EVALUATION OF PROPICONAZOLE (TILT) AND PRUNING IN THE CONTROL OF BLACK SIGATOKA DISEASE IN GHANA

BY

CEPHAS KWASI BODAKPUI

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREEE OF MASTER OF PHILOSOPHY IN CROP SCIENCE

NOVEMBER, 1997

CROP SCIENCE DEPARTMENT
UNIVERSITY OF GHANA
LEGON

D E C L A R A T I O N

I hereby declare that, except for reference to other people's work which have been duly cited. this work is the result of my own original research and that this work has neither in whole nor in part been presented for degree in this University or elsewhere.

.....

CEPHAS KWASI BODAKPUI

(STUDENT)

25-8-99

DATE

.....

DR. K. A. ODURO

(SUPERVISOR)

8-9-99

DATE

.....

PROF. K. AFREH-NUAMAH

(CO-SUPERVISOR)

21/9/99

DATE

ABSTRACT

Pruning of diseased leaves and the use of Propiconazole (Tilt), a foliar fungicide with systemic properties, were two different recommendations made by the Ministry of Food and Agriculture to control black sigatoka, currently an important disease of plantain in the country. The two methods were evaluated from September 1995 to April 1997 at the University of Ghana Agriculture Research Station, Kade.

Four treatments, namely, Tilt (0.125g ai/L), Pruning, Tilt (0.125g ai/L) + Pruning and Control (neither Tilt nor Pruning), were applied in an RCBD experiment.

The average height of the plantain in the different treatments were found not to be significantly different from each other. Using percentage total leaf area attacked, disease severity for the Control treatment was on the average 16% (13.1-19%) while it was 4.6% (2.6-6.2%),5.1% (3.7-6.4%) and 3.8% (2.5-5.0%) for Tilt, Pruning and Tilt+Pruning respectively. The Control was significantly different from the other three treatments which were, however, not different from each other at 5% significance level.

Maturity of plantain was found to delay in the Control. The total number of bunches harvested at 66 weeks were 54, 50, 52 and 47 with bunch weights of

453.0Kg, 392.2Kg, 405.0Kg and 249.1Kg for Tilt, Pruning, Tilt+Pruning and Control respectively. The Control was significantly different from the other three treatments which were however not different from each other (p. 5%). The difference in bunch weight was due to significant difference in weight per finger (0.258Kg, 0.245Kg, 0.252Kg and 0.186Kg), respectively, for the treatments. There was no difference in the number of fingers per bunch which was on the average 26.1 for all the treatments. Correlation analysis gave a negative but a significant association (r = -0.96) between severity of disease and yield. Simple pruning and burning of diseased leaves could be recommended for the control of black Sigatoka in the absence of chemical which may be expensive.

To determine the potency of the chemical (Tilt), a bioassay was conducted in the laboratory. One ml of the following concentrations of Tilt viz. 0.0125g ai/L; 0.025g ai/L; 0.05g ai/L 0.0625g ai/L and 0.075g ai/L were incorporated into 5 different PDA in Petri dishes to form Tilt-ammended PDA media plus a control (only PDA). Ascospores from diseased leaves were ejected onto these plates. Germination in all cases started about 12 hours after ejection. Unipolar and bipolar germ tubes were observed on all the plates but the percentages differ, depending on the concentration of Tilt. The higher the

concentration, the lower the percentage germination. Visible growth was seen with the naked eye after 4 days. Aerial mycelia was whitish with reverse dark on the control plates while the aerial mycelia on Tilt-ammended PDA were gray with reverse dark. No conidia were seen in all cases after several weeks. The bioassay studies confirmed that Tilt was potent.

ACKNOWLEDGEMENT

I with to express my profound gratitude to Dr. K. A. Oduro - Head of Crop Science Department, University of Ghana, Legon and Prof. K. Afreh-Nuamah - University of Ghana Agricultural Research Station (UGARS)- Kade under whose excellent supervision I wrote this thesis. Their constructive criticism, patience and encouragement made this work a reality.

I am indebted to Dr. K. G. Ofosu-Budu and Dr. J. K. Osei both of UGARS-Kade who freely volunteered information during the course of the project. I am also indebted to Mr. A. Ampomah and Mr. J. M. Kokroh - Farm manager and senior Assistant farm manager respectively, at UGARS-Kade, for their valuable help and contributions from the beginning to the end of the field work. I am grateful to Mr. Ayifa and wife, Sister Lydia Larbi, and the workers of UGARS-Kade, for their immense assistance whenever I went there.

My sincere thanks go to my course mates especially Messrs. Michael Assuah, Edmund Botchway, Gershon Wodzrah and Benard Boateng for their good company and encouragement during my stay at University of Ghana.

Finally, I thank Miss Favour Anyam who typed the script. To all who helped in one way or the other, I say, thank you. May the Almighty God bless you.

D E D I C A T I O N

This work is dedicated to my wife, **MRS. JOSEPHINE BODAKPUI**, and my son, **MARIUS EDEM FIIFI BODAKPUI.**

TABLE OF CONTENT

CONTENT **PAGE**

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

INTRODUCTION

Plantain (*Musa paradisica* L) is a major source of carbohydrate for millions of people in the developing world (Stover and Simmonds, 1987; Swennen, 1990). In Ghana it was estimated that the national production between 1986 and 1990 was 5,205,617 metric tonnes (Ministry of Food and Agriculture, PPMED, 1990).

Plantain is affected by a number of diseases caused by viruses, bacteria and fungi. One of the fungal diseases which has caused serious losses of friuts in many countries is the leaf spot (Sigatoka) disease (Wardlaw, 1961). This disease is caused by the fungus *Mycosphaerella sp.* There are two types of the disease, namely, the yellow and black Sigatoka. The yellow Sigatoka, caused by *M. musicola*, was first reported in Fiji in 1912 (Philpott and Knowles, 1913). The black Sigatoka is caused by *M. fijiensis*, (*Paracecospora fijiensis*) and tends to be more serious was observed in 1960 in Fiji. It was named by Rhodes (1964) as black leaf streak, but it was later re-named black Sigatoka (Wardlaw, 1972).

Black Sigatoka disease was observed in some West African countries including Ghana in 1985 (Wilson, 1986) and it became epidemic in the 1990s (Hemeng et al 1995).

Oduro et al (1992) reported that the national incidence of the disease was 62.5% and recommended pruning and burning of diseased leaves as one of the control measures.

Yield losses of plantain due to black sigatoka range from 20% (Pasberg-Gauhl, 1989) up to 50% (Stover, 1983; Mobambo, 1993). Mobambo et al (1993) also reported that plantain yield losses of 33% in the first and 76% in the second cropping cycle had been estimated at Onne, Southeastern Nigeria.

To control the disease in Ghana, Oduro et al (1992) recommended pruning and burning of diseased leaves. In 1994 the Ministry of Food and Agriculture recommended the use of Propiconazole (Tilt), a fungicide, for the control (Boadu, 1994). Tilt 250 EC (Emulsifiable Concentrate) is a broad spectrum foliar fungicide with systemic properties. The active ingredient content is 250g propiconazole per liter. Even though Tilt has been uesd successfully in the Central Americas (Molina and Salas, 1990), there has been conflicting reports from farmers concerning its effectiveness (Afreh-Nuamah, personal communication). There was therefore the need to evaluate the two recommended control measures.

The objectives of this work, therefore, were:

(1) to evaluate the effect of the two recommendations, namely the application of Propiconazole (Tilt) and

Pruning on the severity of black sigatoka and yield of plantain.

(2) to test the potency of the fungicide (Tilt) in the laboratory.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and importance

Sigatoka disease takes its name from the Sigatoka valley in Fiji where severe damage was first recorded in 1912 (Philport and Knowles, 1913). Black Sigatoka was first recorded as black leaf streak in Fiji in 1964 (Rhodes, 1964). However the disease is thought to have originated in the Papua New Guinea - Solomon Island area and was widespread in the Pacific and Southeast Asia long before being recorded in Fiji (Stover, 1976; 1978). Black Sigatoga reached the Central America in the 1970s and it had a devastating effect on plantain production (Bustamente, 1983).

In Africa it was first reported in Zambia in 1973 (Reameakers, 1975), but it was not until the early 1980s that it spread to Central Africa (Wilson and Buddenhagen, 1986). It entered all East African banana-growing countries, except Uganda, between 1986 and 1989, where at least some important East African highland bananas were susceptible (Sebasigari and Stover, 1988; Swennen and Vuylsteke, 1988a). According to Wilson (1986), black Sigatoka was observed in some West African countries including Ghana in 1985. Hemeng et al (1995), however, said the disease became epidemic in the 1990s.

Swennen and Vuylsteke (1988b) reported that all plantain cultivars found in Central and West Africa were susceptible. In addition some of the most popular banana cultivars of East Africa were susceptible (Swennen and Vuylsteke, 1991; Vuylsteke et al, 1993). Mobambo et al (1993) noted that all known plantain cultivars collected from West and Central Africa, tropical America and Asia were susceptible to black sigatoka under Onne (Nigeria) conditions. Shillingford (1975) reported that all edible triploid (genome AAA) banana cultivars are susceptible and when the genome B is introduced (AAB and ABB), resistance in the triploid occurs, but Laville (1983) said the presence of a single genome rarely gives varieties resistance to *Mycosphaerella fijiensis*. Resistance seems to intensify only with genome ABB. However, the work of Foure (1985) has shown that this is not always the case.

2.2 The Pathogen

The fungus which causes the disease is *Mycosphaerella fijiensis*. According to Agrios (1988) *M. fijiensis* belongs to the division of higher fungi called Ascomycotina (Ascomycetes or the sac fungi). They produce sexual spores called ascospores generally in groups of eight within an ascus. Its class is Loculoascomycetes (the ascostromatic fungi). They produce pseudothecia ie

perithecium-like stroma with asci in a separate or single large cavity. Dothideales is its order. The fungus has two stages, the conidia and ascospores stages.

2.2.1 The conidia stage

The formation of conidia begins soon after the enlarging streaks or spots on the leaf have become dark brown but the presence of the darkish acervuli and conidia clusters is more easily detected in the buff-grey centres of older leaf (Wardlaw, 1972). Conidia may be found on both sides of the leaf, but are usually more abundant on the upper surface of unsprayed leaves.
The dissemination of conidia takes place only by action of rain or dew, and wind is effective in removing them (Wardlaw, 1972). The conidium in hyaline to olivaceous brown, straight to slightly curved, obclavate to cylindrical with truncate base thickened hilium, smooth wall and 5 to 11 septa. The size ranges from 33 to 153 x 1.7 to 4.9um (Mulder and Holliday, 1974).

2.2.2 The Perfect or Ascospore stage

Leach (1946) demonstrated the presence of the ascospore state of *Mycosphaerella spp.* Subsequent work showed that during the rainy seasons ascospore infection assumes considerable importance. Ascospore is more resistant than conidium and can be distributed by air

current. It is, therefore, more important in the wide dissemination of the pathogen (Wardlaw, 1972). They are mostly found on the under surface of the leaf. The ascospore is hyaline, two-celled with one cell bigger than the other and has slight constriction at the septum. The size ranges from 13.2 to 19.8 x 3.3 to 4.9um with an average of 15.9 x 4.0 um (Mulder and Holliday, 1974). Jacome et al (1991) indicated that ascospres require free water or nearly saturated environment (RH of 98-100%) for germination and germ tube growth. Conidia, in contrast, germinate in a wider range (RH of 92-100%). Pasberg-Gauhl (1994) reported that *M. fijiensis* is a very slow growing fungus in culture and it is time consuming to produce adequate inoculum for artificial inoculation.

2.3 Disease development and Symptoms

The spores rapidly penetrate the stomata on the lower leaf surface by means of their germinative filament (hyphae) and thus colonise the mesophyll (Meredith, 1970; Stover, 1980; Belalcazar and Merchan, 1991). The evident feature of the disease is the presence of a profusion of small discrete spots on the lamina of leaves with areas of scorched or brown leaf tissue where the spots are closely grouped together. For convenience, Foure (1986) divided lesion evaluation into six (6) different stages as follows:

Stage 1 is the first external symptom of the disease. It appears as a small depigmented spot. These are not visible in transmitted light and can be observed only on the undersurface of the leaf. Stage 2 appears as a stripe generally brown in colour and visible on the undersurface of the leaf. In stage 3, the stripes become longer reaching 2 or 3 cm. Stage 4 of the disease appears on the undersurface as a brown spot and on the upper surface as a black spot. In stage 5, the elliptical spot is totally black and has spread to the undersurface of the leaf. It is surrounded by a yellow halo with the centre beginning to flatten out. Stage 6 is when the centre of the spot dries out, turns grey in colour and is surrounded by a well defined black ring, which is in turn surrounded by a bright yellow halo. These spots remain visible after the leaf has dried out.

