Vol. XXIX JULY 15, 1924 No. 2

JOURNAL OF AGRICULTURAL RESEARCH

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PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION
OF LAND-GRANT COLLEGES

WASHINGTON, D. C.
GOVERNMENT PRINTING OFFICE
1925

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXIX WASHINGTON, D. C., JULY 15, 1924 NO. 2

BACTERIAL PUSTULE OF SOYBEAN[1]

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INTRODUCTION

Diseases of the soybean, *Soja max* (L.) Piper, have for several years been the subject of investigation by the writer and his colleagues at the North Carolina Agricultural Experiment Station (*2, 3, 8, 11, 16, 17*).[2] Among those which have been given special study is a leafspot to which the appropriate name "bacterial pustule" has been applied. This leafspot disease was first briefly described (*4*) in 1922 by Florence Hedges. In this preliminary report, Miss Hedges designated the causal organism *Bacterium phaseoli* var. *sojense* and stated that a paper covering the results of her investigations was in preparation (*6*). It might be anticipated that the present report would confirm in all essential features that of Miss Hedges. Should it contribute nothing new to the knowledge of this disease and its causal organism, nevertheless it is believed to have a definite value since the investigations have been conducted entirely independently. It is the writer's purpose, therefore, to embody in this paper a description of the disease, an account of its relation to other soybean leafspots of bacterial origin, studies on its etiology, and on the morphology and physiology of the causal organism.

HISTORY AND DISTRIBUTION

A definite knowledge of the occurrence of this disease dates from Miss Hedges's isolation, from specimens sent from Texas in 1917, of a yellow organism very closely resembling *Bacterium phaseoli* E. F. Smith. However, a bacterial leafspot of soybean which was assumed to be due to *Bacterium phaseoli* was mentioned as long ago as 1904 (*12*). The isolations upon which this report was based were made two years earlier (*13, p. 280*) from diseased soybeans taken from two localities, one near Charleston, S. C., and the other near Washington, D. C. The organism isolated at that time was not proved by inoculation experiments to be pathogenic, and therefore its relation to the leafspot can not now be satisfactorily determined. This problem is further complicated by the fact that other bacterial organisms have recently been found to be pathogenic to soybean. The first of these diseases to be carefully investigated was a bacteriosis (*9*) which manifests itself by the formation of lesions both on stems and pods. Those on the stems are especially characteristic, since they girdle them in a manner suggesting the blackleg disease of potatoes. The pathogen, *Bacillus lathyri* Manns and Taubenhaus, is identical with the one which causes the "streak" disease of sweet pea, *Lathyrus odoratus* L. It is a yellow organism but has very different morphological and cultural characters from the soybean organism under consideration.

As will be shown later, the appearance of bacterial pustule has little in common in any stage of development with that of bacterial blight as described by Miss Coerper (*1*) or by the writer (*16*). The causal organisms identified as *Bacterium glycineum* by the former and as *Bact. sojae* by the latter are both white and are thus easily separable from the pustule organism; but in the case of old lesions caused by *Bact. sojae* the tissues are always occupied also by a yellow, one-flagellate organism (*16*) which may be a source of confusion as to the primary cause. It might be indicated at this point that although the bacterial blight diseases caused by *Bact. glycineum* and *Bact. sojae* are very similar in appearance, the causal organisms are readily distinguishable and specifically distinct, as has been shown by certain cultural studies (*11, 7, 17*).

Mention has been made in several previously published accounts of bacterial diseases of soybeans in the Orient. A bacteriosis has recently been recorded (*10*) by Miura, a Japanese investigator, as occurring in Manchuria. His description of the disease accords with the appearance of

[1] Received for publication Feb. 25, 1924—issued January, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 68.

bacterial blight, and he furthermore expresses the opinion that the disease in Manchuria is the same as that found in America. He also records the occurrence of this bacteriosis in other parts of China and Japan.

In 1921 another Japanese investigator, Takimoto (*14*), published a comprehensive account of his investigations on a bacterial disease of soybeans which he had had under observation since 1914. This disease manifests itself by the formation of numerous small, angular, dark-brown leaf-spots with chlorotic intervening tissues. Lesions may also form along the veins and extend along them making dark streaks. Black, sunken areas form upon both petioles and stems. These symptoms, as he points out, are quite different from bacterial blight in America. The organism which he isolated was proved to be pathogenic not only to soybean in all stages of its growth, but also to Adzuki bean, *Phaseolus angularis* Willd. Takimoto does not assign a name to the bacterium which he had under observation, but does compare it morphologically and physiologically with published accounts of *Bacterium glycineum*, as described by Miss Coerper, *Bact. sojae*, as described by the writer, and *Pesudomonas glycineum*, as described by Nakano,[3] who isolated the organism in 1916 from collections made in Kumamoto prefecture. He concludes that it is most like *Bact. sojae*, but differs from it in the absence of a capsule, in its failure to effect a change in milk, and in its growth in the closed arm of fermentation tubes containing dextrose, saccharose, and mannite. It is doubtful whether these differences would be confirmed were both forms in the hands of one investigator; and it remains for subsequent investigation to determine the identity of the organism of Takimoto and *Bacterium sojae* and whether either or both are identical with *Pseudomonas glycineum* Nakano.[4] Certainly none of these are like the soybean bacterial pustule, as will become apparent when they are compared with the description of the appearance of this disease and with the cultural characters of the causal organism.

