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A NEW DISEASE OF COTTON. COTTON BOLL-ROT.

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COTTON-BOLL ROT.

A NEW BACTERIAL DISEASE OF COTTON AFFECTING THE
SEEDS, LINT AND BOLLS.

BY J. M. STEDMAN.

During the middle of August, 1893, I received from the Department of Agriculture, Montgomery, Ala., some samples of cotton-bolls supposed to be suffering from the attack of insects. The cotton-bolls were accompanied by a note stating that they had been received from Mr. A. W. Bryant, Stockton, Baldwin county, Alabama, and asked for the name and habits of the insect affecting them, and for the remedies to be used to combat or destroy the same. On the 13th of September, I received a box of diseased cotton-bolls from Mr. W. A. Bryant himself.

A short examination of the bolls and of the numerous insects in them was sufficient to convince me of the fact that the insects were not the direct cause of the disease, but that on the contrary, they were present in order to eat of the already dead and decaying vegetable matter. The insects were Coleoptera (beetles) of the family Nitidulidæ (Sap-suckers), and were present in all stages of development. The larvæ, one of which is represented in figure 7, and the adult beetles, represented in figure 5 and 6, were very numerous, while their pupæ were not uncommon. The larvæ are about one-fourth of an inch in length and are nearly white in color. Figure 7 represents one magnified about five diameters.

A closer examination revealed the presence of two species of adult beetles both of which are about one-eighth of an inch long. Figure 5 represents one of these sap-beetles, *Epuræa æstiva*, magnified six diameters, while figure 6 shows

the other species, *Carpophilus mutilatus*, equally magnified. Both of these beetles are well known among fruit growers in the Southern States, Mexico, and Central and South America. They are widely distributed throughout the south, feeding both in the larval and adult condition upon decaying or injured fruit of all kinds, and are sometimes found sucking the sap from wounded portions of trees. They are common in cotton-bolls that have been injured by the boll-worm, and in decaying heaps of cotton seed. Neither the adult beetles nor the larvæ are known to eat or attack healthy fruit or living vegetable tissue. The presence of these insects, then, in the diseased and decaying cotton-bolls is not surprising, and their presence can have at least only a secondary connection with the true disease in that they may, by their burrows cause, perhaps, a more rapid spreading of the disease.

Neither the beetles nor their larvæ were to be found in all the disease cotton-bolls, but only in such as were greatly damaged by the disease having spread so as to involve nearly the entire contents of the boll and to have caused the tips of the carpels to open slightly. In such bolls I also observed several species of ordinary saprophytic fungi, and in a few cases the fungus, *Colletotrichum Gossypii*, Southworth, that produces the disease in cotton-bolls known as anthracnose.* But no fungi were observed in the bolls that were only slightly diseased or decayed inside.

The presence of fungi and insects in those cotton-bolls only that were greatly diseased and decayed inside, and that had either the tips of the carpels opened or the disease had spread so as to involve a portion of the outer surface of the bolls, together with the entire absence of insects and fungi in all cases where the disease was confined to the contents of the boll, led me to suspect the bacterial nature of the disease in question. Accordingly, pure cultures of the bacteria from the disease inside the closed cotton-bolls were then made by the usual plate culture method, and the inoculations made in both tubes of nutrient gelatine and of agar-

*See Bull. No. 41, On Some Diseases of Cotton, by G. F. Atkinson, p. 40.

agar by means of a sterilized platinum needle. In four days the growth of the bacteria in the gelatine tubes had become very profuse, and had clouded the entire mass of gelatine, giving it a slight greenish hue. The growth of the bacteria in the agar-agar tubes was different. Here the bacteria spread out as a milky cloud around the entire length of the path of the inoculating needle through the agar, and also over the surface of the agar as a more or less white, semi-transparent and glossy growth. See figure 3, which represents the growth as it appears in agar-agar tubes.

That this difference in the growth of the bacteria in the agar-agar and gelatine tubes was not due to a difference in the kind of bacteria in each was proven by the numerous cross inoculations that were made. Fresh agar-agar tubes were inoculated with the bacteria from a gelatine tube culture, and fresh gelatine tubes inoculated with the bacteria from an agar-agar tube culture, in all cases by means of a sterilized platinum needle; and in no case was there any signs of a deviation in the method of growth or appearance of the cultures peculiar to either the agar or to the gelatine as above stated.

