Technical Bulletin 133 December 1938

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CONTENTS

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LEON J. TYLER

THE covered kernel smut, *Sphacelotheca sorghi* (Link) Clinton, is coextensive with the culture of sorghums and is considered the most destructive disease to which they are susceptible (18, 35, 36). It constitutes one of the major problems in production of sorghums in the Southern Great Plains area of the United States (18).

The sorghums as a group vary greatly in susceptibility to the covered kernel smut fungus (29, 30, 33, 35, 36). Varietal experiments with sorghums, in the years 1915 to 1921, inclusive, showed that milo, feterita, and hegari remained almost free from the disease (36). But in 1926, Tisdale, Melchers, and Clemmer (46) reported the occurrence of covered kernel smut in fields of milo and hegari. Their observation suggested that there might be pathogenic differences between strains of the kernel smut organism, and by 1927 they had demonstrated the presence of two, probably three, parasitic or pathogenic strains of *Sphacelotheca sorghi* in nature (45). Ficke and Johnston (18) concluded that the above-mentioned strains could be differentiated on the basis of cultural characters when grown on artificial culture media. Later Melchers, Ficke, and Johnston (31) differentiated two additional parasitic strains of *S. sorghi.*

Tisdale *et al.* (45) suggested that the smut previously reported by them on milo and hegari was a strain of *Sphacelotheca sorghi;* that it might be a hybrid between *S. sorghi* and *S. cruenta*; or that it might be "an intermediate in a series of variants ranging from *S. sorghi* as one extreme to *S. cruenta* as the other." In 1932 Rodenhiser (40) demonstrated that strains of *S. sorghi* and *S. cruenta* differing in pathogenicity could be accounted for, in part at least, through inter- and intra-specific hybridization. In a later report, Rodenhiser (41) definitely proved that physiologic races can arise through inter- and intra-specific hybridization.

The tendency of monosporidial lines to produce sector variants on nutrient agar was reported by Isenbeck (27), and Rodenhiser found that cultures of some monosporidial lines from both inter- and intra-specific hybrid chlamydospores mutated frequently while others remained stable (41).

[1] Presented to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Degree granted June, 1934.

The writer gratefully acknowledges his indebtedness to Dr. E. C. Stakman for suggesting the problem, for his supervision and criticism during the course of investigation, and for his aid in preparation of the report. He also expresses his appreciation for advice and aid from the following people: Dr. J. J. Christensen, Dr. Helen Hart, Dr. E. L. LeClerg, Mr. George E. Hafstad, Mrs. Gertrude M. Tyler, and Mr. Thomas W. Graham.

It is clear from this brief review that there is considerable variation in the loose and covered kernel smuts of sorghum, but it would be desirable to know more about the extent and the cause of the variation. The writer therefore undertook to elucidate some of the basic principles underlying the causes of variability in *Sphacelotheca sorghi* (Link) Clinton.

MATERIALS AND METHODS

The original chlamydospore material used in the following work was collected at Amarillo, Texas, and sent to the Division of Plant Pathology at the University of Minnesota in 1929. Subsequent spore material was obtained by crossing monosporidial lines isolated from the Texas material. The origin of all chlamydospores from which sporidia were isolated is shown in Table 1.

Table 1. Origin of Chlamydospores Whose Sporidial Progeny Were Studied

Chlamydospore	Where obtained
Texas A	Smut collection from Amarillo, Texas, obtained in 1929
Texas B	" " " " " " " "
Texas C	" " " " " " " "
Texas D	" " " " " " " "
Texas H	" " " " " " " "
8A*	Texas A1 × Texas $A4_2$
122B*	Texas $B2_1$ × Texas $C2_3$
22A*	Texas A3 × Texas $A3_1$
22B*	Texas A3 × Texas $A3_1$

* Chlamydospore collection produced in greenhouse by pairing monosporidial lines.

All cultures used were of monosporidial origin, isolated by methods described by Dickinson (13) and Hanna (21). When the single sporidial isolations were made, each chlamydospore was designated by a capital letter, and the individual sporidia were numbered according to their respective positions on the promycelium, beginning at the apex. For example, a culture labeled Texas A1 or Texas A2 originated from cell No. 1 and cell No. 2, respectively, of the promycelium of chlamydospore A of the Texas collection. When successively produced sporidia were isolated from one cell of the same promycelium, they were designated by subnumerals. For example, Texas $A1_1$ refers to a culture obtained from the second sporidium isolated from cell No. 1 of chlamydospore A of the Texas smut. Sectors were designated by numerals enclosed in parenthesis following the original nomenclature of the culture. Thus, culture Texas $A1_1(-1)$ originated as a sector in culture Texas $A1_1$. If a sector appeared in culture Texas $A1_1(-1)$, it would be labeled Texas $A1_1(-1-1)$, and so on. The two cultures, Texas $A1_1(-1)$ and $A1_1(-1-1)$, would be recognized as first and second order sectors, respectively.

The nutrient medium usually used for studying cultural characters was potato-dextrose agar (390 grams potatoes, 10 grams dextrose, 15 grams agar per liter). To induce sectoring, malt-dextrose agar (2 per

cent malt, 1 per cent dextrose, and 1.5 per cent agar) was used. Other nutrient agar media also were used and will be mentioned in connection with certain experiments.

Whenever different smut lines were grown for comparison or differentiation, the nutrient medium was prepared in one batch. Equal amounts were poured into 250 cc. Erlenmeyer flasks, then all were autoclaved simultaneously. The steam pressure was held at 15 pounds for 20 minutes, after which the flasks were allowed to cool at room temperature and inoculated when the surface of the agar became visibly dry. The smut colonies were always grown approximately three weeks before notes were taken.

Minnesota Amber, one of the common sorgo varieties known to be very susceptible to *Sphacelotheca sorghi,* was used for inoculation studies. All seed was soaked in 1:240 formaldehyde solution for 45 minutes, then thoroughly washed in running water and dried. Three methods of inoculation were used: (a) Disinfected sorghum seed was inoculated with dry chlamydospores or with a water suspension of chlamydospores; (b) 24- to 36-hour-old seedlings were inoculated in Petri dishes with paired and non-paired monosporidial lines and after 36 hours were transplanted either to the field or to pots in the greenhouse; (c) seedlings, three to five weeks old (depending on whether they were grown in the field or greenhouse), were inoculated by injecting sporidia into them by means of a hypodermic syringe. The sporidia were obtained by growing each sporidial line for 7 to 12 days in flasks containing approximately 75 cc. of boiled-potato water containing one per cent dextrose. Before inoculating plants, the sporidial suspensions were mixed in any desired combination of lines and also used singly. Effort was made to strike the growing point of the plants with the needle point of the syringe to insure infection in the primary heads.

EXPERIMENTAL RESULTS

Cytology of Germinating Chlamydospores and Sporidia

Brefeld (6), Norton (32), Clinton (11), Butler (8), and Kulkarni (28) have figured germinating chlamydospores of *Sphacelotheca sorghi* but have not described the nuclear phenomena.

Chlamydospores of *Sphacelotheca sorghi* germinate readily on potato-dextrose agar and on malt agar, making it possible to follow the histological technique described by Hanna (22). Fixations were made at intervals of three, six, and nine hours. Figure 1, stages 1-22, illustrates the various stages of germination on potato-dextrose agar; stages 23-29 illustrate so-called abnormal types on malt agar. No such abnormalities were noted in material germinated on potato-dextrose agar. Figure 1, 1-5, shows the swelling of the protoplast, which finally pushes through the spore wall to form the elongated promycelium. The nucleus usually divides within the spore, one nucleus moving into the promycelium and

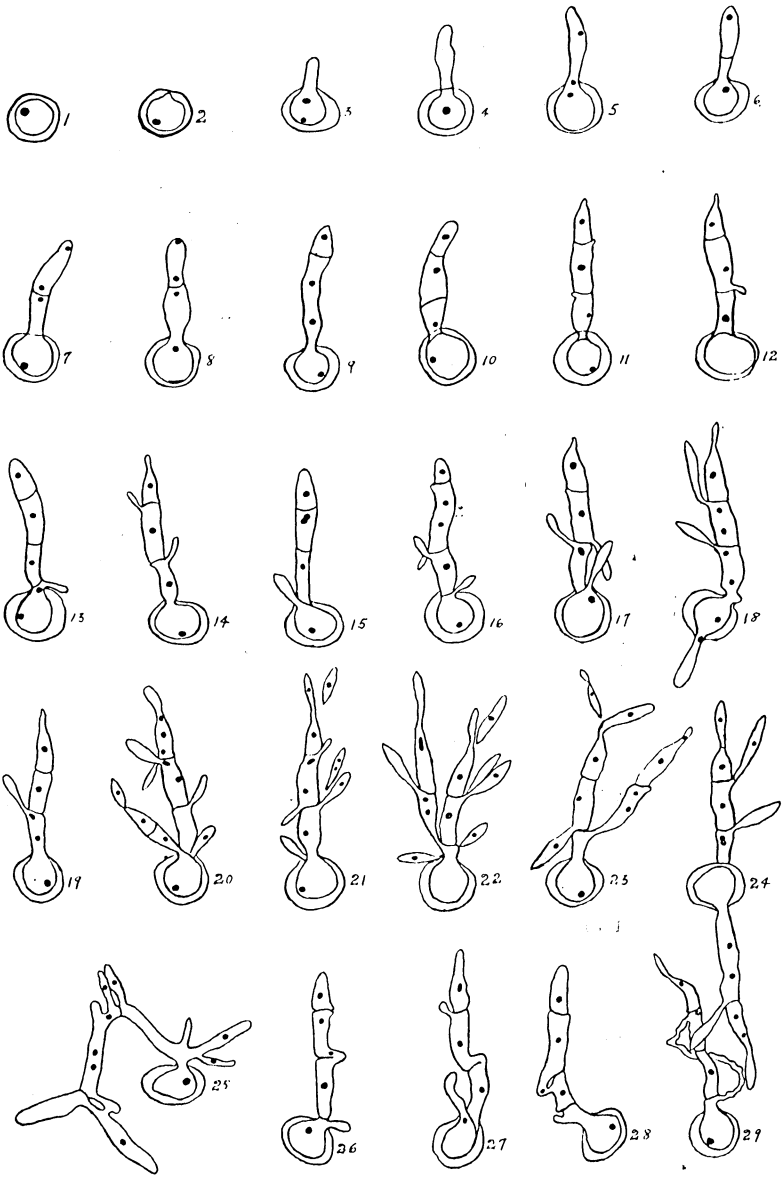

FIG. 1. STAGES IN THE GERMINATION OF CHLAMYDOSPORES OF SPHACELOTHECA SORGHI

Stages 1 to 22, inclusive, illustrate the development of promycelia, the nuclear condition, the formation of septa, and the subsequent production of sporidia and their nuclear condition. Stages 23 to 29, inclusive, illustrate usual although somewhat abnormal types of germination as well as the nuclear phenomena involved.

the other usually remaining within the spore. Figure 1, 6-10, illustrates the formation of septa, the first of which usually is formed between the nucleus of the promycelium and the nucleus of the spore. The nuclei again divide, so that a pair of nuclei appears on each side of the original septum. These pairs of nuclei are usually separated by walls, as in 10. Sometimes only the apical nucleus is walled off, leaving three nuclei about equidistant in the remainder of the promycelium; or the nucleus remaining in the spore may be walled off first. In most of the promycelia observed two septa were formed, but in some there were three. The formation of only two septa results in a three-celled promycelium, so that sporidia arising from the fourth cell seemingly arise from the spore itself at the base of the promycelium, as in Figure 1, stages 15, 16, 17, 20, and 21, or on a separate promycelium-like structure arising from the side of the spore opposite the original promycelium, as in 18 and 24.

Sporidia may bud off at either end of the promycelial segments and sometimes cross each other in their development, as in Figure 1, stages 16 and 20. As the sporidia are formed, the nuclei within promycelial cells divide and one of the resulting daughter nuclei moves into the developing sporidium. At maturity the sporidia become detached, as in 21 and 22. Such sporidia are usually uninucleate. As the sporidia grow and prepare to bud, they may become binucleate and some may even become multinucleate.

From this study it is apparent that cytological phenomena in *Sphacelotheca sorghi* are similar to those in *Sorosporium reilianum* and *Ustilago zeae,* as shown by Hanna (22) and Christensen (9), respectively, and in other smuts of this type, as shown by other investigators. Briefly, the nuclear changes accompanying germination in *Sphacelotheca sorghi* are as follows. The chlamydospore is a zygote containing a diploid nucleus which divides when the promycelium is forming. The division may be either equational or reductional; in any case two nuclei are formed which are separated by a wall. The two nuclei again divide, and each pair of nuclei are again parted by a septum. If reduction division has taken place in either of the two nuclear divisions, the resulting four nuclei will be haploid and sporidia developing from the four haploid promycelial cells will likewise be haploid.

The tendency of the fungus to initiate the dicaryophase quickly (Fig. 1, 25-29) is illustrated by the fusion of sporidia while attached to the promycelium, by the formation of buckle joints with nuclei passing from one cell to the other, and by the formation of long fusion tubes uniting distant cells of the same promycelium.

In studies on sex in *Sphacelotheca sorghi,* Rodenhiser (40) found that the sporidia from a single promycelium belonged to two sex groups, with ratios of 2:2 and 1:3. Figure 1, stage 9, illustrates how a sex ratio of 1:3 could result. After reduction of the fusion nucleus had occurred, one of the resulting pair of nuclei might be walled off at the apex, leaving the other to provide nuclei for the remaining three promycelial cells;

Table 2. Results of Pairing Monosporidial Lines of Sphacelotheca sorghi Derived by Isolating Primary, Secondary, and Tertiary Sporidia from the Promycelia of Nine Germinated Chlamydospores*

Texas A

	A1	A2	A3	A4	$A1_1$	$A3_1$	$A4_1$	$A4_2$
A1	–	+	–	+	–	+	+	+
A2	+	–	+	–	+	–	–	–
A3	–	+	–	+	–	+	+	+
A4	+	–	+	–	+	–	–	–
$A1_1$	–	+	–	+	–	+	+	+
$A3_1$	+	–	+	–	+	–	–	–
$A4_1$	+	–	+	–	+	–	–	–
$A4_2$	+	–	+	–	+	–	–	–

Texas B

	$B1_1$	$B1_2$	B2	$B2_1$	B3
$B1_1$	–	–	+	+	–
$B1_2$	–	–	+	+	–
B2	+	+	–	–	–
$B2_1$	+	+	–	–	+
B3	–	–	–	+	–

Texas C

	$C1_1$	C2	C3	C4
$C1_1$	–	–	+	+
C2	–	–	+	+
C3	+	+	–	–
C4	+	+	–	–

Texas B × Texas C

	$C1_2$	C2	C3	C4	$C2_1$	$C3_1$	$C4_1$	$C2_3$	$C3_2$
$B1_1$	–	–	+	+	–	+	+	–	+
$B1_2$	–	–	+	+	–	+	+	–	+
$B2_1$	+	+	–	–	+	–	–	+	–
B3	–	–	+	+	–	+	+	–	+
$C1_2$	–	–	+	+	–	+	+	–	+

Texas D

	D1	D2	D3	$D4_1$
D1	–	–	+	+
D2	–	–	+	+
D3	+	+	–	–
$D4_1$	+	+	–	–

Texas H

	H1	H2	H3	H4
H1	–	–	+	+
H2	–	–	+	+
H3	+	+	–	–
H4	+	+	–	–

8A

	A1	A2	A3	A4
A1	–	–	+	+
A2	–	–	+	+
A3	+	+	–	–
A4	+	+	–	–

122B

	B1	B2	B3	B4
B1	–	+	+	+
B2	+	–	–	–
B3	+	–	–	–
B4	+	–	–	–

22A

	A1	A2	A3	A4
A1	–	–	+	+
A2	–	–	+	+
A3	+	+	–	–
A4	+	+	–	–

22B

	B1	B2	B3	B4
B1	–	+	–	+
B2	+	–	+	–
B3	–	+	–	+
B4	+	–	+	–

122B × 22A

	B1	B2	B3	B4
A1	+	–	–	–
A2	+	–	–	–
A3	–	+	+	+
A4	–	+	+	+

* Sex reaction of monosporidial lines determined by means of four methods described previously.

thus the latter three cells would normally produce sporidia of similar sex. In chlamydospore 122B (Table 2) such a phenomenon may have occurred, since the four sporidia were isolated from a single, tri-septate, normal-appearing promycelium.

