas in the field, as a leaf blight and as a rot of the upper part of the stalks. Both manifestations may occur on the same plants.

On the leaves the lesions are variously scattered and rather sharply delimited; they range from small, elliptical or oblong spots about 1 by 2 millimeters to long, necrotic stripes 2 to 5 by 100 to 400 millimeters or longer (pl. 1). These longer lesions may coalesce and form extensive necrotic areas that may involve the entire width of leaves or considerable portions of them. Later the badly diseased leaves may become much shredded (fig. 1, *A* and *B*), especially in stormy weather.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 731.

[3] S. M. Pady in correspondence.

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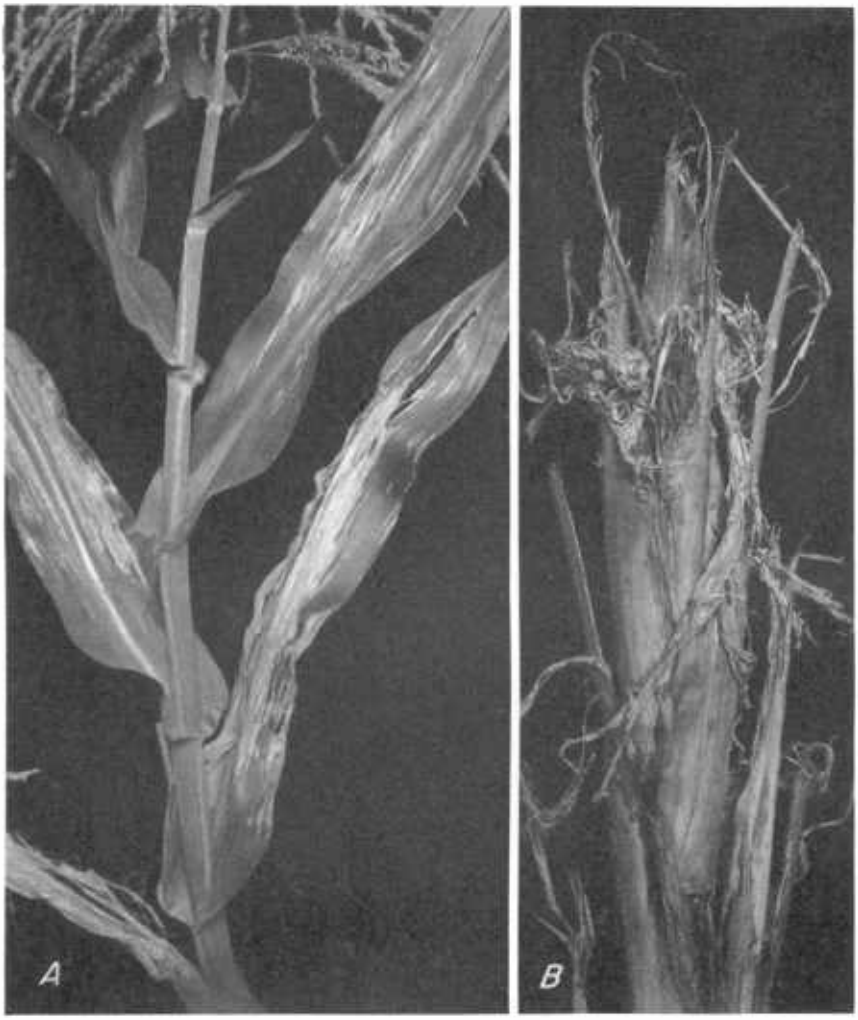


