STUDIES ON THE MODE OF INFECTION OF SORGHUM BY *TOLYPOSPORIUM EHRENBERGII*

by

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TABLE OF CONTENTS

	Page
Acknowledgement	1
List of Figures	2
List of Tables	3
Introduction	4
Literature Review	12
Materials and Methods	17
Results	28
Discussion	41
Summary	49
Literature Cited	50

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LIST OF FIGURES

Figure		Page
1.	A map of Nigeria showing vegetation zones.	8
2.	Typical long smut infection consisting of several widely scattered smut sori.	9
3.	An example of severe long smut infection with many or most florets bearing smut sori.	10
4.	An example of highly localised long smut infection with many sori confined to a small area.	11

LIST OF TABLES

Table		Page
1.	Incidence of long smut resulting from dusting sorghum seed with long smut teliospores before sowing.	28
2.	Incidence of long smut on plants derived from seeds which were germinated and grown for 5 days in a water suspension of sporidia before planting in the field.	30
3.	Incidence of long smut resulting from growing sorghum through the seedling stage in soil heavily infested with germinating teliospores.	31
4.	Incidence of smut on tillers of sorghum plants with smut infected primary heads.	33
5.	The effect of inoculating Lecti panicles at various stages of development with the sporidia, germinating teliospores and ungerminated teliospores of T. ehrenbergii during the rainy season.	36
6.	The effect of inoculating Dwarf Shallu panicles at various stages of development with the sporidia, germinating teliospores and ungerminated teliospores of T. ehrenbergii during the rainy season.	37
7.	The effect of inoculating a mixture of Lecti and Dwarf Shallu panicles at various stages of development with the sporidia, germinating teliospores and ungerminated teliospores of T. ehrenbergii during the dry season.	38
8.	Number of long smut spore balls found on 60 flag leaves of sorghum plants in the boot stage.	40

INTRODUCTION

Sorghum bicolor (L) Moench, an important food crop in northern Nigeria and other parts of the world, is attacked and its potential yield greatly reduced, by numerous disease-causing organisms, among which are the smut fungi which invade and transform the ovaries into smut sori. Tolyposporium ehrenbergii (Kuhn) Pat., the causal organism of long smut, is one of the four smut-causing fungi found on sorghum. The other smuts on sorghum are covered smut, head smut and loose smut.

Long smut of sorghum occurs in many countries in Africa and Asia. Briton-Jones (1922) stated that in Egypt long smut was very common and could be found in almost every crop of sorghum throughout the country, although average yield loss was low (2 per cent). Kulkarni (1918) reported thatin certain of the drier areas of northern India long smut caused heavy losses. Hafiz (1958) reported that in the old Punjab long smut was very common and caused an average yield loss of 5 per cent, although losses from 40 to 60 per cent were observed in individual fields; a record of 90 per cent loss had been observed on sorghum planted on areas used as threshing floors in the previous year. According to Tarr (1962) long smut is common but of little economic importance in Tanganyika (Tanzania) and the Sudan Republic, although a high incidence of infection was commonly observed on Sorghum purpureo-sericeum in the Central Sudan Republic, Kul'pinova (1957) reported that long smut had spread considerably in the middle Asian region of the Soviet Union in recent years and that, although not a major disease of sorghum it could be severe locally.

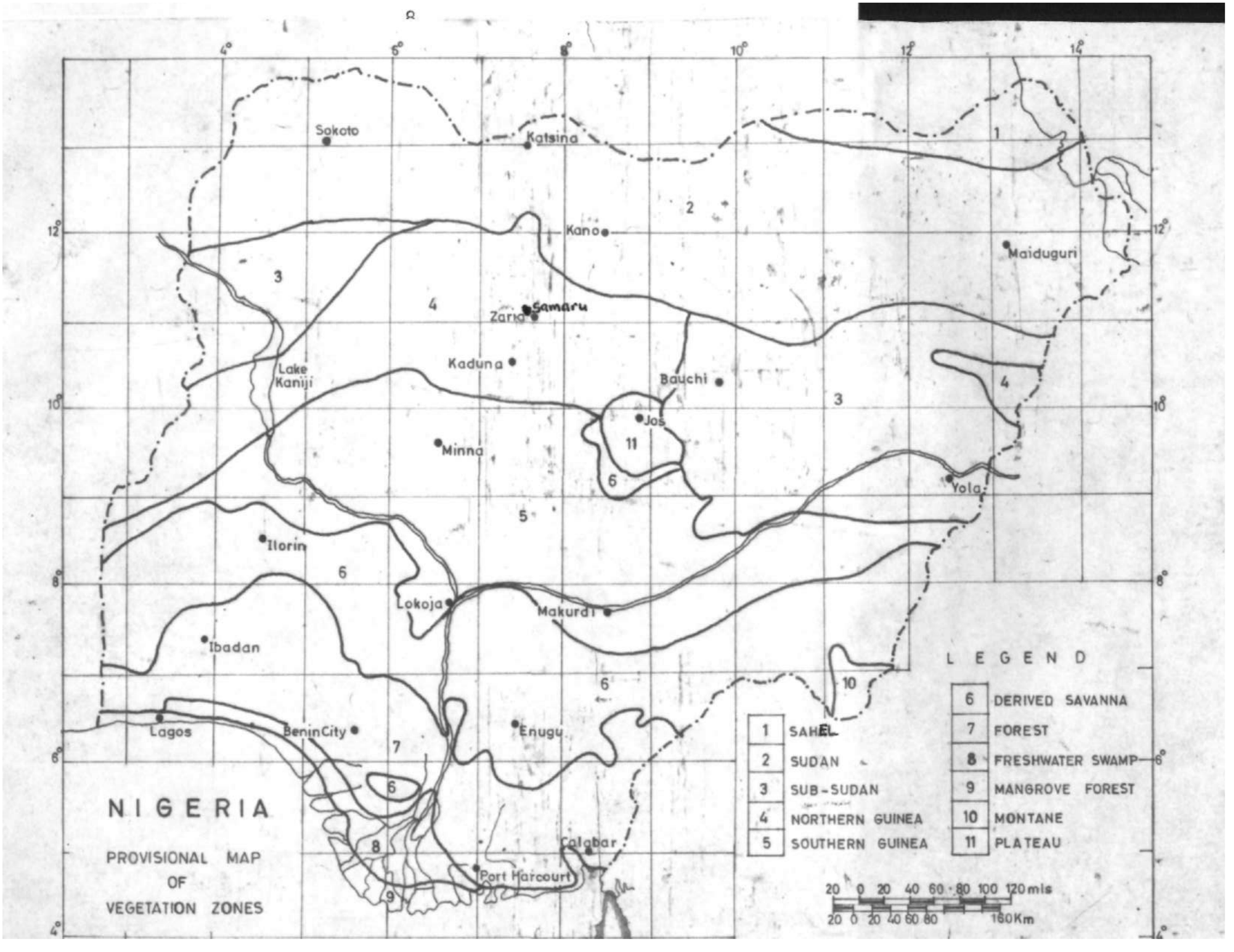
In Nigeria, long smut is confined to the drier parts of the north, namely the Sahel and the Sudan and Northern Guinea Savanna zones (see Fig.I). The Northern Guinea Savanna experiences a lower level of infection than the other two zones. In north-western Sokoto Province of North-west state, long smut is thought to be the most common floral disease of sorghum and is a potential threat to sorghum production. Unless effective control measure are taken, this disease might in the future determine the northern-most latitude of sorghum production at economic level. According to Keay (1968), S. B. King (personal communication) and the author's personal observations, long smut is not as important, in terms of severity and distribution, as the other three smuts that at present occur on sorghum in Nigeria. The fact that covered smut and loose smut are easily controlled by seed dressing (Fischer and Holton, 1957, Tarr, 1962) indicates that in Nigeria covered smut and loose smut offer less potential threat than long smut which is not controlled by seed dressing. Tarr (1962), Sattar and Hafiz (1952), Awoniyi and King (personal communications) have reported observing frequently long smut on sorghum grown from seed treated with copper carbonated and other fungicides. The present author, in 1973, observed severe long smut infection in Kano on sorghum plants raised from seed which had been dressed with a fungicide (Thiram).

