Nº 251

UNIVERSITE DE LOME
FACULTE DES SCIENCES

THESE

Présentée en vue de l'obtention du grade de

Docteur ès Sciences Naturelles
De l'Université de Lomé
Programme: Biologie de Développement
Option: Biotechnologies Appliquées
Spécialité Phytopathologie/ Biologie Moléculaire

Par

Martine ZANDJANAKOU-TACHIN

DISTRIBUTION AND GENETIC DIVERSITY OF *MYCOSPHAERELLA* SPP. OF BANANAS IN NIGERIA

Soutenue publiquement, le 12 Juin 2009

devant la Commission d'examen ainsi composée:

M. Koffi AKPAGANA, Professeur, Université de Lomé, Togo (Président)

M. Abdul GHAFFAR, Professor, University of Karachi, Pakistan (Examinateur)

Dr. Vroh BI-IRIE, Molecular biologist, IITA-Ibadan, Nigeria (Membre)

Dr. Ranajit BANDYOPADHYAY, Pathologist, IITA- Ibadan, Nigeria (Co-Directeur)

M. Y. Mawuena D. GUMEDZOE, Professeur, Univeristé de Lomé, Togo (Directeur)

CERTIFICATION

We certify that Mrs Martine ZANDJANAKOU TACHIN carried out this work in Ecole Supérieure d'Agronomie, Université de Lomè, Togo in collaboration with the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria under our supervision.

Pr Y. Mawuena D. GUMEDZOE
Supervisor
Ecole Superieure d'Agronomie
(Faculty of Agriculture), Laboratoire
de Virologie et de Biotechnologie

Dr. Ranajit BANDYOPADHYAY
Supervisor
Pathology/Mycotoxins Unit,
IITA, Ibadan, Nigeria

Juin 2009

DEDICACE

To my husband Emmanuel S. Tachin
I am deeply indebted to you for your never ending support, staying at my side through the hardest time.

And to my children Katché Volker Felvic & Kotchami Emmafried Martems,
for their patience even during my absences.
May this work be an example of determination for your life!

ABSTRACT

Leaf spot diseases caused by fungi in the genus *Mycosphaerella* are among the greatest threats to sustainable banana production in Nigeria. However information on the relative distribution and genetic diversity of the causal agents in Nigeria is lacking.

Surveys where conducted in 2004, 2005, and 2006 by visiting 100 fields located in four geographical zones named South-south, South-west, South east and North center. The four zones were located in three agroecological zones: derived savanna (DS), humid forest (HF) and southern guinea savanna (SGS) in the bananas production belt of Nigeria.

Ribosomal DNA was then sequenced and compared in 96 isolates of *Mycosphaerella* species collected during the surveys and single nucleotide polymorphism (SNP) was used to identify the species and to determine the genetic structure of the sampled populations. The Arlequin program was used to deduce the population differentiation through analysis of molecular variance (AMOVA). Then the genetic relationships and clustering among the isolates were done by UPGMA methods of NTSYS.

Three varieties of bananas were inoculated with the different *Mycosphaerella* isolates. Aggressiveness of the isolates was determined and data analyzed using ANOVA, *t*-test and correlation procedure. Principal component and cluster analyses assigned isolates into pathotypes.

Data from the three surveys showed that leaf spot diseases are present in Nigeria and the number of infected fields within each year differed significantly (χ^2 = 34·2, df = 2, and P < 0·05) between the HF and DS. Incidence of infected fields was significantly different between geographical zone ($P < 0{\cdot}0001$), and disease severity was similar in HF (27%) and in DS (25·4%).

Using reference GenBank accessions, with intercontinental distributions as controls and shared SNPs in these control accessions, 84 (88·4%) isolates that grouped in 14 haplotypes were identified as *M. fijiensis,* 11 (11·6%) represented by 7 haplotypes were characterized as *M. eumusae*. No *M. musicola* was identified in the Nigerian

collection. The analysis based on previously published species-specific probes designed on actin and β-tubulin gene sequenced confirmed the identification of the two species. A pairwise comparison of the population genetic distances relative to the four geographical zones showed that significant genetic differentiation exists among most populations ($P < 0{\cdot}001$), with an average Fst of 0·126, and a population structure corresponding to the four sampled geographical zones. The intraspecific dissimilarity of *M. eumusae* was 4·6% compared to that of *M. fijiensis* which was 2%. Compared with all the GenBank reference checks, three sequence variations were unique to some Nigerian *M. fijiensis* haplotypes. Twenty-one sequenced haplotypes were identified, geographically mapped and registered in GenBank.

All isolates collected induced symptoms on the tested varieties and there is a significant differences among isolates ($P < 0{\cdot}05$), and isolates by cultivar interaction ($P < 0{\cdot}0001$). Multivariate analyses combined with the Rank-sum method indicated the presence of variations in aggressiveness in the 96 isolates. The cluster analysis partitioned the 85 *M. fijiensis* in nine pathotypes, while the 11 *eumusae* were grouped in seven pathotypes. 80% of the *M. fijiensis* isolates and 20% of the *M. eumusae* were HA. The most aggressive were *M. fijiensis* Iso45, 25, 72, 78, 58, 23, 32 and *M. eumusae* Iso12 and 21. Shannon's index of diversity confirmed considerable pathogenic variation between agroecological zones with values of DS (H' = 1·80) and HF (H' = 1·50).

Results indicate the presence of different haplotype/pathotypes and that *M. musicola* has been replaced by more frequently occurring *M. fijiensis* and *M. eumusae* against which disease management and resistance breeding efforts should be directed in Nigeria.

Keywords: *Musa* species, distribution, *M. fijiensis*, *M. eumusae*, aggressiveness, genetic diversity, pathotype composition.

Word count: 557

RESUME

Les maladies des raies des bananiers sont causées par des champignons du genre *Mycosphaerella* (*M. fijiensis*, *M. musicola* et *M. eumusae*). Ces trois especes sont responsables des plus sérieuses menaces au développement durable de la production des bananes au Nigeria. Cependant, les informations font défaut quant à la répartition relative et la diversité génétique de l'agent pathogène de la maladie.

Des prospections périodiques de terrain ont été effectuées, de 2004 à 2006, à travers des visites de 100 exploitations situées dans quatre zones géographiques: Sud-sud, sud-ouest, sud-est et centre-nord, ces zones étant elles-mêmes situées dans trois régions agro écologiques que sont la Savane arborée (SA), la forêt humide (FH) et la zone soudano guinéenne (SG), lesquelles sont connues pour être des zones de production par excellence de la banane au Nigeria.

L'ADN ribosomal est ensuite séquencé et comparé dans 96 isolats d'espèces de *Mycosphaerella* collectées lors des enquêtes et la presence de simple mutation (SNP) est utilisée pour identifier les espèces et déterminer la structure génétique des populations de l'échantillon. La différenciation de la population a été etudiée par l'usage de logiciel nommé Arlequin et a permis d'estimer l'index de differenciation Fst, et conduire l'analyse moléculaire de variance (AMOVA). Puis, la phylogénie est réalisée à l'aide des méthodes UPGMA de NTSYS.

L'étude de la variabilité pathologique a été conduite à partir de trois variétés de bananes inoculées avec différents isolats de *Mycosphaerella*. L'agressivité des isolats est déterminée et les données ont été analysées grâce à ANOVA et le test de corrélation. Les analyses du principal composant et du cluster ont permis de regrouper les isolats en pathotypes.

Les données des trois enquêtes montrent que les maladies des raies sont présentes au Nigeria et que le nombre de champs infectés diffère chaque année de manière significative ($\chi^2 = 34{\cdot}2$, df = 2, and $P < 0{\cdot}05$) entre la SA et la FH. L'incidence des champs infectés était significativement différente entre les zones géographiques ($P < 0{\cdot}0001$) et la sévérité de la maladie était la meme aussi bien en FH (27%) qu'en SA (25·4%).

En utilisant les accessions de référence de la Banque de gènes, avec des distributions intercontinentales comme isolats de référence, 84 (88·4%) des isolats regroupés en 14 haplotypes ont été identifiés comme étant *M. fijiensis*, 11 (11·6%) isolats, représenté par 7 haplotypes ont été caractérisés comme *M. eumusae*. Aucune espèce *M. musicola* n'a été identifiée dans la collection du Nigeria. L'analyse se fondant sur des sondes spécifiques conçues sur les genes de l'actine et la β- tubuline a confirmé l'identification des deux espèces. Une comparaison par paires des distances génétiques de la population, par rapport aux quatre zones géographiques a montré qu'une différenciation génétique significative existe entre la plupart des populations (*P* < *0·001*) avec un indice de differentiation moyenne *Fst* de 0·126, et une structure de la population correspondant aux quatre échantillons des zones géographiques. La différenciation intra spécifique de *M. eumusae* était plus élevée (4.6%) comparée à celle de *M. fijiensis* (2%). En comparant toutes les souches isolées avec celles du GenBank, trois variations de séquence uniques à certains haplotypes nigérian de *M. fijiensis* ont été identifiées. Les vingt et un haplotypes identifiés, ont été géographiquement cartographiés et enregistrés dans la Banque de gènes.

Le test de pathogénicité a montré que tous les isolats collectés induisent des symptômes sur les variétés testées et il existe une différence significative entre isolats (*P* < 0·05), cultivars, et entre l'interaction isolats X cultivar (*P* < 0·0001). Des analyses multi variantes combinées avec la méthode de Rank-sum laissent voir la présence de variations de l'agressivité dans les 96 isolats. L'analyse par groupe de pathogénicité a reparti les 85 *M. fijiensis* en neuf pathotypes, tandis que les 11 *M. eumusae* ont été regroupés en sept pathotypes. Les souches les plus aggressives étant Iso45, 25, 72, 78, 58, 23, 32 and *M. eumusae* Iso 12 and 21. L'index de la diversité (Shannon index) a confirmé de considérables variations entre les zones agro-écologiques avec des valeurs de *H' = 1·80* pour la SA et *H'* = 1·50 pour la FH.

Le remplacement de *M. eumusae* par de plus fréquentes apparitions de *M. fijiensis* et de *M. eumusae* et la présence de différents pathotypes et haplotypes indiquent que des mesures de contrôle de la maladie doivent être prises pour l'amélioration de la plante et des efforts de sélection variétale doivent desormais considerer les especes *M. eumusae.*

Mots-clés: espèces *Musa*, distribution, *M. fijiensis*, *M. eumusae*, agressivité, diversité génétique, haplotype, pathotype.

ACKNOWLEDGMENTS

I wish to express my sincere thanks and appreciation to my supervisor, Professor Y. M. D. Gumedzoe for his special guidance and supervision during this work. I really thank him for his encouragement and advice throughout this study.

I am deeply indebted to Dr R. Bandyopadhyay, my Supervisor at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, for accepting me as his student and providing me with the ability to push my motivation to research to its extreme, He has assisted me in every way he could.

My profound gratitude and appreciation goes to Dr B. Vroh from Biotechnology Unit, at IITA for his constructive advice, comments, and suggestions, especially for developing my skills in the molecular methods and techniques.

My gratitude extends to Dr P. S. Ojiambo at the University of North Carolina whose comments and answers to my questions shaped some aspects of my study and Dr A. Tenkouano, director at AVRDC, for assisting me especially at the early stage of this project.

My appreciation also goes to all IITA and WARDA Scientists who have contributed in one way or the other to this project. Special thanks to Dr. Tamo Manuelle, Dr Adebola Raji, Dr Richardson Okechuku, Mr Sam Korie and Sam Ofodile, Dr. Gregorio Glen.

My appreciation also goes to my Supervisory committee members and to all Lecturers of the Ecole Supérieure d'Agronomie, Université de Lomé (Togo), and especially to Dr. Kossi Kpémoua.

My special thanks to the entire staff of Pathology and Molecular Biology Unit, IITA, Ibadan, especially, Messrs Olamilekan Ayinde, Greg Ogbe, Owolabi Alaseni, Olamilekan Akintola and Greg Eduviere, Ebere Sunday; Taiwo Adegbesan, Bakare Moshood, Mrs Ruth Oludotun, Miss Sandra Nnadi, Miss Blessing, Miss Tokpe Olaseni, Mrs Bukola Adekoya and Miss. Esther Chukwu for their support, assistance and encouragements.

My sincere thanks go to all my colleagues at IITA, at the Université d'Abomey Calavi and the Université de Parakou especially Drs Afolabi Clement, Afouda Leonard, Agbicodo Eugene, Atehnkeng Joseph, Twizeyimana Mathias, Elie Dannon, Zinsou

Valerien, Avocanh Adolphe, Mrs Olaitan Okechukwu, Dr Kumar Manjula, Miss Cynthia Ijeego Mbah, Miss Mercy Aregbesola, Mrs Vicencia Agbicodo, and all members of IARSAF for their moral support and encouragement.

My gratitude is extended to my colleagues at the Université de Lomé, in Togo, Drs D. Adjata Dodji, Ayisa, Banito Agnassim, Agbeko Tounou for their support

Also I express my gratitude to the Geographical positioning system (GPS) team, especially M. Kunmi Eniayewu R. T. Alabi and, M. Taiwo Omodele

I am grateful to my family in law, particularly Tachin Paul & family for assisting me with their encouragements.

Special thanks to my entire family Zandjanakou and Zodéhougan, especially to my sister Agnès T. Zandjanakou. I am also grateful to all my nieces and nephews.

Special thanks to Ernest and Rosalie Yegnon, and all their children for their constant assistance.

Special thanks to Alice Zandjanakou and Francine Djonontin, for their assistance in the house and support during this project. I cannot forget you.

I acknowledge and thank the Third World Organization for Women in Science (TWOWS) for providing financial support to my research, without which this study could not have succeeded.

I am grateful to IITA, Ibadan, for supporting my research by providing all necessary facilities and some additional funds to allow me to successfully complete this study.

Finally, I wish to thank Almighty God for everything He has done for me throughout all my life and in making this program successful.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

LIST OF APPENDICES

ABBREVIATIONS

AMOVA	Analysis of molecular variance
ANOVA	analysis of variance
AUDPC	Area under disease progress curve
Cm	Centimeter
DNA	Deoxyribonucleic acid
DS	Derived savanna
FAO	Food and Agriculture Organization
Fig	Figure
GPS	Global positioning system
HF	Humid forest
IITA	International Institute of Tropical Agriculture
INIBAP	International Network for the Improvement of Banana and Plantain
IPM	Integrated pest management
ITS1	Internal transcribed spacer 1
ITS2	Internal transcribed spacer 2
Kb	Kilo base
LAI	Leaf area infected
µl	Micro liter
µg	Microgram
Mg	Milligram
Ml	Milliliter
NC	North center
PCR	Polymerase Chain reaction
PDA	Potato dextrose agar
SE	South-east
SGS	Southern Guinea savannas
SNP	Single nucleotide polymorphism
Spp	Species
SS	South-south
SW	South-west
T	tons
UPGMA	Unweighted Pair Group Method with Arithmetic mean

INTRODUCTION

Bananas (*Musa* spp), referred to as banana and plantain in this study, are giant perennial, but monocarpic plants (Fig. 0.1) from two to 15 meters high, that originated in the tropical regions of Southeast Asia (Jones, 2000; Ploetz, 2001; Roux *et al.,* 2008). They constitute a major staple food crop and provide sustenance for millions of people in developing countries. This is due to the crop's contribution to food security, employment, diversification of income sources in rural and urban areas, and to the gross national product (GNP) (Nkendah and Akyeampong, 2003).

