The Perfect Stage of Colletotrichum Falcatum.

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THE PERFECT STAGE OF COLLETOTRICHUM FALCATUM

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Botany

By

Fernando Carvajal
M.S. in Agr., Cornell University (Ithaca, N.Y.) 1941
1943
ACKNOWLEDGMENT

The writer wishes to express sincere thanks and appreciation to Dr. C.W. Edgerton under whose guidance this work was carried on. He also wishes to thank Dr. I.L. Forbes for his assistance, especially in inoculation work; and to Dr. L.H. Flint and Dr. E.C. Tims for their help in the preparation of the manuscript.

The writer also obtained valuable assistance from his wife Mrs. Mona B. Carvajal in the preparation of the manuscript and in typing the dissertation.
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ABSTRACT

Since 1893 the causative organism of the red rot disease of sugarcane has been considered as being *Colletotrichum falcatum* Went., one of the Fungi Imperfecti. The present investigation, however, has demonstrated that *Colletotrichum falcatum* is only one of the stages of a perfect fungus found in the genus Physalospora of the Ascomycetes. Perhaps on account of the behavior of this ascigerous stage in occurring on fading or dead cane leaves as inconspicuous black dots between veins, entirely embedded in the host tissues, the true nature of this fungus was not recognized by previous investigators and therefore was not given its proper taxonomic position.

In this work, besides establishing the true nature of the fungus, several other important facts concerning the life cycle of this pathogen are given.

The perfect stage of the red rot fungus has been found occurring very abundantly under natural field conditions on fading and dead leaves and occasionally on dead young top portions of stems of sugarcane in Louisiana. It was found on the five recognized species of Saccharum and also on the leaves and stems of a grass *Leptochloa filiformis* (Lam. Beauv. The *Colletotrichum falcatum*
stage also appears intermingled with the perfect stage. Dead cane shoots usually are one of the commonest places where the perithecia are found in abundance, even early in the growing season.

The perfect stage of the red rot fungus also was developed from single conidium as well as from single ascospore cultures. Under sterile, controlled conditions, it was developed by inoculating sterile cane leaves and leaf sheaths, corn leaves, Johnson grass leaves, or filter paper. Also it was produced in standing cane in the greenhouse on the inoculated leaves and around the point of inoculation in the cane stalks. Finally it was produced on leaves of standing cane in the field and in the laboratory. The perfect stage also was produced in moist chambers, by placing in them naturally infected cane leaves. As a rule, the conidial stage accompanied the ascigerous stage.

By means of intensive inoculation work with cane stalks and cane leaves of different varieties growing in the field and in the greenhouse as well as with cane cuttings kept in the laboratory, the pathogenicity was thoroughly proved by the single ascospore cultures as well as by several single conidium cultures. The typical symptoms of the red rot fungus were produced by each single spore culture. In these inoculations, the dark and light strains of the red rot fungus, ascospore and conidium cultures, were used. The single ascospore cultures were obtained from asci from perithecia developed entirely under natural field conditions as well as from those developed under sterile, controlled conditions and from those
developed in moist chambers.

Some variation in pathogenicity was found among several single ascospore cultures as well as among some of the single conidium cultures.

Eight ascospore cultures from one ascus and another eight ascospore cultures obtained from a second ascus, both from the same perithecium (which was produced with a single conidium culture), all were identical in severity of pathogenicity, and in producing the typical red rot symptoms upon inoculated cane-stalk cuttings. Also the same ascospore cultures were grown on oatmeal agar plates and in repeated experiments, all of them were identical to each other and to the original single conidium culture which produced the perithecium from which they were isolated.

The red rot fungus was isolated during February by means of ascospores and conidia from leaves and leaf sheath trash found lying on the ground in the cane field.

The importance of the perfect stage of the red rot fungus is due to the production of a large amount of inoculum besides that of the conidial stage for the spread of infection to leaves and stalks of sugarcane under the natural field conditions of Louisiana.

The red rot fungus is homothallic; single ascospore cultures of the dark and light strains produced the perfect stage under sterile, controlled conditions. The hereditary characters of pathogenicity and cultural behavior were kept uniform and stable by a particular isolate (x) through many single conidium generations.
and also through the sexual stage.

In comparative studies in the germination and growth of single ascospores and single conidia on sterile culture media, both kinds of spores reacted in an identical manner. They both produced the typical conidia, setae, the so-called chlamydospores or appressoria, and the mycelial growth proper to 

*Gliotetrichium falcatus* Went.

The dark and light strains of the fungus were actually obtained by means of single ascospore isolations from different perithecia which occurred side by side on dead leaves of sugarcane and of the grass *Leptochloa filiformis*. The perithecia developed entirely under natural field conditions.

No marked difference was found between the perfect stage produced by single ascospore and single conidium cultures of the dark and light strains of the red rot fungus.

The germination of the ascospores from naturally-developed perithecia varied from 85 to 100 per cent.

The perithecia are smooth, dark olive-brown to blackish, imbedded in the host tissue, protruding at maturity by a short papillate ostiole; globose to subglobose or with peculiar forms and shapes according to the proximity or distance of the vascular bundles between which they develop. Perithecia are usually scattered and separated one from another, but sometimes two to four or even more are aggregated together forming a row. When perithecia develop between two vascular bundles which are very
close together, then the perithecia thus formed will show two widths, the smaller one perpendicular to the direction of the bundles and the other longer one parallel to the bundles, while the height remains the same. This is particularly true of perithecia developed under the midrib. But when perithecia develop between distant vascular bundles they often show the same width in both directions as often is seen in sections through the leaf sheath. It is here in the leaf sheath that perithecia of larger dimensions are found.

The perithecia sizes are variable; they are found between (height x width) 100-260 x 85-250 microns. Taking either height or width as diameter, we find that the majority of the perithecia are found with the range 100-200 microns diameter.

The thickness of the perithecial wall is usually from 10-20 microns and is formed by three and sometimes more one-celled layers.

Asci are club-shaped or clavate, short-pedicellate, not fasciculated; ascus wall thickened at its top. The ascus normally contains eight ascospores in an irregular biseriate arrangement. Ascii sizes vary from 51.80-118.40 x 7.4-12.24 microns, but most of the asci fall in the ranges 70-90 x 13-18 microns.

The ascospores are hyaline, guttulate, smooth, one-celled, and uninucleated; elongated, blunt, oval, or egg-shaped, very often bean-shaped, slightly curved or straight. The ascospore measures are found between 12.5-29.6 x 5-11.1 microns, but most fall within
the ranges of 18-22 x 7-8 microns.

The paraphyses are abundant, hyaline, delicate, filamentous, sometimes tending to be clavate at their tops, septated, single, rarely branched; with oil drops within them. Most are from 65-100 x 1.5-4 microns.

For Colletotrichum falcatum, imperfect stage, the commonest measurements found are:

- Conidia .............. 20-50 x 4-8 microns
- Conidiophore ........ 15-25 x 6-9 microns
- Setae ................. 90-225 x 4-6 microns
- Chlamydospore ........ 8-30 microns diameter.

The examination of the Spegazzini material gave the following results:

(1) For Physalo spora:

- Perithecia ............. 100-230 x 80-170 microns
- Perithecial wall ....... 11.1-18.5 microns
- Ascospores
  - Range: ............... 12-22 x 5-10 microns
  - Usual ................. 16-19 x 6.6-8.5 microns

(2) For Colletotrichum:

- Conidia: Range ....... 19.8-32.2 x 3.3-6.8 microns
  - Usual ................. 23-28 x 4-6 microns
- Setae:  Usual ........... 100-200 x 3-6 microns
- Chlamydospores ......... 9-18 microns diameter.

These Colletotrichum measurements are in perfect agreement with the description of Colletotrichum falcatum Went.
The perfect stage of *Colletotrichum falcatum* Went has been described as it occurs in Louisiana. Its description agrees reasonably well with the original description of *Physalospora tucumanensis* Spag. Spagazzini's original type material was obtained from Argentina for purposes of comparison.

As a result of these investigations, it was found that the Louisiana and the Argentinian materials were in agreement in all their morphological features. Both stages, *Colletotrichum falcatum* Went and *Physalospora tucumanensis* Spag., were present in both specimens. Therefore, it is concluded here that the perfect stage of the red rot fungus of sugarcane should be named *Physalospora tucumanensis* Spag.
INTRODUCTION

Red rot has been one of the most serious diseases of the sugarcane plant in the United States and in several other sugar-producing countries of the world. The disease as well as its causative organism was discovered and described by Went in Java in 1893 (46). At that time the organism was named Colletotrichum falcatum Went and was placed in the Order Mеланконiales of the Fungi Imperfecti.

The red rot has been notable for its outbreaks in Java, India, West Indies, and Louisiana. Since the first report of the disease in the United States in 1910 (13), it has been considered as a very important factor in sugar production in Louisiana. It has been studied extensively at various times and has been kept under constant observation.

In the Southern United States in such States as Louisiana, Georgia, Florida, Alabama, and Mississippi, red rot has caused considerable damage to seed cane almost every year.

However, the red rot has been important only in certain regions of the world. In Puerto Rico and Cuba, for instance, in recent years the disease has not had any economic importance. This has been due to the fact that in these tropical countries, planted cane as well as the stubble cane soon germinated and the shoots
rapidly became independent of the mother plant. Also the damage to cane stalks caused by borers was very low and apparently the population of these insects was kept down by certain factors, such as more continuous and more abundant rains. Therefore, the red rot fungus had less chance to get into the cane stalks through the borer holes which constituted the most important avenue of entrance for the fungus. In the subtropical and temperate regions, however, conditions have been different. In Louisiana, for instance, during the cold weather of the winter months often accompanied by freezes, the cane has often ceased to grow and has been always killed back. Under these conditions, the seed-pieces as well as stubble canes have been forced to stop their growth, an unnatural procedure for sugarcane, a tropical plant naturally of continuous growth. During this time the cane has been more subject to invasion and deterioration by pathogenic organisms, especially the red rot fungus.

Under the winter conditions prevailing in Louisiana, the most important effect of red rot has been the serious killing of the buds which has caused the deterioration of infected seed and stubble canes. As a result, there has followed a reduction in germination, a poor stand for the next growing season and a general decrease in yield at harvest time. The red rot has also caused serious losses by decreasing the sucrose content of affected canes. The cane leaves have often been affected and especially in summer and fall a great portion of the leaves of the growing cane have
their entire midribs invaded. The amount of damage caused by
the fungus on the leaves has not been well understood, but it
has been known that the leaves produced a great amount of inoculum
as a potential source of stalk infection.

The red rot also has constituted one of the most important
limiting factors in the growth and release of certain desirable
cane varieties. Red rot accompanied by root rot and mosaic have
caused the discontinuation of the cultivation of several suscep-
tible varieties and their replacement by others of more resistance,
both in the United States and in other countries of the world.
These diseases were responsible for the "running out" of the
"Noble Canes" in Louisiana. These canes were, previous to 1923,
outstanding for their good size, milling qualities, and high
sucrose content. Such canes were Louisiana Purple, Louisiana
Striped, and D. 74. These canes, however, began to be very sus-
ceptible to the various diseases and their yields went down. In
1924 the cane industry in Louisiana had the first severe failure
(21). Young cane plants died out to such an extent that 40 to
50 per cent stands were the rule. An examination of the stubble
in the spring of 1924 showed a high percentage of red rot infection.
Then P.O.J. 234, P.O.J. 213, and P.O.J. 36 varieties were intro-
duced. In 1927 and during 1929-31, there were severe epidemics of
red rot. During this last period, the P.O.J. 213 cane was very
badly affected and began to be abandoned and replaced by the
varieties C.P. 807, Co. 281, and Co. 290. Soon, however, C.P. 807
began to be much affected by the red rot and was therefore
replaced by some newly released, resistant varieties such as
C.P. 28-11, C.P. 28-19, C.P. 29-320, C.P. 29-116. (1, 16, 21,
44)

The red rot fungus has been evidenced as composed of
strains of different pathogenicity upon leaves and stalks of
various cane plants. Abbott in 1933 (1) reported the actual
presence of physiologic forms of *Colletotrichum falcateum* Went,
which were based upon their differences in pathogenicity in the
different cane varieties. He suggested that the sudden failures
of cane varieties in Louisiana, such as that of D.O.J. 213 during
1930-31, were due to the appearance of new physiologic forms of
the red rot fungus.

Actually here in Louisiana, as well as in other southern
states, the red rot has remained a disease of great economic
importance, especially because it has reduced germination and
stands in diseased planted and stubble canes.

The present control of the red rot has been achieved by
the improved breeding programs which produced new resistant varie-
ties of canes. Other important factors in control programs have
been careful selection of the seed cane, digging out of the
diseased stubbles, and better cultural practices, such as having
a good drainage system for the soil.

From 1893 to the present time the causative organism of the
red rot has been considered as being a fungus belonging to the
Fungi Imperfecti. This work describes an inquiry into the nature of the red rot organism itself and comprises an intensive study of the complete life cycle in nature.
HISTORICAL SURVEY

The red rot of sugarcane was discovered in 1892 by Went on the Tjomal estate in Java, and the following year he described the disease as causing rotting of the stalks of the sugarcane plants (46), and gave the outstanding characteristic symptoms for the diagnosis of the disease on the stalks. He named and described the organism causing this malady as Colletotrichum falcatum Went. The disease itself was called "Rood Snot". Went considered it as one of the most serious diseases of sugarcane in Java. Its parasitism was proved by inoculating cane stalks with the fungus from pure culture and life history studies were carried out. The same author continued his studies giving more details of the disease and how it gradually spread in the fields in Java (47). In 1896 (48) he published in English and called the disease "Red Smut". He considered the serious damage to be due to the deterioration of the canes and the diminution of the sugar content. Went referred to the fungus as a simple saprophyte on dead cane leaves, but stated that under special circumstances it became a wound parasite of the cane stalks. He concluded that the natural infection occurred chiefly through the holes made by boring insects and that healthy cane, which had not been damaged, could not be attacked by the fungus with the exception of very young parts of the stem.
In 1893 Massée (33) at Kew, England received stools of sugarcane from Barbados, sent to him by Bovell for the purpose of determining the cause of the root disease which was serious in that region of the West Indies. After examination of the material, he concluded that the disease was due to a parasitic fungus, Colletotrichum falcatum Went. Also, he stated that mycelium of this fungus in the roots produced enormous amounts of a second kind of conidia inside the cane cells. The roots in some instances were decayed by the attack of the fungus, and the mycelium evidently passed from the stalks to the roots. Massée did not give any evidence to support his opinion. Went in Java (48) stated, however, that it was extremely improbable that the so-called "root disease" reported by Massée in the West Indies - a disease which Went thought looked very much like the "Sereh" in Java - was due to Colletotrichum falcatum. Went, who considered this fungus as a saprophyte, felt that its mere presence on diseased canes was not enough evidence for its being considered the real cause of such a disease. Went added that after having cultured the fungus, Colletotrichum falcatum, on different nutrient solutions and also having searched for other forms of the fungus on diseased canes, he had never obtained anything but conidia and chlamydospores.

About 1890 a serious epidemic was destroying the Bourbon cane and putting it out of cultivation in the West Indies. J.H. Hart of Trinidad sent to Kew, England some infected material with the purpose of finding out the true nature of the disease. Later Bovell at Barbados Station sent to Kew more infected material,
consisting of 18 selected canes, each one accompanied by a
description of the supposed cause of the disease. The ascribed
causes included the "shot borer" (Xyleburnus perforans Wall),
"moth borer" (Diatraea saccharalis Fabr.), and "fungus". In
some instances, two or all of these were described as being
present in the same cane. From the examination of these canes,
Massee in 1893 published his report considering Trichosphaeria
sacchari Massee as the actual fungus causing this disease of the
sugarcane in the West Indies which later was called "rind disease".
He described a Melanconium stage as the first stage in the life
cycle of his Trichosphaeria and indicated that the conidia of
Melanconium produced the so-called macro- and microconidia which
in turn gave the ascigerous stage of Trichosphaeria. (33)

But Went in Java in 1896 (48) gave evidence that the macro-
and microconidia, described by Massee as a form of Trichosphaeria
sacchari, were identical with those of Thielaviopsis ethaceticus
Went, the "pineapple" disease fungus of sugarcane in Java. He
stated that Massee had not given enough evidence that the ascigerous
stage, Trichosphaeria sacchari, the macro- and microconidia, and
the Melanconium-stylospores were forms belonging to the same fungus.
Went demonstrated that the macro- and microconidia always gave the
same fungus spores and the same type of disease proper to
Thielaviopsis ethaceticus; while Melanconium-stylospores always
gave rise to the formation of the pycnidia of Melanconium and the
large black spherical conidia typical of this fungus - in culture
as well as in inoculated dead canes.

At the same time that Went was proving that these different stages of Massee's *Trichosphaeria sacchari* were different species of fungi, a report came out from Argentina. Spagazzini (41) described Massee's Melanconium as *Melanconium sacchari* Massee in 1896, with Argentinian specimens (from the provinces of Tucuman and La Plata). He said that as it seemed that this species had been confused with others and that there was some doubt about its synonymy, he would therefore give the diagnostic and description of this organism. He stated that this species was most common in the sugarcane fields, upon living plants as well as upon dead ones, and that, if it had a real genetic connection with the *Trichosphaeria sacchari* Massee, as Massee claimed, it was unusual that he had not found this ascogenous stage.

Thiselton-Dyer (Director of the Royal Botanic Gardens at Kew, England) in 1900 (43) reported on the "rind disease" and "root disease" seriously affecting the Bourbon cane in the West Indies. He denied Went's findings and he believed and fully accepted Massee's wrong ideas regarding the polymorphic stages of *Trichosphaeria sacchari* Massee (32). He stated that the "rind disease" and the "root disease" were really due to the same organism, *Trichosphaeria sacchari* and that *Colletotrichum falcatus* Went, which was reported by Massee as the cause of the "root disease" in the West Indies, appeared to be nothing more than a condition of the *Trichosphaeria* modified by being more or less buried in the
ground.

Howard (34) Mycologist of the Imperial Department of Agriculture for the West Indies was requested by the Imperial Commissioner of Agriculture (for the West Indies) to repeat Massee's work under tropical conditions with a view to ascertaining how far local conditions affected the life history of the fungus *Trichosphaeria sacchari*. He began his investigations in the autumn of 1899. He made single spore cultures of Helanconium; and, beginning in this way, he studied the life history of the fungus Helanconium in pure culture and also carried out inoculation experiments. He made attempts to produce macro- and microconidia from Helanconium spores, but they never were produced in sterile media. But, in a series of experiments dealing with inoculations of supposedly healthy cane stalks with Helanconium spores, several days after inoculation, Helanconium was reisolated from all the inoculated cane while in two cases macro- and microconidia spores were also obtained both from inoculated and checks. This seemed to confirm Massee's work, but it meant that the other fungus (*Chielaviopsis ethacetica*;) was already present in the cane stalks before their inoculation. Howard also made single conidia cultures of the micro- and macroconidia forms isolated from diseased canes with the purpose of studying the life cycle of those forms, as he did with the Helanconium. The micro- and macroconidia forms each produced both kinds of spores but never any other kind of spores. Inoculation experiments with healthy cane using pure
culture of macro- and microconidia produced both kinds of conidia (macro- and micro-) and the disease proper to these forms. In the checks no infection was found. No ascigerous stage was found on diseased canes or on old cultures of macroconidia. Howard's results really confirmed Went's findings in Java.

Howard continued his investigations dealing with the so-called "rind disease" and "root disease" of sugarcane in the West Indies. In 1903 (25) he gave a more complete survey of this problem. Here, he proved definitely the pathogenecity of the conidial stages involved in these two diseases. His results may be summarized as follows:

(1) As a result of inoculation experiments with conidial cultures of Melanconium using healthy canes, he proved and concluded that Melanconium could not be regarded as the cause of the "rind disease" and that it was usually a secondary parasite upon weak, old, or diseased canes.

(2) The so-called macro- and microconidia stages of Tricho-sphaeria were identical with Thielaviopsis ethaceticus Went, the organism which caused a disease of cane cuttings in the West Indies and which was the same as the "pine-apple disease" of Java. It was also parasitic on growing canes. In the cultures (monoconidial) no traces of Melanconium spores or perithecia were found.

(3) Several thousand rotten canes were examined in Barbados and other islands during three years, but in no case were the perithecia of the described Trichosphaeria found.
(4) By the use of pure cultures of the fungi in careful inoculation experiments, he obtained results which led him to conclude that the "rind disease" in the West Indies was identical with the "red smut" of Java and was caused by the fungus Colletotrichum falcatum Went. This fungus was able to infect ripening canes at wounds and at old leaf bases and could also attack the tissues of young canes which were capable of growth and development. He found also that Melanconium infected canes easily at places in which they had been already invaded by Colletotrichum falcatum. Therefore, the part played by Melanconium in the "rind disease" of sugarcane was that of a follower of Colletotrichum falcatum, invading only diseased canes.

Howard stated that the "rind disease" appeared about November or December and increased rapidly in severity up to March and April, when the canes were harvested; that the disease appeared earlier in planted cane than in ratoons; and that on account of this disease, the Bourbon cane was being entirely replaced by other varieties.

Cobb in 1906 (12) in Hawaii gave a full account of the sugarcane diseases occurring there. He stated that "rind disease" was found everywhere in Hawaii as a well-marked disease occurring on the leaves and stalks. But, this observer did not find Colletotrichum falcatum in the Islands. Therefore he did not associate this fungus with the "rind disease" in Hawaii. After him, Lewton-Brain (29) studied the "rind disease" in Hawaii also. He stated that the
disease was caused by *Melanconium sacchari* Massae and produced serious losses to the Lahaina cane (= Bourbon) occurring normally everywhere. He studied the life history of the "rind fungus". By inoculation experiments with pure cultures of *Melanconium* (isolated from rotten canes) he was successful in producing the characteristics of the "rind disease" (typical symptoms and spore pustules). In his experiments, *Melanconium* produced only *Melanconium* in pure culture and in inoculation experiments. He stated that the "rind disease" was quite distinct from the red rot caused by *Colletotrichum falcatus* and that the fungi did not necessarily accompany one another. He never found spores of *Colletotrichum falcatus* on canes attacked by the rind disease, yet the red rot fungus was isolated from dead leaves. He did not consider the rind disease fungus as a markedly parasitic one in his work in Hawaii or in the West Indies. The rind fungus would attack through wounds only those canes whose vitality had been lowered. According to him, the cane vitality was reduced mainly by leaf hoppers, by poor nutrition, and by the attack of other parasitic fungi.

Lewton-Brain (28) while working as Mycologist for the staff of the Imperial Department of Agriculture for the West Indies, gave a report about the "rind disease" (*Trichosphaeria sacchari* Massae). He stated that "rind disease" had caused serious losses in previous years and that in those islands where the Bourbon cane was abandoned, as in Barbados and Northern Islands and replaced by the White Transparent cane (best known of the resistant varieties) and certain seedlings such as B. 147, the "rind disease" was no
longer feared. In his paper there appeared a discussion of the disease in British Guiana, Leeward Islands, Barbados, Trinidad, Jamaica, Granada, and Surinam.

