BACTERIAL SPOT OF COWPEA AND LIMA BEAN[1]

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INTRODUCTION

The bacterial spot disease of cowpeas was first noted in southern Indiana in 1919, but its bacterial nature was not determined until 1921, when it occurred in an experimental plot of cowpeas at La Fayette, Ind. It is a typical spot disease of the leaves, stems, and pods, distinctly different from the other bacterial diseases of cowpeas reported in the literature. The organism which causes this disease also causes a very similar and widespread spot disease of lima beans which has been described recently by Tisdale and Williamson (*23*, *24*).[3] The work herein reported deals mainly with the symptoms of the disease as it occurs on cowpeas, the isolation, characteristics, overwintering, pathogenicity, and dissemination of the causative bacteria, and the identity of the latter with the species causing the lima-bean disease.

HISTORY AND OCCURRENCE

A search of the literature has not revealed any previous description of this disease of cowpeas, although the symptoms of the spot disease of lima beans and cowpeas described by Smith (*18*, *p. 15*) in 1905, and attributed to *Phyllosticta phaseolina* Sacc., closely resemble those of the disease under consideration. A number of cases of bacterial infection of cowpeas have been recorded. Smith and McCulloch (*19*), in 1919, reported a wilt of cowpeas produced by inoculation with *Bacterium solanacearum* E. F. S. Smith (*20*, *p. 280*) mentions a bacterial spot of cowpeas, but does not describe the disease. Rapd (*15*, *p. 3*) in 1920 and more recently Burkholder (*3*, *p. 7*) have reported infection of cowpeas with *Bacterium phaseoli* E. F. S., an organism which, however, differs radically in culture from the organism causing the spot disease. Wolf and Foster (*25*, *p. 452*) have isolated the tobacco wildfire organism (*Bact. tabacum* Wolf and Foster) from small, yellowish leaf spots on cowpeas grown near infected tobacco, but attribute the cowpea lesions to infection about leafhopper wounds. Osmun (*14*) has isolated the wildfire organism from lima beans grown adjacent to tobacco, and Johnson, Slagg, and Murwin (*10*, *p. 177*) successfully inoculated cowpeas with the organism. However, this organism differs culturally from the one causing the cowpea spot disease, and the writers' attempts to inoculate tobacco have failed. The history of bacterial spot as it occurs on lima bean has been given by Tisdale and Williamson (*24*). Chupp has reported to the Federal Plant Disease Survey the presence since 1918 of the disease on lima beans in New York.

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[3] Reference is made by number (italic) to "Literature cited," p. 862.

The disease on cowpeas was first found at Vallonia, Ind., in August, 1919, and was next found at Decker, in southern Indiana, in August, 1920. In August, 1921, it occurred in an experimental plot of Whippoorwill cowpeas at La Fayette, Ind., and has occurred in the writers' plots in 1922, 1923, and 1924. The disease appears to be very widespread, since it has been found in seed from South Carolina, Virginia, and Washington, D. C., and was noted in the field in 1922 near Decker, Ind., Seaford, Del., and Louisville, Ky. Seiyo Ito, who examined the writers' plots at La Fayette, said that the same disease also occurs in Japan.

In 1923 the disease was found in Knox County, Ind., and in Kansas by R. P. White, and in Florida by W. B. Tisdale, who also reported it as a serious trouble in 1924. In 1924 the disease was serious in the field crop (variety, New Era) in Jackson County, Ind., and was more severe in the writers' plots at La Fayette than in the preceding three years. This disease as it occurs on lima beans was noted commonly in gardens in Indiana in 1919 and 1920, and it occurred to a considerable extent in 1923 and 1924.

HOSTS

Greenhouse inoculations and field studies have shown that the following are hosts of this parasite: Cowpea (*Vigna sinensis* [L.] Endl.); catjang (*Vigna catjang* Walp.); hyacinth bean (*Dolichos lablab* L.); Florida velvet bean (*Stizolobium deeringianum* Bort); adsuki bean (*Phaseolus angularis* Wight); lima bean (*Phaseolus limensis* Macf.), as represented by the Large White Pole, Giant Podded Pole, and King of the Garden varieties; bush lima bean (*Phaseolus limensis* Macf. var. *limenanus* Bailey), as represented by the Burpee's Bush and Fordhook varieties; and the dwarf sieva bean (*Phaseolus lunatus* L. var. *lunonanus* Bailey), as represented by the Henderson's Bush variety. Tisdale and Williamson (*24*) had found the varieties Burpee's Bush, Fordhook, King of the Garden, Dreer's Bush, and Henderson's Bush to be susceptible. Natural infection with what is in all probability the same organism was found rather abundantly on the leaves of the common native weed, tick trefoil (*Desmodium canescens* [L.] DC.), in a fallow field near La Fayette, Ind., on August 23, 1924, and has also been found on asparagus bean (*Vigna sesquipedalis* Wight) in field plots.

All of the following 23 varieties of cowpeas tested have proved to be susceptible: Whippoorwill, Brabham, Early Red, Early Black, Early Buff, Taylor, Black, Red Ripper, Iron, Conch, Groit, New Era, Clay, Wonderful, California Blackeye, Early Ramshorn Blackeye, Cream Chowder, Gallavant, Large Blackeye, Arlington, Columbia, Progressive White, and Victor.

SYMPTOMS

On the cowpea leaves, the spots as usually seen in the field are irregularly circular or lobed rather than angular, and are 1 to 4 mm. broad, sometimes larger. Very young spots are small, circular, sunken dots, first water soaked or greasy, later claret-brown in color (*16*) (pl. 1, A). The larger lesions are characterized by a buff center surrounded by a conspicuous maroon or claret-brown margin about 1 mm. in width (pl. 1, B; pl. 2, A). Leaf lesions frequently become

A.—Lower surface of cowpea leaflet, showing small sunken lesions; and, on lower left side, a large lesion extending along a vein. ×2
B.—Upper surface of cowpea leaflet, showing lesions, older than those in A, with light centers and reddish-brown margins. In some the centers have fallen out. The large lesion near left-hand edge has an extension along a vein. ×2

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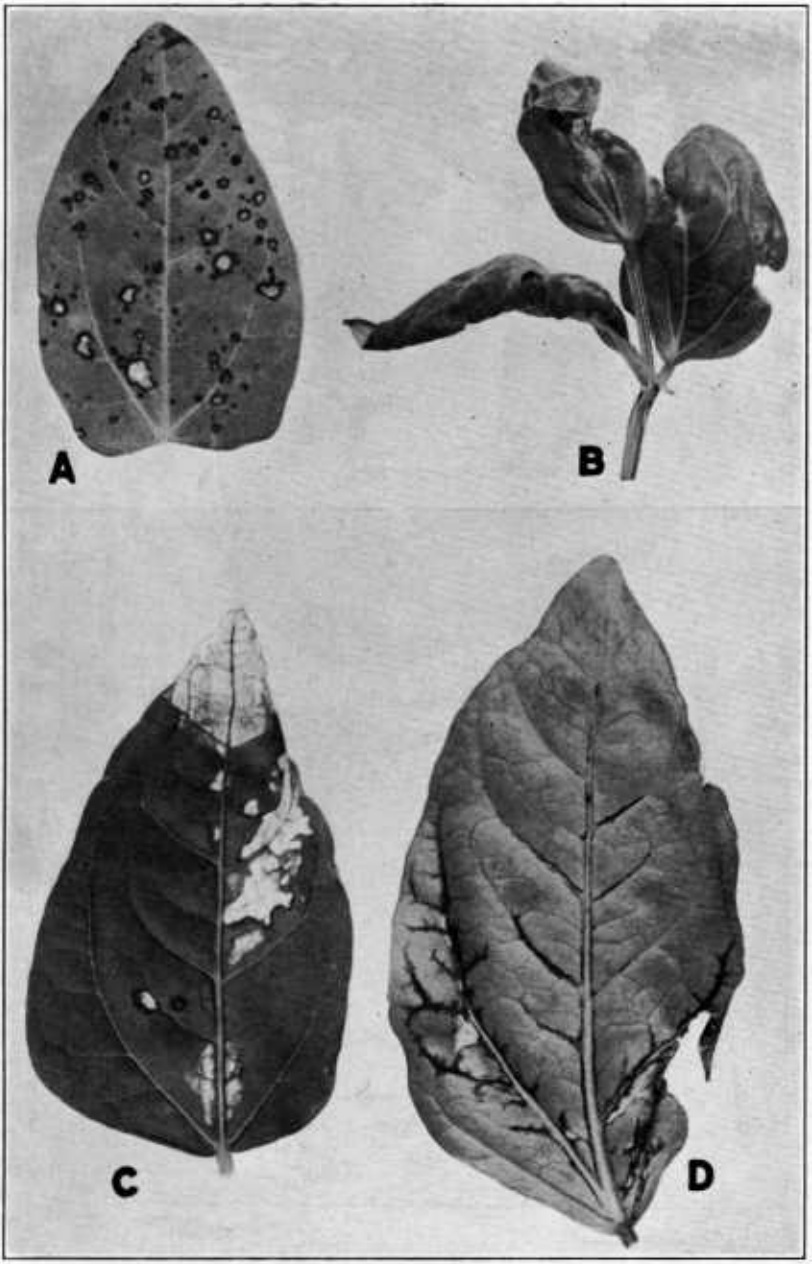