World production is estimated to be 115 million tons annually (FAO 2007) with an exported value of 4.5 to 5 billion US$ per year during 1998–2000 (Ploetz 2000, Marín *et al*., 2003). The plant is cultivated in about 120 countries throughout the tropical and subtropical regions. African production has increased and is now at 32 MT (FAO, 2007). This country, together with Rwanda, accounted for 41% of all plantain and cooking banana production worldwide (Robinson, 1996). In West Africa, plantain is a primary food crop with a total production of 8.6 million tons in 2007. The gross value of the annual production in sub-Saharan Africa was estimated to exceed that of each of the other food crops such as maize (*Zea mays*), rice (*Oriza sativa*), cassava (*Manihot* spp) and sweet potato (*Ipomea batatas*) (Ortiz and Vulylsteke, 1995)**.** Although no African country was ranked among the top ten countries for banana production in the world, eight countries were listed among the top ten world plantain producers, with Nigeria being classified as the fifth highest producer (FAO, 2004).

Bananas are an important food, providing a cheap and easy source of energy. They are rich in minerals, and particularly vitamins A, C, and B6. When compared with apples, they have four times more protein and almost every part of the plant can be used in one way or another. This explains why in India, bananas are popularly known as Kalpatharu, meaning herb with all imaginable uses (Anon, 2005). They are also an important source of fiber, and can be fermented for alcohol production.

The crop also occupies a prime place as a staple food in the diet of the Nigerian populace (Faturoti *et al*., 2007). One green cooking banana is said to have about the

same calorie content as one potato. Fruits are produced year–round, providing an extremely valuable source of food during the off-season (that period of time when all the food from the previous harvest has been consumed, and the next harvest is still some time away). This is an indication of the importance of bananas as a food security crop.

However, in Africa the banana industry is facing different problems related to declining soil fertility, decreasing land sizes, pests and diseases, lack of reliable and clean propagation materials, lack of best practices in banana production (agronomic, post-harvest handling, etc.), lack of reliable and well organized markets, poor infrastructure that limits market accessibility, lack of value-addition from processing, and restrictions on international trade that are very critical to Africa.

The plant is susceptible to a variety of devastating diseases, including *Fusarium* wilt, caused by *F. oxysporum* f. sp. *cubense*, banana bunchy top virus, and *Mycosphaerella*-associated leaf spot diseases (fig. 02). The latest group, attributed to the ascomycete fungi belonging to the genus *Mycosphaerella*, is among one of the most important constraints worldwide (Stover & Simmonds, 1987; Carlier *et al.,* 1996).

Although several species of the causal agent are listed in literature, the most important are *Mycosphaerella fijiensis* (anamorph: *Pseudocercospora fijiensis* (Stewart *et al.,* 1999) that causes black leaf streak disease (black Sigatoka), *M. musicola* (anamorph: *Pseudocercospora Musae*) causal agent of yellow Sigatoka (Stover & Simmonds, 1987), and the more recently identified *Mycosphaerella* species, *M. eumusae* (anamorph: *Pseudocercospora eumusae*) causal agent of *eumusae* banana leaf spot (Carlier *et al.,* 2000b; Crous & Mourichon, 2002).

It was reported that all three species may have originated from the southeast Asian/Australian region which is the centre of origin of *Musa* (Jones, 2000). These diseases are serious threats to banana production worldwide as they reduce the photosynthetic capacity of the plants through necrotic leaf lesions and result in reduced crop yield and fruit quality. The infection process of these *Mycosphaerella* species is similar, except that symptoms develop faster and are more severe on banana infected with *M. fijiensis* and *M. eumusae* than with *M musicola* strains (Balint-Kurti *et al.,* 2001). *M. eumusae* has been reported to affect cultivars that are highly resistant to both *M.*

fijiensis and *M. musicola* (Jones, 2002). Leaf spot diseases (Fig. 0.2) can cause yield losses of up to 50% when infection is high. In West Africa since the 1980s, black Sigatoka has caused yield losses of 30–50% in plantain production (Mobambo, 1993). In Nigeria, average yield losses of 20-80% were reported in low to highly infected plantain fields (Ikotun, 1987).

The annual cost of foliar fungicides used to control black leaf streak alone amount to US$ 1000 per hectare on large plantations (Arias *et al.,* 2003). Moreover, the use of both cultural practices and fungicides has not proved to be sustainable; making the selection and deployment of resistant genotypes to be the most effective strategy in the management of leaf spot diseases of bananas caused by the *Mycosphaerella* species (Marin *et al.,* 2003).

Resistant cultivars that could be used in subsistence situations are available, but they are usually less productive or desirable than those that are susceptible. This situation has begun to change as a result of new, resistant hybrids that are being developed by banana breeding programs. Breeding programs are playing increasingly important roles in subsistence agriculture. In West Africa, the selection criteria in the breeding programs are oriented primarily towards selection for quantitative resistance to *M. fijiensis* (Vuylesteke *et al.,* 1993; Ortiz & Vuylsteke, 1994).

Since durability of host resistance is an evolutionary process that depends on pathogen fitness, recombination, mutation, and migration (Milgroom & Peever, 2003), breeding and deployment of durable resistance require an understanding of the diversity of the pathogen populations. In general, very limited knowledge is available on the genetic diversity of *Mycosphaerella eumusae* and how its variability is distributed within and between populations. Therefore it is difficult to devise quarantine measures and to reliably test and determine whether putatively resistant banana clones will be resistant over a wide geographic area in Nigeria. However, it has been reported that pathogens can evolve to break down resistance (Fullerton and Olsen 1991). It is thus important to research further the evolution of the three *Mycosphaerella* species on resistant hosts**.**

Our research hypothesis states that all the three species are present in West Africa, specifically in Nigeria.

The aims of the present study are to:

(i) study the distribution of the disease within Nigeria,

(ii) detect and identify the *Mycosphaerella* species infecting banana and plantain, using SNP-based assays,

(iii) investigate the genetic structure of the *Mycosphaerella* species infecting banana and plantain in West Africa, and

(iiii) determine the pathogenic variation among isolates.

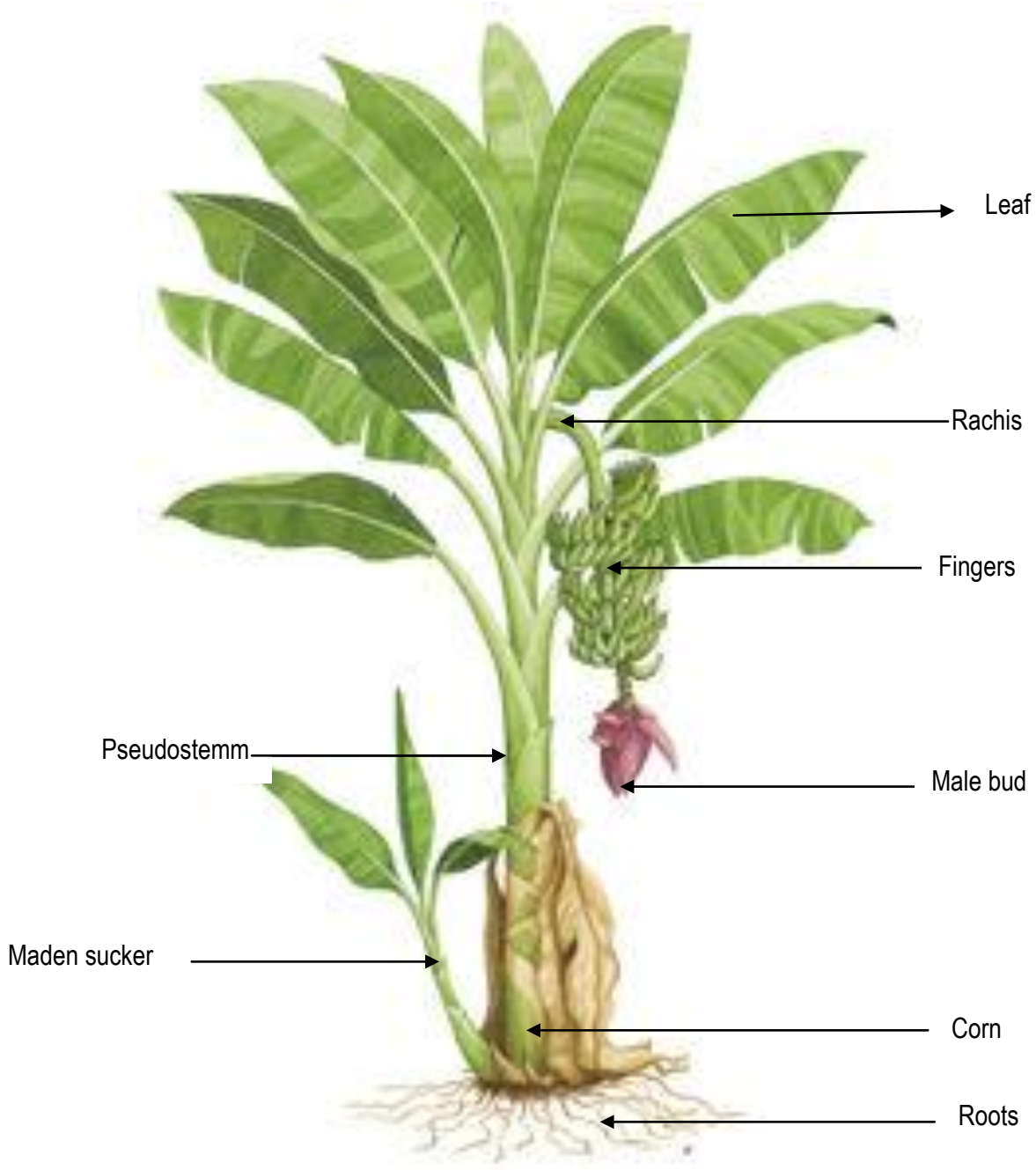


Figure 0.1 Healthy banana tree

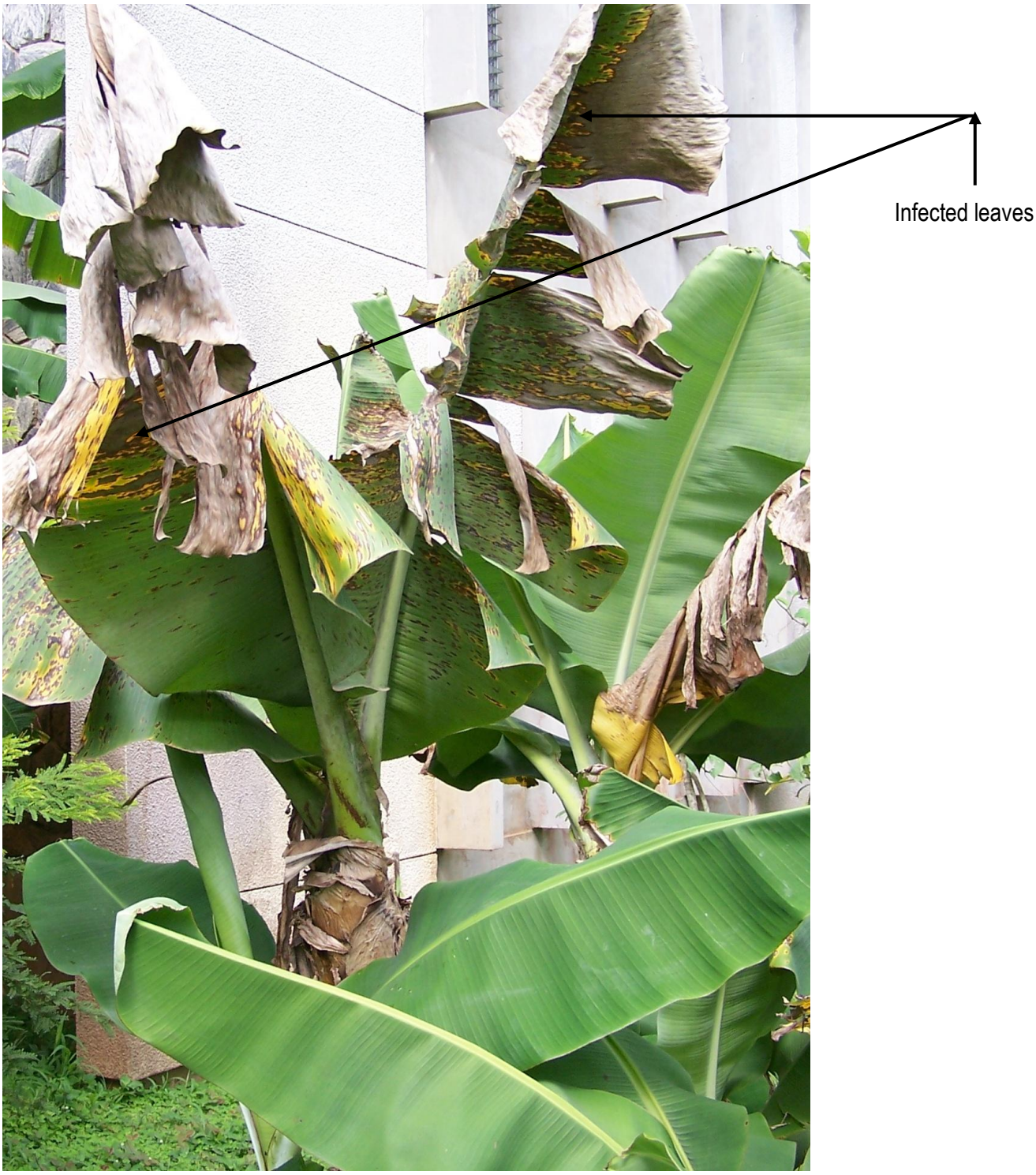


Figure 0.2 Infected banana tree

CHAPTER 1.0: LITERATURE REVIEW

1.1 Taxonomy, Classification, Origin and Distribution of Bananas

1.1.1 Taxonomy and Classification

Bananas belong to the family *Musaceae*. This family contains three genera: *Musa* L., *Ensete* Horan., (Horanonow, 1862) and *Musella* (Franch.) C. Y. Wu (Franchet, 1889; Wu, 1978). *Musa* and *Ensete* belong to the order *Scitaminaceae* (*Zingiberales*) (Jones, 2000). It includes both wild and cultivated seed-sterile species with enormous socio-economic importance, such as bananas and plantains (Hakkinen, 2009). This order comprises perennial plants with six families: *Musaceae, Streliziaceae, Lowiaceae, Zingberaceae, Marantaceae* and *Cananaceae* (Simmonds, 1962; Jones, 2000).

The genus *Musa,* which is the most widely grown, has four sections/series: *eumusae* (2n = 22), *Australimusa* (2n = 20), *Rhodochlamys* (2n = 22), and *Callimusa* (2n = 10) (Cheesman, 1947; Simmonds and Shephard, 1955; Rukazambuga, 1996) of which only *Eumusae* and *Australimusa* have edible clones. The *Eumusae* section is the most widespread, and contains the greatest number of the edible bananas; whereas the second, *Australimusa*, is restricted to the Pacific Islands and is relatively less well-known (Simmonds, 1962).

