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Morphological Features and Germinability of Head and Loose Smut Pathogens in Nigerian Sudan Savanna

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Abstract
There are considerable experimental and field evidences that physiologic race differences exist amongst sorghum smut pathogens infecting both sorghum and maize in many parts of the world. In Nigeria, several researches have been conducted in the areas of epidemiology of many crop plant diseases, but less attention is been paid to some important aspects of the biology of smut fungi. Studies were conducted on the morphological features and germinability of the teliospore samples of Sporisorium cruenta and Sporisorium reilianum; the causal agents of sorghum loose and head smuts; respectively. One composite teliospore sample each of S. cruenta and S. reilianum was collected from 12 locations that were at least 70 km apart on a road network, in the Sudan savanna Agro- ecological zone of Nigeria. Aspects of spore color, length, width, and shape as well as germinablity of the spore in water and PDA of the various samples were determined. The color of both S. cruenta and S. reilianum teliospore was dark in aggregates and dark brown when single. The length and width of S. cruenta and S. reiliana teliosores averaged to 5.7 X 4.1µm while the shape ranged from irregular, spherical, and echinulate. In S. reilianum the mean size of the spore was 9.1 x4.3 µm. Its structure generally ranged between ovoid to spherical and hyaline.in both PDA and water, S. reilianum germinated faster than S. cruenta teliospores with significant (P≥0.05) difference between the various teliospore isolates. This result connotes that there are morphological differences between the various isolates of the same and between different pathogens. The results have shown that both spore isolates of S. cruenta and S. reiliana were alive and that the teliospore of S. reilianum germinated faster and hence might cause disease faster than S. cruenta teliospore in this region.

Keywords: *S. cruenta*, *S. reilianum,* morphology, germination, Sudan savanna

INTRODUCTION
Smut fungi are one of the most important groups of plant parasites, responsible for quiet dramatic yield losses in cereal crops (Kutama *et al*., 2012a,c and Kutama *et al*., 2024,). They comprise of more than 1500 species and their hosts are distributed all over the whole system of angiosperms (Kutama *et al*., 2010, and Kutama *et al*., 2011; Baier *et al*., 2001). Smut fungi

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(Ustilaginomycetes and Microbotryales, Urediniomycetes; Basidiomycota; (Baier *et al*., 2001) are parasites of plants, especially herbs belonging to the Poaceae and Cyperaceae (Piepenbring, 2001). It is recognized that in the early 21st century about 75% of the increase in food production should come from increased yield per unit area (Marley *et al*., 2002a) as increase in acreages is obviously not envisaged (Kutama *et al*.,2024; Aliyu and Kutama, 2007). Therefore, techniques and technologies are urgently required which fit into land use systems of resource-poor farmers and which do not destroy the natural resource base (Van Duivenbooden and Neetson, 1998) and or pollute the environment. Smut diseases of sorghum should become of apparent importance to researchers currently especially with current change in the climate for, the disease may tend to be sporadic in nature because of the prevailing changes in the environmental variables.

Several physiologic races of smut fungi exists (resulting into a lot of variability in many species of smut fungi (Kutama *et al*.,2024;). Knowledge of the morphological features and other aspects of the biology of the pathogens from different locations will help in understanding and explaining the possible differences and causes of the physiologic reactions of the pathogens on different sorghum varieties. Literature is scanty on the epidemiology of the smut diseases in West Africa (Sundaram, 1980). There is a need to study the epidemiology of the diseases so as to gain better insight of possible management strategies for the two smuts. Therefore, the aim of this research was to study the morphology and germinabilty of the two popular smut economically important pathogens in two different media.

MATERIALS AND METHODS

Study Area

The study was conducted in three states representing the Sudan savanna agro ecological zone namely; Kano, Katsina and Jigawa states. Sudan savanna of Nigeria is located in the northern part of Nigeria between latitudes 9°3″—and 12°3″N—and longitudes 4° and 140°3″ E.

