The rots of the cotton boll

Claude Wilbur Edgerton

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Agricultural Experiment Station

OF THE

Louisiana State University and
A. & M. College

BATON ROUGE

The Rots of the Cotton Boll

BY

C. W. EDGERTON, PLANT PATHOLOGIST

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The cotton industry in Louisiana, while not as large as it was a few years back, is still a very important one to the state. In one year, Louisiana produced over a million bales but since the boll weevil has covered the state, there has been a large reduction in the cotton produced. From over a million bales, the production dropped down to 250,000 in 1910. The years 1911 and 1912, however, have shown an increase over the small crop of 1910. In 1911, Louisiana produced a 350,000 bale crop, and in 1912 in the neighborhood of 400,000 bales.

In order to make the crop yield profits to the planter, all of the pests and troubles, even of minor importance, must be taken into consideration. The cotton plant is affected with many troubles, perhaps to a greater extent than the majority of our farm crops. A large number of insects attack the plant in the various stages of its development such as the boll weevil, cotton stainer, boll worm, etc. There are also a number of physiological troubles due to improper fertilization, improper drainage, etc. And finally there are the troubles caused by fungous and bacterial parasites. All of these troubles are important and must be taken into consideration in the future if cotton is to remain a paying crop in the state. In this bulletin, however, only a few of these will be considered.

There are many enemies to cotton culture among the bacterial and fungous parasites and some of these cause troubles that are well known to most planters, such as the cotton wilt, sore shin, leaf blights, and boll rots. All of these troubles are important, but only the latter, the boll rots, and the organism causing them will be considered at this time.

Considerable work has been done upon the fungous and bacterial parasites which damage cotton bolls. While there are only a few real serious diseases, these cause so much damage that they have received a great deal of attention in the past 20 to 25 years.
While the rotting of cotton bolls was recognized as a serious trouble previous to 1890, the causes were unknown and very little research work had been carried on. Wailes (72) in 1854 and Glover (37) in 1855 described some of the boll rots, but they attributed the causes to weather conditions, insects, and other injuries to the bolls. In the early 90's, however, Atkinson at the Alabama Experiment Station studied the troubles for three years and described many of the fungi and bacteria which were responsible for the rots. Since that time a number of workers have added their share to our present knowledge of the diseases and their causes.

**LOSSES CAUSED BY BOLL ROTS.**

The actual annual loss caused by the rotting of cotton bolls is hard to estimate. Different workers at different times have estimated the loss caused by the anthracnose, the most serious of the rot organisms, these estimates ranging from 1 to 50 percent of the crop and sometimes even higher. In Louisiana, from five years observation, the actual loss from all boll rots seems to be between 5 and 10 percent, though there is a great deal of variation from year to year. In wet years, the loss is sometimes greater than 10 per cent, but in dry years it often runs less. But a 5 to 10 percent loss would mean, with a 350,000 bale crop, an actual loss of 17,000 to 35,000 bales, or a money loss, if the cotton is worth 10 cents a pound, of $850,000 to $1,700,000. And if the loss would average the same in the other states of the cotton belt, there would be a loss in the whole country of 750,000 to 1,500,000 bales, or a money loss of $37,500,000 to $75,000,000, counting on a 15,000,000 bale crop.

**CAUSES OF THE BOLL ROTS.**

The rotting of the bolls is caused chiefly by the attack of certain parasitic fungi and bacteria. The principal ones which we have in Louisiana are as follows:

*Glomerella gossypii*, causing a bad rot of the bolls, known commonly as the anthracnose.

*Bacterium malvaecarum*, forming watery spots on the young bolls, these later being invaded by a number of other organisms.

*Diplodia gossypina*, forming a black rot of the bolls.
Fusarium sp., causing a rot of the carpels and also a discoloration of the lint. This is generally recognized in the field by the pink coating of spores on the boll.

Rhinotrichum tenellum and Olpitrichum carpophilum, causing lots of minor importance. These are easily recognized in the field by the thick covering of grey to buff mycelium.

There are also a few others of minor importance which will be mentioned in the following pages but they are of little economic importance.

These fungi are not all of equal importance in the rotting of bolls. Some of them do a large amount of damage, while others are not very common or else only cause a small percent of the loss. The anthracnose is the most common rot, though it is not responsible for all of the loss. Many planters, however, not being able to distinguish the different rots, attribute all to the anthracnose. Furthermore, many writers on the subject of boll rots have counted all rotten bolls in the field as bolls affected with the anthracnose, and held this disease accountable for all of the loss. It is possible that the anthracnose may cause a greater per cent. of the rots in the more Eastern states than in Louisiana, yet these other troubles should certainly be separated in figuring damage done by the anthracnose. In Louisiana, some of these other fungi and bacteria figure quite largely in the total loss.

In counting the bolls in the field to get the actual per cent. of loss caused by the different diseases, many difficulties arise. Some of the bolls may be affected with two or three different disease organisms and it is not always easy to tell just which one was responsible for the damage. Furthermore, if the boll is in an advanced stage of the rot, saprophytic or facultative parasitic organisms may have entirely taken the place of the original parasite. Attempts have been made, however, to get some data on the damage caused by the different organisms. This was done by counting all of the bolls, both diseased and healthy, on a definite number of plants and then by picking the diseased bolls and examining them carefully in the laboratory with the microscope. In Table 1 below, are given the results of some counts made during the past two years. The table shows the total number of bolls in each count, the total number of rotted bolls, and the number
affected with each of the more important boll rot fungi. The
counts made at Baton Rouge were mostly obtained from differ-
ent fields where the conditions of soil and growth were somewhat
different. The counts dated "Aug. 19-23, 1911" and "Aug. 30,
1911" were made in the same field but a few days apart.

In the table, a considerable number of the diseased bolls are
counted twice. Many bolls had more than one fungus on them
and were as a result placed in more than one column. A boll that
had both the anthracnose and Fusarium is counted in both the
anthracnose and Fusarium columns.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Date</th>
<th>Number of plants</th>
<th>Total number of bolls</th>
<th>Number of diseased and per cent</th>
<th>Number with Anthracnose and per cent</th>
<th>Number with Fusarium and per cent</th>
<th>Number with Rhino-orthidium and per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baton Rouge</td>
<td>Aug. 19, 1911</td>
<td>15</td>
<td>180</td>
<td>53</td>
<td>29%</td>
<td>598</td>
<td>18.4%</td>
</tr>
<tr>
<td>Baton Rouge</td>
<td>Aug. 19, 1911</td>
<td>15</td>
<td>128</td>
<td>37</td>
<td>29%</td>
<td>23</td>
<td>28%</td>
</tr>
<tr>
<td>Baton Rouge</td>
<td>Aug. 19-23, 1911</td>
<td>590</td>
<td>7033</td>
<td>1293</td>
<td>18.4%</td>
<td>598</td>
<td>8.5%</td>
</tr>
<tr>
<td>Baton Rouge</td>
<td>Aug. 30, 1911</td>
<td>600</td>
<td>6070</td>
<td>943</td>
<td>15.5%</td>
<td>240</td>
<td>40%</td>
</tr>
<tr>
<td>Baton Rouge</td>
<td>Aug. 31, 1911</td>
<td>20</td>
<td>189</td>
<td>73</td>
<td>38.6%</td>
<td>18</td>
<td>9.6%</td>
</tr>
<tr>
<td>Baton Rouge</td>
<td>Aug. 31, 1911</td>
<td>20</td>
<td>261</td>
<td>78</td>
<td>29.9%</td>
<td>36</td>
<td>14.9%</td>
</tr>
<tr>
<td>Baton Rouge</td>
<td>Aug. 28, 1912</td>
<td>320</td>
<td>3245</td>
<td>456</td>
<td>14.1%</td>
<td>223</td>
<td>6.9%</td>
</tr>
<tr>
<td>Winnfield</td>
<td>Sept., 1912</td>
<td>20</td>
<td>145</td>
<td>16</td>
<td>11.0%</td>
<td>5</td>
<td>4.1%</td>
</tr>
<tr>
<td>Winnfield</td>
<td>Sept., 1912</td>
<td>20</td>
<td>252</td>
<td>18</td>
<td>7.1%</td>
<td>14</td>
<td>5.6%</td>
</tr>
<tr>
<td>Winnfield</td>
<td>Sept., 1912</td>
<td>20</td>
<td>238</td>
<td>35</td>
<td>14.7%</td>
<td>13</td>
<td>5.4%</td>
</tr>
<tr>
<td>Arcadia</td>
<td>Sept., 1912</td>
<td>20</td>
<td>250</td>
<td>15</td>
<td>6.0%</td>
<td>8</td>
<td>3.2%</td>
</tr>
</tbody>
</table>
As is seen by the table the anthracnose, Diplodia, and Fusarium are the predominating fungi on rotting cotton bolls. The damage done, however, is not in proportion to the abundance of the organisms. The Fusarium, while present on as many of the bolls as the anthracnose, does not do nearly as much damage as the latter. It does some damage in rotting the capsular tissue and discoloring the lint, yet the actual loss is much smaller than with the anthracnose.

In the above table, there is seen a considerable difference in the abundance of the organisms in the same field on the different dates of the same year. With only a week to ten days difference in time, there is seen a considerable falling off in the amount of the anthracnose and Fusarium and a rise in the Diplodia and Rhinotrichum. This is due to several things. The falling off of the anthracnose and Fusarium was probably due to a shedding of the diseased bolls, and also to an elimination of these fungi caused by others running them out. If all the bolls that rotted had been tagged and watched, it is probable that a large per cent. of them would have shown the presence of the anthracnose during the earlier stages of the rot, but as the decay advanced, this organism gave place to others on the rotting bolls. The Diplodia acts very different from the anthracnose in this respect. This is a very virulent parasite and when it once gains entrance to a boll stays with it to the end of the season. However, the abundance of the different organisms shows that they all are very important as rot producers and must be considered when a general study is made of all boll rots.

No mention is made of the bacterial rot in the above table for the reason that the identity of the bacterial spot is soon lost on a decayi ng boll. While this is a very important organism in starting decay, the other organisms very soon overrun the spots it develops.

RELATION OF WEATHER TO BOLL ROTS.

While the organisms mentioned above are the primary cause of the boll rots, there are a number of factors which tend to increase or decrease their attack. Perhaps the most important of these is the weather factor. Excessive rainfall and humid conditions favor very materially the development of the rots. While
rots are always present to some extent in any year, they are particularly bad during rainy seasons. It is not unusual for the condition of the cotton crop in the United States to drop off 10-15% during a few weeks of rainy weather. And this deterioration is due, to a large extent, to the rotting of the bolls. These different factors which tend to increase or decrease the rots will be mentioned again during the discussion of the different diseases in the following pages.

THE BACTERIAL ROT.

The presence of bacteria in rotting cotton bolls has been recognized for many years, and it is the common belief, especially in tropical countries, that the trouble known as "Black Boll" is due to bacteria. In Louisiana, there are three species of bacteria common in rotting cotton bolls. One of them, and by far the most important one, is Bacterium malvacearum Smith. The other two have not been determined. One of them produces a deep yellow colony on culture media, while the other produces a white colony. Bacterium malvacearum is a true parasite attacking the uninjured bolls, while the other two develop in injured bolls or bolls already affected by some other organism. Bacterium malvacearum is the one which has received the most study in Louisiana and the one which is most considered in this bulletin, although the other two will be mentioned from time to time.

Another species, Bacillus gossypina Stedman, has been described from rotting cotton bolls in Alabama by Stedman (67), but this organism has not been seen in Louisiana and it seems very doubtful if such a boll-rotting organism really exists.

The diseases of cotton caused by Bacterium malvacearum are known commonly as the Angular Leaf Spot, Black Arm, Blight, Bacterial Boll Rot, and sometimes as Rust, though the latter name should not be used. The disease does not attack the bolls alone, but is also present on the leaves and branches of the cotton plant. While the organism does not do as much actual damage in rotting the bolls as some of the fungi, it is taken up first in this bulletin because the spots it produces often precede the development of the other diseases on the bolls. This bacterium attacks the bolls and forms a spot in which the other organisms develop.
PREVIOUS WORK ON BACTERIUM MALVACEARUM.

The disease caused by *Bacterium malvacearum* was first described by Atkinson from Alabama. He noticed the spots on the leaves and showed that they were full of bacteria, though he did not actually prove that they were responsible for the trouble. In 1891 (5), he described the disease as follows: "It is first manifested by a watery appearance in definite areolate spots which are bounded by the veinlets of the leaf. The spots are sometimes very numerous and frequently conjoined; often the disease follows one or more of the main ribs of the leaf, being bounded on each side by an irregularly zigzag line." In a later publication (7), he gave a fuller description of the disease and called it by the name of the Angular Leaf Spot of Cotton. He noticed that the spots in their earlier stages were filled with bacteria. These were cultured by him and some inoculations tried, but they were without success. Concerning his results, he writes as follows: "The disease usually appears only on the older leaves, those which have passed the prime of their existence. It is quite likely that the bacteria present may easily start the trouble in such leaves, but that they might be unable to enter and disease the younger healthy leaves. This might account for the failure of the inoculations."

For some years after Atkinson studied this trouble, very little more information was published regarding it, not until Smith (63) in 1901 published partial results of some studies he had been making. He isolated the bacteria from the leaf spots and produced the disease by inoculation and gave the organism the name *Bacterium malvacearum*. He also briefly compared this organism with some closely related bacteria that cause other plant diseases. He stated that the disease only attacks rapidly-growing tissue. He also noticed the water-soaked spots which are produced on the cotton bolls by this organism.

During later years, many have noticed the disease and added a few notes in regard to its distribution and severity. From the literature available, the disease seems to be present in Natal (34) and the West Indies (48) besides the United States, and possibly in some other countries, though the published notes are not complete enough to be absolutely sure.
THE SPOTS ON THE LEAVES.

The most noticeable effect of the attack of *Bacterium malvacearum* on the cotton plant is the formation of the spots on the leaves, though these really cause less damage than those on the bolls. The spots are generally seen first on the underside of the leaf. At first they seem to have a slight water-soaked appearance, but otherwise indistinguishable from the healthy leaf. Later the spots extend clear through the leaf and the diseased tissue dries up and dies. It is at this stage that the spots are the most readily seen, as they become brown in color in contrast to the green of the leaf. These spots are generally angular in shape, due to the fact that the bacteria cannot readily cross the veins of the leaf. They are usually not very large, about 1 to 4 mm. in diameter, though sometimes a little larger. No matter what conditions are present, the bacteria seem to die after the spots reach about this size. Sometimes along some of the prominent veins a dead strip will be formed, the outside of this strip being bounded by a zigzag line.

The spots are often very numerous, there often being hundreds on a single leaf. A few spots do not damage a leaf to any extent, but when they are very numerous the affected leaf often withers and falls. This manifestation of the disease is often spoken of as "Rust" in Louisiana, though the name is not the proper one. The disease is not only not a true rust but it is also distinct from the common "Black Rust" or "Red Rust" of the eastern cotton states, the latter trouble being produced by a deficiency of potash in the soil.

THE DISEASE ON THE BRANCHES.

Frequently, in a badly diseased field, the branches of the plants will die for some distance from the tip and turn black, this being followed, of course, by a withering and shedding of the leaves. This trouble, known as the Black Arm, is also supposed to be caused by *Bacterium malvacearum*. Very little work has been done on this phase of the disease in Louisiana. A few cultures made from the inside of these diseased stems have invariably produced a species of Fusarium or else bacteria decidedly different from *Bacterium malvacearum*. This, however,
does not prove that *Bacterium malvacearum* was not the cause of the trouble, as the branches which were used for culturing might have been dead too long before the cultures were made. It is possible that the causative organism had been replaced by saprophytes. As a usual thing, the Black Arm trouble is of little importance in Louisiana.

**THE SPOTS ON THE BOLLS.**

On the bolls, *Bacterium malvacearum* produces spots (Plate II, Figs. 1 and 2), which are in a way quite similar to the spots on the leaves. The affected tissue at first takes on a slightly water-soaked appearance, being in other ways indistinguishable from the healthy portions. These water-soaked appearing spots, generally more or less circular in outline, spread out over the bolls, sometimes covering an area a half centimeter or more in diameter. These often coalesce so that larger spots are formed. As the tissue dies, these spots gradually lose their green color, shrivel up, and become decidedly sunken. Finally they take on a brown or reddish brown color.

While these spots may develop at any place on the boll, they are perhaps the most abundant on the apical portion. They occasionally develop on the lines of dehiscence between the carpels, though they are more frequently found between these lines. Bolls which are in a rapid stage of development are the ones which are most frequently attacked by the disease. Occasionally small spots may be seen on rather old bolls, but these are the exception.

The disease may be confined entirely to the tissues of the carpels or it may extend into the developing lint and seed underneath. There is an internal lining to the carpels which often seems to be impervious to the attack of the bacteria and keeps them from the lint and seed. Other times, however, especially with the aid of small punctures made by sucking insects, the bacteria are able to pass this membrane and attack the soft tissue within.

Just how much damage *Bacterium malvacearum* can do to the lint is difficult to say. Other bacteria and fungi enter the boll with this organism and probably aid materially in the decay. The two other species of bacteria which have been previously
mentioned are almost always present in bolls affected with this
disease, and these, as a general rule, are more abundant than
*Bacterium malvacearum* itself. It is sometimes a difficult matter
to get a pure culture of this organism from inside the bolls on
account of the abundance of the other two. It is probable that
these organisms have as much to do with the decay of the lint
and seed as the original parasite. The lint tissue, when attacked
by these bacteria, disintegrates rapidly if it is still young and
watery (Plate II, Fig. 3). The bacteria, however, are not able
to pass from one lock of the boll to another. If the lint is fairly
well developed and firm, the bacteria do not have a great deal
of affect upon it. Very frequently, there is formed just a small
yellow spot on the surface of the lint just underneath the spot
on the outside of the boll.

The damage actually caused by *Bacterium malvacearum* to
the lint would seem to be quite small. While the spots on the
outside of the bolls are often numerous, many of them, as has
been said, do not penetrate to the lint and many others are on
bolls so old that the lint is not affected. Nevertheless, these
spots are of very great importance, as they allow the entrance of
parasites which do a great deal of damage, such as the anthra-
cnose, Diplodia, and Fusarium. If it were not for these spots,
a considerable percent of the rot caused by these latter organ-
isms would be eliminated. While some of these organisms, as
for example, the anthracnose, can attack the uninjured epidermis,
they do not do so as readily as through wounds. These bacterial
spots form excellent places for the entrance of these fungi. It is
a difficult matter to find an old spot in the field in which some
other organism is not present and doing damage. If it were not
for this, we could place *Bacterium malvacearum* among the boll
rot organisms of minor importance, but, as it is, we must give it
a leading position.

THE CULTURAL CHARACTERS OF THE BACTERIA.