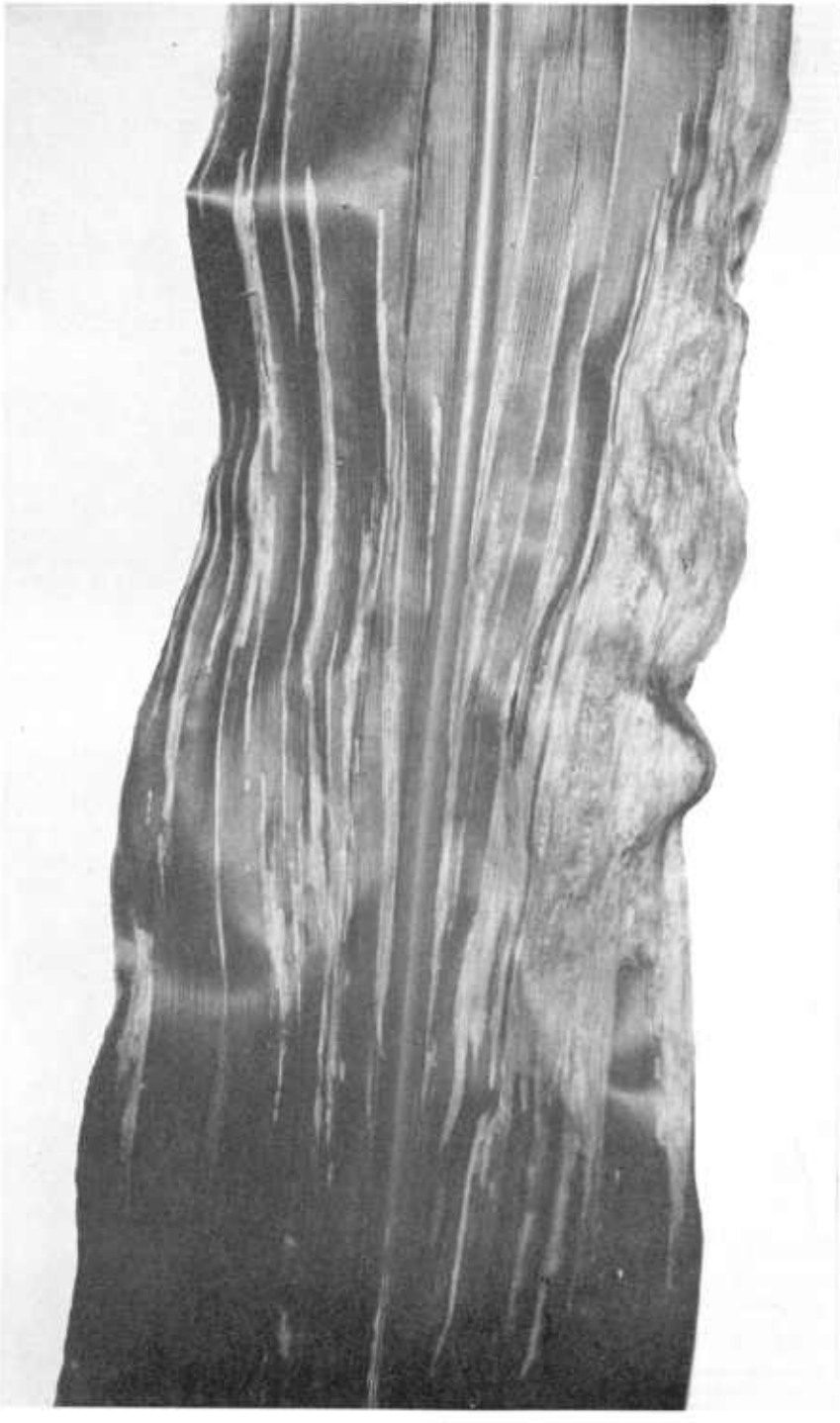
FIGURE 1.—Shredding of corn leaves naturally infected with the leaf blight and stalk rot bacterium: *A*, Early stage, after a heavy rain, Arlington Experiment Farm, Arlington, Va.; *B*, extreme stage, Bay Minette, Ala.

At first the lesions are water-soaked and "olive-green";[4] later the tissues of their centers collapse and bleach first to "warm buff" and later to "light buff" with a delicate edging of "sepia" to "cinnamon-buff." All the leaf lesions are more or less translucent. Sections from leaf lesions examined microscopically in water show abundant streaming of motile bacteria.

The stalk rot occurs usually at or just above the point where the ears are produced, causing a dark-brown to black rot of the internodes and nodes (fig. 2). The outside of the stalk may be discolored with

[4] Throughout the paper, quoted colors are from Ridgway (*12*).

Bacterial Leaf Blight and Stalk Rot of Corn



Upper surface of corn leaf naturally infected with the leaf blight and stalk rot bacterium, showing characteristic lesions, Arlington Experiment Farm, Arlington, Va.

FIGURE 2.—Interior of the upper portion of a cornstalk naturally infected with the leaf blight and stalk rot bacterium, showing the rotting above the ear node, Bay Minette, Ala.

brownish-red streaks. Inside, the rot is black and usually without a particularly foul odor. The pith tissue becomes shredded, as the rot destroys the parenchyma, and leaves the bundles more or less intact. As the rot progresses, the tops die and bleach just before or at the time the tassels emerge (fig. 3, *B*). Most of the plants thus attacked are dwarfed and often develop multiple ears (fig. 3, *A*), all of which are usually sterile and some of which may become rotted.

FIGURE 3.—Tops of corn plants naturally infected with the leaf blight and stalk rot bacterium, showing leaf lesions and multiple ears (*A*) and leaf lesions and killed, bleached top (*B*); both from Arlington Experiment Farm, Arlington, Va.

