1965

Zonate Leaf Spot of Sorghum.

Jack Lemuel Dean
Louisiana State University and Agricultural & Mechanical College

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ZONATE LEAF SPOT OF SORGHUM

Louisiana State University, Ph.D., 1965
Agriculture, plant pathology

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ZONATE LEAF SPOT OF SORGHUM

A Dissertation

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

in:

The Department of Botany and Plant Pathology

by

Jack Lemuel Dean
B.S., Oklahoma State University, 1949
M.S., Oklahoma State University, 1952
May, 1965
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ABSTRACT

Reported here are results of research on zonate leaf spot of sorghum caused by Gloeosporospora sorghi D. Bain and Edg. The emphasis is on resistance and susceptibility to the disease as it relates to a sweet sorghum breeding program being carried out at the U. S. Sugar Crops Field Station, Meridian, Mississippi.

A comparison of field inoculation techniques showed that initial infection and limited lesion development could be obtained without escapes, a requirement for screening segregating populations for resistant forms. However, the reaction of varieties known to show adequate field-resistance was not sufficiently different from susceptible varieties under artificial inoculation to permit a distinction. Attempts to grade the severity of the inoculation procedure to effect a separation of resistant and susceptible varieties were not successful.

A greenhouse technique was developed and used to screen 1509 sorghum importations from the U. S. Department of Agriculture's world collection of sorgo germ plasm. No highly resistant varieties were found.

An attempt to develop a laboratory method for screening the world collection was unsuccessful due chiefly to inability to control "weed" fungi in dishes used for seed germination and seedling inoculation.

The optimum temperature for growth of the zonate leaf spot fungus and for the initial stages of pathogenesis was found to be 80 - 85 degrees F.
By placing infected sorghum leaves in screenwire bags out-of-doors over the winter and re-isolating the fungus in the spring, it was shown that the zonate leaf spot organism is capable of over-wintering on crop debris. Sclerotia were the overwintering organs of the fungus. The fungus was not recovered from leaves held a few inches underground over the winter.

Sclerotia of the zonate leaf spot fungus were observed to germinate by production of sporodochia. Neither storage of leaf borne sclerotia at low temperature nor treatment with soil extract enhanced germination.

Differences in pathogenicity of *G. sorghi* isolates were demonstrated but evidence of host specialization was lacking.

On the day following inoculation, microscopic examination of leaves indicated extensive extracellular mycelial development above the epidermis. Around patches of mycelium, stomata were open wide, whereas stomata not near mycelial patches were tightly closed. Attempts to demonstrate a chemical that would open stomata in culture filtrates of *G. sorghi* were unsuccessful.

By inoculating young and mature leaves of field resistant and susceptible sorgho varieties, either on intact plants or as detached leaves, evidence was obtained supporting the view that only mature leaf tissues of resistant varieties are resistant. Mature-leaf resistance and young-leaf susceptibility would explain why certain varieties remain essentially disease free under natural infection but appear susceptible under the screening techniques used in this work.

A probable disease cycle is as follows. The fungus passes the
winter in the form of sclerotia on crop or weed (Johnson grass) debris. In the spring, these sclerotia germinate to produce sporodochia. Splashing rain brings the spores to lower (mature) leaves of sorgo. Infection, lesion formation, and production of secondary inoculum follow.

Zonate leaf spot is reported from 6 additional states, Arkansas, Alabama, Kentucky, Tennessee, South Carolina, and Maryland, from which it had not previously been reported.

The ability of *G. sorghi* to produce spores and sclerotia through many subculturings was maintained only by transferring from cultures during the short period when young, viable spores were present. Storage of dried sclerotia was a much less laborious method of maintaining cultural characters over extended periods.

When approximately 180 single spore colonies were established from each of 2 original isolates differing in cultural characters, the single-spore colonies from a given original isolate exhibited no cultural variation among themselves and all single-spore colonies were culturally similar to their parent isolates.
INTRODUCTION

Zonate leaf spot of sorghum, caused by Gloeocercospora sorghi D. Bain and Edg., may become severe on sorghum anywhere in the southeastern United States when the season is favorable for its development. At Houma and Baton Rouge, Louisiana, and presumably in other humid areas along the Gulf Coast, the disease is severe almost every year. Severe epiphytotics of zonate leaf spot are also common in eastern Kentucky.

The investigations reported here were begun chiefly for the purpose of devising an efficient technique for producing zonate leaf spot infection of sufficient uniformity to permit individual plant selections for resistance among F3 and subsequent segregating generations of sweet sorghums in the field. When the work was begun there were available at least 3 African importations of sweet sorghum that remained almost free of zonate leaf spot in the field at Houma, Louisiana, when all other varieties were very heavily infected. It was assumed, therefore, that the correct approach would be to devise an efficient means of producing uniform infection in a field of a susceptible variety so that escape of susceptible plants would be prevented. This was readily accomplished. It was then found that field-resistant varieties were also uniformly infected by the method.

The inoculation methods were tested in an attempt to distinguish between resistant and susceptible plants. The next approach was to screen varieties in the U. S. Department of Agriculture's world collection of sorghum germ plasm for parents having sufficient resistance to withstand artificial inoculation. So far, such resistance has not been found.
An attempt was made to determine why field-resistant varieties reacted essentially as field-susceptible varieties under artificial inoculation.
REVIEW OF THE LITERATURE

From lesions of a new leaf spot of sorghum discovered in the fall of 1940 at Baton Rouge, Louisiana, Bain (1, 2) consistently isolated a fungus resembling *Ramulispora sorghi* (Ellis and Everhart) Olive and LaFebvre, the causal organism of sooty stripe of sorghum. This fungus was shown to be the causal organism of a new leaf disease which was called zonate leaf spot. A new genus was erected in the Tuberculariaceae to accommodate the fungus described by Bain and Edgerton in 1943 under the name, *Gloeocercospora sorghi* (4).

Subsequently, zonate leaf spot of sorghum has been reported from South America, West Indies, Asia, and Africa, and from several countries in each of these areas (17). In the United States the disease has been reported from Louisiana, Mississippi, Virginia, Florida, Texas, and Georgia (4, 14, 22, 10, 7). *Gloeocercospora sorghi* on other grass hosts (*Agrostis* spp.) has been reported from Pennsylvania and Rhode Island where it was of some economic importance as a disease of turf grass (21, 20, 19). On these grasses, the disease is known as copper spot.
The known hosts of Gloecercocarpora sorghi are:

Sorghum vulgare Pers. (sorghum) (4)
Sorghum halepense (L.) Pers. (Johnson grass) (4)
Zea mays L. (corn) (4)
Saccharum officinarum (L.) (sugarcane) (4)
Pennisetum glaucum (L.) R. Br. (pearl millet) (11)
Pennisetum purpureum Schumach (napier grass, elephant grass) (18)
Agrostis canina (L.) (velvet bentgrass) (21, 20)
Agrostis palustris Huds. (creeping bentgrass) (19)
Agrostis tenuis Sibth. (colonial bentgrass) (21, 20)
Cynodon dactylon (L.) Pers. (Bermuda grass) (15)

The symptoms of zonate leaf spot were described by Bain and Edgerton (4). Lesions first appear as small reddish or brownish water-soaked spots that sometimes have a narrow, pale-green halo. As the spots enlarge, they become a dark-red (except in certain varieties where they are light-brown) and become somewhat elongate and parallel to the veins. Finally, they form (possibly by coalescence) large, semi-circular, or irregular lesions several centimeters in diameter. Small lesions usually have a light-brown center surrounded by a light-to-dark-red border; but frequently, in larger lesions there may be an alternation of dark and light zones. Leaf lesions may occur along the margins or toward the midrib, or they may cover the entire leaf when infection is heavy. Frequently the young lesions are sufficiently numerous to produce red irregular blotches. Within a few weeks after infection occurs, minute spherical to lenticular sclerotia appear in
the necrotic areas of infected leaves and sheaths. Leaves and sheaths are the only parts of the plant on which symptoms have been observed, although the fungus has been isolated from surface-sterilized seeds and glumes.

In addition to the zonate or irregular spots described by Bain and Edgerton, Wallace and Wallace (19) in Africa and Muntanola (12) in South America have described a different leaf spot caused by *Gloeocercospora sorghi*. These spots, which Tarr (17) reports that he also has seen, are described by Tarr as small, regular oval spots measuring up to about 1/4 inch in diameter or length.

The following description of the fungus comes from the account of Bain and Edgerton (4):

*G. sorghi* sporulates on the host under field conditions but the large, well defined fruiting bodies usually are inconspicuous. When infected leaf material is placed in a moist chamber for 24 to 48 hours, an emergence of fruiting bodies appear in and surrounding necrotic areas. Spores are borne in a sporodochium found on the surface of the leaf above a stomate. The sporodochium arises from hyphae emerging from the stomate; these hyphae branch and form a sporodochial column that, at maturity, is more or less definitely stalked. A bouquet-like structure is produced by the branching conidiophores. The sporodochium does not originate within the tissue and become erumpent. There is no stromatic base above the leaf surface or in the substomatal cavity. The salmon-colored sporodochia, occurring either in dense clusters, or sparsely in and around necrotic areas, are easily visible to the unaided eye.
The length and width of the conidiophores are difficult to determine because they are so densely packed. In general, they appear to be short (5 to 10 microns), hyaline, and either simple or branched.

Conidia, borne in a slimy, pinkish to salmon, matrix, are straight or curved, tapering somewhat from the base to the apex, few- to many-septate, hyaline, and elongate to filiform. Their length varies from 20 to 195 microns and, at the widest place, they are slightly over 3 microns in width.

Black sclerotia, developing within the tissues of the mature leaf lesions, occur at definite intervals and in lines parallel to the veins. Sectioned sclerotia appear round to elliptic, each with a central portion composed of pseudoparenchymatous tissue that is surrounded by a hard layer composed of thick-walled cells. Luttrell gives the dimensions of the sclerotia as 70-98 x 118-134 microns (10).