Although long smut was known and described as early as 1878 (Mason, 1926), its exact mode of infection has remained obscure.

These scientific investigations, namely, Hafiz (1958), Hassan et al (1970), Prasad (1945), Ragab and Mahdi (1966), Ramakrishnan and Reddy (1949), Sattar and Hafiz (1952) and Vasudeva and Seshadri Iyengar (1950) have given different and contradicting views or conclustions on the mode of long smut infection. The variability in symptoms, with respect to incidence and distribution of sori on the sorghum panicle, makes it difficult for one to assume the mode of long smut infection. The mode of infection among the smut fungi in general can be classified into two basic types. One type is localised infection caused by air-borne sporidia or teliospores alighting on the panicles, stems or leaves of the plant. The common maize smut caused by Ustilago maydis is a typical example of a localised type of infection (Fischer and Holton, 1957). The second type is systemic infection and is caused by seed- or soil-borne pathogens which penetrate young seedlings and grow undetected in apical meristematic tissues until the plants head and smut sori form on the panicles. Bunt of wheat, and covered smut, loose smut and head smut of sorghum are typical examples of systemic seedling infection (Fischer and Holton, 1957). It is uncertain whether long smut involves the first, second, or both infection types. In most cases, long smut sori occur individually and are widely scattered on the panicle, each sorus surrounded by normal grain (Fig. 2). A sorus may develop in one member of a pair of florets, whereas the other member may produce a normal seed. This type of infection resembles localised infection caused by wind-blown sporidia. However, in other cases, the long smut sori are numerous and very close together and confined to certain parts of the panicle (Figs. 3 and 4) which resembles

the systemic type of infection observed with covered smut, loose smut and head smut of sorghum.

The purpose of the present investigation was to elucidate the mode of sorghum infection by T. ehrenbergii. A clear knowledge of the mode of infection would not only be of scientific interest but also of practical value to breeders and plant pathologists. This information could serve as the basis for devising the most strategic and sound control measures against this sorghum disease which is a potential threat to the very happiness of the human race.


Sokoto
Katsina
Kano
Maiduguri
Samaru
Zaria
Kaduna
Lake Kaniji
Bauchi
Jos
Minna
Yola
Ilorin
Lokoja
Makurdi
Ibadan
Lagos
Benin City
Enugu
Calabar
Port Harcourt
NIGERIA
PROVISIONAL MAP OF VEGETATION ZONES
LEGEND
1 SAHEL
2 SUDAN
3 SUB-SUDAN
4 NORTHERN GUINEA
5 SOUTHERN GUINEA
6 DERIVED SAVANNA
7 FOREST
8 FRESHWATER SWAMP
9 MANGROVE FOREST
10 MONTANE
11 PLATEAU
20 0 20 40 60 80 100 120 mls
20 0 20 40 60 80 160 Km

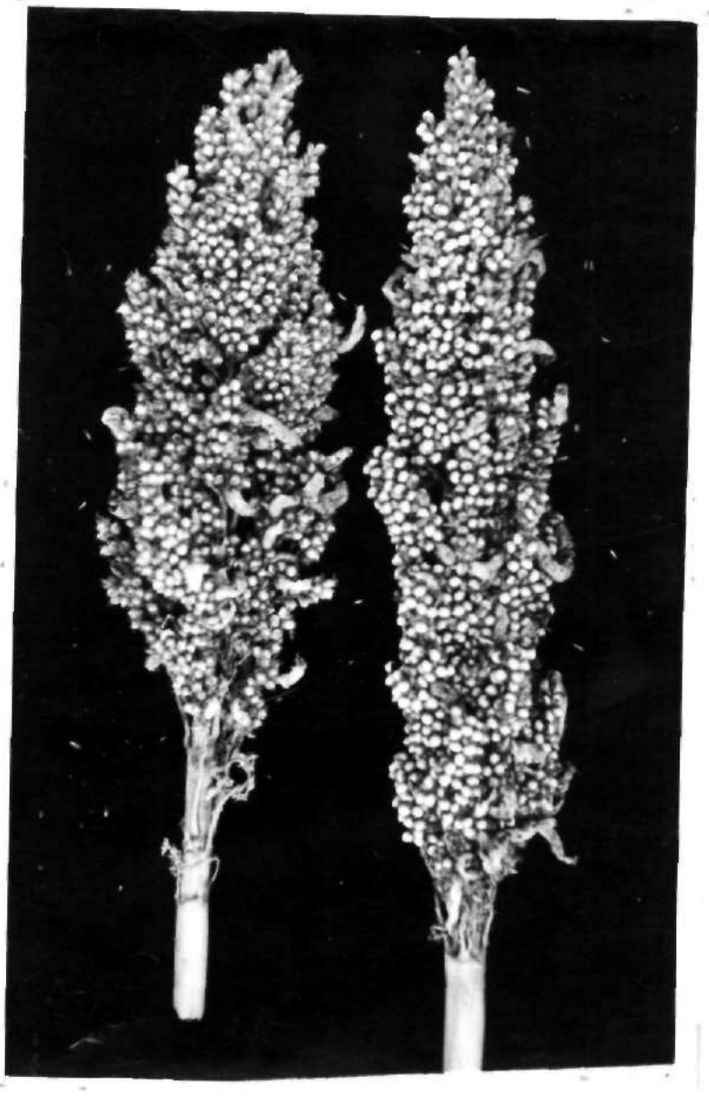


Fig. 2. Typical long smut infection consisting of several widely scattered smut sori.