THE ORGANISM

ISOLATION

The organism has been isolated repeatedly from leaf lesions and also from rotted stalks. Lesions on the leaves were surface-sterilized in 1:1,000 mercuric chloride solution for 30 seconds, washed in sterile water, shredded on a sterile slide, and put into beef-peptone broth. After a few minutes, dilution plates from this were made, with beef-peptone agar as the medium. Isolations from rotted stalks were made by aseptically cutting out bits of the infected tissue along the margins and shredding them into beef-peptone broth. Dilution plates from this were made, with beef-peptone agar as the medium. All plates were stored at room temperature (about 25° to 30° C.), and within 48 hours they showed an abundance of small white bacterial colonies that appeared almost in pure culture. Transfers were made from single colonies.

CULTURAL CHARACTERS

Unless otherwise stated, the incubation temperature at which the organism was grown was 30° C. and the reaction of the media was about pH 7.

Beef-peptone agar plates.—In dilution plates, after 48 hours colonies of the organism freshly isolated from the host are circular, 1 to 4 millimeters in diameter, convex, smooth, white by reflected light, bluish white by obliquely transmitted light, glistening, with margins entire or becoming irregular. In most cases there is a fine cross hatching throughout the colony, but in others there are not definite internal markings. Most of the colonies show a definite clearing of the medium immediately around them; this is accentuated by a heavy clouding of the medium at the outer edge of the cleared area. Others show no clearing. As the medium dries out the colonies become almost invisible.

The smooth colonies that were isolated first were not stable and eventually dissociated into smooth and rough colonies of several types. Some of the rough colonies were irregular in outline, glistening, with or without clearing of the medium, and without characteristic internal markings. Others were round, slightly pebbly rough on the surface, and glistening with coarsely cross-hatched internal markings and slightly fimbriated margins extending into a cleared area around them. Some colonies had a pronounced thickening in the center, which caused a noticeable hump on the surface, coarse inner markings, and a clearing of the medium around them. The margins of many of the smooth colonies were entire at first and later became irregularly lobed or definitely fimbriated, extending in a lacelike pattern into the cleared portion of the medium.

Beef-peptone agar slants.—Growth after 48 hours is moderate, filiform, glistening, white to bluish white, with margins entire or becoming undulated and thin, and in most cases with noticeable clearing of the medium surrounding the streak. The surface ranges from smooth to pebbly rough.

Beef-peptone broth.—After 24 hours cultures show heavy clouding and moderate sediment, which disperses throughout the liquid in slightly stringy whorls when the tube is gently agitated. In older cultures the sediment is heavier and more stringy and clings to the bottom of the tube. There is no pellicle or ring.

Potato cylinders.—After 48 hours there is a wet almost invisible growth and the medium is firm but buff-colored above the water. After 10 days the growth is heavier, dark cream to light brown in color, creamy in texture, and rugose, and the part of the potato cylinder above the water is light brown and softened.

Uschinsky's solution (*9*).—Growth is very heavy after 48 hours, with a heavy pellicle, which falls easily to the bottom of the tube when it is agitated and leaves a slight, irregular ring on the tube at the top of the liquid. At the end of 3 weeks the surface growth is so heavy that strands hang down from the pellicle almost to the bottom of the tube. There is no change in the color of the medium.

Fermi's and Cohn's solutions (*9*).—There is no growth at 20°, 25°, 30°, or 35° C.

PHYSIOLOGICAL CHARACTERS

Carbon metabolism.—The slightly modified formula for Ayers, Rupp, and Johnson's synthetic carbohydrate media (*23*) was used. The alcohols and sugars were added at a concentration of 1 percent and the media were sterilized by steaming 1 hour in an Arnold sterilizer on each of three successive days. Before sterilization bromcresol purple was added as an indicator. The color change from purple to yellow was used as the index of fermentation. An accurate final change in the pH was recorded by the use of a Beckman pH meter. Durham fermentation tubes were used so that the production of gas could be observed at the same time. Six representative cultures, inoculated in duplicate, were used in the tests, which were made three times. Acid was produced with all of the carbohydrates tested, namely, glycerol, saccharose, insulin, raffinose, lactose, maltose, dextrose, galactose, mannitol, and levulose. Gas was not produced.

Temperature relations.—The optimum temperature for growth is 30° C. The maximum temperature at which tests were made with beef-agar slants was 40°. The bacterium grew at this temperature. There was slight growth at 10°, but none at 5°. The thermal death point is 52°.

Relation to free oxygen.—Growth occurs only at the surface or just under the surface of rolled cultures in beef-agar tubes, indicating that the bacterium is an aerobe.

Nitrate reduction.—When Bacto-beef-peptone agar with 0.1-percent potassium nitrate added is the medium for growing the bacterium, there is a rapid reduction of nitrate.

Indole production.—Indole is not produced when the organism is grown in Bacto-tryptone broth. The Ehrlich-Böhme and Gnezda methods (*24*) both gave negative tests.

Ammonia production.—Ammonia is produced in beef-peptone agar and beef-peptone broth. Tests with Nessler's solution were positive after 2 days' growth.

