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Physiologic specialization within Sphacelotheca reiliana (Kühn) Clint. on Sorghum and the Biology of its Chlamydospores in the Soil

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PHYSIOLOGIC SPECIALIZATION WITHIN SPHACELOTHECA REILIANA (KÜHN) CLINT. ON SORGHUM AND THE BIOLOGY OF ITS CHLAMYDOSPORES IN THE SOIL

BY

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Abstract

IBRAHIM AZIZ AL-SOHAILY

Under the Supervision of Professor George Semeniuk

Corresponding to the title, the present study was concerned with two aspects. In the first of these, two chlamydosporous sori from corn were collected from California and Washington and 18 chlamydosporous sori from sorghum were collected from California, New Mexico, Texas and India. Chlamydosporous cultures or paired monosporidial cultures from these were hypodermically injected as desired into seedlings of one or more of four sweet corn varieties and 57 sorghum varieties.

Chlamydosporous cultures from the two corn sources yielded head smut on three sweet corn varieties and not on any of 14 sorghum varieties. Cultures from 14 of 18 sorghum sources yielded head smut on Sugar Drip sorghum and on North Star sweet corn, while cultures from the remaining four yielded head smut only on Sugar Drip sorghum. The 18 sorghum head smut cultures were differentiated on a set of five sorghum varieties, and comprised four races according to sources; (1) California, (2) Poona 1 and Coimbatore 2, (3) Poona 2 and (4) Texas, New Mexico and Coimbatore 1. The difference between the sorghum and the corn head smut fungi accordingly was considered to be varietal, rather than racial as supposed by Reed.

The sorghum and the corn head smut fungi were readily hybridized.

Thirteen monosporidial cultures from four chlamydospores of sorghum head smut and 17 similar cultures from five chlamydospores of corn head smut yielded 108 compatible pairs, of which 105 were pathogenic on Sugar Drip sorghum and Golden Bantam sweet corn, while the other three were not pathogenic. The hybrids were less virulent than were either of the parents on their respective hosts. F_1 chlamydosporous cultures from three hybrids on Sugar Drip sorghum and from two on Golden Bantam sweet corn were pathogenic to both of these crops while a third from another hybrid on Golden Bantam sweet corn was pathogenic only on Sugar Drip sorghum. Intra-compatible sporidia from three F_1 chlamydospores representing two hybrids yielded head smut on both Sugar Drip sorghum and Golden Bantam sweet corn, while similar sporidia from a fourth F_1 chlamydospore representing a third hybrid were non-pathogenic on either of the crops.

In the second part of the study, chlamydospores germinated in soil by forming long multicellular hyphae, the lower cells of which were empty while the apical cells were filled with vacuolated or nonvacuolated protoplasm. The absence of sporidial formation may account for the low number of races found in this pathogen and for the apparent natural stability of the sorghum and corn head smut fungi as separate units.

Chlamydospore abundance in soil affected the per cent incidence of head-smutted sorghum plants. Within limits the per cent incidence of head-smutted sorghum plants was linearly related to the logarithm of the number of chlamydospores in soil. The threshold number of viable spores necessary for infection being estimated at about 800 per gram of soil.

The abundance of infectious chlamydospores in soil declined rapidly after 7 days to sub-threshold levels at a temperature of near-freezing, regardless of soil moisture. The decline at 10°, 20° and 30°C. and at soil moistures near the wilting point, 20%, 40% and 100% of field capacity was less rapid and in most instances did not reach the threshold limit within 30 days.

In supplementary studies sorghum seeds carrying 52,631 or 404,578 chlamydospores per seed failed to yield smutted plants. A dominant form of resistance to head smut was contributed by FC 811 Feterita to a sorghum hybrid.

PHYSIOLOGIC SPECIALIZATION WITHIN SPHACELOTHECA REILIANA (KÜHN) CLINT. ON SORGHUM AND THE BIOLOGY OF ITS CHLAMYDOSPORES IN THE SOIL

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Doctor of Philosophy, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser

Head of the Major Department

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INTRODUCTION

Sorghum head smut is a widely distributed disease of sorghum reported from Africa, Argentina, Brazil, China, India, Italy, Japan, Middle East, New South Wales, Soviet Union and United States. It is the result of infection of seedlings by germinating seed or soil-borne chlamydospores of the fungus Sphacelotheca reiliana (Kühn) Clint., which fungus completely destroys the inflorescence of the growing plant, replacing it with its own body of mycelium and chlamydospores. Corn is also attacked by the fungus and sustains the disease.

The head smut of sorghum as of corn is found largely in warm, dry climates such as in Texas, New Mexico, California and Washington in the United States where losses from it usually are of low order of less than one per cent, but on occasions may reach as high as 60 per cent of the inflorescences. The disease occurs sporadically and unpredictably and no great effort had been directed toward understanding its occurrence or controlling it. However the high incidence of the disease on sorghum in Texas in recent years has posed the question of why this was so, and has directed attention to breeding for resistance, to the possible existence of races of the pathogen, and to the persistence capabilities of the pathogen in the soil. Since little to no information is available on the latter two aspects of the pathogen the present study was concerned primarily with them.

REVIEW OF LITERATURE

The head smut fungus of sorghum was first described as Ustilago reiliana by Kühn in 1875 from a specimen sent him from Egypt by Reil in 1868, but later it was transferred to the genus Sphacelotheca by Clinton as Sphacelotheca reiliana (Kühn) Clint. Since then the fungus and the disease it produces have been studied by a number of investigators with limited results as summarized below.

The fungus is a warm temperature organism with chlamydospores germinating and sporidia multiplying optimally at 28°-30°C., slightly at 17° and 35°C. and not at 15° and 40°C. (18). At best fewer than 15 per cent of the chlamydospores germinate, regardless of age or source (18). Usually within 17 hours on agar media the germinated chlamydospores form promycelia having 3-5 cells (usually 4) and within 24 hours the cells produce a number of uninucleate sporidia (10, 16, 17) that may fuse with one another (14).

Plant infection follows commonly from chlamydospores in the soil (18), infrequently from chlamydospores on the seed (11, 12, 16) and not from chlamydospores sprayed over plants (7) or applied to ovaries (18). Infection does not occur from sporidia smeared on the base of young plants (18). However infection presumably may occur via crown buds when plants are cut back to allow for regrowth to take place (13). With chlamydospores in soil infection occurs over the range of 16° to 36°C. (optimum 28°C.), depending on soil moisture, and more abundantly in dry than in wet soils (6, 21). Young seedlings with plumules not more than two centimeters long were considered by Reed et al. (21) to be the

only ones that would become infected. They planted seeds and seedlings of various ages in a chlamydospore-soil mixture and found that infection was achieved from dry seeds and from 1 to 4-days old seedlings, but no infection resulted from seedlings 5 to 7-days old. Leukel (13), on the other hand, obtained infection of 2 to 9-weeks old seedlings transplanted to chlamydospore-infested soil with highest per cent infection in young seedlings and the least in old seedlings.

Chlamydospores in the soil presumably survive from one year to the next as may be judged from the perpetuation of the disease from one year to the next in certain geographical regions. Such survival however has not been the object of special study by any investigator and presumably it may depend in part on soil temperature and moisture. Reed et al. (21) mixed chlamydospores with soil for as long as 9 days before planting the mixture to seed or young seedlings of sorghum or corn and obtained infection within 3 days using dry seeds and within 5 days using seedlings. Leukel (13), on the other hand, mixed chlamydospores with moist soil 9 weeks before planting the mixture to sorghum seedlings and obtained abundant infection. The factors influencing survival thus are not clear and a special study of this aspect is needed to better understand disease incidence.

Load or concentration of chlamydospores in soil also may influence disease incidence, although no information is available on this point. Presumably the more abundant the chlamydospores are in the soil the better would be the chance that a viable spore will be favorably situated to infect a seedling. Whether more than one viable chlamydospore is

needed for this purpose is not known. Further, chlamydospores which germinate by producing sporidia or by producing infectious germ tubes directly may complicate this relationship.

Physiologic specialization in Sphacelotheca reiliana also would be expected to influence disease incidence although very little is known about this aspect of the fungus. The fungus appears specialized on sorghum and corn such that chlamydospores collected from one host will not infect the other (4, 9, 21), but the sharpness of this distinction is also lost at times under certain unknown conditions (21). Specialization within the sorghum head smut fungus to sorghum varieties is not known although presumably it may occur.

Resistance in sorghum to the sorghum head smut fungus has been observed in all of the major classes, and groups of that crop, which usually is classified agronomically (15) as follows:

I- Annual sorghum (Sorghum vulgare Pers.)

A- Sorgo (sweet or saccharine sorghum)

B- Grain sorghum

1- Milo

2- Kafir

3- Feterita

4- Durra

5- Miscellaneous--Hegari, Darso, Schrock, Shallu and Kaoliang

C- Broomcorn

D- Grass sorghum--Sudangrass

II- Perennial sorghum (Sorghum halepense (L.) Pers.)

A-Johnson grass

Reed and Melchers (20) field tested upwards of 61 varieties at the Amarillo Field Station, Texas, during 1916, 1917 and 1919 and reported the most smut among 17 sorgo and 4 miscellaneous varieties (0 to 28 per cent), the next most among 9 durra variety (0 to 18 per cent), next among 12 kafir varieties, 1 shallu and 1 Sudangrass varieties (0 to 1.8 per cent), less than one per cent among 8 kaoliang varieties, and none among 4 broomcorn, 2 feterita and 3 milo varieties. In a similar manner during 1957 and 1958, Stewart and Reyes (22) tested 90 varieties and hybrids at Refugio and Robstown, Texas, and obtained the most smut among 3 shallu, 1 darso, 3 sorgo, 3 forage, 4 Sudangrass and 11 milo varieties (0 to 25 per cent, average 14 per cent), the next most among 42 kafir varieties (0 to 38 per cent, average 7 per cent) and none among 5 hegari and 2 feterita varieties. Among 21 hybrid sorghum tested by the latter workers, 7 showed between 10 and 20 per cent infected plants, 10 between 5 and 10 per cent infected plants, 2 near one per cent and 2 no smut. One of the hybrids with no smut, RS 630, was considered by them to carry a dominant factor for resistance from its male parent, Combine white feterita. Thus, feterita and hegari varieties at present would appear to constitute one of the important breeding sources of resistance to head smut.

SCOPE OF PRESENT STUDY

The scope of the present study, as indicated by the title of the thesis, covered two aspects of the head smut fungus to sorghum; namely, that dealing with the physiologic specialization of the pathogen, Sphacelotheca reiliana, and that dealing with the biology of the chlamydospores in the soil.

The results of the study are presented in two parts corresponding to each of the above aspects. In the first of these, physiologic specialization within S. reiliana was examined from the standpoint of its occurrence on sorghum and corn, within sorghum, and of the hybridity between those on sorghum and corn. In the second, chlamydospores in soil were examined for manner of germination, survival and concentrations affecting the abundance of head smut incidence. In addition, number of chlamydospores on seeds and Feterita immunity in a hybrid were examined as factors affecting head smut incidence.

MATERIALS AND METHODS

Sources of Head Smut Chlamydospores

Head smut chlamydospores were collected through the mail from various host and geographical sources listed in Table I. Each collection was received as a single sorus wrapped in cellophane or paper bags as a precautionary measure against loss of chlamydospores and mixing with one another.

Source of Sorghum and Corn Seeds

Seeds of 57 sorghum varieties, including 4 hybrid sorghum, 3 Sudangrass and 3 broomcorn varieties, appearing under results in this thesis were kindly supplied by J. R. Quinby, Texas Agricultural Experiment Station, Chillicothe, Texas; F. F. Davies, Department of Agronomy, Oklahoma State University, Stillwater, Oklahoma; and by C. J. Franzke, Agronomy Department, South Dakota State College, Brookings, South Dakota. Seeds of three 60 to 80 days sweet corn varieties first used were kindly supplied by Dr. R. L. Nickeson, Department of Horticulture, South Dakota State College, Brookings, South Dakota; those later used were purchased from the Farmer Seed and Nursery Company, Faribault, Minnesota, Northrup, King and Co., Minneapolis, Minnesota and Joseph Harris Company, Inc., Moreton Farm, Rochester, New York. In all instances where seedlings were inoculated hypodermically the seeds were treated chemically; captan on the corn seeds purchased from the commercial companies; arasan on sorghum seeds and on corn seeds from Dr. R. L. Nickeson. The presence of the chemical on the seeds did not affect disease development from

TABLE I. SOURCES OF HEAD SMUT CHLAMYDOSPORE COLLECTIONS USED IN THE PRESENT STUDY

Collection Designation	Host Variety	Location	Person Supplying Collection
	Sorghum		
New Mexico 1	Combine kafir 60	Clovis	Dr. C.H.Hsi
New Mexico 2	Combine 7078	Pleasant Hill	Dr. C.H.Hsi
New Mexico 3	Hybrid RS 610	Agric.Expt.Sta. Clovis	Dr. C.H.Hsi
Texas 1	?	Near Corpus Christi	Dr. E.C.Gilmore
Texas 2	?	Near Corpus Christi	Dr. E.C.Gilmore
Texas 3	Redbine 60, R 386	Refugio County	Dr. M.C.Futrell
Texas 4	Hybrid 608	Refugio County	Dr. M.C.Futrell
Texas 5	Sumac(Red Top Cone)	Beeville	Dr. M.C.Futrell
Texas 6	Hybrid H. 11027	Refugio County	Dr. M.C.Futrell
Texas 7	Texas blackhull kafir(Dual Purpose)	Beeville	Dr. M.C.Futrell
Poona(India) 1	?	Poona	Head of I.A.R.I. (Division of Mycol. & Plant Path.)
Poona(India) 2	?	Poona	Dr. Abrar M. Khan
Coimbatore (India) 1	?	Coimbatore	Head of I.A.R.I. (Division of Mycol. & Plant Path.)
Coimbatore (India) 2	?	Coimbatore	Dr. Abrar M. Khan
	Sudangrass		
Texas 8	Sudan-Sudax-II (Forage Type)	Beeville Expt. Sta.	Dr. M.C.Futrell
Texas 9	Sudan sweet 72 (S-1)(Forage Type)	Beeville Expt. Sta.	Dr. M.C.Futrell
Texas 10	Sudan sweet 372 (Forage Type)	Beeville Expt. Sta.	Dr. M.C.Futrell
California	Green leaf sudan	Davis	Dr. P.M.Halisky

TABLE I. SOURCES OF HEAD SMUT CHLAMYDOSPORE COLLECTIONS USED IN THE PRESENT STUDY (CONTINUED)

Collection Designation	Host Variety	Location	Person Supplying Collection
	Corn		
California	?	Davis	Dr. P.M.Halisky
Washington	?	Pullman	Dr. C.J.Mankin

such inoculations.

