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Oospore and conidial response of species of Sclerospora

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OOSPORE AND CONIDIAL RESPONSE OF SPECIES
OF SCLEROSPORA

By
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A Thesis Submitted to the Graduate Faculty
For the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Plant Pathology

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The downy mildew, Sclerospora graminicola (Sacc.) Schr., is of world wide distribution upon the green foxtail grass, Setaria viridis (L.) Beauv. Other less widely distributed susceptible hosts include a number of different species in the three tribes Maydeae, Paniceae and Andropogoneae of the family Gramineae. The most important of the other susceptible hosts, in the United States, is Zea mays in the tribe Maydeae.

The fact that this downy mildew will attack corn and that it has a wide spread distribution upon Setaria viridis, a weed in corn fields, makes it a possible menace to the corn crop. It is conceivable that the environmental conditions may be sufficiently favorable in some years in corn growing areas for the mildew to become destructive on some varieties of corn. It seemed desirable therefore to have more information regarding the life history and the influence of environmental conditions upon the development of this fungus.
It is the purpose of this investigation to determine the host relationships and the influence of environmental factors such as temperature, moisture and soil upon this fungus, *Sclerospora graminicola* (Sacc.) Schr.
The fungus, Sclerospora graminicola (Sacc.) Schr., has been placed by early writers in several genera other than Sclerospora viz. Ustilago, Protomyces and Peronospora. It was placed in the genus Ustilago by Magnus (24) in 1879. He collected the fungus on Setaria viridis (L.) Beauv. in Baden, Germany in 1877, but did not publish the fact until 1879. He found that Urban had collected the same fungus near Berlin, Germany in 1875. After comparing the material from both localities, Berlin and Baden, Magnus placed the fungus in the genus Ustilago because of the size, structure and color of the spores and the fungus was named Ustilago urbani Magnus, in honor of Urban. Magnus points out the difficulty of deciding the systematic position of the fungus without knowing how the spores germinate.

About the same time that Magnus published the note on Ustilago urbani Magnus, Schroeter (38) published an article on Protomyces graminicola Sacc. which he held was identical with the fungus described by Magnus. He collected the material in the oospore stage in the vicinity of Rastatt in Baden, Germany on Setaria viridis (L.) Beauv. and Setaria glauca (L.) Beauv. He describes the fungus as occurring throughout the leaves of the host. The leaf parenchyma is
consumed except for the vascular bundles. Then red brown spore masses appear and the leaves become shredded. The conidia appear on the lower surface of the Setaria leaves. The conidiophores stand isolated, erect and are dichotomously branched. They are non-septate short and thick so that the conidia appear in clusters. The conidia are single, broad, oval or elliptical about 20 μ long. Schroeter chose to place it in the genus Protomyces because the material he collected compared most favorably with the descriptions and drawings made from the dry material by Saccardo (36) in 1876 and 1877. He first observed the oospore stage and later the conidia on Setaria. He held that conidial formation exhausted the leaves which led to the formation of the oospore stage. Like Magnus, he was unable to germinate the oospores and hence the fungus seemed more like Ustilago or Protomyces; however he makes the suggestion that the fungus may belong to the sub-genus Sclerospora in the Peronosporae. deBary later (1881) (9) divided the Peronosporae into four distinct genera one of which was Sclerospora.

Passerini (31), (32), described a fungus on Setaria verticillata which he calls Peronospora setariae. The appearance of the conidia on the lower surface of the young
leaves and the glotose, angular, dark ochraceous oospores compare favorably with the fungus described as *Ustilago urbani* Magn., and *Protomyces graminicola* Sacc.

Farlow (12) was the first to describe this fungus on *Setaria viridis* in America. He followed Schroeter's suggestion as to its being an ascomycete and called it *Peronospora graminicola* Schr. He called attention to the fact that the exospore proper is merely a thin film and the thick oogonial wall serves the purpose of the exospore.

The material from which Farlow made his description was sent to him by one of his correspondents in the Western states viz. Dr. L.H. Pammel, who collected the material at LaCrosse, Wisconsin on *Setaria viridis*. Farlow says, "this curious species for which Schroeter has created the subgenus *Sclerospora* has been found in several European countries, but is at present (1884) known only at LaCrosse in this country."

Halsted (15) reported *Peronospora graminicola* Schr. from Ames, Iowa in 1886. He noted that the mildew attacked and deformed the heads of the *Setaria viridis* as well as the leaves. The essential parts of the affected flowers are either abortive or wanting and the flowers are purplish in color. The oospores appear in the palets and flowering glumes as a dark brown powder.
Later Halsted (16, 17) reported that the mildew on Hungarian grass (*Setaria italica* Kth.) produced the same distortions of leaves and flowers as it did on green foxtail grass (*Setaria viridis*).

MacBride and Hitchcock (23) also reported *Peronospora graminicola* Schr. from Ames, Iowa, in 1888 on *Setaria viridis* and *Setaria italica*. This same year Swingle (43) found this mildew at Manhattan, Kansas, on *Setaria italica* (New Golden Wonder Millet) and *Setaria italica* var. *germanica* or Hungarian grass. He said, "this species did considerable damage to a plot of New Golden Wonder Millet and some Hungarian grass mixed with it."

Saccardo's *Sylloge Fungorum* (36) in 1888 described the mildew heretofore called *Peronospora graminicola* as *Sclerospora graminicola* (Sacc.) Schr. The other scientific names were then given as synonyms. All the publications from 1888 to the present have used the name *Sclerospora graminicola* (Sacc.) Schr.

*Sclerospora graminicola* (Sacc.) Schr. has been reported on several hosts from many localities since 1889. The description of the fungus is much the same in all of these reports so only the hosts, the collector and locality of the collection is given in the following summary.
Host

*Setaria viridis* (L.) Beauv.

Schroeter (39) Germany, 1889

Weber (48) Nebraska, 1892

Williams (57) Brookings, S. Dak., 1891

Berlese (2, 3) Italy, Germany, France, North America, 1899, 1902

Traverso (45) Italy, France, Germany, Russia, United States, 1902

Wilson (59) Fayette County, Iowa, 1910

Reglion (35) Germany, 1910

Davis (7) Wisconsin, 1914

Melhus, VanHaltern, Bliss (27) Ames, Iowa, 1926

Weston and Weber (56) United States, Europe, 1923

Weston (52) St. Paul, Minnesota, 1924

*Setaria glauca* (L.) Beauv.

Weber (48) Nebraska, 1892

Wilson (59) Fayette County, Iowa, 1910

Davis (3) Bridgeport, Wisconsin, 1915

*Setaria italica* (L.) Beauv.

Berlese (2) Italy, Germany, France, North America, 1902

Wilson (58) Europe and Asia, Vermont to So. Dakota and Kansas, 1907

Kulkarni (21) India, 1913
Host

Setaria italica (L.) Beauv.
Davis (7) Wisconsin, 1914
Hiura (20, 19) Japan, 1929

Setaria verticillata (L.) Beauv.
Wilson (53) Europe, Asia, United States, 1907

Setaria magna Griseb.
Weber (47) Gainesville, Florida, 1925
Weston and Weber (55, 56) Florida, 1926, 1928

Sea maya L.
Spegazzini (41) Argentina, 1909
Helmus, VanHaltern, Bliss (27) Ames, Iowa, 1928
Helmus and VanHaltern (26) Ames, Iowa, 1925
Weston (55) Wisconsin, 1929

Saccharum officinarum L.
Weston and Weber (56) England, 1928
Helmus, VanHaltern, Bliss (27) United States, 1928

 Panicum miliaceum L.
Helmus, VanHaltern, Bliss (27) United States, 1928

Buchlaena mexicana Schrad.
Helmus, VanHaltern, Bliss (27) United States, 1928

Buchlaena luxurians
Butler (4) India, 1907
Kulkarni (21) India, 1913
Host

Andropogon sorghum Brot.
Kulkarni (21) India, 1913

Pennisetum typhoideum Rich.
Kulkarni (21) India, 1913

Andropogon halepensis Brot.
Sydow and Butler (44) Poona, India, 1912

It is of particular interest that the localities, in which Sclerospora graminicola has been reported on Zea mays, are so widely separated viz., Argentine, South America, and Iowa and Wisconsin in the United States. The earliest report of Sclerospora graminicola on Zea mays was made by Spegazzini (41) in 1909 in Argentine and in the United States by Melhus and Van Haltern (26) in 1925 and 1928 (27). Then Weston (54) in 1929 reported that Sclerospora graminicola (Sacc.) Schr. was collected by Mr. Streats in Wisconsin in 1921.

Kulkarni (21) described many of the same symptoms for Sclerospora graminicola in India on the three cereals - Bajri (Pennisetum typhoideum), Jowar (Andropogon sorghum), Rala (Setaria italica) and a fodder grass (Euchlaena luxurians). The disease is first detected on Bajira by the pale yellow color of the very young plants and the white downy appearance of the lower surface of the second or third leaves. After the
-13-

plant is two months old the disease symptoms are long, narrow streaks and patches on the leaves. These pale yellow streaks become orange and finally turn dark brown after the cells of the attacked part are dead.

Most of the literature on Sclerospora graminicola (Sacc.) Schr. deals with the description of the disease and its host relationship. Infection of the host plant by oospores was observed long before the germination of the oospores was successful. Trelease (46) in 1896 stated that the spores, which originate in the leaf by the process of fertilization, presumably infect new plants in the spring. He did not describe any attempt to produce infection by artificial inoculation with these spores.

Weber (47) in his studies on the seedling infection of Sclerospora graminicola (Sacc.) Schr. on Everglade millet placed diseased tissue containing the oospores on the seeds. After several days, infection appeared on the seedlings which were about two inches tall. He concluded that the infection was probably caused by hibernating mycelium as no germinated oospores were found after diligent search. However, Melhus and VanHaltern (26) produced infection in dent, sugar, pop, and flint corn by placing the oospores on the seed in the soil. Like Weber, they were unable to observe any germinating
oospores, but they concluded that the infection took place before the emergence of the plumule from the soil. Melhus, VanHaltern and Bliss (27) were able to produce infection in Zea mays and Setaria viridis by mixing the oospores with the soil above and below the seed as well as by placing the oospores on the seed. They were not successful in producing germination, under laboratory conditions, with the same collection of oospores that produced the infection.

Weston and Weber (56) sterilized the seed of Setaria magna and the soil, then placed the oospores still embedded in the leaf tissues on the seeds. They observed infection on seedlings four weeks old. Some of the oospore infested leaf tissue of this same experiment was taken from soil, but none of the oospores were seen germinating. These writers also concluded that infection takes place before the seedlings had emerged from the ground. Hiura (20) stated that primary infection took place when the young seedlings of Italian millet were underground. He germinated the oospores from green leaves in soil water, tap or distilled water at a temperature of about 15°C. He found that they germinated equally well whether in the laboratory, the greenhouse or the field from February to November 1928. Hiura mentioned in this report that he had visited the Nishino Experimental farm and found that an experimenter there had germinated the
oospores, but had not yet published his data. A letter from Mr. Hiura to Dr. Melhus stated that "----- Mr. Tasugi who is plant pathologist at the Imperial Agricultural Experiment Station, Nishigahara, Tokyo ---- has not yet (March 1930), published." At least Mr. Hiura had not received word of the publication at that time.

The translation of Gaumann's, Comparative Morphology of Fungi, by Carroll W. Dodge (10) stated that "the germination of the oospores of ----- Sclerospora and Peronospora is through a germ tube which develops in the host into a mycelium." Mr. Dodge in correspondence with Dr. Melhus concerning this statement said that "Gaumann does not give a reference in his bibliography----. The only other possibility is that it may rest on otherwise unpublished observations of Gaumann himself."