Bain and Edgerton (4) reported the optimum temperature for growth of Gloecercospora sorghi around 28° to 30° C.

There is little information on the mode of overwintering and spread of the zonate leaf spot fungus. According to Tarr (17) the fungus is presumed to overwinter in crop debris, infected weeds, and on infected seeds and glumes. That author also states that field observation indicates that the disease spreads by wind and rain, is favored by warm wet weather, and tends to dry out under arid conditions. Zonate leaf spot is believed to have been imported to Venezuela from the United States on infected sorghum seed (5).

Copper spot, the disease caused by G. sorghi on bent grasses, has
been controlled with fungicides (8, 7) but this method has not been used on sorghum, presumably for economic reasons. Apparently on the basis of presumption, Tarr (17) suggests that destruction of infected crop trash, infected wild plants, and volunteer seedlings, combined with crop rotation might be helpful for control of zonate leaf spot. Chemical seed treatment is recommended for preventing shipment of the disease from one country to another.

Resistant varieties would seem to offer the best solution for control of zonate leaf spot but it is doubtful if any commercial variety has a useful degree of resistance. Luttrell reported resistance in Texas milo in Georgia (10). Bain reported Laoti and Shallu only lightly infected in inoculation tests at Baton Rouge, Louisiana (3).
STUDIES OF INOCULATION TECHNIQUES

At the beginning of the work reported here, the sorgo breeding program of the U. S. Sugar Crops Field Station, (Meridian, Mississippi) was in need of an inoculation technique for zonate leaf spot that would assure infection of susceptible individuals in segregating populations, and be sufficiently rapid for inoculation of up to 4 acres of plants. A technique fulfilling these requirements had been developed by LeBeau for anthracnose (9). Consequently, the first attempts to develop a method for zonate leaf spot consisted of substituting G. sorghi inoculum in the needle technique of LeBeau. Briefly the method was to inject a water suspension of inoculum from a compressed air tank (30 pounds pressure) through a hypodermic needle inserted through the rolled leaves of the lower portion of the leaf whorl.

Preliminary Inoculations

In 1952 plots were marked off in a block planting of Sart and the sorgo was inoculated by the needle technique when the plants were about 3 feet tall. Uninoculated plots served as controls. Within a few days it was apparent that all inoculated plants were infected, although lightly. The lesions grew very slowly and never developed into typical zonate spots. The disease did not spread from the inoculated leaves, and as those leaves became senescent the infection essentially disappeared. The lack of escapes, however, was encouraging and there had originally been enough infection to make selection of resistant plants from segregating populations seem feasible. Some idea of the range of infection produced is indicated in Figure 1.
Figure 1. Zonate leaf spot infection resulting from needle inoculation of field-grown plants, ranging from infection confined to needle holes (left) to extensive infection remote from needle holes (right).
Later in the year, sorgo was inoculated by the needle technique in the greenhouse. It soon became apparent that infection by this method was very positive; infection always occurred around the needle punctures if not elsewhere (Figure 2). However, when a resistant variety (MN 960) was inoculated, it was infected to about the same extent as Sart, Rex, and other susceptible varieties tested. On MN 960, as on other varieties, infection was not always confined to wounds made by the needle, but because of the very high resistance that MN 960 was known to exhibit in the field under conditions of natural infection, it seemed likely that the occurrence of wounding with inoculation was responsible for the apparent breakdown of resistance. Consequently, in a field experiment the following year, inoculation techniques were tested on Sart and on MN 960.

Experiment I

The experiment consisted of 24 treatments and 4 replications in a randomized block design. Plots were single rows 10 feet long. The treatments resulted from combinations of 2 types of inoculum, 2 methods of applying the inoculum, 3 times of day of inoculation, and 2 varieties.

One inoculum was a spore suspension prepared by washing spores from heavily fruiting cultures. The other inoculum was prepared by homogenizing the whole colony, plus agar with water in a Waring blender.

One method of applying inoculum was by the needle technique; the other method used the same apparatus except that the hypodermic needle was removed from the end of the hose leading to the pressure tank containing the inoculum. Instead of inserting the needle through the
Figure 2. Infection around needle holes resulting from inoculation by the needle technique. The 2 leaf sections on the right were inoculated with water. (The image is photographically negative)
rolled leaves of the whorl, the needle fitting was used to direct a stream of inoculum into the open leaf whorl without wounding of leaves. Inoculations were made early (8:00 a.m.), near mid-day (1:00 p.m.), and late (6:00 p.m.). The varieties were Sart and MN 960.

An attempt was made to assign disease ratings, but this proved impracticable because of the different methods of applying inoculum and the different wound reactions of the 2 varieties. The needle left punctures in the leaves, definitely marking the site of inoculation. Necrosis bordering the needle wounds and spots clustered in the inoculated region were readily recognized as zonate leaf spot. Application of inoculum without wounding left the site of inoculation less well defined with the less effective treatments and made it more difficult to be sure a given lesion was due to G. sorghi.

NOTE: Sart responds to almost any kind of leaf injury by formation of a dark reddish color at the site of injury, whether it be mechanical injury or parasitic injury. MN 960 appears to lack the ability to form such pigmentation. Injury does cause a color change but tissue darkening seems to be due merely to necrosis. As shown in Figure 3, Sart lesions appear obscured by heavy pigmentation, while MN 960 lesions show considerable detail.

The assignment of numerical disease ratings did not appear to increase the information obtainable from the experiment. Numerical ratings were, therefore, discarded in favor of qualitative descriptions.

It was apparent that more infection resulted from whole-culture than from spore-suspension inoculum, and that needle inoculation produced more infection than inoculation without wounding. Probably, the late afternoon inoculation produced more infection than earlier inoculations when the inoculum was applied without wounding but this
Figure 3. Zonate leaf spot lesions resulting from artificial inoculation without tissue wounding. Left: MM 960. Right: Sart. Sart lesions are much more conspicuous in the field because of the dark red pigment which is absent in MM 960.
difference, if real, was not great. There may have been slightly less infection of MN 960 than of Sart but this difference, too, was small, if real, and definitely not great enough to permit distinguishing resistant from susceptible individuals in segregating populations.

As in the previous year's field test, lesions increased in size very slowly, zonate leaf spots did not develop, secondary spread of the disease did not occur, and with senescence of inoculated leaves the disease became inconspicuous.

Discussion

Clearly the problem was not to produce as much infection as possible but to distinguish between resistant and susceptible varieties. The needle technique gave the heaviest infection but showed little promise of distinguishing resistance. Inoculation without wounding was not clearly superior in this respect but may have been slightly so. In fact, the experiment left the impression that the poorest treatments with respect to amount of infection may have shown the greatest difference between varieties but unfortunately those treatments permitted many escapes in both varieties.

Experiment II

In 1954, a second attempt was made to develop a field inoculation technique that would distinguish resistant from susceptible varieties.

Infection on Sart and on MN 960 was compared with a field test comprising 10 inoculation treatments and 4 replications in a split plot design. Each main plot was 2 rows wide by 16 feet long; one of these rows was Sart, the other MN 960. Inoculation treatments were:
1. Inoculum consisting of homogenized, whole-agar cultures (1 part agar to 20 parts water) injected into leaf whorl with hypodermic needle.

2. As in Treatment 1 but dilution = 1 to 40

3. As in Treatment 1 but dilution = 1 to 80

4. Inoculum as in Treatment 1 but applied to leaf whorl without using needle.

5. As in Treatment 4 but inoculum diluted 1 to 40

6. As in Treatment 4 but inoculum diluted 1 to 80

7. Inoculum prepared by growing fungus on autoclaved whole barley grain, drying the grain and fungus, and grinding in a corn grinder. The resulting fine granular material was sprinkled over the plants.

8. As above but inoculated twice with about 1 week between inoculations.

9. As above but inoculated 3 times at approximately weekly intervals.


Inoculum for Treatments 1 - 6 was increased in 32-ounce prescription bottles, each containing 100 ml. of Difco lima bean agar. Inoculations were made on August 7, August 17, and August 24 when plants were 4 to 6 feet tall and 2-1/2 to 3 months old. Relative disease ratings from 0 to 4 were made 22 days after inoculation. The rating of disease severity was difficult for the same reasons
given previously. However, an attempt was made to simplify the problem by picking out the single most heavily infected leaf on each plant in the plot and assigning a rating to that leaf.

Results

Results of this experiment are shown in Table I. All check (uninoculated plots) were given 0 ratings; this treatment was not included in variance analyses. Sart was assigned significantly higher disease ratings than MN 960. Differences between inoculation treatments were not significant.

Since the purpose of the experiment was to find an inoculation method that would distinguish the disease reaction of Sart from that of MN 960, the differences in disease ratings between Sart and MN 960 were subjected to an analysis of variance but no significance was found. In other words, none of the inoculation treatments was clearly superior to the others for distinguishing zonate leaf spot reactions of Sart and MN 960.

As in previous experiments zonate lesions typical of natural infection did not develop on either variety.

Discussion

At best, the results from this experiment would indicate that resistance of the MN 960 type could be distinguished from more susceptible varieties only by inoculating replicated plots and subjecting the data to analysis of variance. This procedure obviously is not suitable for selecting resistant plants from a segregating population.
### Table I. Zonate leaf spot ratings resulting from various inoculation treatments

<table>
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<th>Treatments*</th>
<th>Varieties</th>
<th>Average</th>
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<td>Sart</td>
<td>MN 960</td>
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<tr>
<td>1</td>
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<td>1.48</td>
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<td>1.30</td>
<td>0.90</td>
<td>1.10</td>
</tr>
<tr>
<td>9</td>
<td>1.48</td>
<td>1.02</td>
<td>1.25</td>
</tr>
<tr>
<td>Average</td>
<td>1.50</td>
<td>0.96</td>
<td>1.23</td>
</tr>
</tbody>
</table>

L.S.D. .01: Treatments - NS
Varieties - .25

*See text for description of treatments
SCREENING SORGO VARIETIES FOR A SOURCE OF RESISTANCE

Since attempts to distinguish the sonate leaf spot reactions of Sart and MM 960 had shown so little promise, a search was begun for parents possessing a type of resistance that would withstand artificial inoculation. This resistance was sought in the U. S. Department of Agriculture's world collection of sweet sorghum germ plasm maintained at Meridian, Mississippi, and at Beltsville, Maryland. The work was begun in 1954 with a field test. Later it became apparent that varieties could be screened faster in the greenhouse than in the field and the program was shifted to the greenhouse.

