STUDIES ON THE *ALTERNARIA* LEAF SPOT OF COTTON; *GOSSYPIUM* SPP., L, INDUCED BY *ALTERNARIA MACROSPORA* ZIMM.

BY

NWANOSIKE MARTIN ROYAL OKECHUKWU
B.Sc. (Hons) BOTANY
IMO STATE UNIVERSITY
OKIGWE, 1991.

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL,
AHMADU BELLO UNIVERSITY,
ZARIA.

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP PROTECTION.

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

June, 1997.

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 higher degree and that other sources consulted are clearly indicated in the references.

Nwanosike, M.R.O
M.Sc. Candidate

12-12-97
Date

Dr. A.A. Adeoti
Major Supervisor

11/12/97
Date

The above declaration is confirmed.

CERTIFICATION

This thesis entitled "**STUDIES ON ALTERNARIA LEAF SPOT OF COTTON; GOSSYPIUM SPP.L, INDUCED BY ALTERNARIA MACROSPORA ZIMM.** by Nwanosike, M.R.O. meets the regulations governing the award of the degree of MASTER OF SCIENCE, Crop Protection of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

Dr. A.A. Adeoti
Major Supervisor — 11th Dec. 1997 Date

Dr. P.S. Marley
Member, Supervisory Committee — 16-12-97 Date

Professor A.M. Emechebe
Member, Supervisory Committee — 11/12/97 Date

Dr. I. Onu
Head of Department — 17/12/97 Date

Professor
Dean of Postgraduate School — 29/4/98 Date

DEDICATION

This thesis is affectionately dedicated to lovers of good and hardwork, particularly my darling and beloved parents, Theophilus and Philomena and my uncle, Mr. Eugene I. Nwanosike in recognition of their sacrifice and relentless efforts throughout the period of this studies.

ACKNOWLEDGEMENT

It interest me to express my honest and utmost gratitude to my major supervisor, Dr. Adeoti, A.A., for his kindly guidance, advice, invaluable suggestions, thorough and critical reading of my manuscripts throughout the period of the work presented in this thesis. His interest in plant pathology gave me the encouragement to undertake this research. I also wish to express my gratitude and esteem to other members of my supervisory committee, Dr. P.S. Marley and Professor Emechebe, A.M. whose meaningful criticism and suggestions contributed immensely to the accomplishment of this intensive research on cotton disease.

The research work was partly funded and supervised by Fibre Research Programme (I.A.R) and therefore, they deserve my sincere appreciation particularly the former programme leader Professor Ogunlela, V.B. and the current one, Dr. A. A. Adeoti.

I am greatly indebted to Dr. and Dr.(Mrs.) Alabi, S. for their affectionate efforts which include supply of cotton seeds for this work, advice and field assistance to ensure that this research work is positively accomplished.

I am grateful to Mr. Auta, Mallam Gumel, James Negedu and Ibrahim Ahmadu for their technical assistance. The deep commitment of Mr. Anyawu Andrew also demands my sincere appreciation and thanks. Matthias Kasa and Theresa Anyalebechi contributed immensely to this achievement and

therefore, I remain grateful to them.

My profound gratitude goes to Mr. and Mrs. R.C. Agbakwuru ("grandfather") and his family, Dr. and Mrs. A. Ezealor and his family (my humble landlord), Chief and Lolo C.N. Agada, and Mr. and Mrs. N. Ihemeje for their relentless efforts in moral and financial assistance.

I shall not fail to thank the entire Oke Ovoro community under the chairmanship of Mr. P. Olelewe and Aboh-Mbaise Progressive union under Mr. Eddy Njoku in Zaria for their concern and assistance to my work and stay in Zaria. This work is uncompleted if I fail to acknowledge the efforts of my friend, Peter, (Mayfair Clinic, Zaria) who took care of my health from 1993 till date without anycharge. He indeed deserved my gratitude.

It is infact impossible to exhaust my deep appreciation and thanks to my beloved and darling parents, Mr. and Mrs. Theophilus Nwanosike, my uncle, Mr. and Mrs. Eugene I. Nwanosike, my brothers and only sister, Caroline Nwanosike - Onwunali whose moral and financial supports sustained me throughout the period of this research.

It is impracticable to acknowledge every one who contributed in one way or the other in this achievement, nevertheless, I express my gratitude and esteem to Mr. and Mrs. Julius Ejike, Mr. and Mrs. S. Ekperi; Mr. and Mrs. Charles Atuegbu and their families for their humility and mostly the help they rendered to me, both financially and

otherwise to enable me achieve and obtain M.Sc. in crop protection. For those whose name did not appear here but contributed, I remain grateful to you all and may God in His infinite mercy bless and reward you abundantly. Amen.

I recognize and appreciated the efforts of Ijeoma Durueke A., whose encouragement and kindness sustained me towards the tail end of this achievement.

Above all, I thank my God whose wonderful protection, goodness and mercies have strengthened me at all times despite the aggressive roaring of the enemy.

Thank you.

ABSTRACT

The incidence and severity of *Alternaria* leaf spot of cotton induced by *Alternaria macrospora* Zimm. in the northern growing zone were investigated. Field trials were conducted at Zaria, Nigeria in 1993 and 1994.

Field survey carried out in 1993 revealed that there was high incidence of the disease in all the four areas visited with an average of 75.13%. Although the disease was presented in all the surveyed areas, the incidence was highest in Daudawa (80.5%). However, the incidence and severity varied considerably between the cotton plants grown at the on-set of the rain and those planted at various times in the rainy season.

Symptomatological studies and pathogenicity test conducted in the screen-house in the Institute for Agricultural Research, Zaria confirmed that the *Alternaria* leaf spot of cotton observed was incited by *A. macrospora* Zimm. The leaf spot was amphigenous with irregular margin, brown at the centre with dark brown advancing border. The conidiophore was single, simple, straight and sometimes flexous with septation while the conidium was solitary, frequently catenate, mostly obclavate and rostrate with size of 127.5/xm (length), 21/µm (width) and beak which was more or less the same size with the body.

The varietal resistance screening trial conducted in the field and screenhouse in 1994 followed the same pattern in

terms of susceptibility to the disease. The ten varieties (comprising local and exotic lines) were susceptible to the disease, but the degree of susceptibility varied. In terms of breeding for resistance, Samcot-9, TXCDP37-HH-1-83, S-295 and GCA-NH-1-83 were found to be advisable and promising while CAM D-E and Tamcot Sp 215 were observed to be tolerant to the disease.

Four fungicides (Benlate, Delsene M, Tecto and Dithane M45) were evaluated both in the laboratory *{in vitro)* and in the field. Delsene M, Tecto and Dithane M-45 were found to completely inhibit mycelial growth (100%) of the pathogen in all the four concentrations in the laboratory, but Benlate had a 100% inhibition at 1.2 kg a.i/ha first and 0.6kg a.i/ha with 86.3% and 67% in the lower concentrations respectively. In the field, four sprays each of the four fungicide at 7-days intervals starting with 5/7 leaf stage were found to reduce incidence and severity of the disease. However, the best result was obtained from Dithane M-45 which reduced incidence from 25.4% to 12.5% and severity from 2.67 to 1.83 at 2.5kg a.i/ha in 1994. This was followed by Delsene M, Tecto and finally Benlate. Their effect resulted in the reduction of incidence and severity of the disease with significant (P = 0.05) increased grain yield when compared with control. Dithane M-45 sprays gave 2572.2kg/ha grain cotton while control was 1388.9kg/ha. Delsene M sprays at 1.2kg a.i/ha were found to control the disease but was highly phytotoxic to

the cotton under field condition. However, Delsene M at *0.6* kg a.i/ha were found to reduce disease incidence and severity without phytotoxicity.

Sowing date trials conducted in 1993 and 1994 showed that July sown crop were not heavily infected. The incidence and severity of the disease was lower and the yield high when compared with the later date of planting (August), although there were no significant (P = 0.05) differences within the treatments in yield for both years. The later planted crops suffered precipitation and this resulted to premature flowering in response to favourable photoperiodism in 1993.

TABLE OF CONTENT

Title Page i
Declaration ii
Certification.....iii
Dedication iv
Acknowledgement v
Abstract viii
Table of Contents xi
List of Tables xv
List of Plates xvii
List of Appendices xviii

CHAPTER ONE

1.0 INTRODUCTION 1
1.1 Origin and Distribution of Cotton 1
1.2 Classification of Cotton 2
1.3 Development of Cotton in Nigeria 3
1.4 Production Zones of Cotton in Nigeria 8
1.4.1 Northern Cotton Growing Zone 8
1.4.2 Eastern Cotton Growing Zone 8
1.4.3 Southern Cotton Growing Zone 8
1.5 Climate and Soil Requirement 10
1.6 Utilization of Cotton 10
1.7 Diseases of Cotton in Nigeria 12

CHAPTER TWO

2.0 LITERATURE REVIEW 15

2.1 Classification and Distribution of *A. macrospora* Zimm. 15

2.2 Symptomatology 15

2.3 Description of the Fungus 16

2.4 Economic Importance of *Alternaria* Leaf Spot of Cotton 17

2.5 Pathogen Survival and Dissemination 18

2.6 Weather Conditions that favour the Disease Development 19

2.7 Effect of the Disease on Cotton 21

2.8 Host Range and Varietal Resistance 21

2.9 Control of *Alternaria* Leaf Spot of Cotton 23

CHAPTER THREE

3.0 PRELIMINARY STUDIES 26

3.1 Materials and Methods 26

3.2 Introduction 26

3.1.2 Symptomatology and Collection of Samples 26

3.1.3 Isolation and Identification of the Pathogen 27

3.1.4 Pathogenicity Test 28

3.1.4.1 Inoculation with Conidia Suspension 28

3.1.5 Spore Size Measurement 29

3.2 Results and Discussion 29

3.2.1 Symptomatology 29

3.2.2 Isolation and Morphological Studies of the Fungus.30

3.2.3 Pathogenicity Studies 32

3.2.4 Spore Size of *Alternaria macrospora* 32

CHAPTER FOUR

4.0 SURVEY FOR THE INCIDENCE OF *ALTERNARIA* LEAF SPOT OF COTTON IN ZARIA AND ITS ENVIRONS 38

4.1 Materials and Methods 38

4.1.1 Areas Surveyed 38

4.1.2 Survey Method 38

4.1.3 Disease Assessment 39

4.2 Results and Discussion 39

CHAPTER FIVE

5.0 SCREENING COTTON CULTIVARS FOR RESISTANCE TO *ALTERNARIA* LEAF SPOT 42

5.1 Introduction 42

5.2 Materials and Methods 42

5.2.1 Screening in the Glasshouse 42

5.2.2 Screening in the Field 44

5.2.2 1 Inoculation of the Plants in the Screenhouse ..43

5.3 Results and Discussion 48

5.3.1 Screening in the glass house 48

5.3.2 Screening in the Field 51

CHAPTER SIX

6.0 EVALUATION OF SOME FUNGICIDES FOR THEIR EFFICACIES IN CONTROLLING *ALTERNARIA* LEAF SPOT OF COTTON 61

6.1 Introduction 61

6.2 Materials and Methods 63

6.2.1 *In vitro* Assessment of the Effect of Four Fungicides on Growth of *A. macrospora* 63

6.2.2 Evaluation of effects of Four Fungicides on the Control of *Alternaria* Leaf Spot under Field Condition 65

6.3 Results and Discussion 67

6.3.1 *In vitro* Result 67

6.3.2 Field Result 75

CHAPTER SEVEN

7.0 EFFECT OF SOWING DATES ON THE INCIDENCE AND SEVERITY OF *ALTERNARIA* LEAF SPOT OF COTTON 82

7.1 Introduction 82

7.2 Materials and Methods 82

7.3 Results and Discussion 84

SUMMARY AND CONCLUSION 89

LITERATURE CITED 95

APPENDICES 104

LIST OF TABLES

TABLE

1. The Trend in Cotton Production for Four Decades. Production Level of Cotton Lint Between 1941-1980....7

2. Incidence of *Alternaria* Leaf Spot of Cotton in Zaria and its Environs. 2nd Week of September to First Week of October 41

3. Ten Cotton Cultivars (both local and exotic) Screened in 1993 and 1994 for Resistance to *Alternaria* Leaf Spot. 47

4. Evaluation of 10 Cotton Cultivars Susceptibility to *Alternaria* Leaf Spot Disease under Screenhouse Condition in 1994 50

5. Evaluation of 10 Cotton Cultivars Susceptibility to *Alternaria* Leaf Spot under Field Condition in 1993 55

6. Evaluation of *Alternaria* Leaf Spot of Cotton at Cotyledon Stage at 3 Weeks after Planting in 1994.....58

7. Evaluation on the Effect of *Alternaria* Leaf Spot on Different Stages of the 10 Cultivars in 1994.....59

8. *Formulation, Chemical Nature, Common and Trade* Names of Fungicides Evaluated in the Laboratory and Field for their Efficacies against *A. macrospora* 62

9. Four Concentrations of each of the Fungicide Assessed *In vitro* 63

10. *In vitro* Assessment of Four Fungicides for their Efficacy in Controlling *Alternaria* Leaf Spot of Cotton 70

11. Effect of Foliar Application of Four Fungicides of Two Concentrations each on the Incidence and Severity of *Alternaria* Leaf Spot of Cotton in 1993...80

12. Effect of Foliar Application of Four Fungicides at Two Concentration each on the Incidence, and Severity of *Alternaria* Leaf Spot and Yield of Cotton in 1994

12. *Effect of Foliar Application of Four Fungicides* at Two Concentration each on the Incidence, and Severity of *Alternaria* Leaf Spot and Yield of Cotton in 1994...81

13. Evaluation of the effect of Date of Planting on the Incidence and Severity of the *Alternaria* Leaf Spot of Cotton in 1993 87

14. Effect of Dates of Planting on the Incidence, and Severity of *Alternaria* Leaf Spot of and Yield Cotton in 1994 88

LIST OF PLATES

PLATES

1. Developmental Stages of *Alternaria* Leaf Spot of Cotton.....33

2. A Section of an Infected Leaf Showing some fully Developed, Coalesced and Spots with Cracked Centre. 34

3. Cultural Characteristics of *Alternaria macrospora* on PDAS at 7-days after Inoculation 35

4. Cultural Characteristics of *A. macrospora* on PDAS at 21-days after Inoculation (front view) 36

5. Solitary Conidia of *A. macrospora* in culture with Mycelia (x50) 37

6. Effect of different Concentration of carbendazim + Maneb on the Mycelial Growth of A. *macrospora* in PDAS Medium.....71

7. Effect of different Concentration of Thiabendazole TBZ on the Mycelial Growth of *A. macrospora* in PDAS Medium.....72

8. Effect of different Concentration of Mancozeb on the Mycelial Growth of *A. macrospora*in PDAS Medium.....73

9. Effect of different Concentration of Benonyl on the Mycelial Growth of *A.macrospora*in PDAS Medium.....74

10. A Cross Section of Cotton Plants Scorched and Killed by Spraying with Delsene M at 1.2kg/ha during the Vegetative Stage.....78

LIST OF APPENDICES

APPENDIX

1. Preparation of Potato Dextrose Agar (PDA) Media. 'a' and 'b' used to Isolate the Pathogen 104

2. Length, Width and Beak of the Conidia Obtained at Random Selection on the Compound Microscope View (x50) 106

3. Temperature, Relative Humidity and Rainfall Distribution in Months During 1993 and 1994 Growing Seasons 109

4. Occurrence of *Alternaria* Leaf Spot of Cotton in Perennial Cotton in Imo State. 110

5. Fungi Associated with *A. macrospora* on Cotton.111

6. Analysis of Variance for the Plant Height(cm), Lesion Diameter (mm), Number of Lesions and Ranking of the *Alternaria* Leaf Spot of Cotton Evaluated in the Screenhouse in 1994 112

CHAPTER ONE

1.0 INTRODUCTION

1.1 Origin and Distribution

Cotton (*Gossypium* Spp) is a very important fibre crop with an uncertain centre for genetic diversity (Purseglove, 1974). However, historical and archeological evidence antecetated that, the use of cotton have an unknown number of centuries (Prentice, 1972). Anons (1976) reported that the upland species of cotton *G. hirsutum* L. and *G. barbadence* grown in Nigeria were originally from Indo-China tropical regions in the old world and South and Central America in the new world. *G. herbaceum* var *africanum* ("wild ancestor") was first domesticated in southern Arabia and is found often as perennial in Ethiopia, southern Arabia and southern Baluchistan (Fryxell, 1979).

In the new world, tetraploid cotton seeds and primitive cotton fabrics have been identified in excavations by Birds Mahler in Norther Peru at Hual Prieta, about 2500BC (Hutchinson, 1959). The earliest record of cotton was in Southern Mexico, where a specimen of a large -bolled cotton plant was found about 3,500 BC (Smith, 1968).

The natural habitat of wild cotton is the arid regions in the tropical and sub-tropical zones where the young plants can grow without having to compete with luxuriant growth of humid climates. The differentiation into varieties within the

species showed that the tetraploid originated long ago, their origin has been placed at various times from cretaceous to the recent geological systems (Munro, 1987). The origin and development of cotton varieties were properly documented (Eckarardt, 1906;Watt, 1907; Gulati and Turner, 1928; Harland, 1938; ; Stebbins, 1947).

1.2 Classification

Cotton belongs to the family Malvaceae, sub-family Hibiscae and genus *Gossypium* with about 30 species of annual subshrubs in tropical and subtropical regions of Africa, Asia, Australia and America (Prentice, 1972 and Purseglove, 1974).

Five cultivated species of cotton are *G. arboreum, G. herbaceum, G. hirsutum G. barbadense and G. tomentosum*; they are classified and divided into two groups: old world and new world species (Stephens, 1942). The old world group has a diploid species 2n=26 (*G. arboreum* and *G. herbaceum*) while the new world has tetraploid species 4n=52 and this included *G. hirsutum G. barbadense* and *G. tomentosa* (Skovsted, 1937; Webber, 1939). Botanical description of various species are documented (Munro, 1987).

G. hirsutum, short-staple American upland cotton is the most cultivated species in Nigeria; grown in July, August and September in the rainy season which last up to November depending on the zone. It is an annual, which grows to a height of 1.0 to 1.5 metres and about 1.5cm in diametre at the base of the stem. Phillip (1977) reported that over 2,000

cultivars have been recorded in the United States and up to 1,937 commercial cultivars has been classified based on the length of lint.