From the fact that in many instances a septum is lacking between cells 3 and 4 of the promycelium, as in Figure 1, stages 18, 19, 21, 22, and 23, a seeming sex reversion in sporidia could take place. For example, in 18 let us suppose that reduction occurred in the second nuclear division. Two non-sister nuclei would be left in the lower part of the promycelium without a wall between them. Through protoplasmic movement it would be possible for the nuclei to change their positions. Through changes in position the haphazard distribution of sexually different nuclei to primary, secondary, and tertiary sporidia isolated from the basal cell of the promycelium might be expected. Possibly such a phenomenon may have occurred in Texas A (Table 2), as two sporidia, A3 and $A3_1$, from the same promycelial cell were of opposite sex.

The cytological details concerned in chlamydospore germination in *Sphacelotheca sorghi,* and illustrated here, agree essentially with those described by Rodenhiser (41). It seems clear, however, that some of the phenomena described later are probably due to irregularities in nuclear behavior such as those just described.

Genetics

Methods for Determining Sex

In genetic studies on *Sphacelotheca sorghi* it is important to know the sexual capabilities of monosporidial lines, since paired lines of opposite sex are prerequisite to normal infection and production of chlamydospores in the host. There are at present at least four methods for determining sex in this smut, two of which have been described by Rodenhiser and are corroborated here. In addition, the writer has adapted two others for use with *S. sorghi* (47).

Chlamydospore Production

Rodenhiser (40) inoculated sorghum plants, grown in the greenhouse, with paired and non-paired monosporidial lines of *Sphacelotheca sorghi* by means of hypodermic injection. Plants inoculated with certain combinations of unisexual lines produced chlamydospores, while other combinations produced none. In no case did plants inoculated with single lines produce chlamydospores. From this he was able to ascertain the sex of certain monosporidial lines and concluded that the organism is heterothallic. The writer also has applied the chlamydospore test to lines of *S. sorghi* and obtained similar results. The technique used was essentially the same as that of Rodenhiser.

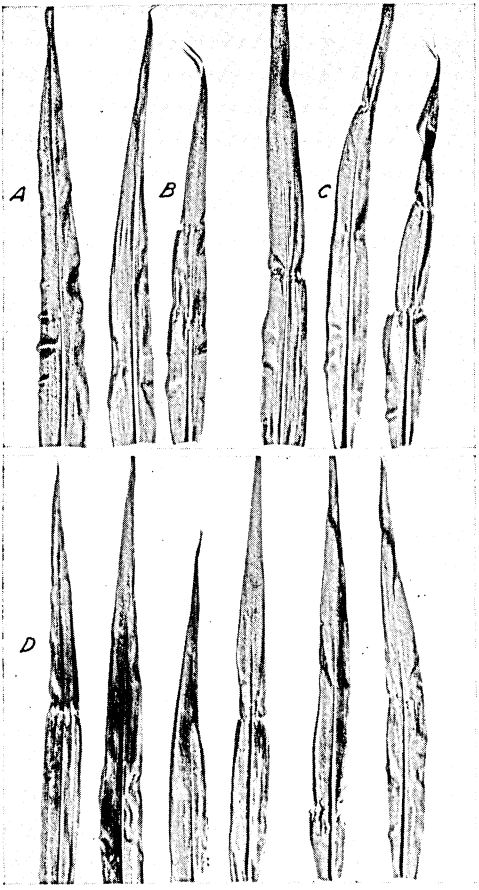

FIG. 2. TWO LEAVES FROM SORGHUM PLANTS INOCULATED WITH SINGLE OR PAIRED MONOSPORIDIAL LINES OF SPHACELOTHECA SORGHI

A, inoculated with monosporidial line Texas $B1_1$ (no chlorosis); B, inoculated with monosporidial line Texas $B2_1$ (no chlorosis); C, inoculated with paired incompatible monosporidial lines Texas $B1_1$ and Texas B3 (no chlorosis); D, six leaves inoculated with sexually compatible lines $B1_1$ and $B2_1$ (note chlorosis and flecking).

Chlorosis

Rodenhiser (40) obtained some evidence that chlorosis in young inoculated plants could be used as indication of sex compatibility between paired lines of *Sphacelotheca sorghi*. He found that certain paired lines of *S. sorghi* and *S. cruenta* produced small chlorotic spots or areas on leaves of sorghum plants four to six days after inoculation. Plants inoculated with certain other combinations or with single monosporidial lines remained normal in appearance. He stated that the chlorotic areas had mostly disappeared after ten days, but a few remained and normal viable chlamydospores were found in them. Only one pair of lines that caused flecking failed to form chlamydospores in the heads.

In November, 1932, the writer observed that sorghum plants inoculated with various combinations of monosporidial lines and with single lines of *Sphacelotheca sorghi* and grown in the greenhouse under humid conditions differed in appearance. There were pronounced chlorotic or flecked areas on the leaves of some plants, while those of others appeared normal. The former produced smutted heads at maturity. Subsequent experiments yielded similar results, and it was concluded that chlorosis or flecking produced by hypodermic injection of certain paired monosporidial lines into young plants may be used as an index of sex differences (Fig. 2). Chlorosis or flecking was never conspicuous, however, in sorghum plants inoculated in the same manner in the field.

The above tests are reliable but are laborious and require considerable time and greenhouse space. Therefore it was desirable to find simpler methods.

Fusion of Sporidia

Fusion of sporidia in mixed cultures of two monosporidial lines has recently been used by several investigators as an index of sex differences between monosporidial lines within certain smut fungi (1, 2, 3, 4, 5, 25, 41, 42, 47). Isenbeck (27) observed rather scanty fusion between sporidia of *Sphacelotheca sorghi* in mixed cultures of monosporidial lines growing on nutrient agar. Recently, Rodenhiser illustrated the fusion of sporidia in *S. sorghi.*

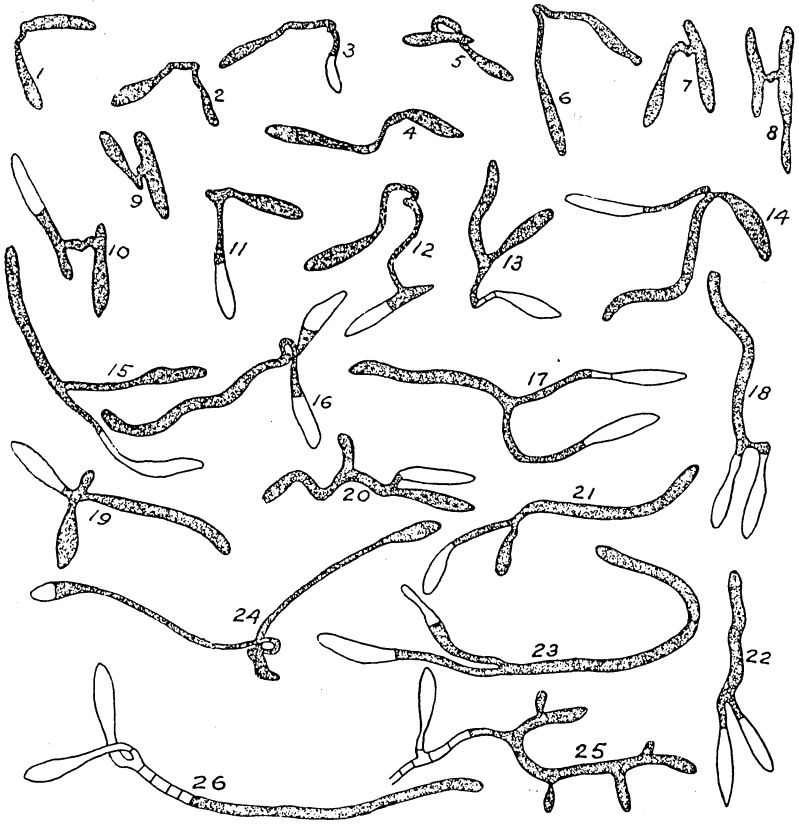

FIG. 3. CAMERA-LUCIDA DRAWINGS OF FUSED SPORIDIA OF SPHACELOTHECA SORGHI

Stages 1 through 12 represent young stages of fused sporidia. Stages 13 through 26 show the development of elongate, thickened fusion hyphae. Stage 25 shows a fusion hyphae beginning to bud and develop branches. Note the peculiar types of fusion and also the lack of protoplasm in one or both sporidia of a fused pair in the older stages.

The writer therefore attempted to ascertain how reliable sporidial fusions are in determining sex differences between monosporidial lines. The primary sporidia were removed from the promycelia of nine chlamydospores of *S. sorghi* and were subcultured on separate cover slips on van Tieghem cells. When visible colonies appeared, they were transferred to tubes of potato-dextrose agar. Enough cover slips containing drops of slightly alkaline malt agar (three per cent malt, two per cent agar) were made to accommodate all possible combinations of lines plus each of the four lines singly from each of the nine promycelia.

After inoculation from the tube cultures of young sporidia, the coverslip cultures were incubated 24 to 48 hours at approximately 27° C. The early stages of fusion could be detected after 24 hours, but only by very careful microscopic observation. The late stages can be observed more easily, since a long fusion hypha can usually be followed to the point of fusion. Large numbers of budding and nonfused sporidia obscure the results; therefore considerable experience and patience are required. Despite the difficulties, however, the sex reactions of 35 monosporidial lines from different chlamydospores were thus established (Table 2). Several types of fusion are shown in Figure 3. In some cases the fusion is H-like; in other cases the sporidia are united by long or short curled fusion tubes; in still others elongated fusion hyphae are produced. The protoplasmic contents from one or both of the fusion sporidia may pass into the fusion hypha. Cultures of young, rapidly developing sporidia seem best for successful fusion.

Bauch Test

A still simpler method for sex determination is the Bauch test, which Bauch (4, 5) used successfully in determining sex differences between monosporidial lines of *Ustilago violacea, U. zeae,* and *U. scorzonerae.* According to him, when lines of opposite sex are mixed on slightly alkaline malt agar (three per cent malt, two per cent agar), the resulting growth will consist of "Suchfäden" and "Wirrfäden" which eventually form a white mycelial mat over all or part of the culture. Paired lines of the same sex and single lines never reacted in such a manner.

Tests were made to determine whether sexually compatible and incompatible lines of *Sphacelotheca sorghi* could be differentiated by the Bauch method. Cultures of primary sporidia from the promycelia of nine chlamydospores of *S. sorghi* were used. The nutrient medium consisted of 30 grams of malt extract and 20 grams of agar made up to one liter with distilled water and made slightly alkaline by the addition of 8 cc. of normal sodium hydroxide per liter of solution. Poured plates of this medium were inoculated in the following manner.

Four monosporidial lines from a single chlamydospore were planted in duplicate on triplicate plates so as to form two concentric circles of cultures in each plate. A transfer was made from line 1 to each of those in the outer circle. The inner circle of cultures served as checks. The

plates were incubated 24 to 48 hours at 27° C. Figure 4 illustrates the results obtained. The chalk-white, pelt-like covering is formed when two mixed lines are compatible, while the inner ring of checks and the mixtures of incompatible lines lack this white growth of enlarged, intertwined hyphae, sometimes with aerial hyphae and conidia. If the cultures are permitted to age, the white pelt disappears, and a grayish-white zone consisting of hyphae growing radially and closely

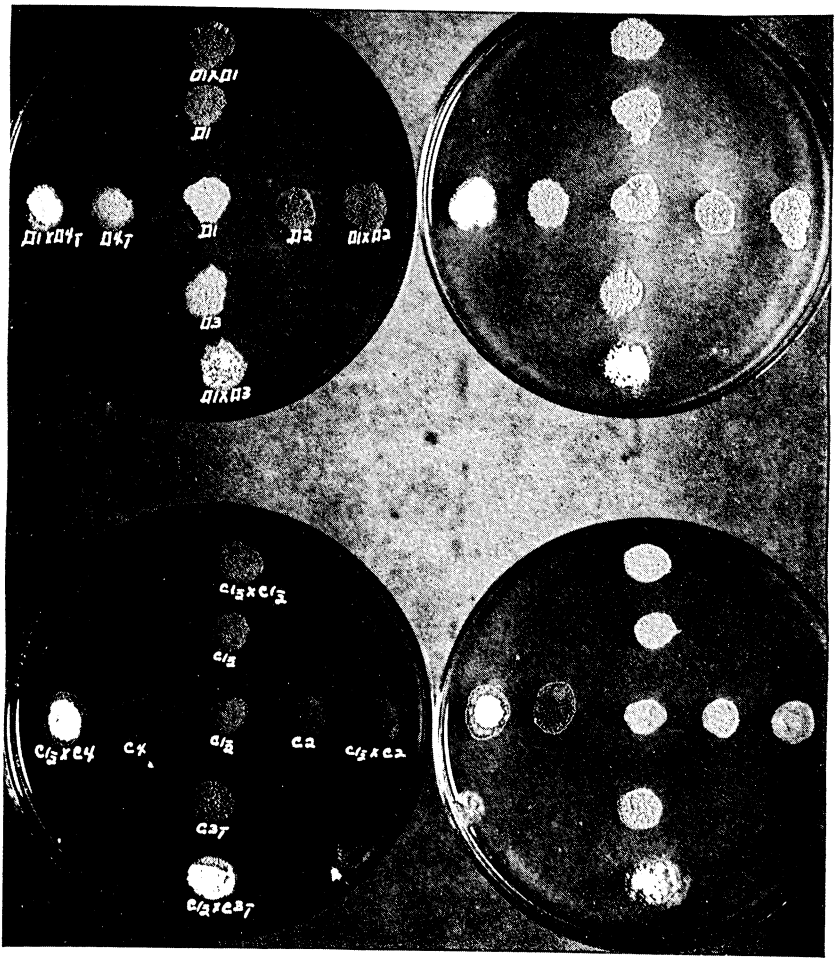

FIG. 4. AGAR PLATES, IN DUPLICATE, SHOWING RESULTS OF THE BAUCH TEST ON FOUR MONOSPORIDIAL LINES FROM A SINGLE PROMYCELIUM OF TEXAS D AND FOUR FROM A SINGLE PROMYCELIUM OF TEXAS C, SPHACELOTHECA SORGHI

In the upper plate line D1 is the tester; material from the center colony is smeared on colonies of lines D1, D2, D3, and $D4_1$ in the outer circle, while colonies in the inner circle are left as checks. It is evident that D1 is like D2 sexually but different from D3 and $D4_1$.

appressed to the substratum appears around the edge of the colony. When the medium is not entirely suitable, the zone of outward-growing hyphae usually is the only indication of sex compatibility between two monosporidial lines. Slightly alkaline potato-dextrose agar and non-nutrient agar were also used, but the results were not so sharp as those obtained on a medium similar to Bauch's. The results from lines of one chlamydospore only are shown in Figure 4, but equally good reactions were obtained with lines from the other eight chlamydospores.