Hydrogen sulfide.—When the test-strip method of ZoBell and Feltham and also the lead acetate agar test (*24*) were used, hydrogen sulfide was formed to a very moderate degree.

Hydrolysis of starch.—Starch is hydrolyzed. Streak inoculations on beef-peptone agar containing 0.2 percent soluble starch grew slowly, but after 10 days a clear zone showed outside the area of growth when the plates were flooded with Lugol's iodine.

Reaction in milk.—Milk is slowly peptonized without coagulation. After 7 days there was a complete reduction of litmus in litmus milk, and after 1½ months the deep-blue color of the litmus had returned. When bromcresol purple was used as an indicator, there was a gradual increase of the purple color, indicating increase in alkalinity. After a month the milk had become completely peptonized, leaving a stringy sediment in the bottom of the tube. No curd was formed.

Gelatin liquefaction.—Gelatin is liquified. On Bacto-nutrient gelatin plates at 25° C., there was definite liquefaction by all cultures after 5 days. Utilization of gelatin was demonstrated also by a modified method of Frazier (*4*) in which 4-percent gelatin was used in beef-peptone agar plates. In test tubes liquefaction was too slow to be detected.

MORPHOLOGY

The bacteria are in the form of short rods, rounded at ends, 0.3μ to 0.7μ by 0.6μ to 1.5μ, averaging 0.6μ by 1.0μ. These ranges and averages are based on the measurement of 50 cells from each of 6 different cultures stained with Ziehl's carbolfuchsin. The rods occur singly, in pairs, or in chains of 3 to 10 cells; they are motile by a single polar flagellum, demonstrated by Casares-Gil's staining method. Capsules can be seen by the use of either Anthony's method or Huntoon's method (*22*). There are no spores, and the cells are not acidfast. The bacteria are Gram-negative when the cultures are grown on beef agar for 1 to 4 days.

INOCULATIONS

The pathogenicity of the bacterium isolated repeatedly from diseased leaves and stalks of corn from different areas was established by artificial inoculations on the following hosts and reisolations of the typical organism from them: Illinois Yellow Dent and Alabama Strawberry dent corn, Golden Bantam and Adams Extra Early sweet corn at Washington, D. C., and Arlington Experiment Farm, Arlington, Va., and U. S. Hybrid 13 dent corn, Golden Giant and Golden Cross Bantam sweet corn, Fulcaster wheat (*Triticum aestivum* L.), Appler oats (*Avena sativa* L.), Oderbrucker barley (*Hordeum vulgare* L.), Abruzzes rye (*Secale cereale* L.), Honey sorgo (*Sorghum vulgare* Pers.), Tift Sudan grass (*Sorghum vulgare* var. *sudanense* (Piper) Hitchc.), *Setaria lutescens* (Weigel) F. T. Hubb. (*Chaetochloa lutescens* (Weigel) Stuntz), and *S. geniculata* (Lam.) Beauv. (*C. geniculata* (Lam.) Millsp. and Chase), at Plant Industry Station, Beltsville, Md. No experiments were conducted to determine the relation of temperature to infection, but the best infections were obtained when greenhouse temperatures were relatively high, about 85° to 95° F. When the temperature was as low as 70° there was little or no infection, and infection was only slight when the temperature was about 75°.

For the inoculations in the greenhouse the plants were grown to the 4- or 5-leaf stage in 4-inch pots usually with 5 corn plants or about 8 to 10 or more small-grain or grass plants per pot. In each inoculation on each host with each culture, the plants in 1 pot were inoculated and corresponding plants in at least 1 pot were similarly treated with sterile water and similarly incubated as controls. In the inoculations in the field, at least 5 corn plants of each variety or strain in the pretasseling stage were inoculated with each culture thus used and at least 5 corresponding plants were similarly treated with sterile water as controls.

Infections were readily obtained by spraying the leaves with water suspensions of the bacterium and holding the plants in a moist chamber for 24 hours. Symptoms appeared within 48 hours when inoculations of this type were made in the greenhouse (fig. 4, *A* and *B*; fig. 5). Corn seedlings were inoculated also by injecting the inoculum into their centers through the leaf sheaths. A summary of the pathogenicity studies of leaf blight and stalk rot at Beltsville is given in table 1.

The lesions produced on corn were typical of those that occur under natural conditions. Young lesions on the small grains appeared as green water-soaked areas surrounded by yellowish tissue. Later they became dark olive-green surrounded by yellowed tissue sometimes bordered with "sepia." Older lesions became buff or light brown, surrounded with yellow and red. In severe infections the leaves sometimes fell over about one-third to one-half the distance from the tip.