In 1906 Butler (8) in a report on the diseases of sugar-cane in Bengal, India, gave a full account of the "red rot", describing it as causing severe losses and wilting of canes. Butler was the first one to use the term "red rot" for this disease instead of Went's "rood snot" or "red smut". Since then the name red rot has been universally accepted in the English-speaking countries because it was most descriptive of this disease. Several other names in other languages have been used, but most of them, such as "enfermedad rosada" and "morve rouge", mean red rot in translation. Butler stated that the active growth of the fungus took place when the cane was rich in sugar and that the presence of the parasite was closely connected with the sugar content, as was apparent from the analyses made. He found a diminution of 45 per cent in the sucrose sugar of diseased stalks, accompanied by an increase in glucose. He proved that the actual consumption of sugar by the fungus was slight compared with its "inverting" action. This was in agreement with the findings of Walker and Went in Java in 1896 (45). Butler stated that the disease spread slowly in the field, but was distributed rapidly when infected seed was used, especially in the case of very susceptible varieties. He believed that the fungus passed from the diseased sets (in the ground) to the young shoots (parts above ground), infecting and killing them by
means of direct mycelial connections. In an examination of some infected, dead shoots, he found actively-growing Colletotrichum in their bases. He attributed the death of these young shoots (for about six inches above ground) to the red rot fungus. Also, the fungus proved to be present in sets shortly after germination. He concluded that this fungus was not a soil-borne organism. Certain cases of soil infection might occur, he thought; but, in general, such infection was of no importance. Butler found stromata of this fungus at the base of the true roots, which appeared to be disorganized by the action of the fungus.

Butler described the fungus and its symptoms appearing in the midrib of the cane leaves, and he considered it as a definite disease; but it was not proved that it was the same red rot fungus of the cane stalks. Besides, he stated that a closely-allied species, Colletotrichum lineola Corda, was very common on the leaves of Andropogon Sorghum (Jawar) where it produced a rather similar appearance. Following Butler's statements, Edgerton (15) in the United States proved by means of cross inoculations that these leaf and stalk diseases of sugarcane were due to the same fungus, Colletotrichum falcatum Went. By inoculations with the related fungi, Colletotrichum lineola (isolated from Johnson grass and broom corn in Louisiana) and Colletotrichum cereale (from wheat and other grasses obtained from Ohio), Edgerton showed that no satisfactory infection nor typical red rot was obtained in the cane stalks.

Before Butler's work, Raciborski in Java (37) described in
1897 the passage of the red rot disease from the planted sets up into the young shoots.

Lewton-Brain (30) recorded the disease as present in Hawaii in 1908 but as being of infrequent occurrence. He made intensive physiological studies of the fungus in the laboratory. He failed to produce the disease by inoculating punctured cane leaves. He considered, as did Went, that the Colletotrichum from the leaves was merely saprophytic. Growing the fungus in sugar solutions in flasks (as Butler had done before) brought out more clearly that the inverting action of the fungus was due to the production of the enzyme "invertase". He proved definitely that invertase was not only contained in the mycelium of Colletotrichum falcatus, but was actually secreted into the medium in which this organism was growing. The main action of the fungus was the conversion of sucrose into invert sugar. The inverted sugar destroyed was very small in quantity and seemed to be all "levulose". The production of "cytase" enzyme (cellulose dissolving substance) also was noted to be quite restricted in amount and seemed to be confined to the tips of the actively growing hyphae. Later Ramakrishnan (36) in India in 1941 continued the work on enzymes produced by Colletotrichum falcatus, using the light and dark strains of the fungus which, he claimed, had originated from a single conidium culture. He stated that both strains produced plenty of invertase. He also claimed that inulase, lipase, and oxidase were formed in equal amounts in both strains; that the dark strain produced more of diastase and pectinase and the
light strain more of trypsin, amylase, and erepsin enzymes; and that emulsin was produced in small amounts by the light strain while the dark strain did not produce any.

In 1910 (13) the red rot disease of sugarcane was reported for the first time in the United States. The first specimen was collected by Edgerton at Audubon Park, New Orleans, Louisiana, in February 1908 (14). It was found later in Georgia, Florida, and Texas. In Louisiana, the disease was more serious in causing a poor stand (14). In 1911 Edgerton (15) gave a full account of this disease, and made the connection between what had been considered as two forms of Colletotrichum occurring on the leaves and on the stalks. By cross-inoculation, he proved definitely that these two forms were the same Colletotrichum falcatum Went. He demonstrated that the fungus from the leaves gave the red rot to the stalks and vice versa. Since that time the major portion of the work with this fungus has been done in this country.

Barber reported in 1911 (6) concerning the damage to sugarcane by the sugarcane borer (Diatraea saccharalis Fab.). He stated that the borer destroyed the eyes, stunted the growth by physical injury to the stem, and made the canes subject to breaking by the wind due to the tunnels and burrows. He also stated that the borer caused the reduction in quantity and quality of the juice of the bored stalks. But, Edgerton (15), by means of chemical analysis, demonstrated that bored cane had a greater per cent of sucrose than healthy ones while the canes infected with both borer and the red rot
disease had a very low per cent of this sugar.

Butler and Khan in 1913 (9) reported that the most serious disease of sugarcane in India was red rot. These authors proved definitely the passage of the mycelium from diseased seed-pieces to the young shoots. They confirmed Edgerton’s work showing that the Colletotrichum from the leaves was parasitic on stalks and vice versa. They succeeded in producing the disease with inoculations to the leaf midribs by puncturing and applying spore suspension in water. When they did not puncture the leaves, only a few inoculations were successful. They concluded that the leaf and stem forms were capable of penetrating uninjured leaves, but that infection occurred much more readily when the leaf was wounded.

Johnston and Stevenson reported red rot occurring in Puerto Rico in 1917 (26) as a disease of the stalks of sugarcane and of the leaves (“red stripe” of the leaf midrib). They found the fungus to be very common in the island but of not much economic importance, acting usually as a saprophyte. It was very common, they said, to find the fungus fruiting on dead leaves, or on the margins and tips of leaves which were dying. By several isolations of the fungus from the leaves and stalks, they found three different forms of Colletotrichum, A, B, and C forms. The Colletotrichum form C was the most prevalent one. They described it as follows:

"Colletotrichum C:
Found on the cane in the field as well as on the cane in damp chambers; profuse cottony white growth in cultures; minute slightly pinkish spore masses arising from tiny black spots on midrib
or blade of leaf; setae absent or commonly present, straight or flexuous averaging 80 μ long; spores thick, falcate, averaging 6.8 x 17 μ.*4

From this description it seems that they were dealing with the so-called light strain of Colletotrichum falcatum which was reported later by Abbott (2, 3).

Edgerton and Moreland in 1920 (18) referred to Colletotrichum falcatum as one of the chief organisms causing seed deterioration in Louisiana. Here, under Louisiana conditions, was denied once again as before (15) Butler's claim in India that the young cane plants became infected directly from diseased seed. According to these authors, this method of infection did not occur in Louisiana nor in the West Indies; but the factors explaining why the same fungus acted differently in different countries was not known.

In temperature studies it was found that the red rot fungus grew well at any temperature from 20-35° C. In experiments it was found that the red rot fungus produced a serious deterioration of the inoculated seed-pieces, decreasing the germination of the eyes about 50 per cent. It was found that the disease developed more rapidly in canes inoculated after cutting than it did in growing cane and that stalks well-infected before cutting did not deteriorate when used as seed so rapidly as those stalks which were inoculated after cutting. Natural methods of infection seemed to be through injuries and through the root band around the nodes. Also the red rot fungus was reported as one of the main factors involved in the stubble deterioration complex in Louisiana (18). The spores of the fungus were washed down by rains, from the aerial parts, and came in contact
with the stubble pieces and infection took place in the nodal and bud regions and in some instances through the cut top surfaces. Many of the eyes were killed before their germination, and some of the young shoots emerging from the ground were killed during the spring. In the spring of 1934 the first severe cane failure in Louisiana occurred in which the young cane plants died out, stands of only 40 to 50 per cent being the rule. An examination of the stubble showed red rot in a very high percentage of cases. Stubble pieces of the varieties P.O.J. 213 and C.P. 807 showed more red rot than any other commercial variety and together with P.O.J. 234 were the most seriously affected by stubble deterioration. While this was true, Co. 281 and Co. 290, for example, were more resistant to this trouble.

In temperature studies on the red rot fungus in Louisiana (44) with petri dish cultures, it was found that growth was very slight at 10° C., quite rapid from 27° to 34° C., and slow at 37° C. This was in agreement with and confirmed previous work in Louisiana (18). Also, temperature studies with cane stalks inoculated with the fungus and kept at various temperatures showed the following results with cane varieties Co. 281 (resistant) and P.O.J. 213 (susceptible). In Co. 281 there was no perceptible reddening at 10° C., very slight rot at 14° C., slight at 19° C., and a moderate reddening at temperatures between 22° and 37° C. Due to the resistance of Co. 281, the temperature effect was not much marked here. But with P.O.J. 213 considerable red rot developed at temperatures
between 14° and 30° C., and at 25° C. or above, dark brown dis- 
coloration also developed. Abbott (3) reported the optimum tem- 
perature for the growth of Colletotrichum falcum to be 30° to 
33.5° C., and Ramakrishnan (36) in India reported that 30° C. was 
the optimum temperature for growth and that the greatest per cent 
germination and growth of the germ tube of Colletotrichum falcum 
in 24 hours was obtained at 32° C.

Physiologic forms of Colletotrichum falcum in Louisiana 
were reported in 1933 by Abbott (1), the distinctions being based 
upon differences in pathogenicity. Tins and Edgerton (44) previously 
manifested the possibility of the presence of specialized or physio- 
logic races of the red rot fungus in Louisiana, and thought that 
the failure of the cane varieties such as P.O.J. 213 and Louisiana 
Purple during the years 1930 and 1931, and the differences in 
severity of the disease in different previous years, strongly sugges-
ted such a possibility. Abbott also found that sugarcane varieties 
possessed two kinds of resistance to the red rot fungus: (1) func-
tional (those which germinated quickly and were vigorous root 
formers, thus preventing the entrance of the fungus through the 
root primordia) and (2) physiological (those in which the develop-
ment of the fungus within the stalk tissues was retarded once 
infestation had taken place).

Physiological specialization was reported to occur with the 
red rot fungus (2). In comparative studies of 85 isolates of 
Colletotrichum falcum, two morphological groups were distinguished
on the bases of the color and texture of the mycelium, one being dark, grey, and velvety - the dark type, - and the other nearly white to light grey and cottony - the light type. The light type L-31 showed a relatively high virulence on resistant variety Co. 281 as compared with other isolations. But, it did not always show the same high virulence on P.O.J. 213 when compared with a large number of isolates. The light and dark types were to be regarded as varieties of *Colletotrichum falcatum*, but the differences in colony appearance did not appear to be correlated with the morphologic characters.

In the development of new sugarcane varieties in the United States by breeding, especial consideration has been given to a number of factors in the seedling tests. One of these has been that the desirable seedlings be tested by inoculations with the red rot fungus, in order to evaluate their susceptibility or resistance (7, 20).

Hinds et al in 1934 (33) showed that borer infestation materially reduced the purity of the cane juice and they had thus confirmed the earlier work of Gross, Allbright, Van Dine, Barber, Stubbs, Morgan, and others which was summarized by Halloway et al (22) and which clearly indicated that both the quality and the quantity of the juice were reduced and the reducing sugars in the juice were increased by injury from borers. Went in Java in the original description of the red rot disease in Java (46) presented juice analyses, showing that even a light infection of the stalks
by the causal fungus resulted in a significant decrease of sucrose and a corresponding increase of invert sugars. These results also were obtained by Edgerton in Louisiana (15). All of these results were confirmed by McKaig and Fort (34).

McKaig and Fort in 1936 (34) reported their work on the chemical composition of the juice of Louisiana sugarcane injured by the sugarcane borer and the red rot fungus. The results of tests in 1931 and 1932 showed that the quality and quantity of the juice extracted from P.O.J. 36-M, P.O.J. 213, P.O.J. 234, and Co. 290 cane varieties were materially reduced by the attacks of the borer (Diatraea saccharalis). The reduction was greater in canes exhibiting a combination of injuries from the borer and the red rot disease. The reduction in quality involved an increase in color and turbidity of the clarified juices and syrups, in the mineral constituents of the juice and in protein and non-protein nitrogenous compounds, especially of the latter.

Chowdhury (11) attempting to determine the relation of relative humidity to the germination of spores of several Indian fungi, tested Colletotrichum falcatum among others. He claimed that the minimum relative humidity at which germination of the red rot fungus occurred was 95 per cent.

Investigations carried out in Louisiana (4, 5) into the nature of the lateral and longitudinal spread of the red rot fungus in sugarcane stalks, demonstrated that spore migration in the ducts of the fibrovascular bundles was the cause of the spread up and down
in stalks inoculated by puncturing. As the injury to cane stalks increased in proportion to the number of spores of Gollototrichum falcatum introduced, it was desirable when studying the comparative pathogenicity of isolates, to use approximately equal spore concentrations in inoculations.

Two types of resistance were reported. First there was morphological resistance to spore spread. This was due to the anatomy of the cane itself. Some varieties, owing to the fact that they had comparatively few vessels extending continuously from one internode to another, showed this type of resistance. Co. 39-116 had the greatest resistance to the longitudinal spread of the disease but lacked marked resistance to the lateral spread. The second type of resistance was physiological resistance. This resistance seemed to be due to the action of the host protoplasm itself which had something that inhibited the easy spread of the mycelium within the tissues. Co. 381 was an example of canes having this type of resistance.

Abbott in 1933 (3) reported on a study of the red rot disease. Most of his work consisted in relative pathogenicity studies of the two races, light and dark, of Gollototrichum falcatum upon commercial cane varieties, cane seedlings, and the other four accepted species of Saccharums besides Saccharum officinarum L. In preliminary trials he found that the presence of the phenolic compounds, in the extracted juices of resistant and very susceptible varieties, were found to be higher in the resistant varieties than in the very susceptible
ones. It suggested that perhaps the amount of phenolic compounds might have a relation to the resistance and susceptibility of cane varieties. Also he made a great number of isolations (dark and light races) of Colletotrichum falcatum from many different places and many different cane varieties (leaves and stalks) in the southern United States, followed by intensive cultural work and inoculation experiments. Dark and light races of Colletotrichum falcatum were isolated also from Sorghum bicolor, Sorghum vulgare, and Brachypodium distachion in Louisiana, and these isolates were indistinguishable in morphology and parasitism from those obtained from leaves and stalks of the sugarcane plant. In inoculation experiments with two cultures of Colletotrichum graminicolum, one obtained from Iowa and another from the Netherlands, no red rot lesions were produced. He concluded that Colletotrichum falcatum and Colletotrichum graminicolum were two different species, and that Colletotrichum falcatum was the one commonly occurring in the leaf midribs of Johnson grass, plume grass, and sorgo in southern Louisiana. Single conidium cultures of the fungus showed constant characters throughout the period of the investigation; mutation or other form of marked variation was not observed.

In India Ramakrishnan (36), making physiological studies of Colletotrichum falcatum in culture, observed that best growth occurred at pH of 4.5 and 5.0. Abbott (3) reported pH 5.5 to be the best for growing the fungus in solid media. Ramakrishnan claimed to have gotten the dark and light strains by saltation phenomena from
a single conidium culture and that these two different types kept their characteristics through several different tests and the two types acted in a somewhat different manner. *Trichoderma lignorum* grown along with *Colletotrichum falcatum*, in five days overgrew the dark strain of *Colletotrichum falcatum* while the light strain was surrounded and only partly overgrown. In both cases the *Colletotrichum falcatum* hyphae had disintegrated in the overgrown portion, the dark strain being more affected. He believed that the best growth of *Colletotrichum falcatum* occurred when the C/N ration was 5/1. According to him, the two strains had similar temperature ranges and thermal death points (conidia killed after five minutes exposure at about 51° C.; but chlamydospores were able to germinate after five minutes exposure at this temperature). Nevertheless, the strains exhibited differences in their sporulation, pathogenicity, amount of growth, and color. A number of enzymes were produced by the two strains. Also he found that setae rarely bore conidia at their tips, in culture. However, no study was made on the behavior of these setae producing conidia.

Chone and Padwick in India in 1942 (10) in about 1,000 isolations made from different areas found that the light strain of *Colletotrichum falcatum* was more predominant and with a heavier sporulation. All isolations made before the epidemic of 1940-1 were of the dark type with sparse sporulation. Therefore, they believed that the new light strain was more virulent than the old dark strain, particularly on Co. 313, and that to this light strain
was due such an epidemic. These findings were quite similar to those obtained in the United States by Abbott (1, 3).

Investigations in Louisiana (17) into the effect of the hot-water treatment on the resistance of the cane to subsequent artificial infection by *Colletotrichum falcatum* showed that in every one of the ten commercial sugarcane varieties used in the tests (these canes representing all degrees of resistance and susceptibility to the red rot fungus within Louisiana canes) the red rot injury was much more extensive in the untreated canes. When canes were inoculated with the red rot fungus prior to the hot-water treatment (20 minutes at 52° C.), no traces of infection were found in any plant treated within two days of inoculation. Those treated three, four, and five days after inoculation showed definite lesions, although they were not so well developed as in the untreated inoculated lots. These results seemed to show that hot water did not entirely destroy the mycelium of the red rot fungus in well-established lesions. When spore suspensions of the red rot fungus were treated at 52° C. for 5, 10, and 20 minutes prior to inoculation into canes, no red rot developed in any of the plants, indicating that spores were killed during the thermal treatment.

Lucas (31) at Louisiana State University indicated, by repeated single conidium and single hypha isolations, that the red rot fungus was able to continue sporulating well in a suitable culture media. *Colletotrichum falcatum* was grown for nine generations through single conidium isolations on Richard's agar and
through hyphal tips for seven generations on oatmeal agar with no loss of vitality. In both cases identical results were obtained.
GEOGRAPHIC DISTRIBUTION AND
IMPORTANCE OF THE RED ROT DISEASE

This disease has been reported from practically every important place where cane is grown commercially. There are several places where the disease has not yet been reported, but this may be due to the lack of plant pathologists or to the unimportant nature of the disease there.

It has been reported from Antigua, Argentina, Barbados, Brazil, British Guiana, Central America, China, Colombia, Cuba, Dominican Republic, Egypt, Fiji, Formosa, Guadeloupe, Haiti, Hawaii, India, Indo-China, Jamaica, Japan, Java, Madagascar, Madeira, Mauritius, Mexico, Peru, Philippine Islands, Puerto Rico, Queensland, Reunion, St. Kitts, St. Lucia, Southern United States (Florida, Mississippi, Alabama, Georgia, Louisiana, Texas), Trinidad, Uganda, Union of South Africa, and Virgin Islands (42). This disease has been noted for sudden outbreaks in Java, India, West Indies, and Louisiana. Yet in several tropical countries this disease is of a minor importance, — as is the case in Puerto Rico.

Since the discovery of this disease in the United States (13), it has been considered as a very important factor in sugarcane production in Louisiana. It has been studied extensively at various times and has been kept under constant observation. The disease
appears every year to a greater or lesser extent, but at certain times it has appeared in epidemic form, producing crop failures. In 1923 and in 1927 there were severe epidemics of red rot, and the Louisiana Purple cane, which was severely affected, was abandoned by many growers. During 1929-32, P.O.J. 213 cane was very badly affected; and, due to its serious deterioration, it also went out of cultivation (44). However, with the introduction of new, resistant cane varieties produced through breeding work at Canal Point, Florida and the cultivation of other resistant varieties such as Co. 281, the disease has been kept more or less under control.

The main losses caused by this disease have been:

1. The great reduction of stand due to a poor germination of the infected seed-stalk-pieces and of the diseased stubble (stubble deterioration).

2. Killing of the young plants.

3. Loss in per cent of sucrose with corresponding increase in glucose and levulose (inverted sugars).

4. Injury to the cane leaves.
SYMPTOMS OF THE RED ROT DISEASE

I. Morphologic Symptoms

The red rot fungus causes its main damage to the sugarcane stalks. On the leaves it attacks mainly the leaf midribs, but also the leaf sheaths and occasionally the leaf blade. It may infect sugarcane roots but here it is of no importance.

1. On the cane stalks

The symptoms in the stalks are difficult to recognize externally, except in severe cases in which the internal tissues are rotted. Then the cane may give marked external symptoms such as shriveling, shrinking, and breaking. There may be loss of the natural color of the rind, with the fungus breaking through the rind and developing its fruiting pustules which appear as black dots (acervuli). This is only seen in severe cases of diseased canes, especially in the top portions or young portions of standing cane which has been killed by this fungus (8, 15). I have noticed these symptoms to be somewhat common here especially on stalks which have been badly infected by both red rot and the sugarcane borer. Diseased stalks which appear healthy upon external examination can be easily recognized as badly infected by means of splitting the stalks longitudinally. When the diseased stems (stalks of standing cane, seed cuttings, or stubble rhizomes) are
split longitudinally, the lesions more commonly are presented as red blotches surrounding whitish areas toward the center of the lesions. The white portions usually extend in a transverse direction. The lesions may appear at the nodes as well as in the internodal regions of the cane stalks. There may be from one single lesion to many lesions present in a stalk of cane. The lesions vary in shape, size, texture, and color tones. Sometimes brownish lesions can be found, especially at the nodes (46, 48).

Natural infection on the stalks is accomplished mainly through wounds or channels caused by boring insects, or other kinds of openings which may give access to the organism. Vent (46) was the first one to state that natural infection occurs mainly through the holes made by boring insects and most of the other workers in other parts of the world agree with him. The spread of the red rot fungus has been described by Raciborski (37) as going internally from the seed-piece to the growing stalk. Raciborski's work was confirmed in India (8, 9, 27) but denied in Louisiana (15) and in the West Indies (39). Leaf scars (25, 46), root primordia, mechanical wounds, and growth cracks (9) also have been pointed out as places of entrance into the stalks for the fungus.

The seed-canes themselves carry the disease in the majority of the cases by means of lesions inside the stalk tissues or on the leaves or leaf sheaths, which are commonly left adhered to the seed-pieces. Infection through the cut ends might occur but it is considered of minor importance (8, 18). In the cane stalks,
longitudinal spread of the disease through the vascular bundles is faster than lateral spread through the parenchyma cells (15). The degree of the spread and severity of the red rot within cane stalks depend upon the degree of susceptibility of the cane variety and on the fungus strain itself and their relation to the environmental factors, such as temperature, moisture conditions, etc. When the conditions are favorable, the fungus will spread rapidly through the vascular bundles and parenchyma tissue. In older lesions the tissue breaks down, dries out, darkens; and dense tufts of mycelium (generally grey in color) may develop in cavities thus formed. Sometimes conidia are developed in these cavities.

Standing cane stalks as well as cuttings, inoculated with a conidial suspension in sterile water (from a pure culture of the red rot fungus) will show the typical symptoms of the disease within a week, more or less. But, the symptoms and progress of the disease will be more severe on cut cane than on standing cane.

Apparently a new type of disease symptom has been found to be produced in cane pieces inoculated with conidial suspension in sterile water of the red rot fungus originated from single ascospore cultures. In the course of my investigations into the relative pathogenicity of several single ascospore cultures from different hosts and different places in Louisiana, it was found that eight to ten days after inoculation, the stalk-pieces being kept at a constant temperature of 25° C., the symptoms produced by some of the ascospores used were markedly different from the typical
symptoms of the red rot lesions caused by the other isolates. On splitting the cane stalks in halves longitudinally, much paling of the tissues was found, turning into a water-soaked appearance, being somewhat uniform throughout the whole length of the stalk-piece. The lesions were mostly dark, and much breaking down of the tissues was found, especially at the nodes, showing a general severe pathogenicity of these isolates. These symptoms were shown in cane varieties Co. 281 and O.P. 34-120. The monosporic cultures used which produced this type of symptom are shown in Table I.

In the beginning it was thought that these symptoms might be due to the use of a great amount of conidia in the suspension applied; but, on the application of a very light spore suspension, the same results were obtained. Also, due to the fact that the same symptom was produced for different isolates using two different cane varieties, Co. 281 (resistant) and O.P. 34-120 (very susceptible), it was concluded that this symptom was mainly due to the fungus strain itself rather than to the cane varieties used.