(a) Short-staple (13/16") produced by CVS of *G.barbadense* and *G. hirsutum*

(b) Medium-staple (7/8"-31/32") most upland CVS of *G. hirsutum* accounting for 20% of world cotton production.

(c) Medium long-staple (11"-13/32") mostly upland CVs of *G. hirsutum* accounting for 65% of world cotton production.

(d) Long-staple (11/18"-15/16") from the upland CVs of *G. barbadense* accounting for 5% of world cotton production.

(e) Extra long-staple (13/18") from CVs of *G.barbadense* accounting for 6% of world cotton production.

1.3 Development of Cotton Production in Nigeria

Before the advent of the American upland cotton in 1912, cotton was among the important crops cultivated in Nigeria, but with the intention to satisfy the local needs for hand weaving and spining industry. The main centres were Kano in the north and Iseyin in the south-west (Oyo State) (Agboola, 1979).

Cotton was exported from south-west of Nigeria as early as 1865 (French, 1926). During that time, cotton industry was based on the low yielding, perennial Asiatic origin type or old world Spp. (*G. herbaceum* and *G. arboreum*) in which commercialization was very limited (Johnson, 1960). Since early years of this century, several varieties have been

introduced from the new world; *G. hirsutum* which grows best in the northern parts of the country; *G. vitifolium* which is suitable for forest areas; and *G. barbadense* which thrives or adapted to the southern savanna. These have displaced the Asian varieties.

Allen long staple, American upland cotton was later introduced from Uganda (Poswal, 1981). The first seeds were issued to farmers in the norther Nigeria in 1916. The British Cotton Growing Association and the Department of Agriculture attempted to encourage farmers to increase cotton production, but response then was generally slow (Choyce, 1968).

Cotton production and export from Nigeria were dominated by western Nigeria between 1850s and early 1920s. However, cotton production in western Nigeria has been at a very low level since 1920s because of heavy insect attack on Allen long staple varieties (Agboola, 1979).

The Commercial cotton growing in Nigeria by the British Cotton Growing Association (BCGA) commenced in 1903. The venture was set up to assist in finding new source of cotton supply to Lancashire cotton industry. It was financed by industries and labour organization in Lancashire and United Kingdom government. Derrick (1976) reported that the introduction of commercial cotton was particularly to provide a commercial crop suitable for mechanized textile factories of Europe.

In Nigeria, the association commenced operation in the

south around Ibadan and Abeokuta with the idea of plantation production. This concept was abandoned because it was not accepted. However, the introduction of the commercial cotton (American Allen) gave encouragement to cotton commercialization and production in the north which has since replaced the southern provinces as major area of production. In 1916, farmers of south-eastern Zaria were using the distributed "Allen seed" after they were made to understand its merit over the local Asiatic types. From here, a gradual step by step "gazetting" of Allen cotton areas started (French, 1926). This process eventually gave birth to the present three cotton growing zones. Some varieties of the Allen resist diseases, mostly the pronounced bacterial blight and increased yield. Recently, most Samaru species were renamed with Samcot hence Samcot-6, Samcot-8, Samcot-9 and Samcot-10; (Alabi, personal communication).

There was progressive increase of cotton production in the northern parts of Nigeria in the decade 1920 to 1929, with about 23,458 bales of cotton produced in 1929. This was followed by a reduction in the early 1930s, with improvement in later half. In 1950s, cotton earned important foreign exchange for Nigeria and in terms of import substitution, the textile industries provided a savings of nearly one billion naira that would have been used in purchasing foreign textile goods (Abubakar, unpublished).

Poswal (1981) indicated that Cotton and Agricultural

Processors Limited (CAPL) reported successive fluctuation in production after world war II and this trend continued till late 1950s and early 1960s with some exceptions in 1957/'58 and 1960/61 seasons. A record crop of about 312,772 bales was made in 1968/69 and an all-time record of 503,640 bales in 1969/70 seasons. This was followed by a drastic fall in production immediately in 1971 (217,267 bales) to 1974 (170,608 bales) the period of prolonged drought. During the 1973/74 to 1975/76 seasons, Nigerian cotton was not traded in the international market due to drought and failure to meet the targeted production level of 300,000 bales of 1968/69. The trend was reversed by 1977 with about 443,195 bales of cotton, but again slumped to an almost all-time record for the decade of 157,280 bales in 1980 season (Table 1). Nigerian textile factories have suffered production set-back as a result of the above fluctuations. Many a time, cotton lint had to be imported to supplement local production (Poswal, 1981). By 1983, production has been on the decline but its usage by the textile mills has been on the increase. Thus the country required over 950,000 bales of lint by 1991 to become self sufficient (F.A.O., 1979).

Table 1: The Trend in Cotton Production for Four Decades.

Production Level of Cotton Lint Between 1941-1980

Decade	Crop Year	No. of Bales	Decade	Crop Year	No. of Bales
	1941	68440		1961	290984
	1942	20000		1962	159704
	1943	No		1963	281209
1941	1944	production	1961	1964	242098
to	1945		to	1965	256913
1950	1946	33800	1970	1966	250318
	1947	32620		1967	288584
	1948	18442		1968	151816
	1949	47742		1969	312772
	1950	61216		1970	503640
	1951	76390		1971	217267
	1952	113427		1972	211519
	1953	95445		1973	269002
1951	1954	140802	1971	1974	170608
	1955	185304	to	1975	281965
to	1956	152707	1980	1976	322038
	1957	141302		1977	443195
1960	1958	237375		1978	204596
	1959	173214		1979	200805
	1960	167513		1980	157280

Source: Cotton and Agricultural Processor Ltd. (CAP), Zaria

1.4 Production Zones of Cotton in Nigeria

Production of cotton is largely confined to the savanna ecological zones and extends from latitude 7°30N to about 13°N. The most suitable area for large scale production and cultivation lies between latitude 9°-12°N mostly in northern Guinea Savanna (N.G.S). The major cotton areas in Nigeria can be divided into three main zones.

1.4.1 Northern Cotton Growing Zone

This comprises Sokoto, Zamfara, Kebbi, Kaduna, Katsina, Jigawa, Kano and northern part of Niger state with 120-170 days of raining season and 300-1150mm annual rainfall. Over 20% crop land are cultivated (NAERLS, 1976). Northern zone produces about 60-65% of the nation's cotton. This zone falls within latitude 10°30N to 13°N.

1.4.2 Eastern Cotton Zone

This zone covers Gombe, Bauchi, Taraba, and Adamawa states with 150-180 days of raining season and about 900-1100mm annual rainfall and Borno and Yobe states with about 90 days raining season and about 380-640mm annual rainfall. The zone accounts for about 30-35% of the nation's total production. The eastern zone lies on latitude 8°N and 11°30'N.

1.4.3 Southern Cotton Zone

This accounts for about 5% of the cotton produced in the country. This comprises areas of southern Niger, Benue, Nassarawa, Plateau, Kwara, and Kogi states. The length of raining season is 170-210 days with annual rainfall of 1100-

1400mm.

The most important cotton producing states are Sokoto, Zamfara, Gombe, Kebbi, Kaduna, Katsina, Jigawa and Bauchi which together account for 90% of the country's total production. Little quantity is produced from the forest zone of Nigeria involving Ekiti, Ondo, Oyo, Osun, Edo and Delta states where Ishan Spp. are commonly grown. These states are called potential cotton growing areas but have insect pest problem due to high humidity. These areas account for about 5% cotton production in Nigeria (NAERLS, 1976). Agboola (1979) reported that 95% of cotton lint was produced from the three zones in 1970. These zone produce average total of 300000 bales of unit of fibre annually and this quantity is not enough for the annual demand of Nigerian textile industries of over 400000 bales (I.A.R, 1976). The mean yield of cotton is still low and is estimated to range between 165-392 kg/ha.

Zaria is the oldest of the former twelve provinces in the northern region in its association with cotton activities. The first British Cotton Growing Association (B.C.G.A) in northern Nigeria was located in Zaria, and about a decade later, another was located in Maigana. At Samaru, the Agricultural Department founded the first experimental station. This is due to the suitability of the soil and rainfall of the area for cultivation of American type of upland cotton.

1.5 Climate and Soil Requirement

Climate is the major factor that influences agricultural production (Munro, 1987). The component of climate are temperature, rainfall and duration of rain, light and relative humidity and aeration. Cotton is a tropical plant but may adapt to temperate climate. Cotton does best with high temperature, abundant, but not excessive soil moisture and high fertility. Engledow (1947) showed that rainfall is the chief determinant of the land suitability for cotton production. Irvine (1974) reported that cotton grows well in areas with moderate annual rainfall of about 650-1250mm. In the United states, Johnson (1926) reported that the most favourable temperature for optimum growth of cotton is 32°C (90°F) with corresponding warm night temperature. Generally, in the tropics cotton is not grown above an altitude of 1500m since night temperature rarely exceeds 15°C and this is too low for crop growth and development.

1.6 Utilization of Cotton

Cotton is one of the most important crops whose product and by-products play vital roles in the socio-economic and political life of a country (Poswal, 1981).

Cotton lint is the most important vegetable fibre in the world today (Purseglove, 1974). It is woven into fabrics alone or combined with other fibres. In early times, wool was the principal fibre of eastern and southern Europe, hemp in northern Europe, flax in Egypt and silk and ramie in China

(Poswal, 1981).

At the end of the 18th century, cotton provided only 41% of the world's raw material compared with 78% from wool (Purseglove, 1974). The invention of the textile mills led to a rapid expansion in the utilization of cotton. By 1890, cotton provided 78.6% of the world's textiles, increasing to about 84.2% in the period of 1924-1928 (Purseglove, 1974). Since then, production has been declining probably because of man-made competition with synthetic fibres. Cotton lint also supplies yarn, cordage, twine and tyre cord.

The fuzz provides linters for use in felts, upholstery, carpet, mattresses, wicks, surgical cotton and in chemical industries, rayon, plastic, lacquers, papers, photographic films, cellulose, explosives and sausage skins (Purseglove, 1974).

Cotton seed is commercially important because of its oil and other products. Commercial cottonseed is approximately 92% dry matter; yielding about 20% semi-drying edible oil which on extraction leaves a 42% protein cotton-cake or meal. Brown and Wares (1958) reported that cotton meal is rich in protein and carbohydrates with about 16-20% and 23% respectively.

Cotton seed oil is yellow and 20-22% by weight obtained by crushing (Irvine 1974), used in lard substitutes as salad and cooking oil, and in margarine manufacture. Low grade oil is used in the manufacture of soap, lubricants, sulphonated

oils and protective coatings. It can also be used as a substitute for more expensive olive oil.

Oyenuga(1968) reported that in the animal industry, the residual seed-cake is a valuable protein supplement for livestock. The Low grade cake is also used as manure while the whole seed may also be fed to livestock.

Cotton seed hulls are used as roughage for livestock and as source for bedding, fertilizer and fuel.

1.7 Diseases of Cotton in Nigeria

Cotton diseases form the major constraint in producing profitable crop and the progress of the disease depends on conditions unfavorable to the crop but favourable to the disease (Munro, 1987). Cotton is host to numerous fungal, bacterial, viral and nematode diseases; restrictions being placed by ecological and climatic differences (Poswal, 1981).

Most of the seedling diseases of cotton have been implicated in the vegetative and flowering stages. Bacterial blight of cotton induced by *Xanthomonas malvacearum* (E.F.M. Smith) Dowson is the only bacterial disease and is considered important in Nigeria with estimated yield loss of 15-20% (Dransfield, 1965).

Weather conditions favouring viral diseases may be quite different. The leaf roll virus in Thailand is transmitted by Aphis and dry weather favours an increase in their population. Viruses causes cotton leaf curl (C.L.C.V) or crumple virus (Munro, 1987; Idris, 1990; Wilson and Brown, 1991).

Nematodes such as root knot caused by *Meloidogyne incognita*, and *M. javanica* and reniform nematode (*Rolylenchus reniformis*) are important diseases of cotton (Sciumbato and Pinckard, 1974). Minton and Ebelhar, (1991) called them parasitic nematodes.

Fungal diseases constitute the bulk of the diseases of cotton, ranging from leaf spots, blights, wilts to bollrot (Munro, 1987). In a check-list comprising fungal diseases associated with cotton in savanna and semi-arid areas of Nigeria, Emechebe et al. (1980) showed the following as important diseases. *Alternaria* leaf spot induced by A. *macrospora* Zimm and other *Alternaria* spp. Arealate mildew (*Ramularia* leaf spot) induced by *R. areala* Ath., Anthracnose (*Colletotrichum gossypii* south), *Ascochyta* leaf spot induced by *Ascochyta* spp., stem blight (*Macrophomina phaseolina* RassiGoid), Stigmatomycosis (*Nematospora gossypii* Ashby and Nowell), Bollrot (*Rhizopus stolonifer*), Collar rot and Wilt (*Sclerotium rolfsii* sac.) and host of fungi isolated from bolls, stems and leaves.

Surveys to the cotton growing fields have shown that *Alternaria* leaf spot of cotton is an important cotton disease with incidence of 10-60% in the north and 40-100% in the eastern cotton growing zones (Adeoti, 1991). Apart from identification (Emechebe et al., 1980) and survey report from cotton growing zones by Adeoti, (1991), information on the *Alternaria* leaf spot of cotton is based on observation rather

than experimental work and the disease sometimes is discussed against the background of work reported from other countries on its effect on yield and on its severity and control.

It is in the light of this, and in order to maximize the farmers output, that the present research is directed at the following objectives:

a) surveying the major cotton producing areas in Zaria and its environs, with a view to assessing the extent of damage due to the disease,

b) isolation and identification of the pathogen and establishing its pathogenicity on cotton,

c) screening some cotton cultivars for resistance to the disease,

d) evaluating some fungicides for their efficacy in controlling the disease and

e) studying the effect of sowing date on the incidence, severity of the disease and yield of cotton.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Classification and Distribution of Alternaria macrospora Zimm

A. macrospora is an imperfect fungus belonging to the form class, Hyphomycetes, form family Moniliaceae and form order Moniliales and they reproduce by conidium. They produce conidia similar to Ascomycetes and are therefore presumed conidial stage of Ascomycetes (Alexopoules, 1962).

It is distributed to almost all the continents where cotton is grown (Cotty, 1987 a&b). *Alternaria macrospora* has been reported on cotton in Australia, Brazil, Central African Republic, China, Congo, Ethiopia, France, Ghana, Guadeloupe, India, Italy, Japan, Malawi, Morocco, Zimbabwe, Romania, Trinidad, Venezuela, Uganda, Zambia and Nigeria (Jones, 1928 and Ellis, 1971). Bashi et al.(1983) and Cotty (1987) reported that *Alternaria* leaf spot of cotton is an important disease of cotton in Israel and United states of America respectively.

2.2 Symptomatology

Alternaria spots are amphigenous on leaves (Ellis, 1971). They form small circular, brown spots each with narrow purple border; these spots may expand and later have grey dry, cracked centres (Jones, 1928). On buds, flowers and bolls small, round lesions are formed on glandular areas altering

with the point of insertion of the bracts on the receptacle. The disease cause some shedding of flowers and young bolls and on older bolls may produce a mummified condition. Munro (1987) showed that in the early stages, the spots are pale green with an irregular margin and may be mistaken for bacterial blight, but as the area enlarges, the colour of the old parts become straw yellow and then rusty brown forming irregular concentric zones in roughly circular lesions. Sciumbato and Pinskard (1974) reported that, initially the lesions are small and first appear on the older plants, brown in colour with reddish purple borders; later, the lesions coalesce resulting in considerable defoliation. The symptom of *Alternaria* leaf spot of cotton is properly documented by Bashi et al. 1983, Cotty and Misaghi, 1984; Rotem et al. 1988.

2.3 Description of the Fungus

A. macrospora was first described by Zimmerman (1904). Conidiophores form singly and sometimes in groups, erect, simple, straight or flexuous. They are almost cylindrical or tapering slightly towards the apex, septate, pale to mid brown, smooth, with one or several conidial scars up to 80μ long, 4-9μ thick (Ellis, 1971). The conidia could be solitary and sometimes in chains of two, straight or curved, obclavate. The body of the conidium is ellipsoidal tapering abruptly to a very narrow beak which is equal in length to or up to twice as long as the body. It is mid to mid-dark reddish brown,

usual minutely verruculose, with 4-9 (normally 6-8) transverse and several longitudinal or oblique septa (Jones 1928, Ellis, 1971 and Sciumbato and Pinckard, 1974). Often, there are slight constriction at the septa, and overall length 90-180 (134μ), 15-22 (17.7μ) thick in the broadest part. The beak is simple, pale, 1-1.5μ thick (Ellis, 1971).

2.4 Economic Importance of Alternaria Leaf Spot Disease of Cotton

Hillocks (1991) reported that *Alternaria* leaf spot of upland cotton is a minor disease in Zambia. Cotty (1987b) identified highly virulent isolates of *Alternaria* spp. in upland cotton (DP90) in Arizona. He further reported a wide spread outbreak of *Alternaria* leaf spot during 1982-1984 and therefore confirmed the importance of the disease in Arizona.

Padagamur,et al. (1989) established a yield loss varying from 16.6 to 60.1% with an average loss of 35.6% in Karnataka, India. This yield loss occurred between 1980-1981 and 1986-87 cotton season with jayadhar cultivar. Hiremath and Savanur (1990) worked on the biochemical changes in cotton in response to *A. macrospora* infection using both healthy and infected leaves from top and lower parts of the plants. Their result showed increases in total sugars, reducing sugars, total phenols and amino acids in diseased bottom leaves and less increase in diseased top leaves and so, they concluded that the pathogen interferes with the crop metabolism particularly

at the lower leaves. Hiremath,et al. (1990) showed yield loss of 5.35-9.48% quintals per hectare. They also proved that difference between the observed and estimated yield loss due to this disease vary from 0-5.95%.

Alternaria leaf spot reduced yield of *G. barbadense* cultivar (Pima 5-5) upto 25% (Bashi, et al. 1983). However, *A. macrospora* only causes substantial losses under conditions of high relative humidity (Pauer, 1990) and Pima cotton is grown commercially in Arizona with minor level of disease in most years (Streets, 1969). Disease severity of < 5% have no impact on the crop yield (Hiremath,et al. 1990).

