

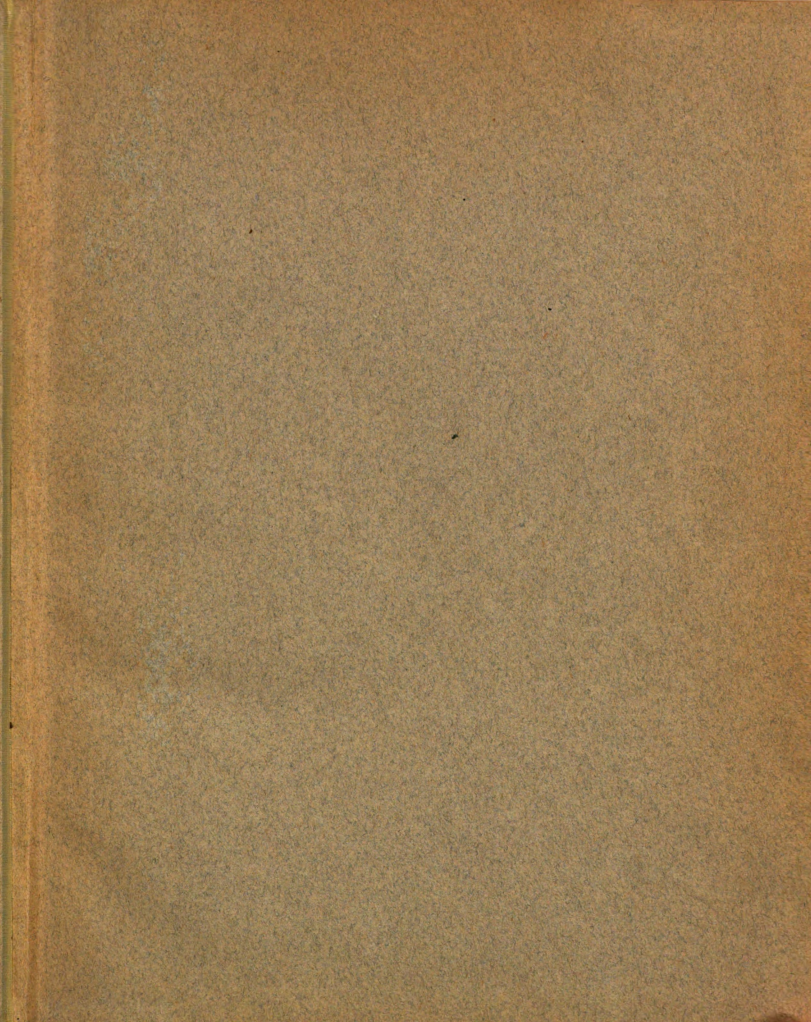
STUDIES ON A BACTERIAL DISEASE
OF LIMA BEANS

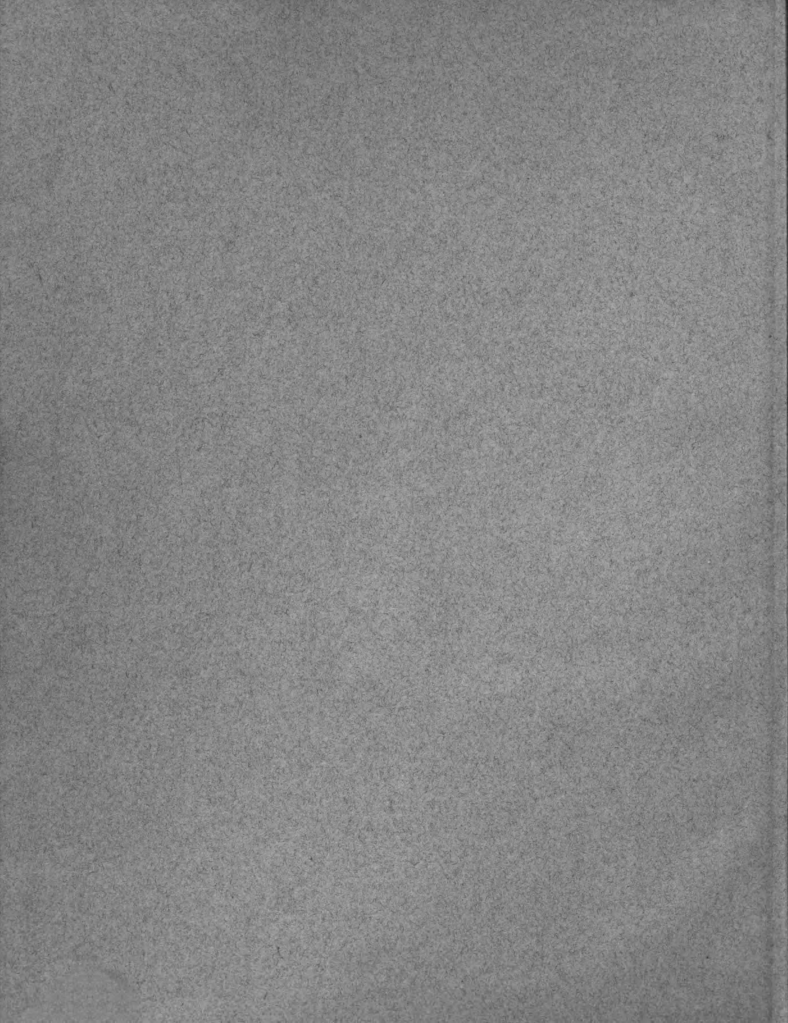
Thesis for the Degree of M. S.

Reuben A. Diettert

1927

Beans - Diseases + pests





STUDIES ON A BACTERIAL DISEASE
OF LIMA BEANS

THESIS

Submitted to the Faculty of the Michigan
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Science.

by
?
Reuben A. Diettert
1927

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STUDIES ON A BACTERIAL DISEASE OF LIMA BEANS

I. INTRODUCTION

Michigan is one of the great bean-growing states. The lima bean(Phaseolus lunatus L.)has been grown for many years and may be considered as one of the important minor crops. The lima beans in this state are grown chiefly for canning purposes, for which they are harvested green. No attempts are made to grow them for seed except for a few cases, such as experimental purposes. This is largely accounted for by the long season required for growing the lima bean to maturity, and the unfavorable effect of the Michigan climate, especially during harvesting time.

One large canning company grows approximately 3600 acres of lima beans each year for canning purposes. Other organizations grow a considerable acreage and lima beans are also grown to some extent in home gardens. The beans for the canning industry are grown chiefly in Montcalm, Oceana, Kent and Clinton counties. Both pole and bush limas are grown. The Ideal Pole and the Henderson Bush are the chief varieties used, although a few other varieties, such as Fordhook, King of Garden Pole and Siebert's Early Pole, are also grown. The seed used for these plantings is grown chiefly in California and Colorado and is obtained through several seed companies.