The classification of bananas is complicated by the numbers of cultivars, which have developed from natural interspecific hybridization (Valmayor *et al.,* 1991). Moreover, several cultivars are designated by different names in different regions by indigenous people and researchers (Chukwu, 1996). All banana cultivars were derived from two main species, which are *Musa acuminata* Colla (syn.s *M. cavendishii* Lamb., Ex Paxt *M. chinensis* Sweet, *M. nana*, *M. zebrine* Van Houtee ex Planch and *Musa balbisiana* Colla. Hybrids of *M. acuminata* and *M. balbisiana* are sometimes given the names *Musa X paradisiaca* L., *Musa X sapientum* L., or perhaps most accurately, *M. acuminata x M. balbisiana Colla*. The group can be divided into two main categories: dessert bananas and cooking bananas.

The first attempt to name the plants was made by using genotypic characterization (Marriot and Lancaster, 1983; Stover and Simmonds, 1987; Robinson, 1996). Each type of banana is given a two to four letter designation consisting of A's representing *acuminata*, and B's, representing *balbisiana.* Based on this method, bananas are grouped into diploids (AA, AB, and BB), triploids (AAA, AAB, ABB, BBB), and tetraploids (AAAA, ABBB). However, the taxonomy of the *Musa* group, including the Rhodochlamys sections, is still very obscure (Häkkinen, 2009). Major banana cultivars include 'Gros Michel', 'Mon Mari', 'Williams', 'Williams Hybrid', and 'Grand Nain'. 'French', 'Horn', Bluggoe' 'Pelipita', and'Saba' are considered the major plantain cultivars.

1.1.2 Origin and Distribution

The banana plant originated from tropical regions of Southeast Asia (Ploetz, 2001; Roux *et al.,* 2008). With human movement, it had spread to other countries such as North Australia. Early Philippine populations probably spread the banana eastward to the Pacific islands, including Hawaii, prior to recorded history. Arab traders were responsible for introducing the plant to Africa; the Portuguese took the banana to the Caribbean and Latin America after obtaining it from West Africa (FAO, 2006). The crop was unknown to Europeans until Portuguese sailors brought them from West Africa to the Canary Islands. Bananas are now grown pantropically in more countries than any other fruit crop in the world.

1.1.3 Production, Agronomy and Socio-Economic Importance

Bananas are the second fruit crop in the world in terms of production, behind the African oil palm. The average yield is about 18,000 lbs/acre in 2004. Although bananas are the world's most exported fruit and the fourth most important food commodity on earth – after rice, wheat and maize – in terms of production value, it is one of the least valuable food crops (Roux *et al.,* 2008).

Production, as well as exports and imports of bananas, is highly concentrated in 130 countries worldwide. These countries are the usual destination for export bananas. World import was about 70 thousand ton in 2004 (Fig. 1.1). The 10 major banana producing countries accounted for about 75% of total banana production in 2007 (Table

1.1) (FAO, 2007). The Latin American and Caribbean region dominated production up until the 1980s, the Asian region taking the lead in banana production.

In 2006, Africa alone accounted for 31% of the world production (Lescot & Ganry, 2008). Africa's share of world trade has risen in the last 20 years from just 3 to 4%; only Cameroon, Côte d'Ivoire and Ghana have significant exports, mostly to France and the UK. Plantain production alone is 34,444,795 million ton with Uganda being first with its contribution of 27 % (Table 1.2) (FAO, 2007).

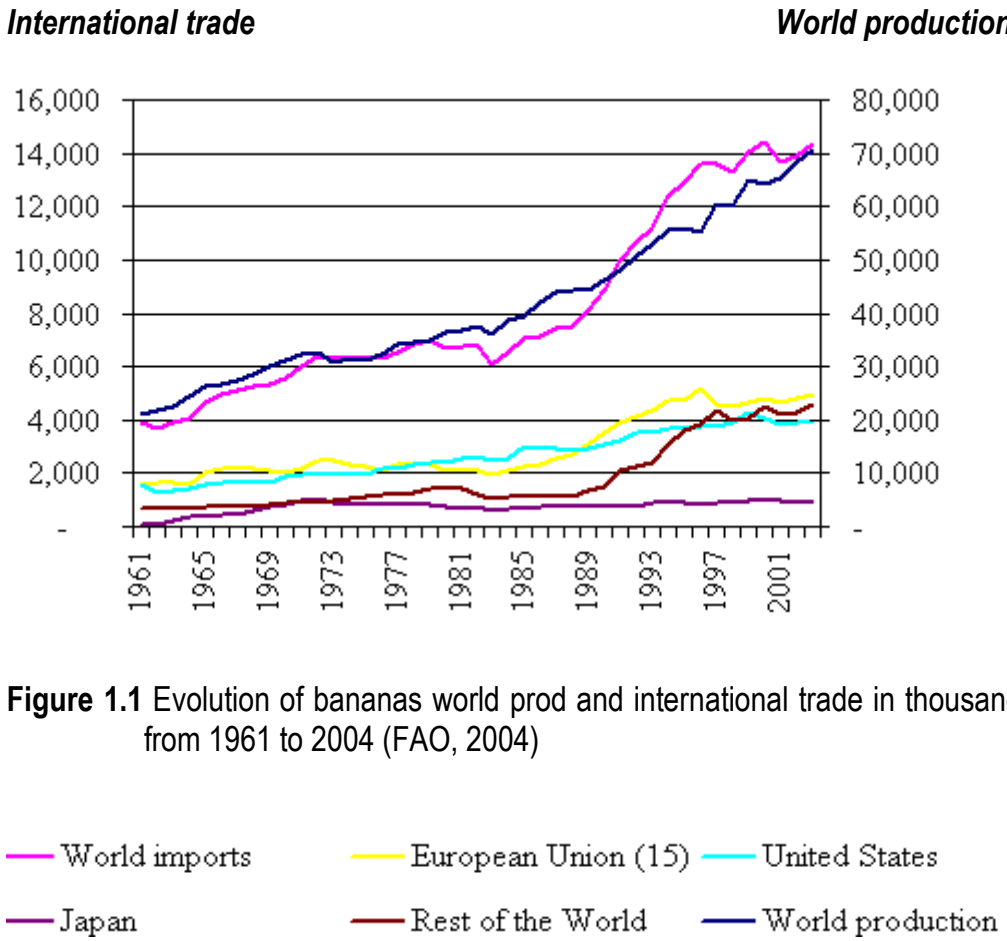


Figure 1.1 Evolution of bananas world prod and international trade in thousands of tons from 1961 to 2004 (FAO, 2004)

Table 1.1 Top 10 banana production countries (Source, FAO, 2007)

Top 10 Countries (% of world production)	
1. India (27%)	6. Indonesia (6%)
2. Ecuador (8%)	7. Costa Rica (3%)
3. Brazil (9%)	8. Mexico (3%)
4. Philippines (9%)	9. Thailand (2%)
5. China (9%)	10. Colombia (2%)
World: 81,263,358 MT	

Table 1.2 Top 10 plantain production countries (Source, FAO, 2007)

Top 10 Countries (% of world production)	
1. Uganda (27%)	6. Peru (5%)
2. Colombia (10%)	7. Côte d'Ivoire (5%)
3. Rwanda (8%)	8. Cameroon (4%)
4. Ghana (9%)	9. Congo (2%)
5. Nigeria (8%)	10. Kenya (2%)
World: 34,444,795 million t	

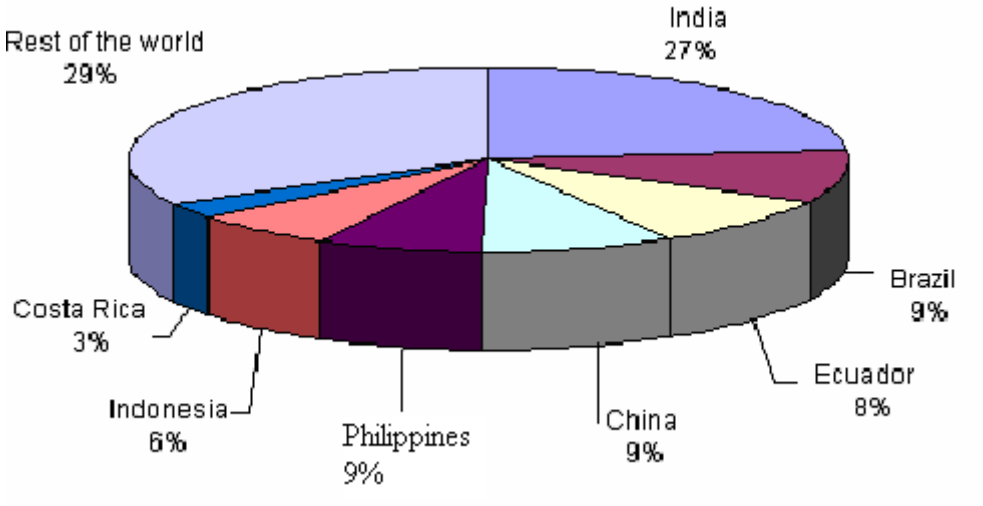


Figure 1.2 Distribution of the world banana production of the 2000-2007 periods (FAO, 2007)

The plants often survive where conflict or natural disasters have adversely affected the production of annual, arable crops (Roux *et al.,* 2008). They require a warm and humid climate with average temperatures between 25and 30°C, rainfall of at least 100 mm per month, and well distributed, dry seasons as short as possible (Swennen, 1990). Organic matter is essential for the development of bananas and plantains. Deep, well-drained alluvial soils are best, but bananas can tolerate a wide variety of soil conditions. Banana plants reproduce asexually by shooting suckers from a subterranean stem. The shoots have a vigorous growth and can produce a ready-for-harvest bunch in less than one year. Suckers continue to emerge from a single mat year after year, making bananas a perennial crop. Fruits can be harvested when about 75% mature which occurs at about 75-80 days after the first hand opens. Harvest may be delayed up to 100-110 days after the first hand opens.

The plant and the fruit feature in several traditional rites especially those associated with fertility. The leaves are used for wrapping and preserving food and cola nuts, and as umbrellas. The pseudo stems, peduncles and peels, when chopped can be used to feed livestock Esiaba, 1987.

The importance of bananas as a food crop in tropical areas cannot be underestimated. In Uganda, for example, annual consumption was 243 kg per capita in 1996, and between 100 and 200 kg in Rwanda, Gabon and Cameroon. In these four countries, bananas account for between 12 and 27 % of the daily calorie intake of their populations. Africa is reported to consume up to 21kg of bananas per capita per year (FAO, 2003). However, the production of the crops has been on a consistent downward trend in recent years. A ten year (1996 - 2005) production figure of the crops showed that land under plantain and banana production increased by 24.6% while yield reduction of 21.8% was recorded during the same period (FAOSTAT, 2006).

In West and Central Africa, about 70 million people derive more than one quarter of their food energy requirement from plantains (Robinson, 1996; Swennen and Ortiz, 1997). The highest consumption rates for bananas were reported to be on the Island of New Guinea and in the Great Lakes region of East Africa, where bananas form a large proportion of

the diet and consumption amounts to 200-250kg/person/year, while in Europe and North America consumption is about 15-16 kg/person/year (Inibap, 1992).

1.1.4 Banana Production in Nigeria

In Nigeria, bananas are intercropped with Cocoyam, yam, cassava, etc. (Njoku, 1990). No real data exist for banana production in Nigeria but plantain production is 2.8 million ton (FAO, 2007) and the country is ranked fourth among the top 10 plantain production countries in the world (Table 1.2). An estimated 70 million people in the region depend on plantains for more than 25% of their carbohydrate and 10% of their calorie intake (Ogungbe, personal communication). The crops are produced mainly by smallholders in the SHF zone, the DS and along the Fadama ecologies. Plantains play an important role in the economy as a staple food and as raw material for the emerging cottage food processing industries. They are consumed both as an energy yielding food and as a dessert.

Being a major staple food crop in lowland Southern Nigeria, the crop is second to cassava in most parts as a cheap source of carbohydrate (Inyang, 1987). The major growing states of the southern region are Akwa Ibom, Edo, Cross River, Imo, Ondo, Ogun, Oyo and Rivers. However, it is also cultivated in Anambra, Benue, Kwara, Lagos and Southern Niger State. Most of the time banana fields are not maintained and crops are kept in fields (Nweke *et al.,* 1988)

Bananas provide employment to several rural dwellers, and local trade in plantain is a lucrative business to several people. Depending on the cultivars, there are used for food (fry, boiled, or chips, roasted or pounded into foofoo). Fruit peels are used for soap manufacture as it is rich in potassium (Ogazi *et al.,* 1986). They are also a raw material for the baking industry, and an alternative source of flour, sugar and flavoring for bread or to prepare beverages such as banana juice. However, farmers' markets depend on the patronage of individuals for their success.

1.2 Pest and Disease Constraints to Banana Production

Pests

Nematodes pose severe production constraints, with losses estimated at about 20% worldwide. Locally, however, losses of 40% or more occur frequently, particularly in areas prone to tropical storms that topple the plants (Tripathi, 2009). The major insect pests are banana weevil (*Cosmopolites sordidus*), stem weevil *(Odoiporus longicollis)*, burrowing nematode (*Radopholus similis*), banana aphid (*Pentalonia nigronervosa*), banana thrips (*Hercinothrips* sp. and *Chaetanaphothrips* sp.), and fruit fly (*Bactrocera kandiensis* and *B. dorsalis*). They are known to attack the plant's underground stems weakening its stability and causing it to break. Losses from nematodes in banana plantations may average 30 to 60% or more where frequent tropical storms topple plants with rotten, nematode-infested root systems.

Diseases

Bananas and plantains are threatened by different diseases ranging from fungi, to bacteria, and viruses. The most economically important fungal diseases include the following:

- The Sigatoka disease complex of banana, caused by three related Ascomycete named *Mycosphaerella fijiensis, Mycosphaerella musicola and Mycosphaerella eumusae* (Stover 1962; Carlier et al, 2000a, b). The main damage by *Mycosphaerella* species is caused by defoliation or the reduction of the photosynthetic capacity of the crop due to necrosis caused by the fungal infection. It is also responsible for the deterioration of the fruit. Originating from Southeast Asia, the diseases have spread to all banana production areas worldwide. The infection process of the three species is the same. Yield losses can reach up to 100% when uncontrolled. Besides the three primary agents of Sigatoka leaf spot disease, numerous additional species of *Mycosphaerella* (or their anamorphs) have been described to occur on *Musa*, but their pathological relevance remains unclear. Some species have been described from diverse hosts, and appear to have hosts other than *Musa*, e.g. *Cercospora apii* (Stewart *et al.,* 1999).

❖ Panama disease (Race 1-4) also known as *Fusarium* wilt (a soil fungus) is caused by the soil-born *F. oxysporum* Schlect. f. sp. *Cubense* (Ploetz, 2000*).* The disease was reported in Australia in 1876 but might have originated from Southeast Asia and now is present in all banana-producing regions except islands in the South Pacific, the Mediterranean, Melanesia, and Somalia. The disease affects the production of a wide range of varieties. The first external symptoms of Panama disease are a yellowing of the oldest leaves or a longitudinal splitting of the lower portion of the outer leaf sheaths on the pseudostem. This is followed by wilt and buckling of leaves at the petiole base. As the disease progresses, younger leaves collapse until the entire canopy consists of dead or dying leaves. A new version of Panama disease, dubbed Tropical Race 4, has been spreading through plantations of race 1 and race 2-susceptible clones in addition to the Cavendish bananas, in Asia and subtropical production areas over recent years, reducing exports and raising the cost of production (Su *et al.,* 1986; Stover and Simmonds, 1987).