2.4 Control

2.4.1 Cultural

The control of the Sigatoka disease has taken different forms since it was first noticed. In Venezuela, cultural methods of disease management such as removal of affected leaves and leaves hanging down the pseudostem to reduce inoculum levels were recommended. These were however either not practised or were being undertaken too infrequently. In addition, leaves with severe necrosis

that had been removed were lying on the ground instead of being stacked in heaps to reduce the surface area of tissue that wouuld be liberating spores (Jones, 1995). In Ghana, Oduro et al (1992) also recommended pruning and burning of diseased leaves.

It has been reported (Bananuku abd Rubaihayo, 1994; Mobambo et al, 1994) that black Sigatoka is more severe on plants grown on soils with poor organic content. Mobambo and Naku (1993) therefore recommended the use of organic matter which helps to enhance plantain growth and thus reduce the effect of black Sigatoka on plantain in backyard gardens. Also, organic nutrients from the disposal of household waste may play a role in reducing black Sigatoka severity on backyard plantain (Mobambo et al, 1993). However the supply of organic matter in large-scale plantain plantation is still a problem (Nweke et al, 1988; Ruhigwa et al, 1994).

2.4.2 Genetic Improvement

The rapid spread of black sigatoka gave impetus to efforts aimed at the genetic improvement of Musa spp. (Persely and DeLanghe, 1987). Molina and Krausz (1988) also said the development of cultivars of banana and plantain resistant to black Sigatoka is imperative to control the disease economically. However, breeding plantain and banana is a very slow and tedious process

due to problems with polyploidy, seed and pollen infertility. Also, low clonal multiplication rates, lack of variability and barriers to sexual hybridization impede genetic improvement.

Due to the apparent lack of resistance in the African Musa gene pool, black Sigatoka has become the overiding constraint to Musa cultivation and possess a major threat to food security in the plantain and banana growing regions of Africa (IITA, 1988). Chemical control was therefore recommended.

2.4.3 Chemical control

Chemical control strategies of the disease are possible and exist but according to Mobambo et al (1993), they are not feasible or are socio-economically unsound because the bulk of the crop produced in Africa is by resource-poor family farmers who cannot afford the expensive imported fungicides. For example, the cost of chemical control of the disease in bananas grown for export in Central and South America is approximately $100 million annually (UPEB, 1985). Ramsey et al (1987), also indicated that the cost of controlling yellow Sigatoka in Northern Queensland, Australia could be as high as 14% of the total production cost.

Different fungicides have been tested and in various degrees, have been found to possess practical value. In

early works in Fiji, the oil Dithiocaramate emulsion was used against Sigatoka, but maneb as a water based spray gave better disease control. The side effect of the oil sprays when used to control Sigatoka in Fiji was that it created conditions for black leaf streak which was found to be an even more serious disease (Firman, 1970). The aerial applications of these oil and fungicide and a greater frequency of application were needed, compared with Sigatoka control in Central America. According to Firman (1972) and Long (1971) oil water emulsion with benomyl gave a better control of the disease than that containing maneb. Other reports have it that, prior to the emergence of black Sigatoka, yellow Sigatoka disease was controlled in Fiji by the use of oil sprays but for black Sigatoka, combinations of fungicides plus oil, for example, manozeb, and benlate were necessary for effective control (Firman and Hoskin, 1970; Firman, 1972). The standard recommendation for the Pacific Islands was either benlate plus oil or dithane M45 or manzate 200 plus oil (Firman, 1976).

Stover and Simmonds (1987) reported that when benlate was used almost exclusively throughout the Pacific Islands during the 1970s, control was at best marginal. The poor levels of control obtained drew concern over the possibility of the development of strains resistant to benomyl and prompted a re-

examination of chemicals for Sigatoka control.

The discovery of the effectiveness of petroleum oil (alone or with added chemical compounds) was made in the French West Indies in control studies on Sigatoka (Cuille, 1965). The action of oils in plant disease control, mostly arising from work on sigatoka disease, was reviewed by Calpouzos (1966). According to him, oil reduces spore germination, germ tube growth and appresorial formation and thus inhibit host tissue penetration. Pont (1970) reported that control was given by oil alone in water emulsions with mancozeb or maneb. Stover (1968) and Garry (1973), also reported that benomyl in such emulsion was effective.

Results from an experiment conducted during 1986 - 1988 at Fermenta Plant Protection Company Research farm in Honduras suggested that good disease control could be achieved using chlorothalonil. Slabaugh (1990) confirmed that black sigatoka disease could be reduced using chlorothalonil when a range of chemicals were evaluated in a series of trials in 1977 at Totokoitu Research Station, Rarotonga, Cook Islands. It was reported that chlorothalonil was particularly effective and convenient chemical to use (ie no oil was applied), but it caused severe eye irritation.

Another problem encountered with the application of fungicides was the development of resistant strains of

the fungus. Fullerton and Tracy (1984) reported that strains of *M. fijiensis*, resistant to benzimidazoles, were first detected in Western Samoa in 1979 and in Tonga in 1983. Benlate was withdrawn from use in Western Samoa in 1981 and in Tonga in 1985 due to resistant strain development. Stover (1977; 1979) reported that in Central America, resistant pathogen population decreased rapidly after benlate was withdrawn but in Western Samoa, the population remained relatively large in most localities. Resistant strains were detected in abandoned plantations where no sprays were applied for many years.

To solve this problem of fungi developing resistance to fungicide, Costa Rica and Honduras reduced drastically the use of fungicide cycles applied per year by implementing four (4) principal strategies. These were:

(i) monitoring the epidemiology of the disease through an adapted early warning disease survey.

(2) the use of systemic fungicide, propiconazole, integrated with other fungicides.

(3) low volume application techniques with good coverage and recovery to obtain miximum efficiency and,

(4) change from overhead to under canopy irrigation which reduced disease incidence and severity (Wielemaker, 1990).

In Ghana, Twumasi et al (1993) reported that bavistin, benlate, dithane M45 and polyram-DF were screen tested by foliar spraying and soil application methods, using different concentrations and time frequencies at Assin-Fosu in the Central Region. Preliminary reports indicate that bi-weekly spraying appeared to be more effective than monthly spraying. Also bavistin gave a better result in controlling the disease than any other fungicide. Polyram-DF was also reported to be promising.

2.5 Propiconazole

In the 1980s a new class of fungicides, the ergosterol biosynthesis inhibitors, came into widespread use. Among them was propiconazole. It is manufactured by CIBA-GEIGY under the trade name TILT. According to the manufacturers Tilt 250 EC is a broad spectrum foliar fungicide with systemic properties for the control of powdery mildew, rust and leaf spot diseases in cereals, plantain, banana and other crops. The active ingredient content is 250g propiconazole per litre. It has the chemical formula 1-{2-{2,4-dichlorophenyl-4-propyl-1,3-dioxolan-zyl-methyl}-1H-1,2,4-triazole. It absorbed by assimilating parts of the plant within one hour. It is transported acropetally in the xylem. Propiconazole acts on the fungal pathogen inside the plant at the stage of first haustria formation. It stops the development of

fungi by interfering with biosynthesis of sterols in cell membrane and belongs to the group of DMI-fungicides (ie demethylation inhibitors). Although the biological mode of action of propiconazole permits protective, curative or eradicative use, best results are achieved if the product is applied when the disease is active but in the early stages of development. Its performance is, however, influenced by such factors as weather and plant type resistance.

Wielemaker (1988) reported that propiconazole was the only triazole compound approved by Costa Rica Environmental Protection Agency (EPA) for foliar sprays on banana. Propiconazole was the first fungicide used to control black leaf streak and listed by the Environmental Protection Agency in the United States in 1984 for use on banana (Stover, 1993). The use of triazole fungicides, however, showed that pathogens displayed increasing resistance to these products. The Fungicide Resistance Action Committee (FRAC) reported that there was a decrease in sensitivity of *M. fijiensis* to Tilt from 1988 to 1992.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Field work

3.1.1 Project site

The research was carried out at the University of Ghana Agriculture Research Station, Kade from September 1995 to April 1997. The mean annual rainfall recorded at the station is 1,386.3mm, with the highest rainfall being recorded in June/July or September while the lowest occured in December. The minimum temperature ranges from 15.3°C to 24.2°C with the maximum ranging from 28.5°C to 33.7°C. The wind force on Beaufort scale is from 0 to 4, that is, mean speed of between 0 to 13 knots but it is between 5-9 knots for the greater part of the year.

3.1.2 Land preparation and treatments

Being a secondary forest, the dominant weed was *Chromolaena odorata*. Therefore the land was prepared by slashing without burning. Lining and pegging were carried out using the 50m tape measure and pegs. The size of each plot was 18 x 10 m^2.

The planting distance within a plot was 3m x 2m, and the distance between individual plots was 6m. Planting holes of 10 x 10 x 30cm^3 were dug, using the chisel.

The randomised complete block design (RCBD) was used. There were four treatments namely; Tilt (0.125g

ai/L), Pruning, Tilt (0.125g ai/L) + Pruning and Control (neither chemical nor pruning). There were 20 plants per plot but records were taken on 15 selected at random. Each treatment was replicated 4 times. Analysis of variance (ANOVA) procedure based on plot means (Kwanchi-Gomez and Gomez, 1984) was carried out for data analysis and Least Significant Difference (LSD) test at 0.05 significance level was used to compare treatment means for each parameter.

3.1.3 Planting Material and Planting

The plantain cultivar used was "Brodeyuo" (Dark pseudostem) which belongs to the False horn (Apentu) AAB group. The split corm technique (Wilson et al, 1985) was used to raise suckers at the nursery for four months. They were uprooted, using the chisel and cutlass, and cut to an average height of about 50cm. Trimming of roots and necrotic lesions were done. Three days after uprooting, the suckers were planted on the field. Dead suckers were replaced.

3.1.4 Research activities and records taken

Plant height was recorded using a graduated pole eight weeks after transplanting and subsequently at 4-weekly intervals. Also, the number of expanded non-drooping leaves were counted starting from the topmost.

The disease severity on the leaves were scored using a 6-grade scale from 0-5 (modified after Gauhl et al, 1993) as follows:

0 -- no symptom,

1 -- less than 1% total leaf area attacked (only streaks and/or up to 10 spots,

2 -- 1 to 5% total leaf area attacked,

3 -- 6 to 15%

4 -- 16 to 33% and

5 -- 34 to 100%

From this, percentage total leaf area per plant attacked by the fungus was estimated using the formula by Gauhl et al (1993) as:

Total leaf area attacked (%) =

$$\frac{LN1 \times 1 + LN2 \times 5 + LN3 \times 15 + LN4 \times 33 + LN5 \times 100}{LN}$$

where LN1 to LN5 = number of leaves with respective grades and LN = total number of leaves.

In addition to the severity determination, the youngest leaf with symptom (YLWS), which gives an idea of how healthy the plant appears (Stover and Dickson, 1970; Stover, 1971) was determined. This was done by recording the number of the youngest leaf of each test plant to have the symptoms counting from the folded leaf downwards. The average of the numbers was calculated for each treatment.

Application of the four treatments were also done when the plants were eight weeks old after transplanting and subsequently at four weekly intervals until the plants were 44 weeks old,when they started flowering and no new leaves were coming.The application was done immediately after the severity determination. The rational for the sequence was to know the initial natural infection and also the effect of the previous treatments on disease severity. The details of the four treatment application were as follows:

a) Pruning - diseased leaves above grade 2 of disease development were removed with cutlass from the test plants. The prunned leaves were gathered outside the field, allowed to wither for 3 days and then burnt.

b) Fungicide application - 5ml of Tilt 250 EC (ai 250 propiconazole per litre) was mixed with 10 litres of water to give a concentration of 0.125g ai/L. This was sprayed onto both surfaces of the leaves. Spraying was first done with Knapsack sprayer (Fig. 1) and later with motorized sprayer (Fig. 2).

c) Tilt + Pruning - diseased leaves above grade 2 of disease development were first prunned and burnt in (a) above. Tilt 250 EC was then applied as in (b) above.

Fig. 1: Using knapsack sprayer to spray short plantain plants with Tilt 250EC.

Fig. 2: Using motorised mist blower to spray tall plantain plants with Tilt 250EC. Note the diseased lower leaves (arrowed).

d) Control - neither Tilt application nor pruning was done.

