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Effect of fungi on the germination of sugar cane

Claude Wilbur Edgerton

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Agricultural Experiment Station

of the Louisiana State University and A. & M. College

Baton Rouge

Effect of Fungi on the Germination of Sugar Cane

By

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Baton Rouge
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1920
EFFECT OF FUNGI ON THE GERMINATION OF SUGAR CANE.

C. W. EDGERTON and C. C. MORELAND.*

One of the greatest losses to the sugar industry of Louisiana is that caused by the poor germination of the seed cane. This is much more important to the planters in this state than it is in tropical sugar countries. In the tropics the planters are usually able to cut their cane in pieces and then plant the pieces but slightly thicker than they want the hills of cane. In contrast to this, the Louisiana planter must put down at least two solid rows of stalks in each furrow to obtain a satisfactory stand. To do this it takes about four to five tons of cane per acre for planting. Or in other words, if the same acreage is maintained, it takes nearly one-sixth of the crop each year for planting purposes.

In the tropics, the young plants come up quickly after the seed is planted, with the result that a comparatively high percentage of germination is obtained. There is some deterioration of seed, especially in those regions in which the soil is badly contaminated with the pineapple disease fungus, Thielaviopsis paradoxa, yet the deterioration averages very much less than it does in the southern United States. According to various statistics (16), the percentage of germination of the eyes or buds in the tropical countries usually runs from 45 to 90%.

Under Louisiana conditions, the cane is planted in the fall or kept in windrow until early spring and there is no chance for satisfactory germination until the warm weather begins in the spring. Instead of a few days or weeks as in the tropics, the cane in Louisiana must lie in the ground for several months. During this period there is continuous deterioration due to the action of various micro-organisms aided to a greater or less extent by unfavorable soil and temperature conditions. As a result, instead of a comparatively high germination of the eyes as is obtained in the tropics, we actually obtain about 10 to 30% germination with an average of about 20%. This means that we have to plant about five times as much cane as would be necessary if we were able to obtain a complete germination of the eyes.

*The authors wish to acknowledge their indebtedness to Mr. W. G. Taggart, Assistant Director of the Sugar Experiment Station at New Orleans, who has helped in all the work at Audubon Park and has materially assisted in obtaining the data given in this bulletin.
In the tropics, also, the planter is able to use cane tops to a considerable extent for planting. The tops seem to germinate better and are less liable to be affected by the various diseases. This, however, cannot be done satisfactorily in Louisiana. On account of the large tonnage that must be handled, the extra labor would be prohibitive. Furthermore, under the conditions existing here, deterioration is more pronounced with the tops than it is with the more mature parts of the stalks. That this is true is shown by the experiments which have been carried on at New Orleans during the past few years. Stalks have been cut in two and the bottom halves and top halves have been planted in separate rows. All the good eyes were counted at planting time and the percentage of germination obtained the following spring. The results of the tests are given in table 1. The figures represent the percentage of sound eyes that grew into plants.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>D 74 Bottom Halves</th>
<th>D 74 Top Halves</th>
<th>PURPLE Bottom Halves</th>
<th>PURPLE Top Halves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10.0%</td>
<td>22.0%</td>
<td>10.0%</td>
<td>18.0%</td>
</tr>
<tr>
<td>1911</td>
<td>36.0</td>
<td>21.0</td>
<td>33.0</td>
<td>28.3</td>
</tr>
<tr>
<td>1912</td>
<td>23.1</td>
<td>23.1</td>
<td>21.8</td>
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<tr>
<td>1913</td>
<td>25.2</td>
<td>17.7</td>
<td>9.9</td>
<td>12.7</td>
</tr>
<tr>
<td>1914</td>
<td>18.7</td>
<td>11.1</td>
<td>22.3</td>
<td>16.3</td>
</tr>
<tr>
<td>1915</td>
<td>12.2</td>
<td>6.5</td>
<td>19.2</td>
<td>21.0</td>
</tr>
<tr>
<td>1917</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>20.9</td>
<td>16.9</td>
<td>19.4</td>
<td>17.8</td>
</tr>
</tbody>
</table>

While the tests show a considerable variation from year to year, the average for the six years shows a greater germination of the eyes on the bottom and more mature halves of the stalks.

The problem of the deterioration of seed sugar cane is an extremely complex one. While the actual rot or decay of the cane is due to the action of various species of fungi and bacteria, there are many factors which influence the rate and extent of the deterioration. Among these should be mentioned the nature of the soil, moisture conditions of the soil at various times during the winter, temperature conditions, depth at which the cane is planted, maturity of cane at time of planting and the condition of cane in regard to infestation with insects and fungi. With
our present knowledge of the problem, it is impossible to estimate the actual importance of any one of these various factors.

The problem of seed deterioration has received considerable attention at different times in the various tropical sugar countries. The various investigators have considered the problem mostly from the standpoint of the organisms that were found developing on the cane and the possibility of treating the cane in order to prevent the entrance of the organisms. In fact, this seems to be the best way of approaching the problem. It is necessary at first to have a knowledge of these organisms and their methods of attack before going deeper into the problem. Investigators who have made a study of the organisms causing seed deterioration include Went and Kamerling in Java, Cobb and Lewton-Brain in Hawaii, Bovell, Howard, Watts and Johnston in the West Indies and Butler in India. The organisms which received particular attention by them were the ones causing the pineapple disease, red rot, rind disease and the root rots. Reference will be made to their work from time to time throughout this article.

The problem of the deterioration of seed sugar cane has been under investigation at the Louisiana Experiment Station for a number of years. The work has included principally a study of the various organisms found on seed cane, the loss caused by them, and their methods of attacking the cane, although various other factors have been kept in mind as far as possible. The experimental work has been carried on in the field and laboratory of the Louisiana Experiment Station at Baton Rouge and in the field at the Sugar Experiment Station, Audubon Park, New Orleans.

METHODS OF WORK.

The work has been carried on along three general lines. (1) Examination of planted cane for the presence of disease organisms. During the course of the investigation, a large amount of seed cane, especially cane which has failed to germinate, has been carefully examined and the different fungi present have been listed and their effect on the cane has been studied. Most of this cane has come from Louisiana but some has come from other southern states. (2) Inoculation experiments. Most of the fungi which seemed to be important have been isolated and inoculation experiments have been carried on in cane at planting time. Also some of them have been used to inoculate growing cane in order
to obtain seed that had been infected for some time. The effect of these different organisms on germination of the cane has been determined. (3) **Effect of disinfectants on seed cane.** Cane has been treated at planting time with different disinfectants such as corrosive sublimate and formaldehyde to see if these would have any effect on the germination.

**Fungi on Seed Cane.**

Cane that has lain in the ground all winter is always more or less infected with various fungi and bacteria. The cane that does not germinate is always badly discolored on the inside, and the outside of the stalks is frequently shrunken and shows the presence of fungus fruiting pustules. The color of the inside of the stalks is not constant but is usually brownish or reddish, though blackish, yellowish or greyish discolorations are not uncommon. The canes in Plate I show a common appearance of deteriorated cane during the late winter and early spring seasons.

The fungi which have been found on such cane and which from their frequency or from their known ability to infect cane seem important include the following: *Colletotrichum falcatum, Melanconium sacchari, Gnomonia iliau, Marasmius plicatus, Thielaviopsis paradoxa, Fusarium sp. (two or more), and Scopularia sp.* A large number of other forms have been seen on cane or have been isolated while making cultures from stalks. These include *Sclerotium rolfsii, Pythium artotragus,* and species of the following genera, *Agaricus, Rhinotrichum, Alternaria, Penicillium, Aspergillus, Meliola, Rhizopus, Cladosporium, Mucor, Saccharomyces, Acremonium, Catenularia, Nectria* and others. While it is possible that some of the latter may have some importance in the deterioration of cane, it does not seem likely and consequently not much attention has been paid to them. They seemed to be mostly saprophytes growing on the already dead tissues of the cane. Bacteria were also present in abundance in most of the canes examined. While it is quite possible that some of these may aid in the deterioration of the stalks and eyes, they did not seem to be particularly important and were not considered to any extent in this investigation.