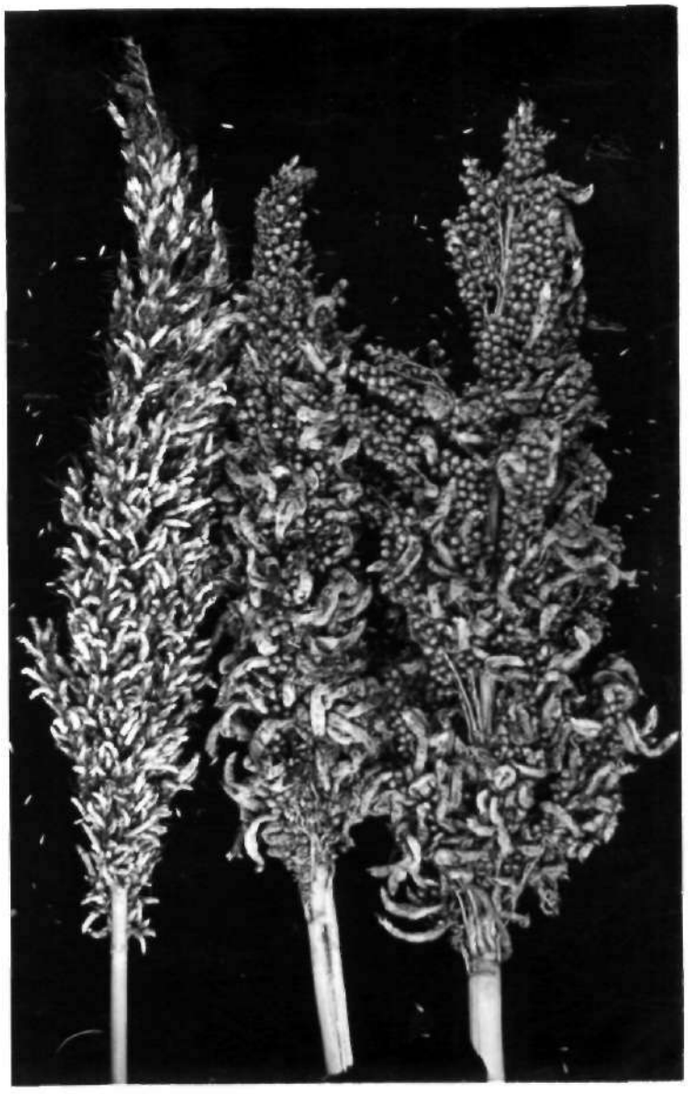


Fig. 3. An example of severe long smut infection with many or most florets bearing smut sori.



Fig. 4. An example of highly localised long smut infection with many sori confined to a small area.

LITERATURE REVIEW

Description of the fungus - A summary of Mason's (1926) account of the evolution of the name, T. ehrenbergii, up to 1926 follows. Long smut of sorghum was first collected in 1876 at Cairo, Egypt, by Schweinfurth, who sent the specimen to Thümen, who recorded it in his Mycotheca Universalis, No. 725, as Ustilago reiliana Kühn F. sorghi-cernui. In 1878, Kühn obtained the specimen from Thümen and, on examination, found it belonged to the genus Sorosporium instead of Ustilago. He described it as Sorosporium ehrenbergii Kühn in Hedwigia, XVII (1878) pp. 12-14. Because the teliospores formed persistent sporeballs, which is characteristic of the genus Tolyposporium, the name of the long smut fungus was changed from Sorosporium ehrenbergii to Tolyposporium ehrenbergii in 1903 by Patouillard in Bull. Soc. Mycol XIX (1903) p. 254. In 1904, Busse collected long smut specimens from sorghum in German East Africa (Tanzania) and gave it the name T. filiferum in Arb. K. Biol. Anst. Land- U. Forstw 4: 319-426. Because the long smut fungus which Busse described and named was identical to that described by Kühn many years earlier, it become necessary to drop Busse's species name, T. filiferum, and adopt Kühn's, T. ehrenbergii. Based on Mason's (1926) account, it is wrong, as Hafiz (1958), Kamat (1933) and Ragab and Mahdi (1966) have done, to cite Busse as the first person to collect and name long smut.

Long smut and the causal fungus have been well described and distinguished from other smut diseases and smut fungi in the description given by the Commonwealth Mycological Institute,

Kew (1965). The sori, which are transformed sorghum ovaries, are irregularly distributed, more or less cylindrical, often curved with tapered ends, and up to 4.0 cm long (mostly 1.0 - 3.0 cm long) and 0.50 - 1.0 cm wide. Each sorus is surrounded by a firm, false membrane of fungal and host tissues which almost always ruptures early from the apex, exposing the teliospores and a bundle of 8 to 10 dark-brown filaments, which are the isolated fibro-vascular bundles of the hypertrophied ovary. Individual spores (teliospores) are tightly bound together in rather solid black masses called spore balls. Sporeballs are rather irregular in size (45.0 - 200.0 μ in diameter) and have a globose to elongated shape. The spores at the surface of spore balls are dark brown with a warty surface. The inner spores are paler in colour, smooth, and globose to subglobose or angular, being 10.0 - 15.0 μ (average 12.0 μ) in diameter. All the spores in a spore ball are capable of germinating. Each spore germinates by producing a promycellium, which laterally and terminally bears four primary sporidia (basidiospores), each sporidium being capable of budding and forming chains or colonies of sporidia. Sometimes, instead of a spore forming a promycellium, a long germ tube is formed which produces lateral sporidia.

Mode of infection - Butler (1918) stated that the life history of T. ehrenbergii was unknown. He observed that infection involved individual ovaries and often the smut sori were very few and widely scattered on the panicle.

He, therefore, was of the opinion that long smut is not typical of a systemic type of infection, but that it is more typical of localized infection. He thus suggested the air-borne nature of infection. Briton-Jones (1922) stated that in nature sorghum infection results from air-borne sporidia brought into contact with flowers at the time of anthesis. Prasad (1945) tried artificial inoculation of sorghum by: 1) dusting sorghum seeds with the smut teliospores before planting, 2) inoculating soil with teliospores before planting clean seed, 3) applying Moor's vacuum method for introducing the smut fungus into the host tissue, and 4) spraying undeveloped sorghum panicles with a suspension of sporidia. Infection was obtained only by spraying young sorghum panicles with a suspension of sporidia. He then concluded that the disease is air-borne and that the sporidia are carried by air to the panicles where they infect florets. Ramakrishnan and Reddy (1949) Vasudeva and Seshadri (1950) and Hassan et al. (1970) concluded from their investigations that long smut is an air-borne disease and that infection results from sporidia alighting on the sorghum panicles at boot stage or shortly after emergence.

On the contrary, Sattar and Hafiz (1952) and Hafiz (1958) stated that infection was obtained by each of three methods of inoculation: 1) dusting sorghum seed with teliospores before planting, 2) planting clean seed in soil previously amended with long smut teliospores, and 3) inoculating sorghum heads at boot stage or after emergence from the boot. They observed that infection was higher if the teliospores were added to the soil

during the previous harvest time than if they were added at the time of sowing. They then concluded that long smut of sorghum is seed-borne, soil-borne and air-borne.

Ragab and Mahdi (1966) tried similar methods of inoculation to those of Prasad (1945), Sattar and Hafiz (1952) and Hafiz (1958), and obtained similar positive results. In addition, they collected clean and apparently healthy sorghum seed from both long smut-infected and long smut-free panicles and, using Popp's (1958) technique, they extracted 600 embryos from kernels obtained from infected heads. These embryos were stained with fungus-staining dyes and observed microscopically for presence of dormant mycelium. Out of the 600 embryos examined, six (1.0 per cent) were found to possess dormant mycelium spread throughout the embryo tissues especially in the region of the scutellum. No account was given of microscopic examination of embryos of seed collected from uninfected heads (control). The remaining seeds from the original stock were surface sterilized and planted in healthy soil. At boot stage, the sorghum heads were bagged with sterilized paper bags to protect the emerging heads from air-borne teliospores or sporidia. Out of the 74 bagged heads, 31 (41.9 per cent) showed long smut infection. Again, no account was given of control plants. From these results they concluded that infection came only from dormant mycelia living in the surface sterilized seed, as supported by the embryo investigation using Popp's (1958) technique.

They did not attempt to show whether or not dormant mycelia of fungi other than T. ehrenbergii also existed in the surface sterilized sorghum seed. Ragab and Mahdi (1966) concluded that long smut is air-borne, soil-borne and seed-borne and that the fungus does not exist just on the seed surface but also within the embryo.

The above review shows the present state of confusion and contradiction on the mode of infection of sorghum by T. ehrenbergii.