Morphological Features of *Sporisorium cruenta* and *S. reiliana* Teliospore Isolates.

Collection of Teliospore isolates

A total of twelve samples each of *S. reiliana* and *S. cruenta* were collected in October, 2021 during a survey of farmers fields, from smutted panicles of different sorghum varieties cultivated in the region. Sample collection was done in locations that were at least 70 km apart on a road network, in Kano, Katsina and Jigawa states, representing Sudan savanna zone (Kutama *et al*., 2013c). The various samples were labeled appropriately in polythene bags and brought and kept in the laboratory in a well ventilated place. The locations of places where samples were collected with date of collection are shown in Table 1 below.

Table 1: Location of Places Where Teliospore Isolates were Collected with Dates

S/N	Place of collection	G.P.S.	State	Date
1	Janguza	$11^0$58.308N $008^0$22.88E	KN	18/10/2021
2	Mainika	$11^0$52.227N $007^0$52.854E	KN	18/10/2021
3	Kankara	$11^0$56.757N $007^0$24.645E	KT	18/10/2021
4	Tashar Biri	$12^0$06.550N $007^0$28.830E	KT	18/10/2021
5	Kofa (Kankiya)	$12^0$27.119N $11^0$28.247N	KT	18/10/2021
6	Kiru	$11^0$28.247N $008^0$12.31E	KN	19/10/2021
7	Kazaure	$12^0$03.234N $007^0$52.123E	JG	20/10/2021
8	Babura	$12^0$22.709N $008^0$30.560E	JG	20/10/2021
9	Gantsawa	$120^0$1.115N $008^0$38.092E	KN	21/10/2021
10	Birnin-Kuda	$10^0$48.892N $009^0$48.569E	JG	22/10/2021
11	Wudil	$11^0$51.843N 008^0.42.411E	KN	22/10/2021
12	Gwaram	$009^0$21.281N $008^0$16.341E	JG	22/10/2021

Key: KN= Kano , JG=Jigawa , KT=Kastina

Determination of Spore Color

Color of the teliospore was determined under a low power compound microscope by carefully removing an aggregate of at least 100 spores, placed on slide containing sterile distilled water and observed under x10 and x40 magnifications. This is followed by carefully reducing the spore density to about 1% and observation was made under x10 and x40 magnifications (Baier *et al*., 2001).

Determination of teliospore size and **shape** (length and width).

To determine the size and shape of the various sample of teliospore, the method described by Kutama *et al.* (2024) was adopted and slightly modified . The length and width of 100 telospores (aggreagate) were measured and the length and width of one spore was also measured. All measurements were done with an ocular micrometer under a compound microspore. Each measurement was replicated three times and the various means obtained.

Germination of Teliospores

Germination in water

An aggregate of 100 teliospores were germinated in cavity slides and kept in moist chamber and incubated at 37±2^{0}C. Germinating spores were observed on hourly basis for 12 hours to detect germination. Spore germination was noted when there was the production of promycelium, germtube or mycelium (Tarr, 1962) which was determined on X10 and X40 magnification. Hours to germinate were also noted.

Germination on Potato Dextrose Agar (PDA) medium

One hundred teliospores from each composite teliospore sample were cultivated on PDA plate and incubated at 37± 2^{0}C for 12 hrs. Germination of the spores was noted whenever there

was growth of pure colony of each of the two fungi. Twelve hours after germination the experiment was terminated and the structure of various germinating spores were observed at X10 and X40 on the compound microscope. Hours to germinate were also noted (Kutama *et al*., 2012).

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) and the various means obtained were separated using least significant difference at 5% level of probability

RESULTS

Morphological Features of *Sporisorium cruenta* and *S. reiliana* Teliospore Isolates

Teliospore color

The color of *S. reilianum* teliospore in aggregate was generally black but dark brown when single spore was observed (Table 2). Similarly, the color of *S. cruenta* teliospore was also black in aggregate but black and dark brown to black in single isolate (Table 2). There was no significant difference ($p>0.05$) in color of the different isolates of the two pathogens even though the various isolates were collected from distant places but within the same agro-ecology.