Harvesting started when the plants were 54 weeks and repeated every 2 weeks thereafter. A bunch was considered mature when a finger had cracked or was beginning to ripen. The experiment was terminated after the 7th successive harvest when the plants were 66 weeks old. At harvest, the number of bunches per treatment and bunch weight (taken by cutting the peduncle above the first hand at the scar of the last bract and below the last hand) were recorded.

However, other components of yield namely, number of hands per bunch, number of fingers (fruits) per bunch and the circumference, length and weight of the middle finger (most of each hand) were also recorded.

3.2 Bioassay Studies in the laboratory

3.2.1 Media Preparation

1.5% water agar (WA) and potato dextrose agar (PDA) were separately prepared by autoclaving at a pressure of 15 lbs psi (1.05Kg/cm^2) and a temperature of 121°C (250F) for 15 minutes.

One ml of each of the following concentrations of Tilt 250 EC were put into separate Petri dishes. These were 0.0125g ai/L, 0.025g ai/L, 0.05g ai/L, 0.0625g ai/L and 0.075g ai/L. Ten ml of molten water agar or PDA were

added to form Tilt-ammended media. These were allowed to set. Each plate represented a treatment and each treatment was repeated four times. There were media which did not contain Tilt 250 EC and therefore served as control.

3.2.2 Isolation of pathogen

Dried and fresh leaf samples showing advance lesions of black Sigatoka (ie necrotic areas characterized by greyish spots, a typical symptom of ascospore-bearing tissue) were collected from the field at the University of Ghana Agriculture Research Station, Kade. A composite sample from several plants were collected. These were air-dried at room temperature for 24 hours. Isolations were done using the ascospore discharged technique described by Stover (1980). Leaf sections about 2x2 cm^2 were stapled to a 9cm-diameter filter papers. These were immersed in sterile distilled water for 5 minutes and then placed in the lids of Petri dishes with the lower leaf surface of the leaf section directly above the Tilt-ammended water agar. The plates were examined every 15 minutes for 3 hours for release of ascospores. The released ascospores were transferred to a corresponding concentration of Tilt-ammended PDA. The plates were periodically examined for contamination and pure cultures transferred onto fresh Tilt-ammended PDA. The cultures

were incubated at room temperature (27-29 C) under continuous light.

3.2.3 Data Collected

Each plate was divided into four sections for counting and measurement of ejected ascospores and also for measuring the length of germ tube. The parameters studied were time for germination after ejection onto media, type of germ tube growth, length of germ tube 12 and 24 hours after ejection and other characteristics of the cultures like colour of the culture.

CHAPTER FOUR

RESULTS

4.1 Field Work

4.1.1 Effect of the treatments on Plant height of Plantain

Plant height increased steadily from planting to flowering (Fig. 3). The average height at 8 weeks after transplanting for Tilt treated plants (TP) was 33.4cm. Control plants (CP) recorded the highest figure of 35.8cm and the prunned plants (PP) 30.6cm. TP and PP recorded values of 36.0cm and 39.8cm each in the 12th and 16th weeks respectively. However, from the 32nd week to flowering, TP gave the lowest values 92.6cm in the 32nd week and 180.1cm at flowering. CP maintained the highest value throughout the experimental period reaching an average height of 226.7cm at flowering. Tilt + Prunning treated plants (TPP) followed with 220.9cm. The average height at flowering for PP and TPP were 222.1cm and 228.0cm respectively.

4.1.2 Effect of Tilt and Pruning on Average number of leaves of plantain infected by black Sigatoka

The average number of leaves for TP, PP, TPP and CP in the 8th week 4.7, 4.4, 4.3 and 4.0, respectively (Table 1). In the 16th week, TP recorded the highest value of 5.8 and maintained this position till flowering, ending

Fig. 3 Plant height of plantain infected by Black Sigatoka.

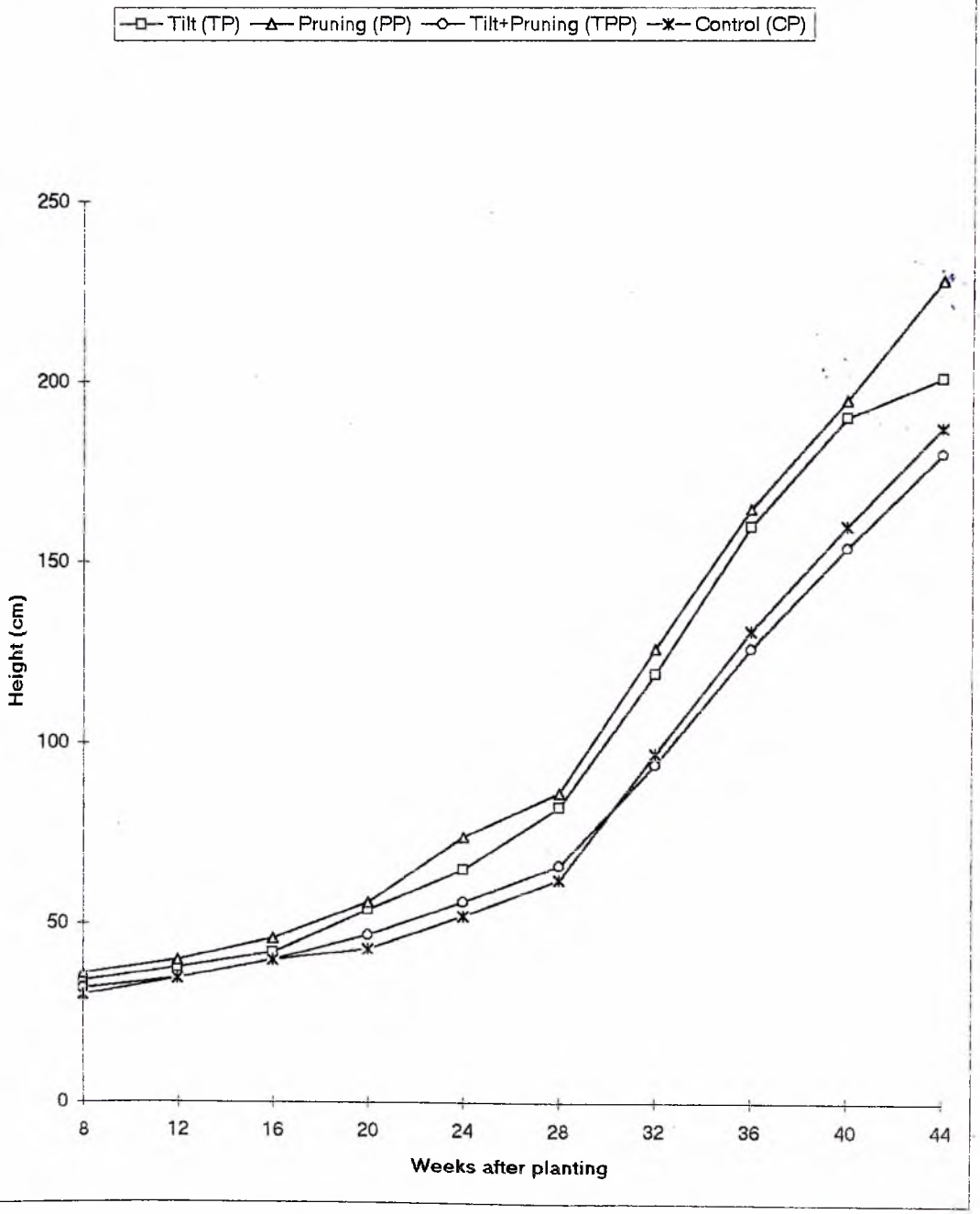


Table 1: Average number of leaves of plantain infected by black Sigatoka from week 8 to week 44.

Treatment	Weeks									
	8	12	16	20	24	28	32	36	40	44
Tilt (TP)	4.7	5.0	5.8	7.1	7.8	8.2	11.8	12.6	14.6	16.6
Pruning (PP)	4.1	4.6	5.0	5.7	6.0	7.1	9.9	9.7	11.7	13.6
Tilt+Pruning (TPP)	4.3	4.6	5.1	5.8	6.2	7.2	11.0	11.4	13.0	14.5
Control (CP)	3.6	4.7	5.0	6.1	6.2	6.9	7.6	8.4	12.1	13.8
LSD $_{(0.05)}$	NS	NS	NS	NS	NS	NS	1.22	2.72	1.69	1.99

NS = Not significant

with 16.6. In the 16th, 20th and 24th weeks, PP recorded the lowest values of 5.0, 5.7 and 6.0 respectively. CP which had the second highest values in the 20th and 24th weeks recorded the lowest values of 6.9, 7.6 and 8.4 in the 28th, 32nd and 36th weeks respectively. In the 40th week, the number of leaves for CP was 12.1 which was higher than the value for PP. The number of leaves in 44th week were 16.6, 13.6, 14.5 and 13.8 for TP, PP, TPP and CP respectively.

4.1.3 Effect of Tilt, Pruning and amount of rainfall on severity of black Sigatoka

The disease was observed when the plants were 8 weeks old. Disease severity, however, fluctuated from the beginning to the end of the experiment and followed the same trend in all the treatments (Fig. 4). From the 8th to 44th week, CP were the most diseased. Throughout the experimental period,the total leaf area attacked in the CP was higher than 13% while it was between 2% to 6.4% for the rest of the treatments.

When the plants were 16 weeks old, the disease severity was higher in CP than the other three treatments (Fig. 5). In week 24, the disease could again be seen more in CP than TP, PP and TPP (Fig.6). In all the treatments, the highest severity was observed when the plants were 32 weeks old and this coincided with the peak

Fig.4 Effects of Tilt, Pruning and amount of Rainfall on Black Sigatoka.

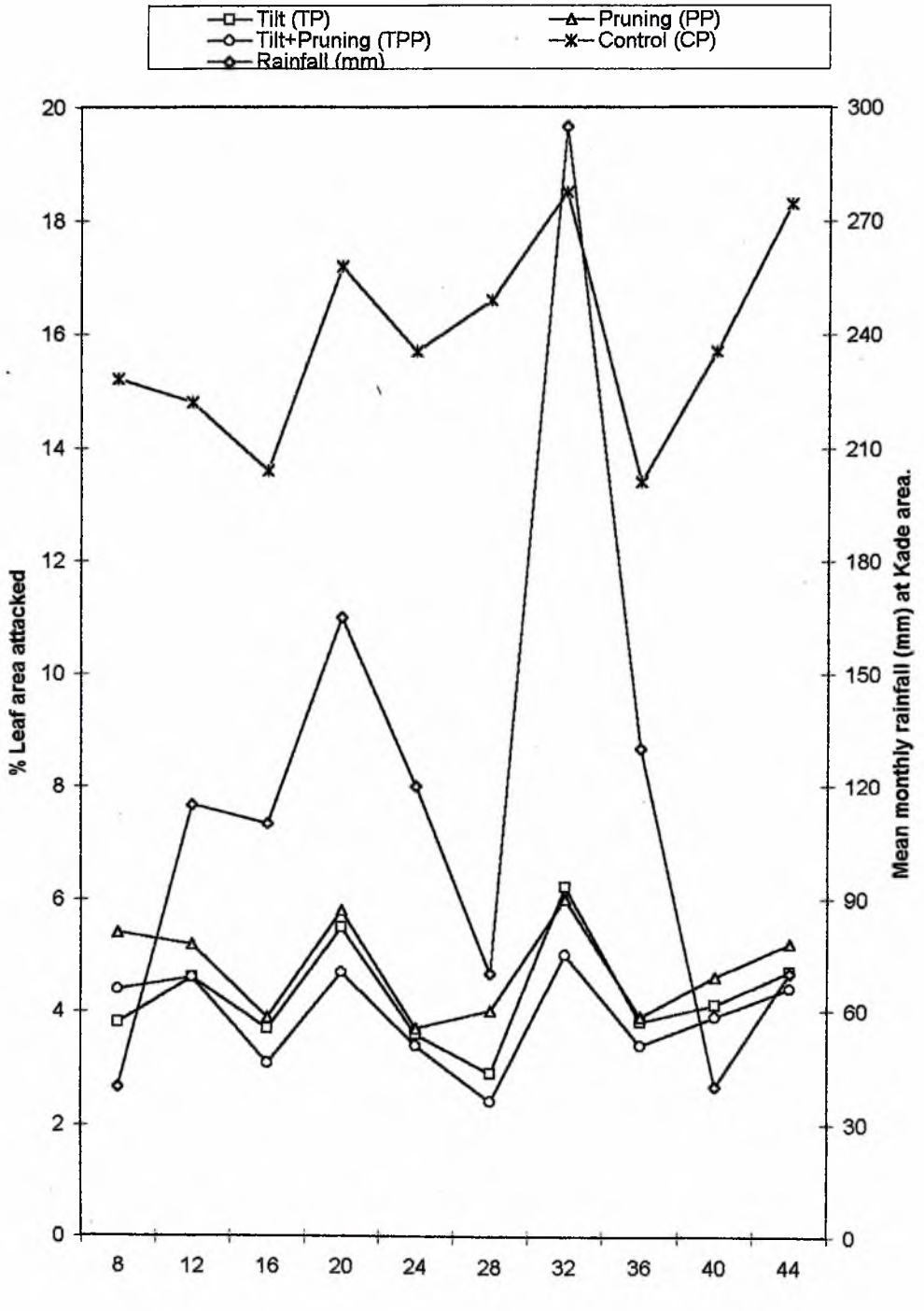


Fig. 5: Effect of Tilt and Pruning on black Sigatoka disease severity of 16-week-old plantain plants at Kade.

