MYCOLOGICAL AND PATHOLOGICAL STUDIES ON THE DOWNY MILDEW OF ITALIAN MILLET

By

Makoto HIURA

[With Plates IX–XIV]

Contents

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Introduction

The downy mildew of Italian millet is a systemic disease caused by a species of Sclerospora. Because of the practical importance of the disease, and also possibly from the characteristic deformity of the affected plants, the disease has long attracted the attention of mycologists as well as of plant pathologists. Consequently, a considerable number of papers concerning this disease have been published up to the present. However, as pointed out by KASAI (61), our knowledge of this disease has been very limited and fragmentary. Most of the earlier studies were really carried out from the mycological side, chiefly describing the causal fungus. From the stand-point of modern plant pathology, therefore, many interesting points in connection with the taxonomy as well as the physiology of the causal fungus, and the influence of environmental factors upon the infection and development of the disease are left entirely untouched.

With the hope of promoting the knowledge of such fundamental principles as may be applied to the general downy mildews caused by species of Sclerospora, and also to other allied diseases, the writer, since 1927, has been endeavouring to solve various underlying problems in the disease under consideration. Some results have already been published, at various times, as preliminary reports. It is intended in this paper to present and discuss in detail all the data obtained in the studies carried out during the past six years.

These investigations were first undertaken at the suggestion of Prof. S. ITO, of the Botanical Institute, Hokkaido Imperial University, Sapporo, Japan, to whom the writer wishes to express his most cordial thanks for kind advice and guidance in carrying on the

investigations. He also wishes to express his heartiest appreciation to Prof. Y. TOCHINAI for valuable suggestions and helpful criticism in revising the manuscript, and to Emeritus Prof. K. MIYABE for unfailing interest and constant encouragement in the work.

The writer wishes to express his sincere thanks to Prof. T. HEMMI, of the Phytopathological Institute, Kyoto Imperial University, Kyoto, for various suggestions and the free use of his library; and to Dr. T. ABE for kindly supplying oospore materials.

Some inoculation experiments and oospore germinations were carried out at the Department of Plant Pathology, University of California, Berkeley. The writer wishes to thank Prof. R. E. SMITH for the use of the green house and laboratory.

The major parts of the environmental studies on seedling infection were performed at the Department of Plant Pathology, University of Nebraska, Lincoln. The writer wishes to make grateful acknowledgment of his indebtedness to Prof. G. L. PELTIER, for so generously furnishing the facilities of the green house and laboratory; and to Prof. R. W. GOSS for valuable suggestions in determining soil moisture.

Certain phases of the environmental studies and of the inoculation experiments were studied in part at the Departments of Plant Pathology and Plant Physiology, University of Wisconsin, Madison. The writer expresses his heartiest thanks to Emeritus Prof. L. R. JONES for his kindness in critically reading a part of the manuscript; and to Profs. B. M. DUGGAR and G. W. KEITT for their courtesies extended during the work.

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Review of Earlier Work

It will be sufficient to give here a brief chronological outline, as the important papers are to be discussed in more detail later.

In 1876, SACCARDO (92, 93), in Italy, described a fungus parasitic on *Setaria verticillata* under the name of *Protomyces graminicola.*

In 1878, MAGNUS (66), in Germany, gave an account of a fungus on *Setaria viridis,* which, according to him, had been first collected by I. URBAN in 1875. MAGNUS tentatively named the fungus *Ustilago* (?) *Urbani.*

In March 1879, PASSERINI (77), in Italy, described a new Peronospora on *Setaria verticillata,* and gave it the name *Peronospora Setariae.* He also pointed out that *Protomyces graminicola* SACC. and *Ustilago (?) Urbani* MAGNUS are the oospore stage of *Peronospora Setariae.*

In June 1879, SCHROETER (98), in Germany, published a paper on a Phycomycetous fungus which was found to be parasitic on *Setaria viridis* and *Setaria glauca.* He believed the fungus to be identical with *Ustilago* (?) *Urbani* MAGNUS as well as with *Protomyces graminicola* SACC. In spite of all his attempts, he failed to germinate the thick-walled, reddish brown spores of the fungus. However, while studying the process of development of these spores, he found the oogonium and antheridium of the fungus. Later, on the same host, he found a conidial form resembling Peronospora, but differing from the latter in the morphology of the conidiophore. He believed that these oosporous and conidial stages belong to one and the same fungus, although he made no experiment to prove it. He proposed, for this fungus, to establish a new subgenus Sclerospora in the genus Peronospora.

In 1881, DE BARY (20) lifted Sclerospora SCHROETER to a distinct genus.

In 1882, SACCARDO (94) made a new combination, *Peronospora graminicola,* for the fungus in question.

In 1884, PRILLIEUX (85), in France, published a short note on *Perospora graminicola,* in which he claimed that FRÉCHOU observed oospores to produce tubes which resemble the hyphae sprouting from the oospores of *Plasmopara viticola.*

In 1884, FARLOW (29), in America, reported the occurrence of *Peronospora graminicola* (SACC.) SCHR. on *Setaria viridis.* But he did not find the conidial stage.

In 1885, TRELEASE (110), in America, reported *Peronospora graminicola* (SACC.) occurring on *Setaria viridis* and *Setaria italica.* He noticed that "conidial branches were sparingly produced (p. 111)."

In 1886, SCHROETER (99) described the characters of the genus Sclerospora, and he also gave a description of *Sclerospora graminicola* (SACC.) which was considered to be parasitic on various species of Setaria. The generic characters given by him are as follows:

"Conidienträger straff aufrecht mit spärlichen graden Aestchen. Conidien eiförmig, am Scheitel mit einer Papille, Schwärmsporen bildend. Oosporen kuglig mit sehr dickem, mit der Haut des Oogons verschmelzendem, mehrschichtigem, braunem Epispor (S. 236)."

In 1886 and 1887, HALSTED (41, 42) reported the occurrence of *Peronospora graminicola* on *Setaria viridis* in Iowa, and described the symptoms of the disease and its causal fungus in some detail.

In 1887, TRELEASE (111) stated that Hungarian grass (*Setaria italica*), and pigeon grass (*Setaria viridis*) were sometimes attacked by *Peronospora graminicola* SACC.

In 1888, SACCARDO (95) described *Sclerospora graminicola* to be parasitic on three species of Setaria, namely, *S. viridis, S. verticillata,* and *S. italica.*

In the same year, HALSTED (43, 44) reported the decline of the foxtail (*Setaria viridis*) downy mildew in a dry season in Iowa, and also mentioned the occurrence of the disease on Hungarian grass.

In 1889, SWINGLE (104) reported *Sclerospora graminicola* (SACC.) SCHROET. on New Wonder millet and Hungarian grass, at Manhattan, Kansas.

In 1892, FISCHER (30) described the genus Sclerospora in his "Phycomycetes."

In 1893, SCHROETER (100) enumerated *Setaria verticillata, S. viridis, S. italica, S. glauca,* and *Panicum Crusgalli* as the hosts of *Sclerospora graminicola* (SACC.) SCHR.

In 1894, SAUNDERS (96) gave a short description of *Sclerospora graminicola* (SACC.) SCHR. on *Chamaerhaphis viridis* and *C. glauca* collected in Nebraska.

In 1897, SHIRAI (101), in Japan, published a paper on the downy mildew of *Setaria italica,* caused by *Sclerospora graminicola* (SACC.) SCHR. The symptoms of both the oosporous and conidial stages of the disease, and the morphology of the causal fungus were described in detail. Methods of control were also suggested.

In 1900, 1901, and 1902, PEGLION (78, 79, 80), in Italy, published successively his investigations on the downy mildew of wheat, which was considered to be caused by *Sclerospora graminicola.*

In 1902, TRAVERSO (107), in Italy, published his comparative studies of Sclerosporas on various grasses. According to him, *Scl. Kriegeriana* MAG. is a synonym of *Sclerospora macrospora* SACC. He also pointed out that the *Sclerospora* fungus reported by PEGLION should be designated as *Sclerospora macrospora.* He was inclined to think that *Sclerospora graminicola* is restrictively parasitic on species of Setaria.

In the same year, TRAVERSO (108) described a new variety of *Sclerospora graminicola,* parasitic on *Setaria italica.* The original description is as follows:

"*Sclerospora graminicola* (SACC.) SCHROET. var. *Setariae-italicae* TRAV.

A typo differt oosporis majoribus, nempe 39–45 μ diam. nec 23–38 (sine tunica oogoniali); tunica oogoniali, magis regulariter sphaerica; 7–11 μ crassa nec 4–6; tota spora denique 53–64 μ diam. nec 29–55. Differt etiam tunica oosporarum tenuiori. Status conidiophorus ignotus videtur (pp. 174–175)."

In 1903, GUÉGUEN (39), in France, briefly reviewing TRAVERSO's paper, doubted the justification for the new variety.

In the same year, BERLESE's (8) monograph on Peronosporaceae was completed. In this book, the genus Sclerospora was described.

In 1907, BUTLER (11), in India, published a paper relating to the Sclerosporas parasitic on *Pennisetum typhoideum, Setaria italica, Andropogon Sorghum,* and *Euchlaena luxurians.* The Sclerospora on Pennisetum was found to have both the oosporous and conidial stages. On Andropogon, and on Setaria, only the oosporous stage

was found, while the conidial stage occurred only on Euchlaena. Investigations were made most extensively on the fungus attacking Pennisetum. His careful comparative studies on the morphology of these Sclerosporas led him to conclude that these fungi are one and the same, *Sclerospora graminicola* (SACC.) SCHROET.

In the same year, 1907, WILSON (127), in America, reported the occurrence of *Sclerospora graminicola* (SACC.) SCHROET. on *Setaria viridis, S. italica,* and *S. glauca.*

In the same year, TRAVERSO (109) published his further studies on *Sclerospora graminicola* (SACC.) var. *Setariae-italicae* TRAV. He found the conidial stage of this fungus to be indistinguishable from that of the type species, *Sclerospora graminicola* (SACC.) SCHR. which parasitizes the other millets. However, from the results of careful field observations, and also of artificial infection experiments, although the latter were not perfectly conclusively, he came to the conclusion that the new variety is truly a form distinct from *Sclerospora graminicola* type, even if the differences in the oospores of the two forms are not considered sufficient for their separation, because the two forms differ in their parasitism.

In 1912, MIYAKE (75), in Japan, reported the occurrence of *Sclerospora graminicola* on *Setaria italica* and *S. glauca* in China.

In 1913, KULKARNI (62), in India, reported a study on the downy mildews of bajri (*Pennisetum typhoideum*), and Jower (*Andropogon Sorghum*). He found not only the difference in the morphology of the conidia and conidiophores of both fungi, but also the difference in the mode of conidial germination between the two, and described the fungus on jower as a new variety of *Sclerospora graminicola,* naming it *S. graminicola,* var. *Andropogonis Sorghi.*

In the same year, S. ITO (56), in Japan, discussed the taxonomy of Sclerospora, and suggested separating the genus Sclerospora into Eusclerospora and Peronosclerospora. The former comprises the species with the conidia which germinate by zoospores, and the latter includes the species with the conidia which germinate by germ-tubes.

In the same year, MASSEE and MASSEE (67) published a book in which *Scl. macrospora* is treated as a synonym of *Sclerospora graminicola* (originally written as *graminis*). The following remarks given by the authors are very interesting:

"Seed of *Saccharum officinale,* obtained from France, was sown in a plot adjoining the Jodrell Laboratory, Kew Gardens. Two of the resulting plants were infested with *Sclerospora graminis,* and

two other plants were badly "smutted" with *Ustilago sacchari.* Neither of these fungi had previously been recorded as occurring in Britain, so that direct infection from spores was out of question, and it is practically certain that in both cases, the source of infection was imported along with the seed."

In 1914 and 1915, DAVIS (18, 19) reported the occurrence of *Sclerospora graminicola* on *Setaria viridis, S. italica,* and *S. glauca* in Wisconsin.

In 1918, BUTLER (12) described the downy mildews of jower and bajra caused by *Sclerospora graminicola* in his book, and predicted the presence of specialization in the fungus.

In 1922, SAWADA (97) recorded the Sclerospora on *Setaria italica* in Formosa to be identical with TRAVERSO's new variety.

In 1924, WESTON (120) reported an extensive study on the nocturnal production of the conidia by *Sclerospora graminicola* on *Setaria viridis.* He gave a thorough description of the conidial stage of the fungus.

In 1925, MELHUS and VAN HALTERN (70) reported that *Scl. graminicola* from *Setaria viridis* can be transferred to *Setaria italica, Panicum miliaceum, Euchlaena mexicana,* and *Zea mays* by means of artificial inoculation.

In 1926, WESTON and WEBER (121) reported the occurrence of *Scl. graminicola* on *Setaria magna* in Florida.

In 1927, BORCHHARDT (9) in Russia reported that ten-year-old oospores of *Sclerospora graminicola,* kept in the laboratory, induced 77.7 per cent infection on *Setaria italica.* The inoculation on wheat and barley gave negative results.

In 1927, KASAI (61), in Japan, gave a historical outline of the downy mildew of Italian millet, and discussed various interesting problems regarding this disease.

In 1927 and 1928, MELHUS and his co-workers (71, 72) published their further studies on the downy mildew of *Setaria viridis* and corn. The physiology of the causal fungus, and some phases of seedling infection were studied.

In 1928, CHAUDHURI (13) reported the importance of the green ear of bajra caused by *Sclerospora graminicola* in the Panjab.

In 1928, WESTON and WEBER (122) published a paper on the downy mildew of the Everglade millet (*Setaria magna*). The symptoms of the disease and the morphology of the causal fungus were fully described.

In the same year, UPPAL and KAMAT (114) reported the results of their inoculations of *Pennisetum typhoideum* with oospores from the same host.

In 1929, HIURA (48, 49, 50, 51), in Japan, published preliminary notes on the downy mildew of the Italian millet. He reported the life-history and physiology of the causal fungus in relation to pathogenesis, together with the results of the inoculation experiments. He succeeded not only in germinating oospores of the causal fungus, but also proved that the fungus from *Setaria viridis* can infect *Setaria italica,* although the percentage of infection is much lower than in the case of the inoculation of *Setaria italica* with oospores collected from the same host, indicating that the fungus on *Setaria viridis* is physiologically different from that on *Setaria italica.*

In 1930, UPPAL (112) reported that *Pennisetum typhoideum* was infected by oospores of the fungus taken from sorghum, but when the latter was exposed to infection by oospores taken from *P. typhoideum* only one infection resulted, while both were readily infected by oospores taken from *Setaria viridis* in America.

In 1930, MITTER and TANDON (73) reported the occurrence of *Sclerospora graminicola* on *Pennisetum typhoideum* in Allahabad, India.

In the same year, 1930, an abstract of TASUGI's study (105) on the causal fungus of the downy mildew of Italian millet was published. He found that the dimensions of the conidia increased in summer, and decreased in autumn. He also described the mode of oospore germination. He is of the opinion that the fungus on *Setaria italica* is distinguished from that on *Setaria viridis* by their specialized parasitism. Certain phases of the physiology of the causal fungus were also studied.

In the same year, HIURA (53, 54) published his further studies on the downy mildew of Italian millet. A simple method for the germination of the oospore was described, and it was also found that the percentage of the germination varies with temperature and also to a great extent with the source and age of the oospore. The results of environmental studies on seedling infection, and of inoculation experiments were also reported.

Later, in the same year, EVANS and HARRAR (26)* reported success in germinating oospores of *Sclerospora graminicola* from *Setaria viridis.*

* There are some mistakes in their citation of the writer's paper.

In 1931, MELCHERS (68) reported the occurrence of the downy mildew of sorghum and maize in Giza, Egypt. The causal fungus was considered to be identical with *Sclerospora graminicola,* var. *Andropogonis Sorghi* KULK. No striking difference in appearance or measurements could be found between the conidial stages on sorghum and on maize.

In 1931, UPPAL and DESAI (115) reported the presence of at least two physiologic forms of *Sclerospora graminicola.* One form attacks *Pennisetum typhoideum,* and the other infects *Setaria viridis, S. italica, S. magna,* and *Euchlaena mexicana.*

In 1931, WESTON (124) published a brief article concerning PRILLIEUX's note on the germination of the oospores.

In the same year, MCGINTY (125) criticized WESTON's note, and pointed out the latter's inaccuracy in reviewing literature.

In 1931, UPPAL (113) reported a new host of *Sclerospora graminicola,* var. *Andropogon-Sorghi.*

In 1931, the Agricultural Experiment Station, Government-General of Chosen (1) reported an account of the downy mildew of Italian millet. The control methods were chiefly studied.

In 1932, CHAUDHURI (14) published his further studies on the downy mildew of bajra (*Pennisetum typhoideum*). He succeeded in infecting young bajra plants with oospores, and also reported success in germinating oospores.

In 1932, UPPAL and DESAI (116) reported two new hosts of the downy mildew of sorghum.

In 1932, WESTON and UPPAL (126), studying thoroughly *Scl. graminicola,* var. *Andropogonis-Sorghi,* established the fungus as a distinct species, and named it *Sclerospora sorghi* WESTON et UPPAL. They also succeeded in germinating oospores.

In the same year, TAKASUGI and AKAISHI (106) published the results of a study on the germination of oospores of *Scl. graminicola* var. *Setariae-italicae.*

Geographic Distribution and Economic Importance

Sclerospora graminicola (SACC.) SCHR., the causal fungus on Italian millet, is known to attack various other cereals and grasses. In 1902, TRAVERSO (107) reported the occurrence of this fungus in the following countries: Italy, France, Germany, Russia and the United

States of America. In 1907, BUTLER (11) found the same fungus in India and East Africa. In 1912, MIYAKE (75) reported the occurrence of the fungus on Italian millet and *Setaria glauca* in China.

In Japan, the downy mildew of Italian millet was reported as early as 1897. The disease is now commonly found from Hokkaido on the north to Formosa on the south, wherever the Italian millet is cultivated. Its occurrence is also known in Korea as well as in Manchuria. It is thus seen that the disease caused by *Sclerospora graminicola* is almost world-wide in its distribution, although so far the fungus has not been found in Holland, and in Scandinavia*.

Since most of the affected plants do not form grains at all, severe losses are often caused by this disease. Several writers have reported serious damage due to this fungus.

SHIRAI (101) reported that the damage to the Italian millet due to the disease was considerable, often reducing the crop to less than 50 per cent, and in the worst cases almost to nothing.

BUTLER (12) stated that the disease caused considerable damage to the Italian millet in Bihar and Madras, India.

MIYAKE (75) mentioned that in the mountain regions of Iichang, Hupei (China), he observed many attacked Italian millet plants with special whisklike upper leaves or abnormal ears, while in the lower plains of the Yangtz river he could not find any such plants.

WESTON (122) stated that serious damage was caused by *Sclerospora graminicola* to the Everglade millet in Florida.

In the summer of 1927, the writer found an outbreak of the downy mildew of Italian millet at Kakudahama-Mura, Niigata Prefecture, Honshu, where there were scattered many small fields of Italian millet. Although the severity of the disease was found somewhat different in different fields, many of them were seriously damaged. While collecting oospore material in some of these disastrously attacked fields, the writer counted the number of the diseased plants of some severely attacked rows. On each row examined, from 80 to 90 per cent of the plants were not uncommonly found to be diseased. Of these diseased plants, 91 per cent exhibited shredded leaves without showing any sign of spike formation. The remainder produced deformed spikes, most of which, however, were entirely sterile, while only a few had some healthy spikelets.

* The writer owes this information to Dr. QUANJER in Holland, and to Dr ERIKSSON in Scandinavia. The writer wishes to express his heartiest thanks to these authors.

The next summer, 1928, the writer again inspected the same fields. However, the disease was not found to be very severe. Several fields were entirely disease-free, so that some search was necessary to obtain oospore material.

The inquiry made in 1930, revealed that about 7 per cent of the Italian millet cultivated over nearly fifty acres in Aichi Prefecture, Honshu, was attacked by the downy mildew.

The Agricultural Experiment Station, Government-General of Chosen (Korea) reported that the downy mildew is found every year throughout the Italian millet growing regions in Korea, and the losses in the worst cases due to this disease in the western parts of Korea amount annually to over 50 per cent of the crop.

The investigations made in the Agricultural Experiment Station of the S. M. R. Co. at Yūgakujō (106) indicate that about 10 per cent of the Italian millet in Manchuria is annually damaged by the downy mildew.

In addition, the present investigations have proved that the fungus from *Setaria italica* can infect several varieties of maize, sorghum, cane, and teosinte. This fact, although formerly quite ignored, may give a new significance to the economic importance of the disease. In the case of these new hosts, affected plants become seriously stunted. Consequently, it is quite possible that, under favorable circumstances, serious damage may be caused by the disease.

Symptoms of the Disease

The causal fungus of this disease has two distinct phases of development in the course of its life-cycle. One is the conidial stage, and the other is the oosporous stage. The symptoms are quite different with the stage. There are also two types of infection: systemic infection, and local or secondary infection. The former may also be called oospore infection, since, so far as known, it is initiated by the mycelia germinated from oospores, while the latter may be called conidial infection, because it is started by the zoospores from conidia.

1. SYMPTOMS PRESENTED BY THE CONIDIAL STAGE

As already pointed out by the writer (48), the symptoms due to the conidial stage have been rather neglected in the earlier works.

a. Systemic Type of Infection

Systemically infected plants manifest themselves in the development of chlorotic conidia-bearing areas on their successive leaves. The conidia and conidiophores are mostly formed on the under surface of the leaves, showing a whitish moldy appearance.

Systemic infections first occur in a very early stage of seedling development. In fields, infections take place before the emergence of the seedlings. Severely infected seedlings grow slowly, and some of them are killed before their emergence. The remainder appear above ground, and give rise to poor leaves on which conidia are formed. The conidia are sometimes produced while the first leaf is still folded. But, if external conditions are unfavorable, for instance if the air humidity is too low, the conidia are not formed, and chlorotic areas are first seen. These chlorotic areas are ready to produce conidia whenever conditions become favorable. As a rule, the infected leaves of seedlings survive longer when conidia are not formed on them. At any rate, severely affected seedlings die sooner or later.

The less severely affected seedlings grow apparently with normal vigor in the early stage of their development. Therefore, it is usually difficult to distinguish the infected seedlings in this stage from the healthy ones either until conidia are produced, or until chlorotic areas are formed, although the leaves of the infected seedlings, in some cases, show a slightly tapered, wrinkled appearance.

These less severely affected seedlings continue to grow, developing new leaves successively, which are in general systemically infected with varying degrees of severity. In case plants are closely planted, the infected plants are liable to show an inferior growth. But, if these infected plants are separately planted, some of them grow to normal size, while others are somewhat smaller than the healthy ones, depending upon the severity of infection.

The most conspicuous feature of the symptoms presented by the conidial stage is seen on the systemically infected leaves of well-grown plants, which are characterized by large laciniate, chlorotic areas. The under surface of these areas is covered with the whitish down of conidia and conidiophores, although under certain conditions, the conidia are also able to be formed on the upper surface of the leaves. The production of conidia and conidiophores may be preceded by chlorotic areas, or vice versa, owing to environmental conditions.

In fields, the conidia and conidiophores are usually formed during favorable dewy nights, and shrivel out in the daytime. Infected leaves may continue to produce conidia for three weeks or more. In the rainy season, however, the chlorotic areas are apt to become water-soaked, and easily succumb to secondary infections due to other saprophytes. Therefore, fine weather is really more favorable for the production of conidia, so far as the continuation of conidial formation is concerned.

The conidia-bearing chlorotic areas vary greatly in their shape and size. On the old lower leaves, the areas appear generally as pallid stripes, stretching parallel to the veins, forming irregular, jagged extensions in the green blades. On the comparatively younger upper leaves of grown plants, discoloration is more extensive, and in most cases the top leaves are almost albicated. This does not mean, however, that the chlorotic areas of the upper leaves are always larger than those of the lower ones. For instance, in a series of affected leaves of a plant, the pallid stripes appearing on the upper leaves are often smaller than those of the lower ones. Sometimes, one or more leaves of a systemically infected plant may escape infection. It is also frequently found that all the leaves lower than the middle of the plant are disease-free, while those located above the middle are successively infected. Most of the conidia-bearing leaves survive as long as the healthy ones.

b. Local Infection

By local infection, restricted spots are developed on the leaves. These spots may be formed separately, or may coalesce into larger areas of irregular margin. The spots are dark purplish in color, and the conidia and conidiophores are sparsely produced on their under surface. The production of the conidia is so scanty compared with what happens as a result of systemic infection, that, without careful observation, it is liable to be overlooked.

In shape and size, the spots produced by local infection vary from irregularly angular specks to elongate streaks running lengthwise on the leaf. The local infection causing these spots is apparently not so injurious to the host as in the case of systemic infection. It is obvious, however, that in case the spots formed by the local infection accumulate, they cause not only considerable damage to the

host, but also play an important rôle in spreading the disease by the abundant production of conidia.

This type of infection has not been found in the writer's experimental field during the past few years. But, in the summer of 1930, a severe occurrence of local infection was found in several fields of Italian millet, at Miyata, Aichi Prefecture, Honshu. The grade of susceptibility to local infection seems to vary with different varieties of the Italian millet.

2. SYMPTOMS PRESENTED BY THE OOSPORE STAGE

One of the most characteristic symptoms of the disease is represented by the symptoms due to the oospore stage, which consists of two different types. One is the shredding of the oospore-bearing leaves, and the other is the peculiar deformity of the oospore-bearing spikes.

a. Shredded Leaves

As has been already stated, the terminal leaves of a mature plant infected systemically become decidedly chlorotic in appearance. An abundant oospore production takes place later in the affected tissues of these albicated leaves. Before the formation of oospores, the conidia are very often produced on these leaves, but at the time of oospore formation, the production of conidia almost ceases. When the oospores are mature, these leaves become brownish in color, and later disintegrated or longitudinally shredded, leaving the fibrous elements in a whisk-like appearance, which may further be tangled. In this way, the oospores are freed to disseminate. Rain and wind seem to hasten this shredding, because it does not take place so markedly in the green house as in the field.

Oospores are also formed in the tissues of the partially albicated areas of the affected leaves near the top of the plant, but as a rule they are not formed in the tissues of the chlorotic portions of the lower leaves which stand long without becoming shredded.

So far as the present investigations have been able to determine, all the plants giving rise to the shredded leaves mentioned above, grow from the seedlings infected by the hyphae from oospores. However, it has been frequently observed under the microscope that conidia are carried by small insects, such as aphides, or thrips which are

found on the albicated terminal leaves, even in the still folded young ones. It seems possible that conidia are carried in this way on young leaves near the growing point. If infection takes place there, a systemic type of infection might be induced. MELHUS and his associates (72) state: "However, the function of the conidia must not be under-estimated because systemic infection of *Setaria viridis* and *S. italica* has been produced by conidial exposure of plants growing in sterile soil. These infected plants have later produced oospores in the typical manner, both upon *S. viridis* and *S. italica*." This statement is interesting, but further extensive investigations are desirable along this line.

b. Deformed Spikes

The deformed spikes are first purplish-green in color, later becoming yellowish-brown, when oospores are formed in the tissues. Giant conidia may be produced on some of these deformed spikes, especially in their early stage of development.

The deformity of the inflorescence may result in entire or partial sterility. Consequently, there occur various kinds of deformed spikes, which, however, may be classified into two main types. The details of the classification are as follows:

I. Main axes of the spike are normal
   1. Spikelet-axes are mostly transformed into leafy structures.
   2. Spikelet-axes are recognizable; primary branches are mostly transformed into clusters of leafy structures.
   3. Spikelet-axes and primary branches are recognizable; secondary branches are mostly transformed into leafy structures.
   4. Floral axes and florets are mostly transformed.
   5. Only florets are transformed.

II. Main axes of the spike are greatly, or somewhat hypertrophied, and suppressed.
   1. Spikelet-axes are chiefly transformed into large, prolonged leafy structures.
   2. Spikelet-axes are hypertrophied, and primary branches are transformed into leafy structures, or spines.
   3. Spikelet-axes, and primary branches are hypertrophied, secondary branches are transformed into leafy structures, or spines.

Two or more kinds of these modifications are usually found in the same deformed spikes.

The tissues of these deformed spikes contain abundant oospores. Most of the leafy structures remain until the time of harvesting, notwithstanding the maturation of oospores, without becoming shredded, differing greatly from the case of oospore-bearing albicated top leaves.

It is noteworthy that many of the deformed spikes are formed on apparently healthy plants showing no chlorotic leaves. According to the examination made in 1930 in the fields at Miyata, 44 of 46 plants bearing deformed spikes were found to be apparently healthy with no chlorotic leaf, while only the remainder showed other symptoms of the disease, giving rise to chlorotic leaves on which the conidia were produced.

Whether the deformed spikes develop on the plants systemically infected by oospores at their seedling stage, or on those infected by conidia secondarily, remains to be determined. But, both cases are of course possible from the theoretical point of view.

Part I. Mycological Investigations

I. MORPHOLOGY OF THE CAUSAL FUNGUS

(I) Descriptive Notes on the Causal Fungus

The Mycelium

The mycelium is non-septate, hyaline, mostly thin-walled and develops intercellularly. The haustoria develop from the intercellular mycelia and invade the attached host cells through the cell wall. They are simple, globose to tubular, or often lobed in shape.

The hyphae located in the mesocotyl of the young seedling, or in the tissues of the growing point can be detected by means of dissection, or simply by pressing the materials under cover glass. However, the hyphae developed in the tissues of chlorotic leaves of the mature plants are generally very inconspicuous even in hand sections, since their wall is particularly thin. Those in the culm may be seen by treating the materials in the 20% KOH solution. The materials are heated in the solution until they boil, then removed on a slide, and macerated by pressing them gently with a needle.

The mycelium develops sparsely in the culm-tissue, while it grows well in the tissues of terminal growing points, spikes and leaves. The hyphae found in the culm are generally slender, with few branches, while those in the leaves are lobed, gnarled, and contorted.

Attempts to trace the mycelium in the culm of the affected plant, have revealed that the hyphae are not found in the old parts of the culm, although they are always found in the mesocotyl of the affected seedlings. The results obtained from several plants showing albicated terminal leaves, about 25 to 35 cm. in height, indicate that the hyphae are found in the tissues of the first to the third internodes from the top of the plants, but not in older lower internodes.

The Conidiophore

When affected leaves are kept under certain favorable conditions, the mycelial branches located in the stomatal cavity grow out through the stomatal slit, resulting in a compact aggregate of knob-like structures. These are conidiophore-initials, from which club-shaped hyphae or young conidiophores are developed. With further elongation, they produce two or more primary branches. Each primary branch, including the tip of the main axis, buds out secondary branches which may develop into tertiary even further. The tips of the branches are crowned with characteristic sterigmata, on which conidia are formed. Plate XIII, Fig. 2, a, shows one of the full-grown conidiophores. Although there are various types of branch systems, Fig. 2, a, represents the most common type. Most of the conidiophores give rise to two side-branches on the main axis. Further branches are formed almost dichotomously.

There is a great variation in the size of the conidiophore. Mature conidiophores produced under favorable conditions are from 210 to 410 microns, commonly 260 to 350 microns in length. The width of the main axis, just below the first side-branch is 18 to 30 microns, mostly 20 to 26 microns.

The shape and size of the conidiophore are greatly influenced by air humidity and temperature. Low humidity and low temperature make the conidiophore shorter, resulting in a stout appearance.

The sterigmata are usually 5 to 13 microns long, having a peg-like appearance with a broad base. Some are rather slender, while

others are more or less stout, depending upon the conditions under which they are produced. The conidiophore shrivels up very soon after the discharge of the conidia.

The Conidium

The conidia are produced as swollen protrusions from the tips of the sterigmata. The mature conidium is hyaline, unicellular, spherical to ellipsoidal, mostly broad-ellipsoidal, with distinct papillae. The size and shape of the conidia vary with temperature and humidity, as well as with the physiological conditions of the host.

Besides these ordinary conidia, giant ones are often found, being mixed among the ordinary conidia in the later growing season of the Italian millet. The giant conidia are also produced on entirely chlorotic terminal leaves as well as on young deformed spikes.