MATERIALS AND METHODS

Several experiments were conducted to study the mode of infection of long smut of sorghum. These have been divided into two basic categories:

1. Experiments designed to study the possible role of early (seedling) infection in which sorghum becomes systemically infected.

2. Experiments designed to study the possible role of late infection (floral) in which infection is localised.

The pathogen used for the inoculation studies was T. ehrenbergii. Teliospores used during the 1972 rainy season were collected at Kano and stored in closed vials in the laboratory at room temperature (20-33°C) for 6 to 9 months before they were used. Teliospores used for most inoculations during the 1972-73 irrigation period were collected at Kano and Sokoto 3 to 5 months before they were used. Teliospores collected at Kano in October, 1971, which possessed very few or no contaminants, were used to supplement the quantity of teliospores used for the irrigation period studies.

Attempts were made to remove common contaminants of long smut spore balls, such as Aspergillus and Penicillium, through the use of chemicals which might have differential action on the contaminants and T. ehrenbergii. To this effect, long smut spore balls were soaked in mercuric chloride at concentrations ranging from 0.025 to 0.10 per cent for periods ranging from 30 seconds to 5 minutes.

Concentrations of sodium hypochlorite ranging from 0.50 to 5.25 per cent for periods of 30 seconds to 5 minutes were also used. Mercuric chloride was observed to have a more toxic effect on T. ehrenbergii than on Aspergillus or Penicillium. Sodium hypochlorite, although toxic to both the contaminants and T. enhrenbergii (judging from the number of spores that germinated as compared to untreated spores). Soaking long smut spore balls in 1.0 to 2.0 per cent sodium hypochlorite for about 3 min. was found to adequately rid them of contaminants even though more than 50 per cent of the long smut teliospores were also killed and their germination retarded. The surface-sterilized long smut teliospores were thoroughly rinsed in sterile distilled water before plating on potato dextrose agar (PDA) for spore culturing and subsequent inoculations.

Sporidia were obtained by dusting teliospores onto PDA in petri dishes and incubating at room temperature (23-33°C). The PDA was made according to the formula given in the C.M.I. Plant Pathologist's Pocketbook (1968) and amended with streptomycin at 50 ppm. Sporidia and teliospores were harvested by adding sterile distilled water onto the surface of the medium on which the long smut spores were growing and agitating with the aid of a sterilized micro-spatula.

Leoti (IS 640) and Dwarf Shallu (IS 453) were the two susceptible sorghum varieties used throughout this investigation, unless otherwise stated. Seed was surface sterilized by soaking in 0.01 per cent mercuric chloride for 3 min. after which it was thoroughly rinsed in distilled water.

On all treatments data were taken on the total number of plants per treatment, the total number of plants infected, and the total number of sori per infected panicle.

Unless otherwise stated, all experiments were conducted at the Institute for Agricultural Research, Samaru, where relatively little natural long smut infection occurs.

1. STUDIES ON EARLY INFECTION

A. Sorghum seed dusted with teliospores before sowing

Covered smut and loose smut of sorghum and bunt of wheat readily occur if seed is dusted with teliospores before planting. To determine if this also occurs with long smut of sorghum, surface-sterilized seed of Leoti and Dwarf Shallu were wetted with water and, coated with dry teliospores of T. ehrenbergii. The inoculated seed were immediately planted in the field. The control consisted of seed which was similarly treated, but with no teliospore coating. This experiment was conducted during the rainy season and repeated during the dry season under irrigation.

B. Sorghum seed germinated in an aqueous suspension of sporidia.

1. Germinated seed dusted with teliospores before planting

Prevost (1939) recommended that in order to obtain a high incidence of bunt of wheat, seed should be soaked until it germinates in a water suspension of germinating teliospores. When the seed has germinated it should be dusted with the smut teliospores before planting. To determine whether Prevost's (1939) recommendation applies to long smut, surface-sterilized seed of Leoti and Dwarf Shallu were soaked for 3 days in an aqueous suspension of

germinating teliospores of T. ehrenbergii and before planting in the field the germinating seed was dusted with dry teliospores. For the control, surface-sterilized seed was soaked for 3 days in sterile distilled water and not dusted with long smut teliospores before planting. This experiment was conducted during the rainy season.

2. Germinated seed not dusted with teliospores before planting.

Early systemic seedling infection of sorghum by Sclerospora sorghi and millet by S. graminicola readily occurs if the conidia and sporangia, respectively, are placed over-night over germinating seed. Also, sugarcane smut caused by Ustilago scitaminea and covered smut of barley caused by Ustilago hordei occur if the buds and seedlings, respectively, are sprayed with teliospores or sporidia, especially under vacuum. To determine whether the sporidia of T. ehrenbergii are capable of causing systemic seedling infection, surface sterilized seed of Lecti and Dwarf Shallu were placed in an aqueous suspension of T. ehrenbergii sporidia in petri dishes and kept in the laboratory at room temperature (23-33°C) for 5 days during which time the seed germinated and the seedlings started to develop. At the end of the 5 days the sorghum seedlings were transplanted into the field. The control consisted of surface-sterilized seed which germinated and grew in sterile distilled water before being transplanted. This experiment was conducted during the rainy season and repeated during the dry season under irrigation.

C. Teliospores applied to the soil before planting sorghum seed.

Bunt of wheat and head smut of sorghum readily occur if the teliospores of the respective smut fungi are applied to the soil before sowing. To determine if systemic seedling infection of long smut can result from soil-borne teliospores, the following two approaches were made.

1. Seed planted immediately after application of teliospores to the soil.

Planting furrows, 2.5 to 4.0 cm deep, were made in the soil in the field. Teliospores were generously applied to the furrows, to make a distinct, black band on the surface of the soil within the furrows. Surface-sterilized Lecti and Dwarf Shallu seeds were planted in the furrows and covered with soil. The control consisted of surface-sterilized seed similarly planted but without the addition of teliospores. This experiment was conducted during the rainy season.

2. Seed planted after initiation of profuse germination of soil-borne teliospores

Teliospores were added to sterilized soil in an open rectangular container and thoroughly mixed. The quantity of teliospores added was large enough as to colour the soil black. The soil-teliospore mixture was kept on a bench in the glass house and maintained moist by watering until considerable germination of the teliospores had occurred as evidenced by the whitish colouring of the soil surface by sporidia.

At this stage surface-sterilized seed of Lecti and Dwarf Shallu were planted in the soil and kept moist by watering until the seed had germinated and seedlings grown to a height of about 24.0 cm. These seedlings were then transplanted into the field. The control was treated in exactly the same manner, except that teliospores were not added to the soil. This experiment was conducted during the rainy season and repeated during the dry season.

D. Growing point region of sorghum seedlings inoculated.

1. Hypodermic injection of the growing point region

Edmunds (1963) and S. B. King (personal communication) for several years successfully produced head smut on sorghum by injecting head smut sporidia into the growing point region of young (10 to 15 cm high) sorghum plants with the aid of a hypodermic syringe and needle. This technique was tested for possible inoculation of sorghum with long smut. The sporidia of T. ehrenbergii used for this were increased by plating for 7 days in PDA or potato dextrose broth containing 50 ppm. of streptomycin in 500 ml Erlenmeyer flasks. Each flask contained 200 to 250 ml potato dextrose broth. The potato dextrose broth was made in the same way and with the same ingredients as PDA except that agar was not added. Streptomycin was added to the cooled potato dextrose broth after sterilization. The control consisted of sorghum plants injected with sterile distilled water. This experiment was performed during the rainy season and repeated during the dry season.