A = Plants sprayed with Tilt.

B = Plants for pruning treatment (diseased leaves yet to be prunned).

C = Previously prunned plants and sprayed with Tilt.

D = Control plant (neither prunned nor Tilt). Note the diseased leaves especially the second plant.

Fig. 6: Effect of Tilt and Pruning on black Sigatoka disease severity of 24-week-old plantain plants at Kade.

A = Plants sprayed with Tilt.

B = Plants prunned only.

C = Plants prunned and sprayed with Tilt.

D = Control plants (neither pruning nor Tilt).

Note most of diseased leaves hanging.

of the rainfall (295mm) in Kade area where the experiment was conducted (Figs. 4 and 7). The lowest severity was recorded at week 28 for TP and TPP.

The youngest leaf with symptom (YLWS) also fluctuated throughout the period as seen in (Fig. 8). Eight weeks after transplanting, TP and PP had the same value of 2.6, while TPP recorded 2.2 and CP 2.3. The highest values of 6.8, 5.0, 5.9 and 3.4 for TP, PP, TPP and CP respectively, were recorded in week 36. These decreased to 5.8, 4.2 and 5.0 for TP, PP and TPP respectively by the 44th week. while CP maintained a constant value of 3.4.

The results meant that at the 44th week after transplanting, on the average, the youngest leaf to have the symptom was 6th leaf for TP while it was 4th, 5th and 3rd for PP, TPP and CP respectively. It meant that the plants treated with Tilt appeared healthier than the control.

4.1.4 Effect of Tilt and Pruning on yield of plantain:

The data on the yield of plantain harvested between week 54 and 66 are presented in Fig. 9 and Table 2. Maturity delayed in the control plants. Thus by the 54th week while 4 and 2 bunches were harvested for TP and TPP respectively, nothing was harvested in either CP or PP. Also, by 56th week, only 2 and 3 bunches were harvested

Fig. 7: Effect of Tilt and Pruning on black Sigatoka disease severity of 32-week-old plantain plants at Kade.

A = Plants sprayed with Tilt showing visible symptom black Sigatoka.

B = Plants prunned only showing no visible symptom.

C = Plants prunned and sprayed with Tilt showing no visible symptom.

D = Control plants (neither pruning nor Tilt) with the lower and hanging leaves showing advanced stages of black Sigatoka.

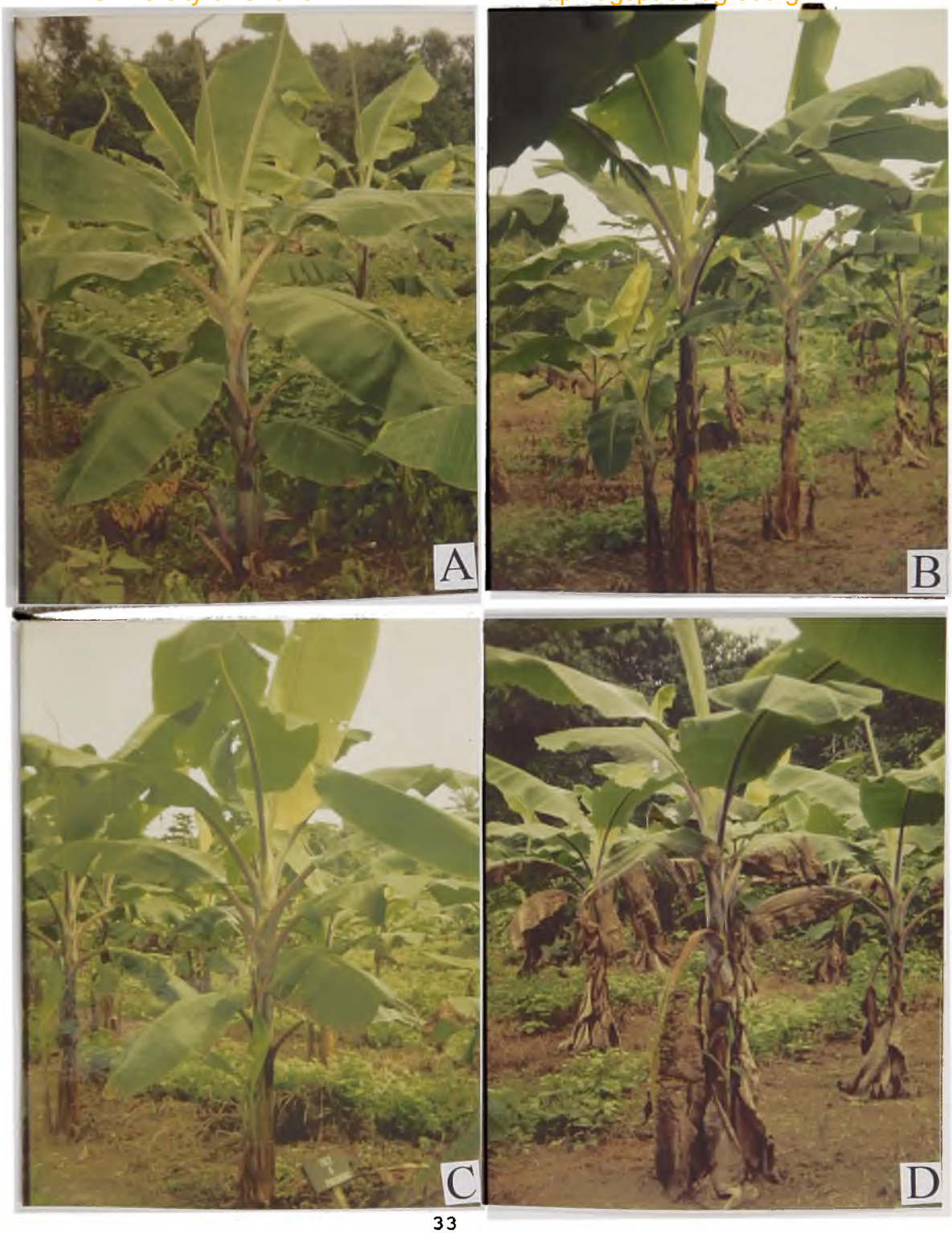


Fig. 8 Effects of Tilt and Pruning on youngest leaf to show symptoms (YLWS) of Black Sigatoka.

