LEPTOSPHAERIA SALVINII, THE ASCIGEROUS STAGE OF HELMINTHOSPORIUM SIGMOIDEUM AND SCLEROTIUM ORYZAE

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INTRODUCTION

Stem rot of rice (Oryza sativa L.), caused by Sclerotium oryzae Catt., has long been known to cause severe losses to rice growers both in the United States and in foreign countries. The fungus was first described from Italy in 1876 by Cattaneo (3). In 1907 Metcalf (8) reported that it had been found in North Carolina and that it "caused a stem rot under water."

In 1921 Tisdale (18) reported the occurrence of the disease in Louisiana and stated that he suspected its occurrence in Arkansas. He also demonstrated the pathogenicity of the fungus by adequate inoculation experiments. In 1926 Young (22) definitely reported the disease in Arkansas.

In 1910 Miyake (9) reported the occurrence of stem rot in Japan. He stated that great losses were caused by this disease because of the failure of the rice grains to fill properly. Subsequent investigations were made by Sakurai (14), Hemmi and Yokogi (7), and others. In Cochinchine the disease was reported by Vincens (21) in 1923. In 1913 Shaw (15) and Butler (2) stated that stem rot occurred in India and that one of the most conspicuous symptoms was the stimulation of tillering, a symptom that has not been observed in the United States. Additional reports of stem rot have been made from various parts of India by Bryce (1), Thompson and Sawyer (17), Rhind (13), and Park (10); from the Philippines, by Teodoro and Bogayong (16) and Reyes (11, 12); and from Bulgaria by Dodoff and Kovachevsky (6).

All the investigators mentioned except Vincens give only the sterile fungus, Sclerotium oryzae, as the cause of stem rot. Vincens (21), on his return to France from Cochinchine, carried on a series of experiments with S. oryzae and reported the development of a number of conidial forms associated with it in culture. These were Verticillium sp., Beauveria oryzae Vincens, forms alpha and beta; Fusarium oryzae Vincens, forms alpha and beta; and Acremonium fuliginosum Vincens. Descriptions and illustrations of these were given, but it was not shown that any of them were connected with the stem-rot fungus, S. oryzae.

The writer (19, 20) has previously reported briefly the results of investigations showing that the stem-rot fungus (Sclerotium oryzae Catt.), has a conidial stage (Helmintosporium sigmoideum Cav.)

1 Received for publication June 27, 1933; issued November 1933. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, and the Arkansas, Louisiana, and Texas Agricultural Experiment Stations.

2 Reference is made by number (italic) to Literature Cited, p. 685.
and also an ascigerous stage (Leptosphaeria salvinii Catt.). The present paper reports these results in full.

**HELMINTHOSPORIUM SIGMOIDEUM, THE CONIDIAL STAGE OF SCLEROTIUM ORYZAE**

In the summer of 1930 the writer found conidia and conidiophores of a Helminthosporium on agar cultures of Sclerotium oryzae and also on some seedlings of rice grown under aseptic conditions in test tubes and inoculated with a pure culture of S. oryzae. Inoculations with sclerotia of S. oryzae on other sterile rice seedlings gave the same results. Transfers made at the same time from the original culture failed to show the presence of any contaminating organism. More extensive inoculations and a detailed study of the conidial stage were then made. In the fall of 1931, 16 commercial varieties of rice grown at the Rice Branch Experiment Station, Stuttgart, Ark., were examined for this Helminthosporium. Spores and conidiophores of the fungus were found on mature stubble of all the varieties.

