STUDIES ON ANTHRACNOSE ON SORGHUM AMD MAIZE CAUSED BY COLLETOTRICHUM GRAMINICOLA (Ces.) Wils. AND ON A MIDRIB SPOT ON MILLET

BY

ABDULLAHI BINDAWA ALIYU

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DEPARTMENT OF CROP PROTECTION
FACULTY OF AGRICULTURE
AHMADU BELLO UNIVERSITY
ZARIA, NIGERIA

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DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented before in any previous application for a higher degree. All quotations are indicated and the sources of information are specifically acknowledged by means of references.

(ABDULLAHI BINDAWA ALIYU) DATE: _____
Candidate

The above declaration is confirmed.

(DR. S.K. MANZO) DATE: _____
(Major Supervisor)

CERTIFICATION

This thesis entitled 'STUDIES ON ANTHRACNOSE ON SORGHUM AND MAIZE CAUSED BY COLLETOTRICHUM GRAMINICOLA (Ces.) Wils. AND ON A MIDRIB SPOT ON MILLET by Abdullahi Bindawa Aliyu meets the regulations governing the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

DR. S.K. MANZO
Chairman,
Supervisory Committee

PROF. A.C. EBENEBE
Member,
Supervisory Committee

DR. S.K. MANZO
Head,
Department of Crop Protection

PROF. DANIEL I. SARROR
Dean,
Postgraduate School

DATE: 16-8-89

AHMADU BELLO UNIVERSITY
ZARIA

DEDICATION

This piece of work is dedicated to mankind.

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ABSTRACT

Field observations on the anthracnose on sorghum and maize in Nigeria had shown variations in the type of symptoms and the location of the symptoms. Confusion existed as to whether a midrib lesion on millet in Nigeria is caused by anthracnose. Because it was not known whether these various symptoms were caused by the one and same fungus, Colletotrichum graminicola, the causal agent of cereals anthracnose, this project was undertaken to clear the doubts or confusions.

Isolates of the pathogen(s) were obtained from sorghum, maize and millet showing the typical various symptoms observed in the field. To determine extent of similarities and/or differences among the isolates morphological features of conidia and setae were studied with the aid of microscopes,. Cultural characteristics on artificial growth media were also studied. Pathogenicity tests of the different isolates using different crop cultivars under glass house conditions were conducted.

Colletotrichum graminicola was readily isolated from a maize and sorghum leaf specimens and never at all from the millet leaf specimens which yielded mainly Curvularia eragrostidis and Phoma sorghina. Statistical analyses of the morphological parameters of the conidia and setae of the different isolates did not show significant differences among the isolates. Various cultural forms were detected but none being specific to a particular isolate.

Pathogenicity tests proved that the millet midrib lesion, a hitherto undescribed millet disease, is not caused by Colletotrichum graminicola but by Curvularia eragrostidis and Phoma sorghina. Two formae speciales were identified from the

isolates of C. graminicola; one form specializing on maize and another specializing on sorghum. Two pathotypes were separated, based on symptoms produced on sorghum, from the form that specialises on sorghum.

The work demonstrated that the same fungus species, Colletotrichum graminicola, is responsible for anthracnose on maize and sorghum in Nigeria.

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CHAPTER 1

INTRODUCTION

Sorghum, maize and millet are the three most important cereal crops grown in Nigeria. They are primarily produced for human consumption and they constitute the staple food for a large portion of the population in the country. Millions of hectares are annualy devoted to them (Abalu, 1977). Between 1960 and 1975 sorghum and millet accounted for about 81% of the total tonnage of cereals produced in Nigeria. In 1976 sorghum alone occupied about 46% of the total area devoted to cereals and formed about 50% of the total cereal grain produced that year (Egharevba, 1978).

In Nigeria, these cereals are cultivated in varied ecological zones, ranging from the relatively wetter Southern Guinea Savanna to the relativeiy dry Sahel Savanna in the north of the country (covering the latittudes 7° to 14°N). Production of sorghum is largely in the Guinea and Sudan Savanna zones while millet is largely restricted to the drier Sudan Sahel Savanna and the bulk of maize comes from the Guinea Savannas.

Variation in ecological condition can affect the biology of an area and bring about variations among organisms found in the different ecological areas. As the above crops are distributed in different ecological zones, there is greater likelihood of the pathogens found in them varying from one ecological area to another.

Many diseases attack sorghum, maize and millet in Nigeria and elsewhere resulting in considerable reduction in yield and quality. Sometimes one disease attacks only one or two or all of the above three cereals. One of the major diseases of sorghum and maize is anthracnose induced by Colletotrichum graminicola. This disease occurs on many cereals and on a very wide range of grasses Selby and Manns, 1909; Wilson, 1914; Sanford, 1935; Choudhury, 1936; Koehler, 1943; LeBeau, 1950; Dickson, 1950; Moseman, 1952; Tarr, 1962; Dale, 1963).

In Nigeria, anthracnose has been described as one of the major foliage diseases, especially on sorghum (Manzo, 1978; Tyagi, 1978). Though observed on maize leaves, anthracnose is at present a minor disease on maize in Nigeria.

A lot of confusion and controversy exist in literature on the nomenclature of the organism(s) responsible for anthracnose on cereals and grasses (Tarr, 1962). The disease is widely considered to be caused by the fungus Colletotrichum graminicola (Ces.) Wilson. In 1914, Wilson lumped together Colletotrichum spp. with falcate conidia recorded on Graminea into one species which he called C. graminicola (Ces.) Wils. The affected preexisting species were C. cereale Manns and C. lineola Corda. C. falcatum Went, the causal organism of the red-rot disease on sugarcane, was excluded. Laboratory and field experiences with the Colletotrichum on these cereals are making researchers wonder whether Wilson's lumping into one species of these Colletotrichum spp on cereals and grasses truly reflects the reality. Opinions among researchers vary as to whether Wilson's C. graminicola is indeed a single species, especially when cultural behaviours on growth media and anthracnose symptoms on the host plant(s) are

taken into consideration. For example, in Nigeria, at least three different symptom types are commonly observed, especially on susceptible sorghum varieties (see plates I - III). The anthracnose lesions on the affected leaf may be restricted to the midrib while the lamina remains free or vice versa. Two forms of lesions on the lamina are commonly observed. With one of the forms, the lesions appear as small and restricted circular to oval spots, 2 - 4 mm in diameter and having a small pinpoint, dark brown centre (acervuli) surrounded by a light brown to tan necrotic areas (see plate 1 I) While with the second form the lesion takes the form, of a wide and diffused patch with a number of black to dark brown spots (acervuli) scattered all over the patch. (see plate 1 P). Lesions on the midrib are commonly the patchy form but the small restricted oval spots are also observed on the midribs. In Nigeria, it is very common to find severe natural infection by anthracnose on sorghum interplanted with maize while the maize remains totally free of infection even though the maize variety is known to be susceptible to anthracnose. Also in Nigeria, some varieties of sorghum hitherto not known to be infected by anthracnose are now oberved to have anthracnose infections (IAR 1985).

These variations in foliar symptoms of anthracnose could lead to mistaken identification of the causal organism especially if only symptoms are used. For example, a midrib lesion similar to that of C. graminicola was found to be caused by the fungus, Glomerella tucumanensis Speg, on some varieties of sorghum in the United States, India and Java (Ramakrishnan, 1963). An anthracnose with zonate type symptoms has been reported on sorghum in India as a distinct anthracnose. (Rajasab and Ramalingam 1981).

Because of the above stated findings and observations on anthracnose and the pathogen, one can not help but wonder whether indeed C. graminicola is the same organism or form(s) of the same organism that is responsible for all the observed anthracnose symptoms on these cereals or whether distinct forms of same organism or distinct species of the same genus are responsible but are being mistakenly considered to be one and same entity as supposed or assumed by Wilson (1914).

The questions raised as a result of the above stated observations, necessitated this research project which has the following objectives:-

1. To determine whether or not isolates of the anthracnose pathogen(s) from the different plant specimens and from the different symptoms or parts of the leaf collected from different locations can be distinguished from one another on the basis of their morphological, cultural and pathological characters, behaviour.
2. To classify forms of the pathogen(s) that may become evident in the course of the investigation.

It is hoped that this study might help in establishing whether or not Wilson's nomenclature holds valid or whether or not Wilson oversimplified the classification of these members on cereals under this genus. The project may prove to be of great value to pathologists and breeders in the development of anthracnose resistant cultivars.

At the start of this investigation, it was thought that the midrib lesions on millet were anthracnose. In the course of the investigation it was clear that these midrib lesions are not anthracnose but a distinct and an undescribed disease. Because of this, the disease on millet is being treated separately

in chapter five of this manuscript.

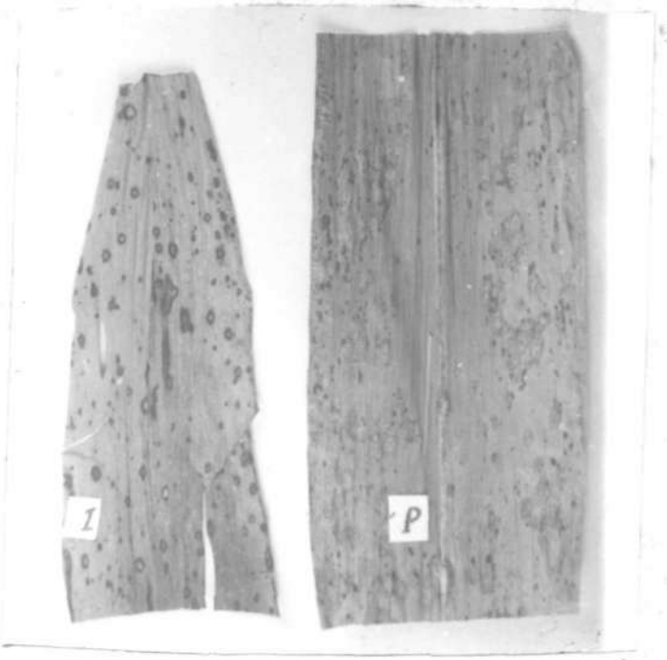


Plate I Isolated-type (I) and patchy-type (P) symptoms of anthracnose caused by Colletotrichum graminicola on sorghum leaves in Nigeria.

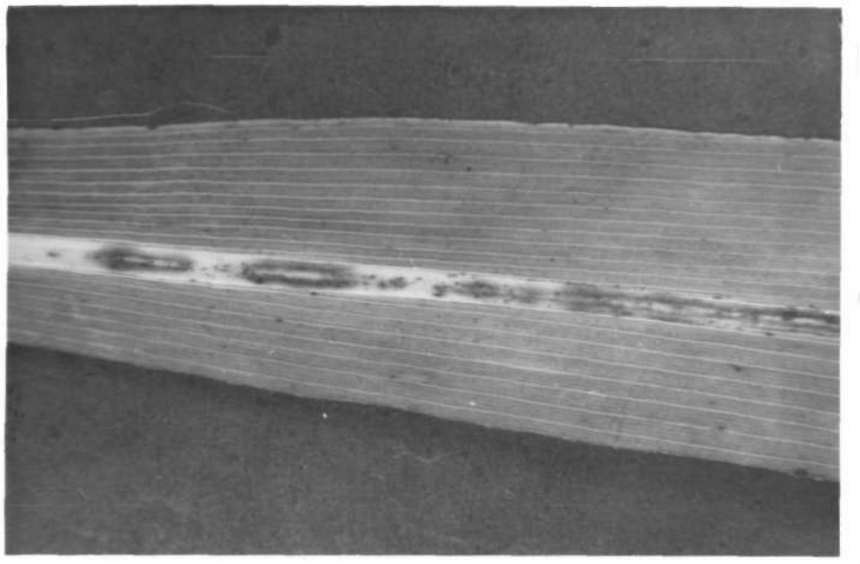


Plate II Sorghum leaf with anthracnose symptoms on the midrib alone, with no symptoms on the lamina.

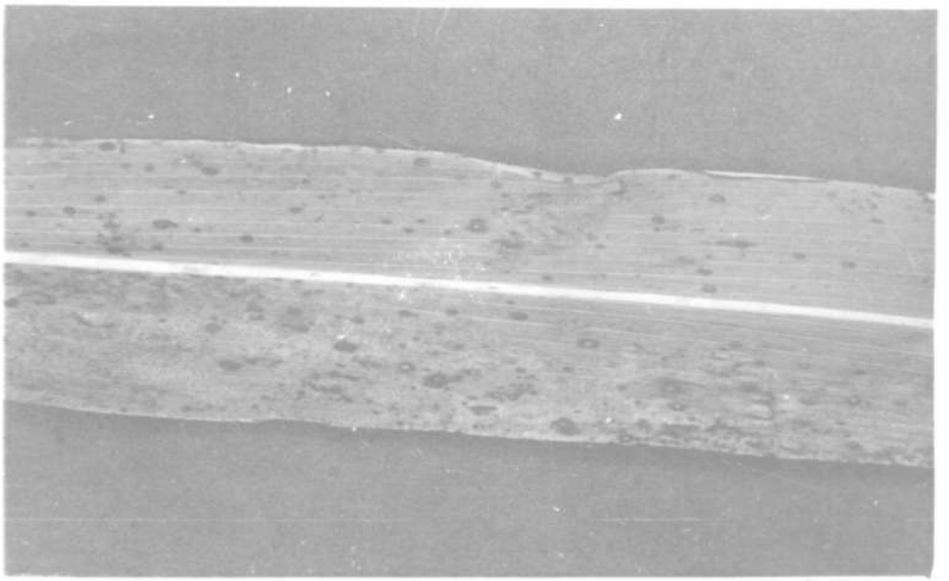


Plate III Sorghum leaf with anthracnose symptoms on the lamina alone; with virtually no symptoms on the midrib.

CHAPTER 2

LITERATURE REVIEW

2.1 Occurance, Distribution, Host Range and Economic Importance of anthracnose.

Anthracnose caused by Colletotrichum graminicola has been widely reported (Selby and Mann, 1909; Wilson, 1914; Sanford, 1935; Tarr, 1962; Manzo, 1978; Tyagi, 1978) as an important disease on a number of cereal crops and a wide range of grasses. Although anthracnose had been known earlier in India and elsewhere, the disease was first described in the United States of America where it was reported on sorghum (Stevens and Hall, 1911; Heald, 1912; Tarr, 1962). Since then the disease has been reported to exist in most sorghum-growing regions of the world (Pastor-Corrales and Frederiksen, 1980).

Anthracnose is one of the most damaging diseases of sorghum, especially on sorghum grown in warm humid regions of the world (LeBeau et al, 1951; Saccas, 1954; Sanford, 1955; Tarr, 1962; Porter, 1966; King, 1969; Sundaram et at, 1972; Berquist, 1973). The disease is favoured by warm humid environment created by frequent rainfall at/a particularly later stage of the host plant growth. Anthracnose may be absent in places with no rainfall or where sorghum is grown under irrigation (Tarr, 1962).