The results of the Bauch test agree perfectly with those obtained with the same monosporidial lines when subjected to the three previously described sexuality tests. In those mixtures which reacted positively in the Bauch test, there were fused sporidia as well as "Suchfäden". There were none in the checks. On the leaves of sorghum plants inoculated with lines that reacted positively in the Bauch test, there was chlorosis and flecking. Later, as the plants approached maturity, chlamydospores were produced. Lines giving negative results in the Bauch test also gave negative results in inoculated plants. The results obtained indicate, as previously reported (41, 47), that this method is entirely reliable for determining the sex reaction of different monosporidial lines of *Sphacelotheca sorghi.*

Segregation for Sex

Bauch (2) found that sporidia of *Ustilago longissima* (Sow.) Tul. might belong to one of many sexual groups. In *U. zeae* (Beckm.) Unger, Hanna (22) has shown that the sporidia from some promycelia belonged to two sex groups, while those borne on others belonged to four. Christensen (9) obtained evidence that indicated the existence of numerous sexual groups in *U. zeae.* Two sexual groups were found in *U. levis* (Kell. and Sw.) Magn. and *U. hordei* (Pers.) Kell. and Sw. by Dickinson (15) and by Holton (25), and Hanna and Popp (23) found two in both *U. levis* and *U. avenae* (Pers.) Jens. Rodenhiser (40) has shown that *Sphacelotheca sorghi* and *S. cruenta* are heterothallic and also pointed out the presence of two sex groups in each species. In a later report Rodenhiser (41) corroborated his previous statements but added that his later results suggest the presence of sterility factors, which, he says, "are obviously a disturbing element in determining the number of sexual groups present." Further, Rodenhiser (40) never found evidence of complete inter-fertility nor intersterility between monosporidial lines from different chlamydospores of either species of kernel smut. Isenbeck (27) found that combinations of monosporidial lines from a single promycelium indicated the presence of two sex groups in *S. sorghi,* but combinations between lines from different chlamydospores suggested the existence of several sex groups.

For the writer's study of sex in *Sphacelotheca sorghi,* the primary sporidia were isolated from nine promycelia, and, in addition, some secondary and tertiary sporidia were isolated from three of the nine. The sporidia from each promycelium were mated in all possible com-

binations with each other and their sex reactions determined by the four methods described previously. The sporidia from each promycelium fell into two sex groups (Table 2).

Likewise, combinations of sporidia from different chlamydospores indicated the existence of two sex groups. However, one of the sporidia from Texas B behaved peculiarly. In Table 2, Texas B, it is seen that $B2_1$, the second sporidium isolated from cell No. 2, is compatible with sporidia from cells 1 and 3, as might be expected. However, primary sporidium B2, the sister of $B2_1$, is compatible only with derivatives of promycelial cell No. 1. The behavior of B2 and $B2_1$ also was consistently different with line B3. Clearly, then, there are more than two sex compatibility groups in *S. sorghi.*

The writer's results show that *Sphacelotheca sorghi* is heterothallic, as was first demonstrated by Rodenhiser (40), since lines injected singly produced no infection in sorghum plants. Evidence was obtained to indicate the existence of two sex groups with ratios on the basis of 2:2 and 1:3. Some evidence was obtained to indicate the presence of three sexual compatibility groups. Further, the results in Table 2 indicate that segregation for sex may take place in either the first or second division of the fusion nucleus. Evidence of a delayed segregation for sex factors beyond that occurring in nuclear divisions within the promycelium was not obtained. Neither was there any indication of complete inter-fertility, nor complete inter-sterility between and within lines tested from different chlamydospores of *S. sorghi.*

Segregation for Cultural Characters

Dickinson (14, 15) found the segregation for cultural characters in *Ustilago levis* to be on a 2:2, 3:1, and 4:0 basis. It was deduced that these ratios might develop from segregation in either the first or second division of the diploid nucleus. He stated, further, that at times a 2:2 ratio was evident for all cultural characters collectively, while their independence was indicated by their segregation in different nuclear divisions within the promycelium.

Hanna (22) and Christensen (9) have shown that segregation ratios for cultural characters in *Ustilago zeae* may differ, that segregation for cultural characters may occur simultaneously with, or independently of, sex. Holton (25) concluded that segregation of factors for cultural characters in *Ustilago levis* and *U. avenae* might extend beyond the second nuclear division in meiosis. Rodenhiser (40, 41) found segregation ratios of 2:1:1, 1:1:1, and 4:0 for color in *Sphacelotheca sorghi* and *S. cruenta* and, further, that segregation for all cultural characters in general may occur independently of each other and of those determining sex.

The segregation ratios for cultural characters in monosporidial progeny of nine chlamydospores of *Sphacelotheca sorghi* studied by the writer are as follows: 2:2 in Texas A, Texas C, 8A, and 22B; 2:1[2] in

[2] Incomplete ratio because sporidia from cell No. 4 of Texas B failed to grow. It probably belongs in the preceding group.

Texas B; 2:1:1 in 22A; 3:1 in 122B; and 4:0 in Texas D and Texas H. In chlamydospores Texas A, 8A, and 122B, segregation for cultural characters and for sex occurred simultaneously, while in the remainder of those studied it took place independently.

Since Holton (25) concluded that segregation of factors for cultural characters might be delayed indefinitely in *Ustilago avenae* and *U. levis*, it seemed advisable to study segregation in *Sphacelotheca sorghi* further.

A definite tendency in monosporidial lines of *Sphacelotheca sorghi* to sector has been noted and discussed elsewhere in this bulletin. Therefore it was decided to investigate the possibility of definite sector trends in derivatives from each of the four promycelial cells of different chlamydospores.

For the purpose of this study, primary, secondary, and tertiary lines from Texas A and Texas D were used. Thirty-one sectors appeared

Table 3. Distribution of First and Second Order Sectors of Sphacelotheca sorghi Into 14 Cultural Types, Chlamydospore Texas A, Lines Grown in Triplicate 250 cc. Erlenmeyer Flasks of Potato-Dextrose Agar

	Cultural types*														
Line	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Texas A1 (—1)	1														
Texas A1 (—1—1)	1														
Texas A1 (—2)										1					
Texas A1 (—2—1)									1						
Texas A1 (—2—2)										1					
Texas A1$_1$ (—1)	1														
Texas A1$_1$ (—1—1)	1														
Texas A1$_1$ (—2)		1													
Texas A1$_1$ (—2—1)												1			
Texas A1$_1$ (—3)													1		
Texas A1$_1$ (—3—1)													1		
Texas A1$_1$ (—3—2)					1										7
Texas A3 (—1)	1														
Texas A3 (—1—1)	1														
Texas A3 (—2)										1					2
Texas A4 (—1)	1														
Texas A4 (—2)						1									
Texas A4 (—2—1)								1							
Texas A4 (—3)											1				
Texas A4 (—3—1)											1				
Texas A4 (—4)								1							
Texas A4 (—4—1)							1								
Texas A4 (—5)								1							
Texas A4 (—5—1)						1									
Texas A4 (—5—2)						1									
Texas A4 (—6)														1	
Texas A4 (—6—1)														1	
Texas A4$_1$ (—1)						1									
Texas A4$_1$ (—2)				1											
Texas A4$_2$ (—1)			1												
Texas A4$_2$ (—1—1)			1												8
Total	7	1	2	1	1	4	1	3	1	3	2	1	2	2	

* Cultural types described elsewhere.

Table 4. Distribution of First and Second Order Sectors of Sphacelotheca sorghi Into 11 Cultural Types, Chlamydospore Texas D, Lines Grown in Triplicate 250 cc. Erlenmeyer Flasks of Potato-Dextrose Agar

Line	Cultural types											
	1	2	3	4	5	6	7	8	9	10	11	Total
Texas D1 (−1−1)					1							
Texas D1 (−2)					1							
Texas D1$_1$ (−1)								1				
Texas D1$_1$ (−1−1)						1						
Texas D1$_1$ (−2)					1							3
Texas D2 (−1)		1										
Texas D2 (−2)							1					
Texas D2 (−2−1)					1							3
Texas D3 (−1)											1	
Texas D3 (−2)				1								
Texas D3 (−2−1)				1								
Texas D3 (−3)	1											
Texas D3 (−3−1)											1	3
Texas D4$_1$ (−1)										1		
Texas D4$_1$ (−1−1)			1									
Texas D4$_1$ (−2)			1									
Texas D4$_1$ (−3)										1		
Texas D4$_1$ (−3−1)									1			3
Total	1	1	2	2	4	1	1	1	1	2	2	

in the lines from chlamydospore A and eighteen from D. Transfers were made from each sector and subcultured separately in triplicate flasks of potato-dextrose agar and held at room temperature for 25 days. At maturity the new colonies were classified on the basis of cultural characters, such as color, luster, topography, consistency, and margin. The sectors from Texas A were distributed into 14 distinct groups (Table 5; Figs. 6 and 7) and those from Texas D into 11 distinct groups. The cultural characters of the lines in each group were distinct from those of all sister groups. The origin and distribution, according to cultural type or group, of the sectors from Texas A and D are shown in Tables 3 and 4.

Table 3 shows that approximately one half of the cultural types produced by Texas A, i.e. 1, 2, 5, 9, 10, 12, and 13, were produced by sectors derived from cultures of sporidia from promycelial cell No. 1; two cultural types, 1 and 10, from cell No. 3; and eight cultural types, i.e. 1, 3, 4, 6, 7, 8, 11, and 14, from cell No. 4. The cultural types derived from cell No. 3 were also produced by cell No. 1, while the eight arising from cell No. 4 were all different from those produced by derivatives of Nos. 1 and 3, with the exception of line A4(−1).

Examination of Table 4 (Texas D) discloses that eleven cultural types were differentiated and that derivatives of each of the four promycelial cells produced approximately one fourth of the total number of cultural types differentiated. With only one exception, D2(−2−1), none of those produced by derivatives of any one cell was duplicated in those produced by any other cell of the same promycelium.

Table 5. Cultural Characteristics of Cultural Types 1 Through 14, Chlamydospore Texas A; Monosporidial Lines Grown on Standard Potato-Dextrose Agar in Triplicate Erlenmeyer Flasks

Type and line	Diameter in mm.	Color	Luster	Topography	Margin and edge
Type 1 A1 (—1—1)	50 mm.	Vinaceous pink; marginal band translucent	Shiny	Flat; membranous alveolate	Marginal band 5 mm. wide, edge irregularly lobate
Type 2 $A1_1$ (—2)	40-50	Center light buff; marginal band salmon-pink	Shiny	Flat; alveolate to reticulate	Edge lobate-lobulate
Type 3 $A4_2$ (—1)	40-45	Central zone pale vinaceous cinnamon with grayish border slightly powdery in center; marginal band translucent	Mostly dull	Flat, membranous veined and reticulate to somewhat pitted	Margin plumose, 3 mm. wide; edge irregular to lobate
Type 4 $A4_1$ (—2)	30-35	Mostly vinaceous cinnamon, slightly lighter toward margin	Wet shiny	Flat; pitted to finely verrucose	Edge finely crenate
Type 5 $A1_1$ (—3—2)	55	Cartridge buff	Shiny	Growth thin and flat; finely reticulate to almost pitted, veinlike toward edge	Edge irregularly dentate
Type 6 $A4_1$ (—1)	55	Light pinkish-cinnamon	Shiny	Slightly umbonate, center somewhat rugose, branched radial folds extending to marginal band	Marginal band smooth, 5 mm. wide; edge slightly wavy and abrupt
Type 7 A4 (—4—1)	50	Extremely pale vinaceous cinnamon	Shiny	Slightly umbonate; shallow pits in center; mostly simple, fine, clockwise radial folds	Marginal band 5 mm. wide, smooth; edge abrupt or entire
Type 8 A4 (—2—1)	52	Light vinaceous cinnamon, slight powdery appearance in center	Shiny	Slightly raised in center; rugose with slightly branched radial folds extending to margin	Marginal band 4 mm. wide, smooth; edge abrupt

Table 5—Continued

Type and line	Diameter in mm.	Color	Luster	Topography	Margin and edge
Type 9					
A1 (—2—1)	55	Dirty ivory yellow	Shiny	Center has coarse, branched, radial folds, smooth band, then unbranched radial folds slightly counter-clock-wise extending to margin	Marginal band 3 mm. wide, smooth; edge abrupt
Type 10					
A3 (—2)	50	Cartridge buff to ivory yellow	Shiny	Center rugose; straight unbranched radial folds extending to margin	Marginal band 2 mm. wide; edge abrupt
Type 11					
A4 (—3—1)	57	Center ivory yellow; white concentric bands, marginal band translucent to ivory-colored	Dull powdery	Center echinulate; straight radial folds extending to margin	Marginal band 7 mm. wide; edge slightly wavy and abrupt
Type 12					
$A1_1$ (—2—1)	52	Ivory yellow to translucent	Shiny	Center reticulate; unbranched radial folds slightly counter-clockwise and extending to margin	Marginal band 5 mm. wide; edge abrupt
Type 13					
$A1_1$ (—3)	52	Ivory yellow to translucent	Wet shiny	Flat; silky counter-clockwise radial folds	Edge diffuse
Type 14					
A4 (—6—1)	42	Tilleul buff	Shiny, glistening in center	Center smooth, viscous glistening; margin has looped folds	Marginal band 7 mm. wide; edge irregularly lobed to dentate

To evaluate the significance of the results reported above, it must be recalled that the ratio of segregation for cultural characters in the primary monosporidial lines from Texas A was 2:2; that is, primary colonies derived from sporidia of promycelial cells A1 and A3 were alike, but different from derivatives of A2 and A4, which also were alike.[3] It may be assumed, therefore, that the nuclei within one pair of promycelial cells contained factors different from those of the other pair, with the result that the derivatives of the first pair tended to produce a series of cultural types through sectoring which were different from those produced by the second pair. This fact appears to support the earlier conclusion that normal segregation for cultural characters had occurred during the nuclear divisions within the promycelium.