Within the last few years the lima beans have been attacked by a bacterial spot disease which has shown indications of becoming an important factor in the lima bean industry in this state. While the disease has probably been prevalent for many years, it was first reported in 1924 as doing some damage in fields in western Michigan. In 1925 the disease became a factor of considerable economic importance. The disease was especially severe in its attack upon the early plantings. A considerable acreage required re-planting due to the failure to secure a favorable stand. A large number of seedlings never developed beyond the opening of the cotyledons, the plumule of the plants being entirely destroyed. Furthermore, a large number of seeds rotted before the emergence of the cotyledons from the soil. This condition has been attributed to the organism later causing the spots on the leaves. The pole limas were attacked to a much greater degree than the bush, the entire first planting requiring re-planting in a number of cases. It was estimated that the disease was at least four times as severe on the pole as it was on the bush limas. The seed of the former was California grown, while that of the latter was grown in Colorado. The disease continued to manifest itself in a typical manner on the leaves, stems and pods of the plants throughout the summer of 1925. However, its effect on these parts of the plant did not injure the subsequent

growth greatly. In 1926 the disease was perhaps the most destructive since its recognition as a distinct disease in Michigan. Approximately 800 acres of the lima beans planted by one of the canning companies required re-planting to secure a stand. The attack seemed to be equally as severe on the bush limas as it was on the pole varieties. The disease continued to develop throughout the season as in the previous year. In 1927 the condition of affairs was practically the same as in 1925. Approximately 800 acres were re-planted, as in the previous year. However, the failure to secure a stand was not entirely due to the bacterial spot disease as in 1926. It was estimated that about 60% of the loss was caused by maggots and wire worms, while about 40% was attributed to the bacterial trouble.

The losses incurred in the spring of 1925 due to this disease marked the beginning of a new problem for the canning companies to deal with. It became apparent that something had to be done to reduce the cost of lima bean production. Samples of the seeds used for the plantings were sent to the Botanical Experiment Station and work was begun on the problem and continued through the summer of 1927. The work was begun mainly with the idea of determining the effect of the disease upon the host tissues, and the possibility of finding some means of controlling the disease. Special attention was given

to the nature of the disease in regard to histological and anatomical effects upon the tissues of the hosts and to the modes of dissemination and overwintering of the causal organism.

II. HISTORY AND OCCURRENCE

The bacterial spot disease of lima beans is comparatively recent in so far as its recognition as a distinct disease and its importance are concerned. Literature up to the present time contains only a few publications(6, 7, 21, 22)with direct bearing on this disease. However, there are several other descriptions of bacterial spot diseases which seem to resemble very much the disease under consideration, although their causes have been attributed to organisms other than that which causes this particular spot disease.

According to Tisdale and Williamson(22), the disease was first found on lima beans in 1917 in some gardens at Racine, Wisconsin, and also in the vicinity of Madison. In August of that year, the disease appeared to be quite severe, especially in the locality of Madison. "Scarcely a plant could be found free from the spotting and some of the plants were effected so badly that most of the blossoms and small pods were shedding."(22, p.141)

* Figures in parentheses are used to indicate the literature citations given in the "Bibliography", page 40.

Chupp(3) has reported the presence of the disease in New York since 1918. According to him the disease has evidently been in existence for many years, but was not fully recognized as a distinct disease until 1917.

A number of investigators have noted and more or less adequately described bacterial diseases of the lima bean. It has been known(17) for quite a number of years that the blight caused by Bacterium phaseoli E.F.S. also attacks lima beans. In 1892 Beach(1) described a bacterial disease of lima beans which occurred in New York. He believed this to be a distinct disease from the one attributed to Bacterium phaseoli. His description of this disease seemingly fits more closely that of the disease under consideration than it does that of the blight caused by Bacterium phaseoli.

In 1892 Halstead(8) reported the presence of a bacterial disease on both common and lima beans on a farm operated by a western seed company, and he mentioned that the disease had been observed on that farm since 1886. In 1898, Sturgis(20) noted the occurrence of a bacterial disease of lima beans in Connecticut, which he described and attributed to Bacterium phaseoli E.F.S. His description also seems to characterize more closely the spot disease than the blight. Manns(9), in 1915, described a bacterial organism which is pathogenic upon certain legumes, including the lima bean. However, he did not describe or illustrate

the disease as it occurs on lima beans, but his description in regard to morphological and cultural characteristics of the organism would lead one to think that it was identical with the organism causing the disease under consideration. In the report of the department of Plant Pathology of Penn. State in 1923, Beach(2)briefly reported the existence of a bacterial disease of lima beans. His description of this disease also characterizes rather closely the spot disease considered in this paper. He indicated the failure of copper fungicides to check this disease.

The organism which causes the spot disease of lima beans also causes a very similar disease of cowpeas, which has recently been described by Gardner and Kendrick (6, 7). According to these investigators, the disease of cowpeas was first noticed in southern Indiana in 1919, but its bacterial nature was not determined until 1921. The spot disease of cowpeas as described by them seems to be quite widespread. A number of other cases of bacterial infection of cowpeas have been reported. In 1905, Smith (15)described a spot disease of cowpeas and lima beans which seems to resemble quite closely the disease under consideration, although he attributed its cause to Phyllosticta phaseolina Sacc. Smith(16)mentions a bacterial spot disease of cowpeas, but does not give a description of it. Coerper(4, 5), in 1919, described a bacterial blight of soy beans caused by Bacterium

glycineum. This blight disease of soy beans is similar in some respects to the spot disease of cowpeas and lima beans. In 1920, Wolf(24)described a similar blight of soy beans which he attributed to Bacterium sojae. Further work, however, will be necessary to determine fully the relationships of the organisms within this group.

The occurrence of the bacterial spot disease of lima beans as it has been observed in Michigan has already been given in the introduction to this paper. It may be added that the disease was also found to be present on lima beans in some home garden plots in the vicinity of North Judson, Indiana, in the summer of 1926. The disease there, however, was not very serious and a large number of plants grew to maturity without becoming infected with the disease. Collections of plants infested with what seems to be the same disease have been made at Rocky Ford, Colorado, from lima beans grown for seed purposes. In regard to the general distribution of the disease, it is quite probable that it occurs wherever the host plants are grown, since the organism is carried over in the seed. The importance of the disease, as evidenced by the degree of infection, varies in different localities and different years, as has already been shown. This variation in regard to the severity of the attack upon the host plants is undoubtedly due to variations in climatic conditions.

III. THE DISEASE

Symptoms

The symptoms of the disease manifested on the various organs of the host plants vary somewhat and will therefore be described separately. Leaves, stems, pods, petioles, peduncles and pedicels may be attacked, although the symptoms on the leaves are usually the most noticeable. On the leaves.