No attempt has been made to work out carefully the cultural
characters of *Bacterium malvacearum* or the other two bacteria
which are associated with it in rotting cotton bolls. The three
species are readily told in all culture media. *Bacterium mal-

vacea produces a raised, smooth, glossy, homogenous growth of a very pale yellow color. One of the associated bacteria produces a growth quite similar to Bacterium malvacearum except it develops a bright yellow color. The other associated form produces a rather slimy white growth. All of these forms grow readily and rapidly on all ordinary culture media. As the cultural characters of all the bacteria-producing plant diseases are being published from time to time in Smith’s Bacteria in Relation to Plant Diseases, it has not seemed necessary to work them out carefully for this bulletin. While a large number of cultures have been made of the different bacteria during the past five years, these have been made mostly to furnish data in regard to their presence or absence in diseased parts of the plant, to provide material for inoculation experiments, or to provide material to work out the resistance of the bacteria to such factors as light or desiccation.

RELATION OF BACTERIUM MALVACEARUM TO LIGHT.

A single experiment to test out the resistance of the bacteria to strong sunlight was tried. On August 10, 1911, the bacteria from a seven-day-old bean agar slope culture was washed off in some sterile water. Loops of this suspension of bacteria were placed on sterilized cover slips and dried. These cover slips were then exposed to strong sunlight in the middle of the day for 1, 5, 10, 15, and 25 minute periods. The cover slips were then dropped into tubes of melted agar, shaken thoroughly, and poured into plates. Plates with bacteria exposed from 1-10 minutes to the light developed an abundance of colonies. One plate with the bacteria exposed for 15 minutes developed nine colonies, while two other plates developed none. One of the plates with bacteria exposed for 25 minutes developed eighteen colonies, another developed eight colonies, and the third did not develop any. It would seem from this that an exposure of strong sunlight for 15 minutes or more is fatal to a large percent of the individuals.
RESISTANCE OF BACTERIUM MALVACEARUM TO DESICCATION.

A few experiments to test the resistance of Bacterium malvacearum to desiccation have been tried. This is an important phase of the problem because of the probability that the bacteria are able to live over from fall to spring in a more or less dry and dormant condition. It is difficult to explain the first spring infections unless the bacteria live over on the seed and infect the young plants when they come up. It is not uncommon at all to see the angular leaf spots on the leaves on very young plants. A very few experiments have been tried to see how long the bacteria would live in a dry condition. In these experiments a suspension of bacteria in sterile water was procured as described above under the relation to light and dried on cover slips and seeds. The cover slips and seeds were kept in sterile petri dishes and some of them were cultured from time to time by dropping them in tubes of melted agar and then pouring into petri dishes. The experiments with the seeds were failures on account of the contamination in the plates. There are so many forms of bacterial and fungous life clinging to the short fibers on the seed that they completely ruined every plate made. Unfortunately there was no opportunity to repeat the experiments with the seed. With the cover slips there was but little trouble with contamination, but we could hardly expect the bacteria to remain alive as long on these as they would on the seed. That germs will live longer on seeds than on cover slips has been demonstrated by Harding, Stewart, and Prucha (38) with the cabbage black rot organism and also by the writer with spores of the cotton anthracnose, as will be discussed later in this bulletin. Two sets of cover slips were made with Bacterium malvacearum. The first set was started in August, 1911. In this experiment the bacteria only lived for a surprisingly short period. A few of the cover slips gave a few colonies at the end of six days, but no colonies were obtained after a longer period. In the second experiment, which was started in August, 1912, the bacteria lived longer than in the first experiment. A few colonies were obtained after thirty-eight days of drying.
INOCULATION EXPERIMENTS WITH  
*BACTERIUM MALVACEARUM.*

Previous to this date, successful inoculations with *Bacterium malvacearum* upon the cotton plant have only been published by Smith (63). He states that the bacteria are able to enter the uninjured leaf through the stomata.

During the past few years a large number of inoculations have been made during the course of this work in Louisiana. The inoculations on leaves and bolls have been made in the greenhouse away from all danger of outside infection. All of the experiments during the first two or three years of the work were failures due to the fact that the conditions favorable and necessary for successful infection were not known. All of the early inoculations were carried on in the winter time and the temperature of the house was too low. Cotton is a hot weather plant and it is evident that the bacteria have adapted themselves to this condition. All of the later inoculations, made in the summer time when the greenhouse was very warm, resulted in good infection. The bacteria used for inoculating purposes were washed off from fresh agar cultures in sterile water. This suspension of bacteria was sprayed on the plants with a small atomizer and the plants covered with bell jars lined with wet paper. The bell jars were kept over the plants for a couple of days and then removed. Other plants sprayed with water alone were used for checks. The dates of these inoculations, the parts of the leaf that were inoculated, and the number of days before spots appeared on the inoculated parts are given in Table 2 below.

**Table 2.**  
**ARTIFICIAL INOCULATIONS WITH BACTERIUM MALVACEARUM.**

<table>
<thead>
<tr>
<th>DATE</th>
<th>No. of Plants</th>
<th>Part Inoculated</th>
<th>Days for Spots to Appear</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 17</td>
<td>8</td>
<td>Whole leaf</td>
<td>16 days</td>
</tr>
<tr>
<td>June 27</td>
<td>10</td>
<td>Whole leaf</td>
<td>9 days</td>
</tr>
<tr>
<td>July 1</td>
<td>2</td>
<td>Whole leaf</td>
<td>10 days</td>
</tr>
<tr>
<td>July 1</td>
<td>4</td>
<td>Upper surface</td>
<td>11 days</td>
</tr>
<tr>
<td>July 1</td>
<td>6</td>
<td>Lower surface</td>
<td>10 days</td>
</tr>
</tbody>
</table>
There was considerable variation in the period of incubation, varying from nine to sixteen days, this variation probably being due to the moisture and temperature conditions. None of the checks developed any of the bacterial spots.

A number of the plants that were used for inoculation had small bolls on them, and in many cases bacterial spots developed on these a few days after they appeared on the leaves.

No inoculations were tried on leaves in the field, it seeming useless to undertake this on account of the abundance of leaves naturally affected with the disease.

FLOWER INOCULATIONS.

Bolls are very readily infected in the field with *Bacterium malvacearum* by placing a suspension of the bacteria into the flower late in the afternoon. The flower shortly afterward closes and dies. The bacteria being kept in a rather moist condition develop on the disintegrating flower parts and come into contact with the apical end of the boll. They penetrate the boll and develop the watery spots which are characteristic of the disease.

A large number of flowers were inoculated in this manner in the summer of 1911. The results have been reported in a previous paper (31), but may be inserted here. The bacterial suspension was dropped into the flowers with a small pipette, perhaps a half a cubic centimeter being dropped in each flower. Checks were also used, water being dropped in these instead of the bacteria. The dates and the results of these inoculations are summarized below in Table 3.

### Table 3.

**FLOWER INFECTION WITH BACTERIUM MALVACEARUM.**

<table>
<thead>
<tr>
<th>DATE OF INOCULATION</th>
<th>June 27</th>
<th>June 29</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of inoculations</td>
<td>36</td>
<td>38</td>
<td>74</td>
</tr>
<tr>
<td>Inoculations with spots in 12-14 days</td>
<td>43%</td>
<td>47%</td>
<td>45%</td>
</tr>
<tr>
<td>Inoculations with spots in 20-22 days</td>
<td>51%</td>
<td>47%</td>
<td>49%</td>
</tr>
<tr>
<td>Inoculations with spots in 29-31 days</td>
<td>68%</td>
<td>73%</td>
<td>71%</td>
</tr>
<tr>
<td>Number of checks</td>
<td>30</td>
<td>37</td>
<td>67</td>
</tr>
<tr>
<td>Checks with spots in 12-14 days</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Checks with spots in 20-22 days</td>
<td>3%</td>
<td>7%</td>
<td>6%</td>
</tr>
<tr>
<td>Checks with spots in 29-31 days</td>
<td>34%</td>
<td>21%</td>
<td>27%</td>
</tr>
</tbody>
</table>
An examination of the table shows that, during the first two weeks, 45% of the bolls developing from inoculated flowers became infected with the disease, while the check bolls remained healthy. After the first two weeks there was an outbreak of the disease throughout the field and the natural infection increased the percent of the disease on both the inoculated and the check bolls in about the same proportion. It is evident that the bolls which became infected from the bacteria in the flowers showed the disease in two weeks' time; otherwise the increase of the disease on the inoculated bolls would have been greater than the checks. The bolls which develop the bacterial disease from the flowers have the spots mostly at the apical ends (Plate II, Fig. 2). Some times as many as fifteen to twenty spots develop near the apex of a boll following flower inoculation.

It seems probable that much of the natural infection with this disease takes place through the flowers. A considerable percent of the naturally infected bolls have the spots at the apical end. The insects which visit the flowers probably carry the bacteria to the flowers.

**INOCULATION OF DIFFERENT BACTERIA BY PUNCTURE.**

Cotton bolls have also been inoculated with the three different bacteria by means of needle pricks. A fine-pointed needle was touched to a pure culture and then gently forced into the boll. Many inoculations with *Bacterium malvacearum* have been made by this method both in the field and in the greenhouse during the course of this investigation. While the number of inoculations with the other two forms have been fewer, there have been enough to show their relation to the boll rot problem.

Inoculations with *Bacterium malvacearum* have in nearly all cases produced the typical water-soaked spots around the points of inoculation (Plate II, Fig. 1), while in no case has either of the other forms produced this appearance. However, the affect on the interior of the punctured bolls has not appeared to be very different when the different organisms have been used.

It is not an easy matter to make puncture inoculations with these bacteria either in the field or the greenhouse and keep
the other forms out. The white and bright yellow bacteria are very common on all parts of the cotton plant, as has been shown by making cultures from the leaves, stems and bolls, and they very often enter the punctured bolls. However, some of the inoculations have remained free from contamination. An experiment started in July, 1911, may be used as an illustration both to show the amount of rot caused by the different bacteria and also the chances of contamination. The bolls were inoculated in the field and examinations made after periods of two and three weeks. The appearance of the inside of the bolls was as follows:

**Checks.**—Contents of punctured locks, variously discolored and some dried up; locks not punctured, healthy in appearance. Direct transfers from nine of these bolls were made to agar slopes with the following results: five gave pure cultures of the white bacterium, two gave a mixture of the white and bright yellow bacteria, one gave a fungus culture, and the other remained sterile.

**Inoculations with the white bacterium.**—The inside of the punctured bolls had about the same appearance as the checks. Direct transfers from five of these bolls gave cultures as follows: two with the white bacterium alone, and three with a mixture of the white and bright yellow bacteria.

**Inoculations with the bright yellow bacterium.**—The inside of the punctured locks had about the same appearance as the checks. Direct transfers from five of these inoculated bolls gave pure cultures of the bright yellow bacterium in each case.

**Inoculations with Bacterium malvacearum.**—The inside of the punctured locks was perhaps very slightly more discolored than the checks; however, the difference in appearance was so small that it is hardly worth considering. Transfers from five of these bolls gave three pure cultures of *Bacterium malvacearum*, one fungus culture, and the other remained sterile.

While some of the bolls became contaminated with other forms, yet enough of them remained uncontaminated to show that there was but very little difference in the rotting ability of the three organisms. All of them seem to be able to rot and discolor the tissue in the punctured locks, while none of them are able to pass from one lock to another without an injury. It may
be well to mention also that bolls that are well advanced do not have the interior rotted by any of these bacteria. After the lint fibers have begun to harden the bacteria will sometimes stain them slightly yellowish at the point of infection, but there is no rot.

The greater damage done by *Bacterium malvacearum* as compared to the other two forms is due to its ability to attack the unjured epidermis of the boll and gain entrance to the interior. The other forms are unable to do this, being able to gain entrance only through an injury. The great importance of all of these forms, however, is not the rot which they, themselves, cause, but it is the formation of decayed tissue, which allows the entrance of boll-rotting fungi, which destroy the whole boll. Thus, in a way, these bacteria are responsible for a considerable percent of the rot.

**RELATION OF INSECTS TO THE BACTERIAL ROTS.**

There are a number of insects that puncture cotton bolls and aid the bacteria in gaining entrance to the boll contents. No attempt has been made to identify these insects, but they seem to be included in a recent bulletin of the Bureau of Entomology (54). Many of these insects make punctures so small that it is difficult to see them on the surface of the boll and not much easier to see when longitudinal cuts are made through them. These punctures, however, form excellent points of entrance for the different species of bacteria. A considerable percent of the water-soaked spots produced by *Bacterium malvacearum*, especially on older bolls, have these small punctures at the center.

These punctures on the bolls are often very numerous. Some of them only extend into the external cells of the carpels, while others pass entirely through the internal lining of the boll (Plate I, Figs. 1 and 6). The puncture is often followed by a development of intumescence tissue (Plate I, Figs. 3, 4, and 5). This development of intumescence tissue is usually spoken of by entomologists as "proliferation" (17, 43). The intumescences are not always developed, however, and it is questionable whether the invading bacteria are not more the cause of their development than the insect punctures themselves.
THE COTTON ANTHRACNOSE.

*Glomerella gossypii* Edgerton.

The cotton anthracnose is another disease which attacks various parts of the cotton plant. It develops spots on the cotyledons, causes a damping off of the young seedlings, grows on dead parts of the stems and leaves, and produces spots on the bolls, and, also, a generalized rot. This disease is the most serious of all of the boll rots.

**PREVIOUS WORK ON THE DISEASE.**

The cotton anthracnose has been studied more than any other boll rot. The disease seems to have been first recognized in 1888, when collections of it were made by the United States Department of Agriculture at Opelousas, Louisiana. There is some of the Opelousas material still preserved in the Bureau of Plant Industry collections at Washington, D. C. Soon after the disease was discovered, studies were made of it by Miss Southworth, of the United States Department of Agriculture, and Atkinson, of the Alabama Experiment Station. The disease was first described by Miss Southworth (66) in 1890, and, as the fungus which caused the disease was new, she gave it the name of *Colletotrichum gossypii*. Very shortly after Miss Southworth's article, Atkinson (6) also published a report on the disease.

For a number of years following these publications, there was but little more work done on this disease. Many papers and bulletins contained notes on it, but they were mostly extracts from the earlier papers. In the past few years, however, a considerable amount of work has been done at the different experiment stations in the south, and our knowledge of the disease is much more complete. Several of the stations have published notes regarding the varietal resistance of the disease. Barre (11) of South Carolina and Lewis (45) of Georgia have shown that the fungus can penetrate the epidermis of the boll without an injury. Barre (11) and Edgerton (31) have shown that the disease may enter the bolls through the flowers. The ascogenous stage of the fungus has been found by Shear and Wood (61) of the United States Department of Agriculture in pure cultures, and by Edgerton (27) of the Louisiana Experiment Station on
the cotton bolls themselves. Several workers, also, have shown that the disease is carried from year to year on and in the seed. A more extended discussion of the work of these different people will be taken up later as the different phases of the disease are discussed.

DISTRIBUTION OF THE DISEASE.

The disease seems to be quite widespread in the different cotton-growing countries. Outside of the United States, it has been reported in Trinidad (3), British Guiana (16), West Indies (47, 48, 49, 50, 65), and possibly in India and Natal. In the United States the disease is present in all of the southern cotton-growing states, but it is doubtful if it is important, or even present, in the sections of the arid southwest, New Mexico, Arizona, and California, where in recent years some cotton has been grown under irrigation.

LOSS CAUSED BY THE DISEASE.

The cotton anthracnose undoubtedly causes a much greater loss to the planters in the cotton belt than any other boll rot. It is difficult to estimate with any accuracy the actual money loss to the state or to the south caused by this disease on account of the variation in severity of the trouble in different years and in different parts of the country. A number of people have, however, attempted to estimate the percent of the crop damaged, and it may not be out of place to give some of the estimates at this time.

Atkinson (7) says that the disease destroyed 10-50% of the crop in places in Alabama, while in other places the damage was very slight.

Barre (11), in an early report, says the loss varied from 40-70% in many places in South Carolina, while in other places the loss was not large. He says that a 1% loss for the whole state would be a very conservative estimate. Even this would mean a loss of $700,000 to the state. In a later report (14), he estimates the loss at 10-60% of the crop, or a money loss of over $1,000,000.
De Loach (20) says that in Georgia a loss of 17% of the crop would be a conservative estimate. This would mean a loss to the state of Georgia of $14,756,000.

Harned (39) says that in some fields in Mississippi the disease caused a loss as large as 25-50% of the crop. He estimated that this disease caused a greater loss to the state in 1909 than the boll weevil.

Hibbard (42), writing also about the disease in Mississippi, says that the loss in some fields was very small, while in others it ran as high as 80-90%.

The above illustrations are sufficient to show that the disease causes an immense loss all over the cotton belt. While there is considerable variation in the estimates, this is to be expected, considering the manner in which the estimates were made.

In Louisiana, estimates have been made at different times during the past five years. It would seem that the actual loss caused by this disease in this state would be from 3-6% of the crop. While the loss is much greater in the river districts, it is generally less in the hilly areas of the northern part of the state. In wet years the loss may run considerably higher than this, but these are offset by the drier years with a smaller loss. While the estimate given here is lower than some of the others from other states, it does not mean that the disease does less damage in Louisiana than in the other states. It is probable that some of the other estimates are too high. If anything, the disease should be greater in Louisiana with the more abundant rainfall.

DESCRIPTION OF THE DISEASE.

THE DISEASE ON THE BOLL.

The appearance of the diseased boll depends more or less upon the manner in which the causative fungus gains entrance to the tissues of the boll. As there are, in general, three ways in which the fungus may enter the boll, there is, of course, considerable dissimilarity in the appearance of the bolls. If the fungus enters through the side of the boll, either without an injury or by the aid of a small wound, such as a small insect puncture, we have the form of the disease which is generally described in articles on the cotton anthracnose (Plate III, Figs.
Spots are developed which are sunken and brownish in color with usually a red border. These spots are at first small, but may reach a considerable size sometimes covering half the boll if conditions for growth are favorable. Usually the center of the spots are covered with a slimy pink mass of spores.

If the fungus gains entrance through the dead pistil of the flower there is either a direct rot proceeding downward from the tip, or else the fungus enters the inside of the boll with only a very small amount of tissue destroyed at the tip (Plate III, Figs. 1, 2, 4, and 5). In either case, there is a splitting of the carpels at the tip and a rot of the contents of the boll.

If the fungus enters the boll in a lock that has been badly rotted by some other organism, as, for instance, *Bacterium malvacearum*, merely a general rot of the whole boll follows.