While these cultures were constant in producing such peculiar symptoms, all the other isolates in contrast produced the typical, well-known red rot lesions on the inoculated cane stalks of the same varieties. Several stalks were inoculated with sterile water to be used as checks. These showed only slight reddening around the point of inoculation as a physiological reaction to injury.
Table I  Origin of Cultures Producing a New Type of Symptom of the Red Rot Disease of Sugarcane

<table>
<thead>
<tr>
<th>Single ascospore culture name</th>
<th>Origin of the isolates</th>
<th>Cane variety inoculated</th>
<th>Number of stalks inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT-14</td>
<td>Leptochloa filiformis leaf from Houma, Louisiana</td>
<td>Co. 281</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C.R. 34-120</td>
<td>3</td>
</tr>
<tr>
<td>RR-1</td>
<td>Kassoer stem, Houma</td>
<td>Co. 281</td>
<td>5</td>
</tr>
<tr>
<td>RR-17</td>
<td>Kassoer stem, Houma</td>
<td>Co. 281</td>
<td>5</td>
</tr>
<tr>
<td>XX-2</td>
<td>Louisiana Purple leaf, Houma</td>
<td>Co. 281</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C.R. 34-120</td>
<td>3</td>
</tr>
<tr>
<td>YY-3</td>
<td>Co. 290 leaf, Houma</td>
<td>Co. 281</td>
<td>5</td>
</tr>
<tr>
<td>YY-7</td>
<td>Co. 290 leaf, Houma</td>
<td>Co. 281</td>
<td>5</td>
</tr>
</tbody>
</table>

2. On the leaves

The lesions on the leaf midrib in the beginning appear as dark reddish blotches on the upper side which soon become elongated and sometimes extend throughout the whole length of the midrib. The older lesions show straw or whitish-colored centers becoming covered with many black dots which are the fruiting bodies (acervuli) of the pathogen. From one to several independent lesions often can be seen on a given leaf. The leaf lesions are the best diagnostic symptoms of the red rot in determining its presence in a given plantation. On these midrib lesions thousands
of conidia are produced which have been considered as the main source of inoculum for the stalk infections (15). No direct mycelial connection ever has been traced between the leaves and the stalks of the cane plant. The lesions on the leaves are usually very common in the infected fields during the later summer and fall months in any cane variety growing in Louisiana. It has been found that different cane varieties are very variable in their susceptibility or resistance to the red rot fungus and that there is no correlation between the degree of susceptibility between the cane stalks and that of the leaves of a given variety. For instance, Co. 281 is very susceptible to the leaf infection but resistant to stalk infection while in F.C.J. 213, C.P. 807, and Louisiana Purple, the stalk tissues are very susceptible to the red rot fungus but generally show little leaf infection (3). The infections on the leaves are frequently originated through some kind of injury such as those made by insects (punctures), but also infection may be accomplished through apparently uninjured epidermis (3). One week after a leaf has been inoculated, the conidial fructifications begin to appear. It has been found that the red rot fungus as well as the grass anthracnose fungus (Colletotrichum graminicolum) also keep on producing spores throughout the winter months on the trash. Hence this infected trash left out in the field along with the infected seed cuttings are the means by which the fungus winters and is ready to cause new infections in the growing cane during the coming spring season.
II. Histologic Symptoms

While the histologic symptoms have been studied by several authors, this subject still needs further investigation.

Under suitable conditions, the spores may germinate and penetrate the host tissue if it has been wounded previously. But, it has also been reported (9) that the penetrating hypha of the fungus can enter the host through the tissues of the leaves without any apparent previous injury. If spores in an aqueous suspension reach, through any wound or other opening, the vessels of standing cane stalks or of fresh stalk cuttings, they will travel through them; and, being thus scattered throughout several different places in the cane stalks, they will germinate there within a few hours. The germ tube will grow on the vessel and eventually the hypha will penetrate the walls of the vessels (mainly through the pits) and grow into the other vascular cells passing finally to the parenchymatic or sugar storage cells. In other cases, through wounds, the infection to the stalks may begin at the parenchymatic tissue, invading later the other tissues too, but the lesions in this case might be localized in one region.

In inoculation experiments on cane leaves, Butler and Hafiz (9) found that even when the leaves were not injured the fungus spore germinated on the leaf and the germ tube formed an appressorium, especially over the motor cells. Then the infecting hypha from this appressorium penetrated directly across the outer epidermal cells, or in some cases down between the side walls of two cells. After
entry, the hyphae may at once branch freely and fill the large motor cells with abundant mycelium; or may penetrate deeply into the leaf tissues passing from cell to cell in the large parenchyma cells between the bundles, but not readily entering these bundles. In some cases the sclerenchyma cells were penetrated. When the leaf tissue was injured, the mycelium first ramified on top of the injury and penetrated freely anywhere in this injured area. In both cases all the invaded tissue developed a bright red color. In less than one week after inoculation, characteristic spores and setae (acervuli) were developed.

Lewton-Brain (30) in studying the progress of the disease in the stalks, stated that the growing mycelium of the red rot fungus probably secretes a substance which is toxic to the living cells of the cane tissue. The hyphae then enters these cells, destroying their contents, and the process is repeated until a considerable area of the cane tissue is killed and the contents of the cells are destroyed, or completely changed. The damage is entirely direct and confined to the destruction of the thin-walled parenchyma cells. The sugar destroyed and the progress of the disease depend upon the resistance of a given variety of cane. The outstanding feature of the attack of the fungus in the stalks is the inversion of the sucrose content into inverted sugar due to the secretion of the enzyme invertase by the fungus. By means of restricted amounts of the enzyme cytase (cellulose-dissolving enzyme) which seems to be secreted by the tips of the actively growing hyphae, the fungus bores its way through the walls
of the host cells, passing from one cell to another. The hyphae appears to be constricted in its passage through the cell wall.

Went (46, 48) by microscopic examination found that the vascular bundles from the diseased lesions (on stalks) were red-colored and gummy along a certain distance from the diseased area. Also, he stated that in any injury to the cane tissue the vascular bundle communicating with the damaged portion will become gummy and red colored. The cells in the diseased portions of the stalk were filled with the mycelium (containing many small oil drops) of the fungus. Frequently the oil drops have persisted, and thus, the direction of the former mycelium could be traced. Howard (25) stated that it is possible that the formation of gum is an adaptation on the part of the cane cells in order to prevent bacteria and fungi from passing into the vessels where the cane is wounded.

In cane stalks (standing or cut) inoculated with the fungus, the lesions first appeared (when the stalks were opened) as small red spots which increased gradually in all directions, especially in the direction of the long axis of the stalk. After the disease had developed for several days, the lesions which were of a deep red color became large and the central portion of some of them lost the red color and became dirty white in color. These white spots surrounded by the red tissue are the main characteristic symptom of this disease used in its diagnosis. These white spots seem to appear faster in the lesions of cut cane than in the standing cane stalks (15, 46). At this stage the tissue of some of the older
lesions breaks down, dries out, becomes dark, and gives off a certain smell of fermentation. The mycelium of the fungus is sometimes found in tufts filling the cavities left by the broken tissues where conidia are sometimes produced, too. In very severe cases, the root primordia, buds of the stalks, and sometimes the entire stalk may be killed. Abbott (3) has stated that within the invaded host cells the mycelium of the fungus may produce conidia too.

In some of my inoculation experiments with cane stalk cuttings (Louisian Purple, Co. 281, No. 33-243), I also found atypical conidia which were produced in the parenchyma cells eight days after inoculation with the red rot fungus. After inoculation the stalks were kept at a constant temperature of 25° C. Cross and longitudinal sections were made through some lesions and examined microscopically. Abundant conidia of variable shapes and sizes were found from almost normal to a very small conidium of sausage shape which was the most prevalent one. These conidia were produced singly or sometimes in chains at the ends of short, slender hyphae. All these conidia which we must consider as atypical were hyaline, unicellular, and uninucleate. They were easily cut off from the hyphae which bore them and therefore it was common to find several of these small conidia free within a single host cell filling it. There is not yet any evidence in regard to the functional behavior of such atypical conidia within the parenchyma cells.
Microscopic examination of longitudinal and cross sections of cane stalk lesions have shown that very often the bright red outer zone of the lesion did not reveal any mycelium while the presence of a gummy, red substance was shown. Also, tissue transfers to agar plates of this outer reddish portion of lesions very often failed to yield the red rot fungus. Several times cultures were made on agar plates by carefully taking out the whole reddened extension of an individual vascular bundle which had been invaded by the fungus. Stalks of Co. 281 and C.P. 33-343 were used one to four days after inoculation with conidium suspension in sterile water. As a result, the fungus generally made its appearance growing out from the center portion of the bundle into the agar, in a circular way, while the other and longer extensions up and down the same red bundle did not give any red rot fungus at all. When longitudinal and cross sections through the whitish or discolored portions of older lesions of cane stalks 10 to 20 days after inoculation were made, abundant fungus mycelium was found in these tissues. Occasionally the so-called chlamydospores were seen sticking against the dead, dried up, or empty parenchyma cells. The red gum seems to have disappeared in this whitish region; and, when tissue transfers were made from these zones to agar plates, the typical red rot fungus was obtained easily. Similar results were obtained in studies of the whitish portions of old lesions on the midrib of cane leaves.

Inoculations on cane leaves were carried out in the field
as well as in the greenhouse and laboratory in order to make some histological studies. On April 24, 1943, 144 cane leaves of the Co. 281 variety were inoculated by means of puncturing the midrib on the upper side for a small space and then applying conidia (originated from single ascospore cultures and from single conidium cultures of the red rot fungus) in suspension in sterile water. The canes were growing in the greenhouse at Louisiana State University. After these inoculations, reddish spots began to appear on the third day, and thereafter the lesions increased in size with time and their centers became pale. On the sixth day many young erumpent acervuli were found in most of the inoculations, especially in the discolored areas on the upper side of the midribs. On the seventh day, free-hand sections (cross and longitudinal) of some of the lesions were made. These showed abundant young growing acervuli, many setae forming and turning brownish, and a great number of tufts of conidiophores, some bearing young conidia, some bearing mature ones. Also later on it was found by microscopic examination that in sections of the lesions through the discolored areas, most of the host cells were affected directly, showing the red rot fungus mycelium spreading and branching, being intra- and intercellular. The cells affected included those of the epidermis, bulliform or motor cells, parenchyma and vascular bundle component cells. Production of atypical conidia directly from the hyphae of the fungus was also actually evidenced as occurring inside of some of the parenchyma cells of the cane leaf. A reddish coloration was
detected especially in the vascular bundles. The parenchyma cells bearing chlorophyll pigments were shown with a pale coloration because of the destruction of the chlorophyll pigments associated with the fungus invasion.

The observations of several authors indicated that the resistance of the cane varieties to the attack of the fungus may be:

(1) Morphological. For example, G.P. 29-116, due to its anatomical behavior, does not permit the fast spread of the disease. However, the protoplasm of the host may be or may not be susceptible.

(2) Physiological. For example, Co. 281, in which the host protoplasm itself is resistant opposing the advance of the fungus within the tissues (1, 3, 4).

Greater abundance of the phenolic compounds in the more resistant varieties than in the susceptible ones may suggest that these compounds may be concerned in the resistance mechanism of the cane varieties (3).
DISCOVERY OF THE PERFECT STAGE OF COLLETOTRICHUM FALCATUM WENT

Early in March, 1941 an acigerous stage was produced upon sterilized cane leaves in test tubes 10 to 20 days after inoculation with conidia from a pure culture of Colletotrichum falcatum. Since that time this ascus stage has been found to be the perfect stage of the red rot fungus and has been produced repeatedly under sterile conditions in the laboratory by means of single conidium and single ascospore cultures as well as by single hyphal tip cultures of the fungus. The fungus was carried on through its entire life cycle under controlled conditions, and an intensive study of its perfect and imperfect stages was made. It was found that the perithecial stage of this fungus belongs to the genus Physalospora of the Ascomycetes.

During May and June of 1941, the perithecial stage was produced again by inoculating leaves of standing cane in the greenhouse. A careful study of these perithecia in different phases of their development in the host plant leaves was carried on by hundreds of free-hand sections (longitudinal and cross) and several microtome sections through the leaves bearing them. A number of microphotographs were taken. As a result of this study, it was
concluded that this Physalospora stage agrees reasonably well with the original description of *Physalospora tucumanensis* Spag., described from Argentinian specimens by Dr. Carlos Spegazzini during the year 1896 ([41]). At this stage in the course of my investigations, however, the question of the true specific name for the perfect stage of the red rot organism could only be definitely solved by getting the original type material of *Physalospora tucumanensis* from Argentina. The type material was obtained and found to be in good condition. Intensive comparative studies of the Louisiana fungus and the Argentinian one were carried on satisfactorily. The fungi were found to be identical, as will be demonstrated later in the course of this work.
In Different Species of Saccharum

After developing the perfect stage under sterile conditions and proving its pathogenicity and genetic connection with the red rot fungus, it also was found occurring very abundantly under natural field conditions on sugarcane. The perithecial stage was first found in the field in July, 1941 on dead and dying leaves previously inoculated. Also in July the perithecial stage of this fungus was found very abundantly on other dead leaves and shoots of the same and other cane varieties in the same plantation (Dalrymple Drive, Baton Rouge, Louisiana). These perithecia were of an entirely natural development. Then by the study of these abundant perithecia found in nature in July, more evidence was obtained that we were dealing with Physalospora tucumanensis Spee. Careful studies of this ascogenous fungus were made. Herbarium specimens and several permanent slides were prepared.

Since that time, the sexual stage of Colletotrichum falcatum as well as its imperfect stage have been found in every cane field regardless of cane variety inspected by the writer in the State of Louisiana.

The ascogenous stage from which single ascospore cultures were obtained has been actually found occurring under natural field
conditions upon leaves and leaf sheaths of the five species of Saccharum recognized today. Such species were *S. officinarum* L. (ascospores isolated from varieties Louisiana Purple and Louisiana Striped), *S. barbati* Jesviet (ascospores isolated from natural variety Chunnee), *S. robustum*, *S. sinense* Roxb. (ascospores isolated from variety Cayena), *S. spontaneum* L. from India (ascospores isolated from varieties Burma and #238 - Houma number) as well as from *S. spontaneum* L. from Java (ascospores isolated from variety Pasoer-ocean). Also it was found occurring on artificial hybrids from which isolation of single ascospores was carried on from such varieties as: Co. 281, Co. 290, C.P. 33-243, C.P. 33-310, C.P. 34-53, C.P. 34-120, C.P. 34-164, C.P. 36-55, C.P. 36-75, C.P. 36-85, C.P. 36-185; and also from the natural hybrid Kassoer cane. Table II shows the number of single ascospore cultures obtained from the above Saccharums, fungus strain obtained, and the place where the material was collected.

From Table II we find that from a total of 497 single ascospore cultures obtained from naturally developed perithecia, the dark strain of the fungus predominated, 357 being dark, and only 140 light. From this total of 497 ascospore cultures obtained, not one failed to produce the typical Colletotrichum falcatum fungus (mycelium, conidia, chlamydomospores, and setae) in pure culture. The pathogenicity of each group of isolates was proved - as will be considered later in another section. Type material of these groups of isolations has been kept dry at the Botany Department.
<table>
<thead>
<tr>
<th>Host</th>
<th>Fungus strain obtained</th>
<th>Total single ascospore cultures</th>
<th>Material collected from</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host material</td>
<td>No.</td>
<td>dark</td>
<td>No.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccharum officinarum L.</td>
<td>3</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Saccharum barberi Jeswiet</td>
<td>13</td>
<td>14</td>
<td>27</td>
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<td>Saccharum robustum</td>
<td>20</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>Saccharum sinense Roxb.</td>
<td>19</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td>Saccharum spontaneum L., India</td>
<td>24</td>
<td>-</td>
<td>24</td>
</tr>
<tr>
<td>Saccharum spontaneum L., Java</td>
<td>15</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>Kassow x, natural hybrid, Java</td>
<td>3</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Artificial hybrid varieties:</td>
<td>2</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Co. 281 (from brown stripes)</td>
<td>10</td>
<td>13</td>
<td>23</td>
</tr>
<tr>
<td>C.P. 33-243</td>
<td>8</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>C.P. 34-120</td>
<td>17</td>
<td>-</td>
<td>17</td>
</tr>
<tr>
<td>C.P. 33-310</td>
<td>12</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>C.P. 34-53</td>
<td>14</td>
<td>-</td>
<td>14</td>
</tr>
<tr>
<td>C.P. 34-120</td>
<td>23</td>
<td>-</td>
<td>23</td>
</tr>
<tr>
<td>C.P. 34-164</td>
<td>2</td>
<td>148</td>
<td>50</td>
</tr>
<tr>
<td>C.P. 36-55</td>
<td>20</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>C.P. 36-15</td>
<td>13</td>
<td>11</td>
<td>24</td>
</tr>
<tr>
<td>C.P. 35-21</td>
<td>21</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td>C.P. 55-25</td>
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<td>5</td>
<td>17</td>
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<tr>
<td>Unidentified varieties</td>
<td>45</td>
<td>4</td>
<td>49</td>
</tr>
<tr>
<td>Winter, unidentified varieties</td>
<td>9</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>TOTALS</td>
<td>357</td>
<td>140</td>
<td>497</td>
</tr>
</tbody>
</table>
The procedure followed in the ascospore isolations was the same in all cases. Fresh, natural material bearing the perfect stage of the organism was used, and a small portion of the surface in which perithecia were embedded in the leaf tissue was thoroughly cleaned by gently rubbing it with a dry handkerchief. Then, with a sterile needle, the perithecium was dug out by gently slipping the tip of the needle between the vascular bundles. The perithecium, thus extracted from the host tissue, was placed in a sterile drop of water on a slide (also sterile); and now the perithecium was crushed with the same needle using the low power of the microscope or using the naked eye. The crushed perithecium, after examination under the microscope to determine whether or not the asci and ascospores were in a good condition, was transplanted to an agar drop hanging in a Van Tieghem cell. Then, by means of a micromanipulator, single ascospores were picked out one by one and each planted in another sterile, separate, single drop of potato dextrose agar. The germination of the ascospores was watched several times daily and on the third day each agar drop containing a single, germinated ascospore, whose mycelium was usually covering the whole drop of the agar by this time, was transplanted to an oatmeal agar slant and kept away from contamination.

The percentage of germination of the ascospores from naturally developed perithecia was generally very high. In the course of these isolations, the germination was found to vary from
65 to 100 per cent because every precaution was taken to select fresh material, to choose only those ascospores having the best appearance, and to take great care to sterilize all the materials and equipment used in this work.

In these isolations dealing with a single perithecium, the whole eight ascospores were isolated a number of times from one single ascus as well as ascospores from other asci from the same perithecium. All single ascospore cultures thus obtained from the same perithecium always were identical with each other.

It has been found that from a single leaf sheath, or from a single leaf blade, or leaf midrib, both culture strains (dark and light) of the red rot fungus were sometimes actually obtained by single ascospore cultures. However, while the two strains were in the same host material, occurring side by side, they were obtained from different perithecia. The presence of both strains is due to the possibility that the same leaf was infected by both strains of the fungus independently. Hence, later on, when the right time came for sexual stage development, the two strains produced their perithecia scattered and intermingled in the same host tissues. This theory, however, suggests further investigation. Nevertheless, in no case did I obtain both strains from the same perithecium.

The majority of the above isolations were made during and toward the end of the growing season of the year 1942 from standing cane material. At only one time were isolations made in winter.
These single ascospore isolations in winter (February 1943) were made from canes, the above ground portions of which were totally killed because of the series of freezes during January and early February, 1943. These ascospores isolated from dead leaves and leaf sheaths produced both strains (light and dark) of the fungus. From this material and also from dead stalks, the red rot fungus, which was actively sporulating, was isolated by single conidium and tissue transfers as well as by ascospores.

Specimens from the United States Department of Agriculture Sugar Experiment Station at Houma, Louisiana, were collected by the writer during a trip made on November 3-4, 1942 in that region. Most of the ascosporic isolations made from this material were taken from leaves (leaf blade and midribs) and leaf sheaths of the cane plant. However, the ascosporic isolations from the Kassoer cane were made from perithecia formed at the top of the dead cane stalk just below the spindle.

At the same time that ascosporic isolations were made, sometimes single conidia isolations were also made.

Perithecia were found occurring fully developed on dead stripes on the leaf blade of Co. 281 cane variety from material collected at the Sugar Experiment Station at Baton Rouge, Louisiana. Single ascospore isolations were made in September 1942 in which 13 dark cultures were obtained. The stripes were found running parallel to the long axis between the green, healthy, living tissue of the leaf blade. These narrow stripes of dying or dead tissue were of sizes varying from one to five millimeters in width and from five
to 40 centimeters in length. Each leaf of the three leaves used presented a few of these stripes which were from a light brown to a dark brown in color. The red rot fungus was found fructifying by means of its sexual and asexual stages in each stripe without showing any characteristic symptoms which have been heretofore known for detecting its presence easily. Perithecia were found here placed between vascular bundles on the upper side as well as on the underside of the stripe, while the acervuli were found in great number on the upper side. In some of the lesions perithecia were more predominant in number on the underside of the stripe. The finding of the sexual as well as of the asexual stage of the fungus naturally occurring on these narrow, brown stripes without showing any apparent injury to the surrounding green, healthy tissue, plus many other field observations on the natural occurrence of the perfect stage of the red rot fungus, inclined me to believe that this organism is in reality a weak parasite, tending to be more saprophytic in nature. Also it is claimed here that the stripes originated because of other causes, such as mechanical injury, sun burn, wind injury, physiologic disturbances, injury by other organisms (mottled stripe, viruses, insects, etc.). We known that the red rot fungus is more likely, initially, to attack the midrib of the leaves than the leaf blade, which is only eventually affected. Here, however, the case suggests that the fungus came as a saprophyte to these stripes when the tissue was dying. Thus it presented an appropriate medium for the pathogen to develop its sexual stage.
Occurrence of Parithecia and Acervuli on Dead Shoots

One of the most common sites of perithecial production in the field is dead shoots of different sugarcane varieties. It is a common thing to find these dead shoots all covered with parithecia and acervuli in all their above ground portions from the ground surface to their tops including leaves, leaf sheaths and sometimes also the stalks. These dead shoots are from a few inches to several feet in height. This fact was observed from late July throughout the summer, Fall, and early winter of 1942 in several cane fields. These shoots grew from some seed pieces (planted in the Fall of 1941) as well as from stubble canes. The dead shoots were commonly found near the base of the normal, growing, healthy canes. One of the most outstanding features was the finding of many of these shoots without the appearance of any characteristic red rot lesion which could indicate the point of entrance of the fungus. Some of these shoots, on the other hand, showed that they had been badly injured by boring insects. These examinations were carried out by cutting the plants at ground level, after which the leaves and sheaths were taken off one by one and carefully studied by splitting the shoots longitudinally in halves. Often it was observed that while the typical, known, red rot lesions did not show up in any of the normal plants in one section of the cane field, the red rot fungus was found flourishing greatly in some of these dead shoots under the healthy sister plants.

It seems to me that this feature is one of the most important
facts concerned in the life cycle of the red rot organism in nature in carrying new infection and in the wide-spread propagation of the disease in the early growing season, carried on through its two stages.

It was not possible to determine the reason for the death of the young shoots upon which the red rot fungus was found. It is possible that a considerable number of things were involved in it. In the first place we have the winter injury which may kill or predispose these young plants to attack by other organisms. The conditions and pathogenic organisms which are involved in the root rot complex as well as in seed cane and in stubble deterioration have to be considered too. We also must take into consideration any other kind of injury, such as insect injury (especially borers) which may kill the young cane plants and permit the entrance of pathogenic organisms such as the red rot fungus. The natural dying out of these shoots may be attributed to nutritional deficiency.