Probably the most characteristic symptom of this disease occurs on the leaves of the plants in the form of a leaf spot. These spots first appear as small, circular, water-soaked, somewhat sunken dots. These enlarge very rapidly the first few days and as the spots become older they may become somewhat lobed or irregularly circular, or oblong. They vary in size from small dots to spots several mm. in diameter. The young spots are somewhat depressed on the under surface of the leaves and the margins of the lesions appear to be considerably lighter in color than on the upper surface. As the spots become older a sort of a crust forms on the reddish-brown margin of the spots on the upper surface of the leaves. This is especially evident after the leaves have been pressed and dried. Occasionally, when the spotting is very severe, a number of spots may coalesce, forming a sort of a blotch. The spots are characterized

by a reddish-brown to purplish color and are sometimes surrounded by a water-soaked area, although this latter character is often missing. Tisdale and Williamson(22, p. 142), however, state that "the spots never at any time become water-soaked". The writer of this paper has found that this is not always the case. In the larger spots the center often becomes lighter in color, somewhat papery in texture, and may finally crack and drop out, thus giving the leaves a ragged appearance. The dried out or bleached centers of these lesions are surrounded by a reddish-brown or maroon border of about one mm. in diameter. The spots may be generally scattered over the entire leaf or they may appear in groups or clusters, especially on the younger leaves.

The disease not only manifests itself as spots on the leaves, but also occurs in lesions along the veins. These lesions may extend several centimeters along the veins and sometimes they are found to occur along the entire length of some of the larger veins. Severe infection along the veins causes a partial drying up of the leaf in the zone of infection. This often results in a curling or distortion of the leaves, especially in the case of young leaves infected in this manner. Sometimes, especially when the cotyledons are very badly infected, the first leaves will fail to develop when the cotyledons open after germination. Very often,

however, such seedlings do not die but after some time produce a sort of a rosette of leaves or witches-broom at the top of the seedling stem. The leaves of the rosette are usually more or less distorted or curled. Such plants always remain stunted and never develop into good pod-producing plants. It has been observed that sometimes the seedlings infected in the manner just described die before any leaves develop. This condition is undoubtedly due to a very early vascular infection of the hypocotyl and epicotyl. Such infections kill the entire seedling, leaving only the open cotyledons which usually wither and die in a short time.

On the stems and petioles.

Evidences of the disease occur on the petioles of the leaves and on the stems of the plants as well as on the leaf blades. The lesions on the petioles and stems are more or less oval in shape instead of being round spots as in the case of the leaf blades. These lesions are sometimes in the form of streaks and occasionally extend some distance along the stems and petioles. The centers of the lesions are somewhat sunken and usually surrounded by water-soaked areas. A number of plants have been found in which the portion of the stem under the ground has also become infected. But in no cases have the roots of infected plants been found to show any symptoms of the disease. Vascular infection of the stems and petioles has been

observed. In such cases the entire stem or petiole seems to become shrunken. The leaves become wilted and finally die and drop off.

On the pods.

Lesions on the sides of the pods are quite similar to those on the leaves. They vary in size from small dots to spots several mm. in diameter. The spots are reddish-brown or purplish in color. They are more often characterized by water-soaked borders and in the case of large spots the center of the spot is somewhat sunken. The pods may also become infected along the sutures. In such cases the lesions are usually in the form of streaks, much like those occurring on the stems and petioles. Severely infected young pods may drop off or there may be a marked constriction or abnormal bending of the pod at the point of infection. Pods have been found in which the entire distal portion had failed to develop due to heavy infection. Pedicels and peduncles have also been found to show infection. In a few instances, in severely diseased plants, reddish-brown spots have been observed on some of the blossoms. But it has not been determined whether these spots were due to infection by the organism causing the spot disease. Isolations of the causal organism have not been made from lesions observed on the peduncles, pedicels and blossoms.

On the seeds.

It is rather difficult to find any characteristic

symptoms of the disease on the seeds. However, it has been observed that seeds taken from severely infected pods are usually considerably stunted or shrivelled and exhibit a discoloration of the seed coat. The veins of the testa of such seeds show a reddish color. However, these symptoms could not be used as a basis for separating healthy from infected seeds, since the organism causing the spot disease has been isolated from the cotyledons of germinating seeds which from all outward appearances would be judged to be healthy seeds. But it has been observed that a selected number of stunted, shrivelled and discolored seeds show a greater percentage of infection upon germination than is found in the same number of selected normal-sized, healthy-looking seeds. This method of separating healthy and infected seeds is not efficient enough to warrant its application in the commercial field.

On the cotyledons.

The cotyledons as well as the leaves, stems, and pods may become infected with the disease. In fact, the cotyledons may often be regarded as the source of infection for the other parts of the plant. Since the organism is seed-borne the cotyledons become infected previous to the infection of the other parts of the plant. Infection of the young leaves or the stem may thus take place when they push their way through the

partially opened, infected cotyledons. The cotyledons of seedlings from infected seed exhibit large dark colored lesions which sometimes mask the entire cotyledon. The lesions may be surrounded by water-soaked areas. Badly infected cotyledons soon become shrivelled or may have constricted areas and may fall off prematurely. Vascular infection of the seedling may occur from infected cotyledons. This type of infection is shown by the wilting or yellowing of the first leaves. In severely diseased cases, as has already been mentioned, the first leaves may fail to develop. The seedling may die after a short time or if it survives the production of a rosette of leaves or witches-broom usually occurs. It has also been observed that in weather conditions in which considerable time is required for germination, severely infected cotyledons may cause a rotting of the seed before germination is completed. This condition is especially evident in cool, wet weather. Further mention of this will be made under another heading.

Temperature and moisture relations

From all observations it appears that the severity of the disease, especially in its early attacks, is largely determined by temperature and moisture conditions. This fact has been brought out by the experiences met with by growers within the last few years. Test plots conducted

under the observation of the writer have shown similar results. At the time of the first planting, about the latter part of May, the weather is usually cool and the soil rather wet. This has been the case, especially when the disease was found to be the most destructive. Later, when the re-planting is done, the weather is much warmer and the soil considerably more dried out. The re-planting is usually done sometime between the middle and latter part of June. Fields planted at this time have resulted in good stands in which the plants were entirely free from the disease. Seed from the same source was used in both the early and late plantings. In the first plantings it takes considerably more time for the seeds to germinate than it does in the case of the re-plantings later in the season. From these observations it seems that in the former case the bacteria develop rapidly in a cool, moist soil and soon overcome the developing young embryo. The tissues of the embryo are at this time especially tender and are thus in a condition in which they will easily become infected. In the case of the later plantings, the seeds germinate within a short time, and it seems that the seedling is well under way before the organisms have much of a chance to do any damage. So, even though the causal organisms may be present, a strong plant and one which is growing rapidly is better able to overcome or survive from the destructive

effects of a parasite than a plant which is under its influence from an earlier time. The disease later in the season is much more severe and spreads more rapidly in the field during cool, rainy weather than it does in hot, dry weather. These circumstances, then, seem to indicate that temperature and moisture are important factors to note in considering the severity of the disease.

Hosts

All of the varieties of lima beans(Phaseolus lunatus L.) tested were found susceptible to the disease. The following varieties were tested: Henderson Bush, Burpee's Bush, Large White Pole, Ideal Pole, Carpinteria Pole, Siebert's Early Pole, Early Leviathan Pole, King of Garden Pole, Fordhook Bush and Burpee's Improved Bush.

Gardner and Kendrick(7) found all of the varieties of cowpeas(Vigna sinensis L. Endl.) tested to be susceptible. The following 23 varieties were tested: Early Red, Whippoorwill, Brabham, 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.