The disease is one of the main yield limiting factors of sorghum production in North, Central and South America, West Indies, Africa and Asia (Pastor - Corrales and Frederiksen, 1979). Anthracnose has a wide host range and it has been recorded on grain and sweet sorghum, Johnson grass, maize, rye, wheat, oat, barley,

various wild sorghums, finger millet, italian millet, broomcorn and many other cultivated and wild genera distributed amongst at least 8 out of the 12 tribes of the family Gramineae (Wilson, 1914; Choudhury, 1936; Sprague, 1950; Ray, 1954; Tarr, 1962; Luke et al, 1963; Schurtleff, 1973; Ullstrup, 1974; Wiese, 1977). Under favourable weather conditions anthracnose can kill or retard the growth of young plants to a considerable extent; leaf destruction and defoliation can occur resulting in serious loss in quality (LeBeau et at, 1951; Burton, 1954). Anthracnose has greatly affected the intensive cultivation of sweet sorghums for sugar and syrup production in the United States of America (LeBeau et al, 1951). In broomcorns, lodging occurs and poor heads are produced (Koehler, 1943; Hsi, 1956; Miller, 1956). The stalk-rot phase of anthracnose can result in severe wilt and stunted plants (Bonning and Walner 1936; Bruehl, 1948; Bruehl and Dickson, 1950; Leukel et al, 1951; Tarr, 1962). In sorghum plants, leaf anthracnose can affect the chlorophyll and carotenoid cotents of the plant (Chiranjee and Tripathir, 1975).

2.2 Symptoms of anthracnose

Anthracnose symptoms on cereals vary considerably with the kind and part of the host plant attacked and are generally more apparent on maturing plants. C. graminicola infects leaves, stems, peduncles, heads and the grain either separately or altogether (Tarr, 1962; Pastor-Corrales and Frederiksen, 1980). These different phases of anthracnose are often manifested as apparently distinct diseases. For the evaluation of host resistance or extent of damage, these symptoms are grouped into three phases, viz: the foliar, the stalk and the floral symptoms (Lohman, et al, 1951; Harris,

et al, 1964; Harris and Fisher, 1973). Very often one phase expresses more than or dominates the other two. Among the three phases, the foliar anthracnose is the most common and it has been reported from many parts of the world. It can occur at all stages of plant growth from the seedling to the mature plant. The stalk-rot and floral phases mainly occur in the maturing plant (Tarr, 1962).

On the leaves, anthracnose infection shows a wide range of symptoms which have been adequately described by several workers (Leukel and Martins 1943; LeBeau, et al, 1951; Dickson, 1956; Harris, 1962; Tarr, 1962; Katsanos, 1967; Kangaswami, 1972; Rao, et al, 1977; Williams, et al, 1978). The characteristic symptoms of anthracnose on leaves of susceptible varieties appear as small sunken, circular to elliptical necrotic spots (3-6mm in diameter) on the lamina of lower leaves and then spread to the upper leaves. The colour of the spots depends on the crop variety being affected. It may be tan, orange, red or blackish purple. In older lesions acervuli are formed and appear as black dots in the centre. Under high humidity the spots increase in number and coalesce to cover much of the leaf area giving a patchy type appearance. The lesions on the midrib of the leaf commonly appear as elliptical to elongate discoloured spots which frequently coalesce to cover the entire length of the midrib. The midrib lesion is more common and severe on older plants (Bruehl and Dickson 1950). Dale (1963) in the United States of America reported that, anthracnose in maize leaves appear first on young plants and the lesions are of variable sizes. They appear linear in shape and taper towards both ends. The lesions have water-soaked appearance.

2.3 Nomenclature

The fungus causing anthracnose on cereals and grasses has been widely referred to as Colletotrichum graminicola (Ces.) Wils. This was as a result of the lumping together of several pre-exisisting species of Colletotrichum on cereals and grasses. Colletotrichum falcatum the causal agent of red-rot of sugarcane, was excluded from this lumping.

A lot of confusion and doubt exist on the correct identity and classification of the anthracnose fungus. According to Von Arx and Muller (1954), C. graminicola and C. falcatum are one species and both are the conidial stage of the ascomycete, Glomerella tucumanensis (Speg) Arx and Muller. Sutton (1968) refuted this combination by Von Arx and Muller after observing some differences in the host range, size and shape of appressoria of C. graminicola on sorghum and maize and C. falcatum on sugarcane, He concluded that C. graminicola consisted of at least two different taxa. Similarly Huguenin (1982), separated the three falcate-spored Colletotrichum species from sorghum, maize and sugarcane on the basis of their electrophoretic patterns. Reports from several workers (Abbot, 1938; Bruehl and Dickson, 1950; Harris and Johnson, 1967; Foster and Frederiksen, 1979; Pastor-Corrales, 1980), on physiologic specialization and variation in morphological, cultural and pathological characteristics of different isolates of the anthracnose pathogen from sorghum and grasses raised some questions on the correct nomenclature of the organism(s). Rajasab and Ramalingam (1981) reported an anthracnose with symptoms that have zonate appearance on sorghum in India, which they named "zonate anthracnose." The symptoms of the anthracnose are, diffused

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spots with numerous acervuli often in concentric zones, which are larger (up to 30 mm diameter) than the typical restricted eye spots (up to 6 mm in diameter) which have few acervuli and induced by C. graminicola. Although found growing side by side on the same leaf with C. graminicola, the zonate anthracnose differed from C. graminicola induced anthracnose in the number of conidia per acervulus, time of appearance of the disease in the field, cultural characteristics on growth media and symptoms. Based on these differences, they proposed that the pathogen causing the zonate anthracnose be designated as a new variety of C. graminicola and named, C. graminicola var zonatum and the disease caused by it be named zonate anthracnose disease of sorghum. Alawode (1982), in Nigeria, also observed that isolates of the anthracnose pathogen obtained from restricted eye-shaped spots on sorghum leaves have distinct cultural, morphological and pathological characteristics from the isolates obtained from the patchy and midrib lesions. Because of these differences between the isolates, Alawode considered the isolates from eye-shaped lesions as a distinct new variety of the pathogen, C. graminicola. He went further to give the name for the type of anthracnose it causes as 'pinpoint' anthracnose. This apparently may be the same anthracnose reffered to elsewhere as eye-shaped anthracnose. On the other hand, isolates from the patchy and midrib lesions that he tested had similar cultural and morphological characteristics but differed only in their pathogenicity, so they were regarded as two different races belonging to the same variety and species of the anthracnose pathogen.

2.4 Morphological Characteristics

The mycelium of C. graminicola consists of slender, branched, colourless and septate hyphae which are both inter and intra-cellular. The hyphae permeate the parenchymatous tissue and sometimes the vascular bundles (Choudhury 1936). The acervuli are small, circular to oval in shape and measure approximately 70 - 300 um in diameter; and usually develop late in the season. Often cells of the acervuli develop as setae, which are brown slightly swollen at the base and tapered at the apex. The setae are unbranched but septate and contribute to the black colour of the acervuli (Tarr. 1962).

The conidiophores are short and colourles and bear conidia at their tips. They are superficial and are mixed with the setae in the acervuli. They are broader at the middle than at the ends and measure approximately 8 - 20 x 4 - 8 u. The conidia are falcate or spindle-shaped with slight curving. They are single celled and hyaline in colour. They are aseptate, terminal and borne singly on the conidiophores. They may contain one to several oil drops. Under abundant moisture, the conidia appear in liquid mass of gelatinous matrix which is orange, salmon to pinkish in colour (Edgerton, 1910). The conidia measure approximately 19.0-29.9 (23.8) u x 3.3-4.8(4.2) u. The conidial dimensions as reported by several workers from different geographical locations and from different host plants vary considerably. In culture the conidia are frequently broader and shorter than on the host and many irregular forms occur. They are typically falcate with pointed ends showing considerable degree of curvature, pointedness and size (Tarr, 1962). For

example, Choudhury (1936) in India, reported the size on maize and sorghum as 4-5 x 18-31 u and 4-5 x 19-30 u respectively, while Rangaswami (1972), also from India reported the size on sorghum as being 3-7 x 21-32 u. Dale (1963) in the United States of America gave the measurements of 4.9 - 5.9 x 21.6 - 39.2 u for isolates from maize. On sorghum from the United States of America, the size was reported as 3-7 x 16-34 u (Katsanos, 1967, The conidial dimensions also vary depending on the origin of isolate on the host plant. For example, Alawode (1982), in Nigeria, gave the dimension of the conidia from an isolate obtained from the patchy symptoms on sorghum leaves as 8.65 x 25.0 u while those obtained from an isolate from the midrib lesions measures 8.0 x 22.0 u and the ones from the restricted spots measured 2.5 x 15.0 u. One wonders whether these different dimensions given by different researchers are due to human errors or are genuine.

2.5 Cultural Characteristics

2.5.1 Growth on Media

Colletotrichum graminicola (Ces.) Wils. grows on a large number of media but grows better on natural than on synthetic media (Choudhury, 1936). A wide variation in cultural characteristics has been observed in features such as the topography, colony formation, colour of aerial growth, substratum colouration, manner of sporulation and/or type of acervuli formation (Choudhury, 1936; LeBeau et al, 1951; Ozolua, 1981).

According to LeBeau et al (1951), the mycelial growth ranged from thin felty to a thick loose cottony type and occasionally aerial growth is very sparse. The colour of the colony also ranges from near white - grey - greenish black to almost black with occasional pink and orange colonies. Submerged mycelium is frequently greenish black but is sometimes white to smoky in colour. Ozolua (1981) in Nigeria studied 15 isolates of the anthracnose fungus from sorghum specimens collected from different ecological locations. He observed that different cultural types of the pathogen exist and the extent of the variation in cultural characteristics depends on the kind of growth medium that is used because a particular cultural characteristic of an isolate observed on a particular growth medium may not be observed on another growth medium. On potato Dextrose Agar with Streptomycin (PDAS) the colony colouration varies greatly. In one of the isolates the colonies were observed to be glaucous grey to pale olivaceous grey in colour with pale luteous substratum. Three of the isolates showed olivaceous grey colonies with substratum colour varying from ochreous to safron orange. Dale (1963) reported growth of isolates from maize in Arkansas, United States. On Potato Dextrose Agar (PDA) the growth of the mycelium was abundant and felt-like with a rose tint in the centre at temperatures between 28° and 32°C and was light grey to white at lower temperatures. On Corn Meal Agar the mycelium was cottony, sparce and various shades of light grey at temperatures at which growth occurred.

The optimum temperature for growth of the fungus in culture is 28°C (Bruehl and Dickson, 1950; Dale, 1963). Also isolates from sorghum, a tropical crop, develop faster at higher temperatures than isolates from cool temperature cereal crops and grasses such

as wheat (Bruehl and Dickson, 1959). Sporulation in culture also is considerably variable. The sporulation was found to be favoured by high humidity and by alteration of light and dark periods rather than by continuous light and continuous dark periods. Ghouse (1975), in India observed that the pathogen grew at temperatures 10 - 35°C but sporulation occurred only at 28 - 35°C on a medium containing KNO_3 as the nitrogen source. The mycelium grew profusely at temperatures 25 - 30°C with maximum sporulation at 30°C. The cultures could be maintained at 10°C. Some isolates of the pathogen produced abundant conidia in pinkish masses or scattered throughout the mycelium while in others the conidia were almost absent. Dale (1963), in United States, observed that the temperatures, 24° through 36°C, favoured sporulation of maize isolates on both PDA and Corn Meal Agar after 48 hours incubation in the dark.

2.5.2 Spore germination and appressoria formation

Spore germination of C. graminicola usually takes place by the protrusion of one or two germ-tubes arising most commonly from the sides and either ends of the conidium (Tarr, 1962). Frequently the conidium becomes bicellular by formation of a transverse septum before germination. The spore germination is favoured by high humidity and presence of sugar or living plant tissue and the optimum temperature for the process has been reported as 25° to 30°C (Choudhury, 1936). In nutritious media the germ-tubes grew into profusely branched mycelia, but in poor media, the pathogen forms many dark brown, mostly spherical or pyriform appressoria 8 - 15u or more in diameter (Choudhury, 1936).

Mishram and Siradhana (1979) reported a reduction in germination of spores inoculated on resistant sorghum varieties; this was attributed to the presence of a leaf diffusate. The morphology of appressoria from germinating conidia have been used to separate Colletotrichum spp from each other. For example, Sutton (1968), was able to separate similar falcate spored Colletotrichum isolates from sorghum, maize and sugarcane on the basis of quantitative and qualitative differences found on their vegetative mycelia.

2:5:3 Penetration and Infection:-

Germination of the conidia under natural condition takes about 24 hours, giving out 1 or 2 germtubes which form appressoria on coming in to contact with the epidermis of the host. Later the appressoria send out germtubes which penetrate directly through the cuticle into the epidermal cells. Entry into the leaf through the stomata has not been observed (Choudhury,1936). The appressoria can remain viable for several weeks (Zwillenberg,1959).

An ultrastructural study of penetration of maize leaves by C. graminicola was conducted by Politis and Wheeler (1973). They observed that after inoculation, the uninucleate spore swells and undergoes mitosis. In each spore a septum forms between the daughter nuclei, and one cell of the resulting 2 celled structures germinates to forms an appressorium with an electron dense outer cell wall. Before penetration, a new inner electronlucent wall forms around the basal part of the appressorium. Six to nine hours after inoculation, the dense wall of the appressorium disappears (without evidence of mechanical disruption) At all stages of infection, the infection peg of the hypha developing from it is

enveloped by a distinctive, electronluscent cell wall which is continuous with the inner wall of the appressorium. The cuticular layers of the host cell wall are depressed inwards and papillae are formed. The plasma membrane of the infected cells and those adjacent to it are disrupted before cell walls are completely penetrated. The whole process of infection is completed in 12 hours by the formation of a septum between the appressorium and the swollen infectious hypha.

Politis (1976) reported that in resistant oat leaves the conidial germination differs. It occurred 10 - 14 hours after inoculation and the appressorium is formed after 14 - 18 hours. Penetration pegs and papillae are present 24 hours after inoculation. In 48 hours the cuticle and epidermal walls are breached but not the underlying papillae. After 72 hours the protoplast of invaded cells appear to be disorganized and the vacuolated electrondense hyphae of the fungus get restricted to the first 2 - 3 adjacent cells.