The time and place of entrance of the red rot fungus into these shoots were not determined. It may enter before or after the death of the shoots. The source of inoculum also is unknown. There seem to be a number of indications that the red rot fungus may pass in some cases from the already infected seed piece or stubble cane into these dead or dying shoots. The following points are of particular interest.

(1) No particular lesion or injury was found on a considerable number of these shoots.
(2) No red rot lesions were detected on neighboring living cane leaves.

(3) The entire stalks of these shoots were rotted by the red rot fungus, which was found to be fruiting through the rind.

**Natural Development and Appearance of the Perithecial Stage on Sugarcane**

The perithecial stage develops on sugarcane on dead and dying tissues, particularly on the leaves and leaf sheaths and occasionally on dead stems. The perithecial bodies begin to form, as inconspicuous, black dots, at the time when the infected leaves are becoming pale.

In infected fields, the dead leaves hanging from the stalk, as well as those already detached from the plant, almost always bear perithecia and conidia. Usually these leaves show lesions of the red rot disease. Sometimes, however, these leaves do not present any apparent lesion which could indicate where the original infection took place. These leaves may or may not be in the neighborhood of other leaves which show lesions of the red rot disease. While the perithecial stage is found only on the dead or dying tissues, the conidial stage is found occurring abundantly upon young to old lesions, as well as on the dead tissues, where they may be intermingled with the perithecia.

As a general rule the perithecia appear to be well distributed and appear as black dots forming rows between the vascular bundles of the midribs, leaf blades, and leaf sheaths. They appear on the upper as well as on the underside of the leaves. Generally
perithecia are formed individually and rarely in groups. Plate V, Figure 3 shows the distribution of the perithecia in the leaf blade of sugarcane — natural development.

Preparations of free-hand sections (cross and longitudinal) observed under the microscope showed young perithecia developing often in the stomatal chamber and usually more abundant on the underside of the leaf. But this is not always the case, as the perithecia are also found developing in places other than the stomatal chamber, such as in parenchyma and epidermal tissues.

When a perithecium develops in the stomatal chamber, the young perithecium increases in size and opens its way by compressing and destroying some of the neighboring cells and then by separating the guard cells and pushing up a papillate ostiolum. The parenchymatous cells are the ones that appear to be more affected in the development of the perithecia while the vascular bundles usually appear to be unaffected morphologically. The perithecia are globose to subglobose or with peculiar forms and shapes according to the place in which they develop. As a general rule the longer dimension of the width of the perithecium is parallel to the direction of the bundles between which it develops because these bundles do not give it enough chance to develop with a uniform diameter — cross and length. The height of the perithecium including the ostiolum may be less than, equal to or longer than the longer diameter of the perithecium.
Occurrence of the Red Rot Fungus in Leptochloa Filiformis

Specimens of the grass, Leptochloa filiformis (Lam.) Beauv. infected with the red rot fungus were collected in the cane field at the United States Department of Agriculture Sugar Experiment Station at Houma, Louisiana, on November 3-4, 1942 by the writer. The grass plants were practically dead and bore perithecia and also acervuli. This grass (common name "red sprangletop") is an annual plant which is common in open and shady places in Louisiana and particularly in the cane fields. It has been reported from several southern states of the United States and is found throughout tropical America also.

The perithecia in this grass are very inconspicuous and appear as very small black points imbedded inside the dead leaf tissues between vascular bundles on the upper and lower sides of leaf sheaths and blades. The acervuli appear anywhere scattered among the perithecia, but more especially near or around the nodes of the stem and also on the leaves. The microscopic examination of this material agrees in all ways with both perfect and imperfect stages of the red rot fungus.

On November 6, 1942, single ascospore isolations were made in the usual way. The percentage of germination obtained was 90 per cent. From these isolations, 20 single ascospore cultures were obtained from five different perithecia. Of these 20 ascospores, 18 were of the dark type (obtained from four perithecia) and two were of the light type (obtained from the fifth perithecium).
The pathogenicity of six of these ascospores was proved by producing the typical red rot symptoms upon inoculated cane stalks of Co. 281. All of them were relatively equal in pathogenicity. Therefore, by the study of the material and by the cultural and pathological characters, it is here proved that the perfect stage of the red rot fungus has been actually found for the first time in a grass other than sugarcane. Also, \textit{Lectochloa filiformis} has been found to be a new host for the red rot fungus.

During the same month of November, abundant material of the same grass was found in the Sugar Experiment Station at Baton Rouge, Louisiana, bearing the two stages of the red rot organism not only on the leaves and leaf sheaths but also on the stems. In the stems, the perithecia were inconspicuous and erumpent through the epidermal tissues by means of their ostiolum, making the surface of the stems somewhat rough when touched.

In all cases the perithecia were found on dead host portions.

Not much effort was directed to the search for the perithecial stage in other kinds of grasses, but it seems likely that it may also occur in some others.

\textbf{Environmental Conditions under which the Perfect Stage Was Found Developing in Nature}

The development of the perfect stage of the red rot organism under natural field conditions does not seem to require any very special or definite favorable conditions. From July 1942 to February 1943, the perfect stage as well as the conidial one had been actually isolated from old and dead leaves of the sugarcane
plant. This period of time involved extremely varied environmental factors, such as mild to hot temperatures in the summer and Fall months and temperatures below zero degrees centigrade in winter. The rain fall was very variable too. However, the most abundant perithecial development has been found during the Fall months when the rains were more abundant and when the disease was much more widely distributed.
DEVELOPMENT OF THE PERFECT STAGE OF THE RED ROT FUNGUS

BY INOCULATION EXPERIMENTS

I. Under Sterile, Controlled Conditions in the Laboratory

Whereas the development of the perfect stage of the red rot fungus under natural field conditions does not require any exceptional feature, its development under sterile conditions in the laboratory needs certain special requirements. Several new techniques were devised and others enlarged upon in the study of the development of the ascogamous stage of this organism.

The perfect stage was produced on sterile leaves and leaf sheaths of sugarcane, corn, Johnson grass, and on filter paper; on longitudinal pieces of cane stalks and on bagasse. This work was carried on by the use of test tubes, flasks, and petri dishes. Each one of the following liquids has been used in order to bring about a moist chamber condition: sterile water, cane leaf infusion, fresh green bean pod infusion, white onion infusion, cane stalk infusion, and cane stalk infusion plus prune juice. About 200 grams of each of these materials were used per liter of water to make the desired infusion, which was then filtered. Two types of inoculations to these sterile materials were used:

(1) by direct transfer of a small piece of agar bearing mycelium of
the fungus from a pure culture to the sterile material, and (2) by
smearing the material to be inoculated with a conidium suspension
in any of the above liquids. Table III shows the cultures with
which the perfect stage was produced.

The age of the cultures used in all the inoculations was
generally from eight to 25 days and they were grown on oatmeal
agar slants. Leaves of several cane varieties were used but no
marked difference was found in the ability of the perithecia to
develop on them.

In all cases in which the perfect stage was developed,
the conidial stage always was present in abundance. The experiments
were carried out by placing the cultures after inoculation on a
table in the laboratory - sometimes receiving only the daylight,
others receiving daylight plus light from an electric lamp of 60
watts during the night. When the electric lamp was used, the cul­
tures were kept at a distance of not less than one meter from it.*
Some were also kept in complete darkness. When the daylight was
used it was never direct, that is not receiving the sun's rays
directly. The temperatures used were those of the laboratory
(room temperatures) and also a constant temperature of 25° C.
Under all of these conditions the perfect stage as well as the im­
perfect was developed. Mature perithecia bearing mature asci and
ascospores were usually developed under these conditions from seven
to 20 days after inoculation. The dark and light color strains
used reacted similarly and no marked differences were found in both
<table>
<thead>
<tr>
<th>Cultures used</th>
<th>Strain</th>
<th>Origin</th>
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<tbody>
<tr>
<td>Single conidia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-c</td>
<td>dark</td>
<td>Single conidium culture from A culture*</td>
</tr>
<tr>
<td>x39, x40, x50</td>
<td>dark</td>
<td>Single conidium cultures obtained from the x culture ** through 39, 40, 50 generations through single conidia</td>
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<tr>
<td>Single hyphal tip</td>
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<tr>
<td>x42, x52</td>
<td>dark</td>
<td>Obtained from the x culture** through 42 and 52 generations through single hyphal tips</td>
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<tr>
<td>Single ascosporia</td>
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<td></td>
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<tr>
<td>A-1 to A-6</td>
<td>dark</td>
<td>Perithecia developed on sterile leaves inoculated with the A culture*</td>
</tr>
<tr>
<td>A-12</td>
<td>dark</td>
<td>Perithecia developed on sterile leaves inoculated with the Houma culture+</td>
</tr>
<tr>
<td>C-f-8</td>
<td>light</td>
<td>Perithecia developed on sterile leaves inoculated with C-f single conidium culture**</td>
</tr>
<tr>
<td>PP-14</td>
<td>dark</td>
<td>Perithecia developed naturally on Leptochloa filiformis at Houma, La.</td>
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<tr>
<td>x40-1 to x40-29</td>
<td>dark</td>
<td>Perithecia produced on sterile leaves with x40 single conidium culture**</td>
</tr>
<tr>
<td>XX-2</td>
<td>dark</td>
<td>Perithecia developed naturally on Louisiana Purple cane leaves at Houma, Louisiana</td>
</tr>
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</table>

* The A culture was obtained from a cane stalk in winter 1942 at the Sugar Experiment Station at Baton Rouge, Louisiana.
+ The Houma culture was obtained from a cane-seedling leaf collected in the Fall of 1941 at the U.S. Department of Agriculture Experiment Station at Houma, Louisiana.
** The x culture was obtained from cane stalks from Napoleonville, Louisiana in the late winter of 1940.
+++ The C culture was obtained from a cane seed-piece (Co. 281) from Rosa, Louisiana during May, 1942.
perithecial stages thus produced. The perithecia were produced embedded in the host tissue erump ing at maturity by means of a short papillate ostiolum the same as it appears under natural field conditions. Sometimes, however, the ostiolum was somewhat elongated and hairy, as indicated in Plate IV, Figure 2. Within the host cells, the perithecia appeared singly or sometimes in groups, similar to those produced in nature. Also perithecia were produced free outside the coal tissue or on whichever material was used. Here again the perithecia were single and sometimes in groups having common walls, and somewhat hairy on the outside, due in part to the mycelium holding these bodies to the substratum. In no case was a stroma ever found; the perithecia had definite walls. Perithecial size was always more or less constant as was also true of their asci and ascospores. The conidial stage also appeared anywhere in the cultures, extending upon the glass walls.

Much care must be taken in order to produce the perfect stage under sterile, controlled conditions. Any contamination of the cultures with bacteria or any other organisms such as fungi, mites, etc. will easily stop and prevent the development of the perfect stage and ruin the experiment.

Sometimes besides normal, fertile perithecia (bearing typical asci and ascospores) there are found other perithecia of abnormal growth. These perithecia for some reason did not reach maturity, that is produce ascospores. Other abnormal perithecia may show some asci of smaller or of much increased dimensions in comparison with
the normal ones and these do not bear any ascospores inside. They only show the presence of disorganized protoplasm, which often is much vacuolated. These asci generally are of abnormal and peculiar shapes.

Sometimes asci are found which bear one or more gigantic ascospores within some portion of the disorganized mass of protoplasm. These gigantic ascospores often show one or two clear, circular regions (free of granules) toward their center. Some other asci do not develop all their ascospores, that is they contain less than the normal number, which is eight. Other abnormal asci may show the full number of ascospores, but these ascospores may differ widely in shape and dimensions.

When a perithecium reaches maturity, generally the ascospores are discharged through the ostiolum, but sometimes they may germinate within the perithecium and inside the ascus which contains them. These ascospores may germinate by sending out germ tubes which will perforate the ascus wall and continue to grow outside, while the original ascospore remains within the ascus. At other times the ascospores germinate by a small bud, in which case the mother cells appear as if holding small ones especially at their ends. They, however, are confined within the ascus.

Thirty-seven single ascospore cultures as well as several single conidium isolates were made from the different cultures described in Table III. All of these new single ascospore cultures which were tried, produced the perfect stage again. The experiment
was repeated several times with the same results.

In all cases, through these several generations through the sexual stage, each typical isolate kept its identity with the parent type as was shown by means of an intensive culture work of the fungus and by pathogenicity studies. No marked variation has been found in the course of my investigations. I must also say here that the x culture, of a well-known strong pathogenicity which has been used previously for testing cane seedlings and which has been cultured since early in the year 1940 (and passed through 50 generations of single conidial cultures and 52 generations of single hyphal tip cultures) has produced the perfect stage easily under sterile conditions. The pathogenicity of this x culture as well as the cultural characters (dark strain of a heavy sporulation) of the single ascospore cultures obtained were in every way identical with the parent type and the previous generations of single conidium cultures. Therefore, this fungus is homothallic, since each independent isolate kept its characters through several generations of conidial, ascospore, or hyphal tip cultures. This homothallism has also been demonstrated by the isolation of all eight ascospores from the same ascus, these proving identical in severity of pathogenicity and in cultural characters.

In summarizing these experiments which were successfully repeated several times, it is here concluded that the very best results were obtained when cane leaves and leaf sheaths were cooked previously in water, and placed in petri dishes (one or two leaf
pieces) on top of wet cotton. Then after sterilization and cooling down, they were inoculated by smearing the surface of the cane leaf piece with a conidial suspension in sterile water by means of a glass bar bent in the form of a loop at the end. Care was taken to draw off any excess water from the petri dishes. The plates were kept on a table in the laboratory receiving subdued daylight (never direct sun rays) at room temperature. This has been carried out successfully at different times throughout the greater part of an entire year. One of the special advantages of this method is the almost complete suppression of the vegetative growth (aerial mycelium) of this fungus culminating in its faster reproduction both sexually and asexually.

When the inoculated leaves were kept in darkness, the vegetative growth, aerial mycelium, was favored, especially when the inoculations were made by means of direct transfers.

As a general rule the inoculations obtained by smearing the conidia on the sterile leaves was more efficient in producing the sexual stage, than when direct transfers were used.

Material bearing perithecia and conidia produced under sterile, controlled conditions in the laboratory has been kept as dry herbarium material.

II. In Inoculation Experiments on Standing Cane

Inoculation experiments were carried on upon standing cane in the field, in the greenhouse, and in the laboratory in order to
determine whether or not the perfect stage of the red rot fungus could be produced in the living host.

1. Leaf Inoculations on Standing Canes in the Greenhouse

Leaf inoculations in the greenhouse on Co. 281 and C.P. 28-19 were made during the month of May, 1942. Over 300 cane leaves were inoculated by puncturing the midrib and applying the conidia from single conidia and single asco spore cultures suspended in sterile water. More than 25 leaves were used as checks. In the inoculated ones, the lesions developed fast within a week and continued to increase and develop abundant conidia. One month more or less after the inoculations, young perithecia began to appear on the lower, older, and dying inoculated leaves (blade, midribs, and leaf sheaths) of the two varieties used. After two months, mature asci and ascospores were found on some of the inoculated leaves which had died while the checks did not develop any lesions at all.

Also cane leaves bearing lesions were cut one week after inoculation and brought to the laboratory where they were placed on wet cotton in large moist chambers. Eight to 13 days after being placed in the moist chambers, abundant mature perithecia were well-developed and 12 single asco spore cultures (A-6-14 to A-6-25) were made. These ascospores were developed from perithecia produced with the single ascospore culture A-6.

2. Stalk Inoculations on Standing Canes in the Greenhouse

Stalks of Co. 281 and C.P. 28-19 varieties were inoculated with single spore cultures during the month of May 1942. Seven days
after inoculation, some of the stalks were cut and split. All bore red rot lesions. It was found that in one of the stalks of Co. 231, several perithecia of the red rot fungus were developing inside right around the inoculating hole. These perithecia were allowed to mature in the laboratory and their identity was thus confirmed. These perithecia were from the single ascospore culture A-1 which was used in this inoculation experiment.

The other halves of cane stalks also bearing the red rot lesions were placed in large test tubes (2" x 12") after wet cotton had been placed in the bottom of the tubes. The top of the test tubes was plugged with dry cotton and then they were left in a vertical position in the laboratory, receiving indirect sunlight. After 13 days the perfect stage bearing mature asci and ascospores became visible in the stalks of Co. 231 and C.P. 28-19. The perithecial stage was produced in separate inoculations made with two single ascospore cultures used (A-1 and A-4) as well as the conidial Houma culture. Under these conditions, perithecia developed mostly around the inoculating hole, but also sometimes upon other separate lesions, especially near the edges of the cuttings. The perithecia formation was generally very scanty and always superficial. More predominant was the production of conidia, setae, and aerial mycelium.

The rest of the stalks were cut and split into halves two weeks after inoculation. All of them showed the definite red rot lesions with white centers. Here several mature perithecia were found developed (with the three cultures mentioned above) around
the inoculating hole in Co. 281 and also in C.S. 28-19 cane stalks.

3. Leaf Inoculations on Cane Plants Growing in Pots in the Laboratory

Leaves of Co. 281 cane variety were inoculated and kept in the laboratory at room temperature receiving indirect sunlight. The experiments were carried out during July and August, 1942.

The inoculations were made by puncturing the upper side of the midrib of each leaf and applying the conidial suspension in sterile water by means of a piece of sterile cotton placed on top of the punctures and then wetting it thoroughly with the suspension. The cotton was removed after 24 hours. Several infections were made on each leaf, and also the inoculum in all cases was drawn down to the leaf sheath from the inoculated leaf. Checks were also made by puncturing the midrib and applying sterile water alone.

In these experiments, two different cultures were used, 0-f and 0-f. 0-f was a single conidium culture obtained from Dr. Abbott's culture L-579 and was obtained from Dr. Abbott by the Botany Department at Louisiana State University in 1940. It was the dark strain type and had been previously isolated by him from Co. 34-79 cane. The second culture used was 0-f, a single conidium culture obtained from the light strain culture 0 (which was isolated from a Co. 281 seed-piece from Poca, Louisiana in May 1942).

One week after the inoculations, definite lesions were developed which continued to develop more and more with the progress of time. No lesions developed on the checks. Two to four weeks later some of the leaves bearing lesions were placed on wet cotton
in large moist chambers. As soon as these leaf pieces were becoming pale, the perithecial bodies appeared everywhere inside the host tissues between vascular bundles. Seven to nine days after the leaves were placed in the moist chambers, many perithecia were matured and single ascospore cultures were made.

From the leaves inoculated with the C-f culture, 30 single ascospore cultures (from C-f-1 to C-f-30) were made from several different perithecia. All proved to be identical with the parent light strain type. From the inoculations with O-f (Abbott's L-579) culture, 21 single ascospore cultures were made from a single perithecium and labelled as follows:

[ascospores C-f-1 to C-f-9, ascus 1]
[same perithecium]
[ascospores C-f-9 to C-f-16, ascus 2]
[ascospores C-f-17 to C-f-21, different asci and perithecia.]

All of these gave identical cultures with the parent type.

In the comparative studies on the perfect stage of the dark and light strains, no marked difference was found. The perfect stage of both strains was produced in the same way. The perithecia, asci, and ascospores are on the average of the same dimensions and shapes. The only notable difference is in the culture color type, one being dark and the other light.

Also the perfect stage was developed one to two months after inoculation on the dead and dying leaves which had been inoculated and left on the plant (standing cane in the laboratory). These perithecia, however, were confined mostly to the leaf sheaths
and underside of the midribs of a few leaves.

4. Leaf Inoculations on Standing Cane in the Field

Cane leaves of C.C. 33-243 and Co. 281 standing canes growing out in the field (Dalrymple Drive Plantation, Baton Rouge, Louisiana) were inoculated in July 1942. Different single conidium and single ascospore cultures involving dark and light types of the red rot fungus were used. The procedure followed in the inoculations was the same as used in the previous experiment. Also a number of checks were made. Here the leaves inoculated were of variable ages from very young ones to those which were old and fading.

After seven days all the inoculations were examined and the typical red rot lesions with conidia and setae present were abundantly produced upon those leaves inoculated with the fungus. The checks showed only a paling or slight reddening just around the puncture.

Perithecia were produced by bringing the inoculated leaves to moist chambers, which contained a layer of wet cotton at the bottom. Some were brought three days after inoculation, others one, two, three, and four weeks after inoculation respectively. In all cases, mature perithecia were produced with all the single conidium as well as with all the single ascospore cultures of the dark and light strains of the red rot fungus in the two cane varieties used. From this material the fungus strains were reisolated by means of several single ascospore isolations - 8 to 15 for each
culture. Several single conidium cultures - 1 to 5 for each culture - were also made. All these isolations resulted in cultures identical with the parent type of culture (dark and light) used in the inoculations.

Also, abundant perithecia were developed upon the inoculated dead and dying leaves (leaf blade, midrib, leaf sheath) right on the standing canes themselves in the field. During the second and third week of the month of July, 1942, mature perithecia began to appear especially in those leaves which were old and fading at the time of their inoculation. In late July most of the inoculated leaves had developed abundant perithecia. Under these conditions all the single conidium as well as the single ascospore cultures of both strains (dark and light) produced the sexual stage in both cane varieties.

Here as well as in the previous inoculation experiments, the perithecia began to develop anywhere on the leaves when they began to get pale and exhausted. The perithecia generally appeared first under the midrib near the inoculating region and very quickly they also developed scattered throughout the leaf. Under these circumstances, no other of the known symptoms of the red rot disease were shown outside of the lesions on the midrib. Therefore it is here concluded that the perithecial development is favored by the dying of the cane leaves; that when the leaves begin to get pale and exhausted, the fungus mycelium will grow extremely fast through the cane leaf tissues, perhaps due to the non-opposition of the host cells and to the more saprophytic nature of this fungus.
In many cases the mycelium will soon cover the whole extension of the cane leaf tissues without forming any apparent known symptom previously used to detect the presence of the fungus within the host. This is confirmed by the prompt development of a great number of perithecia on those leaves. The presence of the mycelium in the leaves before the development of the perithecia was detected by means of the study under the microscope of free-hand sections made through the leaves. In all cases the imperfect stage continued to develop also. No marked difference in the ability of the perithecia to form was found between the two cane varieties used. In the field during wet mornings, masses of ascospores were often seen oozing out of the mature perithecia. These ascospores appeared as small, hyaline to pale pinkish, mucilaginous drops sticking out of the mouth of each ostiolum. Sometimes the same phenomenon was also observed in the laboratory with material in moist chambers. This can be seen clearly with the aid of a hand lens.

At the present time the phenolic compounds are thought to be concerned with the resistance mechanism of the living host cells to the parasitic organism. Dr. Abbott (3) found that phenolic compounds were more abundant in the extracted juices of two resistant varieties than in the two very susceptible ones. Having these things in mind, we are lead to believe that the phenolic compounds may also break down, together with the other components of the host cells, during the natural dying out of the cane leaves and hence the leaf cells lose their resistance to the growth of the fungus.
At this time the fungus will grow very fast without much resistance of the host cells to oppose its progress. It is here suggested that the phenolic compounds which have been reduced to other more simple compounds, taken together with the other cell contents, will constitute a good food for the fungus. Then the fungus, reaching at this time its best development, culminates in the abundant production of the sexual stage as well as the asexual.

In summary, the perfect stage was produced successfully in inoculated cane plants using different single conidium and single ascospore cultures of the red rot fungus of the dark and light strains. As a general rule, perithecia were produced only upon the pale and dying cane leaves while perithecia never were observed upon the green tissue, and their presence is noted by means of the perithecial bodies which appear as inconspicuous, black dots imbedded in the leaf tissues between vascular bundles. No lesions characterized the appearance of the perithecia while the imperfect stage is found more easily upon the typical red rot lesions but also it is always found abundantly intermingled with the perfect stage anywhere it is developing.