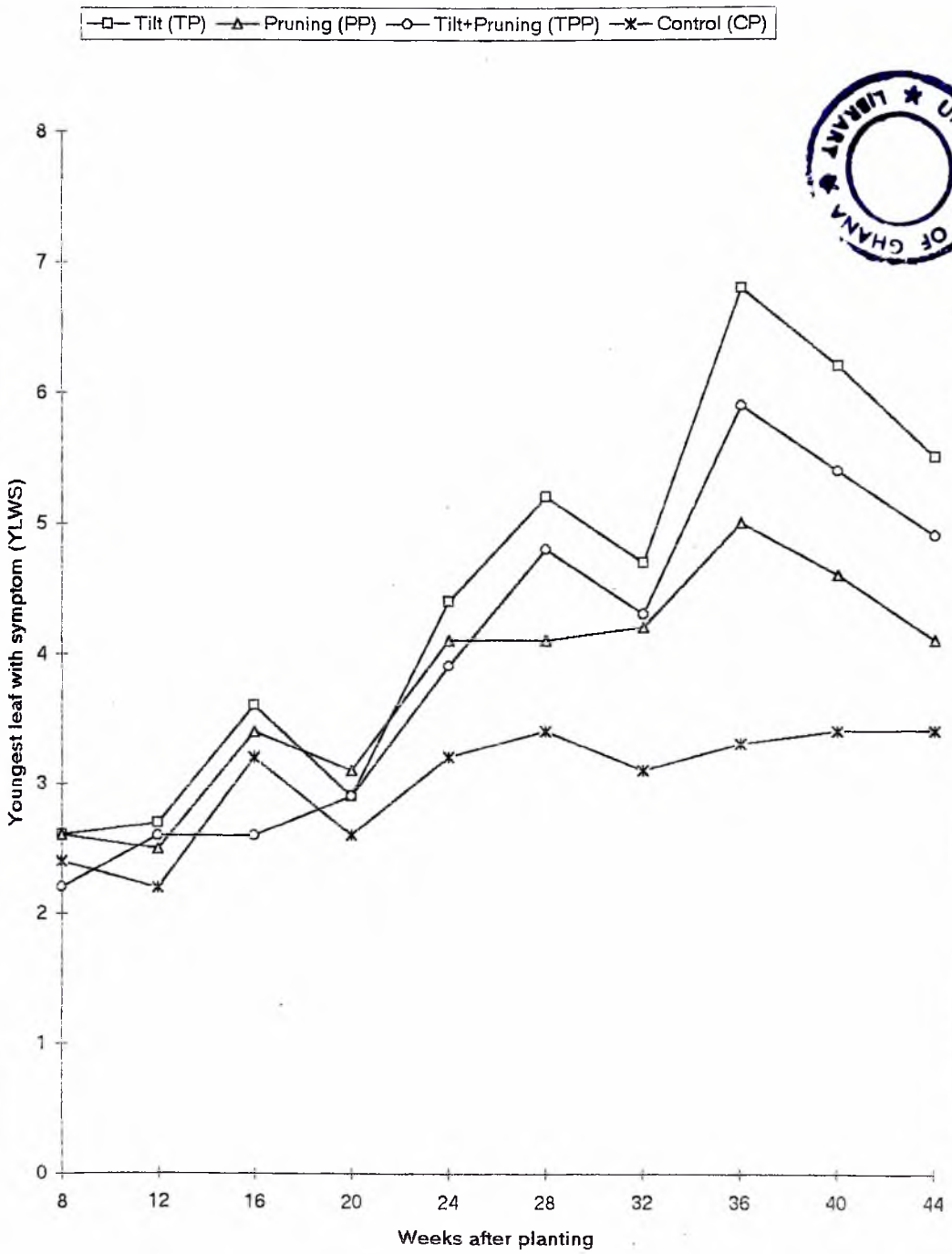


Fig. 9 Number of bunches harvested from week 54 to week 66

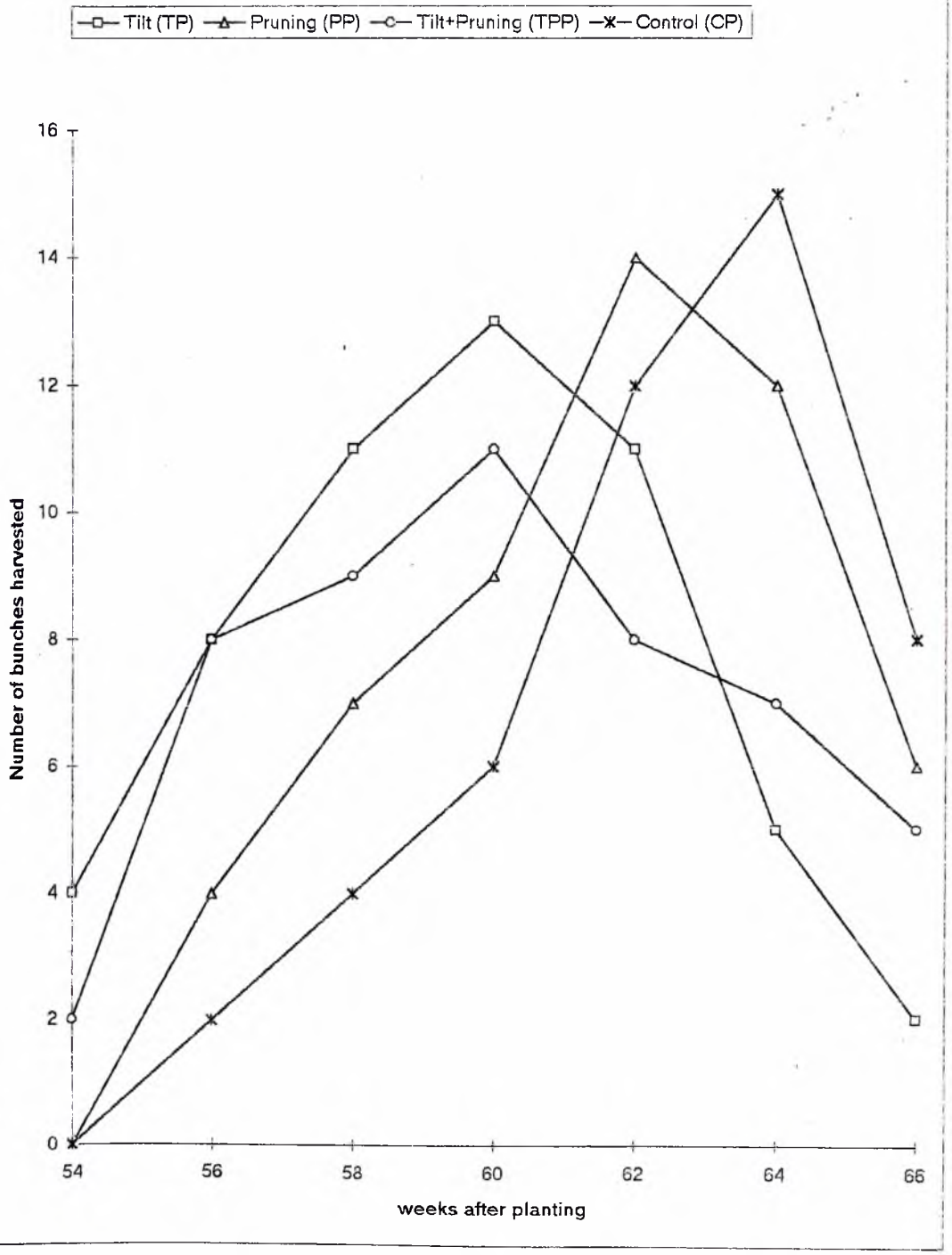


Table 2: Yield and components of yield 66 weeks after transplanting.

Treatments	Total no. of bunches	Total bunch weight (Kg)	Bunch weight (Kg/plant)	No. of hands/ bunch	No. of fruits/ bunch	Middle finger		
						circumf. (cm)	Length (cm)	weight (cm)
Tilt (TP)	54	453.0	8.4	6.4	26.4	12.9	25.3	0.258
Pruning (PP)	52	392.2	7.5	6.1	25.6	12.5	24.2	0.258
Tilt+Pruning(TPP)	50	405.0	8.1	6.3	26.1	12.6	24.8	0.253
Control (CP)	47	249.1	5.4	5.8	25.4	12.1	23.6	0.186
$LSD_{(0.05)}$	NS	NS	0.93	NS	NS	NS	NS	0.05

NS = Not significant.

for CP and PP respectively, while 7 bunches each were harvested for TP and TPP.

By the 60th week, while 13, 11 and 9 bunches were harvested in TP, TPP and PP respectively, only 6 bunches were harvested in the CP. By the 62nd week when the PP reached its peak of 14 bunches, CP had 12, TP 11 and TPP 8.

At 64th week, CP reached its peak of 15 bunches harvested while 13, 7 and 5 bunches were harvested from PP, TPP and TP respectively. At the 66th week, there was a fall of harvest in all the treatments. These were 8, 6, 5 and 2 bunches in CP, PP, TPP and TP respectively. In all, the total number of bunches harvested were 54, 52, 50 and 47 for TP, PP, TPP and CP respectively (Table 2).There was no significant difference in the number of bunches in the four treatments. However, the total weights of 435Kg, 392.2Kg, 405Kg and 249.1Kg in TP, PP, TPP and control showed significant difference between CP and the other treatments. The difference was not due to number of hands per bunch or number of fruits per bunch or the circumference or length of the middle finger. It was rather due to the weight of the fingers. On the average, there were 6.1 hands for PP, 6.4 for TP, 6.3 for TPP and 5.8 for CP. The number of fingers per bunch were 26.4, 25.6, 26.1 and 25.4 for TP, PP, TPP and CP respectively. The circumference of the middle finger

ranged from 12.1cm to 12.9cm, while its length ranged from 23.6cm to 25.3 cm in all the treatments.

4.2. Bioassay in the laboratory for testing efficacy of the Chemical (Tilt)

4.2.1 Ascospore Discharge

It was observed that the discharge of ascospores from the diseased leaves onto the media started 15 minutes after the leaf segments were put in the lids of Petri dishes as described earlier. One hour later, the discharge was completed. The ascospores released were not different from one another (Fig.10). They were hyaline, 2-celled with one cell bigger than the other with a slight constriction at the septum. The sizes ranged from 13.8-19.6um x 3.3-3.5um.

4.2.2 Ascospore germination

Germination started in all cases about 12 hours later (Figs. 11, 12, 13 and 14). However, it could be seen from Table 3 that there was 100% germination for the Control plate. The plates with Tilt concentrations of 0.0625g ai/L and 0,075g ai/L recorded the lowest values of 16% and 5% respectively. This trend was again observed 24 hours later. It was also observed that germination was either unipolar or bipolar, depending on the

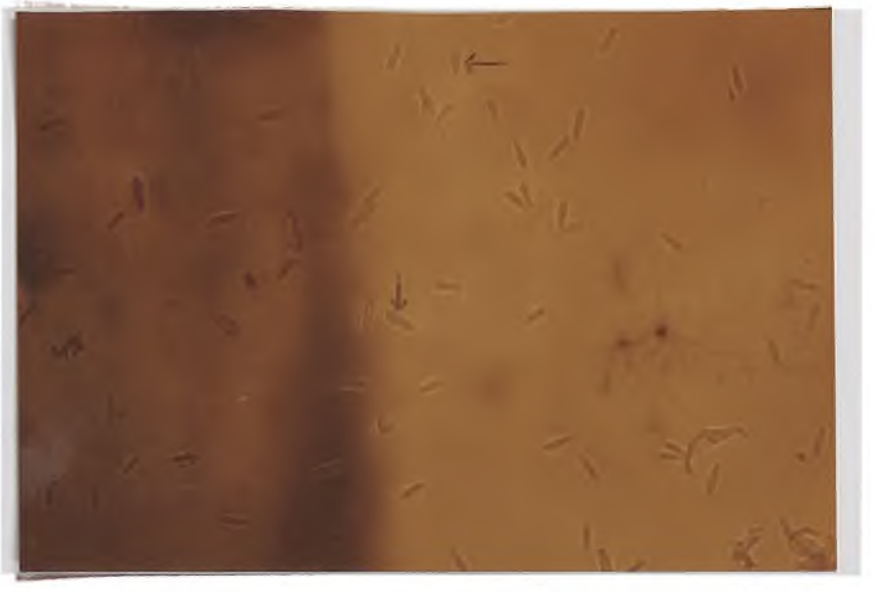


Fig. 10: Ascospores of *Mycosphaerella fijiensis* (arrowed) discharged from diseased plantain leaves on PDA (x 125).

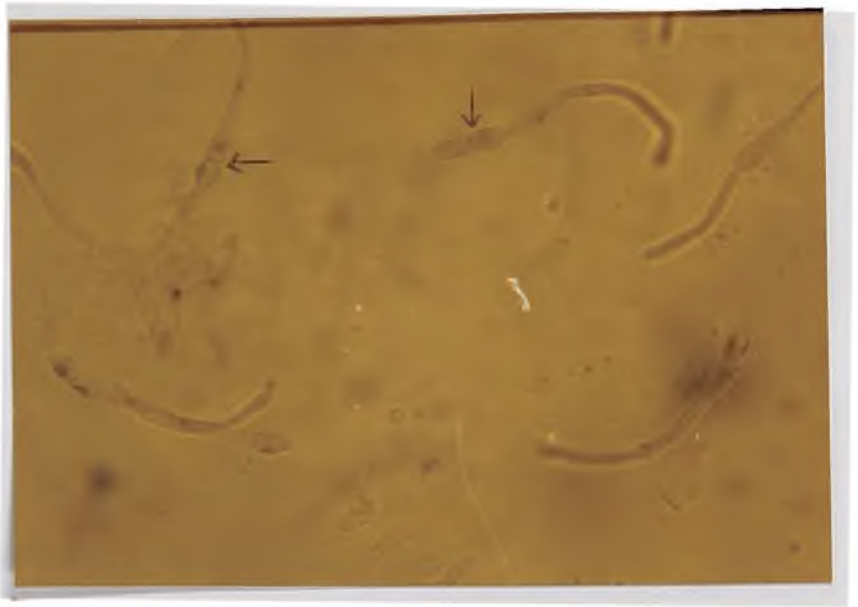


Fig. 11: Germinating ascospores of *Mycosphaerella fijiensis* of plantain on PDA (x 250). Note the arrowed constriction at the septum.

Fig. 12: Germinating ascospores of *Mycosphaerella fijiensis* of plantain on 0.0125g ai/L Tilt-ammended PDA 12 hours after discharge onto PDA (x 125).
Note: Some are unipolar and others bipolar as shown by arrows.

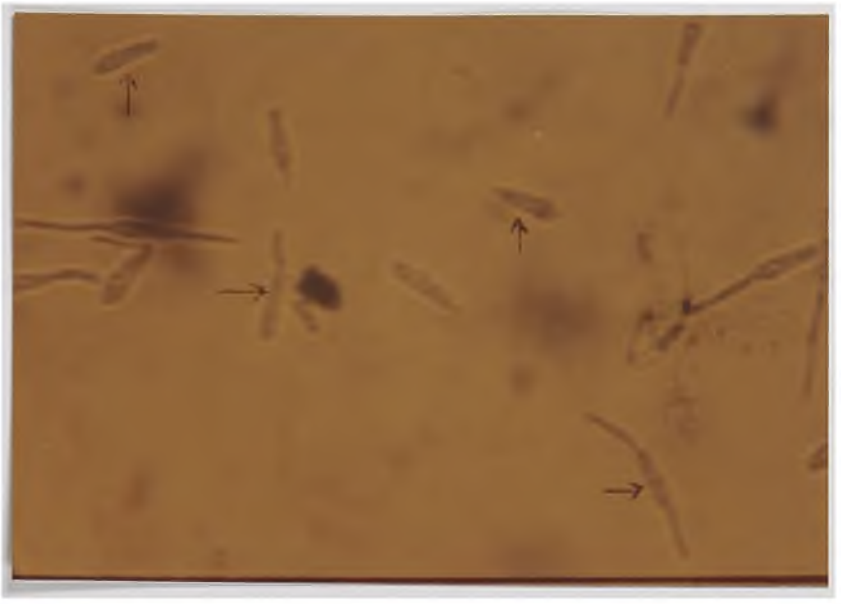


Fig. 13: Germinating ascospores of *Mycosphaerella fijiensis* of plantain on 0.05g ai/L Tilt-ammended PDA 12 hours after discharge.
Note: Some are unipolar, others bipolar and the rest with no germ tube as shown by arrows.

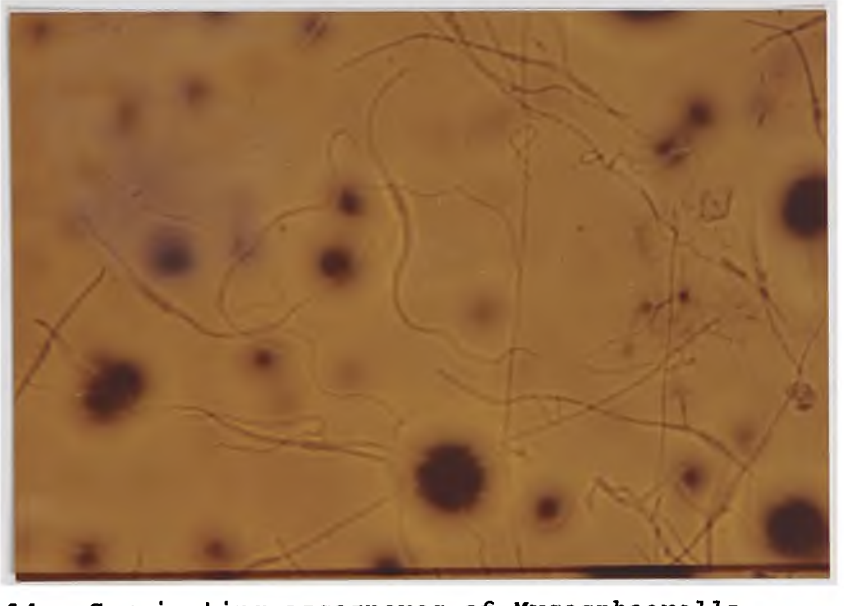


Fig. 14: Germinating ascospores of *Mycosphaerella fijiensis* of plantain on PDA 12 hours after discharged (x 125).