**Colletotrichum falcatum**

*Colletotrichum falcatum* is the cause of the well-known red rot disease of sugar cane. This fungus causes a disease of the
growing cane but it is also very abundant on seed cane and is one of the chief organisms responsible for seed deterioration. This fungus was first reported in the United States in 1911 and has since been found to be well distributed over the sugar sections of this country. It is probable that it has been here for a considerable period. This fungus seems to be present in all tropical sugar countries and in many places it does considerable damage. The red rot disease, as it occurs in Louisiana on growing cane and to some extent on the seed, has been discussed in previous publications (8, 9, 11). The fungus usually gains entrance to the living cane through borer holes or other injuries. The rind tissue is not usually attacked unless it is very young and tender. As the disease is usually confined to the interior of the stalk, it is best told by examining a stalk that has been split lengthwise. The inside of the stalk is much reddened and in this red tissue are elongated white areas at right angles to the main axis of the stalk. The presence of these peculiar white spots is conclusive proof of the presence of the red rot disease (Plate IV, fig. 1; Plate V, figs. 1 and 2).

In seed cane, the presence of the fungus is not always readily told by a microscopic examination. The white spots may be present, but their absence does not mean that the red rot is not present. The action of *Colletotrichum falcatum* in cut stalks is somewhat different than in growing stalks. Furthermore, as this fungus becomes combined with other organisms in the deteriorating cane, the interior of the stalks may assume various colors. The tissues may become reddish, brownish or greyish. The nodal region of a stalk of seed cane affected with this fungus is usually brownish in color and it is from this region that cultures of the red rot are more liable to be obtained, especially if the stalk has been infected for some time. The buds on these infected nodes are usually dead. Figure 2 in Plate III shows split stalks that have lain in moist chamber for a few days. The developing mycelium in the nodal region is that of *Colletotrichum falcatum*.

While the fibrovascular bundles are always reddened in standing stalks that are affected with the red rot, this is not always the case in seed cane. Very frequently the bundles are perfectly colorless or at most but slightly reddened.

The presence of this fungus shows but very little on the out-
side or rind of the affected seed stalks. While many seed stalks, especially immature ones, shrivel to some extent, this is generally due to the action of some other organism than the red rot fungus. About the only evidence of *Colletotrichum falcatum* on the rind is the presence of blackened rootlet buds in the nodal region. There are small thin places in the rind at each node where the young roots emerge. It is apparently through these points that *Colletotrichum falcatum* gains entrance to the seed cane. This method of infection was first demonstrated by Butler (3) in India. From a large number of observations in Louisiana, it seems evident that much of the infection takes place in this manner. Infection through the cut ends of the stalks does not seem important probably because these cut ends quickly become infected with yeasts and various other organisms that prevent the development of the red rot fungus.

There has been some discussion by different investigators regarding the possibility of young cane plants becoming infected with the red rot disease directly from diseased seed. Butler (2) in 1906 claimed that in India the mycelium of *Colletotrichum falcatum* passed directly from the diseased seed into the young plants and he claimed that most of the growing plants became infected in this manner. This question was investigated a few years later in Louisiana and in Bulletin 133 (9), this method of infection was denied for our conditions. While it was admitted in this bulletin that diseased seed cane showed a lower germination of the eyes, it was claimed that the plants that did develop did not become infected from the seed. Following this, the problem was considered in the West Indies (32) with results very similar to those obtained in Louisiana. After this, Butler (3) again worked over the whole problem in India. His results agreed with those he had given in his earlier publication. He showed definitely that the plants do become infected from the diseased seed.

From the various publications on this subject it is evident that for some reason or other, the conditions in India are radically different from those in the West Indies and the southern United States. In our experiments during the last few years in which thousands of diseased stalks have been planted, not a single stalk has been seen which became infected directly from
the seed. Just why the fungus acts differently in the different countries is not known. It may be that there are different strains of the fungus in the various sugar countries or it may be due to varietal susceptibility or climatic conditions.

Colletotrichum falcatum occurs on a large part of the seed cane in Louisiana. It is one of the most widely distributed and common forms found on cane. It is usually easy to obtain cultures of the red rot from even a small sample of seed cane that has lain in the ground during part of the winter.

**Melanconium Sacchari.**

Melanconium sacchari is the cause of the so-called rind disease of sugar cane. This fungus is widely distributed in all sugar cane regions of the world. The early investigators (23) of sugar cane diseases believed that this fungus caused a very serious disease of standing cane. They believed that the deterioration and dying out of some varieties, especially the Bourbon, was mainly caused by this fungus. This belief has been largely discredited. It is the general opinion at present that most of the deterioration noted by these early workers was caused by the red rot and various rootrots and that Melanconium sacchari was merely secondary. However, Johnston (18) still believes that the rind disease is of considerable importance in the West Indies.

In Louisiana it is very unusual to see this fungus on standing cane. Sometimes it may be seen on stalks that are practically dead from other troubles but even this is unusual. However, on the seed cane during the winter months it is very common and abundant. It is often difficult in the late winter to find a stalk that does not show the presence of the organism on some joint. The presence of the fungus is very readily told. The fruiting pustules break out through the rind tissue and are readily seen or can be felt by running the hand over the surface. Affected stalks are usually more or less shriveled due to the decay of the tissues. The inside of the stalk is not very characteristic. As the stalks lose moisture rapidly, a sort of dry rot develops and the tissues do not have the turgid, moist appearance that is present with the red rot. The decaying tissues become whitish, greyish or brownish. A red discoloration is not characteristic of this trouble though some red may be present.
Marasmius plicatus is the cause of the root rot disease of sugar cane in Louisiana. This is not the same fungus that is responsible for most of the root rot troubles in the tropics, though it seems probable that this fungus is present more or less in the West Indies. In the southern United States this fungus has gone under the name of *Marasmius plicatus* since Fulton (13) made a study of it in Louisiana. In a recent article, however, Johnston (17) claims that it is identical with *Marasmius stenophyllus*, a fungus which is common in the West Indies on bananas and to some extent on sugar cane. It is possible that Johnston is right, yet it does not seem that the point is well enough settled to make it advisable to use the other name. It is a little strange that the fungus should be so abundant on sugar cane in Louisiana and to prefer another host in the West Indies. Whether *Marasmius plicatus* is the right name for the fungus is questionable but as it is the name that has been used for some time in Louisiana, it will be used in this bulletin.

*Marasmius plicatus* causes a disease of the growing cane (Plate IV, fig. 2), but as diseased stalks are liable to be used for planting, it is frequently seen on seed cane. It attacks the roots of the growing plants and also grows around the base of the stalks. It grows in and between the lower leaf sheaths of the stalk and the abundant mycelium cements these together so that they remain on the stalk all through the growing season. The presence of the disease is easily recognized in the field by pulling apart the leaf sheaths at the base of the stalks. If abundant mycelium is found between the leaf sheaths and the stalk itself is unaffected, root rot is present. The fungus does not penetrate the rind tissue from the leaf sheaths.

Stalks affected with the root rot are easily recognized in seed cane by the presence of the clinging leaf sheaths and white mycelium. The inside of the stalk is not affected, though mycelium is sometimes found entering the stalk in borer holes and other injuries.

The fungus forms the mushroom fruiting bodies in the field from July to September. It does not fruit on the seed cane.
Gnomonia iliau is the cause of the Iliau or stem rot disease of sugar cane. This disease is only known in Hawaii (25) and Louisiana (10). It was first described from Hawaii where it is commonly called Iliau, meaning hidebound.

The Iliau disease (Plate II, fig. 1) is principally a disease of the growing cane. In some respects it appears very similar to the root rot disease. The lower leaf sheaths are bound together but they are also bound firmly to the stalk itself. This fungus, unlike the root rot fungus, grows into the stalks from the leaf sheaths. As soon as the fungus begins to fruit, it can be readily told from the outside by the numerous black perithecia which develop. The beaks of the perithecia are long, hard and sharp pointed.

The fungus occurs mostly in the north and central parts of the state and is not common in the southern section, though it has been collected as far south as Reserve.