The ordinary conidia produced on well-grown albicated leaves on a dewy mid-summer night are 16 to 32 microns, mostly 20 to 24 microns in length, and 12 to 23 microns, mostly 16 to 19 microns in width. The giant conida are oblong to ellipsoid-cylindrical in shape; 30 to 67 microns, mostly 30 to 40 microns in length, and 19 to 34 microns, mostly 20 to 24 microns in width.

The Oospore

The process of oospore-development has been described by SCHROETER (98), SHIRAI (101), BUTLER (11) and STEVENS (103).

The mature oospores are covered with an oogonial wall of amber to reddish brown in color. The oospore-wall itself is smooth and colorless, or slightly tinged with a yellowish color. The shape of the whole oospore body is generally somewhat spherically polyhedral, while the oospore proper is in most cases spherical.

In the course of the present investigations the writer has found that the diameter of the oospore varies greatly with different materials. The detailed measurements of the oospores obtained from various localities, and from different hosts will be given in a later chapter.

(II) Influence of Environmental factors upon the Size and Shape of the Causal Fungus

It was noticed in the course of the morphological studies of the fungus that the size of the conidia and conidiophores is remarkably variable. Since the size and shape of the conidia and conidiophores are very important from the taxonomical stand-point, it is desirable to determine to what extent environmental factors influence the morphology of the conidia and conidiophores.

Methods

The methods used in these studies consisted simply in measuring and comparing the dimensions of the conidia or conidiophores produced under natural as well as controlled conditions.

At the beginning of these studies, mature conidia were collected in such a manner as carefully to bring the conidia-bearing material in contact lightly with the surface of a water drop on a slide. In this way, mature conidia were readily caught by the water drop because of their easy abscission from the sterigmata. Later, however, a convenient method of catching mature conidia was devised. A piece of an affected leaf was placed on a slide which had been previously smeared with egg albumen and placed in a moist Petri-dish. The cut-piece of leaf was placed on the slide with its conidia-bearing surface on the albumen layer, the direct contact of the conidia with the albumen being avoided, taking advantage of the curvature of the leaf.

In this way the discharged conidia were caught on the albumen layer. If the slide is very slightly smeared with albumen, the discharged conidia remain for ten hours or more without germinating. This method was found to be most ideal.

The conidia of the fungus in question are liable to shrivel as soon as the dew evaporates. Therefore, when the conidia produced under natural conditions were to be measured, they were collected early in the morning, while dew was still present.

Care was also taken to measure the conidia from different parts of the same material in order that representative dimensions might be obtained. As a rule, fifty conidia were measured from one slide, and the results from four or more slides were added to establish a

frequency distribution. Sometimes, the measurements of the first fifty spores seemed to be sufficient for the purpose of classification, because the results of the second, third and fourth sets, respectively, were found to coincide with those of the first fifty spores. In most cases, the measurement of 200 spores was found sufficient to ascertain the extent of the variation of spore-size, although more than 200 conidia were usually measured in the present studies.

1. Studies on the Conidia produced in Nature

In order to learn whether the dimensions of the conidia are variable at different periods of the season, some measurements were made at certain intervals during the growing season of the Italian millet. The results obtained are summarized in the following table.

TABLE 1

Frequency Distribution for the Dimensions of Conidia of the Sclerospora on Italian millet

Series 1

Date	Length		Width	
	Class (μ)	Number	Class (μ)	Number
June 8, 1928	14	19	12	66
	16	120	14	150
	18	102	16	84
	20	53	18	9
	22	14	20	2
	24	3		
	(Total)	312		312
July 4, 1928	18	10	12	1
	20	62	14	2
	22	67	16	67
	24	40	18	103
	26	18	20	26
	28	3	22	1
	(Total)	200		200

TABLE 1 (*Continued*)

Date	Length		Width	
	Class (μ)	Number	Class (μ)	Number
July 30, 1928	18	1	16	39
	20	17	18	91
	22	43	20	92
	24	89	22	21
	26	82	24	1
	28	10		
	30	1		
	32	1		
	(Total)	244		244
Series 2				
May 18, 1929	12	2	10	1
	14	93	12	148
	16	175	14	150
	18	35	16	25
	20	20	18	2
	22	1		
	(Total)	326		326
July 19, 1929	14	2	12	3
	16	8	14	38
	18	48	16	227
	20	186	18	80
	22	66	20	6
	24	30	22	2
	26	15		
	28	1		
	(Total)	356		356

The conidia measured on June 8, 1928, and on May 18, 1929, respectively, were taken from seedlings, while the remainder were obtained from more fully grown plants. It is evident from the data shown in the table that the dimensions of the conidia greatly increase, when the season advances and the plants become larger. These results coincide mainly with TASUGI's findings (105) which have been recently published.

2. Studies on the Conidia produced under Controlled Conditions

In order to determine the main environmental factors influencing the dimensions of the conidia, several sets of experiments were carried out.

(1) Influence of Temperature upon the Dimensions of Conidia

Conidia-bearing leaves cut off from the plants grown in the field or in the green house were thoroughly washed in running water to remove the original sporulation, and then they were cut into a number of small pieces. These pieces were placed in moist Petri-dishes which were subsequently kept in the incubators held at known temperatures. Measurements were made of fresh conidia newly produced. Care was given to measuring and comparing the dimensions of the conidia produced on the pieces from the same leaf, since preliminary tests suggested that the dimensions of the conidia might vary to some extent with different plants grown even under the same conditions.

Experiment 1

Leaves were obtained from about two months-old, well-grown plants. The results are shown in the following table.

TABLE 2

Frequency Distribution for the Dimensions of 450 Conidia produced at Different Temperatures (Experiment 1)

Temp. C.	Length		Width	
	Class (μ)	Number	Class (μ)	Number
13°	12	6	12	70
	14	70	14	239
	16	134	16	131
	18	140	18	9
	20	80	20	1
	22	15		
	24	5		
	(Mean)* : 17.29 μ in length ; 14.36 μ in width (Ratio)** : 1.204			

* Arithmetic mean.
** The ratio of length to width.

TABLE 2 (*Continued*)

Temp. C.	Length		Width	
	Class (μ)	Number	Class (μ)	Number
16°	14	10	12	72
	16	138	14	205
	18	152	16	155
	20	111	18	16
	22	35	20	2
	24	4		
	(Mean): 18.16 μ in length; 14.54 μ in width (Ratio): 1.249			
24°	18	17	14	15
	20	81	16	95
	22	122	18	180
	24	154	20	138
	26	74	22	22
	28	1		
	30	1		
	(Mean): 22.86 μ in length; 18.25 μ in width (Ratio): 1.253			

The results shown in the above table indicate that the dimensions of the conidia are decidedly influenced by temperature, greatly increasing when temperature rises.

Experiment 2

This is essentially a repetition of Experiment 1. The results are shown in the following table.

TABLE 3

Frequency Distribution for the Dimensions of 380 Conidia produced at Different Temperatures (Experiment 2)

Temp. C.	Length		Width	
	Class (μ)	Number	Class (μ)	Number
13°	14	31	12	35
	16	132	14	187
	18	160	16	152
	20	43	18	6
	22	14		
	(Mean): 17.35 μ in length; 14.68 μ in width (Ratio): 1.182			

TABLE 3 (*Continued*)

Temp. C.	Length		Width	
	Class (μ)	Number	Class (μ)	Number
24°	18	38	14	22
	20	62	16	88
	22	110	18	215
	24	97	20	47
	26	55	22	8
	28	18		
	(Mean): 22.65 μ in length ; 17.64 μ in width			
	(Ratio): 1.284			

These results agree well with those of the foregoing experiment, showing that the dimensions of the conidia are much larger at the higher temperature.

Experiment 3

Another experiment was performed at Madison, Wisconsin, U. S. A. The diseased leaves were obtained from a plant over 65 cm. in height. The results are shown in TABLE 4.

TABLE 4

Frequency Distribution for the Dimensions of 360 Conidia produced at Different Temperatures (Experiment 3)

Temp. C.	Length		Width	
	Class (μ)	Number	Class (μ)	Number
13°	14	14	12	18
	16	141	14	196
	18	158	16	143
	20	42	18	3
	22	5		
	(Mean): 17.35 μ in length ; 14.73 μ in width			
	(Ratio): 1.178			

TABLE 4 (*Continued*)

Temp. C.	Length		Width	
	Class (μ)	Number	Class (μ)	Number
16°	14	4	12	2
	16	27	14	144
	18	145	16	176
	20	128	18	37
	22	48	20	1
	24	8		
	(Mean): 19.18 μ in length; 15.39 μ in width			
	(Ratio): 1.246			
18°	16	11	12	6
	18	122	14	142
	20	157	16	152
	22	53	18	57
	24	17	20	3
	(Mean): 19.68 μ in length; 15.49 μ in width			
	(Ratio): 1.270			
24°	16	12	14	22
	18	15	16	165
	20	88	18	133
	22	120	20	40
	24	60		
	26	32		
	28	15		
	30	18		
	(Mean): 22.48 μ in length; 17.06 μ in width			
	(Ratio): 1.318			

The results show that the dimensions of the conidia gradually increase as temperature rises.

The ratio of length to width slightly increases; in other words, the conidia become more elongate as temperature rises.

In the course of Experiment 4, some conidiophores were measured. The size of the conidiophores also increases at higher temperatures; for example, at 12° C.: 230–380 μ, mostly about 220 μ in length and 18–28 μ, mostly about 23 μ in the diameter of the main axis; at 18° C.: 280–430 μ, mostly about 360 μ in length and 20–30 μ,

mostly about 24 μ in diameter; at 25° C.: 300–500 μ, mostly about 400 μ in length and 20–30 μ, mostly about 24 μ in diameter.

(2) Influence of Air Humidity upon the Dimensions of Conidia and Conidiophores

During the investigation on the effect of air humidity upon the production of conidia, it was found that those produced under a certain dryer condition showed a less vigorous appearance than those formed in saturated atmosphere. Microscopic examination verified that the conidiophores of the former were markedly shorter than those of the latter.

In order to obtain more exact information on the effect of air humidity upon the conidiophores as well as the conidia of the fungus, a series of experiment was carried out. The methods of controlling air humidity are given in a later chapter.

Experiment 1

A diseased plant, about 65 cm. tall, growing in a pot, was exposed to a known atmospheric humidity* until the conidia were newly produced. After some of these fructifications were collected for measurement, the diseased leaves in question were wiped with absorbent cotton to clean their surface, and the whole plant was kept in a saturated condition until fresh conidia were again produced. The temperature was maintained at 20° C. throughout the experiment. The results are shown in the following table.

TABLE 5

Frequency Distribution for the Length of 50 Conidiophores produced under both Dry and Saturated Conditions

Relative humidity, 80–84%, 20°C.		Saturated, 20°C.	
Class (μ)	Number	Class (μ)	Number
141–170	5	201–230	2
171–200	13	231–260	5
201–230	20	261–290	8
231–260	8	291–320	17
261–290	4	321–350	12
		351–380	4
		381–410	2

* See the methods of air humidity control given on page 42–43.

TABLE 6

Frequency Distribution for the Dimensions of 440 Conidia produced under both Dry and Saturated Conditions

Relative humidity, 80–84%, 20°C.			
Length		Width	
Class (μ)	Number	Class (μ)	Number
14	27	12	9
16	96	14	172
18	142	16	178
20	155	18	81
22	20		
(Mean): 18.20 μ in length ; 15.50 μ in width (Ratio): 1.174			
Saturated, 20°C.			
16	7	12	8
18	84	14	20
20	210	16	206
22	105	18	173
24	32	20	33
26	2		
(Mean): 20.35 μ in length ; 16.92 μ in width (Ratio): 1.203			

It is self-evident from the results shown in TABLE 5 that the length of the conidiophores under the dry condition is decidedly shorter than that of the conidiophores under the saturated condition. The diameter of the main axis under the dry condition was found to be a little smaller than that formed under the saturated condition, namely, in the former, it ranged from 18 to 26 μ, mostly 20 to 24 μ, while in the latter, it ranged from 20 to 28 μ, mostly 22 to 26 μ. Consequently, the conidiophore of the former showed a stouter appearance in comparison with that of the latter.

The results shown in TABLE 6 indicate that the dimensions of the conidia become slightly smaller, when the atmospheric moisture goes down.

Experiment 2

A potted plant with several chlorotic leaves, about 25 cm. in height was used in this experiment. A negligibly small amount of

conidia was found on some of the chlorotic leaves. Each upper half of three chlorotic leaves on this plant was cut off and put separately in three saturated Petri-dishes. Then, they were placed in the incubator of constant humidity and temperature together with the potted plant bearing the basal half of the chlorotic leaves. In this way, each half of the same leaf was exposed to different atmospheric conditions, and the conidia produced were compared. The results of the measurements are shown in TABLE 7.

TABLE 7

Frequency Distribution for the Dimensions of 300 Conidia produced under both Dry and Saturated Conditions

Relative humidity, 84–79%*, 18°C.			
Length		Width	
Class (μ)	Number	Class (μ)	Number
13	1	10	4
14	4	11	20
15	38	12	53
16	44	13	110
17	62	14	91
18	78	15	22
19	35		
20	26		
21	6		
22	4		
23	2		
(Mean): 17.49 μ in length ; 13.10 μ in width (Ratio): 1.335			
Saturated, 18°C.			
14	1	11	2
15	5	12	35
16	8	13	109
17	29	14	127
18	78	15	27
19	96		
20	57		
21	16		
22	6		
23	4		
(Mean): 18.79 μ in length ; 13.47 μ in width (Ratio): 1.395			

* The methods used for the control of air humidity were different from those in Experiment 1. The detailed description is given on page 46.

Experiment 3

This is practically a repetition of Experiment 2, with the exception of different materials and different air humidities. The results obtained are shown in TABLE 8.

TABLE 8

Frequency Distribution for the Dimensions of 400 Conidia produced under both Dry and Saturated Conditions

Length		Width	
Class (μ)	Number	Class (μ)	Number
Relative humidity, 85–80%, 18°C.			
12	2	10	8
13	5	11	26
14	19	12	154
15	98	13	155
16	130	14	43
17	85	15	13
18	36	16	1
19	18		
20	3		
21	2		
22	2		
(Mean): 16.22 μ in length; 12.61 μ in width (Ratio): 1.286			
Saturated, 18°C.			
14	12	11	16
15	35	12	59
16	64	13	141
17	85	14	144
18	98	15	36
19	67	16	3
20	31	17	1
21	6		
22	1		
(Mean): 17.48 μ in length; 13.35 μ in width (Ratio): 1.309			

The results of Experiments 2 and 3 substantiate those of Experiment 1, demonstrating that the dimensions of the conidia decrease, though not very markedly, when atmospheric humidity becomes low to a certain extent. It is noticeable that the dimensions of the conidia produced under saturated conditions in Experiment 3 are somewhat smaller than those obtained in Experiment 2 (See TABLE 7, Saturated, 18° C.).

(3) Relation of the Growing Stages of the Host Plant to the Dimensions of Conidia

In the beginning of these morphological studies, attention was drawn to the fact that the conidia produced on seedlings are generally smaller than those on more grown plants. It was also found, later, that most of the conidia produced on leafy structures due to the metamorphosis of affected spikes are extraordinarily large. These facts are clearly demonstrated in the following tables.

TABLE 9

Frequency Distribution for the Dimensions of the Conidia produced on Young Seedling

Date	Age	Length		Width	
		Class (μ)	Number	Class (μ)	Number
May 25, 1930 Lincoln, Nebraska	10 days after sowing	12	13	10	6
		14	78	12	133
		16	101	14	115
		18	90	16	62
		20	45	18	14
		22	3		
		(Total)	330		330
		(Mean): 16.52 μ in length; 13.67 μ in width (Ratio): 1.208			
Oct. 14, 1930 Madison, Wis.	14 days after sowing	12	16	10	12
		14	42	12	112
		16	88	14	65
		18	51	16	21
		20	10		
		22	3		
		(Total)	210		210
		(Mean): 16.06 μ in length; 12.90 μ in width (Ratio): 1.245			

TABLE 10

Frequency Distribution for the Dimensions of 300 Conidia produced on Metamorphosed Leafy Structures

Length		Width	
Class (μ)	Number	Class (μ)	Number
24	1	16	1
26	1	18	10
28	10	20	54
30	30	22	90
32	39	24	81
34	48	26	38
36	48	28	18
38	36	30	5
40	28	32	2
42	15	34	1
44	15		
46	12		
48	6		
50	3		
52	3		
54	1		
56	1		
58	1		
60	1		
66	1		

(Mean): 36.85 μ in length ; 23.13 μ in width
(Ratio): 1.593

The results shown in TABLE 9 coincide mainly with the data presented in TABLE 1, indicating that the dimensions of the conidia produced on affected seedlings are particularly smaller than those produced on grown plants in other previous experiments.

The results shown in TABLE 10 clearly indicate that the dimensions of the conidia formed on the metamorphosed leafy structures are especially large. It is apparently difficult to be understood that these phenomena were caused only by the influence of temperature or moisture. Therefore, some experiments were performed in order to obtain more exact information on this subject.

Experiments were first attempted to compare the dimensions of the conidia formed on both seedlings and mature plants growing under the same environmental conditions. For this purpose, the conidia produced on plants of different ages, arising from the seeds sown on different days were measured. Two sets of measurements were made. The results are shown in the following table.

TABLE 11

Comparison of the Dimensions of the Conidia on both Seedlings and Mature Plants

Set I

Data of Measurements	On the seedlings sown on July 25			
	Length		Width	
	Class (μ)	Number	Class (μ)	Number
Aug. 4, 1931	12	1	9	1
	13	6	10	15
	14	14	11	31
	15	23	12	53
	16	46	13	35
	17	35	14	9
	18	14	15	6
	19	8		
	20	2		
	21	1		
	(Total)	150		150
	(Mean): 16.18 μ in length ; 12.05 μ in width (Ratio): 1.343			
	On the plants sown on April 19			
	16	1	11	1
	17	3	12	1
	18	7	13	7
	19	11	14	22
	20	20	15	42
	21	29	16	24
	22	22	17	19
	23	19	18	13
	24	12	19	7
	25	4	20	10
	26	4	21	3
	27	4	22	1
	28	2		
	29	1		
	30	2		
	31	4		
	33	2		
	35	1		
	36	1		
	41	1		
	(Total)	150		150
	(Mean): 22.53 μ in length ; 16.07 μ in width (Ratio): 1.402			

TABLE 11 (*Continued*)

Set II

Date of measurements	On the seedlings sown on Aug. 1			
	Length		Width	
	Class (μ)	Number	Class (μ)	Number
Aug. 10, 1931	12	1	9	1
	13	10	10	23
	14	22	11	60
	15	38	12	76
	16	69	13	66
	17	42	14	17
	18	30	15	13
	19	21	16	3
	20	15	17	1
	21	8		
	22	2		
	23	2		
	(Total)	260		260
	(Mean): 16.67 μ in length; 12.18 μ in width (Ratio): 1.369			
	On the plants sown on April 9			
	20	16	14	11
	21	21	15	37
	22	43	16	55
	23	46	17	72
	24	44	18	43
	25	33	19	28
	26	27	20	22
	27	15	21	2
	28	9		
	29	4		
	30	1		
	31	1		
	(Total)	260		260
	(Mean): 23.78 μ in length; 17.70 μ in width (Ratio): 1.344			

These results clearly indicate that the dimensions of the conidia vary with different growing stages of the host plant, that is, the conidia produced on seedlings are smaller than those on more grown plants.

As shown in the above table, the conidia produced on the plants sown on April 19 were measured twice, namely on August 4, and on August 10. The conidia obtained on August 4, are smaller in size than those obtained on August 10. This might be explained partly by the difference in the temperature in the night of both days, when the conidia were produced, since the minimum temperature was 22° C. on August 4, and 24° C. on August 10. The difference in the growing stage of the host plant, however, might also have some relation to this problem.

Late in July, 1932, giant conidia were found on terminal albicated leaves of diseased plants. These leaves were cut off and washed in running water to get rid of the original conidia. They were placed in moist Petri-dishes, and kept at 18° C. and 24° C. in the incubators. The dimensions of the conidia newly produced were found to be as follows:

TABLE 12

Frequency Distribution for the Dimensions of the Conidia produced on Albicated Leaves at 18° C.

Length		Width	
Class (μ)	Number	Class (μ)	Number
19	2	15	2
20	5	16	11
21	13	17	28
22	18	18	53
23	20	19	42
24	23	20	40
25	41	21	15
26	37	22	8
27	19	23	1
28	11		
29	8		
30	3		
(Total)	200		200

(Mean): 24.75 μ in length ; 18.74 μ in width

(Ratio): 1.321

TABLE 13

Frequency Distribution for the Dimensions of the Conidia produced on Albicated Leaves at 24° C.

Length		Width	
Class (μ)	Number	Class (μ)	Number
19	2	15	1
20	2	16	8
21	8	17	27
22	7	18	42
23	11	19	58
24	13	20	45
25	18	21	33
26	31	22	18
27	45	23	12
28	48	24	4
29	35	25	2
30	21		
31	5		
32	1		
33	2		
34	1		
(Total)	250		250

(Mean): 26.85 μ in length ; 19.48 μ in width

(Ratio): 1.378

The results shown in TABLE 12 indicate that the conidia produced on albicated leaves are much larger than any other conidia previously obtained at temperatures below 20° C. The dimensions of the conidia produced at 24° C. in the present experiment, which are shown in TABLE 13 are also found to be much larger than any of those obtained in the previous experiments. It is evident that the increase of the dimensions of the conidia produced on the albicated leaves is not caused only by the influence of temperature or moisture, but it should be ascribed to some abnormal metabolic conditions of the heavily affected leaves.

(4) Conclusions concerning the Influence of Environmental Factors upon the Size and Shape of the Causal Fungus

1. The dimensions of the conidia greatly increase, when the growing season of Italian millet advances and the plants become larger.

2. The size of the conidia as well as of the conidiophores increases as temperature rises, and the conidia become more elongate at higher temperatures.

3. The length of the conidiophores produced under a dry condition is decidedly shorter than that of the conidiophores under a saturated condition, and the dimensions of the conidia become slightly smaller, when atmospheric moisture goes down.

4. The conidia produced on seedlings are generally smaller than those on more grown plants.

5. The conidia formed on metamorphosed leafy structures are generally very large.

6. Seasonal variation in the size and shape of the conidia and conidiophores is mainly caused by temperature, atmospheric moisture, and some physiological conditions of the host plant.

II. PHYSIOLOGY OF THE CAUSAL FUNGUS

(I) Production of the Conidia

In order to learn the relation of environment to the production of the conidia, some experiments were carried out.

1. Influence of Temperature upon the Production of Conidia

a. Cardinal Temperatures for the Production of Conidia

Methods

Conidia-bearing leaves of *Setaria italica* were collected in the field, and immediately brought to the laboratory. They were washed with running water until the original fructification was removed entirely, and excess water was shaken off. Then, they were cut into pieces of proper length, and put into Petri-dishes moistened by

means of wet filter paper, which were incubated at different temperatures.

Special attention was paid to obtain the material in the evening of a fine day, since preliminary tests proved that affected leaves collected after continued rain were inappropriate as material for the present experiment. Each piece of the affected leaves was placed in Petri-dishes with its under surface to the moist bottom of the Petri-dish.

Experimental Results

The results obtained are shown in the following table :

TABLE 14

Influence of Temperature upon the Production of Conidia

Experiment 1 (July 3, '28)

Temperature C.	No. of diseased leaves			
	1	2	3	4
6°— 7°	0	0	0	0
9°—10°	++	+	+	+
17°—18°	++++++	+++++	+++++	+++++
25°—26°	++	++	++	++
Remarks	(1) The record was taken after 12 hours (2) The sign + indicates the amount of conidia			

Experiment 2 (July 6, '28)

Temperature C.	No. of diseased leaves			
	1	2	3	4
6°— 7°	0	0	0	0
9°—10°	+	+	+	+
17°—18°	+++++	+++++	+++++	+++++
20°—21°	+++++	++++	+++++	++++
25°—26°	++	++	++	+
Remarks	(1) The record was taken after 20 hours			

Experiment 3 (July 7, '28)

Temperature C.	No. of diseased leaves		
	1	2	3
6°— 7°	0	0	0
9°—10°	Trace	+	+
17°—18°	++++	++++	++++
20°—21°	++++	++++	++++
25°—26°	+	++	++
Remarks	(1) The record was taken after 30 hours		

Experiment 4 (July 8, '28)

Temperature C.	No. of diseased leaves				
	1	2	3	4	5
6°— 7°	0	0	0	0	0
9°—10°	++	+	+	++	+
17°—18°	++++++	++++++	+++++	++++++	++++++
20°—21°	++++++	+++++	+++++	++++++	+++++
25°—26°	+++	++	+	++	++
Remarks	(1) The record was taken after 15 hours				

Experiment 5 (July 24, '29)

Temperature C.	No. of diseased leaves		
	1	2	3
9°—10°	+	+	+
14°—15°	+++++	+++++	+++++
17°—18°	+++++	++++++	++++++
19°—20°	+++++	+++++	+++++
24°—25°	+	+	++
27°—28°	0	0	0
Remarks	(1) The record was taked after 10 hours		

Experiment 6 (July 27, '29)

Temperature C.	No. of diseased leaves			
	1	2	3	4
9°—10°	++	+	+	+
14°—15°	+++++	++++	++++	++++
17°—18°	++++++	+++++	+++++	+++++
19°—20°	+++++	++++	++++	++++
24°—25°	++	++	+	+
27°—28°	0	0	0	0
Remarks	(1) The record was taken after 8 hours			

The results of the experiments shown in TABLE 14 indicate that the conidia of the causal fungus can be produced between 10° and 25° C., and the optimum temperature for the production of conidia is about 17°–18° C., while they are abundantly produced between 15° and 20° C.

b. Time required for the Production of Conidia

The purpose of this experiment was to determine the minimum time for the production of conidia at various temperatures. The same methods as in the preceding experiments were used except that in each test, three or four Petri-dishes were kept at each temperature. The results are summarized in the following table.

TABLE 15

Minimum Time required for the Production of Conidia at Different Temperatures

Temp. C.	Time in hours						
	No. of tests	1	2	3	4	5	6
10°		12	10	8	10	12	12
15°		7	6.5	5	5.5	6	8
18°		6	6	4.5	4.5	5	6
20°		6	6	4.5	4.5	5	6.5
23°		—	6	4.5	—	5	—
25°		6	6	5	5.5	6	6.5

The results indicate that the minimum time required for the production of conidia is different in different materials, but as a whole, it is the shortest at 18°–20° C., while slightly longer at temperatures higher than this, and very much longer at temperatures lower than this. At 25° C., the conidia are sparsely produced, and therefore, the time shown in the table may be longer than the actual time, since the sign of sparse sporulation is recognized with difficulty in its early stage.

WESTON (119) reported that *Sclerospora philippinensis,* and *S. spontanea* sporulate within 5 to 7 hours under favorable condi-

tions. MELHUS and his co-workers (72) found that the Sclerospora fungus from *Setaria viridis* sporulates within 4.5 to 8 hours. ARENS (3, 4) reported that it takes 5 days at 9°–10° C. or one day at 12°–13° C. for the formation of the conidiophores of *Plasmopara viticola;* and two days at 5°–6° C. or one day at 7°–8° C. for the production of the conidiophores of *Pseudoperonospora Humuli.*

2. Influence of Light upon the Production of Conidia

MIYAKE (74) who studied *Sclerospora Sacchari* MIY. found that the production of conidia took place at night, and never in the daytime. In the investigation of the Philippine Sclerosporas, WESTON (119) came to the conclusion: "It can be said that the formation of conidiophores in both species of Philippine Sclerosporas —whether on maize, teosinte, sugar cane, sorghum, *Saccharum spontaneum,* or *Miscanthus japonicus* ANDERS.—never takes place except when the surface of the host is covered with a persistent layer of moisture during the night or very early morning."

WESTON (120) found that the production of conidia was also nocturnal in the case of *Sclerospora graminicola* (SACC.) SCHROET. parasitic on *Setaria viridis.* He emphasized the importance of the nocturnal production of conidia in the genus Sclerospora.

Later, MELHUS and his associates (72) found the daytime production of conidia in *Sclerospora graminicola* parasitic on *Setaria viridis,* a finding contrary to WESTON'S conclusion. It seemed desirable, therefore, to determine which of the two conclusions is true in *Sclerospora graminicola* on *Setaria italica.* In this connection, the following experiments were performed.

On July 8, 1928, conidia-bearing leaves were cut and washed in just the same manner as in the foregoing experiments. Excess water was shaken off. Then the leaves were placed in moist Petri-dishes which were kept in the laboratory in a large glass-ware ordinarily used as desiccator. Moist filter paper was placed on the bottom of the Petri-dishes, and the upper parts of them were merely atomized with distilled water. The temperature of the glass-ware was maintained from 19° to 24° C. by means of ice. Under these circumstances, abundant conidia were produced on the cut-leaves in the daytime. The same experiment was later repeated, but in all cases, the results were positive. On July 24, 1929, when the writer was

studying at Berkeley, California, U.S.A., a number of diseased leaves of Italian millet were cut, washed, and kept in moist Petri-dishes, which were placed on a table in the laboratory where the temperature was ranging from 18° to 19° C. Five hours later, abundant conidia were found, which were newly produced in the daytime.

Observations in field as well as in green house have also revealed that on cool cloudy days, conidia are sometimes produced in the daytime.

These results coincide well with those reported by MELHUS and his associates. The writer suspects that the same might be the case with the tropical Sclerosporas.

3. *Influence of Air Humidity upon the Production of Conidia*

There is no doubt about the fact that dew or other moisture is indispensable for the production of conidia, in other words, the saturated moisture is favorable for the production of conidia. However, the relation of air humidity to the production of the conidia in any case of Sclerospora has not been studied in detail. In order to obtain more exact information along this line, the following experiments were conducted preliminarily.

Methods of Experimentation

A simple incubator of constant temperature and moisture was designed with a large wooden case. The temperature and humidity within this case were controlled by means of a mercury regulator, electric lamp, and relay. This incubator was placed in the cellar where the temperature was almost constant during the experimental period. A layer of moist sphagnum was placed over the bottom of the incubator. The electric lamp was partially covered with moist sphagnum in a pan placed in the incubator. By changing the amount of water in sphagnum, on the one hand, and also by using different kinds of electric lamps varying in power, on the other hand, the desired air humidity in the incubator was obtained. A recording hygrometer carefully adjusted was supported at a point higher than the middle of the incubator. Preliminary tests showed that the air humidity in this incubator was not perfectly uniform. Therefore,

special care was taken to secure data from only the diseased leaves lying near the hygrometer. The temperature inside of the incubator was maintained at about 18° C., the optimum temperature for the production of conidia.

Experiment 1 (Oct. 17 '30)

Two plants, about two months old, in a large pot, were used in this experiment. All the original sporulations were wiped off with absorbent cotton. The pot was placed on the bottom of the incubator. The soil of the pot was more or less dry. Each plant had several diseased leaves. But the data were taken from two or three diseased leaves lying near the hygrometer. The results are shown in TABLE 16.

TABLE 16

Effect of Air Humidity upon the Production of Conidia
(Experiment 1)

Temp.: 18° C.; Air humidity: 88%; Experimental time: 8 hours.

Plant no.	Diseased leaves tested	Amount of the conidia produced	Approximate proportion of the conidia-producing areas to the total chlorotic areas
I	(a)	Abundant	1/1
	(b)	Abundant	1/1
II	(a)	Abundant	3/4
	(b)	Ditto	1/1
	(c)	Ditto	1/1

The leaf (a) of plant no. II was situated lowest among the three. The production of the conidia on this leaf was found to be poorer toward the end than on the basal part of the leaf. It is unknown whether this may be explained by the partial difference in air humidity or by some other reason. Anyhow, the results indicate that the air humidity of 88% at 18° C. is sufficient for the production of conidia, if the other conditions are favorable.

Experiment 2 (Oct. 19, '30)

Two plants, about 50 cm. and 82 cm. tall, respectively, were used. The soil of the pot appeared dry. The methods were essentially the same as in the preceding experiment.