A.—Catjang leaflet, showing bacterial spot lesions
B.—Cowpea leaf. Infected early along the veins. Note distortion
C.—Cowpea leaflet. Infected early. Note bleached areas
D.—Lower side of cowpea leaflet, showing invasion along the vein

rather large, bleached, dried areas surrounded by a claret-brown border (pl. 3, B). In old lesions the central tissue dries and may crack or drop out. Frequently the central tissue is invaded by fungi.

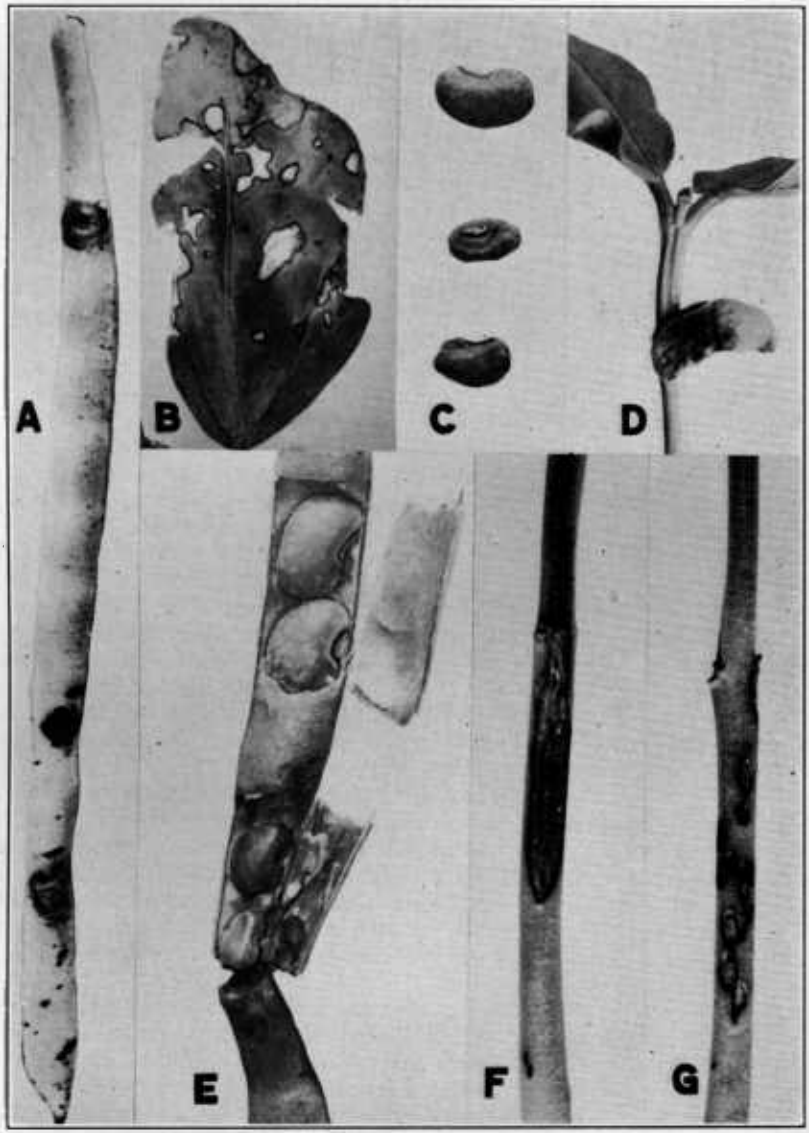
The lesions are not in any way delimited by the veins; on the contrary they frequently extend markedly along the veins (pl. 2, D; pl. 4, D and E). Linear leaf lesions have been noted extending along a vein a distance of 4 to 6 cm. If young leaf lesions are mounted in a drop of water on a slide and cut across with a scalpel, the bacteria may readily be seen under the microscope, oozing out in cloudy masses from the cut edge, especially from the region of the veins.

Infection occurs very readily on young growing leaves, and considerable distortion, curling, tearing, and puncturing of the leaves may be caused by the growth stresses (pl. 2, B; pl. 3, B). Lesions of early inception may thus become maroon-bordered slits and tears in the leaf lamina or along its margin, and vein lesions may cause curvature and crinkling. Lesions on a vein may cause a yellowing or bleaching (pl. 2, C) of distal portions of the leaf.

Lesions on the young leaves of cowpea seedlings under greenhouse conditions first become visible as small sunken pits or craters in the epidermis (pl. 5, C), which are translucent in transmitted light but soon become greasy or water-soaked and later claret-brown in color. At first such lesions are visible only on the lower side of the leaf and as a rule remain more extensive and more conspicuous there than on the upper side of the leaf. Older lesions assume the buff center and the claret-brown border and are surrounded by a narrow light-green halo. Also very small claret-brown lesions occur on the stipules (pl. 6, D). The symptoms on lima-bean leaves, described by Tisdale and Williamson (*24*), are essentially similar to those on cowpea leaves, as are also the lesions on catjang leaves (pl. 2, A). On the leaves of artificially infected velvet-bean plants the lesions were dark brown to black, with some tendency to be delimited by the veins (pl. 6, F). Natural infection of velvet-bean leaves produced lesions more closely resembling those on cowpea. These were irregularly circular, with a tan center and a dark-brown marginal line, and were surrounded by a light-green or yellowish halo in the living tissue. On tick trefoil the lesions showed a tan to light-brown center, with a narrow, reddish-brown marginal line, and were surrounded by a light-green or yellowish halo in the living tissue (pl. 5, B).

On the cowpea stems and petioles the lesions are more or less oval, 1 to 5 mm. long, and Victoria lake in color (pl. 6, D and E). The center is usually sunken, and there may be water-soaked tissue above and below the lesion. Frequently the lesions are much longer, especially on the petioles, and large sunken lesions are formed on the epicotyls and hypocotyls of seedlings (pl. 3, F and G). Similar stem and petiole infection also occurs on lima beans. Catjang leaves have been noticed to break off at petiole lesions.

The lesions on cowpea and catjang pods are irregularly circular and 1 to 8 mm. in diameter, and morocco red, claret brown, maroon, or Victoria lake in color (pl. 3, A). The larger lesions often have a sunken center and a water-soaked outer border. Infection of young pods results in a marked constriction of the pod at the point of infection, and usually in an abnormal bending of the pod at that



A.—Full-grown cowpea pod, showing lesions which have not caused conspicuous constrictions

B.—Cowpea leaflet, showing large lesions with tan centers and reddish brown borders. Such lesions result from early infection and cause malformation and shattering of leaves