**SOURCE OF CONIDIA**

Sterile rice seedlings grown on agar in test tubes were inoculated with cultures of Sclerotium oryzae from the United States, Japan, and India, respectively. In these experiments eight cultures of S. oryzae from the following sources were used:

- Culture no. 1 isolated by V. H. Young, Department of Plant Pathology, University of Arkansas, from diseased rice collected by him in the vicinity of Stuttgart, Ark.
- Culture no. 2 isolated by the writer from diseased rice collected by him in the vicinity of Beaumont, Tex.
- Culture no. 3 received from the Centraal Bureau voor Schimmelcultures, Baarn, Netherlands. This culture was isolated from diseased rice in Japan by K. Nakata, Kyushu Imperial University, Fukuoka, Kyushu, Japan.
- Culture no. 4 obtained from India through the courtesy of M. Mitra, imperial mycologist, Imperial Institute of Agricultural Research, Pusa, India.
- Cultures nos. 5 to 8 obtained from India through the courtesy of B. B. Mundkur, of the Imperial Institute of Agricultural Research, Pusa, India.

In all the inoculations in test tubes the sclerotia were placed on the agar at the side of the seed after the seed had germinated and the seedling had been found to be sterile. Following inoculation, the seedlings were incubated at about 25° C. The seedlings were not wounded.

On January 7, 1930, culture no. 1 was used in making inoculations on five sterile Blue Rose rice seedlings grown singly in test tubes on corn-meal agar. Fifteen days later conidia of a Helminthosporium had been produced on four of the seedlings. By that time all the inoculated seedlings had succumbed to the attacks of the fungus, and typical sclerotia were found in them. The check seedlings in the uninoculated test tubes remained alive and sterile.

On February 10 culture no. 2 was used in making similar inoculations on 26 sterile seedlings of Blue Rose rice. All the seedlings were killed by the fungus and at the end of 3 weeks conidia of a Helminthosporium were noted on 20 of them. Sclerotia were produced in all cases. Five uninoculated check plants remained alive and sterile.

Culture no. 3 was used on February 10 in similar inoculations on seven sterile Blue Rose rice seedlings. The Helminthosporium conidial stage was subsequently found on two of them. Six of the seed-
lings were killed by the fungus. Four uninoculated check plants remained alive and sterile.

On June 7, 1930, culture no. 4 was used in similar inoculations on six sterile seedlings of Fortuna rice. The inoculated seedlings were killed by the fungus but no spores were produced on any plant. Characteristic sclerotia, however, were produced in one plant and in the agar of that tube. The uninoculated check plants remained alive and sterile.

No inoculation of seedlings was made with cultures 5, 6, 7, and 8. However, cultures 5, 6, and 7 produced the typical conidial stage when transferred to corn-meal agar slants with a 4 percent agar base.

The results of these inoculations indicated that under the conditions of the experiments the strains of the stem-rot fungus from the United States, Japan, and India are similar in their ability to produce conidia of a Helminthosporium.

**Cultural Studies**

Cultural studies were made of the Helminthosporium obtained from the inoculations of sterile rice seedlings just described. Corn-meal-agar dilution plates were prepared with the conidia to determine whether they were viable and to study the type of germination.

On February 10, 1930, four plates were poured from the conidia produced from culture no. 2. Germination was noted 12 hours later. Germ tubes had been produced from one or both end cells of the conidia and an arborescent mycelium was developing. Forty-eight hours later the growth was visible to the naked eye. At the end of a week small white spherical bodies had developed in all the cultures. These finally matured and proved to be the typical sclerotia of Sclerotium oryzae, which, when transferred to corn-meal-agar slants, produced typical colonies and sclerotia. The plates were kept under observation for 1 month, but no conidia were produced in any of the cultures.

On August 1, 1930, a second set of four dilution plates was prepared with conidia from transfers of culture no. 3 made June 15, 1930. When growth was visible, 48 hours after the plates were poured, 12 single spores were located with the aid of a microscope. These were then transferred to corn-meal-agar slants. At the end of 2 weeks each of these cultures had produced sclerotia typical of Sclerotium oryzae. For more than 3 months these cultures were examined frequently for conidia, but none was found in any of the 12 cultures.