2.5.4 Pathogenic Variations

The anthracnose fungus is variable in its pathogenicity. Isolates of the pathogen from one host species do not necessarily infect other host species and the capacity of an isolate of the pathogen to attack several species of host plants is not always there (Pasto-Corrales and Frederiksen 1980). C. cereale and C. lineola were among the earlier existing species combined by Wilson (1914) to form the single species Colletotrichum graminicola. Boning and Walner (1936), excluded C. falcatum because of its falcate-shaped conidia and the occassional production of two conidia in

chain-like form. Edgerton (1911) noted that C. cereale, C. lineola and C. falcatum were morphologically and culturally alike as if they are merely different pathogenic forms of one species. Sometimes both C. lineola and C. cereale could be found in the vascular tissues of inoculated host plants but would not produce the typical red rot symptoms of C. falcatum in sugarcane. Similarly Butler and Hafiz Khan (1913), reported that despite the close morphological similarity between C. falcatum and C. lineola, the latter did not infect sugarcane and C. falcatum was only restricted to sugarcane. Despite the considerable morphological similarities that exist between C. graminicola and C. falcatum, the fact that C. graminicola does not infect sugarcane has continued to be used as evidence that the sugarcane and cereal-infecting forms are distinct. LeBeau (1959), reported that isolates from sorghum were not pathogenic to sugarcane but for most part were highly pathogenic to sorghum and other related grasses. Similarly Berquist (1973), in Hawaii, reported that C. graminicola from sorghum was weakly pathogenic to sugarcane but C. graminicola from sugarcane was not at all pathogenic to sorghum. Different pathotypes of C. graminicola exist in different parts of the world. Warren (1983), in the United States obtained nine sorghum isolates of C. graminicola from different regions of the United State and tested them for pathogenicity on six sorghum lines. On the basis of differential responses exhibited by the six sorghum lines to the C. graminicola isolates, the isolates were separated into five physiological races. Data obtained from International Sorghum Anthracnose Virulence studies showed that different population of C. graminicola exist in Nigeria, Brazil and the United States of

America (King and Frederiksen, 1976). In Nigeria between 1979 and 1981, pathogenicity tests with fifteen isolates of C. graminicola were conducted. The collection of the isolates tested was not based on the kind of symptoms on the infected sorghum plants but on the basis of different ecological locations of Nigeria. Based on the differential reaction on test host plants to these isolates, ten (10) pathotypes were separated with none of them being able to infect maize and wheat (Ozolua, 1981; Ozolua et al, 1987). However, Choudhury (1936), in India, rereported that an isolate of C. graminicola from maize readily infected sorghum and maize. Slight infection was also obtained using the maize isolate on millet, barley, wheat and oats. Messian et al (1959), in France, reported that a maize isolate did not infect sweet sorghum, broomcorn, sugarcane and rye; it only infected maize.

Similarly Dale (1963) in the United States, failed to obtain infection on sorghum with a maize isolate collected from Arkansas and an isolate from sorghum did not attack maize. Also Hooker (1974) in Illiois (U.S.A.) reported that maize isolates were only pathogenic to maize and not to sorghum. Zwillenber (1959) could only obtain infection of maize using isolates from maize; no microscopic lesions developed on leaves of wheat, barley, oats and other grasses. Nicholson (1975), in Indiana (U.S.A.), failed to obtain infection of wildcane, Johnson grass, sorghum, tall panicum, and six different foxtails using maize isolates. Wheeler et al (1974) studied 30 isolates of C. graminicola collected from six different states on unspecified crops in United States of America. They readily obtained infection on maize and the three species of sorghum tested (S. bicolar, S. budanense and S. halepense). Isolates from nine other

grass genera did not infect maize. Also oats, barley, wheat, Festuca and Pearl millet were not infected by maize isolates. Also Williams and Willis (1963), in Ohio (U.S.A.), did not obtain infection on other cereals using isolates associated with maize anthracnose in that region. Field observation by Tarr (1962), in the Sudan showed that anthracnose was not found on maize or other cultivated cereals, even though the disease was commonly found on sorghum species.

CHAPTER 3

MATERIALS AND METHODS

3:2 Collection of the Pathogen Isolates

Specimens of sorghum and maize showing the various forms of symptoms of anthracnose referred to earlier and millet leaves showing the midribs lesions almost similar to that of sorghum and maize anthracnose were collected from farms in areas shown in Table 1, in October,1984 and between June and November,1985. These leaf specimen were placed in paper bags and brought to the laboratory where they were air dried on the laboratory bench. Some of the dried leaves were later transferred to a plant press and pressed for proper preservations for future use. Only one type of anthracnose symptom (Patchy type) was found on maize while all the three types (Patchy eye-shaped and midribs lesions) were found on sorghum.

Table 1. Sources of leaf specimens from which isolates of anthracnose and millet midrib lesion pathogens were obtained

Isolate designation	Hostplant	Type of Symptom on host plant	Locality
S-M-Z	Sorghum	midribs lesion	Zaria
S-M-BK	"	"	Birnin Kebbi
S-M-KT	"	"	Katsina
S-M-KB	"	"	Kabba
S-LP-Z	"	Pathcy spots on Lamina	Zaria
S-LP-KB	"	"	Kabba
S-LI-Z	"	Isolated small oval spots on Lamina	Zaria
S-LI-B	"	"	Bauchi
Z-LP-Z	Maize	Patchy spots on Lamina	Zaria
P-M-K	Pearl millet	midribs lesion	Katsina
P-M-Z	"	"	Zaria
P-M-BK	"	"	Birnin Kebbi

3.2.0 Isolation of the anthracnose pathogen from infected leaf specimens in the laboratory

3.2.1. Using humid chambers

The sorghum and maize leaf specimens with the various symptoms of anthracnose were thoroughly washed under tap water. Small pieces from leaves with individual type of symptoms (0.5 - 1.0 cm length) of the washed leaf specimens were cut in such a way as to include little of the apparently healthy and the infected tissues. The cut pieces were surface sterilized by shaking for 5 minutes in 0.5% solution of sodium hypochlorite. They were rinsed 3 times in sterile distilled water, dried with filter papers and then placed in separate Petri dishes containing moist filter papers. The dishes were labelled to identify specimens from the different symptom types and kept on top of the laboratory benches under room temperature (27±2°C) for 2 - 3 days to induce sporulation. The conidia and setae obtained from the specimens were observed under the microscope for determination of morphological characteristics.

3.2.2. Using the growth media, Potato Sucrose Agar with Streptomycin (PSAS)

Infected sorghum, maize and millet leaf specimens were cut into small pieces, surface sterilized and rinsed in same manner as described above. The rinsed pieces were plated in Petri dishes containing PSAS. A total of 200 Petri dishes (9 cm diameter) were used with each dish containing 3 - 4 pieces of infected leaf. The Petri dishes were labelled to identify the different forms, and incubated upright at room temperature 27 ± 2°C) for 5 - 7 days.

Observation was daily carried out for growth of the pathogen from the infected tissues. As soon as growth was evident, advancing hyphae from such colonies were picked using sterilized dissecting needle and transferred to fresh plates containing PSAS and incubated at 27 ± 2°C to ensure that only pure cultures of the isolates were retained. As soon as the isolates started sporulating, small samples were taken and observed under the compound microscope for confirmation of the fungal identity. To further improve purity of isolates to be used, single spores from each isolate in a water suspension on a glass slide, were picked with the aid of sharp sterilized dissecting needle and dissecting microscope (X40 magnification), and individually placed on fresh PSAS in petri dishes.

Some of the pure cultures of the different isolates obtained from the single spores of the pathogen were maintained in slanted PSAS in test tubes covered with cotton wool and placed in refrigerator and deep freezer for preservation. The rest were maincained on Petri dishes on the laboratory benches. Whenever necessary subculturing was done to ensure viability up to the time each isolate was needed either for morphological and cultural studies or pathogenicity tests on differential test plants.

3.3. Observations on the morphological features of the isolates of the Pathogen.

3.3.1. Isolates obtained directly from the host that had been placed in humid chambers.

After 2 - 3 days of placement of fresh infected leaf specimens from the different locality and symptom types in humid chambers (as earlier described), the acervuli formed from the infected parts

were gently scraped using a sharp blade. The scrapings were placed in lactophenol with cotton blue on glass slides. A coverslip was placed on each stained specimen. Each stained specimen of the fungus isolate was observed under the compound microscope at x40 magnification. With the aid of ocular micrometer in a compound microscope the length and width of fifty (50) randomly selected conidia and setae from each of the isolates were measured and recorded. The shape of the conidia from each isolate and the number, if present, of oil droplets in each conidium was recorded. The number of septation per seta was also recorded. The photograph of the setae and conidia of the isolates were taken with the aid of a camera fixed to a compound microscope.

3.3.2. Isolates obtained using growth media (PSAS)

Morphological features of isolates of the pathogen isolated and maintained on growth media (PSAS) in the manner described earlier were observed and recorded using same methods and facilities described in 3.3.1. above. Cultures of 21-dayold were used in the study of the morphological features. This was to ensure that acervuli were present.

3.4. Cultural Studies on Potato Sucrose Agar with Streptomycin

Each of the different isolates earlier referred to were separately grown on PSAS for 7 days at the end of which, 7mm diameter mycelial discs were cut from each plate using a sterilized cork borer. Each disc was placed centrally in a 9-cm diameter Petri dish containing PSAS. For replication purpose, 10 Petri dishes were used for each isolate. The plates were labelled and randomly placed on racks in an icubator at 28 - 29°C and allowed to grow for three weeks.

Daily radial growth was measured using a ruler and also the following cultural features were observed and recorded:-

(i) Colour of the aerial colony and the colour of the substratum. The Commonwealth Mycological Institute (CMI) colour chart by Rayner (1970), was used to described the colours of each colony.

(ii) Shape and growth characteristics of the colony.

(iii) Manner of acervuli formation.

3.5. Pathogenicity tests under glasshouse condition

Eight different varieties of test cereal crops were used for the pathogenicity test of the various isolates of the pathogen. This number consisted of 6 sorghum varieties namely; KSV_4, KSV_{11}, L1499, SK 5912, S18 and SPV 221 which were selected on the basis of their predetermined varying reactions to anthracnose infection under natural field conditions. A maize variety (TZB) which is susceptible to anthracnose and one millet variety that had been earlier observed showing severe midrib lesions almost similar to that of sorghum anthracnose were among the eight differentials used. The millet variety was obtained from a local farmer in Katsina.

Surface sterilized seeds of the differential test plants were sown in clay and plastic pots (35 - 40 cm diameter) containing field soil mixed with one table-spoonful of 15:15:15 compound (NPK) fertilizer. The pots were labelled, kept in the glasshouse and regularly watered. Two weeks after planting the seedlings were reduced to 3 plants per pot and one table spoonful of compound (NPK) fertilizer was added. Regular watering of the plants continued and the plants were allowed to grow until 6 weeks old when they were inoculated as shown below with the different isolates of the pathogen.

3.5.1. Inoculum preparation and Inoculation of Test Plants

The different isolates of the pathogen were grown in the laboratory for 3 weeks in 9-cm diameter Petri dishes containing either PSAS or Oat Meal Agar, which induced greater sporulation. At the end of the 3 weeks when spores had been abundantly produced, 20 ml of sterile distilled water were added into each Petri dish and a suspension of the mycelium and conidia, was made from each Petri dish by carefully scraping with a sterilized glass slide. The suspension was filtered through a double layer of cheese cloth into labelled 500 ml conical flasks. With the aid of a haemocytometer, the concentration of the conidia was determined and adjusted, where necessary to about 5×10^5 conidia/ml. The conidial suspension, so formed, was transferred into labelled plastic hand-held sprayers.

To prevent mixed infection as a result of contamination during inoculation, the plants were brought out of the glasshouse before spraying them. A set each of the 8 test varieties (3-plants per pot) were inoculated with each of the different test isolates of the pathogen by spraying the conidial suspension on the lamina, midrib and the leaf whorls. A set sprayed with sterile distilled water served as control plants. Using stakers as support, both the inoculated and the control plants were immediately covered after inoculation with polyethylene bags which were sprayed from inside with sterile distilled water to create a humid condition necessary for infection to take place. The pots in each set of treatment were labelled and randomly arranged in the glass houses. After every 24 hours the polyethylene bags were carefully lifted for a short while to aerate the plants after which the bags were replaced and sprayed from inside with fine droplets of water to

increase humidity. Three days after inoculation the polyethylene bags were removed and no longer replaced. The plants were periodically sprayed with sterile distilled water until symptoms started showing. Water from the tap was routinely poured on the cement floor of the glass house to help create a warm, humid environment which is needed for the development of anthracnose.

3.5.2. Disease Recording

From the third day after inoculation, the plants were daily observed for appearance of disease symptoms. Symptoms were recorded on the basis of their type and location on the leaves as shown in Table 2. These served as the criteria for disease recording and classification. The pathogenicity test described above was repeated in order to confirm the results of the first test. The two pathogenicity tests were conducted in the glass house only and not in the field, in order to avoid or minimise natural infection from air-borne inoculum. The first test took place in March which is a hot dry month and the second test took place in July which is a warm humid month. This helped to reveal whether dry or wet weather has any determination on the symptom of anthracnose observed on infected plants.

3.6. Data Analysis

The data were analysed using a computer to determine whether or not statistical differences in morphological features exist between the different test isolates of the anthracnose fungus.

Table 2. Types of anthracnose symptoms and their location on the leaf of sorghum and maize as observed in the field and used as criteria to evaluate test isolates.

Location of Symptoms on the leaf	Type(s) of Symptom
Midrib only	Patchy only
Midrib only	Isolated (eye-shaped) only
Midrib only	Patchy and isolated (eye-shaped)
Lamina only	Patchy only
Lamina only	Isolated (eye-shaped) only
Lamina only	Patchy and isolated (eye-shaped)
Both lamina and midrib	Patchy only
Both lamina and midrib	Isolated (eye-shaped) only
Both lamina and midrib	Patchy and isolated (eye-shaped)

CHAPTER 4

RESULTS

4.1 Isolation of the anthracnose pathogen and studies under the compound microscope.

Acervuli, setae and conidia were readily produced and observed under the compound microscope on sorghum and maize leaf specimens especially when leaf specimens were placed in humid chambers. All methods adopted to isolate Colletotrichum from the millet midrib lesions consistently failed. Because of the difficulties encountered in isolating C. graminicola using growth media PSAS, method was adopted, whereby needles were used to transfer observed growth and spores from specimens placed in humid chamber. However at no time and with no method were acervuli, setae or conidia of Colletotrichum observed on the millet midrib lesions.

4.1.1 Length of conidia

The measurements of fifty (50) randomly selected conidia produced on the host tissue after placing on moist chamber and those produced on the growth media PSAS, are presented in table 3. Variations in lengths among conidia were observed. Even though the lengths of conidia of the different isolates varied from one another on a given substrate, the differences were not statistically significant (see appendices 5 and 9). The length of conidia from host plant tissues ranged from 7.7μ to 30.0μ.