Soil Used

The soil used for growing plants in the greenhouse and for studying chlamydospore germination and survival in it was a local Vienna loam of the Chernozem class. In all instances it was nonsterilized. This was also the soil type in the Plant Pathology Experimental Farm and in another plot area one mile west of Brookings.

Greenhouse Conditions

Temperatures of near 25°C. and supplementary fluorescent lighting from near sundown to 2 a.m. were provided in the greenhouse during the fall, winter and spring months. At these times the plants were grown to heading in 6, 7 or 8 inch unglazed clay pots with 5 or 6 plants in each pot.

Field Plantings

The 57 sorghum varieties inoculated in the field were arranged in one area of the Plant Pathology Experimental Farm in single row plots 15 feet long, 3½ feet apart, with 30 to 40 plants per plot. Overhead irrigation was supplied on two occasions to maintain the plants in a proper state of growth. The same area was planted to sorghum the following year in rows similarly spaced.

Monochlamydosporous Cultures

Small quantities of chlamydospores derived from the center of each sorus collection of chlamydospores listed in Table I were immersed

in a one per cent copper sulfate solution in a centrifuge tube to free them of contaminating yeasts, molds and bacteria and the contents were centrifuged for 3 to 5 minutes to settle the spores to the bottom of the tube. After 15-17 hours the liquid in each of the tubes was drained out and the spores were suspended in sterile distilled water. Three-tenths of a milliliter of the water suspension of spores was dispersed by means of a pipette onto the surface of Petri dishes containing potato-dextrose agar, hereafter abbreviated as PDA, and rotated to ensure an even distribution of the spores. The plates were then incubated for 2 days at 30°C. and at that time 3 or 4 germinated spores were picked off with a fine needle and transferred individually to separate PDA slants in test tubes. These multiplied on the agar slants as sporidial mixtures.

Monosporidial Cultures

Monosporidial cultures were obtained from germinating chlamydospores on PDA blocks placed on cover glasses and inverted on Van Teighem cells. As soon as sporidia were formed a single sporidium from each cell of the promycelium of chlamydospore was removed with a micromanipulator and a stock culture of it was established on PDA. The four isolated sporidial lines from each chlamydospore were given an arbitrary number from 1 to 4 and bore no relationship to their actual position on the promycelium of that chlamydospore. In designating monosporidial lines each spore was given a number and the origin of the spore was designated a "C" or "S" indicating a corn or sorghum origin, respectively. A line designated as 3S2 would mean a monosporidial culture 2 taken from sorghum chlamydospore 3.

Bauch Test for Compatability Between Monosporidial Cultures

Cells from all possible pairs of monosporidial cultures were spot mixed on the surface of PDA in Petri dishes and held for 48 hours at 30°C. for colony development. If at the end of that time the colony edge appeared mycelial in any degree, the paired monosporidial cultures were deemed compatible. If at the end of that time the colony edge appeared wholly cellular, the paired monosporidial cultures were deemed incompatible.

Inoculation Method

Except for seed infestation with chlamydospores reported under results of this thesis, the plants in the field and greenhouse were hypodermically inoculated with mixed monochlamydosporous or paired compatible monosporidial cultures in the seedling stage after they had unfolded 3 to 5 leaves. At that time the apical growing point region of each seedling was located at the soil line. A Becton-Dickinson Company hypodermic needle, size 27, attached to a one milliliter syringe charged with inoculum was inserted at the side of each seedling about one-half centimeter above the soil line and pushed down at about a 45° angle from the horizontal until the point of the needle was somewhere in the center of the fold of leaves and slightly above the apical growing point. Enough inoculum, usually about one-half milliliter, was then discharged from the syringe so that a portion of it appeared as a drop at the top of the central whorl of leaves. The inoculation was repeated at another point on the same seedling a few millimeters above the first. In older plants of 6 to 8 leaves in the field, the point of needle insertion

was raised to approximately one to one and one-half centimeters above the soil line, with resulting successful inoculations. The inocula comprised heavy aqueous suspensions of sporidia derived from PDA slants of the above cultures. In no instance in field or greenhouse tests was head smut found on uninoculated check plants.

SIGNS AND SYMPTOMS OF SORGHUM HEAD SMUT

Sorghum head smut expresses itself in the plant as mycelium or more commonly as black chlamydospore masses of the fungus, which are the signs of the disease, and less commonly as alterations in the normal appearance or development of the plant, which are the symptoms of the disease. Signs and symptoms may appear together but usually one predominates over the other, depending on the pathogen and the host and on the ingenuity and visual acuity of the observer. Both arise from an early infection of the plants as seedlings (18) when the apical and axillary buds, stems and certain of the leaves are regionally packed together in embryonic or juvenile form (5). At that time presumably the mycelium invades the region, as observed in corn (14), and becomes disproportionally distributed therein, as has been observed in other smut diseases. Following that, the mycelium separates as pockets within the various tissues and organs wherein it is lodged as these tissues and regions separate with the elongation of the plant in growth (18). Subsequent growth of the mycelium in these tissues and organs with ultimate sporulation of the fungus probably depends on the youthful duration and on the nutritive quality of these tissues and organs for the fungus. Localization of the signs and symptoms of head smut in sorghum thus is a consequence of the above described mode of plant infection.

The most striking and characteristic sign of the disease is the mass or sorus of chlamydospores that appears on the plant at one or more places. Commonly sori appear in place of the inflorescence

panicles as these organs emerge from the whorl of leaves at heading time. Occasionally they may appear on a part of the inflorescence panicle (Figure 2), leaves (Figure 3), nodes (Figure 4) and internodes (Figure 5). They range from pea seed to baseball sizes and from spherical to much elongated shapes. At first they are enclosed in a moderately thick peridium of fungus tissue (Figure 8) but soon this ruptures, exposing the black, powdery mass of spores to the air. Sori replacing the inflorescence panicle commonly carry elongated black strands of host vascular bundles within their mass (Figure 7).

A less commonly observed sign of the disease is the mycelium in the tissues of the host. Such mycelia have been sought and found in the apical and axillary buds of the plants (18). For obvious reasons detection of mycelium in plant tissues is not amenable to routine use.

Symptoms of head smut assume various forms both in the presence and absence of external signs. Usually head smut symptoms are not recognizable as such in the absence of external signs unless the plant is found to carry internal mycelium of the fungus or the plant is known to have been inoculated. Symptoms include such abnormal characteristics as chlorophyll degeneracy in localized areas of the leaf (Figure 3), stunted plants, non-headed plants, proliferated floral parts of the panicle (Figure 1) and partially or completely sterile panicle (Figure 6).

In the present study the commonest sign was the characteristic sorus of chlamydospores replacing the inflorescence and the commonest symptom was the flecking of leaves at the point where the hypodermic needle was inserted into the seedlings. In one extremely susceptible

sorghum variety, Sugar Drip, abundant elongated sori developed on the leaves and the plant failed to head. In a few varieties abundant, elongated sori developed on the leaves and the panicles were smutted. In other varieties the axillary panicles were smutted and the main panicles were non-smutted and sterile (Figure 6). Individual plants scattered among varieties exhibited partially smutted panicles with the other part sterile. An array of signs and symptoms of head smut thus was obtained over the range of sorghum varieties hypodermically inoculated.

Figure 1. Vegetative Proliferation of a Panicle of Sorghum Infected with *Sphacelotheca reiliana*

Figure 2. Vegetative Proliferation on Part of a Panicle Carrying a Chlamydospore Sorus of *Sphacelotheca reiliana*

Figure 1.

Figure 2.

Figure 3. Sporulation of Sphacelotheca reiliana on the Leaves of Sugar Drip Sorghum

Figure 4. Nodal Sori on White Kaoliang Sorghum Infected with Sphacelotheca reiliana

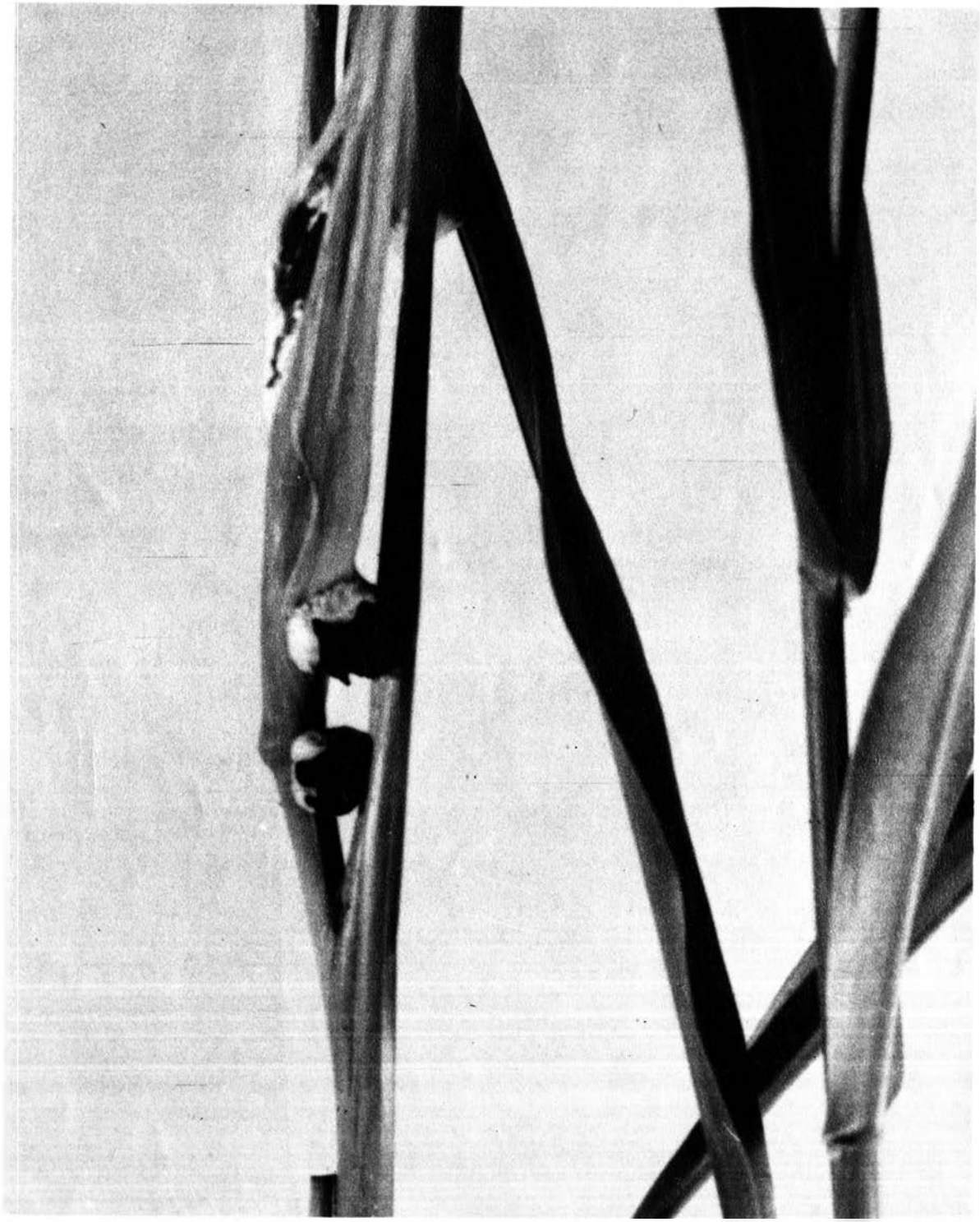

Figure 4.

Figure 5. Internodal Sorus of *Sphacelotheca reiliana* on Manchu Br. Kaoliang Sorghum

Figure 5.