2.5 Pathogen Survival and Dissemination

Bashan (1984) reported that carry over of the disease to new cotton field can be through seed contamination and debris of the previously infected crop (flowers and bolls) where they remain as thick walled conidia. Bashi,et al.(1983) showed that transmission can be through leaves and cotyledons and susceptibility decreases slightly in leaves with age.

A. macrospora survives on post season plant debris left growing at the field edges (Bashan, 1984). The pathogen is transferred from the seed, through the plant stem, to the cotyledon leaves and to sporulate on their surface (Bashan and Levanomy, 1987).

Rotem (1990) showed that overwintering of the pathogen in debris is associated with survival of the debris itself. Ling

(1944) explained that the causal fungus possess spores with higher resistance to desiccation and might have other means of overwintering than contamination or seed infection. Overwintering was better in debris located on the surface of the soil than that buried beneath the surface (Rotem 1978, 1990 and Filajdic and Sutton, T.B., 1995). Jackson (1959) established that the primary source of inoculum is the dead host materials and that buried leaf debris do not produce conidia, but mycelium which can develop and produce conidia in a moistured condition.

A. macrospora is an air-borne spore produced on debris. These spores have low infectivity and causes few lesions in their first generation, however, the second generation of the spores formed on those lesions are highly infectious (Rotem, 1990). Air-borne conidia show circadian periodicity with a peak either at noon or in the late evening (Raghuram and Mallaiah, 1989) and air-borne conidia are wind dispersed (Dransfield, 1966; Bashan and Levanony, 1991). Gregory (1958) reported that 90% of air-borne spores are deposited within 100 metres of their source and this percentage is not likely to be lower with such a large spored fungus as *Alternaria*.

2.6 Weather Conditions that Favour the Disease Development

Bashan and Levanony (1991) reported that sporulation occurred on leaves only during period of high relative humidity (R.H > 95%) and temperature ranging from 20 to 28°C.

Under such condition, mycelia penetration into the internal tissues of the leaves are rapid. Bashi,et al. (1983) showed that the pathogen is able to penetrate and infect within the relatively short dew period.

Heavy rains favoured the disease development (Hiremath,et al. 1988). Cotty (1987) and Olsen and Cotty (1986) reported that in 1982, 1983 and 1984 increased rainfall and reduced daily temperature maxima occurred and this resulted to moderate to heavy levels of *Alternaria* leaf spot in central and southern Arizona cotton. Free moisture and rain pattern increases disease pressure.

Bashi,et al. (1983) showed that temperature between 25-30°C favour infection of this disease and lesion formation was reduced after exposure to 43.5°C for 2 hours. They also observed 40-100% reduction in lesion number when the plants were incubated at elevated temperature compared with plants maintained at 30°C.

Munro (1987) established that nutrient deficiencies can sometimes increase the incidence of leaf spots by reducing the vigor of the crop. Sciumbato and Pinckard (1972) showed that *Alternaria macrospora* is favoured by moisture stress and nutrient deficiencies.

Rotem,et al. (1988) postulated that sunlight triggers the development of symptomless infections into visible lesions. Well fertilized soil with high level of nitrogen makes the crop succulent and prone to infection by leaf spot diseases

(Munro, 1987). Weather conditions that favour the development of *A. macrospora* are documented by Sinclair and Shatla, 1962 and Miller, 1969 ,Watkins, 1981.

2.7 Effects of the Disease on Cotton

Alternaria macrospora causes premature defoliation which resulted to yield loss in *G. barbadense* and when upland cotton is predisposed to infection by environmental or physiological stress, they experience defoliation (Hillocks, 1991; Sciumbato and Pinckard, 1972; Shtienberg, 1991). Hillocks (1991) reported that potassium deficiency is a predisposal factor to infection of this disease in Zimbabwe.

The disease produced toxin(s) *in vitro* and induces typical leaf spot symptoms on cotton (Krishnamohan and Vidhyasekaran, 1989). This disease may cause severe shedding of leaves and bolls and also reduce fibre quality (Bashi,et al., 1983 and Munro, 1987).

2.8 Host-Range and Varietal Resistance

Not much has been reported on the host range of *A. macrospora* causal agent of *Alternaria* leaf spot, however, Bashan and Levanomy (1991) isolated *A. alternata* from leaves of wild beets and this isolate infected cotton as well as wild and cultivated beets. The conidiaphores of the aggressive pathogen emerged only from necrotic areas of leaf tissues and sporulation occurred on leaves only during period of high RH

(> 95%) and temperature 20 to 28°C. They also observed that, plants in cotton fields adjacent to the diseased wild beet plant were infected. Based on that observation, they proposed that, the pathogen on cotton may overwinter on wild beet plants, making them important source of inoculum in epidermis of *Alternaria* blight of cotton. Chandler and Oliver (1979) reported spurred Anoda (*Anoda cristata)* as an important cotton weed in New Mexico and Arizona for 15 to 20 years and that this weed is associated with *A macrospora* with about 810,000ha been infected in United States in 1976.

Heavy infection of this weed with A. *macrospora* was reported in Western Texas (Miller,1973) and Southern United States (Sheperd,1972). Ohr, *et al.*(1977) and Walker and Sciumbato (1979) showed that spurred anoda is an important alternative host of A. *macrospora*, however, they suggested that spurred Anoda isolate may be specialized form of fungus (A. *macrospora*) which might be usefull as potential biocontrol agent for the weed. Other weeds such as velvetleaf, prickly side were reported to show hypersensitive type of reaction to A. *macrospora* while crop species like Hollyhock and Okra ("Clemson spinesless") were found to be susceptible to the fungus by Walker and Sciumbato (1979).

Little or no cotton cultivar has been reported to be absolutely or completely resistance to *Alternaria* leaf spot. However, several reports and suggestions have been made to compare the resistant of some cultivars. Cotty (1987)

reported that Deltapine 61, Acala SJ-5 and McNair 235 (*G. hirsutum*) were more resistant to the disease than CV Deltapine Acala 91. He further compared the resistance of *G. hirsutum* and *G. barbadense* and showed that *G. barbadense* (Pima S-6 and Pima S-5) were more susceptible to *A. macrospora* than *G. hirsutum* cultivars.

Bashi,et al. (1983) reported that susceptibility of Pima S-5 in cotyledons increased with age up to 20 days and decreased thereafter. The leaves susceptibility slightly decreased with age and this was attributed to both genetic and environmental factors. Adeoti,et al. (1994) showed that TXCD P37HH-1-83 was more resistant to *Alternaria* leaf spot at matured stage than the other nine cultivars tested.

2.9 Control of Alternaria Leaf Spot of Cotton

Pauer (1990) stated that no special control measures are taken; but infected materials should be picked and destroyed by burning at the end of the farming/growing season. Heat application will kill the conidiophore of the disease and subsequently destroy the resting thick conidia. This condition is known as "cotton close season" in Nigeria and "cotton free zone" in Southern Tanzania.

The use of fungicides as seed dressing and foliar application have been promising form of controlling *Alternaria* leaf spot irrespective of the fungicidal hazard to man and environment. Mancozeb and Captafol are effective for

Alternaria diseases on cotton. These fungicides reduced disease incidence from 62.1% to 22.8% and 25.5% respectively (Alagarsamy,et al. 1989 and Desai and Patil, 1983). Tebuconozole (as Raxil) and difenocozole (as Dragon) are systemic fungicides. These fungicides have been shown to delay *Alternaria* disease progress by 20-24 days as seed dressing fungicide. The effect persisted for up to 86 days after planting (Shtienberg, 1991). Shtienberg and Dreishpoun (1991) showed that difenoconazole at a rate of 0.125kg a.i/ha and tebuconazole at 0.187kg a.i/ha suppressed *Alternaria* disease to a significant extent. They also showed that Maneb (protective fungicide) at the rate of 2.0kg a.i/ha suppressed the disease with yield differences 6.3 to 26.0% increase. Desai and Patil (1983) recommended the following fungicides for the control of *Alternaria* leaf spot and other leaf spots on cotton; RH 2161 (femapanil), Panocfine (guazatine), Cuman (Zariam), Dithane M-45 (Mancozeb), Du-ter (fentinhydroide) and Blitox (Copper oxychloride). Padaganur and Basavaraj (1984) reported that soaking of cotton seeds for 1-2 hours in Ceresan (phenylmecury acetate) or Duter (fentinhydroxide) is effective for the control of *A. macrospora*. The effectiveness of these chemicals is regulated through soaking time.

Ling (1944) showed that late sowing would postpone maturity and enable the host plants escape the rainy period during late season growth. This will reduce the chances of being infected. However, early sowing in Nigeria enables the

crop to escape the disease and increase yield (Adeoti, Personal Communication).

CHAPTER THREE

3.0 PRELIMINARY STUDIES

3.1 *Materials and Methods*

3.1.1 Introduction

Douglas and Pavek (1971) reported that *Alternaria* species does not produce spores readily under laboratory condition; however, they established an efficient method which have consistently induced sporulation of many isolates of *A. solani*.

Several techniques have been used by scientists to isolate *Alternaria* spp. These attempts varied from multilation of the mycelium (Rands, 1917 and Ludwiq,et al, 1962, Barksdale, 1968), use of different culture media (Charlton, 1953), exposure to sunlight and ultraviolet light (McCallan and Chan, 1944), exposure to flourescent light (Lukens, 1960), dehydration of the mycelium and chemical treatment of the culture (Charlton, 1953). Lukens (1966) reported that light stimulates conidiophores of *A. solani* and inhibits its sporulation, but he further explained that, light temperature below 23°c enhanced sporulation of the conidiophore.

3.1.2 Symptomatology and collection of Samples

Studies of the development of symtoms on *Alternaria* leaf spot of cotton was carried out in 1993 in I.A.R, Zaria research farms. Visit were made to infected fields and observations taken on symptoms of the disease at different

stage of the crop development. Infected leaves were collected in labelled paper envelops and then taken to the laboratory for isolation of the pathogen.

3.1.3 Isolation and Identification of the Pathogen

The leaf lesions were cut off (5mm) close to the margin of the advancing lesion. This was to avoid contamination by saprophytes which would have invaded the central portion of the lesion. The leaf pieces were surface-sterilized with one percent (1%) mercuric chloride ($HgCl_2$) for 15 seconds and then rinsed in three changes of sterile distilled water, dried between filter papers and aseptically plated (3 pieces per plate) on 24 hour-old potato dextrose agar to which streptomycin (10mg/litre) had been added (PDAS) (See appendix 1). Appendix 1 (A and B) contain the two formulations of PDAS media used to induce sporulation of the fungus. This procedure was carried out under sterile condition and the plates were kept on the fumigated laboratory bench.

After 72 hours, fungal hyphae growing out of the plant materials were transferred into six fresh PDAS plates which were then incubated for two days at 26±2°C. Thereafter, heat sterilized 0.3mm-diameter inoculation loop were used to subculture and separate the different hyphal growth into separate plates and then incubated for 10-days at temperature of 28°C.

Detailed examination was carried out to determine the colony characteristics of the organism observed daily during

the 10-day growth period. The pathogen was maintained until the organism sporulated while some were preserved on PDA-slants in McCartney bottle until required. The conidiophore and conidia were also observed under x10 objective lens of the compound microscope.

3.1.4 Pathogenicity Test

Samcot-6 cotton cultivar reported to be highly susceptible (Adeoti, et al. 1994) was used for this test. Ten perforated plastic pots were filled with sand properly mixed with organic manure (cow dung). To each pot were planted four undressed cotton seeds which was thinned down to two after germination. Attempts to reisolate the fungus were made after the leaves which showed evidence of the disease symptoms were surface sterilized in 1% mercuric chloride for 15 seconds.

3.1.4.1 Inoculation with Conidia Suspension

Conidia suspension was prepared from pure culture of the pathogen on PDAS plates. The suspension was obtained by scraping 12-day old mycelial culture of *Alternaria macrospora* on PDAS into sterilize distilled water in 1000ml conical flask, using a scalpel. This was shaken vigorously and then filtered through cheese cloth. The filtrate (conidia suspension) was adjusted to approximately $1x10^6$ spore/ml using hemocytometer and a compound microscope.

At five-leaf stage, the leaves were sprayed with conidia

suspension to run-off using hand sprayer. Inoculation was done in the glass house. The inoculated pots were covered with transparent polyethylene bags (70cm long 30cm wide) immediately after inoculation. They were uncovered after every 12 hours, sprinkled with water and covered again. The essence of this was to maintain humidity and induce germination of the conidia. After 48 hours, the polyethylene bags were finally removed from the plants and observed daily for the disease symptom.

3.1.5 Spore Size Measurement

Temporary slide preparation was made from 13-day old culture and viewed under x10 objective lens of compound microscope. Measurement was done using an ocular micrometer previously calibrated with a stage micrometer. Fifty spores were selected randomly and measured for length, width and length of the beak (See appendix 2). The average of each of the three parameters was multiplied by the constant for x10 objective lens (5.0μ) to obtained the size of the conidia.

3.2 Results and Discussion

3.2.1 Symptomatology

The lesions were amphigenous on leaves (small circular) with narrow dark brown border and rusty brown at the centre of the spot (Plates I and II). The lesion expands and may have a grey, dry, cracked centre which is believed to be as a

result of environmental conditions (Plate II). In most cases, the lesions coalesce and form leaf blight (Plate II). These observations agree with earlier ones by workers on the symptoms of the disease; Sciumbato and Pinckard (1974) reported that older plants first form lesion that were small and brown with reddish purple borders. Later the lesion coalesced resulting in considerable defoliation. Ellis (1971) showed that the lesion were small circular with narrow dark brown border.

3.2.2 Isolation and Morphological Studies of the Fungus

Colony characteristics of the fungus on both PDAS formulations were similar in colour, hyphal growth rate, and number of days to sporulation. The chlamydiospores from the diseased material inoculated on PDAS plates germinated within two days of inoculation and produced hyphae. The hyphae were septate with pale to dark green in colour.

Within five days of inoculation, the colour of the medium changed to light red (Plate III). When the hyphal growth was up to 7cm (21-days), the entire medium completely turned to dark red (PLate IV). Colour change might be as a result of the pathogen utilizing the nutrients in the media. It is also important to note that, invaded portion of the leaf has a red colour (personal observation) under the microscope. Yar'adua (1986) observed a similar colour changed with *A. Porri* in Onion.

The conidiophores were found to be singly, simple, straight and flexuous. They are cylindrical towards the apex, septate, smooth with one or more conidial scars and dark in colour. This description agrees with earlier observation by Ellis (1971).

The conidia were found to attach to the conidiophore with their broad ends. At full sporulation (14-days), conidia are observed to be solitary, frequently catenate, mostly obclavate and rostrate. The body is ellipsoidal with narrow beak. The beak is equal to body length and sometimes, twice as long as the body. The conidia were also observed to occur in chains of two and three which was straight or curved. The conidia have a transverse and oblique septa (Plate V). These observations are similar to those described by Zimmerman, (1904), Jones (1928). Observation showed that the mycelia were of two different types; fluffy white colour covering over the dark green colour when the medium were light red and completely darkgreen colour when the medium was darkred. The fluffy white mycelium might be mistaken for *Fusarium* spp at the initial stage, but as the culture gets older and mycelia length increases, the hyphae completely turned darkgreen without the fluffy white appearance (Plate IV).

A. macrospora was observed to grow slowly on artificial medium.This is demonstrated by Plates III and IV which are 7 and 21-day old cultures respectively. This observation agreed with Yar'adua (1986) who worked on *A. porri* of Onion. Daily

temporary slide preparations showed that sporulation of *Alternaria macrospora* (Samaru isolate) on artificial media occurred between 10 to 11 days. This result contradicted the work of Sciumbato and Pinckard (1971) who reported that *Alternaria macrospora* sporulated within six days on the same media.

3.2.3 Pathogenicity Studies

The first visible symptom appeared between five to six days. Initially, the lesion was a pen-point, similar to bacterial blight except that it was not water soaked. This brown spot later enlarged and the fungus could be seen growing on the lesions. The advancing margin of the lesion was purple to dark brown in colour, circular and brown at the middle. The fungus was re-isolated from the lesions of the inoculated plants. These findings agreed with Ellis (1971) who showed that colonies on leaves are amphigenous and Munro (1987) showed that, the early stages of the spots are pale green with an irregular margin and may be mistaken for bacterial blight.

3.2.4 Spore Size of Alternaria macrospora

The average length and width of 50 spores was 127.5μm and 21μm, respectively. The average length of 50 beaks was 72.4μm, but in most cases, the beak were observed to have the same length with the spore body (See appendix 2). Spores were minutely verruculose (Plate V). This fungus, A. *macrospora* was apparently identical with that described by Jones (1928), Ellis (1971) and Sciumbato and Pinckard (1972).

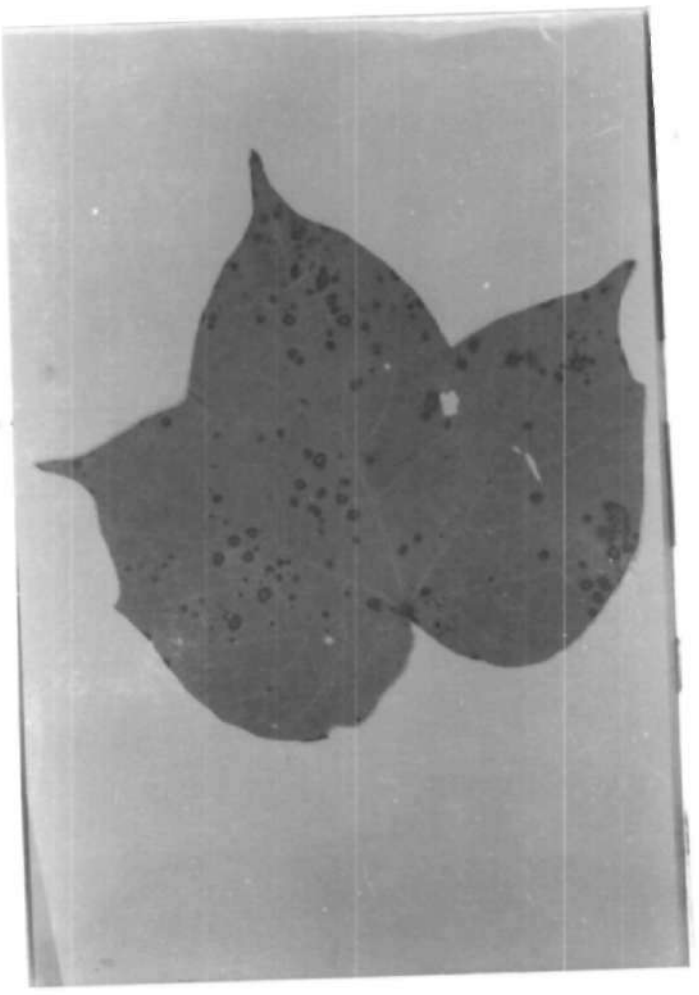


Plate 1: *Alternaria* leaf spot at different stages of development.