The results with Texas D are somewhat confusing. As stated before, the segregation ratio of factors for cultural characters was 4:0; that is, primary colonies produced by the sporidial derivatives of promycelial cells Nos. 1, 2, 3, and 4 were alike. Since the colonies were phenotypically alike, it may be argued that normal segregation for cultural characters had not occurred within the promycelium. The latter argument is nullified by the fact that derivatives of each of four cells of the same promycelium produced a series of cultural types, as a result of sectoring, which differed from those produced by derivatives of each sister promycelial cell. It is concluded, therefore, that although the primary colonies produced by the sporidial derivatives of cells 1, 2, 3, and 4 were phenotypically alike, they were genotypically different. Further, if it is granted that four genotypes existed, one must conclude that they arose through normal segregation of factors for cultural characters within the promycelium.

In general, it has been concluded that normal segregation for cultural characters occurred within the promycelia of the chlamydospores studied, particularly in chlamydospores Texas A and B whose sporidial progeny were studied in detail. The evidence, therefore, indicates that sectors resulted through mutation and not from delayed segregation.

Manner of Appearance of Sectors

Sectoring was first observed in cultures of *Sphacelotheca sorghi* by Ficke and Johnston (18). They observed sectoring in multi- and single-chlamydospore cultures of two or three physiologic races, but not in monosporidial cultures of the same races. Later, Rodenhiser (41) and Isenbeck (27) reported sectoring to occur commonly in some monosporidial lines of *S. sorghi.*

When the monosporidial lines used in the segregation studies reported in the previous section were grown on various agar media, numerous sectors appeared in colonies of many lines on certain media. The manner of their appearance is similar to that reported for *Ustilago zeae* by Stakman *et al.* (43). Figure 5 illustrates the production of sectors in four primary monosporidial lines from a single germinated

[3] Line A2 did not survive in stock culture.

chlamydospore of *Sphacelotheca sorghi.* Some variants appear as fan-shaped sectors when the colonies are quite young. Usually such sectors appear at the edge of older colonies. Some may appear as patches in various parts of old cultures. The production of sectors may escape notice when lines are grown in test tubes; therefore it is necessary to grow them either in Erlenmeyer flasks or Petri dishes. Variants appearing as fan-shaped sectors are relatively easy to isolate in apparently pure condition, particularly if the sectors are mycelioid. Patch sectors can seldom be obtained in pure condition, because there are always many potentially variable sporidia adherent to them. Sectors usually are sporidial and viscous, sporidial and grumose, or mycelioid. The latter types are most prevalent.

The writer attempted to isolate all variants that appeared, whether in patches or sectors. Since the latter were much easier to obtain in a pure state, final efforts were restricted mostly to isolation of the fan-shaped sectors.

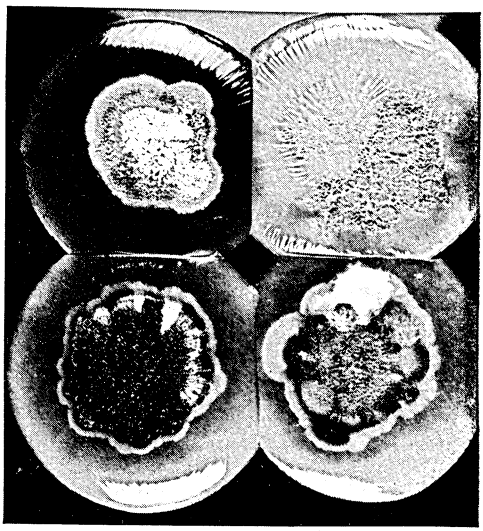

FIG. 5. THE APPEARANCE OF SECTORS IN FOUR PRIMARY MONOSPORIDIAL LINES FROM A SINGLE GERMINATED CHLAMYDOSPORE OF SPHACELOTHECA SORGHI

Note the appearance of numerous fan-shaped sectors which differ in topography, color, type, consistency, and rate of growth.

It is evident from Figure 5 that the sector variants differ considerably in type of growth, rate of growth, topography, etc. A more detailed account of this follows.

Characters of Cultural Types

Sectors are not uncommon in artificial cultures of many cereal smuts (10, 14, 15, 17, 20, 25, 38, 39, 43, 44). The most extensive cultural study of a smut fungus is that of Stakman *et al.* (43) on *Ustilago zeae,* who classified 200 variant lines on the basis of rate of growth, type of growth, size, color, topography, consistency, and direction of growth.

In the present work, more than 100 sectors, which appeared in monosporidial lines isolated from nine chlamydospores of *Sphacelotheca sorghi,* have been studied with respect to cultural differences like those outlined by Stakman *et al.* (43). Table 5 and Figures 6 and 7 show some of the striking differences observed in 14 variant types from Texas A.

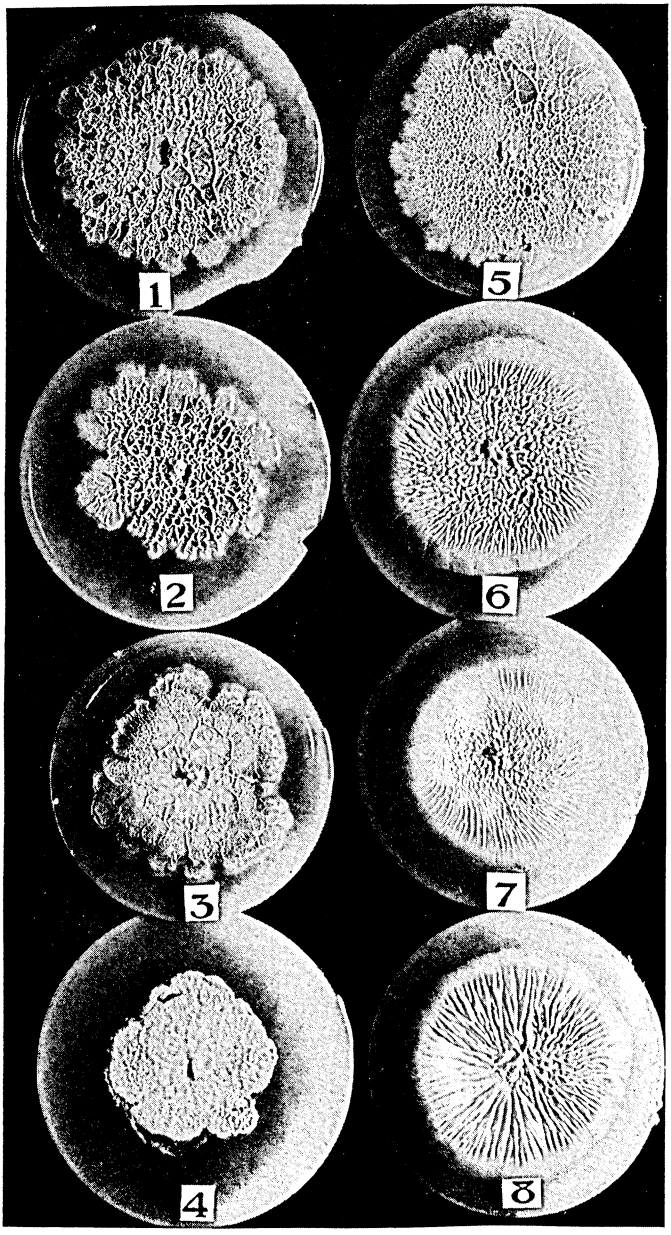

FIG. 6. EIGHT CULTURAL TYPES DERIVED AS FIRST- OR SECOND-ORDER SECTORS FROM PRIMARY MONOSPORIDIAL LINES OF SPHACELOTHECA SORGHI FROM CHLAMYDOSPORE TEXAS A

See Table 5 for detailed description.

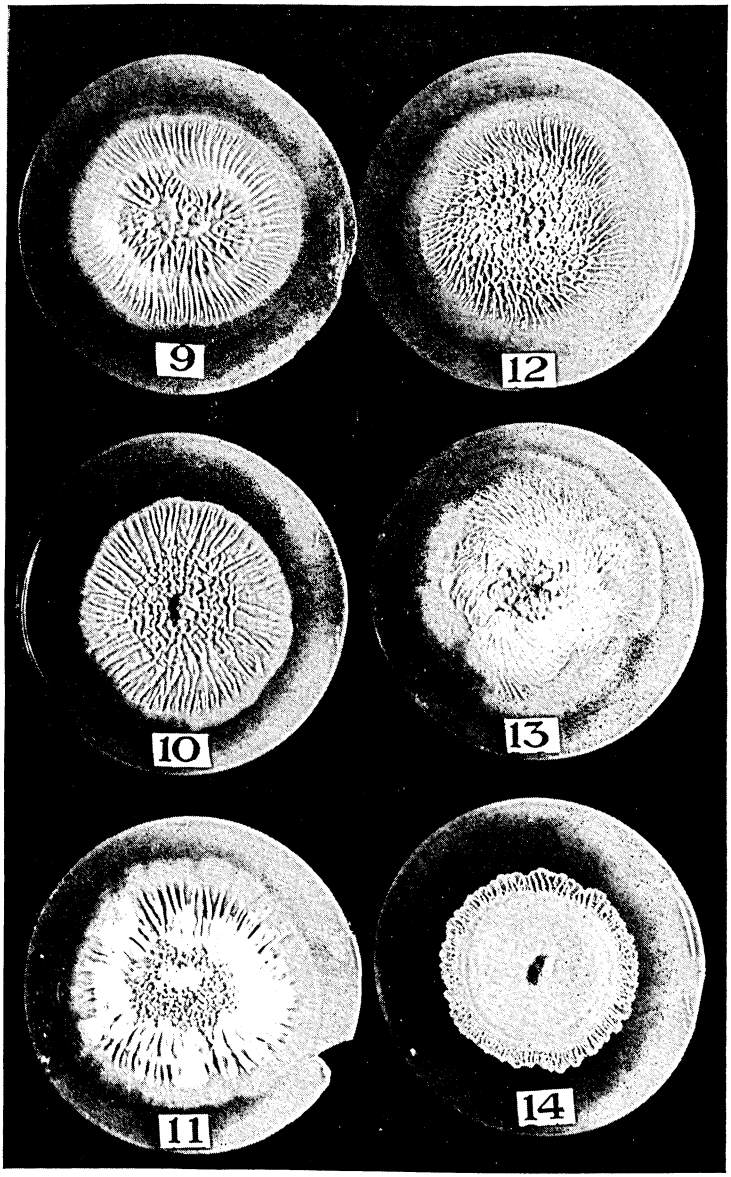

FIG. 7. SIX CULTURAL TYPES DERIVED AS FIRST- OR SECOND-ORDER SECTORS FROM PRIMARY MONOSPORIDIAL LINES OF SPHACELOTHECA SORGHI FROM CHLAMYDOSPORE TEXAS A

See Table 5 for detailed description.

Table 6. Number of Sectors That Developed in Three Monosporidial Lines of Sphacelotheca sorghi on 14 Different Nutrient Media in Triplicate Flasks for Each Medium

Medium	Lines, number of sectors, and growth* of lines			
	Texas $A4_1(-2)$	Texas $A1_1(-3-2)$	Texas $A1(-2-1)$	Total No. sectors
Agar 1.5%	0 Scanty	0 Scanty	0 Scanty	0
Sucrose 1.5%, agar 1.8%	0 Fair	1 "	0 "	1
Sucrose 3.0%, agar 1.8%	0 "	0 "	0 "	0
Dextrose 3.0%, agar 1.8%	1 "	3 "	0 "	4
Sucrose 1.0%, dextrose 1.0%, agar 1.8%	2 "	0 "	0 "	2
Peptone 1.5%, dextrose 1.5%, agar 1.8%	0 Excellent	0 Excellent	0 Excellent	0
Peptone 3.0%, agar 1.8%	0 "	0 "	0 "	0
Sucrose 1.5%, agar 1.8%, $MgSo_4$ .018%	2 Fair	2 Scanty	0 Scanty	4
Sucrose 3.0%, agar 1.8%, $NaNO_3$ .006%	4 "	1 "	0 "	5
Sucrose 3.0%, agar 1.8%, $NaNO_3$ .006%	5 "	1 "	0 "	6
Sucrose 1.5%, agar 1.8%, $Ca(NO_3)_2$ .012%	3 "	1 "	0 "	4
Sucrose 3.0%, agar 1.8%, $(NH_4)_3\ PO_4$ .012%	2 "	3 Fair	0 Fair	5
Sucrose 3.0%, agar 1.8%, $(NH_4)_3\ PO_4$ .012%	1 "	1 "	0 "	2
Sucrose 1.5%, agar 1.5%, $(NH_4)_3\ PO_4$ .006%	0 "	0 Scanty	0 Scanty	0
Sucrose 1.5%, agar 1.5% plus				
$Ca(NO_3)_2$ .005%, $(NH_4)_3\ PO_4$ .005%	0 "	3 Good	0 Good	3
Sucrose 1.5%, agar 1.5%, K_2HPO_4 .01%	1 "	0 Scanty	0 Scanty	1
Total sectors	21	16	0	37

* Growth of lines compared with growth on potato-dextrose agar.
Excellent=colonies practically normal.
Good=colonies medium in thickness, about one half natural size.
Fair=colonies thin, about one third natural size.
Scanty=colonies thin, indistinct, varying in size or diameter.

Uncolored photographs do not show adequately all the cultural differences encountered. An attempt will therefore be made to describe them more in detail. Naturally, each cultural character may vary considerably on various nutrient media and at different temperatures, but if all the cultural characters of one variant type are studied under a certain set of conditions they are distinctive. To simplify matters, the following description of the range for any one character is based on the manifestation of that character on potato-dextrose agar at room temperature.

Either duplicate or triplicate colonies for each line were measured in two directions, and an average of their dimensions was recorded. Colonies of the 105 cultural types studied range in size from 15 mm. to 65 mm., by far the greatest number being from 30 mm. to 50 mm. in diameter.

Ridgway's (*37*) color standards were used to determine color. There was a wide range in color of colonies—white or cretaceous white, gray or black, brown, olive, buff, and pink. A large number of color intergrades occur among the cultures: for example, salmon pink, cinnamon, vinaceous, olive gray, light and dark buff, ochraceous buff, cartridge buff, salmon buff, Tilleul buff, olive buff, chestnut brown, carob brown, and bay. (The color descriptions of 14 cultural types shown in Figures 6 and 7 are given in Table 5.) The luster may be shiny, glistening, or dull.

Colonies of different lines may consist of mycelium, of sporidia, or a mixture of both. Mycelial colonies may produce aerial hyphae and conidia, or they may have only prostrate hyphae. In Figure 6, types 1, 2, 3, 4, and 5 are sporidial, while 6, 7, 8 are mycelial. In Figure 7, type 12 consists of sporidial growth in the central area, while the two outer zones are mycelial.