Bacterial pustule is known to occur in Texas, Virginia, Kansas, South Carolina, and Louisiana (*3*, *4*). It has been collected from a sufficient number of localities in North Carolina to warrant the belief that it is generally prevalent throughout the State.

APPEARANCE OF THE DISEASE

Soybean pustule has not been observed upon the stems and pods, but upon the foliage only. Soybeans in all stages of growth varying from the seedling stage to mature plants are subject to infection. The disease may appear upon the foliage in any stage of maturity, but reaches its most destructive stage of development at the time when the plants have reached their maximum vegetative growth.

The first indication of the disease is the presence of minute elevations on either or both leaf surfaces (figs. 1 and 2). These elevations themselves are light green in color. Soon a yellowish-green halo forms as a border at the base of each elevation. At this stage the lesions are easily distinguished from those produced by *Bacterium glycineum* and *Bact. sojae*, neither of which causes the formation of pustules, and each of which, especially in the early stages, causes the invaded tissues to be translucent. This is followed by the enlargement of the elevations to very prominent pustular outgrowths often extending above the leaf about twice as high as the thickness of the leaf (fig. 3). These raised portions soon collapse and, together with a portion of the surrounding invaded tissues, become brown. Ultimately, the lesions become angular to irregular reddish-brown areas which vary in size from minute specks to large irregular spots. These large areas arise by the fusion of lesions in case initial lesions are abundant. Lesions in all stages of development occur on the same leaflet. This indicates that the initial infections serve as the source of inoculum for secondary infections.

The general aspect of the pustule disease in the late stages (Pls. 1, 2) is like that produced by the rust fungi, whereas bacterial blight lesions are dark brown to brownish-black in color and tend to break out and fall away, thus making perforations or notches in the leaves.

Nakano's original paper has not been seen by the writer, nor does he have a complete reference to it.

These papers were translated by Mr. S. Yonemachu, a special student of textile manufacture at the North Carolina State College of Agriculture and Engineering. Grateful acknowledgment is herewith made of his kindness and courtesy in rendering this service.

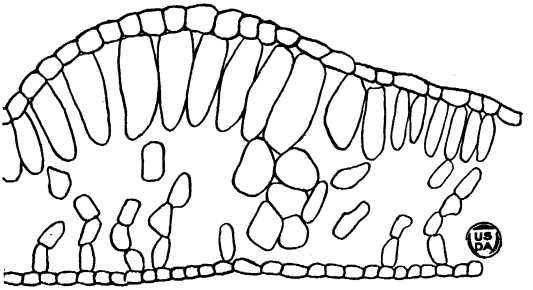


FIG. 1.—Diagram outlined with camera lucida showing beginning of pustule formation on upper leaf surface