Evans and Harrar (11) germinated the oospores from Setaria viridis in much the same way as Hiura by placing them in distilled water in watch glasses at a temperature of about 18°C. They observed hyaline branched germ tubes containing numerous globular bodies.

There are ten species in the genus Sclerospora and one variety, S. graminicola var. andropogoniasorghii. Kul.

Sclerospora magnusiana Sor. was first described in 1889.
by Sorokine (40). He collected this material on *Equisetum* in the vicinity of Orsk, Russia. The description is much like that given by the early writers for *Protomyces graminicolae* Schr., *Ustilago urbani* Magn and *Beronosporea setariae* Pass. for the production of both oospores and conidia. The writer has been unable to find reference to this species in other publications.

*Sclerospora sacchari* T. Miy. is also very much like the type species *S. graminicolae* according to Miyake (28). It occurs on *Zea mays*, *Euchlaena* sp. and *Saccharum officinarum* in Formosa, Queensland, Fiji Islands and the Philippine Islands, where it is called the "leaf stripe" disease of sugar cane by Lee and Medalia (28). The conidial phase of this species is the most destructive. The conidia are said to show nocturnal production and germinate by a germ tube.

*Sclerospora javanica* (Rec.) Palm is described by Palm (30) only in the conidial stage. In Java where it is found it is called the "lyer" or "sleepy" disease on the hosts *Zea mays* and corn-teosinte hybrids. The conidia germinate by a tube which penetrates the leaf through one of the stomata. Infection by these conidia is retained about one day if they are lying exposed on the leaf; however after four days in or on the soil they are not viable.
The conidia are disseminated by the aid of the wind. Studies in the field have proved the presence of conidia in the air and have shown that the conidia are able to travel over a distance of more than two kilometers and still remain viable. The disease is propagated only by means of the conidial fructification.

*Sclerospora macrospora* Sacc. is known and described by Saccardo (37) only in the oospore stage in the leaves of *Alopecurus* sp. The oospores are larger than the type species *Sclerospora graminicola*. The conidia have not been observed.

*Sclerospora maydis* (Sacc.) Butl. attacks young corn plants which soon become chlorotic and fail to develop. The enormous destructively power of this fungus has become an important feature in connection with corn culture in the Philippines. Ten per cent of the crop and above is commonly lost through this disease and according to Baker's report (1) whole fields may be swept clean. Reinking (37) further describes the disease as causing stunted plants, due to the checking of growth and frequently to the shortening of the internodes. Butler (5) states that no oospores have been found. The conidial stage appears on *Buchloea* sp. and *Zea mays*. The conidia germinate by a germ tube.

*Sclerospora philippinensis* Weston was first named and
This fungus is very prevalent in the Philippines, oftentimes in the Orient, and resembles very closely the other Oriental species of S. javanica Palm, S. maydis (Pers.) Butl. and S. sacchari T. My. All of these species are similar in effects and show close relationship in structure and development. They are all characterized by the predominance of the conidial stage with the exception of S. sacchari and oospores in this case were not observed with certainty.

Under favorable conditions for the development of Sclerospora philippinensis whole fields of maize may be destroyed. The maize plants are usually infected as young seedlings and the mycelium is found in practically every part of the host except the roots. The symptoms are much like other Sclerospora species appearing as linear or irregular whitish yellow to pale spots, often entirely discoloring the leaves and more or less deforming the host. The conidia germinate by a tube. The dissemination of these conidia apparently transmits the disease from plant to plant.

Sclerospora spontanea. Weston also a downy mildew of maize in the Philippines has been adequately described by Weston (50). Its destructiveness is much like S. philippinensis, however, its conidia conidiophores are unlike this
this species morphologically. The conidia lack the apical papilla and are greater in length and width than S. Philippinensis. The conidia germinate by a tube.

Sclerospora spontanea has been reported on the following hosts: Zea mays L.; "Bugang" or Saccharum spontaneum L.; and Saccharum officinarum L. It has been transferred to Euchlaena luxurians Schrad. teosinte, and a wild grass Miscanthus japonicus (Thunb.) Anders. The data for these Philippine downy mildews indicates that wild grasses are natural hosts of the oriental form from which they have passed and are passing to susceptible introduced crops such as maize.

The conidia of these two downy mildews, Sclerospora philippinensis and S. spontanea are the dominant and most destructive phase of their life histories. They are said to appear at night when the leaf surface is covered with dew or other moisture and are produced in successive crops over a period covering 75 per cent of the life of the plant. They are dispersed at night by wind and splashing of rain.

Sclerospora farlowii n. sp. was first collected on Chloris elegans at Cochise, Arizona in 1900 by Griffiths (14). It is one of the commonest fungi throughout southern Arizona and northern Mexico. The effect upon the plant is never
serious and has not been observed to cause any reduction in the size of the attacked plants. The fructification appears in the leaf sheaths forming irregular grayish-black discolorations which are darker around the edges of the infected areas. Oospores are found embedded in the tissues of the host, but no mention is made of the conidial stage.

Five of the two species of Sclerospora are known only in the conidial stage viz., S. javanica, S. maydis, S. sacchari, S. philippinensis and S. spontanea. Four of the species are known only in the oospore stage viz., S. magnusiana, S. miscanthi, S. farlowi and S. macrospora. Sclerospora graminicola and S. graminicola andropogonis sorghi are known in both the oospore and conidial stages, but the oospore stage predominates.
THE LIFE HISTORY OF SCLEROSPORA GRAMINICOLA

All of the spore stages of the downy mildew on Setaria viridig have been germinated and their sequence in the life cycle has been determined and so before further discussion of the fungus is given the life cycle will be summarized.

The oospores of Sclerospora graminicola are borne in the fall of the year in the parenchymatous tissue of the leaves of Setaria viridig. This production of the oospores causes the parenchyma to disintegrate leaving only the vascular system and the oospores. The oospores are held loosely in the shredded leaves and are easily scattered by the wind or rain. They fall to the ground where they remain through the winter months. About the same time that the oospores are ripe the seeds of Setaria viridig also mature are scattered on the ground.

In the spring when the seeds of the Setaria viridig begin to grow the oospores germinate.

The oospores push out a germ tube, which enters the host. The mycelium grows with the host and as soon as the first leaves expand the fungus sends out conidiophores through the stomata on the lower surface of these leaves. These conidiophores in turn produce the conidia of the fungus. The mycelium in the old parenchymatous tissues
also produces oospores.

THE DISEASE

Very young seedlings of Setaria viridis unfolding the first leaves do not show symptoms of Sclerospora graminicola. However, these small leaves (1.5 to 3 cm.) will later show the glistening "downy" appearance of the mildew on the under surface. If these small leaves are generally infected they soon shrivel and die. Many such shriveled plants are shown in figure 1. The expanded leaves of the plants shown in this figure are either normal or showed only a small amount of infection.

The conidiophores often appear on both surfaces of these very young first leaves of Setaria viridis.

The young seedlings not destroyed by the mildew continue to grow and the mycelium apparently advances with the growing parts, as manifest by the light yellow streaks between the veins in the leaves. These streaks at first are very narrow, but increase in size as the leaf increases. These same yellow streaks appear on the secondary leaves soon after they unroll and are not confined to any particular portion of the leaf. However, the infection as a white "downy" surface appears only on the lower surface of these
secondary leaves. As the plant matures the fungus reaches all the leaves and oftentimes the inflorescences. The heads are not normal in size and are sterile. The most striking symptom of infection in the inflorescences of *Setaria viridis* is the curling or malformation of the heads and the enlarged glumes of the flowers giving the head a leafy and
ragged appearance. Halsted (15) notes that usually not more than one head in the same plant is affected.

Weston and Weber (56) describe this mildew on the wild grass, Everglade millet (*Setaria magna*), as first showing pallid yellowish markings restricted to the base of the lowest (earliest) leaves. Frequently the young plants are one or two feet high before the fungus invades the later unfolding leaves which become pallid and produce the conidia on the lower surface of these leaves. The small seedlings which are infected early are killed within a few weeks.

The symptoms of *Sclerospora graminicola* on dent corn, pop corn and teosinte are much the same as far as the yellow streaking of the leaves of young plants is concerned. However, the older plants show distinct stunting and spreading which is due to the short internodes. Two diseased plants exhibiting this stunting are shown in foreground of figure 2. These plants are dent corn var. Iodent infected by oospores from *Setaria viridis*. Both of these plants show yellow streaking and have produced conidiophores, while the two larger healthy plants do not show symptoms of the disease.

The infection of popcorn var. Japanese Hulless by oospores of *Setaria viridis* has been described by Melhus, VanHaltern and Bliss (27). They describe extreme shorte-
Fig. 2. Two healthy plants of dent corn var. Iodent and two diseased plants. Note the short spreading habit and yellow streaking of the leaves of the diseased plants.
ing of the internodes, which causes the overlapping of the leaf sheaths on one side and spreads them apart on the other. The transparency of the leaf veins imparts a streaked appearance to the leaves, while the small chlorotic areas between the veins give the typical mottled effect of *Sclerospora graminicola*. Many of these symptoms have been confirmed by the writer. Figure 3 shows a diseased plant of popcorn var. Japanese Hulless infected by oospores of *Setaria viridis*. The diseased plant in this photograph is typical of many plants of popcorn var. Japanese Hulless infected under greenhouse and laboratory conditions. The internodes are short, but the plants do not show as much spreading as the dent corn var. Iodent. The leaves show the yellow streaking and mottling symptoms so typical of this disease.

Another host susceptible to *Sclerospora graminicola* on *Setaria viridis* is teosinte (*Duchlaena mexicana* Schrad.) according to Helhus, VanHaltern and Bliss (27). The symptoms of the disease on teosinte are not as positive as on *Setaria viridis*. The first leaves appear mottled by yellow streaks and blotches the same as noted on the dent corn and popcorn seedlings. The conidiophores are produced on these mottled areas when the plants are placed under conditions of high humidity and low temperature. These pallid areas on the leaves are also described by Weston and Craigie (54) as
Fig. 3. Plants of popcorn var. Japanese Hulless showing two healthy plants and one diseased.
a symptom of *Sclerospora philippinensis* on teosinte. It is imperative that the teosinte plants showing symptoms of disease should produce conidiophores because sometimes these areas that appear yellow at first soon turn red and do not produce conidiophores.

The older diseased plants of teosinte are stunted, figure 4, but do not show the spreading due to shortened internodes as described for popcorn var. Japanese Hulless and dent corn var. Iodent.

The infection by *Sclerospora graminicola* is most commonly systemic because the mycelium in the tissues follows the growing point. However, on Everglade millet Weston and Weber (56) report local infection. The local spots are distinctly defined, somewhat sunken and have an irregular roughened surface. The spots are buff to dull reddish to dark-brown in color and vary in size from a few millimeters to linear streaks.

This mycelium in the host tissue in the mature plant is replaced by oospores that originate on branches of the mycelium within the leaf after fertilization, Stevens (42). These oospores form only in the parenchyma tissue of the leaves and soon disintegrate all the tissue between the veins leaving the vascular tissue not attacked by the fungus. It is the remaining vascular tissue that gives the older in-
Fig. 4. Plants of teosinte showing one healthy and two diseased plants.
fected leaves the shredded appearance such as shown in figure 5. Halsted (15) found that oospores were also formed in the outer glumes of the flowers in the malformed heads. Butler (4) describes the formation of "leaf heads" on Setaria italica in India. These two types of symptoms viz. shredded leaves and malformed heads are shown in figure 5. The local infection spots described by Weston and Weber (56) do not produce oospores in Everglade millet and consequently the leaves do not shred.
Fig. 5. The malformed head and shredded leaves of Setaria viridis containing abundant oospores.
MATERIALS AND METHODS

Materials

The oospore material was collected as shredded leaves of *Setaria viridis* in corn fields near Ames, Iowa in the fall and spring during the years 1924 to 1930, by Dr. I. E. Helhus, Mr. Frank VanHaltern, Mr. Donald Bliss and the writer. This material was stored in a dry condition at a room temperature of 20°C to 25°C. In some instances the oospores were thrashed out of the host material and stored (dry) in bottles. Other oospore material was collected by Mr. Dewey Stewart near Fort Collins and Windsor, Colorado in August 1929.

