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SYMPTOM TYPES OF *COLLETOTRICHUM GRAMINICOLA CESATI* WILS. OF SORGHUM IN THE NORTHERN GUINEA SAVANNA OF NIGERIA

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ABSTRACT

A study of symptom types of *Colletotrichum graminicola* Cesati Wils. of Sorghum was carried out at Samaru, Nigeria using four sorghum varieties, maize and millet. Morphological and cultural characteristics in addition to the pathogenicity studies under screen house conditions were carried out. The occurrence of three symptomatic groups (patchy, midrib and isolated lesions) in Sorghum anthracnose was confirmed. Conidia measurement showed that the symptoms with isolated spots produced the largest conidia (24.0-30.4x8.4-9.4 µm) among the sorghum cultivars. Maize isolates produced larger conidia (22.5-35.0x5.0-8.5 µm) than those of sorghum. Maize produced only one symptom group (patchy). Pathogenicity test in the screen house revealed that all the three isolates from sorghum could only infect sorghum varieties and neither infected maize nor millet. Maize isolates infected both maize and millet, but not sorghum. *C. graminicola* was found to be host-specific. Based on findings in the pathogenicity test studies, it was concluded that isolates of *C. graminicola* from maize were highly pathogenic to both maize and millet, but not virulent on sorghum, while isolates from susceptible sorghum cultivars were highly pathogenic to sorghum varieties, but did not infect maize and millet. Investigation of the host range using more cereals and grasses is recommended.

Key words: Symptom types; *Colletotrichum*; Sorghum; Guinea Savannah
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INTRODUCTION

Sorghum (*Sorghum bicolor* (L) Moench) is a very important food crop in Nigeria and the semi-arid tropics. In Nigeria, production is concentrated in three agro-ecological zones: sahel savanna (400-600 mm annual rainfall), sudan savanna (600-1000 mm annual rainfall) and guinea savanna (1000-1100 mm annual rainfall) (Marley *et al.*, 2002).

Sorghum production in Nigeria and West Africa as a whole is limited by diverse constraints, both biotic and abiotic factors (Marley *et al.,* 2002). Marley (2004) reported the occurrence of 32 diseases of Sorghum in Nigeria. Some of the more common diseases are of fungal, bacterial, viral and nematode origin, of which fungal anthracnose caused by *Colletotrichum graminicola* (Syn. *C. sublineolum*) is the most important foliar disease on both local and improved Sorghum in Nigeria and Africa as a whole (Thomas *et al.,* 1996; Marley *et al;* 2001; Ukpai and Osuoha, 2018).

Sorghum anthracnose was first reported in 1902 from Togo, West Africa (Tarr, 1962; Kalu, 2011). It has been reported to be widely prevalent under hot humid conditions in tropical and sub-tropical regions of the world (Frederiksen and Duncan, 1992).

Sorghum anthracnose is present wherever sorghum is grown in warm humid environment. It is predominant and is considered of primary importance in parts of West Africa including Nigeria. Many land races

and improved varieties are susceptible to the foliar stage of the disease with serious epidemics occurring on farmers and researchers' fields (Thomas, 1996; Marley, 2002; Kalu *et al*., 2011).

Anthracnose occurs on all aerial parts of the plant – leaves, leaf sheath, stalk, panicle and seed. The foliar phase is the most common and characterised by circular to elliptical red spots up to 0.5-5.0 mm in diameter with few or numerous acervuli. These lesions may coalesce to occupy most or all of an infected leaf and may even kill the leaf (Mathur *et al*., 2002: Kalu, 2011). Acervuli of *C. graminicola* develop as brown-grayish to black specks usually arranged in concentric rings. Differences in foliage and grain symptoms may be due to host reaction, physiological status of the host, the environments or pathogenic variability (Frederiksen, 1986; Pande *et al.*, 1993). The variability in foliar symptoms (patchy, mid-rib and pin-point/isolated spots), cultural characters and isolate morphology led to the description of forma-species, *C. graminicola var. isolatum* in Nigeria and other West and Central African sub-regions (Marley and Ogungbile, 2002; Ukpai, 2009) and *C. graminicola var. zonatum* from India which forms diffuse spots (up to 50 mm in diameter), with numerous acervuli in concentric zones (Frederiksen and Duncan, 1992; Ukpai, 2009).