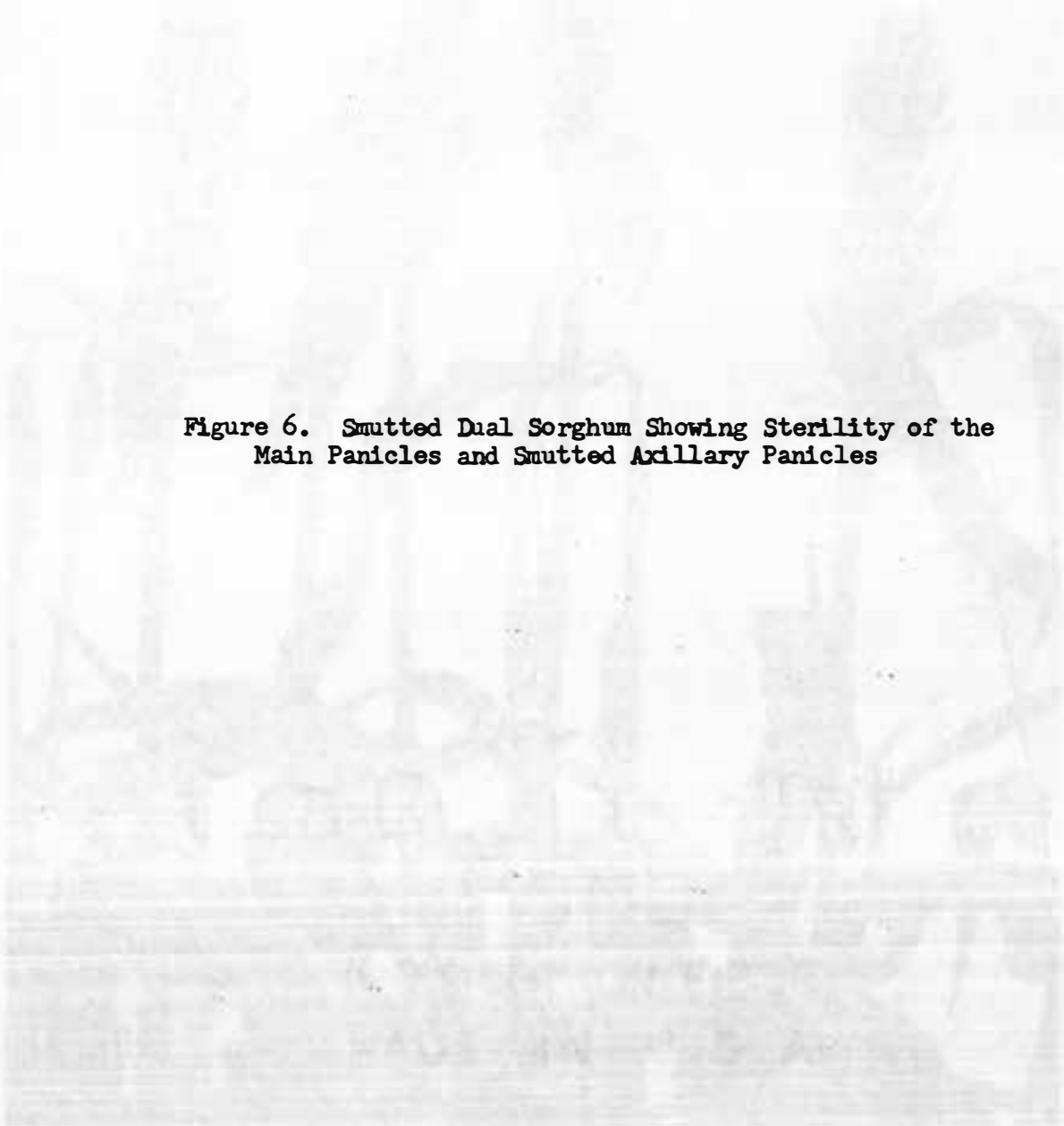

Figure 6. Smutted Dual Sorghum Showing Sterility of the Main Panicles and Smutted Axillary Panicles

Figure 6.

Figure 7. Head Smutted Brown Durra Sorghum Showing Remnants of Host Vascular Bundles

Figure 7.

Figure 8. Head Smutted White Kaoliang Sorghum Showing Peridium of Sphacelotheca reiliana

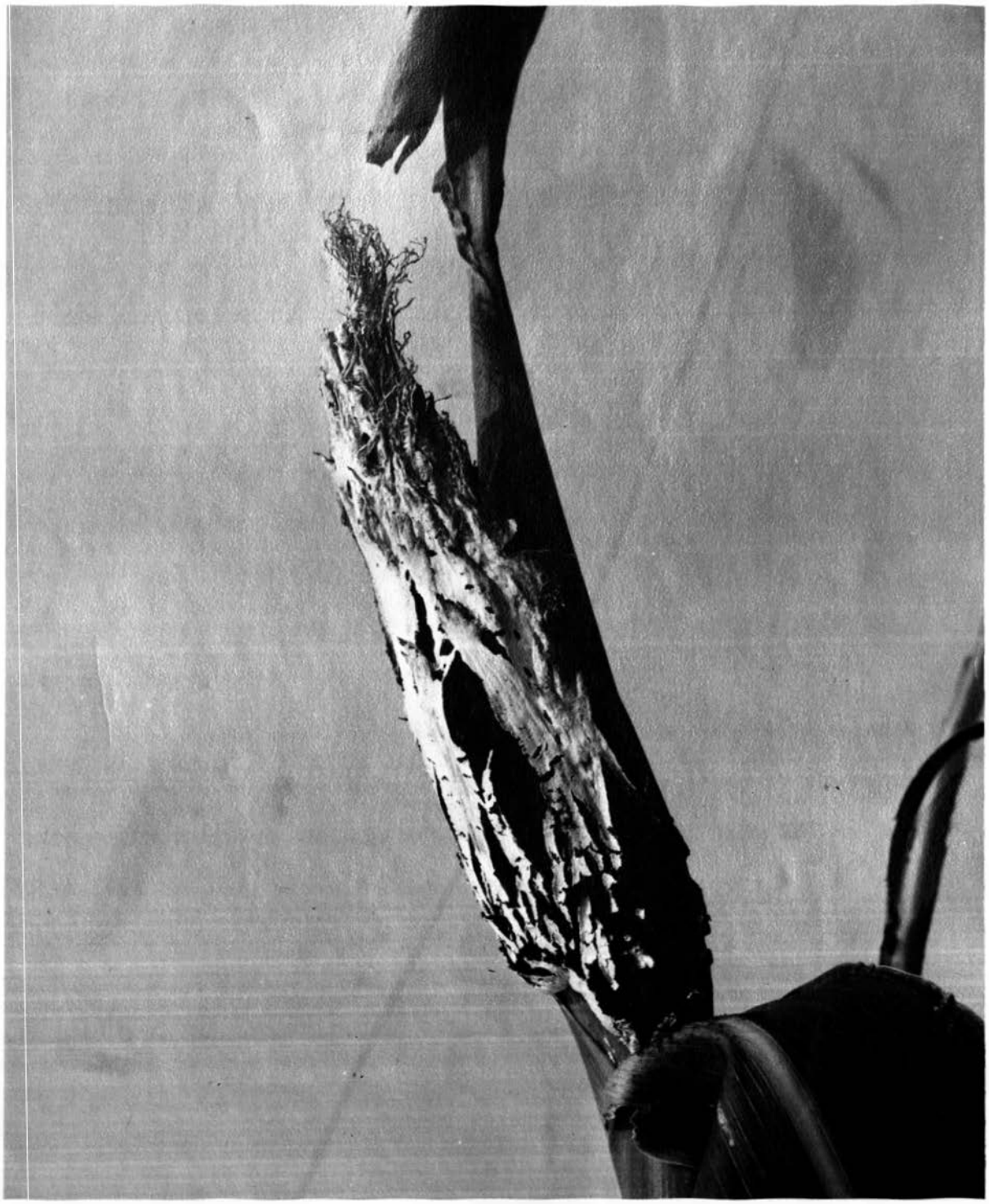

Figure 8.

EXPERIMENTAL RESULTS

Part 1. Physiologic Specialization in Sphacelotheca reiliana with Special Reference to that on Sorghum

Physiologic specialization is a widespread phenomenon among obligately and semi-obligately parasitic fungi and therefore it may be expected to encompass the sorghum and corn head smut fungus complex. Reed (19) already has separated that complex into two groups, one for each of the crops, and these he labelled races. The head smut fungus on Sudangrass, broomcorn and other units of sorghum is tacitly included in the sorghum head smut group. However as the components of the complex have not been sharply defined, a start toward that definition was made in the present study and particularly with respect to the head smut fungus of sorghum.

In previous studies of the complex (21), chlamydospores from corn or sorghum were added to soil for cross infection tests to these crops. Since chlamydospores in soil may fail to infect and they do not lend themselves as such to a detailed study of their specialization, the inoculum used in the present study consisted of 3 to 4 monochlamydosporous and paired monosporidial cultures representing various sources as will be indicated. Such inocula were introduced hypodermically into the plant in the manner described under methods. For that reason the specialization of Sphacelotheca reiliana to sorghum and corn was reexamined and this was followed by an examination of the specialization of the fungus in sorghum and of the hybridity between the sorghum and the corn head smut fungi.

Specialization of Sphacelotheca reiliana to Sorghum and Corn

To test specialization of S. reiliana to sorghum and corn three greenhouse trials were conducted in which monochlamydosporous cultures from several geographically divergent sorghum and corn head smut chlamydospore sources were injected into several sorghum and several 60 to 80-days sweet corn varieties.

In the first of these tests, as already reported (1) a culture of the sorghum head smut fungus from sudangrass in California and two cultures of the corn head smut fungus from corn in California and Washington, respectively were injected into three sorghum and three sweet corn varieties, with results presented in Table II. The one sorghum head smut culture from Sudangrass yielded head smut on all three sorghum varieties and on one sweet corn variety, North Star; while two corn head smut cultures yielded head smut on the three sweet corn varieties and not on the three sorghum varieties. The results from the two cultures of the corn head smut fungus thus agreed with those reported by Mankin (14) and essentially with those reported by Reed et al. (21), while results from the one culture of the sorghum head smut fungus from Sudangrass did not agree with those reported by Reed et al. (21) and by Halisky et al. (9). Since Halisky et al. (9) used Stowells Evergreen and Country Gentleman sweet corn varieties in their tests the resistance of these varieties may have contributed to the failure of getting infection of those varieties from chlamydospores in soil derived from Sudangrass.

In a second test, 18 cultures of the sorghum head smut fungus,

TABLE II. HEAD SMUT DEVELOPMENT IN SWEET CORN AND GRAIN SORGHUM INOCULATED WITH CULTURES DERIVED FROM CORN AND SUDANGRASS HEAD SMUT

	Head Smut Collection		
	Corn		Sudangrass
Host and Variety	California	Washington	California
Sweet Corn			
Golden Beauty	+	+	-
Sugar & Gold	+	+	-
North Star	+	+	+
Grain Sorghum			
Reliance	-	-	+
Dual	-	-	+
Norghum	-	-	+

+ = Smutted Plants
- = Healthy Plants

of which four were derived from Sudangrass, were injected into North Star sweet corn and Sugar Drip sorghum, a highly head smut susceptible variety. As may be seen from the results presented in Table III 14 of the 18 cultures, which included three of four from Sudangrass, infected North Star sweet corn, while all 18 yielded head smut on Sugar Drip sorghum. Thus the peculiar susceptibility of North Star sweet corn to the sorghum head smut fungus from Sudangrass noted in the preceding test was confirmed in this test and was extended to include susceptibility to cultures from other sorghum sources as well. Infected plants of this variety were much stunted (Figure 9), their tassels were leafy structures in most instances (Figure 10) and they either failed to ear or the ears formed were small with some kernels replaced with smut sori.

Since failure of the two cultures of the corn head smut fungus to infect sorghum noted in the first test may have been due to the resistance of the few varieties used, a third test was conducted in which these same cultures and three cultures of the sorghum head smut were injected into 14 sorghum varieties. Included in the sorghum varieties were two Sudangrass varieties, and one of broomcorn, and included in three cultures of the sorghum head smut fungus was one culture from Sudangrass head smut in California. As may be seen from the results in Table IV neither of the two cultures of the corn head smut fungus yielded head smut on any of the 14 sorghum varieties while one of the three cultures of the sorghum head smut fungus yielded head smut on 12 of the 14 varieties. The other two cultures of the sorghum head smut fungus may be considered to have yielded head smut on no more than 10 of the 14

TABLE III. HEAD SMUT DEVELOPMENT IN NORTH STAR SWEET CORN AND SUGAR DRIP SORGHUM INOCULATED WITH 18 CULTURES OF THE SORGHUM HEAD SMUT FUNGUS DERIVED FROM VARIOUS SOURCES

Host and Variety	Sources of Sorghum Head Smut Fungus																		
	Sorghum															Sudangrass			
	Texas							New Mexico			Coimbatore		Poona		Texas			California
	1	2	3	4	5	6	7	1	2	3	1	2	1	2	8	9	10	
Sweet Corn North Star	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+
Sorghum Sugar Drip	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = Smutted Plants
- = Healthy Plants

TABLE IV. SUSCEPTIBILITY OF FOURTEEN SORGHUM VARIETIES TO CULTURES OF SORGHUM AND CORN HEAD SMUT FUNGI

Variety	Sorghum Head Smut Fungus			Corn Head Smut Fungus	
	Sorghum		Sudangrass		
	New Mexico Collection 1	New Mexico Collection 2	California Collection	Washington Collection	California Collection
FC 811 Feterita	-	-	-	-	-
CI 182 Feterita	-	-	-	-	-
Redlan (Grain Type)	+	+	+	-	-
PI 54484 Durra	+	+	+	-	-
Agros 2650 shallu	+	+	+	-	-
PI 62610 White kaoliang	+	+	+	-	-
SA 301 Darso 28	+	+	+	-	-
Sugar Drip (Forage Type)	+	+	+	-	-
PI 19749 Red kafir	+	+	+	-	-
PI 34911 Hegari	-	-	+	-	-
SA 281 Early Hegari	-	-	+	-	-
Sweet Sudan	+	-	+	-	-
FC 33673 Common Sudan	-	-	+	-	-
CI 556 Standard broomcorn	+	+	+	-	-

+ = Smutted Plants
- = Healthy Plants

Figure 9. North Star Sweet Corn Plants. Left Healthy, Right Stunted from Sudangrass Head Smut Inoculation