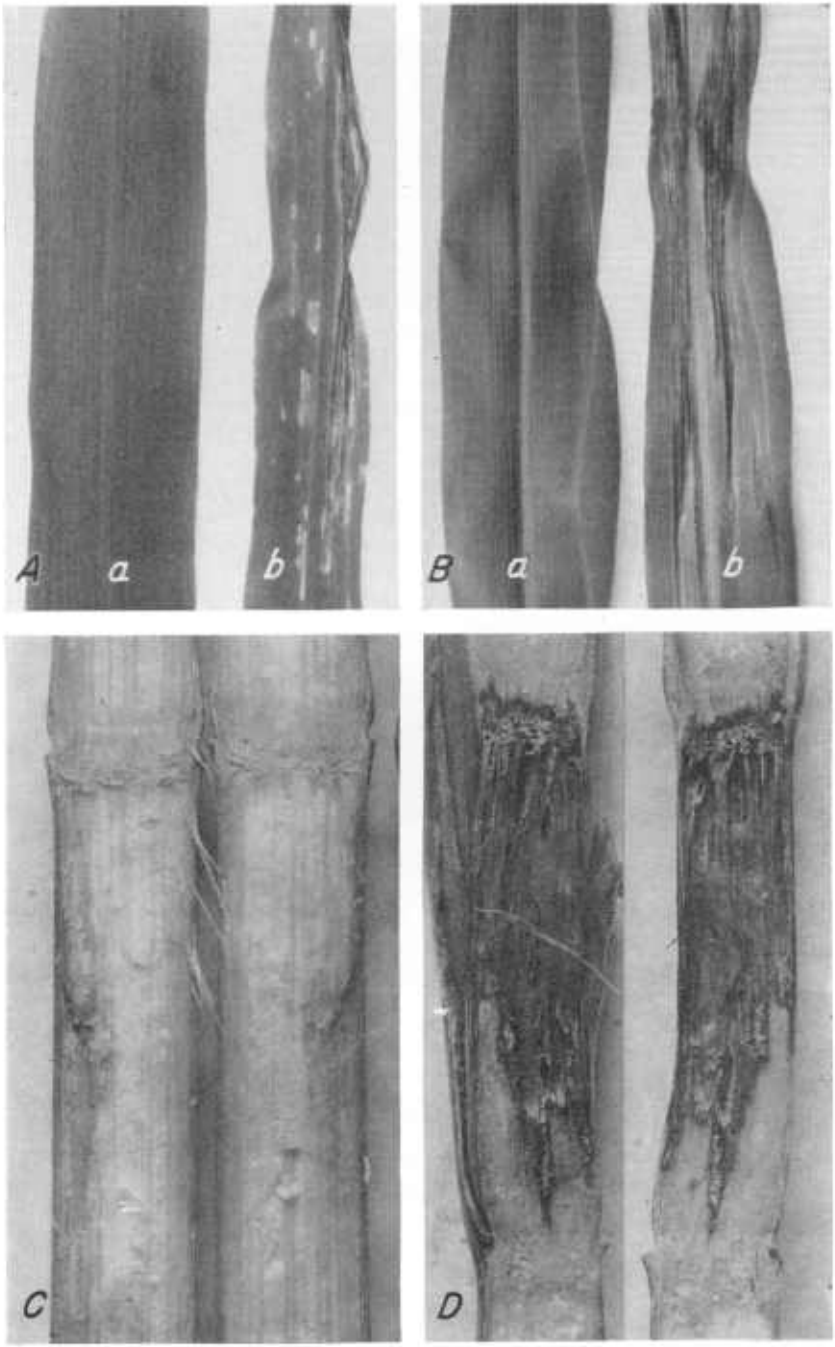


FIGURE 4.—Leaves of dent corn (*A*) and sweet corn (*B*) grown in the greenhouse: *a*, Uninoculated control; *b*, inoculated with the leaf blight and stalk rot bacterium. Split stalks of dent corn grown in the field: *C*, Control, injected hypodermically with sterile water; *D*, inoculated hypodermically with the leaf blight and stalk rot bacterium.