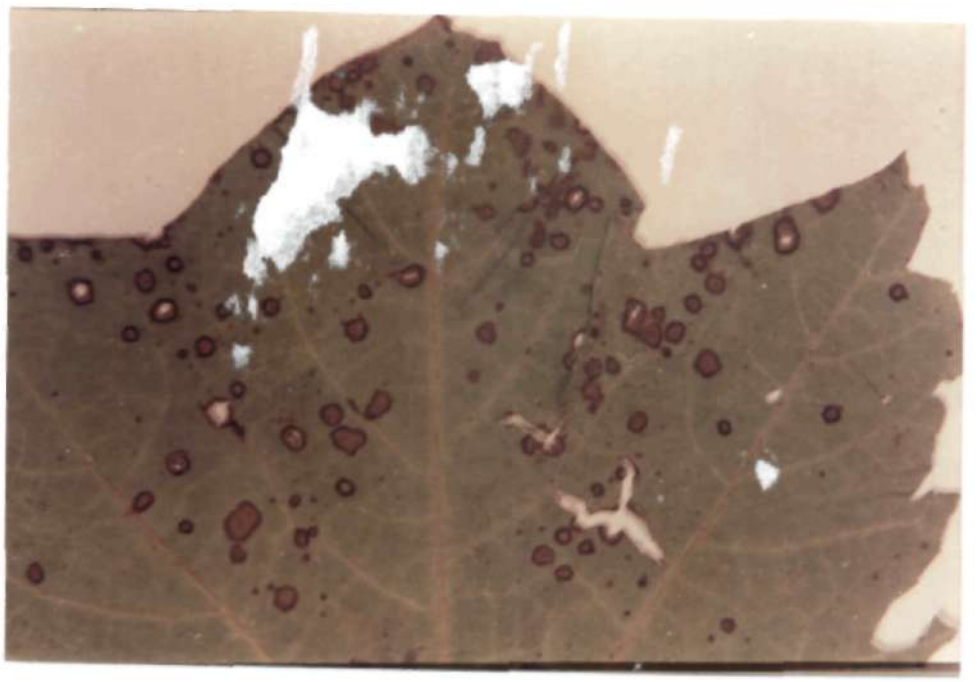


Plate 2: A section of an infected leaf showing some fully developed, coalesced and spots with cracked centres.

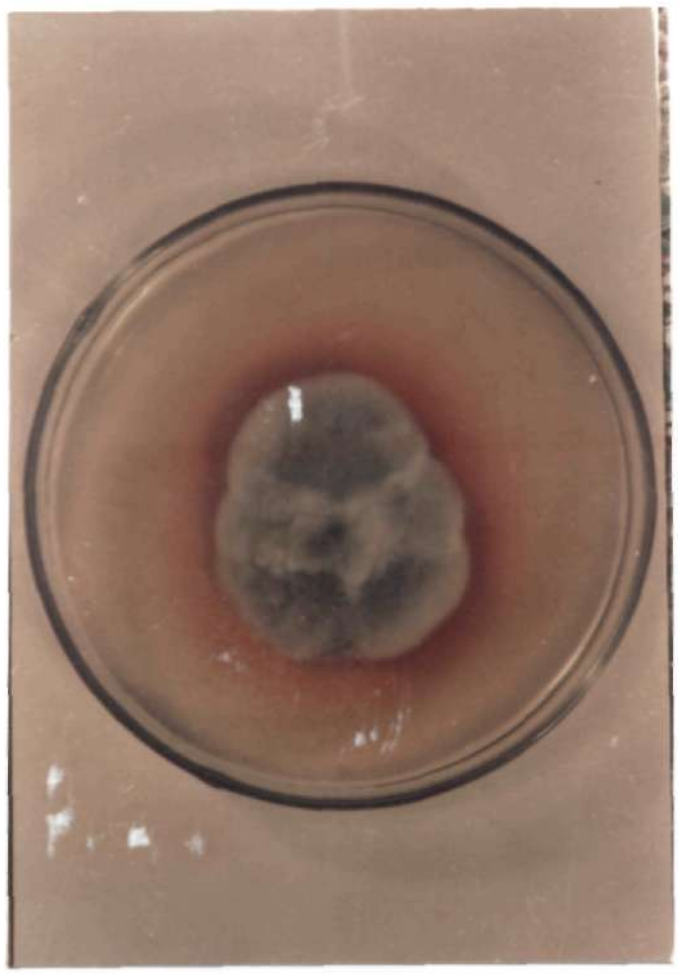


Plate 3: Cultural characteristics of *Alternaria macrospora* on PDAS at 7 days after inoculation.



Plate 4: Cultural characteristics of *Alternaria macrospora* on PDAS at 21 days after inoculation (front view)

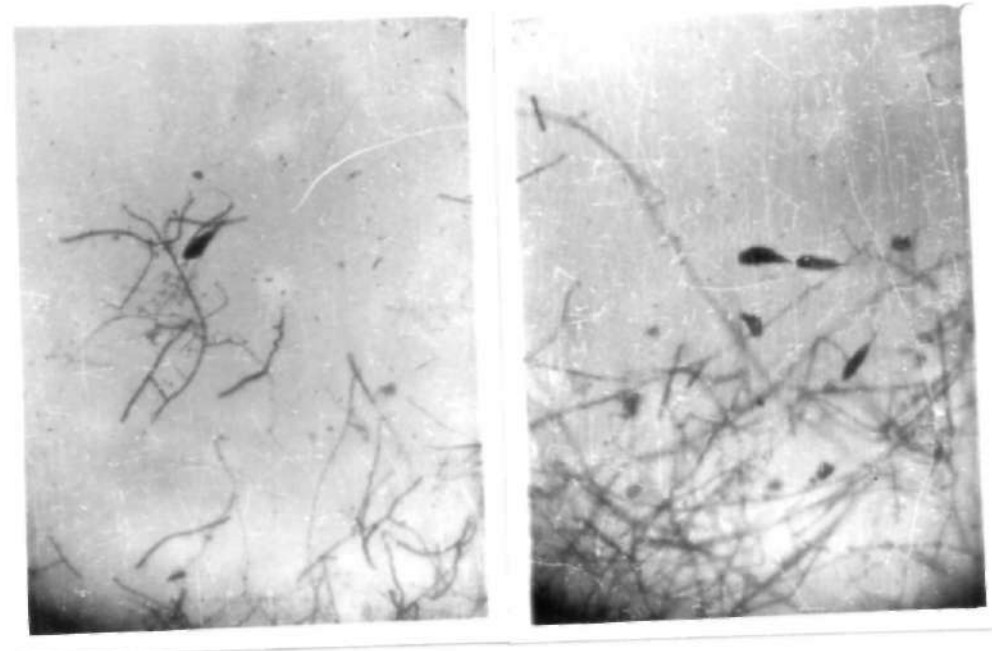


Plate 5: Solitary conidia of *A. macrospora* in culture with mycelia (x100).

CHAPTER FOUR

4.0 SURVEY FOR THE INCIDENCE OF *ALTERNARIA* LEAF SPOT OF COTTON IN ZARIA AND ITS ENVIRONS

4.1 Materials and Methods

4.1.1 Areas Surveyed

The survey for *Alternaria* leaf spot of cotton was carried out in the northern cotton growing zone particularly Zaria and it's environs during the growing season in 1993. The survey was conducted between second week of september and first week of october. The places visited were, I.A.R research farm in Zaria, Daudawa, Maigana and farmers' fields in Basawa.

4.1.2 Survey Method

Two sites with average farm size of 0.3ha were visited in Daudawa, Three in Maigana (0.4ha) and Four in Zaria (0.5ha). In these sites, four, five and six quardrat were thrown respectively. In Basawa, three different commercial farms estimated at 0.4ha, 0.5ha and 0.4ha respectively were visited. The disease incidence was determined by random sampling using quadrate of 2mx2m in size. The number of plots selected in various farms varied according to farm size, hence 5,6,5 quadrants respectively were thrown in the three farms. At the site of a quadrant, a 2mx2m plot was marked out and all the cotton plants within the plots were assessed for the disease incidence and their averages taken.

4.1.3 Disease Assessment

The incidence of the disease were visually assessed in all the farms visited by counting the number of infected plants in each plot and expressing this as a percentage of the total number of the cotton plants in the plots.

4.2 Results and Discussion

The pre-survey field visits showed that cotton was infected by *Alternaria* leaf spot as soon as it germinated (as from the cotyledon stage). Tsror, *et al.* (1990) similarly reported that *A. macrospora* appeared at the early stages of the plant development and was widespread. Infection continues from one stage of the plant growth to another of the crop till when the environmental conditions are unfavourable for the pathogen survival. The unfavourable condition for the pathogen were observed between November and December during which rainfall and relative humidity were reduced and temperature increased to detrimental levels for the pathogen. The disease incidence is wholly affected by the period of the rainfall during the growing season. Four areas visited (Zaria, Daudawa, Maigana and Basawa) recorded average incidence of 75%, 80.5%, 70% and 75% (Table 2).

Field observations showed that the incidence of the leaf spot was generally higher in Daudawa than the other areas visited. The survey further revealed that the farmers do not observed the "close season practice". This therefore enhances

the build up of inoculum (inoculum potential) in the field and hence possible yield loss. It is also possible that, continuous cultivation of cotton over the years in these sites led to build up of the pathogen in the soil or on crop hence the high disease incidence (Table 2).

Alternaria leaf spot of cotton is not confined to Zaria and its environs. The disease is wide spread and has been reported in most of the major cotton growing areas of the world (Ellis, 1971 and Cotty, 1987). However, its occurrence appears to be favourd by the high humidity, rainfall and temperature of 25±2°c in the tropics during the mid-growing season.

Field observation showed that the disease incidence varied considerably between the cotton plants sown at the on set of rains and those planted at various times in the season. However, the disease incidence was relatively high during the vegetative growth stage of the crop.

In this study, it is observed that *Alternaria* leaf spot was to a sgnificant level with disease incidence of 70 - 80.5% in the Northern growing zone of Nigeria. Therefore, proper control mmeasure such as: clearing and burning of the infected crop debris after harvest and crop rotation with other crops that do not share similar disease. These cultural masures has been found to reduce inoculum transfer from one growing season to another.

Table 2: Incidence of *Alternaria* leaf spot of cotton in Zaria and it's environs. 2nd week of september to first week of october, 1993

Town	Location	Average farm size (ha)	Incidence/ site(%)	Incidence /town(%)
Zaria	1		75	
	2	0.5	75	75
	3		74	
	4		76	
Daudawa	1		80	
	2	0.3	81	80.5
Maigana	1		70	
	2		69	
	3	0.4	71	70
Basawa	1		69	
	2		80	75
	3	0.4	75	
SE±	-	-	-	37.6

CHAPTER FIVE

5.0 SCREENING COTTON CULTIVARS FOR RESISTANCE TO *ALTERNARIA* LEAF SPOT

5.1 Introduction

The use of resistant varieties in disease control remains the most feasible, most economical, easiest, safest and the effective means (Agrios, 1978). It saves time and money for farmers and the hazards associated with chemicals are not experienced.

This experiment was therefore designed to identify cotton cultivar(s) that are resistant to *Alternaria* leaf spot, under artificial and field conditions which could be recommended to the farmers or as source of resistance to breeding work.

5.2 Materials and Methods

5.2.1 Screening in the Glasshouse

Fifty plastic pots (12.5cm x 9cm) perforated at the bottom were filled each with 800g heat sterilized sandy clay soil. In each pot, four machine delinted seeds were planted and four weeks after germination, the seedling were thinned down to two per pot. These were arranged on the screenhouse bench in a randomized complete block design, replicated five times with one pot per replicate.

N.P.K.,(15:15:15) fertilizer was mixed with the soil at 60kg N/ha, 40kg P_2O_5/ha and 40kg K_2O/ha which gave 0.160g N/pot 0.107gP_2O_5 pot and 0.107g K_2O/pot.

After three weeks, the remaining 20kgN/ha was applied as top dressing with urea.

5.2.2.1 Inoculation of the Plants in the Screenhouse

Inoculum was harvested from 14-day old sporulating culture. Harvesting of the inoculum and the process of inoculation were described in 3.1.4.1 above. The inoculum suspension were adjusted to $1x10^6$ spore/ml concentration using haemocytometer and a compound microscope and this was used to inoculate the seedlings to run off at five/seven leaf growth stage of the crop.

The inoculated seedlings were observed daily for the disease symptom. The following parameters were assessed; plant height before inoculation; number of days taken for each cultivar to show disease symptom, number of lesions per centimeter square (cm^2) and lesion diameter (mm). The plants were subjectively ranked using scale 1-5, where

Disease Score	Number of leaves infected per pot
1	No disease
2	1 leaf infected
3	2 leaves infected
4	3 leaves infected
5	> 4 leaves infected

This was done by visual observation and number of lesions/cm^2 were assessed by harvesting eight infected leaves

from each pot. Thereafter, the average of the five replicates were taken. The same infected leaf materials were used for lesion diameter (mm) determination, but a micro-gauge apparatus was used to measure the spot diameter per cultivar. The scale used for lesion diameter is as below and was adapted from Cotty (1987). where

0 - 1mm diameter was represented with 0.5mm

1 - 2mm diameter was represented with 1.5mm

2 - 3mm diameter was represented with 2.5mm

3 - 4mm diameter was represented with 3.5mm

4 - 5mm diameter was represented with 4.5mm

The measurements were done 14 days after inoculation and analysis of variance (ANOVA) was used to determine interactions among the treatments.

5.2.2 Screening in the Field

Ten promising cotton cultivars (Table 3) received from a breeder were assessed on the field in 1993 and 1994 for their reaction to *Alternaria* leaf spot of cotton. The cultivars included four Institute for Agricultural Research (I.A.R) released varieties and six others (exotic cultivars) with high disease resistance and yield potentials (Alabi, personal communication).

Machine delinted seeds of each of the ten varieties were sown on 5mx2 ridges plot, replicated three times in a randomized complete block design. The replicates were separated with one meter boarder row while one ridge of bare

ground was used to separate plots. Inter and intra-row spacing was 75cm and 45cm respectively. Four undressed seeds were planted per hole and this was thinned down to two per stand after germination (before disease assessment)

N.P.K (15:15:15) compound fertilizer was applied in two splits at recommended rate of 60kgN/ha, 40kg/P_2O_5/ha and 40kg/K_2O/ha (Dadari, et al., 1994). The first application was carried out at three weeks after germination at the rate of 40kg/N/ha, 40kg/P_2O_5/ha and 40kg/K_2O/ha (0.167kg/plot) while the remaining 20kg/N/ha was applied as top dressing six weeks later as urea. Application method was by making a small hole close to the stands and pouring the required quantity into the holes (spot application). Hoe weeding was carried out two times, at three weeks after planting and three weeks later, thereafter, the ridges were moulded up with tractor.

Inoculation of the field was through natural infection of the crop. Observation were made and data collected on the incidence and the severity of the disease at different stages; cotyledon stage, 5/7 leaf stage, vegetative stage and full vegetative stage. Yield of each treatment (cultivar) was assessed for the two years. In 1994, the yield was ginned, and weight of both the seed and lint was assessed separately after determining boll number per plot at the matured stage.

Cultivars were assessed for percentage disease incidence as described in section 4.1.3. The grading of cultivars for their reaction to attack by *Alternaria* leaf spot was adapted

from Bawa (1992) with scale of 1-5 as stated below.

Disease Score	Percentage of Plants with Leaf Spots
1	No disease
2	1 - 20%
3	21 - 40%
4	41 - 70%
5	More than 70%

The disease severity in each plot was obtained by giving individual plants (stands) within the plot numerical disease ratings as described above, and then calculating the average score for all the plants in a plot as described by Poswal (1981)

$$\text{Mean disease grade} = \frac{\text{Sum of all disease score}}{\text{Total number of plant graded}}$$

Assessment of the disease severity at cotyledon stage under field condition was changed in 1994. This was done by the introduction of two different parameters; number of lesions per leaf assessed through ranking (score) and lesion diameter (mm) obtained with a micro-gauge. The scale was adapted from Cotty (1987).

This was carried out by sampling 20 leaves randomly and the harvested leaves were collected in labelled paper envelopes separately according to replicates of each treatment.

Table 3: Ten cotton cultivars screened in 1993 and 1994 for resistance to *Alternaria* leaf spot

Serial Number	Source of development	Cultivars
1	I.A.R., Zaria	Samcot-6
2	I.A.R., Zaria	Samcot-8
3	I.A.R., Zaria	Samcot-9
4	I.A.R., Zaria	Samcot-10
5	T.A.E.S, USA	CAM D-E
6	T.A.E.S, USA	GCA-NH-1-83
7	T.A.E.S, USA	Tamcot sp 37
8	T.A.E.S, USA	Tamcot sp 215
9	Chad	S-295
10	T.A.E.S, USA	TXCDP 37-HH-1-83

*TAES=Texas Agricultural Experiment Station, USA.

(Source: Alabi, Personal Communication).

5.3 Results and Discussion

5.3.1 Screening in the glass house

First symptom appearance was observed between 4 and 7 days in all the cultivars tested with Samcot-6 appearing after 4 days of inoculation. Samcot-8, Samcot-9, and CAMD-E appeared last at seven days after inoculation. However,there were no significant difference between cultivars in the number of days to the first symptoms (Table 4). Symptoms of the disease varied slightly according to cultivars; some are in rings of red-stained cells, others yellow chlorotic halo, whitish halo and some brown border. Coty(1987) reported similar symptom variation in *G.barbadence* which had rings of red-stained cell and *G. hirsutum* with yellow chlorotic halo. However, the pathogen was re-isolated from the different lessions.