The catjang(Vigna catjang Walp.), the hyacinth bean (Dolichos lablab L), the Florida velvet bean(Stizolobium deeringianum Bort.), the adzuki bean(Phaseolus angularis

Wight) have also been found susceptible to the disease (7). Natural infection with what appeared to be the same organism has also been observed on the common weed, tick trefoil (Desmodium canescens L. DC.) and on the asparagus bean (Vigna sesquipedalis Wight) (7). Inoculations made on garden beans, peas, and soy beans have failed to produce infection. Gardner and Kendrick (7) report similar results with garden beans, soy beans, broad bean, sweet pea, peas, lupine, clovers, cauliflower, tobacco, tomato, and potato.

IV. THE CAUSAL ORGANISM

Isolation

The bacterial nature of the spot disease of lima beans was determined in 1925. Sections made through lesions and observed under the microscope have shown the infected tissues to be swarming with bacteria. The organism has been isolated from lesions on leaves, stems and cotyledons. Various methods were used in making these isolations. The following method was used in making isolations from leaf lesions: The diseased leaves were first washed in running water and then dipped into mercuric chloride (1:1,000), after which they were washed in 90% alcohol, followed by several washings in sterile water. Lesions were then cut out

with a flamed scalpel and crushed in a tube of sterile broth. After several hours dilution plates were poured and the organism obtained in pure culture. Spots from leaves treated as described above were also taken and mascerated in sterile water on a flamed slide. A drop of this was used for pouring dilution plates. The organism was successfully isolated in this manner. Separate sets of dilution plates were made, using potato-dextrose agar, nutrient agar, and lima bean agar. Isolations of the organism from stem lesions were made by employing methods similar to those used in making isolations from leaf lesions. Several methods were used for making isolations of the organism from the cotyledons. The method suggested by Norton and Chen(15)for investigating internal seed infection was first used. This method, however, did not prove itself to be very satisfactory, and no successful isolations of the organism were made by using it. Another method, which proved to be more satisfactory, was then employed. Deep culture dishes were cleaned and sterilized in a hot-air oven. Sand sterilized in an autoclave was transferred to these sterile dishes. Seeds were soaked for 5 minutes in an aqueous solution of mercuric chloride(1:1,000)or in a 1:100 solution of chlorozene. The seeds where then washed in 90% alcohol and rinsed in sterile water. Seeds thus treated were then transferred aseptically to the

TABLE I. Test showing internal seed infection, Feb. 17-22, 1927.

Variety of bean	Time harvested	Number of seeds used in test	Number showing infected cotyledons
Henderson Bush	1925	10	0
Siebert's Early Pole	1925	10	3
Henderson Bush	1925	15	2
Carpinteria Pole	1925	10	3
Ideal Pole	1925	10	2
Early Leviathan Pole	1925	10	1
King of Garden Pole	1925	10	2
Unknown(bush)	1925	25	1
Henderson Bush	1925	10	1
Henderson Bush	1926	25	2
Henderson Bush	1926	20	0
Henderson Bush	1926	15	1
Henderson Bush	1926	12	2
Baby limas	1923	15	0
Baby limas	1923	15	3
Baby limas	1923	25	2
Henderson Bush	1926	50	1
Henderson Bush	1926	25	3
Unknown(bush)	1924	10	1
Unknown(bush)	1924	10	2
Burpee's Bush	1925	10	0
Pole lima	1925	10	2

culture dishes containing the sterile sand. These were kept in the room for five days, after which the seeds were removed under aseptical conditions and examined carefully for infected areas on the cotyledons. A number of lesions were found and isolations of the organism obtained from them. Table I gives the results obtained from these isolations. Similar tests were later conducted in which healthy-looking, normal-sized seeds were used in one series and dwarfed, shrivelled and discolored seeds in another series. Fifteen seeds were used of each lot tested. The findings of these tests are recorded in Table II. The results obtained in these tests, as Tables I and II indicate, show that in general the seeds which are shrivelled and discolored are infected to a greater degree than those which are normal in appearance. These tests also show that there is no appreciable difference in the percentage of viable bacteria in one, two, three and four year old seed.

Inoculation and reisolation

Cultures obtained from isolations made as described above were used for inoculation purposes. Infection of healthy plants has been brought about by spraying them with a water suspension of the organism or by using broth cultures. Cultures of the organism used for inoculation experiments were from 2 to 10 days old. The disease has been reproduced with its characteristic symptoms and the

TABLE II. Tests showing the percentage of bacterial spot infection present in normal and shrivelled or discolored seeds.

* Variety tested	Normal seeds	Shrivelled or discolored seeds
Henderson Bush	2	3
Siebert's Early Pole	0	2
Henderson Bush	0	3
Carpinteria Pole	1	3
Ideal Pole	3	3
Early Leviathan Pole	2	4
King of Garden Pole	3	2
Unknown(bush)	1	1
Henderson Bush	0	0
Henderson Bush	2	2
Henderson Bush	3	2
Henderson Bush	1	3
Henderson Bush	2	2
Baby limas	0	1
Baby limas	1	3
Baby limas	2	3

* Varieties used for tests recorded in Table II are listed in the same order as in Table I.

organism successfully reisolated from typical lesions. Inoculation experiments have shown both older and younger parts susceptible to infection. The inoculated plants were kept in moist chambers or under bell jars from 24 to 72 hours. Typical lesions began to develop in from 2 to 8 days after inoculation. Infections produced by spraying plants from an atomizer with suspensions of the organism have shown that wounds are not necessary for producing infection. This indicates that the organisms probably gain entrance into the host tissues through the stomata.

Morphology

The organism is a short rod with rounded ends, occurring singly or in pairs. It stains readily with any of the ordinary stains, such as Loeffler's methylene blue, Ziehl's carbol fuchsin and gentian violet. It is gram negative. No endospores, capsules, and involution forms have been observed. The cells measure from 0.3 to 0.8 microns in width and from 0.9 to 2.5 microns in length, averaging about 0.5 by 1.9 microns.

Cultural characteristics

The organism grows fairly well on nutrient agar, but best growth occurs on potato-dextrose agar(1-2%) and on lima bean agar. A culture of Gardner's organism was obtained from Purdue University and also one of Tisdale's from the University of Wisconsin. These

cultures were carried in parallel series with the organism under consideration. Tests were made on only the ordinary media, and for a more detailed account of cultural characteristics the work of Gardner and Kendrick(7) and Tisdale and Williamson(22) should be consulted. The tests made and recorded below are practically identical with those made by the investigators just mentioned.

Agar poured plates--

On potato-dextrose agar small, round, glistening, somewhat raised, grayish white colonies appear in about 24 hours. Surface colonies are round with entire margins, while the submerged colonies are somewhat smaller and lenticular. 24 hour surface colonies are about 1 to 2 mm. in diameter, while in 6 days they measure from 5 to 6 mm. in diameter. The surface colonies are more or less smooth but the older colonies may become quite definitely concentrically ridged. Older colonies become somewhat olive-buff in color. There is no change in the color of the medium. Only a slight odor is detected.