Figure 10. North Star Sweet Corn Plants Inoculated with Sudangrass Head Smut Showing Leafy Tassels and Stunted Stalks

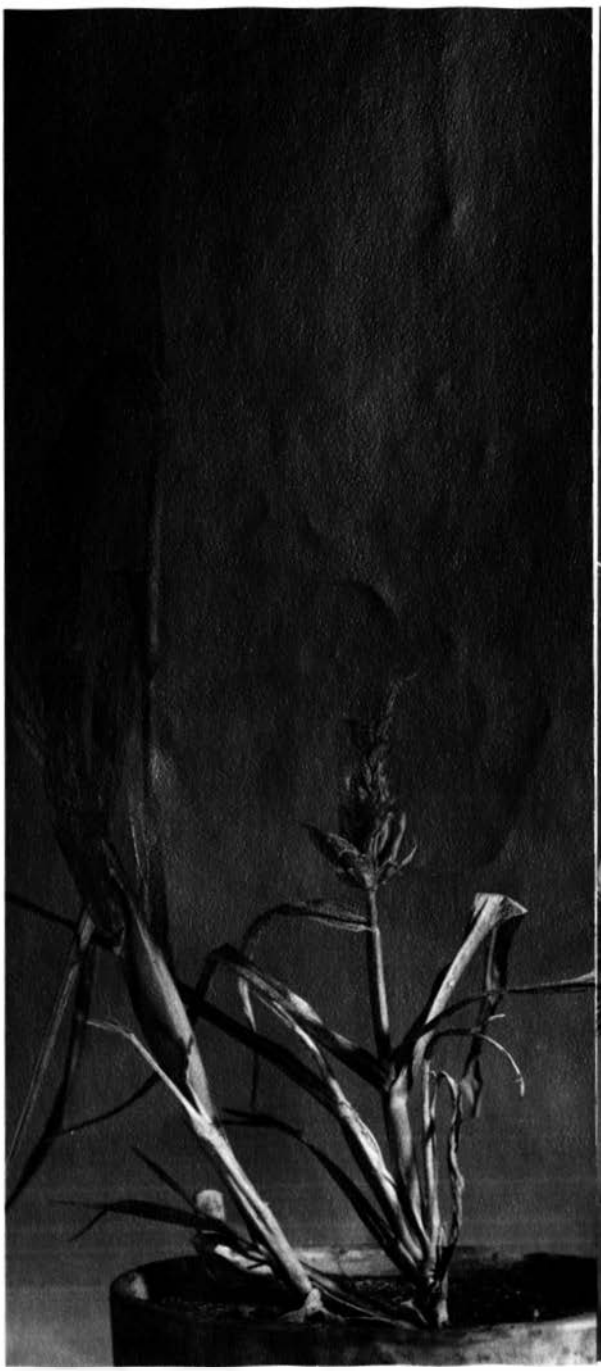

Figure 9.

Figure 10.

varieties for the reason that negative head smut on Sudangrass varieties was considered an unreliable index of their true reaction. Sudangrass seedlings usually were so thin that the writer was never sure that he adequately inoculated them.

Thus from this and the preceding tests one may draw the conclusion that the corn and the sorghum head smut fungi were separate entities to the extent that the corn head smut fungus yielded head smut only on corn while the sorghum head smut fungus yielded head smut on sorghum and at least on one variety of corn.

Races of the Sorghum Head Smut Pathogen

Races within species of plant pathogens is a common phenomenon in plant pathology dramatized most spectacularly by the well known wheat stem rust fungus, Puccinia graminis var. tritici Eriks, and Henn. where over 200 races are known. They are recognized by their ability to parasitize different varieties of wheat, and to parasitize them to different degrees. Their number among smut fungi is less numerous and spectacular (19), but their presence is no less important for at times they have accounted for the sudden outbreak of smut in some crops where new varieties of a crop were introduced. Their existence within the sorghum head smut fungus has not been demonstrated to the writer's knowledge and would be of help if so demonstrated for they might account for the recent increase of sorghum head smut in Texas and provide a base for the plant breeder in the development of resistant varieties.

The existence of such races in the sorghum head smut fungus has already been alluded to in the presentation of the data in Table IV.

In that table a culture of the sorghum head smut fungus from Sudangrass in California yielded head smut on ten varieties of sorghum, while two cultures from sorghum in New Mexico yielded head smut on eight varieties, the negative results on Sudangrass being ignored for the reason stated earlier.

To further appraise the existence of races within this pathogen seven cultures of the sorghum head smut fungus derived from several sorghum varieties from five widely separated geographical areas were injected in the field in the spring of 1959 into 30-40 seedlings each of 57 sorghum varieties. The latter included 4 hybrids, 3 Sudangrass and 3 broomcorn varieties. The plants were observed throughout the growing season and the final smut counts were made in early September after heading was completed. The data for the per cent of smutted plants and for the per cent of smutted heads are presented in Table V. Again the results with Sudangrass were considered unreliable for the reason stated above.

As might be expected from the tillering habit of sorghum, the per cent of smutted plants usually was higher than the per cent of smutted heads, but the difference between these values generally was not great. The average value for per cent of smutted plants was 67.7 and for per cent of smutted heads was 52.2.

The sorghum head smut fungus from one collection of chlamydospores from Sudangrass in California proved most pathogenic, yielding head smut on 50 of 57 entries. The remaining six cultures of the fungus from other sources were divisable into two groups: five being

TABLE V. PER CENT OF PLANTS AND HEADS SMUTTED IN 57 VARIETIES OF SORGHUM INOCULATED WITH SEVEN SORGHUM HEAD SMUT COLLECTIONS

	Head Smut Collections													
	Sorghum												Sudangrass	
	New Mexico						Texas		Coimbatore		Poona		California	
	1		2		3		1		1		1			
Variety	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.
FC 16207 Kalo	90	66	72	54	85	79	84	74	52	36	0	0	40	22
SA 387 Redbine 66	82	84	80	71	80	92	80	60	80	79	0	0	54	46
CI 171 Manchu Br. kaoliang	64	51	72	58	52	40	84	62	40	36	34	30	50	42
SA 7000 Caprock	66	44	65	44	68	36	90	52	76	64	0	0	72	52
CI 616 Schrock	74	62	70	52	90	86	86	48	78	66	0	0	21	15
SA 7078 Combine 7078	86	70	56	48	60	76	78	64	52	50	0	0	38	30
SA 294 Combine shallu	84	62	80	60	59	60	66	46	59	62	40	46	78	50
SA 7005 Plainsman	54	40	60	32	22	30	90	42	72	42	0	0	72	51
Darset	84	78	84	58	92	76	92	58	72	62	0	0	92	66
Redlan (Grain Type)	70	66	88	78	86	85	92	80	88	80	0	0	66	49
44-14 (Grain Type)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sumac (Forage Type)	58	60	56	58	52	58	56	62	29	33	0	0	65	70

TABLE V. PER CENT OF PLANTS AND HEADS SMUTTED IN 57 VARIETIES OF SORGHUM INOCULATED WITH SEVEN SORGHUM HEAD SMUT COLLECTIONS (CONTINUED)

Variety	Head Smut Collections													
	Sorghum												Sudangrass	
	New Mexico						Texas		Coimbatore		Poona		California	
	1		2		3		1		1		1			
	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.
SA 217 Brown Durra	34	26	37	40	50	41	22	14	74	72	40	44	4	6
PI 62610 White kaoliang	24	24	78	68	28	24	94	66	78	66	70	52	84	56
SA 301 Darso 28	76	65	96	90	80	74	96	80	96	84	92	74	96	78
PI 19749 Red kafir	54	58	86	70	58	52	100	90	86	82	0	0	96	68
CI 293 Shuntung Br. kaoliang	64	38	44	26	42	34	90	52	54	44	18	12	34	22
Agros 2650 shallu	96	68	72	40	86	65	100	62	92	46	80	44	82	66
SA 208 Cal. W. Durra	78	81	82	52	96	96	92	60	78	60	80	68	96	90
PI 54484 Durra	88	30	66	25	64	26	72	21	24	21	0	0	100	50
SA Hegari Derivatives	0	0	0	0	0	0	0	0	0	0	0	0	42	32
PI 34911 Hegari	0	0	0	0	0	0	0	0	0	0	0	0	52	52
SA 392 Combine Hegari	0	0	0	0	0	0	0	0	0	0	0	0	68	62
SA 281 Early Hegari	0	0	0	0	0	0	0	0	0	0	0	0	82	36

TABLE V. PER CENT OF PLANTS AND HEADS SMUTTED IN 57 VARIETIES OF SORGHUM INOCULATED WITH SEVEN SORGHUM HEAD SMUT COLLECTIONS (CONTINUED)

Variety	Head Smut Collections													
	Sorghum												Sudangrass	
	New Mexico						Texas		Coimbatore		Poona		California	
	1		2		3		1		1		1			
	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.
Looti milo (Forage Type)	75	72	56	44	60	69	26	24	48	41	0	0	22	26
Sooner milo	45	51	75	48	55	39	82	52	78	58	0	0	76	64
SA 7680 Ryer milo	0	0	0	0	0	0	0	0	0	0	0	0	50	50
SA 370 D.D.Y. Sooner milo	48	24	82	44	68	22	54	52	100	62	0	0	34	28
SA 371 D.D.W. Sooner milo	84	38	100	52	100	42	88	38	28	18	0	0	94	48
Cal. 38 D.D.Y. milo	0	0	0	0	0	0	0	0	0	0	0	0	96	70
SA 385 Combine kafir 60	88	73	66	68	94	80	96	74	86	62	0	0	94	74
TS 45 Pink kafir	58	74	78	84	86	59	44	38	18	18	0	0	70	74
Texas Blackhul kafir	100	66	94	64	92	63	70	46	66	46	0	0	86	48
Combine White feterita	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FC 6601 Spur feterita	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CI 182 Feterita	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FC 811 Feterita	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE V. PER CENT OF PLANTS AND HEADS SMUTTED IN 57 VARIETIES OF SORGHUM INOCULATED WITH SEVEN SORGHUM HEAD SMUT COLLECTIONS (CONTINUED)

	Head Smut Collections													
	Sorghum												Sudangrass	
	New Mexico						Texas		Coimbatore		Poona		California	
	1		2		3		1		1		1			
Variety	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.
TX 04	82	84	86	58	84	54	90	52	80	69	0	0	58	50
TX 07	60	28	50	38	84	75	76	32	68	54	0	0	32	40
RS 630	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Texas 620	96	75	82	68	96	82	66	46	59	62	10	10	62	46
Sweet Sudan	20	12	26	21	32	14	34	24	40	14	14	6	30	26
FC 33814 Piper Sudan	0	0	0	0	9	1	0	0	0	0	0	0	0	0
FC 33673 Common Sudan	0	0	6	6	22	4	14	2	0	0	0	0	25	6
CI 243 Acme broomcorn	60	41	78	70	64	58	90	66	94	60	40	34	94	64
PI 30204 Japanese Dwarf broomcorn	92	52	45	26	96	54	78	24	85	34	76	34	72	36
CI 556 Standard broomcorn	88	60	90	46	92	70	74	44	94	72	75	50	85	78

P. = Percentage of smutted plants
H. = Percentage of smutted heads

intermediately pathogenic, yielding head smut on 46 of 57 varieties; with Poona 1 from India being weakly pathogenic, yielding head smut on 20 of 57 entries. On this basis the seven cultures of the pathogen comprised three races, corresponding to the above groups, as may be seen best on an arbitrarily selected set of differentiating sorghum varieties set out in Table VI. However if one were to separate races on the per cent of plants smutted such that varieties with more than 50 per cent head smut were deemed susceptible and those with less than that amount were deemed resistant then all seven chlamydospore collections would be differentiated as separate races. Such separations of races is commonly done by investigators of smut fungi, but since the per cent of plants smutted usually fluctuates over a wide range from one trial to the next, the writer felt that a more conservative approach was best and limited race separation to the presence or absence of smut.

To determine further whether the three races from the above seven chlamydospore cultures would maintain their race characteristics in a greenhouse test and also whether races existed among eleven other sorghum head smut chlamydospore collections on hand, including three from Sudangrass, the eighteen cultures corresponding to these collections were injected into the set of five race differentiating varieties previously referred to in Table VI.

As may be seen from presence or absence of head smut in this set of differential varieties in Table VII, the 18 cultures corresponding to the same number of chlamydospore head smut collections clearly comprised three races: (1) the California Sudangrass head smut collection; (2) the

TABLE VI. PER CENT HEAD SMUTTED PLANTS IN FIVE DIFFERENTIAL SORGHUMS INOCULATED WITH CULTURES FROM SEVEN SORGHUM HEAD SMUT COLLECTIONS

Collection Source		Sorghum Varieties				Sorghum Hybrid
		CI 182 Feterita	Darso 28	SA 281 Early Hegari	Redlan (Grain Type)	RS 630
Texas	1	0	96	0	92	0
New Mexico	1	0	76	0	70	0
New Mexico	2	0	96	0	88	0
New Mexico	3	0	80	0	86	0
Coimbatore	1	0	96	0	88	0
Poona	1	0	92	0	0	0
California (Sudangrass)		0	96	82	66	0

TABLE VII. PER CENT HEAD SMUTTED PLANTS IN FIVE DIFFERENTIAL SORGHUMS INOCULATED IN THE GREENHOUSE WITH CULTURES FROM 18 SORGHUM HEAD SMUT COLLECTIONS

Collection Source		CI 182 Feterita	Darso 28	SA 281 Early Hegari	Redlan (Grain Type)	RS 630
Texas	1	0	72	0	66	0
Texas	2	0	100	0	84	0
Texas	3	0	100	0	66	0
Texas	4	0	58	0	70	0
Texas	5	0	70	0	90	0
Texas	6	0	70	0	76	0
Texas	7	0	62	0	88	0
New Mexico	1	0	75	0	100	0
New Mexico	2	0	40	0	86	0
New Mexico	3	0	82	0	75	0
Coimbatore	1	0	84	0	92	0
Coimbatore	2	0	66	0	10[1]	0
Poona	1	0	62	0	80[2]	0
Poona	2	0	62	0	60	100

TABLE VII. PER CENT HEAD SMUTTED PLANTS IN FIVE DIFFERENTIAL SORGHUMS INOCULATED IN THE GREENHOUSE WITH CULTURES FROM 18 SORGHUM HEAD SMUT COLLECTIONS (CONTINUED)

Collection Source		CI 182 Feterita	Darso 28	SA 281 Early Hegari	Redlan (Grain Type)	RS 630
Texas (Sudangrass)	8	0	88	0	75	0
Texas (Sudangrass)	9	0	25	0	88	0
Texas (Sudangrass)	10	0	100	0	85	0
California (Sudangrass)		0	58	66	90	0

[1]Represents only one infected plant. No infected plants were obtained in a subsequent field test (Table VIII).