On December 7, 1930, another set of dilution plates was poured with conidia from the transfers of culture no. 3. These conidia were slow in germinating. On the third day, however, germination began; some of the conidia did not germinate until the fourth day. Typical growths were produced. At the end of the first week there appeared in the agar irregular dark sclerotiod bodies with coarse, rather short, dark-colored radiating hyphae. Some of the bodies were completely embedded; others were formed at the surface of the agar. The submerged bodies (fig. 1, A) produced conidia sparsely (fig. 1, B); those on the surface produced conidia abundantly (fig. 1, C).

Soon after the discovery of the conidia all the cultures of Sclerotium oryzae in the laboratory were examined. Of the 30 available tubes of cultures 1, 2, 3, and 4, Helminthosporium conidia were found in 9; these represented cultures 1, 2, and 3 only. Later, conidia were found also in no. 4, as well as in nos. 5 to 8.
Another set of dilution plates was poured with *Helminthosporium* conidia produced on the rice seedlings that had been inoculated in test tubes with culture no. 2, as previously described. From these plates 15 single-spore colonies were transferred to separate tubes of corn-meal agar. In each of the tubes sclerotia were produced but no conidia were found in any of them. Sclerotia were transferred from
these tubes to other tubes of corn-meal agar. Each of these produced sclerotia again and 11 of them also produced typical conidia of *Helminthosporium sigmoideum*.

All the cultures of *Sclerotium oryzae* produced typical spores of *Helminthosporium sigmoideum*.

**INOCULATION EXPERIMENTS**

With *Helminthosporium* conidia from rice seedlings that had been grown in test tubes and inoculated with *Sclerotium oryzae*, inoculations were made on individual leaves of sterile Blue Rose rice seedlings growing singly in test tubes on corn-meal agar. One leaf of each seedling was inoculated by placing on it, by means of a transfer needle loop, a small drop of sterile water in which the conidia were in suspension. Following inoculation, the seedlings were kept in diffuse light in the laboratory. After 3 days the inoculated leaves of two plants showed infection in the form of small brown elongated spots. The further development of the fungus from these spots killed the seedlings in 2 weeks. Typical *Helminthosporium* conidia were produced on the seedlings and sclerotia typical of *S. oryzae* were formed in the crown tissues of the seedlings and on the agar.

On March 21, 1930, leaves of 21 Blue Rose rice seedlings growing in soil in the laboratory were inoculated by placing a loop of a conidial suspension on each leaf. These plants were incubated for 24 hours in a saturated atmosphere at room temperature. Three days later brown lesions were observed on the leaves of 17 of the inoculated seedlings. In tissue platings made from these seedlings *Sclerotium oryzae* was obtained in only one, in which characteristic sclerotia and conidia developed. Later, microscopic examination was made of the remaining seedlings after they had been killed by the fungus, and on one additional plant typical conidia of *Helminthosporium sigmoideum* were found on sheaths just above the crown.

**IDENTIFICATION**

A study of the conidial stage showed that the fungus corresponded in all essential details with *Helminthosporium sigmoideum*, described and illustrated by Cavara (5, pl. 1, fig. 5) in 1889. Cavara's original description (5, p. 185) follows:

_Effusum, atrum; hyphis fertilibus sparsis erectis, rigidiusculis, hinc inde nodulosis 8–10-septatis, simplicibus, olivaceis, 100–150×5μ; gonidiis magnis, falcato-sigmoideis, utrinque obtusis, triseptatis, cellulis mediis crassioribus, granulosis, dilute olivaceis, extimis hyalinis 55–65×11–14μ._


Cavara’s illustrations are here reproduced as figure 2. Cavara (5) reported also the occurrence of *Sclerotium oryzae* from the vicinity of Pavia and referred to a specimen distributed in exsiccati. The writer examined this specimen, in the mycological collections of the Bureau of Plant Industry, United States Department of Agriculture, and found conidia of *Helminthosporium sigmoideum* as well as sclerotia of *S. oryzae*. Portions of conidiophores, also found in this specimen, 

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3 _Briosi, G.,_ and _Cavara, F._ 1. _Funghi parasitie delle piante coltivate od utili essiccati, delineati e descritti._ Fasc. 1, no. 25. 1888.
bore the sharp-pointed sterigmata characteristic of the conidiophores as noted by the writer (fig. 3). The conidia and conidiophores from the Briosi and Cavara specimen were found to be essentially identical with those from the writer’s material.