On nutrient agar colonies appear in about 24-48 hours. They are smooth, flat, glistening, somewhat butyrous, with undulate margins. The colonies on nutrient agar are somewhat smaller than those on potato-dextrose and also somewhat darker in color. Submerged colonies are lenticular. There is no change in the color of the medium.

On lima bean agar colonies appear in about 24 hours. The colonies appear much the same on lima bean agar as on potato-dextrose agar, except that they are somewhat lighter in color, more glistening and butyrous and considerably more raised.

Agar stabs--

In potato-dextrose agar there is slight growth along the upper part of the line of puncture. Surface growth is abundant, spreading over nearly the entire surface in three days. Growth is flat, dull, grayish white, and finely rugose. Margin entire with a definite beveled border. No change in the color of the medium.

In nutrient agar growth is good, but not as abundant as in potato-dextrose agar. The growth is smooth, flat, grayish white, spreading over about half the surface in three days. In older growths there is a definite concentric ridging. There is no change in the color of the medium.

In lima bean agar growth is very abundant, spreading over the entire surface in a few days. Growth is lighter in color than in potato-dextrose agar. The growth is somewhat raised, glistening, butyrous, with a rather lobate margin. Slight growth occurs along the upper part of the line of puncture. A slight greenish pigmentation of the medium occurs.

In dextrose agar surface growth is abundant.

There is slight growth along the upper part of the line of puncture. Growth is creamy white in color, glistening, with entire margin, covering nearly the entire surface in three days. There is no change in the color of the medium.

In lactose agar growth is moderate, smooth, flat, grayish white, spreading over about half the surface in five days. Growth is much the same as in nutrient agar but does not show concentric markings. There is no change in the color of the medium.

In litmus-dextrose agar there is moderate growth, much the same as in lactose agar. The medium turned slightly red in 6-7 days.

Agar slants---

On potato-dextrose agar growth is abundant, flat, smooth, glistening, creamy white, filaform to spreading. Older growths become somewhat ecru-drab to smoke-gray(14) in color. There is no change in the color of the medium.

On nutrient agar growth is moderate, flat, somewhat dull, grayish white at first and turning sayal brown(14) later. Growth is filaform to echinulate. There is no change in the color of the medium.

On lima bean agar growth is abundant, filaform to spreading, somewhat raised, glistening, butyrous, grayish white. A slight greenish pigmentation of the

medium occurs.

On prune-juice agar growth is moderate, filaform to echinulate, flat, glistening, grayish white, much the same as on lima bean agar, but not as abundant. There is no change in the color of the medium.

On corn meal agar growth is very scant, filaform, flat, dull, grayish white. There is no change in the color of the medium.

Gelatin plates--

Small white, circular colonies appear in about 2 days. Liquefaction begins shortly after the appearance of the colonies, producing saucer-shaped depressions. Liquefaction is complete in about 5 days.

Gelatin stabs--

There is no growth along the line of puncture. Surface growth is abundant. Liquefaction begins in about 2 days and proceeds rather slowly. Liquefaction is crateriform at first, becoming stratiform later. Liquefaction proceeds at the rate of about one mm. per day.

Nutrient broth--

Growth is abundant in beef-peptone broth, the medium becoming cloudy within 12 to 24 hours. A slight pellicle is formed which drops to the bottom of the tube after several days. A precipitate is formed which is viscid on agitation. A putrefactive odor is produced.

Litmus milk--

Blue litmus milk is completely reduced and

peptonized in 8-10 days. No curd is formed and only a very slight pink color appears during the reduction of the litmus. The liquid becomes viscid or gelatinous in consistency before digestion begins. When digestion is complete the cleared liquid is somewhat yellowish-green in color and rather thick and oily in consistency. After 10 days it is still in the same condition.

Potato cylinders--

Abundant growth occurs on potato cylinders. The growth is grayish white, glistening, butyrous, spreading over the entire surface in three days.

Uchinsky's solution--

Moderate growth occurs in Uchinsky's solution. The medium turns slightly yellowish-green in color.

Dunham's solution--

Good growth occurs in Dunham's solution. Tests made after 7 and 14 days indicated that no indol was produced.

Nitrate-peptone solution--

There is good growth in nitrate-peptone solution. Tests made of this solution ten days after inoculation showed that nitrates were not reduced, but a good reaction was obtained for the presence of ammonia with Nessler's reagent.

Temperature relations--

Poured agar plates of the organism were incubated

in a differential thermostat. The organism was found to grow in a wide range of temperatures. Growth occurred on plates exposed to temperatures ranging from 10 degrees C. to 45 degrees C. Good growth occurred at temperatures between 20 and 30 degrees C. The best growth was found on plates incubated at temperatures from 24 to 28 degrees.

The thermal death-point was not determined, but according to Gardner and Kendrick(7) it was found to lie between 49 and 50 degrees C.

The organism seems to be quite resistant to desiccation. According to Tisdale and Williamson(22) it was found alive in diseased leaves which had been kept in the herbarium for 2½ years. The organism, however, is very sensitive to desiccation on glass, remaining viable for only a short time.

Taxonomy

The organism causing the bacterial spot of lima beans has been designated by Tisdale and Williamson(22) as Bacterium viridifaciens n. sp. In March 1923 the organism causing the bacterial spot disease of cowpeas was briefly described by Gardner and Kendrick(6). The name given to the causal organism was Bacterium vignae n. sp. When this description of the organism came out in print Gardner and Kendrick received a letter(7) from W. B. Tisdale calling their attention to the similarity of this organism to the one causing the spot disease of

lima beans. A description of the organism causing the disease of lima beans was at that time in print and appeared several months later(22). The symptoms of the two diseases are practically identical in all respects. Furthermore, the organism isolated from lesions on lima beans produced typical infection on cowpeas(7). Likewise, the organism isolated from cowpea lesions produced typical lesions on lima beans(7). Careful studies and comparisons of the cultural characteristics of the two organism reveal their identity. The results of the cross inoculations and the similarity of the cultural characteristics indicate that the two organisms are undoubtedly identical.

The disease on lima beans in Michigan was found to be quite similar to the one which was found by Tisdale and Williamson(22) to occur on lima beans in Wisconsin. Successful inoculations have also been made on cowpeas with the organism obtained from lima bean lesions. Cultures of the cowpea organism obtained from Purdue University and of the lima bean organism from the University of Wisconsin were carried in parallel series with the organism isolated from lima beans in Michigan. The cultural characteristics of the three organisms were found to be practically identical in all respects.

Since the organism causing the lima bean disease has been shown to be identical with the one causing the cowpea disease, the term Bacterium viridifaciens becomes

synonymous with Bacterium vignae, according to the rules of priority.

According to the revision of Migula's classification adopted by the Committee of the Society of American Bacteriologists in 1920(18), the terminology would be Pseudomonas vignae. In a later report(19) of another committee of the same society the genus name Phytomonas was erected to include organisms of this character, whence the name Phytomonas vignae which is used in this paper.