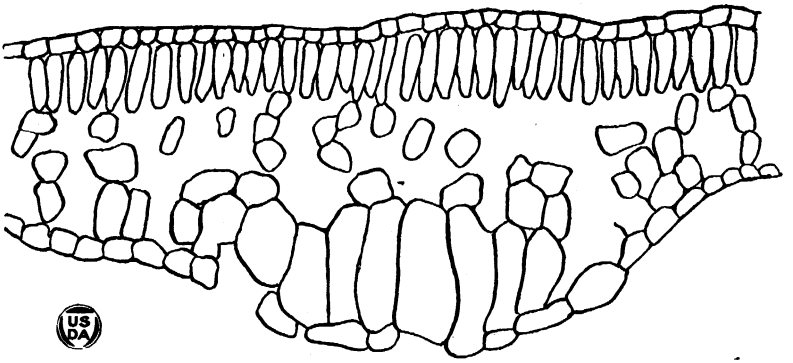


FIG. 2.—Pustule formation involving the tissues of the lower leaf surface

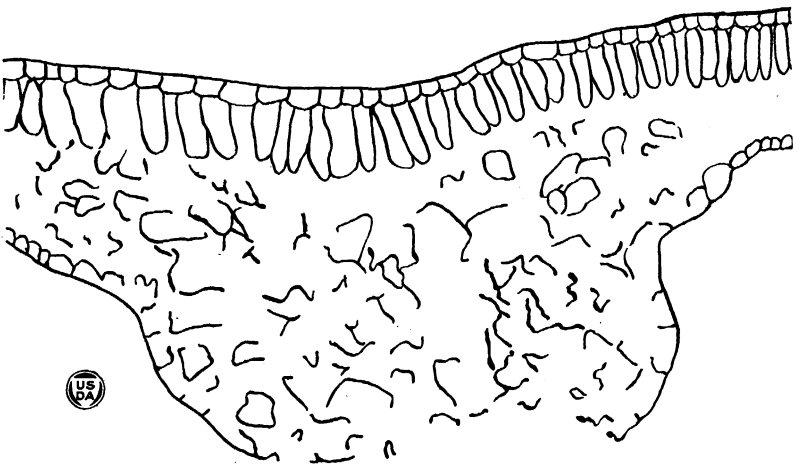
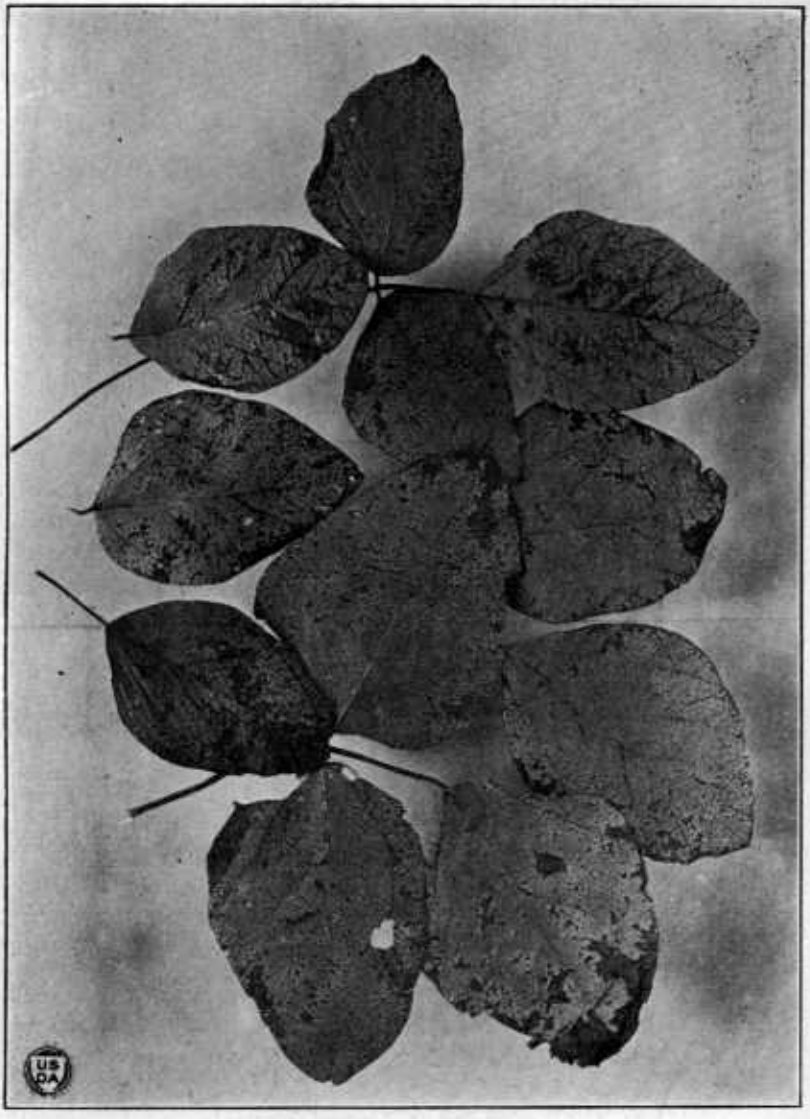
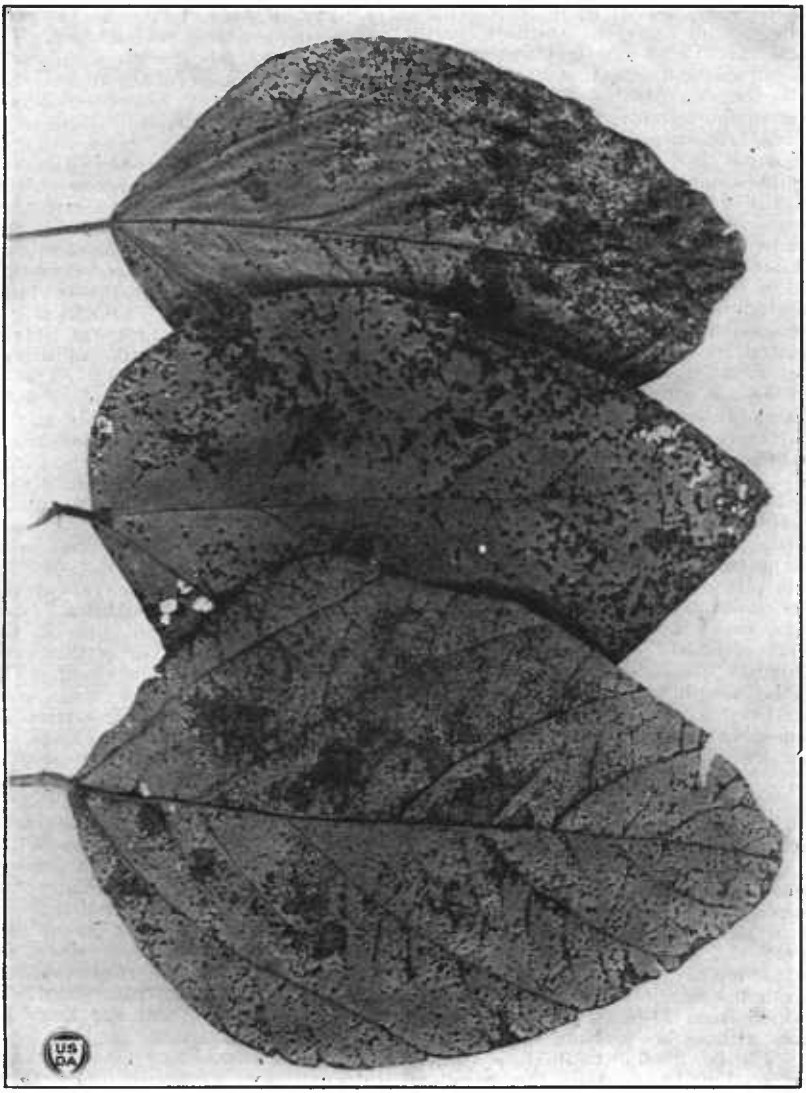