From the cultural studies and artificial inoculations here recorded it is evident that Helminthosporium sigmoideum and Sclerotium oryzae are the conidial and sclerotial stages, respectively, of the same fungus.

LEPTOSPHAERIA SALVINII, THE ASCIGEROUS STAGE OF SCLEROTIUM ORYZAE

On some plants of Blue Rose rice and red rice grown in the greenhouse at Fayetteville, Ark., and infected with Sclerotium oryzae, a few peritheciumlike fruiting bodies were found on the outer sheaths. These fruiting bodies contained spores that were similar to the conidia of Helminthosporium sigmoideum.

Further examination of additional specimens revealed other such fruiting bodies with the spores in asci. These asci were found in various stages of development. The fungus was identified as Leptosphaeria salvinii, which had been described on rice in Italy by Cattaneo (4) in 1879. In 1910, Miyake (9) reported that this fungus had not been found in Japan.

In October 1931, perithecia of Leptosphaeria salvinii were found on a number of rice varieties infected with Sclerotium oryzae at the Rice Branch Experiment Station, Stuttgart, Ark. On these perithecia, conidiophores and conidia similar to those of Helminthosporium sigmoideum were frequently found. This, together with the similarity of the ascospores and conidia and the fact that the perithecia were found on plants infected with Sclerotium oryzae, suggested the possibility that L. salvinii might be the ascigerous stage of H. sigmoideum and S. oryzae, which already had been shown to be connected. With this in mind, cultural studies and inoculation experiments were undertaken.

CULTURAL STUDIES

On November 17, 1931, three perithecia of Leptosphaeria salvinii from Blue Rose rice plants grown in the greenhouse at Fayetteville, Ark., were broken up in separate drops of distilled water to which dilute lactic acid had been added. The spore suspensions were smeared over the surface of slants of tubes of potato-dextrose agar. On the second and third days after the inoculations, 36 single-spore

![Figure 2.—Reproduction of Cavara’s figures (5, pl. 1, fig. 5), the legend for which may be translated as follows: “Helminthosporium sigmoideum n. sp.; a, portion of stem of Oryza with spots caused by the fungus; b, fruiting hyphae; c, spores.”](image-url)
colonies were located with the aid of a microscope and marked. Each colony was transferred to a separate tube of potato-dextrose agar (series 1).

On November 20 three additional perithecia were similarly broken up in distilled water to which a trace of lactic acid had been added. The drops of water were placed in sterile Petri dishes and diluted with cooled melted agar. On the second day thereafter nine transfers of single-spore colonies (series 2A) and three transfers from mycelium produced from two or more ascospores (series 2B) were made, as in the preceding experiment.

![Figure 3. Helminthosporium sigmoideum, X 1,140: A, Conidia and a portion of conidiophore from a test-tube culture on corn-meal agar. B, conidium and conidiophore from inoculated Blue Rose rice seedling grown in soil in the laboratory.](image)

On December 4 dilution agar plates were prepared, as previously described, from perithecia from Blue Rose rice grown in the greenhouse. Three single-spore cultures were secured (series 3).

On December 9, 1931, dilution agar plates were prepared of spores from perithecia produced on red rice in the greenhouse. Three single-spore cultures were secured from these plates also (series 4).