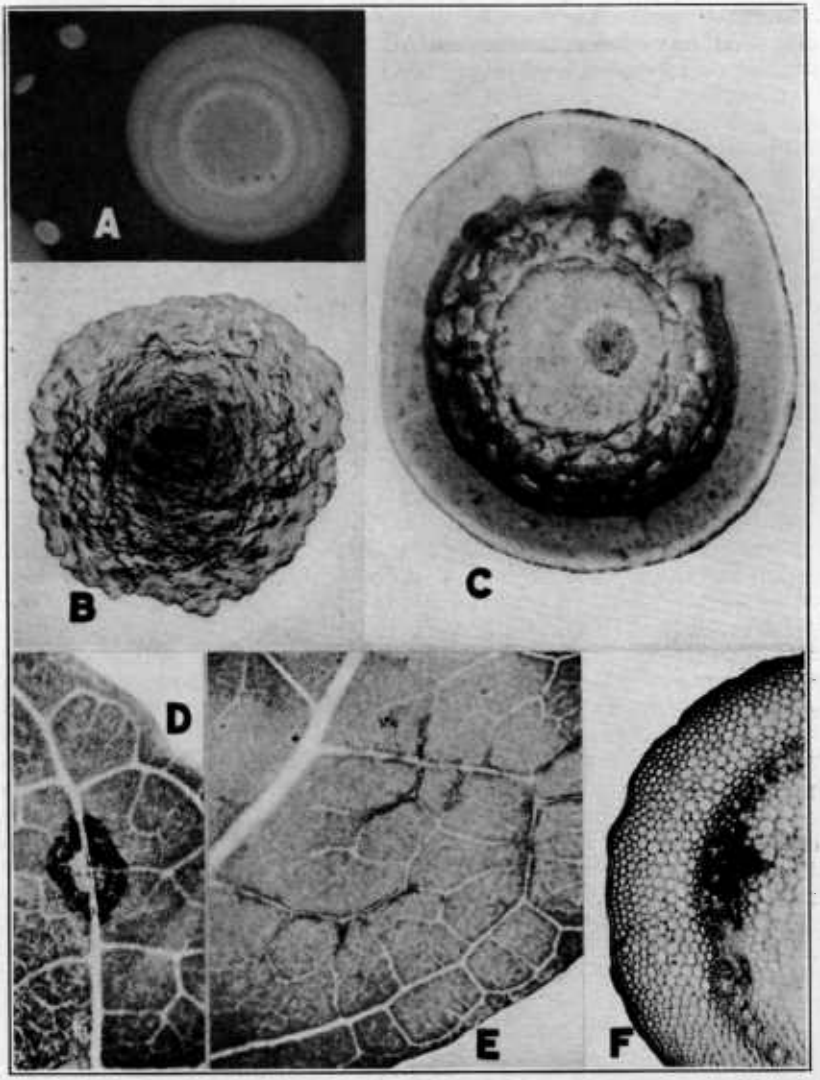
C.—Normal cowpea seed above. Two infected, shriveled, discolored seeds below, for comparison. × 2

D.—Seedling from an infected seed, showing the cotyledon lesion. × 2

E.—Portion of cowpea pod, showing the large normal seeds in the healthy part of the pod and the small, dark, infected seeds under a bacterial-spot lesion. × 2

F.—Portion of seedling stem, showing a large lesion extending down the hypocotyl from the point of attachment of the cotyledon. Such a lesion is the result of invasion from a cotyledon lesion such as is shown in D. × 2

G.—Hypocotyl of seedling enlarged (× 2) to show sunken lesions



A.—Agar-plate surface colony, with smaller submerged colonies at left. × 5
B.—Surface colony on a very dry agar, showing lobed margin and surface sculpturing. × 20
C.—Surface colony such as that shown in A, showing internal pattern. × 17
D.—Cowpea leaf lesion, showing that the veins do not limit the spread of the invasion. × 9
E.—Infection extending along the veinlets in a cowpea leaf. × 9
F.—Cross section of hypocotyl of cowpea, showing bacterial invasion of xylem elements as a result of infection proceeding from a cotyledon lesion. × 34

point (pl. 6, A, B, and C). Sometimes the entire distal portion of the pod fails to enlarge. Large lesions frequently penetrate through the ovary wall to the seed, causing a stunting, shriveling, and dark discoloration of the latter (pl. 3, C and E). However, attempts to separate upon this basis the infected from the healthy in commercial seed, have been unsuccessful. Tisdale and Williamson (*24*) found similar pod lesions and resultant seed infection in the lima bean.

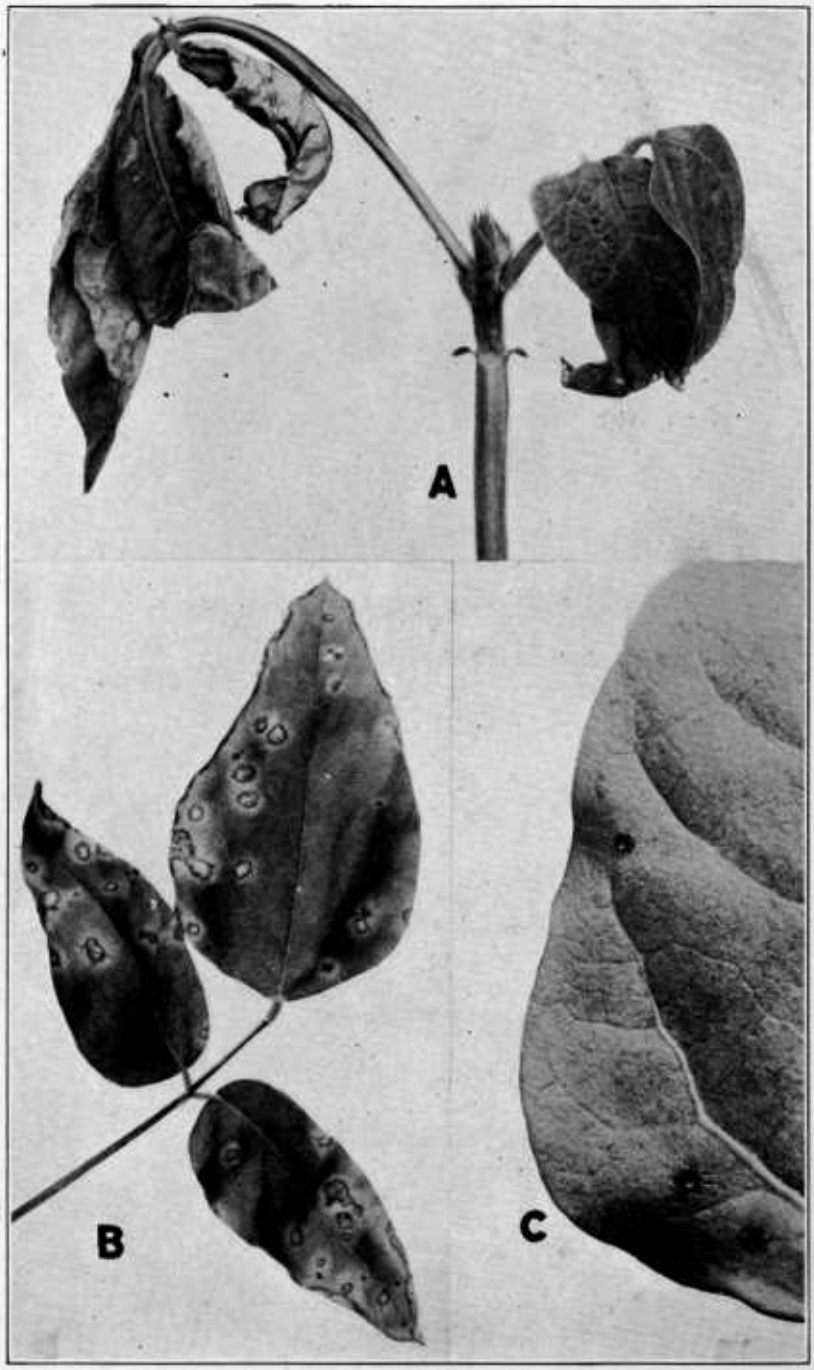
In cowpea seedlings from infected seed the cotyledons bear large lesions which frequently cause a water-soaking or dark discoloration and a shriveling or constriction of a considerable portion of the cotyledon (pl. 3, D). Such lesions are frequently accompanied by a transverse crack or fissure, and may kill the cotyledon prematurely. Oval, maroon lesions also occur on the hypocotyls and epicotyls of such seedlings (pl. 3, F and G).

