THESIS,

A DISEASE OF BROOM-CORN,

FOR THE DEGREE OF

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BY

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Introduction.

During the past six or eight years complaints have been made of a severe damage to the broom corn crop of Champaign County, Illinois. The diseased plants exhibit bright crimson-red blotches on both sides of the leaves and leaf-sheaths, on the stalks and on the flower panicle or "brush". When the plants are badly affected they grow spindling and weak, many of length dying. If the plants are not killed the brush is often injured so as not to bring as good a price as otherwise. Entomologists, after carefully studying the disease, have failed to attribute it to the defoliations of insects. It was thought at one time that the trouble came from injuries by chinch-bugs, but further observations on that insect dispelled the idea. A parasitic fungus described by Professor Burrill occurs in great abundance on broom corn. This was, naturally, suspected and studied. But it could not be connected with the disease as a cause. All the study of this disease having resulted in the proof that none of the suspected causes were
the real ones, attention was called to bacteria. Are they the cause of the
trouble? To answer this question a study of the disease has been
made. The investigations are embodied in this thesis whose object
is to ascertain the relation of bacteria to the disease.

Before entering upon the subject at hand a few words of
explanation must be given in regard to the originality of the work
done lest silence on this point might lead one to infer that the
whole was original with the writer. It is to be understood that
these investigations were the work of a student under the
direction of his instructor. Further than this, nearly all the
essential points developed by me were made out last season by
Mr. Woodworth who was also working under Prof. Burrill's direction.
Had Mr. Woodworth made notes of all he did and placed them
so as to be accessible it is probable that my work would be a
duplication of his under different conditions. Mr. Woodworth's work
was done during the latter part of the summer 1884, from July 26 to
the middle of September, while mine was done in the spring. This made
it necessary to the old material which had been out over winter
for cultures, and also to use plants grown in the greenhouse.

Other supposed bacterial plant diseases were also studied by
me; viz, the potato scab and a disease of young greenhouse plants
such as colens etc. Desiring to study bacteria in connection with
plant diseases I was told that the broom corn disease was the
most likely to give results in the time at my disposal. My first
inoculation experiments tended to prove this fact, so my attention
was wholly turned in this direction.

The general plan of this thesis and of the experiments on
which it is founded is taken from Koch's four postulates in the
study of bacterial diseases, substituting the word plant for animal
which is usually given, these four postulates are substantially as follows:

First, there must be a well defined type of disease so that it
constitutes a specific malady, and in the diseased plant there
must be one or more kinds of organisms in a constant relation
to the host if these be examined at the proper time.
Second, by isolating these organisms from the tissues of the plant, it must be possible to cultivate them in nutrient culture media outside the tissues of the host, and if any one species of microbe is to be proven the cause of a disease it must be obtained as a pure culture and retain its specific character throughout a series of cultivations.

Thirdly, it must be possible by inoculating cultures of the special organism into the plant to produce the specific disease.

And fourthly, in the disease so produced there must be found the same microbe in the same relation to the tissues of the host as in the original case.

Accordingly the subject is treated under the following heads:

I Bacteria in the diseased broom corn

II Culture experiments.

III Inoculation experiments.

IV Detection of the microbe in the artificially produced disease.

V Conclusions.
Nothing is more apparent to the writer than the incompletion of his experiments and studies. It must be stated at the outset that the four postulates have not been completely fulfilled. The only excuse for the presentation of the matter in its present incomplete form is the shortness of the time during which the work could be carried on.
I Bacteria in the Deceased Broom Corn.