❖ Moko disease caused by an aerobic Gram-negative bacterium *Ralstonia solanacearum* (Palleroni, 1984; Yabuuchi *et al.,* 1995; syn. *Pseudomonas solanacearum, Burkholderia solanacearum*). The species is widespread and highly diverse with five races designated by host range (Buddenhagen*,* 1962; Hayward, 1964; He *et al.,* 1983). It can be confused with Panama disease because it causes several of the above symptoms (Stover and Simmonds, 1987). However, unlike Panama disease, Moko causes wilt and chlorosis on plants that are about four months old and younger, and will also discolour internal portions of the fruit. Due to the systemic nature of the pathogen infection, the destruction of the pathogen implies the destruction of the plant. The best means to control the disease is the destruction of all infected plants and the adoption of improved cultural practices to limit the spread of the disease.

❖ Bacterial Wilt (*Xanthomonas* wilt) or bacterial wilt of Abaca was reported to be the most serious disease of bananas in the Davo region of Mindanao in the Philippines (Palo and Calinisan, 1939). Although the appearance of the symptoms is similar to those of Moko, it differs by producing distinctive rusty-brown streaks along the leaf veins of

infected plants (Thwaites *et al.,* 2000). The disease is widely distributed in several banana production fields. Another type called bacterial wilt of Ensete (in comparison with bacterial wilt of Abaca) was reported to affect all growing districts in Ethiopia 60 years ago and was caused by *Xanthomonas musaecearum* now *Xanthomonas campestris musaecearum* (Yirgou and Bradbury, 1968). Necrotic panels develop and a slimy secretion may be apparent. Leaves of younger plants are distorted and wilted. When the disease progresses, older leaves wilt and collapse.

The major viral diseases recorded are the *banana streak virus*, banana bunchy top virus and *banana bract mosaic virus* (Dassanayake & Rathnabharathi, 2002).

❖ *Banana streak virus* was first isolated in 1985 (Lockhart, 1986) and belongs to the Badnavirus group and the virions are bacilliform in shape. It changes the normal appearance of the banana plant. A few banana leaves remain erect at the terminal part of the plant while older leaves drop off. It causes yellow streaks parallel to the veins of the leaves, these turn brown and then black. Internal necrosis can be observed. In Côte d'Ivoire, studies on the AAA Cavendish subgroup cultivar Poyo showed yield losses over two cropping cycles of between 7% on plants with mild symptoms and 90% on plants with severe symptoms (Frison & Sharrock, 1998).

❖ *Banana bunchy top virus* (BBTV) was first reported in Fiji in 1889 although there is controversy about the discovery date. It spread from plant to plant by the aphid vector (*Pentalonia nigronervosa*) and from place to place by people transporting planting materials obtained from infected plants. BBTV is widespread in Southeast Asia, the Philippines, Taiwan, most of the South Pacific islands, and parts of India and Africa. Banana plants that show symptoms rarely bear fruit, and because they are reservoirs of the virus, they must be destroyed. The initial symptoms of *Banana bunchy top virus* consist of dark green streaks in the veins of lower portions of the leaf midrib and the leaf stem (petiole) (Ferreira *et al.,* 2001). BBTV is a destructive pathogen in several banana-cultivating areas of the world (Su *et al.,* 2003).

❖ *Banana bract mosaic virus* belongs to the family Potyviridae; and is a flexuous rod-shaped virus transmitted by aphid vector-*Aphis gossypii*, *Pentalonia nigronervosa*, *Rhopalosipium maidis* (Thomas *et al*., 2000). The disease induces red diamond-shaped patches on the banana flower. Splitting of the base of the pseudostem is also visible. An infected plant produces distorted fruits and bunches.

1.3 *Mycosphaerella* **Leaf Spot Disease of Bananas**

1.3.1 Taxonomic/Nomenclature of the Causal Agent

The three species *M. fijiensis, M. musicola* and *M. eumusae* are the primarily important pathogens of bananas. They are heterothallic Ascomycete species (Stover, 1963; Mourichon & Zapater, 1990; Carlier *et al.,* 2000b). They are characterized by two stages: the perfect and the imperfect stages (Alexoupoulos & Blackwell, 1996). The first stage is characterized by the formation of perithecia, spermogonia and ascospores. The second stage or imperfect stage is characterized by the presence of conidiophores, resulting from asexual reproduction. The fungus *M. eumusae* has *Septoria* as an imperfect stage and *Mycosphaerella* as the perfect stage (Anon., 1995).

The first stage: Perfect stage	**The second stage**: Imperfect stage
Kingdom: Fungi	
Phylum: Ascomycota	Class: Deuteromycetes
Class: Loluco-ascomycota	Subclass: Hyphomycetae
Order: Dothideales	Order: Moniliales
Family: Dothidaceae	family: Dematiaceae
Genus: *Mycosphaerellaceae*	Genus: *Cercospora*

1.3.2 The genus *Mycosphaerella*

The genus *Mycosphaerella* is one of the largest genera of Ascomycete belonging to the *Mycosphaerellaceae* (Capnodiales, Dothideomycetidae) (Schoch *et al.,* 2006). Several thousand species are members of this genus (Crous, 1998; Crous *et al.,* 2001; Aptroot,

2006). The species are complex with a wide range of lifestyles, ranging from saprobes, and plant pathogens to fungal hyperparasites (Hoog *et al*., 1991; Goodwin *et al.,* 2001; Jackson *et al.,* 2004). *Mycosphaerella* species are haploid for the major part of their lifecycle, with a short dikaryotic and diploid phase during sexual reproduction. The dikaryotic phase followed by a diploid phase is restricted to the ascogenous hyphae, which develops into bitunicate asci containing two-celled ascospore within pseudothecia ascomata. Besides the sexual reproduction, some *Mycosphaerella* species also produce haploid conidia in an asexual reproductive cycle. Conidia are produced in closed fruiting bodies or on free conidiophores.

The taxonomy of *Mycosphaerella* is based on morphological characters of both anamorphs and teleomorphs (Crous 1998, Stewart *et al.,* 1999, Crous *et al.,* 2000). Previous studies revealed that the genus was divided in six sections (Barr 1972; Crous *et al.,* 2000) while currently, it is reported to contain close to 3000 species (Aptroot 2006) and at least 7000 additional anamorph species (Crous *et al.,* 2000, 2001, Crous & Braun, 2003; Crous *et al.,* 2007). Recent studies using 28S rDNA sequence data revealed the genus to be polyphyletic, involving at least two families, namely *Mycosphaerellaceae* and *Teratosphaeriaceae* (Schoch *et al.,* 2006, Crous *et al.,* 2007). The genus is also known as monophyletic based on ITS sequence data (Goodwin *et al.,* 2001). To differentiate species, the morphological characters of the conidial apparatus, pigmentation, and nature of the scars and hila are successfully used (Stewart *et al.,* 1999, Crous *et al.,* 2001). However, according to several authors, characters do not have any true evolutionary meaning for the currently accepted anamorph-generic concepts (Stewart *et al.,* 1999; Crous *et al.,* 2007).

Mycosphaerella is characterized by cylindrical asci and mostly uniseriate, thin-walled, often small ascospores. Ascospores are constricted at the septum, with rounded upper ends (Crous *et al.,* 2002).

1.3.3 Host Ranges

Although the list of the hosts of *Mycosphaerella* may be incomplete, following recent references some species are host specific (Goodwin *et al*. 2001; Stukenbrock *et al.,* 2007), while some such as *M. literalis* have multiple hosts (Crous *et al.,* 2004; Jackson *et*

al., 2004). For instance, *M. graminicola* was isolated from wheat (Banke *et al.,* 2004; Stukenbrock *et al.,* 2007). Some species *were* reported to occur on multiple hosts (Jackson *et al.,* 2004). For example, *M. citri*, a major pathogen of *Citrus*, has been isolated from acacia, banana and *Eucalyptus* (Crous *et al.,* 2004). Among the *Mycosphaerella* species attacking *Eucalyptus,* species such as *Teratosphaeria cryptica* (syn. *M. cryptica*) have a broad host range and cause disease on different species across the *Eucalyptus* subgenera *Monocalyptus* and *Symphyomyrtus*; *T. nubilosa* (syn. *M. nubilosa*) shows a narrower host range, infecting only six *Eucalyptus* species within the subgenus *Symphyomyrtus* (Park *et al.,* 2000, Maxwell *et al.,* 2005). Recent analysis revealed that more than 20 species of *Mycosphaerella* or its anamorphs occur on banana, including species from hosts other than banana, such as *Cercospora apii*, *Mycosphaerella citri*, *M. thailandica*, *M. communis*, *M. lateralis* and *Passalora loranthi* (Arzahlou *et al.,* 2007). Some accessions of *M. acuminata* ssp. *banksii*, which is a wild diploid banana that has contributed genetic components to most edible banana clones, are known to be susceptible to at least two of the *Mycosphaerella* species causing leaf spot (Carlier *et al.,* 2000a) and so co-evolution is a strong possibility.

1.3.4 Origin, Distribution, Identification and Symptomatology of Mycosphaerella Leaf Spot Diseases of Bananas

1.3.4.1 Sigatoka or Yellow Sigatoka Disease (SD)

First reported in Java (Zimmermann, 1902), yellow Sigatoka was the most important foliar disease of bananas. From Java, it moved to the Sigatoka Valley in Fiji (Philpott & Knowles, 1913; Massee, 1914) from where it has been given the name. It has been reported that the disease was present in Sri Lanka (1919) and the Philippines (919) (1921). In the 1930, it was found throughout the Central American-Caribbean region. Afterwards it was recorded in Surinam, Guyana and Colombia in South America, Tanzania and Uganda in East Africa, China and West Malaysia. The report from West Africa, India and Brazil was much later in the 1940s (Stover, 1962). It became a serious global epidemic during the next 40 years colonizing major banana production areas. In the 1960s, the disease had spread to all banana-growing countries (Stover, 1962). *M. musicola* (anamorph: *Pseudocercospora musae*) R. Leach ex J.L. is the causal agent of

the disease. The sporodochia developed in the substomatal air chamber and the conidiophores grow through the stomata. Sporodochia are pale to very pale, olivaceous brown, straight or slightly curved, rarely branched, without septa, narrow toward the apex and without conidia scars (Table 1.3). They measure 5-25µm. The conidia are borne terminally and singly on the conidiophores. They are pale to very pale, olivaceous brown, smooth straight or variously curved, sometimes undulate and almost cylindrical to obclavate- cylindrical. The apex is obtuse or sub-obtuse without basal thickened hilum. They usually have 2-5-septates or more and measure 10-80 x 2-6µm (Meredith, 1970; Mülder and Holliday, 1974).

The disease disrupts the photosynthesis pattern of the plant and the physiology of fruit. Yield losses of 25-50% were registered in some places such as Guadeloupe in 1937 (Jones, 2000). Screening trials have shown that some banana wild species in the *Eumusae* section of *Musa* such as *M. schizocarpa*, *M. balbisiana* and *M. acumunata* ssp. *malaccensis, microcarpa*, *samea* and *truncata* are highly resistant to Sigatoka (Cheesman and Wardlaw, 1937; Vakili, 1968). However, Vakili, 1968 reported that some species of *M. acuminata* (*banksii* and ssp. *errans*) are susceptible.

On a vegetative growing banana plant, the most efficient leaves for photosynthesis are the second to fifth, counting down the profile (Robinson, 1996). Lower leaves are less effective due to their age. Although banana plants have the possibility to partially compensate for the loss of photosynthetic assimilation (due to leaf area destruction), it is important that the first five leaves remain free of excessive shade, severe leaf tearing and disease, otherwise assimilation potential is highly reduced (Robinson, 1996). Although studies have proven that Sigatoka has less effect on the vegetative growth of the plant in the tropics (Leach, 1946), it is clearly demonstrated that the effect on the fruit development is very important. There have been a lot of reports and reviews of the reaction of *M. musiscola* in the field (Parham, 1935; Brun, 1962; Simmonds, 1966).

1.3.4.2 Black Sigatoka or Black Leaf Streak Disease (BLSD)

In the early 1960s, a more pathogenic and more aggressive type of disease, black Sigatoka, appeared on the Fiji islands (Rhodes, 1964; Mourichon & Fullerton, 1990). Since then, the disease has spread rapidly to new banana and plantain-growing areas,

being of greatest importance to both commercial banana growers and in countries where banana and plantain are staple crops. The disease reached the Western Hemisphere (Honduras) in 1972 and Africa in 1973. By 1991, it had spread to the Americas, and sub-Saharan Africa, and is now found in most of the humid tropics. It continues to spread to the remaining areas that are free of the disease (Ploetz & Mourichon, 1999). More recently it was reported in Trinidad (Fortune *et al.,* 2005). The distribution in Africa was first reported in Zambia in 1973 where the symptoms resembled black leaf streak disease. However, its identification was not possible when specimens were sent to the UK for positive identification. It was in Gabon in 1978 that an accurate report was done and the location was considered to be the introduction of the disease through planting material from Asia (Frossard, 1980). The disease was reported in Nigeria in 1986 (Wilson & Buddenhagen, 1986). It is still spreading and its widespread distribution in and around the Pacific suggested that it was there long before its discovery in Fiji in 1963 (Meredith, 1970; Stover, 1978).

Black leaf streak is very similar to Sigatoka disease, but more virulent and affects a wider range of varieties and remains a considerable problem in subtropical regions (Carlier *et al.,* 2000a), Balint- Kurti *et al.,* 2001; Jones 2003; Marin *et al.,* 2003). The causal agent of BLSD is the fungi *M. fijiensis* (anamorph: *Pseudocercospora fijiensis* (Stewart *et al.,* 1999). The life cycle of *M. fijiensis* is characterized by two stages: The perfect and the imperfect stages.

The first stage, the perfect stage is characterized by spermogonia that are more abundant on the adaxial surface of the leaf and develop in the substomatal chamber of the stomata. Mature spermogonia contain hyaline rod-shaped spermatia. Perithecia are amphigenous and more frequent on the adaxial surface of the leaf than the abaxial surface. When mature, the perithecia contain the ascospores. The later are biseriate, 1-septate with a slight constriction at the septum, fusoid-clavate with one end larger than the other. They are hyaline, and measure 11.5-15.6 x 2.5-5.0 µm (Meredith and Lawrence, 1969). They constitute the asexual stage of the pathogen.

The second stage or imperfect stage is characterized by the production of conidiophores that can be seen in stage 2 of symptom development. They result from asexual

reproduction. They are single or in small groups, straight or bent and often with several geniculation, pale to medium brown, with 0-5 septates (16.5-62.5 mm, long, 4-7 wide mm). Conidiophores can be wider at the tip with scars. The conidia are single at the apex of the conidiophores. Up to four mature conidia may be attached to a single conidiophore. They are obclavate to cylindro-obclavate, straight or curved hyaline to very pale with 1-10 septates (Table 1.3). They possess a slightly thickened basal hilum and measure 30-132 (long) x 2.5-5 (wide) (Meredith and Lawrence, 1969). All known plantain cultivars collected from West and Central Africa, the tropical Americas and the Philippines, are susceptible to black sigatoka (IITA, 1990).