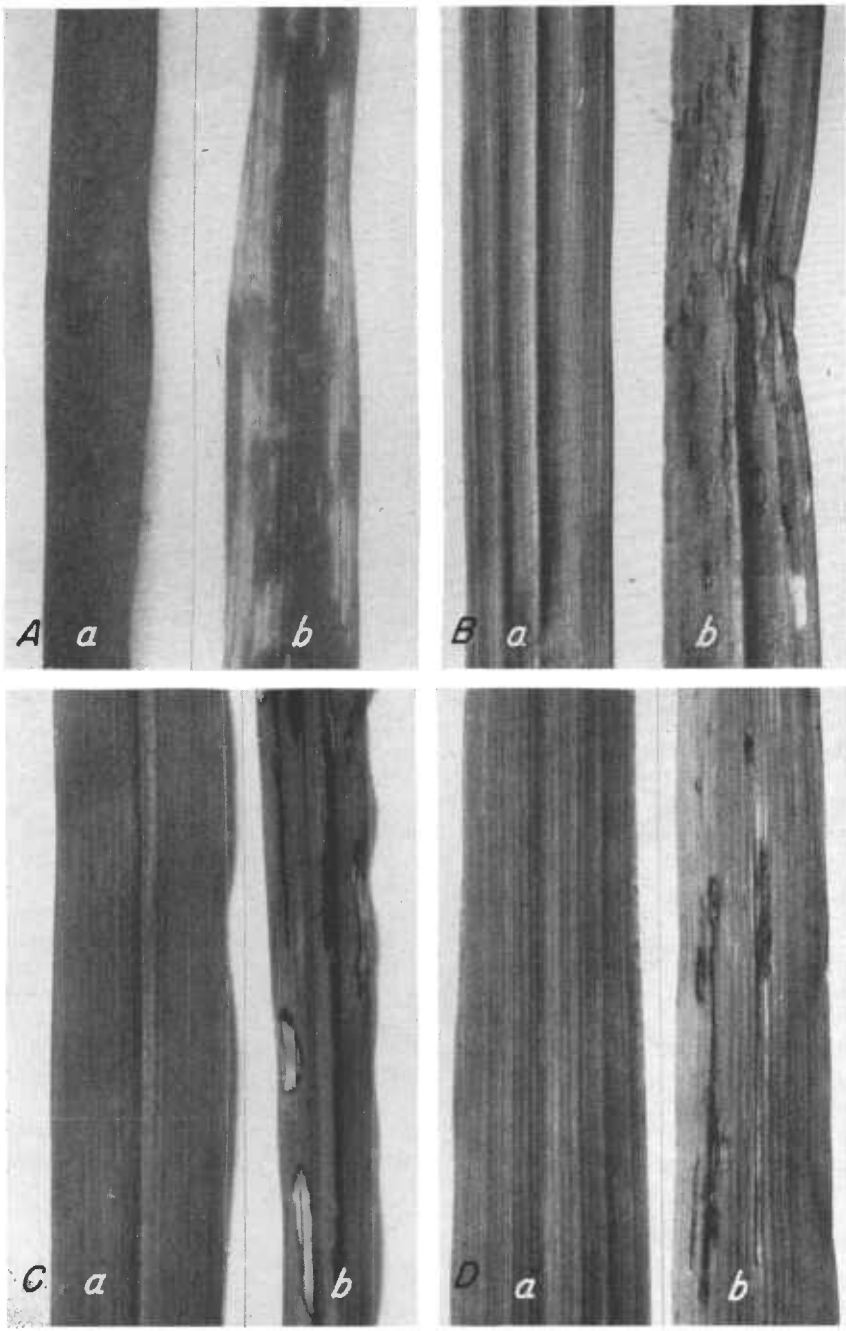


FIGURE 5.—Leaves of wheat (*A*), barley (*B*), Sudan grass (*C*), and *Setaria lutescens* (*D*): *a*, Uninoculated control; *b*, inoculated in the greenhouse with the leaf blight and stalk rot bacterium, showing characteristic lesions.

TABLE 1.—*Summary of infections in the greenhouse on various hosts by 9 cultures of the organism isolated from lesions on corn leaves, Beltsville, Md.*

[Infection ratings: 0, None; 1, trace; 2, slight; 3, moderate; 4, heavy; and 5, very heavy]

Plant inoculated	Infection rating from indicated culture [1]								
	2a	2aD1	2aD3	2aD3 [2]	2aD3r [2]	2b	$2bS_1$	3a	3ar [2]
U. S. Hybrid 13 dent corn	3	5	4	4	3	2	5	4	4
Golden Cross Bantam sweet corn	------	------	4	4	4	------	5	4	5
Golden Giant sweet corn	5	5	5	------	------	5	5	5	------
Fulcaster wheat	4	4	5	2	2	5	5	5	2
Appler oats	4	4	5	2	3	5	5	5	1
Oderbrucker barley	4	4	5	4	4	5	5	5	4
Abruzzes rye	4	4	5	3	3	5	5	5	5
Honey sorgo	1	1	2	4	4	2	2	2	3
Tift Sudan grass	1	2	2	3	5	1	1	2	3
Setaria lutescens	2	3	2	4	4	1	1	1	4
Setaria geniculata	1	1	0	3	3	1	1	0	3

[1] Cultures 2a, 2b, and 3a were isolated from different lesions on leaves of U. S. Hybrid 13 dent corn received from Kansas, July 1943; cultures 2aD1 and 2aD3 from infections resulting from artificial inoculations with culture 2a on U. S. Hybrid 13; culture $2bS_1$ from infection resulting from artificial inoculation with culture 2b on Golden Giant sweet corn; and cultures 2aD3r and 3ar, reisolations from infections on Golden Cross Bantam by 2aD3 and 3a, respectively.