[2]This result is at variance with those obtained in two field tests (Tables V and VIII) where no infection was obtained.

Poona 2 sorghum head smut collection; and (3) the remaining 16 head smut collections from sorghum and Sudangrass, which included the Poona 1 that was differentiated as a race in the field. The Poona 2 sorghum head smut collection of chlamydospores comprised a new race over those revealed in the field test as it was the only collection that produced head smut on RS 630. However the over-all differentiation in the greenhouse of races discovered in the field was not wholly satisfactory as the Poona 1 collection of sorghum head smut chlamydospores infected the Redlan variety of sorghum which it did not in the field, while the Coimbatore 2 collection of sorghum head smut chlamydospores yielded one infected plant of ten inoculated. Since the aberrant results could have been due to error or contamination at the time of inoculation, the entire test was repeated in the field in the summer of 1960.

As may be seen from the results of this field test presented in Table VIII, the 18 head smut collections of chlamydospores comprised four races; namely, (1) the California Sudangrass head smut collection of chlamydospores, (2) the Poona 1 and the Coimbatore 2 sorghum head smut collections of chlamydospores, (3) the Poona 2 sorghum head smut collection of chlamydospores and (4) the remaining 14 head smut collections of chlamydospores from sorghum and Sudangrass. The results of this test confirmed the races of head smut differentiated in the first field test and the Poona 2 race differentiated in the greenhouse test.

Thus the existence of races within the sorghum head smut fungus have been demonstrated to the extent of four in number. The results of this study are reported in the literature (2).

TABLE VIII. PER CENT HEAD SMUTTED PLANTS IN FIVE DIFFERENTIAL SORGHUMS INOCULATED IN THE FIELD WITH CULTURES FROM 18 SORGHUM HEAD SMUT COLLECTIONS

Collection Source		CI 182 Feterita	Darso 28	SA 281 Early Hegari	Redlan (Grain Type)	RS 630
Texas	1	0	92	0	60	0
Texas	2	0	70	0	82	0
Texas	3	0	56	0	58	0
Texas	4	0	96	0	62	0
Texas	5	0	42	0	52	0
Texas	6	0	75	0	50	0
Texas	7	0	40	0	48	0
New Mexico	1	0	75	0	84	0
New Mexico	2	0	80	0	88	0
New Mexico	3	0	65	0	66	0
Coimbatore	1	0	54	0	70	0
Coimbatore	2	0	85	0	0	0
Poona	1	0	78	0	0	0
Poona	2	0	50	0	16	53

TABLE VIII. PER CENT HEAD SMUTTED PLANTS IN FIVE DIFFERENTIAL SORGHUMS INOCULATED IN THE FIELD WITH CULTURES FROM 18 SORGHUM HEAD SMUT COLLECTIONS (CONTINUED)

Collection Source		CI 182 Feterita	Darso 28	SA 281 Early Hegari	Redlan (Grain Type)	RS 630
Texas (Sudangrass)	8	0	52	0	65	0
Texas (Sudangrass)	9	0	45	0	62	0
Texas (Sudangrass)	10	0	72	0	80	0
California (Sudangrass)		0	50	51	84	0

Hybridization of Sorghum and Corn Head Smut Fungi

As noted earlier sorghum and corn head smut fungi were physiologically different from one another to the extent that the sorghum head smut fungus usually did not produce head smut on corn and the corn head smut fungus usually did not produce head smut on sorghum. Since the sorghum head smut fungus has been hybridized in the sorghum plant with Sphacelotheca cruenta and Sphacelotheca sorghi, species of smut fungi infecting sorghum, and have yielded smut sori or mature chlamydospores morphologically different from either parent (23, 24, 25), the possibility exists that hybridization may occur naturally on occasions between the sorghum and the corn head smut fungi in either of their hosts when the two fungi are present together. A demonstration of such possible hybridization would be of value toward understanding host specificity in these fungi particularly if the hybrids could be shown to have the capacity of producing head smut on both sorghum and corn. Obviously hybridization between these fungi should take place in a host plant where infection symptoms and chlamydospore formation may be expressed with final proof of hybridization coming only when the mature chlamydospores are shown to be morphologically and/or physiologically different from those of either parent. Since the morphology of the two head smuts chlamydospores are alike no difference should be expected and found in the morphology of the hybrid chlamydospore from those of the parents.

As with most hybridization studies with smut fungi compatible haploid units (sporidia) of the respective fungi are injected simultaneously into a plant to allow them to conjugate to form the diploid

phase, which phase is the only one that produces chlamydospores. To this end for the present experiment, 13 monosporidial cultures supposedly haploid were derived from four chlamydospores of the sorghum head smut fungus (New Mexico 1) and 17 monosporidial cultures were derived from five chlamydospores of the corn head smut fungus (California), and between them these yielded by the Bauch test 108 compatible pairs as shown in Table IX. This number of pairs was very close to a 1:1 ratio of compatible to non-compatible pairs, although a larger or smaller ratio than this arose with individual chlamydospores of the corn head smut fungus.

The haploid nature of the 30 monosporidial cultures was presumptively proved when all 30 cultures failed to produce head smut on both Golden Bantam sweet corn and Sugar Drip sorghum. On the other hand, 105 of the 108 compatible pairs of monosporidial cultures, when injected simultaneously as a mixture into seedlings, yielded mature chlamydospores on both Golden Bantam sweet corn and Sugar Drip sorghum while the remaining three failed to produce head smut symptoms or mature chlamydospores on either of these hosts. The three pairs (1S1 x 2C1, 3S3 x 1C2, 1S1 x 5C1) may be considered incompatible in the host plants despite their apparent compatibility by the Bauch test.

The disease produced by the 105 compatible pairs of monosporidial cultures was less severe than, and the sorus of chlamydospores produced was typical of that produced in each host by its own wild type fungus. Golden Bantam sweet corn manifested partially smutted tassels and ears (figure 11) while Sugar Drip sorghum manifested smutted heads when

TABLE IX. RESULTS FROM ALL POSSIBLE PAIRINGS BETWEEN 17 MONOSPORIDIAL CULTURES OF THE CORN HEAD SMUT FUNGUS AND 13 MONOSPORIDIAL CULTURES OF THE SORGHUM HEAD SMUT FUNGUS

Corn Head Smut Fungus		Sorghum Head Smut Fungus															
Chlamydospore Designation	Sporidial Number	1S				2S				3S			4S				
		1	2	3	4	1	2	3	4	1	2	3	1	2			
1C	1	+	-	-	+	+	-	+	+	-	+	+	+	-			
	2	+	-	-	+	+	-	+	+	-	+	+	+	-			
	3	+	+	+	+	+	+	+	+	+	+	+	+	+			
	4	-	-	+	-	-	-	-	-	-	-	-	+	-			
2C	1	+	-	-	-	+	-	+	-	-	+	+	+	-			
	2	+	-	-	+	+	-	+	+	-	-	+	+	-			
3C	1	+	+	+	+	+	+	+	+	+	+	+	+	+			
	2	+	+	+	+	+	+	+	+	+	+	+	+	+			
	3	+	-	-	+	+	-	+	+	-	+	+	+	-			
	4	-	-	-	-	-	-	-	-	-	-	-	-	-			
4C	1	+	+	+	+	+	+	+	+	+	+	+	+	+			
	2	-	-	-	-	+	-	-	-	-	-	-	-	-			
	3	-	+	+	-	-	-	-	-	+	-	-	-	+			
	4	-	-	-	-	-	-	-	-	-	-	-	-	-			
5C	1	+	-	-	+	+	-	+	+	-	+	+	+	-			
	2	-	+	+	-	+	-	-	-	-	-	-	-	+			
	3	-	-	-	-	-	-	-	-	-	-	-	-	-			

\+ = Compatible pair
\- = Non-compatible pair

Figure 11. Partially Smutted Tassels of Golden Bantam Sweet Corn Plants Inoculated with a Pair of Compatible Monosporidial Cultures from Sorghum and Corn Head Smut Fungi

Figure 11.

normally it failed to head and carried sori only on its leaves.

Final proof of the hybrid nature of the chlamydospores formed from various compatible pairs of monosporidial cultures was derived from 3-4 monochlamydosporous cultures prepared from each of three different hybridizations on corn and on sorghum. When a composite of these 3 or 4 monochlamydosporous cultures corresponding to each of the 3 corn and 3 sorghum sources were injected into seedlings of Golden Bantam sweet corn and Sugar Drip sorghum, head smut developed as shown in Table X, on both corn and sorghum from all three composite cultures derived from sorghum and two composite cultures derived from corn, while it developed only on sorghum from the other composite culture derived from corn. Thus with these results head smut sori of chlamydospores should be expected to occur in nature on either sorghum or corn with the fungus from each of these sources having the capacity of producing head smut on both or the other of these hosts. Their presence awaits discovery.

A Genetical Interpretation of the Pathogenic Properties of Hybrid Chlamydospores Having the Capacity of Producing Head Smut on Sorghum and Corn

If the above demonstrated ability of hybrid chlamydospores to yield head smut on both sorghum and corn were dependent simply on the presence on homologous chromosomes of an allelic gene governing pathogenicity to sorghum contributed by the parent of the sorghum head smut fungus and of another gene governing pathogenicity to corn contributed by the parent of the corn head smut fungus, then when such hybrid chlamydospores are germinated on agar media to yield sporidia, a segregation of the genes should occur such as to yield two sporidia

TABLE X. PATHOGENICITY OF F_1 CHLAMYDOSPORES DERIVED FROM CROSSES BETWEEN THE SORGHUM AND CORN HEAD SMUT FUNGI TO GOLDEN BANTAM SWEET CORN AND SUGAR DRIP SORGHUM

Host Source of F1 Chlamydospore	Hybrid Constitution	Pathogenicity to Golden Bantam Sweet Corn	Pathogenicity to Sugar Drip Sorghum
Sorghum	1S3 x 4C3	+	+
Sorghum	1S4 x 3C1	+	+
Sorghum	3S3 x 3C3	+	+
Corn	2S1 x 4C1	+	+
Corn	1S3 x 1C3	+	+
Corn	3S2 x 3C1	-	+

+ = Smutted Plants
- = Healthy Plants

containing the gene governing pathogenicity to sorghum and two sporidia containing the gene governing pathogenicity to corn. If such sporidia are mixed in pairs in six possible combinations and injected into sorghum and corn, one of the combinations should produce head smut only on corn, another one only on sorghum and four on both sorghum and corn. However since not all pairs of six possible combinations are compatible with one another a complication is introduced such that this factor determines whether or not hybridization will occur between any two sporidial combinations. If a pair of allelic genes (+, -) governed compatability among the sporidia and it was unlinked to the gene pair governing pathogenicity then a situation would occur where only four compatible combinations would be possible among the four sporidia and these would be classified for pathogenicity as follows: one combination yielding head smut only on sorghum, one only on corn and two on both sorghum and corn. Further, if the pair of genes for compatibility was closely linked to the pair governing pathogenicity to both sorghum and corn then the four compatible combinations of the four sporidia would yield head smut on both sorghum and corn and not on one or the other. On the other hand, if compatibility among sporidia is dependent on additional genes with more or less dosage value effects, as may be derived from the compatibility data in Table IX, and if pathogenicity is still monogenic and linked to the compatibility factors, then all possible combinations among the four single sporidia from a chlamydospore may be expected to yield disproportionate numbers of compatible combinations and with no pathogenicity to both sorghum and corn in some of them.

Such non-dual pathogenic compatible combinations however should be pathogenic to either sorghum or corn since they would be expected to carry a pair of genes for pathogenicity to either sorghum or corn.