Samcot-6 was observed to be highly susceptible using all parameters. This result therefore agreed with the previous observation of Adeoti *et al.*(1994) were they reported that,Sancot-6 was highly susceptible when compared with other cultivars tested. On the other hand, Samcot- 9, TXCP 37-HH-1-83, GCA-NH-1-83 and S-295 were found to show less number of lesions and at the same time restricted lesion expansion. These cultivars might be promising in terms of breeding for resistance. The later three are exotic cultivars and have been reported to be resistant to *A. macrospora* (Alabi, personal communication).

Lesion diameter remain the most reliable criterion for

evaluating host susceptibility because it was objective, simple and permitted rapid separation of susceptible cultivars. Cotty (1987) reported that lesion diameter is an objective and qualitative measure of relative host resistance that is useful in evaluating host susceptibility to *A. macrospora*. Apparently, restriction of expansion of the lesion is an important factor in host resistance to diseases. Although the number of lesion/cm^2 of the leaf area typically followed the same pattern, the technique was time consuming and conidial deposition on the leaves might be uneven despite vigorous shaking of the suspension before inoculation. This is the first report of a qualitative difference in susceptibility among these cultivars in Nigeria.

Table 4: Evaluation of Ten Cotton Cultivars for Susceptibility to *Alternaria* Leaf Spot Disease under Glasshouse Condition in 1994.

Cultivars	Plant Height (cm)	First Symptom Appearance (Days)	Rank	Lesion Diameter (mm)	Number of Lesions Per cm^2
Samcot-8	41.5ef	7	2abc	2.17abc	1.4abc
CAMD-E	30.33abc	7	2.67cd	2.67bc	1.33ab
GCA-NH-1-83	32.5bc	6	1.33a	0.5a	0.33a
Samcot-10	37.5de	6	3.0d	2.83bc	2.0bc
Tamcot Sp 37	39.67e	5	3.0d	1.67ab	1.33ab
Tamcot Sp 215	33.33cd	5	2.33bcd	2.83bc	1.0ab
S-295	30.5abc	5	2.67cd	0.5a	0.33a
TXCDP37-HH-1-83	27.83ab	5	1.33a	0.17a	0.33a
Samcot-9	45.33f	7	1.67ab	0.83ab	0.66a
Samcot-6	27.5a	4	4.0e	3.83c	2.67c
$SED\pm$	2.243		0.37	0.966	0.63
LSD	4.712	NS	0.786	2.03	1.32

Means within the same column followed by the same letter do not diff significantly at P=0.05.

5.3.2 Screening in the field

Result (Tables 5 and 7) show that all the varieties were infected by *A. macrospora*. However, the ten cotton cultivars reacted differently to the disease under same field condition at different growth stages of the crop. The variation in response at different growth stages were attributed to the cultivar genotype since they were exposed to similar environmental condition. Infection was found to be severe when rainfall was high and temperature 25±2°c, (Appendix 3). Under such conditions, the spots were found on both old and young leaves particularly on the local cultivars and CAMD-E; while the other cultivars showed severe infection of the old leaves with same environmental condition. Bashi, *et al.*(1983) reported that free moisture and rain patterns are involved in increased *Alternaria* leaf spot of cotton in Isreal during 1982 - 1984.

Sciumbato and Pinckard (1972) reported that older leaves first form lesions and in most cases, young bolls and leaves are not infected.Spross-Blickle, et al. (1989) and Shienberg et al. (1993) reported that, suscepptability of cotton to *A. macrospora* was highest at the seedling stage, decreased in young plants, and then increased again in with age to a second peak in sensecent plants.

There was no significant difference (P = 0.05) between incidence of the disease on S-295 (15.2%) and Samcot-6 (16.7%) (Table 5) which was earlier shown to be highly susceptible to

the disease at cotyledon stage by Adeoti, *et al.*(1994). Cultivars Samcot-8, Samcot-9, CAMD-E, GCA-NH-1-83, Tamcot Sp 37, Tamcot Sp 215 and TXCDP 37-HH-1-83 did not show any significant difference in the disease incidence too. This result agreed with Bashi (1983) who reported that, cotton seedlings are infected by *A. macrospora* as soon as they germinate.

Samcot-10 which gave the highest disease incidence at cotyledon stage (39.4%) gave the lowest disease incidence (15.5 %) at 5/7 leaf stage of the crop. The observation agreed with phenomenon of "adult plant resistance" as reported by Bashi, *et al.(1983)* who showed that susceptibility in cotton cotyledons to *Alternaria* leaf spot increased with age up to 20 days and decreased thereafter. They further explained that susceptibility in leaves slightly decrease with age. However, the incidence (94.8%) and severity (4.6) were high in the vegetative stage of the crop. Bashi et al. (1983) and Spross-Blickle et al. (1989) reported three epidermic level of the disease on cotton which corresponded to emergence, vegetative and reproductive growth stages of the host

The highest damage was done at the vegetative stage in 1993. At that stage, (Table 5) as the incidence increased, the disease severity on different cultivars also increased. This was attributed to favourable environmental conditions which occurred during that period (Appendix 3). Cotty (1987) reported that *Alternaria* leaf spot of cotton is influenced by

the temperature at which plants are held after infection. Incidence as high as 100% were observed with CAMD-E and disease severity upto 4.83 were recorded on TXCDP 37-HH-1-83 in 1993. Sancot-6 gave 93.7% disease incidence and showed no significant difference (P= 0.05) with other cultivars tested except samcot-8 and Samcot-9. Hiremath, *et al*. (1988) reported that *A. macrospora* development was slow during the vegetative period (Aug - Sept.) and rapid during peakboll development (Nov.). This is contradictory to the findings presented here. During November, environmental factors mostly rainfall have stopped and the temperature falls below 23°c with maximum relative humidity of 36% (Appendix 3). Field observation showed that, above conditions were detrimental to the development of *Alternaria* leaf spot of cotton. However, Shhtienberg (1996) reported that *Alternaria* infections are scattered and that the progress of the epidermic is associated mainly with an increase in number of infected plants. He further explained that crop microclimate is also affected by the region.

Yield was generally low in 1993 due to poor stand count as a result of one week drought experienced immediately after planting and water logging later in the season. However, there were significant difference (P= 0.05) among the treatments. S-295 gave the highest yield of 1041.3 kg/ha and the least of the tested cultivars was Tamcot SP 37 which gave 253.3 kg/ha, samcot-9 and TXCDP 37-HH-1-83 gave relatively high yield; 760

kg/ha and 800 kg/ha respectively despite their disease incidence of 78.9% and 97.9% respectively at the vegetative stage. From the yield result, it is possible that S-295, TXCDP 37-HH-1-83 AND Samcot-9 might be tolerant to *Alternaria* leaf spot.

Table 5: Evaluation of 10 Cotton Cultivars for Susceptibility to *Alternaria* Leaf Spot under Field Condition in 1993

Cultivars	Cotyledon stage	5/7 leaf stage		Vegetative stage		Yield Kg/ha)
	Incidence (%)	Incidence (%)	Severity	Incidence (%)	Severity	
Samcot-8	19.7^{ab}	25.9^{a}	2.5	79.1^{a}	4.3^{abc}	400^{a}
CAMD-E	33.3^{ab}	25.0^{a}	2.03	100^{b}	4.73^{d}	373.3^{a}
GCA-NH-1-83	33.3^{ab}	33.9^{ab}	2.37	90.9^{b}	4.23^{ab}	306.7^{a}
Samcot-10	39.4^{b}	15.5^{a}	2.13	94.8^{b}	4.6^{bcd}	373.3^{a}
Tamcot S37	18.2^{ab}	30.0^{a}	2.5	98.2^{b}	4.53^{abcd}	253.3^{a}
Tamcot Sp 215	28.8^{ab}	38.7^{b}	2.13	96.3^{b}	4.77^{d}	373.3^{a}
S-295	15.2^{a}	34.5^{ab}	2.23	92.9^{b}	4.67^{cd}	1041.3^{b}
TXCDP37-HH-1-83	31.8^{ab}	51.5^{b}	2.1	97.9^{b}	4.83^{d}	800^{b}
Samcot-9	30.3^{ab}	31.8^{ab}	2.13	78.9^{a}	4.17^{a}	760^{b}
Samcot-6	16.7^{a}	37.8^{b}	2.03	93.7^{b}	4.5^{abcd}	413.3^{a}
SED±	10.54	10.01		4.99	0.2	161.21
LSD	22.15	21.03	NS	10.48	0.42	338.7

Means within the same column followed by the same letter do not differ significantly at P=0.05

In 1994, there was no significant difference (P = 0.05) in disease severity between the different cotton varieties screened on the field at the cotyledon growth stage. The result of the ranking and lesion diameter showed no significant (P = 0.005) within the means of each parameter. However, lesion diameter was observed to be simple, objective and separated the means compared to the ranking which was closely related. This observation is similar to Cotty (1987a) who reported that lesion diameter was simple, objective and permitted separation of the means of some cotton cultivars. (Table 6). Cotty (1987) showed that lesion diameter was the

most useful criterion for evaluating host susceptibility.

Disease incidence and severity in 1994 (Table 7) were relatively lower than that of 1993 and the cultivars reaction to *Alternaria* leaf spot were not consistent when compared with what was obtained in 1994. Rainfall was observed to be responsible for the low disease development in 1994. Annual rainfall in 1993 and 1994 were 1061.7 and 911.9 mm respectively but the duration was more in 1994 (Appendix 3). Earlier workers like Hiremath, *et al.* (1988) have also found rainfall to be determinant factor in disease severity.

The phenomenon of "adult plat resistance" was observed with TXCDP 37-HH-1-83 and Samcot-9 which gave disease incidence of 21% and 51.7% respectively at 5/7 leaf stage and 19.7% and 23% respectively at the vegetative stage. This continued to full boll matured growth stage (Table 7).

S-295 was highly susceptible to the disease at both 5/7 leaf stage and vegetative stage when compared with other cultivars tested. Even at the matured stage, the disease incidence (47.9%) and severity (3.0) were also high. Such increase in disease incidence and severity was equally observed in Tamcot Sp 215 and Tamcot SP 37. Cultivar GCA-NH-1-83 gave the lowest incidence (26.7%) and severity (1.67%) at 5/7 leaf stage and disease incidence and severity on the cultivar increased to 41% and 3.33 respectively at the vegetative growth stage. These results therefore showed that the cultivars reacted differently to *Alternaria* leaf spot at

different growth stages. This is similar to the findings by Cotty (1987) who showed that different cultivars reacted differently to *A. macrospora*.

The 1993 and 1994 field results showed that, Samcot-9, TXCDP 37-HH-1-83, and CAMD-E were the least susceptible in forms of disease incidence and severity at different stages of the crop development and therefore, remain promising cultivars for breeding cotton for resistance.

S-295, Tamcot SP 215 and GCA-NH-83 gave a relatively high yield irrespective of their high disease incidence and severity at different stages of the plant growth. Therefore, they can be said to be tolerant to the disease.

Samcot-6 remained high in terms of susceptibility to the disease at all stages even the yield was relatively poor (413.3 and 456.7 kg seed cotton/ha) when compared to other cultivars tested.

Table 6: Evaluation of ten cotton cultivars susceptibility to *Alternaria* leaf spot at cotyledon stage 3 weeks after planting in 1994.

	Severity	
Varieties	Rank	Lesion diameter (mm)
Samcot-8	1.83	4.17
CAMD-E	2.4	3.53
GCE-NH-1-83	2.7	3.47
Samcot-10	2.43	3.53
Tamcot Sp 37	1.67	3.8
Tamcot Sp 215	2.73	3.8
S-295	2.6	3.47
TXCDP37-HH-1-83	2.77	3.03
Samcot-9	2.0	3.6
Samcot-6	2.07	3.53
LSD	NS	NS

Figures followed by the same letter in the same column are not significantly different at P = 0.05

Table 7: Evaluation on the Effect of Alternaria Leaf Spot on Different Stages of 10 Cultivars in 1994

Cultivars	5/7 leaf stage		Vegetative stage		Full boll matured stage		
	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity	Yield (kg/ha)
Samcot-8	49.03^{bcd}	2.33^{abc}	38.47^{a-c}	2.67^{ab}	31.1^{ab}	2.67^{ab}	700a
CAMD-E	30.13^{abc}	2.83^{bcd}	32.42^{a-c}	2.83^{ab}	40.7^{b}	2.83^{a-c}	1998.3f
GCE-NH-1-83	26.17^{ab}	1.67^{a}	40.98^{a-c}	3.33^{bc}	49.7^{b}	2.67^{ab}	807d
Samcot-10	38.5^{abcd}	3.33^{cd}	44.39^{a-c}	2.5^{ab}	29.2^{ab}	2.17^{ab}	510a
Tamcot Sp 37	38.1^{abcd}	2.67^{abcd}	34.92^{a-c}	3.17^{abc}	49.5^{b}	3.0^{bc}	615b
Tamcot Sp 215	48.63^{bcd}	3.0^{bcd}	62.7^{c}	3.67^{c}	52.6^{b}	3.83^{c}	1334.7e
S-295	56.03^{d}	3.67^{d}	64.35^{c}	3.67^{c}	47.9^{b}	3.0^{bc}	1995.7f
TXCDP37-HH-1-83	21.03^{a}	2.0^{ab}	19.74^{a}	2.67^{ab}	8.7^{a}	1.83^{a}	1986.7f
Samcot-9	51.7^{cd}	2.33^{abc}	23.02^{ab}	2.33^{a}	28.6^{ab}	2.67^{ab}	1954.0f
Samcot-6	51.2^{cd}	2.67^{bcd}	59.85^{bc}	3.33^{bc}	35.5^{ab}	2.33^{ab}	456.7a
SED±	11.56	0.533	17.87	0.408	12.97	0.541	36.19
LSD	24.28	1.120	37.55	0.857	27.25	1.137	76.02

Means within the same column followed by the same letter do not differ significantly at P=0.05

Yield performance in 1994 followed similar pattern in 1993. Samcot-9 gave 1954.0 kg/ha, TXCDP 37-HH-1-83 (1986.7 kg/ha), S-295 (1995.7 kg/ha) and CAMD-E (1998.3 kg/ha). Despite the variation in figures there were no significant diffrerence (P = 0.05) within the means (Table 7). Tamcot sp 215 followed with 1334.7 kg/ha regardless of high incidence and severity. Base on these findings, Samcot-9, TXCDP 37-HH-1-83 and S-295 are recommended forbreeding for resistance while CAMD-E and Tamcot sp 215 are tolerant to the disease and therefore are recommended to farmers. These findings agreed with Adeoti, *et al.*(1995) who had earlier identified S-295, Tamcot Sp 215 cultivars as being tolerant under field conditions.

CHAPTER SIX

6.0 *Evauation of some Fungicides for their Efficacies in controlling alternaria leaf spot of cotton*

6.1. Introduction

Fungi*cides* serve as quick and most effective way of controlling fungal disease of plants despite the associated hazards to man and environment. Chemical trials were conducted in 1993 and 1994 to evaluate the efficacies of four fungicides for the control of *Alternaria* leaf spot of cotton. This fungicides were tested in *vitro* in ythe laboratory and on the field as foliar application. The tested fungicides were carefully selected from both systemic and non systemic groups. they are benlate (Benomyl), Delsene M (Carbondazim + Maneb), Tecto (Thiabendazole TBZ) and Dithane M 45 (Mancozeb). The formulation used trade and common names of the fungacides as well as thier chemical composition are given in the table below:

Table 8: Formulation, chemical nature, common and trade names of fungicides evaluated in the laboratory and field for the efficacies against A macrospora

Trade Name	Common Name of fungicides (G.I)	Chemical Nature of fungicides (G.i)	Formulation of Fungicides used
Benlate	Benomyl	Methyl-1-(butylcarbamoyl) benzimidazole-2-yl carbendamate	50 wp*
Delsene M	Carbendazim and Maneb	Carbendazim=(Methyl benzimidazole-2-yl carbamate 10% maneb=(manganese ethylene bisdithio-carbamate)-64%	80 wp*
Tecto	Thiabendazole (TBZ)	2-(thiazol-4-yl) benzimidazole	FL **
Dithane M-45	Manconeb	2.5% Zn ion + 20% Mn as in Maneb	80 wp*

* wettable powder

** Flowable liquid

6.2. Materials and Methods

6.2.1 In vitro assesment of the effect of four fungicides on Mycelial growth of A. macrospora

Four fungicides were assessed for their ability to control the growth of A. macrospora In vitro. Each of the fungucides was evaluated at four concentrations as shown below:

Table 9: Four Concentration of each of the Fungicide tested in vitro

	Concentration levels			
Fungicides	B	C	D	E
Benlate	6g/lit	3g/lit	1.5g/lit	0.75g/lit
Delsene M	12g/lit	6g/lit	3g/lit	1.5g/lit
Tecto	10g/lit	5g/lit	2.5g/lit	1.25g/lit
Dithane M-45	0.5g/lit	6.4g/lit	3.2g/lit	1.6g/lit

The recommended spray concentrations for Benlate, Delsene M, Dithane M-45 are 0.6,1.2, and 2.5kg of commercial product per hectar (ha) respectively while the flowable liquid(FL) Tecto is 1000ml/ha (Adeoti, personal communication). To assessed the fungicides, appropriate concentration of the above formulatiions were incoperated into 250ml of potato dextrose agar (PDA) respectively.

To prepare PDA-fungicide mixture, the following method was adopted. The appropriate quantity of each fungicide was added separately into 500ml conical flask. To each of the wettable powders in conical flask, little volume (10ml) of sterile distile water was added to dissolve the fungicides. Appropriate volume of molten PDA at about 50-55°C was then added to make up to 250ml. The flask was gently shaken to ensure proper mixing of the fungicides and streptomycin solution(2ml, for 1g/200ml solution) was equally incorporated into the PDA-fungicide mixture before pouring to avoid baterial contamination

To ensure that equal quantity of the mediun was poured into each petridish. The following method was adopted: McCartney bottles were sterilized along with the media in the autoclave. Using one 20ml McCartney bottle for each concentration(filling the bttle up to marked point on the neck), 20ml of the medium was poured into each petridish. The whole process was conducted under sterile condition. Seven petridishes were poured for each concentration. The plates

were allowed to set on the bench for twenty four hours undeer sterile condition.