The shredded leaves of *Pennisetum typhoideum* (Sajri) containing the oospores of *Sclerospora graminicola* var. *andropogonis sorghi* were sent to the writer by Dr. B.N. Uppal of Poona, India, in 1927. Oospore material in shredded leaves of millet was obtained from Mr. R. H. Porter and collected at Nanking, China in 1927. The oospore material on Everglade millet, *Setaria magna*, was secured from Dr. James Seal, collected in Florida in 1927.

Some of the seed used for the determination of the host range was obtained from the Office of Forage Crops, the Seed Laboratory of the Bureau of Plant Industry, Washington, D. C. and from the Department of Farm Crops of Iowa State...
College.

The seed of *Setaria viridis* and other grasses in the three tribes *Maydeae*, *Paniceae*, and *Andropogoneae* were collected in the corn fields near Ames, Iowa during October and November of 1927, 1928 and at Ft. Collins, Colorado in 1929.

The seeds used in the experiments to determine the host range of *Sclerospora graminicola* were:

**Tribe Maydeae**

*Zea mays* L. (dent corn var. Iodent (popcorn var. Japanese Hulless (maize from India.

*Euchlaena mexicana* Schrad. – teosinte

*Coix lachryma* – *jobi* L. – Job's tears

**Tribe Paniceae**

*Setaria viridis* (L.) Beauv. – green foxtail

*Setaria glauca* (L.) Beauv. – yellow foxtail

*Setaria italica* (L.) Beauv. – Hungarian millet, *rula

*Setaria italica* Beauv. var. *ruzrofructa* Bailey, Siberian millet

*Pennisetum typhoides* Rich. pearl millet, bajri

*Panicum miliaceum* L. – millet, Proso millet

*Panicum capillare* L. – tumble grass

**Tribe Andropogoneae**

*Andropogon sorghum* Brot. – sorghum, jowar
Tribe Andropogoneae

Andropogon sorghum var. technicus Koern. & Wern.
- broom corn

Holcus sorghum L. var. Durra, Bailey - Durra
Holcus sorghum L. var. caffrorum, Bailey - kaffir
Holcus sudanensis, Bailey - Sudan grass

The conidia used for infection and germination studies
were collected from stock cultures of infected Setaria
viridis growing in the greenhouse. These cultures were ob-
tained by mixing oospores in the top soil or by placing them
directly on the seed at time of planting.

A constant temperature and percentage of soil moisture
was necessary in order to obtain infection under controlled
conditions. The temperature tank used to maintain a constant
temperature consisted of a long wooden tank in the bottom
of which were copper coils. By keeping the water running
through these coils a temperature as low as 8°C. could be
maintained. The higher temperatures were obtained by shutting
off the water through the coils and inserting a small electric
heating unit. This tank is shown in figure 6.

A constant percentage of soil moisture was maintained by
placing the soil of known moisture content into a tin tray
in the tank. These trays were sealed with wax paper before
being placed in the tank. Mr. Henderson (18) found that a
Fig. 6. A constant temperature tank used for infection work with oospores of *Sclerospora graminicola.*

Negligible amount of moisture was lost by this method. Several trays and their parts were pictured in figure 7.

The soil trays pictured in figure 7 are arranged to exhibit the parts of the tray. The tray in the upper left hand side of the picture (fig. 7 A.) is filled with the soil of known moisture percentage, the seeds, and the oospores, the tray is sealed over with the wax paper by
Fig. 7. Soil trays of the temperature tank. A. sealed tray; B. showing wax paper fastened to one side of tray; C. tin strip which fits in tray; D. showing how strip is used to pull out soil and seedlings.

folding over the top as on the tray in the upper right hand side (fig. 7 B.). A strip of tin just as wide as the tray fits inside and extends above the top of the tray (fig. 7 C.) at the ends. By pulling on these extended tabs the soil and the seedlings are easily removed from the tray.
without injury to the very young seedlings. Figure 7 D, shows *Setaria viridiflora* seedlings and soil removed from the tray by the metal strip. These trays may be set up with any percentage of soil moisture and at any temperature. Figure 6 shows several of the trays in position in the temperature tank.

**Methods**

Infection of the various hosts by ooospores was obtained by using the constant temperature tank, a sandy loam soil, viable seeds and viable ooospores. The sandy loam soil was made up to various percentages of moisture content by a method based on the dry weight of the soil. The soil was weighed in 200 to 400 gram lots and then dried at 110°C. for 24 hours. It was again weighed and water added to bring it to the desired soil moisture content.

The sandy loam soil of the various soil moistures was then placed in a tray as shown in figure 7. The seed was next planted on this soil, and the ooospores were either scattered on these seeds or mixed with the soil used to cover the seeds. The tray was next sealed with wax paper and placed in the tank for six to ten days at the desired temperature. At the end of this time the trays were opened and the seedlings transplanted to the greenhouse.
The spores for germination study were scattered over a few centimeters of distilled water in the bottom of Syracuse watch glasses. These glasses were then stacked and placed in a moist chamber, in the incubators or refrigerators at various temperatures. At the end of the time required for germination the watch glass was fastened into a mechanical stage on the microscope where the germination could be easily and quickly examined and the number of germinated spores counted.

The conidia used for germination and infection were collected from the leaves of *Setaria viridis* either in the field or from stock cultures in the greenhouse. The infected leaves that were collected in the field were brought to the laboratory, wiped with a clean cloth, placed in a moist chamber and quickly cooled to 15°C, thus forming a film of moisture on the leaves. The conidia were removed from these leaves into drops of distilled water in van tieghem cells which were placed at different temperatures. After the incubation period the van tieghem cell with the drop was placed on the mechanical stage of the microscope in the same manner as the Syracuse watch glasses. This made it possible to observe accurately the steps for the germination of the conidia.
The conidia used for the infection experiments were removed from other infected leaves into several centimeters of distilled water in an atomizer or flask. This conidial suspension was then sprayed or poured on the young seedlings. These exposed seedlings were incubated at 18° to 25°C. for several hours before being placed on the greenhouse bench.

**EXPERIMENTAL DATA**

The oospore material, already described of *Sclerospora graminicola* on *Setaria viridis*, was used for studies to confirm the germination of the oospores. The effects of various conditions upon germination led to a particular consideration of the relation of the fungus to moisture, temperature, infection and host range.

**Description of Oospores of Sclerospora graminicola**

The oospore of *Sclerospora graminicola* is spherical and enclosed in a thick amber to reddish brown-colored oogonial wall. Stevens (1942) describes the first formation of thickness of this oogonial wall when the nuclei during mitosis are passing into the spireme stage. After fertilization the oospore wall develops rapidly, first appearing as a clearly defined wall, then rapidly increasing in thickness. As the oospore wall thickens the oogonial wall collapses in regular
folds and the periplasm and periplasmic nuclei degenerate. These folds on the outside of the oogonial wall give the spore an irregular contour. Berlese (2) gives the diameter of the oospore and outer wall as 50 to 60 by 40 to 45 μ. Traverso (45) gives the diameter of the oospore as 32.0 μ and the oogonial wall as 5.3 μ which brings the total diameter (37.3 μ) a little smaller than the measurements given by Berlese. Weston and Weber (56) give the measurements for the oogonial wall as 2 to 10 μ thick and the oospore as 30 to 36 μ for the larger limits. They note also that the smooth wall of the oospore is 2 μ thick.

The oogonial wall often shows distinct lines as though the wall was in layers around the oospore. The contents of the oospore appears finely granular with a distinctly denser spot near the center, or as coarsely granular with no particularly dense parts.

Preliminary study of the germination of the oospore disclosed the fact that the spores of other fungi deposited on the irregular surface of the oospores germinated before the oospore germinated and the germ tubes and hyphae of these contaminating spores obscured the germ tube of the oospore. Hiura (20) encountered this same difficulty of contaminating spores. This difficulty indicated that it was necessary to clean the oospores before setting up further germination
studies.

The oospores, after having been threshed out of the shredded leaves, were placed in various chemicals and under various other conditions. After the treatment the viability of the oospores was tested by placing them on seeds of *Setaria viridis* and then noting the amount of infection produced. The chemicals made up to various concentrations included $\text{H}_2\text{SO}_4$, $\text{HCl}$, $\text{CuSO}_4$ and $\text{HgCl}_2$. The result of these tests indicated that where oospores were in two per cent $\text{H}_2\text{SO}_4$ at 50°C for three to five minutes the treatment did not inhibit infection of *Setaria viridis* by these spores under greenhouse conditions. This test included 207 plants showing 4.45 per cent infection. These oospores did not appear to germinate when placed in distilled water at 18°C, which indicates that this method may not be used for cleaning oospores for germination studies.

Evans and Harrar (11) obtained positive germination of the oospores in sterile distilled water at 18°C after cleaning them with a five per cent solution of lactic acid and then washing them in sterile distilled water. The writer was unable to confirm this method of cleaning the oospores, but found that centrifuging the oospores several times in distilled water washed away most of contaminating debris and spores. After the oospores had been cleaned they were allowed
Germination of the Oospores

Oospores collected in August, 1929 at Fort Collins, Colorado and in January, 1930 at Ames, Iowa were cleaned as previously described, by centrifuging, and placed in distilled water at 18° to 20°C. The method used for germination was essentially the same as given by Evans and Harrar (11) viz. oospores floated on a few cubic centimeters of distilled water in Syracuse watch glasses. These glasses were then covered to lessen the amount of evaporation and placed at 18°C. and germination was noted after 48 hours. Only one trial of the oospores collected at Ames, Iowa showed 24 per cent germination. The four trials totalling 226 oospores showed 1.49 per cent germination with oospores collected at Fort Collins, Colorado in August, 1929. Hiura (26) was successful in germinating the oospores in tap, distilled and soil water and calcium nitrate. Evans and Harrar (11) record good germination on agar, malt potato dextrose, corn seedling agar, bouillion and in soil.

The appearance of the contents of the oospore changes as it germinates. At first the contents are finely granular with a darker spot, probably the nucleus, in the center or at one side of the oospore. This dark spot disappears, the contents become more coarsely granular and often dark.
radial lines appear a little while before the spores germinate. Schroeter (38) noticed somewhat the same phenomenon when he sowed the oospores, stored under dry conditions, on water and moist substrata but did not observe a distinct germination process. He states further that the previously dry protoplasm divided equally in the spore under these conditions (water) and took on a granular appearance; later it divided into a number (8 to 12) of round, elliptical divisions. He was not sure whether the appearance was in preparation for swarm spores or only as a process associated with the decay of the protoplasm. Several of the oospores in figure 3 show the darker granular spot and radial lines.

The oospores germinated by a tube which grows rapidly and became very long and branched within a few days after germination takes place. This germ tube appeared finely granular, hyaline, branched, and is non-septate and the protoplasm often is denser in some places than in others. This same condition was noted by Hiura (20) who states that the germ tubes have the contents concentrated on the side of the tube. Evans and Harrar (11) also noted that the germ tubes contained numerous globular bodies and seemed to be empty or devoid of cytoplasm.
Fig. 8. Oospores showing the appearance of the protoplasm before germination and the germ tube.