After the fungus gains entrance to the boll tissue, it travels very rapidly. The affected tissue of the carpel becomes at first brown or even black in color. It later dies, dries out, generally takes on a lighter color and becomes brittle. The inner lining of the carpel becomes yellow and later, frequently, brown. The lint and seed are, also, frequently affected, but the appearance of these depends to a considerable extent on the size and development of the boll when the infection starts. If a boll is attacked when the contents are still soft and watery, the lint and seed will turn brown to black in color and later dry down to a brittle mass. If the boll is older with the lint fibers partially developed, the fungus does not develop as well. It will frequently grow among the lint threads, turning them slightly blackish in color and cementing them together so that when the boll opens the lint will remain in rather a firm mass. These hard masses are frequently picked and ginned, but the fiber is weak and much of it is lost in ginning. If the boll is nearly mature when it is attacked, or if the lint is infected after the boll opens, very little real damage is done unless the weather is very wet, when the growth of the fungus on the lint will slightly discolor it.

The fungus also attacks the seed in diseased bolls. As has been said, if the boll is attacked when it is quite young, the whole inside, including the young seed, is changed into a black, brittle mass. If the boll is not attacked until the lint is fairly well
developed, the seed are not rotted, but the fungus often grows through the seed coats and grows in among the folds of the cotyledons (Plate V, Fig. 1). If a hull is removed from a seed in this condition, small brown spots can occasionally be seen on the kernel, but otherwise it appears to be in a perfectly normal condition as far as can be seen with the naked eye. However, a microscope will show the presence of the mycelial threads within the kernel.

THE DISEASE ON THE OLD STEM AND LEAVES.

The fungus is frequently found growing in injuries and dead spots on both the stems and leaves of the cotton plant. Frequently the bacterial spots on the leaves become infected with the anthracnose. However, the fungus does not seem to be able to spread to any extent on the living parts of these organs. These spots are of considerable importance, as the fungus remains alive in them during the spring and early summer, forming sources of infection when the bolls begin to develop on the plants.

THE DISEASE ON THE COTYLEDONS AND YOUNG STEMS.

On the cotyledons and young stems of seedlings the cotton anthracnose fungus is often present. On the cotyledons the fungus either develops in small reddish or light-colored spots, or else on the margin of the cotyledon (Plate IV, Figs. 2 and 3). A large percent of the young plants in a field will often show dead margins on the cotyledons or else dead spots. Dead spots or lesions develop on the young stems in wet weather. The fungus rots the stem and causes it to "damp off" (Plate I, Fig. 4). This manifestation of the disease however, is not common, most of the dying of young plants in the field being due to another disease, the so-called "sore shin." The spots on the cotyledons and young stems caused by the anthracnose are frequently colored somewhat pinkish by the abundant development of the spores.

DESCRIPTION OF THE FUNGUS.

The fungus causing the anthracnose disease of cotton is very similar to other members of the genus Glomerella in mycelial, conidial, and ascogenous characters. All the different stages of
the fungus are fairly well known at present and have been described at different times. The stages in the life history of the fungus include the mycelium, the secondary spores or appressoria, the conidial pustule or acervulus, and the perithecia or ascogenous stage.

**THE MYCELIUM.**

There is nothing typical or very characteristic regarding the mycelium of the cotton anthracnose fungus. The threads which permeate the affected tissue of the cotton plant are at first hyaline, much septate, and with no marked variation in thickness, but later they often become dark brown in color, especially those which develop around the fruiting pustules.

**THE SECONDARY SPORES OR "APRESSORIA."**

Secondary spores or appressoria are often developed by this fungus. These are small, dark brown, one-celled bodies which develop generally at the end of mycelial threads. They are most frequently developed on the germ tubes of spores which have germinated in water or culture media lacking in some of the required nutrients. These develop abundantly on cotton bolls when the spores are placed on the surface in drops of water (Plate VI, Fig. 2). These appressoria are no different in appearance from those developed by other anthracnoses. These appressoria are resistant to drying and also are able to attach themselves to the surface of the cotton bolls. They do not wash off readily by the rain and are able to germinate and infect the boll at any time when moisture conditions are favorable.

**SPORE DEVELOPMENT.**

The spores of the cotton anthracnose may develop directly on the tips of mycelial threads, or they may develop in special fruiting pustules called acervuli. If the weather is moist, it is not uncommon to see spores developing on the mycelium, especially on bolls that have broken open. They develop very often in this manner on the lint, after the boll opens, but sometimes when the boll is still closed. They sometimes even develop on the surface of bolls in this manner when the bolls remain moist.

The development of the spores in an acervulus, however, is
the most common method. The acervulus (Plate V, Figs. 2 and 3) consists of a more or less well-developed stratum or stroma of mycelium, from which arise short conidiophores. The acervulus generally begins its development underneath the epidermis and by rapid growth ruptures the cells above so that it is exposed to the surface. Occasionally, however, if the weather is very moist, it will begin its development on the surface, thus being exposed from the beginning. The acervuli vary considerably in size and also in the height they rise above the surface. If the atmosphere is moist the acervulus may extend above the surface for a millimeter or more (Plate VI, Fig. 1). If the atmosphere is dry it will extend but very slightly above the surface. Upon the stratum of fungus threads in the acervulus, a layer of very crowded conidiophores develop. These are very short and hyaline and develop the spores at the tips. Also scattered among the conidiophores are usually many long, dark-colored setae with hyaline tips (Plate XII, Figs. 4, 5, 6, and 7). Spores are also commonly developed on the tips of these setae.

The spores (Plate XII, Fig. 1) which develop singly at the tips of the conidiophores are hyaline, one-celled, cylindrical, and generally straight, though sometimes they may be slightly curved. They are rounded at the ends and are usually slightly less in width near the center than toward the ends. When first developed a spore contains a clear, large nucleus near the center, but this is frequently not visible in spores that are older. The spores measure about 3.5-7 x 12-25 microns. They are formed very rapidly, and as there is a mucilaginous substance produced with them, there is usually a pink, slimy mass of spores on the acervulus. The spores germinate readily by sending out germ tubes slightly smaller in diameter than the spores themselves (Plate XII, Fig. 2). The germ tubes may start from any place on the spore, though the first one is usually from near the end. The germ tubes grow very rapidly if the proper nutrients for growth are present.

THE ASCOGENOUS STAGE.

The ascogenous stage of this fungus is known and has been found by the writer on cotton bolls (27). The perithecia and asci are in most ways quite similar to those of other species of
this genus. The perithecia (Plate VI, Figs. 3, 4, 5, and 6) develop very rarely on cotton bolls, but when they do develop they are very abundant. They are dark brown to black in color, subglobose to pyriform in shape, and from 80-120 x 100-160 microns in size. They are usually buried in the host tissue with only the beaks protruding, but occasionally they are found on the surface or but very slightly imbedded. The length of the beak of the perithecium varies from nearly nothing up to 60 microns. The asci (Plate XII, Fig. 9) are very numerous in the perithecia, clavate in shape and from 55-70 x 10-14 microns in size. The ascospores are uniseriate or irregularly biseriate. They are nearly elliptical, though sometimes slightly curved, granular, hyaline and show a clear nucleus at the center similar to the conidia, and are from 12-20 x 5-8 microns in size. Long slender threads, or paraphyses, are also very abundant in the perithecium, these filling the entire perithecial cavity above the asci.

THE COURSE OF THE DISEASE.

As the cotton anthracnose is on all parts of the cotton plant above ground, it matters little what part of the plant we use to start the course of the disease, and it may be well to begin with the very young seedling plant. As has been said, the disease occurs in spots on the cotyledons and very young stems. Acervuli develop in these spots and spores are formed very abundantly. The spores are held together by a mucilaginous substance and will not germinate until they are freed from this. As the plant grows, the spores which were developed on the cotyledons and young stems are carried to the newly formed parts of the plant, the stems and leaves, by insects, by rain, and by the wind. The rain washes the spores out of the acervuli and the wind aids by whipping the upper parts of the plant and bringing them in contact with the ground or the lower part of the stem, where the spores have found lodgment. If a spore happens to come in contact with an injury in a branch or leaf or a spot caused by some other fungus, it quickly germinates, and starts to develop on the dead tissue. The spores may be able occasionally to enter the uninjured tissue of the stem or leaf, but this is, at most, not common. Spores are again formed
on these spots and these may be carried to other parts of the cotton plant. This is the most critical stage in the life of the cotton anthracnose on the cotton plant because of the inability of the fungus to develop well on the healthy leaf or stem. However, the fungus generally is able to develop enough in the dead spots to keep itself alive until the bolls form on the plant. After the bolls begin to form, especially if the weather conditions are suitable, the disease spreads very rapidly. The spores are carried to the young flowers and to the young bolls. They come in contact with insect punctures in the boll, or bacterial spots and very soon develop large anthracnose spots. They are also able, though less rapidly, to attack the uninjured epidermis, especially of young, tender bolls. They may, too, be carried into the open flower by insects, and after germination grow down through the dead pistil into the inside of the boll. After the fungus has gained a foothold in the boll, it develops rapidly, sending the mycelium through the carpel tissue, the lint and seeds. A very few days after the spot shows on the boll, the spores begin to develop, and soon the whole surface is covered with a slimy pink mass of them. The number of spores which may be produced on a single boll in a season is enormous. An estimate was made of the number of spores on a small boll about one and one-half centimeters in diameter. The spores were all washed off in a definite quantity of water, and then the spores in a very small portion of this were counted under the microscope. They were found to be about 40,000,000 spores on the boll. However, as these spores are constantly being produced and washed off, many more than this are really developed on a single boll during the season, possibly as many as 100,000,000 to 500,000,000. A large percent of these spores are washed to the ground and lost, yet enough of them find their way to other bolls and other parts of the plants to increase the infection.

While the fungus is producing the spores on the outside of the boll, it is also sending its mycelium through the contents of the boll and into the seed. If the seed are fairly well advanced toward maturity when the fungus enters them, they are not killed. The fungus ceases its growth when the seed ripens, and dries up and goes into a more or less dormant condition with the seed. This mycelium readily stays alive in the seed until
the following spring when the seed is planted. When the seed
takes up water and begins growth, the fungus also becomes active.
As the cotyledons break out of the seed coats, the disease appears
on them in the form of spots and dead strips along the margin.
These spots rapidly produce spores, which again infect the
various parts of the cotton plant.

The fungus is also carried over the winter to some extent
by means of the spores that find lodgment in the short lint which
remains on the seed after ginning. It is probable also that some
spring infection may be produced by spores that have developed
on old plants of the previous year that have been left in the
field. Most of the spring infection, however, comes from the
seed that have the fungus mycelium alive in them, or the spores
on the surface.

NUMBER OF SPORES ON THE SEED.

The presence or absence of spores on cotton seed is an impor-
tant factor for consideration by the farmer and planter. If
spores can be found abundantly on the outside of the seed, there
is no doubt that many of the seed are affected internally. How-
ever, if no spores are found it is pretty certain that the seed
came from an anthracnose-free field, and that they are perfectly
healthy. The presence or absence of the spores on the outside of
the hulls is quickly told and is a very reliable test for healthy
seed.

Some estimates were made of the number of spores on different
samples of seeds. Three hundred seeds from a sample were
counted out and placed in a flask with 125 cc. of water. The
flask was thoroughly shaken for several minutes to wash the spores
out of the lint. 10 cc. of this solution was then centrifuged
for a few minutes. When the spores were thrown down to the
bottom of the tube, 8 cc. of the clear water above was poured off.
The 2 cc. that remained was again shaken thoroughly. Drops
of this solution of known size were then placed on a slide. By
counting the number of spores in a number of fields of the
microscope, and knowing the size of the field and also of the
cover slip that covered the drop, an estimate of the number of
spores in the whole drop was easily made. From this the number
of spores in the whole 125 cc. of solution was readily obtained.
The number of spores was estimated in three different samples of seed. The number of spores given is the estimated number on 300 seed. The weight of these seed in grams is also given.

**TABLE 4.**

**NUMBER OF SPORES ON SAMPLES OF COTTON SEED.**

<table>
<thead>
<tr>
<th>Source of Seed</th>
<th>Wt. of Seed (grams)</th>
<th>Number of Spores</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. From a Baton Rouge gin, Sept., 1911.</td>
<td>34.7</td>
<td>2,400,000</td>
</tr>
<tr>
<td>2. Toole's cotton from Exp. Sta. plots, only healthy appearing cotton used. Picked Sept., 1911.</td>
<td>35</td>
<td>2,900,000</td>
</tr>
<tr>
<td>3. Toole's cotton, picked in Sept., 1910. Only bolls showing evident signs of the anthracnose were picked.</td>
<td>20.4</td>
<td>7,800,000</td>
</tr>
</tbody>
</table>

The table shows that the number of spores on the seed was very high. Even if only a very small percent of the spores on the outside were able to live until spring, there would still be sufficient to cause a high percent of infection.

Germination tests were made of the first two samples of the above seed in order to find out what percent would really show the anthracnose on the young seedlings and also to find out what proportion would become diseased from an internal infection. The experiment was conducted as follows: 200 test tubes were procured and in the bottom of each was placed some wet absorbent cotton and filter paper. The tubes were then plugged and sterilized. Two hundred cotton seed were then procured and divided into two lots. One lot of a hundred were used without treatment, while the rest were soaked for 25 minutes in a formalin solution made up by mixing 1 cc. of a 40% formalin solution with 99 cc. of water. The seed were then dropped into the test tubes, a single seed to a tube, and covered with sterilized filter paper. The seed were then allowed to germinate, and after the seedlings had reached a height of one to two inches were taken out and examined under the microscope. The number of seed that germinated and the number of seedlings showing the presence of fresh anthracnose spores are given in the following table.
The object of the formalin treatment was to kill the spores that were on the surface of the seed. While it is possible that this strength of formalin would not kill all of the spores, yet it is probable that it would kill the greater percent of them. It was not thought advisable to give a longer treatment for fear of injuring the germinative power of the seed. Even in this length of time, the treated ones show a slightly less percent of germination.

An examination of the table shows several things that are worthy of note—

1. The untreated seeds produced young plants with nearly 100% infection.
2. The young plants growing from the treated seeds show a smaller percent of infection. It is probable that most of the spores on the outside of the seeds were killed, and that those plants which developed the disease came from seeds that were affected internally.
3. A large percent of the seed that did not germinate showed the presence of the anthracnose on the seed coats after lying on the moist cotton. It is possible that the poor germination of these seed was due to a large extent to the action of the anthracnose, either by attacking the seed too early in the boll, or by killing the embryo before it had time to break out of the hulls.
4. The table shows clearly that some of the spores that are on the outside of the seed are still able to germinate and cause infection at planting time.

---

### Table 5.

<table>
<thead>
<tr>
<th>Lot</th>
<th>Treatment</th>
<th>Number germinated</th>
<th>Number not germinated with anthracnose</th>
<th>Number germinated with anthracnose</th>
<th>Number not germinated with anthracnose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1</td>
<td>No treatment</td>
<td>68</td>
<td>65</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>Lot 2</td>
<td>Formalin</td>
<td>61</td>
<td>29</td>
<td>39</td>
<td>17</td>
</tr>
<tr>
<td>Lot 3</td>
<td>No treatment</td>
<td>54</td>
<td>53</td>
<td>46</td>
<td>31</td>
</tr>
<tr>
<td>Lot 4</td>
<td>Formalin</td>
<td>50</td>
<td>19</td>
<td>50</td>
<td>26</td>
</tr>
</tbody>
</table>

*These numbers refer to those used in Table 4.*
Another experiment was tried in the field in the spring of 1912 to see if a further infection of the seed would increase the percent of anthracnose on the young plants. Two rows of Simpkins seed were planted side by side. The seed was known to be badly affected with the anthracnose, but it is questionable whether it was any worse than a large percent of the seed that is used for planting in this region. One row was planted with seed that was not treated in any way, while the other row was planted with seed that was soaked for a few minutes in a heavy suspension of fresh anthracnose spores in water. A few days after the plants were up twenty-five from each row were examined with the microscope. Nineteen out of the twenty-five plants from each row showed the presence of anthracnose spores on the young cotyledons. From this it is evident that there were sufficient anthracnose spores on the seed to begin with and that the addition of more did not increase the infection.

It seems probable, however, that a large part of the infection on the young plants in the field comes from seed that is infected internally. In the experiment described above in which the seed were germinated in test tubes, a high percent of infection developed from spores on the outside of the seed, yet under field conditions we could not expect such results. In the test tubes, the anthracnose had but little competition, while under field conditions there are many other organisms present that must be detrimental to the growth of the fungus. This has been shown to be the case with the bean anthracnose (28), and it is probable that the same principle applies to the cotton anthracnose. Some infection must come from the spores on the seed nevertheless. In the last experiment described above in which the seed was planted in the field it is certain that 19 out of 25 of the seed were not affected internally, and some of this infection must have come from spores on the surface of the seed.

**MICROSCOPIC APPEARANCE OF DISEASED SEED.**

The appearance of seed which are affected with the anthracnose differs considerably, depending mostly upon the age of the seed when first attacked. If the fungus enters the seed coats while the interior is soft and watery there is a complete disintegration of the inside parts. While the outside of the seed
may look in fairly good shape when it is cut open, all that is seen is a mass of fungus threads. Seeds in this condition break up very readily, being easily crushed between the fingers. Most of the seed in this condition are left in the field, as the lint surrounding them is usually worthless; and what are picked are probably mostly broken in ginning.

If the fungus does not enter the seed until the little embryo is fairly well developed and hard there is not this complete disintegration of the tissues. The fungus readily passes through the seed coats into the interior, but the growth on the inside is less rapid than in the young seed and also less rapid than on the outside of the boll tissue itself. If the hull is removed from these seed, often a small darker colored spot is seen on the outside of the kernel, but the difference in color or appearance of this spot is not very marked. If these seed are cut on the microtome and stained, the presence of the fungus is easily ascertained. The fungus forms a network of mycelium between the folds of the cotyledons and is also seen within the tissues of the cotyledons to a slight extent. There is not a very marked disintegration of this tissue, however. The photomicrograph (Plate V, Fig. 1) shows very well the appearance of the mycelium in the diseased seed.

Frequently, also, acervuli develop on the outside of these diseased seed (Plate V, Figure 2). Spores also develop to some extent within the seed, though they are not very common unless the seed is affected while very young. Spores are very rarely seen within such seed as shown in Figure 1 of Plate V, mycelium alone seeming to be present.

The cotton anthracnose and the bean anthracnose are quite similar in the method in which they attack the seed and live over during the winter. There is, however, this difference, which is important: on the bean seed there is always developed a dark-colored spot, which enables anyone to readily pick out the diseased seed; on the cotton seed, however, no such spots develop, and it is absolutely impossible to say from an external examination what seed are infected. Frequently some of the diseased seed are light, but this is not always the case: The bean anthracnose also causes a much greater disintegration of the affected tissue of the seed than the cotton anthracnose.
However, both the fungi are able to live over the winter in a dormant condition in the seeds and begin development again as the seeds germinate.