The consistency depends largely upon the type of growth. The colonies may be slimy, viscid (Fig. 7, type 14), buttery (Fig. 6, types 2, 4, and 5), filamentous (type 13), rubbery and tough (types 6, 7, 8, 9, 10, 11, 12), or membranous (types 1 and 3).

Most of the colonies tend to grow radially, but others, particularly mycelial colonies, tend to grow either clockwise or counter-clockwise.

Most colonies are flat, some are slightly raised, and others are somewhat umbonate.

The surface markings may vary considerably. Mycelial colonies are often waxy, cottony, tufted, felty, granular, or pubescent. Sporidial colonies may be pitted, reticulate, verrucose, or smooth. Mycelial colonies may be verrucose, reticulate, contoured, rugose, or smooth. Rugose colonies may have radial or tangential folds, either fine or coarse. They may be simple or branched, anastomosed or regular. Some colonies may have a marginal zone quite different from the remainder of the colony, but the same general surface markings also characterize the margin. The edge may be entire, lobate, lobate-lobulate, crenate, undulate or wavy, dentate or fimbriate.

Effect of Nutrients on the Production of Sectors

Stakman *et al.* (43) have shown that the environment has considerable influence on phenotypic characters of *Ustilago zeae*. They found "no evidence of slow, cumulative, heritable changes"; neither did they find any evidence of the acquisition of new characters as a result of growth under different conditions. The changes they observed were sudden and they attributed them to mutation. Further, they showed that the frequency of mutation could be influenced considerably by different environmental factors.

In the preceding section on cultural characters it has been shown that many different and distinct cultural types may arise through sectoring in colonies of monosporidial lines of *Sphacelotheca sorghi*. An attempt therefore was made to learn what effect nutrients would have upon the production of sectors.

Three lines were grown on 14 different media of the same formulas as those used by Stakman *et al.* (43). The various media contained different concentrations of several sugars, alone and in combination with other sugars, and various concentrations of sucrose mixed with different percentages of salts of sodium, calcium, phosphorus, potassium, magnesium, etc. The per cent of agar of the medium was also varied.

Results in Table 6 show that numerous sectors developed in some smut lines on different media. For example, Texas $A4_1(-2)$ produced 21 sectors, and Texas $A1_1(-3-2)$ produced 16. Sectors were not produced by Texas A1(—2—1) on any medium. Of the 37 sectors, only seven appeared in cultures grown on various sugars and peptone agar media, while 30 arose in cultures growing on sucrose plus varying percentages of nutrient salts. Most of the sectors appeared in cultures grown on media to which nitrogenous compounds had been added, most of them being rather indistinct.

The extent of growth on different media varied considerably. In general, the growth of the three lines on the plain carbohydrate media was mostly scanty to fair. All grew extremely well on the proteinaceous media, while growth on media containing a carbohydrate plus nutrient salts was mostly fair to scanty. Growth was good on a medium consisting of sucrose plus calcium nitrate and ammonium phosphate.

In a second experiment, 21 different lines were grown on potato-dextrose agar and on malt agar. The results are shown in Table 7. Thirteen of the lines, 1-13 inclusive, arose previously as first- or second-order sectors in primary sporidial cultures, while eight lines, 14-21, are cultures of primary sporidia more recently isolated. In all, 136 sectors were produced, all on the malt agar medium. Forty-four were produced by the old lines, 1-13, which had remained relatively stable for more than a year, while 92 were produced by the newly isolated lines, 14-21. Transfers to potato-dextrose agar were made from each of the 136 sectors. From the 136 sectors, 43 types not previously encountered were obtained. Of these, 14 were produced by the old lines

and 29 by the eight new lines. In the column on the left, Table 7, the growth types of the 21 lines used are indicated. Almost without exception, sporidial cultures produced from one to many sectors. The mycelial types remained comparatively stable. The three columns on the right indicate the growth types prevalent in the new variants with the number of new cultural types produced as sectors, by lines. Of the 43 new cultural types distinguished, 14 were mycelial, 13 sporidial, and 16 were mixed mycelial and sporidial.

Table 7. Number of Sectors Developed in 21 Monosporidial Lines of Sphacelotheca sorghi on Two Different Agar Media in Triplicate Flasks for Each Medium; Also Number and Type of Growth of New Sectors

Lines and type of growth	Medium and number of sectors		Type of growth of new sectors			
	Standard potato-dextrose agar	2% malt, 1% dextrose, 1.5% agar	No. sectors	Mycelial	Sporidial	Mycelial and sporidial
1. Texas $A1_1$ (−2) Sporidial	0	0	0	.....	.....	.....
2. " $A1_1$ (−2−1) Mycelial	0	0	0	.....	.....	.....
3. " $A1_1$ (−3−2) Sporidial	0	3	2	.....	.....	2
4. " A4 (−3−1) Myc.-Spor.	0	2	1	1	.....	.....
5. " $A4_1$ (−2) Sporidial	0	1	1	.....	1	.....
6. " A3 (−2) Mycelial	0	0	0	.....	.....	.....
7. " A1 (−2−1) Mycelial	0	0	0	.....	.....	.....
8. " $A1_1$ (−1) Sporidial	0	7	2	1	.....	1
9. " A4 (−6−1) Sporidial	0	5	2	2	.....	.....
10. " $A1_1$ (−3−1) Myc.-Spor.	0	11	1	.....	.....	1
11. " $A4_2$ (−1−1) Sporidial	0	13	5	2	2	1
12. " A4 (−4) Mycelial	0	0	0	.....	.....	.....
13. " A4 (−5−2) Mycelial	0	2	1	1	.....	.....
14. 122 B1 Sporidial	0	30	4	3	.....	1
15. " B2 Sporidial	0	11	7	2	3	2
16. " B3 Sporidial	0	6	4	.....	2	2
17. " B4 Sporidial	0	10	4	.....	1	3
18. 22 A1 Sporidial	0	9	2	1	1	.....
19. " A2 Sporidial	0	10	1	.....	1	.....
20. " A3 Sporidial	0	7	3	.....	2	1
21. " A4 Sporidial	0	0	3	1	.....	2
Total	0	136	43	14	13	16

It is evident that certain nutrients strongly influence the frequency of sectoring. Carbohydrate media containing little or no mineral salts induced sectoring very little or not at all. No sectors were produced on peptone agar, although only three lines were tested on it. Considerable sectoring was observed on carbohydrate media which contained certain nutrient salts, principally nitrogenous salts, and numerous sectors appeared on malt agar media.

Constancy of Cultural Characters

The writer has observed that cultural characters of some lines may vary widely on different media and at different temperatures. Hence, it is necessary to have complete culture records for each line on different media or to grow them on a well standardized medium that is known

not to favor cultural variation. Even under fairly well standardized conditions, one or more characters may vary considerably.

Temperature has a profound influence on the size of colonies, as shown in Table 8, and topography may be affected considerably. At low temperatures, 10° C. and 15° C., the colonies tend to heap up or become raised; deep, coarse convolutions appear, whereas at higher temperatures they tend to flatten out, are smoother, and cover the surface of the medium more quickly and evenly.

Table 8. The Influence of Temperature on the Growth of Six Lines of Sphacelotheca sorghi on Potato-Dextrose Agar and on Malt Agar, in Triplicate Plates for Each Medium

Lines, and media	Temperature, and size of colonies in mm.				
	10° C.	15° C.	24.5° C.	27.5° C.	30-32° C.
Potato-dextrose agar					
Texas A4 (—6—1)	12	16	35	39	34
Texas A1 (—2—1)	15	35	85	90	80
Texas $A1_1$ (—3—2)	14	22	34	37	33
Texas A4 (—4)	9	16	80	92	82
Texas $A4_1$ (—2)	12	19	30	40	28
122 B3	14	21	35	37	30
Malt agar					
Texas A4 (—6—1)	10	13	25	34	27
Texas A1 (—2—1)	12	30	75	80	70
Texas $A1_1$ (—3—2)	11	17	27	26	30
Texas A4 (—4)	7	12	70	85	65
Texas $A4_1$ (—2)	10	15	22	None	22
122 B3	12	16	27	28	24

Varying amounts of nutrients and the rapidity of evaporation of moisture likewise exert considerable influence on the expression of cultural characters. In the experiments described previously, where certain lines were grown on different media, some individual lines might easily have been mistaken for entirely different lines on the different media. However, when transfers were made from these lines back to a certain nutrient medium, the characters distinctive of each for that medium always reappeared. This was evident in the experiments where lines $A4_1(-2)$, $A1_1(-3-2)$, and A1(—2—1) were grown on several different media. In the case of those lines that sectored freely care had to be taken to transfer from the parent type and not from sectors.

Colonies of the 14 lines shown in Figures 6 and 7 were photographed in November 1932, after having grown for 21 days at room temperature in Erlenmeyer flasks of potato-dextrose agar. Fourteen months later the same lines were again grown under similar conditions, and they were almost identical in appearance. This is rather surprising, since the lines had been grown in test tubes containing potato-dextrose agar and were transferred at least six times during the 14 months. However, 13 of these lines were used in the previous experiments on "Effect

of Nutrients on the Production of Sectors." Tables 6 and 7 show that eight of them produced sectors in one or more of the experiments and that many of the sectors produced were new types. Nevertheless, the parental types were recovered in all cases and when grown on potato-dextrose agar they always regained their distinctive characters. While eight of these lines proved to be unstable, five were stable, as is indicated in Tables 6 and 7. Texas $A1_1(-2)$, $A1_1(-2-1)$, A1(−2−1), A3-(−2), and A4(−4) resisted all attempts to make them sector and therefore remained culturally constant for more than a year.

The constancy of cultural characters in variant lines of *Sphacelotheca sorghi,* which arose as sectors, indicates that these lines are genotypically different from their parents. It is true that the cultural characters in a given line may vary greatly, but the variation is in degree of expression of certain characters as influenced by the environment and is purely temporary. Stakman and his coworkers have shown that cultural characters, sex, and pathogenicity of variants in *Ustilago zeae* are due to genetic factors and are constant except where mutation or abnormal segregation occurs to produce new types (9, 43, 44). The writer's results indicate that cultural characters of haploid lines and sector variants in *S. sorghi* are likewise due to genetic factors and therefore are subject to change in a similar manner.

Intra-specific Hybridization

PATHOGENICITY.—In 1915 Potter (33) demonstrated that *Sphacelotheca cruenta* (Kuhn) Potter and *S. sorghi* (Link) Clinton, the loose and covered smuts of sorghum, were distinctly different macroscopically when parallel inoculations were made on several varieties of sorghum. Potter and Melchers (34) inoculated the seed of varieties representing all commercial types of *Sorghum vulgare.* In general, they concluded that none of the types were immune, although some were highly resistant. Kulkarni (28, 29) inoculated seed of different strains of milo sorghum with chlamydospores of *Sphacelotheca sorghi* and *S. cruenta.* He concluded that dwarf milo is resistant to covered smut and decidedly susceptible to loose smut. He also noted differences in pathogenic effects of the two kernel smuts on the same sorghum varieties. Reed (35) inoculated seed of varieties belonging to the main sorghum groups (broom corn, durra, kafir, kaoliang, milo-feterita, shallu, sorgo, and dorso) with *S. sorghi* and *S. cruenta.* The two smuts were about equally pathogenic except that *S. cruenta* attacked two strains of dorso and *S. sorghi* attacked none. Further tests by Reed and Melchers (36) were made on 250 varieties and strains, comprising perennial sorghum, grass sorghum, broomcorn, sorgo, and grain sorghum. The seed of these varieties and strains was inoculated with spores of *S. sorghi* and planted at different places in southern and eastern United States each year for a period of seven years. All strains of shallu were very susceptible; the sorgos in general proved susceptible,

with high percentages of infection; Sudan was moderately susceptible; broom corn and kafirs were very susceptible; and milo and feterita were very resistant.

As previously mentioned, Tisdale, Melchers, and Clemmer (45) pointed out the presence of parasitic races of covered kernel smut. They inoculated numerous varieties and strains of sorghum with chlamydospores of the smut occurring on milo and with the common type. From these inoculations they were able to demonstrate that smut pathogenic to milo, white yolo, and hegari was different from the common sorgo strain because the latter did not attack milo, white yolo, and hegari. Further, these workers collected smut from milo which resembled both so-called species of kernel smut and suggested that *S. sorghi* and *S. cruenta* may have hybridized. Recently Melchers and his coworkers have demonstrated the presence of at least five parasitic races of *S. sorghi* (31).

Rodenhiser (40) inoculated Reed kafir sorghum with inter- and intra-specific crosses of both kernel smuts. The two species hybridized readily. He made preliminary tests that indicated that the ability of the hybrids to infect differed from that of their parents. More recently, Rodenhiser (41) demonstrated clearly that "pathogenically distinct strains may be produced as a result of hybridization" between and within the loose and covered kernel smuts.

From the literature it is readily seen that studies of pathogenicity, except Rodenhiser's, involved the use of chlamydospore collections, probably comprising many biotypes. In the present studies all chlamydospore lines used were developed on sorghum plants in the greenhouse as a result of hypodermic inoculations with paired, compatible monosporidial lines of *Sphacelotheca sorghi.*

In the fall of 1932 monosporidial lines were obtained and cultured artificially, and sorghum plants were inoculated and maintained in the greenhouse until mature. Chlamydospore collections for field studies were made from 10 different lots of the greenhouse-matured plants, each lot of which had been inoculated with a different pair of monosporidial lines. The 10 chlamydospore lines were selected at random from the numerous smutted lots of plants. Each line bears the number allotted to the particular smutted plot in the greenhouse from which it was collected. Thus, F_1 chlamydospore lines 2, 4, 22, 34, 67, 76, 87, 95, 99, and 122 resulted. The two monosporidial lines which united to produce each of the different chlamydospore lines used as inoculum are listed in Table 9.

In the spring and summer of 1933, field studies were made to test the pathogenicity of the 10 F_1 chlamydospore lines of known parentage. Treated seed of Minnesota Amber sorghum was placed in packets with approximately 200 seeds per packet. Each of the 10 packets of seed was inoculated with spores from a different one of the chlamydospore collections. Other packets containing non-inoculated seed were retained for checks.

A replication of the above was made, except that the treated seed was dipped in a water suspension of chlamydospores. Plantings were made on May 25, 1933, in plots of three parallel rod rows each, and the plots were distributed at random, end to end. All plants were fully mature by August 15, and the total number of healthy stalks and smutted stalks were recorded for each row. The results are given in Table 9. The average percentage infection for each line was considerably higher where seed was inoculated with dry chlamydospores. The difference in percentage of smutted stalks between the plots inoculated by the two methods ranged from 3.0 to 39.3 per cent, the average difference being 22.4 per cent. The percentage of plants smutted by the different lines was consistently higher when seed was inoculated with dry spores in contrast to wet spores. It is possible that the difference may have been due to difference in spore load of the inoculated seeds, for it was observed that seeds inoculated with dry spores were coated so heavily as to cause them to appear dark brown, while those inoculated with the water suspension of spores were not visibly coated. Heald (24) has shown a correlation between percentage of infected plants and spore load in bunt of wheat, *Tilletia tritici* and *T. levis*. It is possible, also, that the spores suspended in water may have germinated too rapidly and therefore failed to cause infection.