Table 2: Color of *S. cruenta* and *S. reilianum* teliospores collected from different locations in Sudan savanna, Nigeria

Isolate	*S. cruenta* aggregate/single spore	*S. reilianum* aggregate/single spore
1.	dark brown (black)	dark brown (black)
2.	dark brown (black)	dark brown (black)
3.	dark brown (black)	dark brown (black)
4.	dark brown (black)	dark brown (black)
5.	dark brown(black)	dark brown (black)
6.	black brown (black)	dark brown (black)
7.	dark brown (black)	dark brown (black)
8.	dark brown (black)	dark brown (black)
9.	dark brown (black)	dark brown (black)
10.	dark brown (black)	dark brown (black)
11.	dark brown (black)	dark brown (black)
12.	dark brown (black)	dark brown (black)

Note: colors in bracket are for single spores

Length, Width and Shape of Teliospores

The length and width of *S. reilianum* teliospores ranged from 6.7x 3.1 µm, from isolate No. 6 from Kiru in Kano state to 11.0x 5.2 µm obtained from isolate No. 12 from Gwaram in Jigawa state suggesting significant (P=0.05) differences between the sizes (Table 3). The mean size of the 12 teliospore samples was registered at 9.1x5.1 µm. the largest teliospore isolate therefore was from Gwaram in Jigawa state. The shape of the teliospore isolates was also significantly different ($p<0.05$) between locations and ranged between ovoid/spherical,hyaline to globose/sub globose and ovoid.

Table 3: Length, Width and Shape of *S. reilianum* teliospore samples collected from the Sudan savanna, Nigeria

Sample	Length(μm)	Width(μm)	Shape
1	8.9	3.2	ovoid/spherical,hyaline
2	9.3	4.1	spherical, hyaline
3	9.4	4.3	globose to sub globose, hyaline
4	8.8	4.3	spherical, loose
5	8.6	5.1	sub globose,hyaline
6	6.7	3.1	irregular,loose
7	9.1	5.6	spherical and hyaline
8	8.7	4.1	ovoid/spherical, hyaline
9	10.2	4.3	ovoid/round, hyaline
10	8.9	3.9	angular (hexagonal),hyaline
11	9.3	4.9	globose/sub globose
12	11.0	5.2	ovoid round and hyaline
Mean	9.1	4.3	
LSD	3.123	2.11	

In the same vein, the length and width of *S. cruenta* teliospores varied significantly ($p<0.05$) between the isolates. Isolate No. 6 from Kiru was the longest (9.1x5.1 μm) while the least were isolates 4, 5, and 11 from Tashar biri, Kofa, and Wudil in Katsina and Kano states respectively and measuring 5.3x 3.5 μm, 5.3x 3.5 μm and 5.3x 4.3 μm respectively. Shape of the teliospores was also significantly different ($p<0.05$) between locations and ranged from irregular and echinulate, elliptical, round to ovoid/spherical and echinulate in shape (Table).

Table 4: Length, Width and Shape of *S. cruenta* teliospore isolates collected from the Sudan savanna, Nigeria

Isolate	Length (μm)	Width (μm)	Shape
1	4.5	3.6	ovoid to spherical
2	5.6	2.6	irregular,slightly echinulate
3	6.0	4.9	eliptical,
4	5.3	3.5	ovoid
5	5.3	3.5	spherical, echinulate
6	9.1	5.1	round or ovoid
7	5.7	4.4	round
8	6.3	6.0	spherical
9	6.2	4.3	irregular
10	5.9	3.8	spherical
11	5.3	4.3	ovoid, echinulate
12	8.3	4.1	spherical and echinulate
Mean	**5.7**	**4.1**	
LSD	**3.012**	**3.112**	

Germination rate

Germination rate also varied significantly between samples and germination media. In *S. reilianum* teliospore commenced germination also 4-5 hours after incubation in water (Table 5) but the rate was generally low; only 4 out of the 12 isolates commenced germination; samples 3, 6, 9, and 10 from Kankara, Kiru, Gantsawa and Birnin-kudu. However, after 12 hours of incubation in water, sample 6 from Kiru had 78.1% germination while the least was sample 3

from Kankara with 53.5%.There was significant difference between the rates of teliospore germination at 5% level of probability.