As diseased stalks may be cut at planting time, affected stalks are sometimes found in the seed. The presence of this fungus on the seed cane can be readily told. The lower leaf sheaths remain firmly attached to the stalks and, differing from the root rot, the rind tissue and also the interior of the stalks are affected.

Thielaviopsis paradoxa.

Thielaviopsis paradoxa is a common fungus in many tropical countries where it is the cause of the so-called pineapple disease of sugar cane. It received this name from the peculiar pineapple odor that is present in the affected stalks. In the tropics, this disease is apparently the cause of a large part of the deterioration of seed cane.

The fungus enters wounds in the rind tissue or at the cut ends and rapidly grows through the whole stalk. The affected region turns darker in color. Frequently there is a central black pipe running lengthwise of the stalk (Plate II, fig. 2). While it is not always possible to tell the presence of this fungus without a microscopical examination, a black discoloration is always suspicious. The intensity of this dark color will be increased if the stalk is cut open and laid in a moist place for twelve to twenty-
four hours. The dark spores of the fungus develop very abundantly under such conditions.

This fungus does not seem to be very abundant in Louisiana. While it is occasionally found in south Louisiana, it does not have the same importance that it has in the tropics. Whether this is due to the fact that it has not become thoroughly distributed in this state or whether it is not adapted to our conditions is not at present known.

**SPECIES OF FUSARIUM.**

Fungi of the genus Fusarium are very common on seed cane in Louisiana. No attempt has been made to determine species in this genus but there are at least two distinct types of Fusaria present on the cane. These two types, for want of better names, are discussed in this bulletin under the names of purple Fusarium and white Fusarium. The purple Fusarium is a very large spored form which produces deep purple fruiting pustules on the cane and also produces the same colored growth on culture media. This fungus is commonly present on the outside of the stalks of seed cane during the late winter and is very easily recognized by the purple fruiting pustules.

The term, white Fusarium, as used in this bulletin, may include one or more species. The cultures which have been used in the investigations were quite similar and were closely related, if not identical with some of the ordinary Fusaria found on various decaying plant tissues. As these forms are all so similar, it seemed impossible for any one who has not made a particular study of Fusarium species to try to differentiate between the strains studied. In these forms, the mycelium is white in culture and the spores are of the common type, much smaller than those of the purple Fusarium.

The white Fusarium is present in almost every stalk of discolored seed cane. Its presence in a stalk can only be told by making cultures in the laboratory, yet it is a fact that it can be obtained from almost any discolored stalk.

**SCOPULARIA SPECIES.**

A species of Scopularia is found very commonly on the outside of stalks of seed cane in Louisiana. It forms patches of
greyish mycelium on the stalks. If the stalks are in a humid place, the conidiophores of the fungus carrying little heads of spores develop very abundantly. Each little grey conidiophore bears a little glistening head of spores. This fungus may also occur on the inside of the stalk in split places, borer holes and other injuries.

On account of the frequent occurrence of this fungus on seed cane an attempt was made to identify it. A specimen was sent to the late Professor G. F. Atkinson of Cornell University, who said that it was probably an undescribed species of the genus Scopularia.

**Inoculation Experiments.**

In order to obtain some accurate data upon the effect of these organisms on planted cane, a series of inoculation experiments have been run during the past ten years. The mere presence of an organism, even if it is present on most of the stalks, does not necessarily mean that it is one of the factors in the poor germination of the eyes. Its real importance can only be ascertained by carrying on some controlled inoculation experiments.

It has been the aim in the experimental work to determine which organisms cause a decrease in the germination of the eyes and to determine the progress of the deterioration produced by each one. In order to obtain this information, inoculation experiments have been carried on under two different conditions. Canes have been inoculated with the several different organisms which seemed to be associated with seed deterioration and planted directly in the field. These tests are described as "Field Experiments." Then canes have been inoculated and placed in beds and covered with soil. These are described as "Bedding Experiments." This work has been conducted at the Sugar Experiment Station, Audubon Park, New Orleans, and at the State Station at Baton Rouge.

**Field Experiments.**

In the field experiments, each test consisted of a series of plots. Generally a plot contained a single row two hundred and ten feet long, though in a few cases the plots were larger. In these plots was planted cane that had been treated or inoculated with
different fungi. The plan of the tests was more or less the same in the different years in which the experiments were run. Check plots were used in which untreated seed was planted. The seed in the other plots was inoculated with pure cultures of the fungi. Generally the stalks were cut in two and the bottom halves were planted in one plot and the top halves in another. This was in order to test out the resistance of the hard ripened tissue at the bottom of the stalks with the soft unripened tissue of the tops.

The stalks were inoculated by three different methods. In some of the tests, the spores of the organisms from pure cultures were sprayed on the stalks after they had been laid in the furrows. In other tests, the stalks were inoculated by placing material from a pure culture in a puncture in the rind. A single puncture was made in each piece of cane. The pure culture was inserted into the stalk with one of the injectors used by veterinarians to inject blackleg pellets into an animal. A portion of pure culture was placed in the end of the needle and this was then pushed through the rind tissue and the culture left in the interior of the stalk. The hard tissue of the rind makes it impossible to use an ordinary needle with any satisfaction, especially if a large number of inoculations are required. Check punctures, in which no organism was used, were also made in the seed in some plots in order to determine the effect of the injury alone. Then in some of the tests, plots were planted with stalks that had been inoculated in the field during the preceding summer, several months before planting time.

In all of the experiments, cane was used just as it came from the field. The ideal way of performing an experiment of this kind would be to have the stalks perfectly free of the organisms which are liable to cause decay and then inoculate them with the ones desired. However, in practice, such a condition is absolutely impossible. These various fungi are present in the field on the stalks and it is impossible to sterilize them with any degree of satisfaction without damaging the eyes. Also the soil is more or less contaminated with the various fungi. In such experiments, then, as we have carried on, we must assume that the organisms are more or less present on all of the stalks but to a considerable greater degree on the inoculated ones. With such a condition, the results must be quite marked or else they are of little value.
The factors which are at work are so numerous and complex that a small variation of a few percent do not show anything. Furthermore, the results must be fairly consistent over a period of years.

In most of the tests carried on, counts were made of all the good eyes in each plot. These were usually made by the same person in order to eliminate as far as possible the personal error. While it is impossible to tell all the good eyes with certainty, especially when the eyes are being counted rapidly, this is possibly a more accurate and satisfactory method than that of measuring the combined lengths of the canes. In the experiments in which the eyes were not counted, the canes were laid end to end and were not overlapped, so that the combined length of the stalks planted in one plot was the same as in the others. In the tests in which the eyes were counted, the number of good eyes to the row ran from 325 to 700, depending upon the year, the variety of cane and the amount of seed put down. The stalks were usually overlapped to a considerable extent with the result that there was a considerable variation in the amount of cane planted in the different rows. The number of good eyes being known, this had no effect on the percentage of germination. Usually the number of good eyes to the row was between five and six hundred. In the other experiments in which the eyes were not counted, the stalks were laid end to end and the variation in the number of eyes was not so marked. Counts were always made of a few of the rows in order to get the average number of eyes.

The cane was planted in the plots in the same way as used by all planters in Louisiana. Two running stalks were planted in each furrow.

Effect of Puncture on Germination.

As many of the inoculations were made by puncture, it is first necessary to know what effect the puncture alone will have on germination. As the needle is liable to carry various organisms from the rind tissue to the inside, the puncture alone might be sufficient to cause some decrease in the germination. While the experiments to test this out were carried along at the time of the various inoculations, to save space in the tables the results
are given by themselves. Two varieties of cane were used, D 74 and Louisiana Purple. The eyes were counted in each test. The results obtained during three years' trial are given in tables 2 and 3. The stalks were cut in two in the first two tests and the germination figured on both top and bottom halves.