Overwintering and Dissemination

Tests have shown that the disease is carried over with the seed and that such seed gives rise to diseased plants. Indications of the systemic nature of the disease makes it seem probable that the organisms are conducted to the seeds through the vascular system. Infection of the seed may occur from pod lesions which penetrate through the pod tissues into the seeds. The organism has also been found to be very resistant to desiccation on the surface of the seed(7). Furthermore, it is possible for the organism to overwinter in refuse of diseased plants, since pathogenic strains of the organism have been isolated(22) from lima bean leaves which had been dried for $2\frac{1}{2}$ years.

In regard to the dissemination of the organism

it is not definitely known how the disease spreads in the field, but it is quite evident that after having made its appearance infection spreads quite rapidly from plant to plant. This is especially true during cool, rainy weather. The following may be suggested as possible methods of dissemination: It is possible that the organisms may be transferred from plant to plant by the splashing of rains, by insects, or by the cultivator, especially after heavy dews. The soil may also be a source of infection in some fields, although data proving this have not yet been obtained. No attempts have been made to isolate the organism from overwintered bean refuse or soil from fields which were infested with the disease. However, it seems possible that the organism may overwinter in this way, especially where refuse of badly diseased plants has been left on the field. The organisms may be carried over in such plants, since isolations of the organism have been made from diseased material which had been kept in the herbarium for several years, as has been mentioned before. If this assumption should be true then it is highly probable that the organisms may be carried to the plants on dust particles. Severe and prolonged winds which may assume the form of sand storms, transport quantities of soil, irrespective of whether it is diseased or not, from one locality to another. The mechanical injury to the leaves and pods which results from this incessant pounding by the sand

grains, not only weakens the plant but also opens the way for subsequent infection with germ-laden soil particles.

V. HISTOLOGY OF DISEASED HOST TISSUES

Since inoculation experiment have shown that wounds are not necessary for infection, it would seem likely that the organisms enter the host tissues through the stomata. Sections through prepared diseased material have shown this to be the mode of entry. Tisdale and Williamson(22) have also found invasion to occur through the stomata.

Sections were made through leaf lesions in various stages of development. Microscopic examinations of these have shown the invasion to be intercellular and involving only the spongy tissue at first. The middle lamella is attacked and the cells finally become discolored and collapse. As the invasion progresses the palisade layer is attacked in a similar manner. Finally the entire thickness of the leaf blade is killed and the center of the lesion becomes dry and may drop out.

The toxicity of the organism was also tested. Broth cultures(3-10 days old) of the organism were centrifuged and injections of the supernatant fluid were made into stems, petioles and veins of leaves. Sterile broth and sterile distilled water were used for checks. No

apparent injury of the tissues resulted from any of these injections.

Vascular infection has also been observed. As has been previously mentioned, there were definite lesions along the veins of leaves. Microscopic investigations of sections through such diseased portions have shown the bacteria to ooze from the cut ends. Longitudinal sections of badly diseased stems and petioles were closely examined and the vascular tissue found to be discolored. Discolored bundles have been traced from petioles into the larger veins of leaves. Such leaves showed definite infection along the veins externally as well as internally. Dark colored bundles have also been traced from infected cotyledons through the epicotyl into the veins of the first leaves.

In the case of badly diseased pods discolored tissues were observed extending from lesions along the dorsal suture through the funiculus into the seed. This is one way in which the organism may gain entrance into the seed and thus be carried over in the seed. Seeds from badly diseased pods showed the veins of the testa to be discolored.

Injections of broth cultures and water suspensions of the organism into the stems, petioles and veins of leaves have resulted in typical vascular infection. No apparent injury of the tissues was observed in the control plants, into which sterile distilled water and sterile

broth were injected.

From the investigations on the systemic nature of the disease, it appears that vascular infection may take place from lesions on the veins of leaves, from petiole and epicotyl lesions, or from infected cotyledons.

VI. CONTROL MEASURES

As yet no definite method for controlling the disease has been developed. The selection of seed from disease-free pods and the use of two and three year old seed in the case of cowpeas have been suggested by Gardner and Kendrick(7). Rapp(12) has also suggested the use of aged seed as a control for bacterial blight. The results of tests recorded in Tables I and II show that in the case of lima beans there is no appreciable difference in the percentage of infection of seedlings from one, two, three or four year old seed. Furthermore, since pathogenic strains of the organism have been isolated(22) from diseased material which had been kept in the herbarium for $2\frac{1}{2}$ years, it seems very likely that the organisms would remain viable even a much longer period in the seeds.

Since the disease is seed-borne, seed disinfection would suggest itself as a possible means of control. However, treatment with formaldehyde and mercuric bichloride solutions of sufficient concentration and duration to kill the bacteria in the tissues would be injurious to

TABLE III. Results of dusting seed-treatments as a control for bacterial spot infection, Aug. 1925

Variety of bean	Treatment	Control *
Large lima	Bayer dust	0%
Large lima	Check	0%
Henderson bush (Colorado grown)	Bayer dust	0%
Henderson bush (Colorado grown)	Check	0%
Henderson bush (Michigan grown)	Bayer dust	0%
Henderson bush (Michigan grown)	Check	0%
Henderson bush (1924 seed)	Semesan $\frac{1}{4}$ %	0%
Henderson bush (1924 seed)	Semesan dust	0%
Henderson bush (1924 seed)	Semesan Jr. dust	0%
Henderson bush (1924 seed)	Check	0%

TABLE IV. Results of liquid seed-treatments as a control for bacterial spot infection, Aug. 1925.

Variety of bean	Treatment	Time	Control*
Henderson Bush (1924 seed)	Semesan $\frac{1}{4}$ % plus 10% alcoholic solution #	10 min.	0%
"	Semesan $\frac{1}{4}$ % plus 20% alcoholic solution	10 min.	0%
"	Formaldehyde (1-240)	30 min.	0%
"	Formaldehyde (1-120)	30 min.	0%
"	Formaldehyde(1-240)plus 10% alcoholic solution	10 min.	0%
"	Uspulun $\frac{1}{4}$ %	30 min.	0%
"	Check		0%

* All treatments were ineffective. Plants showed an equal amount of disease in treated and untreated rows.

Injury of seed resulted from all treatments containing alcohol.

the seed as well as to the testa. Tables III and IV show the results obtained from various seed treatments conducted by R. Nelson in August 1925.

The development of resistant varieties has also been suggested(22)as a feasible means of control. However, inoculation experiments and observations of plants in the field have shown that there is no apparent difference in susceptibility in the different varieties tested. There seems to be no indication of any resistant varieties. Yet the ultimate control of this disease will probably lie in the development and selection of such plants. Another method which is advisable, as in the case of so many plant diseases, is that of rotation of crops. This would seem to be important, since Gardner and Kendrick (7)found the disease on volunteer plants in a lot that was in cowpeas the year before.

Observations and tests have shown that late planting of diseased seed in Michigan will give fields entirely free from the disease. This discovery seems capable of immediate application for the control if not the elimination of this disease. It would then seem to be advisable to make late plantings to secure a clean seed stock. Such seed will give disease-free plants, irrespective of the time of planting, or if the disease is not entirely eliminated the amount of it would doubtless do slight damage. If it seems inadvisable to grow the

commercial seed stock in Michigan, it would be possible to increase clean Michigan grown seed by planting it in the west under conditions to avoid reinfection.