In growth media PSAS, the conidia ranged from 7.8μ to 32.4μ (see table 3).

4.1.2 Width of conidia

The measurements of width of conidia of the different isolates obtained directly from host tissues and from growth media PSAS, are presented in table 6. Data analysis of the means presented in appendices 2 and 6, showed no significant diffirences among the means of width of conidia from the two sources (host tissues and growth media). However the substrate from which the conidia were obtained significantly influenced the width of the conidia. For example the conidia produced on the host were significantly wider than those produced on growth media PSAS, irrespective of isolate type.

4.1.3 Length of setae

Eventhough the lengths of setae of the different isolates varied from one another on a given substrate the differences were not statistically significant (see appendices 7 and 11). As was the case with the width of the conidia, the substrate from which the setae were obtained significantly influenced the length of the setae. The setae produced on the host plant were significantly longer than those produced on growth media irrespective of the isolate. For example while on the host the means of lengths of setae of different isolates ranged from 50.75μ to 68.30μ the range was 54.47μ to 59.57μ on growth media (see table 5).

Table 3. Length (u) of conidia of 10 isolates of Colletotrichum graminicola obtained from the host plant leaf and from potato Sucrose Agar with Streptomycin growth media.

Isolate designation	Length of conidia obtained from			
	Host plant leaf		Culture media	
	Range	Mean*	Range	Mean*
S-M-Z	12.1 - 21.5	17.72[a]	7.8 - 18.5	17.53[a]
S-M-KT	14.5-26.0	17.50[a]	11.0 - 22.0	16.35[a]
S-M-BK	13.2 - 20.2	17.33[a]	16.6 - 32.4	17.55[a]
S-M-KB	8.8 - 17.6	16.97[a]	14.3 - 22.1	16.46[a]
S-LP-Z	15.3 - 30.0	17.64[a]	15.4 - 22.0	17.07[a]
S-LI-B	12.1 - 20.9	17.84[a]	9.9 - 16.5	17.26[a]
S-LP-Z	14.3 - 22.0	16.97[a]	12.1 - 22.0	16.55[a]
S-LP-B	7.7 - 19.5	17.54[a]	9.8 - 21.8	16.89[a]
S-LP-KB	12.1 - 22.1	17.12[a]	11.0 - 18.7	16.74[a]
Z-LP-Z	12.2 - 21.3	16.80[a]	13.2 - 22.0	17.55[a]

* Mean was calculated from measurements of fifty (50) randomly selected conidia.

Means in the same column bearing a common letter are not significantly different at 5% level by Duncan's New Multiple Range Test.

Table 4. Width (u) of conidia of 10 isolates of Colletotrichum graminicola obtained from the host plant leaf and from potato Sucrose Agar Streptomycin culture media (PSAS)

Isolate designation	Width of conidia obtained from			
	Host plant leaf		Culture media	
	Range	Mean*	Range	Mean*
S-M-Z	2.2 - 3.9	3.160[a]	2.2 - 5.0	2.894[a]
S-M-KT	2.2 - 4.0	3.145[a]	2.2 - 3.3	2.952[a]
S-M-BK	2.0 - 3.0	3.066[a]	1.7 - 3.3	2.998[a]
S-M-KB	2.2 - 5.5	2.086[a]	2.2 - 5.0	3.078[a]
S-LI-Z	2.0 - 3.5	3.177[a]	2.2 - 3.6	2.938[a]
S-LI-B	2.2 - 3.3	3.193[a]	1.7 - 3.9	2.924[a]
S-LP-Z	2.2 - 4.4	3.150[a]	2.2 - 3.3	2.890[a]
S-LP-B	2.2 - 3.3	3.110[a]	2.2 - 4.2	2.994[a]
S-LP-KB	2.75 - 4.4	3.251[a]	2.2 - 3.9	2.902[a]
Z-LP-Z	2.4 - 3.3	3.142[a]	2.8 - 4.4	2.944[a]

* Mean was calculated from measurements of fifty (50) randomly selected conidia.

Means in the same column bearing a common letter are not significantly different at 5% level by Duncan's New Multiple Range Test.

Table 5. Length (u) of setae of 10 isolates of Colletotrichum graminicola obtained from the host plant leaf and from Potato Sucrose Agar Streptomycin culture media.

Isolate designation	Length (u) obtained from			
	Host plant leaf		Culture media	
	Range	Mean*	Range	Mean*
S-M-Z	12.56 - 119.9	65.83[a]	22.0 - 56.0	54.69[a]
S-M-KT	50.5 - 120.1	58.75[a]	27.0 - 104.5	54.07[a]
S-M-BK	30.1 - 66.0	63.96[a]	31.9 - 66.0	55.40[a]
S-M-KB	35.3 - 90.2	61.80[a]	23.0 - 56.1	54.47[a]
S-LI-Z	50.0 - 130.0	65.54[a]	22.0 - 165.0	59.57[a]
S-LI-B	35.2 - 112.2	65.76[a]	22.0 - 81.0	56.27[a]
S-LP-Z	0.7 - 104.5	63.20[a]	44.0 - 115.5	57.74[a]
S-LP-B	35.2 - 115.5	66.80[a]	40.3 - 100.2	58.44[a]
S-LP-KB	40.5 - 90.3	62.41[a]	22.1 - 101.2	57.87[a]
Z-LP-Z	30.6 - 95.3	68.30[a]	33.5 - 115.5	58.59[a]

* The average of fifty (50) randomly selected setae formed the mean.

Means in the same column bearing a common letter are not significantly different at 5% level by Duncan's New Multiple Range Test.

4.1.4 Width of setae

As seen in appendices 8 and 12, the width of setae produced on the host was greater than those produced on artificial media irrespective of source of isolate. However no statistical differences were found between the widths of the setae of the different isolates irrespective of the substrate from which the setae were produced.

4.1.5 Shape of conidia and presence of oil globules

The conidia produced by all of the isolates were hyaline, unicellular, vacuolated and curved. Slight variations existed in the curvature and apices of some of the samples. In some, the conidia were straight with blunt ends while in others they were strongly falcate and fusoid with long acute apices (see plate IV). Conidia of the isolate (Z-LP-Z) from maize were longer than those of all the isolates from sorghum. The number of oil globules varied considerably among conidia. As presented in table 7, some of the conidia lack globules while others had 1 - 7 globules. The conidia produced in growth media PSAS, contained more globules than those produced on host plant leaf.

4.1.6 Shape of setae and number of septa per seta

The setae were generally thicked walled and dark coloured to brown. They were slightly swollen at the base (see plate V). They were pointed at the apex and contain two to several septa depending upon the age. The number of septa per seta ranged from 2 to 8. Generally setae of isolates raised on growth media PSAS have more septa than setae from isolates directly from the host plant.

Table 6. Width (u) of setae of 10 isolates of Colletotrichum graminicola obtained from the host plant leaf in potato Sucrose Agar Streptomycin culture media.

Isolate designation	Width (u) of setae from			
	Host plant leaf		Culture media	
	Range	Mean*	Range	Mean*
S-M-Z	2.3 - 8.5	4.108[a]	3.3 - 5.5	3.820[a]
S-M-KT	3.1 - 6.0	4.084[a]	2.2 - 5.5	3.845[a]
S-M-BK	2.0 - 4.1	4.068[a]	2.2 - 4.4	3.968[a]
S-M-KB	3.0 - 5.1	3.914[a]	3.3 - 4.4	3.840[a]
S-LI-Z	3.3 - 5.0	4.086[a]	3.1 - 4.4	4.030[a]
S-LP-B	3.3 - 5.5	4.102[a]	1.7 - 4.4	3.876[a]
S-LP-Z	3.1 - 5.5	4.188[a]	2.2 - 4.4	3.972[a]
S-LP-B	2.2 - 5.4	4.252[a]	2.2 - 5.3	4.066[a]
S-LP-KB	3.1 - 5.5	4.116[a]	3.1 - 6.6	3.932[a]
Z-LP-Z	3.0 - 5.0	4.036[a]	3.0 - 5.5	3.772[a]

* Mean was calculated from measurements of fifty (50) randomly selected setae.

Means in the same column bearing a common letter are not significantly different at 5% level by Duncan's New Multiple Range Test.

Table 7. Number of oil globules in conidia of 10 isolates of Colletotrichum graminicola obtained from host plant leaf and from Potato Sucrose Agar Streptomycin culture media.

Isolate designation	Number of oil globules per conidium obtained from			
	Host plant leaf		Culture media	
	Range	Mean*	Range	Mean*
S-M-Z	0 - 6	1.9	2 - 8	2.8
S-M-KT	0 - 6	2.5	2 - 6	3.1
S-M-BK	0 - 5	3.2	2 - 7	3.5
S-M-KB	0 - 6	2.1	0 - 4	2.7
S-LI-Z	0 - 6	2.1	0 - 6	3.1
S-LI-B	0 - 5	1.5	1 - 5	2.0
S-LP-Z	2 - 5	2.3	2 - 4	3.2
S-LP-B	0 - 5	2.8	0 - 7	3.1
S-LP-KB	0 - 4	2.0	0 - 5	2.9
Z-LP-Z	0 - 3	2.3	2 - 6	4.2

* Mean was calculated from observations of fifty (50) randomly selected conidia.

Table 8. Number of septa in seta of 10 isolates of Colletotrichum graminicola obtained from host plant leaf and from Potato Dextrose Agar Streptomycin culture media.

Isolate designation	Number of septa per seta obtained from			
	Host plant leaf		Culture media (PDAS)	
	Range	Mean*	Range	Mean*
S-M-Z	2 - 8	3.5	2 - 7	3.8
S-M-KT	2 - 4	4.5	3 - 5	4.6
S-M-BK	2 - 5	3.2	2 - 8	3.7
S-M-KB	2 - 5	4.5	2 - 6	4.9
S-LI-Z	2 - 6	3.9	3 - 7	4.0
S-LI-B	2 - 6	3.8	2 - 7	3.7
S-LP-Z	2 - 6	2.7	3 - 5	2.9
S-LP-B	2 - 6	2.5	2 - 8	2.8
S-LP-KB	2 - 6	3.2	2 - 5	3.5
Z-LP-Z	2 - 5	4.1	2 - 7	4.2

* Mean was calculated from observations of fifty (50) randomly selected conidia.

4.2 Cultural Characteristics

Using PSAS the cultural characteristics of the test isolates of C. graminicola from sorghum and maize were studied. The results of the observations on the cultural characteristics of the isolates from the three main sources viz; midrib, isolated-type and patchy-type lesions are separately presented under the sub headings below.

4.2.1 Cultural Characteristics of Isolates from the midrib lesions on sorghum leaves.

The results of observations on the cultural characteristics on PSAS of isolates obtained from sorghum midribs are presented in Table 9. Five different cultural types were identified (see plate VI). The topography of the aerial colony in types 1, 4 and 5 was cottony. The colony was velvety in type 2 and felty in type 3. Except in type 5 where the mycelium was scanty and slow growing, the rest of the cultural types had abundant aerial mycelium and were fast growing (see fig. 1). The colour of the aerial mycelium was of various shades of Grey through Fuscous Black to Hazel. The colour of the sub-stratum was Buff through Fulvous to ochreous. The margins of the colony were smooth and round in types 1, 2 and 3; whereas in types 4 and 5 the margins were smooth and lobate. Abundant acervuli and spores were formed in concentric rings in types 3 and 5. Few acervuli and not in concentric rings were observed in type 4.

Table 9. Cultural characteristics* of isolates of *C. graminicola* causing midrib lesions (anthracnose) on sorghum leaves in Zaria, Katsina, Birnin Kebbi and Kabba on Potato Sucrose Agar with Streptomycin

Isolates designation	Topography of aerial colony	Colony colour and Zonation	Sub-stratum colour and Zonation	Margin	Avervuli formation and sporulation
1	Cottony and dense; flat and fast growing	Iron grey-Dark Mouse grey; zoned	Buff; not zoned	Smooth, Round	Acervuli not observed; very good sporulation
2	Velvet and smooth flat, fast growing	Fuscous Black-Greyish sepia, zoned	Vinaceous Buff-Hazel; zoned	Smooth; Round	Acervuli not observed; scanty sporulation
3	Felty and dense; slightly elavated, moderately fast growing	Dark Mouse Grey-Greyish Sepia; zoned	Fulvous-Ochreous Umber; zoned	Wavy	Many acervuli in concentric rings; very good sporulation
4	Cottony and dense, depressed at the centre and slightly raised towards periphery; fast growing	Fawn-Hazel; Net zoned	Dark Mouse Grey-Cinnamon-Honey; Not zoned	Lobate; frilled	Few and scattered acervuli; very good sporulation
5	Cottony and dense depressed at the centre; flat towards periphery; slow growing	Fuscous black-Greyish Sepia; Zoned	Brick-Buff; Zoned	Smooth; Round	Many acervuli in concentric rings; very good sporulation

*Characteristics observed 6 days after plating isolates on the media.

4.2.2 Cultural characteristics of isolates from the eye-shaped (isolated-type) lesions on sorghum leaves.

Four cultural types were identified under this group of isolates. The results of the observations are presented in table 10.

A more uniform texture of aerial mycelium was observed. In cultural types 2, 3 and 4. The topography of the aerial colony was cottony, while in type 1 it was felty. All the four cultural types have abundant fast growing mycelium (see fig. 3).

The aerial colony and the sub-stratum have Grey-Fuscous Black-Rosy Buff colours. Except in type 3, the sub-stratum of types 1, 2 and 4 were zoned. The margins of cultural types 1, 3 and 4 were wavy while that of type 2 was smooth and round (see plate VII).

Acervuli were abundantly produced in all the four cultural types. Except in cultural type 3 which has scattered acervuli, the rest have their acervuli in concentric form. The sporulation among the cultural types was very good.

Table 10. Cultural characteristics* of isolates of C. graminicola causing isolated-type lesions on sorghum leaves in Zaria and Bauchi, on Potato Sucrose Agar with Streptomycin

Isolate designation	Topography of aerial colony	Colony colour and Zonation	Sub-stratum colour and zonation	Margin	Acervuli formation and sporulation
1	Felty and dense; raised at the centre then flat; moderately fast growing	Fuscous Black-Sepia; Zoned	Sepia-Cinnamon; Zoned	Wavy	Many acervuli in concentric rings; very good sporulation
2	Cottony and dense; Elavated then flat; Fast growing	Vinaceous Buff-Greyish Sepia; Zoned	Fuscous Black-Greyish Sepia; Zoned	Smooth; Round	Very many acervuli in concentric rings; very good sporulation
3	Cottony and dense; Elavated; Fast growing	Greyish Sepia; Not zoned	Fuscous Black-Not zoned	Wavy	Many acervuli in scattered form, very good sporulation
4	Cottony and dense; Elavated then flat; slow growing	White-Dark mouse Grey; Zoned	Brown Vinaceous-Rosy Buff; Zoned	Wavy	Many acervuli in concentric rings; very good sporulation

***Characteristics observed 6 days after plating isolate on the media.**

4.2.3

4.2.3 Cultural characteristics of isolates of *Colletotrichum graminicola* from the patchy-type lesions on sorghum leaves

More cultural types were observed from the isolates obtained from the patchy-type lesion as evidenced by the data presented in Table 11. Eight (8) different cultural types were identified.