FIG. 3.—A large pustule whose cells have collapsed



Bacterial Pustule of Soybean Plate 1

Bacterial pustule of soybean showing natural infection in various stages of development.



Bacterial Pustule of Soybean Plate 2

Leaves, natural size, affected with bacterial pustule

CAUSAL ORGANISM

ISOLATION

In preparations made by macerating lesions in a drop of water, the invaded tissues were found, upon microscopic examination, to be teeming with bacteria. These bacteria swarm out in such abundance as to cloud the water and form a turbid suspension, a phenomenon characteristic of diseases of bacterial origin. If an inoculum is prepared by direct maceration of young lesions in sterile water and a loopful of this inoculum is transferred to an agar plate and spread over its surface with a zigzag stroke, discrete colonies of the pathogen form near the end of the stroke. These can then be selected for transfer to tube cultures. A considerable number of strains were isolated by this procedure during the course of the present investigation. Subsequent comparative study of these strains to determine their cultural characters and their ability to infect soybean showed them all to be identical. Since it was apparent from the preliminary studies that the soybean pustule organism is closely related to *Bact. phaseoli*, several strains were compared with *Bact. phaseoli* isolated from pod lesions of Lima bean, *Phaseolus lunatus* L., and garden bean, *Phaseolus vulgaris* L.; and with a strain of bean blight isolated by Miss Hedges. The following parallel studies of the morphology and physiology of all strains both from soybean and from Phaseolus show that all are practically identical.

MORPHOLOGY

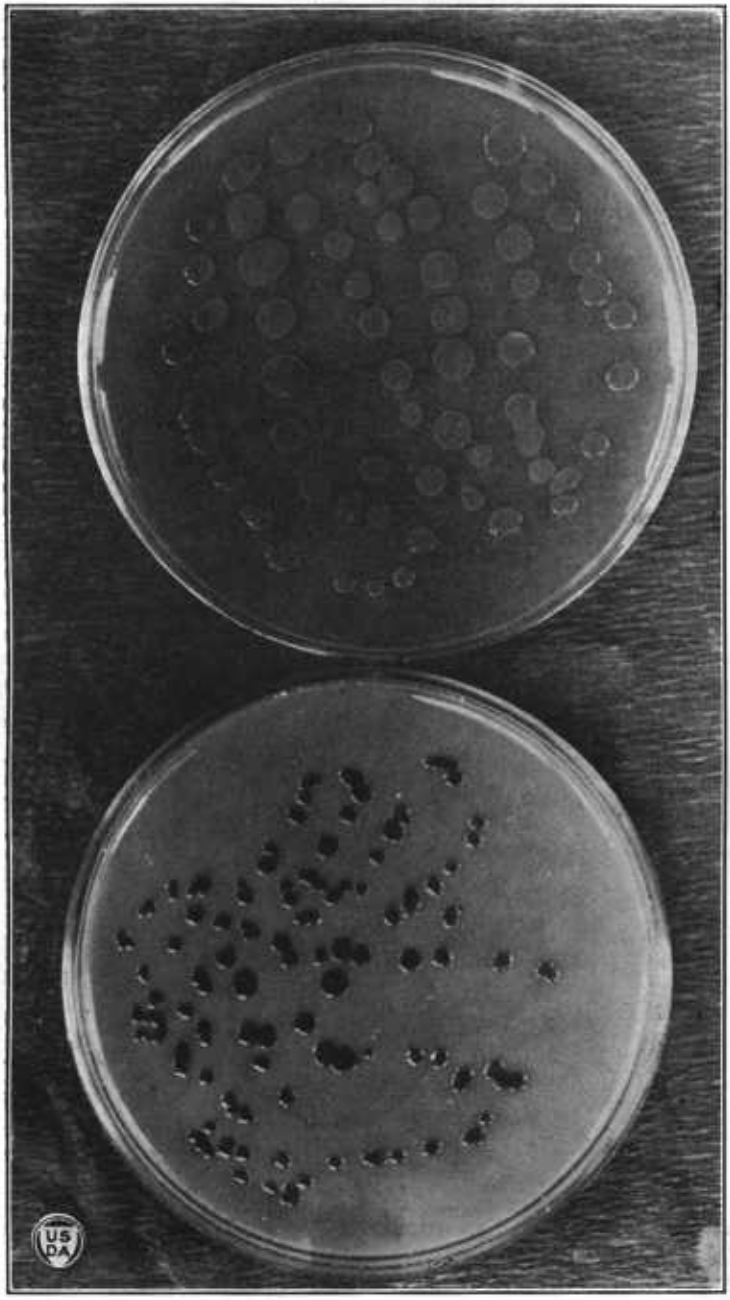
VEGETATIVE CELLS.—The primary cause of soybean bacterial pustule is a yellow rod-shaped organism with rounded ends. When taken directly from lesions it is found to occur singly or in pairs, but tends in bouillon to form short chains. The organism stains readily with all of the more common bacteriological stains. When stained from 24-hour potato-agar cultures with carbol fuchsin, the elements are 1.3 to 2×0.6 to 0.75 μ. Such preparations, too, show the presence of investing material, as is manifest also with Welch's capsule stain; but this envelope can not be interpreted as indicating a well-defined capsule. When stained by Gram's method, the organism is decolorized. Endospores and marked involution forms have not been noted.