In the first attempts at screening varieties for resistance in the greenhouse, elevated relative humidities were obtained by placing plastic covers lines with wet burlap over flats of inoculated seedlings. This procedure resulted in very poor infection. Inoculation tests conducted in a mist chamber at Louisiana State University in the fall of 1955 indicated that good infection could be obtained by that method. Therefore, a mist chamber was installed at Meridian. Results obtained were erratic. In some runs, very heavy infection was obtained, in others almost none. Eventually it was noticed that good infection was obtained on cool, cloudy days and poor infection on hot, bright days. This led to the speculation that high temperatures were responsible for poor results. A series of experiments on temperature relations of the fungus (described elsewhere in this report) was begun. However, it was discovered accidentally that relative humidity and not temperature was the problem.
At first it was assumed that relative humidity could not be a factor in the mist chamber with the atomizer nozzle spraying. But it was observed that very good and consistent infection could be obtained if the cloth walls of the mist chamber were kept wet. A canvas hose (called a "soaker") was wrapped around the top of the mist chamber and water from the hose oozed down over the sides of the mist chamber. After this modification no further difficulty in obtaining infection was encountered.

Since whole agar cultures, with agar included, had seemed to enhance infection in field inoculations, agar and gelatin were tried as additives to the inoculum in greenhouse experiments during the period when difficulty in obtaining adequate infection was being experienced. Both additives had seemed to give slightly increased infection before the relative humidity problem was solved. After installation of the soaker, it was found that 2 percent gelatin added to a spore suspension would result in infection sufficiently heavy to kill most of the plants (Figure 4). This extremely severe infection was considered unnecessary; consequently, most of the screening work was done with spore suspensions in water.

The mist chamber was built to hold 8 greenhouse flats; 8 varieties were planted in rows crosswise in the flats. One row of a variety across a flat was inoculated in a given run. After being atomized with a spore suspension, seedlings were left in the mist chamber about 20 hours. Upon removal from the chamber, small water-soaked lesions were already visible. The plants were checked for
Figure 4. Sart sorgo seedlings 2 weeks after inoculation. 
Left: inoculated with spore suspension in 2 percent gelatin. 
Right: sprayed with 2 percent gelatin.
evidence of resistance about 24 hours after removal from the mist chamber. Any variety that did not show at least moderate infection was rechecked in the next run. Plants were about 1 week old when inoculated.

Sart and MN 960 were included in each flat to serve as check varieties.

A total of 1509 varieties were screened for resistance in these tests. No highly resistant varieties were found. On 2 different occasions, highly resistant entries occurred but these, although bearing MN numbers and maintained in the sorghum germ plasma collection, were not sorghums but related wild grasses.

Although the primary purpose of the greenhouse screening program was to find a source of resistance of a different type from that of MN 960, it would have been of interest to know if MN 960 type resistance could be determined by the screening method. MN 960 was present in every flat as a check and a note was made of any variety that appeared no more heavily infected than MN 960. Admittedly this was a somewhat dubious judgment since it was not even perfectly clear that MN 960 looked more resistant than most varieties, but the attempt was made, nevertheless. Such selected varieties were re-inoculated in a later run and, if they again seemed to show some resistance, they were planted in the field, along with MN 960, at Houma, Louisiana, where natural infection from zonate leaf spot is usually severe. Eleven such varieties were grown at Houma in 1958 and again in 1959.

Unfortunately in both years, very poor stands and growth resulted
and only very crude evaluations of resistance in the field were obtained. It was clear, however, that at least some of the varieties were quite susceptible, indicating that the greenhouse technique probably was not adequate for picking out the MM 960 type of resistance.
ATTEMPTS TO DEVELOP A LABORATORY METHOD OF SCREENING FOR RESISTANCE

In April, 1956, a series of exploratory experiments was conducted in an attempt to develop a laboratory method which would be more rapid and less laborious than the greenhouse method.

**Experiment I**

Sorgo seed were inoculated with the zonate leaf spot fungus by covering the seed in a beaker with a spore suspension. The spore suspension was poured off and the seed layered between moist paper towels. A layer of Sart seed was followed by a layer of MN 960 seed until 4 layers of each were completed. The stack of towels was placed on a piece of glass under an inverted plastic tray to retard evaporation. A second, similarly treated stack of towels containing uninoculated seed served as control.

At the end of 6 days, Sart seedlings from inoculated seed showed slightly more root discoloration than control Sart seedlings but this was vague and not considered useful. "Weed" fungi such as *Apergillus* and *Fusarium* sp. were so abundant around MN 960 seedlings, both inoculated and uninoculated, that comparisons were not possible.

**Experiment II**

In a second experiment, sorgo seedlings were grown in petri dishes in a seed germinator. Twenty-five seed were placed in each dish. The germinator had 8 shelves and each shelf was used for one replication of the 12 treatments listed in the following:
<table>
<thead>
<tr>
<th>Treatment number</th>
<th>Seed treatment</th>
<th>Inoculation</th>
<th>Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>None</td>
<td>MN 960</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>None</td>
<td>Red Top</td>
</tr>
<tr>
<td>3</td>
<td>Phygon</td>
<td>Dip^1/</td>
<td>MN 960</td>
</tr>
<tr>
<td>4</td>
<td>Phygon</td>
<td>Spray^2/</td>
<td>Red Top</td>
</tr>
<tr>
<td>5</td>
<td>Phygon</td>
<td>Dip</td>
<td>MN 960</td>
</tr>
<tr>
<td>6</td>
<td>Phygon</td>
<td>Spray</td>
<td>Red Top</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>Dip</td>
<td>MN 960</td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>Spray</td>
<td>Red Top</td>
</tr>
<tr>
<td>9</td>
<td>None</td>
<td>Dip</td>
<td>MN 960</td>
</tr>
<tr>
<td>10</td>
<td>None</td>
<td>Spray</td>
<td>Red Top</td>
</tr>
<tr>
<td>11</td>
<td>Phygon</td>
<td>None</td>
<td>MN 960</td>
</tr>
<tr>
<td>12</td>
<td>Phygon</td>
<td>None</td>
<td>Red Top</td>
</tr>
</tbody>
</table>

1/ Seed inoculated by dipping in a spore suspension before being placed in dishes on moist filter paper in incubator.

2/ Young seedlings atomized with spore suspension 3 days after seed were placed in incubator.

Dip inoculation resulted in no recognizable infection by *G. sorghii* on either variety. Spray inoculation produced some *G. sorghii* infection on Red Top but there were too many escaped seedlings for the method to be workable. MN 960 seedlings were so over-grown with "weed" fungi that it was impossible to tell whether or not they were infected by *G. sorghii*. Phygon reduced this mold growth slightly but not enough to be of real value.

**Experiment III**

Sart seed were sprouted in dishes in a seed germinator. Ten dishes with 25 seeds per dish comprised each of the 4 treatments listed in the following:

**Treatment 1.** Seedlings sprayed with a spore suspension in a nutrient medium made of 7.5 percent gelatin, 2.5 percent dextrose, and 2.5 percent peptone.
Treatment 2. Seedlings sprayed with the nutrient medium alone.

Treatment 3. Seedlings sprayed with a spore suspension in distilled water.

Treatment 4. Seedlings sprayed with distilled water.

All seeds were treated with arasan SW-X slurry. Inoculation and control treatments were applied 3 days after the seed were placed in the germinator.

Twenty-four hours after inoculation the only noticeable difference among treatments was a more extensive browning of the root systems of the seedlings receiving Treatment 1 (spore suspension in nutrient medium).

Four days after inoculation, G. sorghi was fruiting extensively over roots, coleoptiles, and pericarps of seedlings receiving Treatment 1. Seedlings in the other 3 treatments appeared about alike with no evidence of G. sorghi infection.

Experiment IV

A final experiment in this series was designed to take advantage of the infection obtainable by inoculating with spores suspended in a nutrient medium while testing surface sterilization of seed in Clorox (5-1/4% sodium hypochlorite) as a means of reducing overgrowth of seedlings by molds. Treatments were:

1. Seed surface treated in 50 percent Clorox for 5 minutes

2. Seed not treated with Clorox

The foregoing treatments were applied to seed of Sart and MN 960. After treatment with Clorox, seed were dried and treated with captan. Seed
not treated with Clorox were also treated with captan. Dishes used for germinating seed were hot air sterilized with a filter paper pad in place in the dishes. Ten dishes (replications) of each treatment on each variety (40 dishes total) with 25 seeds per dish were placed in the seed germinator. Three days later all seedlings were sprayed with spores suspended in the gelatin-dextrose-peptone medium previously described.

Five days after inoculation, there were some fruiting lesions of *G. sorghi* on Sart, especially on root systems, but there were many escapes. There may have been a slight increase in germination in both varieties due to Clorox treatment as tabulated below:

<table>
<thead>
<tr>
<th></th>
<th>Clorox treated</th>
<th>Un-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sart</td>
<td>66</td>
<td>49</td>
</tr>
<tr>
<td>MN 960</td>
<td>55</td>
<td>50</td>
</tr>
</tbody>
</table>

Mold growth was slight on Sart treated with Clorox and moderate on untreated Sart. Mold growth was greatly reduced on MN 960 but there was still enough to complicate assessing the amount of infection by *G. sorghi*.