A heat sterilized 0.6cm-(diameter) cork borer was used to transfer the fungus from 7-day old pure culture, onto the centre of each fungicide-agar plate. Check treatment consisting of PDAS plates (A) alone were similarly inoculated for each fungicide concentration. The plates were arranged in randomized complete block design on the laboratory bench at room tempreture of 25±2°C.

The tested rates for each fungicide were designated as 2x(B),x(C),0.5x(D) and 0.25(E) while A is the control (PDAS alone); where x is the above recommended ddose of the fungicide per ha to be dissolved in 250ml of molten PDAS, assumming that the rate per hectare is to be dissolved in 200ml of water.

Fungitoxicity was assessed by measuring the linear (diameter) growth of the fungus after seven days with a ruler (mm). This result was obtained by measuring the average of the horizontal and vertical growth of the mycelia in each of the seven plates.

6.2.2. Evaluation of Effects of Four Fungicides On the Control of *Alternaria* Leaf spot Under Field Condition

The effect of foliar application of two concentrations each of the four fungicides; namely Benlates, Delsene M, Tecto and Dithane M-45 on the incidence and severity of *Alternaria*

leaf spot of cotton were assessed in the wet seasons of 1993 and 1994 at I.A.R. Samaru Zaria. The two rates are the recommended rate, 0.6, 1.2, 1000ml/ha and 2.5kg/ha for Benlate, Delsene M, Tecto and Dithane M-45 respectively and half of the rates; 0.3, 0.6, 0.5lit:ha and 1.25kg/ha. The cultivar usedas test crop was Samcot-6.

The experimental layout after land preparation was randomized complete block design with three replicates. Each plot measured 3m x 5 ridges with 75cm interval and space between stands measured 45cm. One meter border separated one replicate from the other and one ridge guard row separated one plot from the other. Four seeds were planted per hole and was thinned down to two per stand at three weeks after germination. Maize (TZESR variety) was planted on the borders to catch their drift of the fungicides from one plot to another.

Plots were hoe weeded two times at three weeks after germinaton and six weeks later. NPK (15:15:15) fertilizer was carried out using spot application at the rate recommended by Dadari et al. (1994). The first application was done at three weeks after germination at the rate of 40kg N/ha, 40kg P_2O_5/ha and 40kg K_2O/ha (0.34kg/plot) and 20kg N/ha was applied as top dressing six weeks later as Urea. Thereafter the ridges were moulded up with tractor.

The plants were exposed tonatural infection and spraying started at six weeks after germination. Four sprays were

carried out at weekly intervals, the crop was assessed for the disease incidence and severity before and after spraying as described in 4.1.3 and the severity as in 5.2.2. The seed yield was also recorded.

6.3. Results and Discussion

6.3.1. In Vitro Results

Results (Table 11) showed that Delsene M, (carbendazim + Maneb), Tecto (Thiabendazole MBZ) and Dithane M-45 (Mancozeb) completely inhibited mycelia growth of A. *macrosôra* in agar plates even at their lowest tested concentrations (1.5g, 1.25ml and 1.6g of commercial product per 250ml PDAS respectively) (Plats 8, 7 and 8).

Benlate (Benomyl) on the other hand gave 100% growth inhibition at 69kg/ha and 39kg/ha (first and second concentration) compared to the growth of the control (plat 9). However, the last two concentrations, 1.5/ha and 0.75g/ha) of benomyl inhibited mycelia growth to 84.98% and 63.82% respectively. It might be explained that at 0.75 and 0.38kg/ha the fungus is tolerant to benomyl hence the linear growth of 15.02 and 36.18mm respectively.

The most effective fungicides *In vitro* were, Carbendazim, Thiabendazole and Mancozeb. Since benomyl inhibited mycelial growth to 86.3% and 67% at low concentrations, it is considered to be effective in the control of A macrospora in vitro.

These fungicides have been reported by other workers to inhibit the mycelia growth of fungi at different concentrations in vitro. Yar'adua (1986), Alafiatayo (1982) and Obagwu (1996) reported that these fungicides with the exception of Tecto inhibited mycelia growth of *Alternaria Porri* on onions, *Collectotrichum capsici* on cowpea and *collectotrichum capsici* on bambara-nut respectively. Ahonsi (1995) showed that, Delsene M, Benlate and Tecto were effective in the inhibition of false smut of rice induced by *Ustilaginoide virens* while Adeoti (1991), Tologbonshe (1983) showed that Benlate and Delsen M were effective in vitro on *Coniella musaiaensis* (concilla leaf spot of Kenaf), *Cercospora* spp and *Alternaria macrospora* of cotton respectively.

The effectiveness of these fungicides in vitro might be attributed to the direct close contact between the fungus and the fungicides in the agar plate. However, Davis and Diamond (1952) showed that the efficiency of a fungicides in vitro may not hold in the field, rather the results provide simple clues to what may probably occur in the field.

Cushion growth which was fluffy white in colur was observed in all concentrations of Tecto (Plate 7). This growth was specifically on the top of the residual agar transferred with the inoculum and the mycelia was not observed to grow on the media even after two weeks (14 days). Similar observations were made on Benlate at 1.5g/l (Plate 9).

Bawa (1992) reported the effectiveness Benomyl, mancozeb,

Maneb plus carbenddazim on the mycelia growth and sporulation of curvularia eragrostidis in vitro Shimfe (1984) demonstrated that benomyl increasingly inhibited mycelial growth in *Colletotrichum gloeosporioides* with increasing concentration. Komolafe (1984) reported that carbendazim plus Maneb was most effective fungicide against *Curvularia pallescens* since it completely inhibited the pathogen at 20 x 50 ppm^{-1}.

Table 10: *In vitro* Assessment of Four Fungicides for their Efficacy in controlling *Alternaria* Leaf spot of Cotton induced by *A. Macrospora*

Growth of *A. macrospora* 7 Days After Inoculation			
Fungicides	Concentrations of Fungicides in agar (gm/lit)	Colony Diameter (cm)	Growth as % of Growth of Control
Benlate	Control A	6.43	-
	6B	0.0	0.0
	3C	0.0	0.0
	1.5D	0.88	15.02
	0.75E	2.12	36.18
Tecto	Control A	6.43	-
	10ml/lit	0.0	0.0
	5ml/lit	0.0	0.0
	2.5ml/lit	0.0	0.0
	1.25ml/lit	0.0	0.0
Delsene M	Control A	6.43	-
	12B	0.0	0.0
	6C	0.0	0.0
	3D	O.O	0.0
	1.50E	0.0	0.0
Dithane M-45	Control A	6.43	-
	4.8B	0.0	0.0
	6.4C	0.0	0.0
	3.2D	0.0	0.0
	1.6E	0.0	0.0



Plate 6: Effect of different concentrations of Carbondazim plus maneb on the mycelial growth of A. macrospora in PDAS medium

Key: A = Control
B = 2x
C = x
D = 0.5x
E = 0.25x

Where x = Recommended dose of the fungicide.



Plate 7: Effect of different concentration of Thiqbendazo MBZ on the mycelial growth of A. macrospora in PDAS medium

Key: A = Control
B = 2x
C = x
D = 0.5x
E = 0.25x

Where x = Recommended dose of the fungicide.



Plate 8: Effect of different concentration of mancozeb on the mycelial growth of A. macrospora in PDAS medium

Key: A = Control
B = 2x
C = x
D = 0.5x
E = 0.25x

Where x = Recommended dose of the fungicide.



Plate 9: Effect of different concentration of Benomyl on the mycelial growth of A. macrospora in PDAS medium

Key: A = Control
B = 2x
C = x
D = 0.5x
E = 0.25x

Where x = Recommended dose of the fungicide.

6.3.2. *Field Results.*

The results (Table 12 and 13) showed that the four fungicides were effective for the control of Alternaria leaf spot on cotton. In 1993, there were no significant difference among treatment, but considerable reductions of the disease incidence and severity were observed after four sprays at weekly intervals (Table 12). The result (Table 13) showed significant differences (P = 0.05) among treatments after four sprays in both incidence and severity in 1994.

Disease incidence and severity of the unsprayed plots in both year were relatively higher when compared with the sprayed plots. Disease severity as high as 3.27 were observed after sprays in 1993 and in 1994; incidence of 36.1% and severity of 3.33 was observed after the spraying.

Benlate, though effective was not consistent in action, in 1993, benlate (0.6 kg/ha) reduced disease severity fro, 4.1 to 2.5 while in 1994 plots sprayed with the same concentration showed increased disease incidence and severity from 38.33% and 3.0 to 41.4% and 3.17 respectively. This could be as a result of the mode of action of the systemic fungicide. Hill and Walter (1990) reported that, for systemic fungicide to be effective in vivo, they need o be absorbed into the plant tissues and transported acropetally to the meristermatic tissue where they become effective. It is possible that the disease have established before spay schedule started. Moorman; et al. (1989) reported that; Botrytis cinerea

isolated fro, rose plant was tolerant to benomyl and therefore they recommended that mancozeb (non systemic) should beused among other protective fungicides. It is possible that A. macrospora might also be tolerant to benomyl.

Delsene M appeared to offer more disease control at the recommended rate of 1.2 kg/ha (6 g/1). However, there was severe scorching of the leaves which resulted in defoliation and death of the plants (Plant 10). Some stands were completely killed due to the effect of the fungicide. This phytotoxic effect of Delsene M was also reported by Ahonsi (1995) on rice. The phytotoxicity pose a lot difficulties in disease assessment, however, the incidence and severity was reduced to 18.5% and 2.67 repectively after spray (Table 13). The lower concentration (0.3 kg/ha) of the same fungicide were found to reduce disease incidence and severity significantly to 10.9% and 1.69 respectively without showing any form of phytotoxicity on the crop. This therefore should that weekly spray of this fungicide was over dose hence the phytotoxicity. Ito; et al. (1988) reported that, carbendazim at 75%a.i inhibited growth of Ceratocystis fimbriata.. Maneb at 2.0 kga.i/hα suppressed Alternaria leaf spot of cotton significantly and increased yield from 6.3 to 26.0% (Shtienberge and Dreishpoun, 1991).

Tecto was observed to suppress the fungus by reducing the disease severity from 4.23 before spray to 2.73 after spray in 1993: However, there was no significant difference among

treatments (Table 12). In 1994, the disease incidence and severity were significantly reduced fro 54.12% and 3.0 to 4.8 and 1.33 respectively at the rate of 1 lit/ha. This observation agreed with Ahonsi (1995) who reported that, Tecto at 1 lit/ha was effective for the control of false smut of rice.

Dithane M 45 a protective fungicide, reduced severity from 4.13 to 2.77 at the rate of 2.5 kg/ha in 1993. In 1994, the disease incidence and severity was consistently reduced after treatment from 25.4% and 2.67 to 12.5% and 1.83 respectively.

Mancozeb is a contact fungicide whose mode of action is at the surface of the plant unlike systemic fungicides that are absorbed and transported acropetqlly to the meristermatic tissues to be effective. It is therefore possible that its presence at the surfqce of the crop offered enough protection against the air-borne pathogen (A. macrospora). Adeoti (1991), Alabi (1994) and Alagarsamy et al. (1989) showed that, mancozeb was effecive for the control of coniella leaf spot of Kenaf, Collectrotrichum capsici (cowpea isolate) and Alternaria leaf spot of cotton respectively when applied foliarlly.



Plate 10: A Cross section of cotton plants scorched and killed by spraying with Delsene M at 1.2 kg/ha during the vegetative stage in the field.

In terms of yields, there was high significant difference (P = 0.05) among treatment in 1993 zith the highest yield 1488.9 kg/ha recorded in Tecto at 1 lit/ha concentration while the unsprayed plot (control) gave the lowest 588.9 kg/ha. The low yield was attributed to the disease severity (3.27) after spray. In 1994, plots sprayed with the Delsene M at 1.2 kg/ha gave the lowest yield (862.8 kg/ha). This was associated with the scorching effects on leaves which reduced photosynthetic activities of the crop and hence low yield. This increase yield in 1994 was similar to that obtained in 1993. Results (Tqble 12) showed that Tecto, (1925.7 kg/ha) and Dithane m45 (1647.1 kg/ha) gave the bes yield at their respective recommended doses when compared with the check. At 0.6 kg/ha, Delsene m gave a relatively high yield (1647.1 kg/ha) when compared to Tecto and Dithane m45. It is therefore advisable to spray Delsene M forthnightly instead of weekly to avoid phytotoxicity.

Generally, Mancozeb, Thiobendazole TBZ and Carbendazim plus Maneb were found to be effective in reducing the incidence and severity of Alternaria disease of cotton in the field. However, the outbreak of Alternaria disease can not be completely controlled by spraying, but can only slow the progress once the disease has established in the field. It is therefore advisable to spray the field before the disease is observed as a protective measure.

Table 11: Effect of foliar Application of Two Concentration of four fungicides on the incidences ans Severity of *Alternaria* leaf spot of Cotton in 1993

Fungicides	Concentration kg a.i/ha and La.i/ha	Before Fungicides spray		After Sprav	
		Incidence %	Severity	Severity	Seed Cotton yield (kg/ha)
Benlate	0.6 0.3	84.2 84.1	4.1 4.37	2.5 2.7	866.7b 988.9c
Delsene M	1.2 0.6	72.5 80.1	3.93 3.93	2.63 2.97	1233.3cde 1288.9de
Tecto	1 0.5	83.1 78.2	4.23 3.9	2.73 3.0	1488.9e 1133.3bcd
Dithane M-45	2.5 1.25	72.0 71.8	4.13 3.9	2.77 2.73	1355.6de 1422.2e
Control	-	78.2	4.07	3.27	588.9a
SED±		10.59	0.3169	0.504	-
LSD (5%)		NS	NS	NS	271.8

Figures followed by the same letter in the same column are not significantly different at P = 0.05

Table 12: Effect of Foliar Application at Two Concentrations of Four Fungicides on the incidence and severity and yield of Alternaria leaf spot of cotton in 1994.

Fungicides and Concentrations in kg a.i./ha and L a.i/ha	Before fungicide spray		After fungicide spray		Yield (kg/ha)
	Incidence (%)	Severity	Incidence (%)	Severity	
Benlate 0.6	38.33	3.0	41.4c	3.17bc	1176.5c
0.3	52.86	3.0	20.6a-c	2.67a-c	1490.2de
Delsene M 1.2	30.95	3.0	18.5a-c	2.67a-c	862.86
0.6	43.18	3.33	10.9ab	1.69a	1647.1ef
Tecto 1	54.12	3.0	4.8a	1.33a	1725.5f
0.5	37.8	3.0	12.1ab	1.67a	1568.1def
Dithane M 2.5	25.4	2.67	12.5ab	1.83ab	1411.8d
1.25	26.0	1.33	5.6a	1.33a	1647.1ef
Control	50.5	3.33	36.1bc	3.33c	470.6a
SED±	18.31	0.556	12.82	0.666	778.98
LSD	NS	NS	27.18	1.412	187.0

Figures followed by the same letter in the same column are not significantly different at P = 0.05

CHAPTER SEVEN

7.0 Effect of sowing dates on the incidence and severity of Alternaria leaf spot of cotton

7.1 Introduction

Field observation showed that the disease incidence and severity of Alternaria leaf spot of cotton vary considerably between the cotton plant grown at the onset of rains andtthose planted at various times in the rainy season. The disease incidence and severity were found to be lower in the early sown cotton. This same opinion was held by most of the farmers whose farms were visited for the desease assessment. However, some varieties show relatively high desease incidence throughout the growing period.

To determine the most appropppriate time for planting cotton so as to avoid the high epiphytotics of Alternia leaf spot and at the same time good yield, field trial were conducted in 1993 and 1994 in I.A.R, Research farms during the wet seasons. The intention was to evaluate sowing date as a cultural method of controlling the disease in the cotton growing zone of Nigeria (Northern growing zone of Nigeria).

7.2 MATERIALS AND METHODS

The experiment was carried out on I.A.R,research field in 1993 and 1994 with machines delinted cotton seed. Three plantings were made in both years with intervals of two weeks each. The first planting date in each year was determined by

the time rains become established. The date of plantingin both years were 9th July, 23rd July and 6th August. The sowing dates constituted the treatment which were replicated three times in a randomized complete block design. Cultivar Samcot-6 which was reported to be highly susceptible to the diseasewas used as the test cultivator. The field was ploughed, harrowed and ridged one week later. Inter ridge space was 75cm. Four seeds were sown per holeat interval of 45cm and the seedlings were thinned down to two per stand at three weeks after sowing. Each plot measured 5m x5 ridges with one meter border row between replicates and one ridge left between plots.

Each plot was hoe weeded two times, at three weeks after planting and six weeks later. Spot application of NPK (15:15:15:) fertilizer was carried out following Dadari et al (1994). The first application was carried out at three weeks after germination at the rate of 40kg N/ha, 40kg P_2O_5/ha and 40kg K_2O_5/ha (0.56kgNPK/plot) while the remainng 20kg/ha was applied as top dressing six weeks later.

Field inocculation was through natural means and disease incidence and severity was assessed for each growth stage of the crop (cotyledon, 5/7 leaf stage, vegetative and matured stages). This was done following the same proceedure described in 4.1.3 and 5.2 above. To obtain the yield, the crop was harvested through hand picking and weighed according to treatments. The yield in 1994 was ginned, the seed and the

lint were weighed seperately and expressed in percentage. Seedling rot (%) was alsc assessed in 1993.

7.3 RESULTS AND DISCUSSION

Results (Table 14 and 15) followed a similar pattern and consistently showed that the later the planting date, the greater the disease incidence and severity. July sown crops showed significantly (P=0.05) less disease attack than August sown crop in all the stages with the exception of the cotyledon stage in 1994 which gave 93.1% (Table 15). This result agrees with Bashi et al. (1983) which showed that cotton seedling are infected by Alternaria leaf spot as soon as they germinate. It is possible that, environmental factors: Rainfall, temperature and relative humidity was not favourable to disperse and induse infection, hence the less infection of the crops in the month of July.