It is difficult to say who first recognized the presence of the anthracnose within the seed. Atkinson (9) without doubt first recognized the possibility of this. He wrote as follows: "It is quite possible that the mycelium may rest in the tissues of the seed, as in the case of the bean anthracnose, Colletotrichum lindemuthianum." In the years that followed Atkinson's work we find but very little written regarding the disease, though it is very probable that the presence of the disease in the seed was recognized. Shear and Wood (61) in 1907 wrote as follows regarding it: "In the anthracnose of cotton and bean, the disease is known to be transmitted by diseased seed." In recent years, Barre (11) and DeLoach (20) have published bulletins in which the presence of the fungus in the seed is discussed.

MICROSCOPIC APPEARANCE OF DISEASED LINT.

Lint that has been affected by the disease is slightly yellowish in color and generally is very weak. Considerable of it is left in the field, it not being considered of any value; and much of that which is picked is so weak that it is broken during ginning. The presence of this discolored and weak cotton decreases the grade and, consequently, the price.

If these diseased fibers are examined with the microscope they are found to be thin walled, in contrast to the thicker walled fibers in the high-grade cotton. If these fibers are examined from bolls that are not as yet open the cause of these thin walls is seen. The mycelial threads of the fungus enter the fiber strand, especially near the end attached to the seed, and grows for a considerable distance through the fiber cavity. The protoplasm in the fiber is killed and is used for food by the fungus threads. The cellulose wall of the cotton fiber is built up gradually by the protoplasm in the interior. When the fiber first starts to develop the wall is very thin, but gradually thickens and becomes stronger as the boll matures. If the mycelium is killed by the fungus, the formation of cellulose and the thickening of this wall is discontinued. As the lint dries out the mycelial
threads within the lint fibers die and it is often a difficult matter to make them out with a microscope, but they can be seen very easily before the lint is matured.

THE FUNGUS IN PURE CULTURE.

The fungus, *Glomerella gossypii*, is very readily isolated and grown on culture media. It grows very rapidly on all of the ordinary bacteriological culture media. It is easily isolated, either by making dilution cultures in petri dishes with the spores, or by transferring fragments from the inside of the diseased bolls or seeds to tubes of sterile media.

The growth of the fungus in culture media is similar in many ways to that of other anthracnoses, such as the fungus causing the bitter rot disease of apples, yet decidedly dissimilar from some other anthracnose forms, such as the bean anthracnose. On most of the ordinary media, such as bean and potato agars, a floccose growth with considerable white aerial mycelium develops. The colony is at first white, but gradually darkens, especially if there is any sugar present. On special media, such as corn meal agar and oat juice agar, the growth is faster, and the aerial mycelium is so abundant that it forms a dense covering over the medium. On the media, there is a darkening of the growth in color after a few days. Spores develop abundantly in pink masses over the colony, and they also develop profusely on the mycelial threads, generally more profusely than with other anthracnose forms. Often this spore development on the mycelium is so abundant as to give the whole colony a salmon color. On Elfving's nutrient solution, a synthetic medium, as reported in a previous paper, the fungus cells enlarge and break apart into short threads, each cell of which is again able to grow into a new colony.

In most ways the growth of the cotton anthracnose in culture media is similar to *Glomerella fructigenum*, the form causing the bitter rot disease of apples, though the two are dissimilar enough to be generally told apart. However, all the anthracnoses vary so much in culture media that their cultural characters are of but little value. Unless the cultural characters are radic-
ally different, as is the case with such forms as the bean and cotton anthracnoses, their value as specific characters is very doubtful.

RESISTANCE OF SPORES TO DESICCATION.

As spores live over the winter on the seed in a dry condition, data in regard to the actual amount of drying they can stand are important. In the summer of 1911 some experiments were carried on to procure these data. The object of the experiments was to see how long the spores would remain alive when dried on cotton seed and cover slips, and also to see if the presence of the mucilaginous matrix in which the spores are imbedded helps to retain their vitality when dried.

In the first experiment, which was begun in June, cover slips were placed in petri dishes, sterilized, and then divided into two sets. With one set, spores from pure cultures were transferred directly to the cover slips with a sterile needle, and allowed to dry down imbedded in the mucilaginous matrix that surrounded them. For the other set, the spores were washed from the culture in sterile water. This suspension of spores was then centrifuged for a few minutes and the clear liquid above poured off. The spores, being free from the mucilaginous matrix, were then placed on the cover slips and allowed to dry. At different periods these cover slips were dropped into tubes of melted agar, and poured into plates. The plates were examined in about 24 to 48 hours after making and the condition of the germination of the spores noted. The results of this experiment are given in Table 6, below.
TABLE 6.
RESISTANCE OF SPORES TO DRYING.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time dried, Days</th>
<th>Number of plates</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifuged</td>
<td>0</td>
<td>1</td>
<td>97% germination</td>
</tr>
<tr>
<td>Not centrifuged</td>
<td>0</td>
<td>1</td>
<td>97% germination</td>
</tr>
<tr>
<td>Centrifuged</td>
<td>3</td>
<td>1</td>
<td>95% germination</td>
</tr>
<tr>
<td>Not centrifuged</td>
<td>3</td>
<td>1</td>
<td>95% germination</td>
</tr>
<tr>
<td>Centrifuged</td>
<td>14</td>
<td>1</td>
<td>95% germination</td>
</tr>
<tr>
<td>Not centrifuged</td>
<td>14</td>
<td>1</td>
<td>93% germination</td>
</tr>
<tr>
<td>Centrifuged</td>
<td>16</td>
<td>1</td>
<td>95% germination</td>
</tr>
<tr>
<td>Not centrifuged</td>
<td>16</td>
<td>1</td>
<td>85% germination</td>
</tr>
<tr>
<td>Centrifuged</td>
<td>18</td>
<td>1</td>
<td>75% germination</td>
</tr>
<tr>
<td>Not centrifuged</td>
<td>18</td>
<td>1</td>
<td>80% germination</td>
</tr>
<tr>
<td>Centrifuged</td>
<td>20</td>
<td>1</td>
<td>95% germination</td>
</tr>
<tr>
<td>Not centrifuged</td>
<td>20</td>
<td>1</td>
<td>No germination</td>
</tr>
<tr>
<td>Centrifuged</td>
<td>22</td>
<td>1</td>
<td>25% germination</td>
</tr>
<tr>
<td>Not centrifuged</td>
<td>22</td>
<td>2</td>
<td>No germination</td>
</tr>
<tr>
<td>Centrifuged</td>
<td>24</td>
<td>1</td>
<td>45% germination</td>
</tr>
<tr>
<td>Not centrifuged</td>
<td>24</td>
<td>3</td>
<td>1 colony in 3 plates</td>
</tr>
<tr>
<td>Centrifuged</td>
<td>25</td>
<td>2</td>
<td>9 colonies in 2 plates</td>
</tr>
<tr>
<td>Not centrifuged</td>
<td>25</td>
<td>1</td>
<td>No germination</td>
</tr>
<tr>
<td>Centrifuged</td>
<td>27</td>
<td>2</td>
<td>2 colonies in 2 plates</td>
</tr>
<tr>
<td>Not centrifuged</td>
<td>27</td>
<td>2</td>
<td>No germination</td>
</tr>
<tr>
<td>Centrifuged</td>
<td>30</td>
<td>2</td>
<td>3 colonies in 2 plates</td>
</tr>
<tr>
<td>Not centrifuged</td>
<td>30</td>
<td>2</td>
<td>No germination</td>
</tr>
<tr>
<td>Centrifuged</td>
<td>34</td>
<td>2</td>
<td>No germination</td>
</tr>
<tr>
<td>Not centrifuged</td>
<td>34</td>
<td>16</td>
<td>No germination</td>
</tr>
<tr>
<td>Centrifuged</td>
<td>34</td>
<td>2</td>
<td>No germination</td>
</tr>
<tr>
<td>Centrifuged</td>
<td>37</td>
<td>2</td>
<td>No germination</td>
</tr>
<tr>
<td>Not centrifuged</td>
<td>37</td>
<td>15</td>
<td>No germination</td>
</tr>
</tbody>
</table>

This table shows that the spores that were allowed to dry down in their mucilaginous matrix practically lost their ability to germinate in twenty days, only one colony developing after that time, and that on the twenty-fourth day. The spores that were washed free from this mucilaginous substance seemed to remain viable for a longer time, some germinating at the end of thirty days.

In another experiment, started in July, 1911, a set of cover slips were covered with the centrifuged spores and allowed to dry. As a contrast to this, some cotton seed were treated with a suspension of spores from the same culture. These cover slips and seeds were dropped in sterile agar as in the previous experiment and the spores allowed to germinate, when the percent of germination was estimated. The results of this experiment are given in Table 7.
### TABLE 7.

**RESISTANCE OF ANTHRACNOSE SPORES TO DRYING.**

<table>
<thead>
<tr>
<th>Source of Spores</th>
<th>Time dried, Days</th>
<th>Number of Plates</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>1</td>
<td>1</td>
<td>91% germination</td>
</tr>
<tr>
<td>Cover Slip</td>
<td>1</td>
<td>1</td>
<td>97% germination</td>
</tr>
<tr>
<td>Seed</td>
<td>11</td>
<td>1</td>
<td>95% germination</td>
</tr>
<tr>
<td>Cover Slip</td>
<td>11</td>
<td>1</td>
<td>90% germination</td>
</tr>
<tr>
<td>Seed</td>
<td>13</td>
<td>1</td>
<td>95% germination</td>
</tr>
<tr>
<td>Cover Slip</td>
<td>13</td>
<td>1</td>
<td>93% germination</td>
</tr>
<tr>
<td>Seed</td>
<td>14</td>
<td>1</td>
<td>80% germination</td>
</tr>
<tr>
<td>Cover Slip</td>
<td>14</td>
<td>1</td>
<td>No germination</td>
</tr>
<tr>
<td>Seed</td>
<td>20</td>
<td>1</td>
<td>55% germination</td>
</tr>
<tr>
<td>Cover Slip</td>
<td>20</td>
<td>2</td>
<td>14 colonies</td>
</tr>
<tr>
<td>Seed</td>
<td>22</td>
<td>1</td>
<td>Slight germination</td>
</tr>
<tr>
<td>Cover Slip</td>
<td>27</td>
<td>2</td>
<td>No germination</td>
</tr>
<tr>
<td>Seed</td>
<td>27</td>
<td>2</td>
<td>200-300 colonies in 2 plates</td>
</tr>
<tr>
<td>Cover Slip</td>
<td>31</td>
<td>2</td>
<td>52 colonies in 2 plates</td>
</tr>
<tr>
<td>Seed</td>
<td>31</td>
<td>2</td>
<td>75% germination</td>
</tr>
<tr>
<td>Cover Slip</td>
<td>35</td>
<td>2</td>
<td>About 200 colonies in 2 plates</td>
</tr>
<tr>
<td>Seed</td>
<td>39</td>
<td>1</td>
<td>75% germination</td>
</tr>
<tr>
<td>Cover Slip</td>
<td>39</td>
<td>1</td>
<td>55 colonies</td>
</tr>
<tr>
<td>Seed</td>
<td>41</td>
<td>1</td>
<td>Several hundred colonies</td>
</tr>
<tr>
<td>Cover Slip</td>
<td>41</td>
<td>1</td>
<td>No germination</td>
</tr>
<tr>
<td>Seed</td>
<td>44</td>
<td>1</td>
<td>Several hundred colonies</td>
</tr>
<tr>
<td>Cover Slip</td>
<td>44</td>
<td>2</td>
<td>No germination</td>
</tr>
<tr>
<td>Seed</td>
<td>48</td>
<td>2</td>
<td>No germination</td>
</tr>
<tr>
<td>Cover Slip</td>
<td>51</td>
<td>1</td>
<td>No germination</td>
</tr>
<tr>
<td>Seed</td>
<td>51</td>
<td>2</td>
<td>No germination</td>
</tr>
<tr>
<td>Cover Slip</td>
<td>51</td>
<td>2</td>
<td>No germination</td>
</tr>
<tr>
<td>Seed</td>
<td>52</td>
<td>5</td>
<td>No germination</td>
</tr>
<tr>
<td>Cover Slip</td>
<td>55</td>
<td>10</td>
<td>Each plate hundreds of colonies</td>
</tr>
<tr>
<td>Seed</td>
<td>55</td>
<td>5</td>
<td>Many colonies</td>
</tr>
<tr>
<td>Seed</td>
<td>59</td>
<td>2</td>
<td>Many colonies</td>
</tr>
<tr>
<td>Seed</td>
<td>63</td>
<td>2</td>
<td>Many colonies</td>
</tr>
<tr>
<td>Seed</td>
<td>65</td>
<td>2</td>
<td>Over 100 colonies</td>
</tr>
<tr>
<td>Seed</td>
<td>70</td>
<td>6</td>
<td>5 colonies</td>
</tr>
<tr>
<td>Seed</td>
<td>78</td>
<td>5</td>
<td>About 150 colonies</td>
</tr>
<tr>
<td>Seed</td>
<td>81</td>
<td>5</td>
<td>About 15 colonies</td>
</tr>
<tr>
<td>Seed</td>
<td>87</td>
<td>4</td>
<td>4 colonies</td>
</tr>
<tr>
<td>Seed</td>
<td>91</td>
<td>4</td>
<td>No germination</td>
</tr>
<tr>
<td>Seed</td>
<td>94</td>
<td>4</td>
<td>3 colonies</td>
</tr>
<tr>
<td>Seed</td>
<td>98</td>
<td>4</td>
<td>40 colonies</td>
</tr>
<tr>
<td>Seed</td>
<td>106</td>
<td>2</td>
<td>No germination</td>
</tr>
<tr>
<td>Seed</td>
<td>118</td>
<td>2</td>
<td>No germination</td>
</tr>
<tr>
<td>Seed</td>
<td>133</td>
<td>8</td>
<td>One colony</td>
</tr>
<tr>
<td>Seed</td>
<td>139</td>
<td>4</td>
<td>No germination</td>
</tr>
<tr>
<td>Seed</td>
<td>144</td>
<td>12</td>
<td>No germination</td>
</tr>
</tbody>
</table>

In Table 7 it is seen that the spores on the cover slips began to lose their viability after the fourteenth day, though some of
them continued to germinate up to the thirty-ninth day. On the seeds themselves the spores retained their vitality much longer, some germinating at the end of one hundred and thirty-nine days. The seed ran out at the end of one hundred and forty-four days and the experiment was discontinued. Some of the irregularity in germination of the spores from the seed, as shown in Table 7, is due to the presence of bacteria in the plates. It is very difficult to sterilize such seed as cotton and then dry them out quickly, and some of the plates showed enough bacterial development to retard or even prohibit the germination of the anthracnose spores.

That disease organisms will live longer when dried on the seed of the host plant than on other material is known for other diseases beside the cotton anthracnose. Harding, Stewart, and Prucha (38) showed that the bacteria causing the black rot disease of cabbage would live longer on cabbage seed than when dried on other material, and it is very probable that this will be found true with many other diseases.

Just how long cotton anthracnose spores will stay alive while in a dried condition depends to a considerable degree on the time of year they are dried. They evidently retain their vitality much better during the winter months than during the summer. Spores remain alive over winter in large numbers on cotton seed—that is, from picking time in September to November until planting time in April. It is possible that some of these spores may live for some time longer on these seed, but I have failed to get them to germinate in the middle of the summer from seed gathered the preceding fall.

**LENGTH OF LIFE OF THE MYCELIUM WITHIN THE SEEDS.**

Another very important thing to be taken into consideration is the time that the fungus will remain alive within the seed. If the fungus is not able to stay alive as long as the seed itself, it might be possible to eliminate the disease in the field by planting old seed in which the fungus was dead. An experiment was planned and carried through to find out how long the fungus would stay alive in the seed. In the fall of 1910 cotton was
picketed from badly diseased bolls and ginned separately. This seed was saved and planted from time to time and the amount of anthracnose on the young plants was estimated. Cultures were also made from many of the seed at different times as a check on the planting experiments. The seeds were all planted in the greenhouse, where there was no danger from outside infection. The results of these planting experiments are given in Table 8 below.

### Table 8.

**LENGTH OF LIFE OF ANTHRACNOSE IN SEED.**

<table>
<thead>
<tr>
<th>Age of Seed</th>
<th>No. of Seed Planted</th>
<th>% Germination</th>
<th>% Young Plants with Anthracnose</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 months...</td>
<td>500</td>
<td>70.2</td>
<td>9.1</td>
</tr>
<tr>
<td>11 months...</td>
<td>500</td>
<td>70.6</td>
<td>9.9</td>
</tr>
<tr>
<td>13 months...</td>
<td>1000</td>
<td>69.2</td>
<td>0.0</td>
</tr>
<tr>
<td>13.5 months.</td>
<td>1000</td>
<td>12.7</td>
<td>0.0</td>
</tr>
<tr>
<td>14.5 months.</td>
<td>1000</td>
<td>2.3</td>
<td>0.0</td>
</tr>
<tr>
<td>15 months...</td>
<td>1000</td>
<td>9.1</td>
<td>0.0</td>
</tr>
<tr>
<td>16 months...</td>
<td>1000</td>
<td>14.3</td>
<td>0.0</td>
</tr>
<tr>
<td>18 months...</td>
<td>850</td>
<td>52.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

The poor germination of the seed between the thirteenth and sixteenth months was due to the temperature of the greenhouse. The part of the greenhouse where these experiments were carried on was not warm enough for the cotton to germinate, and much of it rotted. That the seed was still good was shown by the much higher germination at the end of the eighteenth month. This was in April when the temperature of the house was again warm.

The cultures made from the seed gave results that corresponded well with the above. Cultures made during the first winter gave a high percent with anthracnose, though the exact percent was not obtained. At the end of eight months transfers from sixty-four seed gave twenty-four cultures with anthracnose. At the end of ten months, however, only one culture was obtained from 200 seed. It was at this time that the decrease is noted in the table above. At the end of eighteen months 200 seed were germinated on sterilized cotton in test tubes, these showing a germination of 63% without any anthracnose.
It would seem from the above experiments that the cotton anthracnose dies out in the seed after about thirteen months in this climate. Barre claims that they live longer in South Carolina and that an occasional seed will be found in the second year to have the fungus alive in it. His results, however, were obtained in the field, his laboratory tests failing to show the presence of any living anthracnose.