Presumably the captan may have been responsible for reduced infection of Sart by *G. sorghi*. In any case it was apparent that any laboratory method that could be worked out for zonate leaf spot resistance screening was likely to be more laborious than the greenhouse method already in use and the project was abandoned.
GROWTH-TEMPERATURE RELATIONS OF THE FUNGUS IN CULTURE

When difficulty in obtaining consistent infection was encountered in the greenhouse mist chamber, high temperatures were suspected of causing the trouble. Consequently, the growth-temperature relationships of the fungus in culture were studied.

Materials and Methods

Gloeosporium sorghum was grown on 50 ml. of Difco lima bean agar in sealed 12-ounce prescription bottles. Because it was not possible to control relative humidity in the various temperature chambers, all bottles were closed with cotton plugs followed by plastic screw caps so that relative humidity would be held to 100 percent inside the bottle.

Three tests of the growth-temperature study were completed with 3 isolates of the fungus, 9 temperatures, and 6 replications (cultures).

Results from the first test were discarded because of 2 problems. In the first test, spores were used to seed the culture flasks, a loopful of spores being deposited at the center of the agar surface. Water condensed on the glass above the agar, then dripped back down and washed the inoculum around somewhat before growth started. This resulted in irregularly shaped colonies which were difficult to measure. The second problem probably resulted from the toxic effects of mercuric chloride adsorbed on the glass of the bottles. (The bottles had been used, several years previously, to store sorghum juice preserved with mercuric chloride). Even at the more favorable temperatures, some
colonies almost failed to grow and others seemed to grow normally.
Subsequently, the bottles were soaked for a week, then washed
thoroughly several times with warm soapy water. Tests indicated
there was then less toxicity in the bottles but that there was still
a problem. Therefore, new bottles were used for tests 2 and 3.

Temperatures were obtained by using 3 refrigerated rooms
(available at the U. S. Horticultural Field Station, Meridian,
Mississippi) and smaller incubators in these rooms and in a non-
refrigerated room. Some of the temperature chambers were completely
dark, others were not. Therefore, cultures at all temperatures were
packed in corrugated cardboard boxes and covered with aluminum foil
to prevent light from reaching any of them.

In tests 2 and 3 a different procedure was used for seeding
the culture bottles. A small "biscuit cutter" was fashioned at the
end of a piece of flattened aluminum wire. With this tool it was
possible to cut a cylinder of agar about 1 mm. in diameter from the
edge of a young, non-sporulating colony. Such a cylinder was pressed
into the center of the agar surface in each culture bottle.

The problem of excessive condensation of moisture on the glass
above the agar was solved by placing the bottles horizontally on a
screenwire rack and covering them with a blanket as soon as they were
removed from the autoclave. An electric fan was used to circulate
air under the bottles. This arrangement promoted cooling of the agar
and retarded cooling of the glass above the agar, reducing condensation.

In test 2, colony diameter was measured after a growth period
of 7 days. At the more favorable temperatures, the colonies had spread all the way across the bottle laterally and were therefore measured in the longitudinal direction of the bottles. In test 3, colonies were measured after 4 days of growth.

Results

Growth-temperature curves for isolates 8, 9, and 11 of test 2 are shown in Figure 5 and for isolates 3, 4, and 11 of test 3 in Figure 6. Figure 7 shows the average curve resulting from both tests and all isolates.

Evidently the optimum temperature for growth (i.e. increase in colony diameter) of G. sorghi in culture was close to 80°F., perhaps slightly more.

The left arm of all curves but 1 in Figures 5 and 6 shows an irregularity. As illustrated in the average curve (Figure 7) there are 2 possible ways of viewing the irregularity. If one considers dotted-line B the regular course, then the irregularity is regarded as a hump at the 60°F. point. If dotted-line A is considered the regular course, the irregularity is regarded as a depression at the 70°F. point.

Sporulation for all isolates, except 4, occurred between 70°F. and 90°F. as indicated in Figure 7. It appeared likely, therefore, that the irregularity represented a depression at 70°F. resulting from the onset of sporulation. This would not necessarily mean that growth was checked by the beginning of sporulation, but perhaps only that there was a change in growth habit.
Figure 5. Influence of temperature on growth in culture of G. sorghi isolates 8, 9, and 11
Figure 6. Influence of temperature on growth in culture of *G. sorghi* isolates 3, 4, and 11
Figure 7. Average growth-temperature curve for 1 test of 4 isolates, and 2 tests of 1 isolate of G. sorghi
The irregularity was not present in the curve for isolate 4 in test 2 (Figure 6). This peculiar isolate produced spores at all temperatures at which it grew. It even produced a few spores at 32° F. The spores at 32 and 40° F. were severely reduced in size, consisting of no more than 2 or 3 cells. There was a gradual increase in spore length and cell number with increasing temperature, and in the range between 70 and 90° F., spores of isolate 4 were indistinguishable from those of other isolates. At 90° F., the highest temperature at which the fungus grew, spores of all isolates were of normal size but many of them contained misshapen (bulbous) cells, interspersed with normally-shaped cells.

The 18 culture bottles (3 isolates x 6 replications) held at 95° F. for 4 days in test 3 were left at room temperature for 3 days following their removal from the 95° F. chamber. At room temperature _G. sorghi_ colonies developed in 7 of the 18 bottles. Apparently the inoculum in 11 of the bottles did not survive 95° F. for 4 days. The 12 flasks which did not produce measurable growth at 32° F. in test 3 were also held at room temperature for 3 days following their removal from the 32° F. chamber. _G. sorghi_ colonies developed in all of these bottles.

_G. sorghi_ occasionally develops zonate patterns on agar surfaces in culture. In this series of experiments isolates 8 and 11 in test 2 produced growth consisting of a series of concentric rings at 80° F. These zonate patterns were not produced at any other temperature. In this case, at least, the zonate pattern was not due to fluctuating
temperature, light, or other known environmental conditions. The thermostat of the 80°F. chamber operated on a temperature differential of less than 1°F. and cycling time was much too short for the zonations to have developed in response to thermostat cycling.

The only isolate producing sclerotia in these experiments was isolate 9 in test 2. In that case all sclerotia were produced between 70 and 90°F., the same range at which spores were produced by most isolates of G. sorghi.
TEMPERATURE-INFECTION STUDIES

Since the optimum temperature for growth of a pathogen in culture is not necessarily the same as its optimum for pathogenesis, determination of one of these optima for G. sorghi led to a question about the other.

In September of 1956 a series of experiments was conducted to determine the optimum temperature for infection. The same temperature chambers used in the experiments on growth-temperature relations of the fungus in culture were used for this work. Temperature fluctuation due to either refrigeration unit or heating element cycling was no more than + or - one degree F. Plants at all temperatures were in complete darkness and relative humidity was held at 100 percent as indicated by water droplets on the foliage at all times.

**Experiment I**

Tracy sorgo plants were grown for 8 days in 4-inch clay pots before they were atomized with a spore suspension and placed in the temperature chambers at 60, 70, and 80° F. The fungus isolate used was the one designated isolate 11 above. Two pots containing about 25 seedlings each were placed at each temperature.

Twenty hours later there was no visible infection at 60°, moderate infection at 70°, and severe infection at 80°. At the end of another 24 hours there was very severe infection at 70° and 80° and a trace of infection at 60° F.

**Experiment II**

This experiment was essentially a replicate of Experiment I
except that 2 isolates of the fungus (4 and 11) were used and the
temperatures 90° and 95° F. were added, as was a noninoculated control
(one pot for each isolate at each temperature). Two pots of seedlings
were inoculated with each isolate of the fungus and placed at each
temperature. One of the fungus isolates (obtained from corn) had
responded atypically to low temperatures in culture (It had produced
spores at 32° F.) and was included here to see if it would exhibit
any peculiar temperature responses on the host. The other isolate was
a typical sorgo isolate, the one used in Experiment I. Degree of
infection was rated 0 to 6 as indicated in the following:

0 = No infection
1 = A few scattered flecks but most seedlings disease-free
2 = A few seedlings disease-free but most with one or more
   flecks
3 = Slightly larger spots; no disease-free seedlings
4 = Still larger spots, some coalescence of spots, all
   seedlings with several lesions
5 = All plants with some dead leaves or leaf tips;
   extensive coalescence of spots
6 = Most of the leaves dead

Results of the experiment are given in Table II.

The corn isolate was not pathogenic on sorgo. The optimum
temperature for infection was about 80° F. This was found previously
to be the optimum for growth of the fungus in culture.
Table II. Disease ratings of sorgo 21 and 48 hours after seedling inoculation with 2 isolates of *G. sorghi* at various temperatures

<table>
<thead>
<tr>
<th>Temperature °F.</th>
<th>Isolate</th>
<th>Disease rating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>21 hours</td>
</tr>
<tr>
<td>60</td>
<td>Corn</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sorgo</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Check*</td>
<td>0</td>
</tr>
<tr>
<td>70</td>
<td>Corn</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sorgo</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Check</td>
<td>0</td>
</tr>
<tr>
<td>80</td>
<td>Corn</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sorgo</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Check</td>
<td>0</td>
</tr>
<tr>
<td>90</td>
<td>Corn</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sorgo</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Check</td>
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</tr>
<tr>
<td>95</td>
<td>Corn</td>
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</tr>
<tr>
<td></td>
<td>Sorgo</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Check</td>
<td>0</td>
</tr>
</tbody>
</table>

*Noninoculated*
Experiment III

A third experiment differed from Experiment II in that corn was the test plant used for the corn isolate, and a corn check was added. One other difference may have been quite important. The plants in Experiments I and II were about a week old and growing vigorously at the time of inoculation. In Experiment III, the plants were almost 3 weeks old, were not growing well, and were slightly chlorotic. This probably accounted for the different result obtained in this experiment.