When the organism from young lesions, from bouillon cultures, or from 24-hour agar cultures is examined for motility, it is seen to possess the power of rather active locomotion. That this movement is due to the presence of a polar flagellum about twice the length of the cell has been demonstrated by several modifications of the method of Loeffler.

CULTURAL CHARACTERS.—The various media employed in the cultural studies were prepared by the methods employed by the writer and his associates in their studies on the physiology of certain plant pathogenic bacteria (*17*). The nutrient broths contained 1 per cent Difco peptone and 0.3 per cent Liebig's beef extract; the nutrient agars the same, with the addition of 1.8 per cent of bacto-agar. The hydrion concentration of cooled media was measured colorimetrically and the media were not heated after adjustment of reaction. The carbon compounds were sterilized in distilled water and added with aseptic precautions to cooled media. The cultures were incubated at room temperatures which approximated 20° to 25° C.

NUTRIENT AGAR.—The colonies may appear in agar plates within 24 hours, but are not prominent, nor do they show the characteristic yellow color until they are 48 hours old. They are circular in outline with entire margins and a glistening surface but with no surface markings. Their consistency varies from nonviscid to slightly viscid. In potato agar or other nutrient agar containing 3 per cent or more of agar, the colonies are convex with internal markings (Pl. 3). With media of a low degree of viscosity, the colonies are flat and spreading with no striking internal convolutions. In agar slants the growth is filiform, spreading at the base of the slant, with an entire or somewhat contoured margin, glistening and translucent. No odor is developed and the agar does not become pigmented.

POTATO CYLINDERS.—On steamed potato cylinders, growth is first manifest by a faint yellowish streak. This becomes abundant within 24 to 36 hours, is spreading, yellow, and has a striking whitish zone along the border of the growth. Within six to eight



Bacterial Pustule of Soybean Plate 3

Bacterium phaseoli var. *sojense* in plate cultures showing difference in appearance due to difference in viscosity of agar. The upper culture contains 1.8 per cent agar, the lower contains 5 per cent agar. All other conditions are identical.

days the cylinder will have collapsed, and when this collapsed tissue is tested with Lugol's solution the starch will be found to have disappeared, showing that the pathogen possesses strong diastatic properties. An ocular demonstration of the ability of the pustule-forming bacterium to hydrolyze starch may be made by growth in plate culture on beef extract agar plus 0.2 per cent of soluble starch. If the plates are flooded with iodine solution after a week's incubation, a broad clear zone surrounding the colonies will indicate the area in which the starch has been destroyed by enzym activity.

MILK.—Plain sterilized milk becomes separated into whey and curd, and the curd is slowly digested. The first evidence of these enzymatic activities is the appearance of a thin layer of clear whey just at the surface and below the pseudozoogloeal surface growth. As the curd forms, it slowly settles. Cultures three weeks old will have cleared with at most a small quantity of curd at the bottom of the culture tube.

LITMUS MILK.—Lavender-colored litmus milk undergoes the same type of clearing and separation into whey and curd as does plain milk. Little or no free acid is formed, or at least is not formed in sufficient quantity to change the color of the indicator.

CARBON METABOLISM.—In the preliminary tests on the fermentative ability of the soybean pustule organism in which only the common sugars were employed, it was apparent that it could not by this means be separated from *Bacterium phaseoli*. Accordingly, use was made in addition of certain rare sugars which have been successfully employed in distinguishing *Bact. glycineum* and *Bact. sojae* (*11*) and also *Bact. sojae* and *Bact. trifoliorum* (*7*). Bouillon to which was added sufficient of the carbon compounds to make a 1 per cent solution was employed to follow the progressive changes in hydrion concentration as an index to fermentative activity. The sugar was added to cooled, sterile bouillon flasked in convenient quantities and was tubed with aseptic precautions in previously sterilized test tubes. All were made from the same stock bouillon and a considerable number of cultures of each strain were made on the same day. This made possible the employment of several tubes of each strain at each reading at each of the consecutive intervals. The results of these fermentation tests are assembled in Tables I to IV.