The germ tube appeared to come from the oospore through the oogonial wall without splitting it. Evans and Harrar (11) observed that the walls of the tubes were a direct continuation of the endospore wall and that they protruded through a small opening in the exospore apparently dissolved by the action of the forming germ tube, at least no pore was ever observed on a dormant oospore.  — germinating
ooospore is shown in figure 9. These spores were floating on distilled water in a Syracuse watch glass.

Fig. 9. A germinating ooospore of Sclerospora graminicola. Note the granular non-septate germ tube.

The germ tubes measure 600 to 700 μ long and the diameter 5 μ. (Evans and Harrar (11). Miura (20) finds that they are usually 6 μ to 8 μ in diameter. Figures 8 and 9 show that there is a slight constriction of the germ tube at the surface of the oogonial wall.
Oogonial Infection in the Field

The mature oospores are shattered from the shredded leaves and fall on the ground along with seeds of *Setaria viridis* and overwinter in the soil. In the spring infection of the young seedlings is produced by the oospores and subsequently conidia are produced in May and June. If these deductions are correct, and they do appear coincident with the life history of the fungus, then a study of the climatological data should give the conditions under which primary infection takes place. The presence of conidia, indicating primary infection, has been noted in the fields near Ames, Iowa on the leaves of seedlings of *Setaria viridis*, during May and June. The climatological data for this period were put in tabular form.

Table 1. Climatological data for Ames, Iowa for May and June of the years 1926, 1927, 1928 and 1929

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature</th>
<th>Ppt. in inches</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Highest</td>
</tr>
<tr>
<td>May 1926</td>
<td>65.6°F. 18.3°C.</td>
<td>95°F. 35.0°C. 34°F. 1.1°C.</td>
</tr>
<tr>
<td>June 1926</td>
<td>66.8</td>
<td>19.4</td>
</tr>
<tr>
<td>May 1927</td>
<td>58.7</td>
<td>15.0</td>
</tr>
<tr>
<td>June 1927</td>
<td>67.0</td>
<td>19.4</td>
</tr>
<tr>
<td>May 1928</td>
<td>63.6</td>
<td>17.2</td>
</tr>
<tr>
<td>June 1928</td>
<td>66.0</td>
<td>18.9</td>
</tr>
<tr>
<td>May 1929</td>
<td>68.4</td>
<td>20.0</td>
</tr>
<tr>
<td>Average</td>
<td>64.2</td>
<td>17.0</td>
</tr>
</tbody>
</table>
During May and June for the years 1926 to 1929 inclusive, at Ames, Iowa the mean atmospheric temperature was 17° C. and the rainfall averaged 2.93 inches. Since the soil was usually several degrees lower than the surrounding air we may surmise that a soil temperature of 15° C. and high moisture content were the conditions under which infection of *Setaria viridis* takes place in the field.

Preliminary experiments, under greenhouse conditions where the temperature was between 15° to 18° C. and the soil at various moistures, indicated that the supposition drawn from the climatological data had merit. The data obtained from these experiments made in 1928 and 1929 with oospores and seeds of *Setaria viridis* collected in 1927 and 1928 showed a comparatively low percentage of infection. There were 236 plants in the experiment with oospores collected in 1927 and 11.5 per cent of these plants were infected. The experiment with 1928 oospores included 1233 plants showing 13.0 per cent of them infected.

The results obtained in these preliminary experiments may be interpreted to mean that in order to obtain high percentages of germination and subsequent infection, it became necessary to determine the optimum soil moisture for the germination of the oospores and seed.
Soil Moisture Necessary for Oospore Infection

A sandy soil was divided into six different lots each made up to have a soil moisture content of 5, 10, 15, 20, 30 or 35 per cent, by the method previously described. The seed of *Setaria viridis* and the oospores were planted in these various soils in the temperature tank trays. The trays were sealed with wax paper so that the moisture did not change for the duration of the experiment which was six to ten days. These first experiments were initiated in temperatures from 11° to 20°C because these temperatures prevailed in May and June when infection took place in the field.

This experiment pointed out that a soil moisture content above 30 per cent was too wet to be favorable for germination of the seed and that 5 and 10 per cent were too dry. This also meant that plants showing possible infection were obtained only in soils with moisture content of 30, 20 and 15 per cent.

The oospores used in the experiment with the different soil moistures at 11° to 20°C were from six to twelve months old. After the plants were two centimeters high (in about 10 days) the trays were opened and the tiny yellow leaves of the covered plants allowed to expand and to become green. At this time they were transplanted to pots, placed on the
greenhouse bench and allowed to grow until the secondary leaves appeared. They were then placed under conditions of high humidity and temperatures, which simulated field conditions and the infected plants produced conidia. Only the plants showing conidia were counted as positively infected. A summary of the data obtained from this experiment is given in Table 2.

Table 2. Oospore infection of Setaria viridis in sandy loam soil of 30, 20 and 15 per cent moisture at 11° to 20°C.

<table>
<thead>
<tr>
<th>Year</th>
<th>Year of exp.</th>
<th>Per cent soil moist.</th>
<th>Temp.</th>
<th>No. of oospores collected</th>
<th>No. of inf. plts.</th>
<th>Per cent inf.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1928</td>
<td>1929</td>
<td>30</td>
<td>15°-18°C.</td>
<td>99</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1928</td>
<td>1929</td>
<td>30</td>
<td>14°-20°C.</td>
<td>56</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1928</td>
<td>1929</td>
<td>30</td>
<td>15°-18°C.</td>
<td>272</td>
<td>71</td>
<td>26.1</td>
</tr>
<tr>
<td>1930</td>
<td>1930</td>
<td>30</td>
<td>15°-18°C.</td>
<td>19</td>
<td>7</td>
<td>36.1</td>
</tr>
<tr>
<td>1928</td>
<td>1929</td>
<td>20</td>
<td>15°-18°C.</td>
<td>252</td>
<td>36</td>
<td>14.1</td>
</tr>
<tr>
<td>1928</td>
<td>1929</td>
<td>20</td>
<td>11°-19°C.</td>
<td>121</td>
<td>1</td>
<td>0.82</td>
</tr>
<tr>
<td>1930</td>
<td>1930</td>
<td>20</td>
<td>15°-18°C.</td>
<td>30</td>
<td>5</td>
<td>16.6</td>
</tr>
<tr>
<td>1928</td>
<td>1929</td>
<td>15</td>
<td>11°-19°C.</td>
<td>238</td>
<td>53</td>
<td>43.2</td>
</tr>
<tr>
<td>1928</td>
<td>1929</td>
<td>15</td>
<td>14°-20°C.</td>
<td>398</td>
<td>92</td>
<td>23.1</td>
</tr>
<tr>
<td>1928</td>
<td>1929</td>
<td>15</td>
<td>15°-18°C.</td>
<td>399</td>
<td>73</td>
<td>18.3</td>
</tr>
<tr>
<td>1929</td>
<td>1930</td>
<td>15</td>
<td>15°-18°C.</td>
<td>81</td>
<td>35</td>
<td>45.2</td>
</tr>
</tbody>
</table>

The data in Table 2 point out that in a 30 per cent soil moisture the highest percentage of infection was 36.1 at temperatures between 15° to 18°C. The infection in 20 per cent soil moisture showed 16.6 per cent as the highest amount at 15° to 18°C. The infection in 15 per cent soil moisture was highest (43.2 per cent) at temperatures between 11° and 18°C. According to the above table, the result of 20 trials, the highest per cent of infection, 43.2 per cent,
was obtained from 12 months old oospores in 15 per cent soil moisture at 15° to 18°C.

The data in table 2 did not show, however, that 19°C. was necessarily the highest temperature at which infection may take place, so, an experiment was initiated using the same oospores and 30 and 20 per cent soil moistures with the exception that the temperatures were between 20° and 25°C. The oospores and seed of *Setaria viridis* were planted in the temperature tank trays and sealed the same as for the previous experiment. The plants were transplanted and allowed to grow the same length of time, viz. until the secondary leaves appeared. A summary of the data obtained from the above described experiment is given in table 3.

<table>
<thead>
<tr>
<th>Year</th>
<th>Year</th>
<th>Per cent</th>
<th>Temp.</th>
<th>No.</th>
<th>No.</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>oospores</td>
<td>exp.</td>
<td>soil</td>
<td>plts.</td>
</tr>
<tr>
<td>1928</td>
<td>1929</td>
<td>30</td>
<td>20°-22°C.</td>
<td>295</td>
<td>13</td>
<td>5.01</td>
</tr>
<tr>
<td>1929</td>
<td>1930</td>
<td>30</td>
<td>20°-22°C.</td>
<td>20</td>
<td>4</td>
<td>20.0</td>
</tr>
<tr>
<td>1930</td>
<td>1930</td>
<td>30</td>
<td>20°-22°C.</td>
<td>22</td>
<td>7</td>
<td>31.8</td>
</tr>
<tr>
<td>1928</td>
<td>1929</td>
<td>20</td>
<td>20°-22°C.</td>
<td>93</td>
<td>2</td>
<td>2.1</td>
</tr>
<tr>
<td>1928</td>
<td>1929</td>
<td>20</td>
<td>25°C.</td>
<td>65</td>
<td>28</td>
<td>45.7</td>
</tr>
<tr>
<td>1929</td>
<td>1930</td>
<td>20</td>
<td>22°C.</td>
<td>366</td>
<td>240</td>
<td>65.5</td>
</tr>
<tr>
<td>1929</td>
<td>1930</td>
<td>20</td>
<td>22°C.</td>
<td>197</td>
<td>177</td>
<td>39.8</td>
</tr>
<tr>
<td>1930</td>
<td>1930</td>
<td>20</td>
<td>25°C.</td>
<td>55</td>
<td>33</td>
<td>63.6</td>
</tr>
<tr>
<td>1928</td>
<td>1930</td>
<td>20</td>
<td>22°C.</td>
<td>36</td>
<td>11</td>
<td>30.7</td>
</tr>
<tr>
<td>1929</td>
<td>1930</td>
<td>20</td>
<td>20°-22°C.</td>
<td>59</td>
<td>24</td>
<td>40.6</td>
</tr>
<tr>
<td>1930</td>
<td>1930</td>
<td>20</td>
<td>20°-22°C.</td>
<td>27</td>
<td>2</td>
<td>7.4</td>
</tr>
<tr>
<td>1929</td>
<td>1930</td>
<td>20</td>
<td>22°C.</td>
<td>20</td>
<td>4</td>
<td>20.0</td>
</tr>
</tbody>
</table>

*Aug. 1929; Oct. 1929; Coll. in Colo. 1929*
The data from the experiment summarized in table 3 confirm the data given in table 2 which shows that the percentage of infection in 30 per cent soil moisture is less than the infection at soil moisture below 30 per cent. However, the data of 15 trials from the second experiment differed from the first in showing that the highest percentage of infection is 89.8 per cent obtained in 20 per cent soil moisture at 22°C. This leads to the inference that the difference in the amount of infection may be due to the difference in temperature, (since 11° to 19°C gave a lower percentage of infection than the same soil moisture (20 per cent) at 20° to 25°C.) a wide host range, and the possibility that a high water content is not essential.

According to Melhus, VanBaltern and Bliss (27) Setaria viridis and popcorn var. Japanese Hulless may become infected by the oospores between the time the testa of the seed broke and the plumule emerged above the surface of the soil. It would appear from this statement that if these germinated seeds were placed in the temperatures unfavorable to the growth of the seed, but favorable for germination of the oospores a higher percentage of infection could be obtained. These deductions led to an experiment in which the same oospore material, as used in the two previous experiments, was placed on seeds of Setaria viridis in 15, 20
and 30 per cent soil moisture. The trays containing these seeds and oospores were placed first at 20° to 25°C for three or four days, then at 15° to 18°C until the seedlings were two to four centimeters high. These exposed plants were then transplanted in the same manner as for the other experiments.