The disease incidence and severity were generally high during the vegetative stage (August - September). During this periods as the incidenece increase from 51.1% to 68.4% the disease severity increase too from 3.67% to 3.83% in 1994. However, there are no significant difference (P =0.05) between the disease severity in the late July sown and August sown crops (Table 15). This might be attributed to high relative humidity (80.1%), optimum temperature (24±2°C) and high rainfall (150.3mm) which occurrd at the time of the year (Appendix 3). This observation agreed with Pauer (1990) who reported that *Alternaria* only cause substantial losses under

conditions of high relative humidity. Sharma and Bedi (1990 also reported that, incidence of wilt (*Fusarium oxysporum f sp. vasinfectum*) was significantly less in May-sown crops than in those sown in April in 1981 and 1982. Bashan, et al. (1991) showed that, sporulation of A. *alternaria* occurred on leaves only during the period of high relative humity (<95%). The result of the work presented here disagreed with Hiremath et al. (1988) who reported that *alternaria* disease development was low during the vegetative period (August to September) and rapid during the peak boll development (November). This is because during the vehetative stage, plant canopy was sufficient enough to trap appreciable number of *Curnidia* in the air. After that period, the crop begin to mature and the disease incidence and severity reduced possibly due to partial defoliation and dry weather conditions.

Yields in 1993 were 513.3 kg/ha and 140 kg/ha for early July and Late July sdown crops respectively. August soown crop with almost 95% germination could not continue after 5/7 laef stage because rainfal stops first week of September. This lack of rainfall resuted to premature flowering in response to favourable photoperiodism. Other factors that are associated with poor yield in 1993 include; temporary drought which occurred one week after planting resulted in poor seedling emergence in the first planting. Water logging later in the season coupled with poor rain distribution between third week of Augus and first week of September. Despite the

variation in yield in 1993 there where no significant difference (P =0.05) within treatments. In 1994, blanket treatment using Dimathioate was given to the crop to control insects and rainfall extended to last week of October on like 1993. This therefore resulted increased yield when compared to 1993 yield. At fiert planting in July, the yield was 1694.0 kg/ha, second planting in July gave 3042.7 kg/ha while the August sown gave 900.1 kg/ha (Table 15). This result indicated that as planting is delayed, the yield decrease. The decrease in yield may be attributed to unfavouarble environmental conditions particularly rain fall, humidity and temperature.

Table 13: Effect of Sowing date on the Incidence and severity of *Alternaria* leaf spot of cotton in 1993

		Cotyledon stage	5/7 leaf stage		Vegetative stage		Yield kg/ha
Sowing date	Seedling rot (%)	Incidence (%)	Incedence	Severity	Incidence	Severity	
09/07/93	6.06b	13.33a	23.0b	2.033	26.33	2.533a	513.3
23/07/93	3.03a	37.58b	30.6b	2.267	36.23	3.033b	140.0
06/08/93	1.82a	16.37a	7.87a	2.0	0.00	0.00	0.00
SED ±	1.838	5.59	4.94	0.1106	9.16	0.0923	250.0
LSD	5.108	15.518	10.379	NS	NS	0.256	NS

Figures followed by the same letter in the same column are not significantly different at P = 0.05

Table 14: Evaluation of the effect of date on the planting on the incidence and severity of *Alternaria* leaf spot of cotton in 1994

Date of planting	Cotyledon stage		5/7 leaf stage		Vegetative stage		Full boll maturity stage		Yield kg/ha
	Incidence (%)	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	
09/07/94	46.7a	2.0	10.5a	2.0a	31.8a	2.83a	16.23a	2.33a	1694.0
23/07/94	93.3b	2.5	10.47a	2.33ab	51.1b	3.67b	22.97a	3.0b	3042.7
06/08/94	66.7ab	2.33	62.03b	3.0b	68.4c	3.83b	66.64b	3.17b	900.7
SED±	12.77	0.544	5.38	0.272	2.76	0.136	9.40	0.167	1692.63
LSD	34.45	NS	14.935	0.755	7.662	0.378	26.09	0.464	NS

Figures followed by the same letter in the same column are not significantly difference at P=0.05

SUMMARY AND CONCLUSION

Alternaria macrospora Zimm. was confirmed to be responsible for the *Alternaria* leaf spot of cotton in the cotton growing areas in Nigeria. This conclusion was based on frequent association between the fungus and the symptoms of th disease, isolation of the fungus from lesions, study of morphological characteristics of the fungus and pathogenicity test; However, other leaf spots were observed and the associated pathogens are listed in Appendix 5.

Field observation showed that *Alternaria* leaf spot starts on any part of the leaf surface, even the bolls and the bracks were attacked at fruiting stage. Field survey for the incidence of *Alternaria* leaf spot in Zaria, Daudawa, Maigana and Basawa in September, 1993 showed that *Alternaria* leaf spot attacked the crop in all farms visited with acargae incidence of 75%, 80.5%, 70% and 75% respectively, which were high enough to attract control measures in order to avoid epidermic of the disease.

The initial symptom appears like pen-dot and is greenish brown in colour. As the disease progresses, it enlarges and produces a grey, dry centre with narrow dark brown border. Depending on the cultivar, the boarder can be purple or red but often narrow. The lesions later coalesced and form *Alternaria* leaf spot blight. Direct observation of a squashed lesion under compound microscope revealed that the invaded portion was red in colour. It was also observed that single conidiophore produce conidia

in chains while flexuous ones bear conidia singly at the tps with their broad base attached to the conidiophore. The conidia was solitary with horizontal and vertical separation and in some casses, the beaks were found to be longer than the body length.

Evaluation of ten cotton cultivars for their reactions to *Alternaria* leaf spot under screen-house and field conditions in 1993 and 1994 showed that all the cultivars tested were susceptible to the disease, but the degree of susceptibility varied. Samcot-9 (1994) showed a constant disease severity (2.33) from cotyledon growth stage to 5/7 leaf stage but reduced disease incidence from 51.7% at the cotyledon to 23% at 5/7 leaf stage. The incidence of cotton to *A. macrospora* was highest in the vegetative growth stage. However, screen house observation showed that, the use of lesion diameter was simple, objective and permitted proper separation of means than other measures of severity with Samcot-9, TXCDP 37-HH-1-83, GCA-NH-1-83, S-295 been resistant to *Alternaria* leaf spot. Tamcot sp 37 and Samcot-8 were moderately resistant while CAMD-E, Samcot-10, Tamcot sp 215 and Samcot-6 were susceptible to the disease.

On the other hand, field observation showed that susceptibility increased from the cotyledon stage and reduced in the seedling stage particularly with Samcot-10 and CAMD-E (Table 5) and Samcot-8, Tamcot sp 37, TXCDP 37-HH-1-83 and Samcot-9 (Table 7). Consequently epidermics developed in three phases, which corresponded closely to

the cotyledon, 5/7 leaf stage (seedling) and vegetative growth stages. During the seedling stage, temperature (Appendix 3) was low and the canopy was open and thus does not create a microclimate favourable for disease development. Accordingly, *Alternaria* infection are scattered and the progress of the epidermic is associated mainly with an increase in the number of the infested plants. During the third phase (vegetative stage) of the epidermic, the development of a dense canopy and high temperature and humidity created a more favourable microclimate for disease development. Consequently, the host becomes progressively more susceptible and the amount of damaged foliage increase rapidly. This therefore resulted to leave senesce and premature dropping of the leaves. Cultivars Samcot-9, TXCDP 37-HH-1-83, S-295 and CAMD-E yielded high and therefore are recommended for farmers. However, in terms of breeding for resistance to *Alternaria* leaf spot, Samcot-9, TXCDP 37-HH-1-83 and GCA-NH-1-83 are recommended base on the experimental error of this study. CAMD-E and Tamcot sp 215 are tolerant to *Alternaria macrospora*, while the other cultivars were susceptible to the disease.

Four fungicides were evaluated for their efficacy in controlling *Alternaria* leaf spot of cotton in vitro and under field conditions in 1993 and repeated in 1994. Laboratory results showed that all the tested fungicides were effective for the diseases control. Tecto, Dithane M 45 and Delsene M completely inhibited mycelial growth of A.

macrospora while Benlate gave 100% growth inhibition at 1.2 Kg a.i./ha and 0.6Kg a.i./ha. However, at 0.3Kg a.i /ha and 0.15 Kg a.i./ha benlate inhibited 86.3% and 67% respectively on the amended agar medium. Field observation followed the same pattern of performance as in agar medium with Tecto giving the best result both in disease reduction and yield followed by Dithane M 45. However, Delsene M was phytotoxic to the crop at 1.2 Kg a.i./ha, but at 0.6 Kg a.i./ha, it was observed to considerably reduce disease incidence and severity and increase in yield. In contrast, application of benomyl spray at 0.6 Kg a.i./ha was not consistent in action. It reduced disease severity from 4.1 to 2.5 in 1993 and increased severity from 3.0 to 3.17 in 1994. The application of sprays of Thiabendazole T BZ, Mancozeb and Carbendazim plus Maneb at 7-14-21 and 28 day intervals in 1993 produced significant difference (P=0.05) in fibre yields among different sprays regime compared to the check. However, Thiabendazole T BZ gave a significantly high yield of 1725.5 Kg seed cotton/ha (1.0 litre a.i./ha). The low yield with Delsene M (826.8 Kg seed cotton/ha) at 1.3 Kg a.i./ha was attributed to the phytotoxicity effect of the fungicide which reduce the photosynthetic area of the crop. This was observed at weekly spray interval of the fungicide. Since the same fungicide perform well at 0.6 Kg a.i./ha by suppressing the disease upto 74.8% and reduced yield to about 71.4% when compared to the check, it is therefore recommended to be applied fortnightly to avoid over dose of the fungicide

to the crop. Consequently, the performance of the fungicide under field conditions were satisfactory, therefore they are recommended. However, Benlate (systemic fungicide) should be mixed with contact fungicides such as Maneb to obtain proper and effective disease controll. Generally, the out-break of this disease cannot be completely controlled by spraying once the disease has established. It is therefore recommended that these fungicides be applied as protective fungicides before the establishment of *Alternaria* leaf spot in the field.

The effect of sowing date was evaluated in 1993 and 1994 in the field to determine the proper time of the year to plant cotton so as to enable it escape severe attack of A. *macrospora*. This was done in three successive times starting from July, 8th at two weeks intervals. Results showed that the later the planting dates, the more severe was the disease on the crop. The early stoppage of rainfall in 1993, resulted in premature flowering of the last planting in August in response to favourable photoperiodism. It is therefore possible that August-sown crops was exposed to high relative humidity, optimum temperature and heavy rainfall which was associated with strong winds that favoured inoculum production, dispersal and deposition as well as infection of later-sown crops at younger stage.

The yield was high in the July-sown crops when compared with August-sown crops. In 1993, early July-sown crop gave 513.3 Kg/ha seed cotton and 140 Kg/ha seed cotton

for late July-sown crop while August yielded nothing due to the fact that the seedlings could not with stand the sudden drought which resulted from early stoppage of rainfall in September. However, in 1994, first and second July-sown crops yielded 1694.0 Kg/ha and 3042.7 Kg/ha seed cotton respectively which were also higher than August-sown crop (900.7 Kg/ha). This therefore indicated that late sown crop produce low fibre and grain yield. Consequently, farmers and researchers are therefore advised to plant seed cotton by June or at most second week of July depending on rainfall establishment. This will help to suppress *Alternaria* leaf spot significantly.

LITERATURE CITED

Adeoti, A.A. (1991). The biology and control of Coniella leaf spot of kenaf, Hibiscus cannabinus, L. induced by Coniella musacaensis B. Sutton Var. Hibici B. Sutton. Ph.D Thesis, A.B.U., Zaria 123pp.

Adeoti, A.A., Alabi, S.O. and Nwanosike, M.R.O. (1995). Cropping Scheme Report, Fibre Research Programme, I.A.R., A.B.U., Zaria. p.28-36.

Agboola, S.A. (1979). An Agricultural Atlas of Nigeria. Oxford University Press, 246pp.

Agrios, G.N. (1978). Plant Pathology. Academic Press New York, 703pp.

Ahonsi, M.O. (1995). Studies on the false smut of rice, Oryza sativa L., induced by Ustilaginoidea virens (CKE) TAK. M.Sc Thesis, A.B.U., Zaria 100pp.

Alabi, O. (1994). Evaluation of yield losses in groundnut caused by foliar diseases. Report on Legume and Oilseeds Research Programme, I.A.R., Zaria. p.15-20.

Alafiatayo, O. (1982). Evaluation of fungicides for the control of cowpea brown blotch disease caused by Collectotrichum capsici (Syd) Butler and Bisby. M.Sc. Thesis, A.B.U. Zaria. 89pp.

Alagarsamy, G. Krishnamohan, G. and Jejarajan, R. (1989). Field evaluation of fungicides against leaf spot of cotton caused by Alternaria macrospora Indian Journal of Plant Pathology, 17(1): 105-106.

Alexopoulos, J. (1962). Introductory Mycology. 2nd edition, John Wiley and Sons, Inc. New York, London, Sydney 613pp.

Anonymous, (1976). Cotton and Fibre Programmes, Report to the I.A.R. Board of Governors on the Institute work, 1977-1978. 30pp.

Barksdale, T.H. (1968). A method of screening for resistance to early blight of tomato seedlings. Physopathology, 58:883.

Bashan, Y. (1984). Transmission of Alternaria macrospora in cotton seeds. Phytopathologische zeitschrift, 10(2):110-118.

Bashan, Y. and Levanony, H. (1987). Transfer of A. macrospora from seed to seedling: Light and scanning electron microscopy of colonization. Journal of Phytopaothlogy 120(1):60-68.

Bashan, Y. and Levanony, H. (1991).[a] Association between A. macrospora and A. alternata, cansal agent of cotton leaf blight. Canadian Journal of Botany 69(12):2603-2607.

Bashan, Y. and Levanony, H. (1991).[b] Wild beets as an important inoculum source of A. alternata, a cause of leaf blight of cotton in Israel. Canadian Journal of Botany, 69(12):2608-2615.

Bashan, Y. and Levanony, H. (1991).[c] Wind dispersal of Alternaria alternata. a cause of leaf blight of cotton. Journal of Phytopathlogy, 133(3):225-238.

Bashi, E.; Sachs, Y.; and Rotem, J. (1983). Relationships between diseases and yields in cotton fields affected by Alternaria macrospora Phytoparasitica, 11:89-97.

Bashi, E.; Rotem, J.; Pinnschmidt, H.; and Kranz, J. (1983). Influence of controlled environment and age on development of Alternaria macrospora and on shedding of leaves of cotton. Phytopathology, 73:1145-1147.

Bawa, A. (1992). Studies on the midrib spot disease of millet in Nigeria. M.Sc. Thesis, A.B.U., Zaria. 102pp.

Brown, B.H. and Wares, O.J. (1958). Cotton. Mc Ga-Hill. pp.18-19.

Chandler, J.M and 1.R. Oliver (1979). Spurred Anoda: A potential weed in southern crops. US. Sci. Ed. Admin; Agricultural Reviews and Manuals, Southern series, No 2, 19pp

Charlton, K.M. (1953). The sporulation of Alternaria solani in culture. British Mycological Society Transaction. 36:349-355.

Choyce, M.A. (1968). A review of cotton development in Nigeria. Samaru Agricultural Newletter, 10 :44-57.

Cotty, P.J. (1987).[a] Evaluation of cotton cultivar susceptibility to Alternaria leaf spot. Plant Diseases, 71 :1082-1084.

Cotty, P.J. (1987).[b] Temperature-induced supression of Alternaria leaf spot in Arizoma. Plant Diseases, 71: 1138-1140.

Cotty, P.J. and Misaghi, I.J. (1984). Zinniol production by Alternaria species. Phytopathology 74:785-788.

Cotty, P.J. and Misaghi, I.J. (1985). Effect of light on the behaviour of Alternaria tagetica in vitro and in vivo. Phytopathology: 75:366-370.

Dadari, S.A., Kumar, V., Miko, S., and Musa, U.T. (1994). Chemical weed control in cotton (Gossypium spp.) + cowpea (Vigna unguiculata) mixture. Indian Journal of Agronomy 39(1):4-8.

Davis, D., and Diamond, A.E. (1952). Altering resistance to diseases with synthetic organic chemicals. Phytopathology 42(10): 563-567.

Derrick, J. (1976). Cotton Research in Nigeria. West Africa, No.3065, pp.416-417.

Desai, S.A. and Patil, S.A. (1983). In Vitro evaluation of different fungicides against Alternaria macrospora Zimm. incitant of leaf spot of cotton. Current Research 11(718):72-73.

Douglas, D.R. and Pavek, J.J. (1971). An efficient method of inducing sporulation of Alternaria solani in pure culture. Phytopathology, 61:239.

Dransfield, M. (1965). Cotton seed dressing in Northern Nigeria. Samaru Research Bulletin No.48.

Dransfield, M. (1966). The fungal Air-spora at Samaru, Northern Nigeria. Samaru Research Bulletin. No.69.

Eckaradt, W.B. (1906). Der Baumwollenabau in Senner Abhangigucit Xom Vlima den Gnen Sen Sonier Abaugebiete, baih. Tropenpflanzer Vol. V11.

Ellis, M.B. (1971). Dematicaceous Hyphamycetes. Commonwealth mycological Institute, England. 608pp.

Emechebe, A.M., Erinle, I.D., Bos, W.S., Tyagi, P.D., Sundaram, N.V., Ebenebe, A.C. and Subbarayudu, S. (1980). A check list of diseases of crops in the savanna and semi arid areas of Nigeria. Samaru Miscellaneous paper No 100,

pp13.

Engledow, F.C. (1947). Agricultural development in the British Colonial Empire. Agricultural Economics Society: London.

Filajadic, N. and Sutton, T.B. (1995). Overwintering of Alternaria mali, the causal agent of Alternaria Blotch of Apple. Plant Diseases, 79(7):695-698.

Food and Agricultural Organization (F.A.O.): Production year book, 1984 Vol.33.

French, C.N. (1926). Cotton Growing Industry in Nigeria. pp.5-13. Empire Cotton Growing Corporation, London.