1.3.4.3 Eumusae Leaf Spot Disease (ELSD)

In the mid-1990s Eumusae leaf spot disease was recognized as a new constituent of the Sigatoka complex of banana diseases (Carlier *et al.,* 2000b; Crous & Mourichon 2002). It is similar to yellow Sigatoka and black streak disease. Presently this disease is known in Southeast Asia and parts of Africa where it affects cultivars that are highly resistant to both yellow and black Sigatoka (Jones 2003; Carlier *et al.,* 2000b). The causal agent named *M. eumusae* (anamorph: *Pseudocercospora eumusae* (Crous & Mourichon, 2002) is related to the two other species. It is characterized by two types of fruiting structures, which are more prevalent in lesions on the upper leaf surface. The first fruiting has a flask-shaped pycnidium, which measures 31-42µm in width at maturity. Conidia associated to the pycnidia are hyaline, filiform and measure 22-41.6µm in length. Pycnidia and conidia are used to identify the asexual stage as Septoria. The pycnidia are smaller and the conidia larger than described for *P. musae* (Punithalingram, 1983). The second fruiting body is a perithecium. The perithecia are globose with short protruding ostiole (42-51µm diameter) and dark brown in colour. Ascopores are two celled as in *M. fijiensis*i and *M. musicola*. The sexual stage is identified as *Mycosphaerella* and is indistinguishable from that of *M. fijiensis* and *M. musicola.* Conidia are 3-5-celled, hyaline, straight or slightly flexuous, with an average size of 40.9µm x 2.1µm (Table 1.3).

The disease has almost the same degree of destruction as Black Sigatoka and the evolution of the disease with its apparent dominance in parts of Asia suggests that it was established before the introduction of black Sigatoka and thus perhaps is able to resist

intrusion by the latter (Jones, 2002). Little is known about the reaction of banana clones to ELSD, but it has been isolated from banana genotypes AA and AAA such as 'Ghuoi Ngu' (AA, syn. 'Sucrier'), 'Kluai Hom Thong' AAA), Pisang Kapas' (AA), 'Anamal' (AAA, syn. 'Gros Michel'), cultivars in the Cavendish subgroup (AAA) and unidentified AAB clones from Nigeria. The disease was found on some varieties known to be resistant to SD and BLSD such as 'Mu Nang' (AA, 'Pisang Lilin'). Currently very little is known about Eumusae leaf disease as far as distribution, genetic diversity and pathogenic variation are concerned.

Table 1.3 Anamorphic characteristics of *Musa* leaf spot fungi

Pathogens	*P. fijiensis*	*P. musicola*	*P. eumusae*
Anamorph	Paracercospora	Pseudocercospora	Pseudocercospora
Sporodochia	Hypophyllous	Amphigenous	Epiphylous
Conidiophores			
Septa	0 to 5	No septate	0 to 3
Colour	Pale brown	Pale to olivaceous brown	Sub-hyaline to pale brown
Shape	Straight or bent	Ampulliform, bottle shape	Subcylindrical
Size	16.5-62.5µm x 4-7µm	5-25 µm	10-25µm x 3-5µm
Conidiogenic loci	Minutely thickened scars	Lacking visible scars	Truncate ends
Conidia			
Colour	Pale green	Pale to olivaceous brown	
Shape	Obclavate to cylindric-obclavate, straight or curved	Smooth, straight or variously curved, cylindrical to obclavate –cylindrical	Subcylindrical, hyaline, straight or slightly flexuous
Septa	1 to 10; commonly 5 to 7	0 to 8; commonly 2 to 5	3 to 8
Size	30-132µm x 2.5-5µm	10-80µm x 2-6µm	(18-)30-50(-65) x (2-)2.5-3 µm
Ascospores			
Shape	Hyaline, two celled, slightly constricted at the septum	Hyaline, two celled, slightly constricted at the septum	Two celled
Size	12.5-16.5µm x 2.5-3.8µm		12.0-16.5 x 3.0-4.5µm

1.3.5 Symptomatology and Spread of the Pathogens

1.3.5.1 Symptoms

The infection process of the three *Mycosphaerella* species is similar, except that symptoms develop faster and are more severe on banana infected with *M. fijiensis* and *M. eumusae* than with *M musicola* strains (Balint-Kurti *et al.,* 2001). Both imperfect and perfect stages play an important role in the infection process. For infection by ascospore to occur, free moisture is required with temperatures (minimum, optimum and maximum) to be 12ºC, 27ºC and 36ºC respectively. The unfurled leaf provides an ideal condition for infection and it is in this microclimate that significant infection occurs. Also, the abaxial surface of the leaves possess several stomata that make this surface suitable for the primary infection (Washington *et al.,* 1998). On infected leaves, depending on the variety of banana and the species of *Mycosphaerella*, symptoms start with light green, narrow specks on the upper surface of the leaves. The specks develop in width running parallel to the leaf veins (Fig. 1.3.A, Appendix 1.1). The leaf then elongates, to become elliptical in shape and turns rusty red or brown. When the leaf is turgids, the lesion is surrounded by a water soaked halo. From there, the lesion turns brown and a young spot is formed. The dark brown center later shrinks and becomes sunken and the halo turns a darker brown. The sunken area and the darker brown halo will form a well-defined ring around the mature spot which will remain as such, even after the leaf is dead. In the presence of a high infection, the necrotic leaf will become whitish grey within a dark border, showing numerous tiny, black, globose fruiting bodies (pseudothecia) containing sac-like structures (asci) filled with ascospores (Fig. 1.3 B). Primary lesions are brown streaks that expand to form large brown spots. This stage of the disease is the most recognisable and can be used to distinguish between the three *Mycosphaerella* leaf spot diseases. In the presence of a low infection, spots are ovoid or elliptical when mature. At this stage, it is possible to distinguish septoria leaf spot from black and yellow sigatoka where the spots are smaller and narrower (Carlier *et al.,* 2000b). With this species, as the disease progresses, spots become grey in the centre but keep a brown border (Fig. 1.3C) (Crous & Mourichon, 2002).

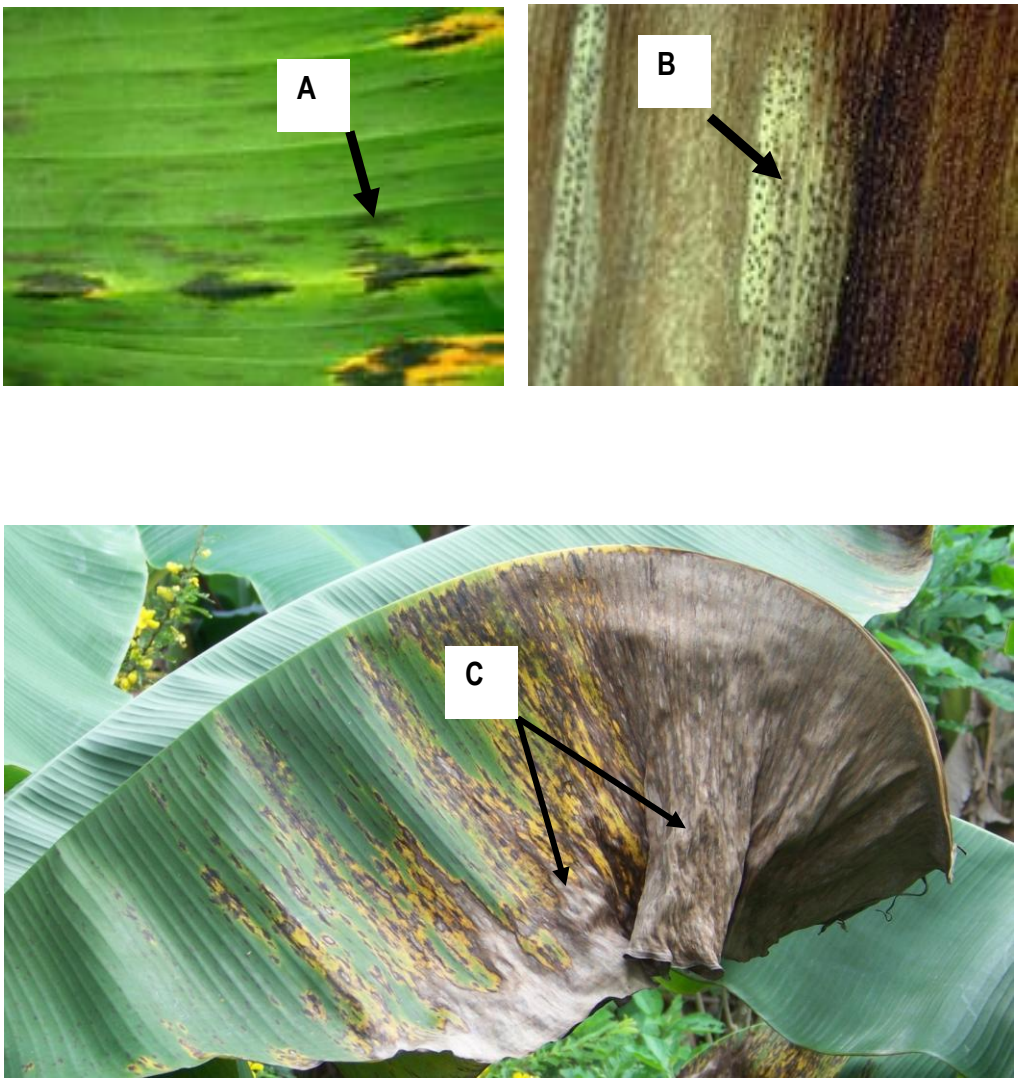


Figure 1.3 Symptoms of leaf spot diseases on banana: symptoms parallel to the leaf vein (A); perithecia containing ascospores (B) and leaf necrosis (C)

Source A, B: Ploetz, 2001

1.3.5.2 Spread of Mycosphaerella *Species on Bananas*

The three ascomycetes produce conidia and ascospores, both of which are infective (Ploetz, 2000). They are produced under high moisture conditions and are dispersed by rain and irrigation water. Ascospores are most responsible for the spread of the disease within plantations due to their small size and greater abundance. Brown & Hovmoller, (2002) reported two forms of disease spread. The first is a rare, unpredictable single-step pathogen invasion involving the transport of spores over long distances. This natural spread, dispersal from an inoculum source can also result from transporting infected plant material and tends to occur more on a continental scale and even on a global scale (Rivas *et al.,* 2004). Ascospores released from pseudothecia of infected plant tissue are carried by air currents over longer distances, than the conidia are dispersed (Meredith, 1970).

The second spread consists of a gradual expansion of the range of the pathogen populations within continents through normal pathogen dispersal processes. Genetic drift through population bottlenecks or founder effects accompanying the spread of the disease may have been the main evolutionary factor in the shaping of the population structure of *M. fijiensis* on a continental scale. Ascospores are reported to be released from lower positioned infected leaves or dead leaves. The disease rapidly spreads on the same plant through vertical air movements or to other plants via horizontal air currents (Craenen, 1998).

Disease dynamic studies based on the rate of disease development in relation to climatic factors have shown that ascospores are the predominant inoculum of black leaf streak disease and that their release is correlated to temperature and rainfall (Jacôme, 2002).

1.4 Socio-Economic Constraints to Banana Production

Farmer fields are generally not-well maintained. Most post-harvest management is informal, with minimal quality preservation. In remote areas, post-harvest losses are higher during the rainy periods. Difficult transport conditions and long travel times reduce product value at the point of sale. Access to credit, information, technical assistance, and innovation systems in participatory form is generally very weak (Lescot & Ganry, 2008).

Production increases slower than the population growth and therefore the demand increases (studies & surveys). In most urban areas in West, Central Africa and East Africa, the general trend is an increase in the prices of plantain. As a consequence, these products are becoming less and less affordable for the less favoured population groups (i.e.: Cameroon, Côte d'Ivoire, Gabon, etc…). As plantain, along with cassava, is considered as a major staple food in Africa, its production remains a major challenge for food security and sovereignty which must be part of the agricultural policies at national and global level.

1.5 Interaction Between the Three *Mycosphaerella* Species

The three species can coexist at leaf, plant and field levels. When *M. fijiensis* first appeared, there was a report that this species might have arisen in Fiji by mutation from *M. musicola* and some reported that *M. fijiensis* may be a physiological strain of *M. musicola*. Other reports stated that the three species arose from similar saprophytic or weakly pathogenic fungi growing on damaged and weakened banana leaf tissue *M. musicola* was reported to replace *M. fijiensis* in several banana production areas.

Ascospore and conidial germ tubes of *M. musicola* grow faster in cooler conditions than those of *M. fijiensis. M. musicola* is normally observed under cooler conditions than black sigatoka (Jones 2000; Marin *et al.,* 2003). However, there is evidence that at altitudes of >1500 m in Colombia and Costa Rica, both pathogens are equally severe (Marin *et al.,* 2003). Yellow sigatoka disease has been largely supplanted by black sigatoka in several banana-producing areas, but remains a significant problem at higher altitudes and cooler temperatures (*Mouliom-Perfoura et al., 1996*). There is no study to adequately demonstrate that *M. musicola* has really disappeared from areas that are dominated by *M. fijiensis* and *M. eumusae*. A recent study in Nigeria has shown that *M. musicola* is not prevalent (Zandjanakou-Tachin *et al*., 2009) although plantain is the preferred crop in that region and is known to be the main host for *M. musicola* (Buddenhagen, 2008, personal communication, 2009).

1.6 Diagnostic Tool

1.6.1 Morphological and Cultural Identification

An extensive description of conventional detection methods applicable to the *Mycosphaerella* species is used and reported by different authors. *M. fijiensis* were differentiated from *M. musicola* and *P. musae* from *Septoria* leaf spot by microscopic means of asexual stages of the fungi on leaf samples and in culture (Meredith and Lawrence, 1969, 1970; Mourichon and Fullerton, 1990*)*. Conventional methods are i) direct microscopic examination of disease material, ii) baiting with plant materials, and iii) isolation of the pathogens from infected plant tissues with water, using general or selective agar media. *M. eumusae* can be differentiated from *M. fijiensis* and *M. musicola* on morphological grounds (Crous and Mourichon, 2002).

All the three species have black stroma-like structures and appear similar. *Mycosphaerella* species are traditionally identified based merely on morphology, which is time consuming and requires taxonomical expertise. Although the presence of a specific *Mycosphaerella* species can be visually based on disease symptoms (Crous, 1998), the presence of several different species on infected leaves usually complicates the process and use of diagnostic tools. Also, ascospore discharge, which is often the successful approach of isolating the *Mycosphaerella* species from necrotic leaf tissue, is usually confounded by the absence of mature perithecia. Identification based on cultural methods is also an option, although it is more complicated by the similarity of the color of the mycelia. The sporulation of the three species is often difficult and growth on cultural media is very slow. On potato dextrose agar (PDA), single inoculated conidia form a colony of 1 cm after a minimum of 32 days of incubation at 26ºC (Meredith, 1970; Zapater *et al.,* 2008). Furthermore, even when obtained in culture, *M. fijiensis*, *M*. *musicola* and *M. eumusae* are not easily distinguishable (Pons, 1990; Carlier *et al.,* 2000a) due to the limited morphological and physiological characteristics that unequivocally differentiate these species (Goodwin *et al.,* 2001).

The inability of traditional methods to rapidly identify plant pathogenic micro-organisms on a routine basis has led to the development of culture-independent, highly specific molecular detection and identification techniques that can aid in disease management. The separation of the three species of *Mycosphaerella* for morphological observation was not accepted by all researchers and was questioned by different authors (Graham, 1969; Wardlaw, 1972). Therefore identification based on molecular markers such as Single sequence repeats (SSR), Amplified fragment length polymorphism (AFLP), Restriction fragment length polymorphism (RFLP), etc., were developed.