A field of broom-corn near the University was visited early in March, and the bright red blisters were noted on the leaves, leaf sheaths, and in many instances on the stalks. The spots have a strong tendency to lengthen in the direction of the veins. This is very pronounced on the midrib and sheaths. Points often project, on a vein, outside the general limit of a spot. By stripping off the leaf sheaths from around the stem the bright crimson spots are found better protected from the weather than elsewhere. For obtaining material for cultures and slides such places were invariably selected because it was presumed that the organism would be in good condition in those places if in any. Repeatedly for examination and for cultures one of the cleanest spots was selected and cut out with the scissors. Placing it in a crucible, cover which had been washed in strong sulphuric acid and potassium chromate cleaning fluid then in alcohol, then in distilled water, it was teased up with a
distilled water recently boiled. Hereafter this will be called teared broom-corn material. A little of the liquid from this material was dried on a cover-glass then washed carefully in potash solution, alcohol, and dilute nitric acid, allowing it to remain several minutes in each. This process was for the purpose of removing as much of the material as possible that might be mistaken for bacteria. Upon staining this slide with a preparation of aniline red and carbollic acid, a considerable number of organisms were found. A short-jointed Bacillus occurs in groups or scattered over definite areas. A slide made from this material always contains great numbers of granules and fragments of tissue which take the stain so that unless there is a considerable quantity of an organism its detection is not easy. My slides made from this material invariably contained large numbers of a short-jointed Bacillus. Upon staining with methyl violet they were more apparent than without the stain. While examining a slide stained with methyl violet numerous granules were seen which it was thought were possibly spores.
But the aniline red stain mentioned above, used especially for exhibiting spores did not confirm this supposition. Of course at the time of year when this was done it was not possible to make slides from the fresh diseased material. This would certainly be a great advantage. No slides could be found which were made from fresh material last season, however, it may be worth something to say that as far as memory goes a short Bacillus resembling those seen in my slides was found in the fresh material.

II Culture Experiments.

In the culture experiments made during the study of this disease two kinds of culture media were employed; namely, solid and liquid. For liquid sterilized beef broth was used and for solid agar-agar. The methods of preparing the media and sterilizing it in the test tubes will be briefly given. To make the beef broth a pound of was stripped of fat and cut up finely. This was placed in a litre
of distilled water and allowed to stand over night in a cool place. The material was then heated in a water bath to the boiling point and kept there a few minutes. It was then strained through flannel cloth, neutralized carefully by adding sodium carbonate testing with litmus paper after every few drops were added, and was made a little more than neutral, that is, slightly alkaline. The liquid was then boiled on a sand bath and filtered through filter paper. The filtrate was then a pale straw-colored liquid perfectly clear and transparent.

For making the solid culture medium 500 cubic centimeters of the beef broth and to this was added 5 grams of ground age
This was allowed to soak three hours and was then placed in the steam sterilizer and kept near to boiling point for six or eight hours. It was then filtered through two thicknesses of flannel cloth. Some of the material was squeezed through with the hands but that made later was forced through by an apparatus giving air pressure by contracting and expanding a rubber bulb containing a valve like a bellows. In case of both beef broth and age-ages part of the
material was stored in small flasks and part put into test tubes. In either case the glass was thoroughly cleaned by washing in soap
suds or if necessary with sulphuric acid and potassium chromate
cleaning fluid, after rinsing in water it was washed in alcohol. A
cotton plug about two inches long was inserted in the mouth of
the tubes. The plugged test tubes and flasks were then placed in
an oven and subjected to a temperature of 150°C. for half an hour
or more. The process of heating is carried on just before the material
is filtered, so that as soon as the beef broth and agar-agar is ready
it is transferred to the test tubes. The filled test tubes were removed
to the water oven which was kept to a boil for at least two hours.
This boiling was repeated for three consecutive days. The test tubes
were then placed in the incubator and kept at a temperature of
from 35° to 37°C. for two days at least. If after this time there was no
growth they were considered sterile and were used for making cultures.
A little teased broom-corn material was made as described
before. A portion of the red-tinted liquid was then taken up by a newly
made capillary tube which was thrust through the cotton plug and
the liquid blown out into the sterilized leaf broth. The result was
invariably a culture. As might be expected cultures prepared in this
er way were seldom pure. Other species exist in the dead leaf presumably
besides the one which causes the disease. Most numerous among
the organisms and the most invariably found in the cultures
was a short thick Bacillus. Micrococci were found in some
cultures also a long Bacillus determined as Bacillus figuranus.
There is little doubt however that these are adventitious.
Again with similar results a portion of the diseased leaf sheath was
cut out and inserted by means of forceps through the cotton plug into
the leaf broth. By using a platinum needle fresh cultures were
made in ager-ager from the teased broom-corn material. In every
case a growth containing the short thick bacillus was the result. In order
to be certain that the growths observed came from what was planted
each of
chicks were carried on. Parallel with my first inoculations a culture
was made in which boiled distilled water, which had been through the
sterilizing process with the beef broth, was used as the inoculation material instead of the teased brown-corn. In not one single instance was there any growth resulting. These checks were tried a few times and discontinued because the perfect results of those tried gave sufficient confidence that whatever growth came was from the material inoculated. The next step was to obtain a pure culture of the Bacillus. The short thick Bacillus was suspected of being the cause of the disease on account of its invariable occurrence in the cultures and because as near as could be ascertained its appearance was the same as those observed in the slides spoken of in section I. The isolation of the microbe was effected by employing plate cultures which proved to be a most admirable way on account of the ease and rapidity of securing the result.