Table 4. Oospore infection of Setaria viridis in sandy loam soil of different soil moisture content in temperatures alternating between 20° to 25°C. and 15° to 18°C.

<table>
<thead>
<tr>
<th>Year</th>
<th>Year</th>
<th>Per cent</th>
<th>Temperature</th>
<th>No. coll.</th>
<th>No. exp. soil</th>
<th>No. moist.</th>
<th>No. da.</th>
<th>No. da.</th>
<th>No. plts.</th>
<th>No. inf.</th>
<th>No. inf.</th>
<th>per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1930</td>
<td>1930</td>
<td>30</td>
<td>20-25°C</td>
<td>4</td>
<td>178</td>
<td>99</td>
<td>55.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1928</td>
<td>1929</td>
<td>20</td>
<td>20-25°C</td>
<td>4</td>
<td>42</td>
<td>10</td>
<td>23.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1929</td>
<td>1929</td>
<td>20</td>
<td>15-18°C</td>
<td>5</td>
<td>40</td>
<td>7</td>
<td>18.5</td>
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<td></td>
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<tr>
<td>1929</td>
<td>1930</td>
<td>20</td>
<td>15-18°C</td>
<td>6</td>
<td>32</td>
<td>7</td>
<td>21.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1930</td>
<td>1930</td>
<td>20</td>
<td>15-18°C</td>
<td>6</td>
<td>35</td>
<td>10</td>
<td>28.5</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1928</td>
<td>1929</td>
<td>15</td>
<td>15-18°C</td>
<td>3</td>
<td>54</td>
<td>9</td>
<td>16.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1928</td>
<td>1929</td>
<td>15</td>
<td>15-18°C</td>
<td>5</td>
<td>45</td>
<td>3</td>
<td>6.6</td>
<td></td>
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</tbody>
</table>

It should be noted that in Table 4, giving the data for infection at alternating temperatures, the percentage of infection (55.6 per cent) is highest in the soil of 30 per cent moisture content at temperatures between 20° and 25°C. for four days and 15° to 18°C. for six days. However, this one trial is not as significant data as the four trials in 20 per cent soil moisture. Alternating the temperature between 20° to 25°C. for three or four days and 15° to 18°C. for five or six days in 20 per cent soil moisture, shows a lower percentage (18.5 to 23.5 per cent) of infection than
when the seeds and oospores remain continuously at 22° to 25°C. where the amount of infection ranged from 7.4 to 89.8 per cent. The infection (0.32 to 16.6 per cent) at continuous temperatures between 11° and 20°C. in 20 per cent soil moisture was less than the infection at the alternating temperatures. It appeared from the foregoing discussion that a higher percentage of infection of *Setaria viridis* by oospores was obtained at temperatures between 22° and 25°C. in 20 per cent soil moisture, for the entire infection period, than is obtained under alternating temperatures. It may be assumed from the above statements that both the seeds and oospores germinate and grow more rapidly at 20° to 25°C. and more slowly at 15° to 18°C. A study of the germination and daily growth of *Setaria viridis* seed may indicate whether or not the assumption is correct.

Seed of *Setaria viridis* was planted in 20 per cent soil moisture and placed in temperatures of 15° to 18°C. and 20° to 25°C. Each day a tray from the temperature tank was opened and the measurements of the length of the plumules of 20 plants were made and averaged. These averages for a period of six days have been arranged in chart form, figure 10.

This chart shows that the seed of *Setaria viridis* had germinated and the plumule was two to three millimeters long
on the fourth day in temperatures from 15° to 18°C and 22°C. The plumules elongated rapidly during the fifth and sixth day and were well above the surface of the soil. The chart, figure 10, also showed that *Setaria viridis* grew more rapidly at temperatures between 15° and 18° than at 22°C.

**Fig. 10.** Chart showing time of germination and rate of growth of seed of *Setaria viridis*. C, at 22°C, and D, 15° to 18°C, and popcorn var. Japanese Hulless, A, at 22°C, and B, at 15°C.
These trials suggest that the highest percentage of infection was obtained at continuous temperatures between 20° and 25°C. and the lowest percentage at temperatures between 11° and 20°C. The amount of infection under conditions of alternating temperatures (20° to 25°C. then 15° to 18°C.) were more nearly like that obtained under continuous low temperatures than like the continuous high temperatures. The information gained from the chart on the rate of growth of the *Setaria viridis* plumules showed that this low percentage of infection under alternating temperatures was to be expected since the seeds had only just germinated on the fourth day when they were transferred to lower temperatures. This indicates that the seedlings did not remain under conditions for high infection (20° to 25°C.) for a long enough period before they were transferred to conditions under which the seedlings grew rapidly, (15° to 18°C.) but the amount of infection was low. If the alternating conditions had been reversed, viz. 15° to 18°C. for five days then 20° to 25°C. for six days or seven days instead of 20° to 25°C. for the first four days and 15° to 18°C. for the last six days, the amount of infection may have been higher.

These data suggest that the optimum conditions for oospore infection of *Setaria viridis* are: a 20 per cent
moisture content of a sandy loam soil and continuous temperatures between 15° and 20°C. for a period of five or six days.

Teosinte, sorghum, cane, popcorn, sweet corn and dent corn have been found by Melhus, ManHaltern and Bliss (27) to be susceptible to Sclerospora graminicola collected on Setaria viridis. Some of these same hosts as well as others were used to determine the amount of infection under controlled conditions of temperature and soil moisture content. The same methods of infection were used, viz., scattering the oospores over the seeds or mixing them with the soil covering the seeds in 20 per cent soil moisture in temperatures of 15° to 25°C.

There were many hosts used, as given on page 33, but only those showing positive infection from 25 trials were given in the following table.

Table 5. Oospore infection of hosts other than Setaria viridis under controlled conditions of soil moisture and temperature.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Popcorn far.</td>
<td>1929</td>
<td>1930</td>
<td>20</td>
<td>18°-22°C.</td>
<td>15</td>
<td>1</td>
<td>6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>1930</td>
<td>1930</td>
<td>20</td>
<td>22°-25°C.</td>
<td>7</td>
<td>1</td>
<td>14.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>1928</td>
<td>1929</td>
<td>25</td>
<td>20°-25°C.</td>
<td>10</td>
<td>2</td>
<td>20.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>1928</td>
<td>1929</td>
<td>20</td>
<td>18°-25°C.</td>
<td>24</td>
<td>1</td>
<td>4.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teosinte</td>
<td>1929</td>
<td>1930</td>
<td>20</td>
<td>22°-25°C.</td>
<td>13</td>
<td>1</td>
<td>7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>1929</td>
<td>1930</td>
<td>15</td>
<td>15°-18°</td>
<td>43</td>
<td>3</td>
<td>6.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. italicata</td>
<td>1928</td>
<td>1929</td>
<td>20</td>
<td>18°-20°</td>
<td>12</td>
<td>1</td>
<td>8.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Although the percentage of infection on the three hosts listed in table 6 is low, it indicates that it is possible to infect hosts other than *Setaria viridis* under the same conditions and with the same oospores. *Setaria glauca* (yellow foxtail), *Setaria italica* var. *rubrofructa* (Siberian millet), *Pennisetum typhoideum* (pearl millet), *Panicum miliaceum* (proso millet), *Panicum capillare* (tumble grass), *Andropogon sorghum* (sorghum), *Holcus sorghum* var. *caffrorum* (kaffir) and *Holcus sudanensis* (Sudan grass) were not susceptible under the conditions given in table 5. However, dent corn var. Iodent and Siberian millet were infected under greenhouse conditions. Although *Setaria glauca* (yellow foxtail) and *Panicum capillare* (tumble grass) were nearly as prevalent in the corn fields as *Setaria viridis* no infection has been observed on these hosts as the result of natural or artificial inoculation. Melhus, VanHaltern and Bliss (27) mention that they were also unable to produce infection on *Setaria glauca*.

**Viability of Oospores**

Oospore material, for infection studies, was collected from several sources and over a period of several years. It was noted in the experiments to determine the optimum conditions for infection by oospores that some of the material
never produced infection on any host used.

Since all these oospores had infected *Setaria viridis* at some time or other it was used as the host upon which to test the viability of the various oospores. The oospores were mixed with the soil covering the seeds. The seeds and oospores were planted in sandy loam soil of 20 per cent moisture content at temperatures of 18° to 25°C. The results of this experiment are presented in tabular form in table 6.

Table 6. Oospores of Sclerospora graminicola of different ages tested for viability on *Setaria viridis* in 20 per cent soil moisture and 18° to 25°C temperature in March, 1930.

<table>
<thead>
<tr>
<th>Year</th>
<th>Locality</th>
<th>Coll. Age</th>
<th>Host collected</th>
<th>Collector (Inf.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct. 1910</td>
<td>Madison, Wis.</td>
<td>19yr.6mo. C. viridis</td>
<td>Melhus</td>
<td></td>
</tr>
<tr>
<td>1924</td>
<td>Ames, Ia.</td>
<td>6yr.</td>
<td>VanHaltern</td>
<td></td>
</tr>
<tr>
<td>Jan. 1925</td>
<td></td>
<td>5yr.2mo. C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov. 1926</td>
<td>Florida</td>
<td>3yr.4mo. C. magna</td>
<td>Seal</td>
<td></td>
</tr>
<tr>
<td>Nov. 1927</td>
<td>Nanking, China</td>
<td>2yr.4mo. Pen. miliacum</td>
<td>Porter</td>
<td></td>
</tr>
<tr>
<td>1927</td>
<td>Poona, India</td>
<td>3yr.</td>
<td>Uppal</td>
<td></td>
</tr>
<tr>
<td>Oct. 1927</td>
<td>Ames, Ia.</td>
<td>2yr.5mo. C. viridis</td>
<td>Howe</td>
<td></td>
</tr>
<tr>
<td>Oct. 1928</td>
<td></td>
<td>1yr.5mo. Pen. typhonideum</td>
<td>Evans</td>
<td></td>
</tr>
<tr>
<td>Dec. 1928</td>
<td></td>
<td>1yr.3mo. Pen. typhonideum</td>
<td>Howe</td>
<td></td>
</tr>
<tr>
<td>Nov. 1928</td>
<td></td>
<td>1yr.4mo. Pen. typhonideum</td>
<td>Howe</td>
<td></td>
</tr>
<tr>
<td>Aug. 1929</td>
<td></td>
<td>7mo.</td>
<td>Ft.Collins, Stewart</td>
<td></td>
</tr>
<tr>
<td>Aug. 1929</td>
<td></td>
<td>7mo.</td>
<td>Colorado</td>
<td></td>
</tr>
<tr>
<td>Jan. 1930</td>
<td></td>
<td>3mo.</td>
<td>Howe</td>
<td></td>
</tr>
</tbody>
</table>

Oospores more than a year and five months (17 months) were not viable according to trials listed in table 6. Melhus, VanHaltern and Bliss (27) found that oospores more than 30 months old were not viable.
Host Range of Sclerospora graminicola

*Setaria viridis* is the most prevalent host of *Sclerospora graminicola* in the corn fields, but there are many other grasses growing as weeds in a corn field which may be possible hosts to the disease, especially since many of them belong to the same tribe.

Hosts susceptible to *Sclerospora graminicola* have been reported by Melhus, et al. (27) in the three tribes *Maydeae*, *Paniceae* and *Andropogoneae*. As many of these grasses as were available were used in experiments with viable oospores to determine the possible host range of the pathogene. In order to verify the previous report or the host range the infection on any host was called positive only when the mildew appeared on the surface of the leaves. After having been exposed to the infection by oospores the plants, whether they showed symptoms or not, were placed in a moist chamber under optimum conditions for the production of the conidia viz. a humidity of 82 to 100 per cent and temperature between 6° and 28°C. No attempt was made to infect the many different varieties of dent corn, sweet corn or popcorn.