Twenty-four hours after inoculation the plants were examined and no infection was noted. After 48 hours there were a few isolated flecks but it was not clear that they were caused by G. sorghi infection. Since 48 hours in the dark, particularly at 95° F., produced severe etiolation, it was not considered worth while to leave the plants in the temperature chambers any longer. All plants were removed and placed on the greenhouse bench. These plants were not examined until 3 days later at which time the sorgo plants (inoculated with the sorgo isolate) which had been incubated at 60°, 70°, 80°, and 90° F. were moderately diseased. The corn was not infected at any temperature indicating that the corn isolate was no more pathogenic on corn than it had proved to be on sorgo in the previous experiment. Most of the sorgo plants which had been held 48 hours in the dark in a water saturated atmosphere at 95° F. were dead when they were re-examined 3 days after their removal to the greenhouse.

Apparently infection did occur in this experiment but most of
the infection developed too slowly to become evident in 48 hours.

Discussion

Results obtained in this series of tests indicated that the optimum temperature for infection of sorghum by *G. sorghii* was the same (about 80° F.) as the optimum for growth of the fungus in culture.

It also became apparent from these results that high temperatures in the mist chamber did not account for the large differences in infection between runs in the work on screening for a source of resistance. Temperatures in the mist chamber were never above 80° F. at night, and all inoculations were carried overnight.
OVERWINTERING OF THE FUNGUS ON SORGHUM LEAVES

On September 23, 1953, heavily infected sorgho leaves were gathered from the field and placed in small screenwire bags constructed by sewing 2 rectangular pieces of screenwire together on 3 sides with a piece of fine wire. One bag was hung on a fence about 3 feet above the ground, another was placed on the ground surface in the fence row, and a third was buried in the soil at a depth of about 3 inches.

On June 22, 1954, these bags were brought into the laboratory and attempts were made to recover the fungus in culture. Cultures of G. sorghi were easily obtained by surface sterilizing (Clorox) sclerotium-bearing fragments of leaf tissue from leaves that had passed the winter above ground, either at the soil surface or hanging 3 feet above it. Because of the general discoloration and decomposition of the underground leaves, it was impossible to find sclerotia or recognize old zonate leaf spots. It was necessary, therefore, to take leaf fragments at random. Eventually about 1000 fragments were plated on lima bean or oatmeal agar, either with or without surface sterilization, but G. sorghi was never obtained.

In a further attempt to recover the fungus from leaves overwintered underground, a greenhouse flat was filled with soil and sown with sorgho seed on a spacing of approximately 1 x 1 inch. After the seed were covered, the overwintered leaf trash was reduced to small fragments by hand crushing and the resulting litter was spread evenly over the surface of the flat. The flat was watered daily.
with a sprinkling can held high to promote splashing, and several times during the course of the experiment, the flat of sorgo was placed in a moist chamber overnight. After the seedlings emerged they were given frequent, careful, examinations. When the seedlings began to become crowded, they were thinned. Any blemishes on the rogued plants, however slight, were removed, surface sterilized, and plated out on lima bean agar.

The plants remaining in the flat were held for about 10 weeks during which time all suspicious leaf spots were cultured. *G. sorghi* was never recovered.
PRODUCTION OF CONIDIA BY SCLEROTIA

According to Tarr (17) germination of sclerotia of *Gloeocercospora sorghi* has not been observed. K. S. Luttrell once questioned whether or not the black bodies produced by *G. sorghi* were truly sclerotia as they had been called or if they were really perithecial initials (personal communication).

Since it had been shown that sclerotia were capable of overwintering in sorghum leaf debris and it was known that *Ramulispora sorghi* (a fungus superficially resembling *G. sorghi*) sclerotia were capable of germinating by production of conidia (13) an attempt was made to germinate *G. sorghi* sclerotia.

**Culture-grown Sclerotia**

In a first attempt (July, 1958) 20 sclerotia were picked from a lima bean agar culture; 10 of these were plated on lima bean agar in a petri dish and 10 were placed in a second petri dish on a moist filter paper mat. All dishes were placed in a dark incubator at 28° C. The sclerotia plated on lima bean agar promptly produced mycelial cultures and were discarded. The sclerotia on moist filter paper were scanned under a stereomicroscope every day for 20 days.

On the fifth day after placement on the filter paper, one sclerotium had produced a typical sporodochium, except that it was somewhat more slender than those usually found in culture or on a leaf. Microscopic examination of the spores revealed that they were entirely typical of *G. sorghi*. No other sclerotium in this test germinated in the 20-day period of observation.
In another test, 10 sclerotia were picked from each of 2 fungus isolates growing on lima bean agar. The 10 sclerotia from one isolate were placed in one dish on moist filter paper. After 10 days no sclerotia had germinated.

In a third test involving 25 sclerotia, 4 sclerotia each produced a small sporodochium on the fourth day, 2 on the fifth day, and one on the sixth day.

In a fourth test involving 25 sclerotia 2 sporodochia were produced on the sixth day, and one on the eighth day.

Leaf-borne Sclerotia

Near the first of October, 1958, an experiment was initiated by collecting sorgo leaves heavily diseased with zonate leaf spot from the field. These were brought into the laboratory and from leaf areas densely occupied by sclerotia, discs were punched out with a paper punch. Each leaf disc contained from 50 to 75 sclerotia. The discs were allowed to dry in an open dish in the laboratory for about 6 weeks, after which they were divided into 3 lots for storage at room temperature, 4° Centigrade, and -4° C. in covered dishes. At certain dates thereafter 3 discs were removed from storage at each temperature and placed on moist filter paper in a covered dish in an incubator at 28° C. After placement in the incubator, the discs were scanned under a stereomicroscope every day for 10 days and the number of germinated (sporodochium-bearing) sclerotia recorded. When a sclerotium was found to have germinated, it was destroyed with the point of a needle so that it would not be counted again at a later examination.
The storage periods, running from 1 to 32 days, are indicated in Table III where the results of the experiment are given.

Germination of sclerotia was erratic and only 3 or 4 percent germinated. Most of the germination occurred on the fourth to sixth days after placement of sclerotia on moist filter paper at 28° C. It is doubtful that either storage period or temperature had any effect on germination although a rather dramatic "burst" of germination occurred among sclerotia that had been stored for 18 days. This happened at all storage temperatures. Since all discs had been stored in the laboratory at room temperature for 6 weeks before the experiment was set up, the 18th day can have no significance for discs stored at room temperature. This indicates the 18th day "burst" was either entirely fortuitous or was caused by some uncontrolled and unknown factor.

From storage at room temperature at 4° C., and -4° C. there were respectively 60, 83, and 13 germinated sclerotia in the experiment. Because of the extreme variation in the data it cannot be concluded that freezing inhibited germination but it is clear that it did not enhance germination.

Two sporodochia arising from a culture-grown sclerotium are shown in Figure 8. This photomicrograph was made from a dry mount (no mounting fluid or cover slip) and shows the intact fruiting structure. Illustrations of the sporodochium of G. sorghi found in the literature (4, 16) apparently show only the few spores remaining after most have been washed away by mounting fluids, killing-fixing
Table III. Number of germinated sclerotia from various temperatures and periods of storage on given days of incubation.

<table>
<thead>
<tr>
<th>Days in storage</th>
<th>Temperature storage</th>
<th>1st</th>
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Figure 8. Germinated sclerotium from culture. Two sporodochia are visible, one projecting toward upper left corner and the other toward lower left corner. 110 X
agents, etc. In the intact sporodochium the spore mass typically has the general shape of a cone and at least the peripheral spores seem to be helically wrapped around the cone. Figure 9 shows the spores taken from the sporodochium near the top in Figure 8. The longest spores are found to be roughly twice the length of the sporodochium if the differences in magnification were taken into account.

The sclerotium in Figure 8 is much larger and more irregular than typical leaf-borne sclerotia, and leaf-borne sclerotia have never been observed to produce more than one sporodochium. Possibly the large, irregular sclerotia produced in culture should be regarded as compound structures.

**Effect of Soil Extracts**

In January of 1959, 10 leaf discs bearing sclerotia were incubated at 28° C. for 14 days on filter paper moistened with tap water. Another 10 discs were treated similarly except that the filter paper was moistened with a soil extract. The extract was prepared by stirring a handful of soil in a beaker of water, allowing the soil to settle for several minutes, and pouring off the supernatant which was used as the extract.

The leaf discs were scanned daily under a stereomicroscope and germinated sclerotia were counted.

Sporodochia were produced on 14 sclerotia with tap water and 6 with soil extract. These figures represent about 2.3 and 1.3 percent germination, respectively.

**Discussion**

It has been shown that sclerotia of *G. sorghi* are capable of
Figure 9. *G. sorghi* spores taken from sporodochium at upper left of Figure 8. 480 X
surviving the winter in sorghum leaf trash above ground at Meridian. It has been shown further that leaf-borne sclerotia are capable of germination by production of conidia.

The very first zonate leaf spots of the season, which are found on sorgo in the field at Meridian, are invariably on lowermost leaves, often on leaves bearing rain-splashed soil residues. In brief, the impression is gained that primary inoculum is rain splashed from the soil.

These considerations make it seem likely that the disease cycle normally consists of overwintering of sclerotia on sorghum or Johnson grass leaf debris, spring (or later) germination of sclerotia by conidial production, rain splashing of conidia to lower leaves with infection, fruiting of the fungus, and secondary spread.

The germination rate of sclerotia was very low in the laboratory. Since in nature, at Meridian, overwintered sclerotia would be associated with soil and would have been subjected to low temperatures, these factors might conceivably have induced a higher germination rate. Low temperature storage and soil extracts failed to increase germination in the laboratory, however.

In some seasons zonate leaf spot first appears at Meridian in early June, although more often it does not appear until fall. Even in years when it does show first in the spring, it often disappears when the lowermost leaves senesce and does not reappear until fall. It may be that the very low percent germination of sclerotia obtained in the laboratory was due to some mechanism possessed by the fungus
for spreading germination over a long season in nature.

In view of the temperature relations indicated for the fungus in culture and in the host, it seems likely that the appearance of zonate leaf spot in the spring, its disappearance in the summer and reappearance in the fall, may be explainable as temperature effects.
Luttrell (11) reported zonate leaf spot of pearl millet and made cross inoculation tests of sorghum and millet with sorghum and millet isolates of Gloeocercospora sorghi. A sorghum isolate produced severe infection on sorghum but only slight infection of millet. A millet isolate produced moderate infection on both sorghum and millet.