As the disease dies out in the seed before the second year, it would seem that the use of old seed for planting might be a satisfactory method for eliminating the anthracnose in the field. Of course, a person would have to be sure that the old seed was good and would show a high percent of germination. There are very few experiments described at present that give us any data on how long cotton seed will remain alive when stored. Tempany (71), writing from results obtained in the West Indies, claims that the seed with which he worked deteriorated very rapidly before the second spring. These results, however, do not agree with the ones described in the previous pages of this bulletin. Duggar (22), also, in Alabama, carried on some field experiments with seed of different ages. In 1896 he planted seed from the 1893, 1894, and 1895 crops and evidently obtained good results in each case. He states that the three plots did not show any difference in yield. It would seem, at least, that a man could plant two-year-old seed with perfect safety, though, of course, it would be best to obtain the percent of germination before planting any considerable acreage.

INOCULATION EXPERIMENTS ON COTTON BOLLS.

During the course of this investigation a large number of bolls have been artificially inoculated with the cotton anthracenose. These inoculations have been made by transferring the spores into a needle puncture and also by spraying the spores on the uninjured epidermis. Successful infection has been obtained by both methods.

As the fungus produces infection more easily through a wound, the most successful method of inoculation is by means of needle punctures. Several thousand bolls have been inoculated in this manner with practically no failures. After the in-
oclulation, the fungus begins development immediately and in the course of a day or two the tissue begins to blacken around the spot of inoculation. If the inoculation is on the side of the boll, the spot enlarges rapidly, and if the weather conditions are favorable and the boll is not too old a general rot follows. If the inoculation is at the tip of the boll the spread of the disease is perhaps more rapid than when the inoculation is on the side. The fungus very soon comes in contact with all the locks of cotton, destroying them simultaneously. Also when the inoculation is at the tip there is generally a splitting apart of the carpels in that region.

A considerable number of bolls have also been inoculated by spraying the spores on the uninjured epidermis. Young bolls, only a few days from the flower, are more susceptible to infection in this manner than older bolls, on account of the softness of the tissue. In the experiments which have been included below in Table 9 the flowers were tagged with the date marked upon them. After different periods, the young bolls were sprayed with a suspension of spores in water. Part of the bolls were covered with paper sacks, while others were left uncovered. The bolls were not protected from natural infection before the inoculations were made. These experiments were conducted in July, 1911.

<table>
<thead>
<tr>
<th>Age of Bolls</th>
<th>Bolls Covered</th>
<th>Bolls Uncovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Affected</td>
</tr>
<tr>
<td>One day ......</td>
<td>12</td>
<td>76%</td>
</tr>
<tr>
<td>Two days......</td>
<td>18</td>
<td>67%</td>
</tr>
<tr>
<td>Two days ......</td>
<td>15</td>
<td>53%</td>
</tr>
<tr>
<td>Five days.....</td>
<td>20</td>
<td>50%</td>
</tr>
<tr>
<td>Six days......</td>
<td>25</td>
<td>32%</td>
</tr>
<tr>
<td>Checks .......</td>
<td>19</td>
<td>21%</td>
</tr>
</tbody>
</table>

An examination of the table shows that the very young bolls were very readily attacked by the anthraenose and a high percent of infection was obtained. As the bolls became older, however, there was a gradual decrease in the percent of infection. The bolls which were covered with paper sacks also show a higher
Percent of infection than the uncovered ones. The checks show a lower percent of infection than the inoculations. The percent of infection on the uncovered checks was practically the same as the percent of natural infection in the field.

In another experiment tried in July, 1911, bolls over half grown were inoculated with the anthracnose by spraying them with spores in water. Part of these bolls were covered with paper sacks after the inoculation and part were left uncovered. Of the thirty-six bolls left uncovered 29% developed the anthracnose; of the thirty-three bolls that were covered 46% developed the disease. Other bolls of the same size that were not inoculated showed a natural infection of only 6%. There is another factor that enters in such an experiment as this, however, and that is the presence of injuries on the bolls which may have been caused by insects and other agencies. There is no way of telling whether these bolls became infected through small insect punctures which are often quite abundant on the bolls, or whether the infection took place through the uninjured surface. This factor would also apply in a less degree to the experiment given in Table 9.

Another experiment to test out the ability of the anthracnose to infect the uninjured epidermis of the boll was tried in the summer of 1912. For this experiment the flowers were covered with paper bags, these being left over the young bolls until they were inoculated. These bolls were all inoculated on the same day, the flowers being covered at different times before, so that there were some of several different ages. After the bolls were sprayed with the spore suspension, the bags were again tied back over them for a period of ten days, when they were examined. The number of bolls inoculated, their age, and the percent of infection is given in Table 10 below.
TABLE 10.

INFECTION EXPERIMENTS WITH BOLLS OF DIFFERENT AGES.

<table>
<thead>
<tr>
<th>Age of Bolls</th>
<th>No. of checks</th>
<th>% of checks diseased</th>
<th>No. of inoculations</th>
<th>% of inoculations diseased</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 days</td>
<td>13</td>
<td>0%</td>
<td>27</td>
<td>77.8%</td>
</tr>
<tr>
<td>7 days</td>
<td>...</td>
<td>...</td>
<td>41</td>
<td>95.1%</td>
</tr>
<tr>
<td>11 days</td>
<td>...</td>
<td>...</td>
<td>38</td>
<td>81.6%</td>
</tr>
<tr>
<td>16 days</td>
<td>1</td>
<td>0%</td>
<td>25</td>
<td>24.6%</td>
</tr>
<tr>
<td>21 days</td>
<td>3</td>
<td>0%</td>
<td>1</td>
<td>0.0%</td>
</tr>
<tr>
<td>26 days</td>
<td>12</td>
<td>8.4%</td>
<td>17</td>
<td>11.8%</td>
</tr>
<tr>
<td>30 days</td>
<td>4</td>
<td>50%</td>
<td>4</td>
<td>0.0%</td>
</tr>
<tr>
<td>32 days</td>
<td>4</td>
<td>25.5%</td>
<td>4</td>
<td>14.3%</td>
</tr>
</tbody>
</table>

By a mistake, no bolls were left for checks for some of the different periods, but there are enough of some of the other periods for a good comparison. This experiment shows that bolls that have been covered with paper bags are easily attacked by the anthracnose up until they are at least eleven days old, and to a less degree up until they are sixteen days or more old. However, after the bolls are from twenty-six to thirty-two days old they are not readily attacked by the anthracnose disease without an injury in the epidermis. The table shows that there was some infection on both the inoculated and check bolls of this age, but this evidently came from outside sources. Some of the bags that staid on the bolls for so many days were torn by the wind or had holes eaten in them by insects and were exposed to outside infection. Also it was impossible to tie the bags on so tight that insects could not crawl up the stem to the bolls. The table shows that the infection was but very little different on either the checks or the inoculated bolls that were twenty-six days or more old.

One point should be borne in mind, however, in the consideration of the above experiment. The bolls in the bags were placed under unnatural conditions. The humidity of the air surrounding them was at most times higher than around uncovered bolls; and, furthermore, the bolls, not being exposed to the sunlight, were more tender and perhaps more susceptible to the disease than uncovered bolls. Yet the very high percent of infection on these bolls shows that the disease can enter the uninjured epidermis.
INOCULATION OF FLOWERS WITH THE COTTON ANTHRACNOSE.

Young bolls also may become infected by spores which have been placed in the open flower. The spore may germinate in the flower and grow on the dead flower parts. Spores develop on the corolla and other flower parts and later come in contact with the young boll underneath. The dead flower parts hold the fungus in a growing condition until weather conditions allow it to infect the young boll. Also the spores may germinate and grow down the dying pistil, thus causing an infection at the tip of the young boll. In a previous article (31) this method has been called "flower infection" in contrast to the first one mentioned above, though this is very similar to the tip infection of young bolls. Experiments in flower inoculation have been carried on for several years at the Louisiana Station. The first successful inoculations were obtained in the summer of 1908, though the percent of infection was not estimated in that year. In the years 1910, 1911 and 1912 further inoculations have been made and the percent of infection obtained. The inoculations were made by dropping a suspension of spores into the flowers late in the afternoon just before or shortly after the corolla closed. These flowers were tagged and the young bolls were examined from time to time for a few weeks following. The results for the years 1910 and 1911 were given in the previous article, but the tables showing the percents of infection, and the portions of the bolls affected will be repeated here. The results for 1910 are given in Table 11 below.

<table>
<thead>
<tr>
<th>Date of inoculation</th>
<th>July 13</th>
<th>July 15</th>
<th>July 18</th>
<th>July 21</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of inoculations</td>
<td>28</td>
<td>38</td>
<td>20</td>
<td>36</td>
<td>122</td>
</tr>
<tr>
<td>Inoculations affected</td>
<td>57%</td>
<td>39%</td>
<td>40%</td>
<td>50%</td>
<td>45%</td>
</tr>
<tr>
<td>Inoculations affected at the tip</td>
<td>7%</td>
<td>14%</td>
<td>20%</td>
<td>28%</td>
<td>17%</td>
</tr>
<tr>
<td>Number of checks</td>
<td>26</td>
<td>24</td>
<td>5</td>
<td>29</td>
<td>84</td>
</tr>
<tr>
<td>Checks affected</td>
<td>4%</td>
<td>4%</td>
<td>0%</td>
<td>0%</td>
<td>2%</td>
</tr>
</tbody>
</table>

The results of the flower inoculations of 1911 are given in Table 12.
TABLE 12.
FLOWER INFECTION EXPERIMENTS IN 1911.

<table>
<thead>
<tr>
<th>Date of inoculation</th>
<th>June 24</th>
<th>June 26</th>
<th>June 27</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of inoculations</td>
<td>46</td>
<td>74</td>
<td>65</td>
<td>185</td>
</tr>
<tr>
<td>Inoculations affected in 15-17 days</td>
<td>24%</td>
<td>19%</td>
<td>...</td>
<td>21%</td>
</tr>
<tr>
<td>Inoculations affected in 23-25 days</td>
<td>24%</td>
<td>28%</td>
<td>29%</td>
<td>28%</td>
</tr>
<tr>
<td>Inoculations affected in 31-34 days</td>
<td>35%</td>
<td>34%</td>
<td>38%</td>
<td>36%</td>
</tr>
<tr>
<td>Number of checks</td>
<td>19</td>
<td>18</td>
<td>29</td>
<td>66</td>
</tr>
<tr>
<td>Checks affected in 23-25 days</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Checks affected in 31-34 days</td>
<td>0%</td>
<td>6%</td>
<td>7%</td>
<td>5%</td>
</tr>
</tbody>
</table>

During 1911, also, each boll was examined carefully for the original point of infection, whether at the tip of the boll, on the line of dehiscence between the carpels, or on the capsular tissue between the lines of dehiscence. The percentage of infection on the different places 23-25 days after the inoculation is shown in Table 13 below. There was no increase in tip infection after this period.

TABLE 13.
POINT OF INFECTION FOLLOWING FLOWER INOCULATION.

<table>
<thead>
<tr>
<th>Date of inoculation</th>
<th>June 24</th>
<th>June 26</th>
<th>June 27</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of inoculations</td>
<td>46</td>
<td>74</td>
<td>65</td>
<td>185</td>
</tr>
<tr>
<td>Total infection</td>
<td>24%</td>
<td>28%</td>
<td>29%</td>
<td>28%</td>
</tr>
<tr>
<td>Infected at tip</td>
<td>13%</td>
<td>23%</td>
<td>15%</td>
<td>18%</td>
</tr>
<tr>
<td>Infected on lines of dehiscence</td>
<td>2%</td>
<td>1%</td>
<td>5%</td>
<td>3%</td>
</tr>
<tr>
<td>Infected between lines of dehiscence</td>
<td>9%</td>
<td>4%</td>
<td>9%</td>
<td>7%</td>
</tr>
</tbody>
</table>

In the summer of 1912, flowers of four different varieties were inoculated with the cotton anthracnose. Of the 207 bolls that developed from these flowers, 57% developed the disease and 17% had the disease at the tip. No bolls were tagged for checks, but the natural infection in the field was between 5 and 10%, showing that the high infection was really due to the inoculation. This experiment was really tried for another purpose and will be discussed more fully under "varietal resistance."

An examination of the results tabulated above show that good infection was obtained in each experiment. They also show that a considerable percent of the bolls first show the infection at the tip. These results are somewhat different from some obtained
in South Carolina by Barre (11), as he claims that on the largest percent of the bolls developing from inoculated flowers, the infection first shows on the lines of dehiscence between the carpels. From the experiments in Louisiana, however, it would seem that no portion of the uninjured epidermis of the boll is any more susceptible than another, and that the large percent of bolls infected at the tip is due to the presence of the dead pistil. The dead pistil presents a condition quite similar to a wound, and infection takes place much more readily in a wound than on the uninjured epidermis.

While the inoculations described above show that bolls can become infected through the flowers, they do not show that this is the usual method of infection. The placing of a half a centimeter of a heavy suspension of spores in a flower is certainly an abnormal condition. Under natural conditions the number of spores that would actually find an entrance into a flower would be small, and, furthermore, unless there was a rain to partially fill the flower with water and supply moisture, the conditions for germination would not be the best. Of course, a spore that actually came in contact with the nectar of the flower would germinate and possibly cause infection, but it is readily seen that the chances for infections are much less than where a large number of spores are placed in water in the flower. This is also borne out by observations in the field. While it is not uncommon to find young bolls affected at the tip, the actual percentage compared to the total percentage of the disease is small.

RELATION OF FLOWER INOCULATION TO SHEDDING.

During the years 1910 and 1911 the number of shed flowers were counted in each of the flower inoculation experiments. It was thought that the development of the anthracnose on the young pistils might cause a large percent of the flowers to drop without developing bolls. The results for the 1910 inoculations are given in Table 14 below. There is included the number of checks and inoculations and also the percent of each that shed without developing bolls.
Table 14.

RELATION OF FLOWER INOCULATION TO SHEDDING.

<table>
<thead>
<tr>
<th></th>
<th>July 13</th>
<th>July 15</th>
<th>July 18</th>
<th>July 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>% shed</td>
<td>No.</td>
<td>% shed</td>
<td>No.</td>
</tr>
<tr>
<td>Checks</td>
<td>39</td>
<td>49</td>
<td>47%</td>
<td>25</td>
</tr>
<tr>
<td>Inoculations</td>
<td>40</td>
<td>50</td>
<td>24%</td>
<td>63</td>
</tr>
</tbody>
</table>

The results for the 1911 inoculations are given in Table 15.

Table 15.

RELATION OF FLOWER INOCULATION TO SHEDDING.

<table>
<thead>
<tr>
<th></th>
<th>June 24</th>
<th>June 26</th>
<th>June 27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>% shed</td>
<td>Number</td>
<td>% shed</td>
</tr>
<tr>
<td>Checks</td>
<td>20</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Inoculations</td>
<td>53</td>
<td>86</td>
<td>73</td>
</tr>
</tbody>
</table>

From the tables it is seen that there were no results obtained that would show that the anthracnose inoculations had very much to do with the shedding of the young bolls. In some of the experiments the check flowers show the highest percent of shedding, while in others the inoculated flowers show a higher percent. During the 1910 experiments, however, a greater amount of water was used in the check flowers than was used of the inoculating fluid for the others and this may have had something to do with the higher percent of shedding of the checks. During the 1911 experiments, about the same amount was used in each case, and the checks show a slightly less percent of shedding. Yet the difference in percent in shedding is so small that it seems certain that the presence of anthracnose spores in the flowers has but very little to do with shedding under natural conditions.

The results obtained in the above experiments do not bear out very well the common idea that rain in the open flowers will cause a shedding of the young bolls. Many farmers firmly believe that the rain falling in the flowers is responsible for the shedding. It is very probable that rainy weather has much to do with shedding, yet it seems probable that this trouble is brought about by the condition of the roots and not by the presence of water in the flowers. The 1910 experiments were
carried on during a rainy period and all the bolls shed badly. The 1911 experiments were conducted during dry weather and the shedding was very slight. If the presence of water in the open flowers was the cause of the shedding, the bolls should have fallen as badly in 1911 as in 1910.

INOCULATION OF SEEDS.

As has been stated in previous pages, spores that are in the lint around the seeds are able to infect the young seedlings during germination. At various times in the course of this investigation, seeds have been immersed in a suspension of spores, and then germinated in order to get infected seedlings, and in practically all cases a high percent of the young plants were diseased. That this is true has long been recognized by botanists and needs little more than mention at this time. It might be well, however, to give the results of some experiments in which the actual percent of diseased seedlings was obtained. Two experiments were tried in the summer of 1911, but as the results in the two were practically the same, they are combined. Seeds absolutely free from anthracnose spores were procured. One hundred and fifty of these were wet with a suspension of anthracnose spores in water and planted; also 150 were planted without treatment for a check. The 150 inoculated seed when planted in ordinary unsterilized soil gave a germination of 85%, with 32% of the young seedlings affected with the disease. The 150 seed used as a check gave a germination of 95% with all of the seedlings healthy. That a greater percent of the young seedlings developing from inoculated seed were not affected was doubtless due to the various other organisms that were in the soil. These various soil organisms hinder the growth of the anthracnose and decrease the percent of the infected seedlings. Inoculated seed in sterilized soil always give more infected seedlings.

VARIETAL RESISTANCE TO COTTON ANTHRACNOSE.

During the past few years, at a number of the stations in the south, counts have been made of the diseased bolls in the variety plots to see if any of the varieties are more subject to the disease than others. Some varieties of cotton, such as Cook's, are gen-
erally conceded to be much more susceptible than others. The results obtained by the other stations have been studied carefully and have been put in table form—Table 16 below. To this table there is also added the results of some similar counts made in Louisiana. This table gives the variety, the years the results were obtained, and the percent of diseased bolls. The numbers in parentheses refer to the publication from which the percents were obtained, and the dates in the Louisiana counts refer to the dates the counts were made.