From the infected cotyledons or from epicotyl lesions, infection of the vascular bundles occurs (pl. 4, F), and the invasion frequently extends up along one or more bundles through the petiole and out into the veins of the first leaf. This type of infection is visible as an internal reddish-brown streak within the vascular bundles, and causes a yellowing or wilting and blighting of all or portions of one or both of the first leaves. The affected portions of such leaves may show a darkened network of veins (pl. 2, C), and such infection has been observed to cause a rather extensive, shiny, brownish discoloration of the lower surface of the leaf along the veins. In some cases a preliminary yellowing of the veinlets and a stunting of the corresponding half of each leaf was observed. In extreme cases the entire seedling may be stunted or may wilt and die. Similar wilting and blighting effects have been noted on the first compound leaves of fieldgrown seedlings (pl. 5, A), and also on older plants as a consequence of early infection of the petiole which had resulted in extensive internal invasion of the vascular tissues. Under such conditions, irregular bleached areas of considerable extent frequently occurred on the leaf blades (pl. 2, C), accompanied sometimes by an extensive reddish discoloration of the lower epidermis.

The tendency to invade the veins has been noticed in the case of lesions on or near the veins of young leaves, in which case a brown streak may be traced out through or along the vein and into its smaller branches (pl. 1, A, B; pl. 2, D). Such infection may cause a yellowing or wilting of portions of the lamina supplied by the infected vein. Thus, although this is typically a spot disease, there are distinct evidences of localized vascular infection, particularly in the seedlings, which results in symptoms of a systemic nature.

In the Early Buff variety of cowpea this disease should not be confused with another spot disease with which a species of Cladosporium is associated. The latter trouble is characterized by smaller purple to black spots on the pods, irregular in outline, and by oval, sunken, purplish spots on the young stems and peduncles. These lesions may have a tan center on which appears a greenish, velvety, fungous growth. Pod infection occurs very early and often causes great distortion of the pods.

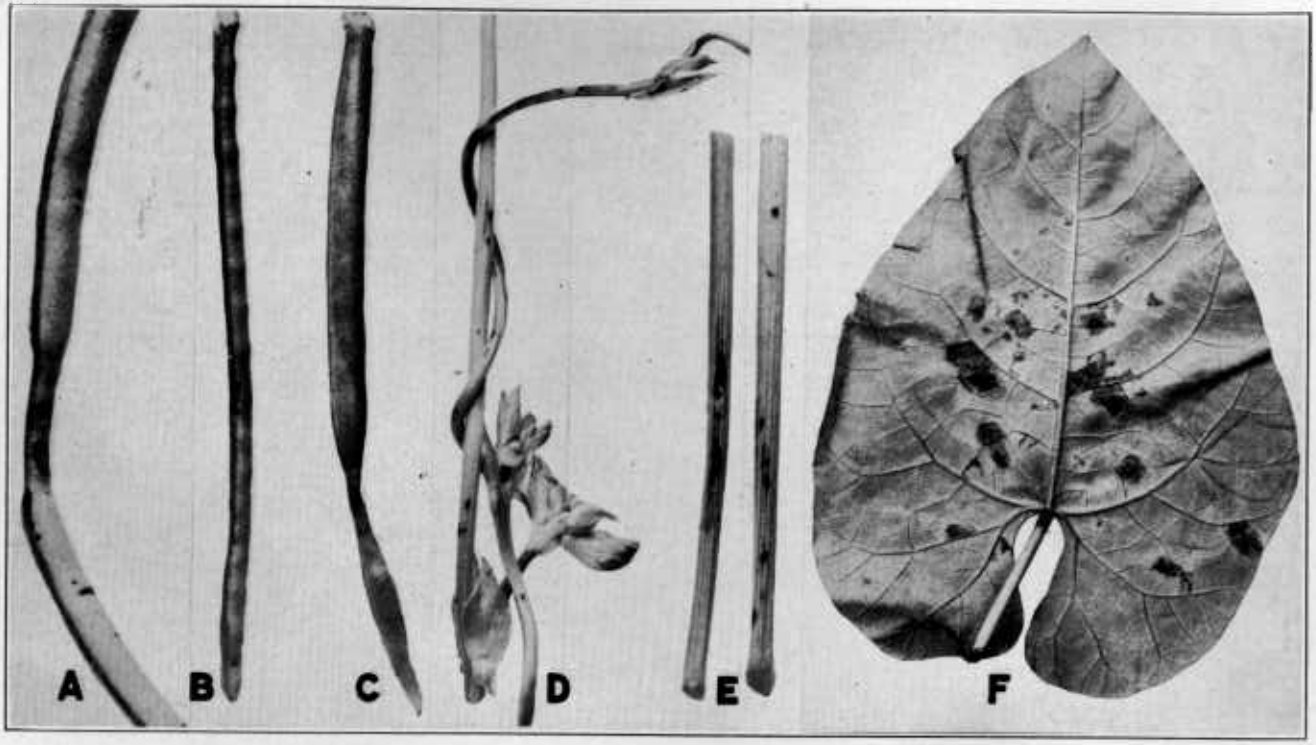
Early stages of the leaf spot caused by *Amerosporium oeconomicum* E. and T., as observed on the New Era variety, somewhat resemble bacterial spot, but the white center, concentric rings, and pycnidia of older lesions serve as distinguishing characters. The



A.—Wilting of leaves of field-grown cowpea seedling as a result of vascular infection in the stem and petiole

B.—Lesions on leaf of tick trefoil resulting from natural infection. The dry centers are tan or light brown, surrounded by a narrow dark-brown margin and a light-green or yellow halo

C.—Craterlike lesions on upper side of a first leaf of a cowpea seedling as a result of seed-borne infection ×3



A.—Portion of immature cowpea pod, showing a constriction due to two lesions resulting from early infection
B.—Young cowpea pod, showing stage at which growth is arrested by infection which results in such constrictions as are seen in A and C
C.—Young cowpea pod failing to enlarge at the infected point
D.—Lesions on stems and bracts of cowpea
E.—Lesions on petioles of cowpea
F.—Lesions on velvet bean leaflet resulting from atomizer inoculation

early stages of the leaf lesions caused by *Cercospora cruenta* Sacc. are more diffuse and less clearly defined. The older lesions are larger than the bacterial lesions, with a darker center and less conspicuous border than the latter, and show some tendency, at least in the Blackeye variety, to be delimited by the larger veins. The abundant sporulation of the fungus is of course a reliable differential characteristic.

ECONOMIC IMPORTANCE

The bacterial spot disease may cause very severe foliage injury to cowpea seedlings and young plants, especially in wet seasons, and not only kills leaves but may even cause the death of many young plants. Tisdale has reported through the Federal plant disease survey that this disease caused a serious defoliation of cowpeas in Florida. In the cowpea crop grown for seed the pod lesions of this disease may cause considerable loss. In the case of lesions constricting and stunting the pod, the number of seeds is reduced, and seeds borne under lesions are stunted or shriveled, are impaired in germinability, and produce diseased and weakened seedlings. Leaf and stem infection on older plants is, as a rule, less destructive. The disease seems to be very destructive to lima beans, especially in its leaf attack (*24*).

CAUSAL ORGANISM

ISOLATION

The bacterial nature of the disease on cowpeas was discovered in the late summer of 1921. On August 26 the surface of a spotted pod was sterilized in a 1:1,000 solution of mercuric chloride and a lesion was cut out with a flamed scalpel and macerated in a drop of sterile water on a flamed slide. This drop of water was plated out in potato-dextrose agar by the loop-dilution method, and after three days at room temperature the plates were evenly seeded with similar grayish-white bacterial colonies. The surface was sliced off from another pod lesion and a portion of the underlying brown tissue of the ovary wall was similarly tested. The plates were evenly seeded with colonies similar to those from the other lesion.

On the same date two leaf lesions were cut out with flamed scissors, immersed first in alcohol to wet the surfaces and then in a 1:1,000 solution of mercuric chloride for a few minutes, rinsed in sterile water, and macerated in drops of sterile water on flamed slides. The loop-dilution plates from both lesions were evenly seeded with grayish-white colonies apparently identical with those in the plates from the pod lesions. Furthermore, 18 leaf lesions were similarly cut out, sterilized, rinsed, and planted in poured plates of potato-dextrose agar. Grayish-white bacterial growth occurred around nine of these. Poured plate isolations made on August 30 from leaf, pod, and stem lesions yielded a similar organism. Numerous transfers were made from typical plate colonies to dextrose-potato agar slants and all appeared to be similar. Successful atomizer inoculations were made in the field and greenhouse, and the organism was reisolated from the lesions produced.