In order to determine whether or not the bacteria of which I had made pure cultures were the cause of the disease in the cotton-bolls, I selected ten healthy cotton plants, and with a sterilized needle, I made two punctures into four healthy cotton-bolls on each of the plants, numbers 1, 3, 5, 7, 9, and labeled each boll. Then by means of the same needle, sterilized and then infected with the bacteria from the pure tube culture, I made two punctures into four healthy cotton-bolls on each of the plants, numbers 2, 4, 6, 8, 10, and labeled each boll. In twelve days all the cotton-bolls inoculated with the bacteria from the tube cultures had taken the disease in varying degrees, and in twenty days they were entirely destroyed; the entire contents of the bolls having rotted, and the outer surface to a more or less extent. On the contrary, the four bolls used as a control experiment on each of the other five plants were perfectly healthy and showed no signs of a disease, except one that had been attacked by a fungus at the place where the needle

had caused an injury, thus enabling the fungus to develop there; but this boll was not affected with the disease in question.

Hence it is demonstrated that this specific bacterium was and is the cause of the disease in question.

From one of the original bolls some diseased tissue including seed was hardened in increasing strengths of alcohol, infiltrated with paraffine in the usual manner, cut into sections which were fastened to the slide by clove-oil-collodion, stained with gentian violet or with carbofuchsin, and mounted in balsam. On examination with a high power (1-24 inch Hom. Imm. Obj. of Winkel) of the microscope, most of the cells in the diseased region of the tissues were found to contain bacteria in abundance. Figure 4 represents a portion of a section of such a tissue as seen under the microscope, and is magnified 800 diameters.

Several cover-glass preparations from the pure cultures of bacteria in both agar-agar and gelatine were made and stained with either gentian violet or with carbofuchsin, and examined with the 1-24 inch Hom. Imm. The appearance of these bacteria as seen under such a high power of the microscope is shown in figure 1, which represents them as magnified 1500 diameters. When magnified equally, the bacteria in the sections of diseased tissue will be seen to be identical in appearance with those from the culture tubes.

Not being able to identify this species of bacteria with any heretofore described, I have named it

BACILLUS GOSSYPINA.

Obtained by Stedman (1893) from the inside of diseased cotton-bolls suffering from a rot of the seed and lint.

Morphology.—Short, straight bacilli, truncate with slightly rounded corners, 1.5 micron long and 0.75 micron broad; usually solitary, sometimes in pairs, and occasionally in chains of from three to four.

Stains readily with the usual aniline colors.

Biological characters.—An aërobic, non liquefying (slight liquefaction in old gelatine cultures), motile bacillus. Forms spores. Grows at the room temperature in the usual

culture media, but more rapidly at 25° to 35° C. In gelatine tube cultures, the growth in three days gives a milky appearance, which spreads from the line of puncture of the inoculating needle, until in five days the entire gelatine becomes milky and assumes a slight greenish color. In agar-agar the growth on the surface appears as a smooth, semi-transparent, milky layer; while the development along the line of the puncture of the inoculating needle through the agar takes place as a cloudy, more or less even growth, gradually becoming thinner at the periphery.

Pathogenic.—Inoculated into healthy cotton-bolls, a disease resulting in a rotting or decaying of the seed and lint is produced in from one to two weeks, which soon involves the carpels, and thus destroys the entire cotton-boll.

This new rot disease of the cotton-boll is readily distinguished from the only disease likely to be confounded with it, namely anthracnose, by the fact that the anthracnose first makes its appearance as small, reddish brown spots on the surface of the boll, which spots enlarge and become dark, gray or pink according to circumstances. Finally, when the spots have attained a considerable size, they will be found to consist of a pink centre surrounded by a dark band, and this in turn surrounded by a dull, reddish brown band. The anthracnose is caused by a fungus, *colletotrichum Gossypii*, Southworth* which originates on, and is usually confined to, the carpels of the boll, and only occasionally infects the lint.

