DETERMINATION OF THE VARIABILITY OF *COLLETOTRICHUM TRUNCATUM*, THE CAUSAL AGENT OF ANTHRACNOSE OF SOYABEAN (*Glycine max* (L.) Merr.) IN ZAMBIA.

BY

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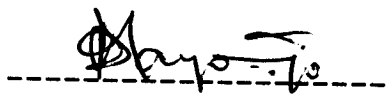
DECLARATION

I, **David Onesmo Mayonjo** hereby declare that this dissertation represents my own work and that it has not been previously submitted for a degree at this or at any other university.



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APPROVAL

This dissertation of **DAVID ONESMO MAYONJO** is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Agronomy (Crop Science) of the Universty of Zambia.

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DEDICATION

Dedicated to my father Mr Onesmo Mayonjo, my wife Flora and my sons Onesmo and Ernest for their patience, endurance and encouragement throughout the course of these studies which kept us separated for two years.

ABSTRACT

Soyabean (*Glycine max* (L.) Merr.) has become an important crop in Zambia. Its increased production is hampered by several foliar diseases of which the anthracnose caused by *Colletotrichum truncatum* appear to be the most prevalent, than red leaf blotch caused by *Dactuliochaeta glycines* reported before.

A study on anthracnose of soyabean disease was undertaken to determine the level of variability that exists in the pathogen populations in Zambia. Ten samples of anthracnose of soyabean were collected from the provinces of Central, Lusaka and Southern. Isolates Ct-01, Ct-02, and Ct-03 were collected from Central province (Kabwe); Ct-04, Ct-05, Ct-06 and Ct-07 from Lusaka province (Lusaka -West and South), and Ct-08, Ct-09, and Ct-10 from Southern province (Mazabuka). Isolation were made following conventional mycological methods and the identification was confirmed by the International Mycological Institute, England.

Isolates were characterized on the basis of acervulus size, colony features and its growth rate on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA). The conidial size, germ tube elongation during conidial germination and pathogenicity of the isolates to three soyabean cultivars were also determined.

The isolate Ct-07 had the largest acervulus diameter and Ct-03 had the lowest. After eight days of growth, Ct-04, Ct-05, Ct-07, and

Ct-09 had greater colony diameter on PDA whereas these isolates produced significantly smaller colonies on MEA. In comparison, Ct-01, Ct-03, Ct-06, and Ct-08 had reduced growth rate and produced smaller colonies on PDA. After eight hours of growth on water agar, isolates Ct-04 and Ct-09 developed the longest germ tubes as compared to Ct-06 and Ct-08. Conidial size also showed significant differences among isolates.

Differences in colour of conidial ooze emanating from the acervulus and the colony margin were also noticed among isolates at 25 °C. The colour of aerial hyphae showed differences on the two media, but the majority of the isolates produced white hyphae which later turned grey.

The isolates differed in their ability to infect soyabean cultivars. Isolates Ct-01, Ct-07 and Ct-09 infected Kaleya, Hernon-147 and Santa Rosa the tested cultivars whereas Ct-02, Ct-04, and Ct-08 infected only Kaleya and Hernon-147. Isolates Ct-05 and Ct-10 attacked only Kaleya and Santa Rosa and isolates Ct-03 and Ct-06 infected only Kaleya and Santa Rosa respectively.

The results indicate that the ten isolates of *Colletotrichum truncatum* possess considerable morphological and physiological variability and therefore their control measures should be directed after knowing which one of the trains of the pathogen is in the population.

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LIST OF ABBREVIATIONS

1.	MEA	Malt Extract Agar
2.	PDA	Potato Dextrose Agar
3.	mm	Millimeter
4.	μm	micrometer
5.	LSD	Least Significant Different
6.	CV	Coefficient of Variation
7	DMRT	Duncan Multiple Range Test
8	Ct	*Colletotrichum truncatum*

INTRODUCTION

Soyabean (*Glycine max* (L.) Merr.) is the world's leading legume crop (Purseglove, 1968;) with oil and protein content estimated at about 20 and 40 percent respectively. It has been exploited primarily as a source of edible oil and as protein-rich meal for livestock. Traditional soyabean foods have been consumed in oriental countries for centuries, but these account for only a small fraction of the world soyabean crop. Many developing countries have recognized the potential of soyabean as a valuable source for supplementing the traditional staples with much needed protein. National programmes are in place in many developing countries to expand the production of soyabean.

The origin of soyabean (*Glycine max* (L.) Merr.) is thought to be from *Glycine ussuriensis* Regel and Maack, which is a slender, prostrate, wild twining legume of Eastern Asia and *Glycine tomentosa* Benth., of Southern China (Purseglove, 1968). *Glycine gracilis* Skvortzor, a semi cultivated species, is considered to be an intermediate between the wild *Glycine ussuriensis* and *Glycine max*.

Soyabean has been cultivated in China since the written documents came in existence. The crop has also been an important food for a long time in Manchuria, Korea, and Japan. As a crop, soyabean was introduced into tropical countries during this century.

Soyabean is believed to be a subtropical plant (Purseglove, 1968), and its cultivation has extended from the tropics to 52° N. Its climatic requirements are similar to those of maize. It is a short day plant and all cultivars of soyabean flower quickly with 14 to 16 hours of darkness (Purseglove, 1968). The maturity period varies from 75 to 200 days.

Soyabean is a relatively new crop in Zambia. As a result of agronomic research and plant selection work, its cultivation has become a popular and a reliable venture (Wellving, 1984) in Zambia. Soyabean production in Zambia has increased from 6,518 tons in 1981 to 27,712 tons in 1992 (Anonymous., 1992) - an increase of 425 percent.

The most common diseases of soyabean in Zambia are bacterial blight caused by *Pseudomonas sp.* and bacterial pustule incited by *Xanthomonas phaseoli*. However, Wellving (1984) reported that the present varieties have satisfactory level of tolerance to these diseases and therefore they do not suffer much loss.

Red leaf blotch of soyabean caused by *Dactuliochaeta glycines* (Stewart) Hartman & Sinclair (1988) has become a serious disease in Zambia (Wellving, 1984). Consequently, the red leaf blotch disease is a major threat to soyabean production in Zambia. In the mid 70s the disease caused severe defoliation in soyabean crops (Javaid

and Ashraf, 1978). Later, in 1977 estimates showed yield losses of up to 50 percent in soyabean crop (Sinclair, 1982). Hartman *et al.* (1987) reported that a yield reduction of 34 percent was quite common in Zambia. The high yield losses due to red leaf blotch appear to be associated with an increase in acreage of soyabean (Datnoff *et al.*, 1987).

In the course of further studies on the red leaf blotch disease of soyabean and its causal organism, collected disease samples from infected leaves yielded *Colletotrichum truncatum* (Schwein.) Andrus & Moore rather than *Dactuliochaeta glycines*. It therefore became apparent that the symptoms of the red leaf blotch disease due to *Dactuliochaeta glycines* could be related to those produced by anthracnose which is incited by *Colletotrichum truncatum*. The yield reduction in soyabean reported earlier could therefore be either due to red leaf blotch or anthracnose or both of them. The yield losses in soyabean reported for the 70's and early 80's in Zambia were based on symptoms alone. No accurate identification of the pathogens has been mentioned in these reports.

Since anthracnose was found to be far more prevalent on soyabean than red leaf blotch when collecting disease samples from infected leaves it was considered important to study the biology of *Colletotrichum truncatum* in greater detail.

The objective of this study was to determine the level of

variability in *Colletotrichum truncatum* (Schwein.) Andrus and Moore a causal organism of soyabean anthracnose in Zambia. Colony characteristics, acervulus and conidial sizes, and germination attributes of conidia among isolates were compared to establish existing variation. Pathogenicity of isolates on selected soyabean cultivars was also determined.

LITERATURE REVIEW

Soyabean (*Glycine max* (L.) Merr.) is a relatively new crop in Zambia. Initially there were few disease problems on soyabeans but by early 1970's bacterial blight became a serious and an important disease in Zambia (Javaid and Ashraf, 1978). In recent years red leaf blotch or Pyrenochaeta leaf blotch of soyabean caused by *Pyrenochaeta glycines* Stewart (= *Dactuliophora glycines* Leakey) has been recognized as another major problem (Datnoff *et al.*, 1987).

Red leaf blotch of soyabean was first described by Stewart (1957) in Ethiopia and later by Leakey (1964) in Rhodesia. Since then the disease has become a major problem in Southern Africa especially Zimbabwe and Zambia. In Zambia it was first reported by Smart (1960) who regarded it as a minor disease. However, by 1977 yield losses of up to 50 percent became common (Sinclair, 1982). Reports of Hartman *et al.* (1987), Datnoff *et al.* (1987) and Hartman and Sinclair (1988) indicate that red leaf blotch on soyabean is a very common and serious disease.