[2] Used in a separate series of inoculations in which infection ratings on some hosts were somewhat different rom those previously obtained.

When cultures from the previously described variant colonies were sprayed on leaves of seedlings of U. S. Hybrid 13 dent corn, Golden Giant sweet corn, and *Setaria lutescens* as described previously, typical lesions resulted. Reisolations from the infected plants gave the same-type colony that was used in inoculating the plants, and sometimes one or two of the other types of variant colonies that have been described, as well as the typical, smooth colonies. This apparently indicates that the organism is not stable and is represented by several types that are pathogenic and may change within the host as well as on artificial media. Throughout these various series of leaf inoculations, the uninoculated control plants remained healthy.

Stalk inoculations were made in the field and in the greenhouse by hypodermic injections of water suspensions of the bacterium into the stalks of plants just before the ears began to form. Injections were made as near to the upper ear node as could be determined. Fourteen different varieties of open-pollinated dent corn were inoculated in the field at Arlington Experiment Farm, Arlington, Va. These showed typical stalk rot (fig. 4, *C* and *D*), top rot, and multiplicity of ears. All were about equally susceptible. Inoculations were made into stalks of dent corn, U. S. Hybrid 13 and U. S. Hybrid 357, in the field at Plant Industry Station, Beltsville, Md., with six different cultures. Typical stalk rot and multiplicity of ears resulted, but no top rot occurred in any of the plants inoculated. Similar stalk inoculations were made into three dent corn single crosses grown in 14-inch pots in the greenhouse with seven cultures. Typical stalk rot and multiplicity of ears were produced in each of the crosses even though the plants did not reach maturity in the greenhouse. The uninoculated controls remained healthy.

One limited series of inoculations also was made on seed. Fifteen kernels each of U. S. Hybrid 13 dent corn and Golden Giant sweet corn were inoculated by soaking them for 15 minutes in a water suspension of each of seven cultures of the leaf blight and stalk rot or-

ganism. These inoculated kernels were planted in a warm greenhouse in 6-inch pots of autoclaved soil, five kernels per pot. In 6 days the seedlings were in the three-leaf stage and some of them showed water-soaked lesions in the leaves. Later, a number of other plants of both the dent and the sweet corn showed conspicuous lesions. The final results, taken 12 days after planting, are given in table 2. Reisolations were made from representative lesions in the leaves of the U. S. Hybrid 13 and Golden Giant plants inoculated with each culture, and the characteristic organism was recovered in each case. All of the uninoculated, control plants remained healthy.

TABLE 2.—*Results from inoculating seed of U. S. Hybrid 13 dent corn and Golden Giant sweet corn with water suspensions of 7 cultures of the leaf blight and stalk rot organism*

Culture	U. S. Hybrid 13			Golden Giant		
	Planted	Emerged	Infected	Planted	Emerged	Infected
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
2a	15	15	27	15	12	42
2b	15	15	27	15	13	77
2c	15	15	60	15	15	60
2d	15	14	21	15	13	46
2e	15	15	47	15	14	50
2f	15	14	36	15	15	53
3a	15	15	40	15	12	58
None (control)	15	15	0	15	15	0

Two series of inoculations were made on vegetables. In each of these, six cultures of the organism previously found to be pathogenic on corn and other hosts were tested for pathogenicity on fresh, clean slices of cabbage, carrot, potato, and turnip. At least four slices of each of these were cut carefully with a sterile knife and placed in each of two large petri dishes. One set was left uninoculated and the other was inoculated by placing a drop of water suspension of each culture separately on the freshly cut surface of each vegetable. Within 5 days a distinct soft rot, with a foul odor, was produced on each of the vegetables. The cultures showed only minor differences in their ability to cause rot. The uninoculated control slices remained sound. Isolations were made from each of these inoculation tests and the typical organism was obtained in each case in which infection occurred.

IDENTIFICATION

The organism described as causing the bacterial leaf blight and stalk rot of corn has been found to agree in all essential respects with that described by Rosen (*14*, *16*) as *Pseudomonas alboprecipitans*. While Rosen described this species as the cause of a bacterial disease of *Chaetochloa lutescens* (*Setaria lutescens*) as found in nature, he reported that under greenhouse conditions it was pathogenic also on *C. geniculata* (*S. geniculata*), *Holcus sorghum* L. (*Sorghum vulgare*), *H. sorghum sudanensis* Hitchc. (*S. vulgare* var. *sudanense*), *Hordeum vulgare*, *Secale cereale*, *Triticum vulgare* Vill. (*T. aestivum*), and *Zea mays*.