Fred, 1966

Fryxell, P.A. (1979). The natural history of the cotton tribe. Texas A and M University Press College station and London.

Gregory, P.H. (1958). A correction. Transaction of British Mycological Society 14:202.

Gulati, A.M., and Turner, A.J. (1928). A note on the early history of cotton. Technical Lab. Bulletin No.17. Indian Central Cotton Committee.

Harland, S.C. (1938). The Genetics of Cotton. Jonathan Cape: London.

Hill, D.S. and Waller, J.M. (1990). Pests and Diseases of tropical crops. Vol.1, Principles and methods of control. Longman Group, Ltd. London, and New York. 175pp.

Hillocks, R.J. (1991). Alternaria leaf spot of cotton with special reference to Zimbabwe. Tropical Pest Management 37(2): 124-128.

Hiremath, R.V., Padaganur, G., and Basavaraj, M.K. (1988). Epidermiology of Alternaria leaf spot of cotton in transition zone of Karnataka. Indian Phytopathology, 41(4): 586-589.

Hiremath, P.C., Advani, M.R., Panchabhavi, K.S., and Hegde, R.K. (1990). Gompertz model for determining the loss in yield of cotton due to Alternaria leaf spot. Karnatakas Journal of Agricultural Sciences, 3(3.4):276-277.

Hiremath, P.C. and Savanur, R.D. (1990). Biochemical changes in cotton leaves in response to Alternaria macrospora infection. Current Research University of Agricultural Sciences (Bangalore) 19(8):142-143.

Hutchinson, J.B. (1959). The application of Genetics to cotton improvement. Cambridge University Press: London.

Idris, A.M. (1990). Cotton leaf curl virus disease in the Sudan. Paper presented at the 42nd International Symposium on Crop Protection, Gent, Belgium, 8 May, 1990.

Institute for Agricultural Research (I.A.R): Samaru Conference Paper 14: 1976.

IAR (1991) Cropping Scheme Report. Adeoti A.A. Fibre Research Programme, A.B.U., Zaria.

IAR (1994) Cropping Scheme Report. Adeoti A.A; Alabi S.O. and Nwonosike M.R.O. Fibre Research Programme, A.B.U., Zaria P. 15-20.

Irvine, F.R. (1974). West African Crops. 3rd Edition, University Press: Oxford, Great Britain. pp.48-54.

Ito, M.F; Bergamin Filho A.; Igue, T. (1988) In vitro sensitivity of Ceratocystis fimbriata to systmic fungicides and its chemical control on Crtalaria juncea. Filopatologia brasileira 13 (1): 35-42.

Jackson, C.R. (1959). Cercospora blight Snapdragon. Phytopathology, 60:190-192.

Johnson, W.H. (1926). Cotton and its production. macmillan: London.

Johnson, R.O. (1960). A decade of Nigerian Cotton 1949-1958. Nigeria Geographical Journal 3(2):1-4.

Jones, C.H. (1928). An Alternaria disease of the cotton plant. Annals of Botany, 42:935-947.

Komolafe, O. (1984). Studies on the biology of Curvularia leaf spot disease of maize Zea mays L.). Fellowship of the Institute of Science and Technology, London 65pp.

Krishnamohan, G. and Vidhyasekaran, P. (1989). Possible involvement of toxins in Alternaria leaf spot development in cotton. Indian Phytopathology, 42(1):99-102.

Ling, L. (1944). Factors influencing the development of cotton diseases. Annal of Applied Biology 31:194-204.

Ludwiq, R.A., Richardson, L.T., and Unwin, O.H. (1962). A method for inducing sporulation of Alternaria solani in culture. Canadian Plant Disease Survey 42:149-150.

Lukens, R.J. (1960). Conidial production from filter paper cultures of Helminthosporum vagans and Alternaria solani. Phytopathology 50:867-868.

Lukens, R.J. (1966). Interference of low temperatures with the control of tomato early blight through use of nocturnal illumination. Phytopathology, 56:1432-1433.

McCallan, S.E.A. and Chan, S.Y. (1944). Inducing sporulation of A. solani in culture. Contrib. Boyce Thompson Institution, 13:323-335.

Miller J.F. (1973). Cotton weeds. Weeds Today 4 (1): 6-9

Miller, J.W. (1969). The effect of soil moisture and plant nutrition on the cercospora - Alternaria leaf blight complex of cotton in Missouri. Phytopathology 59:767-769.

Minton, E.B. and Ebelhar, M.W. (1991). Potassium and aldicarb-disulfoton effects on verticillium wilt, yield and quantity of cotton. Crop Science 31(1):209-212.

Moorman, G.W.; Lease, R.J; Vali, R.J. (1989) Fungicide-resistance to Botrytis from pennsylvania greenhouse. Bulletin, Pennsylvania Flower Growers. No. 392, 1-2.

Munro, J.M. (1987). Cotton 2nd edition, Longman Group U.K. Ltd. 436pp.

National Agricultural Extension and Research Liasion Services (NAERLS, 1976). Cotton production in the Northern states of Nigeria. Extension Bulletin 14.

Obagwu, J. (1996). Studies on the brown blotch disease of Bambara nut (Vigna subterranea L.) Verdc. induced by Collectotrichum capsici (Syd.) Butler and Bisby in the Zaria area of Northern Nigeria. M.Sc. Thesis A.B.U., Zaria. 77pp.

Orh, H.D; Pollack, F.G and Inger, B.F (1977). The occurance of Alternaria macrospora on Anoda cristata in Mississipi. plant Disease Reporter 61 : 208 - 209.

Olsen, M.W. and Cotty, P.y. (1986). Control of Alternaria leaf spot of Gossypium barbadense. Pages 27-28 In: Proceedings Beltroide Cotton Production Research Conference, Las Vegas, NV. 524pp.

Oyenuga, V.A. (1968). Nigerian's Foods and Feeding stuffs. Ibadan University Press. 1968 pp.60-63.

Padagamur, G.M. and Basavaraj, M.K. (1984). Elimination of seed-borne inoculum of Alternaria macrospora Seed treatment. Plant Pathology Newsletter 2(1):20-21.

Padagamur, G.M., Hiremath, R.V. and Basavaraj, M.K. (1989). Estimation of yield loss due to Alternaria leaf spot and blight in cotton. Journal of the Indian Society for Cotton Improvement 14(2): 144-145.

Pauer, G.D. (1990). Alternaria leaf spot in cotton. Farming in South Africa, University of Orange state, Bloemfontein, South Africa. G.9, 1p.

Phillip, T.A. (1977). An Agricultural notebook. Longman.

Poswal, M.A.T. (1981). Studies on bacterial blight of cotton in Northern Nigeria. M.Sc. thesis submitted to Department of Crop Protection, I.A.R., A.B.U. Zaria, Nigeria 144pp.

Prentice, A.N. (1972). Cotton. Longman London.

Purseglove, J.W. (1974). Tropical Crops: Dicotyledons pp.333-364. Longman, London.

Raghuram. M. and Mallaiah, K.V. (1989). Aerial dissemination of Alternaria macrospora pathogenic to cotton. Indian Journal of mycology and plant pathology, 19(1):1-4.

Rands, R.D. (1917). The production of spores of A. solani in pure culture. Phytopathology 7:316-317.

Rotem, J. (1978). Climate and weather influences on epidermics. Pages 317-337 In: Plant disease Vol.2. J.G. Horsefall and E.B. Cowling. eds, Academic Press New York.

Rotem, J. Edidt, J., Wendt, U. and Kranz, J. (1988). Relative effects of A. alternata and A. macrospora on cotton crops in Israel. Plant Pathology 31(1): 16-19.

Rotem, J. Wendt, U. and Kranz, J. (1988). The effect of sunlight on symptom expression of Alternaria alternata in cotton plant pathology 37:12-15.

Rotem, J. (1990). Overwintering of A. macrospora in cotton debris. Phytoparasitica, 18(2):143-152.

Sciumbato, G.L. and Pinckard, J.A. (1974). Alternaria macrospora leaf spot of cotton in Louisiana. Plant Disease Reporter, 58(3):201-202.

Sharma, J.R, and Bedi, P.S. (1990). Effect of sowing dates on wilt of cotton. Plant Disease Research 5(1): 83 - 85.

Shephard, C.G. (1972) Cotton weed control. Mississppi Farm Research 35(10):2.

Shimfe, D.N. (1984). In vitro effect of Benlate, antrocol neem extract on Sclerotium rolfsii and Collectotrichum gloeosporioides M.Sc. Thesis. Department of Botany, University of Jos. 101pp.

Shtienberg, D. (1991). Effects of leaf susceptibility and fungicide seed treatment on leaf spot of Pima cotton caused by A. macrospora. Plant Pathology, 40(3) 415-421.

Shtienberg, D. (1996) Variables Associated with Intensity of Alternaria Leaf Spot in Pima Cotton. Phytopathology 86 (1) 123-128.

Shtienberg, D., and Dreishpoun, J. (1991). Suppression of Alternaria leaf spot in Pima cotton by systemic fungicides. Crop Protection 10(5): 381-385.

Shtienberg, D., Krener, Y; and Dinoor, A. (1993) Influence of physiological age of pima cotton on the need for fungicides treatment to suppress Afternaria leaf spot. Phytopathology 83: 1235-1239.

Spross-Blickle, B., Rotem, J., Lerl, M; and Kranz, J. (1993). The relationship between infection of the cotyledon of Gossypiu, Barbadense and G. hirsutum with Alternaria macrospora and cotyledon ascission. Physiological Mol. of Plant Pathology 35: 293-299.

Skovsted, A. (1937). Cytological studies in cotton. IV. Chromosome conjugation in interspecific hybrids. Ibid. 34:97-138.

Smith, C.E. Jr. (1968). Plant remains. In Byers, D.S. (ed) Prehistory of the tehuacan Valley, 1: Austin, Texas. 220-255.

Stebbins, G.L. Jr. (1947). Evidence on rates of evolution from the distribution of existing and fossil plan species. Ecol. Monogr., 17: 147-158.

Stephens, S.G. (1942). Colchincine produced polyphoids in Gossypium. I. An Auto-tetraploid Asiatic cotton and certain of its hybride with wild deploid species. Journal of Genetics, 44:272.

Streets, R.B.S. (1969). Diseases of the cultivated plants of the south west. University of Arizona Press, Tueson 390pp.

Tsoror, L. Nachmias, A. Yaron, A, Uner, M. (1990). Verticillium with and Alternaria leaf spot in pima cotton. Hassadeh 70(5):681-682.

Walker, H.L. and G.L Sciumbato (1979). Evaluation of Alternaria macrospora as a potential Biocontrol Agent for spurred Anoda (Anoda cristata): Host Range Studies. Weed Science, 27: 612 - 614

Watt, G. (1907). The wild and cultivated cotton plants of the world. Longmans Green: London.

Webber, J.M. (1939). Relationships in the genus Gossypium as indicated by cytological data. Journal of Agricultural Research, 58: 237-261.

Wilson, F.D. and Brown, J.K. (1991). Inheritance of response to cotton leaf crumple virus infection in cotton. Journal of Heredity, 82(6) 508-509.

Yar'adua, A.A. (1986). Studies on Alternaria porri the causal organism of purple blotch disease of onion in Zaria environs. M.Sc. thesis submitted to Crop Protection Department A.B.U., Zaria.

Zimmerman, A. (1904). Beriche Uber Land-u, Forstwirtschcaft Von Deutsch Ostafrika. Bd. 2:24-25.

APPENDICES

APPENDIX 1: Preparation of potato Dextrose Agar (PDA) media.

(a) The PDA media used for this research were always prepared following the procedure given below.

Materials:

Peeled irish potato	200g
Dextrose	20g
Agar	20g
Water	1000ml

The peeled irish potato were weighed and cut into small pieces, washed and boiled for one hour in 1000ml of water. The filtrate was filtered with muslin cloth and the volume made to 1000ml. 20g each of dextrose and agar were added to the filtrate and reboiled to dissolve the agar. The PDA was plugged with cotton wool 500ml conical flask (pyrex) and antoclaved at 121°C for one hour.

The petridishes were sterilized on the oven inside clovered canisters for about 24 hours at temperature of 120°C to ensure adequate sterilization.

Just before pouring the PDA into the petridishes, 5ml of streptomycin solution (prepared by dissolving 1g of the powder in 100ml of sterile water) was pipetted into 500ml of the molten PDA to avoid bacteria contamination.

Between 35-50°C, the molten PDAs was poured under sterile condition. Approximately 15-20ml of PDAs was poured per petridish and the plates were allowed to set for 24 hours before use.

PDA slants were prepared on McCartney bottles. Appropriate volume of the PDA were poured into the bottle after boiling the PDA to dissolve the agar. These were then autoclave at the same temperature and time as above.

(b) Another formulation of PDA used to induces sporulation contain the following, after Douglas and Pavek (1971).

Instant mashed potatoes	15g
Dextrose	15g
Flake agar	23g
Distilled water	1500ml

The preparation was same as above.

Appendix 2:

Length, width and beak of the conidia obtained from random selection on the compound microscope viewed under x10 objective lens.

Serial Number	Length (mm)	Width (mm)	Beak
1	25	3	24
2	25	6	20
3	26	8	6
4	25	5	10
5	24	6	20
6	26	4	18
7	25	6	12
8	26	3	28
9	25	4	10
10	26	4	20
11	26	5	11
12	25	4	24
13	26	3	6
14	26	3	11
15	25	4	12
16	25	4	6

Serial Number	Length (mm)	Width (mm)	Beak
17	25	4	12
18	25	4	20
19	25	6	24
20	23	4	10
21	27	3	20
22	26	4	18
23	25	4	12
24	27	4	28
25	25	3	10
26	25	4	20
27	27	4	11
28	26	5	24
29	27	4	10
30	24	4	11
31	25	3	12
32	26	4	9
33	26	3	12
34	25	4	10
35	25	3	12
36	25	5	15
37	26	7	16

Serial Number	Length (mm)	Width (mm)	Beak
38	24	5	20
39	25	5	20
40	25	4	9
41	25	4	10
42	25	3	10
43	27	4	8
44	27	6	11
45	26	4	12
46	26	3	10
47	26	3	20
48	25	3	15
49	27	3	15
50	26	5	10

$$\frac{\Sigma \text{ Length in mm} \times 5.0\mu}{50} \quad \frac{}{1}$$

where 5.0μ is the constant factor for x10 objective lens.

Appendix 3: Temperature, Relative Humidity and Rainfall Distribution in Months during 1993 and 1994 Growing Season.

Months	1993			1994		
	T (°C)	R.H. (%)	R.F. (mm)	T (°C)	R.H. (%)	R.F. (mm)
January	19.0	21.4	0.0	21.7	20.9	0.0
February	23.2	21.4	0.0	23.8	13.2	0.0
March	26.3	30.6	0.0	28.6	22.7	0.0
April	30.0	38.3	8.3	29.1	42.65	20.4
May	29.9	54.9	113.6	27.8	56.25	120.1
June	26.3	62.3	155.8	25.3	65.25	231.9
July	24.9	67.6	269.0	24.7	74.25	169.2
August	24.0	72.0	300.8	23.8	81.7	218.6
September	24.8	67.5	181.8	24.3	78.45	82.5
October	26.4	58.6	32.4	24.8	70.7	69.2
November	23.9	-	0.0	21.6	35.7	0.0
December	21.6	28.4	0.0	19.1	31.65	0.0
Total			1061.7			911.9
Mean	25.0	43.6		24.6	49.45	

Source: I.A.R., Samaru Meteorological Centre.

Appendix 4:

Occurence of Alternaria Leaf Spot of Cotton in Perennial Cotton in Imo State (Mbaise).

Alternaria leaf spot of cotton induces by *A. macrospora* zimm. is a widespread disease of cotton in the world (Ellis, 1971). However, its occurence is favoured by high humidity and adequate rainfall during the growing season. In Nigeria, the disease is known to be prevalent in the cotton growing zones (1.4) where the crop is widely grown. In the Eastern part of the country however, the disease is not pronounced, but visit made to perennial cotton crops cultivated for medicinal purposes particularly in Mbaise (Imo State) showed that the disease is present at level that requires attention. The author observed and collected some samples and the pathogen was isolated from the leaf lesions in IAR, Samaru, Zaria.

The stands visited were few and in most cases, only one stand was found in a village. This therefore showed that cotton can be grown in such areas and the disease should be properly taken care of since there is no close season in such places. Considering the perennial condition. The places visited were Uvuru, Chikoneze and Nguru (all in Mbaise). Average disease incidence was 100% with severity of about 4.5 using the key in chapter 4.

Appendix 5:

Fungi Associated with Alternaria Microspora on Cotton

Aspergillus spp.

Ulocladium spp.

Helminthosporium spp.

Ramilaria spp.

Curvularia spp.

Nigrospora spp.

Fusarium spp.

Other *Alternaria* spp (like *A. raphani, A. alternata.*

It is therefore possible that any of the above pathogens is responsible for the new leaf spot observed by the author under field condition or they might be saprophytes occupying the invaded portion of the leaves. However, they were isolated pure under artificial media.

Appendix 6:

Analysis of Variance for the Plant Height Lesion Diameter, Number of Lesions and Ranking of the Alternaria Leaf Spot of Cotton Evaluated in the Glasshouse in 1994

(1) Plant Hieght (mm)

Sources	Df	SS	MS	F
Rep	2	0.65	0.325	0.043 NS
Trt	9	1001.72	111.3	14.75 **
Res	18	135.83	7.546	
Total	29	1138.2	-	

(2) Lesion Diameter (mm)

Sources	Df	SS	MS	F
Rep	2	3.95	1.98	1.41 NS
Trt	9	42.67	4.74	3.386**
Res	18	25.18	1.4	
Total	29			

(3) Number of Lesions Per Plot (Convenient unit).

Sources	Df	SS	MS	F
Rep.	2	0.29	0.135	1.41 NS
Trt.	9	16.84	1.87	1.97 NS
Res.	18	17.06	0.95	
Total	29	34.17		

(4) Rank

Sources	Df	SS	MS	F
Rep.	2	0.2	0.1	0.48 NS
Trt.	9	19.2	2.13	10.14 **
Res.	18	3.8	0.21	
Total	29	23.2		

** Significant at 5%

NS Not significant at 5%