The high percentages of infection obtained is of interest since Reed (35) states that, even in the highly susceptible sorgo group, the percentage of infection seldom is above 60 in the United States. Table 9 indicates an average infection for one individual plot of 77.6 per cent, while the highest single row average was 85.0 per cent.

Although the percentages of plants attacked by different lines (Table 9) indicate possible differences in pathogenicity, the data are not considered conclusive. However, a further index of pathogenicity was observed to be the stunting effect of covered kernel smut.

Reed (35) measured sorghum plants, some of which were infected with *Sphacelotheca sorghi* and others with *S. cruenta*. He noted no difference in height of plants infected with *S. sorghi* and non-infected plants. However, he found that plants infected with *S. cruenta* were six to twelve inches shorter than non-infected plants. Kulkarni (28) stated that plants infected with *S. cruenta* were shorter, the stalks were thinner, and in some cases the plants tillered more freely than healthy plants. Rodenhiser's (41) results show that sorghum plants were not always stunted when infected with either of the two kernel smuts, but when stunting occurred it was most evident in plants attacked by smut of *S. cruenta* types.

The writer measured stalk length and head length of random samples of 100 stalks from the check plot and from ten field plots, each infected with one of the 10 F_1 chlamydospore lines of *S. sorghi*. The results are given in Table 10, from which it will be seen that the mean stalk

Table 9. Results of Two Methods of Field Inoculation of Minnesota Amber Sorghum With 10 Lines* of Sphacelotheca sorghi

Smut lines and source	Seed inoculated with dry chlamydospores					Seed inoculated with water-suspended chlamydospores				
	Row	Total no. heads	Total no. smutted heads	Percentage smutted heads	Average	Row	Total no. heads	Total no. smutted heads	Percentage smutted heads	Average
2	a	51	41	80.3		a	37	16	43.2	
A1 × A2	b	73	49	67.1	74.3	b	42	33	78.6	59.0
	c	71	55	77.4		c	31	16	51.6	
4	a	67	28	41.8		a	59	27	45.7	
A1 × A4	b	54	16	29.8	40.2	b	57	20	36.9	35.8
	c	68	32	47.0		c	43	10	23.2	
22	a	73	55	75.3		a	46	21	45.6	
$A3_1$ × A3	b	55	41	74.5	74.0	b	45	23	51.1	48.6
	c	65	47	72.3		c	18	9	50.0	
34	a	78	61	80.7		a	47	15	31.9	
$A1_1$ × A2	b	65	45	70.0	70.7	b	45	16	35.5	31.3
	c	55	34	61.8		c	61	17	27.8	
67	a	56	33	58.9		a	47	35	74.4	
$B1_1$ × B2	b	70	43	61.4	60.5	b	17	8	47.0	57.5
	c	54	33	61.1		c	42	18	42.8	
76	a	82	50	60.8		a	44	17	38.6	
$B1_1$ × $B2_1$	b	67	38	56.7	62.2	b	37	18	48.7	43.4
	c	47	34	59.6		c	64	28	43.9	

Table 9.—Continued

Smut lines and source	Seed inoculated with dry chlamydospores					Seed inoculated with water-suspended chlamydospores				
	Row	Total no. heads	Total no. smutted heads	Percentage smutted heads	Average	Row	Total no. heads	Total no. smutted heads	Percentage smutted heads	Average
87	a	84	62	71.4		a	25	13	52.0	
$C1_2 \times B2_1$	b	56	37	66.0	69.1	b	24	9	32.5	**52.2**
	c	64	42	65.6		c	18	13	72.2	
95	a	83	52	61.9		a	46	18	39.1	
$B1_1 \times C3$	b	60	38	63.3	65.9	b	48	16	33.2	**41.2**
	c	95	67	70.5		c	54	27	50.0	
99	a	67	49	73.1		a	42	16	38.0	
$C1_2 \times C3$	b	58	42	72.4	74.8	b	36	13	36.1	36.2
	c	66	52	78.7		c	38	13	34.2	
122	a	67	57	85.0		a	55	19	34.5	
$B2_1 \times C2_3$	b	47	37	79.1	77.6	b	59	22	37.2	38.3
	c	83	59	71.0		c	45	20	44.4	
Check	a	59	0	0		a	42	0	0	
	b	60	0	0	0	b	45	0	0	0
	c	55	0	0		c	44	0	0	

* The F_1 smut lines used as inoculum were obtained from greenhouse-reared, inoculated sorghum plants.

Table 10. Mean Difference and Range in Lengths and Diameters of Chlamydospores and Smut Sori of 10 Lines of Sphacelotheca sorghi and Mean Differences in Length of Sorghum Stalks and Heads Parasitized by 10 Lines of S. sorghi

Lines	Chlamydospores		Smut sori				Sorghum stalks		Sorghum heads	
	Mean diameter* and range in μ		Mean length† and range in mm.		Mean diameter‡ and range in mm.		Mean length and range in cm.		Mean length and range in cm.	
Brown peridia										
2	5.727	4.8–6.5	5.139	2.3–9.4	1.886	1.3–2.7	209.92	100.0–271.0	16.88	12.0–23.0
4	5.652	4.7–6.8	5.094	2.8–8.0	1.945	1.4–2.4	210.43	80.0–277.0	15.24	7.0–20.5
22	5.730	4.8–6.7	4.735	2.3–8.9	1.993	1.3–2.7	215.24	81.0–273.0	15.69	7.5–24.0
34	5.726	4.1–7.2	4.712	2.3–9.4	1.964	1.3–2.4	212.44	40.0–266.0	15.75	6.5–22.0
Gray peridia										
67	4.581	3.6–5.3	3.884	2.1–6.5	2.050	1.2–2.8	202.63	85.0–274.0	13.40	6.0–21.0
76	4.478	3.7–5.3	4.043	2.5–7.3	1.880	1.0–2.9	191.09	92.0–263.0	12.51	4.0–19.5
87	4.396	3.2–5.3	3.722	2.2–6.4	1.815	1.0–2.7	213.41	130.0–286.0	13.03	5.0–22.0
95	4.830	3.2–6.1	3.532	1.9–6.6	1.959	1.0–3.3	145.90	67.0–246.0	9.28	5.0–22.0
99	4.340	3.6–5.4	4.009	2.3–7.0	2.003	1.3–2.7	203.74	82.0–275.0	12.70	6.0–21.0
122	4.316	3.3–5.2	3.541	1.9–5.9	1.844	1.3–2.8	220.20	121.0–283.0	13.82	6.5–19.5
Check							241.90	139.0–286.0	20.00	11.0–24.0

* Minimum significant difference between lines, 0.14 μ at the five per cent point; 0.16 μ at the one per cent point.
† Minimum significant difference between lines, 0.24 mm. at the five per cent point; 0.31 mm. at the one per cent point.
‡ Minimum significant difference between lines, 0.09 mm. at the five per cent point; 0.12 mm. at the one per cent point.

length of healthy plants was 21.7 cm. greater than that of stunted plants in the plot least affected and 96.9 cm. greater than the mean stalk length of stunted plants in the most severely affected plot. The mean length of healthy heads was 20.0 cm., or 3.2 cm. greater than the longest smutted heads and 10.72 cm. greater than the mean length of the shortest smutted heads. Stunting was particularly evident in the plants smutted by lines 76 and 95. In general, the individual stunting effects of lines 2, 4, 22, and 34 on sorghum heads were about the same but less than those of lines 67, 76, 87, 95, 99, and 122. (See Fig. 10.) The latter results are noteworthy because lines 2, 4, 22, and 34 were derived from a single and different chlamydospore than were the remaining six lines.

Peridial Color in Smut Sori

Kulkarni (28) states that the peridial membrane of *Sphacelotheca sorghi* is of two sorts of colors: brownish, turning to dull gray, and shining gray, which turns to pale gray on maturity. Reed (35) stated that the membrane of *S. cruenta* appeared light gray, but the color of the peridial membrane of *S. sorghi* was not mentioned. Melchers, Ficke, and Johnston (31) described the peridial color of five physiologic races of typical *S. sorghi* on different varieties. Forms 1 and 4 were brown on all varieties. Forms 2, 3, and 5 were brown on some and white on others. From their results, it appears that peridial color is dependent on the host reaction to different physiological races.

Rodenhiser (40) stated that there were intergrading color types in inbred lines of *S. sorghi* as well as in hybrid lines derived from species crosses. Of greater significance are the more recent results reported by Rodenhiser (41). He crossed *S. sorghi* (reddish-brown peridia) with *S. cruenta* (gray peridia), and the F_1 hybrid was of the loose smut type with gray peridia. He also back-crossed the F_1 hybrids with the *S. sorghi* parent and found that the progeny segregated into three color groups, reddish-brown, grayish-brown, and gray.

The writer observed striking differences in peridial color on smutted plants grown in the previously mentioned field plots. Two color groups were prevalent, reddish-brown and silver-gray (Fig. 8). Furthermore, it was found that the peridia of sori in plants smutted by chlamydospore lines 2, 3, 22, and 34 were reddish-brown, while those plants smutted by lines 67, 76, 87, 95, 99, and 122 were silver-gray. Since the reddish-brown lines were originally obtained by crossing monosporidial lines of Texas A, and the silver-gray lines by crossing monosporidial lines of Texas B and of Texas C in various combinations, it is not surprising that two distinct color groups were differentiated. But it is noteworthy that chlamydospores Texas A, B, and C, from which the two color groups were derived, came from a single smut ball with brown peridium. To explain the presence of the two kinds of chlamydospores in a single smut ball, it is suggested that mutation occurred during the parasitic

Table 11. The Degree of and Time Required for Germination of Chlamydospores in F_1 and F_2 Lines of Sphacelotheca sorghi; Cultures Incubated at 25° C.

No. hours after test was started	Chlamydospore lines and degree of germination*									
	2	4	22	34	67	76	87	95	99	122
A. F_1 chlamydospores of *S. sorghi* produced in the greenhouse by inoculating Minnesota Amber sorghum with different pairs of monosporidial lines. Test May 13, 1933										
3	Tr	Tr	Tr	0	0	0	0	0	0	0
4	G	G	G	F	0	0	0	0	0	0
7	Sp	Sp	Sp	G;Sp.0	Tr	Tr	Tr	Tr	Tr	Tr
10	Sp+	Sp+	Sp+	Sp	G	G	G	G	G	G
12	Sp+	Sp+	Sp+	Sp	Sp−	Sp+	Sp−	Sp−	Sp−	Sp−
B. F_2 chlamydospores subsequently produced in the field on Minnesota Amber sorghum by inoculating seed with F_1 chlamydospores. Test November 28, 1933										
3	Tr+	Tr+	Tr+	Tr	0	0	0	0	0	0
4	G;Sp.0	G;Sp.0	G;Sp.0	G;Sp.0	0	0	0	0	0	0
5	E;Sp.0	E;Sp.0	E;Sp.0	E;Sp.0	Tr	Tr+	Tr	Tr	Tr+	Tr
6	Sp	Sp−	Sp+	Sp.0	G	G	F	Tr+	G	G
8	Sp+	Sp+	Sp+	Sp−	G;Sp−	E;Sp+	G;Sp−	G;Sp.0	Sp−	G;Sp.0
C. Replication, using F_2 chlamydospores from same lots collected for use in (B). Test December 21, 1933										
3	Tr+	Tr+	Tr	Tr+	0	0	0	0	0	0
4	G;Sp.0	G;Sp.0	G;Sp.0	G;Sp.0	0	0	0	0	0	0
5	Sp−	E;Sp.0	E;Sp−	E;Sp−	Tr+	Tr+	Tr+	Tr+	Tr	Tr
6	Sp+	Sp−	Sp	Sp	G	G	G	G	G	G
8	Sp+	Sp+	Sp+	Sp+	Sp−	Sp+	Sp−	Sp−	Sp−	Sp−−

* 0=no germination
Tr=trace of germination
F=fair germination
G=good germination
E=excellent germination
Sp=sporidia present, few present −; very few − −; numerous +
Sp.0=no sporidia present

phase of the fungus, or that two physiologic races of *Sphacelotheca sorghi* parasitized a single culm, finally became closely associated, and formed chlamydospores in the same ovary. Another possibility is that the two color groups differentiated here had their origin through hybridization in nature prior to collection of the original chlamydospore material with which this work was started.

Hardness of Smut Balls

The writer has noticed differences in hardness of smut balls in the case of the brown and gray peridial smut lines. Smut balls of the lines with brown peridia were harder and less easily broken than those with gray peridia. Melchers, Ficke, and Johnston (31) indicated varying degrees of rupturing in the smut sori of five physiologic races. Flor (20) noted differences in hardness of smut balls in races of *Tilletia levis* and *T. tritici*. Further studies are needed to determine whether this may be a constant difference between strains of *Sphacelotheca sorghi*.

Differences in Time Required for Chlamydospore Germination

Preceding the inoculation of sorghum seed with the F_1 chlamydospore lines for the 1933 field work, germination tests were made with chlamydospores of each line to ascertain whether they were viable. All lines had developed in the greenhouse at the same time and under the same conditions. They were collected at the same time and stored under the same conditions. The technique used was as follows: Chlamydospores of each of the F_1 lines were dusted on drops of potato-dextrose agar on triplicate glass coverslips which were then inverted over van Tieghem cells in moist Petri dishes and kept at approximately 25° C. The results are given in section A of Table 11.

Spores of all lines with reddish-brown peridia—2, 4, 22, 34—began to germinate in about three hours, while those with silver-gray peridia—67, 76, 87, 95, 99, 122—required seven hours. Sporidia were produced in the first group in from five to seven hours, while in the second group the time required for sporidial formation was 8 to 11 hours after the tests were started. The final percentage of germination was high throughout, but spores of the reddish-brown group of lines are clearly about four hours faster than those of the silver-gray group. That this difference is due to genetic factors is shown by the fact that similar results were obtained when germination tests were made of F_2 chlamydospores resulting from inoculating plants with the above-mentioned F_1 lines. (See Table 11.)

The writer's results with *Sphacelotheca sorghi* support those of Holton (26) who showed that two races of *Tilletia tritici* differed to some extent with respect to time required for chlamydospore germination.