The hosts found to be susceptible to *Sclerospora graminicola* are given in table 7. This table confirms many hosts in the list of susceptible ones given by Melhus et al. (27).
However, in the tribe *Maydeae*, dent corn var. Iodent and in the tribe *Paniceae* the Hungarian millet, (*Setaria italica*) (rula, in India) were added to the list.

Table 7. Susceptible hosts of *Sclerospora graminicola*.

<table>
<thead>
<tr>
<th>Tribe</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Maydeae</em></td>
<td><em>Zea mays</em> L.</td>
</tr>
<tr>
<td></td>
<td>-dent corn</td>
</tr>
<tr>
<td></td>
<td>-var. Iodent</td>
</tr>
<tr>
<td></td>
<td>-popcorn</td>
</tr>
<tr>
<td></td>
<td>-var. Japanese Hulless</td>
</tr>
<tr>
<td></td>
<td><em>Euchlaena mexicana</em></td>
</tr>
<tr>
<td></td>
<td>-teosinte</td>
</tr>
<tr>
<td><em>Paniceae</em></td>
<td><em>Panicum miliaceum</em></td>
</tr>
<tr>
<td></td>
<td>-common millet</td>
</tr>
<tr>
<td><em>Setaria</em></td>
<td><em>viridig</em></td>
</tr>
<tr>
<td></td>
<td>-green foxtail</td>
</tr>
<tr>
<td><em>Setaria</em></td>
<td><em>italica</em></td>
</tr>
<tr>
<td></td>
<td>-Hungarian millet (Rula, in India)</td>
</tr>
<tr>
<td></td>
<td>-Siberian millet</td>
</tr>
<tr>
<td><em>Pennisetum</em></td>
<td><em>typhoideum</em> Rich.</td>
</tr>
<tr>
<td></td>
<td>-pearl millet (Bajri, in India)</td>
</tr>
</tbody>
</table>

The hosts reported as susceptible to *Sclerospora graminicola* include six genera in the three tribes of the *Gramineae*, *Maydeae*, *Paniceae* and *Andropogon*. The following is a list of the plants susceptible to *Sclerospora graminicola* according to Helius et al. (27), Weston and Weber (56) and the writer.
Tribe **Maydeae**

*Zea mays* - (dent corn)

- Silver King
- Reid's Yellow Dent
- Silvermine
- Iajap Striped
- Hardin County White
- MacArthur's Golden King
- Walden Dent
- Toleuming
- Edward's White King
-Golden Jewel
- Iowanda
- Golden Murdock
- Workman
- King Yellow Victor
- Iowa 119
- Iodent

*Zea mays* - (sweet corn)

- Golden Bantam
- Golden Dawn
- Narrow Grain Evergreen
- Crosby
- Golden Giant

*Zea mays* - (popcorn)

- Baby Golden
- Black Beauty
- Japanese Hulless
- American Wonder
- Yellow Pearl
- May's Golden

*Euchlaena mexicana* - teosinte
*Euchlaena luxurians* - fodder grass

Tribe **Paniceae**

*Setaria viridis* - green foxtail
*Setaria glauca* - yellow foxtail
*Setaria magna* - Everglade millet
*Setaria verticillata* - whorled millet
*Setaria italica* - Italian millet, rale in India

*Setaria (millet)*

- White French
- Siberian
- White Wonder
Tribe Paniceae

Setaria italica var. germanica
German or Hungarian millet

Fenugreek typhoidum - pearl millet, bajri in India

Panicum miliaceum - common millet

Tribe Andropogoneae

Andropogon sorghum (sorghum)
Red Amber
Black Amber
Orange
Sumac

Saccharum officinarum (cane)
Black amber
Orange
Sugar Cane

Andropogon halepensis - Johnson grass

Oospore Infection of Sclerospora graminicola var.
andropogonis sorghi Kul.

The oospores of Sclerospora graminicola var. andropogonis-
sorghi were collected in 1927 at Poona, India by Dr. B. N.
Uppal.

The oospores were sprinkled on the seeds which were plant-
ed in pots in a sandy loam soil. The pots were then placed
on the greenhouse bench at a temperature of 18° to 20° C.
These experiments were carried on during March, April and
May of 1928. The oospores had been collected in October of
1927 which made them five to seven months old, at which age
they were viable according to the data given in table 6.
The hosts given in the following table were only those that
showed infection.
Table 8. Oospore infection of popcorn and dent corn with Sclerospora graminicola var. andropogonis sorghi Kul, at 18° to 20°C, on the greenhouse bench in sandy loam

<table>
<thead>
<tr>
<th>Host</th>
<th>No.</th>
<th>Plts</th>
<th>No.</th>
<th>Inf.</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Popcorn var.</td>
<td>19</td>
<td>5</td>
<td></td>
<td></td>
<td>26.3</td>
</tr>
<tr>
<td>Japanese Hulless var.</td>
<td>11</td>
<td>3</td>
<td></td>
<td></td>
<td>27.2</td>
</tr>
</tbody>
</table>

Two trials, 19 plants, out of the nine trials made to infect popcorn var. Japanese Hulless with the oospores of Sclerospora graminicola var. andropogonis sorghi that showed infection. There were twenty trials on dent corn var. Iodent only two of which showed infection. These data are given in table 8.

The twenty-two trials of Pennisetum typhoideum using the sandy loam soil and oospores of Sclerospora graminicola var. andropogonis sorghi showed no infection. However, one trial of 10 plants using moistcotton as a substrate showed 50 per cent infection. Ninety-two plants of Setaria viridis growing on the cotton substratum showed 6.52 per cent infection.

Dr. E.N. Uppal also sent seeds of Zea mays, Setaria italica Scribn. (raja or Hungarian millet) and Andropogon sorghum Bro. (jowar or sorgum). These seeds were planted with the oospores in the same way as those given in table 8, that is, by placing the oospores directly on the seed.
Four trials were made on maize, 24 on Setaria italica (rala) and eight on Andropogon sorgaeum but no infection developed. Other hosts exposed that did not show infection were: teosinte, Sudan grass, Proso millet, tumble grass (Panicum capillaire) and dwarf milo. Later trials with these same oospores in 1929 gave no infection.

A few experiments were also carried on with the oospores which had been collected on Setaria viridis in 1926 and 1927 on Setaria magna from Florida and on Panicum miliaceum (millet) from Nanking, China. These oospores were threshed out and stored under the same conditions as the oospores gathered in 1927, viz., in glass bottles at room temperature 20° to 25°C. Neither of these oospores produced any infection of Setaria viridis.

The experiments for infection with Sclerospora graminicola from Florida and China were carried on previous to the work on the controlled conditions of soil moisture and temperature. These oospores, collected in 1927, were not viable in 1930 when experiments under controlled conditions were conducted.

**Infection Experiments with Oospores on Substrates Other than Soil**

It was thought that the soil and soil water played an important part in the germination of the oospores of Sclerospora graminicola. This was proven otherwise when Melhus
et al. (27) infected *Setaria* plants with oospores on moist cotton instead of soil as a substrate. The cotton substrate was formed in a funnel with a wick running through the tube into the tap water. In other experiments the cotton was placed in new flower pots and the seeds and oospores planted in the same manner as in the funnels. These funnel and pot cultures were held in the greenhouse at 18° to 20°C.

Pfeffer's nutrient solution was used instead of tap water in the preliminary experiment, but the germination of the seed seemed to be no better than with tap water, so it was used in all subsequent experiments.

Table 9. Oospore infection of various hosts on a moist cotton substratum.

<table>
<thead>
<tr>
<th>Host</th>
<th>No. trials</th>
<th>No. pts.</th>
<th>No. pts. inf.</th>
<th>Per cent inf.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Setaria viridis</em></td>
<td>3</td>
<td>36</td>
<td>12</td>
<td>30.5</td>
</tr>
<tr>
<td><em>Euchlaena mexicana</em></td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Setaria italic</em> (rra)</td>
<td>2</td>
<td>34</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td><em>Panicum miliaceum</em></td>
<td>3</td>
<td>41</td>
<td>3</td>
<td>7.3</td>
</tr>
<tr>
<td><em>Andropogon sorghum</em></td>
<td>2</td>
<td>66</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Popcorn var. Jap. hulless</td>
<td>3</td>
<td>23</td>
<td>6</td>
<td>26.0</td>
</tr>
</tbody>
</table>

The trials, recorded in table 9, show that *Setaria viridis* and *Zea mays* are the most susceptible hosts among the seven exposed to infection. Under the conditions of the experiment, it is quite clear that infection must have taken place shortly after the plumule emerges from the seed because the oospores
were resting on the seeds. It was also worthy of special emphasis in connection with these trials that the popcorn var. Japanese Hulless was almost as generally infected as the Setaria viridis which suggests that the difference in susceptibility may not be great.

It has been stated by Helius et al. (27) that infection of Setaria viridis and corn plants by oospores may take place any time after the plumule breaks thru the testa until it appears at the surface of the soil. In order to determine in what stage of the development of the plumule it was most susceptible to oospore infection, seeds were germinated on moist filter paper at temperatures from 18° to 22°C. The oospores were placed on the plumules and the seeds returned to the moist filter paper and kept at temperature of 16° to 18°C for 43 hours. These plants were then transplanted to sterile soil in test tubes and placed on the greenhouse bench. The infection was noted after 22 days.

The plumules of Setaria viridis that became infected were from one-half to five millimeters in length. The one-half millimeter plumule was just beyond the broken testa, table 10. The other hosts, teosinte, popcorn, rala and jowar, susceptible to Sclerospora graminicola were infected the same way as Setaria viridis but showed no infection under the same conditions.
Table 10. Infection of germinated seeds by placing the oospores of *Sclerospora graminicola* on the plumules.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Length of plumule</th>
<th>No. plts.</th>
<th>No. inf.</th>
<th>Per cent inf.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. viridis</em></td>
<td>1.3 mm.</td>
<td>50</td>
<td>3</td>
<td>6.0</td>
</tr>
<tr>
<td><em>S. viridis</em></td>
<td>0.5-5 mm.</td>
<td>50</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Teosinte</td>
<td>2-5 mm.</td>
<td>10</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Popcorn var.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japanese Hulless</td>
<td>2-3 mm.</td>
<td>30</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Rala</td>
<td>1-2 mm.</td>
<td>3</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Jowar</td>
<td>1-2 mm.</td>
<td>5</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

The oospores when placed on the root tips (one to eight mm.) of 65 plants of the same hosts as given in table 10 produced no infection. This would seem to indicate that the initial infection took place in the plumule and not in the root.

**Conidial Production of *Sclerospora graminicola***

Although the oospore stage of *Sclerospora graminicola* is dominant in the life history of the fungus, the conidial stage is also important. The branched conidiophores arise from the mycelium in the host tissue and emerge through the stomata. The conidia are borne on the sterigmata at the tips of these branches. These conidiophores and conidia appear under certain conditions of moisture and temperature as observed in the field by Melhus, et al. (27) and Weston (52) but no one has defined the exact conditions of humidity and temperature for sporulation. Some of the experiments
bearing on this problem are presented. Leaves of *Setaria viridis*, showing the glistening white conidial sporulation on the lower surface of the leaves, were collected in the field and carried to the laboratory in moist chambers. These leaves were wiped with a damp cloth so as to remove all the old conidiophores and conidia. The cleaned leaves were placed on a moist filter paper in a petri dish and incubated at 15° to 18°C. This resulted in forming a thin film of moisture on the leaf surface. This moisture film is very essential since it simulates the dew or rain on the leaves in the field.