The topography of aerial colony was cottony in cultural types 1, 5 and 7. A velvet and slow growing aerial mycelium was observed in types 2, 3 and 8. Cultural types 4 and 6 have felty aerial colony. While cultural type 4 was fast growing, type 6 was slow growing.

Various shades of grey colours were observed among the cultural types. The most conspicuous ones are Grey, Rosy, Buff and Vinaceous. The sub-stratum colours were from vinaceous Buff to Brown vinaceous and Hazel.

The colony margins were not the same but varied among the cultural types. The margins were wavy in cultural types 1, 4 and 5 and lobed in 2 and 3. In types 6, 7 and 8 the margins were smooth and round. Except in type 7, acervuli and very good sporulation were observed among all the cultural types.

Table II. Cultural characteristics* of Isolates of Colletotrichum causing patchy type lesions (anthracnose) on sorghum leaves in Zaria, Kabba and Bauchi on Potato sucrose Agar Streptomycin

Isolate designation	Topography of aerial colony	Colony colour and zonation	Sub-stratum colour and zonation	Margin	Acervuli formation and sporulation
1	Cottony and dense; raised at the centre then flat; fast growing	White-Fuscous Black-Greyish Sepia Zoned	Vinaceous Buff-Hazel; zoned	Wavy; frilled	Many acervuli in concentric rings; very good sporulation
2	Velvet and dense; elavated; Moderately fast growing	Rosy Buff-Buff; not zoned	Dark Mouse Grey-Cinnamon; zoned	Lobed	Few acervuli in scattered forms; very good sporulation
3	Velvet and smooth; elavated; slow growing	Greyish sepia-Dark Mouse grey; zoned zoned	Violaceous Black-Dark Mouse Grey;	Lobed	Few acervuli in concentric rings; scanty sporulation
4	Felty and dense; slightly raised at the centre then flat; fast growing	Dark Mouse Grey-Brown Vinaceous, zoned	Brown Vinaceous-Fawn; zoned	Wavy	Few acervuli in scattered form. Very good sporulation
5	Cottony and dense; depressed at the centre then raised; fast growing	Purplish Grey-Vinaceous Buff; not zoned	Dark Mouse Grey-Pale Purplish Grey; zoned	Wavy	Many acervuli in concentric rings; very good sporulation
6	Felty and dense; flat, slow growing	Dark Mouse Grey-Greyish Sepia; zoned	Greyish Sepia; zoned	Smooth; Round	Many acervuli in concentric rings; very good sporulation
7	Cottony and loose; flat; fast growing	Purplish-pale Purplish Grey: zoned	Greyish Sepia; zoned	Smooth; Round	Acervuli not observed; scanty sporulation
8	Velvet and smooth; flat; slow growing	Pale Purplish-Greyish Sepia; zoned	Dark Mouse Grey-Greyish Sepia; zoned	Smooth; Round	Many acervuli in concentric rings; very good sporulation

* Characteristics observed 6 days after plating isolates on media.

4.2.4 Cultural characteristics of isolates of Colletotrichum graminicola from the patchy-type lesions on maize leaves.

Observations on cultural characteristics of isolates of C. graminicola which cause patchy-type lesions of anthracnose on maize leaves are presented in Table 12. Three cultural types were identified.

Considerable variation was observed in the topography of the cultural types. In type 1, the topography was felty to wooly, whereas in types 2 and 3 it was velvety. All the three cultural types were slow in growth (see fig. 2). The colour of the aerial mycelium among the three cultural types was Greyish sepia to Brown vinaceous to Fuscous. Zones were observed in the colonies (see plate VIII). The sub-stratum colour was consistent among the cultural types. Brown vinaceous through Fuscous to Blood colour were observed. The three cultural types have lobed margins.

Abundant acervuli and good sporulation were observed among the cultural types.

Table 12. Cultural characteristics* of isolates of *C. graminicola* causing patchy type lesions on maize leaves in Zaria; on Potato Scurose Agar with Steptomycin

Isolate designation	Topography of aerial colony	Colony colour and Zonation	Sub-stratum colour and Zonation	Margin	Acervuli formation and sporulation
Z-LP-1	Felty and wooly; smooth; elavated; very slow growing	White-Greyish Sepia; zoned	Brown vinaceous-Fuscous Not Zoned	Lobed	Many acervuli in scattered form; very good sporulation
Z-LP-2	Velvet and smooth; loose; scanty aerial mycelium; very slow growing	Brown vinaceous-Fuscous; Zoned	Brown vinaceous-Fuscous; zoned	lobed	Many acervuli in concentric rings; very good sporulation
Z-LP-3	Velvety at centre; Felty towards periphery; Elavated then flat; very slow growing	Fuscous-smoke Grey zoned	Brown vinaceous-Blood colour; not zoned;	lobed	Many acervuli in scattered forms; very good sporulation

*Characteristics observed 6 days after plating isolates on the media.

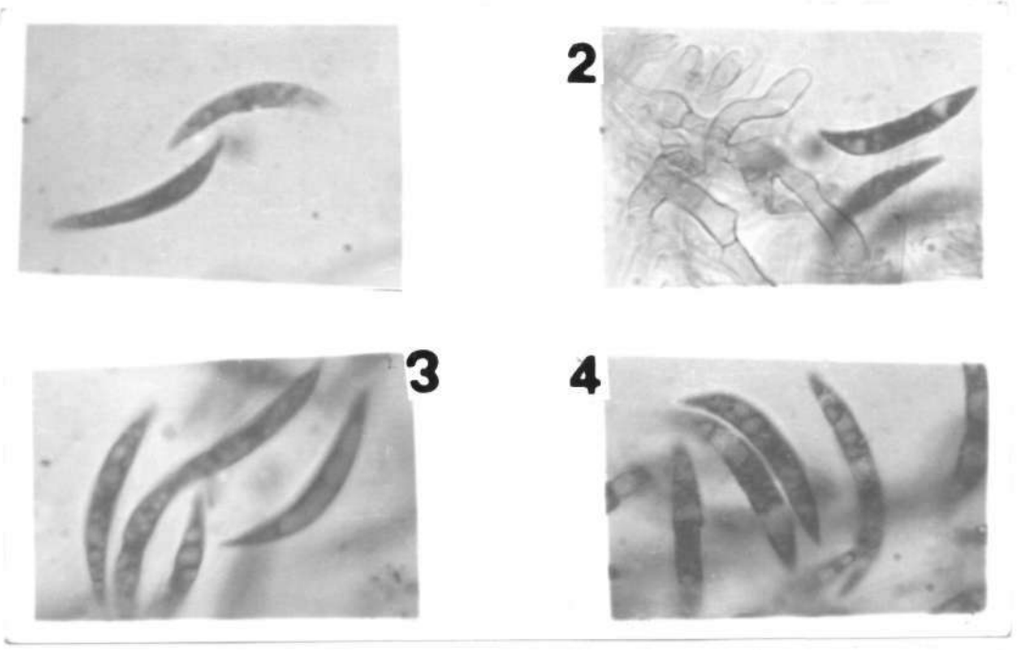


Plate IV Conidia of Colletotrichum graminicola

(1) conidia from the isolated-type symptoms on sorghum leaves

(2) conidia from the patchy-type symptoms on sorghum leaves

(3) conidia from the midrib-type symptoms on sorghum leaves

(4) conidia from the patchy-type symptoms on maize leaves

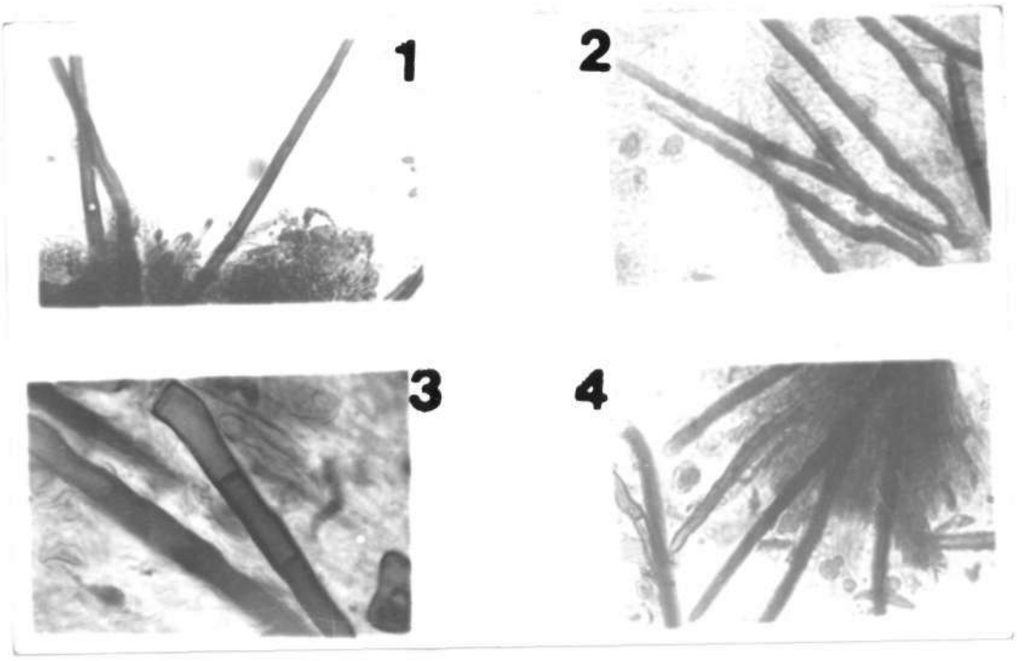


Plate V Setae of Colletotrichum graminicola

(1) Setae from isolated-type symptoms on sorghum leaves

(2) Setae from patchy-type symptoms on sorghum leaves

(3) Setae from midrib-type symptoms on sorghum leaves

(4) Setae from patchy-type symptoms on maize leaves

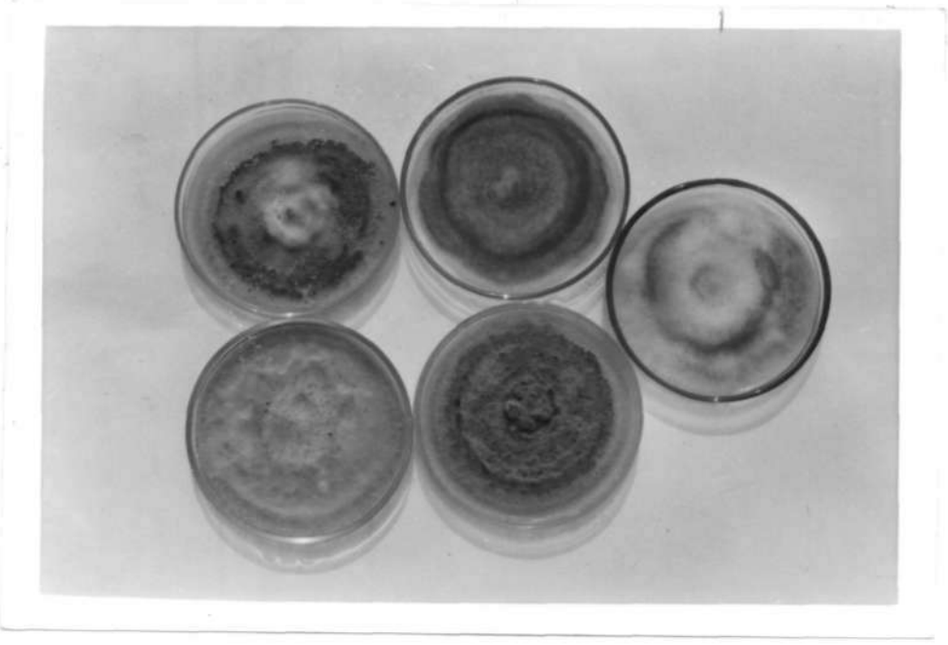


Plate VI Cultural characters of isolates of Colletotrichum graminicola from the midrib-type symptoms on sorghum leaves on Potato Sucrose Agar Streptomycin.

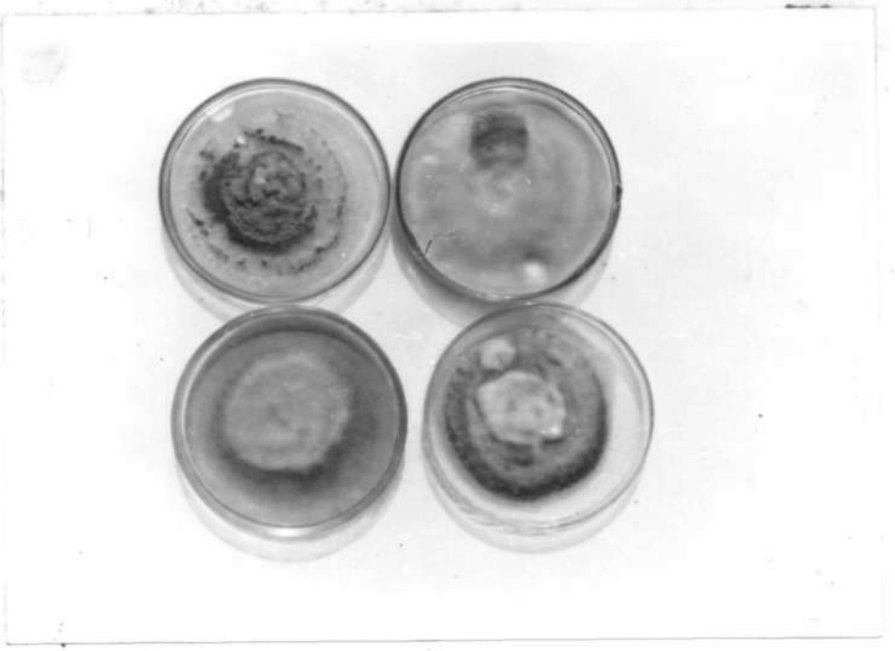


Plate VII Cultural characters of isolates of Colletotrichum graminicola from the isolated type symptons on sorghum leaves on Potato Sucrose Agar Streptomycin.