TABLE 17

Effect of Air Humidity upon the Production of Conidia
(Experiment 2)

Temp.: 18° C.; Air humidity: 84–86%; Experimental time: 12 hours.

Plant no.	Diseased leaves	Amount of the conidia produced	Approximate proportion of the conidia-bearing areas to the total chlorotic areas
I	a	Fairly abundant	1/8
	b	Fairly abundant	1/3
II	a	Fairly abundant	3/4
	b	Fairly abundant	1/3

The results indicated in the table show clearly that conidia are produced in the air humidity of 84–86 per cent at 18° C., although the amount produced is considerably reduced. It was ascertained in this experiment that vigor of the production of conidia is different not only in different leaves, but also in different portions of the same leaf, since conidia were not uniformly produced on the chlorotic area where, in all probability, the air humidity was uniform.

After records had been taken, the plants were subsequently kept in a saturated condition on the floor of the cellar. When they were examined five hours later, the whole chlorotic areas of all the diseased leaves tested were found to be covered with abundant conidia.

Experiment 3 (Oct. 20, '30)

Two plants grown in a large pot were used. The pot had been watered before the experiment. One plant had three albicated leaves, while the other had four such. The pot was placed on a stand in

order to bring these leaves near the level of the hygrometer. The air humidity gradually rose from 78 to 82 per cent at about 18° C. during the twenty hours of the experiment. The humidity curve showed that the leaves were exposed to the air humidity of 80 to 82 per cent for nearly fifteen hours. No conidia were produced on any of these chlorotic leaves, although when the plants were kept under a saturated condition after the end of this experiment, all of these leaves were covered with abundant condia.

Experiment 4 (Oct. 22, '30)

This experiment was essentially a repetition of Experiment 3 with different plants. The results are shown in TABLE 18.

TABLE 18

Effect of Air Humidity on the Production of Conidia
(Experiment 4)

Temp.: 18° C.; Air humidity: 80–82%; Experimental time: 15 hours.

Plant no.	Diseased leaves tested	Amount of the conidia produced	Approximate proportion of the conidia-bearing areas to the total chlorotic areas
I	a	None	—
	b	Very slight	1/8
	c	Fairly abundant	1/5
II	a	None	—
	b	Very slight	1/10
	c	None	—

The results obtained in the present experiment were positive in the production of conidia, notwithstanding the conditions were almost similar to the preceding one. It may be safely concluded from the results of Experiments 3 and 4 that the relation of air humidity to the production of the conidia varies with different leaves as well as with different portions of the same leaf, and consequently,

the minimum air humidity for the production of conidia will vary with different leaves or plants, and different portions of a leaf.

Experiment 5 (May 26, '32)*

In this experiment, a glass box with a door, 58 cm. sq. in the area of the bottom, and 79 cm. in height, was used for controlling the air humidity. A recording hygrometer was placed on the bottom of the box, and another hygrometer was hung along the wall of the box. Both hygrometers had been carefully adjusted with an aspiration psychrometer. This glass box was then placed in an incubator held at 18° C., the optimum temperature for the production of conidia. Preliminary tests proved that the air humidity in this glass box can be changed at will, if water is properly atomized over the bottom, and on a part of the wall. A diseased plant was uprooted, and its roots were inserted in water in an Erlenmyer's flask. This flask was then placed in the glass box, so that the diseased leaves were situated about 8 to 10 cm. above the recording hygrometer, but a little lower than the hanging hygrometer. The results are presented in TABLE 19.

TABLE 19

Effect of Air Humidity upon the Production of Conidia
(Experiment 5)

Temp.: 18° C. Air Humidity: 85–80%. Experimental time: 19 hours.

Plant no.	Diseased leaves tested	Amount of the conidia produced	Approximate proportion of the conidia-bearing areas to the total chlorotic areas
I	a	Fairly abundant	1/5
	b	Ditto	1/4
II	a	Fairly abundant	1/5
	b	Ditto	1/4

* Experiments 5 and 6 were performed at Gifu, Japan, while Experiments 1–4 were carried out at Madison, Wis., U.S.A.

Experiment 6 (May 28, '32)

Another experiment was performed by the same method used in Experiment 5. The results are shown in TABLE 20.

TABLE 20

Effect of Air Humidity upon the Production of Conidia
(Experiment 6)

Temp.: 18° C. Air humidity: 84–78%. Experimental time: 11 hours.

Plant no.	Diseased leaves tested	Amount of the conidia produced	Approximate proportion of the conidia-bearing areas to the total chlorotic areas
I	a	Fairly abundant	1/4
	b	Ditto	1/6
	c	Ditto	1/4
II	a	Fairly abundant	1/3
	b	Slight	1/8

During these experiments, no appreciable differences were observed between the two hygrometers. The results mainly coincide with those obtained in the previous experiments.

Experiment 7 (Oct. 1, '30)

In the autumn of 1930, the temperature of one of the green houses belonging to the Department of Plant Pathology, Madison, Wis., U. S. A. was frequently ranging from 17° to 19° C. for twelve hours or more. The writer took advantage of this temperature for a preliminary study on the effect of intermittent air humidity upon the production of conidia. Diseased plants, the sporulation on which had been wiped off, were covered with moist galvanized cans for a definite period of time, and then the cans were taken out, the plants being exposed to the air of the green house for ten hours, the air humidity in which was ranging from 68 to 74 per cent. Some of these covered plants were left as control, and kept moist long enough

for the production of conidia. During the course of this experiment, uncovered diseased plants placed in parallel showed no production of conidia. The results are shown in TABLE 21.

TABLE 21

Effect of Intermittent Air Humidity upon the Production of Conidia

Series	No. of plants	Total no. of diseased leaves	Conidial production		
			3 hrs. in moist cans	4 hrs. in moist cans	10 hrs. in moist cans
I	2	7	None		
II	2	8		None	
III	1	5		None	
Control	3	13			Abundant

The results indicate that the growth of conidiophores and conidia is apparently inhibited as soon as the atmospheric moisture falls to 68–74 per cent.

ARENS (3, 4) reported that conidiophores of *Plasmopara viticola* are produced in 4 days in the air humidity of 75–85 per cent at 15° C., and conidiophores of *Pseudoperonospora Humuli* are produced in 3 days in the air humidity of 70–75 per cent at 20°–22° C. or in 2 days in the air humidity of 80–85 per cent within the same range of temperature.

(II) DISCHARGE OF THE CONIDIA

WESTON (119) who studied *Sclerospora philippinensis* apparently first discovered the fact that the conidia of the Sclerospora are actively discharged. The limit of projection was determined approximately to be about 1 to 2 mm. MELHUS and his associates (72) also demonstrated the discharge of the conidia in *Sclerospora graminicola* parasitic on *Setaria viridis*.

It happened occasionally in the field that whitish spore prints were observed on healthy leaves lying very near the conidia-bearing leaves of Italian millet. It seems hardly possible to account for this situation by mere passive adhesion of the conidia, since the areas of

spore prints corresponded to the conidia-bearing areas of the diseased leaves, and in addition, spore prints were often found in a horizontal direction to the conidia-bearing areas.

In order to demonstrate the discharge of the conidia of the fungus in question, some experiments were conducted.

Methods of Experimentation

Conidia-bearing leaves were cut into pieces of proper size, and placed in large Petri-dishes moistened by means of filter paper, with the conidia-bearing surface turned upward. Pieces of slide glass or cover glass were placed as supporters on both ends of each piece of these conidia-bearing leaves, and a glass plate of proper size was then put on both supporters to hang over the conidial layer. The under side of this glass plate had been smeared with egg albumen. The distance between the conidial layer and the under side of the glass plate was changed by substituting higher or lower supporters. In the present experiments, various heights were obtained by piling up pieces of slide glass or cover glass. After a certain period of time, the glass plate was examined macroscopically or microscopically.

Experiment 1 (July 8, '28)

The purpose of this experiment was to demonstrate primarily the vertical discharge of the conidia. The distance between the conidial layer and the under surface of the glass plate smeared with albumen was about 1.5 mm. The duration of the experiment was 12 hours. The results obtained are presented in TABLE 22.

TABLE 22

Showing the Discharge of the Conidia of *Sclerospora graminicola* on Italian millet (Experiment 1)

Temperature C.	Amount of discharged conidia					
	No. of diseased leaves					
	1	2	3	4	5	6
17°–18°	++++	++++	++++	+++	+++	++++
25°–26°	++	++	++	+	+	++

The results shown in the above table indicate that the conidia are vertically discharged across a distance of 1.5 mm. It is also evident from the data that the amount of discharged conidia varies not only with different leaves, but also with different temperatures. This seems to be reasonable from the results of the preceding experiments in relation to the production of conidia.

The diseased leaves collected after continued rain were found to be unsuitable. Practically, well-grown, thick, chlorotic leaves which are ready for the production of abundant conidia are suitable for the present experiment.

Experiment 2 (July 13, '28)

This experiment was performed to determine the extreme distance of the spore discharge. Proper diseased leaves were washed to take off the original sporulation, and pieces of the leaves were placed in Petri-dishes with the spore-traps mentioned above. They were kept in an incubator held at 18° C., the optimum temperature for the production of conidia, and the results were recorded after ten hours.

TABLE 23

Vertical Distances of the Spore Discharge in *Sclerospora graminicola* on Italian millet (Experiment 2)

Vertical distances (mm)	Discharge of the conidia						
	No. of diseased leaves tested						
	1	2	3	4	5	6	7
1	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+
3	+	−	+	−	−	+	−
4	−	−	−	−	−	−	−

The results obtained in the present experiment and shown in the above table indicate that the conidia are generally discharged up to 2 mm. upward, and some of them are discharged up to 3 mm.,

but not to 4 mm. vertically. It was found that the amounts of discharged conidia decreased as the vertical distances became larger.

These results coincide mainly with those reported by MELHUS and his associates (72).

Experiment 3 (July 13, '28)

This was undertaken to determine whether or not the discharge of conidia can occur in the daytime. The Petri-dishes containing the materials and spore-traps were kept in a large glass receptacle ordinarily used as a desiccator held at 19°–24° C. in the same way as in the case of the study on the influence of light upon the production of conidia. The distance between the conidial layer and the under surface of the plate glass was about 1.5 mm. Proper diseased leaves were carefully chosen. The results were always positive in three sets of experiments.

(III) GERMINATION OF THE CONIDIA

1. Mode of Conidial Germination

It was discussed and emphasized by S. ITO (56) that the mode of conidial germination is very important for the taxonomy of the genus Sclerospora. The mode of conidial germination in the typical *Sclerospora graminicola* parasitic on *Setaria viridis* is well known, but that in the fungus parasitic on Italian millet has not yet been fully studied. It has therefore been an interesting question to learn whether or not there is any difference in the mode of conidial germination between the typical *S. graminicola* and the Sclerospora fungus parasitic on Italian millet.

As already reported by the writer (48), the conidia, as a rule, germinate by liberating zoospores. Most of the conidia liberate five to twelve zoospores, and giant conidia give rise to far more zoospores. The zoospores are ejected through the apical pore resulted from the breaking of the papilla. They leave the conidia one by one, or are first forced out as an aggregate, and after a short period separate. The zoospores are usually kidney-shaped with two cilia. Their shape and size gradually change while they are swimming. The size increases after liberation, but then decreases gradually toward the

end of the swimming stage. The dimensions of the zoospores are 8 to 16 microns in length, and 5 to 9 microns in width. The zoospores swim in the medium, first slowly, then promptly, and again slowly toward the end of the swimming stage. Finally, they come to rest, and develop into spherical bodies, the so-called resting spores. Frequently zoospores fail to form cell wall, and degenerate.

Each resting spore germinates by a germ-tube which does not branch as a rule. Two or more zoospores often join together and swim in mass. Such zoospore-masses usually grow into compound resting spores which are larger than the ordinary ones. The compound resting spores originating from two zoospores germinate in two different ways: one, by a large germ-tube which gives rise to a branch, the other, by producing two ordinary germ-tubes from the beginning. Incomplete cleavage seems also to cause large compound resting spores. When these germinate, they quite resemble the conidia germinating by germ-tube, and it is often difficult to distinguish one from the other. But the former are usually spherical, and their wall is thin, while the latter retain their normal shape. Compound resting spores are more frequently found at high temperatures near the maximum for the germination of the conidia.

The germination of the conidia by germ-tube was first thought to be directly related to certain environmental factors. Further attempts artificially to induce the germtube germination have, however, so far failed.

2. *Influence of Temperature upon the Germination of Conidia*

Constant temperatures lower than the room temperature were secured by regulating the temperature inside of refrigerators with electric bulbs. In this way the desired constant temperatures ranging from 7° to 20° C. were obtained readily. In order to treat the fungus at temperatures lower than 5° C., a large block of ice was put into a refrigerator kept at about 6°–7° C., which was covered by a layer of saw dust, and the culture-dishes were placed directly upon the layer of saw dust on the ice block. Preliminary tests showed that if the thickness of saw dust is properly adjusted, the surface of the saw dust can be held at about 2° C. to 4° C. for ten hours or more.

All the thermometers were adjusted with a standard thermometer. A maximum and minimum thermometer was also used to

ascertain the range of temperature fluctuation in the incubator during the course of these experiments. The variation did not exceed one degree.

For the purpose of obtaining fresh conidia, infected leaves were cut off, washed in running water, placed in moist Petri-dishes, and kept at the optimum temperature for the production of conidia until an abundance were newly formed.

A drop of distilled water was placed on a clean slide, and fresh conidia produced on diseased leaves were carefully brought in contact with the surface of the drop. In this way, abundant conidia were easily liberated in the drop. From this spore-suspension, one or two smaller drops of almost uniform size were subsequently transferred onto the other clean slides by means of a small dropper or of a glass rod. These slides were immediately placed in moist Petri-dishes which were kept at different constant temperatures for a definite period of time.

(*1*) *Time required for the Germination of Conidia at Different Temperatures*

Preliminary tests were first made to get a general idea of the time required for the germination. Four or more Petri-dishes, each of which contained one slide bearing a drop of the spore-suspension were kept at each temperature. Tests were made at four or five different temperatures at the same time. The results are summarized in the following table.

TABLE 24

Time required for the Germination of Conidia at Different Temperatures

No. of tests	Time required at different temperatures (C.)						
	6°—7°	9°	12°	15°	20°	25°	30°
1	5.00	2.40*	1.15	0.50	0.45	0.45	0.55
2	4.30	2.40	1.10	0.50	0.40	0.40	0.50

* 2.40 means two hours and forty minutes.

TABLE 24 (*Continued*)

No. of tests	Time required at different temperatures (C.)						
	6°—7°	9°	12°	15°	20°	25°	30°
3	5.00	2.30	1.20	1.00	0.50	0.50	0.55
4	5.00	2.30	1.20	0.50	0.40	0.40	1.00
5	4.00	2.20	1.10	1.00	0.40	0.40	1.00
6	4.00	2.20	1.20	1.00	0.40	0.35	0.50
7	4.30	2.00	1.30	0.50	0.35	0.35	0.55
8	4.30	2.20	1.20	0.55	0.35	0.40	0.55
9	4.30	2.30	1.30	0.45	0.30	0.30	0.55
10	5.00	2.30	1.30	0.55	0.40	0.40	1.00
11	5.00	2.30	1.20	0.55	0.40	0.40	0.55
12	5.00	2.30	1.15	0.55	0.40	0.35	0.55
Average	4.40	2.27	1.20	0.54	0.40	0.39	0.55

The results shown in the above table indicate that the time required for the germination of the conidia varies considerably, ranging from 30 minutes to 5 hours, at different temperatures. The time required was found to be shortest at 20° C. and 25° C., and it became longer at temperatures lower than the optimal, it being also prolonged at 30° C. In the course of these experiments, it has been observed that the zoospores are inactive at 6°–7° C. and at 30° C.

(2) *Percentage of the Germination of Conidia at Different Temperatures*

Tests were always made at five or more different temperatures at the same time. Two or more drops of spore suspension were subjected to each temperature. The number of germinated spores in these drops was counted in several different fields under a microscope, and the sum was taken as representing the prevailing condition at a given temperature. The results are presented in the following table.

TABLE 25

Percentage of Germination of Conidia at Different Temperatures

Temp. C.	No. of tests	1	2	3	4	5	6	7	8	9	10	11	12	T*
2°—4°	Experimental hours	8	10	10	10	10	24	24	27	27	27	10		
	No. of spores counted	312	282	520	452	384	370	333	391	528	480	425		4477
	No. of germinating spores	0	0	0	0	0	0	0	0	0	0	0		0
	% of germination	0	0	0	0	0	0	0	0	0	0	0		0
5°—7°	Experimental hours	8	8	10	10	8	24	24	8	8	8	8		
	No. of spores counted	186	212	815	482	188	520	415	255	189	145	315		3722
	No. of germinating spores	114	112	0	0	85	0	0	107	78	60	0		556
	% of germination	61	53	0	0	45	0	0	42	41	41	0		15
10°	Experimental hours	4	4	5	4	4	4	4	4	4	4	4	4	
	No. of spores counted	177	187	200	321	201	214	182	131	163	240	210	231	2457
	No. of germinating spores	141	164	165	229	147	153	168	118	138	165	140	165	1893
	% of germination	80	88	83	71	73	72	92	90	85	69	67	71	77
15°	Experimental hours	3	3	3	4	4	3	4	4	4	4	4	4	
	No. of spores counted	127	156	295	288	143	112	292	171	139	192	108	228	2251
	No. of germinating spores	122	132	250	246	134	94	241	135	106	126	89	164	1839
	% of germination	96	85	85	86	94	84	83	79	76	66	82	72	82

TABLE 25 (*Continued*)

Temp. C.	No. of tests	1	2	3	4	5	6	7	8	9	10	11	12	T*
18°	Experimental hours	3	3	3	4	4	3	4	4	4	4	4		
	No. of spores counted	130	200	245	187	166	173	175	333	258	145	164		2176
	No. of germinating spores	120	190	238	165	139	143	148	285	228	123	135		1914
	% of germination	92	65	97	88	84	83	85	86	88	85	82		88
20°	Experimental hours	3	3	3	4	4	4	4	4					
	No. of spores counted	143	232	118	216	156	252	309	257					1683
	No. of germinating spores	115	200	98	160	126	212	276	218					1405
	% of germination	80	86	83	74	81	84	89	85					83
25°	Experimental hours	3	3	3	4	4	4	4	4	4	4			
	No. of spores counted	274	98	166	106	176	232	274	260	149	144			1879
	No. of germinating spores	232	80	144	86	145	190	224	215	119	124			1559
	% of germination	85	82	87	81	82	82	82	83	80	86			83
30°	Experimental hours	3	3	3	4	4	4	4	4	4	4	4	4	
	No. of spores counted	158	132	228	248	127	150	210	255	108	288	83	220	2207
	No. of germinating spores	82	65	0	0	67	80	120	148	50	0	45	0	657
	% of germination	52	49	0	0	53	53	57	58	46	0	54	0	30
32°	Experimental hours	3	3	3	5	5	5	5	4	4	6	6	5	
	No. of spores counted	230	113	212	380	415	400	400	320	250	500	320	500	4040
	No. of germinating spores	9	4	8	0	0	0	5	2	3	0	0	0	31
	% of germination	39	35	38	0	0	0	12	06	12	0	0	0	08

* T means total.

It is evident from the results shown in the table that the percentage of conidial germination varies with different materials. However, it seems likely that the minimum temperature for the germination of the conidia lies at about 5°–7° C., the optimum temperature at about 18° C., and the maximum at about 30°–32° C. These results do not coincide well with those obtained in the foregoing experiments concerning the relation of temperature to the time required for the germination of conidia.

It may be worthy to note that better results have been obtained in a number of individual tests at 10°–15° C. than at 20° and 25° C., although the average percentage of germination has been lower at the former temperatures than at the latter.

(3) *The Duration of Motility of Zoospores at Different Temperatures*

Methods

Fresh conidia were germinated at 18° to 20° C., and the resulting zoospore suspensions, 1.5 hours after sowing of the conidia were quickly transferred to clean slides which were subsequently placed in moist Petri-dishes, and subjected to known temperatures. Several dishes were kept at the same temperature in each experiment. Preliminary tests showed that the period of motility of zoospores is greatly influenced by the size of the drop of the suspension, and also by the presence of mixtures in the medium. Therefore, special care was given to obtain pure zoospore cultures in distilled water. The size of the drop of the zoospore suspensions was about 5 to 6 mm. in diameter. Tests were always made at five different temperatures. The results are shown in the following table.

TABLE 26

Period of Motility of Zoospores at Different Temperatures

Temp. C.	Period of motility of zoospores (hours)													
	Number of tests													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
9°—10°	24	24	20	20	30	30	35	40	35	40	28	30	24	18
15°—17°	20	20	18	20	30	26	38	24	24	28	28	28	20	18
19°—20°	6	7	6	6	14	14	8	8	10	10	5	5	4	4
25°—26°	4	5	4	4	4	2.5	3	3.5	4	4	3.5	3.5	2.5	2
29°—30°	5/6	5/6	2/3	0.9	0.9	2/3	2/3	1/2	1/2	1/2	1/2	0.4	1/2	0.4

The results obtained in these tests clearly indicate that the period of motility of zoospores decreases sharply as temperature rises. The numerals given in TABLE 26 are to be understood as being smaller than the actual duration of zoospore-motility, because most of the conidia sown at 18° C. are usually germinated in about 40 to 50 minutes after sowing.

3. *Viability of the Conidia*

In July, 1928, the writer experienced frequent failure in germinating the conidia collected in the experimental field. This led him to make a series of experiments about the viability of the conidia. A part of the results has been already published in a preliminary report (48). The problem will be considered here in detail.

Experiment 1 (July 5, '28)

Diseased leaves were collected early in the morning while dew was still present, and immediately brought to the laboratory where the temperature was about 24° C., and the air humidity was 78 per cent. Germination experiments were made at 17° C. with various intervals of time, and the percentage of the germination of conidia was determined three or four hours after sowing. The results are presented in TABLE 27.

TABLE 27

Viability of the Conidia of *Sclerospora graminicola* on Italian millet (Experiment 1)

	No. of tests	1	2	3	4	Total
Control	No. of spores	414	276	312		1002
	No. of germinating spores	323	224	252		799
	% of germination	78	81	81		80

TABLE 27 (*Continued*)

	No. of tests	1	2	3	4	Total
After 5′	No. of spores	232	228	248	78	786
	No. of germinating spores	76	87	81	19	263
	% of germination	33	38	33	24	33
After 10′	No. of spores	254	246	196	211	907
	No. of germinating spores	19	17	14	17	67
	% of germination	7	7	7	8	7
After 15′	No. of spores	520	480	500	924	2424
	No. of germinating spores	2	10	0	4	16
	% of germination	.4	2.1	0	.4	.7
After 20′	No. of spores	752	690	811	735	2988
	No. of germinating spores	0	0	0	0	0
	% of germination	0	0	0	0	0

The dew first found on the layer of conidial fructification was apparently dried up in 7 minutes after the start of the experiment. The results shown in the above table indicate that the percentage of conidial germination greatly decreased very soon after the dew on the materials was apparently dried up.

Besides the above-mentioned, some tests were made to germinate the conidia after 25′, 30′, 60′, 90′ and 120′, respectively, but no germination occurred.

Experiment 2 (July 6, '28)

In this experiment, the conidia produced in moist chambers were used. The materials were prepared in the same way as in the foregoing experiments for the production of conidia. When new conidia were abundantly produced, the materials were exposed to the air of the laboratory where the temperature was 26° C., and the relative humidity was 60 per cent. The germination of conidia was tested in drop cultures at several intervals of time. The results are presented in TABLE 28.

TABLE 28

Viability of the Conidia of *Sclerospora graminicola* on Italian millet (Experiment 2)

	No. of tests	1	2	Total
Control	No. of spores	117		117
	No. of germinating spores	113		113
	% of germination	97		97
After 5′	No. of spores	198	188	386
	No. of germinating spores	195	187	382
	% of germination	99	99	99
After 10′	No. of spores	116	227	343
	No. of germinating spores	107	223	330
	% of germination	92	98	96
After 15′	No. of spores	101	143	244
	No. of germinating spores	95	101	196
	% of germination	94	71	80
After 20′	No. of spores	420	293	713
	No. of germinating spores	47	32	79
	% of germination	11	11	11
After 25′	No. of spores	438	392	830
	No. of germinating spores	5	0	5
	% of germination	1.1	0	.6

In this experiment, water droplets on the materials dried up apparently in 13 minutes after the start of the experiment. In spite of the difference in the temperature and humidity of the air, the conidia were found to be more long-lived than those in the preceding experiment.

It is noteworthy that in this experiment, water droplets were found to remain on the layer of conidial fructification for about 6 minutes longer than in Experiment 1. However, the viability of the conidia after droplets apparently vanished, agrees mainly in both experiments. These facts suggest that the viability of the conidia is extended over as long a period as dew or droplets of water remain on the conidial layer. In order to determine this point, further experiments were conducted.

Experiment 3 (July 8, '28)

The materials were obtained by the same method as in Experiment 2. In the present experiment, some of the materials were atomized as soon as they were exposed to the air of the laboratory where the temperature was 27° C., and the relative humidity was 60 per cent. The viability of the conidia on either atomized or non-atomized materials was compared. The results are presented in TABLE 29.

TABLE 29

Viability of the Conidia of *Sclerospora graminicola* on Italian millet (Experiment 3)

Non-atomized series

	No. of tests	1	2	Total
After 10′	No. of spores	269	247	516
	No. of germinating spores	251	241	492
	% of germination	93	98	95
After 15′	No. of spores	219	299	518
	No. of germinating spores	113	140	253
	% of germination	52	47	49
After 30′	..... No conidia germinated			

Atomized series

After 10′	No. of spores	219	204	423
	No. of germinating spores	217	201	418
	% of germination	99	99	99
After 15′	No. of spores	438	216	654
	No. of germinating spores	434	214	648
	% of germination	99	99	99
After 30′	No. of spores	380	230	610
	No. of germinating spores	369	207	576
	% of germination	97	90	95

In the non-atomized series, water droplets on the conidial layer almost vanished after 15 minutes, while in the atomized series, they were still slightly visible after 30 minutes.

The results indicate that the conidia die very soon after the conidial layer loses moisture, and if the conidial layer retains moisture, the conidia live longer.

In this connection, the length of time in which the conidia can carry their viability under moist conditions must be an interesting question. If conidia-bearing leaves are kept in moist conditions, new conidia are successively produced, adding to the old conidia on the conidial layer, so far as the other factors are favorable for the production of the conidia. Therefore, it is difficult to study the viability of the conidia under such conditions. Because of this obstacle, only fragmentary experiments were carried out. The results are summarized as follows:

(1) Affected leaves bearing fresh conidia were kept at 27°–28° C. for 12 hours, and then the conidia were germinated at 17° C. The results are presented in the following table.

TABLE 30

Showing the Viability of the Conidia kept at 27°–28° C. for 12 Hours

Set I

No. of tests	1	2	3	Total
No. of spores	470	346	391	1207
No. of germinating spores	126	100	50	276
% of germination	27	29	13	23

Set II

No. of spores	110	86		196
No. of germinating spores	52	38		90
% of germination	47	44		46

(2) Leaves bearing fresh conidia were kept at 27°–28° C. for 35 hours. None of the conidia germinated at 17° C. in three sets of experiments.

(3) Leaves bearing fresh conidia were kept at 2°–4° C. for 24 hours. A number of conidia germinated at 17° C., but the zoospores were found to be very inactive from the beginning.

(4) Conidial suspensions were kept at 2°–4° C. for 30 hours. None of the conidia germinated under these conditions. These drop cultures were then transferred to an incubator held at 18° C., and a number of conidia germinated within 50 minutes. The zoospores were found to be inactive, and compound zoospores were commonly found.

(5) Some of the discharged conidia caught by the albumen layer on a slide in a moist Petri-dish at 18° C. were found to survive for about ten hours.

These results indicate that the conidia can survive much longer in moist conditions than in dry conditions.

(IV) Germination of the Oospore

1. Mode of the Germination of the Oospore

Oospores germinate by germ-tubes which later grow into vegetative mycelium. The germ-tubes are hyaline, non-septate, variously branched, and as a rule, contain large globular bodies. The oospore, in most cases, grows out one germtube. However, two or more germ-tubes are occasionally found at the same opening of an oospore. Up to this time, no conidia or sporangia have been found to be formed on the germ-tube, or on its branches.

The germ-tube grows very rapidly. In repeated measurements, some of the germ-tubes were found to grow at the rate of 10 to 15 microns in five minutes at about 24° C. It has often been observed that the contents of the oospore are moving out into the germ-tubes, or the contents in the germ-tube are moving toward its tip. If oospores ready to germinate are sown in such a manner as described by the writer (53), the germ-tubes grow abundant aerial hyphae showing a whitish moldy appearance.

The diameter of the germ-tube ranges from 3 to 11 microns, mostly 3.5 to 5 microns. Most of the large germ-tubes give rise to

two or more side-branches shortly after germination, while smaller germ-tubes are liable to produce branches after they elongate to some extent. Hyphal branches grow often at almost right angles to the main axis.

No distinct germ-pore is seen in a dormant oospore.

2. *Some Factors influencing the Germination of the Oospore*

a. Methods used for the Germination of the Oospore

It has already been reported by the writer (50, 51) that oospores may be germinated by ordinary methods for spore germination. It was, however, highly desirable to discover a special method for the successful germination of the oospores for the purpose of solving various phases of oospore physiology as well as of related pathological problems. To meet this need, the writer has described a simple method, the essential procedure of which is briefly presented in the following citation:

"The method consists of placing a layer of moistened cotton in the two parts of a Petri dish. Then a small piece of moist filter-paper on which small amounts of oospore powder are placed is put upon the surface of the moist cotton in such a way that the filter paper will partly, but not entirely, touch the cotton. Both the cotton and filter-paper must be drained of excess moisture before the oospores are added to the dish. It is essential that the space between the two layers of cotton in the dish be about one half the height of the dish. Small blocks of 2 per cent agar-agar, on which the oospores are scattered over the surface just as the agar is hardening, can be substituted for the filter paper."

In further studies, it has been found that if the cotton (absorbent cotton is most convenient) is properly drained of excess water, it is not necessary to place the filter paper on the moist cotton in such a special way as originally described. The space between the two layers of cotton in the dish may also be greater than mentioned above.

In some trials, two layers of filter paper were used instead of absorbent cotton, but it gave no good results especially at high temperatures over 30° C.

The idea of scattering oospores over agar-agar* has been found very satisfactory for the study on the relation of temperature to oospore germination, since more reliable percentages of germination are obtainable, because, by the filter paper method, the oospores on the periphery of the mass germinate better, and consequently the percentage of the germination to be obtained, may easily be influenced through the process of removing the material on a slide for counting.

In scattering oospores over agar-agar, care should be taken of the temperature of congealing agar. Of course, too high temperature must be avoided. A tepid condition before hardening is suitable. The thickness of oospore-bearing agar-layer should be about one millimeter.

In some cases, melted agar was first poured into one part of a Petri-dish up to about one half its height, and oospores were then scattered as usual, while the other part of the Petri dish was fitted up with moist absorbent cotton. This has been found, however, somewhat inferior to the method described above.

Besides the methods mentioned above, other attempts to germinate oospores were also made. A brief summary may be worth reporting. The methods tested are as follows:

(1) Pieces of oospore-bearing moist filter paper were supported at various heights (3–10 mm.) in Petri dishes fitted up with moist absorbent cotton. The supporter was made of wire. The idea was derived from Dr. TASUGI's method for the germination of the oospore.

(2) A small cavity was prepared by means of wire-gauze in properly moistened soil in pots, and pieces of moist oospore-bearing filter paper were

(a) placed directly on the bottom soil of the cavity;

(b) supported at various heights (3–10 mm.) in the cavity.