Plate cultures were made as follows: Glass plates about three by four inches were sterilized in the oven by heat. A damp chamber was prepared by setting a low bell jar in a glass dish and pouring a one per cent solution of corrosive sublimate in
water into the lower dish so as to cover the bottom. The upper dish then fits into the liquid. The plates were supported on glass dishes which elevated them out of the water. The agar-agar having been melted by holding the tube in boiling water, was then incubated in the ordinary way with a capillary tube. The glass plate was then taken from the oven and held by the edges in the left hand. The plug was quickly removed from the test tube and the contents poured out on the plate. A sterilized glass rod was used to spread the material. As soon as made the plate was placed in the clamp chamber. Plate cultures were made as quickly as possible after the materials were exposed to the air and the process was conducted in the bacteriological laboratory in which special precautions were taken to make the air as free as possible from floating germs.

Plates made in the manner described very seldom contained any colonies which looked like adventitious ones. Occasionally these did occur but were easily seen hence did not interfere
A plate culture was made as described above and placed in the incubator with the temperature at 36°C. Upon opening at the end of forty eight hours the plate was found to be nearly covered with an irregularly lobed growth very characteristic. It grew upon the surface of the agar-agar as a grayish-white wrinkled stratum, on the margins much lobed with radiating branches varying from a millimetric to three or four times that breadth. Some of the lobes were linear or spatulate others digitately branched at the end. Occasionally two slender lobes lying parallel were within a hair's breadth of each other. In many cases adjacent branches coalesced. Fig. is an outline drawing to show the characteristic lobing.

From this plate culture fresh cultures were made at once in tubes of agar-agar. The lobed appearance was visible on the growths covering the surface of the material in the tubes but it could not attain the extent of branching that it did in the plate culture. Slides were made from the plate and from the tubes. They showed the lobed growth to be a pure culture of a short jointed Bacillus horti.
referred to as the brown-corn Bacillus. The organisms occur in in
chains composed of couples, in couples or singly. The joints are cylindrical
with rounded ends. Those in chains stain deep violet, those in couples
or singly usually have a pale centre and dark spots at the
poles. All stages seem from solid Bacilli to those appearing as a simple
elliptical empty sack. Some have a very small pale spot occasionally
with a constriction of the walls opposite others have the opening larger.
No motions were observed which even of a ciliary nature. Size

The appearance of the organisms under a Yells one
fifteenth homogeneous immersion lens is shown in Fig. The solid
stained Bacilli were most frequently in chains as this shows.

Another plate culture was made verify the fact. It gave
the same peculiar lobed appearance and the same pale central Bacillus.
III Inoculation Experiments.

For the purpose of inoculation experiments out of the ordinary season of the plants' growth and, it was hoped, out of the season of growth for the disease brown-corn plants were grown in the University greenhouse in early spring. Some were planted in pots others were grown in boxes and afterwards transplanted in pots about six inches deep. When the inoculation experiments were commenced the plants were about four inches high and the leaves were scarcely a quarter of an inch broad. To find out whether or not the disease was directly transferable from the old leaves portions of the diseased brown-corn, which had been out of doors over winter, were teased up with a little distilled water for a few minutes in the same manner as for making a culture. My first inoculation was made after by taking a little of this material and painting it on with a camel's hair brush. Dots of it were made on the margin of the leaves. Where one dot was placed distilled water was painted and where two dots the brown-corn material.
These inoculations were by no means satisfactory in the results they revealed. After withering them two weeks a number of red spots appeared on the inoculated leaves. But these leaves being very small ones had been outstripped by the younger leaves and used soon to die. More than this an occasional red spot was observable on the checks which had not been touched to my knowledge with germ material. However the inoculated leaves on these first plants had upon them a much greater number of red spots than was observed upon any of the checks. So that although not much dependence could be placed upon them they still tend to show that the disease is transmissible.

My next inoculation was made April 11, using the same material as in the first. One plant was painted all over with the liquid another was punctured and torn with a needle and then the material painted on. A check for this was carried on by innoculating another plant in the same way and painting on distilled water.