Weston (52) finds that the nocturnal production of conidia takes place at temperatures ranging from 43° to 65°F. (6° to 18°C.) while the leaves are covered with moisture.

After an incubation period at 15° to 18°C. for five to 32 hours the second crop of conidia appeared. However, under these conditions the leaves must be covered with a film of moisture. If this film is absent irrespective of other conditions the conidia will not appear. The following table, 11, shows the number of hours required for a crop of conidia to be produced on the cleaned leaves of *Setaria viridis*. 
Table 11. Time required for the second crop of conidia to appear after infected leaves have been cleaned and placed in moist chambers at 15° to 18°C.

<table>
<thead>
<tr>
<th>No.</th>
<th>Time leaves trials placed in m. ch.</th>
<th>Time conidia appeared</th>
<th>Time conidia appeared</th>
<th>Time conidia appeared</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>9:15 A. M.</td>
<td>3:00 P. M.</td>
<td>5 hr.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9:35 A. M.</td>
<td>3:00 P. M.</td>
<td>1 hr. 25 min.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12:00 M</td>
<td>7:15 P. M.</td>
<td>7 hr. 15 min.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3:00 P. M.</td>
<td>9:00 P. M.</td>
<td>6 hr.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11:00 A. M.</td>
<td>9:00 P. M.</td>
<td>10 hr.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3:00 P. M.</td>
<td>9:30 P. M.</td>
<td>6 hr. 30 min.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11:00 A. M.</td>
<td>9:30 P. M.</td>
<td>10 hr. 30 min.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4:00 P. M.</td>
<td>9:00 A. M.</td>
<td>11 hr.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11:00 A. M.</td>
<td>9:00 A. M.</td>
<td>21 hr.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11:00 A. M.</td>
<td>3:00 A. M.</td>
<td>32 hr.</td>
<td></td>
</tr>
</tbody>
</table>

Second day

The conidial material collected in the greenhouse did not dry between the time they were collected and when they were used in the laboratory, so it was not necessary to have them produce a second crop of conidia. This material from the greenhouse during the fall and winter months when conidia were not available in the field, was obtained from stock cultures of *Sclerospora graminicola* on *Setaria viridis*. These stock cultures were made by placing viable spores on the seeds. In order to obtain conidia, the plants were placed in petri dish moist chambers and kept under the conditions observed in the field, viz., relatively high humidity and comparatively low temperature.

**Humidity and Temperature Experiments for Conidial Production.**

The plants showing symptoms of the disease were placed
in moist chambers on the greenhouse bench and a recording thermo-hygrograph placed in the chamber in order to record the temperature and humidity. The experiments were set up between 5 and 6 P.M. and the readings for sporulation were made at 8 A.M. the following morning. Sometimes electric lights (75 to 200 watt) were hung above the plants in the chamber. Thus the same humidity and temperature conditions were maintained in darkness and in light. The following table gives the moisture and temperature data for the above experiment with plants of *Setaria viridis* and illustrates the conditions under which sporulation occurs.

Table 12. Thermo-hygrograph records in moist chambers where the production of conidia on leaves of *Setaria viridis* occurred.

<table>
<thead>
<tr>
<th>Time</th>
<th>Percent humidity</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 P.M.-4 A.M.</td>
<td>88 100</td>
<td>51°F 10.6°C</td>
</tr>
<tr>
<td>2 P.M.-8 A.M.</td>
<td>89 94</td>
<td>59 15.0</td>
</tr>
<tr>
<td>9 P.M.-5 A.M.</td>
<td>91 100</td>
<td>43 6.1</td>
</tr>
<tr>
<td>6 P.M.-6 A.M.</td>
<td>82 100</td>
<td>54 12.2</td>
</tr>
<tr>
<td>6 P.M.-4 A.M.</td>
<td>95 100</td>
<td>47 8.3</td>
</tr>
<tr>
<td>6 P.M.-8 A.M.</td>
<td>96 98</td>
<td>50 10.0</td>
</tr>
<tr>
<td>4 P.M.-8 A.M.</td>
<td>98 100</td>
<td>50 13.3</td>
</tr>
<tr>
<td>4 P.M.-2 A.M.</td>
<td>83 89</td>
<td>51 10.6</td>
</tr>
<tr>
<td>5 P.M.-1 P.M.</td>
<td>98 100</td>
<td>48 8.9</td>
</tr>
<tr>
<td>2nd da.</td>
<td>6 P.M.-5 A.M.</td>
<td>99 100</td>
</tr>
</tbody>
</table>

* Indicates a light hung above the moist chamber for the duration of the experiment.

The foregoing table, 12, gives the maximum and minimum temperature and humidity in moist chambers where conidia were successfully produced on infected leaves of *Setaria*.
The temperature between 43° and 83°F. (6° and 28°C.) and a percentage humidity of 82 to 100 were the most favorable conditions for conidial production. It will be noted that four of the trials in the table were with the light. When these conditions prevail during either darkness or light the conidia will appear on the lower surface of the infected leaves. The greatest amount of sporulation appeared on the leaves at temperatures between 60° and 65°F. (15° and 18°C.) in 95 to 100 per cent humidity. However, the degree of sporulation depended upon whether the leaves showed symptoms of heavy or light infection. The following photograph, figure 11, of a recording thermo-hygrograph chart for one week is typical of those from which the data in table 12 was compiled.

Fig. 11. Chart of recording thermo-hygrograph of the temperature and humidity record for one week for conidial production.
It will be noted on the chart, figure 11, that as the humidity increased the temperature decreased. This, of course, takes place soon after the moist chamber is set up in the evening. Then during the time the humidity is fairly constant the temperature varies from the maximum to the minimum. When the moist chamber is opened the humidity decreases and the temperature increases rapidly. Hence the readings for the data in table 12 describe only the period of relatively constant humidity.

The record shown on the thermo-hygrograph for Tuesday evening and Wednesday morning may be used to illustrate this point. The humidity has rapidly reached its high point at 6 P. M. and the temperature at this time is 70°F. The rapid decrease in humidity, when the chamber was opened, began at 8 A. M. and the temperature at this time was 55°F. The temperature and humidity were read for each two hours between the time 6 P. M. on Tuesday and 8 A.M. on Wednesday. From this data the maximum and minimum temperatures and humidity were determined and are shown in table 12.

**Discharge of Conidia**

The writer has observed that the mature conidia are discharged from the conidiophores. This has also been noted and described by Melhus et al. (27) who stated that they were shot from the sterigmata of the conidiophores 1.5 mm. to
2.5 mm. If a cover slip is placed over the leaves showing the newly formed conidia and conidiophores, the conidia which have been discharged will form a thin white film on the cover slip. Often the leaves beneath infected ones will be covered with this same white film of conidia. This is one method of spreading the organism although it is not a very important one, because of the limited conditions of moisture and temperature necessary for the production and germination of the conidia. Weston and Weber (56) suggest "that the zoosporangia themselves are not resistant and do not survive long as such, they are produced in crop after crop by successive growth of the mycelium and continually accomplish infection and reinfection perpetuating the disease."

Cropping of Conidia

The plants infested with Sclerospora graminicola which were kept as stock cultures in the greenhouse produced several crops of conidia. The same plants were placed in the moist chamber on the greenhouse bench for several nights in succession and each time new conidiophores and conidia appeared. Hiura (19) observing conidial production on Italian millet finds that they are produced night after night on the same leaves for a period of three weeks or more. In order
to determine whether there actually were successive crops of conidia produced, each crop was removed and examined under the microscope. This examination showed a distinct difference between new and old conidia. The two types are illustrated in figure 12. The new and viable conidia are finely granular and almost hyaline, while the old conidia are coarsely granular and dark in color. These latter conidia do not germinate and hence are called the dead conidia.

Fig. 12. The new and viable conidia are shown beside the piece of conidiophore and the dead conidia are near the lower corner of the plate.

During June 1928 at Ames, Iowa, the writer collected a great deal of conidial material in the field just after rains. The moisture from the rain assured the collector of
a moisture film on the leaves and subsequent production of conidia. According to the climatological data (34) between June 4 and 9 the rains occurred about every other day, June 5, 7 and 8. The conidial material was collected during the mornings of June 6, 8 and 9. This material showed both new and dead conidia indicating that a new crop appeared after the old had died. The moisture factor was essential not only for the production of the conidia of *Sclerospora graminicola*, but also for conidial infection since the conidia and zoospores germinate only in the presence of moisture. Melhus (25) found this to be true of other Phycomycetes and says that the spread of a fungus by zoospore infection is directly dependent upon the presence of water on the foliage of the plant.

**Description of Conidia**

The conidia of *Sclerospora graminicola* are oval with a rather thick hyaline cell wall and a small papilla at the end opposite the point of attachment to the conidiophore. The conidia collected on stock cultures in the greenhouse measured 19 to 20μ by 12 to 16 μ. These measurements correspond closely with those of Weston and Weber (56) and Weston (52) which are 19 to 22.9 μ by 13 to 16.9 μ; with Berlese (2) of 20 μ by 15 to 18 μ and Schroeter (39)
with 20 μ by 15 to 18 μ. Melhus et al. (27) found two sizes of conidia viz., "large spore-like structures which measured 43 by 18.6 μ, while ordinary conidia measure 14 to 23 μ by 11 to 17 μ". These two sizes of conidia have not been observed by the writer. Weston (52) mentioned that a Japanese investigator also noted large conidia measuring 38.4 to 57.6 μ by 19.2 to 24 μ but he suggests that this investigator may not have been dealing with Sclerospora graminicola alone.

Germination of the Conidia

The viable conidia for germination studies, were scraped from the leaves, placed in a hanging drop of distilled water in a van tieghem cell, and held at different temperatures. The following table 13 indicates the various temperatures at which conidial germination occurred.

Table 13. The temperature and time necessary for germination of the conidia in van tieghem cells in drops of distilled water.

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>9°C</td>
<td>-</td>
<td>3 hr. 35min</td>
<td>30°C</td>
<td>/</td>
<td>1 hr. 25 min</td>
</tr>
<tr>
<td>15°C</td>
<td>/</td>
<td>1 hr.</td>
<td>35°C</td>
<td>-</td>
<td>2 hr.</td>
</tr>
<tr>
<td>23°C</td>
<td>/</td>
<td>30 min.</td>
<td>30°C</td>
<td>/</td>
<td>1 hr. 25 min</td>
</tr>
<tr>
<td>25°C</td>
<td>/</td>
<td>1 hr. 25 min.</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

"dried up"

The conidia germinate in distilled water in temperatures ranging from 15°C to 30°C and the optimum appears to be between 20°C and 23°C. The zoospores germinate best at
the same optimum temperatures as the conidia. One trial showed that the zoospores would germinate at 35°C.

The first change in the process of conidial germination is in the appearance of the protoplasm. The outer edge of the protoplasm next to the cell wall becomes irregular, then faint cleavage lines appear from this irregular margin. These cleavage lines divide the protoplasm into the zoospores and soon after they are formed the entire contents begin to move in the conidium. Then, suddenly, the conidial wall opens at the papillate end and the zoospores very quickly emerge enmass.

This method of germination of the conidia of Sclerospora graminicola is like the method described by Nishimura (29) for another mildew, Plasmopara halstedii. He finds "that before germination in the conidium slight indentations are found along the margin of the previously smooth protoplasmic mass. The indentations become more and more visible as the primordial zoospores develop in size. The size of the conidia increases and the protoplasmic mass of the primordial zoospores swells as a result of the absorption of water. This swelling hastens the formation of cleavage furrows, which form the exterior and work inward by means of the fusion of adjacent vacuoles." This swelling of the conidium just before the formation of the zoospores was not observed in the conidia.
of Sclerospora graminicola. Figures 13 and 14 show a series of photographs and drawings of the steps in the germination of the conidia. Figure 11 was made from living conidia in a drop of distilled water in a van tieghem cell at 23°C.