When the inoculated leaves bearing the lesions of the red rot were put in moist chambers, the best perithecial development was obtained when the moist chambers were placed on a table in the laboratory at room temperature receiving subdued daylight (never the direct sun's rays).
DEVELOPMENT OF THE PERFECT STAGE WITH LEAVES
FROM THE FIELD BROUGHT TO MOIST CHAMBERS

I. Naturally-Infected Cane Leaves Brought to Moist Chambers

Naturally-infected leaves of more than 12 different varieties of cane bearing red rot lesions were brought from the field and placed on wet cotton in large moist chambers on several occasions during the summer and Fall of 1943. As a general rule, mature perithecia were formed in all those leaves seven to 20 days after being placed in the moist chambers. A total of 85 single ascospore cultures were made from this material. Light and dark culture types were obtained. Sometimes eight ascospores were isolated from a single ascus. The cultures obtained from each of these eight ascospores from the same ascus always were identical.

II. Apparently-Healthy Cane Leaves Brought from the Field to Moist Chambers

On November 13, 1942, young cane leaves, shoots, and tops of the varieties Co. 281, Louisiana Purple, and C.P. 33-243 were cut and brought to the laboratory where they were placed in large, sterilized moist chambers without directly wetting the material. The cane field from which this material was collected (Dalrymple
Drive, Baton Rouge, Louisiana) was infected with the red rot disease and there had been no rain for some days previous to the cuttings. Before putting the material into the moist chambers, care was taken to select material free from visible lesions of any kind; and only healthy, young, vigorously growing, green tissues were chosen. This experiment resulted in the prompt appearance of the red rot fungus on a few of the leaves of the three cane varieties placed in the moist chambers but not in all of them. Ten days after the leaves had thus been placed in the moist chambers, however, the perfect as well as the imperfect stages of the red rot fungus were found in abundance. Here the fungus made its appearance on the paling leaves without showing any of its typical well-known symptoms and its presence was only detected by the evidence of its two stages.

In some of the leaves of Louisiana Purple, perithecia began to appear on the typical yellow spots on the leaves caused by the presence of the mosaic virus. On November 27, 42 single ascospore cultures (ZZ-1 to ZZ-42) were made from a single peritheciurn. Eight ascospores from a single ascus were obtained resulting in cultures ZZ-1 to ZZ-8 while the rest of the ascospores were from several asci. All the ascospores produced identical cultures and were of the dark strain type of the red rot organism.

From this we may conclude that although no sign of the fungus was visible to the naked eye when the leaves were cut, the fungus must have been present nevertheless on or in the leaves before being placed in the moist chambers. If the fungus mycelium was within the
leaves, it may have been in a latent condition. This fact brings up a very important problem which should be studied in the near future.
PROOF THAT PHYSALOSPOR A IS THE PERFECT STAGE OF

COLLETOTRICHUM FALCATUM VENT

PATHOGENICITY OF THE SINGLE ASCOSPORE CULTURES

First, we have already taken into consideration that single conidium cultures of Colletotrichum falcatus Vent produced the perithecial stage under sterile, controlled conditions. All of the single ascospore isolations which were made from the perithecia thus produced, gave the typical red rot fungus in agar culture (conidia, setae, mycelium, etc.). With single ascospore cultures, the perithecial stage was again produced on sterile materials in the laboratory.

Second, all of the single ascospore cultures isolated from the Physalospora found on dead and dying leaves of the five species of Saccharum and a grass Leptochloa filiformis produced the typical Colletotrichum falcatus cultures without any exception. With several different ascospore cultures which were isolated from naturally developed perithecia on Saccharum and Leptochloa filiformis, the perfect stage was produced again under sterile controlled conditions in the laboratory.

Third, by means of an intensive inoculation work, the pathogenicity of the single ascospore cultures (obtained by natural development and also under sterile controlled conditions) was
thoroughly proved upon cane plants of different varieties. Comparative pathogenicity studies among different isolates of single ascospore cultures, as well as of some single conidiium cultures of the red rot fungus have been successfully carried out from April to December 1942.

I. Inoculation Experiments in Standing Canes (Stalks and Leaves) Growing in the Greenhouse

1. First Experiment

The first leaf inoculations were made on Co. 281 cane leaves on April 24, 1942 using six single ascospore cultures (A-1, A-2 from the same ascus, A-3, A-4, A-5, and A-6 from different asci) which were obtained from perithecia developed on sterile cane leaves with the Colletotrichum falcatum culture A. Twenty-four leaves were inoculated with each culture (total of 144 leaves) and ten leaves were inoculated with sterile water as checks. The inoculations were made by puncturing the upper side of the leaf midrib, after which a small piece of cotton was applied. Then the conidial suspension in sterile water was applied to the cotton, wetting it thoroughly by means of separate, sterilized droppers. Some inoculum was drawn down to the leaf sheath. In some leaves one or two additional inoculations were made at different distances from each other. The cotton was removed after 34 hours. As a result of these inoculations, reddish spots began to appear on the third day; and with time the lesions increased in size and in some lesions the centers began to discolor and to turn to a dirty whitish
color. After one week more of the lesions showed the presence of the asexual fructifications of the fungus. Conidia and setae appeared especially on the discolored areas on the upper side of the midrib. Histologic studies with several free-hand sections through the lesions were also made. All of the leaves which were inoculated with the fungus gave positive results - they developed the characteristic lesions of the red rot fungus. On the other hand, the checks showed only a weak reddening just around the punctures. The plants were kept under observation to note the progress of the disease. The perithecial stage also developed in some of the dead and dying leaves of the standing canes. All of the six cultures produced perithecia. The six cultures used were similar in severity of pathogenicity.

2. Second Experiment

A second experiment was also carried out with similar successful results. On May 7, leaves and stalks of the varieties C.P. 28-19 and Co. 281 were inoculated with two single ascospore cultures, A-1 and A-4 and also with a conidial culture (Houma culture, isolated from a leaf of a cane seedling). Three stalks and 16 leaves of each variety were inoculated with each one of the cultures. The leaves were inoculated according to the same procedure as used in the first experiment described above. The stalks were inoculated by boring a hole at one internode and the inoculum (conidia in suspension in sterile water) was applied directly by means of droppers. Three stalks and eight leaves of each variety
were inoculated with sterile water only, to be used as checks.

Seven days after inoculation, the inoculated cane stalks (which were split in halves for observation) and leaves gave positive results showing the production of the typical red rot symptoms for each of the cultures used. All of the cultures were similar in severity of pathogenicity and symptoms developed. In one of the stalks (Co. 281 inoculated with A-1) several perithecia were found developing around the point of inoculation; perithecia also developed later in the other stalks.

From the cane inoculated in these two experiments, the fungus was reisolated by means of tissue transfers from leaves and stalks and also by single ascospore isolations from the perithecia produced in the host. All these isolations developed the typical red rot fungus (conidia, mycelium, setae, etc.) in pure cultures on agar.

Therefore, by means of artificial inoculations on cane stalks and leaves of plants growing in the greenhouse, the pathogenicity of cultures originated from single ascospores (which were produced under sterile, controlled conditions) was demonstrated upon varieties Co. 281 and C.F. 28-19 of standing cane under greenhouse conditions.

II. Inoculation Experiments in Standing Canes (Stalks and Leaves)

1. Leaf Inoculations

On July 6 and July 21, 1942, leaf inoculations with the red rot fungus were made using cane varieties Co. 281 and C.F. 33-343
growing in the Dalrymple Drive Plantation, Baton Rouge, Louisiana. Eleven single spore cultures were used, six of which were single conidium cultures 12 days old and the other five were single asco—spore cultures 15 days old. With each one of the cultures 16 leaves of each cane variety were inoculated; and, as checks, eight leaves of each cane variety were "inoculated" with sterile water. The procedure followed in these inoculations was the same as used before. Table IV gives the cultures used, their color strain, and origin.

In general, seven days after these inoculations the typical symptoms of the red rot lesions were developing and conidia and setae also were abundantly produced from all the cultures used. The checks showed paling or slight reddening around the punctures. During the succeeding days, the red rot lesions were much elongated and in some of the leaves they covered the whole length of the leaf midrib.

In the study of the relative pathogenicity among the asco—sporic cultures, x—40—15 was found to be the most severely pathogenic, A—12 and A—13 about equal and next to x—40—15, while A—7 and A—10 were moderately pathogenic. Among the conidial cultures, x—39, B—h, and C—g were more severely pathogenic (similar to x—40—15), while D—h and E—g were next (similar to A—12 and A—13), and G—f was moderately pathogenic (about equal to A—7 and A—10. Plate III.

Figure 2 shows cane leaves of Co. 281 inoculated with single conidium cultures and a check, 55 days after the inoculation. Plate III.

Figure 1 shows cane leaves of Co. 281 inoculated with single asco—spore cultures, 40 days after inoculation.
Table IV  Origin of the Cultures Used in Leaf Inoculations on July 6 and 21, 1942

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<thead>
<tr>
<th>Cultures</th>
<th>Color strain</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single ascospore</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-7</td>
<td>dark</td>
<td>Perithecia produced on sterile leaves with the Baton Rouge culture A</td>
</tr>
<tr>
<td>A-10</td>
<td>dark</td>
<td></td>
</tr>
<tr>
<td>A-12</td>
<td>dark</td>
<td>Perithecia produced on sterile leaves with the Houma culture</td>
</tr>
<tr>
<td>A-13</td>
<td>dark</td>
<td></td>
</tr>
<tr>
<td>x-40-15</td>
<td>dark</td>
<td>Perithecia produced on sterile leaves with the 40th generation (through conidia) of the x culture</td>
</tr>
<tr>
<td><strong>Single conidium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-h</td>
<td>dark</td>
<td>C.P. 28-19 seed-piece, Rosa, Louisiana</td>
</tr>
<tr>
<td>G-g</td>
<td>light</td>
<td>C.P. 28-19 seed-piece, Rosa, Louisiana</td>
</tr>
<tr>
<td>D-h</td>
<td>dark</td>
<td>Co. 281 seed-piece, Sug. Expt. Sta., Baton Rouge, Louisiana</td>
</tr>
<tr>
<td>E-g</td>
<td>light</td>
<td>Louisiana Purple seed-piece, Sug. Expt. Sta., Baton Rouge, Louisiana</td>
</tr>
<tr>
<td>x-39</td>
<td>dark</td>
<td>Thirty-ninth generation through single conidia of the x culture (from cane stalks, Napoleonville, Louisiana)</td>
</tr>
</tbody>
</table>
From these results it was obvious that there was some variation in the pathogenicity of the single conidium cultures as well as in the ascospore cultures when inoculated upon leaves of Co. 281 and C.P. 33-243 cane varieties.

Without exception, when the infected cane leaves were dead or dying, a profuse amount of perithecia always developed upon them.

By means of single ascospore and single conidia isolations, each fungus culture was reisolated and the original culture type, either light or dark, was actually obtained.

2. Stalk Inoculations

These were the first of the field stalk inoculations on standing cane varieties C.P. 33-243 and Co. 281 with single ascospore cultures. The inoculations were carried out on August 22, 1942. The plantation was situated at Dalrymple Drive, Baton Rouge, Louisiana. Four different stalks of each variety were inoculated with one of the different cultures. Eight stalks for each variety were "inoculated" with sterile water to be used as checks. The inoculations were made in the usual way by boring a hole at one of the internodes of each stalk and applying the conidial suspension in sterile water by means of separate sterile droppers. Table V shows the origin and color strain of the single ascospore cultures used in these inoculations.

In order to study the results, the cane stalks were cut 16 days after these inoculations and brought to the laboratory, where they were split longitudinally. Then the relative pathogenicity
Table V  Origin of the Cultures Used in Stalk Inoculations on August 22, 1942

<table>
<thead>
<tr>
<th>Ascospore culture&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Color strain</th>
<th>Age of culture in days</th>
<th>Amount of conidia in suspension</th>
<th>Origin of the ascospores</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-7-3</td>
<td>dark</td>
<td>10</td>
<td>many</td>
<td>Ascospores</td>
</tr>
<tr>
<td>A-10-4</td>
<td>dark</td>
<td>12</td>
<td>many</td>
<td>from perithecia</td>
</tr>
<tr>
<td>A-12-5</td>
<td>dark</td>
<td>13</td>
<td>many</td>
<td>developed from</td>
</tr>
<tr>
<td>A-13-1</td>
<td>dark</td>
<td>12</td>
<td>many</td>
<td>artificial</td>
</tr>
<tr>
<td>B-h-3</td>
<td>dark</td>
<td>11</td>
<td>many</td>
<td>inoculations</td>
</tr>
<tr>
<td>C-f-11</td>
<td>light</td>
<td>7</td>
<td>many</td>
<td>on cane leaves</td>
</tr>
<tr>
<td>C-g-2</td>
<td>light</td>
<td>12</td>
<td>moderate</td>
<td>in the field.</td>
</tr>
<tr>
<td>D-h-1</td>
<td>dark</td>
<td>11</td>
<td>many</td>
<td></td>
</tr>
<tr>
<td>E-g-6</td>
<td>light</td>
<td>10</td>
<td>many</td>
<td></td>
</tr>
<tr>
<td>x-40-15-6</td>
<td>dark</td>
<td>12</td>
<td>many</td>
<td></td>
</tr>
<tr>
<td>Q-5</td>
<td>light</td>
<td>12</td>
<td>many</td>
<td>Ascospores</td>
</tr>
<tr>
<td>R-2</td>
<td>dark</td>
<td>12</td>
<td>many</td>
<td>from natural infection and development in the field.</td>
</tr>
</tbody>
</table>

* These cultures were grown on oatmeal agar slants, and only one test tube was used of each for the above inoculations.
was studied. The results obtained are given in Table VI. After this the red rot fungus for each culture was reisolated by means of tissue transfers from those lesions found from 1\(\frac{1}{2}\) to 2\(\frac{1}{2}\) feet distant from the point of inoculation. As a result of these re-isolations, the original culture type (dark or light) was obtained in each case. Photographs were taken of Co. 281 and C.P. 33-243 split stalks in order to show the type of lesions obtained from these inoculations.

The results indicated that all the single ascospore cultures used, though of different origin, showed the typical red rot lesions in stalks of Co. 281 and C.P. 33-243 standing cane plants growing in the field, when examined 16 days after inoculation. By means of the Q-5 (light strain) and R-2 (dark strain) single ascospore cultures obtained from the perfect stage of the red rot fungus (grown under natural field conditions), the occurrence of the sexual stage in nature was for the first time established. Some variation was noticed in the pathogenicity upon the two varieties inoculated among the several single ascospore cultures used.

A second experiment of the same nature was made in order to determine and to prove definitely the occurrence of the sexual stage of the red rot organism under natural field conditions and to determine the relative pathogenicity by inoculating cane stalks (standing cane growing in the field) with single ascospore cultures.

On September 3, 1942, four stalks of C.P. 33-243 and four
Table VI  Results of the Single Ascospore Culture Inoculations of Standing Cane, Obtained 16 Days Later

<table>
<thead>
<tr>
<th>Ascospore culture</th>
<th>Cane variety</th>
<th>Type of red rot lesions</th>
<th>Relative pathogenicity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-7-3</td>
<td>Co.281</td>
<td>Few lesions as A-10-4.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C.P.33-243</td>
<td>Typical red rot lesions at nodes.</td>
<td>xx</td>
</tr>
<tr>
<td>A-10-4</td>
<td>Co.281</td>
<td>Bundles red thru 5 joints, a few small outbreaks.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C.P.33-243</td>
<td>Lesions more definite than in Co.281.</td>
<td>xxx</td>
</tr>
<tr>
<td>A-12-5</td>
<td>Co.281</td>
<td>Typical red rot lesions, tho' small.</td>
<td>xxx</td>
</tr>
<tr>
<td></td>
<td>C.P.33-243</td>
<td>Lesions large, nodes necrotic.</td>
<td>xxx</td>
</tr>
<tr>
<td>A-13-1</td>
<td>Co.281</td>
<td>Same as A-12-5.</td>
<td>xxx</td>
</tr>
<tr>
<td></td>
<td>C.P.33-243</td>
<td>Same as A-12-5.</td>
<td>xxx</td>
</tr>
<tr>
<td>B-h-3</td>
<td>Co.281</td>
<td>Distinct lesions, many narrow ones.</td>
<td>xxx</td>
</tr>
<tr>
<td></td>
<td>C.P.33-243</td>
<td>Severe red rot, especially at nodes; lesions large.</td>
<td></td>
</tr>
<tr>
<td>C-f-11</td>
<td>Co.281</td>
<td>Distinct red rot; some lesions rather large. Similar to Q-5.</td>
<td>xxx</td>
</tr>
<tr>
<td></td>
<td>C.P.33-243</td>
<td>Some lesions; rot (red discoloration) of inoculated internode.</td>
<td>xx</td>
</tr>
<tr>
<td>C-g-2</td>
<td>Co.281</td>
<td>Severe red rot. Same as Q-5.</td>
<td>xxx</td>
</tr>
<tr>
<td></td>
<td>C.P.33-243</td>
<td>Red discol. confined mainly to internode of inoculation.</td>
<td>xx</td>
</tr>
<tr>
<td>D-h-1</td>
<td>Co.281</td>
<td>As A-10-4 but less pronounced.</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>C.P.33-243</td>
<td>Some distinct lesions though small.</td>
<td>xx</td>
</tr>
<tr>
<td>E-g-6</td>
<td>Co.281</td>
<td>Severe red rot. Lesions as with Q-5.</td>
<td>xxx</td>
</tr>
<tr>
<td></td>
<td>C.P.33-243</td>
<td>As with C-f-11 in C.P. 33-243.</td>
<td>xx</td>
</tr>
<tr>
<td>x-40-15-6</td>
<td>Co.281</td>
<td>Typical lesions throughout 5 joints.</td>
<td>xxx</td>
</tr>
<tr>
<td></td>
<td>C.P.33-243</td>
<td>Severe red rot, especially at nodes.</td>
<td>xxx</td>
</tr>
<tr>
<td>Q-5</td>
<td>Co.281</td>
<td>Distinct red rot lesions throughout 5 to 6 joints, very parasitic.</td>
<td>xxx</td>
</tr>
<tr>
<td></td>
<td>C.P.33-243</td>
<td>Definite lesions few. General rot in internode of inoculation.</td>
<td>xxx</td>
</tr>
<tr>
<td>R-2</td>
<td>Co.281</td>
<td>Typical severe red rot, as with Q-5.</td>
<td>xxx</td>
</tr>
<tr>
<td></td>
<td>C.P.33-243</td>
<td>Severe red rot. Nodes necrotic.</td>
<td>xxx</td>
</tr>
<tr>
<td>Checks</td>
<td>Co.281</td>
<td>Reddening around the point of inoculation only, no red rot lesions.</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>C.P.33-243</td>
<td>Reddening around the point of inoculation only, no red rot lesions.</td>
<td>0</td>
</tr>
</tbody>
</table>

* 0: Negative results.
  x: Very weak pathogenicity.
  xx: Weak pathogenicity.
  xxx: Definite pathogenicity showing the beginning of darkening and breaking down of the lesions, especially at the nodes.
  xxxx: Very severe case of pathogenicity.
stalks of Co. 281 were inoculated by boring a hole and applying the inoculum as in the previous experiment. For checks, seven cane stalks of each variety were punctured and "inoculated" with sterile water. The location of the plantation was on Dalrymple Drive, Baton Rouge, Louisiana. Table VII shows the origin, color strain, and age of each of the single spore cultures used. All cultures were grown on oatmeal agar slants, and only one test tube of each was used for the inoculations. In order to determine the results, the inoculated canes were cut 11 days after inoculation and brought to the laboratory where they were split longitudinally and studies.

The results of the pathogenicity studies on Co. 281 were as follows:

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check</td>
<td>Discoloration around the puncture.</td>
</tr>
<tr>
<td>Cg-9</td>
<td>Reddening of the vascular bundles through four joints; no outbreaks, not red rot.</td>
</tr>
<tr>
<td>x-40-15-5</td>
<td>Numerous typical red rot lesions in six joints; very pathogenic.</td>
</tr>
<tr>
<td>S-13</td>
<td>Small lesions through seven joints; like W-19, but more severe.</td>
</tr>
<tr>
<td>T-15</td>
<td>Typical red rot lesions throughout five joints. Very pathogenic.</td>
</tr>
<tr>
<td>T-16</td>
<td>Reddening of the vascular bundles through five joints; some very small lesions.</td>
</tr>
<tr>
<td>U-7</td>
<td>Typical red rot in seven joints; very pathogenic.</td>
</tr>
<tr>
<td>U-25</td>
<td>Small, scattered lesions throughout seven joints.</td>
</tr>
<tr>
<td>V-20</td>
<td>Red rot lesions throughout seven joints.</td>
</tr>
<tr>
<td>W-19</td>
<td>Lesions very small throughout five joints.</td>
</tr>
</tbody>
</table>
Table VII  Origin of the Cultures Used in Stalk Inoculations on September 3, 1942

<table>
<thead>
<tr>
<th>Cultures</th>
<th>Color</th>
<th>Age of culture strain</th>
<th>Amount of conidia in days</th>
<th>Isolated from:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single ascospore</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-13</td>
<td>dark</td>
<td>17</td>
<td>moderate</td>
<td>C.P. 36-85</td>
</tr>
<tr>
<td>T-15</td>
<td>light</td>
<td>17</td>
<td>moderate</td>
<td>C.P. 36-185</td>
</tr>
<tr>
<td>T-16</td>
<td>dark</td>
<td>17</td>
<td>moderate</td>
<td>C.P. 36-185</td>
</tr>
<tr>
<td>U-7</td>
<td>light</td>
<td>17</td>
<td>moderate</td>
<td>C.P. 34-164</td>
</tr>
<tr>
<td>U-25</td>
<td>dark</td>
<td>17</td>
<td>many</td>
<td>C.P. 34-164</td>
</tr>
<tr>
<td>V-20</td>
<td>dark</td>
<td>15</td>
<td>moderate</td>
<td>C.P. 36-55</td>
</tr>
<tr>
<td>W-19</td>
<td>dark</td>
<td>15</td>
<td>moderate</td>
<td>C.P. 36-75</td>
</tr>
<tr>
<td>W-24</td>
<td>light</td>
<td>15</td>
<td>moderate</td>
<td>C.P. 36-75</td>
</tr>
<tr>
<td>Y-7</td>
<td>dark</td>
<td>13</td>
<td>moderate</td>
<td>C.P. 34-53</td>
</tr>
<tr>
<td>Z-7</td>
<td>dark</td>
<td>9</td>
<td>moderate</td>
<td>C.P. 34-120</td>
</tr>
<tr>
<td>x-40-15-5*</td>
<td>dark</td>
<td>22</td>
<td>moderate</td>
<td>Co. 281</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(artificial inoculation)</td>
</tr>
<tr>
<td>Conidial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cg-9*</td>
<td>dark</td>
<td>13</td>
<td>many</td>
<td>grass (Poa annua)</td>
</tr>
</tbody>
</table>

* x-40-15-5 of a known pathogenicity and Cg-9 of the so-called Colletotrichum graminicolum of unknown pathogenicity upon sugarcane were used for comparative studies of relative pathogenicity with the rest of the cultures. The rest of the cultures originated from single ascospores isolated from asci which came from perithecia developed entirely under natural field conditions at the Sugar Experiment Station at Baton Rouge, Louisiana, collected and isolated in August 1942.
Typical red rot lesions throughout five joints; very severe case, breakdown at the nodes and bigger lesions.

Lesions throughout six joints.

Red rot lesions throughout seven joints.