1.6.2 Molecular Methods

Although phenotypic characteristic can be used to study the genetic diversity of an organism, different molecular markers are used. More recently, DNA fingerprints, generated with the polymerase chain reaction (PCR), have been used to identify genetic diversity among strains of closely related plant pathogenic organisms (Alexander *et al.,* 2004; Cooke *et al*., 2007).

Contemporary approaches to identify, diagnose and study *Mycosphaerella* species as well as other plant pathogens have moved overwhelmingly towards those that exploit nucleic acid sequence differences between species. Prior to the introduction of PCR, nucleic acid-based diagnostics usually involved the use of hybridization probes, and they are still in use as alternatives to PCR for the identification of plant pathogens such as *Mycosphaerella* species. PCR is a method for synthesizing (amplifying) millions of copies of specific DNA sequences identified by two short oligonucleotides (primers) using a thermostable enzyme (Taq DNA polymerase) and repeated cycles of denaturation, polymerization and elongation at different temperatures (Mullis & Faloona, 1987; Ward *et al.,* 2004). The whole process is repeated several times, so that after 30-35 cycles (approximately two hours) millions of copies of the sequence are produced. As the primer is specific, the amplification of an appropriately sized fragment can be used as an indication of the presence of a specific organism. The presence of the amplified DNA is usually checked by agarose gel electrophoresis, but alternative detection formats include using colorimetric (Mutasa *et al.,* 1996) or fluorometric assays (Fraaije *et al.,* 1999). PCR

is known as the most important and sensitive technique presently available for detecting plant pathogens (Ward *et al.,* 2004) and in particular for *Mycosphaerella* spp. (Johanson *et al*., 1994; Johanson & Jeger, 1993; Lievens & Thomma, 2005; Waalwijk *et al.,* 2004).

A current invention provides Internal Transcribed Spacer (ITS) DNA sequences that show variability between different fungal pathotypes. Such DNA sequences are useful in the method as they are used to derive primers for use in PCR-based diagnostic assays. These primers generate unique fragments in PCR reactions in which the DNA template is provided by specific fungal pathotypes and is thus used to identify the presence or absence of specific pathotypes in host plant material before the onset of disease symptoms. In particular, it provides the ITS DNA sequences from an unknown species of *Mycosphaerella*, as well as ITS-derived diagnostic primers for the detection of this species of *Mycosphaerella* and for differentiating it from other *Mycosphaerella* species such as *Mycosphaerella fijiensis* and *Mycosphaerella musicola*.

To differentiate *M. fijiensis* from *M. musicola*, PCR was used (Johanson & Jeger, 1993; Johanson *et al.,* 1994). For instance, during an outbreak of the disease in several plantations in Australia in 2001, PCR assay was used to detect and eradicate the disease (Henderson *et al.,* 2003). To ensure the robustness of PCR diagnostics and to facilitate the development of new diagnostic assays for *Mycosphaerella* spp. in Australia, it has been suggested that the region incorporating the ITS1, 5.8S ribosomal gene and ITS2 will be cloned and sequence information will be required (Henderson *et al.,* 2003).

Sequence analysis of the ITS region of nuclear ribosomal DNA was used to confirm the classification of the two species as separate (Johanson & Jeger, 1993; Johanson *et al.,* 1994). Another approach, based on the integration of DNA phylogeny data with morphological traits, facilitates the identification of species. ITS sequences and housekeeping genes like the actin, translation elongation factor 1-α, β-tubulin and histone H3 genes have frequently been applied in studying phylogenetic relationships among *Mycosphaerella* species and their anamorphs (Crous *et al.,* 2001, 2004, 2006, Banke *et al*. 2004, Verkley & Starink-Willense 2004, Feau *et al.,* 2006).

A traditional technique such as restriction fragment length polymorphism (RFLP) was used to study the divergence of *M. fijiensis* and *M. musicola* in 1994 (Carlier *et al.,* 1994).

To identify and analyze the genetic diversity in *Mycosphaerella* spp., some authors later used RFLP (Rivas *et al.,* 2004; Hayden *et al.,* 2005) and single sequence repeats (Neu *et al.,* 1999; Molina *et al.,* 2001; Perea *et al.,* 2005). An alternative and perhaps more powerful approach to distinguish between related species is to employ single nucleotide polymorphism (SNP) analyses, since closely related species may differ in only a single base pair for a target gene (Cooke *et al.,* 2000; Lievens *et al.,* 2006).

A SNP is a unique nucleotide base difference between two DNA sequences. SNPs located in known genes provide a fast alternative to analyze the fate of agronomically important alleles in breeding populations, thus providing functional markers (Fusari *et al.,* 2008). Currently, the ribosomal RNA (rRNA) gene and ITS regions are among the most commonly used genomic targets for PCR products and SNPs that differentiate closely related species and strains. Such SNPs were employed previously to detect *Mycosphaerella* species (Carlier *et al.,* 2000b; Goodwin *et al.,* 2001; Crous *et al.,* 2004; 2006; Hunter *et al.,* 2006). The ITS regions provide attractive targets because they are highly stable, can be easily amplified and sequenced with universal primers, occur in multiple copies, and possess conserved as well as variable sequences (White *et al.,* 1990).

1.7 Economic Impact of *Mycosphaerella* Leaf Spot Diseases

Bananas are produced by small-scale farmers for local consumption and in some countries for export. They are major sources of carbohydrate in the diet of the population. In Africa, the plant is used for different purposes. In East Africa, bananas contribute considerably to food security. *Mycosphaerella* species are responsible for major economic losses of bananas (Ploetz 2000; Crous & Mourichon 2002). Yield losses due to their attack can be as much as 20-50% (Stover, 1983; Pasberg-Gauhl, 1989).

In Brazil, the second largest producer of bananas in the world, the complex Sigatoka diseases can cause yield losses of up to 100% on susceptible varieties from the Cavendish (*Musa* cv. AAA) and Prata (*Musa* cv. AAB) groups (Cordeiro & Pires, 2002). Yield loss of up to 50% and more were recorded making the disease a major threat to the

farm economy in West and Central Africa (Jeger *et al.,* 1995). In Nigeria, black sigatoka disease outbreak was recorded in 1986 (Faturoti et al., 2007).

The complex Sigatoka diseases endangered the food security of small scale-farmers as well as substantially increasing the market price of this fruit (Meredith, 1970; Stover, 1972). Uncontrolled, the diseases are very destructive. They are also capable of inducing physiological changes, resulting in premature ripening of the fruits and decreasing the number of fruit per bunch with lower fruit weight (Jones, 2000, Marin *et al.,* 2003). Therefore, in areas where the diseases are present, *M. fijiensis* can cause yield losses of up to 33 to 76 % (Romero & Sutton 1997: Jones, 2003). In Africa, owing to a lack of numerous commercial plantations, it is not always easy to determine accurately the percentage of losses. In West Africa of 33-50% from yield losses due to black sigatoka were reported (Mobambo *et al.,* 1993). In Nigeria, yield losses are estimated to be 20% in well-maintained plantations and up to 80% in non-maintained fields (Ikotun, 1987). *M. fijiensis* is classified as high risk as the pathogen has a short generation time, sporulates abundantly, and has a sexual cycle that enabled the development of new resistance phenotypes (Ploetz, 2000).

According to Cordeiro (1990), the cost of disease-control using five applications per year of systemic fungicides plus mineral oil accounted for 9% of the total production cost estimated at US$ 1350 ha/year. This cost increased in 2002 because of the increased requirements of seven applications per year making the cost to be about 10% of total production costs (Codeira & Pires de Matos, 2002). In Cuba for instance, the cost per hectare in 'Cavendish' plantations varied between US $134 and US $801 (Perez *et al.,* 2002).

1.8 Disease Management

1.8.1 Chemical Control

Generally, the complex Sigatoka diseases are controlled in large plantations by frequent applications of fungicide. Generally, more application is required, increasing the impact on the environment and health of banana workers. Bordeaux mixture was used in the 1930s and has been replaced by several other generations of protectants and followed

by systemic fungicides (Ploetz, 2001). Currently, a sterol, biosynthesis inhibitor, tridemorph, and different sterol demethylation inhibitors- most importantly propiconazole-- and the methoxyacrylate, azoxystrobin, are the fungicides most commonly used. The cost of chemicals is very high and unaffordable by small-scale farmers. Although fungicides improved over the years, the pathogens develop resistance. In recent years researchers have been working intensively to understand the disease and to find new agrochemicals because the fungus quickly develops resistance to new fungicides. It is worth mentioning that fungicides are used in combination or alteration with broad-spectrum, protectant fungicide such as dithiocarbamates and chlorothalonil. These fungicides are also mixed with petroleum-based spray oils that can retard the development of the pathogens. Sometimes they are mixed with water to give a better and superior disease control.

1.8.2 Biological and Integrated Pest Management Control

Due to the restriction of some elaborate resistance varieties to be freely exchanged (such as *Banana streak virus* (BSV) dissemination), a panel of World Bank experts suggested the development of biological control options to complement disease resistance (Persley & George, 1999). With an increasing global demand for organically grown crops, there is little research on how to manage the disease using biological control. The use of quitinolytic bacterium *Serratia marcescens* and effective microorganisms (EM) to evaluate black sigatoka in Costa Rica have resulted in some success. Also, there is evidence that epiphytic mycelia present on leaf-surface fungi have an inhibition effect on the germination of *M. musicola* (Meredith, 1970).

The most common practices undertaken to control the spread of the disease is to reduce the inoculum levels in the field by removing the affected leaves. Good drainage systems that can take the groundwater out of the plantation with sufficient spacing also help to reduce the relative humidity inside the crop.

Farmers have successfully used Integrated Pest Management (IPM) to combat Black Sigatoka in some regions such as the Dominican Republic where bananas are planted in dry areas (Lopez, 1999). This has allowed the country to expand its production of organic

bananas from 2000 t in 1993 to over 60 000 t in 2000, and to become the world's number one exporter of organic bananas. The IPM method also involves the use of fertilizers. Plastic sleeves, or diapers, that separate the hands on each bunch during the growing period and reduce the amount of scarred fruit and rejects may also inhibit somewhat the spread of black sigatoka.

The need to comply with the phytosanitary and quality control requirements of import markets, as well as with bilateral and multilateral environmental agreements, has driven innovations on pest and disease control. Quality assurance schemes, specifically those related to low pesticide content, are increasingly being demanded by consumers in the major importing countries. Therefore, low pesticide pest control techniques, such as IPM that uses biological control, pest eradication and the prevention of pest proliferation gained importance during the 1990s.

Another IPM method is agroecological intensification which replaces chemical inputs by ecological processes. It is mainly based on the introduction of more diversity in the agrosystem to limit pathogen development, and to improve soil structure and biological fertility, and nutrient availability. Moreover, it has been suggested that using rotation, associated plants, cover plants, associated crops, and spatial organization of the culture are suitable techniques for controlling the disease (Côte *et al.,* 2008).

1.9 Breeding for Resistant Varieties

The use of genetically resistant cultivars appears to be the most viable long-term solution for controlling Black Sigatoka (Pereira et al. 1999). Developing new *Musa* cultivars presents special difficulties for plant breeding because of high levels of male and female infertility, polyploidy, and different genomic combinations in germplasm, and the need to retain those features in the final progeny.

Breeding for varieties resistant to leaf spot diseases started at the Fundation de Investigacion Agricola (FHIA) in La Lima in 1984, taking over from the Chiquita Brand in 1959. FHIA has developed different dessert, plantain and cooking hybrids that have been tested by the International *Musa* Testing Programme (IMTP) of the International Network for the Improvement of Banana and Plantain (INIBAP). Several varieties provided by

INIBAP through IMTP proved to be resistant to black leaf streak disease in Southern Asia (Molina & Fabregar, 2002). FHIA series were listed as the most resistant to this disease in that region but have not been generally accepted by the consumer.

The International Institute of Tropical Agriculture (IITA) breeding program at Onne station, Nigeria, has crossed Black Sigatoka resistant bananas with landrace plantain (AAB) to produce Tropical *Musa* Plantain hybrid (TMPx) and Tropical *Musa* Banana hybrid (TMBx). The most common crosses used by IITA involve the use of black sigatoka susceptible triploid (AAB), French plantain 'Obino l'Ewai' and 'Bobby Tannap' with the female parent crossed with wild diploid banana 'Calcutta 4' and 'Pisang Lilin' as the male parent (Vuylsteke *et al.,* 1993).

However, to ensure that the resistance being utilized in the breeding program is of a durable nature, it is necessary to have an understanding of: (1) the genetic nature of the resistance in parent lines, in particular, whether it is controlled by a single major gene (specific or vertical resistance), or several minor genes with additive effects (general or horizontal resistance); and (2) the range of pathogenic diversity in populations of the pathogen, and its capacity for change.

1.10 Pathogenic Variability

Some resistant banana varieties have become susceptible to some *M. fijiensis* isolates in certain regions after several seasons of widespread commercial fungicide use. For instance, *Acuminata* spp. *burmannicoides* accession 'Calcutta 4', Yangambi Km 5' (AAA, Syn. 'Ibota Bota') produced a susceptible reaction when inoculated with isolates from the Pacific Islands and Papua New Guinea (Fullerton & Olsen, 1995; Mouliom Perfoura, 1999). Similar situations were reported with FHIA 23 that displayed a resistant reaction in Nigeria, but became susceptible when introduced in Côte d'Ivoire (Kobenan *et al.,* 2004). Jacôme and Schuh, 1993) have reported that six isolates of *M. fijiensis* from Honduras have induced different levels of disease severity on Grand Nain AAA, Cavendish subgroup. Moreover, Romero and Sutton, (1997b) found that *M. fijiensis* varies in its aggressiveness when isolates from different geographical zones were used.

1.11 Host-Pathogen Interaction

When a plant is attacked by a pathogenic infection, immediately, local defense reactions and delayed systemic responses are activated in order to counteract the pathogen's attack. Among the early local responses, the hypersensitive response (HR) leads to a local programmed cell death in order to deprive the pathogens of their nutrition base (Pontier *et al*., 1998). This defense strategy is based on the pathogen's recognition and cell-to-cell communication in the tissue adjacent to the site of infection. Later on, the plant can develop systemic acquired resistance (SAR) leading to resistance throughout the whole plant in an unspecific manner towards a broad spectrum of pathogens. In the case of Mycosphaerella species, fungus enters leaf through stomata of susceptible cultivars and establishes a biotrophic relationship. Hyphae move to adjacent cells or adjacent stomata 3-4 weeks later necrotic lesions appear. The fungus becomes saprophytic. This demonstrates that signal perception in initial pathogen recognition and signal transduction to initiate further defense responses is essential for plants to counteract phytopathogens (Nürnberger & Scheel, 2001).

1.12 Genetic Diversity Among *Mycosphaerella* Leaf Spot

Genetic diversity refers to any variation in the nucleotides, genes, chromosomes, or whole genomes of organisms. Genetic diversity at its most elementary level is represented by differences in the sequences of nucleotides (adenine, cytosine, guanine, and thymine) that form the DNA (deoxyribonucleic acid) within the cells of the organism. Therefore, knowledge of the genetic population structure and the evolution of the pathogen is an important issue in breeding and managing disease resistance. This knowledge is useful as it helps to identify potential sources of resistance, which are present in the location where the diversity of the pathogens and the host are high. It will also help to ensure that the diversity of pathogens used for screening for resistance is representative of the one in the regions where resistant hosts are intended to be used (Carlier *et al.,* 2002).