Table 5. Germination rate of 12 *S. reilianum* teliospore sample in water collected from the Sudan savanna, Nigeria

	Hours to germinate											
Isolate	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12
1	0	0	0	0.0	5.0	20.1	22.1	30.3	35.2	50.3	53.2	54.1
2	0	0	0	0.0	4.7	19.3	23.1	26.1	30.3	53.5	53.5	55.3
3	0	0	0	5.0	10.0	19.5	26.1	28.2	32.5	52.3	53.1	53.5
4	0	0	0	0.0	10.1	19.3	24.1	26.1	28.2	51.9	53.1	55.2
5	0	0	0	0.0	6.0	20.1	23.3	23.1	27.2	58.9	60.2	63.2
6	0	0	0	5.5	11.3	30.1	32.3	26.1	40.1	63.8	68.3	78.1
7	0	0	0	0.0	4.5	30.1	34.1	24.1	28.1	49.7	49.7	60.2
8	0	0	0	0.0	6.1	20.1	25.1	26. 3	29.2	51.8	52.3	65.5
9	0	0	0	2.0	7.0	20.8	23.1	24.5	30.1	48.3	60.1	65.2
10	0	0	0	2.0	10.1	20.3	23.0	23.1	29.1	47.2	60.1	64.1
11	0	0	0	0.0	7.3	20.1	24.0	28.0	31.5	49.3	50.1	61.1
12	0	0	0	0.0	2.8	23.1	24.0	28.0	30.1	50.1	53.1	54.0
Mean	**0**	**0**	**0**	**1.2**	**7.0**	**21.9**	**25.3**	**27.9**	**32.0**	**50.1**	**55.6**	**65.9**
LSD	**NS**	**NS**	**NS**	**12.5**	**20.2**	**17.8**	**20.3**	**20.5**	**20.3**	**23.1**	**21.5**	**22.1**

NS= Not significant

In *S. cruenta*, germination in water commenced after 4-5 hours of incubation (Table 5). Although the rate was generally low at that period and the fact that some isolates such as Nos. 3, 8, and 9 from Kankara, Babura and Gantsawa, did not germinate at the hour, germination was generally faster in this experiment. In *S. cruenta*, sample 7 from Kazaure produced 62.1% germination after 12 hours of incubation in water while the least was obtained from samples 9, 10, and 12 from Gantsawa in Kano state and Birnin-kudu and Gwaram in Jigawa state each with 50.1% germination. This showed that the germination rate was significantly different ($p<0.05$) at 12 hours after incubation.

Table 6. Germination rate (%) of 12 *S. cruenta* teliospore samples in water collected from the Sudan savanna, Nigeria

	Hours to germinate											
Isolate	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12hrs
1	0	0	0	0	2.0	3.0	14.5	40.1	43.2	45.1	50.1	60.5
2	0	0	0	0	1.0	4.0	17.6	40.1	44.2	45.0	50.2	52.3
3	0	0	0	0	0	3.0	20.1	40.1	44.2	50.1	50.2	53.2
4	0	0	0	0	2.0	4.0	30.0	40.5	45.1	50.2	50.5	53.1
5	0	0	0	0	1.0	4.0	25.0	42.1	42.1	50.2	50.5	61.1
6	0	0	0	0	1.0	5.0	20.3	43.2	43.2	50.3	50.7	52.1
7	0	0	0	0	1.0	4.0	30.2	44.1	44.1	50.1	51.2	62.1
8	0	0	0	0	0.0	5.0	25.1	44.1	44.1	50.1	51.1	52.1
9	0	0	0	0	0.0	6.0	24.1	45.5	45.5	75.1	51.3	50.1
10	0	0	0	0	1.0	1.0	23.1	42.5	42.5	45.1	50.1	50.1
11	0	0	0	0	1.0	1.0	28.1	40.5	40.5	45.3	52.0	55.6
12	0	0	0	0	1.0	2.0	24.1	40.5	40.5	45.5	50.2	55.1
Mean	**0**	**0**	**0**	**0**	**0.9**	**3.5**	**23.5**	**38.5**	**43.3**	**47.9**	**50.7**	**55.2**
LSD	**NS**	**NS**	**NS**	**NS**	**20.1**	**12.3**	**20.2**	**23.3**	**28.3**	**33.1**	**20.5**	**23.1**