### TABLE 16.

**Varietal Resistance to Cotton Anthracnose.**

<table>
<thead>
<tr>
<th>Variety</th>
<th>Alabama (24) 1909</th>
<th>Alabama (24) 1910</th>
<th>Georgia (20) 1907</th>
<th>Georgia (20) 1908</th>
<th>Georgia (46) 1909-10</th>
<th>Georgia (44) 1909</th>
<th>South Carolina (12) 1909</th>
<th>Louisiana, Aug. 19-23, 1911</th>
<th>Louisiana, Aug. 30, 1911</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bank Account</td>
<td>3.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Barber</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.9</td>
<td>8.1</td>
</tr>
<tr>
<td>Cleveland</td>
<td>4.6</td>
<td>3.4</td>
<td>23.0</td>
<td>10.0</td>
<td>13.6</td>
<td>5.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooks</td>
<td>23.1</td>
<td>31.0</td>
<td>44.0</td>
<td>9.4</td>
<td>5.8</td>
<td>9.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooks (Steinheims)</td>
<td>8.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooks No. 206</td>
<td>9.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooks (Hall)</td>
<td>28.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn Silk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Covington Toole</td>
<td>9.4</td>
<td>1.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dickson</td>
<td>11.5</td>
<td>3.3</td>
<td>24.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egyptian</td>
<td>15.4</td>
<td>16.0</td>
<td>15.0</td>
<td>8.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Georgia’s Best</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Griffin Rust Proof</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hasting’s Sure Crop</td>
<td>10.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herlong</td>
<td>10.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hites</td>
<td>12.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>King</td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Money Maker</td>
<td>0.4</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortgage Lifter</td>
<td>9.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natchez</td>
<td>18.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oziens Long Staple</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pride of Georgia</td>
<td>10.0</td>
<td>3.4</td>
<td>8.0</td>
<td>17.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosser No. 1</td>
<td>27.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Russell</td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simpkins</td>
<td>10.0</td>
<td>3.4</td>
<td>8.0</td>
<td>17.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toole</td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triumph</td>
<td>8.7</td>
<td>3.3</td>
<td>26.0</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Union Big Boll</td>
<td>9.0</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90-day cotton</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The data from all of the stations were evidently not obtained in the same manner. Some of the figures represent all the rot whether it was known for a certainty to be caused by the anthracnose or not. In the publications giving these results, however, the authors state that the rot was mostly due to the anthracnose. Then some of the figures were obtained by counting the empty burs and the diseased dried up bolls after the cotton was picked, assuming that practically all of the rot was due to the anthracnose. The Louisiana figures were obtained by counting all of the bolls on twenty plants and then by examining all of the diseased bolls with the microscope. All the diseased bolls on which the anthracnose spores were found were included as anthracnose bolls whether this was the predominating fungus or not and whether the bolls were badly injured or only slightly. The total percent of rot caused by all boll-rotting organisms was about double the amount caused by the anthracnose for most of the varieties on the first count, and considerably more on the second count.

If the figures in the table are studied it will be seen that we know but very little regarding the actual resistance of the different varieties. Some varieties show a very high percent of anthracnose in some places and very low in others. Furthermore, the same variety often shows a wide variation on the same piece of ground, as illustrated by the different selections and lots of Cook's cotton. From the figures in the table, one would naturally draw the conclusion that there is but very little difference in the susceptibility of the different varieties, and that the different percents of the disease is due to the condition of the seed rather than to the variety itself. If clean, healthy seed were used for planting, the cotton bolls would be relatively free of the disease, but if badly diseased seed were planted naturally a higher percent of the bolls would be diseased. And this conclusion would in the main be true. A high percent of diseased seed will result in a high percent of diseased bolls, and clean, healthy seed in a low percent of disease no matter what variety is grown. This does not show conclusively, however, that there may not be a slight difference in susceptibility of the different varieties. This can only be shown by carefully inoculating bolls or flowers of different varieties and by counting the number
of diseased bolls. There seems to be no data published that were obtained in this manner. Some results, however, have been obtained during the season of 1912 at Baton Rouge. Four different varieties growing side by side were selected and used for inoculation experiments. The flowers were inoculated with a suspension of spores in water as has been previously described under “flower infection.” This seems to be the most satisfactory method we have for inoculating the young bolls. As the row of each variety was short, the flowers for several different days were inoculated and these are all combined in the table below. All of the bolls that grew from these inoculated flowers were examined from time to time and the percent of infection was obtained. The results were as follows:

### Table 17.

**Resistance of Different Varieties to Inoculation**

<table>
<thead>
<tr>
<th>Variety</th>
<th>No. of bolls</th>
<th>No. diseased</th>
<th>% diseased</th>
<th>Diseased at tip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toole</td>
<td>26</td>
<td>20</td>
<td>77%</td>
<td>35%</td>
</tr>
<tr>
<td>Cook</td>
<td>64</td>
<td>46</td>
<td>72%</td>
<td>14%</td>
</tr>
<tr>
<td>Simpkins</td>
<td>88</td>
<td>37</td>
<td>42%</td>
<td>16%</td>
</tr>
<tr>
<td>Cleveland</td>
<td>29</td>
<td>14</td>
<td>48%</td>
<td>14%</td>
</tr>
</tbody>
</table>

In this experiment there was a considerable difference in the amount of infection on some of the different varieties. The Toole and Cook cottons show a much higher percent of infection than the Simpkins and Cleveland. There were, however, not enough bolls of the Toole and Cleveland varieties for a good test, due to the excessive shedding of the inoculated flowers. But if these are not taken into consideration, there still remain the Cook and Simpkins with a considerable number of bolls and with a large difference in the amount of infection. As the inoculations were made at the same time and as the different varieties were growing side by side, it is difficult to explain the variation in amount of infection in any way other than that some of the varieties are more susceptible than others. This also coincides with the general belief that the Cook cotton is very susceptible to the disease. But this table shows that none of the varieties are very resistant, even a 40% infection showing low resistance.
RELATION OF GLOMERELLA GOSSYPII TO OTHER ANTHRACNOSES.

All of the members of the genera, Gloeosporium and Colleto-trichum, that have ascogenous stages belonging to the genus Glomerella are in many ways very similar. In this group are the cotton anthracnose, the bean anthracnose, the apple bitter rot, the fig anthracnose, the anthracnoses of various fruits and vegetables and greenhouse plants, and many other forms more or less common on wild and cultivated plants. The morphological characters of all of these are indistinguishable, or, at most, show but slight variation. The conidiophores are the same in shape and size, the setae are similar in appearance, though the relative number varies somewhat, the perithecia can not be told apart, and there is but little difference in the ascospores. To be sure, there are some differences in the color of the spots and the appearance of the colonies on culture media, but these characters, unless they are very striking, are of but little value as specific characters. These forms from different hosts all grow readily on culture media, but the growth characters are not constant. Two different cultures from the same host do not always have the same cultural characters, and very often cultures from different hosts are more alike than two from the same host. The variation in growth is so striking that these characters are usually of but little value in separating the different ones. Occasionally a cultural character is quite pronounced, as, for instance, the black growth of the bean anthracnose, and may be of specific rank, yet there are other forms that also produce a black growth, as, for example, a form that grows on Melilotus indica in Louisiana. Most of the common forms, including the cotton anthracnose, produce a whitish to greyish growth and the cultural characters are not usually distinct enough to tell them apart.

The similarity of the different anthracnose forms presents a difficult problem in the determination of the species. In former years it was the custom of many botanists to determine the species by the hosts upon which they were found growing; and if a form was found on a new host it was usually described as a new species. Consequently we have at the present time a large
number of described species with very similar morphological characters. In recent years, since so many of these names have been found to be synonymous, not so many new species have been described.

From a physiological standpoint, some of the forms show striking differences. Some forms are confined to a single host and will not grow on other hosts, while other forms grow on quite a wide range of host plants. The ability or inability of a form to pass from one host to another is a very important point in its life history. Whether two forms morphologically similar but growing on two distinct hosts are separate species from a taxonomic standpoint is not easy to answer; but from a pathological standpoint, they must certainly be considered as distinct. The pathologist and the planter must know that the form from Plant A will not pass to Plant B and produce a disease there. These physiological forms must be named in some manner, and as it is not convenient to call them varieties and use trinomials, it seems advisable to keep them as distinct species, at least until we have a more complete knowledge of the whole group.

The cotton anthracnose seems to be distinct from the other anthracnoses. There are even slight morphological differences which may represent specific characters. The production of setae in the acervuli is usually much more abundant in the cotton anthracnose than in other forms. Spores are also borne on the tips of the setae, which has not been noticed, at least, with other forms. Then there seems to be a slight difference in the shape of the ascospores which may be an important specific character.

From a physiological standpoint, the cotton anthracnose is quite distinct from other forms. Cross inoculation experiments which have been tried at various times show that it does not grow readily on many hosts other than cotton and that the anthracnoses from other hosts do not readily attack cotton. These experiments have been carried on at various times during the past five years. Some of them have been described in previous bulletins where other anthracnose diseases were under discussion, but the results may be repeated at this time.

Experiment March 22, 1909. (28). Young bush bean, alfalfa and cotton plants in pots in the greenhouse were sprayed with a
suspension of bean anthracnose spores and the plants protected with bell jars. The results were as follows:

On bush beans—abundant infection.
On alfalfa—no infection.
On cotton—no infection.

Experiment February 10, 1909. (28) Planted healthy wax beans, different lots of which were wet with suspensions of spores of the bean, fig and cotton anthracnoses, in pots in the greenhouse. The amount of germination and the amount of disease on the young plants is given in the following table:

<table>
<thead>
<tr>
<th>Anthracnose</th>
<th>No. of seed</th>
<th>No. of plants</th>
<th>No. with spotted cotyledons on germination</th>
<th>No. spotted Feb. 23</th>
<th>No. spotted Feb. 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bean</td>
<td>25</td>
<td>21</td>
<td>9</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Cotton</td>
<td>25</td>
<td>24</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fig</td>
<td>25</td>
<td>18</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Both the cotton and the fig anthracnoses were able to attack the semi-living cotyledons of the germinating beans, and in one case with each, cotyledons spotted with these were carried above the ground. These spots, however, did not look at all typical of the bean anthracnose spots, though they contained spores in abundance. These spots did not develop further, the cotyledons finally fell off and the plants were left clean. The bean anthracnose, however, produced excellent infection.

Experiment November 25, 1910. (29) Half-grown figs were gathered and placed in moist chambers in the laboratory and were inoculated with anthracnoses from the following hosts: fig, Melilotus indica stem (this had caused a stem rot of the Melilotus); cotton, silver maple leaf (this had caused a blight of the maple leaves), and pepper. The anthracnoses from the fig, maple leaf and pepper took readily on the fig, producing a rot and abundant spore formation. The forms from Melilotus and cotton took very slowly on the figs and did not produce spores until nearly a month after the inoculation.
Experiment December 28, 1910. (29). Apples were procured and placed in moist chambers in the laboratory and inoculated by puncture with anthracnoses from the following hosts: fig, apple, cotton, pepper, *Populus deltoides*, silver maple, clover, bean, and *Melilotus indica*. These inoculations were examined frequently and on January 27 the final notes were taken. The ones that had formed lesions perfectly typical of the bitter rot were the forms from the apple, fig, pepper and maple. The forms that had produced no infection whatever were cotton, *Melilotus*, clover, and bean. The form from *Populus* produced a slightly decayed spot, but no acervuli.

Experiment summer, 1910. (29). A large number of cotton flowers were inoculated with anthracnose spores from cotton and figs. While the bolls developing from the cotton anthracnose infected flowers showed an infection of about 40%, the bolls from the fig anthracnose infected flowers showed none.

Experiment May 16, 1910. Inoculated two varieties of alfalfa, Arabian and Ecuadorian, with pure cultures of the anthracnoses from *Melilotus indica*, cotton, fig, and bean. The inoculations were made in the stems by needle punctures. These were examined on May 25 and the following condition of the plants was found:

On the Arabian alfalfa, the *Melilotus* anthracnose took in every case forming large lesions, some 3-5 cm. in length. The lesions were sunken and covered with black acervuli, and often the stems broke at these points. On the Ecuadorian alfalfa, the fungus took slightly forming sunken lesions from a half to one centimeter long. The stem was not weakened enough to break.

On the Arabian alfalfa, the cotton anthracnose showed up slightly forming small lesions near the point of inoculation. No lesions developed on the Ecuadorian alfalfa.

The anthracnoses from the bean and fig produced no spots on either variety of alfalfa.

Experiment July 19 and 21, 1911. Inoculated young okra pods on the plants with pure cultures of the anthracnoses from the bean, pepper, apple, cottonwood, *Manihot*, *Melilotus*, and cotton, the inoculations being made by needle puncture. On August 10, when the inoculations were examined for the last time there were no diseased spots except on two of the pods in-
oculated with the cotton anthracnose and these spots were small, not more than a centimeter in diameter, though they were producing spores. About thirty other pods inoculated with the same culture showed no infection.

Experiment July 14 and 15, 1911. Inoculated cotton bolls with pure cultures of the anthracnososes from the bean, pepper, apple, Manihot, cottonwood, Melilotus, and cotton. About forty bolls were inoculated with each fungus, the inoculations being made by needle puncture. These bolls were examined from time to time and their appearance noted.

Bolls inoculated with the cotton anthracnose showed the effect of the fungus in a very few days. A dead spot developed around the point of inoculation and this gradually spread over a large area of the boll. The capsule tissue turned black and dried up and spores were produced very abundantly. The disease spread to the inside of the boll and destroyed the lint and seed or else changed it to a rather dark solid mass. All of the locks in the boll were affected. The other anthracnoses developed but very little on the bolls. Th dead tissue around the point of inoculation merely dried up and there was no spreading out into the adjacent tissue. If the needle passed clear into the lint, the latter was generally discolored and became slimy, but this was only in the single lock inoculated. This condition, however, always follows a puncture with a sterile needle. Intumescence tissue or proliferations also developed abundantly in these bolls, while they were entirely absent from the bolls inoculated with the cotton anthracnose. The appearance of some of the bolls on July 21 is shown in Plate IV, Figure 1. As may be seen, only the boll inoculated with the cotton anthracnose (d) shows the disease.

Experiments July 22 and 31, 1911. Healthy cotton seed was treated with spores of the different anthracnoses and then planted in the greenhouse. The spores were immersed in a heavy suspension of the spores in water so that they were thoroughly inoculated. Two different sets of seed were used, but as the results with the two were practically identical, they are combined in the table below. As the young plants came up they were watched and if any showed any traces of leaf spots or stem rots, they
were examined for the presence of spores. The following table shows the percent of germination and the percent of the young plants that were diseased.

**Table 19.**

**Effect of Different Anthracnoses on Cotton Seed.**

<table>
<thead>
<tr>
<th>Anthracnose</th>
<th>No. of seed</th>
<th>No. germinated</th>
<th>% germinated</th>
<th>No. diseased</th>
<th>% diseased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton ......</td>
<td>150</td>
<td>128</td>
<td>85%</td>
<td>41</td>
<td>32%</td>
</tr>
<tr>
<td>Fig ..........</td>
<td>50</td>
<td>41</td>
<td>82%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Mellilotus ..</td>
<td>150</td>
<td>142</td>
<td>95%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Pepper ......</td>
<td>150</td>
<td>135</td>
<td>90%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Apple ......</td>
<td>150</td>
<td>130</td>
<td>87%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Bean ..........</td>
<td>50</td>
<td>45</td>
<td>90%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Manihot ......</td>
<td>150</td>
<td>126</td>
<td>84%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Check ......</td>
<td>150</td>
<td>142</td>
<td>95%</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

In these experiments it is seen that only the seed that were inoculated with the cotton anthracnose itself developed diseased plants, the other fungi evidently being unable to infect the young cotton plants.

The results of all of the above inoculation experiments point conclusively to one thing, and that is that the cotton anthracnose is at least physiologically distinct from the other common anthracnoses. The other anthracnoses will not infect the cotton plant, and the cotton anthracnose will not grow readily on other plants. To be sure, it will attack a few plants to some degree, as, for instance, the Arabian alfalfa and okra, but it does not grow with the vigor on these plants that it does on cotton. Taubenhaus (70) in a recent paper has also shown that the cotton anthracnose will attack the foliage of the sweet pea and cause a blight. This does not show, however, that the fungus is not distinct from the other anthracnoses. The inability of the other forms to attack the cotton plant shows that the cotton fungus is distinct in some way from these. It is not surprising that the cotton anthracnose would attack the sweet pea and it is possible that other plants will be found which can be inoculated by this form while, they are in a tender vegetative condition, especially if the plants are kept in the laboratory or greenhouse, where the relative humidity is high. The cotton anthracnose may
have developed from the apple bitter rot fungus or it may not, a point that is immaterial to the question at present. As it exists today, it is certainly widely divergent from the other forms in many of its physiological characters and to some degree in its morphological characters, and it seems to the writer that it should be kept distinct from the others.

**THE DIPLODIA ROT.**

The Diplodia rot is chiefly a disease of the bolls, though occasionally the stems of the cotton plant are affected. The disease is caused by the fungus *Diplodia gossypina* Cke.

**PREVIOUS WORK ON THE DISEASE.**

The fungus, *Diplodia gossypina*, was described in 1879 by Cooke (18). His description is as follows:


Whether this was the original description is perhaps questionable. It is possible that this fungus is the same as some of the Diplodia species described from other hosts. This point however, has not been studied. The fungus was again described by Ellis and Bartholomew (32) from cotton stems from Alabama as *Botryodiplodia gossypii*. Some of this material was distributed as No. 1510, Fungi Columbani. The spores illustrated in Plate XIII, Figure 2, are from some of this material and are seen to be identical with those of *Diplodia gossypina* (Plate XIII, Fig. 1) from the bolls.

Atkinson (10) reported *Diplodia gossypina* as occurring on cotton bolls in Alabama, but he makes no mention of it doing any damage.

Shear (60) also reports *Botryodiplodia gossypii*, stating that it is the conidial stage of *Botryosphaeria fuliginosa*. Experiments at the Louisiana station, however, show that a Macrophoma is the conidial stage of the Botryosphaeria (see Botryosphaeria rot in following pages).
Practically nothing has been written of this fungus from a pathological standpoint. Evidently it has been considered as only one of the minor troubles and no attention has been paid to it. This may be due to the fact that the fungus is not so abundant in the eastern states as it is in Louisiana. In the latter state it certainly does enough damage to be classed among the important cotton fungi. Practically nothing is known of the distribution of this disease throughout the cotton-growing countries as there is nothing written on it. The fungus is well distributed throughout the state of Louisiana.

LOSS CAUSED BY THE DISEASE.

There are no published data on the loss caused by this disease in the United States. During the past few years, some data have been collected at the Louisiana station regarding the loss in this state alone and these have been given in Table 1 on a previous page. This table shows a considerable loss throughout the state due to this disease. While there is considerable variation in the amount of the disease in different localities and in different fields, it is probable that a loss of 2% would be a conservative estimate for the whole state. In some localities, the loss would run under this, while in some it would run higher. Occasionally one notices fields where the loss would run around 10%. The disease is worse in the alluvial soils and bluff lands than it is in the hills of north Louisiana. A loss of 2% on a 350,000-bale crop would mean a loss of 7,000 bales, or a money loss, figuring cotton at 10 cents, of $350,000.

THE DISEASE ON THE BOLL AND STEM.

The Diplodia rot is principally a disease of the bolls. The appearance of these bolls (Plate VII) is very characteristic. For about a day after the infection first shows the diseased epidermis is smooth and of a brown color. But the fungus develops so rapidly that bolls in this condition are not often seen. Very soon the boll takes on a black appearance and becomes papillate with the very numerous pycnidia. The spores develop rapidly and on coming out of the pycnidia form a smutty coating over the bolls. If a boll is touched with the hand, the spores
cling to the fingers like the spores of certain smuts. The boll also dries out very rapidly and becomes more or less brittle. If the boll is attacked before it is half-grown, it does not open, though sometimes there is a slight splitting apart of the carpels at the tip. If the boll is attacked when it is older, it sometimes partially opens, exposing the hard, black masses of lint and seed. The fungus grows into the lint and seed and destroys them, turning the entire contents of the boll into a black, hard mass. These black masses are very seldom picked, and if they are, they are worthless, as the lint is weak and is torn to pieces in the gin. The seed is also worthless. The kernel is usually destroyed so the seed will not germinate. These diseased bolls dry up on the plant and do not fall off, so that it is very easy to find them in the field after all of the cotton has been picked, or even in the following spring if the plants have not been cut and destroyed.