Experiment I

In 1952 and 1953, 104 isolates of G. sorghi were collected from sorghum and Johnson grass at locations widely scattered over the southeastern states. Ten of these isolates were selected for host specialization tests. Although selections were largely arbitrary, 3 came from Johnson grass and 7 from sorghum, and an attempt was made to include as great diversity of cultural types as possible and to have selections represent widely separated geographic locations.

G. sorghi isolates may produce only mycelium in culture, mycelium and conidia, mycelium and sclerotia, or mycelium, conidia, and sclerotia. Furthermore, some isolates that produce sclerotia do so very quickly, while conidia are still present. Other isolates that produce sclerotia may not do so until conidia have undergone autolysis and disappeared. There are all stages between these 2 extremes and culture medium has an important influence upon the fungus (Figures 10, 11, and 12). However, the concept of "delayed sclerotium production" has some validity and will be used here. In this paper, cultural types will be symbolized as follows:
Figure 10. Isolate 1 on oatmeal agar. Note small sclerotia

Figure 11. Isolate 1 on sorghum juice agar. Note large sclerotia

Figure 12. Isolate 2 on oatmeal agar. Note absence of sclerotia
M = mycelium only
MC = mycelium and conidia
MS = mycelium and sclerotia
MCS = mycelium, conidia, and sclerotia with sclerotia appearing early
MC-S = mycelium, conidia, and sclerotia with sclerotial production delayed

The preceding symbols apply to Difco lima bean agar cultures grown at approximately 28° C.

The following table provides information about the isolates selected:

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</tr>
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Sorgo varieties inoculated were Sart, Wiley, MN 1056, MN 960, MN 1054, MN 2754, MN 1996, MN 87C, Mer. 53-1, and 49-45-2WC. Sart, Wiley, and MN 1056 were known to be susceptible to xonate leaf spot under field conditions. MN 876 had seemed to show slightly less infection from artificial inoculation than most varieties. All the other varieties had received low disease ratings under field conditions at Houma, Louisiana.

Month old sorgo growing in the greenhouse was inoculated in the leaf whorl with a hypodermic needle and syringe. The needle was pushed through the lower part of the rolled leaves just above the growing point.
Inoculum consisted of whole agar cultures, including agar, blended with water in a Waring blender.

Four plants of each sorghum variety were inoculated with each isolate of the fungus; a different needle and syringe was used for each isolate.

The plants were examined frequently over a 2-week period following inoculation.

Results

Sharp differences in pathogenicity of isolates were apparent a few days after inoculation. Isolates 39 and 77 produced more infection than did the other isolates; 13 and 46 were non-pathogenic; 88 was weakly pathogenic; and all other isolates were moderately pathogenic. It was not possible to distinguish severity of reaction among varieties within isolates.

Experiment II

Johnson grass, Sart and MN 960 sorghum, and C. P. 36-111 sugarcane were inoculated with the same 10 isolates used in Experiment I. Plants in 2 pots (with 2 to 6 plants in each pot) were inoculated with each isolate using the needle-and-syringe technique described previously.

Pathogenicities of the isolates on Sart, MN 960, and Johnson grass were relatively the same as in Experiment I. Very little infection occurred on sugarcane with any isolate; the small amount of infection present was confined to the margins of the punctures made by the needle and this did not spread in 3 weeks following inoculation. Three weeks after inoculation, however, G. sorghi was re-isolated from tissues bordering needle punctures.
Experiment III

In the spring of 1956 corn, Sart sorghum, and 3 varieties of pearl millet were inoculated with 3 isolates of G. sorghi, 1 from corn (the same corn isolate used in temperature studies) at Baton Rouge, Louisiana, 1 from pearl millet at Blairsville, Georgia, and 1 from sorgho at Meridian, Mississippi.

One row of each of the 5 test plants was grown across each of 6 greenhouse flats.

Inoculum was prepared by homogenizing whole lima bean agar cultures with water in a Waring blender until it would pass easily through a small atomizer. Two-week-old plants were placed in a mist chamber and sprayed with inoculum. The mist was turned on and the plants left in the chamber for about 28 hours.

Two runs of this same procedure were completed with the same results.

Results

The sorghum isolate produced moderate infection on sorghum but no infection on corn or on any variety of millet. The millet isolate produced light infection on sorghum but no infection on corn or on any variety of millet. The corn isolate produced no infection on any test plant.

Experiment IV

Although MN 960 remains almost free of zonate leaf spot under natural conditions which cause heavy infection of most other varieties, occasional fully developed lesions are found on the variety. Presumably these relatively rare lesions could be the result of infection by a
relatively scarce race of the fungus especially pathogenic on MN 960. The evident failure of the hypothetical race to build up to any noticeable degree over a period of years, could be explained as a result of the very restricted cultivation of MN 960. The variety is not grown commercially.

In the fall of 1956, 2 isolates of the zonate leaf spot fungus from naturally infected MN 960 (1 from Houma, Louisiana, and 1 from Meridian) were compared for pathogenicity on Sart and on MN 960 with 4 isolates from susceptible sorgo varieties.

The experiment consisted of 4 isolates on 2 varieties in 2 replications. A plot in the experiment consisted of a half flat of seedlings. Sart was planted in 1 end and MN 960 in the other end of each of 8 flats. A spore suspension of each isolate was atomized onto seedlings in 2 plots after which the flats were set into a mist chamber and left for about 24 hours. At the time of inoculation, the 2 plots in a flat were separated by a large cardboard partition held in place temporarily to prevent cross contamination. The experiment was conducted in 2 runs; each run compared the 2 MN 960 isolates with 2 of the isolates from susceptible varieties.

Readings on severity of infection were made 48 hours after inoculation. Only 2 levels of infection were distinguishable among the plots and these were designated mild and severe.

Differences in disease rating for a given isolate either between varieties or between replications did not occur. One MN 960 isolate and 1 isolate from a susceptible variety was rated mild; all other isolates were rated severe. There was no evidence that the MN 960 isolates were more pathogenic than the other isolates on MN 960.
Discussion

In this series of experiments were found differences in pathogenicity among fungus isolates, and differences in resistance among test-plant species. There was, however, no evidence of a hosts-by-varieties interaction to indicate host specialization among fungus isolates.
In February of 1960, 10-day-old sorghum seedlings were being screened for zonate leaf spot resistance in a mist chamber in the greenhouse. The plants growing in greenhouse flats were atomized with a spore suspension and placed in the mist chamber around 9:00 a.m. They were removed at 7:00 a.m. the next morning and left on the greenhouse bench until noon when a few leaves were pinched off and taken into the laboratory for examination.

Unsectored pieces of leaf were mounted in a water solution of phloxine B under large cover slips and examined microscopically.

The few fungus spores that were still recognizable and the fungus hyphae on the leaf surface were stained a brilliant red. Healthy leaf tissue was unstained. Cell walls of injured cells were stained. Cytoplasm was brownish in injured cells; chloroplasts in these cells were disintegrated.

Scattered over the leaf surface were small mats or patches of mycelium. In most cases the original spore body which had produced the mat was no longer recognizable. Often, but not always, there were injured cells or patches of cells under or near these mycelial mats. In a few cases mycelial strands were observed growing through open stomata. These strands were unstained below the stomatal aperture and it was not possible to see mycelium in the host tissue.

Under and around all mycelial mats, all stomata were wide open (Figure 13). Stomata not in the immediate vicinity of a concentration of mycelium were tightly closed (Figure 14). The open stomata were not
Figure 13. Open stoma in vicinity of a surface mycelial mat of *G. sorghi* during early stage of pathogenesis.
Figure 14. Closed stoma in area remote from mycelial mat of *G. sorghi*.
confined to regions of visibly injured epidermis; most of them were in regions of what appeared to be perfectly healthy tissues.

Twenty-four hours after the observations just reported, more leaves were collected from the same flats of seedlings and examined by the same technique. Now there was extensive tissue injury, and many visible, but unstained, fungus hyphae in the host tissue. Optical properties of the leaves were poor and it was not possible to determine further details. Sporophores were beginning to form in some stomatal apertures.
TESTING FOR A STOMA-OPENING SECRETION OF GLOEOCERCOSPORA SORGHI

Since Gloeocercospora sorghi is known to penetrate the host through stomata (4), the finding of open stomata around concentrations of hyphae in the initial stages of infection led to the speculation that one aspect of the parasite's mode of attack was the secretion of a substance that opened stomata making penetration easier. Further, it seemed possible that the mechanism of resistance in MN 960 might consist of its ability to resist or nullify the stoma affecting chemical.

**Experiment I**

The purpose of this experiment was to demonstrate, if possible, a stoma-opening chemical in a culture filtrate of *G. sorghi*.

The fungus was grown in an oatmeal broth for 4 days. The broth was passed twice through filter paper; the resulting filtrate was tested against sorgho leaves without dilution.

Young and mature leaves of MN 960 and Sart were cut into sections about 1 x 2 cm. and floated on the culture filtrate and on distilled water in covered petri dishes.

Sections were examined microscopically every half hour until 3 examinations had been made and then every hour until 3 more examinations had been made. Almost all stomata were closed and there were no apparent differences between controls and filtrate treated leaves.

**Experiment II**

For this experiment a culture filtrate was obtained in the same way as in Experiment I. The undiluted filtrate was atomized onto young
Sant seedlings in the laboratory. Similar seedlings were atomized with tap water as controls.

The first leaves were removed for microscopic examination 15 minutes after the plants were sprayed. Leaves were removed for subsequent examinations at hourly intervals until 4 examinations had been made.