**TABLE 2.**

**Effect of puncture on germination percentage— Variety D 74.**

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Bottom Halves</th>
<th>Top Halves</th>
<th>Whole Stalks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Punctured</td>
<td>Check</td>
</tr>
<tr>
<td>1914</td>
<td>25.2%</td>
<td>23.1%</td>
<td>17.7%</td>
</tr>
<tr>
<td>1915</td>
<td>18.7</td>
<td>20.9</td>
<td>11.1</td>
</tr>
<tr>
<td>1916</td>
<td>9.4</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>22.0</td>
<td>22.0</td>
<td>14.4</td>
</tr>
</tbody>
</table>

**TABLE 3.**

**Effect of puncture on germination percentage— Variety Purple.**

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Bottom Halves</th>
<th>Top Halves</th>
<th>Whole Stalks</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Punctured</td>
<td>Check</td>
</tr>
<tr>
<td>1914</td>
<td>9.9%</td>
<td>8.8%</td>
<td>12.7%</td>
</tr>
<tr>
<td>1915</td>
<td>22.3</td>
<td>20.7</td>
<td>16.3</td>
</tr>
<tr>
<td>1916</td>
<td>14.5</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>16.1</td>
<td>14.8</td>
<td>14.5</td>
</tr>
</tbody>
</table>

An examination of the tables shows considerable variation in the different tests. Some tests showed a higher germination in the checks and some showed a lower. Averaging the whole set of ten tests, the checks show an average germination of 15.8% while the punctured stalks show an average of 14.8%. A variation of one percent in such an experiment comes well within the limits of the variation we might expect. While it is possible that puncturing may decrease the germination to a slight extent, this decrease is evidently not sufficient to account for the results obtained with some of the fungus inoculations to be discussed later.

**Inoculations with Colletotrichum Falcatum.**

As *Colletotrichum falcatum* seemed to be the most important of the organisms causing deterioration of seed cane in Louisiana, it has received the most attention. The inoculation and planting experiments under field conditions have been run over a period
of seven years. The inoculations have been made mostly by puncture, though some tests have been made by spraying the spores directly on the canes in the furrows. The results of the puncture inoculations with D 74 and Purple cane are given in tables 4 and 5. Counts were made of the good eyes in all years excepting 1917.

### TABLE 4.
**Effect of puncture inoculations with Colletotrichum falcatum on germination percentage—Variety D 74.**

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Bottom Halves</th>
<th>Top Halves</th>
<th>Whole Stalks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Inoculated</td>
<td>Check</td>
</tr>
<tr>
<td>1911</td>
<td>10.0%</td>
<td>5.0%</td>
<td>23.1</td>
</tr>
<tr>
<td>1913</td>
<td>23.1</td>
<td>9.3</td>
<td>17.7</td>
</tr>
<tr>
<td>1914</td>
<td>25.2</td>
<td>10.8</td>
<td>11.1</td>
</tr>
<tr>
<td>1915</td>
<td>18.7</td>
<td>15.6</td>
<td></td>
</tr>
<tr>
<td>1916</td>
<td>12.2</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>1917</td>
<td>12.2</td>
<td>3.0</td>
<td>6.5</td>
</tr>
<tr>
<td>Average</td>
<td>17.8</td>
<td>8.7</td>
<td>14.6</td>
</tr>
</tbody>
</table>

### TABLE 5.
**Effect of puncture inoculations with Colletotrichum falcatum on germination percentage—Variety Purple.**

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Bottom Halves</th>
<th>Top Halves</th>
<th>Whole Stalks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Inoculated</td>
<td>Check</td>
</tr>
<tr>
<td>1913</td>
<td>21.8%</td>
<td>8.0%</td>
<td>10.4%</td>
</tr>
<tr>
<td>1914</td>
<td>9.9</td>
<td>5.6</td>
<td>12.7</td>
</tr>
<tr>
<td>1915</td>
<td>22.3</td>
<td>16.2</td>
<td>16.3</td>
</tr>
<tr>
<td>1916</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1917</td>
<td>19.2</td>
<td>12.8</td>
<td>21.0</td>
</tr>
<tr>
<td>Average</td>
<td>18.3</td>
<td>10.7</td>
<td>15.1</td>
</tr>
</tbody>
</table>

An examination of the tables shows that the results obtained are remarkably consistent. In every case, the percentage of germination of the checks is above that of the inoculated and in most cases considerably above. Averaging the whole nineteen tests, it is found that the check stalks germinated 16.1% and the inoculated ones only 8.2%. Inoculating the stalks with *Colletotrichum falcatum* decreased the germination of the eyes nearly
fifty percent. The tables also show that D 74 was injured more than the Purple and that the tops of D 74 were nearly ruined by the inoculation. The soft, unripened tissues of the tops seem to be less resistant than the ripened tissues at the bottom of the stalks.

During the years 1911, 1912 and 1916, some experiments were run in which the spores of *Colletotrichum falcatum* were sprayed upon the uninjured stalks of seed cane after they were laid in the furrows. In these tests four different varieties were used, D 74, D 95, Louisiana Striped and Louisiana Purple. In all, sixteen different tests were made. The stalks were cut in two and the bottom and top halves were tested separately. The results of these experiments were extremely variable. Out of the sixteen tests, eleven showed a higher germination in the checks and five showed a higher germination in the inoculated. Averaging all of the tests, the checks showed a germination of 20.8% and the inoculated a germination of 18.3%. While the inoculations may have decreased the germination to this slight extent, the difference is not sufficient to be conclusive. It is probable that there were spores of the red rot fungus on all the canes before they were planted. If it had been possible to use canes that were free of spores, probably more striking results would have been obtained.

As *Colletotrichum falcatum* causes a disease of the growing cane, it is evident that a considerable amount of cane affected with the red rot disease is used for planting. In order to find out what effect the presence of this disease in the stalks has upon germination, tests were run during four seasons. Enough stalks were inoculated in the field by puncture during July, August and September of these years to plant a plot along with the other inoculations. In 1913, 1914 and 1915, the stalks were cut in two and only the bottom halves were used for planting. This was necessary in order to have cane in the same condition as in the other plots. In 1916, the whole stalks were used, as whole stalks were used in all of the experiments of that season. As all of the inoculations in the growing cane were made in joints near the ground, the bottom halves showed most of the infection at planting time. The good eyes were counted in all of these tests. The results obtained in the different years are given in table 6.
TABLE 6.
Effect on the germination percentage caused by inoculating grown cane with Colletotrichum falcatum.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>D 74</th>
<th>PURPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Inoculated</td>
</tr>
<tr>
<td>1913</td>
<td>23.1%</td>
<td>18.8%</td>
</tr>
<tr>
<td>1914</td>
<td>25.2</td>
<td>18.7</td>
</tr>
<tr>
<td>1915</td>
<td>18.7</td>
<td>22.9</td>
</tr>
<tr>
<td>1916</td>
<td>9.4</td>
<td>14.0</td>
</tr>
<tr>
<td>Average</td>
<td>19.1</td>
<td>18.6</td>
</tr>
</tbody>
</table>

*In 1914, the Purple cane that was inoculated in the field was from another plot than that from which the check cane was obtained, and it is evident that it was much stronger and better cane and superior for seed purposes.

Contrary to expectations, it was found that cane inoculated in the field with Colletotrichum falcatum did not show any marked decrease in germination. The average of the four years tests showed that it germinated practically as well as did the seed in the check plots. A possible reason for this will be considered on a later page.

Inoculations with Melanconium Sacchari.

The seed inoculation experiments with Melanconium sacchari have not been as extended as those with Colletotrichum falcatum. The field experiments with this fungus at Audubon Park were conducted during only two years, 1912 and 1914. Other than this, however, the bedding experiments have given considerable information regarding the effect of this fungus on planted cane.

The inoculation experiments at Audubon Park were carried on with two varieties, D 74 and Louisiana Purple. The stalks were cut in two and the top and bottom halves placed in separate plots. In 1912, the spores from pure cultures were sprayed on the stalks in the furrow, while in 1914 the stalks were inoculated by puncture. The results of the inoculations are given in table 7.