TABLE I.—*Fermentation of various carbon compounds by the organisms from soybean pustule (initial P_H 7.0)*

Carbon compound	Age of culture and P_H concentration				
	3 days	5 days	7 days	11 days	13 days
Dextrose	7.0	7 0	7.0	7.4	7.6
Saccharose	7.0	7.0	7.0	7.4	7.6
Lactose	7.0	7.0	7.0	7.2	7.4
Maltose	7.0	7.0	7.0	7.2	7.4
Glycerin	7.0	7.0	7.2	7.6	7.8
Arabinose	7.0	7.0	7.0	7.2	7.4
Xylose	7.0	7.0	7.2	7.4	7.6
Rhamnose	7.0	7.0	7.2	7.6	7.8
Dextrin	7.0	7.0	7.0	7.4	7.6
Salicin	7.0	7.0	7.0	7.4	7.6
Mannitol	7.0	7.0	7.0	7.6	7.8
Inulin	7.0	7.0	7.2	7.6	7.8
Galactose	7.0	7.0	7.2	7.4	7.8

TABLE II.—*Fermentation of the same various carbon compounds by Bact. phaseoli from snap bean (initial P_H 7.0)*

Carbon compound	Age of culture and P_H concentration				
	3 days	5 days	7 days	11 days	13 days
Dextrose	7.0	7.0	7.2	7.2	7.4
Saccharose	7.0	7.0	7.2	7.2	7.4
Lactose	7.0	7.0	7.2	7.6	7.6
Maltose	7.0	7.0	7.0	7.2	7.4
Glycerin	7.0	7.0	7.2	7.4	7.6
Arabinose	7.0	7.0	7.2	7.4	7.6
Xylose	7.0	7.0	7.2	7.4	7.6
Rhamnose	7.0	7.0	7.2	7.4	7.6
Dextrin	7.0	7.0	7.2	7.4	7.6
Salicin	7.0	7.0	7.2	7.4	7.6
Mannitol	7.0	7.0	7.2	7.4	7.6
Inulin	7.0	7.0	7.2	7.4	7.6
Galactose	7.0	7.0	7.2	7.4	7.6

TABLE III.—*Fermentation of various carbon compounds by Bact. phaseoli from Lima bean (initial P_H 7.0)*

Carbon compound	Age of culture and PH concentration				
	3 days	5 days	7 days	11 days	13 days
Dextrose	7.0	7.0	7.2	7.4	7.6
Saccharose	7.0	7.0	7.2	7.4	7.6
Lactose	7.0	7.0	7.2	7.4	7.6
Maltose	7.0	7.0	7.2	7.4	7.6
Glycerin	7.0	7.0	7.2	7.4	7.6
Arabinose	7.0	7.0	7.0	7.2	7.4
Xylose	7.0	7.0	7.2	7.4	7.6
Rhamnose	7.0	7.0	7.2	7.4	7.8
Dextrin	7.0	7.0	7.2	7.4	7.6
Salicin	7.0	7.0	7.2	7.4	7.6
Mannitol	7.0	7.0	7.2	7.4	7.8
Inulin	7.0	7.0	7.2	7.4	7.8
Galactose	7.0	7.0	7.2	7.2	7.4

TABLE IV.—*Fermentation of various carbon compounds by strain of Bact. phaseoli from Washington, D. C. (initial P_H 7.0)*

Carbon compound	Age of culture and PH concentration				
	3 days	5 days	7 days	11 days	13 days
Dextrose	7.0	7.0	7.0	7.2	7.4
Saccharose	7.0	7.0	7.0	7.2	7.4
Lactose	7.0	7.0	7.0	7.2	7.4
Maltose	7.0	7.0	7.0	7.2	7.4
Glycerin	7.0	7.0	7.0	7.4	7.4
Arabinose	7.0	7.0	7.0	7.2	7.4
Xylose	7.0	7.0	7.0	7.2	7.4
Rhamnose	7.0	7.0	7.0	7.2	7.4
Dextrin	7.0	7.0	7.0	7.2	7.6
Salicin	7.0	7.0	7.0	7.2	7.6
Mannitol	7.0	7.0	7.0	7.2	7.4
Inulin	7.0	7.0	7.0	7.4	7.6
Galactose	7.0	7.0	7.0	7.2	7.4

It is evident from an analysis of the data presented in these tables that the soybean pustule organism and the several strains of *Bact. phaseoli* are identical so far as concerns their inability to utilize any of these carbons as the source of energy and that they can not therefore be separated on their fermentation relations. The increase in alkalinity, as appears in all cultures with all sugars, develops from the decomposition of the proteins, as shown by growth in plain bouillon.