Most of the stomata were closed and there was no apparent difference between plants sprayed with culture filtrate and those sprayed with water.
DEVELOPMENT OF RESISTANCE WITH TISSUE MATURITY

Before maturity (heading) sorghum plants have a built-in moist chamber (the so-called cornucopia or leaf whorl) i.e., the cup formed by the bases of the blades of the partially expanded leaves. In all attempts to develop a field method of inoculation, inoculum in some form was applied to the open leaf whorl in order to take advantage of this moist-chamber effect. When infection occurred in these tests, it was always on the young leaves. Natural infection, in all cases noted, began toward the bottom of plants on the older leaves and then progressed upward. MN 960's resistance was originally discovered under conditions of natural infection and had not withstood artificial inoculation. When small plants were inoculated in the greenhouse, all leaves were immature.

These considerations suggested the possibility that young leaf tissues of all varieties are susceptible to infection but that resistance develops with maturity of leaf tissues in certain varieties such as MN 960.

In order to test this hypothesis, it is necessary to grow plants long enough so that there are some mature leaves on the plants. Such leaves must remain free of spots and blemishes except in inoculated areas so that it is possible to assume with reasonable certainty that any spots produced are in fact zonate leaf spot. This has proved extremely difficult. Several field and greenhouse tests were abandoned and several were impossible to interpret because of extraneous spots due either to parasitic disease, insecticidal spray injury or unknown causes.
Experiment I

Two field plots of Sart and 2 of MN 960 were available for this test.

The plan was to inoculate upper, middle, and lower leaves of plants of both varieties with a spore suspension, and then wrap those leaves with a plastic wrap (Saran wrap) to prevent drying before infection could occur. It was considered necessary to wait until near sundown to apply the treatments to prevent heat building up excessively under the plastic wrap.

Due to the great difficulty of handling the plastic wrap (2 hands were not enough) only 2 plants, 1 Sart and 1 MN 960, were inoculated before it became too dark to work. Three leaves, young, intermediate, and mature were inoculated on each plant. The mature leaf was not as old as desired but older leaves were already spotted and unusable.

Thirty-six hours after inoculation it was apparent that infection had occurred and that it was lighter on the mature MN 960 leaf than elsewhere. On the sixth day after inoculation the inoculated Sart plant was discovered to have been broken over during the previous night by a trespassing cow. The inoculated leaves from both plants were detached and taken to the laboratory where lesion lengths were measured.

A straight edge was moved down a leaf over the inoculated area and each time the edge became tangent to a spot, that spot was measured. This was continued until 10 spots on each leaf had been measured.
Results of these measurements are given in Table IV. Also shown in the table are the number of lesions in the inoculated areas of the leaves.

It is apparent that the mature MN 960 leaf had much fewer and smaller lesions than the mature Sart leaf while there was little difference between varieties in number or size of lesions on the young leaves. The intermediate MN 960 leaf was intermediate in size and number of lesions between the young and mature MN 960 leaf while the intermediate Sart leaf was not noticeably different from either the young or old Sart leaf. There may have been a slight real increase in lesion size on the old Sart leaf over the younger Sart leaves but the data are too meager to be taken seriously.

**Experiment II**

In July of 1962, 2 young (incompletely expanded) and 2 mature (fifth leaf from youngest visible leaf) leaves were detached from Sart and from MN 960 plants growing in the field. These leaves were intermediately placed upright in large glass cylinders (Figure 15). The bases of the blades stood in about an inch of water. Each leaf occupied a separate cylinder.

In the laboratory the leaves were removed from their cylinders, atomized with a *G. sorghi* spore suspension and returned to the cylinders which were then covered with petri dish lids.

Twenty-four hours later there were abundant watersoaked spots on all leaves except the 2 mature MN 960 leaves; there were 3 spots on 1 of these leaves and none on the other. Thirty-two hours after inoculation the mature Sart leaf was badly watersoaked and necrotic.
Table IV. Length (mm.) of zonate leaf spot lesions on Sart and MN 960 leaves of 3 different ages, 6 days after inoculation

<table>
<thead>
<tr>
<th>Lesion number</th>
<th>Young leaf</th>
<th>Intermediate leaf</th>
<th>Mature leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sart</td>
<td>MN 960 (120)</td>
<td>Sart</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>21</td>
<td>27</td>
</tr>
<tr>
<td>7</td>
<td>26</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>19</td>
<td>26</td>
</tr>
<tr>
<td>9</td>
<td>24</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>24.9</td>
<td><strong>18.8</strong></td>
<td><strong>23.1</strong></td>
</tr>
</tbody>
</table>

1/. Numbers in parentheses give the total number of lesions in the inoculated area of the leaf.
Figure 15. Method of incubating detached, inoculated sorgo leaves
The young leaves of both varieties were heavily infected but holding up better than the mature Sart leaf. The mature MN 960 leaves continued to show few or no lesions.

Forty-eight hours after inoculation, all leaves were deteriorating and the experiment was ended. The mature MN 960 leaves were in much better condition than the other leaves and their deterioration was not due to *G. sorghi* attack; they were becoming yellow streaked.

**Experiment III**

This experiment was similar to Experiment II except that MN 1054 was substituted for MN 960, uninoculated checks were added, and 2 leaves were placed in each cylinder. This resulted in a total of 8 cylinders, i.e. 2 varieties x 2 leaf ages x 2 inoculation treatments (inoculated and control).

Infection and deterioration of leaves progressed at about the same rate as in the preceding experiment and the results were similar.

Lesion counts were made 30 hours after inoculation. When the number of lesions per leaf was greater than 200 the result was tabulated simply as 200+.

All inoculated leaves in the experiment had in excess of 200 lesions except the 2 mature leaves of MN 1054, 1 of which had 6 and the other 9 lesions.

**Experiment IV**

In the previous tests with detached leaf sections, the tissues deteriorated so rapidly that only the very early stages of lesion formation could be observed. In the next test of this series, an
attempt was made to prolong the life of the leaf sections. A further objective was to reduce the size of the leaf sections and their containers with a view toward the development of a laboratory technique for screening segregating populations for resistance of the MN 960 type.

Leaf sections measuring about 25 x 75 mm. were taken from young and mature leaves of Sart and MN 960 plants growing in the greenhouse. These leaf sections were floated on tap water or 1 percent sucrose in tap water in petri dishes. Inoculation was accomplished by placing a loopful of spore suspension on each section. Uninoculated controls of each variety for each leaf age and substrate were also prepared. One leaf section occupied 1 dish. There were 2 replications of all treatments.

Eighteen hours after inoculation small watersoaked lesions had appeared at the inoculation site of several of the sections but the infection bore no apparent relationship to treatment or variety. Only 2 treatments showed no infection (mature MN 960 on 1 percent sucrose, and young Sart on tap water) and only 2 treatments showed infection in both replications (young Sart on 1 percent sucrose, and mature Sart on 1 percent sucrose). In all other treatments 1 replication showed infection and the other did not.

The sucrose solutions did not prolong the life of the leaf sections, and with or without sucrose, deterioration of these small sections was more rapid than in previous tests with large sections.

**Experiment V**

Ordinarily sorgo plants meeting the requirements for experiments involving young and mature leaf tissues on the same plant cannot be
grown in the greenhouse during the season of the year when temperatures in the greenhouse are low enough for zonate leaf spot work. At those seasons, the plants head so quickly that a reasonable spread in leaf age does not occur. In the present experiment that obstacle was removed by keeping the plants under a flood light at night. It had been determined previously that sorghum plants could be grown indefinitely without heading by this method.

In February and March of 1965, Sart and MN 960 plants in clay pots were grown to a height of about 3 feet at which time they had about 9 visible leaves. The plants were sprayed thoroughly with homogenized, whole-culture inoculum and then placed in a mist chamber for approximately 20 hours. After that they were placed on the greenhouse bench and observed for a period of about 3 weeks. This experiment was performed twice using 2 Sart and 2 MN 960 plants at each run.

Upon removal from the mist chamber, 20 hours after inoculation, young leaf tissues of both varieties bore many small water-soaked spots. On MN 960 these spots were present on 4 or 5 leaves down from the youngest visible leaf tip. On Sart they were present on all leaves on the plant. Within 5 or 6 hours after removal from the mist chamber, a few very small spots appeared on lower (old) leaves of MN 960. By this time most spots on all parts of both plants had lost their water-soaked appearance and were becoming pigmented (dark red) on Sart, merely dark on MN 960 apparently as a result of necrosis, not of pigment formation. (This difference in color reaction to injury between the 2 varieties was discussed on page 12).
Three days after inoculation, lesions on young leaves of Sart and MN 960 were essentially alike except for color, whereas on mature leaves, Sart lesions were much larger than MN 960 lesions (Figure 1b). Seven days after inoculation, Sart lesions which had originally appeared on young leaves were about the same size as lesions which had originally appeared on mature leaves, and lesions in both situations had expanded considerably. On MN 960, however, lesions which had originally appeared on old leaves had undergone practically no enlargement, whereas lesions which had originally appeared on young leaves had enlarged only slightly (Figure 17). By this time the leaf which was the youngest on the plant at the time of inoculation was now about sixth in order from the youngest visible leaf on the plant. The lesion size illustrated in Figure 17 did not increase further on either variety in the next 2 weeks after which the plants were discarded.

Discussion

The evidence obtained in this series of experiments seemed to indicate that both young and mature leaf tissues of Sart were susceptible to zonate leaf spot whereas only young tissues of MN 960 were susceptible. The small amount of lesion development (increase in lesion size) which has been obtained on MN 960 by artificial inoculation has been obtained only by inoculation of young leaf tissues. A much greater development was obtained by inoculation of either young or mature tissues of Sart.

Results of these studies, however, were not entirely satisfactory. In the first place, a few well developed, zonate lesions have been observed on MN 960 in nature, and secondly, large, well developed zonate
Figure 16. Sart and MN 960 leaves 3 days after inoculation with G. sorghi. Left to right: Young Sart, young MN 960, mature Sart, mature MN 960. Approximately life size.
Figure 17. Sart (left) and MN 960 (right) 7 days after inoculation with G. sorghi. Approximately life size.
lesions have seldom been produced on Sart by artificial inoculation and never in experiments designed to study resistance in relation to tissue age. These facts leave the issue somewhat clouded.