To summarize, since we are dealing here with a disease that is seed-borne, systemic and correlated in its occurrence with a type of growth condition, the most available control measures at present seem to reside in the use of seed from as clean fields as possible together with the best of farm practices.

VII. SUMMARY

1. The literature on the disease under consideration has been reviewed.

2. The bacterial spot disease of lima beans described in this paper has been observed in various localities in Michigan. It has also been found to occur in Wisconsin, Indiana, New York and Colorado.

3. A similar disease of cowpeas, caused by the same organism, has been reported.

4. The disease of lima beans is best characterized by its appearance on the leaves. The spots are more or less circular with dried out centers and definite reddish-brown borders. The spots may be surrounded by water-soaked areas.

5. The stems, petioles and pods may also become infected. The stem and petiole lesions are more often in

the form of streaks.

6. Severe infection of the cotyledons has been observed. The disease is most destructive to the seedlings when it first occurs on the cotyledons. Other organs of the plant become infected from diseased cotyledons.

7. A considerable curling or distortion of the leaves may occur. Deformity of pods and discoloration and shrivelling of seeds have been observed.

8. Temperature and moisture conditions seem to play an important part in the severity of the disease.

9. The disease becomes a factor of considerable economic importance, especially when the cotyledons are severely attacked. This usually requires the replanting of a considerable acreage of beans.

10. The hosts include all the varieties of lima beans tested, a large number of varieties of cowpeas, velvet bean, hyacinth bean, asparagus bean, adzuki bean, catjang, and the common weed, tick trefoil.

11. The causal organism has been described by Gardner and Kendrick as Bacterium vignae n. sp. It was later described by Tisdale and Williamson as Bacterium viridifaciens n. sp. According to the rules of priority, the latter becomes synonymous with Bacterium vignae. According to the latest revision of Migula's classification, the combination now stands as Phytomonas vignae n. sp.

12. The organism has been successfully isolated from diseased lesions on the cotyledons, leaves and stems.

13. The disease has been reproduced with its characteristic symptoms and the organism reisolated from the lesions produced.

14. Inoculation without wounds has been successful. The organism enters the host tissues through the stomata.

15. Both old and young parts of the plants are susceptible to infection.

16. Tests have shown that the disease is carried over with the seed.

17. Vascular infection of the stems, petioles and veins of leaves has been observed, and a careful study of these diseased tissues has been made.

18. Vascular infection may result in seed infection.

19. No definite control measures have been developed.

20. Seed treatments have been ineffective.

21. Inoculations of healthy plants with the organism causing the bacterial spot disease and observations of plants in the field have shown no indication of resistant varieties.

22. The use of disease-free seed and the rotation of crops have been suggested as feasible means of control.

23. The selection of seed from clean fields and late planting to obtain such fields are advisable.

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IX. BIBLIOGRAPHY

1. Beach, S. A.
Blight of lima beans. N.Y. Agr. Exp. Sta.
Bul. 48:331. 1892.
2. Beach, W. H.
Report of the department of plant pathology
Penn. State. Penn. State Bul. 181:14-16.
1923.
3. Chupp, C.
Manual of vegetable-garden diseases. 647 p.,
illus. The Macmillan Co., New York. 1925.
4. Coerper, Florence M.
Bacterial blight of soy bean. Jour Agr.
Research 18:179-194. 1919.
5. —————
Bacterial blight of soy bean. Phytopathology
10:182. 1920.
6. Gardner, M. W., and Kendrick, J. B.
Bacterial spot of cowpea. Science 57:275.
1923.
7. ————— and Kendrick, J. B.
Bacterial spot of cowpea and lima bean. Jour.
Agr. Research 31:841-863, illus. 1925.
8. Halstead, B. D.
A bacterium of phaseolus. N. J. Agr. Exp.
Sta. 13th Ann. Rpt., 1892:283-285. 1893.
9. Manns, T. F.
Some new bacterial diseases of legumes and
the relationship of the organisms causing
the same. Del. Agr. Exp. Sta. Bul. 108:
1-44. 1915.
10. Munn, M. T.
Seed-borne plant diseases. Seed World 5,
No. 10:20-21. 1920.
11. Norton, J. B. S., and Chen, C. C.
Some methods for investigating internal
seed infection. Phytopathology 10:399-
400. 1920.
12. Rapp, C. W.
Aged bean seed, a control for bacterial
blight of beans. Science 50:568. 1919.

13. Rapp, C. W.
Aged bean seed, a control for bacterial blight of beans. *Phytopathology* 10:183. 1920.
14. Ridgway, R.
Color standards and color nomenclature. Washington, D. C. 1912.
15. Smith, C. O.
The study of the diseases of some truck crops in Delaware. *Del. Agr. Exp. Sta. Bul.* 70: 16 p., illus. 1905.
16. Smith, E. F.
An introduction to bacterial diseases of plants. 688 p., illus. W. B. Saunders Co., Philadelphia and London 1920.
17. _____
Description of Bacillus phaseoli, n. sp., with some remarks on related species. *Proc. Amer. Assoc. Adv. Sci.*, 46th meeting, 1897, p. 288-290. 1898.
18. Society of American Bacteriologists.
Committee on characterization and classification of Bacterial types. The families and genera of the bacteria. Final report. *Jour. Bact.* 5:191-229. 1920.
19. _____
Bergey's manual of determinative bacteriology. 461 p. Baltimore. 1923.
20. Sturgis, W. C.
A bacterial blight of lima beans. *Conn. Agr. Exp. Sta. 22d Ann. Rpt.*, 1898, p. 262-263. 1899.
21. Tisdale, W. B., and Williamson, M. M.
Bacterial leaf spot of lima bean. *Phytopathology* 11:52. 1921.
22. _____ and Williamson, M. M.
Bacterial spot of lima bean. *Jour Agr. Research* 25:141-154, illus. 1923.
23. Wolf, F. A.
A bacterial spot of velvet bean. *Phytopathology* 10:73-80, illus. 1920.
24. _____
Bacterial blight of soy bean. *Phytopathology* 10:119-132. 1920.

X. EXPLANATION OF PLATES

Plate I

Leaves showing typical spot lesions. Note the dried out centers of the lesions, surrounded by dark colored borders. Also note the water-soaked areas around some of the lesions.

Plate II

Severely spotted leaves, about natural size. Note size and shape of spots. Also note the water-soaked areas around some of the lesions.

Plate III

Severely spotted leaves, collected from a diseased field near Edmore. Note the dried out centers of the lesions.

Plate IV

Leaf showing old lesions in which a number of spots have coalesced. Note the crust formed on the surface of the lesions.

Plate V

Under surface of a leaf showing spots in early stages of development. Note the depressed areas.

Plate VI

Leaf showing spots resulting from greenhouse inoculation with a culture of the organism isolated from cotyledon lesions. About three weeks after inoculation.