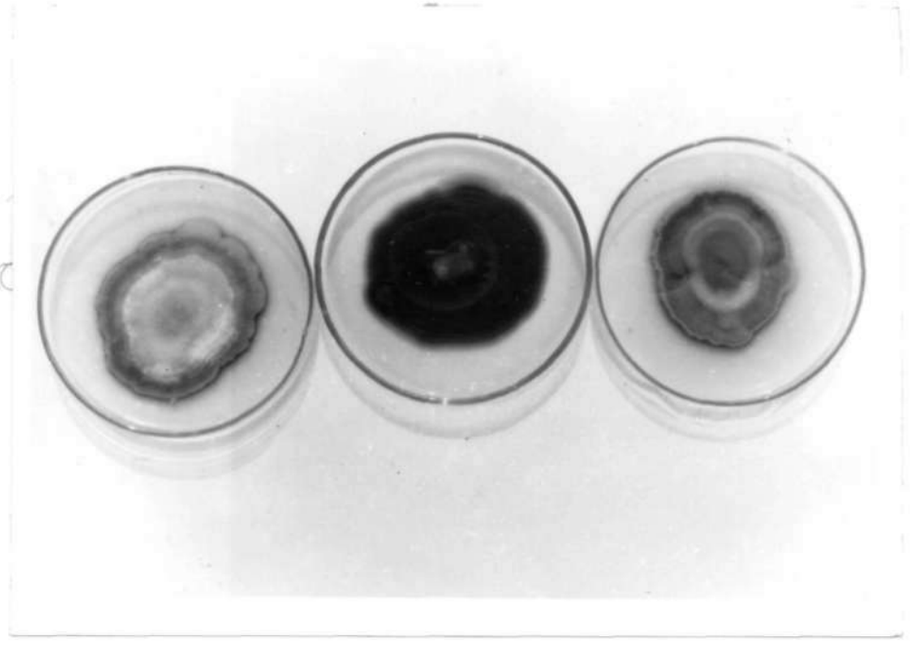


Plate VIII Cultural characters of isolates of Colletotrichum graminicola from the patchy type symptoms on maize leaves on Potato Sucrose Agar Streptomycin.

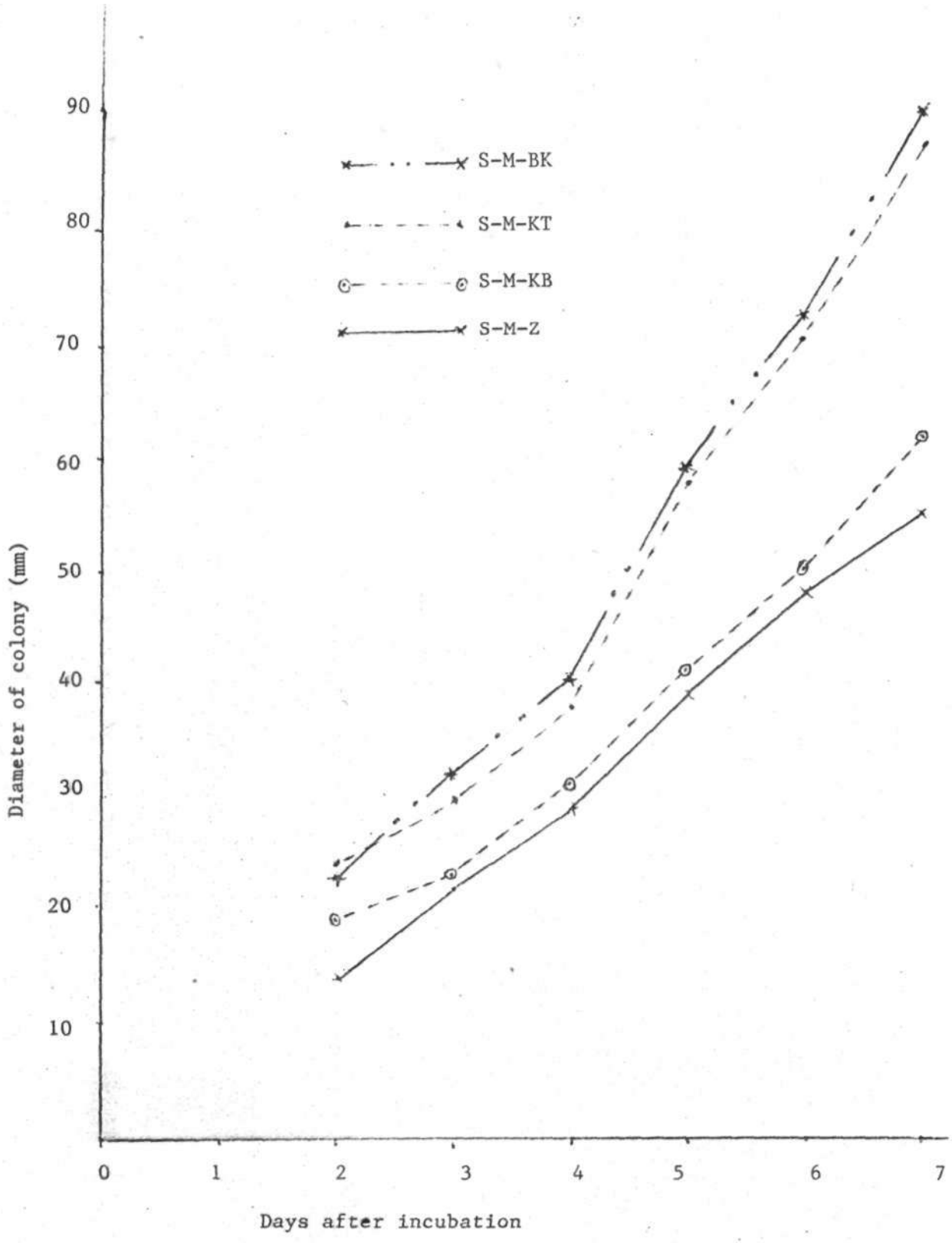


Fig. 1 Growth rate on Potato Sucrose Agar Streptomycin at 29 - 30°C of four isolates, S-M-BK, S-M-KT, S-M-KB and S-M-Z of C. graminicola which cause midrib lesion on sorghum leaves in Nigeria.

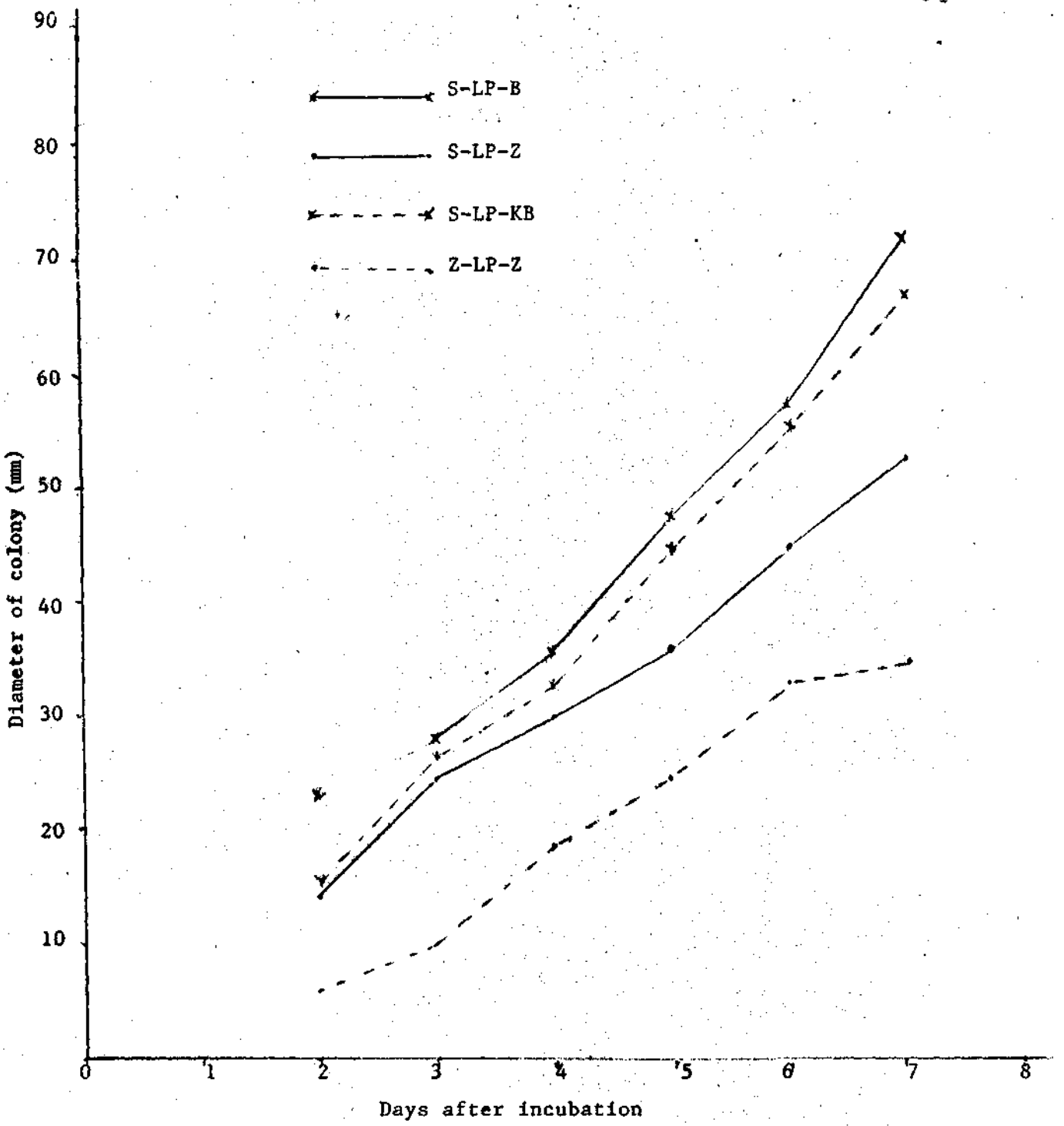


Fig. 2 Growth rate on Potato Sucrose Agar Streptomycin at 29 - 30°C of three isolates S-LI-B, S-LP-B, S-LP-Z, S-LP-KB and one isolate Z-LP-Z of Colletotrichum graminicola which respectively cause patchy-type lesions on sorghum and maize in Nigeria.

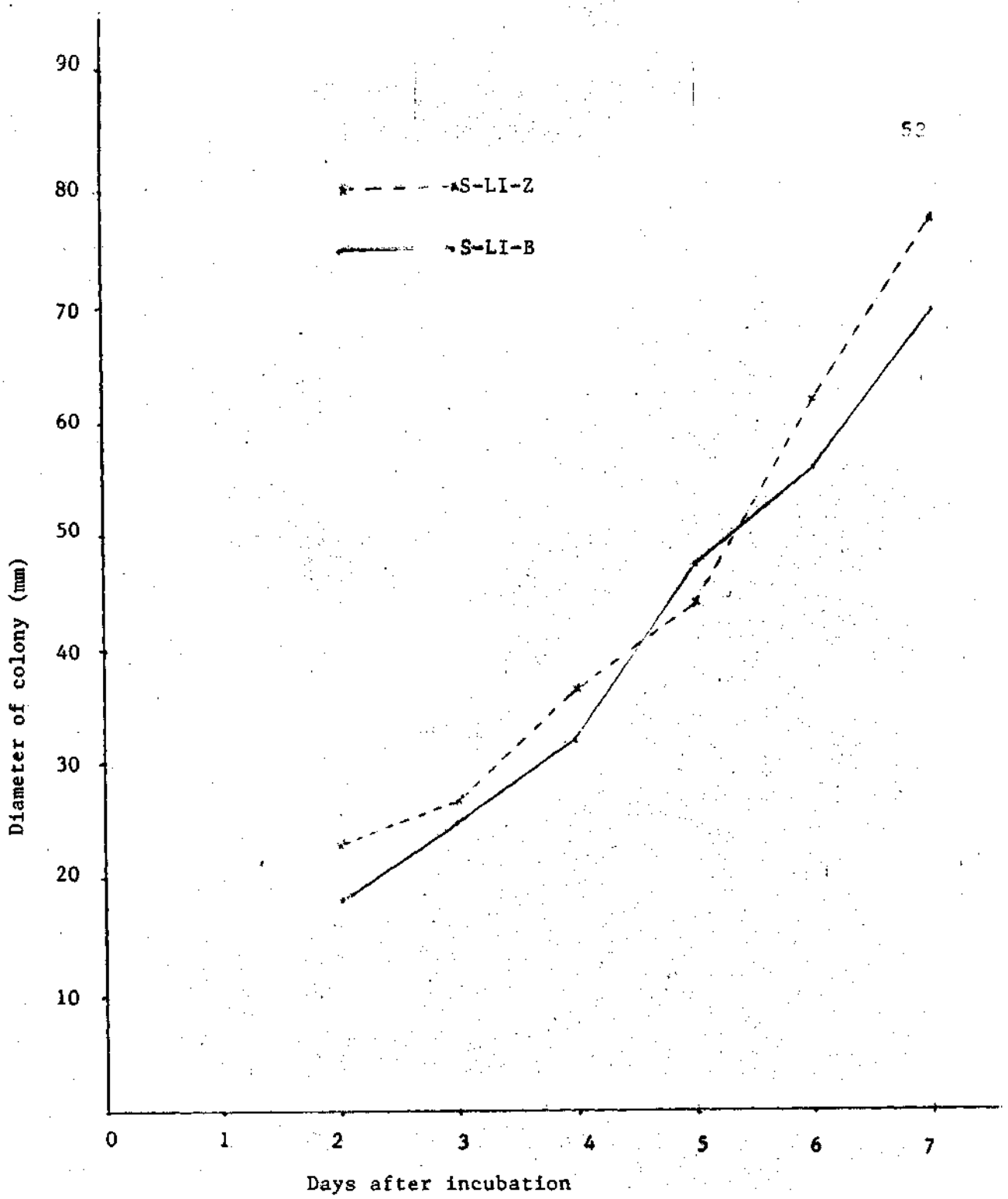


Fig. 3 Growth rate on Potato Sucrose Agar Streptomycin at 29-30°C of two isolates S-LI-Z and S-LI-B of Colletotrichum graminicola causing isolated-type lesions on lamina or sorghum leaves in Nigeria

4.3 Pathogenicity test.

None of the isolates that originated from sorghum infected maize or millet. They only infected sorghum. Distinct symptoms types were produced on sorghum by the different isolates. Maize was only infected by the isolate, Z-LP-Z, which originated from maize leaves. This isolate from maize did not infect sorghum and millet; it only infected maize.