A photograph (Plate I, Figure 1) was made in order to show the different types of lesion caused by the different monosporic cultures upon Co. 281 cane stalks.

The results of the pathogenicity studies on C.P. 33-243 were as follows:

**Check**
Discoloration around puncture.

**Cg-9**
General reddish discoloration at the internode of inoculation.

**S-13 W-19**
All of these cultures gave typical red symptoms for this variety of cane.

**T-15 W-24**
General necrosis in the inoculated internode, including the two proximal nodes, occurred. Also some lesions were observed in a few joints above and below the inoculated internode. S-13, Y-7, Z-7, and U-25 were severely pathogenic.

As the above results show, it has been determined and proved that the sexual stage of the red rot fungus occurs under natural field conditions. Due to the pathogenicity of the several single ascospore cultures used in these artificial inoculations, typical red rot lesions were obtained on standing cane stalks of Co. 281 and C.P. 33-243 growing out in the field. Some degree of variation in pathogenicity was found among the single ascospore cultures used. The conidial culture of *Colletotrichum graminicolum* which was also
used in these inoculations did not produce the red rot lesions.

A third experiment was carried out on September 12, 1942 in order to test the relative pathogenicity of ten single ascospore cultures (which were isolated from perithecia developed upon sterile cane leaves in the laboratory from the single conidium culture x-40) upon standing canes of the Co. 281 variety. Four stalks were inoculated with each culture, and the same procedure as in the two previous inoculations was followed. All cultures were ten days old and grown on single, separate oatmeal agar slants. Four stalks were punctured as checks. The single ascospore cultures used were:

- x-40-1
- x-40-2
- x-40-7
- x-40-8
- x-40-15
- x-40-21
- x-40-16
- x-40-22
- x-40-23
- x-40-26

The suspensions used for inoculating were heavy in amount of conidia. The location of the cane field was Dalrymple Drive, Baton Rouge, Louisiana.

The results of these inoculations were observed eight days later on September 22, 1942. In the checks only a reddish discoloration around the point of inoculation was produced. All the single ascospore cultures produced the typical red rot symptoms upon Co. 281 stalks (standing cane). The lesions had spread up and down through five to seven joints. Dark, necrotic areas and breaking-down of tissues (especially at the nodes of the inoculated joints)
were observed. As a conclusion, no difference in pathogenicity was found among any of the ten single ascospore cultures which were used here and had originated from perithecia produced with the conidial culture x-40 (the fortieth generation of the x culture obtained through single conidial isolation). A photograph was taken of the inoculated stalks, showing the lesion types. This experiment shows clearly the stability and uniformity of the hereditary characters of pathogenicity by ascosporic cultures obtained through the sexual stage of the red rot organism.

III. Inoculation Experiments in Cane Cuttings Kept in the Laboratory

Several experiments were made by making inoculations in cane stalk cuttings of different commercial varieties, particularly Co. 281. Single ascosporic and conidial cultures of the red rot fungus were used. Also some single conidium cultures of Colletotrichum graminicolum isolated from several different grasses were used along with the red rot organism in order to determine their pathogenicity. All of these isolates (ascospores and conidia of the red rot fungus as well as conidia of Colletotrichum graminicolum) used in these inoculations as well as in most of the other inoculations were grown on single oatmeal agar slants which were kept on a table in the laboratory receiving subdued light (never direct rays of the sun), protected from contamination, and kept at the prevailing room temperature. All these experiments were carried out from September to December 1942 at the Botany Department.
Louisiana State University. The same procedure was followed with each experiment. The cane stalks were cut in the field and brought to the laboratory and inoculated the same day. Only those healthy, more or less straight stalks, free of borer holes or of any other lesion or disease, were chosen. The stalks were cut into pieces containing from three entire internodes (four nodes) to seven internodes and ranging from two to four feet in length. They were washed thoroughly with soap and water, cleared of leaf sheath portions, rinsed with fresh water and permitted to dry on the surface. The inoculations followed immediately. Extreme care was taken to keep the inoculated pieces from contamination. The inoculations were made by means of boring a hole in one of the internodes which was located toward the center of the cane piece and applying immediately the conidial suspension in sterile water. A number of checks were "inoculated" with sterile water in each case. Every precaution was taken to prevent any mixture of the cultures; individual sterile droppers were used for each culture. After inoculation, the cut ends of the stalks were waxed with melted paraffin in order to reduce drying out of the cane pieces through the exposed ends and to prevent further contamination of any kind. Inoculated pieces were wrapped in new brown wrapping paper. Afterwards the inoculated stalk-pieces were kept in darkness in a room at a constant temperature of 25° C. for several days, the time varying with the experiment. After several days of incubation, the canes were brought out and split longitudinally by means of a knife. The pathogenicity of each individual culture was studied and judged
according to the type of lesions presented in each stalk-piece. Following this, the original fungus culture was reisolated from the lesions a number of times. Usually photographs were taken of split stalks, showing the type of lesions in each experiment. The photographs are on file at the Botany Department of Louisiana State University. A more detailed description and the results of each experiment follow.

1. First Experiment

On September 5, 1942, five stalk-pieces of the variety C.P. 33-243 were inoculated with each one of the following single ascospore cultures which were of the dark type and originated from perithecia produced on sterile leaves:

A-10  Isolated from perithecia produced with the A culture (Baton Rouge).

x-40-1  

x-40-2  

x-40-7  Isolated from perithecia produced with culture x-40-8  x-40, a single conidium culture (40th generation x-40-15  through conidia of the x culture).

x-40-16  

x-40-21  

x-40-22  

x-40-26  

x-40-15-1  Isolated from perithecia produced with the single ascospore culture x-40-15. It is the second ascosporic generation after the 40th single conidia generation of the x culture.

Also five stalks were inoculated with C-9, a conidial culture of Colletotrichum graminicolum (grass anthracnose) originally obtained from leaves of the grass Echinochloa walteri (Parah) Heller, collected near University Lake, in Baton Rouge, Louisiana.
Eight pieces were punctured as checks and tap water was placed in the bore. All the cultures were 22 days old and were grown on individual oatmeal agar slants.

The stalks were examined and studied on September 12, the seventh day after the inoculations. The following pathogenicity readings were made:

<table>
<thead>
<tr>
<th>Check</th>
<th>Light red discoloration around point of puncture.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cg—9</td>
<td>Some light red discoloration in the internode inoculated. Not red rot.</td>
</tr>
<tr>
<td>A—10</td>
<td>Nodes necrotic, dark. Some typical lesions in internodes.</td>
</tr>
<tr>
<td>x—40—1</td>
<td>Nodes necrotic. Red rot.</td>
</tr>
<tr>
<td>x—40—2</td>
<td>Nodes necrotic, brown. Red rot.</td>
</tr>
<tr>
<td>x—40—7</td>
<td>Nodes necrotic, dark. Much dark mycelium throughout internodes and nodes.</td>
</tr>
<tr>
<td>x—40—8</td>
<td>Nodes necrotic. Red rot.</td>
</tr>
<tr>
<td>x—40—15</td>
<td>Typical red rot (distinct lesions), otherwise as with x—40—7.</td>
</tr>
<tr>
<td>x—40—16</td>
<td>Nodes necrotic. Red rot.</td>
</tr>
<tr>
<td>x—40—21</td>
<td>Nodes necrotic. Red rot.</td>
</tr>
<tr>
<td>x—40—22</td>
<td>Nodes necrotic, brown to black. Internodes a light red. Some distinct lesions but mostly general discoloration. Red rot.</td>
</tr>
<tr>
<td>x—40—26</td>
<td>As with x—40—7.</td>
</tr>
<tr>
<td>x—40—15—1</td>
<td>As with x—40—7.</td>
</tr>
</tbody>
</table>

As the above results show, all the single ascospore cultures were pathogenic, producing the typical red rot symptoms. No marked difference was found in their pathogenicity upon O.C. 33-243 stalk
cuttings. On the other hand, the conidial culture of *Colletotrichum graminicola* Cg-9 did not produce the red rot symptoms.

2. Second Experiment

On September 11, 1943, an interesting experiment was carried out testing the relative pathogenicity upon Co. 281 cane stalk cuttings of the eight ascospores from an ascus of the sexual stage of the red rot organism. Altogether 17 single ascospore cultures were used (all dark type). They were:

- O-f-1 to O-f-8 from ascus 1
- O-f-9 to O-f-16 from ascus 2
- 0-15-6 from a single perithecium which was produced by a single conidium culture O-f which was obtained from Abbott's culture L-579 (dark type).
- x-40-15-6 obtained from a perithecium produced by a single ascospore culture x-40-15 (and this was obtained from a perithecium produced by the 40th conidial generation of the x culture).

The inoculations were made with these cultures which were 11 days old and grown on oatmeal agar slants. Five stalk cuttings were inoculated with each one of the 17 cultures used while ten stalks were inoculated with sterile water as checks.

The results of the inoculations were obtained ten days after the inoculation on September 21 from which we have the following pathogenicity reading:

**Check:** Red discoloration around the point of inoculation.

**x-40-15-6:**

Pathogenicity of this culture was already well known and hence used here as a check of pathogenicity. A severe red rot was produced by it - very pathogenic, darkening and breaking-down of tissues where tufts of dark mycelium were naked.
Ascus 1: O-f-1 to O-f-8 and
Ascus 2: O-f-9 to O-f-16:

Severe red rot (very pathogenic) was produced in all the pieces inoculated with each one of these cultures. Nodes dark and necrotic, breaking-down of host tissues at many lesions especially at the nodes. No apparent or marked difference was found in the pathogenicity for any of the 16 ascospore cultures from the two asci, from the same peritheciurn, upon Co. 281 cane cuttings. Also no differences have been observed (by repeated experiments) among these 16 ascospores growing in pure culture on oatmeal agar plates. It shows a conservative stability in the genetic constitution, the cultural and pathological characters of this fungus isolate being so uniformly equal and stable as might be expected of this homothallic red rot organism.

Two photographs were taken of split stalks showing the lesions.

Plate II shows the lesions produced by the eight ascospores from ascus 1 (O-f-1 to O-f-8) and checks.

3. Third Experiment

On September 18, 1942, the relative pathogenicity of several single ascospore cultures obtained from leaves of different cane varieties was studied through inoculations on stalk cuttings of the variety Co. 281. Each culture used was ten days old and was grown on a single oatmeal agar slant.

The ascospore cultures were obtained in the following manner. Infected leaves (bearing the conidial form of the fungus) were collected in the field on August 28, 1942, brought to the laboratory, and placed in moist chambers, where peritheciurn developed. Eleven days after these leaves had been so placed in the moist chambers, single ascospore isolations were made; and from these ascospore cultures, the ones used here were chosen. Besides these ascospore cultures, one single conidium culture Cg-11-8 of Colletotrichum
graminicolum, the x-40-13 single ascospore culture (of well-known pathogenicity) and II-10 single ascospore culture were used. Five stalk cuttings were "inoculated" with sterile water as checks. The cultures used, together with their origin, may be seen in Table VIII.

The results which were obtained on September 26, eight days after the inoculations, were the following:

Check    Red discoloration around the point of inoculation.

Cc-11-8  Light red bundles (narrow, elongated lesions), mainly in the internode of the inoculation. No outbreaks of red rot.

BB-12    (light strain) Severe red rot throughout the stalks. Light buff colored. Very pathogenic.

BB-15    Red to brown lesions, smaller than with BB-12 and less pathogenic.


DD-7     About the same as CC-5.

EE-5     Typical red rot as with x-40-13 although lesions were smaller.

FF-11    About the same as CC-5.

GG-2     About the same as CC-5.

HH-9     Severe red rot throughout the stalks. Nodes dark, necrotic.

II-10    Red rot, though not so severe as with most other isolates.

x-40-13  Severe red rot throughout the stalks. Nodes dark, necrotic.

All of the single ascospore cultures produced the typical red rot lesions upon Co. 261 stalk cuttings. Some variation in pathogenicity
### Table VIII  Origin of the Cultures Used in Cut Stalk Incubations on September 18, 1942

<table>
<thead>
<tr>
<th>Cultures</th>
<th>Color</th>
<th>Strain</th>
<th>Isolated from leaves of:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single ascospore</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB-12*</td>
<td>light</td>
<td>C.P. 36-19</td>
<td></td>
</tr>
<tr>
<td>BB-15*</td>
<td>dark</td>
<td>C.P. 36-19</td>
<td></td>
</tr>
<tr>
<td>CC-5*</td>
<td>dark</td>
<td>C.P. 36-44</td>
<td></td>
</tr>
<tr>
<td>DD-7*</td>
<td>dark</td>
<td>C.P. 36-54</td>
<td></td>
</tr>
<tr>
<td>EE-5*</td>
<td>dark</td>
<td>C.P. 36-59</td>
<td></td>
</tr>
<tr>
<td>FF-11*</td>
<td>dark</td>
<td>C.P. 36-83</td>
<td></td>
</tr>
<tr>
<td>GG-2*</td>
<td>dark</td>
<td>C.P. 36-100</td>
<td></td>
</tr>
<tr>
<td>HH-9*</td>
<td>dark</td>
<td>C.P. 36-105</td>
<td></td>
</tr>
<tr>
<td>II-10+</td>
<td>dark</td>
<td>Co. 281</td>
<td></td>
</tr>
<tr>
<td>x-40-13**</td>
<td>dark</td>
<td>Co. 281</td>
<td></td>
</tr>
<tr>
<td><strong>Single Conidium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG-11-8**</td>
<td>dark</td>
<td>Isolated from Sorghum stems</td>
<td></td>
</tr>
</tbody>
</table>

* Developed in moist chambers.
+ Natural development on brown stripes on the leaf blade.
** Produced under sterile conditions with the x-40 conidial culture.
++ Culture of *Colletotrichum graminicolum*. 
was noticed among them. The single conidial culture of *Gallotto-
trichum graminicolum* did not produce red rot.

4. Fourth Experiment

On November 12 inoculations were made on 30 stalk cuttings in order to test the pathogenicity and to study the relative parasitism of several single ascospore cultures of the red rot fungus which were originally isolated from naturally developed perithecia. Besides these ascosporic cultures, two single conidium cultures also were used. One was x-51 of known pathogenicity which represents the 51st generation through single conidium cultures of the x culture of the red rot fungus, and the other Cg. 15-a, single conidium culture of the grass anthracnose (*Gallotto-trichum graminicolum*) originally isolated from cultivated Johnson grass. The cultures used, of which two were light and the rest dark, were all 23 days old and each one came from a single oatmeal agar slant tube. They were the following ones:

**Single ascospore cultures:**

- 00-2 (light)  00-19
- 00-4 (light)  00-37
- 00-9  00-40
- 00-15  00-43

**Single conidium cultures:**

- x-51
- Cg. 15-a

On November 20, eight days after inoculation, the following results were noted upon the splitting of the cane cuttings:

**Check**  Only light reddening around the point of inoculation, rest of a pure, fresh, white color.
Cg. 15-a Did not produce red rot; only very weak lesions in the inoculated internode, especially reddening along bundles.

x-51 Produced the most pathogenic red rot; used as check of pathogenicity.

00-2 (White) Very pathogenic red rot, many lesions in whole stalks, darkening and break-down of nodes, especially near the inoculation.

00-4 (White) Like 00-2.

00-9 Typical red rot lesions; not so pathogenic as 00-2.

00-15 Typical red rot lesions; but here much break-down of nodes (more than of the internodes), and besides the lesions were accompanied by a certain water-soaked coloration of breaking tissues.

00-19 Typical red rot lesions; moderately pathogenic.

00-27 Typical red rot lesions; moderately pathogenic.

00-40 Very pathogenic; typical red rot lesions.

00-43 Moderately pathogenic; typical red rot lesions.

As the above results show, the pathogenicity of all these single ascospore cultures was proved as being positive and the relative pathogenicity described. Some variation in the pathogenicity of these single ascospore cultures was noticed. Colletotrichum graminicolum. Cg. 15-a, did not produce red rot.

5. Fifth Experiment

On November 19, 1942, stalk cuttings of the variety 0c. 281 were inoculated in order to test the pathogenicity and to study the relative pathogenicity of six single ascospore cultures of the red rot fungus isolated from the grass Leptochloa filiformis (Lam.) Beauv. (natural development, collected at U.S. Department of Agriculture
Sugar Experiment Station at Houma, Louisiana, November 3–4, 1943). Five stalks were used for each culture and besides 12 stalks were "inoculated" with sterile water to be used as checks (total 42 stalks inoculated). The cultures used were:

- PP-3
- PP-7
- PP-11 (light)
- PP-14
- PP-16
- PP-18

Of these cultures, only PP-11 was of the light type, while the others were of the dark type. Each culture was 13 days old and had been grown in a single oatmeal agar slant.

On November 28, nine days after the inoculations, the results were observed upon the splitting of the canes. The check showed only reddening around the point of inoculation. All the six single ascospore cultures produced the typical red rot symptoms (red lesions with whitish centers). All of them showed themselves to be very pathogenic; many lesions being scattered throughout the whole stalk-piece, causing darkening and breaking-down of tissues, especially at the nodes. All of them showed themselves to be very similar in severeness of pathogenicity. However, the culture PP-14 gave to the entire internal tissues of the inoculated stalks a pale or water-soaked appearance.

6. Sixth Experiment

On November 19, 1942, stalk cuttings of the variety Co. 281 were inoculated with single ascospore cultures of Physalospora (isolated from different Saccharums collected at the U.S. Department of Agriculture Sugar Experiment Station at Houma, Louisiana) in order
to test their pathogenicity. Five stalks for each culture were inoculated. Also 12 stalks were "inoculated" with sterile water to be used as checks. A total of 103 stalks were inoculated. Each one of the cultures used was a single original colony that came from a single ascospore and was grown in a single agar slant. The origin and age of the cultures used are found in Table IX.

Table IX  Origin and Age of the Cultures Used in Stalk-Cutting Inoculations on November 19, 1942

<table>
<thead>
<tr>
<th>Single ascospore cultures</th>
<th>Age in days</th>
<th>Isolated from:</th>
</tr>
</thead>
<tbody>
<tr>
<td>GQ-4, GQ-7, GQ-9</td>
<td>11</td>
<td>Saccharum spontaneum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>India, #238</td>
</tr>
<tr>
<td>RR-1, RR-17, RR-21</td>
<td>13</td>
<td>Kassoer, natural hybrid Java</td>
</tr>
<tr>
<td>SS-3, SS-6, SS-15</td>
<td>12</td>
<td>Saccharum sinense, var. Gayana</td>
</tr>
<tr>
<td>TT-3, TT-11, TT-12</td>
<td>12</td>
<td>Saccharum robustum</td>
</tr>
<tr>
<td>UU-5, UU-7, UU-13</td>
<td>13</td>
<td>Saccharum spontaneum &quot;Burma&quot;, Malaya Peninsula</td>
</tr>
<tr>
<td>WV-2, WV-4, WV-11</td>
<td>12</td>
<td>Saccharum spontaneum,Java var. &quot;Pasoer-ocean&quot;</td>
</tr>
</tbody>
</table>

Of all these cultures, only two were light, RR-1 and RR-21, while the rest were of the dark type.

On November 30, eleven days after the inoculation, the stalks were split and examined. The check showed only a slight reddening around the point of inoculation, while all the stalks
inoculated with the ascosporic cultures showed the typical red rot symptoms. All of them were extremely pathogenic and approximately equally so. All the stalks inoculated with the fungus presented many lesions throughout their whole length. Darkening, break-down, rotting of the nodes, and internodal lesions were noted.

Cultures RR-1 (light) and RR-17 (dark) produced a somewhat different type of symptom. There was much paling of the tissues, turning into a water-soaked appearance throughout the whole length of the internal tissues of the stalk pieces, and independent dark necrotic lesions were scattered throughout the stalks.

In conclusion, the pathogenicity of these single ascospore cultures of Physalospora from different Saccharums was proved and the fungus demonstrated to be the same red rot fungus. All of them were very pathogenic upon Co. 281 stalk pieces. A somewhat different type of red rot symptom appeared to be shown by the RR-1 (light type) and RR-17 (dark type), as was stated above.

7. Seventh Experiment

On November 23, 1942, inoculations were made on Co. 281 cane cuttings in order to determine the relative pathogenicity of several of the single ascospore cultures of the red rot fungus perithecia isolated from different Saccharums obtained at Houma, Louisiana, which were collected during November 1942. The material consisted of dead leaves. Five stalk cuttings were inoculated with each culture of the fungus and 12 stalks were "inoculated" with sterile water to be used as checks (total inoculated stalks 72).
Each one of the single ascospore cultures used was a single original colony grown in a single oatmeal slant nine days. Also, three different single conidium cultures, x-51, Cg. 11-h, and Cg. 14-e, were used. The x-51 culture (51st generation through single conidia of the x culture) was used here for its well known strong pathogenicity in order to compare it with the others. The origin, age, and color strain of the cultures used are found in Table X.

Table X The Origin of the Cultures Used in Stalk-Cutting Inoculations on November 23, 1942

<table>
<thead>
<tr>
<th>Cultures</th>
<th>Color strain</th>
<th>Age in days</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single ascospore</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW-2</td>
<td>dark</td>
<td>9</td>
<td>Saccharum barberi</td>
</tr>
<tr>
<td>WW-14</td>
<td>dark</td>
<td>9</td>
<td>from natural variety</td>
</tr>
<tr>
<td>WW-21</td>
<td>dark</td>
<td>9</td>
<td>&quot;Chunnee&quot; (leaves)</td>
</tr>
<tr>
<td>XX-2</td>
<td>dark</td>
<td>9</td>
<td>Saccharum officinarum</td>
</tr>
<tr>
<td>XX-5</td>
<td>dark</td>
<td>9</td>
<td>from natural variety</td>
</tr>
<tr>
<td>XX-6</td>
<td>dark</td>
<td>9</td>
<td>La. Purple (leaves)</td>
</tr>
<tr>
<td>YY-3</td>
<td>dark</td>
<td>9</td>
<td>Co. 290 variety</td>
</tr>
<tr>
<td>YY-7</td>
<td>white</td>
<td>9</td>
<td>artificial hybrid</td>
</tr>
<tr>
<td>YY-9</td>
<td>dark</td>
<td>9</td>
<td>(leaves)</td>
</tr>
<tr>
<td>Single conidium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>x-51</td>
<td>dark</td>
<td>12</td>
<td>x-50</td>
</tr>
<tr>
<td>Cg. 11-h</td>
<td>dark</td>
<td>30</td>
<td>Cultivated Sorghum (stem)</td>
</tr>
<tr>
<td>Cg. 14-e</td>
<td>dark</td>
<td>30</td>
<td>Johnson grass (leaf)</td>
</tr>
</tbody>
</table>

On December 1, 1942, eight days after the inoculations, the canes were split and the following symptoms were noted:

Checks Showed only slight reddening around the point of inoculation, while the rest was of a white, fresh, fleshy coloration.
Cg. 11-h  Reddening at the inoculation internode. No red rot. A few vascular bundles showed lesions up and down in the internode of inoculation.

Cg. 14-e  The same as Cg. 11-h.

x-51  Very pathogenic, typical red rot lesions.

WW-2  Very pathogenic, many lesions through whole stalk lengths. Typical red rot.

WW-14)  Very pathogenic but different type reaction of host. Lesions dark, tissue pale; much break-down at nodes. The main symptoms different from the common red rot were the paling or somewhat water-soaked appearance of the whole cane piece. It is referred to as a new symptom of the red rot disease on this variety of cane.

WW-21)  Very pathogenic; typical red rot.

XX-2  Typical red rot.

XX—5)  Very pathogenic; typical red rot.

XX-6)  Both about equally pathogenic.

YY—3)  Just like XX—2; very pathogenic.

YY—7)  Different reaction type.