Table 3: **Percentage germination of *M. fijiensis* ascospores discharged from diseased plantain leaves on different concentration of Tilt-ammended PDA.**

Treatment	Germination		
	after 12 hours	after 24 hours	at both ends
Control	100	100	100
0.0125g ai/L	85	92	85
0.025g ai/L	67	70	80
0.05g ai/L	48	57	48
0.0625g ai/L	16	24	17
0.075g ai/ L	5	13	7

concentration of Tilt in the Tilt-ammended medium. Germ tubes were straight to slightly curved.

No measurement could be taken 24 hours after germination for the control plate and the plate with 0.0125g ai/L Tilt (Table 4). This was because the mycelia grew faster and interwoven, making it impossible to trace individual mycelia from one end to the other (Fig. 15).

4.2.3 Culture Characteristics

No visible growth was observed with the naked eye until after 4 days. The culture on the control plate was compact with whitish aerial mycelia. The underside was, however, dark (Fig. 16). The culture on the plate with 0.0125g ai/L Tilt had grey velvety surface with a black underside (Fig. 17).

Table 4: Average length of germ tube of *M. fijiensis* ascospores discharged from diseased plantain leaves (um).

Treatments	12 hours	24 hours
Control	160.0	Over grown*
0.0125g ai/L	146.0	Over grown*
0,025g ai/L	90.0	91.0
0.05g ai/L	77.0	85.0
0.0625g ai/L	52.0	53.0
0.075g ai/L	43.0	43.0

* Not possible to measure since mycelia were interwoven.

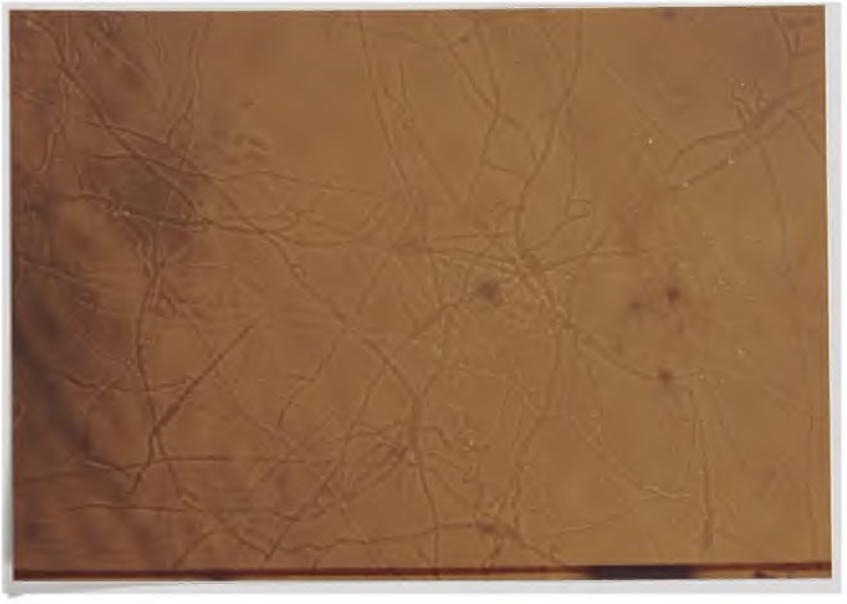


Fig. 15: Germinating ascospores of *Mycosphaerella fijiensis* of plantain on PDA 24 hours after discharged (x 125).
Note: The mycelia grew faster and interwoven, making it impossible to trace individual mycelia from one end to the other.

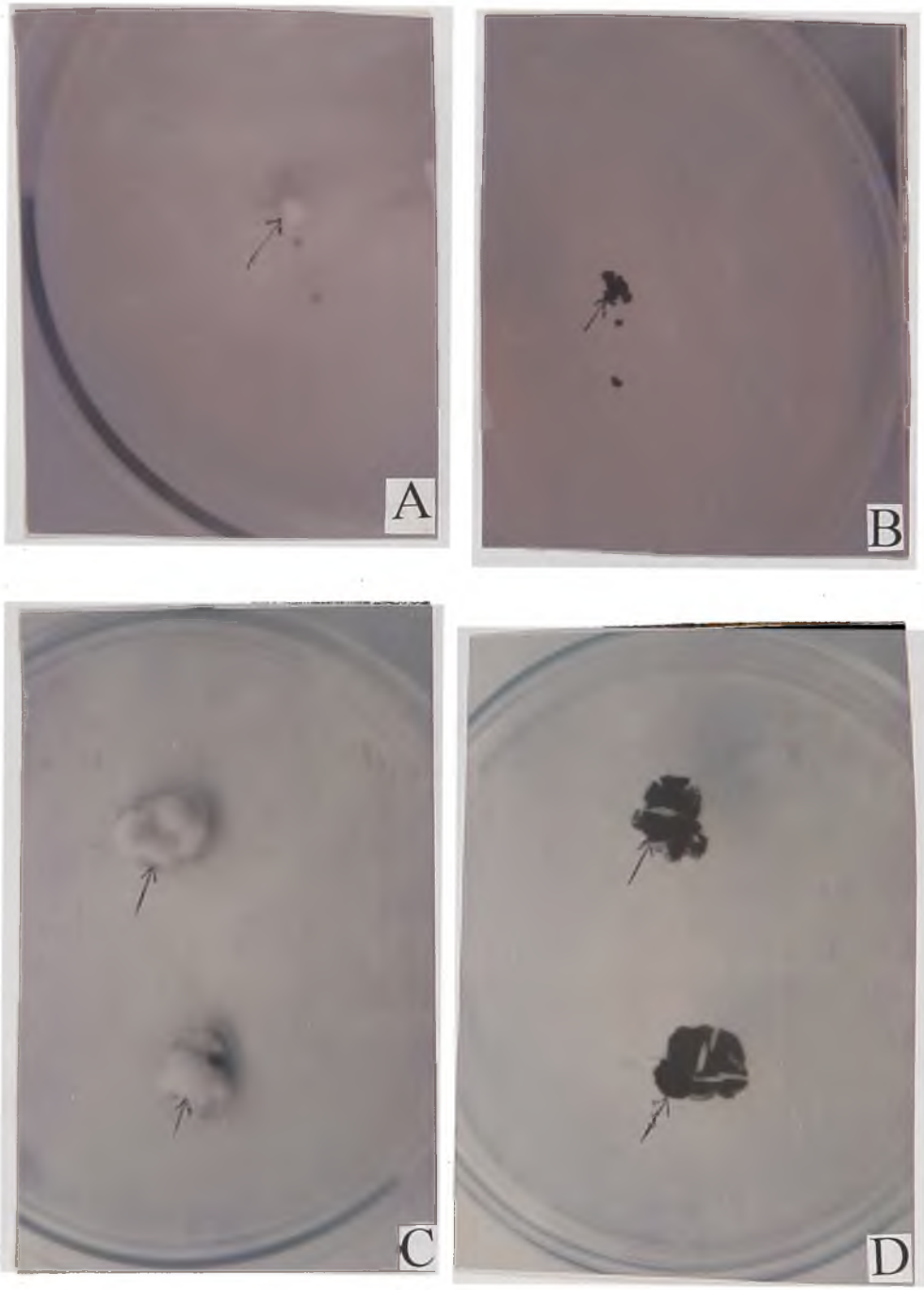
Fig. 16: Cultures of *Mycosphaerella fijiensis* from diseased plantain leaves on PDA.

A = 2-week-old culture with whitish aerial mycelia.

B = 2-week-old culture with reverse (underside) of mycelia black.

C = 4-week-old culture with whitish aerial mycelia.

D = 4-week-old culture with reverse (underside) of mycelia black.



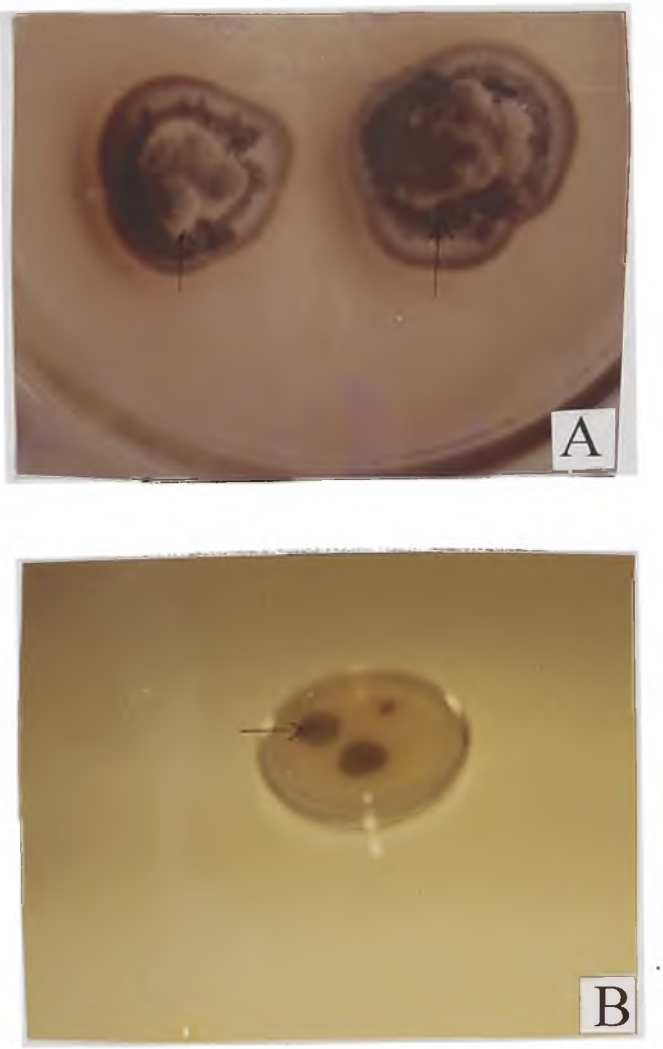


Fig. 17: Cultures of *Mycosphaerella fijiensis* from diseased plantain leaves on 0.0125g ai/L Tilt-ammended PDA.
Note: Greyish aerial mycelia (A) and reverse (underside) black (B).

CHAPTER FIVE

DISCUSSIONS

5.1 Effect of Tilt and Pruning on height of plantain

Statistically, there was no difference between the average height values recorded for the four treatments from transplanting to the 28th week. This shows that neither the disease nor the Tilt and/or Pruning had any effect on the height of the cultivar used in the experiment which is said to be on the average about 180cm tall at flowering (Nsiah, personal communication).

From week 8 to week 24, there was no difference in the average number of leaves recorded for all the treatments. However, significant differences were noticed from week 32 to week 44. The differences might be due to the black Sigatoka disease which causes the leaves to dry out faster than they otherwise would have done. According to Mwashayenyi (1994), the higher the number of leaves on banana, the heavier the fruit bunch. This, he said, may be linked with dry matter accumulation pattern of the plant, suggesting that plants with larger number of leaves also have higher photosynthetic ability which reflects in heavier bunch weight. He further stated that at least, 8 leaves were required to sustain a bunch. The lowest average number of leaves recorded at the time of flowering (week 44) was 13.6 for plant prunned (PP). It could be said that all the plants in the experiment could

sustain a bunch. However since the number included diseased leaves at different stages of disease development their photosynthetic ability will not be the same. This difference might account for the difference in bunch weight at harvest.

5.2 Effect of Tilt, Pruning and amount of rainfall on severity of black Sigatoka and yield of plantain at Kade

The percentage total area attacked on the Control plant was significantly different from the other three treatments which were not significantly different from each other at 5% significance level. This means that chemical treatment by Tilt or cultural practices like pruning could significantly reduce the severity of the disease. Among the three treatments, however, severity was as follows: plants prunned (PP) was the highest, followed by Tilt treated plants (TT) and Tilt + Pruning treated plants (TPP) in that order. On the average, severity was 16% of the total leaf area for Control plants (CP) while it was 4.6%, 5.1% and 3.8% for TP, PP and TPP respectively. The disease was severe in the CP while it was rare to mild in the other treatments when the modified scale of Gauhl et al (1993) is used.