All the sides of the cavity were covered with moist soil, and the pots were kept at various temperatures. All the results verified

* For the sake of convenience, this may better be called, "agar-agar method", while the other, "filter paper method".

that these methods were not specially superior to the writer's simple method which is useful even at such a high temperature as 35° C.

b. Influence of Temperature upon Oospore Germination

(1) Time required for the Germination of the Oospore at Different Temperatures

The filter paper method was used. Cotton and filter paper were moistened with distilled water. In the course of several preliminary tests to get a general idea at each temperature, it was found that the time required for germination varies greatly with different sources of the oospores. In each test several dishes were kept at the same temperature. The results are shown in the following table.

TABLE 31

Time Required for the Germination of the Oospore at Different Temperatures

Temp. C.	Source	Time in hours — No. of tests							
		1	2	3	4	5	6	7	8
10°-11°	A	—	216	200	200	216	200	192	200
15°-16°	A	96	96	85	85	92	88	72	88
20°-21°	A	52	52	45	45	50	45	42	48
	B	58	60	56	—	—	—	—	—
25°-26°	A	35	38	32	35	38	35	30	35
	B	40	40	40	—	—	—	—	—
27°-28°	A	—	—	—	—	—	32	28	32
30°-31°	A	30	30	28	28	30	28	24	28
34°-35°	A	—	—	—	26	—	26	22	24

Source A is the material collected in 1929 in our experimental field, and Source B is that collected in 1929 at Anjo, Aichi Prefect., Honshu. The numerals in the above table indicate the shortest time required for germination in each test. It is often found that even

in the same culture, some oospores may germinate 5 to 10 hours later than the earliest.

The results show that the time required for germination becomes shorter as the temperature rises. Source A showed 28 to 36 per cent germination at 25° C., while Source B, 3 to 10 per cent at the same temperature. As seen from the table, the time required for germination is longer with the materials showing a lower percentage of germination. It may be quite possible that a shorter time may be obtainable according to the oospores to be tested. The results of the tests made by Mr. KAWADA showed that in some cases, oospores germinated within 24 hours after sowing at 25° C. Similar results have been reported by the Agricultural Experiment Station, Government-General of Chosen (1). It is noticeable that the time required for germination varies greatly at different temperatures.

(2) Percentage of the Germination of the Oospore at Different Temperatures

The agar-agar method was used throughout the experiments. Owing to the results obtained in the experiments concerning the time required for oospore germination, the counts were made after a proper period of time at each temperature, respectively. The results are shown in TABLE 32.

TABLE 32

Percentages of Oospore Germination at Different Temperatures

Temp. C.	Experimental hours	No. of tests	1	2	3	4	Total
15°–16°	100	No. of spores	710	660	530	666	2566
		No. of germinating spores	80	75	64	78	297
		% of germination	11	11	12	12	12
20°–21°	49	No. of spores	532	602	633	510	2277
		No. of germinating spores	194	234	261	171	860
		% of germination	36	39	41	34	38
25°–26°	42	No. of spores	720	687	482	564	2453
		No. of germinating spores	223	192	156	204	775
		% of germination	31	28	32	36	32

TABLE 32 (*Continued*)

Temp. C.	Experimental hours	No. of tests	1	2	3	4	Total
29°-30°	37	No. of spores	605	740	591	558	2494
		No. of germinating spores	200	230	408	205	843
		% of germination	35	31	35	37	34
34°-35°	34	No. of spores	789	687	530	658	2664
		No. of germinating spores	47	72	56	69	244
		% of germination	6	10	11	10	9

Experiment 2 (May 5, '30)

10°-11°	220	No. of spores	605	682	515	630	2432
		No. of germinating spores	1	3	2	4	10
		% of germination	0.2	0.4	0.4	0.6	0.4
15°-16°	102	No. of spores	640	450	500	660	2250
		No. of germinating spores	14	15	14	15	58
		% of germination	2.2	3.3	2.3	2.3	2.6
20°-21°	60	No. of spores	738	692	474	567	2471
		No. of germinating spores	41	34	20	26	121
		% of germination	5.6	4.9	4.2	4.6	4.9
25°-26°	48	No. of spores	545	531	682	905	2663
		No. of germinating spores	33	28	32	40	133
		% of germination	6.1	5.3	4.7	4.4	5.0
30°-31°	38	No. of spores	482	438	460	575	1955
		No. of germinating spores	30	26	17	37	110
		% of germination	6	6	4	6	6

Remarks: The oospores used in this experiment were collected in 1928, and were nearly 20 months old. The time required for germination was found a little longer than usual, for instance, 54 hours at 20°C.

Experiment 3 (May 14, '30)

10°-11°	200	No. of spores	625	572	650	600	2447
		No. of germinating spores	11	3	8	4	26
		% of germination	2	1	1	1	1

TABLE 32 (*Continued*)

Temp. C.	Experimental hours	No. of tests	1	2	3	4	Total
15°–16°	82	No. of spores	600	576	544	562	2282
		No. of germinating spores	105	95	84	94	378
		% of germination	18	16	15	17	17
20°–21°	53	No. of spores	526	483	587	564	2160
		No. of germinating spores	115	112	125	142	494
		% of germination	22	23	21	25	23
25°–26°	38	No. of spores	586	495	699	490	2270
		No. of germinating spores	136	114	177	126	553
		% of germination	23	23	25	26	24
29°–30°	31	No. of spores	465	500	581	530	2076
		No. of germinating spores	99	57	141	120	417
		% of germination	21	11	24	23	20
34°–35°	30	No of spores	578	603	518	475	2174
		No. of germinating spores	44	60	52	36	192
		% of germination	8	10	10	8	9
38°–39°	38	No. of spores	800	832	915	850	3397
		No. of germinating spores	0	0	0	0	0
		% of germination	0	0	0	0	0

Experiment 4 (May 14, '30)

Temp. C.	Experimental hours	No. of tests	1	2	3	4	5	Total
15°–16°	102	No. of spores	580	751	655	574	620	3180
		No. of germinating spores	28	28	25	28	21	130
		% of germination	5	4	4	5	3	4
20°–21°	60	No. of spores	560	598	582	593	698	3031
		No. of germinating spores	26	35	36	39	34	170
		% of germination	5	6	6	7	5	6

TABLE 32 (*Continued*)

Temp. C.	Experimental hours	No. of tests	1	2	3	4	5	Total
25°–26°	48	No. of spores	584	592	557	720	637	3090
		No. of germinating spores	34	31	25	23	39	152
		% of germination	6	5	4	3	6	5
29°–30°	32	No. of spores	692	486	748	593	690	3209
		No. of germinating spores	38	21	56	27	39	181
		% of germination	5	4	7	5	6	6

Remarks: The oospores used in this experiment were collected at Anjo in 1929. Germination has been found not very good.

Although the results shown in the above table are somewhat irregular, it is evident that the oospores germinate at temperatures between 11° and 34° C. The optimum temperature was found different with different materials, but it may be generally concluded that it lies between 20° and 25° C., although high percentages of germination have been obtained between 15° and 30° C.

c. Dormancy of the Oospore

When oospores begin to scatter from the shredded leaves in the field, they are apparently mature being complete in morphology, but so far as their germination is concerned, most of them seem to be immature or dormant, since the percentage of oospore germination increases after a certain lapse of time.

In September, 1928, oospores were floated over a water drop on a slide and kept in a moist chamber. No oospores were found to germinate. Some materials of the same source were kept until the next spring, and in May, 1929, germination experiments were made on them. Many oospores were found to germinate in this case.

The oospores collected in September, 1929, showed a poor germination in October of the same year, while a much better germination was observed in March, 1930, and a less germination in September of the same year. Similar results were also obtained with the oospores collected in 1930.

In order to obtain more exact information, some experiments were carried out. In August and September, 1931, oospores were

collected at Miyata, Aichi Prefecture at certain intervals. Some of the materials were kept outdoors, and the remainder in the laboratory. Germination tests were made at intervals by means of the filter paper method at 25° C. The results are summarized in the following table.

TABLE 33

Relation of the Dormancy of Oospores to Their Germination

(a) Oospores kept outdoors

Date of germination tests		The oospores collected on			
		Aug. 11	Aug. 24	Sept. 6	Sept. 21
Sept. 25, '31	No. of spores	—	—	—	—
	No. of germinating spores	None	A few	A few	A few
	% of germination	—	—	—	—
Nov. 30, '31	No. spores	510	656	566	502
	No. of germinating spores	0	7	25	26
	% of germination	0	1	4.4	5.2
Feb. 13, '32	No. of spores	546	620	518	456
	No. of germinating spores	56	54	138	54
	% of germination	10	9	27	12
May 26, '32	No. of spores	630	540	520	480
	No. of germinating spores	33	21	42	26
	% of germination	5	4	8	5

(b) Oospores kept in the laboratory

Sept. 26. '31	No. of spores	—	—	—	—
	No. of germinating spores	None	A few	None	None
	% of germination	—	—	—	—
Jan. 21, '32	No. of spores	—	522	548	502
	No. of germinating spores	None	24	40	10
	% of germination	—	5	7	2
May 18, '32	No. of spores	—	466	508	454
	No. of germinating spores	None	81	150	72
	% of germination	—	17	30	16

The oospores used in the last experiment with oospores kept outdoors were brought into the laboratory two days before the experiment, and were dried on a table, since they had been considerably wet. Examinations revealed that some of the wet oospores were germinating. The low percentage of oospore germination in this experiment may have some relation to this condition.

These results coincide with those of the previous experiments, indicating that the percentage of oospore-germination increases with a certain lapse of time.

It is also noteworthy that the percentage of the oospore germination varies not only with different sources, but also with the conditions under which the oospores are kept.

3. *Longevity of the Oospore*

Little has been known on the longevity of the oospores of *Sclerospora graminicola* (SACC.) SCHROET. In 1927, BORCHHARDT (9) reported that the oospores of *Sclerospora graminicola* kept for ten years in the laboratory were viable and gave rise to infection on *Setaria germanica*. In 1928, MELHUS and his co-worker (72) reported that oospores of *Sclerospora graminicola* on *Setaria viridis* kept for 30 months under dry conditions in the laboratory were found to be viable.

Recently, TAKASUGI and AKAISHI (106) reported that the oospores of *S. graminicila* on Italian millet are viable for eight years in the laboratory conditions.

When the writer was studying in the Department of Plant Pathology, Minnesota Agricultural College, St. Paul, some germination experiments were carried out with the oospores of *Sclerospora graminicola* kept in the Mycological Herbarium. Three different sources of the oospores on *Setaria italica* kept for 10 to 14 years, and two different sources of those on *Setaria viridis* kept for 11 years were tested. No oospores were found to germinate.*

In the course of the present investigation, it has also been found that some oospores collected in 1929 germinated very well after 19 months when kept in the laboratory condition, but the percentage of germination decreased markedly after sixteen months.

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* The writer is greatly indebted to Dr. E. C. STAKMAN, and Dr. L. DOSDALL for the free study of the specimens.

At the beginning of May, 1930, seeds of Italian millet were inoculated with oospores in a sandy soil, and 82 per cent of infection was obtained. This infested soil was left to dry in the laboratory, and eight months later, seeds of Italian millet were again sown in this soil. It followed that 37 out of 346 plants were infected, i.e., the percentage of infection was 11. These results indicate that some oospores which had not germinated in the first experiment, germinated in the second. This is quite interesting as an indication that in nature the oospores in the soil may germinate at different times according to the soil conditions as well as to the internal conditions of the oospores themselves. The longevity of the oospores proved in a dry condition in the laboratory may not be applied directly to that of the oospores in the soil in nature.

The Chosen Agricultural Experiment Station (1) reported that the viability of the oospores in soil is greatly reduced after two years. In this connection, the following data were obtained in the experiment made by the writer.

In 1927 and 1929, abundant oospores were placed on the soil in large porcelain pots which were left in our experimental field. Late in autumn 1932, inoculation experiments were made with these infested soils. Eighty-four plants out of 168, that is, 50 per cent became infected in the soil infested in 1929, but none of 282 plants was infected in the soil inoculated in 1927. In other words, oospores were viable in nature at least for three years, but not for five years. This means that the rotation of crops in a 5-year-cycle may be enough to avoid the downy mildew of Italian millet, but three years' interval is clearly inadequate for this purpose.

(V) Conclusions concerning the Physiology of the Causal Fungus

1. The conidia of the causal fungus can be produced between 10° and 25° C., and the optimum temperature for the production of conidia is about 17°–18° C., while they are abundantly produced between 15° and 20° C.

2. The minimum time required for the production of the conidia is different in different materials. However, the time is as a whole the shortest at 18°–20° C.

3. The conidia of the fungus can be produced in the daytime. Observations in field as well as in green house have also revealed that on cool cloudy days, conidia are frequently produced in the daytime.

4. The relation of air humidity to the production of conidia varies considerably with different leaves as well as with different portions of the same leaf, and consequently the minimum air humidity for the production of conidia varies with different leaves. So far as the present investigation is concerned, the minimum air humidity for the production of conidia may be considered to be about 80–85 per cent at 18° C.

5. The conidia are readily discharged up to 2 mm. upward, and some of them are discharged up to 3 mm., but not to 4 mm. vertically. The discharge of the conidia may also occur in the daytime.

6. The conidia, as a rule, germinate by liberating zoospores, but occasionally they germinate by germ-tube.

7. The time required for the germination of conidia varies considerably, ranging from 30 minutes to 5 hours, at different temperatures. It is shortest at 20° and 25° C., and it becomes longer at temperatures lower than the optimal, it being also prolonged at 30° C.

8. The minimum, optimum, and maximum temperatures for the germination of the conidia are about 5°–7°, about 18°, and about 30°–32° C., respectively.

9. The period of motility of the zoospores varies greatly with different temperatures, decreasing as the temperature rises. The period of motility has been found to be 18–40 hours at 9°–10° C., while 25′–50′ at 29°–30° C.

10. The conidia are extraordinarily short-lived. They may die in about 15 to 20 minutes after dew has apparently dried up. However, the conidia may survive much longer in moist conditions.

11. Oospores germinate by germ-tubes which are hyaline, non-septate, and as a rule contain large globular bodies. The germ-tubes grow very rapidly. Some of them have been found to grow at the rate of 10 to 15 microns in five minutes at about 24° C. The diameter of the germ-tube ranges from 3 to 11 microns, mostly 3.5 to 5 microns. Hyphal branches are liable to grow at almost right angles to the main axis.

12. Various methods to germinate oospores have been tested, but the one described previously by the writer (53) has been ascertained to be the best.

13. The time required for the germination of the oospores varies with different temperatures as well as with different materials. It becomes shorter as the temperature rises, and is generally longer with the materials showing a lower percentage of germination.

14. The percentage of oospore germination also varies with different temperatures as well as with different materials. The optimum temperature for the germination of oospores seems to be between 20° and 25° C.

15. The percentage of oospore germination increases with a certain lapse of time, indicating that the oospores have a dormant period.

16. Oospores are viable in nature at least for three years, but not for five years.

III. TAXONOMY OF THE CAUSAL FUNGUS

There are two different opinions on the scientific name of the Sclerospora parasitic on Italian millet, *Setaria italica*. Certain investigators are of the opinion that the fungus on *Setaria italica* is distinct from that parasitic on other Setaria species, and accept TRAVERSO's new variety, *Sclerospora graminicola* (SACC.) SCHROET. var *Setariae-italicae*, while others consider the fungus on *Setaria italica* to be identical with the type form, *Sclerospora graminicola* which parasitizes all other Setaria species.

At the beginning of the present investigation, the writer happened to discover that among many shredded leaves of the Italian millet collected in a field at Kakudahama-Mura, Niigata Pref., a number of specimens had smaller oospores than the remainder. The dimensions of these smaller oospores were found to be identical with those of the typical *Scl. graminicola*, but those of the remainder were considered to be nearly identical with TRAVERSO's new variety.

It was also found, on the other hand, that among the oospore specimens from *Setaria viridis* collected in Kyoto, Japan, there were some specimens having oospores larger than the usual ones. These findings led the writer to make a thorough study on the taxonomy of the fungus under consideration.

Further efforts were directed in two ways. One was to make a comparison of the oospore-dimensions of the Sclerospora from various hosts, and localities, and the other was to make inoculation experiments in order to obtain information relating to host range.

(I) Measurements of the Oospore

1. *Source of Materials*

a. Oospores from *Setaria italica*

Locality	Date	Collector	Sign
Anjo, near Nagoya, Aichi Pref., Japan	Aug. 11, '29	S. Morino	Anjo-29
Green house, Berkeley, Calif., U. S. A.	Nov. 20, '29	M. Hiura	Berk-29
Experimental field of our college, near Gifu, Japan	Aug. 28, '28	M. Hiura	Col-28
Ditto	Aug. 29, '29	M. Kawada	Col-29
Mandan, North Dakoto, U.S.A.	Sept. 28, '16	B. H. Humphrey J. T. Sarvis	Dak-16
Albosaggia (Sondrio), Italy	Aug. — '02	G. B. Traverso	Ita-02
Kakudahama-Mura, near Maki, Niigata Pref., Japan	Aug. 25, '27	M. Hiura	Kak-27
Ditto	Aug. 25, '28	M. Hiura	Kak-28
Coimbatore, Madras Pres., India	Oct. 30, '12	W. McRae	Mad-12
Ditto	Jan. — '30	S. Sundararaman	Mad-30
Yugakujo, Manchuria	Sept. 5, '31	H. Takasugi	Man-31
Koshurei, Manchuria	Aug. 15, '26	Y. Akaishi	Man-26
East Lansing, Michigan U.S.A.	Sept. 9, '96	C. F. Wheeler	Mich-96
Virginia, Minn., U. S. A.	Aug. 28, '19	J. J. Christensen	Minn-19
Miyata, Aichi Pref., Japan	Aug. 15, '30	S. Kawada	Miy-30
Nagano, Japan	Aug. 25, '27	J. Murata	Nag-27

b. Oospores from *Setaria viridis*

Ames, Iowa, U. S. A.	Sept. — '30	I. E. Melhus	Ames-30
Bruce, S. Dakota, U. S. A.	Sept. 2, '30	A. G. Johnson	Dak-30
Tarn, France	Aug. 26, '30	E. Mayor	Fran-30
Kyoto, Japan	Sept. 17, '27	T. Abe	Kyo-27 b
Ditto	Aug, 31, '27	T. Abe	Kyo-27 a
Ditto	Sept. 22, '28	M. Hiura	Kyo-28

b. (*Continued*)

Locality	Date	Collector	Sign
Kyoto, Japan	Aug, 30, '29	S. Kawada	Kyo-29
Ditto	Sept. — '30	S. Kawada	Kyo-30
Ditto	Sept. — '31	M. Hiura	Kyo-31
Hutchinson, Minn., U. S. A.	Aug. 18, '19	J. J. Christensen	Minn-19 c
St. Paul, Minn., U. S. A.	July 19, '19	J. J. Christensen	Minn-19 a
Minneapolis, Minn., U. S. A.	Aug. 3, '19	J. J. Christensen	Minn-19 b
St. Paul, Minn., U. S. A.	Sept. 4, '30	C. C. Allison	Minn-30
Cautou de Vaud, Switzerland	Sept. 9, '07	D. Cruchet	Suis-07
Helvetia, Jverdon, pr. Neuchatel, Switzerland	Aug. 16, '11	D. Cruchet	Suis-11
Berlin, Germany	Aug. — '19	P. Sydow	Ber-19

c. Oospores from *Setaria glauca*

Lichterfelde, Berlin, Germany.	July—'96	P. Sydow	Lich-96
Zehlendorf, Berlin, Germany	Sept. — '81	P. Sydow	Zehl-81

d. Oospores from *Setaria magna*

Homestead, Florida	May 22, '31	S. Hawkins	Hom-31

e. Oospores from *Pennisetum typhoideum*

Poona, Bombay Pres., India	Oct. — '30	B. N. Uppal	Bom-30
Nagpur & Akola, India	Nov. 13, '30	J. F. Dastur	Ako-30

f. Oospores from *Holcus Sorghum*

Coimbatore, Madras Pres., India	Sept. 1, '30	S. Sundararaman	Mad-30
Poona, Bombay Pres., India	Oct. — '30	B. N. Uppal	Bom-30

2. *Results of Oospore Measurements*

The results obtained are tabulated in the following pages.

TABLE 34

Frequency Distribution for the Diameter of the Oospore of *Sclerospora graminicola*

(a) Diameter of the Oospore from *Setaria italica*

Sign	Class in microns														Total	Mean
	24	26	28	30	32	34	36	38	40	42	44	46	48	50		
Kak-28 a					2	9	13	48	52	80	52	23	18	3	300	41.54
Nag-27				1	3	2	10	27	66	62	22	6	1		200	40.58
Ita-02					2	12	27	81	90	67	40	1			320	39.81
Mad-30 c					3	4	28	65	60	31	27	2			220	39.51
Dak-16					1	8	21	49	111	72	15	3			280	39.23
Miy-30 b			1	5	6	11	15	37	58	43	20	4			200	39.40
Berk-29				6	14	20	30	41	63	51	30	5			260	39.07
Kak-27			1	2	10	31	105	131	114	60	36	8	2		500	38.70
Miy-30 a			1	2	6	15	44	65	46	22	16	3			220	38.41
Minn-19					2	6	40	85	43	20	2	2			200	38.39
Col-28		1	1	5	23	31	41	59	88	42	25	4			320	38.37
Col-29 a		1	1	4	15	21	28	48	53	30	17	2			220	38.26
Man-30				5	6	22	50	69	51	30	6	1			240	38.01
Anjo-29 a				2	14	30	58	61	80	21	13	1			280	37.98
Anjo-29 b					2	25	59	73	48	11	2				220	37.65
Mad-30 a		2	8	10	17	20	37	48	35	14	6	3			200	36.73
Mich-96				1	36	54	73	66	18	2					250	35.83
Mad-12 c				4	30	51	68	43	3	1					200	35.29
Col-29 b		1	2	12	41	43	75	30	18	4	2				210	35.16
Kak-28 b		1	8	36	72	104	103	57	15	3	1				400	34.54
Man-26			6	24	44	46	87	21	10	2					240	34.48
Kak-28 c			6	22	75	89	63	12	2	1					270	33.70
Mad-30 b		2	12	34	45	59	38	8	2						200	33.03
Mad-12 a		4	11	38	64	56	30	7							210	32.62
Mad-12 b	1	4	12	29	74	54	22	4							200	32.41

(b) Diameter of the Oospore from *Setaria viridis*

Sign	Classes in microns														Total	Mean
	24	26	28	30	32	34	36	38	40	42	44	46	48	50		
Kyo-29		1	1	3	4	24	32	67	47	18	3				200	37.77
Minn-19 a					2	27	60	72	54	5					220	37.49
Kyo-27 b				3	13	25	47	52	35	23	2				200	37.39
Minn-19 b				1	7	32	55	81	61	3					240	37.36
Kyo-31		1	3	8	15	19	57	47	41	17	5	5	2		220	37.34
Kyo-28 a		1	1	2	6	28	52	66	37	4	2	1			200	37.10
Suis-11					6	23	81	85	32	3					230	37.07
Minn-20					2	63	82	80	19	4					250	36.50
Kyo-30			1	2	18	33	74	68	13	1					210	36.17
Minn-30			1	4	21	23	76	61	12	2					200	36.10
Ames-30			2	5	14	48	59	52	17	3					200	35.96
Dak-30				3	22	49	65	53	17	1					210	35.89
Kyo-28 b			2	14	27	41	50	39	29	7	1				210	35.78
Fran-30			2	8	22	50	63	52	11	2					210	35.56
Minn-19 c			1	15	60	71	58	24	1						230	34.14
Suis-07		1	8	43	48	62	52	20	15	1					250	33.83
Kyo-27 a	3	5	7	20	46	53	37	17	9	1					198	33.59
Ber-19	4	6	15	29	58	52	21	11	1						197	32.38

(c) Diameter of the Oospore from *Setaria glauca*

Lich-96					1	3	35	85	61	12	3				200	38.50
Zehl-81				4	5	45	89	64	3						210	36.03

(d) Diameter of the Oospore from *Setaria magna*

Hom-31		8	17	41	60	43	31	13	5	2					220	32.68

(e) Diameter of the Oospore from *Pennisetum typhoideum*

Bom-30 a			1	9	18	20	70	66	13	3					200	36.14
Bom-30 b			4	13	27	42	48	40	21	5					200	35.46
Ako-30		9	19	46	52	60	28	13	3						230	32.49

(f) Diameter of the Oospore from *Holcus Sorghum*

Bom-30			3	7	12	75	74	28	1						200	36.98
Mad-30		4	9	45	66	81	32	3							240	32.66

It is evident from the results of measurements tabulated above that the diameter of the oospores of the Sclerospora parasitic on *Setaria italica* as well as on other species of Setaria, varies greatly with different sources of materials. Oospores in some materials from *Setaria italica* are somewhat larger than those from other species of Setaria. But, it is noticeable that the oospores in other materials from *Setaria italica* are considerably smaller than those in some of the materials from *Setaria viridis*, or other species of Setaria.

(II) Host Range

From the present status of our knowledge in relation to specialization in plant pathogenic fungi, it seems rather natural to expect the occurrence of specialization in *Sclerospora graminicola* hitherto known as parasitic on various species of Setaria.

In order to determine to what extent this is the case with the fungus in question, a series of inoculations was made on various graminaceous plants. Oospores were used as the source of inoculum throughout the experiments. The methods will be detailed in a later chapter. The results are presented in the following pages.

1. *Inoculation Experiments with the* italica-*fungus** *on Various Graminaceous Plants*

(*a*) *On Varieties of Setaria italica*

Experiment 1 (Aug. 20, '30)

Variety	Pot	No. of plants	No. of infected	% of infection
Echigo	a	22	15	68
	a	99	82	83
Siberian millet	a	85	47	55
	a	78	36	46
Common millet	a	58	29	50
	b	57	35	61
	c	76	41	54

* This means the fungus parasitic on *Setaria italica*.

Experiment 1 (*Continued*)

Variety	Pot	No. of plants	No. of infected	% of infection
German millet	a	67	20	30
	b	48	11	23
	c	32	6	19
Hungarian millet	a	35	0	0
	c	42	0	0

The oospores used in this experiment were collected in 1929 in the experimental field of the Gifu Agricultural College, and they germinated very well. All the pots were kept for a few days after inoculation in a cellar at about 19°–25° C.

Hungarian millet germinated very poorly, and the time required for emergence was found to be 2–3 days longer than that of the other varieties. Unless otherwise stated, the production of conidia was taken tentatively as the sign of infection.

Experiment 2 (Aug. 26, '30)

Variety	Pot	No. of plants	No. of infected	% of infection
Common millet	a	31	8	26
	b	36	8	22
	c	36	10	28
Siberian millet	a	56	6	11
	b	72	4	6

The oospores used in this experiment were obtained from the same source as in the above experiment. But the percentage of their germination was found to be lower than that of the oospores used in Experiment 1. The lower percentage of infection as shown in the above table might be in part ascribed to the smaller percentage of oospore germination, since the other essential conditions were quite similar in both experiments.

Experiment 3 (Dec. 30, '30)

Variety	Pot	No. of plants	No. of infected	% of infection
Echigo	a	120	23	19
	b	156	45	29
Hungarian millet	a	152	0	0
	b	135	1	0.7
	c	208	0	0
White wonder	a	147	0	0
	b	120	0	0

The oospores used in this experiment were collected at Miyata, Aichi Prefecture, and their germination was not very good. However, the results clearly show that Hungarian and White Wonder are anyway very resistant to the infection of the fungus in question.

Experiment 4 (Jan. 10, '31)

Variety	No. of plants	No. of infected	% of infection
Hungarian millet	89	1	1.1
White Wonder	27	0	0

Experiment 5 (Jan. 10, '31)

Hungarian millet	82	1	1.2
White Wonder	36	0	0
Miyata*	80	14	18

* This is a susceptible variety cultivated at Miyata, Aichi Prefecture, Honshu, Japan.

In Experiments 4 and 5, the oospores collected in 1930, at Miyata were used. The results clearly show that Hungarian millet and White Wonder are very resistant to the fungus in question.

Experiment 6 (Jan. 22, '31)

Variety	No. of plants	No. of infected	% of infection
Sato-damashi	120	40	33
Neko-ashi	184	36	20
Meaki-damashi	117	22	19
Sogen-mochi	82	38	46
Echigo	75	23	31
Kan-hoku 115	154	20	13
Heinan 163	115	22	19
Hei-nan 23	192	30	16
Chosen	30	14	47
Okuaka	77	10	13
Akauchikiri	89	13	15
Shina 75	169	18	11
Heihoku 277	78	14	18
Shina 29	117	12	10
Kotaro	148	4	2.7
Kanhoku 11	68	2	2.9
Heihoku 312	100	2	2.0
Kannan 133	134	2	1.5
Shina 129	117	1	0.9
Wobai	100	1	1.0
Kogen 87	97	1	1.03
Shina 39	138	0	0
Shina 45	69	0	0
Shina 70	82	0	0
Shina 111	101	0	0
Heihoku 320	85	0	0
Heihoku 402	130	0	0
Kokubo	189	0	0
Inagoshirazu	166	0	0
Oawa	78	1	1.3
Kanhoku 195	82	0	0
Heinan 144	112	0	0
Awa-ocho	61	1	1.6
Hozan-akakuki	88	0	0

The oospores used in this experiment were obtained from the same source as in Experiment 2. 200 seeds of each variety were

sown, but their germination was unfortunately very poor in certain varieties. This invalidates greatly the value of the results of this experiment. It is however evident from the results shown in the above table that there are various grades of susceptibility among different varieties.

(*b*) *On Green Foxtail, Setaria viridis*

Experiment 1 (May 2, '30)

	No. of plants	No. of infected	% of infection
Pot 1	315	2	0.6
Pot 2	182	1	0.6
Experiment 2 (May 10, '30)			
Can 1	80	0	0
Can 2	66	1	1.5
Can 3	78	0	0
Can 4	77	1	1.3
Experiment 3 (May 12, '30)			
Can 1	67	2	3.0
Can 2	62	1	1.6
Can 3	53	1	1.9
Experiment 4 (July 5, '30)			
Pot 1	52	2	3.9
Pot 2	53	4	7.6
Pot 3	45	1	2.2
Experiment 5 (Aug. 26, '30)			
Pot 1	215	0	0
Pot 2	182	0	0
Experiment 6 (Aug. 30, '30)			
Pot 1	310	0	0
Pot 2	330	2	0.6

The results reveal that the infection of green foxtail seedlings by the fungus under consideration is very low. The infected seedlings in Experiments 1 and 4 were left in the pots to observe the process of their further growth. It was found that all the affected plants became dwarfed, sprouting poor side-branches; neither were conidia produced on the third and subsequent leaves, nor were oospores formed. The oospores used in this experiment were obtained in 1929. They were found to germinate very well.

(*c*) *On Varieties of Corn*

Experiment 1 (Oct. 18, '29)

Variety		Pot	No. of plants	No. of infected	% of infection
Pop corn	White Rice	1	32	0	0
Dent corn	Improved Leaming	1	23	0	0
Sweet corn	Golden Cream	1	28	0	0

Experiment 2 (Nov. 21, '29)

This was practically a repetition of Experiment 1. The results were apparently negative, since there was no sign of infection observed and no conidia were produced. In these experiments, the oospores collected in 1928 at Kakudahama-Mura, Niigata Pref. were used, and their germination was found to be fairly good. The seeds were obtained from a seed store in San Francisco, Calif., U. S. A.

Experiment 3 (March 24, '30)

Variety		No. of plants	No. of infected	% of infection
Pop corn	Japanese Hulless	24	1	4.2
Dent corn	Leaming	37	0	0
	Silvermine	44	0	0
	Reids' Yellow	45	0	0
Sweet corn	Golden Bantam	62	0	0
	Country Gentleman	50	0	0

The seed was obtained from a seed store at Lincoln, Nebraska, U. S. A. The oospores used as inoculum were collected in 1928, at Kakudahama-Mura, Niigata Pref. The germination of these oospores was found to be rather poor. The soil was apparently much moistened at the beginning of the experiment.