The new rot disease of the cotton-boll, on the contrary, originates within the boll, and does not make itself visible, as a rule, until the entire or nearly entire contents of the boll has become involved and decayed, when the carpels may become affected and show signs of decay in places. The cotton-boll rot is caused by a bacterium, *Bacillus gossypina*, Stedman, and first appears as a small black or dark brown area on some of the young and developing seed and lint inside the boll near the petiole. This area gradually enlarges and causes the affected parts of the seed and

* See Bull. No. 41, On "Some Diseases of Cotton," by G. F. Atkinson p. 40.

lint to decay or rot, and ultimately spreads so as to involve all the seed and lint within the boll, and may then even affect portions of the carpels. Figure 2 shows a diseased boll cut open, the seed and lint being affected. If the boll becomes diseased early in its growth, say four weeks before it is ripe, the disease will cause the entire boll to rot before the carpels can open at all. If, however, the disease appears later, when the boll is full size or nearly so, and the seed and lint nearly developed, the carpels may open or separate slightly at the tips, and thus admit the small sap-beetles that will enter and feed upon and breed in the decaying contents of the boll, and thus help to disintegrate it. Saprophytic and other fungi finding here a suitable pabulum may now appear and infest the decaying boll. Of course these diseased bolls can never mature lint or seed.

Should the disease appear still later when the boll has partially opened, or is nearly ready to open, the rot may affect only a few seed and a small portion of the lint before the boll opens and dries. In this case the boll would appear nearly normal and a large portion of the lint and seed would be perfect, especially that exposed to view, while that nearest the petiole would be affected. This is really the most serious condition so far as the cotton growers at large are concerned, since it is probably here that the great danger of spreading the disease to unaffected areas is to be found. In the other cases the contents of the boll is either wholly or more or less destroyed, and the boll fails to mature or develop lint; and if it opens it is but slight, and the boll is known to be diseased or imperfect and is never picked. But when the disease is so slight as to allow picking, the effected seed and lint is mixed unconsciously and taken to the gin, where the seed becomes mixed with seed from unaffected district; and thus all the seed that passes through the gin is liable to be infested with the germs of the rot disease, and finally to become distributed to distant parts of the country. Too great a precaution in regard to this method of spreading the disease can not be taken. The cause of the disease has been shown to be a micro-organism (bacteria) of extreme minuteness, and one that is found in innum-

erable numbers in the diseased tissues; and since the presence of a single one of these bacteria may cause the disease, we should guard against dangers of contamination.

Although it has never been demonstrated, yet it seems probable that the bacteria present in the diseased seed, lint and carpels, after they fall to the ground and become disintegrated, are liberated and find their way to the roots of the cotton plant which they enter, and pass up through the plant to the bolls, inside of which they find conditions suitable for their development. Or the seed may be unaffected but the lint left attached to it may contain the bacteria, which would thus be in close connection with the young cotton plant when it germinates, and then could find its way into the roots. And it also seems very probable that those seed which are affected with the bacteria, but not in sufficient quantities to prevent their germination, may produce young plants with the rot bacteria already within their tissues (seed leaves), and thus these bacteria may then easily find their way into the bolls when they appear. But it seems to me even more probable that the bacteria are carried by the wind or insects from the soil to the flowers, where they remain attached to the moist and viscid stigma or in the nectar; and that they not only thus readily find their way into the young and developing bolls, but that they even multiply in the nectar or on the stigma; and that the insects which visit the flowers are thus contaminated and inoculate other flowers. This seems even more probable since we know of certain other bacterial diseases of plants, as pear blight, that is thus carried from one tree to another, and from one flower to another on the same tree. This explanation of the spread of the disease helps us over one difficulty, namely, the fact that the disease is principally confined to the middle and top crop. For if the bacteria are in the young cotton plant before the bolls are formed, one would expect the first or lower crop to be equally affected. If the bacteria enter by way of the flowers, we could explain the scarcity of the disease in the lower or first crop of bolls by the supposition, that the insect which carries the disease from one flower to another does not appear until

the flowers of the middle crop are beginning to open. The lower crop would have simply the wind to introduce the disease, while the middle and top crop would have in addition the greater agency, insects. An effort will be made this summer to determine whether or not the bacteria do normally enter the bolls through the flower, and also to determine the insects which carry the disease from one flower to another. Experiments are now being conducted to determine the truth of the other four supposed methods of the distribution and entrance of the bacteria into the interior of the cotton-bolls.