Plate VII

- A. Leaf showing several large lesions.
- B. Infected bean seedling. Note the lesions along the upper part of the stem. Note also the dried up cotyledon and the rosette of leaves formed at the top of the seedling stem. A curling of the leaves is also shown.

Plate VIII

- A. Leaf showing lesions along the veins.
- B. Infected pod.
- C. Leaf with spots in early stages of development.

Plate IX

Under surface of a leaf, showing infection along the veins.

Plate X

- A.** Healthy-looking, normal sized seeds.
- B.** Diseased, shrivelled seeds. Note the discoloration of the veins of the testa.

PLATE I

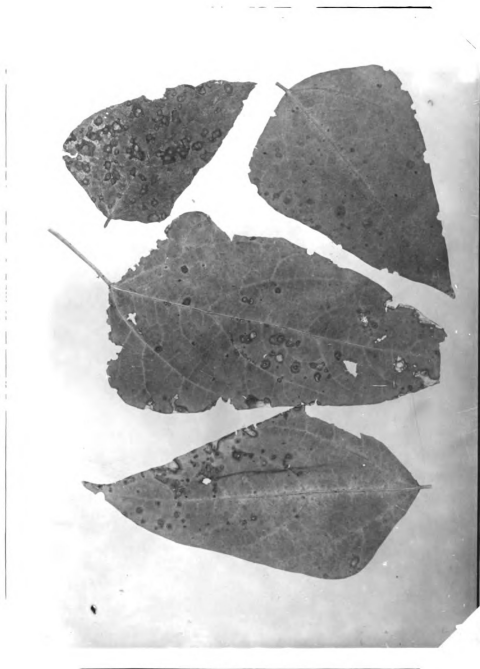


PLATE II



PLATE III



PLATE IV



PLATE V

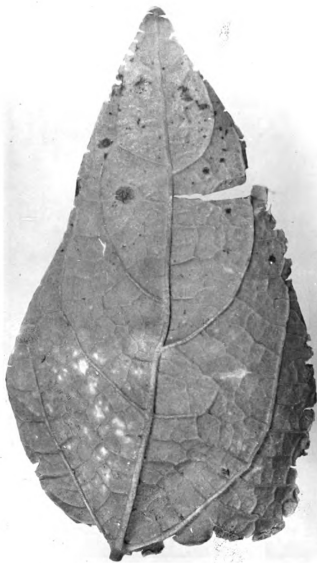


PLATE VI



PLATE VII

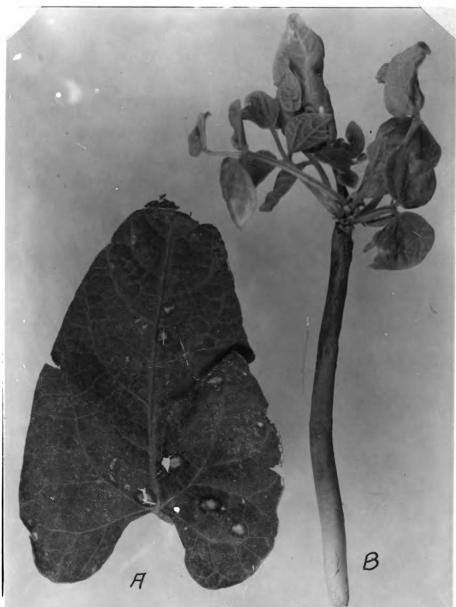


PLATE VIII

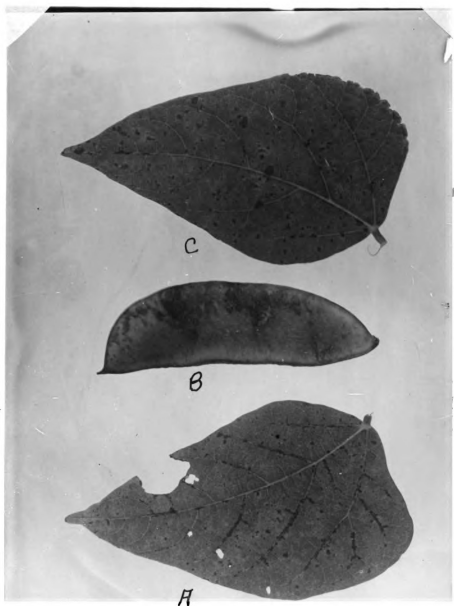
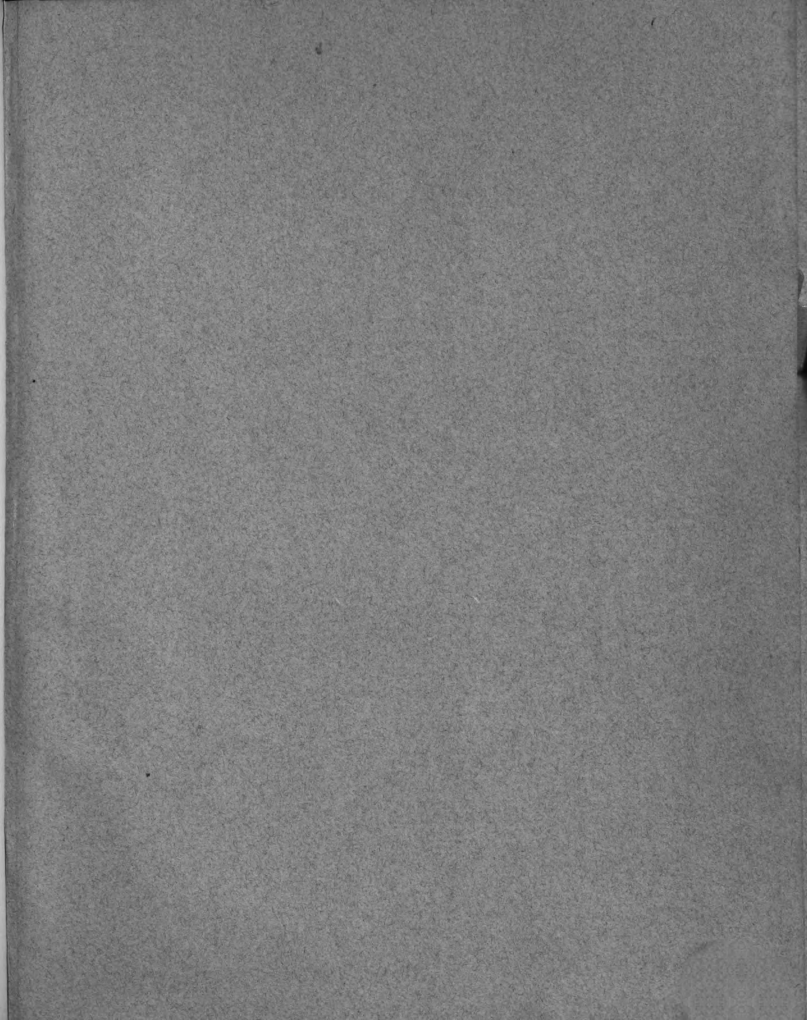


PLATE IX



PLATE X

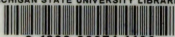




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