To analyze in a preliminary way the factors for pathogenicity expression in such hybrid chlamydospores, two chlamydospores designated A and B from a cross 3S3 x 3C2 and one each designated C and D from crosses 3S1 x 4C1 and 3S2 x 2C1, respectively, all obtained from Golden Bantam sweet corn, were germinated on an agar medium and the 4 sporidia produced by each were isolated and cultured. Sub-cultures of the 4 monosporidial cultures so established from each chlamydospore then were brought together in all possible combinations and evaluated for compatability by the Bauch test on PDA, and for pathogenicity on Golden Bantam sweet corn and Sugar Drip sorghum. The results obtained are presented in Table XI with (+) signs indicating compatability or pathogenicity (head smut production) and (-) signs indicating no compatability or no pathogenicity. As may be seen from this table there were more compatible combinations among the monosporidial cultures than there were pathogenic combinations, with variations being evident in this relationship among the chlamydospores. The variations were such that one of them (C) had no pathogenic combinations and another of them (B) had as many pathogenic combinations as compatible combinations. The surprising aspect of the results was that in all instances the pathogenic combinations were pathogenic to both sorghum and corn and there was no combination that was pathogenic to either sorghum or corn alone. Since pathogenic combinations of monosporidial cultures can express their pathogenic

TABLE XI. COMPATIBILITY BETWEEN AND PATHOGENICITY OF PAIRED SPORIDIA DERIVED FROM F_1 CHLAMYDOSPORES

Hybrid Chlamydospore		Sporidial Combinations	Compatibility	Pathogenicity to	
Designation	Constitution			Golden Bantam Sweet Corn	Sugar Drip Sorghum
		1 x 2	+	-	-
		1 x 3	+	+	+
A	3S3 x 3C2	1 x 4	+	+	+
		2 x 3	+	+	+
		2 x 4	+	+	+
		3 x 4	-	-	-
		1 x 2	-	-	-
		1 x 3	+	+	+
B	3S3 x 3C2	1 x 4	+	+	+
		2 x 3	-	-	-
		2 x 4	-	-	-
		3 x 4	-	-	-
		1 x 2	+	-	-
		1 x 3	+	-	-
C	3S1 x 4C1	1 x 4	+	-	-
		2 x 3	-	-	-
		2 x 4	+	-	-
		3 x 4	-	-	-
		1 x 2	+	-	-
		1 x 3	+	+	+
D	3S2 x 2C1	1 x 4	+	+	+
		2 x 3	+	+	+
		2 x 4	+	+	+
		3 x 4	+	-	-

+ = Compatible and/or Pathogenic
- = Non-compatible and/or Non-pathogenic

capabilities only when they are compatible, the possibility exists that detection of pathogenic capabilities of sporidial combinations to either sorghum or corn alone, or even to both sorghum and corn in some instances, may be obscured by the incompatibility of those combinations.

Therefore to see what genetic explanation might be invoked to interpret the data in Table XI, the same information was arranged in a lattice shown in Table XII for each hybrid chlamydospore, with (+) indicating compatible combinations of monosporidial cultures and (p) indicating pathogenic combinations of such cultures to both sorghum and corn. Assuming hybrid chlamydospores contain allelic factors designated C and S for pathogenicity to corn and sorghum respectively, as suggested in the introductory portion of this section, 2 of the 4 monosporidial cultures that arose from them would then contain either a C or an S factor and the other two would contain the other factor as designated along the top and side of each lattice in Table XII. Accordingly, if C and S factors together determine pathogenicity to sorghum and corn, C and C factors together determine pathogenicity to corn only and S and S factors together determine pathogenicity to sorghum only, then the four combinations among monosporidial cultures in the upper right hand corner and those in the lower left hand corner of each of the lattices should be pathogenic to sorghum and corn, while 2 of the 4 combinations in the upper left hand corner should be pathogenic only to corn and 2 of the 4 in the lower right hand corner should be pathogenic only to sorghum. The other two combinations in each of these corners represent selfing and therefore they would not be compatible with themselves.

TABLE XII. COMPATIBILITY AND PATHOGENICITY OF ALL POSSIBLE PAIRINGS BETWEEN MONOSPORIDIAL LINES FROM EACH OF FOUR F_1 CHLAMYDOSPORES

A
3S3 x 3C2

	1	2	3	4
1	-	+	+(p)	+(p)
2	+	-	+(p)	+(p)
3	+(p)	+(p)	-	-
4	+(p)	+(p)	-	-

B
3S3 x 3C2

	1	2	3	4
1	-	-	+(p)	+(p)
2	-	-	-	-
3	+(p)	-	-	-
4	+(p)	-	-	-

C
3S1 x 4C1

	1	2	3	4
1	-	+	+	+
2	+	-	-	+
3	+	-	-	-
4	+	+	-	-

D
3S2 x 2C1

	1	2	3	4
1	-	+	+(p)	+(p)
2	+	-	+(p)	+(p)
3	+(p)	+(p)	-	+
4	+(p)	+(p)	+	-

+ = Compatible
+(p) = Compatible and pathogenic
- = Neither compatible nor pathogenic

Thus in viewing the four lattices one sees that the monosporidial combinations represented by the lower left and upper right hand corners for chlamydospores A and D were fully compatible and pathogenic to sorghum and corn while those for chlamydospore B were one-half compatible and correspondingly pathogenic, while those for chlamydospore C were three-fourth compatible and non-pathogenic. Obviously some interferring factor or factors must be invoked to account for the abberant results in each of these quartiles of the lattices for chlamydospores B and C, and in addition, to account for the pathogenicity failures to only corn or sorghum by the compatible combinations in the upper left hand and lower right quadrants, respectively, of each of these lattices. Of course, one of these factors would be compatibility which did not segregate as a single pair of allelic genes but as a "dosage" modification of it with all four chlamydospores. Another factor applicable to all four chlamydospores would be an inhibitory gene to pathogenicity expression as contributed by each parent going into a cross. If such a factor existed in a heterozygous state in both the sorghum and the corn head smut fungi and the corresponding factor H in the sorghum head smut fungus was an allele of a similar factor I in the corn head smut fungus, then combinations of these factors such as HH or II from compatible monosporidial cultures with pathogenicity factors only toward sorghum or corn, respectively, would have suppressed that pathogenicity, while a combination of HI from compatible monosporidial cultures with pathogenicity factors to sorghum and corn would not have suppressed that pathogenicity to both unless a rare factor A had been present that

synergized their action. With such reasoning from the results depicted in the four lattices the factors contributing to head smut production by hybrid chlamydospores and by their parents may be understood, giving order to the results that otherwise would appear unintelligible.

Part 2. Biology of Sorghum Head Smut Chlamydospores in Soil

In addition to the inherent physiological variations within Sphacelotheca reiliana expounded in the preceding section, there are other sources of variation that also may contribute to the variable incidence of sorghum head smut; namely, (1) the viability of chlamydospores, (2) manner of chlamydospore germination in the soil, (3) the survival capability of chlamydospores in the soil, (4) the number and distribution of viable chlamydospores in the soil, (5) the erratic performance of seed borne chlamydospores to produce head smut, (6) the direct influence of temperature and soil composition on infection, and (7) the resistance the host offers to the progress of infection. As each one of these items could command extensive studies in their own right, the present investigation of these variation sources was of a preliminary nature limited to items 2, 3, 4, 5 and 7.

Method of Chlamydospore Germination in the Soil

In view of the previously cited capacity of hybrid chlamydospores from crosses of sorghum and corn head smut fungi to yield head smut on both corn and sorghum, the question arose as to why such chlamydospores are not found in nature in head smut sori on either of these crops. Obviously one reason for this may reside in the way the chlamydospores

germinate in the soil and infect the plant. According to generally held concepts, hybridization must occur because chlamydospores in the soil are believed to germinate in the same manner as they do on glass surfaces or agar media in the laboratory. That is, the dikaryotic chlamydospore produces a short promycelium within which a diploid nucleus divides meiotically and mitotically to form four haploid nuclei segregated for compatibility, pathogenicity and other characters and each nucleus subsequently divides repeatedly to yield many sporidia (10, 16, 17). Fusion between compatible sporidia from different nuclei then occurs in the soil or after hyphal penetration of the plant and yields a dikaryotic mycelium that eventually produces a head smut sorus of chlamydospores.

To observe the manner of germination in the soil, chlamydospores from both sorghum and corn head smut sori were dusted onto glass slides, covered with water-permeable cellophane and buried for 3-4 days in moist soil at 50% of its water holding capacity in plastic boxes at 30°C. In the very first and in subsequent tests with different soil samples and with different soil moisture, as already reported (3), the chlamydospores germinated by producing long, branched hyphae instead of short promycelia, which is the invariable situation described above on glass surfaces or agar media in the laboratory. The manner of germination in soil is illustrated in Figure 12 from the drawings made with the aid of a camera lucida. Germination types 6, 7, 8 and 9 of this figure were more frequent than those in the other figures of this plate. The hyphae resembled those formed when compatible monosporidial cultures are brought together on an agar medium. Generally the apical portions

Figure 12. Manner of Chlamydospore Germination of Sphacelotheca reiliana in Soil

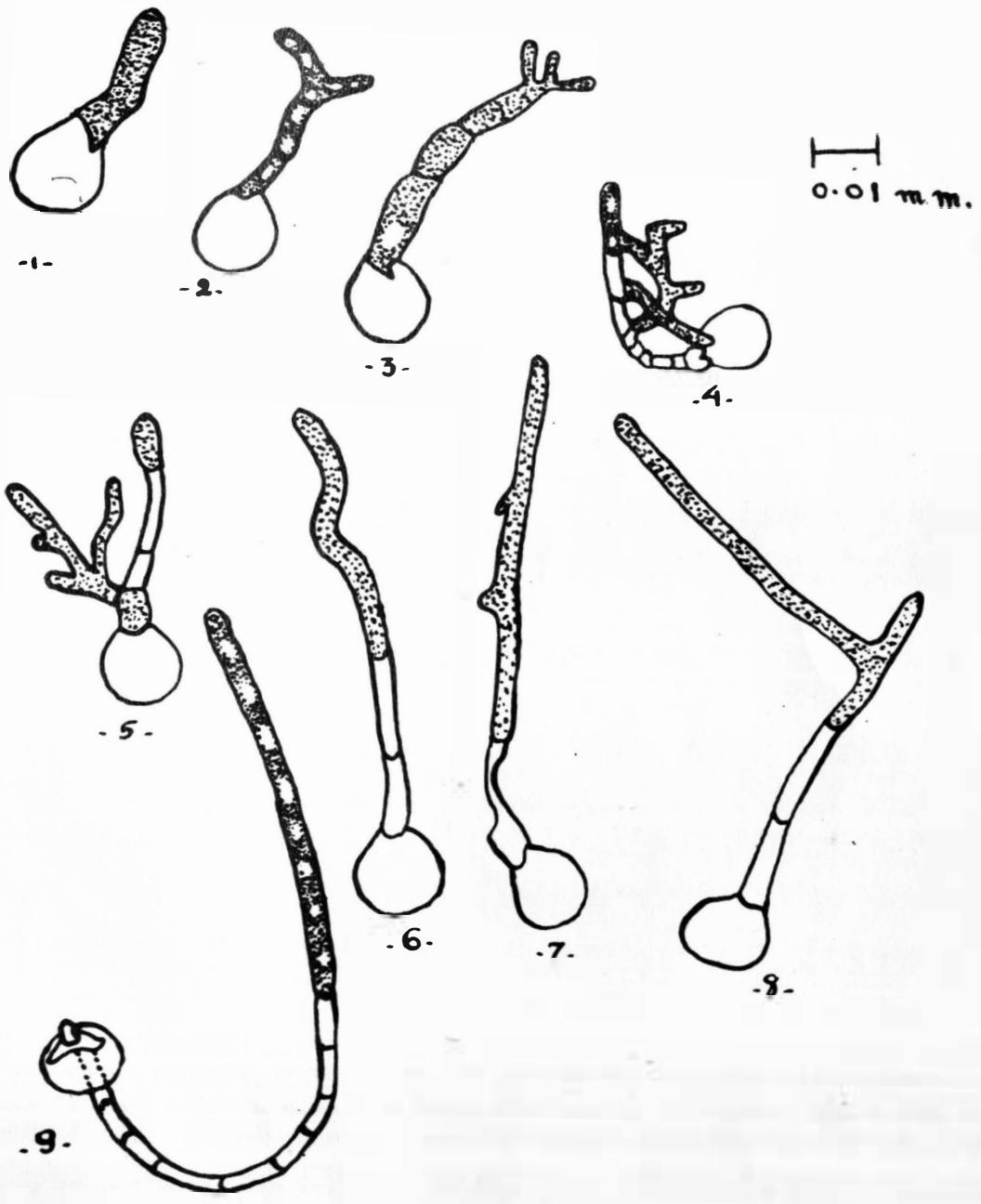

Figure 12.

of the long branched hyphae were filled with vacuolated or nonvacuolated protoplasm and they were cut off by cross walls from the lower portions of the hyphae. The lower portions of the hyphae were devoid of protoplasm and often collapsed.

Although the nuclear condition of the hyphae was not determined the hyphae presumably contained the diploid complement of chromosomes necessary for the hyphae to infect the plant directly and yield head smut sori. With this manner of germination hybridization would be excluded unless an interchange of haploid nuclei took place via hyphal fusions between dikaryotic mycelia in the soil or in the plant following multiple infections by mycelia from different chlamydospores. As fusion between hyphae from different chlamydospores were not seen on glass slides buried in soil, the hyphal manner of chlamydospore germination in the soil environment may be taken tentatively as evidence for the scarcity or absence of hybridization in nature.

Survival of Sorghum Head Smut Chlamydospores in Soil

As mentioned under Review of Literature, Reed et al. (21) and Leukel (13) mixed chlamydospores with moist soil and from it obtained head smut on corn and/or sorghum within a maximum of 5 days or within 9 weeks thereafter, respectively. Under some natural conditions as in the semi-arid, warm areas of Texas, New Mexico, California and Washington, the chlamydospores presumably survive in the soil from year to year as seen from the yearly occurrence of the disease in those areas. In the more humid, cool areas the disease seldom is found and one reason for that may be the nearly complete failure of chlamydospores in or on the

soil to overwinter in those areas. Such failure was brought out by the following two field experiments conducted in areas immediately adjacent to Brookings, South Dakota.