The results described were about the same as those described above that is, not very definite. The margins of the torn places became red
in some cases and in others did not. There was much less redden-
ning on the cheek but still there was some. Most of the torn places on the
cheek dried up and turned brown. A much larger number of red
specks came on the leaves inoculated than with the liquid containing
the microbes than upon any of the other plants. There was no exception
to this statement in any of my experiments.

Next a culture of leaf broth was tried. This was made by inserting
a fragment of the old diseased material through the cotton plug into the
broth. It was not a pure culture but contained the brown corn
Bacillus, Bacillus lichenans and a spherical microbe was that occurred in
pairs. April 16th, this culture was painted on the principal leaves of a
plant, with a camel's hair brush. This plant was watched through the
next week. The disease made its appearance as minute red specks
just one week after inoculation. The specks were barely visible to the
naked eye but increased in size and number during the next week.

So that, two weeks the great number of red spots on the inoculated
leaves made it evident that the inoculation had been effective. The
younger leaves which had opened out during this time were free from
the disease or very nearly so. Fig. shows the appearance of one of these
leaves. On this leaf with a small lens 235 distinct red specks were
counted. On other leaves 198, 148, 122, spots respectively were counted.
No such abundance was to be found on the plants not inoculated.
Another plant was inoculated at the same time with the same material,
but some of the leaves were punctured and torn with a needle. The
plant gave results identical with the one from which the leaf shown in
Fig. came. The redness came around the wounds but also came in
abundance on the uninjured spots. Since more or less redness occurs around
any wound in these plants, and the inoculation comes just the same
without a wound, it is more satisfactory to produce the disease by simply
painting on the culture. Fig. shows the appearance of a leaf on which
was torn with a needle. Another leaf on the same plant contained 180 red spots
and several more hand over a hundred upon them.

The next step was to circumcise an area and paint the
culture on within this to see if the disease would appear only when the
culture was placed. A small area was marked off with ind. lines and a culture of the broom-corn Bacillus painted on within the area. One of these areas was on the upper side of a leaf and another on the underside of the leaves. When this inoculation was made the plants were at least a foot high. The disease came out distinctly on the inoculated area in one week. There were a few spots on this leaf outside the inoculated area but they were very few and scattered. The appearance of the leaf one week after inoculation is shown in Fig. Most of the spots at this time had distinct margins but occasionally some of them, especially those located on the midrib, were lengthened out and diffused. On one area of a little less than one fourth inch square there were 45 distinct spots when the disease had showed itself, while there were only one visible spot on the underside of the same leaf outside the inoculated area.

Upon examining this area with a one inch objective it was found that the smaller spots were exactly in the place of stomates. Broom-corn has its stomates in parallel rows and at rather regular
intervals, therefore, if a spot is not very large so as to cover several stomates, it can be told where it originated. Spots were seen in all quadrations from a small one between the guard cells to those too large to assign to any particular stomate. Fig. shows several of these stomates as seen under a one inch objective. Fig. shows the way they appear under a one fifth objective. Fig. shows a stomate with just a little color between the guard cells and Fig. shows one where the entire stomate has become colored. This appears to show that the disease gains entrance through the stomates. It indicates that the ordinary epidermal cells are a guard against their entrance but that the stomate do not keep them out.

Another inoculation was made with some of Mr. Woodworth's leaf broth cultures preserved in a sealed bulb of glass since last season. This was placed on a leaf inside a square area marked lines and the margin of the leaf. One on the upper side of a leaf and another on the lower. The broom-corn plants were about a foot high and the leaves nearly an inch broad. This plant was not watered the
second day after inoculation and it being very hot and sunny, when it was examined on the morning of the third day it was very dry and somewhat wilted. The leaves that were inoculated showed a very definite area covered with spots within the limits marked by ink lines. The plant was not at all seriously injured but was just suffering a little for maturing. It recovered very shortly after it was watered. Probably the quickness with which the disease took effect was due, at least in part to the drought to which the plant was subjected. The consequent weakening of the vitality of the plant would presumably give a better opportunity for any parasite to obtain a footing. The conditions were not at all dissimilar to those which repeatedly take place in the field. Everyone has noticed how tender plants wilt and die off on dry sunny days.