YY—9)  Very pathogenic. Typical red rot.

As may be seen from the above, all the single ascospore cultures of the red rot fungus, as well as x—51 single conidium culture, were comparatively very pathogenic, producing many lesions throughout the whole length of the inoculated stalks of Co. 261. The chief symptoms were darkening, break-down, rotting at the nodes and internodal lesions. On the other hand, the two single conidium cultures of Colletotrichum graminicolum from two different grasses did not produce the red rot symptoms. A different and peculiar type of symptom was produced with the red rot fungus cultures, XX—2, YY—3, and YY—7, giving a pale to water-soaked appearance to the whole internal portion of the cane pieces, where dark necrotic red rot.
lesions appeared scattered, especially at the nodes. These cultures were all very pathogenic.

8. Eighth Experiment

On December 9, 1942, stalk cuttings of the variety Co. 281 were inoculated with some of the single ascospore cultures of Physalospora which had developed in moist chamber from young green cane leaves, which did not have any apparent lesion, collected out in the field (Dalrymple Drive, Baton Rouge, Louisiana). These ascosporic cultures were ZZ-17, ZZ-27, and ZZ-37, which came from the same peritheciun. Two other cultures were also used: PP-14, single ascospore culture from Leptochloa filiformis (used because of its known pathogenicity, and in order to compare it with the others) and Cg. ll-m, which was a single conidium culture of the grass anthracnose Colletotrichum graminicolum originally isolated from stalks of cultivated sorghum (at Sugar Experiment Station at Baton Rouge, Louisiana) and associated with a rotting of internal tissues. Five stalks were inoculated with each of the above cultures, while eight stalks were "inoculated" with sterile water to be used as checks. The total number of stalk cuttings inoculated was 33. All the cultures were 12 days old and each one was grown in a single oatmeal agar slant. All were of the dark type.

On December 18, nine days after the inoculations, the canes were split in halves and the following symptoms were noted.

Checks Reddening around the point of inoculation only.
Reddening at the point of inoculation. Reddening of some vascular bundles (as elongate and narrow lesions) at the inoculated internode and also in the other internodes and nodes but not so abundant. No red rot symptoms produced.

All produced the red rot typical symptoms throughout the stalk tissues. Much break-down at tissues, especially near the inoculating internode. All the three were about equally moderately pathogenic.

Typical red rot lesions. More pathogenic than ZZ-17, ZZ-27, and ZZ-37. The cane internal tissues showed a general water-soaked appearance.

By means of diseased host tissue transfers (from lesions other than those found in the internode of inoculation), all original cultures which had been used were actually reisolated in pure culture. Conidia and setae were produced in all of them.

Some of these split diseased cuttings were placed in moist chambers for seven days. They were placed on a table in the laboratory receiving only subdued light. In the case of those which had been inoculated with the red rot fungus, the organism grew very fast and was very prolific upon the sectional face of the stalks. On some of the more developed lesions, aerial mycelium, conidia, and setae were found to be developing 24 hours after they had been placed in the moist chambers. In the case of Colletotrichum graminicolum (Cg. 11-m) the fungus growth was very poor, almost confined to the inoculated internode and consisted almost entirely of scarce aerial mycelium, although conidia and setae also were produced.

In conclusion, the single ascospore cultures of Physalospora used here all produced the typical red rot lesions upon Co. 261
stalk cuttings as well as PP-14, which had been isolated from leaves of *Leptochloa filiformis*. *Colletotrichum graminicolum* (Cg. 11-m) did not produce the red rot lesions.

9. Ninth Experiment

On December 18, 1942, stalk cuttings of the varieties Co. 281 and C.P. 34-120 were inoculated with the following cultures: PP-14, XX-2, ZZ-17, ZZ-37, ZZ-37, which were single ascospore cultures and Cg. 11-m (*Colletotrichum graminicolum*) and x-50 which were single conidium cultures. Of these cultures, XX-2 (from dead Louisiana Purple leaves, U.S. Department of Agriculture Sugar Experiment Station, Houma, Louisiana) and x-50 (50th generation through single conidia of the x culture used here for its well-known strong pathogenicity) were the only ones which were not used in the eighth inoculation experiment.

With each culture of the fungus, three stalk cuttings of each variety were inoculated, while three stalks of each variety were "inoculated" with sterile water as checks. All the cultures were nine days old and each one was grown in a single oatmeal agar slant tube. All were of the dark color type.

On December 28, ten days after inoculation, the canes were split in halves and the following symptoms were noted on Co. 281 and C.P. 34-120 varieties.

| Check           | Only light reddening around the point of inoculation while the rest of the tissue was of a fresh, fleshy-white color. Same for both cane varieties Co. 281 and C.P. 34-120. |
FP-14  Typical red rot lesions scattered throughout the cane pieces. More break-down of tissues at the internodal or nodal regions near to the inoculation point. There was a water-soaked appearance tendency. Equally for both varieties. Very pathogenic.

XX-2  Typical red rot lesions throughout the stalk cuttings. Much break-down of tissues. Some showed a clear water-soaked appearance of the invaded tissues, more marked than with PP-14. Equally pathogenic for both varieties. This culture was the most pathogenic of the ones used here.

ZZ-17) Typical red rot lesions throughout the stalks were produced for all of them. Much break-down of tissues especially near the inoculating internode. All of them were equally moderate in pathogenicity for both varieties.

ZZ-27) ZZ-37) Cg. 11-m No red rot lesions. Reddening of tissue mostly at the inoculated internode, while reddening of some vascular bundles throughout the whole stalks. Same for both varieties.

x-50  Typical red rot lesions throughout the stalks. Same for both varieties. This culture was the second in degree of pathogenicity, following XX-2 which was most pathogenic of the cultures used here.

In conclusion, all the single ascospore cultures of Physalospora were pathogenic, producing the typical red rot symptoms upon Co. 281 and C.p. 34-120 cane stalk cuttings. Culture XX-2 (single ascospore culture) was the most pathogenic one and x-50 was next. The symptoms of the lesions produced by each individual culture upon these two cane varieties were similar. Cultures PP-14 and XX-2 produced, besides the typical red rot lesions, a clear water-soaked appearance throughout the entire internal tissues of the inoculated stalks in both varieties, thus confirming the results obtained in previous inoculation experiments.
10. Summary of Experiments 1 - 9

These nine experiments dealing with stalk cuttings made with the single spore cultures of the red rot fungus proved all the isolates to be pathogenic. Results were obtained generally one week after inoculation. The typical red rot symptoms appeared upon the inoculated cane stalk cuttings up and down from the point of inoculation. Among the single ascospore and single conidium cultures, some were found to be more virulent than others. No marked differences in the pathogenicity of the light and the dark strains of the fungus were found.

The pathogenicity of the single ascospore cultures (dark and light strains) was thoroughly proved. Some were of a natural origin, while others were obtained in the laboratory (under sterile conditions and in moist chambers). Some of the ascospores of a natural origin were from the five different species of Saccharum obtained from different places in Louisiana and some were isolated from the grass Leptochloa filiformis.

By means of the first and second experiments, the stability and the uniformity in the hereditary characters of pathogenicity were clearly shown among all the ascospores obtained through the sexual stage of the red rot fungus originated from a given single conidium culture. The 16 ascospores obtained from two asci (0-f-1 to 8, ascus 1; 0-f-9 to 0-f-16, ascus 2) from the same peritheciurn produced by the 0-f single conidium culture, all were identical in severity of pathogenicity and produced identical red rot lesions.
10 days after the inoculations upon Co. 281 cane cuttings. The equality in pathological characters of the eight ascospores from one ascus, taken in conjunction with the identity and constancy of cultural behavior upon oatmeal agar plates, demonstrated the homothallism of the red rot fungus.

The single ascospore cultures, Co-15, PP-14, RR-1, RR-17, XX-3, YY-3, and YY-7 all produced similar red rot symptoms and were of an equal and very severe pathogenicity upon the inoculated cuttings (Co. 281, C.P. 34-100 canes). About one week after the inoculation with the above cultures, the stalks presented a general water-soaked appearance throughout all the internal tissues, with dark necrotic lesions scattered throughout.

The single conidium cultures of the grass anthracnose, *Colletotrichum graminicolum* (which were isolated from different grasses in Louisiana in 1942) did not produce the red rot symptoms. Inoculation with such cultures showed only a reddening of the vascular bundles appearing as very narrow, reddish, elongated lesions, especially at the internode of the inoculation.

In all cases in which an attempt was made to reisolate the fungus from lesions produced by a certain culture, the original culture type was obtained again.

IV. Cane Cuttings Inoculated and Planted in Dry Soil in the Greenhouse

The inoculations were made on August 12, 1942, by Dr. Forbes, in order to study the relative pathogenicity of different red rot
isolates. Five stalks of Co. 281 and five stalks of O.P. 33-343 were inoculated with spore suspension of seven-day-old oatmeal agar cultures of four single ascospore cultures and one single conidium culture. After inoculation the stalk cuttings were planted in dry, loose soil in the greenhouse.

The single ascospore cultures used were:

A—7, A—10, A—12 .......... dark strain

G—f—30 ..................... light strain.

The single conidium culture O—f (dark) was used as a check.

On August 20, the stalks were brought to the laboratory and some were split and their pathogenicity recorded by Dr. Forbes. On the same day he gave me from two to three stalks of each culture inoculation and also checks which were not split. I kept them until August 24 and then all of them were opened and I obtained the following results. All the inoculations made with the above six single spore cultures showed severe pathogenicity through their red rot lesions up and down from the point of inoculation in both varieties. Of these, G—f—20 (light) was the most severe, especially upon Co. 281 in which developed many large lesions throughout the whole length of the stalks, including both ends.

All of the cultures produced internal break-down of tissues which was especially marked at the nodes, darkening and drying out. Sometimes a hole was formed within the lesions, and in this cavity the fungus mycelium was actively growing, forming tufts. On the same day (August 25) reisolations were made. After the stalks were split,
tissue transfers from different lesions 1\(\frac{1}{2}\) to 2\(\frac{1}{2}\) feet up from the point of inoculation were made to acid oatmeal agar plates. As a result, the original culture type (of the six used) was actually obtained.

A photograph was taken (Plate I, Figure 2) in order to show the type of lesions developed 12 days after inoculation (eight days in dry soil and four in the laboratory).
IDENTIFICATION OF THE PERFECT STAGE OF

COLLETOTRICHUM FALCATUM WENT

After an intensive study, it has been shown definitely, by microtome and free-hand sections through cane leaves bearing perithecia of different ages, that the perfect stage of Colletotrichum falcatum Went is an Ascomycete which belongs to the genus Physalospora. The results were based upon the microscopic examination of the perithecia which had developed entirely under natural field conditions as well as those developed under sterile, controlled conditions. A great number of mature as well as immature perithecia were crushed in a drop of water for microscopic examination.

The perithecia in nature are from dark olive-brown to blackish, subglobose, smooth, sunken in the dead host tissue, and protruding at maturity by means of their oothecium which become papillate. The perithecia have a definite perithecial wall which is membranous to leathery, without a stroma. Asci are clavate or club-shaped, do not form a definite fascicle; they are interspersed with hyaline, continous or rarely branched paraphyses. An ascus is regularly eight-spored. Ascospores are hyaline and elongated or blunt. The ascus wall is thickened at the top portion at maturity,
becomes very elongated, and ruptures circumscissily; thus the ascus finally frees the ascospores.

The above characteristics placed the perfect stage of the red rot fungus of sugarcane definitely in the genus Physalospora of the Ascomycetes.

We have here the case of a parasitic fungus which has the imperfect stage in the genus Colletotrichum and the perfect stage in the genus Physalospora and not in the genus Glomarella.
DESCRIPTION OF THE RED ROT FUNGUS

I. The Perfect or Sexual Stage

As has been stated above, the perithecial stage of the red rot organism (including the dark and light strains) belongs to the Physalospora genus.

1. The Perithecia

The perithecium develops naturally on fading and dead tissues, preferably on cane leaves, and occasionally on the cane stalks. It is also found on dead leaves and stems of the grass Leptochloa filiformis. Perithecia, dark brown to black, are imbedded in the host tissues, protruding at maturity by a short, papillate ostiolum through the stomatal openings or through the epidermal cells. Perithecia are globose to subglobose or with peculiar forms and shapes according to the place in which they developed. In general they are between the vascular bundles and are usually scattered and separated from one another. (See Plate V, Figure 3.) Sometimes, however, two to four and more are aggregated, forming chains between bundles, often having common walls as seen in a longitudinal section. Plate IV, Figure 3 shows a longitudinal section through perithecia imbedded in a cane leaf blade of Co. 281.
Table XI gives us various measurements of the maximum and minimum as well as the averages of height and width of mature perithecia developed under different conditions.

Studying the above table we can see that usually the width of the perithecia in a longitudinal section of the cane leaf is larger than in a cross section, while the height remains the same for both cross and longitudinal sections.

In material bearing perithecia which has developed entirely under natural field conditions, the perithecia of largest dimensions, especially in height, are usually found in the leaf sheath.

These variations in the perithecial sizes depend on the place in which the perithecia developed, and it is usually correlated with the proximity or distance of the vascular bundles. In the development of the perithecia the parenchymatic cells are the ones more affected, being pressed and also destroyed, while the vascular bundles are usually not much affected, as is clearly shown in any section through the host tissue and perithecia. When perithecia develop between two vascular bundles which are very near each other, the perithecia will have a small dimension (or width) perpendicular to the bundles, while they may be very elongated in a direction parallel to the bundles, as is the usual case with perithecia developed under the midrib of the leaf. Compare Plate IV, Figure 1, a cross section, with Figure 3, a longitudinal section, both sections made on Co. 281 leaf blade.

The leaf sheath and the lamina of the cane leaf contain many
Table XI  Measurements in Microns of the Height and Width of Perithecia of the Red Rot Fungus of Different Origins

<table>
<thead>
<tr>
<th>Minimum to maximum perithecial sizes</th>
<th>Average perithecial sizes</th>
<th>Number of perithecia</th>
<th>Section fresh material</th>
<th>Conditions of perithecial development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height 129.5-166.5 x 185.0-232.0</td>
<td>148.0 x 200.9</td>
<td>10</td>
<td>Longitudinal Leaf blade Co. 290</td>
<td>Sterilized leaves inoculated</td>
</tr>
<tr>
<td>Width 161.0-166.5 x 160.5-251.6</td>
<td>138.6 x 187.6</td>
<td>20</td>
<td>Longitudinal Leaf blade Co. 281</td>
<td>Infected leaves in moist chambers</td>
</tr>
<tr>
<td>Height 118.4-166.5 x 85.1-111</td>
<td>143.6 x 99.9</td>
<td>20</td>
<td>Cross Leaf midrib Co. 281</td>
<td>Natural field conditions</td>
</tr>
<tr>
<td>Width 155.4-203 x 85.1-140.6</td>
<td>176.1 x 125.1</td>
<td>20</td>
<td>Cross Leaf sheath Louisiana Purple</td>
<td>Natural field conditions</td>
</tr>
<tr>
<td>Height 129.5-259 x 92.5-192.4</td>
<td>191.6 x 151</td>
<td>48</td>
<td>Cross Leaf sheath Co. 281</td>
<td>Natural field conditions</td>
</tr>
</tbody>
</table>

* In measuring the height of the perithecia, the ostiolum was also taken into account.
parallel vascular bundles. But the leaf sheath is thicker between the upper and inner epidermis, and the bundles are spaced further apart and separated by larger parenchyma cells than in the blade. In a cross section through the leaf sheath we often see the parallel bundles embedded in fundamental parenchyma. Some of the cells of the parenchyma become broken down naturally, and so large air cavities are often found between the vascular bundles of the leaf sheath. All these conditions of the leaf sheaths taken together favor a more free development of the perithecia, and therefore perithecia developed here often show larger dimensions than those developed in the leaf blade.

In summary we can say that the perithecia of the red rot fungus usually have one height and two width dimensions (cross and longitudinal) and that these dimensions have a large range depending on the host tissues in which they developed. The same variations in perithecial sizes are even found in a single leaf of any given cane variety. In the course of these studies the same approximate range in variation was found in perithecia produced in leaves in the field which were previously inoculated with single ascospore cultures (dark and light strains of the red rot fungus).

In cross section through perithecia embedded in the leaf sheath, the more common sizes (height and width) are usually: 150-200 x 110-180 microns while the commonest sizes of perithecia in the leaf midrib are 120-150 x 90-120 microns and in the leaf blade, 120-150 x 100-150 microns.
In conclusion we can state here that the most common size of the perithecial diameter is found to be between 100 and 200 microns.

2. The Perithecial Wall

Each perithecium possesses a definite perithecial wall, which is very uniform in thickness and shape, but sometimes appears somewhat slightly modified, especially at the sides of the ostiolum. The perithecial wall is smooth on the outside. The component cells of this wall are of a pseudoparenchymatic nature; they have a coloration from dark olive-brown to blackish. Each cell has thick walls and sometimes shows within heavy oil contents, especially at early stages of the perithecial development. By means of the microscopical study of many cross and longitudinal sections, it was found that the perithecial walls were usually three one-celled-layers thick, sometimes four and occasionally more. Just inside the perithecial wall there is a thin layer of very small, hyaline cells which are very rich in granular contents and are those which give rise to the contents inside each perithecium. See Plate IV, Figures 1, 2, and 3.

The thickness of the perithecial wall has been found to be very uniform when they developed within the host tissues. The thickness of the perithecial wall was obtained from 118 different perithecia and it was found to vary normally from 10 to 20 microns with an average of 13.14 microns. These measurements were taken from cross and longitudinal sections (free-hand and microtome) through the
leaf blade, midrib, and leaf sheath of different varieties of sugarcane bearing mature perithecia; some developed naturally and some others developed in the laboratory under sterile conditions and in moist chambers.

3. The Ostiolum

The ostiolum of the peritheciun is short and usually papillate, has its canal beautifully provided with abundant hyaline paraphyses which may be stained with eosin or cotton blue solutions. Very rarely setae and conidia were seen developing from the outer side of the mouth of the perithecia. The height and width of the ostiolum vary but in the majority of cases they are found within the following range, 15–40 \( \times \) 20–50 microns (height \( \times \) width). Sometimes the ostiolum has its height and width equal, sometimes either one can be larger than the other.

4. The Ascus

The asci are club-shaped or clavate, short-pedicellate, not fasciculated, found arising from the bottom as well as from the sides of the inner perithecial wall. Asci are interspersed with the paraphyses. The ascus wall is thickened at its top portion; at maturity the ascus becomes much elongated and the ascus wall ruptures circumcisally, finally culminating in the liberation of the ascospores. This process is done in an extremely fast manner. An ascus may contain from two to eight ascospores, eight being the most common and normal number, and in biseriate arrangement. See asci in Plate V, Figures 1 and 2.
Two hundred and fifty-five (255) ascis, which were obtained from perithecia of different origins, were measured. Of the 255 ascis, 208 were from perithecia developed entirely under natural field conditions on cane leaves of many varieties in Louisiana; 25 from perithecia developed on infected leaves which were put in large moist chambers (not sterile), and the remaining 22 ascis were from perithecia developed under sterile, controlled conditions. All the measurements were made upon fresh, living ascis with mature ascospores which were placed in sterile water. The minimum and maximum size of the length and width among the 255 ascis were from: 51.80-118.40 x 7.4-19.24 microns, and the average for the same number (255) was: 80.34 x 13.01 microns. Most of the ascis fell in the ranges: 70-90 x 13-18 microns.

5. The Ascospores

The ascospores are hyaline, irregularly biseriate in arrangement inside the ascus; they are smooth, one-celled, and uninucleate, with granular and oily contents inside, and usually with one (and sometimes more) clear circular refractive region, without granules, which is located toward the center of each ascospore. The ascospores generally are very uniform in shape, sizes, and structure, but sometimes considerable differences are found among different specimens. The ascospores are elongated, blunt, oval, egg-shaped, very often bean-shaped, slightly curved or straight. See Plate V, Figures 1 and 2. The ascospores are discharged out of the mature perithecium through the ostiolum. Sometimes on wet mornings in the field we can see a small drop of a whitish to a pale pinkish color
sticking to the mouth of the peritheciurn. This drop is composed of a mucilaginous substance, usually bearing within it many ascospores which were expelled from the mature peritheciurn. The same condition can be observed by placing leaves bearing mature peritheciurn in a moist chamber.

Certain abnormalities in the ascospores were observed in those taken from peritheciurn found on leaves which were collected out in the field. Before this material was collected, several weeks had passed without any rain during the hot summer weather. Sometimes one or a few gigantic ascospores were found in an ascus surrounded by cytoplasm of granular structure bearing oil globules. These ascospores were wider than usual. Some asci were greatly and abnormally developed without bearing a single ascospore, thus presenting a mass of seemingly disorganized protoplasm. Sometimes within a single ascus there were very small ascospores and even the ascospores within the same ascus were of variable dimensions. Also it was frequently found in this material that some ascospores had begun to germinate showing a bud-like protuberance usually at one of their ends, inside the ascus, giving the impression that the ascus had more than eight ascospores. But by taking the ascospores carefully out of those asci, they were shown to be really germinating. In general the ascospores were hyaline but in a very few cases some were of an extremely weak, yellowish coloration.

Normally the ascospores germinate rather fast when placed in water or in any nutrient solution or agar nutrient. They
germinate by sending out a hyaline germ tube, usually from both ends of the spore. Sometimes the germ tubes grow from both ends at once, at other times one of the two may be slightly delayed. Also germ tubes, one, two, or even more, have been observed coming out at any other place from the ascospore. Once they germinate they grow extremely fast, and it was often seen that from ascospores three to five hours after being placed in a drop of potato dextrose agar (150–20–30 per liter of water) in Van Tieghem cells, the germ tube began to be septate, and to produce branches. Some germinating ascospores form a cross septum in the middle of the ascospore; some form these cross walls after germination, and some do not form the cross wall at all.

From two to three days after a single ascospore had been placed in a potato dextrose agar drop in a Van Tieghem cell, the drop was overgrown by the much branched mycelium and the typical falcate conidia began to be formed, usually accompanied by dark setae and some very small sausage-shaped atypical conidia. Also a great abundance of the so-called chlamydospores or appressoria were produced, commonly against the glass walls, under the agar or outside of the agar. They are thick-walled, similar to resting bodies, with large oil globules, produced intercalary or at the ends of hyphae. Each appressorium often shows toward the center a clear region which is the compressed portion that is attached to the glass.

In comparative experiments in the germination of single
ascospores and single conidia in separate drops of agar, no difference was found between these two kinds of spores; they behaved in a similar manner as described above. However, it seemed that the ascospores had a higher percentage of germination than the conidia.

Several experiments were made germinating ascospores by means of placing them in a drop of sterile water on a glass slide, adding cover glasses and putting them in moist chambers for observation. The chambers were kept on a table in the laboratory at the prevailing room temperature and receiving subdued day-light only. The majority of the ascospores germinated rather quickly, but here the results were not the same as in the case of the ascospore germination on nutrient media. After 24-72 hours it was usual to find that many of the ascospores, especially those near the edges of the cover slide, had formed a cross wall and had an appressorium at the end of each germ tube. In some the appressorium was formed just attached to the outside of the spore. Sometimes one spore had at one end an appressorium and at the other a long and ramified mycelium. It was not rare to find germinated ascospores which had formed several appressoria originated from a single germ tube. Appressoria may stop the growth or may germinate directly by giving rise to another appressorium or a hypha. Sometimes it was found that two appressoria were united and had a common wall at the place where they met and each one was from a different ascospore. Occasionally some ascospores which did not germinate became thick and dark to black cell-walled, with their contents slightly olive brownish
and granulated, showing big oil drops inside. Some formed a cross wall at the middle. After several days the slide glass and cover slide were separated and washed and observed under the microscope and it was observed that the so-called appressoria were strongly sticking against the glass slide as well as to the cover slide.