It is not clear whether fungal isolates from foliage, grain and stalk are different or whether variation in symptomology on different plant parts is due to the age of the plant, organ specificity of the particular isolate, environmental conditions or combination of these factors. Thus Sorghum lines resistant to foliar anthracnose may develop heavy grain infection. And there is weak but significant negative correlation between foliar and grain anthracnose ratings of hybrids, suggesting that resistance to foliar and grain anthracnose are independent characters (Mathur and Thakur, 1998). It is thus suspected that some isolates may be specific for foliage or grain antrachnose (Totla, 1990). However, leaf and stalk isolates from Sorghum in Texas were indistinguishably analysed with molecular markers, suggesting that there is no genetic basis for organ specificity (Rosewich, 1996).

The occurrence of *C. graminicola* on infected leaves, stems, peduncles, head and grain may take place separately or simultaneously with other infections. In India, for example, several foliar fungal diseases are common (Sharma, 1980 ; Kalu *et al*., 2011). These include zonate leaf spot (induced by *Gloeocercospora sorghi*), gray leaf spot (caused by *Cercospora sorghi*), rust (caused by *Puccinia purpurea*), leaf blight (caused by *Excerohilum turcicum*) and rough leaf spot (caused by *Ascochyta sorghi*). Environmental conditions that favour infection by one of these fungi may favour infection by other fungi; this means that multiple infection will be found and may be more common than generally perceived (Ukpai, 2009). Often these different phases of anthracnose are manifested as different diseases (Bindawa, 1987; FAO, 2015). For this reason, it is not uncommon to find in literature discussion of the peduncle and head symptoms referred to as red rot aspects of the disease. Most often only one symptom is present or predominates over the other two, and leaf is sometimes the most common and at other times it is none-existent.

Based on symptoms, two distinct types of anthracnose on sorghum were reported by Bindawa (1987): the restricted eye anthracnose induced by *C. graminicola* (Ces.) Wilson, which forms diffuse type of spots; at maturity the zonate anthracnose appears. On the other hand, Alawode (1982) in Marley and Ogungbile (2002) reported three types of symptoms on sorghum namely, patchy, mid-rib and isolated spots. He observed that the first group (patchy spots) infected the lamina; the second group (mid-rib lesion) infected the midrib, while the isolated spots (pin- type lesions) infected both the mid-rib and the lamina on Sorghum. It was only the last group that was pathogenic to maize and millet. This study was aimed to investigate the symptom types of *Colletotrichum graminicola* Cesati. Wils. of sorghum in the Northern Guinea Savanna of Nigeria.

MATERIALS AND METHODS

Isolation and identification of pathogens from infected leaf samples were carried out using the method of Gwary *et al.* (2003) in the laboratory and screen house of Department of Crop Protection, Institute for Agricultural Research, Samaru (11^0 11^0, 07^0 38^1E; Altitude 686 above sea level), near Zaria, Kaduna State, Nigeria.

Samples of anthracnose-infected sorghum leaves were collected from susceptible cultivars, BES (SAMSORG 4), ICSV400 (SAMSORG 41), and ICSV 111 (SAMSORG 40) which readily produced the symptoms from IAR field. Leaves were collected with moist filter paper and placed in humid chambers. To isolate the pathogen from the tissues, small pieces of preserved infected parts of the leaves from the identified symptoms were surface-sterilised by immersing in 1% sodium hypochlorite solution for one minute and then rinsed in sterile distilled water (SDW). Each of the three categories was separately lifted using sterilised forceps and placed in Petri dish containing Potato Dextrose Agar Streptomycin (PDAs). Fungal growth in PDAs was daily observed and isolates suspected to be *Colletotrichum graminicola* were later sub-cultured by picking fragments of hyphae/spores and placing with the help of forceps in fresh PDAs. The sub-culturing was in order to get pure cultures of the pathogen. The pure isolates were later stored in slants of PDAs in McCartney bottles at temperature between 20 - 25^0C until required for use.

Morphological and cultural studies of the pathogen

Spore growth, size of culture, colour, colony diameter, presence or absence of acervuli and seatae were noted. Morphological and cultural studies of the pathogen were carried out using the method of Gwary *et al*. (2003). To obtain spore size, 50 conidia were randomly selected, then length and width were measured in micrometres (µm), using the stage micrometer and eye-piece ocular scale of the compound microscope. Spore counts were determined using a haemocytometer under the microscope at x40 objective lens. The shapes of conidia were also noted. Furthermore, the mycelial growth for each of the isolates was determined by averaging the horizontal and vertical lengths of mycelia after 7 days of growth. For colour determination, each culture was visually observed and noted. Finally, presence or absence of seatae and acervuli was determined by adjusting different views of the microscope and recording by using positive (+) or negative (-) signs, respectively. Relevant photographs of the organisms were taken.