Following the descriptions of symptomatology of red leaf blotch of soyabean, as reported by Hartman *et al.* (1987), it was found that anthracnose which is caused by *Colletotrichum truncatum* is much more prevalent than red leaf blotch of soyabean in these three provinces namely Central, Lusaka, and Southern (Mazabuka). It is

not clear whether red leaf blotch and anthracnose have any relationship or whether the two diseases produce entirely different symptoms on soyabeans in Zambia.

According to Dhingra (1975), anthracnose of soyabean was first reported from Korea in 1917, and has currently been observed in all major soyabean-growing areas of the world. Anthracnose is not a major disease of soyabeans in cooler areas. However, in warmer, tropical and subtropical regions, it may cause considerable damage by reducing stand, seed quality and yield (Dhingra, 1975).

The anthracnose fungus is an unspecialized parasite of leguminous and non-leguminous plants (Allen, 1983). Its host range is therefore wide. Symptoms of anthracnose are variable on infected host plants.

Anthracnose is a complex disease caused by several fungi which produce similar symptoms. It may be caused by either *Colletotrichum dematium* (Pers. ex Fr.) Grove var. *truncata* (Schw.) Arx. (Syn.: *Colletotrichum truncatum* (Schw.) Andrus and W. D. Moore; *C. glycines* Hori; *C. caulivorum* Heald and Wolf; *C. viciae* Dearn and Overh.; *Vermicularia truncata* Schw.; and *V. polytricha* Cke.) or *Glomerella glycines* (Hori) (Dhingra, 1975).

Colletotrichum truncatum causes anthracnose of soyabean, limabean and also the brown blotch of cowpeas (Allen, 1983). It has also

been reported to infect such malvaceous plants as *Abutilon theophrasti* (Hepperly *et al.*, 1980).

The pathogen *Colletotrichum truncatum* can become a serious disease if favarouble weather conditions, especially heavy rains, continue for a protracted period. Under such conditions both young and old plants are attacked and the resulting losses may be quite serious (Toler and Wester, 1966; Wheeler, 1969; and Manandhar *et al.*, 1988).

Colletotrichum truncatum is the most widely distributed of anthracnose causing fungi in the world (Dhingra, 1975; Allen, 1983). Another, fungus *Glomerella glycines*, was discovered in North Carolina in the mid-1920's, and it was the first fungus reported to be the causal agent of soyabean anthracnose in the United States of America. The difference between these two pathogens is that with *C. truncatum*, the plants are susceptible to infection at any stage of development while with *G. glycines* only the old plants are susceptible (Dhingra, 1975).

Allen (1983) reported that brown blotch of cowpea in Zambia is caused by *Colletotrichum truncatum*. Javaheri (1985), also mentioned that anthracnose of soyabean occurs in Zambia and is caused by *Colletotrichum dematium*. Both diseases are widely distributed in the country but our knowledge of their symptoms, host range and their effect on plant growth and yield are not fully understood. It

is interesting to note that even Kannaiyan and Haciwa (1993), did not make any mention of anthracnose of soyabean in their comprehensive work on diseases of legume crops in Zambia.

2.1. Symptoms of Red leaf blotch:

It is not clear whether red leaf blotch and anthracnose have any relationship or whether the two diseases produce entirely different symptoms on soyabeans in Zambia. It has a wide host range and symptoms are variable on host plant.

Hartman *et al.* (1987) reported that the lesions of red leaf blotch caused by *Dactuliochaeta glycines* occur on the foliage, petioles, and stems and are often associated with the primary leaf veins. On unifoliolate leaves, the lesions are dark red to brown, circular to angular and one to three millimeters in diameter. However, on the trifoliolate leaves, the lesions are dark red on the upper surface and reddish brown with dark margins on the lower surface. The lesions enlarge, coalesce and form irregular blotches of 3 - 20 millimeters in diameter. The lesions can merge to form larger blotches of up to four centimeters. The leaf spots are often surrounded by a yellow ring. The necrotic tissue frequently drops out, giving a shot - hole appearance to the leaves. The disease causes premature defoliation and senescence of infected plants.

2.2. Symptoms of Anthracnose:

Soyabean plants have been reported to be susceptible to *Colletotrichum truncatum* at all stages of development and symptoms appear at early reproductive stages (Sinclair, 1991). Dhingra (1975) reported that stems, pods and leaves may be infected without showing external symptoms. However, during periods of heavy rainfall, the disease manifests itself as irregularly-shaped brown areas on the foliage and other organs of the shoot system.

Several workers have described the symptoms of *Colletotrichum truncatum* on different leguminous hosts. Tiffany and Gilman (1954), Allen (1983) reported that the symptoms of anthracnose induced by *Colletotrichum truncatum* on cowpea and bean involve the development of purplish to reddish-brown blotches on petioles, leaf veins, stems, peduncles and pods. However, vascular necrosis of leaves is a key characteristic of bean anthracnose (Allen, 1983). Tiffany (1951), reported that as the soyabean plants approached maturity, defoliation of leaves was also common.

2.3. Morphology and physiology of *Colletotrichum truncatum*:

Dhingra (1975), described *Colletotrichum truncatum* as being characterized by having crowded, black acervuli on well developed stromata. The acervuli are oval to elongate, hemispheric to truncate-conical and erumpent with numerous needle-like,

intermixed, long and short setae 60 to 300 X 3 to 8 micrometer. Most conidia are borne singly on the conidiophore. The conidia are bluntly tapered, curved, aseptate, hyaline, and measure 17 to 31 and 3 to 4.5 micrometer. Upon germination, conidia normally produce one or two germ tubes.When the germ tubes come in contact with a solid object, they produce dark, sticky appressoria and direct penetration might follow later on.

The organism grows well on potato dextrose agar at 28 to 34 °C, produces a whitish colony which eventually turns smoky black, containing abundant acervuli (Dhingra, 1975). Tiffany and Gilman (1954) reported that the vegetative mycelium of *Colletotrichum truncatum* becomes olive green to black with little aerial mycelium at first but later, as it becomes older, a grey aerial mycelium overgrows the acervuli.

Holliday (1980) reported that the fungus produces conidia in mucilaginous masses which are often pinkish and fairly conspicuous on the typically sunken, irregularly outlined necrotic lesions called anthracnose, on the leaves and stems. Tiffany and Gilman (1954) reported that *Colletotrichum truncatum* may produce two types of conidial masses which were either cream or pinkish in colour.

2.4. Control measures of *Colletotrichum truncatum*:

The pathogen is known to be seed-borne and is carried to the next

cropping season in crop residue (Holliday, 1980). The control measures of anthracnose include planting of disease free seed or seed treatment with recommended fungicides and to deeply plough the crop residue. The disease can also be controlled by foliar sprays of fungicides.

Reports from Nigeria show that brown blotch of cowpea can be controlled by a mixture of Benomyl and Permethrin (Oladiran, 1990). Kaushal and Paul (1989) reported that the leaf extracts of *Cannabis* sp., *Pinus longifolia*, *Eupatorium* sp. and *Lantana indica* had an inhibitory effect on the spread of *Colletotrichum truncatum*. Farmers can therefore incorporate these inhibitory plants in the soil to control the spread of disease.

Sinclair (1986) reported that changing the planting dates can help the crop to mature during a low rainfall period, which in turn reduces the plant's contact with seed-borne fungi such as *Colletotrichum truncatum*.

Some soyabean cultivars have been reported to be resistant to *Colletotrichum truncatum* and this was associated with the presence of latent infection in soyabean plants (Nelson, 1979).

Following the importance of soyabean in Zambia, most farmers control fungi using fungicides, since they were not aware of other control measures for specific disease such as anthracnose.

MATERIALS AND METHODS

3.1. Collection of *Colletotrichum truncatum* samples:

Disease samples were collected from three provinces (Central (Kabwe), Lusaka (South and West) and Southern (Mazabuka) provinces which are located in the same agroecological zone (zone II). Soyabean leaflets showing symptoms of anthracnose were collected at the reproductive stage of the plants.

Specimens of infected soyabean leaves were collected from commercial farm during January to March 1994. Records of host cultivar and date of collection were always made at the time of sample collection. Infected leaflets were removed from soyabean plants, transferred to unused polyethylene bags and taken to the plant pathology laboratory for processing. Samples were occasionally stored overnight in a fridge before isolating the fungus next day. It is a total of ten samples were collected as shown in Table 1.

3.2. Isolation of *Colletotrichum truncatum*:

Lesions from infected soyabean leaflets were cut out with a sterilized scalpel. The leaflets were disinfected in 10 percent Jik (Commercial Sodium hypochlorite) for 10 minutes and then placed on potato dextrose agar (PDA) in sterilized plates after rinsing them in distilled water. The inoculated plates were incubated at 25 °C.