2. Application of germinating teliospores to the cut ends of sorghum seedlings

Leukel and Martin (1950), successfully obtained loose smut on sorghum when young sorghum plants were cut just above the growing point and a water suspension of loose smut teliospores applied to the cut ends. This technique was employed by the author to determine whether long smut infection could be obtained by applying a water suspension of germinating long smut teliospores to cut ends of sorghum seedlings. Plants were inoculated between 17 and 18 h Nigeria time in order to avoid excessive drying of the inoculum. The control consisted of plants which were cut, but not inoculated. This experiment was conducted during the rainy season.

E. Smut incidence on tillers of sorghum plants with smut-infected primary heads

As a rule, the panicles of tillers of sorghum plants whose primary heads are smutted with covered smut, loose smut or head smut (all of which are seedling infecting and systemic) are also smutted. If long smut infects seedlings and infection becomes systemic, tillers of long smut-infected sorghum plants would be expected to also bear long smut sori. This was investigated at Kano by obtaining data on long smut infection of the tillers of 25 sorghum plants whose primary heads showed relatively high levels of long smut infection (at least 35 sori per panicle). The smutted primary heads of these plants were cut to encourage tillering.

For comparative purposes, a similar investigation was conducted at Samaru on 25 sorghum plants infected with covered smut and 25 sorghum plants infected with loose smut.

F. Systemic smut mycelium in branches of smutted sorghum panicles

It was hypothesized that, if panicle branches of plants with systemic smut infection were sectioned, stained and observed under the microscope, systemic smut mycelium might be observed. If this were true, then smut mycelium should be present in branches of sorghum panicles infected with covered smut, loose smut or head smut. Also, if a high level of long smut infection is the result of systemic infection, then systemic long smut mycelium should be present in sections of the infected panicle branches. If smut mycelium were not present in long smut-infected panicle branches, but were present in panicle branches infected with covered smut, loose smut or head smut, then one would expect that the high level of long smut infection was not due to systemic infection but rather to local infection. To test this hypothesis, sorghum panicle branches with severe infections of covered smut, loose smut or long smut were sectioned (10.0 - 12.0 μ thick) using a hand microtome. Branches from smut-free panicles (control) were similarly sectioned. The dyes, iron haematoxylin, Pianese stain, cotton blue in lactophenol, and trypan blue were separately used in an attempt to get one that would differentially stain the fungal mycelium and the surrounding host tissues. The stained sections were observed under the microscope for smut mycelium.

G. Dormant mycelium in sorghum embryos

Ragab and Mahdi (1966) reported that they observed and demonstrated the presence of dormant mycelium of T. ehrenbergii inside the embryos of sorghum seeds collected from long smut-infected panicles. Their method of investigation was repeated by the author with little or no modification to find out if their conclusions could be verified. To this end, 200 to 300 long smut-infected panicles of Lecti, Dwarf Shallu and five other unknown sorghum varieties were collected from the field (mainly at Kano) and the seed removed from paired florets in which one of the florets produced a long smut sorus. This seed was surface-sterilized and divided into three lots. From the first lot of approximately 600 seeds, 420 embryos were extracted using Popp's (1958) technique. The extracted embryos were stained with either cotton blue in lactophenol or 0.1 per cent trypan blue and observed under the microscope for the presence of fungal mycelia. The control consisted of 450 embryos from seeds collected from smut-free panicles which were extracted and stained using the same technique.

A second lot of about 700 seeds was planted under irrigation in the field. Half of the plants from these were bagged at the boot stage and the remaining half left unbagged. The control received the same treatment except that the seed came from smut-free panicles.

A third lot of 100 seeds, each seed aseptically broken into two halves, was placed on PDA containing 50 ppm streptomycin. Fungi which grew out from these seeds were isolated and identified to the genus level. The control, which consisted of 100 surface-sterilized seeds from smut free panicles, was treated in the same manner.

II. STUDIES ON LATE INFECTION

A. Floral inoculation

Sorghum panicles were inoculated at each of four stages of development:

1) boot stage (panicle completely enclosed by the sheath of the flag leaf), 2) preanthesis stage (panicle emerged from the boot but florets not yet open), 3) early anthesis stage (floral opening beginning) and 4) early seed set stage (male and female floral parts withered and seeds beginning to form). Each of the four stages was inoculated with water suspensions of three types of T. ehrenbergii inocula: 1) sporidia, 2) germinating teliospores, and 3) ungerminated teliospores. Inoculation at the boot stage was done by injecting inoculum into the boot with a hypodermic syringe and needle. Inoculations at the other three stages were done by spraying inoculum onto the panicles using a de Vilbiss adjustible tip atomizer. All inoculated panicles were bagged. The control consisted of panicles which were inoculated with sterile, distilled water. Some uninoculated and unbagged panicles remained in the field.

B. Spore balls on flag leaves

It has been commonly observed at Samaru and Kano that millet and sorghum panicles develop millet smut (Tolyposporium penicillariae) and long smut, respectively, in fields where these smuts have been known to exist, even if the panicles are bagged at the boot stage. Working on the assumption that long smut is not systemic or seedling infecting, one has then to explain how infection of bagged panicles occurs naturally in the field. It was thought that such infection could arise from spore balls or teliospores blown onto the flag leaves and washed into the boot by rain water. To investigate such a possibility, the flag leaves of 60 sorghum plants at early boot stage growing in a field at Kano, where natural long smut infection commonly occurs, were separately washed with distilled water and the washing collected in small vials. The washings were separately centrifuged for 10 min. at medium speed in a table model clinical centrifuge and the precipitate removed and microscopically examined for number of long smut spore balls.

RESULTS

I. STUDIES ON EARLY INFECTION

A. Sorghum seed dusted with teliospores before sowing

1. Sorghum seed was dusted with the teliospores of T. ehrenbergii before sowing in the field during the rainy season. Table 1 shows the results of such a treatment. Less than 1.0 per cent infection resulted from this treatment on both Leoti and Dwarf Shallu. Also no appreciable difference in the incidence of long smut infection occurred between the treated and the control.

Table 1. Incidence of long smut resulting from dusting sorghum seed with long smut teliospores before sowing.

Variety	Treatment	Total No. of plants	Total No. of plants infected	Percent infection	Average No. of sori per infected panicle
Leoti	Seed dusted with teliospores	450	3	0.7	3
	Seed free of teliospores (control)	284	2	0.7	4
Dwarf Shallu	Seed dusted with teliospores	224	1	0.5	6
	Seed free of teliospores (control)	215	0	0.0	0

The experiment was repeated during the dry season under irrigation. Out of 182 plants that resulted from seed dusted with teliospores and 122 control plants, none showed any long smut infection.

B. Sorghum seed germinated in an aqueous suspension of sporidia

1. Germinated seed dusted with teliospores before planting

Seed of Leoti was germinated in an aqueous sporidial suspension of T. ehrenbergii for three days and then dusted with teliospores of the same fungus before planting in the field. Out of the 154 treated plants none showed any long smut infection, while one plant out of the 86 control plants showed long smut infection with 12 sori.

2. Germinated seed not dusted with teliospores before planting

Seed of Leoti and Dwarf Shallu were germinated and allowed to grow in an aqueous suspension of long smut sporidia for 5 days. Without further treatment, the young seedlings were planted in the field. The results, shown in Table 2, indicate a very low level of infection with little or no difference between the treated and the control.