In the first of these a field area 200 feet long by 160 feet wide on North Range 1 of the Plant Pathology Farm east of the South Dakota State College Campus was planted in 1959 to 57 sorghum varieties including three each of Sudangrass and broomcorn, and hypodermically inoculated with monochlamydosporous cultures from seven widely different collections of chlamydospores. The plantings and inoculations were for the purpose of the test previously reported under heading "Races of the Sorghum Head Smut Fungus." The average per cent of plants smutted was 67.7 per cent and of heads smutted 52.2 per cent. The chlamydospores had ample opportunity to reach the soil immediately supporting the plants and to become wind-borne to the soil some distance away from them.

In mid-September all of the plants in the plot were cut off with a binder, piled and covered in a dump area and the soil was immediately plowed to a depth of 6 inches. In mid-June, 1960 the same width area and that adjoining it for 150 feet to the north was planted to Reliance sorghum, a head smut susceptible variety, while that adjoining it for 265 feet to the south was planted to 30 different varieties of sorghum. In September 1960 the entire area of sorghum was free of head smut except for one head smut sorus appearing in the center of the area previously supporting inoculated plants and for another head smut sorus appearing on the Agronomy Department Plots one-half mile to the east

of that area. As sorghum head smut is not known to occur even occasionally within a radius of one hundred miles of Brookings, the possibility is strong that the two closely adjacent instances of head smut occurrence arose from the inoculum supplied by the inoculated plot. Nevertheless the surprising aspect of the test was that only two plants in the immediate and surrounding area became infected.

In the other test which was conducted at the same time as the first, finely screened chlamydospore material collected from the large plot of infected plants mentioned above was raked in to a depth of one inch in four rectangular plots, each 5 feet by 4 feet. Approximately 700 ml. of these chlamydospores were applied on October 4, 1959, to each of these plots located one mile west of Brookings in an area protected on the north and west by a shelter-belt of trees. Two of the plots, which were adjacent to one another, were 10 feet away from a large cottonwood tree, while the other two plots, which also were adjacent to one another, were located approximately 700 feet to the east from first two and 100 feet clear of the nearest tree. In June of 1960, the plots were shallowly spaded, raked and planted to approximately 100 seeds each of head smut susceptible Dual sorghum arranged in four rows. Seedling emergence was good in all of the plots but plant development was much retarded because of a deficiency of moisture in the two plots close to the cottonwood trees. Head smut failed to appear in the two plots close to the cottonwood trees, while it appeared on one plant in one and on six in another of the two plots 100 feet away from the nearest tree. Head smut also failed to appear on sorghum in a nearby one acre field

150 feet south of the latter two plots. In this test where superabundant amounts of spores were added to the soil, the disease thus either failed to appear or it appeared in very low amounts. This again points to the low overwinter survival of chlamydospores in humid cool regions.

To ascertain in a preliminary way what relation temperature and moisture would have on the survival capability of chlamydospores in soil, a laboratory experiment was then set up in which 10 per cent by weight of sorghum head smut chlamydospores, collected from the above mentioned inoculated sorghum field plants, were uniformly mixed with a local soil and stored at set conditions. For this purpose 200 lots from one large mixture were placed in tightly covered glass jars and held at -1.5°, 15°, 20° and 30° C. with soil moisture at near wilting point, 20, 40 or 100 per cent of field capacity. At intervals of 3, 7, 14 and 30 days thereafter the mixture from one randomly selected jar representing each of the different temperature and moisture conditions was emptied into three shallow 8 cm. wide plastic dishes. These were planted with 20 to 25 seeds of the head smut susceptible Double Dwarf Shallu sorghum at the 3-day interval, and because of a seedling blight disease on this variety, to Dual sorghum seeds at all later intervals. The planted mixtures were moistened to nearly equal amounts and transferred to a warm greenhouse room where they were kept moist for seedlings development. When seedlings attained nearly the 3-leaf stage they were lifted from the mixture, transplanted to potted soil in the same greenhouse room and held there until heading time for the appearance of head smut. The per cent of plants smutted in this experiment are shown in Table XIII. At zero

TABLE XIII. PER CENT HEAD SMUTTED SORGHUM PLANTS DEVELOPING IN CHLAMYDOSPORE INFESTED SOIL STORED FOR DIFFERENT PERIODS AT DIFFERENT MOISTURES AND TEMPERATURES

Storage Period	Soil Moisture and Temperature															
	W.P.				20% of F.C.				40% of F.C.				F.C.			
	-1.5C.	10C.	20C.	30C.	-1.5C.	10C.	20C.	30C.	-1.5C.	10C.	20C.	30C.	-1.5C.	10C.	20C.	30C.
3 Days	3.4	36.3	11.6	48	9.6	17.1	20	71	55	9.0	16.1	53.5	27.2	10	25	20
7 Days	0	0	6	7.1	16.6	26.6	64.4	31.2	7.9	20	48.3	36	23	4.3	21.4	28
14 Days	0	2.8	2.6	9	0	3	0	0	0	3.6	5.9	6	0	0	9	10
One Month	0	0	9.5	5.2	0	9.6	16.1	2.8	0	5.4	4.5	3	0	0	12.9	13.7

W.P. = Wilting point

F.C. = Field capacity

time the original chlamydospore-soil mixture planted to Double Dwarf Shallu sorghum yielded 73 per cent smutted plants, while the soil alone yielded no smutted plants.

As may be observed from these data in the table, chlamydospores survived at least 30 days at all moistures but not at all temperatures. They survived for a shorter period at -1.5°C. than at any of the other temperatures. Since spore-soil mixtures were not replicated at the various storage conditions, no differential pattern of survival is evident from an interaction of temperature and moisture effects. Accepting per cent of smutted plants as related to the abundance of viable chlamydospores in the soil, one might consider the abundance of viable chlamydospores to have declined rapidly after 7 days at soil moistures of 20, 40 and 100 per cent of field capacity and after 3 days at wilting point soil moisture. In some measure the data may be taken to point to freezing temperature as having a marked influence on survival of chlamydospores.

Percent of Sorghum Plants Sustaining Head Smut as Related to the Abundance of Sorghum Head Smut Chlamydospores in the Soil

With scarcity and decline of sorghum head smut incidence noted in the preceding field and laboratory experiments, the question arose as to whether or not these were the result of a decline in abundance of viable chlamydospores in the soil over that present at the start. To provide an answer to that question the following experiment was conducted.

Weighed quantities of finely-screened chlamydospore material collected from the 1959 inoculated sorghum field plot previously referred

to, were each mixed with 10 grams of talc and then with enough air-dry, finely-screened soil to make a total weight of 500 grams. The chlamydospore-soil mixtures thus prepared were evenly watered to 40 per cent of field capacity and apportioned to three shallow cans each where 10 seeds of Double Dwarf shallu were planted to a one-inch depth. The shallow cans were immediately transferred to a warm greenhouse bench where they were left covered with translucent plastic sheets until most of the seedlings attained a 3-leaf stage of development. At that time the plastic covers were removed and the seedlings were transferred to potted soil in the same greenhouse area. The per cent of plants smutted at heading time is recorded in Table XIV.

As may be seen from the table, head smut failed to appear on sorghum plants when the number of chlamydospores was 1.6×10^4 or less per gram of soil mixture while it appeared on 25 per cent of the plants when the number was 1.6×10^5, on nearly 50 per cent of the plants when the number was 1.6×10^6, and on nearly 90 per cent of the plants when the number was 1.6×10^7 or more. Within this range of chlamydospore numbers the per cent of smutted plants was nearly linearly related to the logarithm of the number of chlamydospores in the soil and curvilinearly related to the distance those chlamydospores were apart in the soil. A positive answer was thus provided to the question raised above, with the additional item that a threshold of slightly more than 16000 chlamydospores per gram of soil, corresponding to a center to center distance between chlamydospore of less than 400μ, was needed for the start of head smut appearance. However as only about 5 per cent at most

TABLE XIV. THE RELATION BETWEEN PER CENT OF HEAD SMUTTED SORGHUM PLANTS AND THE AMOUNT OF INOCULUM IN SOIL

Weight of Inoculum in Mixture		Number of Spores per Gram of Mixture (1)	Average Distance Spores were Apart in Mixture (2)	Number of Plants in Test	Plants Smutted
Grams per 500 Grams Mixture	%				%
0.0005	0.0001	1.6×10^3	833 μ	21	0
0.005	0.001	1.6×10^4	400 μ	27	0
0.05	0.01	1.6×10^5	185 μ	20	25
0.5	0.1	1.6×10^6	90 μ	21	47.6
5	1	1.6×10^7	40 μ	16	93.7
25	5	8×10^7	20 μ	20	90.0
50	10	1.6×10^8	10 μ	22	90.9
Check		0		16	0

(1) By sample count, 1 gram of inoculum contained 1.6×10^9 chlamydospores.
(2) Calculated by dividing 10,000, the number of microns in 1 cm., by the cube root of the number of chlamydospores in 1 gram of mixture. One gram of the mixture was found to occupy a volume of 1.0 cubic centimeter.

of the above chlamydospores were observed to germinate on agar media or in soil in an earlier experiment the threshold number of germinable chlamydospores accordingly should be reduced to about 800 per gram with a corresponding maximum distance of about 1087 μ between their centers. Since chlamydospore diameters average 12 μ such distances would mean approximately 73 chlamydospore diameters. The biological significance of this fact is not known.

Chlamydospores on Seeds as a Source of Inoculum for Head Smut Appearance in Sorghum

Sorghum seeds infested with chlamydospores of the sorghum head smut fungus have yielded few smutted plants in the trials of some investigators (11, 16) and none in others (7, 12, 18). On the other hand, corn seeds infested with chlamydospores of the corn head smut fungus have yielded smutted plants more abundantly and consistently than sorghum seeds infested with its fungus. Since corn seeds generally are larger than sorghum seeds and may hold a larger quantity of chlamydospores than sorghum seeds hold, the thought occurred to the writer in the light of the results from the preceding experiment that large quantities of chlamydospores on sorghum seeds may make the difference between getting few to no smutted plants and getting many smutted plants.

To test this, two lots of chlamydospore-infested Double Dwarf Shallu sorghum seeds were prepared. One lot was shaken with an excess of dry sorghum head smut chlamydospores collected from the inoculated sorghum field plants previously described. The other lot was heavily

coated with a high concentration of these chlamydospores in an aqueous solution of Methocel (Dow methylcellulose). In both instances the seeds were blackened by the spores they carried. The seeds of the first lot carried an average of 52,631 chlamydospores per seed and those of the second lot carried an average of 404,578 chlamydospores per seed. Neither lot, however, yielded head-smutted plants when 60 seeds were planted in the field at each of four adjacent locations. The number of chlamydospores on sorghum seeds thus is not the factor that determines failure or near failure of head smut incidence on the crop from chlamydospore-infested seed.

Head Smut Resistance of an F_1 Hybrid Between an Immune and a Susceptible Variety of Sorghum

Out of eight hybrid sorghums and their parents pointedly examined by Stewart and Reyes (22) on chlamydospore-infested fields in Texas, only hybrid RS 630 proved free of head smut while the other seven showed head smut incidence less than, equal to or greater than that of the common susceptible parent. The common susceptible parent in each instance was Combine kafir 60 (9.3 per cent field smut incidence) while the male parents in each instance were different from one another, ranging in field smut incidence from 0.0 to 37.8 per cent. The smut free condition of RS 630 in the field was attributed by these investigators to a completely dominant form of resistance contributed by the male parent, Combine white feterita.

Since feterita sorghums as a group are widely held to be immune or highly resistant to head smut, the thought occurred to the writer

that another feterita variety as male parent might yield the same or a different result especially when inoculated hypodermically with monochlamydosporous cultures. Accordingly, an F_1 hybrid was prepared from Combine kafir 60 as male-sterile female and FC 811 Feterita as male and the seedlings were inoculated hypodermically with a monochlamydosporous culture derived from New Mexico 1 collection of sorghum head smut. In this instance the female parent was extremely susceptible to and the male parent was entirely free from head smut when inoculated with seven different sources of monochlamydosporous cultures including the one above, as shown in Table V. The F_1 plants also proved free of head smut in the present test and this then confirmed the dominant type of resistance in RS 630 noted by Stewart and Reyes (22) and extended the source of this dominance to another variety of feterita sorghum.

DISCUSSION

As is apparent from the results of the present study, the variability of Sphacelotheca reiliana, the biology of its chlamydospores in the soil, and host susceptibility to it are important factors that control the incidence of head smut in sorghum, as well as in corn.

The species appears to be a complex one, comprised of forms that are specialized to sorghum and to corn, and to certain varieties of sorghum. Presumably it comprises forms that are specialized to certain varieties of corn, but this aspect of its specialization was not investigated.

Reed et al. (21) first noted specialization of the species to sorghum and corn, and later Reed (19) assigned race status to the two forms. In this thesis the writer referred to each of the forms as separate fungi, not as races, for two reasons; namely, (1) races were demonstrated within the sorghum head smut form, and (2) most chlamydospore collections from sorghum head smut were pathogenic to North Star sweet corn. If races could also be demonstrated within the corn head smut form, and proven different from those of the sorghum head smut form, then a basis would be provided for viewing the difference between these forms as varietal. If that were so, and on the basis of priority, the sorghum head smut form would become S. reiliana (Kühn) Clint., as first applied in 1875 to the fungus on sorghum, and the corn head smut form would become S. reiliana (Kühn) Clint., forma zeae Pass., as first applied in 1876 to the fungus on corn. The corn head smut fungus then would be a variety of the one on sorghum, as first supposed by Passerini.