TABLE 7.
Effect of inoculations with Melanconium sacchari on germination percentage.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Variety</th>
<th>How Inoculated</th>
<th>Bottom Halves</th>
<th>Top Halves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Check</td>
<td>Inoculated</td>
</tr>
<tr>
<td>1912</td>
<td>D 74</td>
<td>Spray</td>
<td>36.0%</td>
<td>41.7%</td>
</tr>
<tr>
<td>1912</td>
<td>Purple</td>
<td>Spray</td>
<td>33.0</td>
<td>25.6</td>
</tr>
<tr>
<td>1914</td>
<td>D 74</td>
<td>Puncture</td>
<td>25.2</td>
<td>18.8</td>
</tr>
<tr>
<td>1914</td>
<td>Purple</td>
<td>Puncture</td>
<td>9.9</td>
<td>19.9</td>
</tr>
</tbody>
</table>
In the experiments at Audubon Park, inoculating the cane with *Melanconium sacchari* had very little effect on the germination of the eyes. Averaging all the results given in the table, it is found that the checks showed a germination of 23.0% and the inoculated cane a germination of 22.6%. The table also shows considerable variation in the results. In some cases the checks show a higher germination and in others a lower. From the results that have been obtained thus far, it can only be said that there is no evidence that *Melanconium sacchari* is of much importance in the deterioration of cane. It seems to attack dead stalks and rot the tissues rapidly but it does not seriously injure living cane. In fact, it may be of some importance in the soil in getting rid of the old stalks in the field.

**Inoculations with Marasmius Plicatus.**

*Marasmius plicatus* has never seemed to be a fungus that would cause a deterioration of the cane or one that would decrease germination to any extent. It develops on the outside of the stalks, between the leaf sheaths, around the buds and between the bud scales, but it does not enter the stalks except to follow in some injury such as a borer hole. Consequently this fungus has not been used in the field experiments to test out its effect on germination.

Experiments have been carried on at various times in the field to prove that the disease is carried over to new plants from the infected cuttings, both naturally and artificially infected. While these tests have shown that infected seed will produce infected plants, there was no evidence at any time that there was any important decrease in germination.

**Inoculations with Gnomonia Illiau.**

The experiments with *Gnomonia Illiau* have been similar to those with *Marasmius plicatus*. No direct experiments have been carried on to determine the effect on germination. Many inoculations, however, have been made to determine whether the disease is carried over on the seed. In none of these experiments was there any marked decrease in germination. While the fungus would pass up to the young plant from the infected seed, it did not seem to kill the eyes before germination. Frequently when
the infection was severe the young plants were killed when they were a few inches high, though generally the plants would live on through the season in a stunted, diseased condition.

*Inoculations with Species of Fusarium.*

Both the purple and white Fusariums have been used in the inoculation experiments to test out their effect on germination. With the purple one, inoculations were made both by puncture and by spraying the spores on the surface of the stalks. The results of the inoculations with this form are given in table 8.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Variety</th>
<th>How Inoculated</th>
<th>Bottom Halves</th>
<th>Top Halves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Check</td>
<td>Inoculated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Check</td>
<td>Inoculated</td>
</tr>
<tr>
<td>1914</td>
<td>D 74</td>
<td>Puncture</td>
<td>25.2%</td>
<td>12.6%</td>
</tr>
<tr>
<td>1914</td>
<td>Purple</td>
<td>Puncture</td>
<td>9.9</td>
<td>13.9</td>
</tr>
<tr>
<td>1915</td>
<td>D 74</td>
<td>Spray</td>
<td>18.7</td>
<td>18.2</td>
</tr>
<tr>
<td>1915</td>
<td>Purple</td>
<td>Spray</td>
<td>22.3</td>
<td>19.9</td>
</tr>
</tbody>
</table>

With the purple Fusarium, the results obtained were variable. Sometimes the checks gave a higher germination and sometimes a lower. In only one case was there a marked decrease in the germination of the inoculated cane and consequently it seems likely that this result was due to some other factor. The experiments as a whole seem to show that this fungus is of minor importance in the deterioration of cane seed.

The experiments with the white Fusarium were only conducted during one season. In the fall of 1917, plots were inoculated by puncture with this fungus. Counts were not made of the good eyes but the stalks were laid end to end. The results obtained are given in table 9.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Variety</th>
<th>Bottom Halves</th>
<th>Top Halves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Check</td>
<td>Inoculated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Check</td>
<td>Inoculated</td>
</tr>
<tr>
<td>1917</td>
<td>D 74</td>
<td>12.2%</td>
<td>6.5%</td>
</tr>
<tr>
<td>1917</td>
<td>Purple</td>
<td>19.2</td>
<td>18.0</td>
</tr>
</tbody>
</table>
The results obtained with the white Fusarium, as far as they go, are perfectly consistent. In each test, the inoculated cane shows a lower germination than the check. However, it is impossible to draw conclusions from an experiment of this kind that has only been carried on for one year. Yet the results of these tests, coupled with the fact that this Fusarium is so extremely common in cane in all stages of deterioration, make it seem likely that this fungus is of some importance in decreasing the germination of the eyes.

Inoculations with Scopularia.

Inoculation experiments have been carried on during two years with the species of Scopularia that is so common on deteriorating cane. The results were variable and in no case was there any marked decrease in germination. Consequently it seems evident that this fungus is only of minor importance in the deterioration of seed cane.

BEDDING EXPERIMENTS.

Along with the field inoculation experiments that have been discussed above, bedding experiments were conducted during three seasons. In these experiments, good seed cane of the different varieties was obtained and divided into several piles. The canes in the different piles were then treated in different ways or inoculated with the different fungi. They were then placed in furrows and covered with a plow or hoe. There were from fifteen to forty stalks in each pile or bed. It was easy to open the beds and examine the canes in the spring or at various times during the winter and to study the effect of the inoculation or treatment. The inoculation experiments in the field only gave the actual effect on the germination percentage, while these tests gave information regarding the progress of the fungi in the stalks.

In most sections in the south, outside of the regular sugar belt, seed cane is carried over the winter in beds and then the good cane is selected out at planting time in the spring. In the sugar belt of Louisiana, this practice is not necessary as the temperature does not go low enough to hurt the cane planted in the field. Frequently in the sections where the cane is bedded, the
loss of cane during the winter months is very high. The farmers say that the cane took the dry rot, or the wet rot or the red rot. This condition is not peculiar to Louisiana. The same condition exists in the syrup districts of Georgia. According to reports, the losses are sometimes severe in that state. Specimens which have been received from there show the same predominating fungi in the cane as are present in Louisiana. Colletotrichum falcatum appears to be the most common and destructive form present.

The bedding experiments to be discussed in this bulletin were carried on during three seasons, 1912, 1916 and 1917. In each case the tests were started in the fall and continued through the winter into the spring of the next year. The cane was bedded in the Experiment Station garden at Baton Rouge.

**Experiments Started in 1912.**

In the 1912 tests, the cane was treated and bedded on October 4. Four different varieties of cane were used, D 74, D 95, Louisiana Purple and Louisiana Striped. The D 95, Purple and Striped varieties were each divided into five piles of twenty to twenty-one stalks. One pile was left untreated as a check. The second pile was treated with benetol, 1-50, for about five minutes and the solution allowed to dry. The third pile was dipped in 4-4-50 Bordeaux Mixture and the solution allowed to dry. The fourth pile was sprayed with a suspension of spores of Melanconium sacchari. The fifth pile was sprayed with a suspension of spores of Colletotrichum falcatum. Not having enough of the D 74 cane to treat as above, it was divided into four piles of twenty stalks each. The first three piles received the same treatment as the first three piles of the other varieties, but the fourth pile was sprayed with a suspension of spores of both Melanconium sacchari and Colletotrichum falcatum. The good eyes were counted on all the stalks in each lot, the number of eyes running from 140 to 201 to the pile.

This cane was then covered and was not again disturbed until the first week of the following May when it was dug up and examined. At this examination the eyes that had germinated
were counted and the germination percentage obtained. The results with the differently treated canes are given in table 10.