![Image of conidia showing cleavage lines just prior to germination.](image)

Fig. 13. Photograph of conidia showing cleavage lines just prior to germination.

Soon after these cleavage lines appear in the protoplasm the entire mass begins to move around within the conidial wall. Then very suddenly the conidium breaks at the papillate end and the zoospores come out en masse. Almost immediately the mass breaks into irregular shaped zoospores. They swim off by means of cilia with a rapid rotary motion. Steps in the germination showing these irregularly shaped zoospores are shown in figure 14.
Figure 14 shows the various steps in the germination of the conidia. No. 1, finely granular, viable conidium; No. 2, the slightly irregular margin of the protoplasm and the cleavage lines dividing the protoplasm into zoospores; No. 3 and 4, cleavage lines and No. 5, the spore content emerging from the papillate end of the conidium. In No. 6, two of the zoospores have broken away from the third one emerging through the opening in the conidial wall. Sometimes this last zoospore is unable to get out of the conidium as shown in No. 7.
Sometimes only two or three of the zoospores come out of the conidium and the third or fourth remain. In figure 15 A, a zoospore is shown in the conidial case unable to find its way out. Another such zoospore is shown in figure 15 B, emerging through the small opening at the papillate end of the conidium.

Fig. 15. Germinating conidia
A. A zoospore left behind in the conidial case unable to emerge.
B. A zoospore emerging through the opening in wall of the conidium, note the constriction of the zoospore.

Many times the zoospores do not separate entirely but remain fastened together while swimming about as shown in figure 16. They swim around for several minutes before
breaking apart and assuming the irregular shape of zoospores just escaped from the conidium.

The zoospores vary in size as well as in shape as shown in figure 17. They swim around in the drop for about five hours before changing from this irregular to the regular oval shape. This figure also shows rather indistinctly the long cilia.

The empty conidial case is easily seen after the zoospores leave the conidium. The papilla has disappeared and left only a small opening with thickened boarders of the conidial wall. Figure 18 shows this opening and the thickened portion of the conidial case.
Fig. 17. Showing the irregular shapes assumed by zoospores soon after emerging from the conidium. Note the variation in size from very small ones to the giant one in the center of this figure.

Fig. 18. The conidial wall after zoospores have emerged. Note the opening, as indicated by the dark edge, opposite the papillate end of the conidium.
Gregory (13) describes the emergence of the zoospores of *Plasmopara viticola* through the papillate end of the conidium. He says this opening is probably brought about by the dissolution of the wall at this point and not by its breaking, since no remnant of the wall can be found after evacuation. Weston (52) believes the opening is caused by the gelatinizing of the apical papilla.

The number of zoospores which emerge from a conidium seems to vary. Many hanging drop cultures have been observed to determine the exact number. These experiments point out that three zoospores from a single conidium is the most frequent number and four the next most frequent number. However, in one experiment five zoospores were observed to issue from a single conidium.

The length of time for the germination of the conidia varies from 30 minutes to 90 minutes. The maturity of the conidia when they were placed in the hanging drops in the van tieghem cells determined the time required for germination.

**Description of Zoospores**

The zoospores leave the conidium en masse through an opening at the papillate end. This divided mass of protoplasm remains at the opening of the conidium for a very few
seconds then the zoospores move off as irregular shaped masses of protoplasm. After swimming around with a rapid rotary motion for several hours the zoospores become regularly shaped masses of protoplasm. After swimming around with a rapid rotary motion for several hours the zoospores become regularly oval. The motion is maintained by two long cilia which measure twice to several times the length of the zoospore. These two cilia seem to arise from the same position at the end of the zoospore; shown in figure 19.

Fig. 19. Zoospores showing two cilia. These zoospores were heavily stained to show cilia clearly and hence obscured the cell contents.
Germination of the Zoospores

The zoospores after emerging from the conidium swim around very actively for about five hours at 20° to 23°C. At the end of this time they become quiescent; lose their cilia, and become spherical. Two such zoospores are shown in the lower part of figure 19. The zoospores remain in this resting condition for several minutes and then send out germ tubes such as are shown in figure 20. The germ tube continues to grow and the protoplasm leaves the zoospore to go into the tube.

Fig. 20. Germinating zoospores, showing the protoplasm leaving the spore.

This method of zoospore germination is described for *Plasmopara viticola* by Gregory (13). As mentioned be-
fore sometimes one of the zoospores does not escape in which case they may germinate within the conidium. Figure 21 shows the germ tube of the zoospore pushing out thru the opening.

![Germinated Zoospore](image)

**Fig. 21.** A germinated zoospore in the conidial case. The germ tube is pushing out through the opening.

**Conidial Infection**

*Setaria viridis* is the most susceptible and prevalent host for *Sclerospora graminicola* in the cultivated fields in Iowa, hence it was used for the conidial infection studies. Conidia were available on the stock cultures of *Sclerospora graminicola* maintained in the greenhouse. The mature and viable conidia were placed in distilled water at 23°C. and after one hour many of them had germinated.
and the zoospores were swimming around in the suspension.

Plants of *Setaria viridis* varying from 3 mm. to 1.5 cm. were sprayed with this suspension and in other cases the suspension was poured on the soil around the plants. These inoculated plants were placed in a moist chamber at 18° to 23°C. and after 12 to 24 hours they were removed from the moist chamber and put upon the greenhouse bench where they grew for six to ten days. Any time after this growth period the plants were placed under favorable conditions for sporulation. These conditions are: humidity between 82 to 100 per cent and a temperature between 6° and 28°C. Only those plants that produced conidia were counted as infected.

Table 14 gives the percentage of infection of *Setaria viridis* when the above methods of inoculation were used for conidial infection.

**Table 14. Infection of *Setaria viridis* by spraying the plants with conidial suspension or pouring the suspension on the soil around the plants then placing them in a moist chamber.**

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cm.</td>
<td>Sprayed</td>
<td>22°C.</td>
<td>24 hr.</td>
<td>18</td>
<td>3</td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td>1 cm.</td>
<td>&quot;</td>
<td>17°C.</td>
<td>10 hr.</td>
<td>61</td>
<td>4</td>
<td>6.25</td>
<td></td>
</tr>
<tr>
<td>1 cm.</td>
<td>&quot;</td>
<td>17°C.</td>
<td>16 hr.</td>
<td>34</td>
<td>1</td>
<td>2.94</td>
<td></td>
</tr>
<tr>
<td>1 cm.</td>
<td>&quot;</td>
<td>15°C.</td>
<td>12 hr.</td>
<td>58</td>
<td>5</td>
<td>8.99</td>
<td></td>
</tr>
<tr>
<td>1.5 cm.</td>
<td>&quot;</td>
<td>15°-18°C.</td>
<td>5 da.</td>
<td>48</td>
<td>28</td>
<td>58.0</td>
<td></td>
</tr>
<tr>
<td>3 mm.</td>
<td>Poured on soil</td>
<td>14°-16°C.</td>
<td>24 hr.</td>
<td>45</td>
<td>8</td>
<td>17.7</td>
<td></td>
</tr>
<tr>
<td>3 mm.</td>
<td>&quot;</td>
<td>15°-18°C.</td>
<td>4 da.</td>
<td>70</td>
<td>5</td>
<td>7.1</td>
<td></td>
</tr>
</tbody>
</table>
The foregoing table, 14, indicates that the percentage of infection by conidia in most cases is small. However, the fact that conidial infection is possible on young seedlings gives some evidence that it may be a factor in dissemination of the disease. Under field conditions the conidia may be scattered to the leaves covered with moisture or fall in the moist soil around the seedlings, but it is doubtful if these conditions prevail long enough in the field for the conidia and zoospores to germinate and cause infection.

Nishimura (29) in his investigations with Plasmopara halstidisii finds that zoospores from conidia can infect the roots of sunflowers. This may be the method of infection with Sclerospora graminicola when the conidial suspension is poured on the soil at the base of the plants, but it does seem probable as shown by the following experiment. Seedlings of Setaria viridis with radicles four centimeters long were suspended so that the radicles were in the conidial suspension. The seedlings of Pennisetum typhoideum (radicles 1 to 3 cm.) and popcorn var. Japanese Hulless (radicles 6 to 8 cm.) were also suspended above the conidial suspension. There were eight plants of Setaria viridis and Pennisetum typhoideum and five popcorn plants taken from the conidial suspension and transplanted in a sandy loam soil in the greenhouse. None of these three hosts were infected through the
Does Conidial Infection Become Systemic?

Setaria viridis plants, infected by conidia were allowed to grow for several weeks in the greenhouse. These plants at time of inoculation were three millimeters to one centimeter high. After the secondary leaves appeared these plants were placed under favorable conditions for sporulation. Figure 22 shows that the conidia appeared on the second and third leaves of these plants. It will be noted that the downy covering is continuous over the leaves and not in spots or streaks. This appearance suggests that the infection by conidia becomes systemic and not local.
Fig. 22. Lower surface of *Setaria viridis* leaves showing conidia. These plants were inoculated with a conidial suspension when 3 mm. to 1 cm. high.
The behavior of the three spore stages of Sclerospora graminicola (Sacc.) Schr. have been studied as to method of germination and infection.

The oospores used were viable after being stored seventeen months. They were stored in the laboratory at temperatures between 20° and 25°C.

The oospores are produced in the fall of the year from systemic mycelium in the tissues. They measure 30 to 36 μ and are enclosed by a heavy oogonial wall.

The oospores germinate by the production of a tube which is hyaline, non-septate and branched. Germination took place in distilled water at 18° to 20°C.

A sandy loam soil of 15 or 20 per cent moisture content and temperatures between 18° and 23°C are the conditions most conducive to oospore infection on Setaria viridis (L.) Beauv., Euchlaena mexicana Schrad., Zea mays L. (popcorn var. Japanese Hulless) and dent corn var. Iodent.

The plumule of Setaria viridis is most susceptible when one-half to five millimeters long. This infection becomes systemic.

Infection by Sclerospora graminicola (Sacc.) Schr. was obtained on two genera in the tribe Baeideae, Zea and Euchlaena and the two genera Panicum and Setaria in the tribe Paniceae.
Infection by *Sclerospora graminicola* var. *andropogonis sorghi* Kul. was obtained on *Zea mays* L. (popcorn var. Japanese Hulless and dent corn var. Iodent), *Setaria viridis* (L.) Beauv. and *Pennisetum typhoideum* Rich.

The host range of *Sclerospora graminicola* (Sacc.) Scar. includes two genera in the tribe *Sorghae*, three in *Paniceae*, and two in *Andropogoneae*.

The conidia germinate by zoospores. The number of zoospores from a single conidium is usually three but sometimes four. The germinating conidium first shows slight indentations at the periphery of the spore and later cleavage lines appear from these indentations to the center of the protoplasm. The entire contents of the conidium begins to move within the conidial wall, which is followed by emergence of the zoospores.

The zoospores are biciliate and motile. They remain active for five hours at 20° to 23°C, then become quiescent and germinate by a tube.

The most favorable conditions for infection by conidia are, a moisture film on the first healthy turgid leaf of susceptible plants at temperatures ranging from 18° to 25°C. Conidial infection may become systemic. No local infection was observed.
The production of conidia on the surface of infected host plants takes place in 82 to 100 per cent humidity and within a temperature range of 6° to 28°C. in darkness or in light. The turgid leaves must be covered with a film of moisture.

Successive crops of conidia develop on the same infected host tissues.
ACKNOWLEDGMENTS

The writer wishes to express her deep appreciation for the kind assistance and criticism given her in this study by Dr. W. E. Loomis and Dr. J. C. Gilman of the Botany Department at Iowa State College, as well as to many other members of that department.

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