Table 2. Incidence of long smut on plants derived from seeds which were germinated and grown for 5 days in a water suspension of sporidia before planting in the field.

Variety	Treatment	Total No. of plants	Total No. of plants infected	% infection	Avg. No. of sori per infected panicle
Leoti	Seed germinated in sporidial suspension	330	4	1.2	3
	Seed germinated in sterile water (control)	186	2	1.1	4
Dwarf Shallu	Seed germinated in sporidial suspension	292	1	0.3	9
	Seed germinated in sterile water (control)	140	1	0.7	5

C. Teliospores applied to the soil before planting sorghum seed

1. Seed planted immediately after application of teliospores to the soil.

Seed of Leoti and Dwarf Shallu were sown immediately in soil in the field to which dry, ungerminated long smut teliospores had been generously applied. Out of a total of 215 plants in treated soil and 154 control plants in untreated soil, none showed any long smut infection.

2. Seed planted after initiation of profuse germination of soil-borne teliospores

Seed of Leoti and Dwarf Shallu were planted and grown through the seedling stage in soil heavily infested with germinating long smut teliospores before being transplanted into the field. The results shown in Table 3 indicate a very low level of infection with little or no difference between the treated and the control.

Table 3. Incidence of long smut resulting from growing sorghum through the seedling stage in soil heavily infested with germinating teliospores.

Variety	Treatment	Total No. of plants	Total No. of plants infected	% infection	Average No. of sori per infected panicle
Leoti	Teliospore infested soil	123	1	0.8	8
	Teliospore free soil (control)	85	3	3.5	2
Dwarf Shallu	Teliospore infested soil	96	3	3.1	2
	Teliospore free soil (control)	70	0	0.0	0

D. Growing point region of sorghum seedlings inoculated

1. Hypodermic injection of the growing point region

Lecti and Dwarf Shallu seedlings (10 to 15 cm tall) were inoculated at the growing point region by injecting an aqueous suspension of long smut sporidia with the aid of a hypodermic syringe and needle. No long smut infection occurred on any of the 162 plants inoculated with sporidia during the rainy season and neither was there any long smut infection on any of the 104 control plants.

No long smut sori were observed on any of 55 Lecti plants which were inoculated with sporidia during the dry season and neither were any long smut sori observed on any of the 43 control plants.

2. Application of germinating teliospores to the cut end of sorghum seedlings

Young Lecti and Dwarf Shallu plants were cut just above the growing point and germinating long smut teliospores were applied to the cut ends. Out of a total 88 inoculated plants and out of a total of 55 control plants, none showed any long smut infection.

E. Smut incidence on tillers of sorghum plants with smut-infected primary heads

Data were obtained on the incidence of smut on tillers of sorghum plants whose primary heads were infected with covered smut, loose smut or long smut. The results in Table 4 show that almost all the tillers of plants with primary heads infected with either covered smut or loose were smutted.

On the contrary less than 50 per cent of the tillers of long smut-infected plants showed smut infection. It was observed during the investigation that all or almost all the florets on the panicles of tillers infected with either covered smut or loose smut were smutted, but only a few florets were smutted on panicles of most tillers infected with long smut.

Table 4. Incidence of smut on tillers of sorghum plants with smut-infected primary heads.

	Long smut		Loose smut		Covered smut	
Stand	Total no. of tillers	Total number infected	Total no. of tillers	Total No. of tillers infected	Total No. of tillers	Total No. of tillers infected
1	2	1	3	3	3	3
2	2	0	5	5	3	3
3	3	0	5	5	2	2
4	1	1	4	3	1	1
5	4	2	6	6	1	1
6	3	0	3	2	2	2
7	1	1	3	3	2	2
8	2	0	3	3	2	1
9	2	2	4	4	3	3
10	2	2	2	2	4	3
11	1	0	6	5	3	3
12	3	0	3	3	3	2
13	3	1	7	7	3	3
14	3	2	5	5	1	1
15	2	1	4	4	2	2
16	2	2	3	3	2	2
17	2	1	4	4	3	3
18	5	2	3	3	2	2
19	3	3	3	3	2	2
20	4	1	2	2	1	1
21	5	3	2	2	1	1
22	2	0	5	4	3	3
23	1	1	6	6	3	3
24	3	3	3	3	3	3
25	2	0	4	4	2	2
Total	61	29	98	93	57	54

F. Systemic smut mycelium in branches of smutted sorghum panicles

Branches of sorghum panicles highly infected with loose smut, covered smut or long smut, and branches from smut-free panicles, were sectioned with a hand microtome and stained before observing under the microscope for presence of systemic smut mycelium. No differences were observed between branches from smutted or smut-free panicles regardless of the smut involved. Also, no differences were observed among branches of panicles infected by the three smut diseases. In short, nothing informative was obtained with respect to presence or absence of smut mycelium in panicle branches.

G. Dormant mycelium in sorghum embryos

Four hundred and fifty embryos of seed collected from smut-free panicles and 420 embryos of seed collected from paired florets, in which one of the florets produced a long smut sorus, were extracted and stained using Popp's (1958) technique and then observed under the microscope for the presence of fungal mycelium. Dormant fungal mycelium was found in 18 embryos from the smut-free panicles and 24 embryos from long smut infected panicles.

When seed from paired florets, in which one floret produced a long smut sorus, were planted in the field under irrigation, none of the 385 plants showed any symptom of long smut infection. Also, long smut symptoms were not observed on any of the 280 control plants derived from seed collected from smut-free panicles.

Sorghum seed collected from smut-free panicles and from paired florets in which one floret developed a long smut sorus were separately surface-sterilized and thoroughly rinsed with sterile distilled water. Each seed was aseptically split into two halves and placed on PDA in Petri dishes. From both seed lots, the following genera of fungi were isolated: Aspergillus, Chaetomium, Curvularia and Fusarium. No fungus resembling T. ehrenbergii was isolated from any of the two seed lots.

II. STUDIES ON LATE INFECTION

A. Floral inoculations. Sorghum panicles were inoculated at each of four stages of development:

a) boot stage, b) pre-anthesis stage, c) anthesis stage d) early seed set.

Each of these four stages was inoculated with each of 3 types of T. ehrenbergii inoculum:

a) sporidia, b) germinating teliospores, c) ungerminated teliospores.

Tables 5 and 6 show the results of such inoculations done during the rainy season on Leoti and Dwarf Shallu, respectively. Table 7 shows the results of such inoculations done during the dry season on a mixture of Leoti and Dwarf Shallu plants.

Table 5. The effect of inoculating Leoti panicles at various stages of development with the sporidia, germinating teliospores and ungerminated teliospores of T. ehrenbergii during the rainy season.

Treatment	Stages of development											
	Boot			Pre-Anthesis			Early Anthesis			Early seed set		
	Total No. of plants	% of plants infected	Average No. of sori per panicle	Total No. of plants	% of plants infected	Average No. of sori per panicle	Total No. of plants	% of plants infected	Average No. of sori per panicle	Total No. of plants	% of plants infected	Average No. of sori per panicle
Sporidial water suspension	70	12.9	6	65	7.7	7	65	6.2	3	55	0.0	0
Sterile distilled water (control)	45	0.0	0	40	0.0	0	40	5.0	1	40	0.0	0
Aqueous suspension of germinating teliospores	60	76.7	21	60	35.0	9	60	11.7	5	55	0.0	0
Sterile distilled water (control)	50	0.0	0	40	2.5	4	45	4.4	2	45	0.0	0
Aqueous suspension of ungerminated teliospores	60	0.0	0	60	0.0	0	60	1.7	3	60	0.0	0
Sterile distilled water (control)	40	0.0	0	50	2.0	4	40	0.0	0	40	0.0	0

Table 6. The effect of inoculating Dwarf Shallu panicles at various stages of development with the sporidia, germinating teliospores and ungerminated teliospores of T. ehrenbergii during the rainy season.