Table 1. The localities of collection of samples of *Colletotrichum truncatum* (Schwein) Andrus and Moore.

PROVINCE	CULTIVAR	FARM	ISOLATE CODE
Central	Hernon-147	CRF, Kabwe	Ct-01
Central	Hernon-147	Chinsanto, Kabwe	Ct-02
Central	*	Golden Valley Res.Chibombo	Ct-03
Lusaka	Kaleya	Lilayi, Lusaka south	Ct-04
Lusaka	Kaleya	Zambia -China, Lusaka West	Ct-05
Lusaka	Kaleya	Kashima, Lusaka West	Ct-06
Lusaka	Santa Rosa	Masdar Chanyanya,Lusaka West	Ct-07
Southern	Kaleya	Pinkines, Mazabuka	Ct-08
Southern	Gazelle	Hamapula, Mazabuka	Ct-09
Southern	*	Chisoba, Mazabuka	Ct-10

* The name of soyabean cultivar has not yet been established.

Culture plates were examined every day, under a dissecting microscope to establish growth and acervulus development. This procedure was followed in this study after the report of Sinclair (1986), who observed that the presence of acervulus was the key to know whether the crop was infected with *Colletotrichum truncatum* and not *Dactuliochaeta glycines*. Therefore there was no need to look for other pathogens in the culture.

Plates indicating acervulus development were selected and from these single acervulus was removed and subcultured on PDA. Plates were incubated at 25 °C for three days and thereafter stored in a fridge at 10 °C until required in the current studies.

3.3. Purification of *Colletotrichum truncatum* isolates:

Mature acervuli (one - three) were removed from the colony, crushed with a sterilized glass rod and suspended in a tube of ten milliliter capacity containing five milliliter sterilized water to produce a conidial suspension. The tube was vigorously shaken by striking it repeatedly against the left hand palm to get a homogenous conidial suspension. The suspension was adjusted to yield two to six conidia per inoculating needle-loop by adding more water or acervuli to the tube. With the aid of an inoculating needle-loop, conidial suspension was applied to lines drawn at the bottom of a water agar plate. The plates were incubated at 25 °C for 24 hours. Germinating conidia were located next day under a

stage microscope. They were then removed by cutting out the block of agar on which a conidium had germinated and transferred to a water agar plate. The isolated conidium was allowed to grow at 25 °C to produce a colony of the pathogen. Using the above procedure a pure culture of each of the ten isolates was obtained.

3.4. Acervulus and conidial size among *Colletotrichum truncatum* isolates:

Measurements of acervulus and conidial size were made from eight day old cultures on water agar. Acervuli were transferred to a drop of water on a microscope slide and their size determinations made at a magnification of X100. For conidial measurements, a homogenous suspension of conidia was prepared in sterile water and transferred to microscope slides before microscopic determinations were made at a magnification of X100. The experiment was done following randomized complete block design of one factor (isolates) with three replications.

3.5. Colony growth among *Colletotrichum truncatum* isolates :

Colony growth in isolates of *Colletotrichum truncatum* was studied on two different media - Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA). Both media were sterilized and their pH adjusted to 6.5 and then poured into Petri-plates of nine centimeter diameter. From a pure culture of each isolate of

Colletotrichum truncatum, a disc of inoculum was cut out with a sterilized cork borer number three and transferred to the center of a plate of either PDA or MEA. For each isolate, three replicates were maintained for PDA and MEA respectively. The plates were incubated at 25 °C and colony diameter recorded at two days intervals using graduated ruler (mm) and was measured at the bottom of the Petri-plates. The colony colour was determined following Munsell soil colour charts (1975) and margin type was described following the terminology given by Hawkworth, Sutton and Ainsworth (1983) in their dictionary of the fungi. Initiation of acervulus development and the colour of conidial cirrhus (ooze) were also recorded for each isolate. The isolates were randomized in a split plot design whereas media were considered as main plots and isolates as subplots, each with three replications.

3.6. Conidial germ tube growth among *Colletotrichum truncatum* isolates:

Acervuli from a 10 day old pure culture were crushed in sterilized water on a slide and transferred to a glass tube. Conidial suspension was drawn from the tube using an inoculating needle-loop and transferred to the tap water agar plates. The conidial suspension was smeared on to the medium surface within a two centimeter circle drawn at the bottom of a plate and incubated at 25 °C. The number of conidia in each circle ranged from 50 to 100. The length of the conidial germ tube was recorded at 10X10

magnification with light microscope. At two hour interval, random tracing of ten different conidia on water agar were located and their germ tubes measured for length in micrometer. Each isolate was replicated thrice, following the randomized complete block design.

3.7. Pathogenicity of *Colletotrichum truncatum* isolates to soyabean cultivars:

Three soyabean cultivars, Kaleya, Hernon-147 and Santa Rosa were used in pathogenicity tests. These are the three cultivars which are commonly grown by farmers in Zambia. Three seeds of each soyabean cultivar were sown in a pot of 9.5 centimeter diameter. The plants were kept under the polyethylene cover to isolate them from other pathogens. Each soyabean cultivar was replicated three times and inoculated with the ten isolates of the pathogen. The layout of the plots followed a Split plot design and the treatments were randomized in each replication. The cultivars were used as main plots and isolates as subplots.

Soyabean plants at the true second leaf stage were inoculated with *Colletotrichum truncatum* following the procedure described by Hunger and Brown (1987). This technique involves inoculation of plants with an agar block of the pathogen culture taken from the margin of a growing colony. The ten day old culture was cut out with a cork borer number three. The culture agar block of the

pathogen was placed on the soyabean leaflet after scratching it with a scalpel, followed by spraying of the soyabean plants with water. Before inoculation, the plants were kept overnight in humid environment. Soon after inoculation, the plants were covered by plain polyethylene sheet to maintain a saturated atmosphere. The plants were incubated under the same plain polyethylene sheet. Temperature of the incubation environment was 25 ˚C (± 1) (Appendix VII). The response of cultivars to the isolates of the pathogen was considered on the basis of symptoms and the lesion size after 15 days of inoculation.

3.8. Data Analysis:

Data were subjected to statistical analysis using computer programme. The mean separation was done by Duncan Multiple Range Test (DMRT) (Gomez and Gomez (1984). Correlation analysis between colony diameter after eight days of growth on PDA and leaf lesion size for three soyabean cultivars was also determined using computer.

RESULTS

4.1. Symptoms of anthracnose of soyabean:

The lesions were found associated with the primary and secondary leaf veins and sometimes in between the veins. They were dark red to brown of either circular or angular shape,having one to four millimeters diameter and at times larger up to two centimeters diameter. The upper surface spots were dark red to brown while those on the lower surface were reddish brown with dark borders. In some cases the lesions were surrounded by a yellowish colouration. The larger lesions were in the form of a brown blotch.

4.2. Characterization of *Colletotrichum truncatum* isolates:

The isolates showed great variability in terms of acervulus diameter and conidial size. The isolate Ct-07 had the largest acervulus diameter (264 micrometer). This was followed by the isolate Ct-08 (250 micrometer) while isolates Ct-04, Ct-05, Ct-09, and Ct-10 had smaller diameter averaging 223 micrometer; and isolates Ct-01, Ct-02, and Ct-06 had 162.4 micrometer. Isolate Ct-03 had the smallest diameter of 73.3 micrometer. The acervuli were either oval or spherical in shape (Table 2) (Appendix II).

The conidial length also varied considerably among the isolates. The isolate Ct-04 produced longest conidia (31 micrometer) and this

The isolate Ct-04 produced longest conidia (31 micrometer) and this was followed by the isolate Ct-10 (30 micrometer). Isolates Ct-01, Ct-02, Ct-06, Ct-07, Ct-08, and Ct-09 had relatively smaller conidia of 24.55 micrometer. The shortest conidia which measured 15 micrometer in length (Table 3) were observed in isolate Ct-03 (Appendix III).

The width of conidia among isolates of *Colletotrichum truncatum* fell into two groups. The wider conidia occurred in isolates Ct-04, Ct-05, Ct-07, and Ct-09 (7.25 micrometer) while the narrower conidia were found in isolates Ct-01, Ct-02, Ct-03, Ct-06, Ct-08, and Ct-10 (2.72 micrometer) (Table 3) (Appendix IV).

Table 2. Shape and size of acervulus of *Colletotrichum truncatum* (Schwein) Andrus and Moore isolates grown on water agar at 25°C [a].