Successful isolations were made later from seedling cowpeas grown in sterile soil from diseased commercial seed. The organism was obtained from lesions on cotyledons, first leaves, epicotyl, and hypocotyl, and from vascular infection in the epicotyl. In 1923 the

organism was isolated also from lima beans grown in home gardens and from those exposed to infection in the experimental plots at the Purdue Experiment Station.

For the subsequent detailed study three strains were used, one an isolation from a cowpea-leaf lesion made August 26, 1921, and two were reisolations made in September, 1921, from cowpea-leaf lesions produced by field inoculations. These strains were tested for purity by poured plates and for pathogenicity by inoculations.

MORPHOLOGY

The organism is a rather small rod with rounded ends, and occurs singly or in pairs. The rods stain more readily in gentian violet than in Ziehl's carbol fuchsin or rose bengal. In agar cultures 24 hours old the cells varied in width from 0.44 to 0.66 μ, and in length from 1.10 to 2.34 μ, with an average of about 0.54 by 1.47 μ.

To obtain actively motile cells for the flagella stain, a piece was cut out of a 48-hour-old culture on potato-dextrose agar at the base of the slant and dropped into a sterile water blank. After four hours an examination of a hanging drop showed an abundance of motile cells and indicated that the organisms had diffused throughout the liquid. Smears were made from this water culture, and the flagella were stained by Van Ermengem's method. One to five flagella were found at one or both poles. Considerable variation was found, but more frequently there were three or four flagella at one pole and fewer at the other. Five flagella were noted at each pole in some instances. In another culture the predominating condition was that of one or two flagella at only one end of the rod. A few flagella were measured and averaged 6.5 μ in length.

Endospores, capsules, and involution forms have not been noted. In water suspensions 30 hours old the cells become swollen and vacuolated. The organism is gram negative.

CULTURAL CHARACTERS

The organism grows well on potato agar with 1 or 2 per cent dextrose and no peptone, and this medium has been generally used. Equally good growth occurs on lima-bean agar without dextrose. Unless otherwise specified, the cultures were incubated at room temperature. The reaction of the media as expressed in Fuller's scale was adjusted by titration, using as the neutral point the first permanent but faint pink color with phenolphthalein. Inoculations were made from water suspensions unless otherwise stated. Cultures of *Bacillus coli* and *Bacterium glycineum* Coerper were carried in parallel series in part of the tests.

AGAR POURED PLATES.—On + 10 beef-peptone agar, colonies appeared in 24 hours; and in 56 hours surface colonies were 2 mm. in diameter, and round, raised, glistening, and grayish white. Submerged colonies were smaller and lens shaped. In six days surface colonies were round with an entire margin, raised, and smooth, with a finely granular internal structure showing faint concentric markings (pl. 4, A). The color was grayish white in reflected light and slightly fluorescent in transmitted light.

Surface colonies on potato-dextrose agar incubated four days at 27° C., were 3 to 4 mm. in diameter, and in one week were 5 to 7 mm. in diameter. The colonies were round, and the margin entire (pl. 4, A); the surface was smooth or concentrically ridged; and the elevation varied from raised to pulvinate, with the central portion often higher, making the colony umbonate. The colonies had a finely granular appearance, with a more or less concentric pattern, as

illustrated in Plate 4, C, and were grayish white in reflected light and greenish fluorescent in transmitted light. The colonies which emerge after the agar surface has dried slightly seem to pile up more conspicuously. Colonies on agar that has dried out to some extent have a scalloped margin and sculptured surface, as shown in Plate 4, B. Submerged colonies are lens-shaped, white, and very small (pl. 4, A). Colonies on the underside of the agar are thin, transparent, and greenish fluorescent. The agar is unchanged in color. Only a slight odor is noticeable.

AGAR STABS.—In +10 beef-peptone agar, growth along the stab was scanty and filiform, and on the surface was restricted but piled up. In potato-dextrose agar, growth occurred in the stab along the upper part only, and this was slight; but on the surface the growth was abundant, spreading, flat, and dull with a rugose zone. Growth on this medium was much more vigorous than on the beef-peptone agar.

AGAR SLANTS.—On +10 beef-peptone agar, the growth was moderate, spreading, flat, smooth, and grayish white. On potato-dextrose agar the growth was abundant, spreading, flat, dull, finely rugose, and grayish white. The margin was entire, with a definite beveled border. There was no change in the color of this medium in either case. On lima-bean agar a slight greenish pigmentation of the medium occurred.

GELATIN PLATES.—In two days flat, circular, white colonies, producing saucer-shaped zones of liquefaction, were present. Liquefaction had proceeded to completion the third day.

GELATIN STABS.—The liquefaction was napiform in 2 days, infundibuliform in 3 days, stratiform in 7 days, and complete in 14 days, with a white flocculent precipitate.

POTATO CYLINDERS.—On steamed potato cylinders growth was rapid and abundant, grayish white, and somewhat iridescent, smooth, and glistening, and had spread over all the moist surface of the substratum. There was no change in the color of the potato tissue.

MILK.—Clearing of the milk without coagulation began at the top in 2 days and was complete in 33 days. Throughout this period the cleared liquid was of a pale greenish-yellow color, and the liquid became viscid or gelatinous in consistency.

LITMUS MILK.—Pale-blue litmus milk was completely decolorized in seven days, but no pink color appeared. Digestion proceeded as noted above and was complete in 26 days. The cleared liquid was slightly yellowish green.

BROM CRESOL PURPLE IN MILK.—Brom cresol purple produces a light bluish color in milk and becomes yellow if the hydrogen-ion concentration is increased. Seven days after inoculation the color was unchanged, except that it was even more purplish in the upper cleared portion. After 21 days the blue color remained. Accordingly, there is no acid production in milk.

METHYLENE BLUE IN MILK.—In this medium a deeper blue color was noted at the end of two days, while after seven days the milk was completely decolorized except for the cleared portion at the surface, which was greenish. After 33 days the color was maize yellow, except for a thin greenish layer at the surface.

REDUCTION OF NITRATES.—In fermentation tubes containing 1 per cent potassium nitrate in a 2 per cent Difco peptone solution, there was good growth in the open arm and none in the closed arm. No gas was formed. At the end of a month the liquid in the open arm showed a slightly yellowish green. Test-tube cultures in the same medium were tested with Trommsdorf's reagent at 14 and 33 days after inoculation, and no nitrites were detected. With Nessler's reagent, a strong positive test for ammonia was obtained. Apparently nitrates were not reduced, and ammonia probably was produced from the peptone.

CARBON METABOLISM.—To test for acid and gas production with different carbon sources, 2 per cent solutions of dextrose, saccharose, maltose, lactose, mannite, and glycerin were made up in a 2 per cent Difco peptone solution. Cultures were run in duplicate, first in the ordinary U type of fermentation tube and later in a simpler and more convenient type of fermentation tube (*9, fig. 47*) consisting of a smaller inverted test tube within a larger one, a type very satisfactory where it is not necessary to measure the quantity of gas. In all cases there was abundant growth in the open arm and none in the closed arm. No gas was formed. Titration with N/20 sodium hydroxide at the end of 17 days in one series and 33 days in the other revealed no marked change in acidity as compared with the sterile control tubes. The lactose and glycerin cultures were slightly less acid than the controls in one series.

In order to determine more accurately the changes in true acidity in these media, three series of six test tubes each were made up, one series containing brom cresol purple, one brom thymol blue, and the other phenol red in a concentration of 0.0016 per cent. These media were adjusted to about P_H 7.3 as indicated by the blue-green color of brom thymol blue and the red of the phenol red. The dextrose and saccharose cultures with brom thymol blue and phenol red became yellow (more acid) seven days after inoculation. All the other cultures slowly became less acid than the controls. There was, therefore, acid production from dextrose and saccharose, but none from the other carbon sources. Probably the decrease in true acidity was due to ammonia produced from the peptone.

AGAR WITH SUGARS.—In litmus-dextrose, litmus-maltose, and litmus-lactose agar slant cultures there was no evidence of acid production. In two days the maltose and lactose cultures were blue under the stroke, and in seven days all cultures were bluer throughout than the controls. However, in a later repetition of this series, in which saccharose, mannite, and glycerin were also tested, a pink color developed in the dextrose and saccharose tubes, indicating acid production.