Treatment	Stages of development											
	Boot			Pre-Anthesis			Early Anthesis			Early seed set		
	Total No of plants	% of plants infected	Average No.of sori per panicle	Total No.of plants	% of plants infect	Average No. of sori per panicle	Total No. of plants	% of plants infected	Average No. of sori per panicle	Total No.of plants	% of phnts infected	Average No. of sori per panicle
Sporidial water suspension	40	17.5	6	40	22.5	5	40	7.5	5	30	0.0	0
Sterile distilled water (control)	25	0.0	0	25	4.0	3	25	0.0	0	25	0.0	0
Aqueous suspension of germinating teliospores	40	77.5	18	35	48.6	9	35	22.9	4	35	0.0	0
Sterile distilled water (control)	30	0.0	0	30	6.7	2	30	3.3	6	25	0.0	0
Aqueous suspension of ungerminated teliospores	30	3.3	7	30	0.0	0	30	0.0	0	25	0.0	0
Sterile distilled water (control)	35	0.0	0	35	2.9	3	25	0.0	0	30	0.0	0

Table 7. The effect of inoculating a mixture of Leoti and Dwarf Shallu panicles at various stages of development with the sporidia, germinating teliospores and ungerminated teliospores of T. ehrenbergii, during the dry season.

Treatment	Stages of development											
	Boot			Pre-Anthesis			Early Anthesis			Early seed set		
	Total No of plants	% of plants infected	Average No. of sori per panicle	Total No. of plants	% of plants infected	Average No. of sori per panicle	Total No. of plants	% of plants infected	Average No. of sori per panicle	Total No. of plants	% of plants infected	Average No. of sori per panicle
Sporidial water suspension	30	36.7	7	30	26.7	6	30	30.0	3	30	0.0	0
Sterile distilled water (control)	20	0.0	0	20	0.0	0	20	0.0	0	20	0.0	0
Aqueous suspension of germinating teliospores	60	90.0	22	45	55.6	8	40	15.0	4	40	0.0	0
Sterile distilled water (control)	40	0.0	0	35	2.9	3	35	2.9	2	35	0.0	0
Aqueous suspension of ungerminated teliospores	45	0.0	0	45	0.0	0	45	0.0	0	40	0.0	0
Sterile distilled water (control)	36	0.0	0	35	0.0	0	35	0.0	0	35	0.0	0

All the 3 tables show that most long smut infection was obtained at the boot stage. This was followed in decreasing order by pre-anthesis stage and then early anthesis stage. No infection was obtained at early seed set stage. Germinating teliospores produced more infection than sporidia at all susceptible stages of panicle development. Ungerminated teliospores produced no infections on any of the four stages of panicle development. Most infection, as shown by the 3 tables, occurred at boot stage when germinating teliospores were used as the inoculum.

During both the rainy season and the dry season it was observed that a few scattered long smut sori developed on unbagged sorghum panicles in the field, especially the panicles that were very near those sprayed with germinating teliospores or sporidia.

B. Spore balls on flag leaves. From the washings of individual sorghum flag leaves at early boot stage in a field at Kano, where natural long smut infection commonly occurs, the results, shown in Table 8 were obtained.

Table 8. Number of long smut spore balls found on 60 flag leaves of sorghum plants in the boot stage.

Total number of spore balls found	No. of flag leaves	Percentage of total
0	22	36.7
1	10	16.7
2	12	20.0
3	2	3.3
4	5	8.3
More than 4*	9	15.0
Total	60	100.0

*Up to 17 spore balls were found on an individual flag leaf.

From this table it can be seen that only 36.7 per cent of the flag leaves washed were free of long smut spore balls. Also 15 per cent of the flag leaves had more than 4 spore balls. Plants with up to three or more spore balls formed 26.6 per cent of the total number of flag leaves examined.

DISCUSSION

In general, the results of the present investigation support the view that long smut of sorghum is a local, floral-infecting disease. The results do not support the view that long smut infection can be initiated during the seed or seedling stage and that the fungus subsequently grows systemically within the plant.

Experiments were designed to study the role of seedling infection on the occurrence of long smut. These consisted of:

1) dusting sorghum seed with teliospores before planting,
2) germinating sorghum seed in an aqueous suspension of sporidia before planting,
3) planting seed in soil infested with teliospores,
4) inoculating young sorghum plants at the growing point region with sporidia or germinating teliospores,
5) planting seed which were removed from paired florets in which one member of the pair developed a long smut sorus, and
6) obtaining data on incidence of long smut infection on tillers of plants whose primary panicles were smutted.

All these experiments gave negative results indicating the improbability of systemic seedling infection. Tables 1, 2, 3 and 4 show the results of some of the experiments designed to study early seedling infections. In the cases where long smut infection occurred on a few of the control plants in the field, infection was thought to have come from air-borne sporidia or teliospores.

A higher incidence of long smut infection among control plants would have been expected if the experiments had been conducted in areas of high natural long smut infection, such as Kano state and Sokoto province instead of at Samaru where the incidence of natural infection is very low.

Hafiz (1958) Sattar and Hafiz (1952) and Ragab and Mahdi (1966) concluded from their investigations that long smut can be both seed-borne and soil-borne and that T. ehrenbergii is capable of, and does cause, systemic seedling infection. However, their results which supported the view of systemic infection originated, in the author's mind, from two basic shortcomings in their work. One shortcoming was that they either did not use controls or the controls which they used were inadequate. The second shortcoming was their assumption that sorghum panicles bagged before emergence from the boot were entirely protected from infection by air-borne spores. If these investigations had had adequate controls they might not have observed differences between treatments and controls in the seedling infection studies. The mere fact that sorghum panicles which were bagged before emergence from the boot developed long smut sori (no controls kept) forced these investigators into believing that infection was systemic and could only have been initiated during the seed or seedling stage. The author's investigation at Kano (Table 8) in which spore balls of T. ehrenbergii (ranging between 1 and 17) were detected on sorghum flag leaves at early boot stage shows that spore balls or teliospores can be blown from the soil by wind and deposited on the flag leaves at boot stage.

These spores can then easily be washed by rain water into the boot through the neck of the boot and deposited on the enclosed, developing panicle long before emergence from the boot and before bagging is done. In such a case bagging of the sorghum head before emergence from the boot would not completely eliminate the possibility of an air-borne infection. The author strongly suspects that the investigators who observed long smut symptoms on bagged panicles did the bagging after long smut spores had already entered the boot. High moisture content in the boot especially during the rainy season will facilitate the germination of long smut teliospores and the development of infection hyphae. It can be argued that bagging of sorghum heads in fields which are infested with long smut spores will not prevent the occurrence of long smut, but rather may actually encourage smut infection because of the increase in humidity brought about by the presence of the bag on the sorghum head. A higher number of smut sori per infected panicle is usually observed on bagged heads than on unbagged ones. From experiments conducted at Kano, S. B. King (personal communication) observed that panicles which were bagged had more long smut sori than unbagged panicles.