	ACERVULUS			
	SHAPE	DIAMETER (µm)		
ISOLATES		Minimum	Maximum	Means
Ct-01	Spherical	100	140	130.0 e*
Ct-02	Spherical	150	230	180.0 d
Ct-03	Spherical	50	90	73.3 f
Ct-04	Spherical	160	270	217.0 c
Ct-05	Spherical	170	310	230.0 bc
Ct-06	Spherical	150	210	177.3 d
Ct-07	Oval/spherical	220	300	264.0 a
Ct-08	Spherical	220	280	250.0 ab
Ct-09	Oval/spherical	150	270	220.0 c
Ct-10	Spherical	200	230	225.0 c
Average		157	233	196.66
LSD	3.23			
CV (%)	5.03			

* Means followed by the same letter were not significantly different at 0.01 probability level according to Duncan Multiple Range test.

[a] Means of 10 observations from each replication.

Table 3. Measurements of conidial size of *Colletotrichum truncatum* (Schwein) Andrus and Moore isolates produced on water agar at 25 ° C [a].

Isolates	Length and width of conidia (μm)					
	Length			Width		
	minimum	maximum	mean	minimum	maximum	mean
Ct-01	20	30	25.0 ab*	2	5	3.00 b**
Ct-02	20	30	25.0 ab	2	6	3.67 b
Ct-03	10	25	15.0 c	1	4	2.00 b
Ct-04	25	39	31.0 a	5	10	7.00 a
Ct-05	30	31	29.7 ab	5	10	7.67 a
Ct-06	20	25	24.0 ab	3	5	3.00 b
Ct-07	20	30	25.0 ab	5	10	7.33 a
Ct-08	20	30	23.0 b	2	4	1.67 b
Ct-09	21	33	25.3 ab	5	10	7.00 a
Ct-10	30	31	30.0 ab	2	5	3.00 b
Average	21.6	30.4	23.13	3.2	6.9	4.53
LSD			6.59			2.20
CV (%)			15.16			20.67

* Means followed by the same letter were not significantly different at 0.01. probability level according to Duncan Multiple Range Test.

** Means followed by the same letter were not significantly different at 0.05 probability level according to Duncan Multiple Range Test.

[a] Means of 10 observations from each replication.

4.3. Colony growth of *Colletotrichum truncatum* isolates:

The colony diameter of *Colletotrichum truncatum* differed significantly on the second day of growth in the media (Appendix I). Isolates, Ct-09 and Ct-04 had the highest growth rate with an average diameter of 29.7 millimeters; isolates Ct-01 and Ct-03 had the lowest diameter of 9.15 millimeter; while other isolates for example Ct-02, Ct-05, Ct-06, Ct-07, Ct-08, and Ct-10 did not differ markedly in their growth on PDA (Fig. 1).

On the fourth day of growth on PDA, isolate Ct-09 produced the largest colony with a average diameter of 69.3 millimeter followed by isolates Ct-04 and Ct-05 (average diameter 48.7 millimeters) while isolates Ct-02, Ct-07 and Ct-10 produced smaller colonies (average diameter 38.3 millimeters). Isolates Ct-01, Ct-03, Ct-06, and Ct-08 produced small colonies of 29.25 millimeter diameter (Fig. 1).

On the sixth day of growth on PDA, isolates Ct-09, Ct-04, and Ct-05 had the largest colony diameter of 75.3 millimeters and these were followed by isolates Ct-02, Ct-07, and Ct-10 which produced relatively smaller colonies of 57.9 millimeter diameter and the remaining isolates Ct-03, Ct-06, Ct-08 and Ct-01 developed smallest colonies averaging 43.57 millimeters in size (Fig. 1).

After eight days of colony growth on PDA, isolates Ct-04, Ct-05,

Ct-07, and Ct-09 developed largest colony diameter (average 82.18 millimeters); isolates Ct-02 and Ct-10 produced relatively smaller colonies of 65.45 diameter while Ct-01, Ct-03, Ct-06, and Ct-08 had slower growth rates and hence produced much smaller colonies of 53.4 millimeters diameter (Fig. 1).

Examining the pattern of growth of *Colletotrichum truncatum* isolates on MEA, it was noted that there was a general reduction in growth of colonies in all isolates. This trend remained unchanged compared to PDA on second, fourth, sixth and eighth day of observations, (Fig. 2)

The studies reveal that the medium PDA was better in facilitating growth of *Colletotrichum truncatum* isolates than medium MEA.

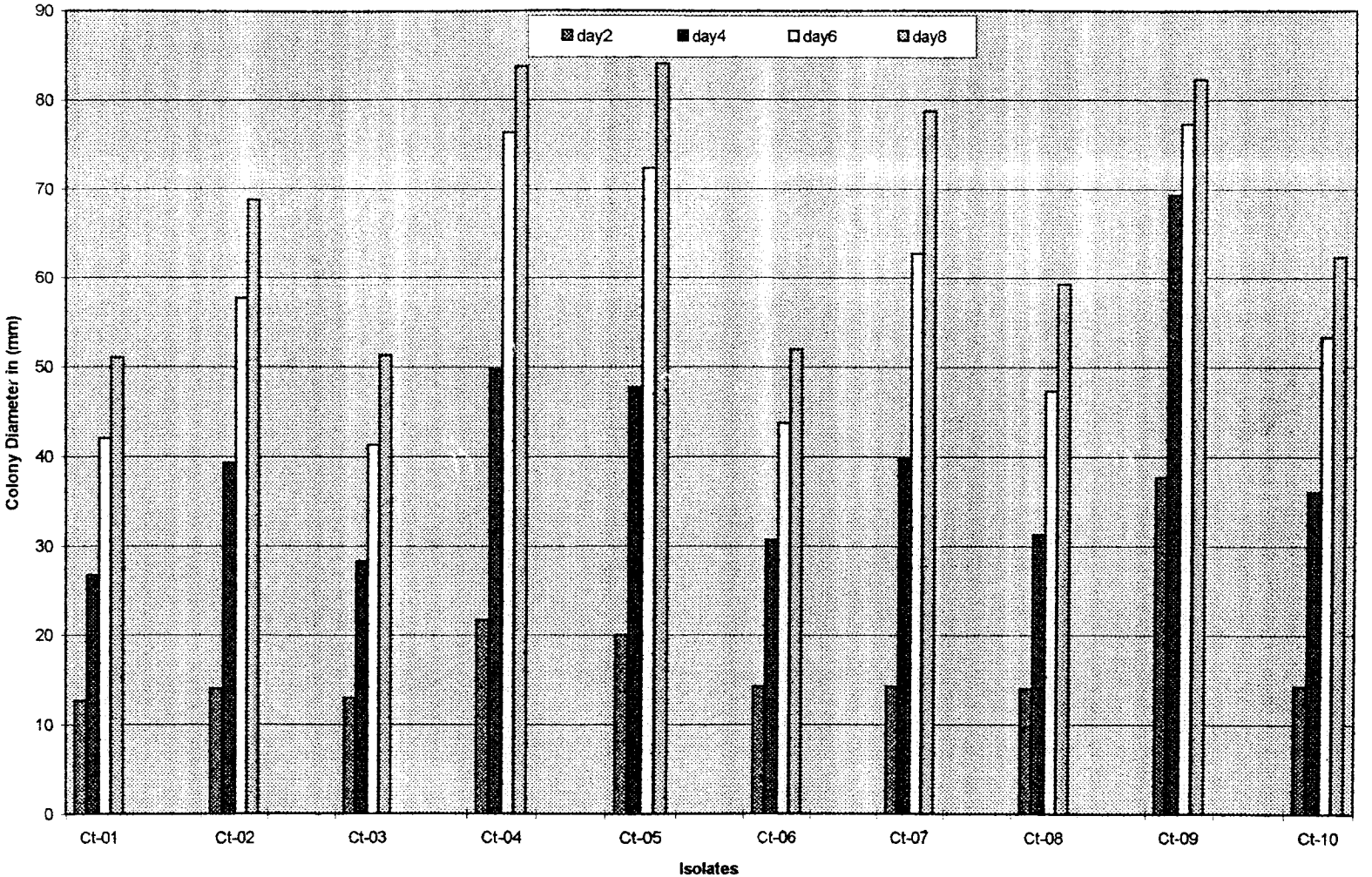

Figure 1. Growth pattern of *Colletotrichum truncatum* isolates on PDA in relation to time (Days).