NS= Not significant

On PDA however, *S. reilianum* germinated faster on PDA than *S. cruentum* (Table 8). The various isolates germinated at different rates revealing a significant difference ($p<0.05$). On

the other hand, in *S. cruenta,* germination commenced 7 hours (Table 7) after incubation while *S. reilianum* germinated 6 hours after incubation (Table 8). Germination was generally good at 12 hours after incubation. *S. cruenta* isolate No. 5 from Kofa in Katsina state had a germination rate of 65.1% while *S. reilianum* isolate No.3 from Kankara had 62.1% after 12 hours. There was significant difference between the germination rates of the various isolates ($p<0.05$).

Table 7. Germination rate (%) of 12 *S. cruenta* teliospore isolate in PDA collected from the Sudan savanna, Nigeria

	Hours to germinate											
	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12
1	0	0	0	0	0	0	0	0	10.5	22.5	45.6	55.1
2	0	0	0	0	0	0	0	2.0	13.1	26.5	50.1	57.2
3	0	0	0	0	0	0	0	2.1	10.8	24.6	50.2	53.1
4	0	0	0	0	0	0	0	2.3	10.5	23.5	48.3	56.0
5	0	0	0	0	0	0	0	0	11.1	26.1	45.0	65.1
6	0	0	0	0	0	0	0	0	16.2	26.0	50.3	58.2
7	0	0	0	0	0	0	0	1.0	12.1	28.1	56.1	56.5
8	0	0	0	0	0	0	0	1.0	11.0	24.1	48.2	55.3
9	0	0	0	0	0	0	0	2.0	15.2	25.0	46.3	56.2
10	0	0	0	0	0	0	0	1.0	12.0	20.0	482	60.2
11	0	0	0	0	0	0	0	1.1	11.0	23.1	53.1	53.5
12	0	0	0	0	0	0	0	0.0	13.1	25.1	46.0	56.2
Mean	**0**	**0**	0	0	0	0	0	0.7	11.7	25.3	48.9	57.6
LSD	**NS**	**NS**	NS	NS	NS	NS	NS	20.1	20.3	22.1	22.2	20.2

Table 8: Germination rate (%) of 12 *S. reilianum* teliospore isolate in PDA collected from the Sudan savanna

					Hours	**to**	**germinate**					
Isolate	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12
1	0	0	0	0	0	0	0	5.9	40.1	45.1	50.1	53.1
2	0	0	0	0	0	0	0	10.0	30.0	40.9	52.0	50.0
3	0	0	0	0	0	0	0	11.0	31.1	44.3	51.1	62.1
4	0	0	0	0	0	0	0	14.0	28.5	42.1	50.1	52.3
5	0	0	0	0	0	0	2.0	12.3	25.8	43.1	50.2	50.2
6	0	0	0	0	0	0	1.0	12.1	39.2	44.0	50.5	51.1
7	0	0	0	0	0	0	1.0	12.1	28.1	42.5	49.0	50.0
8	0	0	0	0	0	0	0	11.2	25.1	40.5	45.0	50.0
9	0	0	0	0	0	0	5.0	11.0	25.0	44.1	46.7	51.2
10	0	0	0	0	0	0	0	12.0	24.1	42.1	45.3	50.1
11	0	0	0	0	0	0	1.0	12.1	25.6	43.1	52.2	52.0
12	0	0	0	0	0	0	0	11.9	28.1	44.1	50.2	52.0
Mean	0	0	0	0	0	0	0.6	9.7	25.3	43.1	47.1	51.7
LSD	NS	NS	NS	NS	NS	NS	NS	20.5	21.2	23.1	22.1	21.2

NS= Not significant

Morphological features of S. *reilianum* and S. *cruenta* teliospores.