The high concentration of long smut sori at the panicle apex, shown in Fig. 4, suggests that a spore ball may have been deposited at the apex of the panicle where teliospores germinated causing a highly localized infection. This type of phenomenon is also very common on millet panicles infected with Tolyposporium penicillariae.

Millet smut is believed to be an air-borne, locally infecting disease (Bhatt, 1946; Fischer and Holton, 1957), and severe infection on bagged millet panicles is common. The author, therefore, suggests that millet smut and sorghum long smut have the same mode of infection.

Ragab and Mahdi (1966) reported that they observed and demonstrated the presence of long smut mycelium inside the embryos of kernels removed from long smut-infected panicles. The results which they presented and those which have been presented in this report cast doubts on the validity of their conclusions. When they extracted the sorghum embryos using Popp's (1958) technique they detected dormant mycelia in one per cent of the 600 embryos. They did not report on whether or not they observed dormant mycelia in embryos serving as controls (i.e., embryos of kernels from smut free panicles). The mycelia they observed could have been the mycelia of fungi other than T. ehrenbergii. They did not attempt to isolate and identify the fungi in embryos of seed from long smut infected panicles. Ragab and Mahdi planted some of the surface-sterilized seeds from long smut-infected panicles and bagged panicles before emergence from the boot. However, there were apparently no controls for this. They observed that 31 out of 72 (41.9 per cent) bagged panicles developed long smut sori, and concluded from this that infection came from dormant T. ehrenbergii mycelium in the seed. The presence of mycelium found in the embryos, they felt, added support to their conclusions that sori developed as a result of systemic infection.

There appears to be no correlation between the 1.0 per cent embryos found to have dormant mycelium and the 41.9 per cent long smut infection found in the field on bagged panicles. Since Popp's technique is a reliable method for detecting dormant mycelium in embryos, it would be expected that the incidence of long smut infection in the field resulting from dormant mycelium in embryos would be only as high as, and not higher than that observed in embryos. Therefore, the 42-fold increase in the incidence of long smut infection in the field as compared to that observed in the laboratory and recorded by Ragab and Mahdi (1966) is a significant increase. It is likely that all or most of the long smut infection in the field recorded by Ragab and Mahdi (1966) was the result of infection from sources other than dormant long smut mycelium in sorghum embryos.

The author failed to obtain a single long smut infected plant out of 395 plants which originated from seed taken from paired florets in which one member of the pair developed a long smut sorus. These results cast further doubt on Ragab and Mahdi's (1966) claim that dormant mycelium of T. ehrenbergii occurs in seed collected from long smut infected panicles and that this mycelium is capable of, and does cause, systemic seedling infection.

Using Popp's (1958) technique, the author observed dormant mycelium in 18 out of 450 embryos from smut-free panicles and 24 out of 420 embryos from smut infected panicles. On plating surface-sterilized and split seed from the two seed lots on PDA the writer isolated from both lots, Aspergillus sp. Chaetomium sp.,

Curvularia sp., and Fusarium spp. The mycelia observed in embryos using Popp's technique likely belonged to this same group of fungi. It is, therefore, possible that the dormant mycelia which Ragab and Mahdi (1966) observed in embryos were fungi other than T. ehrenbergii. In the writer's opinion Ragab and Mahdi have not provided convincing evidence to support their claim that dormant mycelium of T. ehrenbergii occurs in sorghum seed obtained from infected panicles and that each seed can yield a systemically infected plant. Further verification of their claim is needed before it can be accepted as scientific truth.

The fact that positive results, with respect to long smut infection, were only observed during inoculation of the panicles, further confirms earlier claims by Butler (1918), Hassan et al (1970), Ramakrishnan and Reddy (1949) and Vasudeva and Seshadri Iyengar (1950) that long smut is purely an air-borne disease which infects locally and not systemically. The author's results of floral inoculations, shown in Tables 5, 6, and 7, indicate that in nature long smut infection can occur at the boot, pre-anthesis and anthesis stages, and no infection occurs during or after seed set. The results agree with those of Prasad (1945), Hafiz (1958) and Ragab and Mahdi (1966) who reported that ungerminated teliospores are incapable of causing infection when used to inoculate any stage of floral development.

The reason for the failure of laboratory-stored, dry, ungerminated teliospores to cause long smut infection may be that in nature these teliospores require some period of exposure to certain treatments in the soil before they can easily germinate and cause infection. It is known that teliospores of Sphacelotheca reiliana, the causal organism of sorghum head smut, require some period of treatment in the soil before significant infections can occur (Halisky, 1963).

Having found that long smut is air-borne and not systemic, one may then account for how infection of the type in Figures 2 and 3 came about. The type of infection in Figure 2 involving few and scattered sori is probably brought about by paired or fused compatible sporidia or individual infection hyphae which are carried by air and deposited on individual florets. The type of infection in Figure 3 invloving a high concentration of sori is probably brought about by germinating teliospores of a spore ball or spore balls. The ball which consists of germinating teliospores or teliospores just about to germinate may be blown by wind and deposited on a flag leaf where they are washed into the boot and onto the developing panicle. Or, germinating teliospores in a mass may be deposited on parts of the panicle where numerous sori will form. In addition to the presence of suitable environmental conditions for spore germination, invasion, and establishment of T. ehrenbergii, the severity of long smut depends directly on the amount of inoculum deposited at a suitable location on the sorghum plant.

This in turn depends on the availability of inoculum in the soil of the area and the degree to which the wind blows inoculum onto the sorghum plant.

The finding that long smut of sorghum is not seedling infecting but is strictly an air-borne, floral infecting disease is of great significance agriculturally. This means that seed dressing with fungicides, a cheap and practical way of controlling covered smut and loose smut of sorghum (Tarr, 1962), will not control long smut. This has, in fact, as stated earlier, been the experience of many investigators and agriculturists. Mono-cropping, especially of long smut susceptible sorghum varieties, could result in the build up of T. ehrenbergii inoculum leading to an increase in the incidence of long smut.

Identification of long smut resistant germ plasm and the development of long smut resistant sorghum varieties is necessary. Host plant resistance will be the most effective and lasting measure for the control of long smut of sorghum. A screening program for resistance is likely to require artificial inoculation of material to reduce chances of escape. Based on the author's results, a screening program using artificial inoculation should involve inoculum consisting of germinating teliospores applied to panicles at the boot stage.

SUMMARY

The mode of long smut (T. ehrenbergii) infection was investigated. Previous investigations by others on this subject have produced contradicting results and conclusions. The author found overwhelming evidence to support the theory of the air-borne nature of infection, and none in support of the theory of seed or seedling infection and subsequent systemic invasion of the sorghum plant.

The present investigation has shown that inoculating sorghum panicles with sporidia or germinating teliospores of T. ehrenbergii at the boot stage, pre-anthesis stage or early anthesis stage results in the occurrence of long smut. Inoculating at boot stage and using germinating teliospores results in the highest level of infection.

The present investigation has, however, shown that the following treatments do not give rise to long smut:

1. Coating sorghum seed with teliospores before planting.
2. Adding teliospores to soil before planting.
3. Allowing sorghum seed to germinate and grow for some days in an aqueous suspension of sporidia or germinating teliospores before transplanting into the field.
4. Inoculating the growing point region of young sorghum plants with sporidia or germinating teliospores using a hypodermic syringe and needle.
5. Inoculating sorghum panicles after seed set with T. ehrenbergii inoculum.
6. Spraying sorghum panicles at all stages of development with ungerminated teliospores.

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