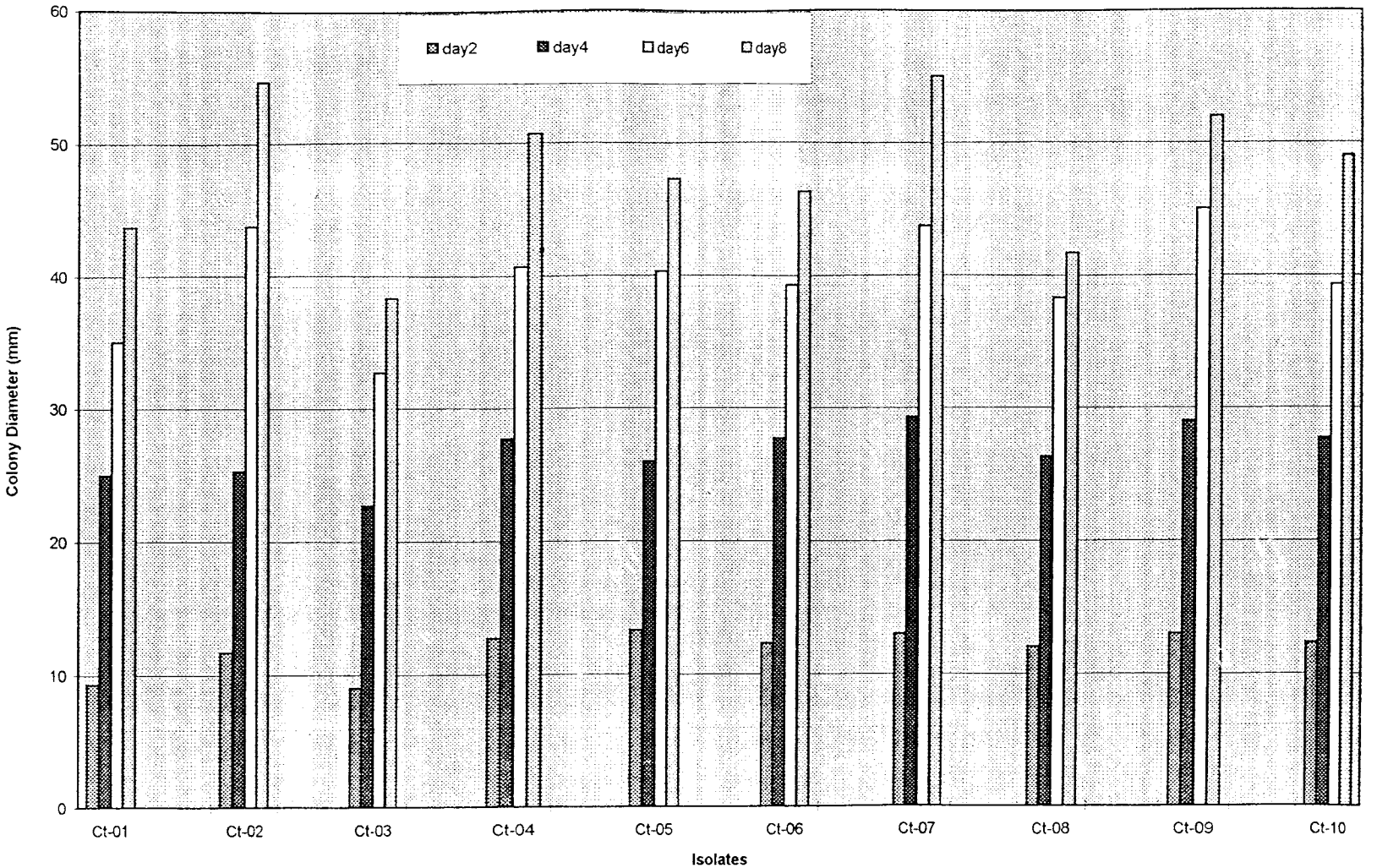

Figure 2. Growth pattern of *Colletotrichum truncatum* isolates on MEA in relation to time (Days).

4.3.1 Colour of aerial and submerged mycelium in isolates of *Colletotrichum truncatum:*

The aerial mycelium was white which changed to grey as colonies grew older in all isolates on PDA. The same colour changes were also observed in isolates Ct-04, Ct-05, Ct-06, Ct-07, and Ct-10 on MEA. However, the isolate Ct-01 developed at first brown aerial hyphae which changed to grey while in the isolate Ct-03 the aerial hyphae were first yellowish to whitish and then greyish and in the isolate Ct-09 the aerial hyphae were grey to brown and in Ct-02 reddish yellow to grey to brownish (Table 4 and 6).

The colour of submerged hyphae in all isolates showed differences of colour on the two media as shown in table 5 and 7.

4.3.2 Production of acervulus in isolates of *Colletotrichum truncatum*:

Isolates differed considerably in respect to time taken to produce acervulus on the two media used in the study. The results are shown in Table 8.

4.3.3 Colony margin and conidial ooze colour in isolates of *Colletotrichum truncatum*:

The results are described in Table 9. The colour of conidial mass was yellowish in isolates Ct-04, Ct-05, Ct-07, Ct-09 and Ct-02 and pinkish in Ct-01, Ct-03, Ct-06, Ct-08, and Ct-10. On PDA colony margins of Ct-04, Ct-05, Ct-07, Ct-09, and Ct-02 were sinuate while those of Ct-01, Ct-03, Ct-06 and Ct-08 were dentate and that of Ct-10 crenulate. On MEA colony margins of Ct-06 and Ct-08 were crenate while Ct-01, Ct-02, Ct-03, Ct-04, Ct-05, Ct-07, Ct-09, and Ct-10 all were sinuate.

Table 4. Colour of aerial hyphae of *Colletotrichum truncatum* (Schwein) Andrus and Moore isolates on Potato Dextrose Agar (PDA) at 25 ° C.

Isolate	After culturing for			
	2	4	6	8 days
Ct-01	Whitish	whitish	brownish	brownish
Ct-02	Whitish	greyish	greyish	greyish
Ct-03	Brownish	whitish	greyish	greyish
Ct-04	Whitish	whitish	whitish	whitish
Ct-05	Whitish	whitish	whitish	whitish
Ct-06	Whitish	whitish	whitish	whitish
Ct-07	Whitish	whitish	whitish	whitish
Ct-08	Whitish	whitish	whitish	whitish
Ct-09	Whitish	whitish	brownish	brownish
Ct-10	Whitish	whitish	greyish	greyish

Source of colony colours: Munsell soil colour charts (Appendix VIII).

Table 5. Colour of submerged hyphae of *Colletotrichum truncatum* (Schwein) Andrus and Moore isolates on PDA at 25 °C.

Isolate	After culturing for			
	2	4	6	8 days
Ct-01	Brownish	brownish	brownish	brownish
Ct-02	Olive green	olive green	olive green	olive green
Ct-03	Reddish	greyish	greyish	greyish
Ct-04	Yellowish	whitish	whitish	whitish
Ct-05	Whitish	brownish	brownish	brownish
Ct-06	Whitish	whitish	greyish	greyish
Ct-07	Whitish	brownish	brownish	brownish
Ct-08	Whitish	whitish	whitish	whitish
Ct-09	Whitish	whitish	pinkish	pinkish
Ct-10	Whitish	olive green	olive green	olive green

Source of colony colours: Munsell soil colour chart (Appendix VIII).

Table 6. Colour of aerial hyphae of *Colletotrichum truncatum* (Schwein) Andrus and Moore isolates on Malt Extract Agar (MEA) at 25 °C.

Isolate	After culturing for			
	2	4	6	8 days
Ct-01	Brownish	whitish	greyish	greyish
Ct-02	Reddish	greyish	brownish	brownish
Ct-03	Yellowish	whitish	greyish	greyish
Ct-04	Whitish	whitish	greyish	greyish
Ct-05	Whitish	whitish	greyish	greyish
Ct-06	Whitish	whitish	grey-brown	grey-brown
Ct-07	Whitish	whitish	greyish	greyish
Ct-08	Whitish	whitish	greyish	greyish
Ct-09	Greyish	greyish	brownish	brownish
Ct-10	Whitish	whitish	greyish	greyish

Source of colony colours: Munsell soil colour chart (Appendix VIII).

Table 7. Colour of submerged hyphae of *Colletotrichum truncatum* (Schwein) Andrus and Moore isolate on MEA at 25 °C.

Isolate	After culturing for 2	4	6	8 days
Ct-01	Reddish	brownish	brownish	brownish
Ct-02	Reddish	olive green	olive green	olive green
Ct-03	Reddish	greyish	greyish	greyish
Ct-04	Olive green	olive green	olive green	olive green
Ct-05	Olive green	olive green	olive green	olive green
Ct-06	Whitish	whitish	olive green	olive green
Ct-07	Olive green	olive green	olive green	olive green
Ct-08	Whitish	whitish	olive green	olive green
Ct-09	Olive green	olive green	olive green	olive green
Ct-10	Whitish	whitish	brownish	brownish

Source of colony colours: Munsell soil colour chart (Appendix VIII).

Table 8. Acervulus production in isolates of *Colletotrichum truncatum* (Schwein) Andrus and Moore on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) at 25 ° C*.

	MEDIUM					
	PDA			MEA		
	Days after culturing			Days after culturing		
Isolate	2	4	6	2	4	6
Ct-01	*				*	
Ct-02			*			*
Ct-03	*			*		
Ct-04	*					*
Ct-05	*					*
Ct-06	*			*		
Ct-07	*					*
Ct-08	*				*	
Ct-09	*					*
Ct-10	*					*

* The day when acervulus formation started.