Pathogenicity Test

Pathogenicity test under screen house condition was carried out using the method of Gwary and Asala (2006).
Four sorghum varieties (BES/KSV4/SAMSORG 4, ICSV111/SAMSORG 40, ICSV400/SAMSORG 41 and KSV8/SAMSORG 14), one maize variety, TZB and a millet variety, Dakace local were used in the pathogenicity studies (Gwary *et al*., 2003). The seeds were surface-sterilised and sown in plastic pots (35-40 cm diameter) containing sterilised soil mixed with one table spoonful (16 grams) of 15:15:15 NPK compound fertilizer. Three replicates (3 pots) of each sample or treatment were used. The pots were labeled, kept in the screen house and watered regularly. Two weeks after sowing, the seedlings were reduced to three plants per pot and one spoonful of 15:15:15 NPK compound fertilizer was added again to each pot. Regular watering was continued and plants were allowed to grow for 6 weeks after which inoculation with the different isolates of the pathogen was carried out.

Inoculum preparation and inoculation of test plants

Inoculum preparation and the inoculation of test plants were done using the methods employed by Gwary *et al*. (2003), Gwary and Asala (2006) as well as Ukpai (2009). The different isolates of the pathogen were grown in the laboratory for two weeks in 9 cm diameter Petri-dishes containing PDAs which produced sufficient sporulation. At the end of two weeks, when spores had been abundantly produced, 20 ml of SDW was put into each Petri-dish and suspension of the mycelium and conidia was made from each Petri dish by carefully scrapping with sterilised glass slide. The suspension was filtered through a double layer of cheese cloth into labeled 500 ml conical flasks. The concentration of conidia was determined and adjusted where necessary with the aid of haemocytometer to about 50 conidia/ml. Each test plant was sprayed with the inoculum suspension until run off. Some inoculum was suspended in syringes and injected to the different portions of the leaf for better contact. The control/check plants were sprayed with SDW. The temperature and relative humidity in the screen house were recorded.

Both inoculated and control plants were covered with polyethylene bags (70 x 40 cm size) which were sprayed with SDW to create humid condition necessary for the anthracnose infection. The pots in each treatment were arranged in completely randomised design. The polyethylene bags were carefully lifted after 24 hours to aerate the plants, sprayed with water to increase the humidity and replaced.

The polyethylene bags were finally removed three days after inoculation. Plants were periodically sprayed with SDW until symptoms started appearing. Tap water was periodically poured on the floor of the screen house to help create and maintain a humid environment necessary for the disease development. Observation for the appearance of disease symptoms commenced two weeks after inoculation.

RESULTS

Results of the symptom type studies showed that three major symptom groups exist in this pathogen, namely patchy lesions, isolated lesions and midrib lesions. The results indicated that variations exist among these symptom groups in terms of their cultural, morphological and pathogenic characteristics. Variations were found in size, shape and colour of conidia and seatae among other features. The growth of the three different isolates as observed in potato dextrose agar streptomycin (PDAs) showed that the patchy lesions (isolate A) was dull white in colour with acervuli, the growth was cottony/loose acervuli in concentric rings and moderately fast-growing (acervuli emerging in 3-4 days), the conidia number was 55, 000 and seatae were present.

Table 1 shows that the symptom with isolated lesions (isolate B) was grey in colour, acervuli fluffy and somewhat scattered and moderately fast-growing (emergence within 5 days). Conidia were up to 73,000 in number and seatae were also observed.

In the case of midrib lesion (isolate C), growth in PDAs was greenish-grey in appearance and acervuli were somewhat cottony, appraised at the centre and sometimes fluffy, acervuli emergence was within 6 days, conidia numbered up to 66,000 and setae were also observed.

The isolate from maize closely resembled the sorghum isolate with isolated symptoms in having grey colour and producing acervuli with fluffy scattered growth, but differed in being produced between 2-10 weeks among other characterstics (Table 1).