On sorghum, the two isolates S-LI-Z and S-LI-B, which originated from the eye-shaped (isolated-type) lesions on leaves of sorghum consistently produced only the eye-shaped (isolated-type) lesions on all the sorghum cultivars irrespective of season of year in which inoculation was carried out. At no time did these isolates produce the patchy-type symptoms on the lamina of any of the sorghum cultivars. In addition to producing isolated type (eye-shaped) lesions on lamina of sorghum they also produced the midrib lesion similar to those isolates that originated from sorghum midribs. Except in one case (S-LI-B on L 499) with one of the two isolates no differential reaction was expressed by the sorghum cultivars to the two isolates. All the sorghum cultivars were susceptible and all expressed only the eye-shaped type lesions on the lamina.

Isolates that originated from the patchy-type lesions on sorghum only produced the patchy-type lesions (symptoms) on the sorghum cultivars irrespective of the season of the year in which the inoculation was carried out except in the case with one isolate, S-LP-B, on cultivar KSV_{11} which produced both patchy and eye-shaped (isolated) lesions.

In addition to infecting the lamina and producing virtually only the patchy-type symptoms these isolates also infected the midribs of the sorghum cultivars and caused midrib lesions similar to those produced by isolates which originated from the sorghum midribs. No evidence of differential reactions to the isolates was shown by the different sorghum cultivars tested with regards to the patchy type infection symptoms.

Isolates S-M-Z, S-M-BK, S-M-KB and S-M-KT which originated from sorghum midrib lesions produced midrib lesions on all, except two ($^{S}18$ and $^{L}1499$) of the sorghum cultivars. The sorghum cultivars S_{18} remained free of infection by the isolate, S-M-Z. While all the isolates that originated from the sorghum midribs produced midrib lesions on the test sorghum cultivars, not all the isolates produced both patchy-type symptoms and isolated type symptoms in addition to the midrib lesions on the test sorghum cultivars. For example the isolates S-M-Z, S-M-KT and S-M-BK were able to cause midrib lesions on all the sorghum cultivars except S_{18} and $^{L}1499$ where they produced only the eye-shaped (isolated-type) lesions. They did not produce the patchy-type lesions. On the other hand the isolate S-M-KB which caused midrib lesions was also able to cause patchy-type lesions on four of the sorghum cultivars in one of the two trials. It did not cause the isolated type (eye-shaped) lesions on any of the cultivars.

In none of the trials did the test sorghum cultivars react differentially to isolates of a given pathotype i.e. isolates which cause the eye-shaped type lesions and the pathotypes that cause the patchy-type lesions.

Table 13. The pathogenicity of 10 isolates of Colletotrichum graminicola on eight differential test plants.

Isolates	Cultivars of test plants inoculated with the 10 isolates of Colletotrichum graminicola lesions produced and the location of the lesions on the host plant leaf																							
	SK 5912 (Sorghum)			SPV 221 (Sorghum)			KSV11 (Sorghum)			KSV4 (Sorghum)			S18 (Sorghum)			L1499 (Sorghum)			TZB (Maize)			Millet (Pearl)		
	M	LI	LP*	M	LI	LP*	M	LI	LP*	M	LI	LP*	M	LI	LP*	M	LI	LP*	M	LI	LP*	M	LI	LP*
S-M-Z	+	+	-	+	+	-	+	+	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-
S-M-KT	+	+	-	+	+	-	+***	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-
S-M-BK	+	+	-	+	+	-	+	+	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-
S-M-KB	+	-	-	+	-	-	+	-	+***	+	-	+***	-	-	+	-	-	+***	-	-	-	-	-	-
S-LI-Z	+	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-
S-LI-B	+	+	-	-	+	-	-	+	-	-	+	-	-	+	-	***+	+	-	-	-	-	-	-	-
S-LP-Z	+	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-
S-LP-B	+	-	-	***+	-	+	-	***+	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-
S-LP-KB	+	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-
Z-LP-Z**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	***+	-	-	-

(+) = infection (-) = no infection

* M = midrib; I LI = Lamina isolated; LP = lamina patch

** All isolates except Z-LP-Z which was obtained from maize, were obtained from sorghum plants from different locations

*** Symptoms produced in only one of two trials.

CHAPTER 5

DISCUSSION

At the start of this project it was thought or assumed that the midrib lesions on millet was similar, in terms of causality to those on sorghum and maize, i.e. they are all symptoms of anthracnose caused by C. graminicola.

That C. graminicola was always isolated from the midrib lesions on sorghum and maize but not from millet shows that C. graminicola unlike claims by others is not responsible for the midrib lesions observed on millet. This is further supported by the fact that C. graminicola isolated from sorghum maize consistently failed to infect millet whereas the Curvularia sp. isolated from the millet midrib lesions readily infected millet and reproduced the usual lesions. It is on this basis that the midrib lesion on millet is being separately described as a new disease since it has hitherto not been described (see Chapter 6).

A According to the rules and guides of Botanical Nomenclature, species are designated from one another on the basis of morphological differences or distinction. Because computer analysis of morphological properties of the conidia and setae of the different isolates from the different symptoms on sorghum and maize failed to prove statistical morphological differences between the isolates and the fact that all the spores

were hyaline strongly suggest that the fungus causing the anthracnose on sorghum and maize, irrespective of sympton type, is the same and one species i.e. Colletotrichum graminicola as earlier proposed by Wilson (1914). However, the results of pathogenicity tests show that pathological distinction exists between the form that infects maize and the one that infects sorghum. Isolates of C. graminicola from maize did not infect sorghum a they only infected maize; and isolates from sorghum did not not infect maize. This observation agrees with the results of similar cross inoculation reported by several workers (Dale, 1963; Ozolua, 1981). These findings on pathogenic differences somewhat explain why anthracnose is sometimes not observed on maize in a sorghum-maize mixture in the field even though the sorghum shows severe infection. The reason for this lack of infection on maize is that the form specializing on sorghum is distinct from the one specializing on maize. Since the isolates from the sorghum and the maize were indistinguishable morphologically but only distinguishable in their pathogenicity and cultural characteristics, they are, therefore, regarded as special forms (formae speciales) of the same species, C. graminicola described by Wilson 1914.

Designations such as Puccinia graminis f. sp. tritici, P. graminis f. sp. avenae and P. graminis f. sp. secalis are in use to separate or identify members of the species P. graminis, which cause rust respectively on wheat, oats and barley. This system of classification is herein used to classify the forms of C. graminicola that specialize on sorghum and maize. The designation Colletotrichum graminicola f. sp. sorghi is herein proposed to identify the forms that specialize on or adapt to

sorghum. While C. graminicola f. sp. zea-mays is used to identify the forms that specialize on or adapt to maize.

Conventionally pathogenic (physiological) races are designated on the basis of differential pathogenicity of different isolates of the same species of a pathogen on differential cultivars of the same host species. In this system of designation, one race either infects or does not infect a particular host cultivar but all the races producing virtually the same symptom on the infected host cultivars. In this present study, two basic distinctions were apparent in the C. graminicola that specializes on or adapts to sorghum (i.e. C. graminicola f. sp. sorghi). Some isolates of the C. graminicola f. sp. sorghi consistently produce only the isolated eye-shaped symptoms on the host while others produced only the patchy-type symptoms irrespective of the host cultivar or environmental conditions. These isolates could, therefore, be distinguished from one another only on the basis of the symptoms produced on the infected sorghum plant, and not on the basis of one cultivar being or not being infected by the isolate as is conventionally used in pathogenic race designation. For this reason the two types of the C. graminicola f. sp. sorghi are considered somewhat not identical to pathogenic races since the variation is not based on ability or inability to infect certain differential cultivar but purely on symptom type. They also do not qualify for the designation formae specialos because the pathogenic adaptation is not based on differences on host species or host genus. These two pathogenic types are herein referred to as pathotypes of C. graminicola f. sp. sorghi as distinct from race or forma specialis.

Even though variation was observed in the curvuture and pointedness of the conidia from the different isolates, the variation was not consistent for any particular isolate. Because of the inconsistency in shape of the conidia from the sorghum and maize isolates, the variation observed is not considered worthy for nomenclatural purposes. A similar argument obtains in the case of the number of oil globules per conidium length and septation of each setae of each isolate. The fact that more globules were found in conidia obtained from the growth media (PSAS) than from the host, irrespective of isolate type indicates that the number of globules may be determined by the substrate. This is also the case for the number of septa per seta.

The fact that in a given isolate a number of cultural characteristics or cultural types were observed and these characteristics were not specific to a particular isolate suggest that these cultural variations can not be given any weight for nomenclatural purposes. These observation and conclusion agree with those of Choudhury, (1936); LeBeau (1951) and Ozolua, (1981). Measurements by different persons of conidia and setae from different substrates may result in mistaken conclusion on similarity or otherwise of the same isolate.

The fact that no evidence of pathogenic races were obtained in this study does not necessarily mean that for sure they are absent in Nigeria. Probably if much greater number of isolates were obtained and evaluated a more definite conclusion would have been made regarding absence or presence of races in the different pathotypes or formae speciales.

This work has now cleared the doubt as to whether or not Wilson oversimplified the taxonomy of the anthracnose fungus in cereals. The findings from this study have fully supported the nomenclature provided by Wilson (1914) and the conclusion that the sorghum and maize anthracnose is caused by the one and same fungus, Colletotrichum graminicola. In addition, this work has provided very strong evidence that formae speciales exist in Colletotrichum graminicola and that the forms specializing on sorghum are distinct from the forms specializing on maize.

Table 14. Reaction of sorghum cultivars to isolates of two pathotypes of Colletotrichum graminicola

Pathotype	Isolates used for inoculation	Cultivars of sorghum											
		SK_{5912} Location of infection		SPV_{221} Location of infection		KSV_{11} Location of infection		KSV_{4} Location of infection		S_{18} Location of infection		L_{1499} Location of infection	
		Lamina	Mid rib	Lamina	Mid rib	Lamina	mid rib	Lamina	mid rib	Lamina	mid rib	Lamina	Mid rib
Pathcy-type symptom	S-LP-Z	-	+	+	-	+	-	+	-	+	-	+	-
	S-LP-KB	-	+	+	-	+	-	+	-	-	-	-	-
	S-LP-B	-	+	+	+**	+	-	+	-	+	-	+	-
Isolated-type symptom	S-LI-Z	+	+	+	-	+	-	+	-	+	-	+	-
	S-LI-B	+	+	+	-	+	-	+	-	+	-	+	*+

** Symptoms produced in only one of two trials.

Key: (+) = infection

(-) = no infection

CHAPTER 6

CURVULARIA MIDRIB SPOT - A NEW DISEASE ON MILLET

6.1 INTRODUCTION

Midrib lesions on millet leaves are commonly observed in the field in the Sudan and Northern Guinea Savanna regions of Nigeria. These midrib lesions look almost similar to lesions that are caused by the anthracnose fungus, Colletotrichum sp. on sorghum and maize midribs. These spots on millet are initially circular to oval in shape and may extend or coalesce to cover a great length of the midrib. The spots range from light brown to dark brown in colour and often show -dots and in some cases the midribs split up exposing the inner tissues of the midribs. With time these spots become necrotic and in severe cases may cause premature death of the leaf or cracking and breaking of the leaves at the punkered spots.

It has all along been assumed that this type of midrib lesion on millet is an anthracnose caused by C. graminicola similar to that on sorghum and maize. However, critical observations on these lesions on freshly collected and incubated leaves consistently showed absence of acervuli with setae while those on sorghum and maize consistently showed acervuli and setae. Precence of acervuli with setae is a common characteristic of Colletotrichum. The absence of acervuli and setae on the millet midrib lesions raised doubts as to whether indeed the lesions on millet are caused by C. graminicola. This doubt was further increased when attempts to isolate, using media, Colletotrichum from midrib lesions on millet, sorghum and maize consistently resulted in

failures in the case of millet but consistently a success in the case of sorghum and maize.

These observations and experiences created a curiosity to have a more thorough look at the millet lesions with a view to scientifically determine or confirm the causality. This formed the basis for the work here-in reported.

6.2 Literature Review

From the efforts made to obtain literature on this disease, it is clear that little or no information is available on it. This disease on millet, which also occurs in Niger Republic, Mali and Burkina Faso, is considered or assumed by the scientists in these countries to be an anthracnose, caused by Colletotrichum sp. (S. K. Manzo, personal communication). The author failed to find any literature documenting this disease and especially its causality. This disease is not recorded among the identified diseases on millet in either Bailey's (1960) "A check-list of plant diseases in Nigeria," including the addendum to that list, or in "A check-list of diseases of crops in the savanna and semi-arid areas of Nigeria" compiled in 1980 by Emechebe et al. This disease is not listed among diseases of millet in the ICRISAT'S monograph on diseases of millet" and in the "Index of plant diseases in the United States" in the agricultural handbook No. 185 of 1978 of the United States Department of Agriculture. However Sundaram (1980), in Nigeria, encountered this disease on millet and described the symptoms without properly Koch postulating. He concluded that the disease was a form of anthracnose cause by a Glomerella sp. The name Glomerella was given because of the difficulty or failure encountered in isolating Colletotrichum from the lesions (S.K. Manzo, personal communication).

6.3 Materials and Methods

In the rainy seasons of 1985 and 1986 trips were made to three locations (see table 15) in Northern Nigeria. Specimens of millet showing the midrib lesions earlier referred to were collected from farmers' fields in each of the locations.

Table 15. Locations from where specimens with midrib lesions were collected

Town	Ecological location	Specimen designation
Katsina	Sudan savanna	P-M-K
Birnin Kebbi	"	P-M-bK
Zaria	Southern Guinea savanna	P-M-Z

The collected and labelled specimens were taken to the laboratory, where some were dried under a plant press for future use, if the need should arise, while the unpressed materials were variously handled as shown below to isolate and identify the causal pathogen.

6.3.1 Observation of collected specimens using a binocular microscope in the laboratory

Materials freshly collected and not previously placed in humid chambers and also those which were first placed in humid chambers for 2 - 3 days were carefully observed under a binocular microscope (x40) for possible presence or absence of acervuli, setae and conidia of *Colletotrichum*. (to suggest whether it was anthracnose or not).

6.3.2 Isolation of the pathogen(s) using humid chambers

The specimens were carefully washed with tap water in the laboratory. The lamina of each leaf was removed leaving only the infected midribs. Cut pieces of the midribs containing the lesions were surface sterilized in 0.1% milton solution for 5 minutes and rinsed 3 times in sterilized distilled water. They were surface dried using filter papers. Some were placed in separate labelled Petri dishes containing moist filter papers while some were kept for placement in PSAS. The plates were kept on the laboratory benches under room temperature (27±2 °C) for 2 - 3 days to induce growth and sporulation. Using a sharp blade and dissecting pins, scrapings were made from the midrib lesions. The scrapings were placed on a microscope slide containing lacto-phenol with cotton-blue. With the aid of a compound microscope, the stained scrapings were carefully observed for fungal spores and other sporulating structures with the hope of identifying the organism(s) associated with the midrib lesion.