Table 9. Colony margin and conidial ooze colour in isolates of *Colletotrichum truncatum* (Schwein) Andrus and Moore after ten days of growth on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) at 25 ° C.

	PDA		MEA	
Isolate	margin	ooze	margin	ooze
Ct-01	Dentate	pink	sinuate	pink-grey
Ct-02	Sinuate	pale-yellow	sinuate	pale-yellow
Ct-03	Dentate	pink-grey	sinuate	pink-grey
Ct-04	Sinuate	yellow	sinuate	none
Ct-05	Sinuate	yellow	sinuate	none
Ct-06	Dentate	pink-	crenate	none
Ct-07	Sinuate	yellow	sinuate	none
Ct-08	Dentate	pink-grey	crenate	pink-grey
Ct-09	Sinuate	yellow	sinuate	none
Ct-10	Crenulate	none	sinuate	pink-grey

Source of margin type: Dictionary of the fungi by Hawksworth *et al.* (1983).

Source of Colours: Munsell soil colour chart (Appendix VIII).

4.4. Conidial germ tube growth among *Colletotrichum truncatum* isolates:

Length of germ tubes of conidia incubated for eight hours were recorded for the ten isolates and the results shown in Table 10 reveal marked differences among them.

Isolates differed considerably in their growth during phases I and II. Growth rates were of the same magnitude in both phases for isolates Ct-04 and Ct-10, though the former appears to be more vigorous than the later. In all other isolates considerably more growth was noted in period II. On the basis of growth of germ tube per hour, significant differences were found in most isolates (Table 11) (Appendix V).

Microscopic examination of conidial germ tube elongation showed a remarkabe growth behaviour in isolates Ct-01, Ct-03, Ct-06, and Ct-08. The conidial germ tubes of these isolates grew forward at first and then abruptly turned backward, thus producing germ tubes with a zig-zag course.

Table 10. Conidial germ tube growth (in μm) among isolates of *Colletotrichum truncatum* (Schwein) Andrus and Moore on water agar at 25 ° C.

Isolate	Time in hours		
	4	6	8
Ct-01	10.83[a]	14.67[a]	26.67 [a]
Ct-02	6.67	13.73	29.50
Ct-03	9.67	15.47	34.00
Ct-04	7.17	27.73	48.00
Ct-05	10.23	17.23	41.50
Ct-06	5.10	12.67	24.17
Ct-07	10.90	18.33	41.33
Ct-08	13.23	14.67	24.83
Ct-09	11.17	25.60	46.77
Ct-10	10.00	21.67	33.33

[a] Means of 10 observations from each replication

Table 11. Conidial germ tube length (μm) among isolates of *Colletotrichum truncatum* (Schwein) Andrus and Moore at different phases of growth and growth rate per hour at 25 °C on water agar.

	Mean germ tube growth between		
	4 and 6 hours	6 and 8 hours	
Isolates	Period I	Period II	Growth/hour [a]
Ct-01	3.84	12.00	7.92 cd
Ct-02	7.06	15.77	11.41 abcd
Ct-03	5.80	18.53	12.16 abcd
Ct-04	20.56	20.27	20.41 a
Ct-05	7.00	24.27	15.63 abc
Ct-06	7.57	11.50	9.53 bcd
Ct-07	7.43	23.00	15.21 abcd
Ct-08	1.44	10.16	5.80 d
Ct-09	14.43	21.17	17.80 ab
Ct-10	11.67	11.66	11.66 abcd
LSD	8.598		
CV (%)	0.00		

[a] Means followed by the same letter were not significantly different at 0.01 probability level according to Duncan Multiple Range Test, being the average of the two growth periods.

4.5. Pathogenicity of isolates of *Colletotrichum truncatum*.

In pathogenicity tests, isolates of *Colletotrichum truncatum* differed in their ability to cause infection in soyabean cultivars. Isolates Ct-01, Ct-07 and Ct-09 developed infections on Cultivars Kaleya, Hernon-147 and Santa Rosa; isolates Ct-02, Ct-04 and Ct-08 infected only Kaleya and Hernon-147; isolates Ct-05 and Ct-10 caused infection in Kaleya and Santa Rosa while Ct-03 infected only Kaleya and Ct-06 only Santa Rosa (Table 12) (Appandix VI).

Examining Table 12, it is noticed that isolates do not produce lesions of the same size on soyabean cultivars. Lesion size showed a range of 1.67 to 12.33 millimeter. Isolate Ct-04 produced the largest lesion on Hernon-147 (12.33 millimeter) and this was followed by isolates Ct-01 and Ct-06 both of which produced lesions of 8.33 millimeter on cultivar Santa Rosa.

It is also noted (Table 12) that soyabean cultivar Kaleya is more susceptible to most isolates while cultivars Hernon-147 and Santa Rosa possess some degree of resistance against four isolates of *Colletotrichum truncatum*. The correlation analysis between colony diameter on PDA after eight days of growth, with lesion size on Kaleya and Hernon-147 cultivars was positively correlated (+ 0.658) and (+ 0.438) respectively. Cultivar Santa Rosa was negatively correlated with colony diameter on PDA (- 0.190) with negative slope.

Table 12. Lesion size among isolates of *Colletotrichum truncatum* (Schwein) Andrus and Moore on soyabean cultivars after 15 days from inoculation.

Isolates	Lesion size in mm [a]		
	Cultivars		
	Kaleya	Hernon -147	Santa Rosa
Ct-01	2.67 def*	3.00 cdef	8.33 b
Ct-02	3.00 cdef	5.00 cd	0.00 f
Ct-03	3.67 cde	0.00 f	0.00 f
Ct-04	6.33 bc	12.33 a	0.00 f
Ct-05	0.00 f	0.00 f	8.33 b
Ct-06	5.67 bcd	0.00 f	4.67 cde
Ct-07	5.67 bcd	1.67 ef	4.67 cde
Ct-08	3.00 cdef	4.67 cde	0.00 f
Ct-09	4.33 cde	6.00 bcd	5.00 cd
Ct-10	5.00 cd	0.00 f	5.33 bcd
LSD	2.823		
CV (%)	35.86		

* Means followed by the same letter were not significantly different at 0.01 probability level according to Duncan Multiple Range Test.

[a] Average of three replications.

DISCUSSION

The symptoms of red leaf blotch of soyabean as described by Hartman *et al.* (1987) appear to be closely related to those described for anthracnose of soyabean by Dhingra (1975) and Allen (1983). The presence of reddish brown spots and necrotic lesions on the veins of the soyabean leaf could lead to the confusion whether the disease is red leaf blotch or anthracnose of soyabean. The proper identification of the causal organisms and their symptoms on soyabean are very important in so far as the two diseases are concerned.

Isolates of *Colletotrichum truncatum*, the cause of anthracnose of soyabean in Zambia, possess considerable variability both in morphological and physiological terms.

Morphological and physiological variability was ascertained on the basis of growth of isolates on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA), and on colony colour, acervulus diameter, conidial colour *en masse* (conidial ooze), conidial size and rate of conidial germination and pathogenicity of *Colletotrichum truncatum* to selected soyabean cultivars.

The acervulus shape and size varied among isolates and are comparable with those reported by Holliday (1980) as spherical or oval with diameter of 90 -300. The variation in shape and size of

acervulus seems to be an inherent feature of isolates of *Colletotrichum truncatum*.

The size of conidia also varied and measurements again are comparable with those reported by Dhingra, (1975); Tiffany and Gilman (1954), and Boyette (1988). However, four isolates Ct-04, Ct-05, Ct-07, and Ct-09 showed much greater variability in conidia width than reported previously. These differences among isolates of *Colletotrichum truncatum* clearly signify their inherent variability.

The results on growth rate of *Colletotrichum truncatum* on PDA resemble those reported by Dhingra, (1975) and Tiffany and Gilman (1954). In general there was higher growth rate in PDA than in MEA especially on 6th and 8th day observations. This could be attributed to the presence of necessary amounts of carbon in PDA (Cole and Kendrick, 1981).

Different growth rates among isolates of *Colletotrichum truncatum* again appear to be influenced by their inherent characters. Growth rate was higher in isolates Ct-04, Ct-05, Ct-07, Ct-09, Ct-02, and to some extent Ct-10 than in isolates Ct-01, Ct-03, Ct-06 and Ct-08 in PDA. Isolates with high growth rate might have used nutrients in the medium more efficiently than those with poor growth rate. Isolates with poor growth rate showed a remarkable type of hyphal growth, whereas their hyphae grew forward and then turned backward

from germinating conidia which could resulted in the reduction of colony diameter. The observed phenomenon cannot be explained by existing knowledge.