The fungus may also attack the lower part of the stem of the cotton plant in the fall. Pycnidia develop and the stem becomes black, having a similar appearance to the boll. However, it is questionable whether the fungus is not more of a saprophyte on the stem than a parasite.

The disease is not known on the other parts of the cotton plant.

MICROSCOPIC APPEARANCE OF DISEASED BOLLS.

When the fungus gains entrance to the boll tissue in an insect puncture or wound of some kind, it does not usually spread in all directions from this injury, as is generally the case with the other boll rot organisms. If the spores are placed in a needle puncture, the fungus does not seem to be able to attack the living cells around the puncture. It first starts a rot on the inside of the boll, the fungus threads growing rapidly in all directions in the immature lint and seed until they come to the line of dehiscence between the carpels. At this place there is a break in the hard internal lining of the boll and there is a thin fold of parenchymatous tissue which extends for a short distance inwards. In Plate IX, Figure 1, is shown a section across the line of dehiscence in a healthy boll, showing the thick internal
lining of the boll and the fold of delicate abscission tissue. The fungus threads are not able to penetrate the boll lining, but they very readily attack this fold of parenchymatous tissue. The cells are killed by some secretion of the fungus threads and the fungus enters the dead cells. It seems probable that the fungus only enters the cells that have been killed by the secretion and this is probably the reason why the disease does not spread from the point of infection. After the fungus enters the carpel tissue that has been killed by the toxic secretion, it spreads very rapidly and soon breaks out to the surface. In Plate IX, Figure 2, is shown a cross section of the line of dehiscence of a boll showing the first stage of the disease, and this may be compared with the illustration from the healthy boll. The fold of parenchymatous tissue is entirely destroyed and the disease is seen to be spreading in the carpel tissue as shown by the dark color. The diseased tissue of the carpel always turns brown in color even before it is invaded by the fungus threads, and often many of the dead cells have the protoplasmic contents precipitated around the cell wall (Plate IX, Fig. 3). The fungus threads enter these dead cells and disintegrate the tissue very rapidly. By the time the fungus is fruiting, the mycelial threads make up a large percent of the entire mass.

**SCRIPION OF THE FUNGUS.**

The fungus, *Diplodia gossypina*, is a member of the order *Sphaeropsidales*. Only two stages of the fungus are known, the mycelial and the pycnidial. No ascogenous stage has ever been recognized as belonging to this fungus.

**The Mycelium.**

There is nothing very characteristic about the mycelium of this fungus. The threads are at first hyaline, but very soon become of a brown color. These brown threads are very abundant in the older diseased portions of the boll.

**The Pycnidial Stage.**

The pycnidia or fruiting organs of the fungus are black, flask-shaped structures which produce the spores. They are
generally somewhat subglobose or conic in shape, though often they are more or less irregular. On cotton bolls in the field under normal conditions, the pycnidia (Plate VIII, Figures 3 and 5) develop underneath the epidermis and become erumpent, generally only the beaks or ostioles of the pycnidia being exposed to the surface. However, if the bolls are placed in a moist chamber, the pycnidia will develop on the surface (Plate VIII, Figure 4). Figures 1-5 of Plate VIII show pycnidia of this fungus that have developed under different conditions. These show the variation in size and shape. A short beak may either be present or lacking. The beak, however, is usually present on pycnidia that have developed in cultures (Plate VIII, Figure 2).

The conidiophores arise from pseudoparenchymatous tissue around the base and the sides of the pycnidium. These are short, simple, and hyaline. The spores are cut off singly from the tips of the conidiophores. The spores (Plate VIII, Figure 1) are at first oblong-elliptical, hyaline, and one-celled, but later they take on a deep brown color and become two-celled, and occasionally three-celled. If the fungus is developing rapidly, the spores are pushed out of the pycnidium in the single-celled and hyaline state. With bolls in a moist chamber, it is not uncommon to see long white strings of these single-celled spores above the ostioles of the pycnidia. These hyaline spores germinate readily without turning dark colored or becoming septate (Plate VIII, Figure 4). However, if the boll is kept in a moist condition, these spores turn black and become septate. Under ordinary field conditions, the spores are usually black and two-celled as they come out of the pycnidia. The spores are about 20-33 x 12-7 microns in size. Plate XIII, Figure 1, shows spores from Diplodia gossypina from bolls, and Figure 2 shows spores from a stem of the cotton plant. These latter spores were taken from material distributed by Ellis and Bartholomew as Botryodiplodia gossypii. The spores show that the two fungi are the same. The spores develop in the pycnidia very soon after the boll becomes affected and continue to develop for several months if the plants are left in the field. Fresh spores can be found on the bolls during the winter and the following spring.
If dried up diseased bolls are brought in from the field in the spring and placed in moist chamber, fresh spores develop very rapidly.

THE COURSE OF THE DISEASE.

The course of the disease seems to be quite simple. The spores develop very abundantly on the diseased bolls. These spores are very light and are blown about the field by the wind or are carried by insects which feed in the diseased bolls. These spores find their way to other bolls and if there are any insect punctures or injuries on these bolls, they very readily germinate and infect them. The fungus does not seem to be able to infect the boll through the uninjured epidermis, and this is very fortunate for the southern planter. If the fungus could penetrate the uninjured epidermis of the boll, this disease would take practically all that the boll weevil leaves. There is no other fungus, not even the anthracnose, that attacks the boll with such vigor after it has once gained an entrance to the tissue.

The disease gradually increases in the field during the summer and fall months, and by the time winter sets in there are a large number of diseased bolls on the plants. These bolls are not picked and spores keep on forming in the pycnidia during the winter. Spores also keep on forming on old bolls that have been broken off and are lying on the ground. It is also probable that the fungus grows saprophytically on various parts of the cotton plant during the winter and perhaps also on other dead plants. By these means the fungus is enabled to keep alive until the following year, when the bolls again begin to form. The spores of the fungus are also long lived, as those that are formed in the fall are still able to grow in the following spring. After the bolls begin to form again the following summer, these old spores and the spores that have developed on the old bolls and stems are blown to them by the wind or carried by insects. These again start the disease and it spreads through the field for the rest of the season. There are two weak points in the life history of this fungus—first, the inability of the fungus to infect the uninjured epidermis, and, second, the apparent inability of the fungus to be carried through the winter on the
seed. Seed that are attacked by the fungus are not able to germinate. To be sure, there are often a few Diplodia spores on the lint of the seed, but these are not able to infect the young plant and it is not likely that they have anything to do with the infection of the bolls the following year.

THE FUNGUS IN PURE CULTURE.

*Diplodia gossypina* is very easy to culture either by making dilutions of the spores in plates or by transferring fragments of the diseased boll to tubes. The fungus is a very rapid grower in pure culture, generally covering a petri dish in three to five days or sometimes even sooner. The fungus forms a dense growth of dark-colored mycelium on most of the ordinary culture media. The mycelium is, of course, at first hyaline, but gradually changes to a brown color. Pyenidia generally develop in cultures after a few days, these developing in small clumps scattered over the surface (Plate VIII, Figure 1). These develop a few spores, but never in abundance like they develop in pyenidia on the bolls. The pyenidia do not develop well on artificial media if the percent of acidity is high, though the mycelium will grow on agar that tests +38 by Fuller's scale. The spores germinate very readily in artificial media or in water, generally sending out a germ tube in about three hours if the weather is warm.

RESISTANCE OF SPORES TO DESICCATION.

On account of the long period from fall until the time when bolls again begin to form the following summer, it would seem probable that the fungus spores must retain their ability to germinate also for a considerable time. If the spores were short lived, the fungus would have difficulty in maintaining itself through the winter and spring months. Some experiments were carried on to see how long the spores would remain alive while dried. These were carried on in two ways: by letting the spores dry down on sterile cover slips, and also by preserving the diseased dry bolls with spores. The spores were placed in sterile agar after definite periods and the ability of the spores to germinate was determined.
The experiment with cover slips was started on July 6, 1911. Spores were taken directly from a diseased boll and placed in a drop of sterile water and allowed to dry to the glass. Some of these slips were cultured at stated intervals. The results of this test are given in Table 20.

### Table 20.

<table>
<thead>
<tr>
<th>Time dried, Days</th>
<th>No. of Plates</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 days</td>
<td>1</td>
<td>100% germination.</td>
</tr>
<tr>
<td>13 days</td>
<td>1</td>
<td>90% germination.</td>
</tr>
<tr>
<td>25 days</td>
<td>1</td>
<td>70% germination.</td>
</tr>
<tr>
<td>43 days</td>
<td>6</td>
<td>Fair germination.</td>
</tr>
<tr>
<td>56 days</td>
<td>1</td>
<td>40% germination.</td>
</tr>
<tr>
<td>67 days</td>
<td>4</td>
<td>Fair germination in 2 plates, none in others.</td>
</tr>
<tr>
<td>75 days</td>
<td>4</td>
<td>2 plates, slight germination; others none.</td>
</tr>
<tr>
<td>78 days</td>
<td>4</td>
<td>Few colonies in each plate.</td>
</tr>
<tr>
<td>84 days</td>
<td>4</td>
<td>One plate, 30 colonies; others none.</td>
</tr>
<tr>
<td>90 days</td>
<td>4</td>
<td>One plate, 15 colonies; others none.</td>
</tr>
<tr>
<td>95 days</td>
<td>8</td>
<td>One plate, 20 colonies; others none.</td>
</tr>
<tr>
<td>101 days</td>
<td>8</td>
<td>Five plates had a few to germinate.</td>
</tr>
<tr>
<td>115 days</td>
<td>4</td>
<td>Slight germination in two plates.</td>
</tr>
<tr>
<td>130 days</td>
<td>3</td>
<td>Germination doubtful.</td>
</tr>
<tr>
<td>136 days</td>
<td>4</td>
<td>Some spores showed germ tubes.</td>
</tr>
<tr>
<td>151 days</td>
<td>4</td>
<td>About 20% of spores showed germ tubes.</td>
</tr>
<tr>
<td>160 days</td>
<td>3</td>
<td>A few spores showed germ tubes.</td>
</tr>
</tbody>
</table>

The spores that were dried in this manner on cover slips showed fair germination for from one to three months. The spores did not germinate well after they had been dried over 100 days. The table shows that some of them still germinated at 160 days, but the germination was not typical. A short germ tube was put out by the spore, but this did not grow into a colony. It stopped growing after it had reached a length of a few microns.

Two sets of diseased bolls were dried in the laboratory and the spores cultured from time to time. The first set was dried about November 1, 1910. The spores were cultured at the end of the following periods: 3.5 months, 6 months, 8 months. There was good germination in every test, though perhaps the percent of germination was slightly less at the end of the 8 months period. Unfortunately these spores were not again cultured, but this shows that the spores were capable of living through the winter.
and at least up until the first of July. If these spores had been in the field, they would have still been viable when the young bolls started to develop.

The second set of bolls were brought into the laboratory in August, 1911. They were thoroughly dried, and on August 18 put in small wooden boxes. Spores were cultured from time to time with the following results.

**Table 21.**

<table>
<thead>
<tr>
<th>Time dried</th>
<th>No. of Plates</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 days</td>
<td>1</td>
<td>98% germination.</td>
</tr>
<tr>
<td>33 days</td>
<td>2</td>
<td>Good germination.</td>
</tr>
<tr>
<td>43 days</td>
<td>2</td>
<td>Good germination.</td>
</tr>
<tr>
<td>61 days</td>
<td>2</td>
<td>Good germination.</td>
</tr>
<tr>
<td>88 days</td>
<td>2</td>
<td>Good germination.</td>
</tr>
<tr>
<td>137 days</td>
<td>2</td>
<td>Good germination.</td>
</tr>
<tr>
<td>157 days</td>
<td>2</td>
<td>60% germination.</td>
</tr>
<tr>
<td>187 days</td>
<td>2</td>
<td>40% germination.</td>
</tr>
<tr>
<td>207 days</td>
<td>2</td>
<td>20% germination.</td>
</tr>
<tr>
<td>247 days</td>
<td>2</td>
<td>Fair germination.</td>
</tr>
<tr>
<td>279 days</td>
<td>2</td>
<td>Few spores germinated.</td>
</tr>
<tr>
<td>297 days</td>
<td>2</td>
<td>Germination feeble; germ tubes short.</td>
</tr>
<tr>
<td>311 days</td>
<td>2</td>
<td>Germination feeble; germ tubes short.</td>
</tr>
<tr>
<td>370 days</td>
<td>8</td>
<td>Germination feeble; a few short tubes.</td>
</tr>
</tbody>
</table>

This table shows that some of the spores were able to live for nine months in a dry condition and germinate, but after that length of time the spores lost their ability to do more than merely send out short germ tubes.

From these tables and experiments it is seen that this fungus can live from the fall until the following summer if necessary. Even if the cotton plants were all destroyed in the fall, we could look for the disease to break out the following year on account of the spores that were formed the preceding fall. But if the cotton plants are left in the field until spring, there will be more of the spores and there would be a better chance for infection when the bolls again formed.

**INOCULATION EXPERIMENTS.**

A large number of inoculation experiments have been carried on with this fungus. These have included puncture inoculation
in the bolls, inoculation of the bolls without injury, flower inoculations, puncture inoculations in stems, and inoculation of the seed before planting.

**Inoculation of Bolls by Puncture.**

During the course of this investigation, several thousand bolls have been inoculated by needle puncture, using both pure cultures and spores taken directly from the diseased bolls. These inoculations have been made both in the field and in the greenhouse. Practically every inoculation has been successful. The fungus would rot the lock inoculated and then break out to the surface of the boll on the line of dehiscence between the carpels. In a very few days after the inoculation, the pycnidia and spores would be forming and the boll would be entirely affected. In the greenhouse, the fungus would often grow back on the stem of the boll and into the branch of the cotton plant, forming a small canker around the point of attachment of the boll. The greater humidity of the greenhouse probably made the plant tissue softer and also furnished better conditions for growth.

**Inoculations of the Bolls Without Injury.**

Many bolls have been inoculated by spraying them with a suspension of Diplodia spores in water. Usually these bolls were covered with paper sacks after the inoculation to prevent drying out and also to prevent outside infection. No satisfactory positive results were obtained in any case. Occasionally with old bolls a few bolls would take the disease; but the percent was so small that it was very probable that these happened to be bolls with small insect punctures on them. In the summer of 1911, 197 bolls just one day from the flower were inoculated in this manner. These bolls were so small that they were practically free from insect punctures. In no case did the Diplodia disease develop on any of them. From all the experiments, it would seem that the disease does not infect the boll normally through the uninjured surface.
FLOWER INOCULATIONS.

In the summer of 1911, 130 flowers were inoculated with the Diplodia by dropping a suspension of the spores in them about the time the flowers were closing. In no case did the bolls that developed from these flowers show any sign of the disease. However, many of the old flower parts and the young bolls that were shed developed a good growth of the fungus. The fungus was able to grow on the dead tissue, but could not infect the living tissue of the young boll.

INOCULATION OF BRANCHES BY PUNCTURE.

In the greenhouse, a number of attempts were made to inoculate the disease into the young branches of the cotton plants by needle punctures, but these were not very satisfactory. Occasionally the fungus would grow for a very short distance from the point of inoculation and fruit, but the growth was very weak. The stem did not seem to be injured to any extent.

INOCULATION OF SEED.

In order to see if there is any chance of the disease being carried over from year to year on the seed, seed have been treated with a suspension of spores and planted in the greenhouse. One hundred seed were used, eighty-four of the seed germinated and grew into young plants. These were kept for several weeks without the Diplodia showing at any time on any part of them. Also seed from diseased bolls have been used a number of times for planting. Usually these seed did not even germinate, but occasionally, if the seed were taken from bolls that were not badly rotted, some of them would grow. However, the young plants were always perfectly healthy and continued so.

THE FUSARIUM ROT.

A species of Fusarium is very commonly found on cotton bolls, especially in wet weather (Plate IX, Figure 4). This species has not been determined, but it seems to be the common one that grows on a wide range of organic matter, perhaps the one that is commonly called *Fusarium roseum*. While the fungus
is very common on rotting bolls, as may be seen by the figures in Table 1, it does not usually cause a great amount of damage. It grows chiefly as a saprophyte, growing on the old parts of the bolls that have been killed by insects or by other fungus diseases. Occasionally, however, it will grow from the dead portions into the living parts of the boll and become a facultative parasite. The greatest damage that it does is to grow into the mature or nearly mature lint and discolor it. The lint may also be slightly decayed so that it is weak and breaks badly during ginning.

A boll affected with this fungus can often, though not always, be told in the field by the pink covering of spores. The spores wash off readily by rains and then a microscope is necessary to tell whether it is present. There is usually nothing characteristic about the rot. The fungus follows up the other rots and insect attacks and does not form a characteristic rot by itself. While the anthracnose also produces a pink covering of spores, the pink color of the Fusarium is of a lighter shade and the surface of the diseased spot is usually not slimy.

The Fusarium, like the anthracnose, is able to grow down into the kernel of the seed and remain in a dormant condition over the winter. The spores of the fungus also remain alive on the lint on the seed. When the seed is again planted in the spring, the mycelium in the kernels form spots on the cotyledons (Plate IX, Figure 5) and the spores also germinate and infect the young plant. A large percent of the young seedlings in the spring often have the Fusarium spots on the leaves or stems.

INOCULATION EXPERIMENTS.

Many inoculation experiments have been tried with this fungus, both in the flowers and on the bolls. Inoculation made by spraying a suspension of the spores in the flowers or on the bolls have always given negative results, showing that the fungus cannot cause an infection without a previous injury. Furthermore, inoculations made by puncture in the bolls have not produced good infection. It would seem that the fungus must have enough dead tissue to get a good start before it can infect the boll.
COURSE OF THE DISEASE.

The course of the disease is quite similar to the anthracnose. The fungus spreads from boll to boll in the summer and fall by the rain, winds and insects. It passes the winter by the spores that find lodgment in the lint on the seed and also by the mycelium that enters the kernel of the seed. When the seed is planted in the spring, the mycelium within the seed starts to grow, as the seed absorbs water, and develops spots on the cotyledons. Also, the spores on the seed germinate and infect the young plants. The young stems of the plants are often affected, especially if the weather is very moist (Plate IX, Figure 5). The stems are rotted off and the young plants die. This is not common in the field, though it is very readily brought about if the seed are planted under bell jars in the greenhouse, where the humidity of the surrounding air is very high. Spores are developed in abundance in these spots on the young plants and are carried to other parts of the plants. In any injury on the plant or on any dead part, the fungus will grow and produce spores. The fungus does not attack the living tissue of the stem or leaves, but there are always enough injuries and dead parts to keep the fungus alive until the bolls again begin to form.