A repetition of this experiment gave precisely the same result. The second plant was kept well watered and the disease appeared after five days. The curious thing about these experiments with Mr. Hardworth's preserved cultures was that when the bulbs were broken to inoculate the plants, cultures were made in beef broth using a
small quantity of the material, and these gave absolutely no growths. Slides were made from the bulbs at the same time and these gave very unsatisfactory examinations. The organisms seem to have lost their shape and substance and to be dead. A few indistinctly show the form of a short-pointed Bacillus. The puzzle is — why should this culture produce the disease and not grow in beef broth?

There is a possibility that in making the cultures, the small amount of the material used contained none of the organisms, and that were some in the bulb in good condition. Another possible explanation is that the microbes were all dead but that the beef broth contained the specific poison resulting from their growth, and this caused the observed effects. In these experiments it is seen that the rest of the exposed foliage serves as a check on the inoculations.

To test whether beef broth had anything to do with causing the reddened appearance, marked areas of the plant were inoculated, in the same manner as in the previous cases, with sterilized beef broth. No result came from this experiment after twelve days, showing that
there must be something besides the broth to which the results must be attributed. The only difference between the two kinds of broth used was that the one contained the Bacillus and its products and the other did not. Either the microbe or its poisonous products, then, must be held to be the cause of the observed effects.

IV Detection of Bacteria in the Artificially Produced Disease.

For the detection of the Bacillus in the inoculated leaves sections of the leaves were cut and examined while freshly mounted in water! The sections were made by immobilizing a fresh leaf in paraffine and cutting on the microtome. The last ones were one one-hundredth of an inch thick. They were examined with a one-fifth, and with a one-fiftieth fold's homogenous immersion objective. It seemed impossible to find the Bacilli in the cells of the host with certainty. This was mainly an account of the numerous granules having about the size and shape of short-jointed Bacilli. In some instances a few
undoubtedly. Bacilli were found either in the cells or floating out near
the section. These have the characteristics of the cultivated forms
except the pale centre. This may easily have escaped observation
because the material was not stained. Many of the granules were
imbibed in the protoplasm and were motionless. Within the
discolored area, in certain cells, which were filled with a clear
liquid and contain little protoplasm, there were great numbers of
these granules in active swarm-like motion. These cells swarming
with granules were seen in several instances but never outside
the reddened area. The red color was seen in the stomate
guard cells and in some instances, in others it had spread to the
adjoining cells only and in still others it had reached considerable
distance. The protoplasm becomes red first then the cell-wall.
Other sections were stained with methyl violet but the tissue
itself took the stain so deeply as to obscure any organisms present.
Another method of search for the organisms was to cut one
of the red spots, tease upon a cover-glass with a little sterilized beef-broth
dry, stain with aniline blue, and mount in balsam. This was not satisfactory in its results. All the organic matter stains a deep blue. The spots were of necessity very small, not larger than a square millimetre so that an abundance of organisms was not expected. But it was only occasionally that a Bacillus could be found and then not certainly the one the pale centre figured in the plate.

The difficulties of detecting bacteria in plant tissues are much greater than in animal, because the various processes of staining color the tissues as well as the microbes. The difficulties may be right overcome and the organism seen, but at any rate this was not done with any degree of certainty in these experiments.

**Conclusions**

Before drawing a conclusion we will sum up the results and see as to how the four postulates have been fulfilled. An organism was first found in abundance in the
diseased leaves. The malady is perfectly definite so that there
is no doubt about its being a specific disease. The disadvantages
of detecting the organism in the old diseased leaves will partially
excuse the poor quality of the slides demonstrating the presence
of the short Bacillus. The first postulate, then, is only fairly well
fulfilled.

The uniform presence of the short-jointed Bacillus with the
pale centre in the cultures have indicated strongly that this is the
organism connected with the disease. By means of solid culture
media this Bacillus has been isolated and pure cultures of it obtained.

The second and third postulates are closely connected. The
pure culture is to be obtained (first postulate) which will produce
the disease (third postulate). This we have done. The disease
was produced from the old material directly, from cultures of it,
and from cultures made while the diseased was in progress last
season and preserved over winter in a sealed bulb.

The fourth postulate is the main place in the otherwise
strong chain of evidence. It is believed that if these inoculations could have started the disease in large and juicy plants where it could have developed more luxuriantly, that the obstacles could have been overcome and this postulate fulfilled. However, what one believes must not have the least weight in drawing a scientific conclusion. We cannot say, then, with absolute certainty that the male central Bacillus is the cause of the disease. We can say that the disease is transmissible directly and from cultures.