Molecular markers have been used to investigate the genetic composition of fungal population (Groppe & Boller, 1997; Bucheli *et al.,* 2001). Restriction fragment length polymorphism (RFLP) was used to study and characterize the population of *M. fijiensis* and *M. musicola* at regional and global levels (Carlier *et al.,* 1994, 1996; Müller *et al.,* 1997). From this study, it was reported that *M. fijiensis* can maintain a high genetic diversity. Also, the population structure of *M. musicola* in Australia, examined using single copy RFLP, displayed moderate levels of gene diversity (Hayden *et al.,* 2003; Hayden *et al.,* 2005). Very few studies were done to determine the genetic diversity of *M. eumusae*, the new-discovered pathogen.

Mycosphaerella species have been reported to express a high genetic variability due to sexual reproduction and a short life cycle, leading to numerous generations per year and a high level of genetic recombination (Manzo-Sanchez *et al*., 2005). Moreover, *Mycosphaerella fijiensis* isolates collected from Nigeria revealed that genetic variability does exist on all levels, i.e., within and among lesions, plants, cultivars and locations (Müller *et al*., 1997).

CHAPTER 2.0: MATERIALS AND METHODS

2.1 Surveys for Incidence, Severity and Prevalence of Sigatoka Disease in Nigeria

2.1.1. Surveys

Data on the distribution of Sigatoka diseases were collected by surveying fields in four geographical zones in Nigeria, between 18 and 28 November 2004, 24-31 August 2005, 3-8 May, 27 June - 2 July 2006. A total of 22 States were visited and 100 fields were sampled to represent the banana production belt. The four sampling regions were:

South-South or SS (Akwa Ibom, Bayelsa, Delta, Edo, River and Cross River states)

South-East or SE (Abia, Anambra, Ebonyl, Enugu and Imo states)

South-West or SW (Ekiti, Ondo, Osun and Oyo states)

North-Center or NC (Abuja, Benue, Kwara, Kogi, Nassarawa, Niger, and Plateau states)

The States in SS and SE regions are in the HF agro-ecological zone except for Enugu which is in the DS zone, all States in SW and NC regions are in the DS (Fig. 2.1).

Generally, bananas are grown as a subsistence crop in the rear gardens of households either alone or intercropped. Fields were chosen randomly and fields without at least ten plants were not surveyed. Generally, in Nigeria, bananas are grown as a sustenance crop in the rear gardens of households either alone or intercropping. Fields were chosen randomly and fields that were not represented by at least ten plants were not surveyed. All banana fields along routes with at least ten kilometers distance were visited for the evaluation of the disease.

The HF lies within latitudes 4°25 and 7°3 N and longitudes 2°4’ and 9°1’ E and is characterized by a bimodal rainfall distribution, averaging from 1500 to 2000 mm annually, and maximum temperatures varying from 27·4°C to 31·9°C. The DS lies within latitudes 6°8’ and 9°3’ N, and longitudes 2°4’ and 12°2’ E and has a bimodal rainfall distribution, averaging from 1300 mm to 1500 mm per year with maximum temperatures varying from 25 to 35°C.

From each field, latitude, longitude and elevation were recorded using a hand-held Global Positioning System device named Magellan GPS (eTrex GPS, Garmin Corporation, Olathe, KS) and the map was elaborated.

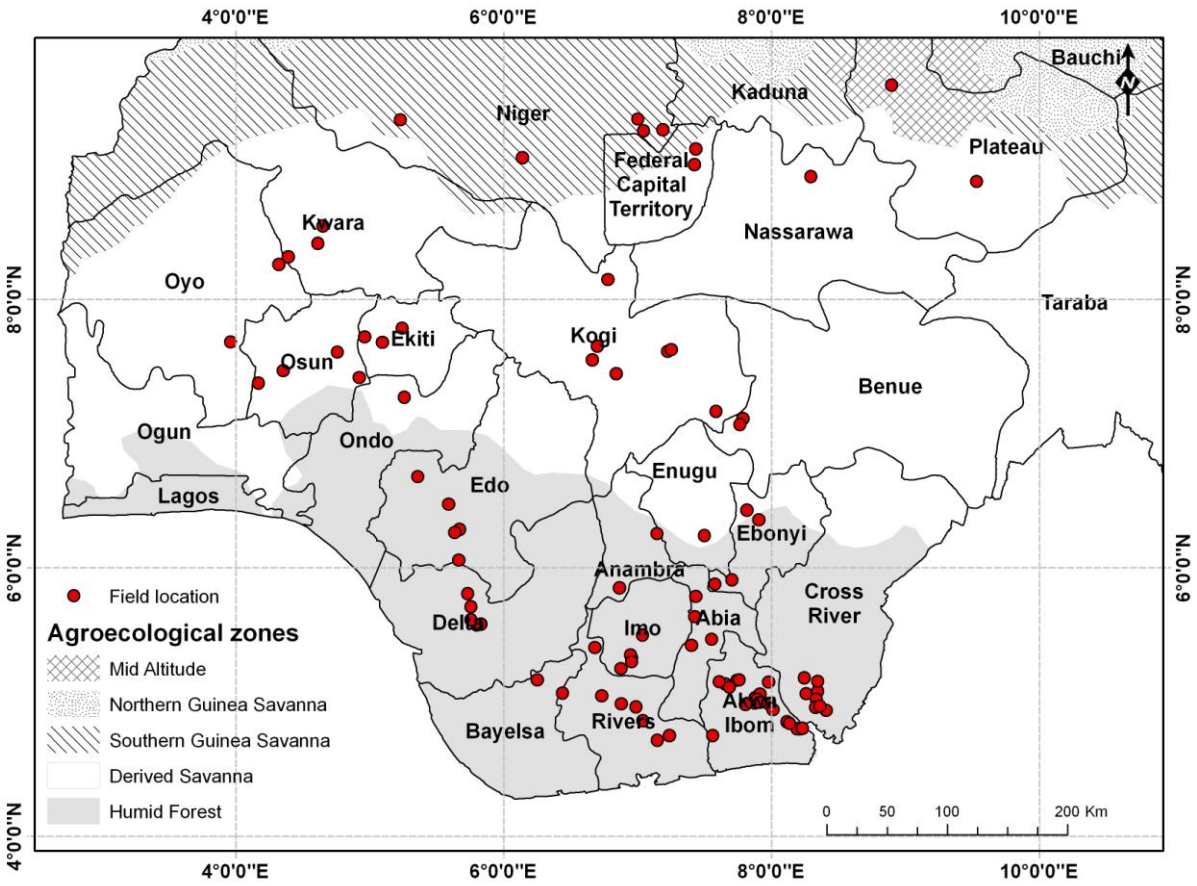


Figure 2.1 Map of Nigeria showing locations visited

2.1.2 Field Evaluation for Sigatoka Severity and Prevalence

To evaluate the disease severity in each field, 20 plants were assessed and from each plant represented by two leaves the upper and lower parts of the plant were randomly scored. Leaves that were totally necrotic or hanging were not considered. The scoring of the disease severity was determined by recording the leaf area infected using the seven-grade scale (0-6) modified from Stover & Dickson (1970) (Table 2.1 & Fig 2.2). Then disease severity was converted to a percentage using the mid-point method (Campbell & Madden, 1990).

Table 2.1 Disease assessment key (0-6 scale) for estimating the infection caused by *Mycosphaerella* species on a single plant

Rating scale	Description
0	No visible symptoms of the disease
1	Less than 1% (only streaks or up to ten spot of the leaf with disease symptoms
2	1 to 5% of the leaf area with symptoms
3	6 to 15% of the leaf area with symptoms
4	16 to 33% of the leaf area with symptoms
5	34 to 50% of the leaf area with symptoms
6	51 to 100% of the leaf area with symptoms

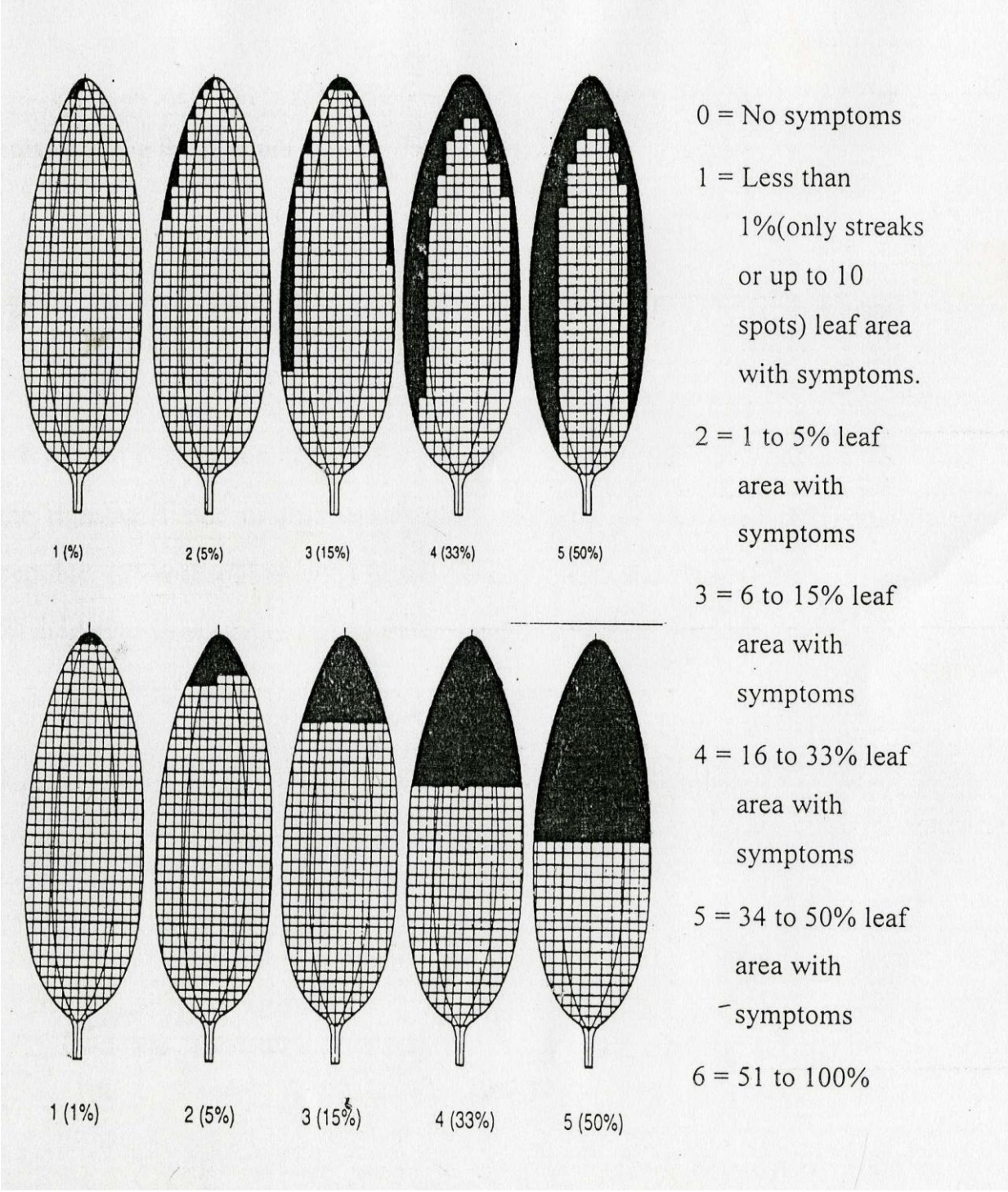


Figure 2.2 Assessment key for estimating the percentage of leaf area infected (LAI), spotted disease grades 1 to 5 on banana leaf areas, proposed and adopted by Stover 1971; Gauhl et al. 1993

2.2. Sample Collection

2.2.1 Fungal Isolation

During the different field surveys (2004, 2005 and 2006), infected plant materials were collected from naturally infected varieties of banana and plantain in four regions of the banana production belt of Nigeria. Leaves from each location were packed together in paper bags and labeled for laboratory isolation.

In the laboratory, a single ascospore of the fungus was isolated from the infected leaf samples, using the method described by Stover (1976). Portions of collected leaves measuring 20x10 cm bearing pseudothecia were cut and incubated in plastic bags with moist towels to allow the maturation of perithecia of the fungus. After 24 hours, the leaves were surface sterilized with 0.5% sodium hypochlorite. 2x5 cm of leaf pieces were cut, stapled to filter papers, immersed in tap water for five minutes, then transferred to the lids of Petri dishes and left to discharge ascospores over 1% water agar (Plates 2.1 a, b). The plates were incubated for one day. In the presence of mature perithecia, ascospore discharge could start after one hour. Under the microscope, a single ascospore was transferred to new media (PDA or V8 juice media) (Appendice 2.2) and incubated under UV-light 24/24h at 25-30°C until the colony developed fully. A total of 96 isolates were collected from the four geographical zones. The list of the 96 isolates collected from all agroecological zones visited and their location coordinates are in the Appendix 2.3

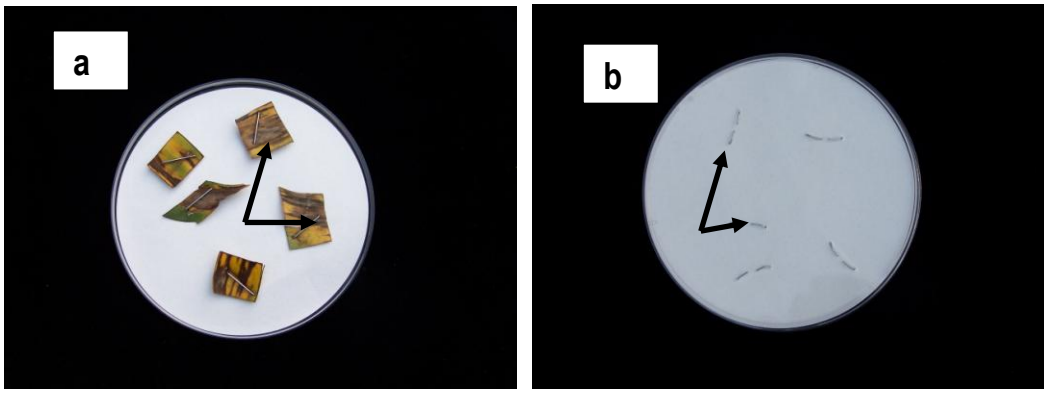


Plate 2.1 Ascospore discharged technique, leaves pieces with mature perithecia stapled on filter paper (a) and place in the lid of Petri dish. The lid is turned over the plate, containing 1% water agar (b)

2.2.2 Fungal Identification: Morphological and Molecular Detection

2.2.2.1 Morphological Identification

Isolates were identified morphologically by recording the color of the mycelia, the form, size and number of septa of each conidium (Stover, 1962).