6.3.3 Isolation of the pathogen(s) using growth media (PSAS)

Some of the surface sterilized and rinsed midribs referred to above were plated in separate Petri dishes containing PSAS. The plates were labelled and kept on the laboratory benches under room temperature (27±2°C). Fungal colonies that grew out of these lesions were observed and transferred to fresh media (PSAS). Samples were taken from these colonies and observed under the compound microscope (x40) with the hope of identifying the organism(s). The pure cultures obtained from series of transfers on media were separately maintained for pathogenicity test. For

identification of the fungi isolated, the following texts or monographs were referred to:

(1) A CMI classification handbook on fungi

(2) Dematiaceous hyphomycetes

(3) The genera of the imperfect fungi

For confirmation of the identity of the fungi, fully labelled pure samples were sent to the Commonwealth Mycological Institute (CMI) Kew, London.

6.3.4 Determination of the causality of the millet midrib lesions - Koch postulation

To determine the causality of the millet midrib lesions, the cultures of the two most commonly isolated fungi (Curvularia sp and Phoma sp) were grown on PSAS and prepared as shown below and used to inoculate a local millet variety which was obtained from Katsina and which had been observed in the field to be susceptible to this disease. In addition to the millet variety, one maize variety (TZB) and one sorghum variety (SK 5912) were inoculated with isolates of these fungi. These plants used for the inoculation were raised and maintained in the glass house in similar manner as earlier described.

6.3.5 Inoculum preparation and Inoculation of test plants

Isolates of a fungus which was commonly isolated and identified by the author as Curvularia sp and another identified as Phoma sp. were raised on PSAS. When the cultures had grown for 3 weeks during which growth was abundant and sporulation also abundant in the case of the Curvularia sp., separate suspensions

of the test isolates were prepared by adding sterilised distilled water on to the cultures and carefully scraping with sterilized glass slides. The suspensions were filtered through two layers of cheese cloth into labelled conical flasks and adjusted with the aid of a haemocytometer to give approximately 2×10^5 spores per ml (in the case of the Curvularia sp). The adjusted suspensions were carefully transferred into labelled plastic hand-held sprayers.

To avoid mixed inoculation, the test plants were brought out of the glass house before inoculating them. For the inoculation, three sets of the differential test crops were sprayed with the spore suspension, in the case of Curvularia sp., or with mycelial suspension in the case of the Phoma sp. Scrapped mycelia of the test fungal isolates were also applied on the lamina, midribs and leaf whorls of the test plants. The check (control) plants were sprayed with sterilized distilled water. In order to create a humid condition and encourage infection, the inoculated and check plants were covered with polythylene bags and the bags were sprayed on the inside with sterilized distilled water. The pots were duely labelled and placed in the glass house. Fine droplets of water were sprayed on the plants every morning. Two days after inoculation the polyethylene bags were removed to aerate the plants. Regular spray of water on the plants continued until symptoms started showing.

Leaves of the plants showing the typical symptoms of the midrib lesions were detached from the plant and taken to the laboratory where they were surface sterilized, rinsed and plated

on PSAS in same manner as earlier described. The fungal organism(s) that grew from these plated infected materials were isolated and compared with the original one(s) used for the inoculation of the healthy plants in order to meet the requirement of Koch postulate. The re-isolated organism(s) were grown in pure state on media (PSAS) and used to inoculate healthy plants for further confirmation of pathogenicity or causality.

6.4 Results

6.4.1 Observation of collected specimens using a binocular microscope in the laboratory

None of the collected diseased leaf specimens from the different locations observed under the binocular microscope either soon after collection from the field or after maintaining in humid chamber for 2 - 3 days, showed any evidence of the presence of acervuli, setae or conidia of Colletotrichum.

6.4.2 Isolation of Pathogen(s) from humid chambers and PSAS

Isolations made from humid chambers and PSAS consistently yielded a species of Curvularia and a Phoma species (see table 16). The frequency of isolation of the Curvularia sp. from the midrib lesions was higher from plant specimens collected from Katsina and Birnin Kebbi (Sudan Savanna). The Phoma sp. was more frequent in specimens collected from Zaria areas (Northern Guinea Savanna). CMI identified the Curvularia sp. as Curvularia eragrostidis (Henn) J.A. Meyer; the CMI number given to it was Her. IMI No-305703.

6.4.3 Pathogenicity tests to determine causality

Sorghum and maize used in the pathogenicity tests consistently remained free of the midrib-lesion infection. Millet

got infected readily with symptoms appearing 5 - 8 days after inoculation in the case of the Curvularia eragrostidis and 10 - 14 days after inoculation in the case of Phoma sorghina.

The characteristic symptoms of the lesions caused by the Curvularia eragrostidis are circular to oval lesions approximately 4 - 12 mm in length and 2 - 4 mm in width. These can coalesce to cover a large area or the whole length of the midrib while the lamina remains totally free of infection. At an early stage of the disease the colour of the lesions are brownish or may look translucent and water-soaked and as they get older the colour turn necrotic dark brown. Cracking or splitting often occurs on the lesions along the midrib. Millet plants inoculated with the Phoma sorghina produced small circular to elliptical spots at intervals along the midrib. The spots measured 3 - 7 mm in length and 1 - 3 mm in width. Little or less punkering was observed along the midrib of the plants infected by the P. sorghina than those caused by C. eragrostidis. Lesions caused by P. sorghina showed greater water soaking. In most cases symptoms started at the base of the leaf and extended upward. The colour of the spots is light brown at the initial stage and later turn dark brown. Throughout the study the control plants remained free from infection.

6.4.5 Re-isolation of pathogen(s) from the artificially inoculated plants.

A re-isolation of the pathogen(s) from the reproduced symptoms on the artificially inoculated plants was readily achieved using PSAS.

Lesions on the inoculated plants yielded the same Curvularia eragrostidis and Phoma sorghina. Those inoculated with Curvularia yielded only Curvularia while those inoculated with Phoma yielded only Phoma. A repeat inoculation with the isolated pathogens also readily reproduced the earlier described symptoms.

Table 16. The kinds and frequency of organisms isolated from lesions on midribs of millet collected from different locations in Nigeria

Specimen designation	Location where collection was made	Number of Isolations made	Frequency of isolation of		
			Curvularia Sp.	Phoma Sp.	others*
P-M-K	Katsina	300	236	16	8
P-B-BK	Birnin Kebbi	103	93	5	5
P-M-Z	Zaria	534	21	402	21

*Others - This consisted mainly species of, Helminthosporium, Fusarium, Aspergillus and Penicillium.

6.5 Discussion

Inspite of the fact that the diagnostic symptoms of the midrib lesions on millet observed from the various location are somewhat similar to the midrib lesions commonly observed on sorghum and maize which are known to be caused by the cereal anthracnose fungus, Colletotrichum graminicola, these midrib lesions

on millet are definately different from those on sorghum and maize in terms of causality. This conclusion is arrived at because the acervuli and setae which are the usual characteristic features of the form-genus Colletotrichum were completely lacking from the millet midrib lesions. Also Koch postulations carried out during this work definitely confirmed that Curvularia eragrostidis and Phoma sorghina are responsible. It is likely that ecological location may determine the type and species of the causal organisms. While Curvularia eragrostidis was consistently isolated from the Sudan savanna region, Phoma sorghina was more frequent in the Guinea savanna. Further investigations will provide information on the locational effect on the type of pathogen responsible for the millet midrib lesion.

To the best of the knowledge of the author, this is the first time that the causality of the millet midrib lesion is being scientifically determined. It is on this basis that these new diseases are being formally given the name, "Curvularia midrib spot" in the case of that caused by Curvularia eragrostidis and "Phoma midrib spot" in the case of that caused by Phoma sorghina.

SUMMARY AND CONCLUSION

Anthracnose caused by Collectotrichum graminicola on sorghum and maize was investigated. A midrib spot on millet leaves which looks somewhat similar to that caused by C. graminicola on sorghum and maize was also investigated. Isolates of the pathogen(s) were obtained from sorghum, maize and millet leaf specimens showing various typical symptoms in the field. Morphological, cultural and pathogenic studies were conducted.

The investigation in the present study has shown that:-

1. Isolation from sorghum and maize leaf specimens consistently yielded Colletotrichum graminicola while that from the millet leaf specimens yielded mainly Curvularia eragrostidis and Phoma sorghina.

2. Statistical analyses of dimensions of conidia and setae of C. graminicola from the various isolates did not differ significantly among the isolates.

3. The various forms of C. graminicola detected on potato Sucrase Agar Streptomycin culture media were not specific to a particular isolate.

4. Pathogenicity test with C. graminicola isolates from sorghum and maize revealed that isolates from maize did not infect sorghum and isolates from sorghum did not infect maize.

5. Since the isolates of C. graminicola from the sorghum and the maize were similar morphologically but only differ in their pathogenicity and cultural characteristics they are regarded as special forms (formae speciales) of the same C. graminicola described by Wilson 1914.

6. Two pathotypes were apparent in the C. graminicola that specialises on sorghum based on the symptoms produced on sorghum. One of the pathotypes consistently produced only the isolated-type symptoms irrespective of the host cultivar or environmental conditions. The other pathotype consistently produced only the patchy-type symptoms irrespective of the host cultivar or environmental conditions.

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APPENDIX

1. Analysis of Variance for the interaction between length of conidia of 10 isolates of Colletotrichum graminicola from host plant leaf and from Potato Sucrose Agar Streptomycin Culture media.

Source of variation	DF	SS	MS	F
Type	1	26.73	26.73	2.276(NS)
Isolate	9	86.54	9.84	0.837(NS)
Type·Isolate	9	59.21	6.58	0.560(NS)
Error	980	11512.58	11.75	
Total	999	11687.05		

2. Analysis of variance for the interaction between width of conidia of 10 isolates of Colletotrichum graminicola from host plant leaf and from Potato Sucrose Agar Streptomycin culture media.

Source of variation	DF	SS	MS	F
Type	1	9.8188	9.8183	24.319**
Isolate	9	0.3452	0.0384	0.095(NS)
Type·Isolate	9	2.3349	0.2594	0.643(NS)
Error	980	395.6707	0.4037	
Total	999	418.1696		

3. Analysis of variance for the interaction between length of setae of 10 isolates of Colletotrichum graminicola from host plant leaf and from Potato Sucrose Agar Streptomycin culture media.

Source of Variation	DF	SS	MS	F
Type	1	14262.2	14262.3	31.545**
Isolate	9	4107.8	456.4	1.009(NS)
Type Isolate	9	1198.4	133.2	0.295(NS)
Error	980	443084.3	452.1	
Total	999	46 265 2.8		

4. Analysis of variance for the interaction betwen width of setae of 10 isolates of Colletotrichum graminicola obtained directly from leaf and from Potato Sucrose Agar Streptomycin culture media.

Source of variation	DF	SS	MS	F
Type	1	7.5169	7.5169	9.477**
Isolate	9	5.7537	0.6393	0.806(NS)
Type Isolate	9	1.508	0.1679	0.212(NS)
Error	980	777.3074	0.7932	
Total	999	792.0888		

5. Analysis of variance for the length of conidia of 10 isolates of Colletotrichum graminicola obtained directly from the host plant leaf

Source of variation	DF	SS	MS	F
Isolate	9	50.018	5.558	0.618(NS)
Error	490	4404.522	8.989	
Total	499	4454.540	8.927	

6. Analysis of variance for the width of conidia of 10 isolates of Colletotrichum graminicola obtained directly from the host plant leaf

Source of variation	DF	SS	MS	F
Isolate	9	1.2533	0.1393	0.321(NS)
Error	490	212.5677	0.4338	
Total	499	213.8210	0.4285	

7. Analysis of variance for the length of seate of 10 isolates of Colletotrichum graminicola obtained directly from the host plant leaf

Source of variation	DF	SS	MS	F
Isolate	9	3507.9	389.8	0.875(NS)
Error	490	218157.9	445.2	
Total	499	221665.8	444.2	

8. Analysis of variance for the width of setae of 10 isolates of Colletotrichum graminicola from the host plant leaf

Source of variation	DF	SS	MS	F
Isolate	9	3.0807	0.3423	0.381(NS)
Error	490	440.3356	0.8986	
Total	499	443.4163	0.8886	

9. Analysis of variance for the length of conidia of 10 isolates of Colletotrichum graminicola grown on Potato Sucrose Agar Streptoymycin culture media

Source of variation	DF	SS	MS	F
Isolate	9	97.73	10.86	0.749(NS)
Error	490	7108.05	14.51	
Total	499	7205.78	14.44	

10. Analysis of variance for the width of conidia of 10 isolates of Colletotrichum graminicola grown on Potato Sucrose Agar Streptomycin in culture media

Source of variation	DF	SS	MS	F
Isolate	9	1.4268	0.1585	0.424(NS)
Error	490	183.1030	0.3737	
Total	499	184.5290	0.3698	

11. Analysis of variance for the length of setae of 10 isolates of Colletotrichum graminicola grown on Potato Sucrose Agar Streptomycin in culture media

Source of variation	DF	SS	MS	F
Isolate	9	1798.3	199.8	0.435(NS)
Error	490	224926.4	459.0	
Total	499	226724.7	454.4	

12. Analysis of variance for the width of setae of 10 isolates of Colletotrichum graminicola grown on Potato Sucrose Agar Streptomycin in culture media

Source of variation	DF	SS	MS	F
Isolate	9	4.1838	0.4649	0.676(NS)
Error	490	306.9718	0.6877	
Total	499	341.1556	0.6837	

Appendix 13. Colony diameter (cm) of 10 isolates of *Colletotrichum graminicola* plated on Potato Sucrose Agar Streptomycin for 7 days.

Isolate	Growth rate in days 2	3	4	5	6	7	Average
S-M-Z	1.4	2.2	2.9	3.9	4.8	5.5	3.5
S-M-KT	2.4	3.0	3.8	5.8	7.1	8.7	5.2
S-M-BK	2.3	3.2	4.0	5.9	7.3	9.0	5.2
S-M-KB	1.7	2.3	3.1	4.1	5.0	6.2	3.7
S-LI-Z	2.3	2.7	3.7	4.4	6.2	7.8	4.5
S-LI-B	1.8	2.5	3.2	4.7	5.6	7.0	4.1
S-LP-Z	1.4	2.5	3.0	3.6	4.5	5.2	3.3
S-LP-B	2.3	2.8	3.6	4.8	5.8	7.3	4.4
S-LP-KB	1.5	2.7	3.3	4.5	5.6	6.8	4.0
Z-LP-Z	0.6	1.0	1.9	2.5	3.3	3.5	2.1

Note (NS) = Not statistically significant even at 5% level of significance.

* = Statistically significant at 5% level of significance (P = 0.05).

** = Statistically significant at 1% level of significance (P = 0.01).