So far as my observations and experiments are concerned, I have never been able to induce the rot bacteria to develop the disease or cause pathological disturbances in any part of the cotton plant other than the interior of the bolls, although they will live and even multiply to a slight extent within the tissues of the other parts of the plant.

All the facts in the case go to show that the cotton plants naturally become affected either by the rot bacteria entering the roots from the soil, or that the plants begin their existence as affected ones by the bacteria having entered the cotyledons (seed leaves) of the seed while still within the boll, or that the bacteria are carried by the wind or insects from the soil to the flowers, and from one flower to another, and enter the bolls in this way. It hardly seems probable that the bacteria could be blown by the wind or carried by other agencies upon the surface of the cotton-bolls and enter by that route, since the rot disease always makes its first appearance as a small diseased area of the seed and lint inside the boll near the petiole, and only later involves the carpels, and makes itself apparent on the exterior. Nevertheless, the bacteria may enter in this way and migrate to the seeds, for we have no definite proof to the contrary.

The rot disease seems to be principally confined to the middle and top crop, and makes itself manifest to the ordinary observer about the first of August. It is usually pretty evenly distributed over a field, and as yet is not as troublesome to river plantations as to high lands. That this rot disease is a very important one can be seen from the fact

that it is damaging the cotton crop to the extent of 35% in certain parts of the State, and is on the increase and spreading. Mr. A. W. Bryant writes me that he has counted as many as nineteen diseased bolls on one stalk, and there were no doubt many more that were not diseased enough to appear on the exterior.

As regards the remedies and precautions to be taken in fighting this disease, it will be readily understood from the nature of the disease as above described, that the remedy must be a preventive one; and that we can not resort to any thing like spraying the plants with a fungicide or other chemicals, since we would kill the plants before the seat of the disease could be reached. We can then do nothing towards curing a boll once diseased, but we may help the cotton plant as a whole, and lessen the chances of having other bolls diseased, if we will remove the diseased bolls. But since the bacteria in the diseased tissues are not readily killed by such natural means as cold of winter or heat of summer, drying or becoming wet, nor by the decaying of the tissues in which they are found, but are simply liberated and thus allowed to work through the soil to infest other cotton plants, we must, therefore, carefully preserve the diseased bolls and burn them, and not allow one to fall to the ground and remain there. If the diseased bolls are not picked and burned, but are simply allowed to remain on the cotton plant, they will sooner or later fall to the ground, and thus distribute millions of new bacteria in the soil, and rapidly increase the chances of having diseased bolls next season. It will not answer to leave the diseased bolls on the stalk after the cotton is picked, since the rain will wash the decayed and affected interior of the bolls out, and distribute it upon the soil. The diseased cotton-bolls should all be picked off and burned just as soon as discovered, or at least during the first picking of the lint, and ever afterwards as discovered.

It is a simple matter to carry a second bag in connection with the one used in picking lint, and to place in the second bag all diseased bolls as discovered, and to put them into small heaps and burn them. By this means the rot disease

can be greatly lessened. Of course all cotton that appears to be imperfect in the boll should be glanced at, to see that it is not diseased farther in the boll, before it is placed with the good lint, otherwise diseased seeds will find their way to the gin and be distributed and planted.

When the cotton field is badly affected with the cotton-boll rot disease, it would be advisable to plant some other crop there for two years, and to use other fields previously occupied by a different crop for the raising of cotton. In this way the bacteria in question might be gotten rid of.