Support for a varietal difference between the two fungi may be derived, perhaps, from the performance of hybrids between them, as noted in the present study. The hybrids from 105 of 108 compatible pairs of their monosporidial cultures proved pathogenic to both Sugar Drip sorghum and Golden Bantam sweet corn, with three of these hybrids being non-pathogenic to either. The pathogenic hybrids were less virulent to both of these crops than were either of the wild-type parents to their respective crop. Compatible sporidia from four of the F_1 hybrid chlamydospores either were non-pathogenic to either of these crops or they were like the hybrid in pathogenicity and virulence to both of them, with none being like the parents. Similarily, F_1 chlamydosporous cultures from three hybrids on Sugar Drip sorghum and two on Golden Bantam sweet corn yielded head smut on both of these crops, while another one from Golden Bantam sweet corn yielded head smut only on sorghum. A somewhat parallel instance to this situation has been reported by Fischer (8) for six hybrids between Ustilago bullata and U. hordei. In these hybrids, parental pathogenic segregants were not obtained from five, while a pathogenic segregant to one parent was obtained from the sixth. Lysis of promycelia and sporidia was the rule among hybrid chlamydospores from U. bullata X U. hordei, while it was absent in the hybrid chlamydospores from our cross. The sporidia formed from hybrid chlamydospores in our cross were larger than those of the parents, and elongated.

The importance to disease incidence of pathogenicity factors within the sorghum, or even the corn, head smut races, and of the presence or absence of resistance factors in the host, may be derived from certain

contrasting situations revealed by the present study. Sorghum hybrid RS 630 was found immune from head smut in Texas, and in this study, from 17 chlamydospore collections of sorghum head smut from five widely-separated geographical areas. It proved susceptible to a chlamydospore collection of sorghum head smut designated Poona 2. North Star sweet corn, susceptible to the corn head smut fungus, was considered immune from the sorghum head smut fungus. It proved susceptible to 14 of the above 18 chlamydospore collections from sorghum. A chlamydospore collection from Sudangrass head smut in California proved pathogenic on 50 of 57 sorghum varieties, and on North Star sweet corn, while a chlamydospore collection from sorghum head smut from India, designated Poona 1, proved pathogenic on 20 sorghum varieties, and not on North Star sweet corn. From these situations one may infer that races are the basic units of S. reiliana, and that these races may be grouped into two varieties, with each variety carrying one or more major genes for pathogenicity to either sorghum or corn. A host variety, such as North Star sweet corn, lacking resistance factors to these genes would be susceptible to races of both varieties, while a host variety, such as Golden Bantam sweet corn, having resistance factors to the sorghum and not the corn head smut fungus would be resistant to the one and not the other.

The failure to differentiate races among sorghum head smut chlamydospores collected from Texas and New Mexico in the present study may be due to any one of three causes: (1) the sorghum varieties used were inadequate to differentiate all races; (2) the criterion of 'some or no' head smut development on the varieties was too severe for all race

differentiation; and (3) the nuclear products from chlamydospore germination normally do not hybridize in nature. Cause 2, which encompasses cause 1, may be valid, but in the opinion of the writer it is untenable for the reason that a less severe criterion, as per cent of plants infected, is subject to the vagaries of inoculative procedure and of environmental control. Cause 1 may be valid and should be explored to learn if it is so. Cause 3 appears valid on the evidence of a direct manner of chlamydospore germination in the soil, as observed in this study, and on the apparent absence of chlamydospore collections from either sorghum or corn that would yield head smut equally on both of these crops, excluding North Star sweet corn. Cause 3 would account for a low number of races found, for the occurrence of one or at most few races within a region, for the natural stability of those races, and for the natural stability of the sorghum and the corn head smut fungi. It would also point to new susceptible varieties as the cause of the recent increase of sorghum head smut in Texas.

With a change to susceptible varieties, the inoculum may be expected to become more abundant in the soil and, as shown in the present study, the per cent of smutted plants may be expected to increase. Furthermore, the inoculum may be expected to persist in greater abundance in Texas soils, where temperatures seldom become freezing, than in areas such as South Dakota, where the top soil is frozen from mid-November to the end of March.

SUMMARY AND CONCLUSIONS

Sphacelotheca reiliana, the cause of head smut in sorghum and corn, was examined first for forms specialized to varieties of sorghum and next for behavior of its chlamydospores in the soil, as these relate to disease incidence. For the first, 18 chlamydosporous sori from sorghum were collected from California, New Mexico, Texas and India, and two chlamydosporous sori from corn were collected from California and Washington. Chlamydosporous and paired monosporidial cultures from all or some of these collections were injected as desired into seedlings of a select number of 57 sorghum or of three sweet corn varieties to establish pathogenicities. For the second, chlamydospores were derived either from the above collections or from many inoculated sorghum varieties from a local plot.

Chlamydosporous cultures from the two corn sources yielded head smut only on the three sweet corn varieties and not on any one of 14 sorghum varieties. Cultures from 14 of 18 sorghum sources yielded head smut on Sugar Drip sorghum and North Star sweet corn, while cultures from the other four sources yielded head smut only on Sugar Drip sorghum. Cultures from all 18 sorghum sources were differentiated in the field and greenhouse into four races, corresponding to their source; namely, (1) California (Sudangrass), (2) Poona 1 and Coimbatore 2, (3) Poona 2, and (4) Texas (including Sudangrass), New Mexico and Coimbatore 1. Of seven cultures from the same number of sorghum sources, one from California Sudangrass proved most pathogenic, yielding head smut on 50 of 57 sorghum varieties, and on North Star sweet corn, while another from

India (Poona 1) was least pathogenic, yielding head smut on 20 of 57 varieties, and not on North Star sweet corn. The culture from California Sudangrass was the only one used to establish the susceptibility of North Star and the resistance of Golden Beauty and Sugar and Gold sweet corn varieties to cultures of sorghum head smut. The difference between the sorghum and the corn head smut fungi thus could be considered to be varietal, rather than racial as supposed by Reed.

Thirteen monosporidial cultures from four chlamydospores of the New Mexico 1 sorghum head smut collection and 17 similar cultures from five chlamydospores of the California corn head smut collection yielded 108 Bauch-test-proved compatible pairs between these collections. Of these, 105 yielded head smut on both Sugar Drip sorghum and Golden Bantam sweet corn, while the other three did not yield head smut on either of these crops. The hybrids were less virulent on either of these crops than were either of the wild-type parents on their respective crop. Chlamydosporous cultures of F_1 chlamydospores from three hybrids on Sugar Drip sorghum and from two on Golden Bantam sweet corn yielded head smut on both of these crops, while a similar culture from one hybrid on Golden Bantam sweet corn yielded head smut only on the sorghum. Intra-compatible sporidia from three F_1 chlamydospores, representing two hybrids, yielded head smut on both Sugar Drip sorghum and Golden Bantam sweet corn, while none yielded head smut on only one or the other of these crops. The intra-compatible sporidia from a fourth F_1 chlamydospore, representing a third hybrid, were non-pathogenic to either of the two crops. The hybrids thus combined the pathogenicity

factors from both parents, and in the F_2, these factors either failed to segregate, they segregated only to one parent, or they were lost. This behavior may be taken as further evidence to support a varietal-type difference between the sorghum and the corn head smut fungi, rather than racial.

Chlamydospores of the sorghum and corn head smut fungi germinated in soil by forming branched, septate hyphae with dense, vacuolated or non-vacuolated protoplasm at hyphal tips. Sporidia were not formed. The manner of germination was considered a possible cause of the relatively few number of races of the pathogen found in this study and of the apparent stability of the sorghum and corn head smut fungi as separate units in nature.

Chlamydospore abundance in soil affected the per cent incidence of head-smutted sorghum plants. Within limits, the latter was linearly related to the logarithm of the number of chlamydospores in the soil and curvilinearly related to the distance those chlamydospores were apart in the soil. The threshold number of viable spores necessary for infection was estimated at about 800 per one cubic milliliters of soil, or a distance between chlamydospores of about 73 chlamydospore diameters.

The abundance of infectious chlamydospores in soil declined rapidly within 7 days to sub-threshold levels at temperatures near freezing, regardless of whether the soil moisture was near the wilting point, 20%, 40% of field capacity, or at field capacity. The decline at 10° to 30°C. at these same soil moistures was less rapid and in most instances did not reach the threshold limit within 30 days.

Chlamydospores on sorghum seeds in numbers of 52,631 or even 404,578 per seed did not prove to be an inoculum source for head smut development in that crop. No head-smutted plants developed from such seed.

The dominant form of resistance to head smut contributed by Combine white feterita in sorghum hybrid RS 630 was extended to include another feterita variety; namely, FC 811 Feterita.

LITERATURE CITED

(1) Al-Sohaily, I. A., and C. J. Mankin. 1960. Reaction of corn and sorghum to corn and sudangrass head smut. Plant Disease Reptr. 44:113-114.

(2) Al-Sohaily, I. A., and C. J. Mankin. 1960. Races of head smut of sorghum. (Abstr.) Phytopathology 50:627.

(3) Al-Sohaily, I. A., and C. J. Mankin. 1960. Method of chlamydospore germination of Sphacelotheca reiliana in soil. (Abstr.) Phytopathology 50:627.

(4) Bressman, E. N., and H. P. Brass. 1933. Experiments with head smut of corn in Western Oregon. Phytopathology 23:396-403.

(5) Chen, C. H. 1937. The development of vascular tissues and the initiation of the inflorescence in Holcus sorghum. Iowa State Coll. J. Sci. 12:217-221.

(6) Christensen, J. J. 1926. The relation of soil temperature and soil moisture to the development of head smut of sorghum. Phytopathology 16:353-357.

(7) Clinton, G. P. 1900. The smuts of Illinois' agricultural plants. Ill. Agr. Expt. Sta. Bull. 57:289-360.

(8) Fischer, G. W. 1951. Induced hybridization in graminicolous smut fungi. I. Ustilago hordei x U. bullata. Phytopathology 41: 839-853.

(9) Halisky, P. M., D. G. Smeltzer, and B. R. Houston. 1959. Head smut of sudangrass and sorghum in California. Plant Disease Reptr. 43:1084-1090.

(10) Hanna, W. F. 1929. Studies in the physiology and cytology of Ustilago zeae and Sorosporium reilianum. Phytopathology 19: 415-441.

(11) Kellerman, W. A. 1891. Experiments with sorghum smuts. Kans. Agr. Expt. Sta. Bull. 23:95-101.

(12) Kellerman, W. A., and O. E. Jennings. 1902. Smut infection Experiments. Ohio Nat. 2:258-261.

(13) Leukel, R. W. 1956. Studies on sorghum head smut. Plant Disease Reptr. 40:737-738.

(14) Mankin, C. J. 1953. Studies of the biology of Sphacelotheca reiliana causing head smut of corn. Doctoral dissertation, State College of Washington. 66 pp.

(15) Martin, J. H. 1936. Sorghum improvement. U. S. Dept. Agr. Yearbook for 1936, pp. 523-560.

(16) McAlpine, D. 1910. The smut of maize and its treatment. J. Dept. Agr. Victoria 8:290-298.

(17) Norton, J. B. S. 1898. A study of the Kansas Ustilagineae, especially with regard to their germination. Trans. Acad. Sci. St. Louis 7:229-241.

(18) Potter, A. A. 1914. Head smut of sorghum and maize. J. Agr. Res. 2:339-372.

(19) Reed, G. M. 1935. Physiologic specialization of the parasitic fungi. Botan. Rev. 1:119-137.

(20) Reed, G. M., and L. E. Melchers. 1925. Sorghum smuts and varietal resistance in sorghums. U. S. Dept. Agr. Bull. 1284. 56 pp.

(21) Reed, G. M., M. Swabey, and L. A. Kolk. 1927. Experimental studies on head smut of corn and sorghum. Bull. Torrey Botan. Club 54:295-310.

(22) Stewart, R. B., and L. Reyes. 1958. Head smut of sorghum and varietal reaction. Plant Disease Reptr. 42:1133-1140.

(23) Tyler, L. J., and C. P. Shumway. 1935. Hybridization between Sphacelotheca sorghi and Sorosporium reilianum. Phytopathology 25:375-376.

(24) Vaheeduddin, S. 1936. Hybridization between Sphacelotheca cruenta and Sorosporium reilianum. (Abstr.) Phytopathology 26:111.

(25) Vaheeduddin, S. 1942. The pathogenicity and genetics of some sorghum smuts. Minn. Agr. Expt. Sta. Tech. Bull. 154. 46 pp.

TABLE V. PER CENT OF PLANTS AND HEADS SMUTTED IN 57 VARIETIES OF SORGHUM INOCULATED WITH SEVEN SORGHUM HEAD SMUT COLLECTIONS (CONTINUED)

	Head Smut Collections													
	Sorghum												Sudangrass	
	New Mexico						Texas		Coimbatore		Poona		California	
	1		2		3		1		1		1			
Variety	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.	P.	H.
Sugar Drip (Forage Type)	62	-	70	-	59	-	58	-	66	-	40	-	68	-
African Millet (Forage Type)	64	47	74	52	68	60	86	60	78	58	84	68	75	58
Norghum (Kalo Type)	66	60	86	72	78	58	75	68	92	66	2	2	54	40
Norkan (Dual Grain and Forage Type)	74	64	64	50	75	54	80	52	72	40	50	24	62	40
Reliance	36	36	36	40	50	52	76	68	50	45	68	46	36	34
Rancher (Black Amber Forage)	58	72	92	68	82	84	86	54	86	76	66	54	88	90
Double Dwarf shallu	69	82	86	58	88	76	84	60	82	78	80	77	82	68
Yellow Endosperm	16	18	15	14	10	15	28	18	7	4	0	0	18	12
Dual	75	65	100	68	96	80	94	72	96	66	78	58	96	76
Redbine 60 (Kafir Type)	40	58	32	30	40	46	35	25	12	24	0	0	66	54