The colour of aerial and submerged hyphae also showed variation and appear similar in nature to those reported by Tiffany and Gilman (1954), but were slightly different to those reported by Dhingra (1975). The change in the colour of the aerial and submerged hyphae could be a phenomenon controlled by either age or altering physiology. It is difficult to explain.

Differences of time taken in the production of acervulus could arise either due to age or nutrition. Since acervuli in most cases developed near the center and away from the colony margin, the role of utilized nutrients might be considered to be of great importance in the manifestation of this behaviour. The investigations of Griffin (1981), together with that of Cole and Kendrick (1981) who reported that sporulation occurs where the nutrient levels are reduced in the colony support this view. The differences in sporulation were shown much more in MEA than in PDA. In this study, sporulation could also be associated with growth rates. The isolates with poor growth rates, sporulated earlier on MEA and could probably be related to zig-zag growth type which might lead to earlier exhaustion of nutrients of the medium.

The colour of conidia *en masse* varied and are comparable with those

reported by Tiffany and Gilman (1954), Holliday (1980), and Boyette (1988).

Differences again existed among isolates in the margin type which was either sinuate or crenate. The former type was observed in 80 percent of the isolates whereas the later type was found in 20 percent cases on MEA. Sinuate type of margin was found in 50, dentate in 40 and crenulate in 10 percent of all the isolates studied on PDA. The variation in margin types among isolates could also be associated with growth rates. Isolates having higher growth rate developed sinuate type of edge while those with poor growth rates produced either dentate or crenulate margin on PDA. The ability of the isolates to differ in the contour of the margin again, demonstrates their variability.

Isolates showed different growth rates of germ tubes. Two isolates had the same growth rate in period I and period II though overall growth was highest in Ct-04 and significantly less in Ct-10. In other isolates the growth was generally less during period I but it increased several fold in period II. Significant differences were also recorded among isolates on the basis of their growth on hourly basis. The tendency of anthracnose isolates to differ, in their growth pattern and growth rate of germ tubes per hour basis, again demonstrates that tremendous variability exists in the populations of *Colletotrichum truncatum* in Zambia.

On pathogenicity of *Colletotrichum truncatum*, Sinclair (1991) reported that most soyabean plants are susceptible to this pathogen at all stages of growth. The disease symptoms may occur at any time on soyabean plants but in most cases will appear during early reproductive stages. In this study, however, isolates showed differences in their ability to cause infection. The Kaleya cultivar was found to be very susceptible to all isolates of *Colletotrichum truncatum*. Only few isolates were able to infect all the three soyabean cultivars. Soyabean cultivar Hernon-147 and Santa Rosa have shown to possess some degree of resistance against the pathogen since only four isolates were able to infect them. Sinclair (1991) reported that soyabean cultivars which show no symptoms after inoculation with *Colletotrichum truncatum* might experience latent infection which was key to the presence of resistance in such cultivars. The presence of resistance in soyabean to *Colletotrichum truncatum* was also reported by Nelson (1979) who showed that the latent infection was a non-race-specific or horizontal-type of resistance, since such resistance is effective to some degree against most or all races of the pathogen involved. In this study all the ten isolates were able to infect the original host plant from which it was isolated except for the isolate Ct-06 on cultivar Kaleya.

The variation in pathogenicity among isolates of *Colletotrichum truncatum* on soyabean cultivars could also be due to wide host range. It has been reported by Herperly *et al.* (1980) that

isolates which came from weeds were more virulent to soyabean than those isolated from soyabean itself.

From these studies carried out, the *Colletotrichum truncatum* isolates collected from three provinces could be grouped into four categories based on their growth rate on PDA. The first category were faster growers which include isolates Ct-04, Ct-05, Ct-07, and Ct-09 with yellowish ooze colour and sinuate margin type. The second category was moderately faster grower isolate Ct-02 with pale yellow ooze colour and sinuate margin type. The third group was slower grower isolate Ct-10 with pinkish-grey ooze colour and crenulate margin type.The last category were slowest growers which include isolates Ct-01, Ct-03, Ct-06, and Ct-08 with pinkish ooze colour and dentate margin type.

Among four isolates which were collected from Lusaka province, three of them were faster growers, these might pose a threat to soyabean production in this province as there was positive correlation between colony growth rates and lesion size on the Kaleya and Hernon-147 cultivars of soyabean. Between these two cultivars, Kaleya was strongly correlated with colony growth rate of *Colletotrichum truncatum* isolates on PDA than Hernon-147.
Depend on the population levels of the slower growers among *Colletotrichum truncatum*, cultivar Santa Rosa could be a solution as there was negative correlation between colony diameter and lesion size.

CONCLUSION

Isolates purified by following single conidial isolation, of *Colletotrichum truncatum* showed considerable morphological and physiological variability. Morphological variation was found in relation to acervulus and conidial sizes and colony features. The growth rate was better in PDA than in MEA. The characteristics of the isolates on PDA for faster growers accompanied by other features like sinuate margin and yellow ooze colour. The moderately growers had sinuate margin with pale yellow ooze colour, while the slower growers had dentate margin type with pinkish ooze colour. On pathogenicity of isolates collected from Lusaka province, three out of four were faster growers and able to cause larger lesions on Kaleya and Hernon-147 cultivars. This means that the solution to the farmers in these provinces could be cultivars Santa Rosa. Cultivar Kaleya is not a desirable one as it was susceptible to all isolates of *Colletotrichum truncatum*.

Existence of variability of this magnitude in *Colletotrichum truncatum,* necessitates that soyabean cultivars have to be regularly screened and only those possess some resistance to the pathogen should be recommended for cultivation which possess some resistance to the pathogen. This will avoid unnecessary usage of fungicides as the only control measures hence protecting the Environment. A research should be done on effect of anthracnose to soyabean production as well as symptoms of these two diseases.

REFERENCES

Allen, D. J. (1983). The Pathology of Tropical Food Legumes. John Wiley and Sons Ltd. Chichester, New York, Brisbane, Toronto, Singapore.

Anonymous, (1992). Agricultural Statistics. Ministry of Agriculture, Food and Fisheries Planning Division Statistics Section. Lusaka. Zambia pp. 3.

Boyette, C. D. (1988). Host range and virulence of *Colletotrichum truncatum*, a potential mycoherbicide for hemp Sesbania (*Sesbania exaltata*. Plant Disease. Vol. 75 p. 62-64.

Cole, G. T. and Kendrick, B. (1981). Biology of Conidial Fungi. Vol. 2. Academic Press. New York, London, Toronto, Sydney, San Francisco.

Datnoff, L. E., Naik, D. M. and Sinclair, J.B. (1987). Effect of red leaf blotch on soyabean yields in Zambia. Plant Disease Vol. 71 (2) p. 132 - 135.

Dhingra, O. D. (ed.). (1975). Compendium of Soyabean Diseases. American Phytopathological Society. St. Paul. MN. Anthracnose of soyabean. pp. 27 - 29.

Gomez, K. A. and Gomez, A. A. (1984). Statistical Procedures for Agricultural Research (2nd edition). John Wiley and Sons. New York, Chichester, Brisbane, Toronto, Singapore.

Griffin, D. H. (1981). Fungal Physiology. John Wiley and Sons. New York, Chichester, Brisbane, Toronto, Singapore.

Hartman, G. L., Datnoff, L. E., Levy, C., Sinclair, J. B., Cole, D. L. and Javaheri, F. (1987). Red leaf blotch of soyabeans. Plant Disease. Vol. 71(2) p. 113 - 118.

Hartman, G. L. and Sinclair, J. B. (1988). *Dactuliochaeta,* a new genus for the fungus causing red leaf blotch of soyabeans. Mycologia. Vol. 80(5) p. 696-706.

Hawksworth, D. L., Sutton, B. C. and Ainsworth, G. C. (1983). Ainsworth and Bisby's Dictionary of the Fungi. Commonwealth Mycological Institute. Kew, Surrey. pp. 445.

Herpperly, P. R., Kirkpatrick, B. L. and Sinclair, J. B. (1980). *Abutilon threophrasti*: Wild host for three fungal parasites of soyabean. Phytopathology. Vol. 70. p. 307 - 310.

Holliday, P. (1980). Fungus Diseases of Tropical Crops. Cambridge University Press. London, New York, NewRochelle, Melbourne, Sydney. pp. 94 - 109.

Hunger, K. M. and Brown, D. A. (1987). Colony Colour, Growth, Sporulation, Fungicide Sensitivity and Pathogenicity to *Pyrenophora triciti-repensis*. Plant Disease Vol. 71. p. 970 - 910.

Javaheri, F. (1985). Report of Seven Months Consultancy Work. Government of Republic of Zambia. Department of Agriculture. Zambia agricultural research extension project (ZAMARE/USAID).