By the last of January 1932 all of the 54 cultures of Leptosphaeria salvinii (series 1 to 4) had produced abundant mycelium and sclerotia typical of Sclerotium oryzae. On January 23, 1932, one culture from series 2A and one from series 2B produced conidia typical of Helminthosporium sigmoideum. By the last of February 1932, 15 of the colonies from all four series of isolations had produced conidia typical of H. sigmoideum.
Conidia from isolation series 1 and 2 were transferred on February 26, 1932, to corn-meal-agar slants. They had germinated by the following day, and nine single-spore colonies were located with the aid of a microscope and transferred to individual tubes. By the middle of April, five of the nine cultures had produced sclerotia typical of *Sclerotium oryzae*. No sclerotia were produced by the other four cultures. No conidia were produced in any of the nine cultures during the period that they were under observation.

Ascospores of *Leptosphaeria salvinii* from rice stubble collected at Stuttgart, Ark., intermittently from October through February germinated readily when placed in tap water at room temperature. Some of the ascospores of the October collection germinated readily the following May in distilled water at room temperature.

The characteristics of the cultures from ascospores were essentially identical with those from either conidia or sclerotia.

Numerous unsuccessful attempts have been made to produce the ascigerous stage in artificial culture, by using cultures originating from each of the three stages.

**INOCULATION EXPERIMENTS**

Blue Rose rice plants were grown in autoclaved virgin soil from the vicinity of Stuttgart, Ark., in three 3-gallon stoneware jars. The seeds were prepared for sowing by removing the hulls, surface-sterilizing them for 5 minutes in 1:1,000 mercuric chloride solution, and then rinsing them in tap water. About 10 plants were grown in each jar.

On June 21, 1932, when the plants were 6 to 8 inches high, they were inoculated with sclerotia from pure cultures originating from ascospores of *Leptosphaeria salvinii*. These cultures were obtained from stem-rotted Blue Rose and Rexoro rice plants grown at Stuttgart in 1931. No plants were left uninoculated as controls, because in numerous trials plants grown similarly without inoculation had always remained free from stem rot. Inoculation was made by placing sclerotia in contact with the plants at the water line without wounding. On August 8, 1932, all culms that had not become infected as a result of the first inoculation were reinoculated with sclerotia from the same sources as were those in the first inoculation. When the reinoculations were made the outer sheaths of a number of the plants had been killed by the stem-rot fungus as a result of the first inoculation, and the typical symptoms had been produced. Examination of the dead sheaths a week later showed mature perithecia of *L. salvinii*. In addition, large numbers of conidiophores and conidia of *Helminthosporium sigmoideum* were found on the outer sheath surfaces, and sclerotia typical of *Sclerotium oryzae* were found embedded in the tissues of the sheaths.

It is evident from the results here set forth that *Leptosphaeria salvinii* is the ascigerous stage of *Helminthosporium sigmoideum* and *Sclerotium oryzae*. 
TECHNICAL DESCRIPTION OF *LEPTOSPHAERIA SALVINII* CATT., 1879 (4, p. 126–127; pl. XV, figs. 1–3)

Synonym for sclerotial stage, *Sclerotium oryzae* Catt., 1876 (3); synonym for conidial stage, *Helminthosporium sigmoideum* Cav., 1889 (5).

**MYCELUM**

Hyphae white to olivaceous, septate, profusely branched, 2 µ to 5 µ in diameter. In culture, mycelium white at first, later smoky to black at surface of medium. On host, mycelium white inside culm, olivaceous outside. Numerous irregular olivaceous appressoria form on culm; range from 14 µ to 30 µ by 8 µ to 24 µ.

**SCLEROTIAL STAGE**

Sclerotia spherical or nearly so, black at maturity, surface nearly smooth, at times covered with cottony web of white mycelium; 180 µ to 280 µ, mostly 230 µ to 270 µ. Habitat, in sheaths and culms of *Oryza sativa* (fig. 4) and *Zizaniopsis miliacea* (Michx.) Doell and Asch.