Size of Smut Sori

According to Clinton (12), the sori or smut balls of *Sphacelotheca sorghi* form "oblong to ovate bodies 3 to 12 mm. in length." Kulkarni

(28) describes the smut sori as follows: "The smut ball is an elongated, sometimes slightly bent when long, thickened, club-shaped body, tapering to a point at the free end. Sometimes it assumes an oblong shape with a blunt end." He states that they vary from 3 to 12 mm. in length and 2 to 4 mm. in width. Melchers, Ficke, and Johnston (31) classified the relative length of smut sori from five physiologic races as long,

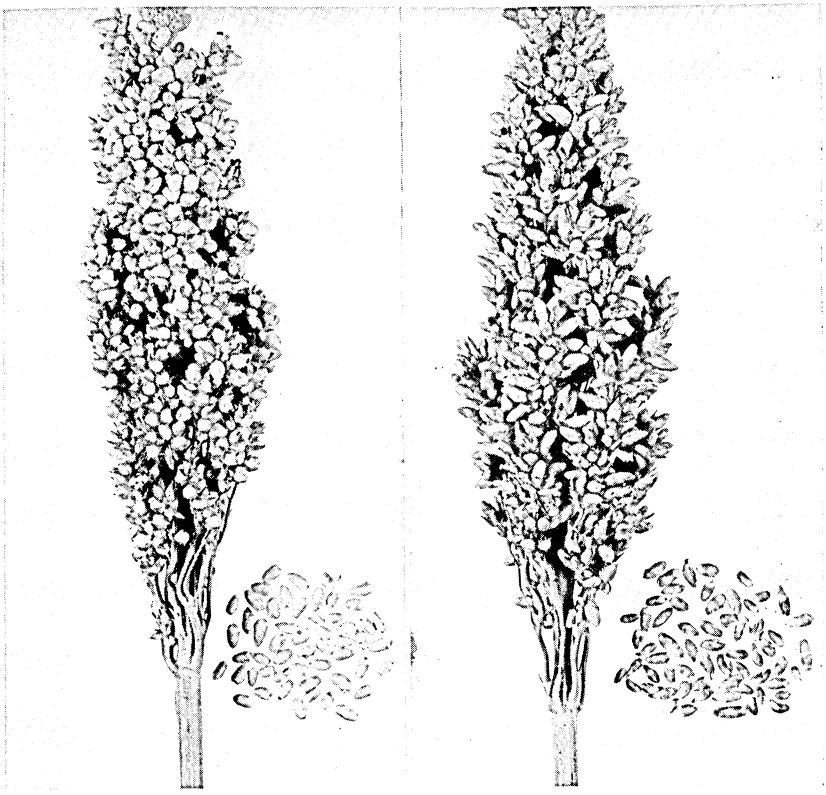

FIG. 8. HEADS OF SMUTTED MINNESOTA AMBER SORGHUM SHOWING TWO DISTINCT COLOR TYPES OF THE PERIDIA

Left, smutted head typical of the gray peridial group; right, smutted head typical of the brown peridial group. Note the apparent differences in length of gray and brown smut sori.

medium, or short, and based the classes on difference in protrusion of the sori beyond the glumes. Races 1, 2, 4, and 5 developed intermediate types, while those of Race 3 were long. They give no figures, but concluded that "it is doubtful whether the five physiologic forms of *S. sorghi* can be separated solely on the basis of observable differences in sori."

Table 12. Analysis of Variance of Smut Ball Size in 10 Lines of Sphacelotheca sorghi

Variation	Sum of squares	Degrees of freedom	Mean squares	*Z*
		Length		
Between lines	848.2409	9	94.24898	2.4268*
Within lines	727.4099	990	0.7348	
Total	1575.6508	999		
		Diameter		
Between lines	5.0825	9	0.5647	0.7813*
Within lines	117.1773	990	0.11836	
Total	122.2608	999		

* Value of *Z* exceeds the one per cent point.

Table 13. Analysis of Variance in Diameter of 10 Chlamydospore Lines of Sphacelotheca sorghi

Variation	Sum of squares	Degrees of freedom	Mean squares	*Z*
Between lines	375.38084	9	41.7090	2.5747*
Within lines	239.66740	990	0.2421	
Total	615.04824	999		

* Value of *Z* exceeds the one per cent point.

Among F_2 lines 2, 4, 22, 34, 67, 78, 87, 95, 99, and 122 there appeared to be differences in size of smut balls, and these differences seemed correlated with peridial color. Accordingly, the length and diameter of a random sample of 100 smut sori were recorded for each of the 10 F_2 lines. The data were analyzed by Fisher's analysis of variance method (19).

Lengths and diameters of smut sori from different lines are shown in Table 10 and Figure 9, and the analysis of variance in Table 12. The value of *Z* in these experiments was found to exceed Fisher's calculated value of *Z* at the one per cent point (19). Minimum significant differences[4] between means are 0.31 mm. for length and 0.12 mm. for diameter at the one per cent point, and 0.24 mm. and 0.09 mm., respectively, at the five per cent point.

A comparison of the mean smut sori lengths in Table 10 shows that the gray peridial group is significantly different from the brown peridial group, even at the one per cent level of significance. When the mean differences in the diameters of the sori are compared (Table 10), significant differences are found between certain lines, but there is

[4] The standard of significance here used is based upon the standard error of the difference between two means. This is represented by the formula $\sqrt{\frac{O^2 \times 2}{N}}$ where the standard deviation squared (O^2) is equal to the mean square for error found in the analysis of variance in each experiment and *N* equals the total number of replications in a given mean. The minimum significant difference between any two means is obtained by multiplying the standard error of the difference between two means by the "t" value corresponding to the number of degrees of freedom for error at the one or five per cent points, depending on which is selected as the minimum level of significance. See Fisher's Table of "t" (19).

no significant difference in the diameters of the gray and brown peridial groups as groups. However, it should be noted that lines 87 and 122 are significantly different from the others at the one per cent point.

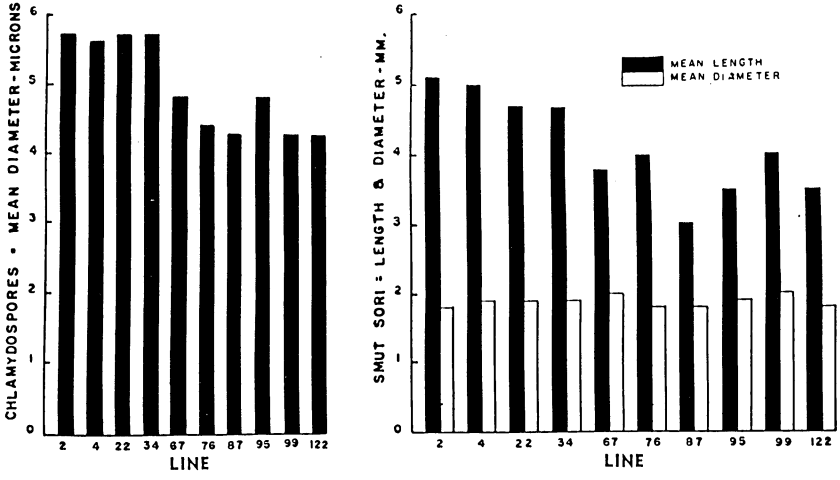

FIG. 9. MEAN DIAMETERS OF CHLAMYDOSPORES AND MEAN LENGTHS AND DIAMETERS OF SORI OF TEN LINES OF SPHACELOTHECA SORGHI.

Left, mean differences in diameter of chlamydospores; right, mean differences in length and diameter of smut sori.

Because of the highly significant differences in length of the lines in the gray peridial group as compared with those with brown peridia, it must be concluded that these two groups are morphologically distinct. The writer's results gain greater significance since Holton (26) was able to distinguish two physiologic races of *Tilletia tritici* on the basis of size and shape of the smut balls. One produced large, elongate sori while the other produced small, rounded sori. He stated further that these differences were sufficient to identify each form positively.

Size of Chlamydospores

Clinton (12) gives the range in size of chlamydospores of *Sphacelotheca sorghi* as 5.5μ—8.5μ. Potter (33) states that they average less than 6.0μ, while Kulkarni (28) says they range between 4.0 and 6.0μ. The range in size of spores given by Melchers, Ficke, and Johnston (31) is from 5.0 to 9.0μ, and they stated that no significant differences were noted in size of chlamydospores of five physiologic races.

Morphologic differences between so-called physiologic races of smut fungi are not unusual. Flor (20) has found physiological and morphological differences between races of *Tilletia levis* and *T. tritici*, such as size, color, and character of spore wall, and hardness of bunt balls. Christensen (9) found statistically significant differences in size of spores of *Ustilago zeae* from different localities and sources.

On microscopic examination of chlamydospores developed in the field by the 10 F_1 chlamydospore lines used in the previous studies, there appeared to be differences in size. Random 100-spore samples of each of the 10 lines were measured. One measurement was made on each spore, since they are mostly spherical or nearly so. The mean diameters and range are shown for each spore collection in Table 10 and Figure 9. A similar statistical analysis was made as in the preceding section for size of smut balls.

Since the value of Z exceeded the one per cent point, the data for differences in size were likewise highly significant (see Table 13). The required mean differences in diameter for significance between any two means at the five per cent and one per cent points were found to be 0.14μ and 0.16μ, respectively. From a comparison of means in Table

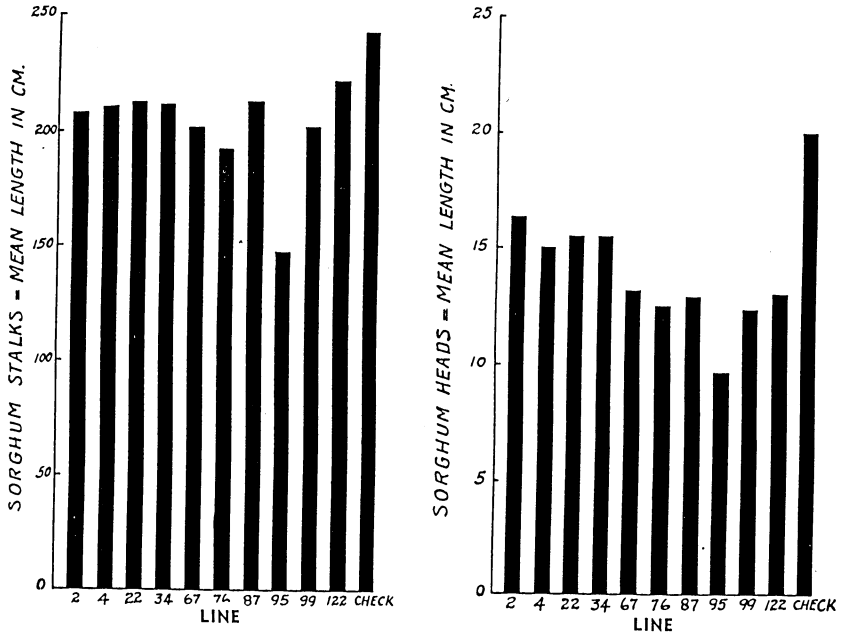

FIG. 10. MEAN LENGTHS OF STALKS AND HEADS OF SORGHUM PARASITIZED BY TEN LINES OF SPHACELOTHECA SORGHI.

Left, mean differences in length of stalks of Minnesota Amber sorghum parasitized by ten lines of *S. sorghi*, plus non-smutted check; right, mean differences in length of heads of Minnesota Amber sorghum parasitized by ten lines of *S. sorghi*, plus nun-smutted check.

10, it will be seen that the brown peridial group is distinctly different in spore size from the gray peridial group. Therefore, the two groups are considered different from each other on the basis of size of chlamydospores.

It is also evident that there are significant differences in spore size between members of the gray group. For example, lines 87, 99, and 122

are significantly different from lines 67 and 95 at either the 5.0 or 1.0 per cent minimum levels of significance. There are no significant differences in size of chlamydospores between members of the brown group. These results are noteworthy because, as explained before, the members of the brown group were originally derived from inbred lines of a single chlamydospore, resulting in uniformity in size of chlamydospores of the four reddish-brown lines. On the other hand, the individual lines of the gray peridial group were originally derived from inbred and outbred monosporidial lines from two chlamydospores and resulted in nonuniformity of chlamydospore size in the gray lines.

In general it must be concluded that intra-specific hybridization between monosporidial lines may result in the production of morphologically distinct races of *Sphacelotheca sorghi,* on the basis of chlamydospore size.

Shape of Sterile Cells

According to Clinton (12) and Potter (33), the sterile cells of the sorus in *Sphacelotheca sorghi* are chiefly elongate and narrow in diameter and adhere in thread-like groups, while in *S. cruenta* they are more nearly spherical and separate easily into small groups. Rodenhiser (40) found both types of sterile cells in inter-specific hybrids, in intra-specific crosses, and in microscopically distinct specimens of both species. The writer observed that smut balls with gray peridia were more susceptible to mechanical injury than those with brown peridia and were therefore more like *S. cruenta.* A microscopic examination was made, according to Rodenhiser's technique,[5] of sterile cells in sori from each of the previously mentioned F_2 lines in an attempt to determine whether or not two species of kernel smut were actually represented. The sterile cells in sori of the brown lines were elongate and definitely of the *S. sorghi* type. In the sori examined from the gray lines, occasional spherical sterile cells of the *S. cruenta* type were found, but it is emphasized that the narrow elongate sterile cells greatly predominated in these lines. Therefore, based upon structure, they were more nearly of the *S. sorghi* type.

DISCUSSION AND CONCLUSIONS

In general, the life cycle of *Sphacelotheca sorghi* is like that of several species of *Ustilago,* but there is far greater variability and more deviation from type in the sorghum smut than has been supposed. The chlamydospore of *S. sorghi* appears to be a zygote containing a single diploid nucleus. When the spore germinates, the diploid nucleus undergoes meiotic division and each cell of the three- or four-celled promycelium usually contains a single haploid nucleus. Sporidial buds are then abjointed from each cell of the promycelium, resulting in the successive production of sporidia which typically are uninucleate and should be

[5] Personal correspondence with Dr. H. A. Rodenhiser.

haploid. The conjugation of two sporidia of opposite sex is prerequisite for infection and ultimate production of chlamydospore zygotes in sorghum plants.

It is clear from the studies made by the writer that the cytological phenomena in *Sphacelotheca sorghi* do not conform to type nearly so much as is suggested by Brefeld's (6) work and some subsequent investigations. In fact, chlamydospore germination in *S. sorghi* is so variable that one is scarcely justified in picturing a normal type.

Segregation for sex was studied in the sporidial progeny of nine chlamydospores of *Sphacelotheca sorghi.* The methods previously used for sex determination were rather cumbersome and time-consuming. Consequently, the writer tried the Bauch test and found it to be entirely reliable when made on a satisfactory medium. Factors for sex usually segregated in the ratios of 2:2, or 1:3, indicating the existence of two sex groups in *S. sorghi.* However, the behavior of certain inbred monosporidial lines indicates that there are more than two sexual compatibility groups in *S. sorghi.*

Segregation of factors for cultural characters seems considerably more complicated than that of factors for sex. The writer found four ratios: 2:2, 4:0, 3:1, and 2:1:1. Factors for cultural characters sometimes segregated at the same time as those for sex, but sometimes they segregated independently. Some evidence was obtained which suggested strongly that segregation was completed within the nuclear divisions of the germinating chlamydospore and that delayed segregation is not the cause of later variation, such as sectoring in monosporidial lines grown on nutrient media.