To obtain more accurate information on this point, the three sulphone phthalein indicators, brom cresol purple, brom thymol blue, and phenol red, were used in a triplicate series of slant cultures with dextrose, maltose, and lactose. These media were adjusted to a P_H of about 7.0 as evidenced by the grass-green color of brom thymol blue. In the dextrose cultures containing brom cresol purple and brom thymol blue a yellow color developed, indicating acid production. No increase of hydrogen-ion concentration was indicated at any time with the other two sugars and most of the cultures became slightly more alkaline. In a later repetition of this test, saccharose, mannite, and glycerin were also included and distinct acid production occurred only in the dextrose and saccharose cultures. Parallel series of cultures of *Bacillus coli* produced the yellow-acid color with all of the indicators. These tests show that there is acid production from dextrose and saccharose.

ACTION ON STARCH.—There were no signs of diastatic action on either potato or corn starch. No halos or cleared zones appeared about the colonies in plates of beef agar to which starch was added.

TESTS FOR INDOL, SKATOL, AND AMMONIA.—Cultures in beef-peptone bouillon gave no test for indol at 7, 26, and 33 day intervals when tested with potassium nitrite and sulphuric acid and no test for skatol when tested with nitric acid and potassium nitrite. A positive test for ammonia was obtained after 7 and 33 days with Nessler's reagent.

FERMI'S SOLUTION.—Growth occurred in Fermi's solution, accompanied by the formation of a greenish-yellow pigment throughout the medium.

USCHINSKY'S SOLUTION.—Good growth occurred in Uschinsky's solution in the case of two of the three strains tested, and a greenish-yellow pigment was produced.

COHN'S SOLUTION.—No growth occurred in Cohn's solution.

BLOOD SERUM.—The growth in stroke cultures on plain solidified blood serum in 10 days was abundant, spreading, flat, smooth, and glistening, and showed a brownish tinge. A slight liquefaction of the medium along the stroke was noted after 17 days, and at the end of 40 days there was a general liquefaction of the upper part of the slant, and a brownish-yellow discoloration of the medium.

On slants of Loeffler's blood serum, growth was more rapid than on plain blood serum, and in two days was abundant, spreading, flat, and rugose. In 10 days the medium showed a slight brownish color and slight liquefaction under the stroke. At the end of 40 days the medium was almost completely liquefied, and was clay color according to Ridgway's chart (*16*).

TOLERATION OF SODIUM CHLORIDE.—In tubes of beef-peptone bouillon neutral to brom thymol blue, 4 per cent of sodium chloride was tolerated and 5 per cent inhibited growth. In a series neutral to phenolphthalein, 5 per cent of sodium chloride was tolerated. Evidence was obtained that the use of hydrochloric acid in adjusting the reaction increased the inhibitory properties of sodium chloride.

TOLERATION OF ACIDS AND ALKALIES.—Tubes of beef-peptone bouillon were adjusted to +30, +25, +20, +18, +15, +14, +12, +10, +5, 0, −5, −10, −15, −20, −30, and −40 Fuller's scale by the use of hydrochloric acid and sodium hydroxide. The three strains grew in the +10, +5, 0, −10, and −15 tubes, and two strains grew in the +12 tubes. In a later series growth occurred in the +15 and −25 tubes. Growth seemed most vigorous in the +5 media. A greenish-yellow pigment was formed in the alkaline cultures.

In order to determine the tolerance of true acidity as indicated by the hydrogen-ion concentration, a duplicate series of beef-broth tubes was made up, one with hydrochloric acid and the other with malic acid, and adjusted by means of the sulphone phthalein indicators. No growth occurred in the tubes acidified to P_H 4.1 with either acid, but retarded growth occurred in both cases in the tubes acidified to P_H 4.5. Good growth occurred in the P_H 5.0 tubes. Growth occurred in tubes rendered alkaline to P_H 8.5 with either sodium hydroxide or ammonium hydroxide.

TEMPERATURE RELATIONS

The organism grows throughout a wide range of temperatures. Slant and plate cultures on potato agar incubated in moist chambers at 3° C., 9°, 12°, 15°, 20°, 23°, 27°, 30°, and 35° showed that the organism did not grow at 3° or 35°, but grew slowly at 9°, and 12°, moderately at 15°, 20°, and 35°, and rapidly at 23°, 27°, and 30°, with a fairly distinct optimum at 27°.

In determining the thermal death point, water suspensions from agar slant cultures were subjected to 10-minute exposures to a series of temperatures in a water bath and tested by loop transfers to agar slants (*12*). The thermal death-point was found to lie between 49° and 50° C.

EFFECT OF FREEZING

Heavy water suspensions from slant cultures were placed in test tubes, frozen in an ice-salt mixture, and held in a refrigerator. At intervals tubes were removed and thawed out and plates poured. The approximate number of living bacteria per cubic centimeter as indicated by the plate counts is shown in Table I.

TABLE I.—*Effect on bacteria of freezing in water*

Time frozen	Approximate number per cubic centimeter		Time frozen	Approximate number per cubic centimeter	
	Strain A	Strain B		Strain A	Strain B
0 [a]	12,635,000	106,714,000	5 days	2,000	149,000
4½ hours	3,192,000	35,285,000	8 days	250	2,400
1 day	2,777,000	13,050,000	11 days	360	540
2 days	27,000	1,224,000	15 days	20	0
4 days	4,000	21,000			

[a] Original suspension.

The results in Table I show that the organism is slowly killed by freezing in water.

EFFECT OF SUNLIGHT

Plates poured from tubes inoculated with a suspension of the organisms, and each partly shaded with black paper attached to the glass, were placed on a cake of ice and exposed to the afternoon sun for periods of varying length. Ten and fifteen minute exposures did not reduce the number of colonies, while 25, 30, and 45 minute exposures greatly reduced the number of colonies, and exposures of 60 minutes or more resulted in complete sterilization.

RESISTANCE TO DESICCATION

The organism is very sensitive to desiccation on glass. Drops of a water suspension of the organisms were allowed to dry on sterile cover slips, and tests were made by inserting the cover slips into agar

slants. No growth was obtained from smears that had been dry 40 minutes, and in most instances no growth was obtained from smears that had just dried.

To test the resistance of the organism to drying on cowpea seeds, small quantities of seed were placed in Petri plates, moistened, sterilized in the autoclave, and allowed to become air-dry. A water suspension of the organisms was then poured over this sterilized seed and allowed to dry. The seed was tested at intervals by planting in agar-poured plates and the organisms were found alive during the following four months. Furthermore, it has been found that the organism lives over winter in the seed, so that it is evident that it is highly resistant to drying on and in cowpea seeds.

Tisdale and Williamson (*24, p. 150*) found the organism alive in lima-bean leaves dried 2½ years. In the writers' experience the organism has not generally been found viable in the older tan-centered leaf lesions on cowpeas; it has a tendency to be rather short lived on potato-agar slants, much more short lived than certain yellow organisms such as *Bacterium phaseoli* E. F. S. and *Bact. vesicatorium* Doidge.

TAXONOMY

The organism causing bacterial spot of cowpea is not identical with any of the previously described bacterial parasites of cowpea, being clearly differentiated in its chromogenesis and other salient characters. It was briefly described in March, 1923, (*6*), and given the name *Bacterium vignae* n. sp. Upon the appearance of this preliminary note the writers received a letter from W. B. Tisdale calling attention to the marked similarity of this organism to the causal organism of the lima-bean disease, a description of which was in press at that time and appeared four months later (*24*). The causal organism of the latter disease was designated *Bacterium viridifaciens* n. sp.

The lima-bean disease was found rather commonly in gardens about La Fayette, Ind. The causal organism was isolated from pod lesions and proved to resemble closely the cowpea organism. With the organism isolated from lima beans, abundant and typical infection of cowpea seedlings grown under a cloth cage in the field was obtained August 15, 1923, along with infection of lima-bean seedlings. In another cage, typical infection of lima-bean seedlings was obtained with one of the strains isolated from cowpeas. Later a culture of Tisdale's organism was obtained from the University of Wisconsin, and with it successful inoculations of cowpea seedlings with the production of characteristic bacterial spot lesions were obtained in the greenhouse in April, 1924. These cross inoculations indicated beyond doubt the identity of the two organisms.