We may sum up briefly as follows :

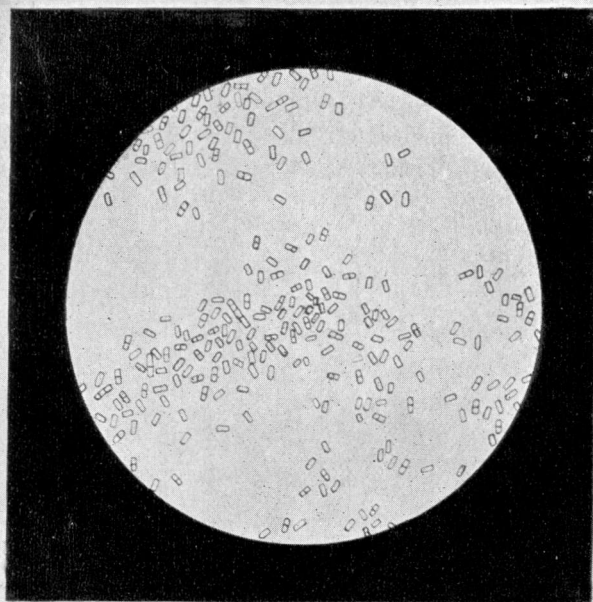
a. The *cotton-boll rot disease* is caused by a bacterium (*Bacillus gossypina*, *Stedman*) which works within the boll, causing its contents (seed and lint) to decay. And since the bacteria are inside the tissues, it would be useless to spray the plant with any chemicals at present known, since we would kill the plant before the diseased region could be reached.

b. The disease is multiplied in and carried from one crop of cotton to another, and also to unaffected areas, by means of the diseased tissues, with probably the help of the wind and insects.

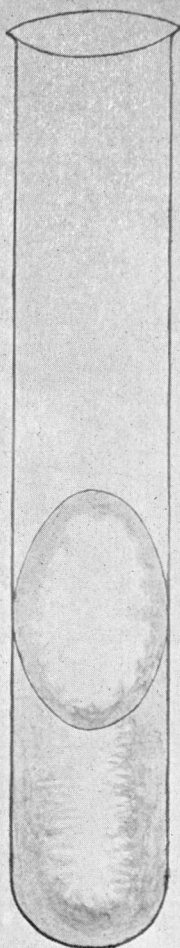
c. The bacteria may possibly enter the cotton plant from the soil, through the roots, although it is possible they may enter through the epidermis of the boll; but more probably they were already in the seed-leaves of the seed, or enter the bolls from the flower.

d. All diseased cotton-bolls should be picked off and *burned* just as soon as discovered, or at least while the lint is being gathered, and the field gone over again immediately after the last picking of the lint.

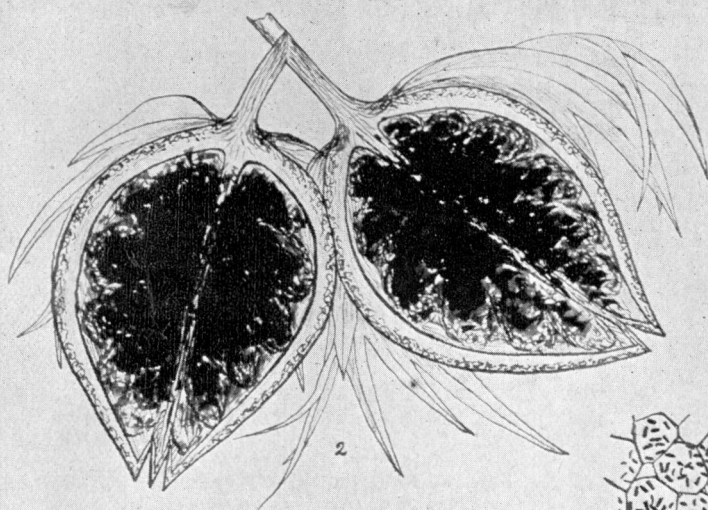
e. Cotton seed coming from a gin known to have ginned cotton from an affected district should not be planted in unaffected districts.



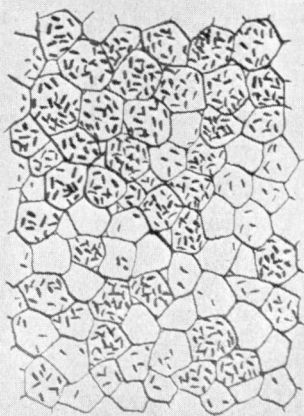
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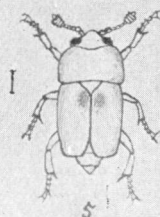
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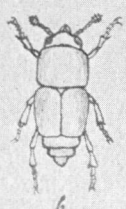
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