Experiment 4 (May 8, '30)

Variety		No. of plants	No. of infected	% of infection
Pop corn	Japanese Hulless	27	17	63
	Yellow Pearl	25	18	72
Dent corn	Leaming	25	10	40
	Improved Leaming	23	9	39
	Reids' Yellow	33	16	49
	Silver King	34	21	62
Sweet corn	Golden Bantam	30	29	97
	Country Gentleman	33	20	61
	Evergreen	32	16	50

The seed was obtained from the State Agricultural College, Ames, Iowa, U. S. A. by the kindness of Prof. I. E. MELHUS. The oospores used in this experiment germinated very well.

Experiment 5 (May 12, '30)

Variety		No. of plants	No. of infected	% of infection
Pop corn	Japanese Hulless	44	13	30
	Yellow Pearl	33	23	70
Dent corn	Leaming	25	14	56
	Reids' Yellow	30	8	27
	Silver King	30	18	60
Sweet corn	Golden Bantam	34	31	91
	Country Gentleman	35	12	34
	Evergreen	30	22	73

This experiment was practically a repetition of Experiment 4, and the results obtained coincide closely with those of the latter.

Experiment 6 (Aug. 26, '30)

Variety		No. of plants	No. of infected	% of infection
Pop corn	White Rice	67	15	22
Sweet corn	Golden Bantam	54	44	81
	Country Gentlemen	88	52	59
	Bantam Evergreen	48	11	23
	Golden Cream	50	24	48

The seeds were obtained from a seed store in Madison, Wis., U. S. A.

The results of the above-mentioned experiments indicate that various varieties of corn are susceptible to the *italica*-fungus. It is also noticeable that there is a considerable difference in susceptibility among different varieties. Conidia were, in general, produced more abundantly on the more susceptible varieties. Golden Bantam and Japanese Hulless showed the most abundant production of conidia.

(*d*) *On Other Graminaceous Plants*

Experiment 1 (March 24, '30)

Plant name	Pot	No. of plants	No. of infected	% of infection
Black Amber Cane	a	36	0	0
	b	35	0	0
Red Amber Cane	a	48	0	0
	b	38	0	0
Teosinte	a	32	0	0

The seeds were obtained from the Agronomy Department of the State Agricultural College, Lincoln, Neb., U. S. A.

Experiment 2 (May 8, '30)

Plant name	No. of plants	No. of infected	% of infection
White Kafir	41	9	22
Black Amber Cane	45	8	18
Red Amber Cane	45	13	29
Teosinte	48	25	52

Experiment 3 (Aug. 20, '30)

Plant name	No. of plants	No. of infected	% of infection
Black Amber Cane	84	14	17
Red Amber Cane	132	68	52
Early Amber Cane	56	6	11
South Amber Cane	42	6	14
White Kafir	74	13	18
Feterita	38	0	0
Milo Maize	45	4	9
Japanese millet	81	0	0
Yellow Foxtail	85	0	0
Teosinte	77	50	65
Broom Corn millet	54	0	0

Experiment 4 (Aug. 26, '30)

Plant name	No. of plants	No. of infected	% of infection
White Kafir	45	12	27
Milo Maize	48	6	13
Feterita	42	0	0
Broom Corn millet	65	0	0
Yellow Foxtail	78	0	0
Japanese millet	73	0	0

Experiment 5 (Jan. 20, '31)

Plant name		No. of plants	No. of infected	% of infection
Sorghum	Orange	48	4	8
	Sumac	52	9	17
	Planter	36	14	39
	Honey	56	0	0

The seeds used in Experiments 2–5 were obtained partly in Madison, Wis., and partly in Washington, D. C.

It is evident from the results given above that cane, kafir, teosinte and sorghum are susceptible to the *italica*-fungus, while Japanese millet, broom corn millet, yellow foxtail, and feterita are apparently not infected.

Besides the above-mentioned experiments, the oospores sent from India were inoculated on several susceptible varieties of Italian millet and of corn. But, no infection occurred owing apparently to the unsuitable oospores.

2. *Inoculation Experiments with the viridis-Fungus* on Various Graminaceous Plants*

(*a*) *On Italian millet*

Experiment 1 (Nov. 6, '28)

Variety	Pot	No. of plants	No. of infected	% of infection
Echigo	a	38	2	5.3
	b	45	1	2.2
	c	56	4	7.1
	d	62	1	1.6

The oospores used in this experiment were collected in Kyoto in late September, 1928.

* The "*viridis*-fungus" means the fungus parasitic on *Setaria viridis*.

Experiment 2 (May 3, '29)

Variety	Pot	No. of plants	No. of infected	% of infection
Echigo	a	10	0	0
	b	8	0	0
	c	12	0	0
	d	20	3	15
	e	12	0	0

Five other pots were prepared as controls in this experiment, which were all free from disease until the end of the experiment. In the control pots, 50 to 80 seedlings emerged. Many of the seedlings in the inoculated pots were attacked by a species of Helminithosporium. This is the reason why the number of the seedlings shown in the above table is so small.

Experiment 3 (June 2, '29)

Variety	Porcelain dish	No. of plants	No. of infected	% of infection
Echigo	a	250	8	3.2
	b	153	1	0.6

This was a repetition of Experiment 2, excepting that porcelain dishes were used instead of pots. One control dish was prepared. The results indicate that the *viridis*-fungus can infect Italian millet, although the percentage of infection is very low.

Experiment 4 (March 11, '30)

In this experiment, two pots were inoculated with the oospores collected in 1929, in Kyoto, Japan. The results were negative. Since these oospores germinated in a small number, the negative results might be connected with this poor germination of the oospores.

Experiment 5 (Jan. 14, '31)

Variety	No. of plants	No. of infected	% of infection
Common millet	53	28	53
Echigo	45	2	4.4
Japanese millet	35	0	0
Broom corn millet	40	0	0
Green foxtail (Control)	188	180	96

The oospores used in this experiment were collected by Prof. I. E. MELHUS, at Ames, Iowa in September, 1930. They germinated very well. It is evident from the table that the variety, common millet is much more susceptible than the variety, Echigo brought from Japan.

It is noteworthy that the seedlings of green foxtail were decidedly infected.

(*b*) *On Other Graminaceous Plants*

Experiment 1 (Jan. 16, '31)

Variety	No. of plants	No. of infected	% of infection
Brom corn millet	51	0	0
Japanese millet	52	0	0
Yellow foxtail	185	0	0
Kin-enokoro*	14	0	0
Enokorogusa*	19	0	0
Murasaki-enokoro*	6	0	0
Honey sorgho	20	3	15
Orange sorgho	45	8	18

* Kin-enokoro: *Setaria glauca*; Enokorogusa: *Setaria viridis*; Murasaki-enokoro: *Setaria viridis*, var. *purpurea*. All these seeds were collected in Japan.

The inoculum was taken from the same oospore source as used in the above experiment. The germination of Kin-enokoro, Enokorogusa and Murasaki-enokoro was very poor, and therefore, the results for these three kinds of plants are not very reliable.

Experiment 2 (Jan. 20, '31)

This was a repetition of the above experiment with the exception of different plants.

Variety	No. of plants	No. of infected	% of infection
Japanese millet	68	0	0
Yellow foxtail	218	0	0
Broom corn millet	92	0	0
Golden Cream	20	0	0
White Rice	21	0	0
Japanese Hulless	54	9	17
Bantam Evergreen	23	0	0
Golden Bantam	31	0	0
Green foxtail (Control)	132	115	87

(c) *Comparative Inoculations with the* viridis-*Fungus from Different Localities.*

Experiment 1 (Jan. 13, '31)

Fungus	Plant	No. of plants	No. of infected	% of infection
Iowa-fungus	Common millet	73	24	33
Minnesota-fungus	Ditto	77	47	61
S. Dakota-fungus	Ditto	67	47	70
Kyoto-fungus	Ditto	90	1	1.1

Experiment 2 (Jan. 21, '31)

Fungus	Plant	No. of plants	No. of infected	% of infection
Iowa-fungus	Green foxtail	51	47	92
	Hungarian millet	74	1	1.4
Minnesota-fungus	Green foxtail	82	82	100
	Hungarian millet	105	38	36
S. Dakota-fungus	Green foxtail	10*	5	50
	Hungarian millet	96	2	2.1
Kyoto-fungus	Green foxtail	68	8	12
	Hungarian millet	72	0	0

* Many seedlings were wilted by the attack of Fusarium.

The oospores used in these experiments germinated very well except those of the Kyoto-fungus. The results of the experiments indicate that the percentage of infection is somewhat different with the fungi from different localities. For instance, Hungarian millet is more susceptible to the Minnesota-fungus than the others.

3. *Symptoms on the Host-Plants other than* Setaria italica

During the inoculation experiments in relation to host range, the symptoms on the following plants have been observed.

(1) On green foxtail

The symptoms presented in the seedling stage of the host plant are quite similar to those of the Italian millet. Later, however, the infected plants become dwarfed, giving rise to poor side-branches. Conidia are found on the first or second leaf of the seedling. So far, no conidia and oospores have been found on stunted mature plants.

(2) On corn

Within eight to twelve days after sowing, chlorotic streaks or spots are seen on the infected leaves, and conidia are produced on the chlorotic areas, when the external conditions are favorable. The amount of conidia produced differs according to the susceptibility of the variety. On such a susceptible variety as Golden Bantam or Japanese Hulless, abundant conidia are produced, while on resistant varieties, such as Leaming and Reids' Yellow, often no conidium is produced. All the affected plants become stunted later.

(3) On teosinte

Affected seedlings show a stunted appearance from the beginning. Small reddish purple flecks are usually found on the leaves and stems. Conidia are sparsely produced on some of the affected leaves.

(4) On cane

Affected seedlings become stunted. Red-purplish streaks or flecks are commonly seen on the leaves or stems. Conidia are rarely produced.

(5) On sorghum

Affected seedlings become stunted. Reddish purple streaks are seen. Conidia are very rarely formed.

4. Conclusions regarding Host Range

(1) The susceptibility of Italian millet to the *italica*-fungus collected in Japan varies greatly with different varieties. Some are very susceptible, while others are apparently immune.

(2) The *italica*-fungus collected in Japan can infect green foxtail, *Setaria viridis*, but the percentage of infection has always been found to be very low.

(3) The *italica*-fungus can infect various varieties of corn, and the grade of susceptibility varies with different varieties.

(4) Certain varieties of sorghum, cane, and teosinte are susceptible to the *italica*-fungus, while yellow foxtail, Japanese millet, broom corn millet, and feterita are apparently immune.

(5) The *viridis*-fungus collected in Japan and in the United States of America can each infect certain varieties of Italian millet.

(6) Certain varieties of sorghum, and corn are susceptible to the *viridis*-fungus, while yellow foxtail, Japanese millet, and broom corn millet, are apparently immune.

(7) The *viridis*-fungus collected in Minnesota is different in its infecting power from that obtained in three other localities.

(8) It has been found that the percentage of seedling infection is influenced to a great extent by the percentage of oospore germination.

Part II. Pathological Investigations

I. LIFE-HISTORY OF THE CAUSAL FUNGUS AS RELATED TO PATHOGENESIS

It has long been believed that the oospores are the source of the primary infection. This presumption, however, has been only recently proved experimentally.

In 1925, MELHUS and VAN HALTERN (70) reported the success of artificial inoculation on *Setaria viridis* and corn with oospores of the Sclerospora from *Setaria viridis*.

When the writer began these investigations, no experimental information had been published about the life-history of the Sclerospora parasitic on *Setaria italica*, and it was thought to be significant to make some inoculation experiments.

Experiment 2

In August, 1927, abundant oospores were collected in Niigata Prefecture. Some of them were after a while mixed with the soil in pots outdoors, and others were kept in the laboratory as dry specimens. A part of the latter was also mixed in the soil in pots later on January 31, 1928, and another part in the soil in the field on February 7, 1928.

The seed of Italian millet, variety Echigo was sown in the infested pots on April 1, April 15, May 1, May 15, June 1, June 15, July 1, and July 15, respectively. Besides these, seeds of Echigo and Mukodamashi were sown in the infested field on May 17, 1928.

In the morning of June 6, 1928, the conidial production was first found simultaneously on some seedlings sown on April 1, April 15, May 1, May 15 and May 17. As we had heavy frost toward the end of April, the seedlings sown before this time were somewhat injured, and as a result the plants sown earlier were only slightly larger than those sown later.

The sign of infection was also found on June 12 on some seedlings sown on June 1, and on July 5 on some sown on June 15, but no infection was recognized on any seedlings sown on July 1 and July 15. During these experiments, control plants remained healthy. It was noticed that variety Echigo was more susceptible than variety Mukodamashi.

Experiment 2

The purpose of this experiment was to determine whether infection due to oospores can take place even in autumn. The plants were removed from some infested pots used in Experiment 1, and the pots were left outdoors. On October 1, 1928, seeds of variety Echigo were sown in these pots, and were held under observation. Seedling infection was found to occur on October 21.

Experiment 3

In order to determine whether or not the oospores can induce the primary infection within the year they were produced, another experiment was carried out.

At the end of September, 1928, abundant oospores were collected from plants artificially inoculated. On November 2, of the same year, the seed of variety Echigo was sown in four infested pots. They were first placed outdoors, but after five days, removed into the green house where the temperature ranged from 25° to 10° C. On November 16, the sign of infection was found in all the infested pots, while controls remained free from the disease.

From the results of the experiments mentioned above, the following points may be concluded:

(1) The oospores of the fungus in question cause seedling infection.

(2) The oospores can induce seedling infection within the year of their production.

In the course of these inoculation experiments, it was presumed that seedling infection may take place before the emergence of seedlings from the ground, and the affected seedlings become systemically infected.

In order to determine whether or not the oospores can infect the seedling through the aerial parts, some inoculation experiments were carried out in May, 1929 in Berkeley, Calif., U. S. A. and in June, 1932 in Gifu, Japan.

Oospore suspension was atomized on the seedlings of various growing stages, and the inoculated seedlings were kept in moist chambers for two or three days. No infection occurred in any of the experiments.

In connection with the life-history of the causal fungus, some inoculation experiments with conidia were also made. However, the secondary infection by zoospores occurred very rarely as already reported by the writer (50). This coincided well with the results of field observations. Later it has been found that the secondary infection may take place on certain varieties in the field.

In this connection, it is interesting that CHAUDHURI (13) has succeeded in infecting young plants of bajra, *Pennisetum typhoideum*, by placing loopfuls of oospore suspensions on both surfaces of the leaves of the plants.

II. STUDIES ON SEEDLING INFECTION

(I) Relation of the Growing Stages of the Seedling to Infection

As already stated, the primary infection on young seedlings takes place in the soil before their emergence. It is very desirable to determine the critical point of the seedling infection decisively for the advancement of the environmental studies. For this purpose, several series of inoculation experiments have been carried out.

Methods of Experimentation

Unless otherwise stated, the seeds of the Italian millet were germinated at 30° C. in a saturated environment, and at the time of inoculation, the seedlings were transferred to moist Petri-dishes or glasses, in both parts of which a layer of moist absorbent cotton had been placed. Since the growth is rapid at this temperature, it was found very easy to obtain the seedlings at various stages of development in a short period of time. For the inocula, germinating oospores, 45–48 hours after sowing at 29°–30° C., were used throughout the experiments.

Before inoculating, a small mass of germinating oospores was immersed in a drop of sterilized distilled water on a slide by means of a fine needle for the purpose of separating and also of properly moistening the oospores.

Special attention was paid to bringing the inoculum into direct contact with the parts of the seedlings. If the inoculum contains too much water, germinating oospores are liable to be suspended in water drops on the inoculated parts. Such a situation should be carefully avoided. However, it was found that when the seedlings are grown to a certain extent, the coleoptile and the mesocotyl increase their character of repelling water, and consequently water drops are more easily formed on the parts inoculated.

All the inoculations were made in the laboratory where the temperature ranged about 18° to 20° C. during the night, and 23° to 27° C. in the daytime. Every inoculation was performed at 5 to 7 p.m. in order to take advantage of cooler temperatures during the first period of infection. Thirty-five to forty-eight hours after inocu-

lation, the covers of the Petri-dishes or glasses were removed, and the seedlings growing in moist absorbent cotton were placed under large bell-jars for the purpose of preventing rapid evaporation of water. Water was also added at intervals when necessary, and the cotton was always kept suitably moistened during the course of the experiments. All the cultures were placed on a table near the window of the laboratory until the end of the experiments. Twelve days after inoculation, the cultures were kept in moist chambers for one night, and records were taken. In case infection was suspected on any seedlings, those were continued in moist chambers two or more nights.

Sometimes, it was found that conidial production took place on severely infected seedlings as early as five days after inoculation. The air humidity within the bell-jars appeared to be high enough for the production of conidia on severely infected seedlings. To make the results surer, however, the seedlings were kept in moist chambers as mentioned above, before final records were taken. A susceptible variety Echigo was used throughout.

Experimental Results

Coleoptile, mesocotyl and root of the seedling of Italian millet were exposed to infection. In order to determine which of these may permit the entrance of the fungus, inoculations were attempted separately on each part.

1. *Coleoptile Series*

Experiment 1

The seedlings as high as 6 to 8 mm. were inoculated on the coleoptile, and kept for 48 hours in a saturated atmospheric moisture. Two of the three dishes inoculated, were placed on a table in the laboratory during the experimental period, while the other, dish III, was kept in an incubator at 20° C. The results are shown in the following table.

TABLE 35

Occurrence of oospore infection on coleoptiles
(Experiment 1)

Plants	Dish I	Dish II	Dish III	Total
Plants inoculated	30	30	24	84
Plants infected	5	3	3	11
% of infection	16.7	10.0	12.5	13.1

It was found in this experiment that thirty seedlings in one dish were too many for inoculation, because the germinating oospore masses inoculated earlier, began to dry before the remainder were inoculated, and it was found very troublesome to supply water again.

The above results clearly show that the fungus can infect the seedling through its coleoptile when the seedling is 6 to 8 mm. in height.

Experiment 2

In this experiment, the coleoptiles just emerged from the seeds at 20° C. were inoculated. The dishes were kept for 48 hours at the laboratory temperature (19°–25° C.). When the covers were removed, the seedlings were grown to 1–1.5 cm. in height. Oospore masses were mostly found on the coleoptile, but a few on the mesocotyl. The latter seedlings were discarded. The results are shown in the next table.

TABLE 36

Occurrence of oospore infection on coleoptiles
(Experiment 2)

Plants	Dish I	Dish II	Total
Plants inoculated	19	18	37
Plants infected	10	9	19
% of infection	52.6	55.00	51.4

Remarks:
1) In Dish I, one seedling was attacked by Helminthosporium, and one attacked by Fusarium.
2) In Dish II, one seedling was attacked by Fusarium.

The seedlings attacked by Helminthosporium or Fusarium are included in the numerals of the table mentioned above, because the first leaves were grown to such an extent that conidia could be produced. It was frequently noticed in other experiments, that some of the infected seedlings almost wilted due to Fusarium, showed evidence of infection, when they were removed to a saturated chamber.

The results of this experiment confirmed the coleoptile-infection proved in Experiment 1, and moreover higher infections took place. These results seem to indicate that the coleoptile-infection by oospores occurs more readily in younger seedlings.

Experiment 3

This experiment was practically a duplicate of Experiment 2. The results are shown in the following table.

TABLE 37

Occurrence of oospore infection on coleoptiles
(Experiment 3)

Plants	Dish I	Dish II	Total
Plants inoculated	9	11	20
Plants infected	6	7	13
% of infection	66.7	63.6	65.0

Remarks:
1) In Dish I, two seedlings became wilted before records were taken, and these are not included above.

The results of this experiment are in accord with those obtained in Experiment 2, substantiating the conclusions that (1) infection takes place through the coleoptile, and (2) the percentage of infection is much higher when the coleoptiles are inoculated at a very early stage of growth.

Experiment 4

The purpose of this experiment was to determine the occurrence of infection at later stages of coleoptile development. The methods used were essentially the same as in the preceding experiments, excepting that when the seedlings had reached desired height, they were kept for a while at 12° C. to control the over-growth until the inoculum would be ready, since the growth of the seedlings is very slow at this temperature.

Two sets of inoculation were made. In Set 1, the seedlings were 4–5 mm. in height, and their coleoptiles and mesocotyls were 3–4 mm., and 1–2 mm. in length respectively, while in Set 2 the seedlings were 9–13 mm. in height, and their coleoptiles and mesocotyls were 4–5 mm. and 5–8 mm. in length. The results of the inoculations are shown in the following table.

TABLE 38

Occurrence of oospore infection on coleoptiles
(Experiment 4)

Set 1

Plants	Dish I	Dish II	Total
Plants inoculated	20	20	40
Plants infected	15	13	28
% of infection	75.0	65.0	70.0

Set 2

Plants inoculated	20	20	40
Plants infected	4	3	7
% of infection	20.0	15.0	17.5

When the covers were removed, the seedlings of Set 1 were 1–1.5 cm. and those of Set 2 were 2.5–4 cm. in height respectively.

The results clearly show that the coleoptiles increase the degree of resistance to infection as they grow taller.

Experiment 5

In order to determine the maximum stage of coleoptile development for infection, the following experiments were made. The seedlings were inoculated in proper glasses, instead of Petri dishes, because the seedlings were too tall. The results are shown in the following table.

TABLE 39

Occurrence of Oospore Infection on Coleoptiles
(Experiment 5)

Height of seedlings	2–2.5 cm.			3–4 cm.		
Glass	1	2	3	1	2	3
Plants inocul.	15	15	15	10	15	20
Plants infect.	0	0	0	0	0	0

Reviewing all the results obtained in the preceding experiments, the following conclusions may be drawn:

(1) Infection by germinating oospores may take place through the cells of the coleoptile of the seedling.

(2) The coleoptile is very susceptible to oospore infection from its earliest stage of development until the seedlings grow to about 0.5 cm. tall, and then they become very resistant when their heights reach 2 cm. or more.

2. *Mesocotyl Series*

In order to determine whether the fungus can infect the seedling through the mesocotyl the following experiments have been performed. The methods used were essentially the same as those described in the experiments of coleoptile series, excepting that the inoculum was placed on the mesocotyl instead of on the coleoptile.

Experiment 1

In this experiment were used the seedlings 4–5 mm. high, with mesocotyls about 1–2 mm. in length. The inocula were applied near the basal portion of the mesocotyls. The results obtained are shown in the following table.

TABLE 40

Occurrence of Oospore Infection on Mesocotyls

(Experiment 1)

	Dish I	Dish II	Dish III	Dish IV	Total
Plants inocul.	20	15	20	20	75
Plants infect.	11	10	13	10	44
% of infection	55.0	66.7	65.0	50.0	58.7

When the covers were removed, the seedlings were grown to 1–1.5 cm. in height. It was frequently found that severely infected seedlings were bent at the inoculated portion of the mesocotyl, and the growth of the mesocotyl was greatly retarded. Most of such seedlings showed irregularly watersoaked, transparent lesions in their first leaves.

The results demonstrate that the fungus readily infects the seedling through the mesocotyl, and it is noteworthy that high percentages of infection took place in all dishes.

Experiment 2

In this experiment, seedlings grown at 20° C. when 6–10 mm. in height were inoculated on their mesocotyls which were 3 to 5 mm. long. The inocula were applied to the middle portion of the mesocotyl. The results are presented in the next table.

TABLE 41

Occurrence of Oospore Infection on Mesocotyls
(Experiment 2)

	Dish I	Dish II	Dish III	Total
Plants inocul.	20	20	20	60
Plants infect.	5	8	5	18
% of infection	25.0	40.0	25.0	30.0

When the covers were removed thirty-five hours after inoculation, the seedlings were grown to 2.5 to 4 cm. in height. All the inocula remained a little above the basal portion of the mesocotyl.

The results are in accord with those obtained in Experiment 1, showing that the fungus can infect the seedling through the mesocotyl. It is noteworthy that the percentages of infection in this experiment were lower than those in Experiment 1.

Experiment 3

In order to obtain further information about the mesocotyl infection, inoculations were made in parallel on the following seedlings in various stages:

(1) Set 1: seedlings 8 mm. tall

(2) Set 2: seedlings 10 mm. tall

(3) Set 3: seedlings 15 mm. tall

(4) Set 4: seedlings 25 mm. tall

The inocula were applied to the basal portion of the mesocotyl. Large Petri-dishes were used for Sets 3 and 4. The results are presented in the following table.

TABLE 42

Occurrence of Oospore Infection on Mesocotyls
(Experiment 3)

Set 1 (Seedlings 8 mm. tall)

	Dish I	Dish II	Total
Plants inoculated	10	10	20
Plants infected	4	3	7
% of infection	40.0	30.0	35.0

Set 2 (Seedlings 10 mm. tall)

Plants inoculated	10	15	25
Plants infected	2	3	5
% of infection	20.0	20.0	20.0

Set 3 (Seedlings 15 mm. tall)

Plants inoculated	10	15	25
Plants infected	0	0	0

Set 4 (Seedlings 25 mm. tall)

Plants inoculated	10	15	25
Plants infected	0	0	0

The results clearly indicate that the mesocotyl increases its resistance to infection as it grows older. If we compare these results with those in Experiment 1 and 2, a gradual increase of resistance in older seedlings is more clearly seen. It is also recognizable that there is no marked difference in susceptibility between the seedlings grown at 20° C. and those at 30° C. (Compare Experiments 2 and 3).

Experiment 4

In order to determine further the latest stage of mesocotyl development for infection of the fungus, two more sets of experiments were attempted. Glasses were used instead of Petri-dishes, because the seedlings were expected to grow too high to be held in the latter.

In Set 1, seedlings 2 to 2.5 cm. tall were inoculated at the basal part of the mesocotyl. Two glasses were kept at 20° C., and the other two at 30° C., for 35 hours after inoculation.

In Set 2, seedlings 3 cm. tall were inoculated at the middle part of the mesocotyl in one glass, and at its basal part in the other glass. The inoculated seedlings were placed in the laboratory from the beginning to the end of the experiment. The results are shown in the next table.

TABLE 43

Occurrence of Oospore Infection on Mesocotyls

(Experiment 4)

Set 1 (2–2.5 cm. tall)

	20°C.			30°C.		
Glass	1	2	(Total)	1	2	(Total)
Plants inocul.	15	15	30	15	15	30
Plants infect.	0	0	0	0	0	0

Set 2 (3 cm. tall)

	Inoculated at the basal portion	Inoculated at the middle portion
Plants inocul.	15	15
Plants infect.	0	1
% of infection	0	6.7

When the covers were taken off, the seedlings kept at 30° C. were found to be twice as tall as those grown at 20° C. The results show that infection may take place even when the seedlings grow to 3 cm. in height, although the percentage of infection is very low.

Further studies are necessary to determine the maximum stage of mesocotyl development for infection, but it is quite evident from the results obtained in Sets 3 and 4 of Experiment 3, and also from the results obtained in the present experiment that the mesocotyl greatly increases its resistance to infection when the seedling grows over 15 mm. in height.

The results of Set 2 shown in TABLE 43 indicate that infection took place only when the seedlings were inoculated at the middle part of the mesocotyl, while no infection took place when the seedlings were inoculated at the basal portion of the mesocotyl. Whether this situation came accidentally, or whether it is of significance in mesocotyl infection will be another interesting theme of study.

From all the results obtained in the experiments of mesocotyl infection, the following conclusions may be drawn:

(1) Infection due to oospores takes place through the mesocotyl of the seedling.

(2) The relation of the stage of mesocotyl development to infection is similar to that of coleoptile development, and the younger mesocotyl is more readily invaded by the fungus than the older one.

3. *Root Series*

In order to determine whether the fungus can infect the seedlings through the root, the following experiments were made. In this series, the seedlings were kept at 20° C. during the period of inoculation.

Experiment 1

The inoculum was carefully applied to the primary roots which had grown to two to three mm. in length. Such materials were secured at the 24th hour after sowing at 30° C. The coleoptile was still invisible in these seedlings. The results are shown in the following table.

TABLE 44

Occurrence of Oospore Infection on Roots
Experiment 1

	Dish I	Dish II	Total
Plants inoculated	25	20	45
Plants infected	7	6	13
% of infection	28.0	30.0	28.9

When the covers were removed after forty-eight hours, the inocula remained just where they had been applied. It may be of interest to note that all the infected seedlings in this experiment showed a decidedly poor growth. After ten days, the length of the affected seedlings was about half that of the healthy ones. The results show that infection may take place through the young root.

Experiment 2

This was practically a duplicate of Experiment 1, excepting that primary roots little older than those in Experiment 1 were inoculated. The primary roots were 3 to 4 mm. long, and the coleoptiles developed a little on all the germinating seeds. The results are shown in the following table.

TABLE 45

Occurrence of Oospore Infection on Roots
Experiment 2

	Dish I	Dish II	Total
Plants inoculated	23	20	43
Plants infected	5	2	7
% of infection	21.7	10.0	16.3

In this experiment, it was also observed that the affected seedlings grew far more poorly than the healthy ones. The results of this experiment substantiate those of Experiment 1, demonstrating that the fungus can infect the seedling through the root. The percentage of infection, however, was lower than in Experiment 1.

Experiment 3

In order to determine the relation of various stages of root development to infection, four sets of inoculation experiments were carried out, the lengths of the roots inoculated being as follows:

Set 1: primary roots 1.5 to 2 mm. long (22 hours after sowing, no coleoptile developed.)

Set 2: primary roots 4 mm. long (30 hours after sowing, no coleoptile developed.)

Set 3: primary roots 8 to 10 mm. long (34 hours after sowing, coleoptile already developed.)

Set 4: primary roots 15 mm. long (38 hours after sowing, coleoptile already developed.)

Seed germination was made in an incubator held at 30° C., and the inocula were applied to the middle part of the primary roots. The results obtained are shown in TABLE 46.

TABLE 46

Occurrence of Oospore Infection on Roots
(Experiment 3)

Set 1 (Primary roots 1.5–2 mm. long)

	Dish I	Dish II	Total
Plants inoculated	15	15	30
Plants infected	8	11	19
% of infection	53.3	73.3	63.3

TABLE 46 (*Continued*)

Set 2 (Primary roots 4 mm. long)

	Dish I	Dish II	Total
Plants inoculated	10	15	25
Plants infected	2	2	4
% of infection	20.0	13.3	16.0

Set 3 (Primary roots 8–10 mm. long)

Plants inoculated	20	15	35
Plants infected	1	0	1
% of infection	5.0	0	2.9

Set 4 (Primary roots 15 mm. long)

Plants inoculated	15	15	30
Plants infected	0	0	0
% of infection	0	0	0

It is evident from the results shown in the above table that infection takes place at very early stages in the development of primary roots. The growth of the affected seedlings was generally very poor.

Judging from the results obtained in the foregoing experiments regarding the root infection, the following conclusions may be drawn:

(1) The fungus can infect the seedling through the young roots.

(2) In the early stage of development of the primary root, from the beginning up to the time when they reach about 3 mm. in length, the roots are very susceptible.

(3) The resistance to infection of primary roots greatly increases when they grow to 15 mm. long.

(4) The growth of the seedlings affected through the roots is generally very poor.

(II) Incubation Period for the Seedling Infection caused by the Mycelium from Oospores

It is evident from the experimental data concerning the relation of the stage of seedling development to infection that 48 hours are sufficient to give rise to infection by the mycelium from oospores. In order to determine the minimum period of incubation required for infection, a series of experiments was carried out.

Young seedlings 3 to 4 mm. high were inoculated at the base of the mesocotyl with germinating oospores 45 to 48 hours old. Moist Petri-dishes containing inoculated seedlings were kept at 20° C. for 20 hours, 25 hours, and 30 hours, respectively. After these periods of inoculation, the covers were taken off, and the oospore-masses in water drops were wiped off from the seedlings, since otherwise they might remain long in action in moist condition. The seedlings were then placed on a table in the laboratory as usual. This experiment was started on April 28 and terminated on May 10, 1930. The results are shown in the following table.

Table 47

Incubation Period for Seedling Infection by the Mycelium from Oospores

	20 hours' incubation		
	Dish 1	Dish 2	Total
Plants inoculated	15	10	25
Plants infected	9	5	14
% of infection	60	50	56

TABLE 47 (*Continued*)

	25 hours' incubation		
	Dish 1	Dish 2	Total
Plants inoculated	15	10	25
Plants infected	12	6	18
% of infection	80	60	72
	30 hours' incubation		
Plants inoculated	15	10	25
Plants infected	10	6	16
% of infection	67	60	64

Another experiment was carried out to get further information along the same line. The results are given in the next table.