GAS PRODUCTION.—These tests were conducted by using fermentation tubes filled with portions of the same solutions as were used in the fermentation tests. These tubes were sterilized prior to filling and were incubated for 48 hours after being filled to determine their freedom from contamination. They were then inoculated in sets of four with each of the several strains. No gas was developed in the case of any of the 13 carbon compounds, and growth was sharply limited to the open arm in all media with each of the strains.

NITROGEN METABOLISM.—Unfortunately, no considerable significance has been attached to the nitrogen metabolism of bacteria in relation to the determination of species. This matter has been based largely upon their fermentative ability. It would appear that plant pathogenic bacteria, especially those which attack few or no carbon compounds, as is the case with those under consideration, might be separated upon the basis of differences in nitrogen metabolism were methods of study known. The writer's attempts in this direction have been limited to the employment of a few nitrogenous compounds, added as nutrients to a stock synthetic agar. This agar was prepared according to the following formula: Distilled water, 1,000 cc.; magnesium sulphate, 0.5 gm.; dipotassium hydrogen phosphate, 1.0

gm.; potassium chloride, 0.5 gm.; ferrous sulphate, 0.01 gm.; agar, 20 gm.

DIGESTION OF CASEIN.—Evidence in addition to that obtained in milk cultures of the ability of the organism from soybeans and *Bact. phaseoli* to digest casein was obtained by adding 1 per cent casein to the stock agar in poured plate cultures. After a week's incubation wide halos, in which the casein was entirely dissolved, had formed around the colonies, thus demonstrating the ability of these organisms to form erepsin.

DIGESTION OF ASPARAGIN.—Stock agar plus 1 per cent of asparagin was used in these tests. The indicator consisted of a sufficient quantity of 4 per cent solution of rosolic acid, and sufficient NaOH was added to give the medium a decided orange color and a reaction of about P_H 6.0. In poured plate cultures incubated for about 10 days, the orange color gave way to a beautiful brilliant red. This change begins with a halo around each colony and comes to involve the entire plate. The change in color is due to the liberation of ammonia in the decomposition of asparagin as a result of the activity of the enzym amidase.

DIGESTION OF SERUM.—Blood serum added to stock agar in poured plate cultures was planted with the several strains of bacteria. This medium in cultures 7 to 10 days old serves as a satisfactory means of demonstrating the ability of these organisms to liquefy blood serum.

LIQUEFACTION OF GELATIN.—Growth in stab cultures on gelatin is slow, but within a period of two weeks the gelatin to a depth of about a centimeter will have become liquefied. Liquefaction begins as an infundibuliform area.

REDUCTION OF NITRATES.—Nitrate broth consisting of 1 per cent peptone, 0.3 per cent beef extract, and 0.1 per cent potassium nitrate supports abundant growth. No indication of nitrites was secured when the tests at appropriate intervals were made with sulphanilic-acid solution or with naphthylamine acetate solution.

INDOL PRODUCTION.—No indication of indol was secured by either the Salkowski, Vanilin, or Ehrlich test.

THERMAL DEATH-POINT.—In determining the thermal death-point, tubes of bouillon P_H 6.6 were inoculated from vigorously growing bouillon cultures and subjected for 10 minutes in the usual manner to various trial temperatures. As a result of these tests, and under these conditions, the thermal death-point was found to be 50°C.

RESISTANCE TO DESICCATION

An inoculum of the soybean organism from cultures on potato cylinders 48 hours old was suspended in sterile water and drops of this suspension were transferred to sterile cover glasses kept in sterile Petri dishes. After desiccation at laboratory temperatures certain of these cover glasses were, at definite intervals, inserted into tubes of nutrient broth. Growth appeared after 18 days' desiccation, hence the organism may be regarded as very resistant to drying.

PATHOGENICITY

Only a few pathogenicity trials were made. Pure cultures from young transfers on potato agar were suspended in sterile water, and this bacterial suspension was applied with an atomizer. When inoculations were made late in the afternoon and the plants were covered until the following morning with bell jars, a large number of centers of infection developed. The first evidence of infection was noted within four to six days, appearing as tiny elevations. Within a week later these had developed into typical lesions. From these the original organism was reisolated in trials with several of the strains. Plants in all stages of development from those with the first pair of true leaves to plants with mature foliage are subject to infection with pure cultures. Both garden beans and Lima beans have been inoculated in the same manner, and in some cases with a portion of the same inoculum; but no evidences of infection have been observed on these inoculated plants.

When *Bacterium phaseoli* was employed as an inoculum on Phaseolus and on soybeans, an abundance of typical bean-blight lesions developed upon both the foliage and pods of Lima bean and of garden bean but no evidence of pathogenicity to soybeans was noted.

Miss Hedges has reported (*4*, *5*) the production of infection on soybeans and several varieties of garden beans following inoculation with pure cultures of the soybean pustule organism. The spots on Phaseolus were like those caused by *Bact. phaseoli* with no evidence of pustule formation such as occurs on soybean. She furthermore has found that *Bact. phaseoli* from Phaseolus is only weakly pathogenic to soybean.