Also conidia which had germinated in sterile water under the same conditions as the ascospores, acted in an identical manner as the ascospores, and the same features were found for them.

In order to determine the actual size of the ascospores, 891 ascospores were measured. The ascospores were of different origins. Eight hundred and thirty (830) were from perithecia developed entirely under natural field conditions of Louisiana and from leaves of several cane varieties; 36 were from perithecia developed under sterile, controlled conditions and 25 from perithecia developed in the moist chamber. The minimum and maximum sizes found among the 891 ascospores (length x width) was:

12.5-29.6 x 5.0-11.1 microns

and the average was:

20.4 x 7.5 microns.

Most of the ascospores fell within the range of 16-22 x 7-8 microns. Usually the ascospore length and width were very uniform, especially the width.

The ascospore measurements were made of living mature spores which were put into sterile water between slide and cover glass.

6. The Paraphyses

The paraphyses are very abundant, hyaline, delicate.
filamentous, of different shapes and sizes, sometimes tending to be elavate at their tops, single and rarely branched, usually septated, especially toward the top end; straight or slightly curved in several ways but especially at their upper part; generally surpassing the asci in size. The paraphyses are usually full of numerous oil drops, some of which have the diameter of the width of the paraphyses. In general the paraphyses do not take the stain, eosin or cotton blue. The direction of the paraphyses is toward the center and toward the ostiolum of the peritheciun. See Plate IV, Figures 1, 2, and 3.

The sizes of the paraphyses are found to be (length x width) 55-120 x 0.7-6.5 microns, but more regularly 65-100 x 1.5-4.0 microns.

In summarizing, the measurements found for the sexual stage of the red rot fungus were as follows:

- Perithecial diameter: 100 - 300 microns
- Asci (length x width): 70-90 x 13-18 microns
- Ascospores (length x width): 18-22 x 7-8 microns
- Paraphyses (length x width): 65-100 x 1.5-4.0 microns
- Perithecial wall thickness: 10 - 20 microns

II. The Imperfect Stage

The imperfect stage of the red rot fungus has been thoroughly studied since 1893 when Went (46) described it as Colletotrichum.
**falcatus Went.** This stage has an acervulus as fruiting body which usually erupts through the host epidermis, leaves or stems.

The name of the imperfect or conidial stage is the one by which the red rot organism has previously been known. In Table XII the measurements are given which had been given by different authors for the imperfect stage of the red rot organism.

<table>
<thead>
<tr>
<th>Author and year</th>
<th>Conidia</th>
<th>Conidio-</th>
<th>Setae</th>
<th>Chlamydo-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Went 1893, 1896</td>
<td>25 x 4</td>
<td>20 x 8</td>
<td>100-300 x 4</td>
<td></td>
</tr>
<tr>
<td>(46, 48)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Howard 1903 (25)</td>
<td>30-45 x 5</td>
<td></td>
<td>100-150 x 4</td>
<td>15-25</td>
</tr>
<tr>
<td></td>
<td>25 x 2.5*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butler 1906 (8)</td>
<td>20-30 x 5-7</td>
<td></td>
<td></td>
<td>15-25 diameter</td>
</tr>
<tr>
<td>Abbott 1938 (3)</td>
<td>16-48 x 4-8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carvajal 1943</td>
<td>20-50 x 4-8/</td>
<td>15-25 x 6-9**</td>
<td>90-225 x 4-6</td>
<td>8-30 diameter</td>
</tr>
</tbody>
</table>

* Howard found these conidia of smaller dimensions.
/ Conidia falling outside these ranges considered atypical.
** Very variable
1. The Conidia

The conidia, pointed at both ends with somewhat rounded apices may be straight but more commonly are falcate, one-celled, hyaline, mostly measuring 20-50 x 4-8 microns. For convenience conidia with dimensions above or below those given here are considered as atypical. The conidia usually contain a refractive circular region toward the center. The "falcate shape" of the conidia gave to this stage its specific name. Conidia always contain characteristic refractive granules. Conidia are usually produced by short hyaline conidioshores. Usually the conidia appear in masses as globose to semispherical small pinkish drops which are held by the setae under favorable conditions. Conidia also can be produced from isolated hyphae inside the parenchymatic host tissue as well as in sterile agar media. Also conidia can be produced by setae. See Plate V, Figure 4.

The range in the size of conidia is very variable. Besides the typical conidia described above, there is another type which must be regarded as atypical. The atypical conidia are usually very small. They may be straight, falcate, pointed, or sausage-shaped (very common). They vary in length from a very few microns (which are difficult to see) to nearly that of the normal conidia. The atypical conidia are found in pure culture of single ascospore and single conidia cultures on agar plates of the red rot organism, as well as in the parenchyma host cells. They are usually produced singly or in chains, by slender hyphae. They are one-celled and hyaline.
2. The Conidiophores

The conidiophores which produce the typical conidia are very variable in size. Most of them are found in the range 15-25 by 6-9 microns. They arise usually from stromata and are one-celled and colorless, but darken with age.

3. The Setae

Setae are stiff and pointed hairs, usually straight but sometimes slightly curved, dark brown to black in the lower portion and lighter toward the upper end. They arise from the stroma and usually among many conidiophores. Under favorable moisture conditions, setae may eventually produce typical conidia at their tips. When setae produce conidia the upper end is still lighter and we can see and stain the living protoplasm within the setae. But when setae reach maturity, this protoplasm becomes transformed, dark and solidified and then the setae cannot produce conidia any more, thus becoming more sharply pointed at the upper ends. Then each seta is like a bristle or sterile hair. In Plate V, Figure 4, there is shown a seta producing conidia from a pure single ascospore culture. Each seta has several cells. The majority of the setae have from 4 to 8 cross walls or septa and are usually from 90-225 x 4-6 microns in size.

4. The Chlamydosporas

The so-called chlamydosporas or oppressoria as they are often also named, are characteristic bodies, usually thick-walled, one-celled, dark olive-green to dark brown, intercalary, but mostly
terminal in the hyphae. They are very variable in size and shape. They contain large oil globules. The majority of the chlamydo-
spores have a diameter of 8-30 microns.

5. The Mycelium

The mycelium of the red rot fungus is usually hyaline when young and fast-growing but may become dark with age. It is thin-
walled, usually delicate, septated, branched, bearing granular con-
tents inside and including characteristic refractive oil drops.
When it was found that the sexual stage of the red rot fungus agreed reasonably well with the original description of *Physalospora tucumanensis* Spag. (41), it then seemed desirable to study the original Spegazzini type material. At our request Dr. Juan C. Lindquist, of the "Colecciones Micologicas", curator of the Instituto de Botanica "Spegazzini" at the "Universidad Nacional de la Plata", kindly sent us this material. On August 31, 1942, the Spegazzini material was received at the Botany Department of Louisiana State University. It consisted of a cane leaf sheath piece of three centimeters long by six millimeters wide in good condition for examination. The specimen was labelled "No. 418. Separado del Tipo, Physalospora tucumanensis Spag., Sobre Saccharum officinarum, Tucuman, R.A., IV-1894".

The Latin diagnosis of *Physalospora tucumanensis* Spag. was given by Dr. Spegazzini (41) in 1896 with material collected in Tucuman, Republic of Argentina and it is quoted here:

*Physalospora tucumanensis* Spag.

Diag. Maculae nullae v. totum folium araecenti-pallescens; perithecia hypophylla laxae gregaria, saepius seriata globosa parvula (110-130 u. diam.) glabra immersa, epidermide tecta eaque subadnata.
ostiolo vix papillato epidermidem perforante sed non
v. vix suberato coronata, membranaceo-coriacea,
contextu subopaco parenchymatico olivaceo donata;
asci subclavulati antice rotundati, postice breviter
cuneato-pedicellati (80-90 u x 18 u) octospori
paraphysibus filiformibus obvallati; sporae oblique
distichaee ellipsoidae saepeius tenuiter inaequilaterales,
in juventute saepe grosse iguttulatae, utrinque ob-
ustiusculae (18-22 u x 8-10 u) continuee, hyalineae
laeves.

Hab. Non rara in vaginis vetustis v. foliis
languentibus, La Florida, San Pablo, Nueva Laviera,
Provincia Tusuman, Jan. et Febr. 1895.

Spegazzini (41) stated that he did not find any other
genetic stage of this fungus. He said that this organism was a
true parasite but it could not be considered as injurious to the
sugarcane plant. He described this parasite as developing mainly
in the outer portion of old and almost dry leaf sheaths as well as
in the elder and almost dead cane leaves. He stated that it was
easy to determine whether or not a leaf was attacked by this fungus
because of the pale color and because of the presence of the peri-
thesia, which appeared as small black dots arranged in rows or
in longitudinal series between the veins of the leaves.

Up until the present, five species of Physalospora have
been reported occurring upon sugarcane in different countries
in the world. These species are:

Physalospora eucrypta Petrark. On dead leaves,
Philippines Islands. 1923. (35). Imperfect
stage unknown.

Physalospora paraguayae Speg. On rotten cane stalks,
Paraguay. 1884. (42). Imperfect stage unknown.

Physalospora rhodina (Berk. and Curt.) Gke. Of world
wide distribution, producing "Dry Pot" of cane.
1889. (42). Imperfect stage is a Diplodia.
Physalospora sacchari (Berk. and Br.) Sacc. On leaves, Queensland (Australia). 1891. (38). Imperfect stage unknown.

Physalospora tucumanensis Spag. On dead and dying leaves, Argentina. 1896. (41) Imperfect stage is Colletotrichum falcatus.

Of these five, Physalospora tucumanensis, which is the one with which we are concerned, is the only one of larger dimensions, especially as regards ascospores and asci. Therefore, it is easy to differentiate Physalospora tucumanensis from the other four.

An interesting experiment which might be made in the near future would be to get single ascospore cultures of Physalospora tucumanensis, Physalospora paraguaya, and Physalospora sacchari in order to determine the type of spores which might be produced by them in pure culture and therefore to determine whether or not such Physalosporas have different or the same conidial stages. In line with this work another point which could be investigated would be the relationship of these Physalospora with Physalospora tucumanensis.

I. Examination of Physalospora Tucumanensis Spag.

Type Material

A careful examination of the Argentinian original material was made. Over a hundred free-hand sections were made of Spagazzini's material, which was held in pith and cut with a razor blade under the binocular. The sections made were mostly cross sections through the perithecia and host. Several permanent slides were made.
I. Studies of Physalespora

Perithecia were dark olive-brown to blackish, imbedded in the host tissue, communicating with the outside by a short, papillate ostiolum provided with periphyses in its canal. Perithecia were smooth, globose to subglobose, but often showed peculiar shapes according to the proximity or distance of the vascular bundles, where they were developed; usually scattered, rarely in groups of two, three, or more in a line parallel to the bundles. The perithecial sizes were variable. The measurements of these perithecia were as follows:

(Height by width) 100-230 x 80-170 microns, with an average of 175 x 123 microns.

The thickness of the perithecial wall varied usually from 11.1 to 18.5 microns. It was formed usually by three layers, each layer one-celled, and some portions had four or more layers. The component cells were thick-walled and of a dark olive-brown to blackish coloration.

The asci and the paraphyses were usually dried out and sticking to the perithecial side walls. The asci were usually eight-spored in an irregular biseriate order. The asci were thickened at their tops, clavate, short pedicellate, not fasciculate, with paraphyses intermingled. Asci measured from 75-85 microns x 11-13 microns. The paraphyses were abundant, usually septate, filamentous, hyaline, often clavate at their tops, single, rarely branched.

The ascospores were hyaline, guttulate, smooth, one-celled, straight or slightly curved, elongated, oval, bean shaped. From
189 ascospores of this Spegazzini material which were measured, I obtained a minimum and maximum (length and width) of 12-22 x 5-10 microns; and an average of 16 x 7.03 microns. The majority of the ascospores, however, were found to vary from 16-19 x 6.6-8.5 microns.

Plate VI, Figure 2 shows a perithecium in a cross-section of the leaf sheath where paraphyses and an ascus appear in its interior. In the same plate, Figure 1 shows a typical perithecium, but almost empty, in a cross-section of the leaf sheath.

2. Finding of Colletotrichum falcatum Vent in Spegazzini's Type Material

In an examination of Spegazzini's material, a Colletotrichum stage was found on both sides of the leaf sheath and intermingled with the perithecia of Physalospora tucumanensis. In the shape, size, and texture of the conidia, conidiophores, setae, and chlamydospores, this Colletotrichum was found to be identical and in perfect agreement with Colletotrichum falcatum Vent. Most of the conidia were stuck to and lying flat on the cuticle. Some conidia had germinated by a germ tube; some by an appressorium at one of the ends. See Plate VII, Figures 1 and 2.

The results of the measurements found for the Colletotrichum found in Spegazzini's material are as follows:

Setae: 100-200 x 3-5 microns

Appressoria: 9-18 microns (diameter)

Conidia (56 measured): 19.8-32.2 x 3.3-6.3 microns but more usually 23-28 x 4-6 microns. The average size for the 56 measured was 25.1 x 5.4 microns.
These measurements are in perfect agreement with the description of *Colletotrichum falcatum* Went.  

In Plate VI (photomicrographs made from the Spegazzini material), Figure 1 shows setae and several appressoria from germinated conidia and conidia all lying dry on a piece of the cuticle of the cane leaf sheath. Figure 2 shows two conidia and also a piece of stromata with fragments of setae lying dry in a portion of the cuticle. As can be easily seen, these photomicrographs show the characteristic features proper to *Colletotrichum falcatum* Went.

II. Relationship of *Physalospora Tucumanensis* Spag. to the Perfect Stage of the Red Rot Fungus

After the perfect stage of the red rot fungus had been described as it occurred under laboratory conditions as well as naturally in the cane fields in Louisiana, and after a careful study of the original type material of *Physalospora tucumanensis* Spag. obtained from Argentina, it was found out that these two, the Louisiana material and the Argentinian material, agreed reasonably well in their morphological features. Both stages, *Colletotrichum falcatum* Went and *Physalospora tucumanensis* Spag., were present in both specimens. Therefore, as a result of these investigations, it is concluded here that the perfect stage of the red rot fungus of sugarcane should be named *Physalospora tucumanensis* Spag. Therefore the true taxonomic position of the causative organism of the red rot disease of sugarcane is as follows:
Class: ............ Ascomycetes
Order: ............ Sphaeriales
Family: ............ Pleosporaceae
Genus: ............ Physalospora
Scientific name:  Physalospora tucumanensis  Spag.
SUMMARY

1. A general historical survey of the literature dealing with the red rot disease of sugarcane was made.

2. The morphological and histological symptoms of the red rot disease were discussed.

3. The perfect stage of Colletotrichum falcatum Went has been discovered; and therefore, the complete life cycle of the red rot organism has been determined for the first time. The perfect stage belongs to the genus Physalospora of the Ascomycetes.

4. The perfect stage was found occurring abundantly in nature as inconspicuous black dots sunken in the host tissues, between vascular bundles in the upper and lower surfaces of fading and dead leaves and leaf sheaths of the five recognized species of Saccharum. Also it was found in a grass, Leptochloa filiformis. It was developed also on sterile cane leaves and in moist chambers in the laboratory. The conidial stage generally accompanied the perfect stage.

5. The red rot fungus is homothallic; single ascospore cultures of the dark and light strains produced the perfect stage under sterile, controlled conditions. The hereditary characters of pathogenicity and cultural characters were kept uniform and stable by a particular isolate (x), through many single conidial generations.
and also through the perfect stage.

6. No marked difference was found between the perfect stage produced from single ascospore and from single conidium cultures of the dark and light strains of the red rot fungus.

7. Comparative studies were made of the germination and growth of single ascospores and single conidia in sterile culture media such as potato dextrose agar drops, slants and plates of oatmeal agar. Both kinds of spores reacted in identical ways. They both produced the typical conidia, setae, the so-called chlamydoconidia or appressoria, and the mycelial growth proper to the red rot fungus of sugarcane.

8. The dark and the light strains of the red rot fungus were actually obtained by means of single ascospore isolations from different perithecia which occurred side by side on dead leaves of sugarcane and of the grass Leptochloa filiformis. The perithecia developed entirely under natural field conditions.

9. The germination of the ascospores from naturally developed perithecia was found to vary from 85-100 per cent.

10. Atypical conidia have been produced from single conidium as well as from single ascospore cultures. The most prevalent type of atypical conidia were those of very small dimensions, mainly sausage-shaped, one-celled, hyaline, uninucleate, which were produced singly, or sometimes in chains, at the ends of short, slender hyphae, occurring inside the parenchyma cells of inoculated cane leaves and cane stalk cuttings as well as in sterile agar media.
11. The red rot fungus was isolated during February by means of its sexual and asexual stages from leaves and leaf sheath trash found lying on the ground in the cane field.

12. The perfect stage of the red rot fungus has been found to have importance in the production of a large amount of inoculum for further infections of leaves and stalks of the sugar-cane plant under the natural field conditions of Louisiana.

13. The pathogenicity of many single ascospore cultures was thoroughly proved by means of intensive inoculation work with leaves and stalks of the sugarcane plant, including several different varieties. The single ascospore cultures used were from naturally developed perithecia as well as from perithecia developed in the laboratory under sterile, controlled conditions.

14. None of the single ascospore cultures used in the inoculation work failed to produce the typical red rot symptoms on sugarcane.

15. Some variation in pathogenicity was found among several single ascospore cultures, as well as among some of the single conidium cultures of the red rot fungus.

16. Of eight single ascospore cultures from one ascus and another eight single ascospore cultures from another ascus, (both ascii from the same perithectum, which was produced from a single conidium culture) all were identical in severity of pathogenicity and produced the same typical red rot lesions upon cane stalk cuttings. Also the same ascospore cultures were grown on oatmeal
agar plates and in repeated experiments all of them were identical
to each other and to the original single conidium culture which
produced the peritheciun from which they were isolated.

17. Apparently a new type of red rot symptom was found:
a general water-soaked appearance of the entire internal tissues
of the inoculated stalk cutting; observed where dark necrotic
lesions were scattered throughout. This peculiar type of symptom
was produced by the following single ascospore cultures: 00-15,
FP-14, RR-1, RR-17, XX-2, YY-3, and YY-7. Of all these, only
RR-1 was of the light culture type while the rest were of the dark
type of the red rot fungus.

18. Several single conidial cultures of Colletotrichum
graminicolum (grass anthracnose), isolated from different grasses
in Louisiana, failed to produce the typical red rot symptoms when
inoculated into cane stalks. They produced a slight reddening
of the tissues near the point of inoculation and usually a slight
reddening of the vascular bundles which appeared as very narrow,
elongated lesions up and down in the inoculated internode as well
as in other nodes and internodes.

19. The peritheciun of the red rot fungus are smooth,
dark olive-brown to blackish, imbedded in the dead host tissue,
protruding at maturity by a short, papillate ostiole, globose to
subglobose, or with peculiar shapes according to the proximity
or distance of the vascular bundles between which they developed.
The peritheciun sizes are variable. They are found between
(height x width) 100-360 x 85-250 microns; but taking either height or width as diameter of the perithecia, we find that most of the perithecia fall in the range of 100-300 microns in diameter. The thickness of the perithecial wall is usually 10-30 microns and is made up of usually three but sometimes more one-celled layers. Asci are club-shaped or clavate, normally containing eight ascospores. Most of the ascus sizes fall in the ranges of 70-90 x 13-18 microns. The ascospores are hyaline and one-celled. The majority fall within the ranges of 18-22 x 7-8 microns. Paraphyses are always present in abundance.

20. The perfect stage of Colletotrichum falcatum Went has been described as it occurs in Louisiana. Its description agrees reasonably well with Physalospora tucumanensis Speg. Spagazzini's original type material of Physalospora tucumanensis was obtained from Argentina and an intensive study of it was made.

21. As a result of these investigations, it was found that the Louisiana material and the Argentinian material were in agreement in all their morphological features. The Colletotrichum falcatum stage as well as the Physalospora tucumanensis stage was present in both specimens. Therefore, it is concluded here that the perfect stage of the red rot fungus of sugarcane should be named Physalospora tucumanensis Speg.
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BIOGRAPHY

Fernando Carvajal B. was born June 4, 1913 in Zapote, San Jose, Costa Rica. After he had completed his primary and secondary school education (11 years), he entered in 1935 the "Escuela Nacional de Agricultura" (today a dependency of the University of Costa Rica) at San Pedro de Montes de Oca where he received the degree of "Bachiller en Ciencias Agrícolas" in 1937 and the degree of "Ingeniero Agronomo" in 1939, and became a member of the "Sociedad Nacional de Ingenieros Agrónomos".

From January that year until October 1939, he was in charge of the Plant Pathology Section of the "Instituto de Defensa del Café" in San Jose. In October of 1939, he left Costa Rica to enter the Graduate School at Cornell University, Ithaca, New York, taking special work in Mycology and Plant Pathology. From this University he received the degree of Master in Science in Agriculture in September 1941. In September of the same year he left Cornell University to enter the Graduate School at Louisiana State University in order to continue work in Plant Pathology, involving problems of crop plants of his country. He is now a candidate for the Ph. D. degree at this University.
DESCRIPTION OF PLATES AND FIGURES

Plate I  Red rot in sugarcane stalks following inoculations with pure cultures.

Figure 1. Stalks of Co. 281 in the field inoculated with single ascospore cultures. Inoculated September 3, photographed 11 days later.

Figure 2. Stalks of Co. 281 and C.P. 33-243 inoculated with single ascospore cultures and a conidial culture. Cane inoculated and planted in dry soil August 12, photographed 12 days later.

Plate II  Red rot in cane cuttings of Co. 281 inoculated with cultures from the eight ascospores from a single ascus. Inoculated September 11, photographed 10 days later.

Plate III  Red rot on cane leaves following inoculation with pure cultures.

Figure 1. Leaves inoculated with single ascospore cultures on July 21, photographed 40 days later.

Figure 2. Inoculations with single conidia cultures on July 6, photographed 55 days later.

Plate IV  Cross sections of perithecia of Physalospora tucumanensis from Louisiana.

Figure 1. Photomicrograph from leaf blade of Co. 281, natural infection (cross section).

Figure 2. From leaf blade sterilized, inoculated with pure culture, and kept in moist chamber (cross section).

Figure 3. From leaf blade, natural infection (longitudinal section).
Plate V  
**Physalospora tucumanensis**, from Louisiana.

**Figure 1.** An ascus not stained, produced naturally. Photomicrograph.

**Figure 2.** Young and mature asci, ascospores, and paraphyses. Natural development. Stained (with cotton blue).

**Figure 3.** Perithecia in leaf blade of Co. 281. Natural development. x 54.

**Figure 4.** A seta producing a typical conidium at its tip, from a single ascospore culture. Stained. Note the protoplasmic contents within the stained seta.

Plate VI  
**Physalospora tucumanensis** Spag., from type specimen of Spegazzini Collection.

**Figure 1.** A cross section of leaf sheath through a perithecium.

**Figure 2.** A cross section of leaf sheath through a perithecium. Note an ascus and paraphyses inside.

Plate VII  
**Physalospora tucumanensis** Spag., from Type specimen of Spegazzini Collection.

**Figure 1.** Setae and appressoria of *Colletotrichum falcatum* on the cuticle.

**Figure 2.** Conidia and portions of setae and stromata of the conidial stage.
PLATE VI

Figure 1

Figure 2
EXAMINATION AND THESIS REPORT

Candidate: Fernando Carvajal

Major Field: Botany

Title of Thesis: THE PERFECT STAGE OF COLLETOTRICHUM FALCATUM

Approved:

[Signatures]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination:

May 3, 1943