The so-called irregularities found in cytological phenomena suggest that variation in segregation ratios for sex and for cultural characters in *Sphacelotheca sorghi* may be expected. It seems improbable that a sex ratio of 1:3 would result from normal segregation. It would seem logical to attribute such a ratio to irregularities in nuclear divisions and in formation of septa within certain promycelia.

Sector variants appear frequently in cultures of some monosporidial lines on some media. Sectors did not appear to a great extent on ordinary potato-dextrose or peptone agar media, but on malt agar and plain sugar media plus nutrient salts, particularly nitrogenous salts, sectoring is very common. There are decided differences in the tendency of different monosporidial lines to produce sectors. In general, newly isolated lines sector more freely on an appropriate medium than do older lines, particularly if the latter are mycelioid.

Since media exert a profound effect on sectoring, it is evident that lines having arisen as sectors in artificially cultured monosporidial lines of *Sphacelotheca sorghi* must be grown and compared on many different media over a sufficiently long period of time before justifiable conclusions can be drawn concerning their genotypic differences. In studying the phenomenon of sectoring in *S. sorghi,* therefore, the same pre-

cautions are necessary as those pointed out by Stakman *et al.* for *Ustilago zeae* (43).

The evidence concerning the nature of sectoring in smut fungi is conflicting; workers, therefore, have come to no general agreement. At present there are several explanations offered, the principal ones of which are mutation, delayed segregation, and heterocaryosis (7, 9, 10, 15, 16, 17, 25, 43, 44).

It seems improbable to the writer that sectoring in *Sphacelotheca sorghi* is due in any great measure to heterocaryosis. Although cytological studies show that promycelia are sometimes incompletely septate and that at times several nuclei may be present in a single promycelial cell, the sporidia abjointed by promycelial cells which were fixed and stained were never observed to have more than one nucleus. Older sporidia preparing to bud often have several nuclei. Such necessarily limited observations do not remove the possibility of heterocaryosis, but they do suggest that if it occurs at all it plays only a minor part.

Studies of segregation for cultural characters in *Sphacelotheca sorghi* indicate that some normal sort of segregation certainly takes place in the first two nuclear divisions within the promycelium, that it is not confined to a single meiotic division, and that it may take place independently of segregation for sex characters. Single monosporidial lines were always haploid sexually, yet the majority of those studied were culturally unstable and produced some sectors. A detailed study of the distribution of sectors produced by monosporidial lines from two chlamydospores showed that segregation certainly had taken place in nuclear divisions within the promycelia and spores, and that this segregation had a definite bearing on the sectors later produced by the haploid lines from these spores. Therefore, it seems improbable that delayed segregation played any part in sectoring in lines of *S. sorghi* studied by the writer.

The origin, by mutation, of so large a number of sectors in cultures of smut fungi may seem surprising, but one must remember that there are millions of individuals in a smut culture which consists largely of sporidia. Therefore, the apparent rate of mutation is not nearly so high as it seems. Also, the simultaneous appearance of sectors of the same cultural type in a monosporidial line or the same type in different monosporidial lines from the same promycelium does not seem surprising. Closely related lines must have some factors for cultural characters in common, and if there are forces which operate to cause mutation in a certain character-promoting gene, then closely related lines having some common characters might easily be influenced to mutate in the same direction. Such a conjecture is not necessarily true, but the theory seems tenable.

Further, if chlamydospore and sporidial lines of *Sphacelotheca sorghi* are in a state of high genetic impurity, a condition that Brierley (7) suggests for fungi in general, the chances for a high rate of sectoring due to mutation seem even more probable. In the case of results obtained

with *S. sorghi,* where certain monosporidial lines remained culturally constant for more than a year and then suddenly produced new cultural types through sectoring on artificial media, it is difficult to conceive of segregation having been delayed for so long a time. It is more probable that sectoring of the type described in cultures of *S. sorghi* by the writer is due to mutation—mutation in the sense that Stakman *et al.* (43) used the term.

Various workers have shown, as stated previously, that there are parasitic races of *Sphacelotheca sorghi* and that different varieties of sorghum react differently toward *S. sorghi.* Further, it has been shown that inter- and intra-specific hybridization results in new smut lines which differ in physiological and morphological characters. Hybridization of monosporidial lines of the common sorghi strains used in the present studies likewise showed that new lines may arise which differ both in physiological and morphological characters.

Evidence was obtained by the writer which definitely showed that F_1 chlamydospore lines, produced as a result of inbreeding and outbreeding between monosporidial lines from different chlamydospores, may vary with respect to their stunting effects during the parasitic phase. Furthermore, these same lines differ with respect to color of peridia, hardness of smut balls, and in time required for chlamydospore germination. Results also show that these differences are heritable in the F_2 generation. Observations and measurements made of smut balls and chlamydospores from lines in the F_2 generation showed that these lines differed distinctly with respect to size. Further, morphological differences were definitely correlated with physiological differences. For example, small chlamydospores and short, ovoid smut balls were associated with gray peridial color, while larger spores and elongated, slender smut balls were associated with brown peridial color.

In general, it must be concluded that *Sphacelotheca sorghi* is highly variable and consists of an indefinite number of physiological and morphological entities which may arise as a result of irregularities in cytological phenomena, through hybridization, and through mutation.

SUMMARY

1. Germinating chlamydospores of *Sphacelotheca sorghi* have been described and figured. Cytological phenomena associated with chlamydospore germination show that there is far greater variability and more deviation from normal type than has been supposed. In its essentials, the life cycle of *S. sorghi* is like that of several species of *Ustilago.*

2. Sporidial fusions and the Bauch test proved to be reliable and expedient methods for determining sex in *Sphacelotheca sorghi.* Evidence was obtained, in support of Rodenhiser's findings, that the sexual compatibility of paired lines may be determined soon after sorghum plants have been inoculated.

3. No delay in segregation of factors for sex was observed; segregation of such factors apparently was complete in the first or second nuclear divisions of the germinating spore. Two sex groups were found, and the factors for sex were found to segregate in the ratios of 2:2 and 1:3. Some results obtained indicated that there are more than two sexual compatibility groups in *S. sorghi.*

4. Four ratios of segregation of factors for cultural characters were found: 2:2, 4:0, 3:1, and 2:1:1. The segregation of factors for cultural characters takes place independently of those determining sex.

5. When artificially cultured, *Sphacelotheca sorghi* comprises an indefinite number of cultural types which may differ in one or more of the following cultural characters: size, color, type of growth, direction of growth, consistency of colonies, topography, and type of margin. Further studies are needed to ascertain the pathogenicity of paired, compatible lines which arose as sectors and thus establish the importance of new lines arising through mutation.

6. Nutrients appear to affect the rate of sectoring. Malt agar and plain sugar media plus nutrient salts, principally nitrogenous salts, appear to induce sectoring. Potato-dextrose, plain sugar, and peptone agar media did not induce sectoring to any extent in the lines tested.

7. Some lines sector abundantly, others do so only rarely. Fourteen lines remained culturally constant for more than a year when grown on potato-dextrose agar. However, eight of them produced one or more sectors of new cultural type when later grown on malt agar.

8. Lines of *Sphacelotheca sorghi* which arose through intra-specific hybridization between monosporidial lines from different chlamydospores differed in the following physiological and morphological characters: pathogenicity as indicated by stunting, color of peridia, size and hardness of smut balls, size of chlamydospores, and length of time required for chlamydospore germination.

LITERATURE CITED

1. Bauch, R. Kopulationsbedingungen und sekundäre Geschlechtsmerkmale bei *Ustilago violacea.* Biol. Centralbl. **42**:9-38. 1922.
2. ————. Ueber *Ustilago longissima* und ihre Varietät *macrospora.* Ztschr. Bot. **15**:241-279. 1923.
3. ————. Untersuchungen über die Entwickelungsgeschichte und Sexual Physiologie der *Ustilago bromivora* und *Ustilago grandis.* Ztschr. Bot. **17**:129-177. 1925.
4. ————. Rassenunterschiede und sekundäre Geschlechtsmerkmale beim Antherenbrand. Biol. Centralbl. **47**:370-383. 1927.
5. ————. Die Sexualität von *Ustilago scorzonerae* und *Ustilago zeae.* Phytopath. Zeit. **5**:315-321. 1932.
6. Brefeld, O. *Ustilago tulasnei* auf *Sorghum vulgare* (*U. sorghi* Link), Untersuchungen aus dem Gesamtgebiete der Mykologie, Heft 12. Die Brandpilze III. p. 120-122. 1895.
7. Brierly, W. B. Biological races in fungi and their significance in evolution. Ann. App. Biol. **18**:420-434. 1931.

8. BUTLER, E. J. Fungi and disease in plants, pp. vi and 547, pls. 5, figs. 201. Thacker, Spink & Co., Calcutta. 1918.
9. CHRISTENSEN, J. J. Studies on genetics of *Ustilago zeae*. Phytopath. Zeit. **4**:129-188. 1931.
10. ———— and E. C. STAKMAN. Physiologic specialization and mutation in *Ustilago zeae*. Phytopath. **16**:979-999. 1926.
11. CLINTON, G. P. Broom-corn smut. Ill. Agr. Exp. Sta. Bul. **57**:289-360. 1897.
12. ————. Ustilaginales. North American Flora **7**:1-82. 1906.
13. DICKINSON, S. A method of isolating and handling individual spores and bacteria. Proc. Roy. Soc. Med. London **19**:1-4. 1926.
14. ————. Experiments on the physiology and genetics of the smut fungi. Cultural characters. Their permanence and segregation. Proc. Roy. Soc. London, Ser. B, **103**:547-555. 1928.
15. ————. Experiments on the physiology and genetics of the smut fungi. Cultural characters. 2. The effect of certain external conditions on their segregation. Proc. Roy. Soc. London, Ser. B, **108**:395-423. 1931.
16. ————. The nature of saltation in *Fusarium* and *Helminthosporium*. Minn. Agr. Exp. Sta. Tech. Bul. 88. 1932.
17. EDDINS, A. H. Pathogenicity and cultural behavior of *Ustilago zeae* (Beckm.) Ung. from different localities. Phytopath. **19**:885-916. 1929.
18. FICKE, C. H., and C. O. JOHNSTON. Cultural characteristics of physiologic forms of *Sphacelotheca sorghi*. Phytopath. **20**:241-249. 1930.
19. FISHER, R. A. Statistical methods for research workers. 4th ed. 307 pp. Oliver and Boyd, London. 1932.
20. FLOR, H. H. Studies on physiologic specialization in *Tilletia tritici* and *T. levis* in the Pacific Northwest. Jour. Agr. Res. **47**:193-214. 1933.
21. HANNA, W. F. A simple apparatus for isolating single spores. Phytopath. **18**:1017-1021. 1928.
22. ————. Studies in the physiology and cytology of *Ustilago zeae* and *Sorosporium reilianum*. Phytopath. **19**:415-442. 1929.
23. HANNA, W. F., and W. POPP. Relationship of the oat smuts. Nature **126**: 843-844. 1930.
24. HEALD, F. D. The relation of spore load to the percentage of stinking smut appearing in the crop. Phytopath. **11**:269-278. 1921.
25. HOLTON, C. S. Studies in the genetics and the cytology of *Ustilago avenae* and *Ustilago levis*. Minn. Agr. Exp. Sta. Tech. Bul. 87. 1932.
26. ————. Differences in size and shape of smut balls of two physiologic forms of *Tilletia tritici* (Abst.). Phytopath. **23**:16. 1933.
27. ISENBECK, K. Untersuchungen über die Physiologie von *Sphacelotheca sorghi*, den gedeckten Körnerbrand von Sorghum. Phytopath. Zeit. **8**:165-182. 1935.
28. KULKARNI, G. S. Smuts of Jowar (sorghum) in the Bombay presidency. Agr. Res. Inst. Pusa Bul. 78. 1918.
29. ————. The susceptibility of dwarf milo sorghum to smut. Phytopath. **11**:252. 1921.
30. MELCHERS, L. E. Belated development of kernel smut *(Sphacelotheca sorghi)* in apparently healthy sorghum plants. Jour. Agr. Res. **47**:343-350. 1933.
31. MELCHERS, L. E., C. H. FICKE, and C. O. JOHNSTON. A study of the physiologic forms of kernel smut *(Sphacelotheca sorghi)* of sorghum. Jour. Agr. Res. **44**:1-11. 1932.

32. NORTON, J. B. S. A study of the Kansas Ustilagineae especially with regard to their germination. Trans. Acad. Sci. St. Louis 7:229-241. 1898.
33. POTTER, A. A. The loose kernel smut of sorghum. Phytopath. 5:149-154. 1915.
34. ———— and L. E. MELCHERS. Resistance of sorghum types to covered kernel smut (Abst.). Phytopath. 8:71. 1918.
35. REED, G. M. Varietal resistance and susceptibility of sorghums to *Sphacelotheca sorghi* (Link) Clinton and *Sphacelotheca cruenta* (Kühn) Potter Mycologia 15:132-143. 1923.
36. REED, G. M., and L. E. MELCHERS. Sorghum smuts and varietal resistance in sorghums. U. S. D. A. Bul. 1284. 1925.
37. RIDGWAY, R. Color standards and nomenclature. A. Hoen and Co., Baltimore, Md. 1912.
38. RODENHISER, H. A. Physiologic specialization of *Ustilago nuda* and *Ustilago tritici*. Phytopath. 16:1001-1007. 1926.
39. ————. Physiologic specialization in some cereal smuts. Phytopath. 18:955-1003. 1928.
40. ————. Heterothallism and hybridization in *Sphacelotheca sorghi* and *S. cruenta*. Jour. Agr. Res. 45:287-296. 1932.
41. ————. Studies on the possible origin of physiologic forms of *Sphacelotheca sorghi* and *S. cruenta*. Jour. Agr. Res. 49:1069-1086. 1934.
42. SLEUMER, H. O. Ueber Sexualität und Zytologie von *Ustilago zeae* (Beckm.) Unger. Ztschr. Bot. 25:209-263. 1932.
43. STAKMAN, E. C., J. J. CHRISTENSEN, C. J. EIDE, and BJORN PETURSON. Mutation and hybridization in *Ustilago zeae*. Minn. Agr. Exp. Sta. Tech. Bul. 65. 1929.
44. STAKMAN, E. C., L. J. TYLER, and G. E. HAFSTAD. The constancy of cultural characters and pathogenicity in variant lines of *Ustilago zeae*. Bul. Tor. Bot. Club 60:565-572. 1933.
45. TISDALE, W. H., L. E. MELCHERS, and H. J. CLEMMER. Strains of kernel smuts of sorghum, *Sphacelotheca sorghi* and *S. cruenta*. Jour. Agr. Res. 34:825-838. 1927.
46. ————. A strain of sorghum kernel smut which infects milo and hegari (Abst.). Phytopath. 16:85. 1926.
47. TYLER, LEON J. Methods for determining sex differences in *Sphacelotheca sorghi* (Link) Clinton. Phytopath. 24:691-693. 1934.