**TABLE 10.**

Germination percentage of bedded canes in 1912-13.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>D 74</th>
<th>D 95</th>
<th>Purple</th>
<th>Striped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check</td>
<td>35.5%</td>
<td>11.0%</td>
<td>13.7%</td>
<td>22.6%</td>
</tr>
<tr>
<td>Benetol, 1-50</td>
<td>28.6</td>
<td>11.0</td>
<td>14.4</td>
<td>20.8</td>
</tr>
<tr>
<td>Bordeaux Mixture</td>
<td>26.6</td>
<td>6.5</td>
<td>14.6</td>
<td>29.1</td>
</tr>
<tr>
<td>Inoculated with <em>Melanconium sacchari</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculated with <em>Colletotrichum falcatum</em></td>
<td>16.2</td>
<td>22.5</td>
<td></td>
<td>21.0</td>
</tr>
<tr>
<td>Inoculated with <em>M. sacchari and C. falcatum</em></td>
<td>24.3</td>
<td></td>
<td>10.7</td>
<td>14.6</td>
</tr>
</tbody>
</table>

The table shows a consistent decrease in germination in all the beds inoculated with *Colletotrichum falcatum* but variable results with *Melanconium sacchari*. This experiment alone would not be conclusive but in connection with the field experiments at Audubon Park, it helps to confirm the statement that *Colletotrichum falcatum* is responsible for considerable deterioration of seed cane.

All of the stalks were examined as they came out of the beds. The various fungi found on seed cane at that season of the year were present. *Colletotrichum falcatum, Melanconium sacchari*, and species of Fusarium and Scopularia were found in all of the beds and there was really little difference in the general appearance of the different lots of cane. There was also no material difference in the appearance of the split canes. They were all more or less reddened and soured. *Colletotrichum falcatum, Melanconium sacchari* and species of Fusarium were obtained from tissue cultures made from the interior of the stalks.

A series of cultures was also made from the dead eyes of a number of stalks from each pile. A single direct transfer was made from the interior of the eye or from the tissue at the base of the eye. After the cultures had developed, they were examined and the organisms present determined. The results are given in table 11.
TABLE II.
Organisms in cultures made from dead eyes of bedded cane.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of cultures made</th>
<th>ORGANISMS PRESENT</th>
<th></th>
<th></th>
<th></th>
<th>Other fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bacteria</td>
<td>C. falcatum</td>
<td>M. sacchari</td>
<td>Fusarium</td>
<td>fungi</td>
</tr>
<tr>
<td>Check</td>
<td>40</td>
<td>40</td>
<td>4</td>
<td>0</td>
<td>23</td>
<td>5</td>
</tr>
<tr>
<td>Benetol, 1-50</td>
<td>42</td>
<td>42</td>
<td>4</td>
<td>0</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td>Bordeaux Mixture</td>
<td>36</td>
<td>35</td>
<td>1</td>
<td>0</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>Inoculated with M. sacchari</td>
<td>32</td>
<td>31</td>
<td>2</td>
<td>4</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Inoculated with C. falcatum</td>
<td>28</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Inoculated with M. sacchari and C. falcatum</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>187</td>
<td>185</td>
<td>11</td>
<td>4</td>
<td>105</td>
<td>44</td>
</tr>
</tbody>
</table>

The table shows that the predominating organisms in the dead buds of cane in the spring are species of bacteria and Fusarium. It is reasonable to expect that the eyes at that time would be filled with various saprophytic forms. There was very little difference in the fungous flora of the buds from the different beds.

Experiments Started in 1916 and 1917.

In the years 1916 and 1917, the bedding experiments were carried on in a somewhat different manner. Instead of spraying spores on the stalks in the beds, each stalk was inoculated by puncture. Then at frequent intervals throughout the winter, some of the stalks were dug up, cut open and examined. While this procedure prevented the obtaining of any germination percentage, it permitted a study of the activities of the different forms in the cane. In both years only one variety, the Louisiana Purple, was used.

In 1916 the seed cane was placed in eight beds with forty stalks to the bed. One bed was used as a check, the stalks being punctured but not inoculated. The other seven beds were inoculated by puncture with the following organisms: Colletotrichum falcatum, Melanconium sacchari, Gnomonia iliau, Marasmius plicatus, Scopularia sp., purple Fusarium and some bacterial forms that had been found abundant in deteriorating cane.

In 1917, the seed cane was placed in eight beds with twenty
stalks to the bed. In one bed, the stalks were punctured but not inoculated; in a second bed, the stalks were inoculated with *Colletotrichum falcatum*; in a third bed, the stalks were inoculated with the white Fusarium; and in a fourth bed, the stalks were inoculated with both the Colletotrichum and the Fusarium. The stalks in the other four beds were soaked in formaldehyde solution, 1-100, for forty minutes and then treated in the same manner as the stalks in the first four beds. As the formaldehyde did not seem to have any particular effect, this really made forty stalks with each treatment.

The cane was examined every week or ten days and some stalks from each bed were split open. Careful notes were taken on the appearance of the cane and a series of photographs was made to show the gradual progress of the rots that developed.

The check stalks developed a certain amount of discoloration in the punctured joints. The tissue would first become somewhat reddened around the puncture and this discoloration would gradually extend back through that joint. Any injury in cane is followed by the development of a red color. The injury allows the entrance of various yeasts and bacteria which set up a fermentation. This discoloration, however, did not usually pass through the node except in a few cases where *Colletotrichum falcatum* happened to gain entrance accidentally. Late in the winter and early spring, many of the check stalks became more or less reddened throughout but this was due to the entrance of various fermentation forms which had gained entrance to the interior at various points in the rind tissue. These points of entrance could usually be readily told. In cane planted in the field, it is difficult to find a stalk in the early spring that is not more or less reddened. In the experiments with bedded cane, general reddening did not develop from the point of puncture in the check stalks.

The stalks inoculated with *Melanconium sacchari*, purple Fusarium, *Gnomonia iliau*, *Marasmius plicatus*, *Scopuloria sp.* and the bacteria developed very much as did the check stalks. There was nothing to show that any of these forms had attacked the cane to any extent. Rarely did the discoloration in the inoculated joint pass through the nodal tissue. While the dis-
coloration possibly developed slightly faster in the inoculated cane than it did in the check, by the end of the winter there was no difference in the appearance of the stalks. In Plate VI are shown stalks inoculated with these forms as compared to the check.

The effect of *Colletotrichum falcatum* in seed cane, however, was perceptible very shortly after the inoculation. The fungus grew rapidly through the internodes and was able to pass easily through the nodal tissue. A week after the inoculation, the fungus had invaded three joints of the stalk, the inoculated and the two adjoining ones. After this period the development of the rot was somewhat slower. This may have been partially due to the cooling of the soil caused by the approach of the winter season, though perhaps other factors may also have been present. However, there was a gradual development of the rot, until by spring usually the greater part of the stalk showed the presence of the fungus. It was also noticed that as the fungus approached the end joints of the stalk, its development was much impeded. Frequently the end joints of the stalk would not be infected with the fungus. This was probably due to the counteracting effect of other organisms such as bacteria and yeast which had entered through the cut ends. On account of this, frequently the inoculated stalks would only show developing buds on the two end joints. This same condition has also been noted in the fields.

The development of *Colletotrichum falcatum* in seed cane inoculated at planting time is somewhat different than its development in growing, living stalks. The appearance of red rot in growing stalks is very characteristic (Plate IV, fig. 1, and Plate V, figs. 1 and 2). The fungus grows rapidly through the fibrovascular bundles, causing them to change to a deep red in color. At places, the fungus will break out of these bundles and spread out through the other tissues. These areas are at first turned red in color but later transverse white spots appear in the red tissue. The red fibrovascular strands and the peculiar white spots in the red areas are very characteristic of the red rot disease in growing cane. In the growing stalks, also, the spread of the disease through the stalk is rather slow. With the varieties commonly grown in Louisiana, it takes several months for the fungus to spread through four or five joints of the stalk (9).
Seed cane that was inoculated at planting did not develop the characteristic red bundles or the definite white spots that are so common in growing cane affected with the red rot disease. While red bundles were sometimes present they were also often absent. The fungus was not held in the fibrovascular bundles; it apparently was able to grow rapidly through any of the tissues of the cane stalk. The affected regions first became slightly reddened or else had a watersoaked appearance. Later the affected areas took on various shades of red, brown, grey and white. After the tissues had been diseased for sometime, they usually became brownish or else greyish white. The colors were so extremely variable that it is impossible to state any definite characteristic. Frequently, also, there were small black areas in the nodes and sometimes in the bundles of the internodes. The nodal tissue always became diseased either changing to a brown or a greyish white. The buds were killed in nearly all cases. Typical stalks at different stages in the development of the fungus are shown in Plates VIII and IX.