**CONIDIAL STAGE**

Conidiophores dark colored, septeate, erect, simple or sparsely branched, 4 µ to 5 µ by 100 µ to 175 µ; conidia borne singly on sharp-pointed sterigmata, fusiform, typically three-septate, simply curved or slightly sinuous; intercalary cells Prout's brown, densely granular; terminal cells lime green, less granular than intercalary cells; apical cell frequently longer and less acutely pointed than basal cell; spores occasionally constricted at middle septum, 9.9 µ to 14.2 µ by 29 µ to 49 µ, mostly 11 µ to 12.5 µ by 34 µ to 40 µ. Habitat, on leaves and culms of *Oryza sativa* (fig. 3 and fig. 1, D) and *Zizaniopsis miliacea*.

**ASCIGEROUS STAGE**

Perithecia dark, globose, embedded in outer tissues of sheath, 202 µ to 481 µ, average diameter 381 µ, beak rather short (30 µ to 70 µ), frequently half the diameter of perithecium in width. Beak nonprotruding, tip flush with surface of outer epidermis of sheath, visible to naked eye; ascii narrowly clavate, walls almost invisible and deliquescent by the time spores mature, short-stalked, 90 µ to 128 µ by 12 µ to 14 µ, mostly 103 µ to 125 µ by 13.5 µ; ascospores biseriate, normally eight in ascus (rarely only four), three-septate when mature, usually somewhat constricted at septae, particularly at middle septum, brown, two end cells usually lighter in color and contents less granular than middle cells, fusiform, somewhat curved, 38 µ to 53 µ by 7 µ to 8 µ, mostly 44 µ to 48 µ by 8 µ (figs. 5 and 6). Habitat, in sheaths of *Oryza sativa*.

**HOSTS AND GEOGRAPHIC DISTRIBUTION**

The sclerotial and conidial stages of *Leptosphaeria salvinii* occur on all commercial varieties of rice, red rice, and *Zizaniopsis miliacea* in the rice-growing areas of Arkansas, Louisiana, and Texas. The sclerotial stage occurs in most foreign countries where rice is grown. The conidial stage is known to occur only in Italy and the United States, but no doubt it is present in the other countries where the disease occurs. The ascigerous stage has been found on seven commercial varieties of rice in Arkansas, Louisiana, and Texas, on a rice selection (C.I. no. 4559) in Arkansas and Louisiana, and on commercial rice in Italy.

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*Ç. I. denotes accession number of Division of Cereal Crops and Diseases.*
FIGURE 6. — *Leptosphaeria salvinii*, perithecial stage: A. Surface view of a portion of a sheath of Rexoro rice, showing the tips of the beaks of the perithecia appearing as small black specks. × 314. B. Perithecium showing tip of beak (a) flush with epidermis of sheath (b). × 50. C. Asci in various stages of development. × 320. D. Asci showing biseriate arrangement of spores. × 432. E, Ascospores. × 900.
As Tisdale (18) has pointed out, the sclerotia are known to overwinter on old rice straw and stubble in the field and to serve as inoculum for the growing rice plants during the following summer. Indications are that these overwintered sclerotia also produce abundant conidia while floating on the surface of flood water. These conidia are readily disseminated on the surface of the water and come in contact with the sheaths and there germinate and cause infections. On the infections, whether originating from sclerotia or conidia, secondary conidia develop, which serve to spread the infection still further. Sclerotia are formed in abundance in all the infected tissues later in the season. Under certain conditions the perithecia form in the infected sheaths about the same time as the sclerotia; under other conditions they appear to form somewhat earlier.

Viable ascospores have been found on old rice stubble as late in the winter as January. It has not been possible to determine whether they live through the winter and function as inoculum for the following crop because the sheaths carrying the perithecia disintegrate so completely during the winter that no perithecia have been found in the spring.

**SUMMARY**

*Leptosphaeria salvinii* Catt. has been found to be the ascigerous stage of *Sclerotium oryzae* Catt. and of *Helminthosporium sigmoideum* Cav., and the various stages of the fungus are described and illustrated. The hosts, geographic distribution, and seasonal development of the fungus are discussed.

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