As stated previously, the bacterial leaf blight and stalk rot on dent corn occurs in nature in a number of Southern and Central States.

The organism isolated was shown to be pathogenic on essentially the same hosts reported as susceptible to *Pseudomonas alboprecipitans* by Rosen.

In addition to the similarity in host range, the cultural, physiological, and morphological characters of the organism under study are in essential agreement with those of *Pseudomonas alboprecipitans* Rosen (*16*) (*Bacterium alboprecipitans* Rosen (*16*), *Phytomonas alboprecipitans* (Rosen) Bergey et al. (*2*)).

COMPARISON WITH OTHER BACTERIAL DISEASES OF CORN

The bacterial leaf blight and stalk rot of corn caused by *Pseudomonas alboprecipitans* as described differs distinctly from baterial wilt caused by *Bacterium stewarti* E. F. Sm. in both symptoms and causal organism. In the former the lesions on the leaves are definite spots or stripes, usually with a "sepia" margin; the stalk rot is limited to the upper part of the plant; and the causal organism is white. In the latter the leaf lesions tend to be long and rather narrow with wavy margins, streaking the leaves. From these lesions the bacterium spreads to the stalk, especially in sweet corn, produces a systemic infection, chiefly in the vascular system, and is yellow.

The conspicuous difference between the stalk rot of corn caused by *Pseudomonas alboprecipitans* and that described by Rosen (*13*, *15*, *17*, *18*) as caused by *Phytomonas dissolvens* Rosen is that the former is confined to the upper part of the stalks and the latter to the lower part. While both of these organisms are white, they differ in certain physiological characters, particularly as regards gas formation. *P. dissolvens* grows more rapidly, forms large colonies, and produces gas in various carbohydrate media, whereas *Ps. alboprecipitans* forms smaller colonies and no gas.

The bacterial stalk rot described by Ark (*1*) as caused by *Phytomonas lapsa* Ark (*Pseudomonas lapsa* (Ark) Burk. (*3*)) differs distinctly from that caused by *Ps. alboprecipitans* in that the corn plants attacked by the former commonly fall to the ground and the causal bacterium produces fluorescence in Uschinsky's, Fermi's, and Cohn's solutions.

The bacterial disease on corn described by Kendrick (*7*, *8*) as caused by *Bacterium holci* Kendrick (*Pseudomonas holci* Kendrick) occurs only on leaves as small circular, elliptical, or irregular spots, at first water-soaked and later brown with a narrow darker brown to reddish-brown border. The organism, which is now considered the same as *Ps. syringae* van Hall (*3*), is white and fluorescent and produces a greenish pigment in some media; therefore it is distinctly different from *Ps. alboprecipitans*.

Nonparasitic injuries to corn leaves caused by sodium nitrate having been accidentally thrown into the leaf whorl on hasty examination may sometimes be confused with leaf lesions caused by *Pseudomonas alboprecipitans*. While the sodium nitrate may cause bleached spots and stripes, these usually do not show the characteristic "sepia" borders; nor do they show bacterial streaming when examined microscopically. Likewise, if plated, they do not yield the characteristic bacterium.

DISCUSSION

On account of the sporadic occurrence of leaf blight and stalk rot and the lack of specific information on the source of inoculum in

nature, no study of control measures was made. From the standpoint of economic importance thus far, control measures seem scarcely needed. It is not known, however, how severe the disease might become under some set of conditions especially favorable for its development. Therefore the disease should be watched.

While it has been shown that the pathogen may be seed-borne if inoculated onto the seed just before planting, there is no information to indicate that it is seed-borne under natural conditions. On the other hand, the pathogen has been identified as *Pseudomonas alboprecipitans*, which was originally isolated from *Setaria lutescens*, a common weed in cornfields. There is the possibility, therefore, that the pathogen may spread from this host to corn under field conditions. Since the identification of the pathogen there has been no opportunity to make field observations on this point.

SUMMARY

A bacterial leaf blight and stalk rot of corn has been found to occur sporadically in various Southern and Central States, where it has been of minor importance.

The disease attacks both the leaves and the stalks of corn.

The causal organism is a white, Gram-negative, motile bacterium identified as *Pseudomonas alboprecipitans* Rosen.

Inoculation experiments have shown that the disease may be readily induced on corn, wheat, rye, barley, oats, and *Setaria lutescens* by spraying the leaves with water suspensions of the bacterium. Light infections were obtained in the same way on *S. geniculata*, Honey sorgo, and Sudan grass.

Stalk infection occurred when water suspensions of the organism were inoculated into the stalks of corn previous to tasseling.

Kernels of both dent corn and sweet corn soaked in water suspensions of the bacterium and then planted in sterile soil in the greenhouse produced infected seedlings, while corresponding controls remained healthy.

Variants of the typical colonies showed pathogenicity on corn and *Setaria lutescens*.

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