It is difficult to explain the difference in development of *Colletotrichum falcatum* in growing stalks and in seed cane. It may be that growing cane is able to produce some counteracting substance which tends to keep the fungus confined to the fibrovascular bundles and to keep it from spreading rapidly in the other tissues of the stalk. There is a development of gum around the infected areas in growing stalks but it is not known whether this tends to check the development of the fungus or not. If the growing cane is able to produce some counteracting substance which cannot be produced by the cut cane, this would explain the variation in the development of the fungus. This would also account for the fact that cane inoculated some time previous to the cutting did not deteriorate when used as seed as rapidly as the stalks that were inoculated after cutting.

The white Fusarium did not seem to develop rapidly when alone. It spread quickly through the inoculated internode but was not able to pass readily through the nodal tissue, though in time it did pass into the adjoining joints. This fungus colored the internal tissues of the stalk a deep red. Red fibrovascular bundles also were fairly abundant. Stalks inoculated with this fungus are shown in Plate IX, figures 1a and 2a. When double inoculations were made with this fungus and *Colletotrichum*
falcatum, the Fusarium could usually be reisolated from the affected joints.

Cultures were readily obtained from the stalks inoculated with Colletotrichum falcatum and the white Fusarium. Direct transfers were usually made from the stalks at each examination in order to make certain that the fungi were developing and were responsible for the deterioration.

The results obtained from the bedding experiments are in perfect agreement with the field tests. Both series of experiments show that Colletotrichum falcatum attacks the seed cane and causes a rapid deterioration accompanied by a poor germination of the buds. Furthermore it would seem from the experiments conducted that it is the chief seed destroying fungus at present in Louisiana. While there may be many factors at work in cane deterioration, there is little question but that Colletotrichum falcatum is responsible for a considerable portion of the loss.

**Temperature Relation.**

The relation of temperature to the organisms which occur on seed cane is of considerable interest. The cane is usually planted while the ground is still warm, but during most of the winter months while the cane is lying dormant the temperature of the soil is comparatively low. It is interesting to compare the temperature of the soil during the different months with the rate of growth of the fungi at different temperatures.

The rate of growth of three of the fungi at different temperatures was determined. The organisms were grown in petri dishes in an incubator which could be regulated for different temperatures. A single large batch of bean pod agar was made and used in all of the plates in order that there would be no variation in the medium. The organisms used in the test were Colletotrichum falcatum, Marasmius plicatus and Gnomonia iliau. While the two latter forms do not seem to be responsible for much of the seed deterioration, they are important because they live over winter on the seed cane and attack the young shoots in the spring. The rate of growth was determined by measuring the increase in diameter of the colonies and finding the average daily increase over a period of four or five days. In figure 1 are shown graphically the results of this test. The curves
show the average daily growth in millimeters at the different temperatures. The temperatures at which the cultures were grown were 13°, 18.5°, 22.5°, 24°, 27°, 35° and 37.5°C.

The fungi were able to grow at all of the temperatures tried but the optimum for all was somewhere in the neighborhood of 27°C. At higher or lower temperatures, the growth was not as rapid. Colletotrichum falcatum and Marasmius plicatus seemed to grow well at any temperature between 20° and 35°C, but the latter temperature seemed to be too high for Gnomonia iliau.

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Fig. 1. Curves showing rate of growth of different fungi at different temperatures. a and b. Two different cultures of Colletotrichum falcatum. c. Marasmius plicatus. d. Gnomonia iliau.
Fig. 2. Mean soil temperatures, average of two and a half years.

New Orleans, La.

Audubon Park,

October, November, December, January, February, March, April.
In order to determine the temperature of the soil in which the cane was planted or bedded, a soil thermograph was located in the field at Audubon Park, New Orleans, and one in the Experiment Station garden at Baton Rouge. These automatically recorded the soil temperatures at a depth of three inches. These instruments have been in operation over two complete seasons and a part of a third. In figure 2 are charted curves showing the average weekly mean temperature of the soil at the two stations for this period. The weekly mean temperature was obtained by averaging the highest and lowest recorded temperatures for the week.

The curves show that the temperature of the soil drops rapidly after the middle of October and that for a period of two or three months during the winter, the temperature is too low for a satisfactory development of the fungi. A suitable temperature for the development of the organisms is not reached until nearly the first of March. As a matter of fact, seed cane ordinarily shows the greatest deterioration during the late winter and early spring. The red rot fungus will develop rapidly in the stalk during the fall if it gains entrance at planting time but it seems probable that most of the natural infection takes place slowly during the winter months and then a rapid development occurs with the approach of warm weather.

The effect of cold on the development of *Colletotrichum falcatus* was easily seen in the inoculated stalks of the bedded cane. During weeks of severe weather there seemed to be but little change in the appearance of the cane.

**Treatment of Seed Cane.**

As most of the organisms which cause deterioration of seed cane are on the stalks at planting time, it would seem that something might be accomplished by treating the seed with some disinfectant before planting. Experiments along this line have been carried on at many of the tropical experiment stations with varied results. In a recent bulletin, Johnston (16) has compiled the results which have been obtained in the various countries. Conditions in most of the tropical countries are somewhat different than in Louisiana. Most of the experiments in these countries have had the primary object of controlling the pineapple
disease fungus, *Thielaviopsis paradoxa*, a fungus which seems to be of only minor importance in Louisiana at present.

In Louisiana, seed treatment experiments have been conducted during two seasons. The disinfectants which were used were formaldehyde and corrosive sublimate. The formaldehyde solution was made by mixing one pint of commercial formaldehyde with thirty gallons of water, the same proportion as is used in the ordinary potato scab treatment. This solution was placed in tanks and the seed cane was immersed in it for thirty to forty-five minutes. The cane was planted without drying. The corrosive sublimate solution was made to the strength of 1-1000. It was made with the ordinary commercial antiseptic tablets, one tablet being added to each pint of water. The seed cane was placed in the solution for five minutes and then taken out and planted without washing. As corrosive sublimate is a powerful antiseptic and will remain after drying, there appeared to be no necessity for a longer treatment.

The first test of these solutions was made in the fall of 1917. Two varieties of cane were used and the top and bottom halves of the stalks were planted separately. On June 1, 1918, the stalks that had grown were counted and the percentage of germination was obtained. The results are given in table 12.

**TABLE 12.**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>D 74 Bottom Halves</th>
<th>D 74 Top Halves</th>
<th>PURPLE Bottom Halves</th>
<th>PURPLE Top Halves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check, no treatment</td>
<td>12.2%</td>
<td>6.5%</td>
<td>19.2%</td>
<td>21.0%</td>
</tr>
<tr>
<td>Formaldehyde, 45 minutes</td>
<td>15.2</td>
<td>10.2</td>
<td>29.5</td>
<td>23.7</td>
</tr>
<tr>
<td>Corrosive sublimate, 5 minutes</td>
<td>15.3</td>
<td>14.7</td>
<td>29.7</td>
<td>21.5</td>
</tr>
</tbody>
</table>

As is shown in the table, in 1917 there was an increased germination in all of the plots that were planted with treated cane. Further than this, the field examination that was made of the plots showed that the plants which grew from the treated canes, especially those treated with corrosive sublimate, were more vigorous and grew off faster than those in the untreated plots.
In the fall of 1918, the experiment was again tried but on a larger scale. Three varieties, Purple, D 74 and L 511, were used and six to nine rows were planted in each plot. Also in this experiment whole stalks were used for planting. The corrosive sublimate treatment was used with all varieties but the formaldehyde was used with only the L 511 cane. After the cane had all germinated in the following spring, the stalks were counted and the percentage of germination was obtained. The results of the test are given in table 13.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>D 74</th>
<th>Purple</th>
<th>L 511</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check, no treatment</td>
<td>19.7%</td>
<td>27.0%</td>
<td>25.6%</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>17.9</td>
<td>25.6</td>
<td></td>
</tr>
<tr>
<td>Corrosive sublimate</td>
<td></td>
<td></td>
<td>28.6</td>
</tr>
</tbody>
</table>

In the 1918 test, as is shown in the table, the germination percentage did not seem to be materially affected by the treatment. In three cases, the check plot showed a higher germination and in one case a lower, but in no case was the variation very marked.