NUMBER OF SPORES ON THE SEED.

Estimates of the number of Fusarium spores were made from two lots of seed, the same as those used in the anthracnose experiments (see Table 5). The first lot of seed was obtained from a cotton gin at Baton Rouge; 300 seed, weighing 34.7 grams, were picked out at random. The number of spores was estimated in the same manner as with the anthracnose spores (see Table 5). On these seed were 3,600,000 Fusarium spores. The second lot of seed was from some picked on the Experiment Station farm. Only the cotton from apparently healthy appearing bolls was picked; 300 of these seed, weighing 35 grams, had 1,000,000 spores in the short lint fibers.

These seed were germinated in sterilized test tubes with the following results:
From these experiments it is seen that ordinary cotton seed carries a large number of Fusarium spores and that these spores are capable of infecting the young plants as the seeds germinate.

**THE RHINOTRICHUM ROT.**

A species of Rhinotrichum, probably *Rhinotrichum tenellum* B. & C., is also often quite common on cotton bolls in the field, though it does not do a great amount of damage. The fungus only attacks bolls which are quite badly rotted with other fungi. The bolls attacked by this fungus (Plate XI, Figures 5 and 6) are very characteristic in appearance. They become covered with a heavy dense mat of white mycelium, sometimes a couple of milimeters in thickness. The fungus grows on the carpel tissue and also in among the lint fibers. It also grows through the seed coats into the kernel. However, the affected seed does not carry the disease over the winter. The young cotyledons are never affected with the fungus.

The fungus is a member of the *Hyphomycetes*. The conidiophores are produced in the mycelial mat on the surface of the boll. The conidiophores (Plate XIII, Figure 5) are long and somewhat enlarged at the apex, where the spores are borne. The spores are borne on short sterigmata which remain after the spores drop, giving the conidiophores a very characteristic appearance. The conidiophore proliferates so that there may be several of the enlarged cells with the sterigmata. The spores (Plate XIII, Figure 6) are single celled, hyaline, elliptical to ovate, 9-15 x 13-20 microns. This fungus has been reported on cotton bolls by Atkinson (10) from Alabama. This fungus grows readily in pure cultures, forming a dense white mat on the media, and producing an abundance of spores.

Inoculation experiments in healthy bolls, even by puncture, have not been successful, showing that the fungus is more saprophytic than parasitic. The fungus does some damage, however, in destroying and discoloring the lint.
THE ÖLPITRICHUM ROT.

The fungus *Olpitrichum carpophilum*, Atk., was first described from cotton bolls by Atkinson (8). The fungus is not as abundant as the Rhinotrichum, though it is not usually difficult to find. Bolls affected with this fungus (Plate XI, Figure 1) look quite similar to those affected with the Rhinotrichum. There is formed a dense mat of mycelium over the diseased portions of the boll. However, this mat of mycelium is of a buff color in contrast to the white of the Rhinotrichum. This fungus only grows saprophytically on dead and diseased portions of the boll, but it does do a small amount of damage in discoloring and destroying the lint.

This fungus is also a member of the Hyphomycetes and perhaps quite closely related to the Rhinotrichum. However, instead of the small sterigmata, there are formed rather large flask-shaped structures (Plate XIII, Figure 10) on which the spores are borne. The spores (Plate XIII, Figure 8) are single celled, hyaline, elliptical to ovate, very irregular in size, 20-40 x 13-28 microns. The fungus grows fairly well in pure culture, but not as rapidly as the Rhinotrichum.

THE BOTRYOSPHAERIA ROT.

A fungus which has been provisionally determined as *Botryosphaeria fuliginosa* (Mongeot & Nestler) E. & E., is occasionally found on cotton bolls in Louisiana. Atkinson (10) also collected some of this fungus in Alabama and sent it to Masse for determination, who determined it as *Botryosphaeria berengeriana* DeNot. However, Ellis and Everhart (33) give this name as a synonym of *Botryosphaeria fuliginosa*. It is probable that the latter name is not the correct one from the point of priority, but the question of nomenclature will not be considered.

Two stages of this fungus have been found in Louisiana, as has been reported in a previous article (30), a pycnidal stage belonging to the genus *Macrophoma*, and the perfect or ascozogenous stage. The pycnidal occurs during the summer and is followed by the ascozogenous stage in the fall. The boll affected with this fungus turns black, dries up, and become covered with the fruiting pustules.
The pycnidia (Plate X, Figures 3 and 4) develop profusely on the surface of the diseased boll, usually almost superficial, though sometimes they may have the base slightly surrounded by fungal or host tissue. They are black in color and about 110-300 x 140-210 microns in size. The spores (Plate XIII, Figure 11) are developed abundantly on short conidiophores and are pushed out of the pore at the apex of the pycnidium. If weather conditions are favorable, these spores remain in white strings protruding from the pycnidia. The spores are hyaline, one-celled, cylindric to slightly ellipsoid or ovoid, coarsely granular, 14-33 x 7-10 microns, usually about 21-25 x 8 microns. The spores are never septate nor dark colored and are much more variable in shape and size than those of Diplodia gossypina.

The perithecia are borne in a manner similar to the pycnidia and are quite similar in appearance (Plate X, Figure 4). They are black in color and are about 190-360 x 250-320 microns in size. A short beak may be present or lacking; if present, it may have a length up to 100 microns. The asci (Plate XIII, Figures 13 and 14) are fairly abundant in the perithecia, though not crowded. They are large, about 100-130 x 20-25 microns, and have very thick walls, especially near the apex. The ascospores (Plate XIII, Figure 15) are hyaline, ellipsoid, slightly granular, and about 20-27 x 10-16 microns in size. The small threadlike paraphyses are very abundant.

The identity of the two forms has been proven by inoculation experiments. The fungus takes readily on bolls by puncture inoculations. The fungus has been cultured both from conidia and ascospores and the disease again produced by inoculation with both cultures. Shear (60) believes that the form of Botryosphaeria fuliginosa that occurs on branches of various trees is connected with a Sphaeropsis or Diplodia stage and also that the imperfect stage is the same as Diplodia gossypina. The experiments in Louisiana have failed, however, to bear out this contention. It is very possible, however, that the Botryosphaeria occurring on branches of different trees is specifically different from the one on cotton bolls.
THE SCHIZOPHYLLUM ROT.

A very peculiar and interesting rot (Plate X, Figure 1) was found once in a field near Baton Rouge in the summer of 1910. Some twenty or thirty bolls were found within a small radius that were affected with Schizophyllum alneum (L.) Schroet. This is a fungus that occurs usually on dead branches, and occasionally as a parasite upon some trees, but it has not been known to be parasitic on soft herbaceous plants, such as cotton. The fungus was growing parasitically on the bolls and not as a saprophyte on dead bolls. A number of cases were noted where the sporophores of the fungus were pushing out through the lines of dehiscence between the carpels of perfectly green bolls. It is very probable that the bolls became affected through insect punctures or other injuries. This disease is of no importance except from a scientific standpoint.

OTHER FUNGI ON COTTON BOLLS.

Besides the fungi that have been mentioned in the previous pages, a number of others are sometimes found on cotton bolls. These, however, are mostly saprophytic and are of very little importance as boll rots. Some of them are found commonly on decaying bolls, while others are only rarely seen. It seems best to make a mention of the ones that have been seen in Louisiana.

A species of Volutella is very common on the outside of the old bolls in Louisiana, especially in wet weather. The fungus fruits abundantly and the abundant setae which develop are sometimes mistaken for those of the anthracnose. The spores of the Volutella, however, are curved and cannot be mistaken for the anthracnose. The fungus merely helps in the distintegration of the boll tissue, growing saprophytically. The species of the Volutella has not been determined.

Sclerotium rolfsii Sacc., a fungus which is not known to produce spores of any kind, is occasionally found on bolls as a true parasite (Plate XI, Figure 2). This is only found on bolls that touch the ground. The fungus lives in the ground and attacks a wide range of plants. If it comes in contact with a boll that touches the ground, the mycelium very soon runs over and through it and forms the sclerotia over the surface. The boll
turns brown in color and does not open. No spore stage is known for this fungus, yet it seems very certain that it belongs to the group of the Basidiomycetes. Clamp connections are formed very abundantly on the mycelium and these are only known in this group.

A species of Nectria has been found once on rotting cotton bolls in Louisiana. A specimen was sent to Professor F. J. Seaver, who wrote of it as follows: “Your specimen corresponds in gross appearance with Nectria brassicae Ellis and Sacc., which species has been reported on various (mostly herbaceous) plants and occurs as far south as Louisiana. The spores, however, vary considerably and I am not sure but that your species may be distinct.” The fungus is of no importance as a boll-rotting organism.

The common Rhizoctonia, or, as it is now known, Corticium vagum, var. solani Burt, occurs sometimes on cotton bolls, especially on those that touch the ground. It causes a disintegration of the whole boll tissue. Its rarity, however, makes it of no importance as a boll rot.

Rhizopus nigricans, one of the black moulds, occurs very frequently on bolls that have been first attacked by other fungi.

Other fungi which have been seen but not studied are species of Penicillium, Alternaria, Mucor, Periconia, Stilbella, Aspergillus, Sphaeronomella (Plate XI, Figure 4) Cephalothecium, Trichothecium (Plate XI, Figure 8), Sporotrichum (Plate XI, Figure 7), and Phyllosticta (Plate XI, Figure 3).

CONTROL OF COTTON BOLL ROTS.

Although the different boll rot diseases cause a great loss to the cotton industry, very little has been done in the past to control them. Many farmers believe that the rotting of the bolls is one of the things that cannot be helped. The diseases are not easy to control, yet it is believed that the percentage of rot can be decreased if the proper precautionary methods are followed. Each disease has weak points in its life history and if advantage is taken of these, certain methods of management will tend to decrease the rot. There is no simple cure-all for these troubles. The only hope is that the farmer will be able to pre-
vent the outbreaks or epidemics which so often occur. There are a number of things which must be considered and these will be treated separately.

DESTRUCTION OF STALKS.

As a number of the diseases live over the winter on the dead parts of the cotton plants, the destruction of the stalks will tend to decrease the amount of infectious material in the fields. Some farmers make a practice of doing this anyway, as it is recommended for the control of the boll weevil.

ROTATION OF CROPS.

While it is impossible to destroy all infectious material in a field by the destruction of the stalks, it can be more nearly accomplished by a careful rotation of crops. If cotton is not grown in a field for a year, some of the very destructive diseases, such as the anthracnose, will be entirely killed out before the following year. The rotation of crops will not only put the ground in better shape and make it more fertile, but will leave the field freer of disease germs.

CULTIVATION AND MANAGEMENT OF THE CROP.

Since the arrival of the boll weevil, much has been written on the methods of planting and cultivating cotton. The same methods which are advocated for the control of the weevil are the best for the control of the boll rots. Small, quick-maturing cotton that is cultivated frequently to insure a rapid growth is less affected by boll rots than the large rank-growing late cottons.

DESTRUCTION OF INSECTS.

Insects aid the boll rots in two ways: by puncturing the bolls, thus forming injuries that aid infection, and also by carrying the boll rot organisms from plant to plant. Insect control does not come under the scope of this bulletin, but everything possible should be done to free the fields of insect pests. This does not include the boll weevil alone, but also a number of other sucking insects which puncture the bolls.
SELECTION OF COTTON FOR SEED.

As some of the diseases are carried from year to year in the seed, special care should be taken in saving seed. The selection of cotton seed is a phase of the cotton industry which has been slighted too much in the past in the cotton-growing states. Every planter realizes that care should be taken in selecting corn for seed, but when it comes to his cotton crop, nearly anything goes. His cotton is picked from wilted plants, from plants badly affected with the boll rots, from ill-shaped plants, from poor producing plants, and from good plants, and it is all ginned together and the seed planted the following year. To be sure, there are difficulties in the selection of cotton seed. Perhaps the most important is the trouble in getting a small amount ginned separately. But it is believed that it is possible to get around these difficulties. If the cotton industry is to thrive as it should, more care must be exercised by the planter in saving his seed. The farmer cannot depend on some seed-grower in another state or locality to grow his seed for him, because the chances are the varieties that thrive in another section of the country are not the best for him. Each farmer should select his own seed and see that seed is only saved from prolific, early, and healthy plants. This means that the picking of the cotton that is to be saved for seed must be done with intelligent labor.

In selecting cotton for seed, several things must be kept in mind.

1.—The cotton must be early. If the planter selects his cotton from the first picking each year, he will gradually eliminate the late-maturing cotton.

2.—The cotton must be selected from plants free from the wilt or black root disease. As wilted plants mature their cotton earlier than healthy plants, special care must be taken in the first picking. Any plant that shows any signs of the wilt should not be saved for seed.

3.—The cotton seed should come from prolific and well-shaped plants.

4.—Cotton seed should not be saved from bolls that show any sign of a rot. It is not believed that it is possible to entirely eliminate the boll rots by seed selection, but they can be reduced.
The method for seed selection should be about as follows: When the cotton is ready for the first picking, a man able to recognize the cotton wilt and other diseases and also who can be depended upon to pick the cotton from only early prolific plants, should pick over the field in advance of the rest of the pickers. He should pick the cotton from only the good, healthy plants, leaving the rest for the other pickers. The cotton which he picks should be ginned separately and the seed saved for planting, while the seed from the rest of the cotton can go to the oil mill. Any one that is not thoroughly acquainted with the cotton wilt should write to the Experiment Station for Bulletin 96 on this subject.

USE OF TWO YEAR OLD SEED.

Under the discussion of the cotton anthracnose, it was shown that this disease dies out on the seed before the second year. As this is perhaps the most serious of the cotton boll rots, it would seem advisable to use two year old seed for planting if it were available. There is one thing which should be borne in mind, however. A test of this two year old seed for germination should always be made before planting. If two year old seed will give a good germination test, there seems to be no reason why it could not be used for planting. It is possible that this may be the final solution of the cotton anthracnose problem.

SUMMARY AND CONCLUSIONS.

The results and experiments which have been discussed in this bulletin may be summed up as follows:

1. Cotton boll rots are chiefly due to bacteria and fungi.
2. The loss caused by these troubles is very great, running up into the millions of dollars a year for the whole country.
3. The most important organisms causing rots are Bac-
terium malvacearum, Glomerella gossypii and Diplodia gossypina.
4. Bacterium malvacearum produces watery spots on the leaves and bolls.
5. These watery spots are later invaded by other rotting or-
ganisms.
6. *Bacterium malvacearum* can enter the uninjured epidermis of leaves and bolls, as has been proven by inoculation experiments.

7. The period of incubation of the bacterial disease on the leaves seems to be between nine and sixteen days.

8. The young bolls can be inoculated with *Bacterium malvacearum* by spraying the bacteria into the open flowers.

9. The cotton anthracnose disease, produced by the fungus *Glomerella gossypii*, causes a greater loss than any other boll rot.

10. The anthracnose fungus produces spots on the bolls which are followed by a general rot, and also produces spots on the cotyledons and a "damping off" of the young plants.

11. The anthracnose lives over from fall until spring by spores which lodge in the short lint on the seed and also by mycelium within the seed.

12. Both the conidial and ascogenous stages are known for the anthracnose fungus.

13. The anthracnose spores live for several months on cotton seed, but for a much shorter period when dried on cover slips.

14. The anthracnose dies out in the cotton seed before the second spring after they are picked.

15. Cotton bolls may be infected with the anthracnose in wounds or the mycelium may penetrate the uninjured epidermis.

16. Young bolls may be infected without an injury until they are at least half grown.

17. Anthracnose spores placed in the open flowers will germinate and infect the young bolls. The bolls may be affected through the dead pistils or spots may develop at any place on them.

18. Anthracnose spores in the flowers do not seem to have much effect on the shedding of the young bolls.

19. Anthracnose spores on seeds germinate as the seeds germinate and infect the young plants.

20. Some varieties of cotton, such as the Cook, seem to be slightly more susceptible to the anthracnose than the other varieties. However, there is no variety that is very resistant.

21. *Glomerella gossypii* seems to be distinct from other anthracnose fungi.
22. *Diplodia gossypina* causes a black rot of the cotton bolls.

23. Only the pycnidial stage is known of *Diplodia gossypina*.

24. The Diplodia only attacks bolls through wounds.

25. The spores of Diplodia are very resistant to drying and are able to live over from fall until the following summer.

26. The Diplodia very quickly destroys the whole boll after it has once gained an entrance.

27. A species of *Fusarium* also does some damage as a boll rot.

28. The *Fusarium* lives over from fall until spring by spores on the seed and also by mycelium within the seed.

29. The *Fusarium* only attacks bolls that have been previously attacked by other boll rotting organisms.

30. Other species of fungi, such as *Rhinotrichum tenellum*, *Olpitrichum carpophilum*, *Botryosphaeria fuliginosa* and several others, are sometimes found on cotton bolls.

31. For the control of the cotton boll rots, care should be taken in destroying the sources of infection, in the raising of early prolific cotton, in the selecting of cotton seed, and in the use of two year old seed for planting.

**SPECIMENS OF FUNGI.**

Specimens of several of the fungi which have been discussed in this bulletin have been deposited in the Bureau of Plant Industry collections at Washington, D. C. These include the following: *Glomerella gossypii*, both the conidial and ascogenous stages (by mistake, in a previous publication (27), it was stated that the type material of the ascogenous stage was deposited in the United States National Herbarium), *Diplodia gossypina*, *Olpitrichum carpophilum*, *Schizophyllum alneum*, and *Nectria brassicae*.

**BIBLIOGRAPHY.**

The following publications are only the ones which have been seen. There may be some more publications on boll rots which are important but no references to them have been found. A few publications not treating of boll rots have been included as they have been mentioned in the text of this bulletin. The num-
bers in parenthesis in the text refer to the numbers in the bibliography.

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EXPLANATION OF PLATES.

Plate I, Figure 1. Angular leaf spot of cotton produced by inoculation with *Bacterium malvacearum*. Natural size. Figure 2. Cross section of carpel of cotton boll showing small insect punctures. Natural size. Figures 3 and 4. Cross sections of carpels of cotton bolls showing the intumescence tissue or proliferations which follow insect punctures and bacterial infection. Natural size. Figure 5. The intumescence tissue as seen from the inside of the boll. Natural size. Figure 6. The appearance of the insect punctures on the internal lining of the cotton boll. Natural size.

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Plate III.
Plate X.