Table 1: Cultural characteristics of three isolates of *Colletotrichum graminicola on* PDAs

Isolate type	Colony colour	Presence of acervulli	Type of growth	Days before acervulli emerge	Number of conidia	Presence of setae
Patchy lesion	Dull white	++	Cottony/loose acervuli in concentric rings, moderately fast growing	3-4	55,000	++
Isolated lesion	Grey	++	Fluffy growth with scattered acervuli, moderately fast growing	5	73,000	++
Mid-rib Lesion	Greenish grey	++	Cottony dense appraised acervulli at the centre sometimes fluffy and scattered fast growing	6	66,000	++
Maize isolate	Grey	++	Loose fluffy growth with scattered acervuli moderately fast growing	2-10	75,000	++

Mean was calculated from measurement of fifty (50) conidia and setae. ++ Presence of the particular structure

The graphic illustration of rate of growth in PDAs shown in Figure1 clearly indicated much variation among the isolates. Thus, *C. graminicola pr*oducing isolated symptoms showed the highest growth (9 cm) within a period of 7 days both in terms of growth speed and size. This was closely followed by the mid-rib isolate (8.8 cm), followed by maize isolate (8.0 cm) and patchy isolate (6.8 cm).

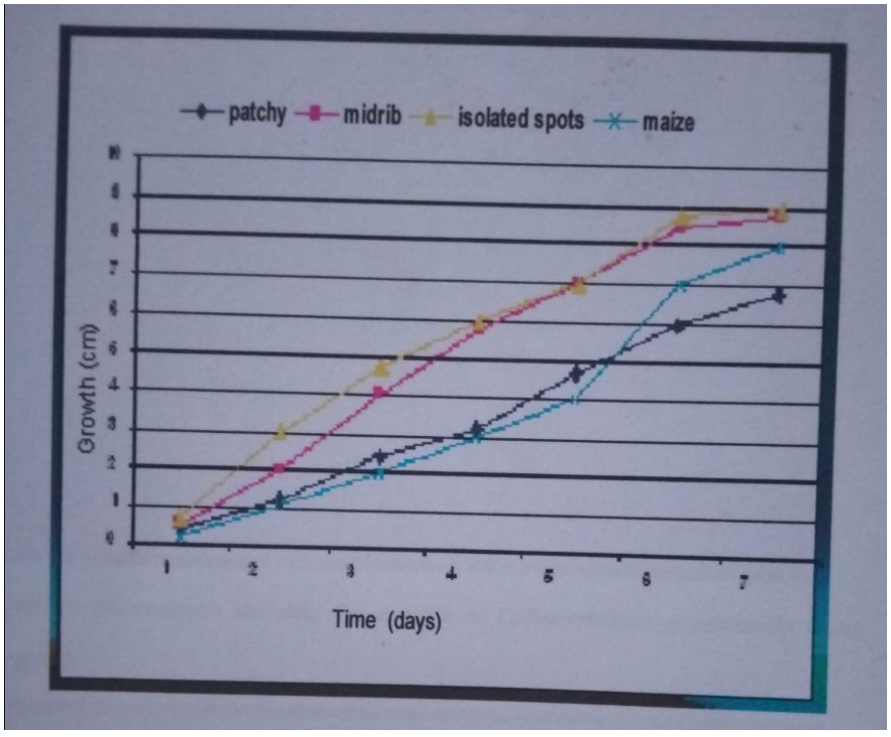

Fig.1.Rate of growth of *C. graminicola* in PDAs

Table 2 shows that in the patchy lesions (isolate A), conidia were simple, straight to curved, more-or-less blunt at both ends and produced in pinkish masses. In the case of isolate B (isolated lesions), conidia were hyaline, single-celled, falcate to vacuolated, curved with pointed ends produced in pinkish masses. While in the case of isolate C (midrib lesions), conidia were hyaline and single-celled, falcate, vacuolated, curved with pointed ends also produced in pinkish masses. In the case of maize isolate, conidia were hyaline, straight to more-or-less curved. Generally, the seatae in all the categories appeared long, slightly tapered at both ends and hyaline in colour. It should be noted that unlike sorghum, only one symptom group was consistently observed in maize and that was the patchy type on the lamina.