Javaid, I. and Ashraf, M. (1978). Some observations on soyabean diseases in Zambia and occurrence of *Pyrenochaeta glycines* on certain varieties. Plant Disease rep. Vol. 62. pp. 46 - 47.

Leakey, C. L. A. (1964). *Dactuliophora*. a new genus of Mycelia Sterilia from Tropical Africa. Trans. Br. Mycol. Soc. Vol. 47 p. 341- 350.

Kannaiyan, J. and Haciwa, H, C. (1993). Diseases of food legume crops and the scope for their management in Zambia. Plant protection bulletin Vol. 41(2) p. 73 - 90.

Kaushal, R. P. and Paul, Y. S. (1989). Inhibitory effects of some plant extract on some legume pathogens. Legume Research Vol. 12(3) p. 131-132. Abstract ATA v. 16(4) 1991. p. 90.

Manandhar, J. B., Hartman G. L. and Sinclair J. B. (1988) Soyabean Germ Plasm Evaluation for Resistance to *Colletotrichum truncatum*. Plant Disease. Vol.72 p.56-59.

Munsell soil colour charts, (1975). Munsell colour Macbeth a Division of Kollmorgen Corporation 2441 North Calvert Street Baltimore, Maryland 21218. Quoted in part from U. S. Dept. Agriculture Handbook 18-Soil Survey Manual. United State of America.

Nelson, R. R. (1979). The evolution of parasitic fitness. Pp. 23-46 In: Horstall, J.G. and Cowling, E. B. (Eds). Plant Disease. An Advanced Treatise. Vol. 4. How Pathogens Induce Disease. . Academic Press, New York.

Oladiran, A. O. (1990). The effect of fungicides and insecticides singly and in combination on the control of brown blotch, pod borer infestation and yields of *Vigna unguiculata* (L) Walp. Tropical Pest Management (UK) Vol. 36(4) p. 397-402.

Purseglove, J. W. (1968). Tropical Crops Dicotyledous. Vol. 1 and 2 Longman House Burnt Mill, Harlow, Essex, England. pp. 265-272.

Sinclair, J. B. (Ed) 1982). Compendium of soyabean diseases. 2nd ed. American Phytopathological Society. St. Paul. MN. pp. 104.

Sinclair, J. B. (1986). Soyabean disease control strategies for the tropics and subtropics. In: Shanmugasundaram, S. (Ed.). Soyabean in tropical and subtropical cropping systems . Proceedings of a symposium . Tsukuba, Japan. 26 -September to 1 October 1983. pp. 251 - 254.

Sinclair, J. B. (1991). Latent Infection and soyabean plants and seeds by fungi. Plant Disease. Vol. 75. (3). p. 220-224.

Smart, J. (1960). A guide to soyabean cultivation in Northern Rhodesia. Rhod. Agric. J. 57: p. 459 - 463.

Stewart, R. B. (1957). An undescribed species of *Pyrenochaeta* on soyabean. Mycologia Vol. 49. p. 115 - 117.

Tiffany, L. H. (1951). Delayed sporulation of *Colletotrichum* on soybean. Phytopathology. Vol. 41. p. 975 - 985.

Tiffany, L. H. and Gilman, J. C. (1954). Species of Colletotrichum from legumes. Mycologia. Vol 46. p. 52 - 75.

Toler, R. W. and Wester, R. E. (1966). A survey of lima bean diseases in the south, 1965. Plant Disease Repotr. Vol. 50. p. 316 - 317.

Wellving, A. H. A. (1984). Seed Production Handbook of Zambia. Department of Agriculture. SIDA. p. 234- 238.

Wheeler, B. E. J. (1969). An Introduction to Plant Diseases. Wiley. London. pp. 374

APPENDICES

APPENDIX 1. Analysis of Variance for mean of squares of colony diameter in relation to time for the isolates of *Colletotrichum truncatum* (growth in mm).

Source of Variation	Degree of freedom	Mean squares [a] T1[b]	T2	T3	T4
Replication	2	33.65	44.317	5.85	5.55
Medium	1	498.817**	2496.150**	4646.400**	5684.267**
Error	2	25.017	23.750	4.050	4.017
Isolate	9	107.491**	300.269**	444.156**	495.711**
MediumXIsolate	9	74.780**	213.706**	203.474**	177.489**
Error	36	21.185	33.737	13.098	9.383

a Mean squares of colony diameter

b T1, T2, T3, and T4 refer to 2, 4, 6, and 8 days after subculturing.

** The F values were significantly different at 0.01 probability level

APPENDIX II. Analysis of Variance for Acervulus diameter for isolates of *Colletotrichum truncatum* in μm.

Source of Variation	Degree of freedom	Sum of squares	mean square	F values
Replication	2	180.47	90.233	0.92 ^{ns}
Isolates	9	91671.33	10185.704	104.24 **
Error	18	1758.87	97.715	

** Significantly different at 0.01 probability level.

^{ns} Difference not significant.

APPENDIX III. Analysis of Variance for conidial length of *Colletotrichum truncatum isolates*.

Source of Variation	Degree of freedom	Sum of squares	Mean square	F values
Replication	2	156.47	78.233	5.29 *
Isolates	9	568.30	63.144	4.27 **
Error	18	266.20	14.789	

* Significantly different at 0.05 probability level.

** Significantly different at 0.01 probability level.

APPENDIX IV. Analysis of Variance for conidial width (in μm) of ten isolates of *Colletotrichum truncatum*.

Source of Variation	Degree of freedom	Sum of squares	Mean square	F values
Replication	**2**	**8.87**	**4.433**	**5.05 ***
Isolates	**9**	**156.80**	**17.422**	**19.85 ****
Error	**18**	**15.80**	**0.878**	

* Significantly different at 0.05 probability level.

** Significantly different at 0.01 probability level

APPENDIX V. Analysis of Variance for mean of squares of germ tube length for isolates of *Colletotrichum truncatum* against time.

Source of Variation	Degree of Freedom	Mean squares [a] T1[b]	T2	T3
Replication	**2**	**5.633**	**28.924**	**26.908**
Isolates	**9**	**17.551****	**70.291****	**226.703****
Error	**18**	**4.756**	**9.305**	**32.767**

** The F value were significant different at 0.01 probability level.

[a] Means of squares of germ tube size.

[b] T1, T2, and T3 refers to 4, 6, and 8 hours after growing in water agar.

APPENDIX VI: Analysis of Variance for soyabean cultivars and *Colletotrichum truncatum* isolates pathogenicity.

Source of Variation	Degree of freedom	Sum of Squares	Mean Square	F value
Replication	**2**	**11.82**	**5.911**	**4.75***
Cultivars (a)	**2**	**6.69**	**3.344**	**2.69***
Error (a)	**4**	**4.98**	**1.244**	
Isolates(b)	**9**	**169.17**	**18.796**	**11.21****
CultivarsXIsolates	**18**	**658.20**	**36.567**	**21.81****
Error(b)	**54**	**90.53**	**1.677**	

* Significantly different at 0.05 probability level.

** Significantly different at 0.01 probability level.

APPENDIX VII. Temperature in the shelter where the plants were placed at the time of inoculation in ˚C.

Date	Minimum	Maximum
10.6.94	8	30
11.6.94	8	35
12.6.94	8	35
13.6.94	6	38
14.6.94	6	31
15.6.94	6	31
16.6.94	6	27
17.6.94	6	48
18.6.94	6	47
19.6.94	6	45
20.6.94	5	45
21.6.94	5	40
22.6.94	6	46
23.6.94	10	45
24.6.94	12	30
25.6.94	12	30
26.6.94	12	30
27.6.94	12	30
28.6.94	12	30
29.6.94	13	32
30.6.94	13	32
1.7. 94	12	30
2.7. 94	13	34
3.7. 94	12	34
4.7. 94	12	30
5.7. 94	12	30
6.7. 94	14	32
7.7. 94	10	45
8.7. 94	10	45
9.7. 94	12	38
10.7.94	13	37
11.7.94	12	35
12.7.94	13	38
13.7.94	12	38
14.7.94	13	37
15.7.94	13	35

APPENDIX VIII. The Munsell soil colour chart.

HUE	VALUE/CHROMA	COLOUR NAME
10R	5/6, 5/8	RED
	4/6, 4/8	RED
10YR	8/1, 8/2	WHITE
	8/6, 8/8	YELLOW
	7/6, 7/8	YELLOW
	6/1, 5/1	GREY
	5/2	GREYISH BROWN
	5/3	BROWN
7.5YR	8/4, 7/4	PINKISH
	7/2	PINKISH GREY
5Y	8/3, 8/4	PALE YELLOW
	7/3, 7/4	PALE YELLOW
	5/3, 5/4	OLIVE
	5/6, 4/3	OLIVE
	4/4	OLIVE