The fluctuation of disease severity observed

generally followed the rainfall pattern at Kade area during the research period. The highest severity was observed in week 32 when the average monthly rainfall was at its peak of 295mm. This confirms earlier report by Jacome et al (1991) that severity of black Sigatoka generally increases with increasing rainfall. The ascospores of *M. fijiensis* which cause considerable infection require free water or nearly saturated environment (RH=98 - 100%) for germination and germ tube growth (Jacome and Schuh, 1992).

The differences noticed in the youngest leaf with symptom (YLWS) values between CP and the other three treatments from week 28 to week 44 were significant at 5%. One characteristic of the disease is that older leaves become more diseased than younger ones. Thus if the plantain has 10 leaves and the youngest leaf to have the disease is 6, it means the first five younger leaves will be free from the disease while only the remaining older five leaves will have the disease with increasing severity from the 6^{th} to the 10^{th}. On the other hand, if the youngest leaf to have the disease is 3, it means only two leaves will be clean while the remaining eight older leaves will be diseased and the plant will look more diseased than the previous one. In this experiment, the control plants (CP) had YLWS of 3 while Tilt treated plants (TP) had 6 indicating that the control looked more

diseased.

When harvesting started 54 weeks after transplanting, no bunch was harvested from either PP or CP. By 62nd week, 50% of bunches have been harvested for the CP, while 65%, 76% and 87% bunches have been harvested for PP, TPP and TP respectively. The results indicate that black Sigatoka could delay the maturity of plantain. When the harvesting was stopped at the 66th week after transplanting there was no significant difference between the number of bunches in the control and the other treatments. However, the bunch weight recorded for CP was significantly lower than the weights recorded for the other three treatments. The differences in bunch weights were due to significant differences in weight per finger (ie individual fruit weight) which were 0.258Kg, 0.245Kg, 0.253Kg and 0.186Kg for TP, PP, TPP and CP respectively. This is in line with observation by Baiyeri and Mbah (1994), that bunch weight correlates with finger weight but weakly correlate with finger number. There was no significant difference in the number of hands per bunch. Also the number of fingers per bunch were not significantly different from each other.

Correlation analysis indicated a negative but significant association ($r = 0.96$) between severity of black Sigatoka disease and yield. This implies that as disease severity increases yield is reduced. The

differences in yield between the CP and the other treatments are 203.9Kg (45.0%) for TP, 143.1 Kg (36.5%) for PP and 155.9Kg (38.5%) for TPP. These agree with what Stover (1983) and Mobambo et al (1993) said that plantain yield loss up to 50% could be recorded in the first cropping cycle. Both delay in maturity and reduction in yield weight of fingers because of the disease may be due to its adverse effect on the photosynthetic efficiency of the plant.

5.3 Efficacy of Tilt as evidence in Bioassay studies:

The ascospore morphology observed conformed to the description of *M. fijiensis* by Mulder and Holliday (1974). The ascospores that were ejected from the diseased plantain leaves were therefore of *M. fijiensis*. However, germination started about 12 hours after discharge, instead of the 24 hours reported by Mulder and Holliday (1974). That no visible colony was observed with the naked eye until after 4 days confirmed what Natural (1990) said that colonies from single spore isolates on artificial media grows very slowly and become visible 4-6 days after incubation on PDA at 22-25°C under continuous light. The whitish aerial mycelia with reverse (under) side dark for the control (only PDA) plate is in line with what Mulder and Holliday (1974) observed. In the

case of 0.0125g ai/L Tilt-ammended PDA, these aerial mycelia later turned olivaceous gray. The colonies were compact, raised with non-circular margin. Natural (1974) said this is possible if there is toxin in the culture. There was no appreciable growth in the other Tilt - ammended PDA plates. Tilt concentration of 0.075g ai/L and above in a medium can therefore stop the growth of *M. fijiensis* since it was this concentration that had only 5% of ascospores germination 12 hours after discharge and 7% germination 24 hours after discharge.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

From the above data, it could be said that

i. black Sigatoka could delay maturity of plantain and reduce bunch weight.

ii. pruning and burning of infected leaves was effective in the control of black Sigatoka disease.

Farmers should be encouraged to use it as an alternative to chemical control which is expensive and environmentally unfriendly, and therefore cannot be sustained by the peasant farmers in Ghana.

The following recommendations could be made:

1. the experiment should be repeated.
2. different cultivars of plantain should be used.
3. the land area used should be increased.
4. the experiment should be multilocational.
5. two or more fungicides should be used and
6. the experiment should be extended into the second cropping season.

CHAPTER SEVEN

LITERATURE CITED

1. Agrios, G. N. 1988. Plant Pathology. 3rd Edition. Academic Press N. Y. 803 pp.

2. Baiyiri, K. P. and Mbah, B. K. 1994. Growth and yield correlation in False horn plantain (*Musa* AAB cu Agbagba) in a sub-humid zone of Nigeria. MusAfrica 5:3-4.

3. Bananuka, J. A. and Rubaihayo, P. R. 1994. Backyard banana cultivation in Uganda. Infomusa 3(2):17-18.

4. Belalcazar, S. and Merchan, V. 1991. Capitulo VI: Control de enfermedades Pp. 241-297 in El cultivo del Platano en el Tropico. (Belalcazar, S.; Toro Meza, J. C. and Jaramillo, R. eds) ICA, Manual de asistencia tecnica No. 50 ICA, Cali, Colombie.

5. Boadu, K. A. 1994. Agriculture Ministry distributes chemicals to contain plantain disease. Daily Graphic. March 18, 1994 Pg. 16.

6. Bustamente, M. 1983. Impact du Cercospora noir sur la production du plantain au Honduras. Fruits. 38: 330-332.

7. Calpouzos, L. 1966. Action of oil in control of plant disease. Ann. Rev. Phythopathology, 4:369-390.

8. Cuille, J. 1965. Aerial application of mineral oil. PANS 11:281-282.

9. Firman, I. D. 1970. Possible side effects of fungicides on banana and coffee diseases. Nature Lond. 225: 1161-1164.

10. Firman, I. D. 1972. Black leaf streak in banana in Fiji. Annals of Applied Biology. 70:19-24.

11. Firman, I. D. 1976. Black leaf streak of banana. South Pacific Commission Adisory Leaflet No. 1 3pp.

12. Firman, I. D. and Hoskin, P. 1970. Spraying banana in Fiji to control black leaf streak disease. Annals of Applied Biology 66:239-300.

13. Foure, E. 1985. Les cercosporioses du bananier et leurs traitements. Comportment des varietes. Etude de la sensibilite varietale des bananiers et plantains a *Mycosphaerella fijiensis* Morelet au Garbon maladie des raies noire (suite III). Fruits 40(6):393-399.

14. Foure, E. 1986. Varietal reactions of bananas and plantains to black leaf streak disease. *In*: Persley, G. J. and De langhe, E. A. (Eds). Banana and plantain Breeding Strategies: Procedings of an international workshop held at Cairns, Australia. 13-17 October, ACIAR Procedings No. 21:110-113.

15. Fullerton, R. A. and Tracy, G. M. 1984. Tolerance of *Mycosphaerella fijiensis* to benomyl and carbendazim in the Pacific Islands. Trop. Agric. (Trinidad) 61:133-136.

16. Gauhl, F.; Pasberg-Gauhl, C.; Vuylsteke, D. and Ortiz, R. 1993. Multilocational evaluation of black Sigatoka resistance in banana and plantain. IITA Research Guide 47, 59 pp.

17. Garry, J. 1973. Methods with systemic oil fungicide. Fruits 28:671-673.

18. Hemeng, O. B.; Banful, B. and Twumasi, J. K. 1995. Plantain production in Ghana. *In*: Ortiz, R. and Akoreda, M. D. (Eds). Plantain and Banana production and research in West and Central Africa - Procedings of a Regional workshop sponsored by the IITA, High Rainfall Station, Onne. River State, Nigeria 23-27 Sept., 1995.

19. IITA, 1988. IITA Annual Report and Research Highlights 1987/88. Ibadan, Nigeria. International Institute of Tropical Agriculture. 161 pp.

20. Jacome, L. H.; Schuh, W. and Stevenson, R. 1991. Effect of temperature and relative humidity on germination and germ tube development of *Mycosphaerella fijiensis* var *difformis*. Phytopathology 81:1480-1488.

21. Jacome, L. H. and Schuh, W. 1992. Effect of leaf wetness duration and temperature on development of black Sigatoka disease on banana infected by *Mycosphaerella fijiensis* var *difformis*. Phytopathology 82:515-520.

22. Jones, D. 1995. Consultancy report to INIBAP adapted for infomusa. INFOMUSA 4(1):13-14.

23. Kwanchi-Gomez, A. and Gomez, A. A. 1984. Statistical Procdures for Agricultural Research. 2nd Ed. John Wiley and Sons. Philippines. 680pp.

24. Laville, E. 1983. Les cercosporioses du bananier et leur traitment. Comportement des varietes. Generalites Fruits 38(3): 147-151.

25. Leach, R. 1946. Banana leaf spot (*Mycosphearella fijiensis*) on the Gros Michel variety in Jamaica. Kingston, Jamaica. Government Printer. 118pp.

26. Long, P. G. 1971. Control with benomyl. Pl. Dis. Rept. 55:10-12.

27. Meredith, D. S. 1970. Status of major banana diseases. Rev. Pl. Pathol. 49:539-540.

28. Mobambo, K. N. 1993. Factors influencing the development of black Sigatoka disease on plantain hybrids. Ph.D. Thesis. 127pp. River State Univ. of Science and Technology, Port Harcourt, Nigeria.

29. Mobambo, K. N.; Gauhl, F. and Pasberg-Gauhl, C. 1993. Plantain response to black Sigatoka in backyard gardens and field in South Eastern Nigeria. MusAfrica 3:3-4.

30. Mobambo, K. N.; Gauhl, F.; Vuylsteke, D.; Ortiz, R. Pasberg-Gauhl, C. and Swennen, R. 1993. Yield loss in plantain from black Sigatoka leaf spot and field performance of resistance hybrids. Field Crops Research 35:35-42.

31. Mobambo, K. N. and Naku, M. 1993. Situation de la cercosporiose noire des bananiers et plantains (*Musa* spp) sous differents de culture a Yangambi, Haut-Zaire. Tropicultura 11:7-10.

32. Mobambo, K. N.; Pasberg-Gauhl, C.; Gauhl, F. and Zuofa, K. 1994. Influence of crop management and soil on plantain (*Musa* spp, AAB group) response to black Sigatoka infection in South-Eastern Nigeria. Tropicultura 12:18-20.

33. Molina, G. and Krausz, J. P. 1988. A phytotoxin activity in extracts of broth cultures of *Mycosphaerella fijiensis* var *difformis* and its use

to evaluate host resistance to black Sigatoka. Plant Disease 73:142-143.

34. Molina, A. B. and Sales, J. A. 1990. Evaluation of sensitivity of *Mycosphaerella fijiensis* to Propiconazole. *In*: Fullerton, R. A. and Stover, R. H. (eds). Sigatoka leaf spot disease of banana. Procedings of an international workshop held at San Jose, Costa Rica, March 28-April 1, 1989. montpellier, France. INIBAP, 1990: 90-99.

35. Mulder, J. L. and Holliday, P. 1974. *Mycosphaerella fijiensis*. CMI Descriptions of Pathogenic Fungi and Bacteria No. 413. 12pp.

36. Mwashayenyi, E. 1994. Banana production and research in Zimbabwe- Paper written for the inaugural meeting of the steering committee of IITA's East and Southern Africa Regional Centre for the improvement of cassava, banana and Plantain (ESARC), Kampala, Uganda 28*th* Feb.-2*nd* March, 1994. MusAfrica 5:7-8.

37. Natural, M. P. 1990. An update on the development of an in vitro screeing procedure for resistance to Sigatoka leaf diseases of banana. *In*: Fullerton, R. A. and Stover, R. H. (eds). Sigatoka leaf diseases of bananas: Procedings of an international workshopheld at San Jose, Costa Rica. March 28-April 1, 1989. Montpellier, France. INIBAP 1990, 208-230.

38. Nweke, F. I.; Njoku, J. E. and Wilson, G. F. 1988. Productivity and limitations of plantain (*Musa* spp. cv AAB) production in compound gardens in South Eastern Nigeria. Fruits 43:161-166.