TABLE 48

Incubation Period for Seedling Infection by the Mycelium from Oospores

	10 hours' incubation		
	Dish 1	Dish 2	Total
Plants inoculated	10	12	22
Plants infected	0	0	0
	15 hours' incubation		
Plants inoculated	10	10	20
Plants infected	2	0	2
% of infection	20	0	10
	20 hours' incubation		
Plants inoculated	10	10	20
Plants infected	3	5	8
% of infection	30	50	40

The results given in the above table indicate that 15 hours' incubation may be sufficient to induce seedling infection.

(III) Relation of Age of the Mycelium germinated from Oospores to Seedling Infection

The mycelium germinated from the oospores is delicate in appearance, suggesting its short-lived nature. If it is exposed to the air of the laboratory, it shrivels up soon. In order to determine how long the mycelium retains its contagious power under moist conditions, some experiments were carried out.

The methods used were essentially the same as those already described, excepting that mycelia of different ages were used as the inocula. The oospores were germinated in Petri-dishes at 30° C. After forty-eight hours, the dishes were removed to the laboratory where the temperature was ranging from 18° to 28° C. during the course of the experiments.

Experiment 1

The mycelium, three days old*, was inoculated on the base of the mesocotyl of 2-day-old seedlings. The length of the mesocotyl was 1 to 2 mm. at the time of the inoculation. The inoculated seedlings were kept at 20° C. for forty-eight hours. When the covers were taken, the seedlings were grown to about 1.5 to 2 cm. long. The results are shown in the following table.

TABLE 49

Seedling Infection due to the 3-day-old Mycelium germinated from Oospores

(Experiment 1)

	Dish 1	Dish 2	Total
Plants inoculated	15	17	32
Plants infected	2	3	5
% of infection	13	18	16

* This means 3 days after the sowing of the oospores.

Experiment 2

Four-day-old hyphae were inoculated in this experiment. Young seedlings 3 to 4 mm. high were inoculated on their mesocotyls. The inoculated seedlings were left on a table in the laboratory. None of 30 seedlings inoculated in two dishes was infected.

Experiment 3

This was practically a repetition of Experiment 2. The results obtained are presented in the following table.

TABLE 50

Infection of the 4-day-old Mycelium from Oospores
(Experiment 3)

	Dish I	Dish II	Total
Plants inoculated	10	10	20
Plants infected	2	0	2
% of infection	20	0	10

Experiment 4 (May 1, '30)

In this experiment, 5-day-old and 6-day-old hyphae were inoculated on very young seedlings. Of 40 seedlings inoculated, none of them was infected.

The results obtained in these four experiments indicate that the hyphae retain their contagious power within a 4-day period.

(IV) Influence of Temperature upon the Behaviour of the Mycelium from Oospores as related to Seedling Infection

It is worth while to determine the possible relation of temperature to the behaviour of the mycelium germinated from oospores

with special reference to seedling infection. Some experiments were carried out for this purpose.

The methods used were exactly the same as already described. The seedlings, 3 to 5 mm. long were inoculated at the basal portion of the mesocotyl. The following three sets of inoculations were made at different temperatures:

(1) Set 1: at 30° C., (2) Set 2: at 20° C.,
(3) Set 3: at 15° C.

The inoculated seedlings were kept for forty hours at each temperature, respectively; then removed to the laboratory, and covered with large bell-jars. The results are shown in the next table.

TABLE 51

Influence of Temperature upon the Behaviour of the Mycelium from Oospores as related to Seedling Infection

Set I (30°C.)

	Dish I	Dish II	Total
Plants inoculated	20	20	40
Plants infected	16	13	29
% of infection	80	65	73

Set II (20°C.)

Plants inoculated	20	20	40
Plants infected	15	15	30
% of infection	75	75	75

Set III (15°C.)

Plants inoculated	20	20	40
Plants infected	16	14	30
% of infection	80	70	75

As seen from the table, high percentages of infection took place in all sets. The results clearly show that the infecting power of the mycelium from oospores is not greatly influenced between 15° and 30° C. so far as the percentage of infection is concerned.

It may also be of interest to note that the seedlings infected at 30° C. showed conidial production earlier than those infected at the lower temperatures. This may be ascribed to the fact that the growth of the seedling is more rapid at 30° C. than at lower temperatures, inasmuch as a certain amount of seedling growth is prerequisite to the production of conidia.

(V) Influence of Soil Factors upon Seedling Infection

A preliminary experiment was first performed to obtain a general idea upon the relation of soil temperature and soil moisture as well as soil type to seedling infection.

Two series of experiments, one with coarse sand and the other with sod soil, were carried on in parallel. In each series, four pots 10 cm. in diameter were used. Each two of these pots were placed in large Petri-dishes, into which water was poured to a depth of about 3 cm. Water was frequently added so as to keep a constant level during the course of the experiment. As a result, the soil surface of these pots always looked to be wet. The other two pots were placed on the sand layer on the floor of the green house, and a small quantity of water was added to the pots when the soil surface appeared to be too dry. The surface usually looked dry, however, because of very rapid evaporation. The temperature of the green house ranged from 16° to 26° C. in the course of this experiment.

The millet seeds were sown 2.5 cm. deep in the coarse sand and 2 cm. deep in the sod soil. Two hundred seeds were planted in each pot. Inoculations were made in the following manner. A quantity of rather dry sod soil or coarse sand was put in each porcelain pot, and sufficient water added to make the soil wet. After drainage, the seeds were placed uniformly on the soil, and the powdery oospores were scattered over them. Then, the seeds were buried, and water was applied by means of an atomizer in order to make the soil on

the seeds properly moist. Two weeks after sowing, all the pots were kept over-night in a saturated condition, and the affected seedlings showing the formation of conidia were counted. The results are recorded in TABLE 52.

TABLE 52

Influence of Soil Factors upon Seedling Infection

(a) The sod soil series

	Pot	Time for emergence	No. of Seedlings	No. of infected	% of infection
Wetter pots	1	6.5 days	31	0	0
	2	6.5 days	42	0	0
Dreyer pots	1	5 days	90	74	82.2
	2	5 days	113	79	69.9

(b) The coarse sand series

Wetter pots	1	5 days	196	26	13.3
	2	5 days	194	13	6.7
Dreyer pots	1	4.5 days	198	63	31.8
	2	4.5 days	198	56	28.3

It is evident from the results shown in the above table that soil moisture plays an important rôle in relation to seedling infection, and that a comparatively dryer condition is more favorable for the infection than a very wet condition. It is also seen that the percentage of seedling infection is influenced by the kind of soil. Under the conditions of this experiment, the difference of infection between wetter and dryer pots was more pronounced in the sod soil series than in the coarse sand series. Seed germination was found to be poorer in the sod soil, especially in wet pots. It is also noteworthy

that the infection took place more prominently in the dryer pots of the sod soil than in those of the coarse sand.

These results suggested the importance of studying analytically the relation of soil temperature, soil moisture, and soil type to seedling infection, respectively. Further experiments were, therefore, undertaken along these lines.

1. *Influence of Soil Temperature upon Seedling Infection*

Methods of Experimentation

For the purpose of controlling soil temperatures, three kinds of apparatus were used. The first is the soil temperature tank based, in principle, upon the so-called Wisconsin temperature tank. Galvanized iron pots were surrounded by water, the temperature of which was regulated by means of electric heaters or cold running water. The second is the glass temperature chamber located in a cold glass house where the temperature was kept at about 2° C. The temperature chambers were heated and controlled by electricity.* The third is the preparation-dishes which were kept for a definite period of time in incubators at different temperatures. The details of experimentation are given in the description of each experiment.

(*1*) *Experiments with Soil Temperature Tanks*

The soil temperature tanks equipped in a glass house were used. In order to avoid direct sunshine, a shade of thick paper was supported above the whole series of tanks. This was done because preliminary tests had shown that such direct sunlight on warm days changed the temperature of surface soil, which is particularly important in connection with the present investigation. On the other hand, attention was also given to the ventilation of the tank room in the daytime to make the air temperature as uniform as possible, while in the night the temperature was automatically regulated to

* These Experiments were conducted in the Department of Plant Pathology, University of Nebraska, U.S.A.

some extent by means of a steam heater. Thus, constant soil temperatures were secured fairly well.

Four different tanks held at temperatures of about 16°, 20°, 25°, and 30° C., respectively were used. The tank held at 16° C. was controlled by running water, the others by electric lamp and mercury regulator. Each tank accommodated four galvanized iron cans with three small pots in each can. Consequently, each tank contained twelve small pots in which inoculations were made.

The galvanized cans were filled with sod soil about four-fifths from the bottom so that the soil surface might be lower than the water level in the tank. The small pots were filled with sandy loam so as to bring the soil surface of both cans and pots to an equal levels. In these small pots, the seeds were sown and inoculated. The temperature record was taken from the soil in these small pots. Special care was paid to the measurement of temperature. All the thermometers had been previously adjusted with a standard thermometer. These were inserted directly into the soil about 3–4 centimeters deep, since it was noticed that soil temperature was somewhat different at different depths in the galvanized iron pots, especially in those kept at high temperatures.

One hundred seeds were placed on the soil of each pot, and oospore suspension was applied to the seeds by means of an atomizer. After the first application of oospore suspension, a small quantity of soil was added until the seeds were buried, and again oospore suspension was atomized upon this until the soil became moist; this was then repeated once more, and a quantity of soil was finally added to bury the seed to a depth of about 2 cm. Three porcelain pots thus prepared were immediately arranged in the soil of each galvanized can.

Since evaporation is rapid at the higher temperatures, the surface soil was carefully observed and sufficient additional water sprinkled upon it from time to time to maintain a slightly moist condition. No other attention was given to soil moisture in as much as previous experiments had shown the above method to insure good results.

The following table gives the outcome from this experimental series. In each case, the variation of soil temperature was kept within 1.5° C. of that stated.

TABLE 53

Influence of Soil Temperature upon Seedling Infection

Temp. C.	Can	I			II			III			IV			
	Pot	1	2	3	4	5	6	7	8	9	10	11	(12)*	Total
16°	No. of plants	61	3	76	13	40	29	32	58	18	37	9	(45)	376
	No. of infected	28	0	55	4	20	14	6	17	10	17	5	(0)	176
	% of infection	46	0	72	31	50	48	19	29	56	46	56	(0)	47
20°	No. of plants	68	52	76	54	78	56	46	70	76	68	55	(88)	699
	No. of infected	34	40	40	34	45	37	36	50	53	55	39	(0)	463
	% of infection	50	77	53	63	58	66	78	71	70	81	71	(0)	66
25°	No. of plants	65	84	56	58	80	72	85	60	84	82	85	(93)	811
	No. of infected	35	52	20	36	55	47	65	39	60	45	68	(0)	522
	% of infection	54	62	36	62	69	65	76	65	71	55	80	(0)	64
30°	No. of plants	86	75	85	89	92	83	84	86	78	90	82	(95)	930
	No. of infected	30	17	25	20	20	18	9	10	14	21	12	(0)	196
	% of infection	35	23	29	22	22	22	11	12	18	23	15	(0)	21

* Pot (12) is control; the seedlings in this pot are not included in the total sum of each temperature set.

In this experiment, the time required for the earliest emergence of seedlings was found to be: 6.5 days at 16° C.; 3.5 days at 20° C.; 3 days at 25° C.; and 2 days at 30° C., respectively.

On the 12th day after the emergence of most of the seedlings, the pots were kept for 24 hours in moist chambers in which the temperature ranged about from 18° to 20° C., the optimum temperature for the production of the conidia. It was found that the production was much less conspicuous on the seedlings in this experiment than in the field. As a result, close observations were often necessary to recognize the conidia produced.

Fewer seedlings emerged in the tank held at 16° C. than in the other tanks. However, in the tank held at 20° and 25° C., more seedlings than shown in the above table appeared, although some of them unfortunately became wilted by the attack of Fusarium before records were taken. A few wiltings were also found in the tanks at the other temperatures.

The results shown in the table indicate that fairly high percentages of infection take place between 16° and 30° C., and that the optimum temperature for infection apparently lies about 20° C. While the results obtained in the 16° C. tank are most irregular, it should be not overlooked that a very high percentage of infection was obtained in pot 3. This is significant as indicating that high infection may take place at 16° C., if other conditions are especially favorable.

(2) *Experiments with Glass Temperature Chambers*

The relation of the seedling development to infection had already been studied, and special attention was paid to soil temperature during the critical period of infection. The detailed description of the methods used is given below.

A sod soil containing water to the extent of 42 per cent of the maximum water holding capacity was put into pots which had been immersed in water just before inoculation. One hundred seeds were uniformly placed on the soil surface, and sterilized water was slightly atomized upon the seeds. Dry oospore-inoculum was then scattered upon the seeds, and a small amount of the soil was added to the pots until the seeds were buried entirely. The same inoculum was again scattered over the soil surface. Finally enough soil was added to bury the seeds about 2 cm. deep, and the soil surface was then softly pressed. The pots inoculated in this way were removed to the constant glass temperature chambers kept at different temperatures, and immediately covered with moist bell-jars in order to check the loss of water. This condition of soil moisture had been previously proved by preliminary experiments to be favorable for the seedling infection. Thermometers were inserted into the pots about 4 cm. deep. When most of the seedlings became 1 to 1.5 cm. tall, the bell-jars were taken off. After this, water was added to the pots when they appeared dry. The results are shown in the following table.

TABLE 54

Influence of Soil Temperature upon Seedling Infection

Temp. C.	Pot	No. of plants	No. of infected	% of infection	Time required for emergence
11°	1	66	0	0	15 days
	2	60	0	0	
15°	1	64	28	43.8	6 days
	2	70	30	42.9	
20°	1	63	53	84.1	4 days
	2	68	54	79.4	
24°	1	52	37	71.2	3 days
	2	61	43	70.5	
29°	1	83	21	25.3	2 days
	2	85	36	42.4	
34°	1	84	2	2.4	1.7 days
	2	85	1	1.2	

The pots kept at 11° C. were removed to a warmer green house after the bell-jars were removed, to make the seedlings grow more rapidly. The results shown in the table clearly indicate that the optimum soil temperature for infection lies near 20° C., coinciding with the results of the soil temperature tank experiment. The wilt caused by an attack of Fusarium occurred on some seedlings grown at 20° and 24° C.

(3) *Experiments with Preparation Dishes*

The details of this method are fully described in the next chapter dealing with soil moisture relations. All the inoculated dishes were kept in incubators held at different temperatures for the critical period of infection. The moisture content of the soil at each temperature was held at approximately 40 per cent of the maximum water holding capacity at the beginning of the experiment. The results are shown in the following table.

TABLE 55

Influence of Soil Temperature upon Seedling Infection

Temp. C.	Dish	No. of plants	No. of infected	% of infection	Average %
15°	1	68	46	67.6	70.9
	2	62	46	74.2	
20°	1	82	67	81.7	78.5
	2	85	64	75.3	
25°	1	84	61	72.6	69.5
	2	86	57	66.3	
29°	1	94	50	53.2	55.1
	2	93	53	57.0	

The results coincide in the main with those obtained in previous experiments, demonstrating that high percentages of infection take place between 15° and 29° C., although the optimum lies near 20° C. A few seedlings wilted due to an attack of Fusarium sp. at 20° and 25° C.

Another experiment was performed to determine more exactly both the minimum and maximum soil temperatures for seedling infection. The dish method as well as the glass temperature chamber method as described above was used. The results are presented in the following table.

TABLE 56

Influence of Soil Temperature upon Seedling Infection

Temp. C.	Dish or pot	No. of plants	No. of infected	% of infection
9°–10°	Dish 1	38	0	0
	Dish 2	42	0	0
	Pot 1	68	0	0
	Pot 2	57	0	0
11°–12°	Dish 1	39	0	0
	Dish 2	40	0	0
	Pot 1	65	2	3.1
	Pot 2	68	0	0

TABLE 56 (*Continued*)

Temp. C.	Dish or pot	No. of plants	No. of infected	% of infection
34°	Dish 1	47	2	4.3
	Dish 2	45	0	0
	Pot 1	95	3	3.2
	Pot 2	45	0	0
36°	Dish 1	45	0	0
	Dish 2	46	0	0
	Pot 1	92	0	0
	Pot 2	88	0	0

The results indicate that infection takes place at as low as 11° C., and as high as 34° C., but it does not occur at 9° C. or 36° C.

From all the results obtained in the experiments on the relation of soil temperature to seedling infection, it may be concluded that the seedling infection takes place between 11° C. and 34° C., and the optimum temperature lies at about 20° C., although high percentages of infection occur between 15° and 30° C.

2. *Influence of Soil Moisture upon Seedling Infection*

Methods of Experimentation

Sod soil (loam) containing 19 per cent of moisture in terms of the dry soil basis, was stored in a dry air-tight galvanized can. The maximum water holding capacity of this soil was determined by means of HILGARD's cup. The cups were allowed to stand in the water for 60 minutes at 25° C., and then removed onto the table to drain for 15 minutes in a moist bell-jar. The soil was dried at 110° C. for 24 hours. The maximum water holding capacity determined in this way was found to be 68 per cent in terms of dry soil basis on the average of three cups. The acidity of this soil, when 50 grms. of air-dry soil dissolved in 100 cc. of distilled water, was pH 7.54 as determined by means of hydrogen electrode.

For the preparation of different soil moistures, two methods were used. One consisted of adding definite amounts of water to

definite amounts of the soil by means of an atomizer. The other consisted of mixing by hand definite amounts of water and soil. Both methods have strong and weak points. By the former method, it is somewhat difficult to make moisture uniform throughout the soil, and moreover, it takes much time to inoculate several dishes. However, the soil tilth prepared in this way is comparatively natural. By the latter method, the tilth of the soil is greatly changed, and in addition, high moisture soils can not be prepared, because the soil becomes muddy. The details of the method will be described further in each experiment.

Oospore inoculations were made in preparation dishes. The seeds inoculated were buried about 1.5 cm. deep. As soon as the inoculations were made, the dishes were placed in saturated chambers, for which KOCH moist chambers with ground glass lids, 6.5 cm. high and 17.5 cm. in diameter, were used. These were the same kind as the vessels first used by G. L. PELTIER (81) as "relative humidity chambers." In order to make the chambers moist, a quantity of distilled water was poured, or moist absorbent cotton was placed on the bottom, and two layers of moist filter paper were also fitted on the underside of the lids, the mouths of which had been tightly stoppered.

When it was necessary, a thermometer was inserted through the mouth of the lid, and the soil temperature in the dish could be measured. When most of the seedlings were grown 1 to 2 cm. tall, the dishes were removed from the moist chamber and were saturated with water, because it was found by preliminary tests, that otherwise a few seedlings occasionally would emerge later. Large bell-jars were also used to cover the seedlings when evaporation was rapid. Before the records were taken, the seedlings were kept in a moist condition one or more nights.

Experimental Results

Experiment 1

The proportion of soil and water for this experiment had been preliminarily studied. A definite quantity of the standard soil (actual moisture, about 19% in terms of the dry soil basis) was first put in a preparation dish. One hundred seeds were quickly placed on the soil surface, and in order to make oospores adhere

easily to the seeds, they were slightly moistened by means of an atomizer. Then, a definite quantity of dry oospores was scattered upon the seeds, and subsequently a certain amount of the soil was added. A definite weight of water was then atomized on the soil as uniformly as possible, and a definite weight of the soil was again added, and again a definite weight of water was atomized. In this way, the seeds were sown about 1.5 cm. deep. Preliminary experiments have shown that if water is added in this manner, the soil becomes apparently uniform in moisture after three or five hours standing.

The initial determination of soil moisture was made ten hours after inoculation from two control-dishes, while the final determination was made when the seedlings were removed from the moist chamber.

Two sets of trials were conducted. One set was kept at 20° C. in an incubator. The other was placed on the table in the laboratory. The results are shown in the following table.

TABLE 57

Influence of Soil Moisture upon Seedling Infection
(Experiment 1)

(a) The 20° C. series

Average actual soil moistures		Dish	No. of plants	No. of infected	% of infection	Average %
Init. (%)	Fin. (%)					
18.6	—	1	0	0	0	
		2	0	0	0	
26.1	21.6	1	93	66	71.0	
		2	95	55	57.9	
		(Total)	188	121		64.4
35.5	31.8	1	97	43	44.3	
		2	98	38	38.8	
		(Total)	195	81		41.5
44.4	39.1	1	98	26	26.5	
		2	98	20	20.4	
		(Total)	196	46		23.5

TABLE 57 (*Continued*)

(b) The room temperature series

Average actual soil moistures		Dish	No. of plants	No. of infected	% of infection	Average %
Init. (%)	Fin. (%)					
18.6	—	1	0	0	0	
		2	0	0	0	
26.1	22.7	1	97	47	48.5	
		2	98	51	52.0	
		(Total)	195	98		50.3
35.5	33.3	1	93	38	40.9	
		2	96	33	34.4	
		(Total)	189	71		37.6
44.4	38.9	1	98	26	26.5	
		2	99	15	15.2	
		(Total)	197	41		20.8

The soil containing 18.6 per cent moisture was the standard soil without the addition of water. The results shown in the above table are fairly uniform in both series, indicating that an actual soil moisture of about 22 to 26 per cent, namely 32 to 39 per cent of the maximum water holding capacity, is the most favorable for seedling infection, although fairly good infections take place in the higher soil moistures tested.

In the course of this experiment, it was found that the time required for the emergence of the seedling was shorter at higher moistures, for example, in the 20° C. series, it was about 3 days at the actual moisture of 39.1 to 44.4 per cent, while 4 days were required at that of 21.6 to 26.1 per cent.

Experiment 2

The methods used were essentially the same as in the 20° C. series of the preceding experiment. In this trial, which was practically a duplicate of Experiment 1, the preparation dishes were

covered by two sheets of moist filter paper, and the determination of soil moisture was made only once on the third day after inoculation, because it was thought that one determination at nearly the most important period of infection might be a better criterion than the determination made at the presumed initial and terminal periods of seedling infection. The results are presented in the following table.

TABLE 58

Influence of Soil Moisture upon Seedling Infection
(Experiment 2)

Actual soil moisture	Dish	No .of plants	No. of infected	% of infection	Average %
22.4%	1	40	25	62.5	
	2	38	16	42.1	
	(Total)	78	41		52.6
25.0%	1	45	36	80.0	
	2	47	32	68.1	
	(Total)	92	68		73.9
28.6%	1	42	37	88.1	
	2	41	30	73.2	
	(Total)	83	67		80.7
41.5%	1	48	5	10.4	
	2	50	6	12.0	
	(Total)	98	11		11.2

The results are generally accordant with those obtained in Experiment 1. The soil with 28.6 per cent moisture, namely 41 per cent of the maximum water holding capacity, appeared to be the most favorable to infection, and a little less favorable in the 25

per cent moisture, that is 37 per cent of the maximum water holding capacity.

From the results of both Experiments 1 and 2, it may be concluded that the optimum soil moisture for seedling infection is about 37 to 41 per cent of the maximum water holding capacity of the soil, since the determination of soil moisture in Experiment 2 is considered to be more reasonable than that in Experiment 1, in which only the initial and final soil moistures as mentioned above were measured.

3. *Relation of Kinds of Soils to Seedling Infection*

Methods of Experimentation

Small iron cans, about 6 cm. in diameter, and 8 cm. in height, were covered with paraffin over their inside-surface. Four kinds of soils were obtained from the Department of Soils, University of Wisconsin, U. S. A., through the courtesy of Dr. E. J. GRAUL.* For the preparation of the soils of different moistures, definite quantities of soil and distilled water were mixed by hand. Five kinds of soil moistures, namely, 20%, 30%, 40%, 50% and 60% of the water holding capacity, were first prepared with each soil, but some of them were discarded because they became too muddy, or were apparently too dry. Inoculations were made as follows:

A quantity of soil was put into a paraffined iron can, and the surface leveled. A quantity of the seed (a susceptible variety, Siberian millet) was then uniformly placed on the soil, and oospore powder was scattered over the seeds. Finally soil was added until the seeds were buried about 2 cm. deep. Each can prepared in this manner was immediately covered with two sheets of moist filter paper, and placed in moist Petri-dishes to prevent the loss of water.

The temperature of the laboratory was ranging from 18° to 23° C. during the critical period of seedling infection. After five days, the cans were removed from the moist chambers, and the filter papers were also taken off. Then the cans were saturated with sterilized water, and brought into the green house. Twelve days later, all the seedlings were kept one night in moist chambers at 17° C., and records were taken. The results are presented in the following table.

* The writer is very grateful to Dr. E. J. GRAUL.

TABLE 59

Relation of Different Soils to Seedling Infection

Soils	Moisture (%)	Can	No. of plants	No. of infected	% of infection
Fine sand, rich in organic matter (pH : 7.4; M.W.H.C.*: 36%)	20	1	60	2	3.3
		2	45	3	6.7
	30	1	145	13	9.0
		2	156	11	7.1
	40	1	140	23	16.4
		2	123	11	9.0
	50	1	152	12	7.9
		2	145	4	2.8
Sandy loam (pH : 6.2; M.W.H.C.*: 38%)	20	1	5	1	20
		2	10	0	0
	30	1	143	50	35.0
		2	96	31	32.3
	40	1	59	18	30.5
		2	107	37	34.6
Silt loam (pH : 6.1; M.W.H.C.*: 68%)	20	1	15	1	6.7
		2	50	2	4.0
	30	1	65	3	4.6
		2	87	7	8.1
	40	1	115	20	17.4
		2	92	9	9.8
Clay loam (pH : 6.4; M.W.H.C.*: 73%)	30	1	122	20	16.4
		2	150	14	9.3
	40	1	128	21	16.4
		2	157	35	22.3

* M.W.H.C. means maximum water holding capacity.

During the course of this experiment, sun-light in the green house was very poor, and the conidia were sparsely produced. The affected seedlings gave rise to broad, somewhat rhombic leaves. These symptoms have not been found on the affected seedlings in the field.

The oospores used in this experiment germinated moderately, and the percentage of infection in general was not very good.

While there are some variations under the same conditions, it is evident from the results in general that the percentage of seedling infection may vary with different kinds of soils, confirming the results of the previous experiment described on page 117.

It is also noticeable that higher percentages of infection were obtained in the 30–40 per cent moisture, confirming the conclusion of previous experiments.

Another experiment was also undertaken with two kinds of alkali soils* to obtain information on the influence of soil alkalinity upon seedling infection. The methods used were essentially the same as in the above-mentioned experiment. The results are presented in the following table.

TABLE 60

Influence of Soil Alkalinity upon Seedling Infection

Soils	Moisture (%)	Can	No. of plants	No. of infected	% of infection
Alkali soil (a) (Sandier) pH : 8.82; M.W.H.C. : 57%	30	1	109	23	21.1
		2	76	15	19.7
		(T)*	185	38	20.5
Alkali soil (b) pH : 8.15; M.W.H.C. : 50%	40	1	81	21	25.9
		2	60	12	20.0
		(T)	141	33	23.4

* T means total.

Both the alkali soils were gray in color, and very light. With 40 per cent moisture, soil (a) became too muddy for experiment, and therefore 30 per cent moisture was used. The oospores used in this experiment were obtained from the same source as in the preceding experiment. If these results are compared with those of the preceding experiment, it is evident that seedling infection takes place in alkali soils as much as in ordinary soils.

* These soils were kindly sent to the writer from Dr. T. J. DUNNEWALD, Laramie, Wyoming, to whom the writer wishes to express his heartiest thanks.

4. Depth of Sowing and Seedling Infection

In order to learn how the depth of sowing influences the seedling infection, a preliminary test was carried out.

A fertile loam prepared by the Department of Plant Pathology, University of Wisconsin, was used in this experiment. The maximum water holding capacity of this soil was determined by HILGARD'S cup to be 69 per cent in terms of the oven-dry soil basis. The hydrogen-ion concentration of this soil was pH 6.4 as determined by the quinhydrone electrode.

The water content of this soil was adjusted to be 40 per cent of the maximum water holding capacity. A quantity of this soil was put into large Petri-dishes. After leveling the surface, the seeds of Italian millet, variety Echigo, were uniformly placed on it. The seeds were slightly moistened by means of an atomizer before dry oospores were scattered over them. Then the soil was added to bury the seeds at various depths. The dishes inoculated in this manner were covered with two sheets of moist filter paper, and kept in saturated chambers for the critical period of seedling infection.

Three series of experiments, including four kinds of depth of sowing, were conducted. The temperature of the head house where these experiments were made was ranging from 17° to 24° C. The soil conditions in this experiment were carefully regulated to be as favorable as possible for seedling infection. The results are shown in TABLE 61.

TABLE 61

Influence of Depth of Sowing upon Seedling Infection

Series	Depth of sowing	No. of plants	No. of infected	% of infection
I	1 cm.	155	20	12.9
	2 ,,	123	24	19.5
	3 ,,	150	13	8.7
II	1 ,,	98	8	8.2
	2 ,,	133	49	32.0
	3 ,,	146	19	13.0
	4 ,,	67	5	7.5
III	1 ,,	122	6	4.9
	2 ,,	122	11	9.0
	3 ,,	152	6	4.0
	4 ,,	88	2	2.3

It is evident from the results given above that the percentage of infection varies considerably with different series, but it is coincident in all the series that the depth of 2 cm. is most favorable for seedling infection, and the depths of 1, 3 and 4 cm. are evidently less favorable. Seed germination was found to be very meagre at the depth of 4 cm. These results coincide mainly with those recently reported by the Chosen Agricultural Experiment Station (1).

General Discussions and Conclusions

1. *Influence of Environmental Factors upon the Morphology of the Causal Fungus*

It is a well-known fact that in certain parasitic fungi the form and size of their fructification are variable under various environmental conditions, although the degree of this morphological modification may vary with different fungi. This is really a disadvantageous fact from the stand-point of taxonomy which is chiefly based upon the morphological characteristics of the fructification. It is necessary for the classification of such fungi which are variable in their morphology to take the physiological characters into consideration more than the morphological ones. A familiar example is offered by the genus Fusarium as well as by the genus Phytophthora. In these genera, the classification is made more satisfactorily on the basis of cultural characters on certain media. However, the same method cannot be adopted for the so-called pure parasites, such as downy mildew fungi, in which the fungus in question is included, since, so far they have proved impossible of artificial cultivation. It is therefore very complicated to determine the systematic details of any pure parasite, if the morphology of its fructification is variable under various environmental conditions.

For this reason, it must be the primary thing to test to what extent the morphology of the fructification of such pure parasites is influenced by various external conditions.

So far as the downy mildew fungi are concerned, however, little has been done on the investigation of the variability of conidia

and conidiophores, the morphology of which is quite important for their specific as well as generic distinction.

In 1923, GÄUMANN (34) called attention to the variability of the conidia of *Peronospora Brassicae* parasitic on *Raphanus sativus*. He made five sets of experiments with varying combinations of temperature and humidity, and found that in his experimental Set III (a), in which the plants were covered with a bell-jar moistened by means of wet filter paper, and placed under the sunlight of July*, the mean values of the dimensions of the conidia were found to be 23.4 microns by 21.2 microns, while in Set IV (b), in which the plants were covered with moist bell-jar, and placed near the cellar window at about 13° C., the corresponding values were 18.7 microns by 17.9 microns; in other words, the difference in length was 4.7 microns, and in width 3.3 microns. However, he was of the opinion that the influence of such extreme environment upon the dimensions of the conidia is of no importance from a practical point of view, since, in nature, the fungus can scarcely be exposed to such extreme conditions. No attempts were made to determine the influence of warmth and moisture separately.

In 1929, HIURA (52) who studied the downy mildew of cucumber, demonstrated that the dimensions of the conidia of the causal fungus increase as the growth of the host plant progresses with a rise of temperature.

In 1929, ARENS (3, 4), experimentally proved that the conidiophores of *Plasmopara viticola,* and of *Pseudoperonospora Humuli* become smaller at low temperature, and also in low humidity.