Young inoculated tissues of MN 960 will not remain young while one waits to determine the final extent of lesion development on young tissues. Therefore, there seems to be no way of determining how much of MN 960's resistance is due to aging of tissues and how much is due to resistance present even in young tissues. Furthermore the failure to get normal lesion development even on Sart raises a question about the meaning of the observations on MN 960.

For a long time, failure to separate zonate leaf spot resistance of the MN 960 type from susceptibility of the Sart type was regarded as due to a breakdown of MN 960 resistance under artificial inoculation. But failure to get full lesion development on Sart makes it equally reasonable to regard the failure as due to unnatural expression of resistance in Sart under artificial inoculation.

Needed are experiments carried out under carefully controlled environments to determine what conditions permit development of typical zonate leaf spot lesions on susceptible varieties. Once this were established, inoculations of young and mature tissues of resistant and susceptible varieties might lead to clarification, especially if a really wide spread in tissue age on the same plant could be obtained. The spread in tissue age which occurs in the field on large plants has not been utilized in the experiments reported here.

In spite of failure to obtain completely adequate confirmation
of the mature-tissue-resistance hypothesis, some evidence to support it has been obtained. Mature-tissue resistance would account for the apparent high resistance of MN 960 under field conditions, and the failure of all inoculation techniques tested for resistance screening to give the desired results. In nature, the first zonate leaf spots develop on the lowermost (mature) leaves on a plant and progresses upward. If these lower leaves are resistant, as suggested for MN 960, inoculum probably would seldom reach susceptible young leaf tissues. When zonate leaf spots have been found on MN 960, the variety has been growing in plots surrounded by susceptible varieties. It is therefore possible that inoculum splashed to young MN 960 tissues from another variety.

Mature tissue resistance could also explain how it was possible to study the inheritance of resistance to zonate leaf spot (6). In that experiment both resistant and susceptible parents showed heavy initial infection at the inoculation site. However, for some reason (presumably favorable weather), there was much secondary spread of the disease that year and by harvest time, it was possible to distinguish between resistant and susceptible plants.
NEW RECORDS OF ZONATE LEAF SPOT

From 1952 to 1959 detailed disease notes were made each year in 13 to 20 sorghum variety tests distributed throughout the southeastern United States. In the course of these tours, commercial plantings of sorghum encountered along the way were also examined for the presence of disease and frequent stops were made to examine Johnson grass growing along roadsides. These surveys and other travel have revealed zonate leaf spot in several states from whence it has not previously been reported. The disease was observed in Texas, Arkansas, Louisiana, Mississippi, Tennessee, Alabama, Kentucky, Georgia, South Carolina, Florida, Oklahoma, and Maryland. So far as has been determined by a survey of the literature, C. sorghi has not previously been reported from Arkansas, Alabama, Kentucky, Tennessee, South Carolina, and Maryland.

I have found zonate leaf spot in every state in which I have had an opportunity to look for it and there is good reason to believe that it occurs in many others.
HANDLING THE FUNGUS IN CULTURE

In the course of the work reported in the foregoing, the growth of *G. sorghi* was compared on several culture media; the best sporulation occurred on oatmeal and lima bean agars as reported by Bain (3). Good sporulation was also obtained on Difco bean pod agar. Sorghum juice agar was notable for the robust sclerotia it produced (Figure 11), but sporulation did not occur. Sporulation was absent or very slight on potato dextrose, corn meal, malt extract, and prune agars. Difco lima bean and oatmeal agars were adopted for routine culturing and inoculum increase.

Considerable difficulty was experienced in maintaining sporulation of isolates. Sporulation was maintained without loss of vigor for many transfers provided fresh spores were transferred at each passage. However, on oatmeal agar, peak spore production was reached in a few days and spores began to lyse within a few days more. Maintaining sporulation on oatmeal agar requires more frequent transfer than on lima bean agar because spores remain viable longer on lima bean agar. Recently it has been learned that half strength oatmeal agar is similar in this respect to lima bean agar.

Cell contents and septa of fresh *G. sorghi* spores are difficult to discern in water mounts under any magnification including oil immersion and the spores do not easily take up cotton blue stain. The cytoplasm of older spores takes up cotton blue and the septa are sharply delineated. Still later, the cytoplasm seems to break up into droplets, and again becomes difficult to stain with cotton blue.

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Septa are no longer apparent (Figure 18). Soon after this stage the spore walls collapse, and finally spores cannot be found. On full strength oatmeal agar, if fresh spores are seeded heavily over the entire agar surface, peak sporulation may be reached in 3 to 4 days and spore lysis begins in 5 or 6 days.

Most isolates of *G. sorghii* from nature have the ability to produce spores and sclerotia. This ability may be lost quickly in culture and is usually lost after several passages if cultures are allowed to become old before transfers are made. In the work reported here, the usual pattern was loss of sporulating ability first and then loss of ability to produce sclerotia. Frequently, the next step was loss of the isolate because, non-sclerotial colonies live no longer than a few weeks without transfer. An unsuccessful attempt was made to prolong the life of a strictly mycelial colony by storage under sterile mineral oil in the refrigerator. The culture failed to survive 4 weeks.

On several occasions, mycelial isolates were compared with spore-bearing and sclerotium-bearing isolates for pathogenicity on sorgo. If homogenized whole agar cultures were used for inoculum, there was no evidence of a loss of pathogenicity associated with loss of ability to produce spores or sclerotia. Upon re-isolation from the host tissues, the mycelial isolates were still mycelial only.

The best way discovered for maintaining isolates without loss of ability to produce all organs was by storage of sclerotia. This could be done either by storing leaf sections bearing sclerotia
Figure 18. Spores from week-old culture of *G. sorghii* on oatmeal agar. Stained cytoplasm (cotton blue) reveals location of septa in 1 spore. Other spores in advanced stages of lysis.
(Figure 19) or sclerotia produced in culture. If the leaf method was chosen, a culture could be started at any time by surface sterilizing leaf fragments briefly in Clorox and plating on agar. When the culture method was used, sclerotium-bearing colonies were simply allowed to dry up completely at room temperature. To re-establish a colony a fragment of dried agar bearing sclerotia was plated on oatmeal or lima bean agar. By both methods, sclerotia were found viable after 12 months storage. Sclerotia stored at room temperature for 5 years failed to produce growth when plated on oatmeal agar. No intermediate periods were tried.
Figure 19. Sclerotia of *G. sorghi* in sorghum leaf. 20x.
SINGLE SPORE CULTURES

Spores of *G. sorghi* are multicellular and upon germination, several cells of a spore produce germ tubes. Nothing is known about the nuclear condition of the fungus. I thought it might be interesting to know how much variation could be found among single spore isolates established from a single colony.

In the fall of 1955, 2 isolates were chosen. One of these, Isolate A, produced both spores and sclerotia in culture; the other, Isolate B, produced only spores.

Suspensions of spores in sterile water were washed over the surface of 4 percent water agar in petri plates; the spores were picked from this surface by means of a fine glass needle formed into a ball at the tip. The work was done under a stereomicroscope at a magnification of about 90x. An attempt was made to place 9 single spores in each plate of oatmeal agar. From 6 to 9 colonies grew on each plate indicating probably that some spores did not detach from the needle. About 180 single spore colonies were eventually established from each of the original isolates, A and B.

All colonies resulting from spores of Isolate A produced abundant spores and sclerotia; all colonies from spores of Isolate B produced abundant conidia and no sclerotia. (See Figure 20).
Figure 20. Single spore colonies of *G. sorghi*
Left: from sclerotium-producing isolate
Right: from conidium-producing (but non-sclerotium-producing) isolate
SUMMARY

1. Field-resistant and field-susceptible sorghum varieties reacted similarly to several methods of inoculation with Gloeosporium sorghi.

2. Screening a total of 1509 sorghum importations failed to reveal any with a type of resistance that would withstand artificial inoculation.

3. Attempts to screen seedlings for zonate leaf spot resistance by a rag doll or dish technique failed because of inability to control "weed" fungi.

4. Optimum temperature for growth of the zonate leaf spot fungus in culture and for the initial stages of pathogenesis was about 80° F.

5. G. sorghi sclerotia overwintered in sorghum leaf debris. Sclerotia germinated by conidial production.

6. Differences in pathogenicity among G. sorghi isolates were found but evidence of host specialization was lacking.

7. At least part of the resistance of certain sorghum varieties appeared to develop with maturation of leaf tissues.

8. During initial stages of infection, mycelial mats formed on host epidermis. Stomata near these mats were open whereas stomata not near the mats were closed. Attempts to demonstrate a stoma-opening chemical in culture filtrates of the fungus were not successful.

9. Zonate leaf spot of sorghum was found in 6 states from which it had not previously been reported.
10. **Maintaining isolates of the zonate leaf spot fungus without change in cultural characters was best accomplished by storage of dried sclerotia up to one year.**

11. **G. sorghi** colonies established from single spores were all culturally identical with their parent isolates.
LITERATURE CITED


VITA

Jack Lemuel Dean was born in Haskell County, near Keota, Oklahoma, on March 15, 1925. He finished high school at Stigler, Oklahoma, in 1943 and went into the U. S. Navy. For the next 2 years he attended classes at Tulane University in New Orleans, Louisiana. He was discharged from the Navy in 1946 and entered Oklahoma State University where he was granted a B.S. Degree in 1949 and an M.S. Degree in 1952. Toward the end of 1951 he went to work for the U. S. Department of Agriculture at the U. S. Sugar Crops Field Station, Meridian, Mississippi, where he is presently employed. He is a candidate for the Ph. D. Degree in May, 1965.
EXAMINATION AND THESIS REPORT

Candidate: Jack Lemuel Dean

Major Field: Plant Pathology

Title of Thesis: Zonate Leaf Spot of Sorghum

Approved:

[Signatures]

Date of Examination:

May 6, 1965