In the meantime, however, the cowpea strain, the strain isolated from lima beans at La Fayette, Ind., and the original lima-bean strain from the Wisconsin laboratory were carefully compared as to a large number of cultural and physiological characters and found practically identical. These tests included the ordinary media such as gelatin, agars, and milk with indicators, agar and bouillon with the six carbon sources and the three hydrogen-ion indicators previously mentioned, toleration of sodium chloride, toleration of acidity, blood media, and Cohn's, Fermi's, and Uschinsky's media. Furthermore, the published description of *Bacterium viridifaciens*

is practically in complete accord with the writers' earlier description and the data herein recorded. While the writers failed to detect an increase in acidity in the dextrose and saccharose fermentation-tube cultures by titration, they obtained striking evidence of acid production with these sugars in the cultures containing the hydrogen-ion indicators. The only difference in group number is due to the writers' failure to class the organism as fluorescent. Since the cowpea and lima-bean organisms are thus shown to be identical, the binomial *Bacterium viridifaciens* becomes synonymous by priority rules with the previously published name, *Bacterium vignae*.[4] (Group Number, 5322–31131–2232).

This organism shows some resemblance to certain other plant pathogenes, such as *Pseudomonas pisi* Sackett (*17*), which it resembles culturally but from which it differs in morphology and pathogenicity, and *Pseudomonas maculicolum* McCulloch (*13*), which it resembles culturally and in type of lesion produced but from which it differs in pathogenicity. It differs both in culture and in pathogenicity from *Bacterium glycineum* Coerper, *Bact. trifoliorum* Jones, Williamson, Wolf, and McCulloch, and *Bact. tabacum* Wolf and Foster, although the Virginia strain of *Bact. trifoliorum* was found to be pathogenic to lima bean and velvet bean (*11, p. 486*). The organism differs radically in morphology from *Aplanobacter stizolobii* Wolf (*26*), the causal organism of bacterial leaf spot of the velvet bean. Attempts to infect cowpeas with *Pseudomonas pisi* have given only negative results, corroborating Sackett's (*17, p. 18*) conclusion that cowpeas were not a host for that organism.

PATHOGENICITY OF CAUSAL ORGANISM

Infection of young, healthy cowpea plants has been produced at will by spraying (from an atomizer) with a water suspension of a young agar slant culture. This has been done in the field and more frequently in the greenhouse, where the plants could be held in a moist chamber for one or two days after the inoculation. The same has been done with young lima-bean plants.

The writers have found, as did Tisdale and Williamson (*24*), that the organism tends to lose its virulence in culture rather rapidly and that best results are obtained with recently isolated or reisolated strains.

The incubation period for leaf infection of cowpeas and lima beans under greenhouse conditions is two to four days, and in the field in August lesions have become visible two days after inoculation. Wounds are not necessary for infection, and the abundance of lesions indicates that the mode of entry into the host tissue is undoubtedly stomatal.

Cowpea seedlings and the younger leaves of older plants have proved much more susceptible to infection than older parts of the plant. It seems possible that this fact may be correlated with a lower hydrogen-ion concentration in the young leaves, a condition found to exist in clover by Haas (*8, p. 350*) and later in pole beans by Gustafson (*7*). In the writers' work with bacterial spot of

[4] According to Migula's classification, Buchanan's revision (*2, p. 48*), and the revision adopted by the committee of the Society of American Bacteriologists (*21, p. 203*), the combination would be *Pseudomonas vignae* n. sp., while in a later report of another committee of the same society (*22, p. 188*), the name has already been changed to *Phytomonas vignae*.

tomato (*5, p. 148*) a correlation was found between the resistance of ripe fruit to infection and the higher hydrogen-ion concentration in the ripe fruit as compared with the leaves and green fruit, and in that disease also the younger parts of the plant were more susceptible to infection.

Clevenger (*4, p. 238*) found that the hydrogen-ion concentration of cowpea leaves was slightly lower than in the stems, and that it showed a diurnal variation (*4, p. 233*). A maximum concentration (P_H 5.27) occurred in the leaves at 10.30 a. m. and a minimum (P_H 5.81) at 1 a. m., while in the stems a maximum (P_H 5.04) occurred at 6.30 a. m. and a minimum (P_H 5.32) at 9 p. m. The writers in their cultural tests found that the parasite tolerated a P_H of 4.5 and grew well at P_H 5.00, so it is evident that the cowpea leaves and stems are well within the limit of tolerance. The lower acidity of the leaves in the night may favor infection at that time.

Atomizer inoculation has been successful upon all of the varieties of cowpea, sieva bean, and lima bean tested, including most of the varieties mentioned in the previous discussion of hosts of this disease, and upon the catjang, hyacinth bean, adsuki bean, and Florida velvet bean (varieties, Bunch and 100-Day). The organism was successfully reisolated from each host species. The three *Phaseolus limensis* varieties—Burpee, Fordhook, and Large White Pole—have appeared to be more susceptible than Henderson's Bush Lima, which, according to Bailey (*1, p. 396*), is a different species. In fact, in the writers' field plots in 1924, Henderson's Bush Lima showed considerable resistance. While the cowpea varieties have been considered equally susceptible, the varieties Early Red, Clay, California Blackeye, Iron, Groit, and Whippoorwill were more severely diseased than any of the other 12 varieties in the 1923 plots. In the 1924 plots the varieties Early Red, Groit, Whippoorwill, New Era, and Catjang were more severely diseased than Blackeye, Iron, and Early Buff. Rather light natural infection occurred on the foliage of velvet beans grown adjacent to cowpeas in 1924, and the organism isolated resembled *Bacterium vignae* in culture, and in inoculation tests produced typical lesions on cowpeas.

The organism isolated from the lesions on *Desmodium canescens* resembled the cowpea organism in culture, and in cross-inoculation tests in a field cage and in the greenhouse produced typical bacterial spot infection on cowpeas. The organism was successfully reisolated, and although its morphology and cultural characters have not yet been studied by the writers, it seems safe to assume that the Desmodium organism is *Bacterium vignae*. The importance of this weed as a possible source of infection is not known.

Unsuccessful attempts have been made to inoculate garden beans (five varieties), soy beans (five varieties), broad bean, sweet pea, peas (seven varieties), lupine, clovers (white, Bokhara, crimson, annual sweet, mammoth red, and alsike), cauliflower, tobacco, tomato, and potato.

RELATION OF PARASITE TO HOST TISSUE

As atomizer inoculation of cowpeas without wounds is successful, it seems likely that the tissues are entered by way of the stomata. Tisdale and Williamson (*24, p. 151*) found that the lima-bean leaf was invaded through the stomata. Microscopic examination of the

leaf lesions shows that the invasion is intercellular and tends to be restricted to the mesophyll during the early stages. In fact the mesophyll may be rather extensively involved without any apparent injury to the palisade tissue. The advance invasion is in the mesophyll layers adjacent to the lower epidermis. The cells involved become a dense reddish brown and soon collapse. The lower mesophyll layers may thus become discolored and collapsed while the upper mesophyll layers and the palisade layer remain apparently uninjured. Later the entire thickness of the lamina is killed and dries out at the center of the lesion.

In cowpea seedlings vascular infection has been frequently observed. The organism may gain entrance to the vascular system from infected cotyledons, epicotyl lesions, petiole lesions, or from lesions on the veins of the leaf. Reddish-brown bundles were traced from the infected cotyledon or epicotyl down into the hypocotyl and up through the epicotyl into the leaf veins (pl. 4, F). In water mounts, the bacteria were seen to ooze from the cut ends of these reddish-brown spiral vessels of the epicotyl, and in cross section these vessels appeared to contain bacteria. The organism was cultured from these internally discolored vascular bundles at some distance from the lesion where it had entered the host. From lesions on the larger veins of the seedling leaves, vascular infection may extend outward a distance of a centimeter or more, and in some cases the reddish-brown vascular elements, being in the xylem, are more clearly visible from the upper leaf surface.

A microscopic examination of unstained razor sections of these internally discolored veins showed that the reddish-brown color was localized in the walls of certain spiral vessels and that this color was very intense, constituting an excellent stain for the walls of the vessels even under the higher powers of the microscope. The stain was also noted in the tubes of the small veinlets in the affected areas. Stained strips of collapsed cells also occurred in the dorsal cortex of the veins and in the mesophyll along the veins.