In 1930, TASUGI (105) reported that the dimensions of the conidia of *Sclerospora graminicola* increase in the conidia produced in mid-summer, and decrease in those formed late in the growing season of the Italian millet.

According to the writer's investigations made in 1928, the dimensions of the conidia of *Peronospora Schleideni* parasitic on *Allium fistulosum* are very variable under different environmental conditions. The dimensions greatly increase in the warm rainy season during June and July. It has also been found that the dimensions of the conidia of *Peronospora effusa* parasitic on spinach are

* Direct sunlight was avoided.

also influenced by external factors, although, so far as determined, the degree of variability has been found not so great as in the case of *Peronospora Schleideni.*

Fragmentary as this information is, it is sufficient to show that the conidia and conidiophores of the downy mildew fungi are variable under various environmental conditions, although this line of study has hitherto been rather neglected, and it may be valuable to give further fundamental contributions to the taxonomy of the downy mildew fungi.

In the present experiments the following salient points have been manifested:

(1) There is seasonal variation in the shape and size of the conidia and conidiophores of the causal fungus.

(2) This seasonal variation is mainly caused by the following three causal factors, namely:

(a) The conidia and conidiophores increase in dimension as temperature rises.

(b) The conidia and conidiophores decrease in dimension as atmospheric humidity goes down.

(c) The dimensions of the conidia are to a certain extent influenced by the physiological conditions of the host plant.

These findings have an important relation to the morphological classification of the species belonging to the genus Sclerospora as well as of other related downy mildew fungi. In this connection, WESTON's criticism on SHIRAI's description of the downy mildew fungus of the Italian millet is interesting. WESTON (120) states,

"In Japan, however, SHIRAI (24, fig. 16, 17) described the conidiophores as much larger (100 to 240 μ by 12 to 19 μ) and with a somewhat more elaborate branching system; but the fact that he found conidia of two strangely different size-classes (24 to 28.8 μ by 16.8 to 19.2 μ, and 38.4 to 57.6 μ by 19.2 to 24 μ) arouses the suspicion that he was not dealing with *S. graminicola* alone, and to some extent invalidates his characterization of the species (776)."

Later, MELHUS and his associates (72) found large conidia of *S. graminicola* parasitic on green foxtail, and confirmed that SHIRAI's finding is not strange.

The present investigation has shown that the production of giant conidia is chiefly related to the physiological conditions of the host plant and also to high temperatures.

2. *Oospore Germination*

The germination of the oospores of *Sclerospora graminicola* (SACC.) SCHROET. has long been an interesting subject for investigation. At the very beginning of the study of this fungus, as early as 1878, MAGNUS (66) * realized the importance of studying the mode of oospore germination. However, he could not succeed in germinating oospores, and therefore, was compelled to name the fungus *Ustilago(?) Urbani.*

SCHROETER (98) also intended to study the mode of oospore germination. He sowed oospores in water or on a moist substratum at various times from April to July. Under these conditions, the protoplasm of the oospores first became granular, and later divided into about 8 to 12 rounded to elliptical bodies, but no further development was seen.

PRILLIEUX (85) reported that M. FRÉCHOU observed the oospores germinating by tubes which resembled the germ-hyphae of the oospores of *Plasmopara viticola.*

SHIRAI (101) also tried to germinate the oospores of *Sclerospora graminicola* parasitic on Italian millet, but no positive result was obtained.

BUTLER (11) who studied the germination of the oospores obtained from *Pennisetum typhoideum,* stated: "After trials extending two years, I have not succeeded in germinating the spores."

MELHUS (69) reported that in his attempts to germinate oospores of Peronosporaceae, including *Sclerospora graminicola,* difficulties were encountered due to a parasite, *Rhizophidium pollinis,* which attacked the ripe oospores.

KULKARNI (62) mentioned that the oospores resisted all his attempts to germinate them in the laboratory.

Later, in 1928, MELHUS and his co-workers (72) reported that all their attempts to germinate oospores gave only negative results. They finally came to the conclusion: "It is, therefore, probable that

* The reference to MAGNUS in the writer's note (53) should be discarded.

only small percentages of the oospores germinate at any time and this may well account for the failure of workers to actually observe the germination of oospores under the microscope." In the same year, WESTON and WEBER (122) also reported the failure of their attempts in germinating oospores.

These references will be sufficient to show how the germination of the oospores in question has been a puzzling problem giving a challenge to mycologists as well as to plant pathologists. A consideration will be given here to PRILLIEUX's note on the germination of the oospores, which has recently been very highly estimated by WESTON (124).

A. FISCHER (30) was apparently the first to call attention to PRILLIEUX's statement, but he did not give much credit to this note.

Even at the present time, when the germination of the oospores has been decisively cleared up by the writer (50, 51, 53), TASUGI (105), and, EVANS and HARRAR (26), whose contributions have been made independently, it seems difficult to judge from PRILLIEUX's non-scientific description* whether or not M. FRÉCHOU did actually observe true germ-tubes of the oospores in question, because, in many cases, analogous hyphae have been found attaching to oospores, which may be easily mistaken for true germ-tubes. In addition, his statement is greatly invalidated in that, although its essential part consists of a comparison between both hyphae from the oospores of *Sclerospora graminicola* and *Plasmopara viticola,* it is entirely questionable, whether or not PRILLIEUX himself actually observed true germ-tubes of the oospores of *Plasmopara viticola.* In 1833, he (83, 84) claimed the discovery of the mode of the oospore germination of *Peronospora viticola,* but his claim has not been accepted by later investigators, because the figure given by him clearly shows that what he found is entirely different from those discovered by GREGORY (36, 37) and RAVAZ and VERGE (88) to whom credit is to be given at present, since ARENS (2) also has recently substantiated these authors' statements.

From all the considerations mentioned above, PRILLIEUX's statement, interesting as it is, seems to be far from a scientific contribution.

* " M. ERÉCHOU observed the oospores producing a tube like the germ-tubes of the oospores of *Peronospora viticola,* but was not able to follow their further development."

In this connection, the following statement given by E. GÄUMANN (35) is worthy of being mentioned: "Die Keimug der Oosporen erfolgt....., bei *Sclerospora* und *Peronospora* durch einen Keimschlauch, der in der Wirtspflanze zu einem Myzel auswächst (DE BARY 1865)." In the spring of 1930, the writer consulted with Dr. GÄUMANN on the original paper of this statement, and received a letter informing that he would try to find it, as he could not recall it to his mind. Later, in 1931, when the writer visited Dr. GÄUMANN in his laboratory in Zürich, he kindly told the writer that he still could not find that paper. It is hoped that the question will be cleared up sooner or later.

At any rate, the germination of the oospores of *Scl. graminicola* is now not a puzzling problem at all. Success in germinating the oospores by the writer's method has also been recently reported by CHAUDHURI (14)*, and WESTON and UPPAL (126).

The essential thing for the success in germinating oospores is to obtain oospores ready for germination. It was first thought by the writer that high temperatures were essential for oospore germination, but it has been later found that the most essential thing lies in the oospores themselves (53), and if they are suitable, they may be easily germinated by the ordinary method for spore germination under laboratory conditions.

Most of the oospores tested have been found to germinate much better in the next spring, although some of them kept in the laboratory have often failed to germinate for unknown reasons. The percentage of oospore germination has also been found to be very different even among the materials collected at the same time in the same field.

The failure of previous workers in germinating oospores may be in part explained by the reason that their germination tests might probably have been done with oospores not ready for germination, and in part, that the methods employed by them were unsuitable.

MELHUS and his co-workers (72) reported that oospores matured in July have produced heavy infection on green foxtail seedling within 10 days. The results of the present investigation shown in TABLE 33 indicate that the percentage of oospore germination increases with a certain lapse of time. Some internal conditions of the oospores themselves seem to be prerequisite for their germination. It

* The mode of the oospore germination described and figured by this author is somewhat different from the normal type. Further investigation is desirable.

seems to be probable that such prerequisite conditions are connected with the dormancy or after-ripening of the oospores, since preliminary tests have shown that the percentage of oospore germination is greatly increased when oospores are kept in a saturated atmospheric moisture at low temperatures (0°–5° C.)* for two months. Further investigation concerning the nature of the dormancy of the oospore is now in progress.

3. *Taxonomic Consideration*

When TRAVERSO (108) first gave a description of *Sclerospora graminicola,* var. *Setariae-italicae* TRAV. parasitic on *Setaria italica,* he was ignorant of the conidial stage of the fungus, and the only reason for the establishment of this new variety was that the oospores of the fungus on *Setaria italica* were found to be much larger than those of the type form, *Sclerospora graminicola,* which parasitizes on the other Setaria species.

Late, in 1907, he (109) found the conidial stage of this fungus to be morphologically indistinguishable from that of the type form. But he was still convinced that the fungus on *Setaria italica* should be distinct from the type form, because the results of his field observations and artificial inoculations strongly suggested that the form on *Setaria italica* could be biologically different from the type form. He was also aware of the papers of GUÉGUEN (39), SWINGLE (104), MACBRIDE and HITCHCOOK (65), SHIRAI (101), WILSON (127), and BUTLER (11), who all considered the fungus on *Setaria italica* to be morphologically identical with the typical *S. graminicola.* Consequently, he was compelled to weaken the first reason for the establishment of the new variety, as seen in the following statement:

"Even if the differences which I have indicated in the oospores are not considered sufficient for their separation, it must, however, be still granted that we have to deal with two separate forms because of the specialization of their parasitism, a fact which cannot be ignored after the studies of ERIKSSON, ARTHUR, KLEBAHN, etc., on the Uredineae, of SALMON on the Erysiphaceae, and the like."

It is thus seen that TRAVERSO's opinion of his new variety was changed, and he himself emphasized the physiological specialization in the pathogenicity of the new variety.

* The electric current was cut twice a month, and the temperature of the refrigerator rose to about 10°C. each time.

BUTLER (12) was apparently the second to believe the existence of physiological specialization in *Sclerospora graminicola,* but in his book he did not adopt TRAVERSO's new variety for the Sclerospora on *Setaria italica.*

In 1922, SAWADA (97) reported the Sclerospora on *Setaria italica* in Formosa to be identical with *Scl. graminicola,* var. *Setariae-italicae* TRAV. on the morphological basis of the oospores. This was followed by HARA (45), KASAI (61), and others.

In 1929, the writer (48) having discovered two kinds of shredded leaves collected in the same field, bearing larger and smaller oospores, pointed out that the scientific name of the fungus in question should be determined by further investigations, it being unreasonable to deal with the taxonomy of the fungus only on the basis of the dimension of the oospore.

In the same year, the writer (50, 51) reported that the oospores from *Setaria viridis* could infect Italian millet, but the percentage of infection was very low.

In 1930, TASUGI (105) expressed his opinion on the name of the fungus parasitic on Italian millet, and adopted TRAVERSO's variety name because of the specialization of parasitism, although he recognized that the oospores of the fungus were not very different from those of the fungus on *Setaria viridis.*

The results of the present investigation seems to give some clue to the scientific name of the fungus in question. For the sake of clearness, the dimensions of the oospores, the morphology of the conidial stage, and the specialized parasitism of the fungus will be discussed separately.

(*1*) *Dimensions of the Oospore*

It is evident from the data presented in TABLE 34 (a) that the dimensions of the oospores obtained from *Setaria italica* vary greatly with different sources of materials. This is also, to some extent, the case with the oospores from other related hosts. If we compare these data with the measurements in the description of TRAVERSO (108), it is self-evident that those given by him are far from representative of the fungus on *Setaria italica.* The same thing can be said about his measurements for the oospores from other species of Setaria (See (b)–(f) in TABLE 34). Generally speaking, the

oospores from *Setaria italica* are somewhat larger than those from other related hosts, but such variabilities are of course insufficient for the establishment of a new variety.

So far as the oospore dimensions are concerned, therefore, it may be well said that there is no sound basis for the continuation of TRAVERSO's new variety. This view is also justified by TRAVERSO's own statement as cited above.

(2) *The Conidial Stage*

SHIRAI (101) was the first to describe the conidial stage of the fungus on *Setaria italica*. He found the conidia and conidiophores to be larger than those of *Sclerospora graminicola* given by previous workers. TRAVERSO (109) found the conidial stage of the fungus to be morphologically indistinguishable from that of the type form, but he considered the morphology of the conidial stage to be not very important in the separation of species of Peronosporaceae.

In 1924, WESTON (120) studied the conidial stage of the Sclerospora on *Setaria viridis*, and pointed out that the description of the conidial stage given by previous workers is limited and imperfect. He gave the size of the conidia as 11 to 34.9 microns, mostly 17 to 18.9 microns in length; 9 to 20.9 microns, mostly 13 to 14.9 microns in width.

In 1928, MELHUS and his co-workers (72) gave the size of normal conidia from *Setaria viridis* as 14 to 23 by 11 to 17 microns, and that of larger conidia to be 43 by 18.6 microns.

In the same year, WESTON and WEBER (122) described the conidial stage of the fungus on *Setaria magna* as 13 to 36.9, mostly 21 to 22.9 microns in length; 11 to 24.9, mostly 15 to 16.9 microns in width.

In 1930, TASUGI (105) demonstrated the dimensions of the conidia to be extremely variable at different periods of the season. He found the conidia from Italian millet to be larger than those obtained from *Setaria viridis*.

The data of the present investigation have indicated that the dimensions of the conidia are influenced by various factors, such as temperature, air humidity, and the physiological conditions of the host plant. In view of these facts, therefore, a mere comparison of the dimensions of the conidia obtained from different host plants

may be of little significance for a taxonomical purpose. During the course of the inoculation experiments, the writer took opportunities of comparing the conidia of the fungus originally taken from *Setaria italica* with those from *Setaria viridis*, both having been produced on seedlings of the same variety, and grown under the same conditions.

The following four cases were studied.

(1) The conidia produced on Italian millet, variety Echigo.

(2) The conidia produced on Italian millet, variety common millet.

(3) The conidia produced on green foxtail.

(4) The conidia produced on corn, variety Japanese Hulless.

As has been tentatively published by the writer (54), the conidia of the *italica*-fungus have sometimes been found slightly larger than those of the *viridis*-fungus, but in no cases, have such marked differences as of taxonomic importance been found.

From these data together with those obtained by previous workers, we have no reliable reason, at present, to separate the fungus on *Setaria italica* from the fungus on *Setaria viridis* on the basis of the morphology of the conidial stage.

(3) *Physiological Specialization*

The existence of physiological specialization in *Sclerospora graminicola* has been believed by TRAVERSO (109), and BUTLER (12), but experimental evidences have been very recently furnished by the writer (51), TASUGI (105), and UPPAL and DEASI (115). The evidences obtained by the present investigation are summarized as follows:

(1) The percentage of infection due to the *viridis*-fungus collected in Kyoto, was always lower than that due to the *italica*-fungus, when the variety Echigo of Italian millet was inoculated.

(2) The percentage of infection due to the *viridis*-fungus collected in Ames, Iowa, U. S. A. was always higher than that due to the *italica*-fungus, when green foxtail was inoculated.

(3) The green foxtail plants infected by the *italica*-fungus become stunted later, and no conidia are produced again. Such symptoms are not seen of the green foxtail infected by the *viridis*-fungus.

These findings are sufficient to indicate the existence of physiological specialization between the *italica*-fungus and the *viridis*-fungus. It must, however, be borne in mind that the *italica*-fungus used in these experiments was collected in certain places of Japan, and it might be different from the *italica*-fungus in other localities. In this connection, it is of special importance that common millet, a variety of the Italian millet, *Setaria italica,* which is widely cultivated in the United States of America, has been found to be very susceptible to both the *italica*-fungus and the *viridis*-fungus. This means that there are at least two physiologic forms in the Sclerospora parasitic on *Setaria italica.*

UPPAL and DESAI (115) reported the occurrence of two physiologic forms of *Sclerospora graminicola.* One form infects *Pennisetum typhoideum,* while the other attacks *Setaria viridis, S. magna, S. italica,* and *Euchlaena mexicana.*

MELHUS and his co-workers (72) reported that *Setaria glauca* is apparently immune to the *viridis*-fungus. The present investigation has also demonstrated that *Setaria glauca* is immune to the *italica*-fungus as well as to the *viridis*-fungus. These facts may be considered as a proof that the Sclerospora on *Setaria glauca* is physiologically different from both the *italica*-fungus and the *viridis*-fungus.

The results of the present investigation have also revealed that there is a possibility of the existence of physiologic specialization within the *viridis*-fungus.

It is thus seen from the data mentioned above that the physiologic specialization in *Sclerospora graminicola* is very complicated.

From these considerations, the following salient points in relation to the taxonomy of the fungus in question may be suggested:

(1) *Scl. graminicola,* var. *Setariae-italicae* TRAV. has no reliable basis for its continuation from a morphological standpoint.

(2) There are at least two physiologic forms in the Sclerospora fungus parasitic on *Setaria italica.* One form can infect *Setaria viridis* but only very slightly, while the other infects *Setaria viridis* severely. This fact seems to be enough to prompt the abandonment of such a superficial view as that the *italica*-fungus in general can be considered as a distinct variety, because of its biological difference from the typical *Scl. graminicola* which develops on other species of Setaria.

(3) For the sake of avoiding confusion, therefore, it is, at present, better to retain *Sclerospora graminicola* (SACC.) SCHROET. for the scientific name of all the Sclerospora forms parasitic on Italian millet.

Whether or not there are more physiologic forms within the *italica*-fungus is an interesting future problem. The new variety proposed by TRAVERSO may be considered to be identical with one of the physiologic forms discovered by the writer. If it is convenient from the pathological stand-point to give a scientific name to these physiologic races, the writer would like to name the one attacking green foxtail (*Setaria viridis*) very slightly *Sclerospora graminicola*, f. *italicae*, and the other one attacking green foxtail very severely *S. graminicola*, f. *viridis*.

4. *Seedling Infection*

The primary infection of many of the downy mildews caused by the fungi belonging to Peronosporaceae has been supposed to be induced by the oospores in the soil. However, the details of seedling infection by the oospores have never been reported. This has been because of the difficulty of germinating oospores on the one hand, and also because of various other obstacles on the other hand. The results of the present investigations on the seedling infection of *Scl. graminicola* may be of special significance as giving some new information on this unsolved phase of the problem in the downy mildews.

The results presented in TABLE 30–46 clearly indicate that every organ of the seedling exposed to infection allows the entrance of the fungus if the seedling is very young. Histological examinations have revealed that the mycelium can penetrate the epidermal cell of the young part of the seedling. When the seedlings are grown to about 2 cm. in height, they become exceedingly resistant to systemic infection. The time required for this degree of the critical growth of the seedling varies greatly at different temperatures as shown in the following table which represents the results obtained with the same variety of Italian millet used in the above-mentioned experiments.

TABLE 62

Rate of the Growth of Seedlings at Different Temperatures

Temp. C.	Time required for the first appearance of primary roots (hours)		Time required for the growth of the seedling to 5 mm. in height (hours)		Time required for the growth of the seedling to 2 cm. in height (hours)	
	Range	Average	Range	Average	Range	Average
15°–16°	70–77	73.5	120–144	132	144–168	156
20°–21°	34–40	37.0	65– 77	71	96–120	108
25°–26°	22–27	24.5	45– 55	50	72– 84	78
29°–30°	18–22	20.0	30– 38	34	48– 60	54

The numerals given in the above table were obtained from 50 grains of Italian millet germinated in moist Petri-dishes kept at known temperatures.

As seen from the table, the rate of the growth of the seedling increases as temperature rises, and the time required for the first appearance of the primary roots and for the growth of the seedlings to 5 mm. as well as to 2 cm. in height increases as temperature falls. This means that the critical period for seedling infection lasts longer at lower temperatures, so far as the seedlings only are concerned.

According to DICKSON and HOLBERT (24, 25), wheat and corn plants grown at different temperatures are widely different in their chemical constituents, and such changes due to temperature are closely related to the infection by *Gibberella Saubinetii*. It is unknown at present whether such is also the case with the seedling of Italian millet. However, if it is taken into consideration that infection takes place only while the seedling is quite young, it seems to be likely that the extent of the influence of temperature upon the chemical composition of such a young seedling may not be so great as in the case of the seedling blight of wheat and corn mentioned above. The results presented in TABLE 41–42 support this view to some extent, since it is clearly demonstrated that the seedlings grown at both 20° and 30° C. are almost equally infected, when they are inoculated under the same conditions.

Another factor concerning the relation of temperature to seedling infection is the behaviour of the mycelium from the oospores germinated at different temperatures. The results shown in TABLE 51 indicate that the infecting power of the mycelium germinated from oospores is not actually influenced by the temperature between 15° and 30° C.

The results given in TABLE 31 show that the time required for the germination of the oospores is widely different at different temperatures, and it becomes longer as temperature falls, as in the case of the seed germination and the growth of the seedling. For the determination of the actual critical period for seedling infection at different temperatures, therefore, it is important to compare the time required for the germination of the oospores with that for the germination of the seeds as well as for the growth of the seedling. The following table has been prepared from the results shown in TABLE 31 and 62.

TABLE 63

Actual Critical Period for Seedling Infection at Different Temperatures

Temp. C.	Time required for the first appearance of primary roots (hours)	Time required for the germination of the oospores (hours)		Time elapsing between the 1st appearance of primary roots and the attainment of 5 mm. height (hours)	Actual critical period for seedling infection (hours)
		Range	Average		
15°–16°	73.5	72–96	84	58.5	48
20°–21°	37	42–52	47	34	24
25°–26°	24.5	30–38	34	25.5	16
29°–30°	20	24–30	28	14	6
Sign	a		b	c	d

Remarks:

Actual critical periods have been calculated as follows;

$$d = c-(b-a)$$

It is evident from the table that the time required for the germination of the oospores is longer than that for the germination of the Italian millet seed. As a consequence, the actual critical period

for seedling infection is shorter than the time estimated theoretically from the side of the seedling. At any rate, the critical period for seedling infection is evidently longer at lower temperatures, and so far as the infection between 15° and 30° C. is concerned, higher percentages of seedling infection must take place at lower temperatures, if other conditions are similar.

The relation of temperature to the germination of oospores is also an important matter for the interpretation of the relation of temperature to seedling infection. The results of the physiological studies on the fungus indicate that the cardinal temperatures for the germination of the oospores are about 34°, 25°–20° and 11° C. respectively, although high percentages of germination take place between 15° and 30° C.

The results shown in TABLES 53–55 indicate that the optimum soil temperature for seedling infection lies at about 20° C., while the maximum and minimum soil temperatures are about 34° and 11° C., respectively. It is thus seen that the cardinal temperatures for the germination of the oospores coincide with those for the seedling infection occurring on the Italian millet.

Judging from these facts, the relation of soil temperature to seedling infection appears to be greatly influenced by the effect of temperature upon the germination of the oospores, and also by the influence of temperature upon the critical infection period of the seedling.

MELHUS and his co-workers(72) concluded from the results of their preliminary experiments that the optimum soil temperature for seedling infection lies at about 15° to 16° C. In this connection, it may be interesting to note that a high percentage of seedling infection was obtained in one occasion (pot 3) at 16° C. as seen in TABLE 53. This indicates that high infection may take place at 16° C., if other conditions are especially favorable. It should, therefore, be borne in mind that the optimum soil temperature for seedling infection as determined by these experiments may vary to a certain extent with other associated variable conditions.

The relation of soil temperature to plant diseases due to various soil-borne micro-organisms has been extensively investigated by JONES and his associates. Their contributions have been recently published in a coalesced form(60). The importance of the study on this line of phytopathology, and the complexity of the problem in relation to environment have been clearly demonstrated and discussed in

detail by them. Similar investigations on other plant diseases have been yearly accumulating. Among such previous investigations, those on certain smuts will be worthy of being considered here, on account of the similarity of the mode of seedling infection to that of the downy mildew in question.

Although there have been published a considerable number of investigations concerning the influence of soil environments upon the seedling infection of various smuts, some such studies as performed under controlled conditions may be particularly referred to.

In 1922, HUNGERFORD(55) stated that low soil temperatures, 9° to 12° C., and high soil moisture, about 22 per cent, with a moisture equivalent of 20.7, were found to be very favorable to seedling infection by *Tilletia tritici*.

In 1924, WOOLMAN and HUMPHREY(130) published a paper on the physiology and control of bunt of wheat. The cardinal germination temperatures for *Tilletia tritici* were found to be respectively, 0° to 1° C., 18° to 20° C., and 25° to 29.1° C., while high percentages of infection took place at soil temperatures between about 5° and 16° C., and a soil moisture, 22.6 per cent, in terms of dry soil, was found to be favorable for infection. The authors did not state why high infections took place at soil temperatures lower than the optimum germination temperature for the chlamydospores. The results of their experiments concerning varietal resistance to bunt indicated that the differences in growth-rate are varietal characters not necessarily related to bunt resistance.

In the same year, FARIS(28) demonstrated the importance of low soil temperatures, 5° to 10° C. in relation to the infection of wheat seedlings by *Tilletia tritici* and *Tilletia laevis*. The character of the substrata and the percentage of moisture in both potting soil and quartz sand tested were not closely related to the amount of infection. He also found that the optimum soil temperature for seedling infection varies with different varieties, all other conditions being the same. The results of his experiments upon the rate of germination of seeds indicated that the more vigorous seedlings are more susceptible to infection. He also pointed out that there is a similarity of parasitic behaviour between smuts and rusts which are fundamentally different from such a fungus as the seedling blight organism, *Gibberella Saubinetii*, of wheat and corn.

According to the investigations cited above, and also to those of earlier workers(129), it seems generally true that low soil tempera-

tures are favorable for the seedling infection of wheat by *Tilletia tritici*. This is not in accord with the results of the investigations on the relation of temperature to the germination of the chlamydospores, since the optimum temperature for chlamydospore germination has been found by previous workers (129), (86), to be about 16°–18° C. The reason why such lower temperatures are favorable for infection has not yet been fully explained.

In 1924, GRIFFITHS (38) reported that the seedling infection of wheat by *Urocystis tritici* took place between 6° and 23.5° C., the optimum being 21.5° to 23.5° C. The most favorable stage of development of the host plant for infection was found to be before the seedling comes through the ground, or before the coleoptile is broken.

In the same year, NOBLE (76) reported that soil temperatures ranging from 14° to 21° C. were optimum for infection of wheat seedlings by *Urocystis tritici*, while some plants became infected at as high as 23° to 25° C., and as low as 5° C., but no infection occurred at 29° C. In green house experiments, he found that wheat seedlings became infected when seed was sown in infested soil constantly watered for five weeks. According to the same author's investigation, the optimum temperature for germination of the spores of *Urocystis tritici* was between 18° and 24° C. A comparison of the optimum temperature for spore germination with that for seedling infection shows a remarkable coincidence, suggesting that soil temperature may influence disease development primarily through its direct influence upon the fungus.

In 1921, WALKER and JONES (117) reported that a high percentage of onions grown on smutted soil were infected at soil temperatures ranging from 10° to 25° C. They also found that high percentages of infection resulted over a wide range of soil moisture. Later, WALKER and WELLMAN (118) studied the relation of temperature to spore germination and growth of the onion-smut fungus, *Urocystis cepulae*. The optimum temperature for chlamydospore germination, and vegetative growth of the mycelium was found to lie between 13° and 22° C. Above 25° C. there was a marked reduction in the amount of spore germination, and the growth of the hyphae became very meagre. From these data, they were inclined to believe the direct influence of temperature upon the parasite to be a primary factor in limiting the infection.

In 1923, BARTHOLOMEW and SEYMOUR(6) reported that a soil temperature of 18° to 22° C. was found to be optimum for the infection of oat seedlings by *Ustilago avenae* at every soil moisture tested. This optimum temperature coincided directly with the optimum for germination and growth of both host and fungus. Low soil moistures were accompanied by relatively high percentages of smut infection.

In 1924, REED and FARIS(89, 90) reported that soil temperature, soil moisture, and soil reaction are interdependent factors influencing the infection of oats by *Ustilago levis* as well as of sorghums by *Sphacelotheca sorghi* and *S. cruenta*. The highest percentage of infection of oats by *Ustilago levis* occurred at the soil temperature of 25° C., while those of sorghums by *S. sorghi* and *S. cruenta* took place between 15° an 25° C. In all cases, lower soil moistures were found to be favorable, and slightly acid or neutral soils were most favorable for the infections.

In 1927, JOHNSTON(58) reported that soil temperatures of 62° to 66° F. were apparently the most favorable for infection of oats by *Ustilago avenae* and *U. levis,* and the highest percentages of infection were obtained when the soil moisture was below 30 per cent of the water holding capacity of the soil.

In 1924, FARIS(27) reported that high percentages of infection of barley seedlings by *Ustilago hordei* occurred over a wide range of soil temperatures from 5° to 30° C. An increase in the soil moisture resulted generally in an increase in the infection in the acid soil tested, but this was not true of the neutral soil, nor of the alkaline soil.

In 1926, CHRISTENSEN(15) reported that the optimum soil temperature for infection of sorghum seedlings by *Sorosporium reilianum* was found to be 28° C. High soil moisture (25 per cent on dry soil basis) materially reduced the percentage of smutted plants at all temperatures and narrowed the thermal range of infection. He was inclined to consider that soil moisture and soil temperature determine the amount of the occurrence of smut by affecting the pathogene directly, rather than by influencing the host plant.

It is evident from these references that most of the previous investigations on the relation of soil factors to the seedling infection by the smut fungi mentioned above are merely descriptive, recording the phenomena discovered, and full explanations are mostly lacking. Consequently, without further investigations, it is difficult to give

here any general conception regarding the nature of the influence of soil factors upon the seedling infection by these smuts. However, it is noticeable that, excepting the case of *Tilletia tritici* and *T. laevis*, the occurrence of seedling infection is apparently influenced greatly by soil factors which affect chiefly the causal fungus rather than the host plant.

The same tendency is recognizable with the oospore infection of the downy mildew of Italian millet in question, the causal fungus of which, so far, has been known as one of the so-called pure parasites.

The results shown in TABLE 57 and TABLE 58 indicate that moderate or rather low soil moistures are favorable for the infection of Italian millet seedlings by *Sclerospora graminicola*. While no full explanation of this phenomenon can be made at present, the following data concerning the behaviour of the causal fungus may give some clue to the problem:

(1) Oospores do not germinate in oxygen-free air.

Preliminary tests were made to determine this point. Sufficient amounts of oxygen-absorbing solution were put in a small Petri-dish. The under side of the cover was fitted with moist absorbent cotton on which were placed oospore-bearing agar-agar blocks prepared as already described. The dish was then placed in the oxygen-absorbing solution in a larger air-tight vessel, so as to make the lower edge of the cover of the small Petri-dish inside come in contact with the oxygen-absorber in the vessel. The whole experimental set was kept at 25° C. for 48 hours. An alkaline pyrogallol solution* was used for the absorption of oxygen. In all cases of three experiments attempted, no oospores germinated. In another experiment, a moist Petri-dish containing agar-agar blocks was placed in an air-tight vessel which contained an alkaline pyrogallol solution. The cover of the Petri-dish had been slightly opened. No germination of oospores occurred. In this experiment, the moisture conditions in the Petri-dish were found to be suitable for oospore germination, and therefore it was concluded that the germination of oospores was inhibited by the absence of oxygen.

(2) The percentage of oospore germination is low in high soil moistures.

* Dissolve 15 grms. of pyrogallol in 15 c.c. of water, and mix this with 135 c.c. of saturated solution of potassium hydroxyde.

This has been confirmed by the following experiments. A piece of filter paper dipped in water, and fully drained was placed on the soil, and dry oospores were scattered upon it. The oospores were then buried with the soil, and different amounts of water were added. This procedure was done in a Petri-dish which was held at 25° C. for 40 hours. The percentage of oospore germination was as follows:

TABLE 64

Influence of Soil Moisture upon Oospore Germination

Soil moisture in terms of the percentage of the M.W.H.C.	No. of spores	No. of germinating spores	% of germination
34%	324	36	11
	450*	34	8
58%	310	27	8
	402*	20	5
70%	326	10	3
	412*	14	3

* The oospores were placed between two sheets of filter paper

It is evident from the results shown in the above table that percentages of oospore germination are low in high soil moistures. These results suggest that the decrease in oospore germination may be an important cause of the reduction in seedling infection at the high content of soil moisture. That the seedlings emerge from the soil much earlier in the case of the higher soil moistures may also play a rôle in this decrease (See page 127).