The differences in regard to cross inoculation are no doubt due to conditions of inoculation. This opin-

ion is supported by the fact that it has been impossible to separate the soybean organism from bean blight morphologically and culturally. It seems advisable, therefore, to regard the soybean organism as a variety of *Bact. phaseoli.*

RELATION OF PARASITE TO HOST TISSUE

Lesions which were appropriately fixed in alcohol, embedded, sectioned, and stained with alcoholic methylene blue were employed in histological studies. Entrance of the parasite is very manifestly effected through the stomates which occur on both leaf surfaces, as evidenced by the fact that the substomatal chamber and the intercellular spaces of the tissues immediately surrounding the stomata in the case of young lesions are densely filled with bacteria (fig. 4). In the older lesions, after the host cells have collapsed, the bacteria are not confined to the intercellular spaces, but occur within the cell cavities. These phenomena are entirely in accord with those known to occur in the case of nearly all plant bacterial pathogens which invade parenchyma. So far as pustule formation is concerned, however, the writer's observations are not in agreement with the statement made by Miss Hedges (*4*) that the pustules show both hypertrophy and hyperplasia. The preparations in hand show that these elevations arise wholly from hypertrophy and without hyperplasia. Any or all of the tissues, epidermal, palisade parenchyma and mesophyll, may be involved. The physiological phenomena which attend these hypertrophic changes are dependent no doubt upon certain enzymatic activities which involve both the cell walls and the cell contents. The parasite and host relationship and thus the proximate cause of pustule formation is believed to be analagous to that which obtains in the case of citrous canker caused by *Pseudomonas citri* (*15*). In the case of this organism evidence has been advanced that both cytolytic and diastatic enzyms are secreted. Through their activity these modify the osmotic properties of invaded cells and thereby are responsible for their enlargement.

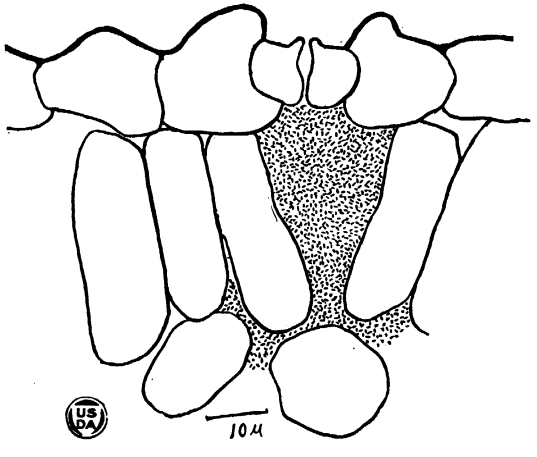


FIG. 4.—Invasion of substomatal cavity by *Bact. phaseoli* var. *sojense*

RÉSUMÉ OF SALIENT CHARACTERS

On the basis of the foregoing studies, *Bact. phaseoli* var. *sojense* is a rod-shaped organism occurring singly, in pairs, or catenulately. The cells measure 1.3 to 2.0×0.6 to .75 μ, are motile by means of a single polar flagellum, possess no well-defined capsule nor endospores, are strictly aerobic and Gram-negative. Colonies on nutrient agar are circular, raised, smooth, shiny, yellow and have an entire or slightly lobed margin. The organism is capable of liquefying gelatin and blood serum, digesting casein and asparagin, is strongly diastatic, very resistant to drying, nonnitrate reducing, and forms neither acid nor gas from the various carbon compounds. Its thermal death-point is approximately 50° C. It has not been possible in culture to distinguish it from *Bact. phaseoli.* According to the 1920 descriptive chart

of the Society of American Bacteriologists its group number is 5322–31135–1333.

SUMMARY

(1) The present investigation concerns a leafspot disease of soybean called bacterial pustule, which is generally prevalent in North Carolina. It is known to occur also in Texas, Louisiana, South Carolina, Virginia, and Kansas.

(2) The disease is distinct from bacterial blight and from the diseases of bacterial origin which have been described in the Orient.

(3) Bacterial pustule appears to be confined to the foliage. Lesions are manifested by the presence of pustular outgrowths on either or both leaf surfaces. They are light green at first, but at maturity collapse and become dry and reddish brown, and the tissues surrounding the lesions become chlorotic.

(4) The disease is caused by an organism to which the name *Bacterium phaseoli* var. *sojense* was first tentatively given by Miss Hedges. This organism is herein fully described and found to be morphologically and culturally indistinguishable from *Bact. phaseoli* E. F. Smith. It forms yellowish colonies on nutrient agar, is flagellate, is unable to utilize any of the carbon compounds tested except starch but can utilize a number of proteins including gelatin, casein, blood serum, and asparagin. According to the descriptive chart of 1920 of the American Society of Bacteriologists, its group number is 5322–31135–1333.

(5) When the organism in watery suspension is applied to uninjured soybean, foliage infection is evident within four to six days. Under the same conditions of inoculation garden beans and Lima beans failed to become infected.

(6) The parasite gains entrance through the stomates and passes thence into the intercellular spaces. The pustules arise by hypertrophic changes of any of the parenchymatous tissues.

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