When the freshly cut edges of leaf lesions were examined in water mounts, the bacteria were seen to ooze from the cut ends of the veins. In cross section the bacteria were noted in the intercellular spaces of the parenchyma of the veins but were not actually demonstrated within the spiral tubes at any distance from the leaf lesion. The organism very evidently shows a preference for the vein tissues and travels more rapidly in the veins than in the mesophyll. In older leaves the tendency for internal vascular invasion is not as marked, although linear surface lesions of considerable length often occur along the veins (pl. 1, A, B; pl. 2, D).

Shallow surface lesions on the epicotyl, hypocotyl, and petioles were found to be limited to the outer cortical layers. The cells in the epidermal and cortical layers were collapsed and dense dark red in color. In large hypocotyl lesions there was evidence of deeper invasion, not in a solid front, but in the shape of ramifying intercellular penetration. There was marked hypertrophy of the cortical cells immediately beneath the epicotyl lesions, and in many cases rather extensive hyperplasia had resulted from the formation of cross walls in these enlarged cells, suggestive of an attempt to occlude the lesion with a cork layer. Very early infection of the

epicotyl results in linear lesions occupying deep grooves and in cross section it was seen that these lesions extended inward through the cambium to the pith and were accompanied by extensive hyperplasia of the latter tissue. These lesions result in a break or uncompleted segment in the vascular cylinder, and, as a result of enlargement of the rest of the epicotyl tissues, become sharply sunken channels or grooves. Shallower lesions were noted which had not interrupted or impaired the cambium and which had occasioned a marked hyperplastic response.

TRANSMISSION OF THE DISEASE THROUGH SEED

Owing to the abundance of cowpea-pod lesions, many of which were found to penetrate through the pod tissues into the seed, actual infection of the seed by this organism may occur very readily and very generally (pl. 3, C and E). Furthermore, it was found that the organism could endure long periods of desiccation on the surface of the seed, being found viable after four months of drying.

In order to determine the possibility of seed transmission of the disease, a number of seeds selected from diseased pods collected in the fall of 1921 were sown in pots of sterilized soil in the greenhouse on March 8, 1922. Among 123 seedlings grown from seeds borne directly under pod lesions, 18 showed bacterial spot infection; and among 198 seedlings grown from seeds not borne directly under lesions, 5 showed infection. These tests proved that the disease was carried with the seed and that infected or contaminated seed gave rise to diseased seedlings.

This primary infection consisted of lesions on the hypocotyl, epicotyl, cotyledons, and first leaves. In some cases lesions had originated at the point of attachment of a cotyledon and extended down into the hypocotyl (pl. 3, F) and up into the epicotyl. Infected cotyledons tended to remain attached longer than normal ones. In one case there was one lesion on each first leaf, each located on the under side of the midrib at a point corresponding exactly with the location of the other. Considerable local vascular infection and localized wilting occurred among these seedlings. The organism was successfully isolated from these seedlings.

Similar tests in pots of sterilized soil were made with 20 lots of commercial seed from two sources representing 16 varieties. The shriveled, discolored seeds were tested separately. The seed was planted April 26, 1922, and the results as noted on May 12 are given in Table II.

The results given in Table II show that the infection was present in 15 of the 20 commercial seed lots tested, and occurred in the seed from both sources and in seed of normal appearance as well as in shriveled or discolored seed. Most of the infected plants represented primary seed-borne infection. The organism was isolated from a number of the seedlings. The high incidence of infection in some of the seed lots, coupled with the large proportion of the lots showing infection, indicates the great extent to which the disease may be present in cowpea seed. The appearance of a seed can not be depended upon to indicate whether or not it may transmit the disease.

TABLE II.—*Tests of commercial cowpea seed samples for the presence of bacterial spot infection*

Variety	Source [a]	Seeds normal in appearance		Seeds shriveled or discolored	
		Number of plants	Number infected	Number of plants	Number infected
Early Red	A	14	0	32	0
Early Black	A	19	0	22	2
Early Buff	A	28	0	32	2
Conch	A	12	0	20	0
Taylor	A	23	0	35	1
Brabham	A	28	1	37	0
California Blackeye	A	23	16	24	11
Vigna catjang	A	18	0	34	2
New Era	A	28	1	36	4
Do	B	13	0	19	0
Whippoorwill	A	30	0	24	4
Do	B	21	0	16	0
Iron	A	19	4	31	10
Do	B	20	0	23	0
Groit	A	29	7	35	0
Do	B	17	0	14	0
Red Ripper	B	21	0	29	2
Black	B	24	0	21	1
Wonderful	B	28	0	30	6
Clay	B	26	6	23	3

[a] Two sources designated A and B.

To determine the effect of age of the seed upon the presence of bacterial spot infection, 125 plants were grown in sterilized soil in the greenhouse in March, 1923, from the same lot of seed of the Whippoorwill variety with which the experimental plot had been planted in 1921. The disease had appeared in this field plot very evidently as a result of seed-borne infection, but did not appear on the greenhouse plants grown in 1923. This would indicate that the extra two years of storage had eliminated the organisms.

TABLE III.—*Effect of one extra year of storage on bacterial spot disease transmission in infected seed in certain commercial seed lots listed in Table II, determined by planting some of the seed in sterilized soil in the greenhouse in March, 1923*

Variety	Source of seed [a]	Planted April, 1922		Planted March, 1923	
		Number of plants	Number infected	Number of plants	Number infected
Early Black	A	41	2	66	0
Early Buff	A	60	2	37	0
Vigna catjang	A	52	2	131	0
New Era	A	64	5	63	0
Iron	A	50	14	35	0
Groit	A	64	7	89	0
Red Ripper	B	50	2	19	0
Wonderful	B	58	6	29	0
Clay	B	49	9	23	0

[a] Two sources designated A and B.

The results in Table III indicate that one year of storage had eliminated the seed infection in every variety tested. While the age of this seed is not known with certainty, it is very probable that it came from the 1921 crop. Although these pot tests represent only a small number of seed, it would seem that seed two and three years old is freer from infection than one-year-old seed, as was found by Rapp (*15*) in the case of bean bacterial blight.

CONTROL

Since the disease is seed borne in the case of cowpeas, prevention of it would primarily require the use of disease-free seed. Disease-free seed can be obtained in small quantities by seed selection from disease-free pods. The use of two or three year old seed should result in decreased primary infection.

In 1924 the disease was found on volunteer plants in a plot that was in cowpeas the year before. Therefore crop rotation would seem advisable.

SUMMARY

Bacterial spot of cowpea is characterized by reddish-brown lesions on the leaves, stems, pods, and seeds.

The hosts include all varieties of cowpea tested, catjang, hyacinth bean, asparagus bean, adsuki bean, velvet bean, lima bean, bush lima bean, dwarf sieva bean, and the common weed, tick trefoil. Henderson's Bush Lima bean has shown some resistance.

On cowpeas the young growing organs are most susceptible, and infection of such organs results in considerable distortion and shattering of the leaves, deformity of the pods, and discoloration and stunting of the seeds. In seedlings and young leaves localized vascular infection and localized wilting may occur.

The causal organism is a motile rod bearing one to five flagella at one or both poles, first described as *Bacterium vignae* n. sp. It is identical with *Bacterium viridifaciens* n. sp. described by Tisdale and Williamson.

The colonies on agar are grayish white and, in transmitted light, slightly fluorescent. Gelatin is liquefied.

Acid is produced only with dextrose and saccharose. Greenish pigment formation occurs in milk, alkaline broth, and in Fermi's and Uschinsky's solutions.

Atomizer inoculation without wounds is successful. The invasion in cowpea leaves is intercellular, and the mesophyll is most extensively involved. Vein tissues are preferred and vascular invasion may occur in seedlings and young leaves. Epicotyl lesions may be accompanied by hypertrophy and hyperplasia of the underlying cortical or pith cells.

Cowpea-pod infection results in seed infection. Seeds from infected pods gave rise to infected seedlings when planted in sterilized soil. Commercial seed of a number of cowpea varieties planted in sterilized soil also gave rise to infected seedlings. Similar seed stored an extra year seemed to be comparatively free from infection.

As control measures with cowpeas, the selection of seed from disease-free pods, or the use of seed two or three years old, and crop rotation are suggested.

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