The results shown in TABLE 52 and 59 indicate that the percentage of seedling infection varies to some extent with different kinds of soils. TABLE 60 shows that the seedling infection takes place in alkali soils tested. The seedling infection by this fungus seems to take place over a wide range of soil reactions. According to TASUGI(105) pH. 3.1 is most favorable for the germination of oospores.

The results of inoculation experiments on different varieties of Italian millet evidently show that there is a promising possibility of obtaining resistant or immune varieties by means of selection or breeding. For such work, inoculations with oospores are of primary

importance, and as a consequence, it is fundamental to study the influence of soil factors upon the seedling infection by oospores. The results obtained in the present studies on this line will serve as a contribution to the prophylactical base of the disease.

Summary

1. The downy mildew of Italian millet is commonly found from Hokkaido on the north to Formosa on the south, wherever the Italian millet is cultivated. Its occurrence is also known in Chosen (Korea) as well as in Manchuria.

2. There are two types of infection, namely systemic infection and local infection. The former is caused by the mycelium germinated from the oospores in the soil, while the latter is the secondary infection due to the zoospores from the conidia.

3. The conidial stage of the causal fungus is found in all stages of development of the host plant, from seedlings to mature plants. The conidia and conidiophores appear as whitish downs on the under side of affected leaves, although they are also formed sometimes on the upper surface of affected leaves.

4. The conidia-bearing areas of the leaves become chlorotic sooner or later. The chlorotic areas of the systemically infected leaves are usually large and laciniate, while those due to the secondary or local infection are restricted or spotted.

5. The symptoms caused by the development of the oospore stage consist of two entirely different types. One is the characteristic shredding of the oospore-bearing leaves, and the other is the peculiar deformity of the oospore-bearing spikes.

6. The dimensions of the conidia greatly increase, as the season advances and the host plants grow large.

7. It has been experimentally proved that the dimensions of the conidia gradually increase as the temperature rises, and the size of the conidia becomes slightly elongate with a rise of temperature.

8. The conidia and conidiophores decrease in size, when atmospheric moisture goes down to a certain extent.

9. The conidia produced on seedlings have been found smaller than those on well-grown plants.

10. The conidia formed on metamorphosed leafy structures are generally very large.

11. Seasonal variation in the morphology of the conidia and conidiophores is mainly caused by temperature, atmospheric moisture, and some physiological conditions of the host plant.

12. The conidia of the causal fungus can be produced between 10° and 25° C., and the optimum temperature for the production of conidia is about 17°–18° C., while they are abundantly produced between 15° and 20° C.

13. The minimum time required for the production of the conidia is different with materials. However, the time has been found to be the shortest at 18°–20° C.

14. The conidia of the fungus can be produced in the daytime. Observations in field as well as in green house have also revealed that on cool cloudy days, conidia are often produced in the daytime.

15. The relation of air humidity to the production of conidia varies with different leaves as well as with different portions of the same leaf, and consequently the minimum air humidity for the production of the conidia varies with different leaves. However, the minimum air humidity for the production of conidia may be considered to be generally 80–85 per cent at 18° C.

16. Conidia are readily discharged up to 2 mm. upward, and some of them are discharged up to 3 mm., but not to 4 mm. vertically. The discharge of the conidia may also occur in the daytime.

17. The conidia, as a rule, germinate by liberating zoospores, but occasionally they germinate by germ-tube.

18. The time required for the germination of conidia varies with different temperatures, ranging from 30 minutes to 5 hours. It has been found shortest at 20°–25° C.

19. The minimum, optimum, and maximum temperatures for the germination of the conidia are about 5°–7°, about 18°, and about 30°–32° C., respectively.

20. The period of motility of the zoospores varies greatly with different temperatures, decreasing as the temperature rises. The period of motility has been found to be 18–19 hours at 9°–10° C., while only 25′–50′ at 29°–30° C.

21. The conidia are extraordinarily short-lived. They die very soon after the layer of conidial fructification loses moisture. However, the conidia survive much longer in moist conditions.

22. The oospores germinate by germ-tubes which are hyaline, non-septate, and as a rule contain large globular bodies. The germ-tube grows very rapidly. Some of them have been found to grow at

the rate of 10 to 15 microns in five minutes at about 24° C. The diameter of the germ-tube ranges from 3 to 11 microns, mostly 3.5 to 5 microns. Hyphal branches are liable to grow at almost right angles to the main axis.

23. Various methods to germinate oospores have been tested, but the one described previously by the writer has been found to be the best.

24. Time required for the germination of oospores varies with different temperatures as well as with different materials.

25. The optimum temperature for oospore germination seems to be between 20° and 25° C.

26. The percentage of oospore germination increases with a certain lapse of time, indicating that the oospores have a dormant period.

27. No seedling infection has been found to occur in the soil infested with oospores about five years ago, but abundant infections have taken place in the soil inoculated with oospores about three years ago.

28. The dimensions of the oospores of *Scl. graminicola* obtained from various localities as well as from different hosts have been compared. The diameter of the oospores from *Setaria italica* has been found to vary greatly with different sources of the materials. Generally speaking, the oospores from *Setaria italica* have been found to be somewhat larger than those from other species of Setaria. However, the diameter of the oospores from some materials of *Setaria italica* has been found to be smaller than those from other species of Setaria.

29. There are various grades of susceptibility to the disease among different varieties of Italian millet.

30. The fungus from *Setaria italica* can infect green foxtail but with difficulty, so far as the present inoculations are concerned.

31. The fungus from *Setaria italica* can infect various varieties of corn, cane, teosinte and sorghum, but cannot infect yellow foxtail, Japanese millet, broom corn millet, and feterita.

32. The fungus from *Setaria viridis* collected in Japan and in the United States of America can each infect certain varieties of Italian millet.

33. Common millet is susceptible to both the fungi from *Setaria italica* and *S. viridis,* but Echigo, a variety of Italian millet

is very susceptible to the fungus from *Setaria italica,* while it is fairly resistant to the fungus from *S. viridis.*

34. The *viridis*-fungus collected in Minnesota has been found to be different in its infecting power from that obtained in three other localities. This may suggest an occurrence of physiologic specialization within the *viridis*-fungus.

35. The symptoms of the affected plants other than Italian millet are somewhat particular according to the kind of the plant. The details have been described in the text.

36. The coleoptile of the Italian millet seedling is subject to infection by oospores. The critical period for the infection extends from the beginning of the development of the coleoptile until the seedlings are grown up to about 5 mm. in height. When the seedlings grow up to about 2 cm. in height, infection takes place with difficulty.

37. The fungus can infect the seedling through the young root, when the primary root grows to about 3 mm.

38. The fungus can infect the seedling through its mesocotyl. The mesocotyl increases its resistance to infection as it grows longer.

39. If young seedlings, about 3 to 4 mm. in height, are inoculated at the base of the mesocotyl with the mycelium from oospores, 15 hours' incubation is sufficient for successful seedling infection.

40. The mycelium germinated from oospores retains its contagious power up to 4 days after the sowing of the oospores.

41. If young seedlings are inoculated with the hyphae from oospores, the occurrence of seedling infection is not greatly influenced by the temperatures between 15° and 30° C.

42. The optimum soil temperature for seedling infection lies at about 20° C., although infections take place between 11° and 34° C.

43. The optimum soil moisture for seedling infection is about 37 to 41 per cent of the maximum water holding capacity of the soil.

44. The percentage of seedling infection has been found to vary somewhat with different types of soil.

45. The seedling infection has been found to take place in alkali soils tested.

46. Experiments have shown that about 2 cm. depth of sowing is most favorable for seedling infection.

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P. S.

After the manuscript of the present paper had been completed, the following papers concerning the downy mildew of the Italian millet were published:

HIRATA, E. and TAKEUCHI, H.: On the morphological and physiological characters of *Sclerospora graminicola* on *Setaria italica*. Ann. Agric. Exp. Sta. Gov.-Gen. Chosen, **6**, No. 3, 1932.

TASUGI, H.: Studies on the physiology of the conidiophores, conidia and oospores of *Sclerospora graminicola* (SACC.) SCHROET. on the Japanese millet (*Setaria italica* (L.) BEAUV.). Journ. Imp. Agr. Exp. Sta. **2**, No. 2, 1933.

TAKASUGI, H. and AKAISHI, Y.: Studies of the downy mildew on Italian millet in Manchuria (First report). About the germination of oospores. Res. Bull. Agr. Exp. Sta. S.M.R. Co. No. 11, 1933.

The essential points of these papers were each reported previously in a preliminary form, and have been cited in the present paper (1, 105, 106). A more detailed discussion on these authors' investigations will be given in near future.

Description of the Plates

Plate IX

Figs. 1–3 Corn seedlings attacked by the *italica*-fungus
Fig. 1 Golden Bantam
Fig. 2 Golden cream
Fig. 3 White Rice
Fig. 4 A conidia-bearing leaf of the Italian millet
Figs. 5–6 Early stages of shredded leaves

Plates X–XII

Various types of deformed spikes bearing oospores

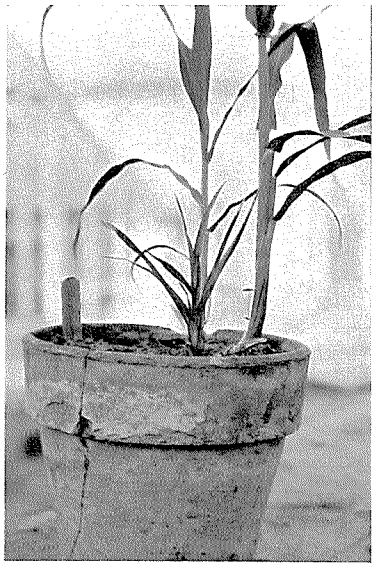
Plate XIII

Fig. 1 Conidia of the causal fungus
a Conidia produced on well-grown leaf at 24° C.
b Conidia produced on well-grown leaf at 13° C.
c Conidia produced on metamorphosed leafy structure in nature
Fig. 2 Conidiophores of the causal fungus
a Conidiophores produced under a saturated condition at 20° C.
b Conidiophores produced in the air humidity of 80–84 per cent at 20° C.
Fig. 3 Various types of germinating oospores

Plate XIV

Microphotographs of germinating oospores

1



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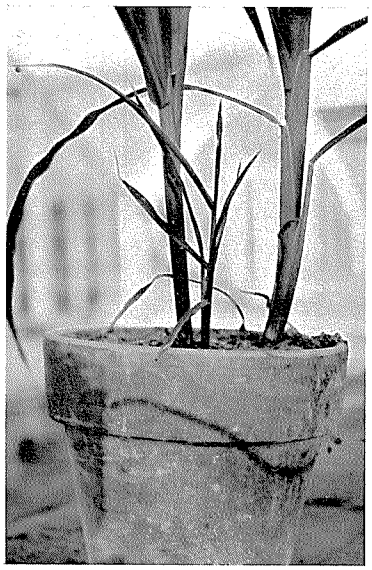

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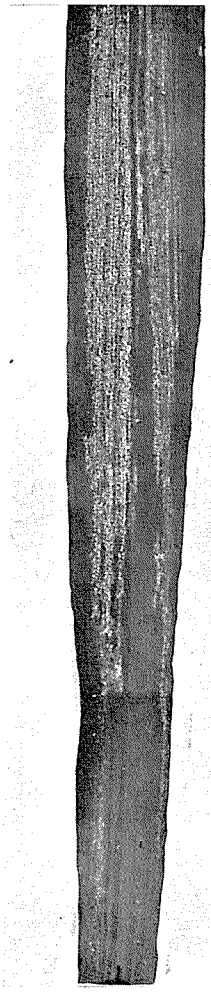

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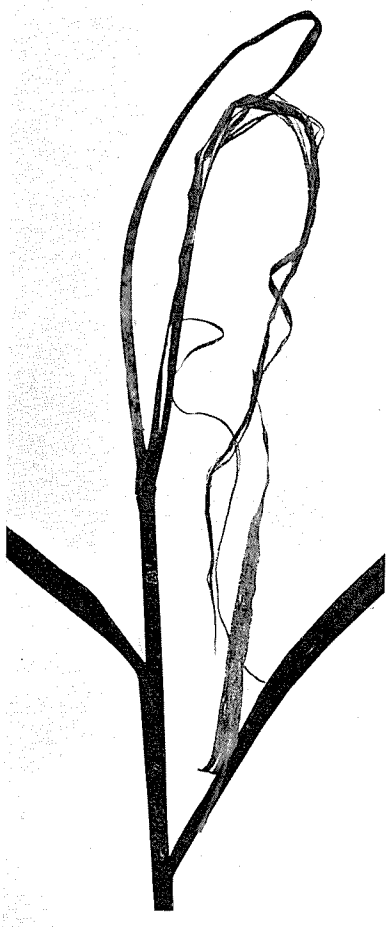

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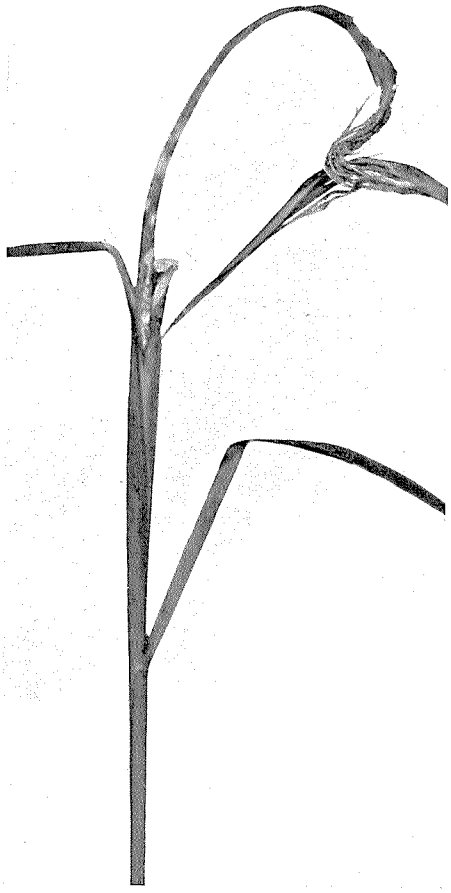
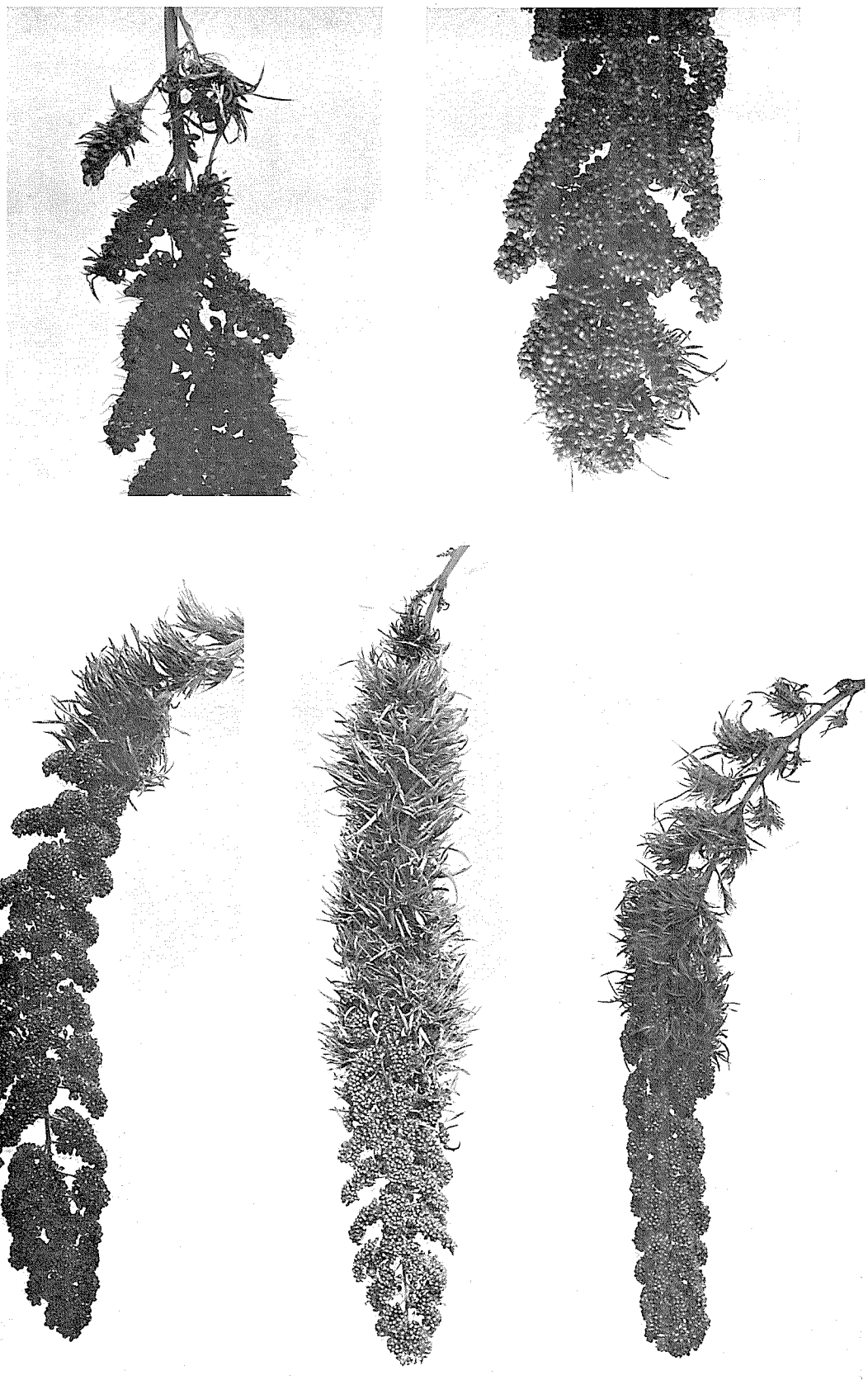


6

M. Hiura et S. Kawada Photo.



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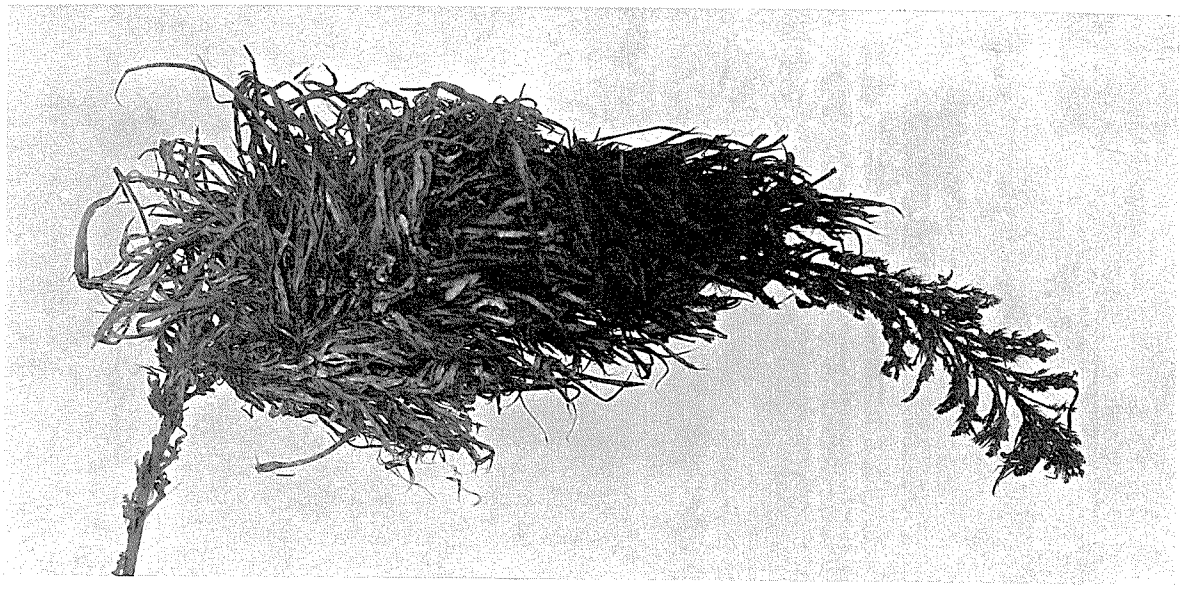

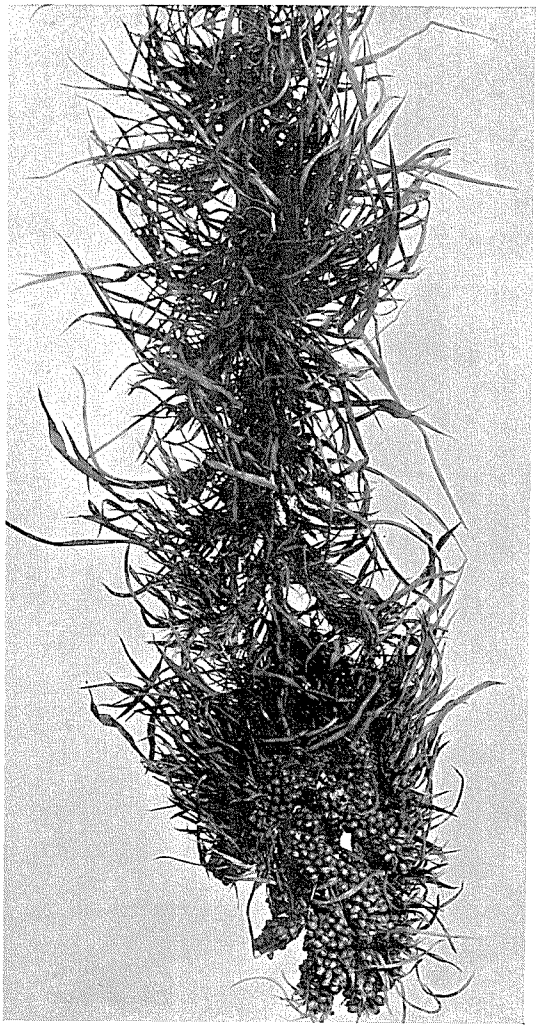

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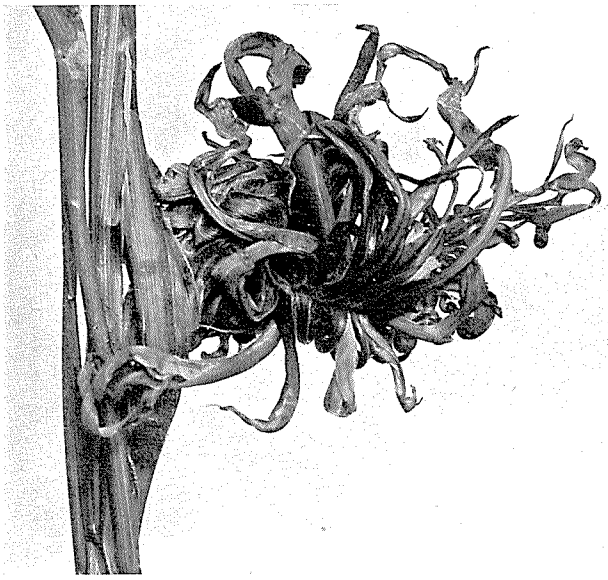
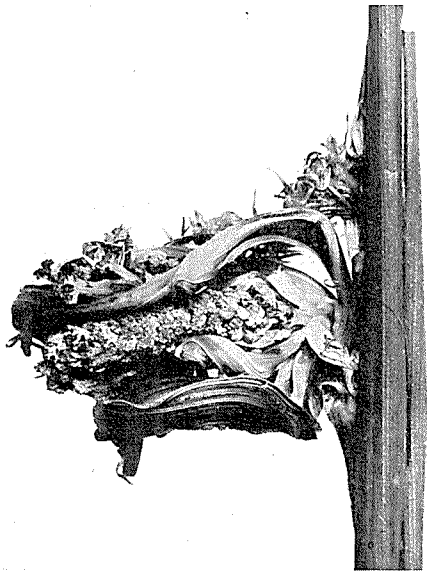




S. Kawada Photo.

Fig. 1

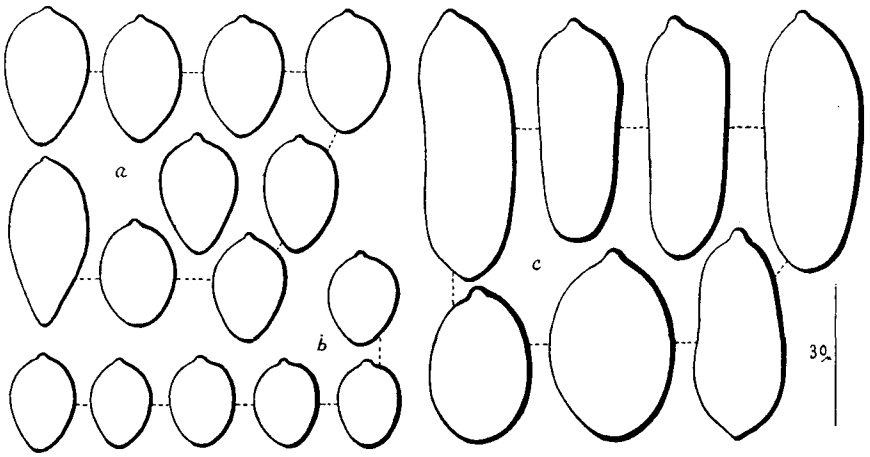


Fig. 2

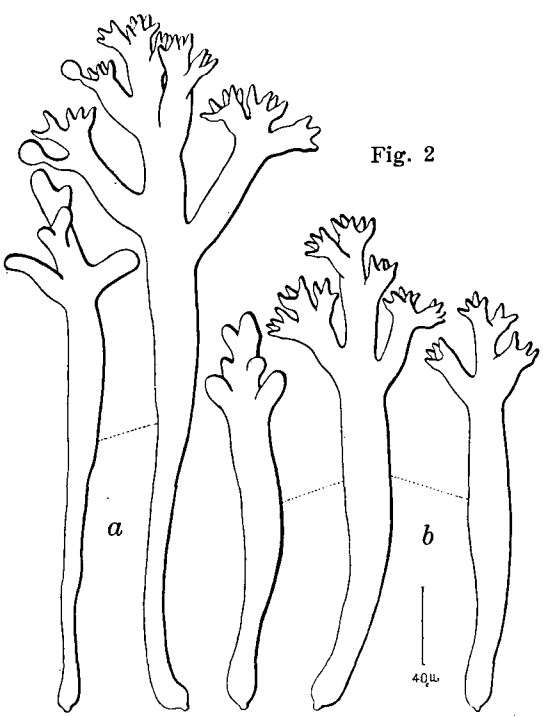
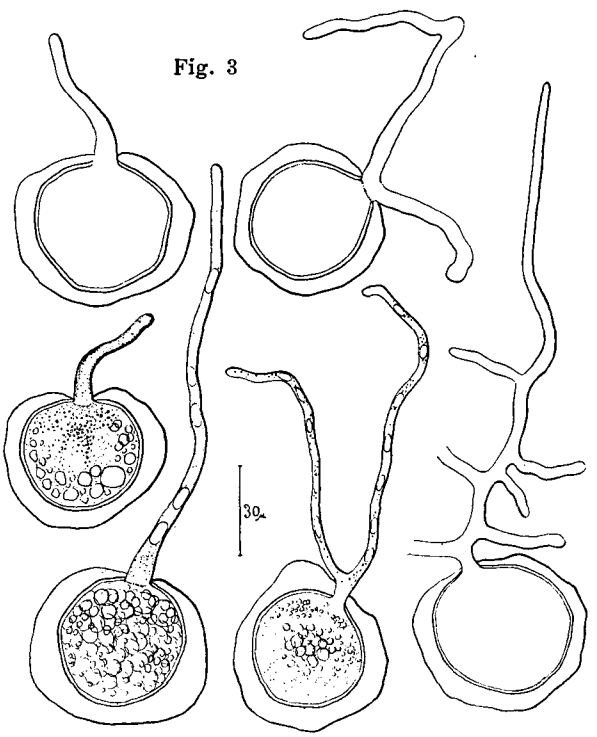
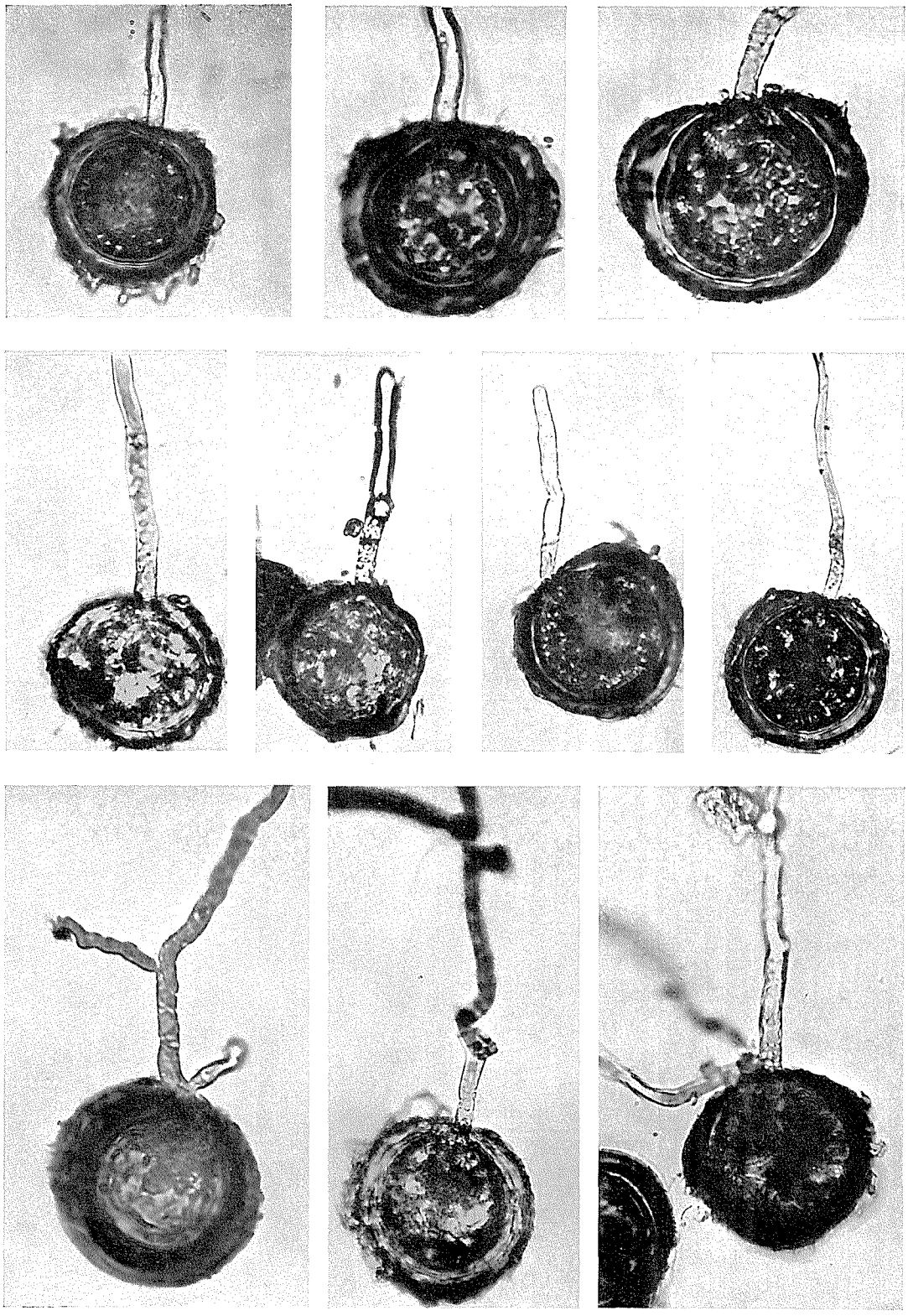


Fig. 3



M. Hiura del.



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