39. Oduro, K. A.; Twumasi, J. K.; Paa-Kwesi Entsie and Fenteng, F. 1992. Incidence and severity of black Sigatoka disease of plantain in Ghana. Technical Report on Nation-wide survey of plantain disease in Ghana. 37pp.

40. Pasberg-Gauhl, C. 1989. Untersuchungen zur symtoment wicklung and Bekampfung der schwarzen sigatoka-Krankheit (*Mycosphaerella fijiensis*, Morelet) an Banana (*Musa* spp) in vitro und in Freiland. Gottinger Beitrage zur Land-u. Forst wirt-schaft in den Tropen U. Sub tropen Heft 40. Gottingen (Germany). 142pp.

41. Pasberg-Gauhl, C. 1994. Symptom development of black Sigatoka leaf spot on young or adult banana and plantain plants after natural inoculation. *In*: Gemmill, B. and Goad, C. (eds). IITA procedings on biological and integrated control of Highland banana and plantain pests and diseases. IITA, Cotonou, Benin, 4:263-275.

42. Persely, G. J. and De Langhe, E. A. 1987. Banana and plantain breeding strategies. Procedings of an international workshop held at Cairns, Australia, 13-17 October, 1986. ACIUAR Procedings No. 21:187pp.

43. Philpott, J. and Knowles, C. M. 1913. Report on a visit to Sigatoka. Department of Agriculture, Fiji. Pamphlet No. 3. 21pp.

44. Pont, W. 1970. Fungicides against banana leaf diseases. Od. J. Agric. Sci.96:709-710.

45. PPMED, 1990. Production estimates for plantain in Ghana, 1987-90. Data File, Policy Planning Monitoring and Evaluation Division (PPMED) Ministry of Food and Agriculture Accra. 11pp.

46. Reamakers, R. 1975. Black streak-like disease in Zambia. PANS 21:396-400.

47. Ramsey, M. D.; Vawdrey, L. L. and Schipke, L. G. 1987. Evaluation of systemic and protectant fungicides for the control of Sigatoka leaf spot (*Mycosphaerella musicola*. Leach) of bananas in North Queensland Aust. J. Exp. Agric. 27:919-923.

48. Rhodes, P. L. 1964. A new banana disease in Fiji. Commonwealth Phytopath. News 10:38-41.

49. Ruhigwa, B. A.; Gichuru, M. P.; Spencer, D. S. and Swennen, R. 1994. Economic analysis of cut-and-carry, and alley cropping for plantain production in Southeastern Nigeria. Agro Forestry Systems 26:131-138.

50. Sebasigari, K. and Stover, R. H. 1988. Banana diseases and pests in East Africa. Report of a survey made in Nov. 1987. INIBAP/88/02. Montpellier, France, INIBAP 1988. 15pp.

51. Slabaugh, W. R. 1990. New approaches for chlorothalinol use in strategies to control *Mycosphaerella fijiensis*. *In*: Fullerton. R. A. and

Stover, R. H. (eds). Sigatoka leaf spot diseases of bananas. Procedings of an international workshop held at San Jose, Costa Rica. March 28-April 1, 1989. Montpellier, France. INIBAP 1990:159-166.

52. Shillingford, C. A. 1975. Cultivar susceptibility in sprayed and unsprayed plants. Trop. Agric. (Trinidad) 52:157-159.

53. Stover. R. H. 1968. Action of oil on life cycle of pathogen. Can. J. Bot. 46:1495-1496.

54. Stover, R. H. 1971. A proposed international scale for estimating intensity of banana leaf spot. (*Mycosphaerella musicola*. Leach) Trop. Agric. (Trinidad) 43:117-129.

55. Stover, R. H. 1976. Distribution and cultural characteristics of leaf spot pathogens on banana. Trop. Agric. (Trinidad) 53:111-114.

56. Stover, R. H. 1977. Behaviour of benomyl tolerant strains of black Sigatoka pathogen in the field. Procedings American Phytopathological Society 4:180-187.

57. Stover, R. H. 1978. Distribution and possible origin of *Mycosphaerella fijiensis* in South East Asia. Trop. Agric. (Trinidad) 55:65-68.

58. Stover, R. H. 1979. Field observation of benomyl tolerance in ascospores of *Mycosphaerella fijiensis* var *difformis*.Transactions British Mycological Society 73:518-519.

59. Stover, R. H. 1980. Sigatoka leaf spots of banana and plantain. Plant Disease 64:750-755.

60 Stover, R. H. 1983. Effect du cercospora noir sur les plantains en Amerique Centrale. Fruits 38:326-329.

61. Stover, R. H. 1993. Changes in the sensitivity of *Mycosphaerella fijiensis* to Tilt. Informe (UPEB), (PAN), SPA 1993 Vol 16(97):41-44.

62. Stover, R. H. and Dickson, J. D. 1970. Leaf spot of banana caused by *Mycosphaerella musicola*. Methods of measuring spotting, prevalence and severity. Trop. Agric. (Trinidad) 47:289-302.

63. Stover, R. H. and Simmonds, N. W. 1987. Bananas. 3rd Ed. Longmas Scientific and Technical, Essex, UK and J. Wiley and Sons, NY 468pp.

64. Swennen, R. 1990. Plantain cultivation under West Africa conditions. A reference manual, IITA, Ibadan, Nigeria. Amarin printing Group Co. Ltd. Thailand. 24pp.

65. Swennen, R. and Vuylsteke, D. 1988a. Bananas in Africa. Aspects of diversity and prospects for improvement. IITA/CNR/IBPGR/UNEP workshop on Crop Genetic Resources in Africa held at Ibadan, Nigeria, 17-20 Oct. 1988. 21pp.

66. Swennen, R. and Vuylsteke, D. 1988b. Plantains in West and Central Africa. 4th West and Central Africa Root Crops workshop held at Lome, Togo 12-16 Dec. 1988. 18pp.

67. Swennen, R. and Vuylsteke, D. 1991. Banana in Africa : Diversity, uses and prospects for improvement; *In*: Ng, N. Q.; Perrino, P.; Attere, F. and Zedan, H. (eds). Crop Genetic resources in Africa Vol 2:151-160.

68. Twumasi, J. K.; Hemeng, O. B.; Oduro, K. A. and Fenteng, P. 1993. Efficacy of some selected fungicides for the control of black Sigatoka disease of plantain in Ghana. Abstracts. 2nd National Roots and Tuber crops and plantain workshop. October 21st -22nd, 1993. K. T. I. Kumasi. 66pp.

69. Union de Paises Exportadores de Banano (UPEB). 1985. Investigadores buscan desarrollar variedades resistentes a la Sigatoka negra. Inf. Mens 9(72):40-41.

70. Vuylsteke, D.; Swennen, R. and Ortiz, R. 1993. R Registration of 14 improved tropical *Musa* hybrids with black sigatoka resistance. Hortscience 28:957-959.

71. Wardlaw, C. W. 1961. Banana diseases including plantain and abaca. 1st Edition. Longmans. U. K. 670 pp.

72. Wardlaw, C. W. 1972. Banana diseases including plantain and abaca. 2nd Edition. Longmans. London.878pp.

73. Wielemaker, F. 1988. Leaf emission rate determination in Rio Frio, Costa Rica. Internal Standard Fruit Company Research Report. 37pp.

74. Wielemaker, F. 1990. Practical notes on black Sigatoka control. *In*: Fullerton. R. A. and Stover, R. H. (eds). Sigatoka leaf spot diseases of bananas. Procedings of an international workshop held at San Jose, Costa Rica. March 28-April 1, 1989. Montpellier, France. INIBAP 1990:107-114.

75. Wilson, G. F. 1986. Status of banana and plantain in West Africa. *In*: Persley, G. F. and De Langhe, E. A. (eds). Banana and plantain breeding strategies: procedings of an international workshop held at Cains, Australia, 13-17 Oct., 1986. ACIAR Procedings 21:29-35.

76. Wilson, G. F. and Buddenhagen, I. 1986. The black Sigatoka threat to plantain and banana in West Africa. IITA Research Briefs 7:3-5.

77. Wilson, G. F.; Vuylsteke, D. and Swennen, R. 1985. Rapid multiplication of plantain: an improved field technique. Pages 24-26. *In*: international cooperation for effective plantain and banana research, procedings, 3rd IARPB meeting, Abidjan, Cote d'Voire. 27-31 May, 1985. IARPB/INIBAP, Montpellier, France.

APPENDIX 1. ANOVA table for average number of leaves 28, 32, 36 & 44 weeks after planting.

A. 28 weeks

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	4.69	1.56	1.48
Block	3	2.93	0.98	0.92
Error	9	9.54	1.06	
Total	15	17.1		

B. 32 weeks

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	40.19	13.40	47.52**
Block	3	1.63	0.54	1.92
Error	9	2.53	0.28	
Total	15	44.36		

C. 36 weeks

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	40.15	13.38	9.53**
Block	3	4.31	1.44	1.02
Error	9	12.64	1.40	
Total	15	57.10		

D. 40 weeks

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	20.49	6.83	6.14*
Block	3	3.40	1.13	1.02
Error	9	10.01	1.11	
Total	15	33.89		

E. 44 weeks

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	21.85	7.28	4.70*
Block	3	6.83	2.20	1.42
Error	9	13.40	1.55	
Total	15	42.40		

APPENDIX II **ANOVA table for Youngest Leaf with Symptom (YLWS) 28, 32, 36, 40 and 44 weeks after planting.**

A. 28 weeks

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	7.07	2.36	10.90**
Block	3	0.37	0.12	0.57
Error	9	1.95	0.22	
Total	15	9.38		

B. 32 weeks

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	5.63	1.88	12.97**
Block	3	0.34	0.11	0.79
Error	9	1.30	0.14	
Total	15	7.28		

C. 36 weeks

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	24.02	8.01	42.88**
Block	3	0.17	0.06	0.30
Error	9	1.68	0.19	
Total	15	25.87		

D. 40 weeks

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	17.83	5.94	42.44
Block	3	0.42	0.14	0.99
Error	9	1.26	0.14	
Total	15	12.72		

E. 44 weeks

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	10.83	3.61	26.34**
Block	3	0.65	0.22	1.59
Error	9	1.23	0.14	
Total	15	12.72		

APPENDIX III ANOVA table for average bunch weight.

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	27.27	9.15	13.45**
Block	3	4.47	1.50	2.21
Error	9	6.08	0.68	
Total	15	37.82		

APPENDIX IV ANOVA table for average number of fingers per bunch

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	33.75	11.25	2.74
Block	3	21.53	7.18	1.75
Error	9	36.92	4.10	
Total	15	92.20		

APPENDIX V ANOVA table for length of 'middle finger'

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	11.57	3.85	1.58
Block	3	11.32	3.77	1.55
Error	9	21.85	2.42	
Total	15	44.74		

APPENDIX VI ANOVA table for weight of 'middle finger'

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	0.0065	0.0022	1.187*
Block	3	0.0022	0.0007	0.402
Error	9	0.0163	0.0018	
Total	15	0.02		

APPENDIX VII ANOVA table for disease severity from week 8 - 44.

A. week 8

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	9.925	3.308	109.511*
Block	3	0.096	0.032	1.062
Error	9	0.272	0.032	
Total	15			

B. week 12

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	8.400	2.800	33.758
Block	3	0.052	0.017	0.211
Error	9	0.746	0.083	
Total	15			

C. week 16

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	9.940	3.313	92.922
Block	3	0.042	0.014	0.390
Error	9	0.321	0.036	
Total	15			

D. week 20

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	12.442	4.147	67.233
Block	3	0.039	0.013	0.210
Error	9	0.555	0.062	
Total	15			

E. week 24

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	13.011	4.337	221.621
Block	3	0.092	0.031	1.567
Error	9	0.176	0.20	
Total	15			

F. week 28

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	17.071	5.690	500.979
Block	3	0.117	0.039	3.430
Error	9	0.102	0.011	
Total	15			

G. week 32

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	10.103	3.368	271.442
Block	3	0.005	0.002	0.147
Error	9	0.113	0.012	
Total	15			

H. week 36

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	9.313	3.104	119.750
Block	3	0.092	0.031	1.186
Error	9	0.233	0.026	
Total	15			

I. week 40

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	11.010	3.670	659.832
Block	3	0.004	0.001	0.253
Error	9	0.050	0.006	
Total	15			

J. week 44

Source of variation	Degree of freedom	Sum of squares	Mean square	Fcal
Treatment	3	15.918	5.306	276.597
Block	3	0.066	0.022	1.145
Error	9	0.176	0.019	
Total	15			