Teliospore color

There was no significant difference ($p>0.05$) between the color of the various isolates of the two pathogens both in aggregate and when single. *S. relianum* spores were black and dark

brown in aggregate and single form respectively. This is evident in some earlier reports that when mature, the spores of *S. relianum* are somewhat opaque and dark brown in aggregate and single (Tarr, 1962; Sigh, 1998). The color of *S. cruenta* in both aggregate and single form was also black and dark brown as demonstrated by Singh (1998) Mehrotra and Aggarwal, (2003) that *S. cruenta* spores were generally dark brown to black in color. The similarity in the color of the various spores might be attributed to the fact that the isolates were collected from the same agro-ecological zone from different sorghum varieties or that differences in sorghum variety had no significant effect on the spore color (Kutama *et al*., 2010c). Moreover, the similarity in color may also be attributed to the fact that both pathogen belongs to the same genus (*Sporisonium*) (Tarr, 1962) in pearl millet smut pathogen; *Tolyposporium penicilliariae* for, they both made collections from various agro-ecologies in India and Nigeria respectively.

Length, width and shape of teliospores

Significant difference ($p<0.05$) were obtained on the length, width and shape of the various isolates of both *S. creunta* and *S. reilianum*. Differences in length, width and shape of the teliospore isolates might not be unconnected with the fact that samples were obtained from different sorghum varieties. The statistically significant difference between the different isolates of different region or area signifies that the two pathogens may differ in their physiological activities on different sorghum varieties as demonstrated by Kutama *et al.* (2024) that differences in structure of the smut fungi could be used to explain their physiological race differences and therefore is an important tool in the taxonomy of smut fungi.

Germination rate

The germination rate of the various teliospores varied between isolates. The various isolates germinated at different rates revealing a significant difference (P[=0.05). The difference in the rate germination in both water and on PDA obtained in this result could be attributed to so many factors. Marley *et al*. (2002b) suggested that the patchy distribution of morphological characters existing in the smut pathogens phylograms, points to their variability and their dependence on the host morphological traits. The result obtained in this study on *S. reilianum* shows that the pathogen spores commenced germination 4-5 hrs but slowly. The overall germination of *S. reilianum* in water varied from low to high after 12 hrs of incubation. This agrees with the work of Kutama *et al*. (2024) who reported very low frequency of germination of *S. reilianum* in soil. However, *S. cruenta* commenced germination later than *S. reilianum* which is an indication of species differences (Kutama *et al*., 2010c; and Kutama *et al*., 2022).
On the other hand, both *S. cruenta* and *S. reilianum* germinated at around 7-8 hrs after incubation with *S. reilianum* being 1 hour earlier than *S. cruenta*. The early germination of *S. relianum* might be attributed to either of its higher virulence than S.cruenta or due to differences in their taxonomic positions. Kutama *et al.* (2024) and Hadiza *et al.* (2021) reported that the difference in the taxonomy of the smut pathogens might be responsible for their difference in germination rate. This also agrees with the report of Fredriksen and Osorio (1998) that teliospore germination pattern in *S. reilianum* differs due to occurrence of variable degree of dormancy in any teliospore population.

Conclusions

The results of this research have indicated that morphological differences exist between isolates of the same and or different smut pathogens. In the same vein, both the isolates were viable which means they can stay for a long period on the host plant tissues. The results of this study have also clearly shown that *S. reilianum* germinated faster in both in water and on PDA growth media than *S. cruenta* hence, could be more virulent in this agro-ecological zone (savanna) under optimum environmental conditions.

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