Table 2: Conidia and setae size (µm) of three different isolates of *Colletotrichum* from sorghum and maize

	Conidia		Setae	
Isolate	Range	Mean	Range	Mean
Patchy lesion (A)	16.3–27.5x 3.0-5.2	17.8x2.4- 5.0	72.2-113.4x3.50-6.9	75 x 3.71
Isolated Lesion (B)	24.0-30.4x 8.4-9.4	25.0x8.5	118-123x8.6-9.5	120x8.75
Mid-rib lesion (C)	20.0-28.4x 6.54-9.3	22.3x7.4	148.2-150.0x 8.4-9.8	149.0x8.75
Maize Isolate	22.5-35.0 x5.0-8.5	31.4x7.5	112-120x6.5-9.0	114 x 8.50

Each value is the mean of four different observations from four cultures. µm = millimicron

Plate 1: Micrograph conidia *C. graminicola* conidia from sorghum

Plate 2: Micrograph of *C. graminicola* conidia from maize

Table 3 shows the pathogenicity test conducted in the screen house. All the three isolates from susceptible sorghum variety did not infect either maize (TZB) or the millet (Dakace local) used in the study both in the mid-rib and on the lamina. Whereas all the sorghum varieties (except SAMSORG 14/KSV8) were infected, the control or check plants were not infected. Furthermore, the pathogenicity test with maize isolate of *C.*

graminicola showed a negative reaction or no infection with all the sorghum varieties both on lamina and in the mid-rib, while maize and millet were all infected. The symptom type result showed that C. *graminicola* isolate from maize was highly pathogenic to both maize and millet, but not sorghum. Similarly, isolates of *C. graminicola* from susceptible sorghum variety were highly infective on sorghum varieties, but did not infect maize and millet.

Table 3: Pathogenicity of three isolates of *C. graminicola* from sorghum on four sorghum varieties, maize and millet

	SAMSORG 14		SAMSORG 4		SAMSORG 41		SAMSORG 40		MAIZE (TZB)		MILLET (Dakace local)	
Isolates	LAM	MR	LAM	MR	LAM	MR	LAM	MR	LAM	MR	LAM	MR
Isolate (A)	-	-	+	+	+	+	+	+	-	-	-	-
Isolate (B)	+	-	+	-	+	-	+	-	-	-	-	-
Isolate (C)	+	-	+	-	+	-	+	-	-	-	-	-

\+ = susceptible reaction
\- = no reaction/ no disease
LAM = lamina
MR = mid-rib

Plate 3: Sorghum leaf inoculated with isolate A/patchy lesions of *C. graminicola* in screen house

Plate 4: Sorghum leaf inoculated with isolate B/Isolated lesions of *C. graminicola* in screen house

Plate 5: Sorghum isolates inoculated with isolate C/Mid-rib lesions of *C. graminicola*

DISCUSSION

The results of this study established the existence of three symptom types in *Colletotrichum graminicola* in sorghum (patchy, isolated and mid rib lesions) and host-specificity of these isolates by examining their pathogenicity in different sorghum cultivars, maize and millet. No correlation could be established between either morphological and cultural characteristics or pathogenicity of the various isolates examined. Morphological variation was reflected in the dimensions and shape of conidia in terms of curvature and pointedness, the length and septation of the setae among other features. This systematic study of the morphological characteristics resulted in the confirmation of the three symptom types earlier reported by Alawode *et al.* (1983), Bindawa (1987) and Ukpai (2009). Also, a wide variation in cultural characteristics such as growth, type of colony, colour of colony, mycelium and sporulation was observed in the present study. Variations in cultural characteristics in *C. graminicola* are well documented in classical studies (Ferreeira *et al*., 1985; Frederiksen, 1986; Marley and Ajayi, 2002), among others.

It should be noted that morphological races distinguishable on the basis of conidial dimensions have been well studied in different pathogens including *Helminthsoporium gramineum* (Ferreira *et al*., 1985). Similarly, cultural races are known in different fungi such as *Colletotrichum falcatum* and *C. lagnerium* (*Phytophthora parasitica* var. nicotiana (Frederiksen, 1986 ; Frederiksen and Odvody, 2000).

It was noted from the study that the isolate from maize could not infect sorghum and those from sorghum could neither infect maize nor millet. The present finding is in conformity with Marley and Ogungbile (2002) and Marley *et al*. (2002), who reported that maize isolates of *C. graminicola* from Akansas failed to infect sorghum and an isolate from sorghum did not infect maize. Ozolua *et al.* (1986), White *et al.* (1987), Beckman *et al*. (1999) and Ukpai (2009) confirmed the host-specificity of both sorghum and maize isolates of *C. graminicola.* On the contrary, Alawode *et al.* (1983) and Bindawa (1987) from Nigeria reported that

isolates of *C. graminicola* from maize readily infected sorghum and that one of the isolates from sorghum (patchy type) readily infected maize.