The germination percentage, however, does not tell the whole story of this experiment. Notes were also taken on the vigor of the developing stalks and at the end of the season, the tonnage, sucrose content and other data were obtained.

Shortly after the plots came to a stand in the spring, a marked difference was noted between the rows of L 511 and Purple cane that were treated with corrosive sublimate as compared to those not treated. The stalks from the treated seed were larger and had a healthier appearance than those from the untreated seed. With the L 511 variety, this difference was apparent all through the summer. At the last examination shortly before the grinding season, the plants in the treated plots were taller and the stalks were considerably larger and better developed. With the Purple variety, as the season advanced, the difference became less noticeable until at grinding time the rows in the untreated and treated plots seemed to be about the same. The plots of D 74 cane did not at any time show the variation in vigor as was noticed with the other varieties.
Why the different varieties did not show the same variation in vigor is not perfectly clear. It is possible, however, that the Mosaic disease had something to do with this. Both the Louisiana Purple and the D 74 varieties showed practically 100% infection with this disease and as this trouble seems to have a tendency to check growth of the canes, especially the older ones, its presence may partially explain the variation noted with the different varieties. The D 74 cane, especially, seemed to be seriously injured by the Mosaic. The L 511 cane is somewhat resistant to the Mosaic disease and does not seem to be seriously affected by the trouble.

The soil conditions in the treated and untreated plots were as nearly identical, as far as is known, as it is possible to have them. The plots were side by side.

In November, during the grinding season, the plots of the L 511 and the Purple varieties were cut, weighed and ground and the number of stalks counted. The D 74 cane was used for seed purposes and the data was not obtained for this variety. The data obtained with the first two varieties is given in table 14.

### TABLE 14.

<table>
<thead>
<tr>
<th>Variety</th>
<th>TREATMENT</th>
<th>Stalks per acre</th>
<th>Tons per acre</th>
<th>Percent sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>L 511</td>
<td>Check</td>
<td>15,481</td>
<td>9.316</td>
<td>14.28</td>
</tr>
<tr>
<td>L 511</td>
<td>Corrosive sublimate</td>
<td>16,885</td>
<td>13.729</td>
<td>14.14</td>
</tr>
<tr>
<td>L 511</td>
<td>Formaldehyde</td>
<td>19,353</td>
<td>11.430</td>
<td>14.29</td>
</tr>
<tr>
<td>Purple</td>
<td>Check</td>
<td>18,109</td>
<td>12.242</td>
<td>10.98</td>
</tr>
<tr>
<td>Purple</td>
<td>Corrosive sublimate</td>
<td>19,496</td>
<td>12.535</td>
<td>10.71</td>
</tr>
</tbody>
</table>

As is shown in the table, the data obtained during the grinding season bear out the general observations made during the growing season. With the L 511 cane, the yield on the formaldehyde treated plot was over two tons greater than on the check and on the corrosive sublimate treated plot over four tons greater. On the latter plot, the increase in tonnage was practically fifty percent. With the Purple cane, the corrosive sublimate treated plot with a slightly lower germination percentage gave a slightly greater tonnage. There were but small differences in the analyses of the canes on the different plots.
Summary.

The principal points discussed in this study of the effect of fungi on the germination of sugar cane, a project which has been in progress for a period of ten years, are summarized below.

1. Sugar cane does not germinate as well in Louisiana as it does in the tropics, the average germination being about 20%. This necessitates heavy planting and consequently materially increases the cost of the crop.

2. The buds, or eyes, on cane tops do not germinate quite as well as do those on the bottom halves of the stalks. This seems to be due to the more rapid deterioration of the soft tissues of the cane tops.

3. A great many factors are instrumental in the deterioration of seed cane but one of the important ones is the action of certain parasitic fungi.

4. A great many different fungi are found on seed cane but the more abundant or more serious ones are Colletotrichum falcatum, Melanconium sacchari, Gnomonia illiau, Marasmius plicatus, Thielaviopsis paradoxa and species of Fusarium and Scopularia.

5. Colletotrichum falcatum, which also causes the disease of growing cane known as the red rot, is very common on seed cane and produces a serious deterioration. The germination percentage of the eyes of stalks inoculated by puncture with this fungus at planting time is reduced about 50%. The natural method of infection in such cane seems to be through injuries and through the root buds around the nodes.

6. Colletotrichum falcatum develops in cane that has been inoculated after cutting more rapidly than it does in growing cane. Also the infected stalks have a somewhat different appearance.

7. Stalks that have a good infection of red rot before cutting do not deteriorate when used as seed as rapidly as stalks that are inoculated after cutting.

8. In Louisiana, there is no evidence that Colletotrichum falcatum passes up into the young stalks from the diseased seed.

9. Melanconium sacchari is very abundant on deteriorating cane but does not seem to decrease the germination percentage.

10. Marasmius plicatus and Gnomonia illiau occur on seed but
there is no evidence that they reduce the germination percentage. They do, however, pass up on to the young stalks from the seed.

11. *Thielaviopsis paradoxa* occurs very sparingly in Louisiana and as yet does not seem to be responsible for much deterioration.

12. Two types of Fusarium are abundant on seed cane. The purple species does not seem to be important in the deterioration of seed. The white species may reduce the germination percentage to some extent.

13. A species of Scopularia is abundant on seed cane but is of little economic importance.

14. The optimum temperature for growth of several of the fungi that were studied is around 27°C. Most of the forms make but slow growth at the normal temperature of the soil during the winter season.

15. Seed cane has been treated at planting time with formaldehyde and corrosive sublimate. While the tests have not been continued long enough to be conclusive, the results are encouraging. In the best test, a 50% increase in tonnage was obtained from a plot treated with corrosive sublimate.

**Bibliography.**


**Explanation of Plates.**

Plate I. The ordinary appearance of deteriorated or dead seed cane during the late winter and spring season.

Plate II. Fig. 1. A cane stalk infected with *Gnomonia iliau*. Fig. 2. A stalk infected with *Thielaviopsis paradoxa*.

Plate III. Fig. 1. Cane stalks infected with *Melanconium sacchari*. Fig. 2. Split stalks of seed cane infected with *Colletotrichum falcatum*. These stalks were placed in a moist chamber for a few days in order for the fungus to develop some mycelium.

Plate IV. Fig. 1. A split stalk of growing cane infected with *Colletotrichum falcatum*. Fig. 2. A stalk of sugar cane infected with *Marasmius plicatus*.

Plate V. Stalks of growing cane artificially inoculated with *Colletotrichum falcatum*. Fig. 1. A very old infection. Fig. 2. A younger infection.

Plate VI. Seed cane inoculated at planting time with different organisms. Stalks photographed during the winter season. Fig. a. Check, stalk punctured but not inoculated. Fig. b. *Melanconium sacchari*. Fig. c. *Scopularia sp*. Fig. d. Purple *Fusarium*. Fig. e. *Marasmius plicatus*. Fig. f. *Gnomonia iliau*. Fig. g. Species of bacteria.
Plate VII. Effect of planting cane inoculated with *Colletotrichum falcatum*. Two rows on the left of the center, not inoculated; two rows on the right of the center, inoculated.

Plate VIII. Development of *Colletotrichum falcatum* in inoculated seed cane. Fig. 1a. Check, stalk not inoculated but punctured. Fig. 1b. Inoculated stalk eight days after inoculation. Fig. 2. Inoculated stalks three weeks after inoculation.

Plate IX. Development of *Colletotrichum falcatum* and the white Fusarium in inoculated seed cane. Fig. 1. Three weeks after inoculation. Fig. 2. Two months after inoculation. c. Check stalk, not inoculated. b. Stalks inoculated with *Colletotrichum falcatum*. a. Stalks inoculated with the white Fusarium.
PLATE V.