It was also observed that *C. graminicola* pathogen from a susceptible variety of sorghum such as BES/KSV4 (SAMSORG 4) was pathogenic to other sorghum varieties like ICSV 111 (SAMSORG 40), ICSV400 (SAMSORG 41) and to a lesser degree KSV8 (SAMSORG 14), a resistant variety as shown earlier in a study by Ukpai (2009). The reaction between *C. graminicola* from sorghum with the variety SAMSORG 14/KSV8 was similar to those with maize and millet.

Studies earlier carried out on pathogenic specialisation in *C. graminicola* have shown that the fungus has a wide range of virulence among cultivated and wild species of cereals and grasses including barley, oats, corn, rye, sorghum, sudan grass, Johnson grass, wheat and a host of others (Levy, 1991; Ukpai, 2009, Ukpai and Osuoha, 2018). Marley (2004) reported that *Colletotrichum falcatum* on sugar cane could not infect sorghum and *C. lineola* also could not infect sugar cane, that is, both were host-specific. Also, Marley (2004) reported that there was host-specificity among different isolates of the fungus and that only isolates from the same or closely related species were pathogenic on given hosts. Isolates from several hosts had a wide range of pathogenicity under identical conditions.

The foregoing report is indeed very similar to the findings in the present research in which all the replicate samples of *C. graminicola* from maize did not show any virulence except between maize and millet. Marley *et al.* (2001) suggested that *C. graminicola* as constituted by Wilson in 1914 consisted of several pathogenic races. Frederiksen and Odvody (2000) reported that corn isolates in Illinois were pathogenic to corn, but not sorghum. In Hawaii, *C. graminicola* from sorghum was weakly pathogenic to sugar cane, but *C. falcatum* (*Glomeralle tucumanensis)* from sugar cane was not pathogenic to sorghum. Here in Nigeria, Bindawa (1987) studied the pathogenicity of sorghum isolates on maize and wheat and observed that none of the 15 isolates tested infected maize or wheat seedlings.

Further evidence on host-specificity of *C. graminicola* from certain host species can be distinguished by unique morphological characters. Results obtained from the present study corroborate those of Marley (2004) and Marley *et al*. (2001) who reported that conidia from maize isolates were larger than those of sorghum. Alawode *et al*. (1983) reported that appressoria produced by sorghum were consistently smaller and not as strongly lobed as those from maize. These differences were used as the primary basis for separating maize and sorghum isolates into different species (Mathur and Thakur, 1998). Variation in conidia size between sorghum and maize isolates is similar to the findings in the present study, where conidia size ranged from 16.3-30.4 µm for sorghum isolates and 22.5-35.0 µm for maize isolates.

Finally, on the basis of an International Sorghum Anthracnose Virulence Nursery grown in countries in North, South and Central America, the Carribean region, Asia and Africa, it has been suggested that physiologic races exist within the isolates of *C. graminicola* that infect sorghum (Ferreira and Warren, 1985; Ferreira *et al.*, 1985). It has been reported that the variety wiley, which is highly resistant elsewhere, was infected by *C. graminicola* in Venezuela. The varieties Mn-960 and Tam - 428 which were found to be highly susceptible to the isolates in Nigeria were shown to be resistant in America. On the other hand, BTX 398, pioneer Brand 846 and C-424, which were highly susceptible in America, showed less infection in Nigeria. Thus, the present study on symptom types clearly confirmed that pathogenic races exist in *C. graminicola* based on host preference and specificity.

CONCLUSION

The occurrence of three symptomatic groups (patchy, midrib and isolated lesions) in Sorghum anthracnose was confirmed in this study. It was also noted from the study that isolates of *C. graminicola* from maize were highly pathogenic to both maize and millet, but not virulent on sorghum, while isolates from susceptible sorghum cultivars were highly pathogenic to sorghum varieties, but did not infect maize and millet. The findings of this research should be useful to Nigerian sorghum growers and farmers especially in the Northern guinea Savannah in carefully planning breeding programme for incorporating resistance against anthracnose. The development of integrated management strategy that combines host plant resistance with cultural control to reduce the number of surviving propagules in the field is recommended as a measure in curbing losses from anthracnose infection thereby contributing towards the attainment of food security in Nigeria. Investigation of the host range using more cereals and grasses is also recommended.

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