2.2.2.2 Molecular Detection

2.2.2.2.1 DNA Extraction, Gel Electrophoresis and Quantification

The list of the A total of 96 isolates were obtained and used (Table 2.2). Fungal material from each isolate was scraped from 30-day old cultures, dried in an oven at 50°C for one hour, and DNA was extracted using the Promega Wizard@Genomic DNA purification kit (Promega, Madison, Wisconsin, USA) with slight modification (Appendix 2.1; 2.2)

2.2.2.2.2 PCR-Amplification and Sequencing of Ribosomal DNA

A portion of the ribosomal DNA including the internal transcribed spacer (ITS) I and II was amplified in the 96 isolates using RedTaq reagent (Sigma, St Louis, USA) and three PCR primers (R635, MF137 and MM137) (Table 2.2) designed on the ITS1 and the 25S subunit (Johanson & Jeger, 1993). PCR reactions were performed on a PTC-200 thermocycler (MJ Research, USA) programmed for an initial denaturation of 4 minutes at 94°C, followed by a touchdown program from 60°C to 51°C with a -1°C increment per cycle, followed by 30 cycles (1 min at 94°C, 45 sec at 51°C, 1 min 30 sec at 72°C), and a final extension of 5 minutes at 72°C. The PCR products from each isolate were ethanol-precipitated and sequenced twice (in forward and reverse directions) using BigDye version 3.1 on a 3100 Sequencer (Applied Biosystems, Forster City, USA). Individual sequences were quality-trimmed based on the phred 20 cut off scores and assembled for each isolate.

2.2.2.2.3 Sequence Alignment

The alignment of sequences was performed using the Clustal-W from the European Molecular Biology Laboratory – European Bioinformatics Institute (www.ebi.ac.uk), manually

edited with the BioEdit program (www.mbio.ncsu.edu/BioEdit/bioedit.html) and subjected to the scoring of the absence/presence of SNPs and Indels in the sequence alignment. The SNPs were scored in

the partial sequence of ITS1, the complete sequence of the 5.8S ribosomal RNA gene, the complete sequence of ITS2, and the partial sequence of the 25S ribosomal RNA gene as bracketed by the PCR primers (Johanson & Jeger, 1993).

2.2.2.2.4 Sequence Comparisons and Identification of Mycosphaerella *Species*

Registered ribosomal DNA sequences with an intercontinental scale distribution and covering the PCR-amplified region described above were gathered from GenBank and included in the sequence analysis as control checks for *M. fijiensis* (acc# DQ865944, AY923765, AY923764, AY923763, AY266151, AY923762, AY266152), *M. musicola* (acc# DQ019356, AY923756, AY646497, AY646494, AY646480, AY646475, AY646473, AY646465, AY646493), *M. eumusae* (acc# DQ019338, AY923760, AY923757, DQ019337, DQ019336, AY923758), and *M. musae* (DQ019353, DQ019354). The sequences of our isolates were aligned on the sequences of the controls and only species-specific sequence variations common to worldwide control accessions were used to identify the *Mycosphaerella* species in our collection. Additional tests of identification and confirmation of the *Mycosphaerella* species were conducted with the PCR primers designed on actin and β-tubulin gene sequences (Table 2.2). In this case, PCR conditions were as described by Arzanlou *et al*. (2007), except that 30 ng of genomic DNA were used instead of 1 ng.

2.3 Statistical Analyses

Data for all isolates and loci were compiled into a binary table that was used for clustering individual isolates and for population statistics. The Arlequin program version 3.11 (Excoffier *et al.,* 2005) was used to assess the population differentiation through the analysis of molecular variance (AMOVA) and F_{ST} indices (Wright, 1969). F_{ST} is a measure of population

differentiation based on genetic polymorphism data, such Single nucleotide polymorphism (SNP) or Microsatellites (SSR).

The significance of the F_{ST} was determined by permutation tests described by Excoffier *et al*., (1992). The genetic differentiation between populations described by F_{ST} was estimated using the estimator θ (Weir & Cockerham, 1984). To determine the genetic relationships and clustering among the *Mycosphaerella* isolates, a distance matrix was generated from the sequence data and subsequently grouped by Unweighted Pair Group MA method using the NTSYS program version 2.02j (Rohlf, 1998). The strength of the clustering was assessed by bootstrap analysis with 1000 iterations using the Winboot program (Felsenstein, 1985).

Table 2.2 Primers used for PCR

Primers & Probes	Nucleotide Sequence 5' to 3'	Authors
MF137	GGCGCCCCCGGAGGCCGTCTA	Johanson & Jeger, 1993
MM137	GCGGCCCCCGGAGGTCTCCTT	Johanson & Jeger, 1993
R635	GGTCCGTGTTTCAAGACGG	Johanson & Jeger, 1993

2.4 Pathogen Variability

2.4.1 Inoculum Preparation, Inoculation Technique and Disease Assessment

Inoculums were prepared by scraping and crushing 30-day-old fungal material of each isolate in a mortar containing distilled water. The suspension was passed through two layers of cheesecloth to remove larger culture fragment and hyphae. Prior to inoculation a drop of 1% Triton X-100' was added to the spore suspension, then sterile water was added to adjust the concentration to $1x10^6$ mycelia bits/ml, determined using the haemocytometer.

Three well-known varieties selected because of their reaction to *Mycosphaerella* spp. in previous assays (Twizeyimana *et al.,* 2007): Agbagba (ABB False Horn plantain, susceptible and local variety), Dwarf Valery (AAA dessert banana, susceptible, worldwide variety) and Calcutta-4 (AA, resistant improved variety), were collected from IITA's banana farm or screen-house, when available. Mycelia suspension obtained from pure cultures of the isolated pathogens was inoculated by putting 40µl of the suspension at four points on the abaxial side of the leaf sections measuring 3x4 cm cut from each variety and kept on 1% technical agar amended with 5 ppm gibberellic acid, following the method developed in Twizeyimana *et al.,* (2007). The experimental unit consisted of four leaves per isolate. The Petri plates were sealed with parafilm (Laboratory film, Menasha, WI 54952) and transferred to an incubator at 25°C with 12/12 h light/dark cycle and arranged in a completely randomized design with two replications. Disease severity evaluation was done every 5 days for 8 weeks by recording the leaf area infected using the 7-grade scale described above. The entire experiment was performed twice.

2.5 Data Analysis

2.5.1 Disease Severity

For each year and each agroecological zone, the disease severity was summarized with the standard error of the means to compare the disease severity statistically within each zone. To examine whether the frequency of infected fields varied significantly among agroecological zones, the frequency of Sigatoka infected in each zone and each year was

recorded and Pearson's χ^2 test was conducted. The analysis excluded the fields in the SGS as they were showing very low level of infection or for most of the time no disease such as leaf spot was recorded.

2.5.2 Aggressiveness of M. fijiensis *and* M. eumusae *and Pathotypes Determination*

Data related to the leaf area infected of the two tests were recorded every 5 days for 60 days and data collected from the two rounds were subjected to t-test. The t-test showed no significant difference ($P < 0{\cdot}6192$), the two data sets were then averaged and the mean used to perform the subsequent analysis (AUDPC, PCA, Rank-sum). For each isolate, the area under disease progress curve (AUDPC) was calculated from the percentage disease severity values, according to Shaner & Finney (1977): AUDPC = $\sum_{i=1}^{n}$ in which Y_i is the percentage leaf area infected on the ith observation, X_i is the date of observation in days after inoculation and n is the number of disease severity readings taken. ANOVA was carried out on the data to determine the significance of the effects of isolates, genotypes and isolates x genotypes interaction, using the GLM procedure in SAS, 2003 version 9.1.

2.5.3 Ranking Method and Multivariate Analysis

To determine the aggressiveness of individuals, isolates were partitioned into various classes of aggressiveness using a Rank-sum method based on the means AUDPC of each isolate across the 12 scoring points (scoring every 5 days for 8 weeks). The Rank procedure of SAS (SAS 2003, version 9.1) with option average handling ties was used to assign ranks to isolates in each variety from the smallest to the largest (Okechukwu *et al.,* 2008). The sum of the ranks (Xn) was calculated for each isolate and compared with the grand mean (Gn) of the rank sums across all the 96 isolates (Gn). Deviation of each isolate from the Gn was calculated as [(Xn-Gn)/standard deviation] x 2. Deviations to the right (positive) of the Gn on the mean distribution curve were rated aggressive while deviations to the left (negative) of

the grand mean distribution were rated less aggressive. An isolate is considered aggressive (HA) if it is more than 0·5 standard deviations from the Gn, moderately aggressive (MA) if it is between -2·5 and 0·5 and less aggressive (LA) if it is less than -2·5 standard deviations from the Gn.

PROC UNIVARIATE analysis was conducted to determine the distribution of the AUDPC. Based on the departure from the grand mean for AUDPC, across all the 96 isolates and the three cultivars, the AUDPC was categorized in three groups: the first, second and third groups represented AUDPC that were one standard deviation below the grand mean, one standard deviation around the grand mean and one standard deviation above the grand mean, respectively (Fig. 2.3). The first class which is defined as LA was assigned a value of 1, while the second is assigned a value of 2 and classified as MA and the third class were assigned to a value 3 representing the A category. The MULTIVARIATE analysis of the isolates was done by the principal component analysis (PCA) to deduct a plot. Cluster procedure was also used to assign isolates to pathotype. SAS correlation procedure was used to study the effect of the two pathogens species on the three varieties of bananas.

2.5.3 Pathogenic Diversity for Aggressiveness

To determine the pathogenic diversity within different zone, Shannon Index (Shannon & Weaver, 1949) was used as defined as follow: $H' = -\Sigma p_i \ln(p_i)$, where p_i is the fraction of individuals belonging to the i-th species. The genetic richness d that is represented by the number of different types divided by the total number of individuals in a sample and evenness evaluated by the index of E_5 (the relative abundance of different types in the sample) (Ludwig & Reynolds, 1988) were also calculated using the formula: d = Ssub/nsub and $Es = H'/n_i$ with S_{sub} = the number of different pathotypes, n_{sub} = the size of the smallest sample, H' represents the Shannon's index and ni the number of isolates of the i-th pathotype.

CHAPTER 3.0 RESULTS

3.1 Survey

3.1.1 Distribution and Disease Severity of Leaf Spot Diseases in Nigeria

From the survey it was confirmed that bananas are widely grown in the different geographical zones. Leaf spot diseases were well established in almost all the fields visited, except in the Southern Guinea Savannah (SGS) where the diseases were less distributed and sometimes absent. It is important to mention that for the reason mention above, the last zone does not represent a collection zone. Most of the farmers were growing only local varieties that are highly susceptible to Sigatoka infection.

The analysis of the survey revealed that the number of infected fields within each year differed significantly (χ^2 = 34·2, df = 2, and P < 0·05) between the HF and the DS. In 2004 100% of the fields visited in HF were infected. In 2005, 66% of the field were infected in HF and 34 in DS. In 2006, 31% were infected in HF and 69% in DS. In general, more fields were surveyed in the HF (66%) and fewer in the DS (34%) across the 3 years (Table 3.1, 3.2). In 2005, more fields were visited in the HF (67%) and fewer in the DS (33%). In 2006, more fields were surveyed in the DS (69%) and fewer in the HF (31%). This can explain the high value of the χ^2 = 34·2. The difference between expected and observed infected fields is small in 2005 and the contribution of this deviation on the χ^2 value is minimal.

The disease severity in 2004 is lower (33·2%) than in 2005 in the HF (43·1%) and in the DS (45%). In 2006 the disease severity is generally low, with 5·1 for the HF and 5·8 for the DS (Table 3.2). In general, the disease severity is similary in the HF (27·1%) and in the DS (25·4%).

Table 3.1 Pearson's χ^2 test for number of fields infected with Sigatoka in two agroecological zones in Nigeria from 2004 to 2006

	Agroecological zones		
Year	Humid forest	Derived savannah	Total
2004	29/18 (6·7)	0/11 (11·0)	29
2005	18/17 (0·06)	9/10 (0·1)	27
2006	12/24 (6·0)	27/15 (9·6)	39
Total	59	36	95

Observed number of fields/expected number of fields (contribution to χ^2 = square difference

Table 3.2 Summary statistics of Sigatoka (*Mycosphaerella* spp.) disease incidence and severity in different agro-ecological zones in Nigeria from 2004 to 2006

		Disease incidence (%)		**Disease severity (%)**	
Year	N	HF	DS	HF	DS
2004	29	100	0	33.2 ± 2.5	.
2005	27	66	34	43.0 ± 2.6	45.0 ± 7.7
2006	39	31	69	5.1 ± 1.6	5.8 ± 1.8
Mean		66	34	27.1 ± 2.2	25.4 ± 4.0

HF: Humid forest, DS: Derive savanna

3.2. Identification of *Mycosphaerella* Species

3.2.1 Based on Morphological Parameters

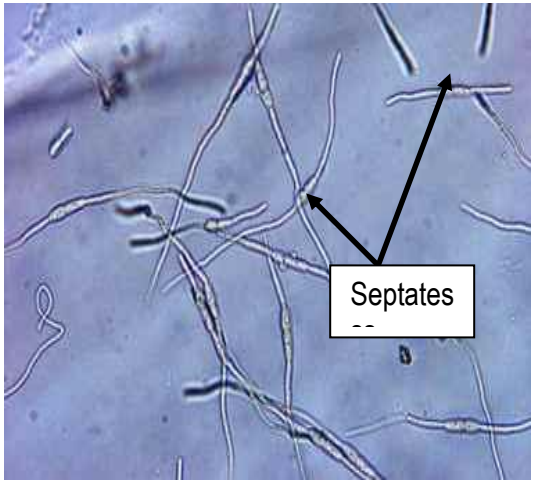
The pathogens isolated were morphologically identified on culture as *Mycosphaerella* species. The two species on PDA or V8 have the same characteristics. The color varied from pale grey, pink-dark grey to grey-brown with a velvet surface (Plates 3.1 a, b, c). It was not easy to differentiate *M. fijiensis* from *M. eumusae*, the ascospore representing the sexual stage were also similar for both species. They are two-celled, hyaline and biseptate with a slight constriction at the septate (Plate 3.2). The asexual stage is characterized by the production of conidia (Plate 3.3 a, b, c). *M. fijiensis* isolates was easily identified as the conidia are long with 5 to 8 septates: one end bigger than the other, bottle shape and possessing a scar. However, it was not easy to differentiate between *M. eumusae* although it appeared smaller with 4 to 6 septates (Plate 3.3 a, b, c) and curved, possessing no scar.

3.2.2 Based on SNPs and Indels

From the initial 96 isolates sequenced, 95 isolates that provided phred quality trimmed sequences were subjected to further analysis. We obtained 661 nucleotides of the ribosomal DNA in the 95 isolates. A total of 65 sequence variants (average of 10.1 variants/100 nucleotides), composed of 55 SNPs and 10 Indels were present. The 5.8S subunit (76 nucleotides) and the 25S subunit were well conserved, with an average of only 3.1 polymorphic sites/100 nucleotides compared to the ITS1 and ITS2 that showed an average of 27.4 variants/100 nucleotides.

Mycosphaerella species that were included in the alignment were identified without ambiguity using species-specific SNPs or Indels. All the *Mycosphaerella* isolates in our study shared species-specific identifiers only with the reference checks of *M. fijiensis* (Uganda, Colombia, the Philippines, Cook Islands, Indonesia, and Papua New Guinea) or the reference checks of *M. eumusae* from Sri Lanka, Thailand, Vietnam, and Nigeria. Eleven isolates (11.6%) in our

study were *M. eumusae* based on unique sequence features (Fig.3.1), the remaining 84 isolates (88.4%) shared specific polymorphic sites that only differentiate *M. fijiensis* from the other species. *M. musae*, represented by GenBank accessions DQ019353 and DQ019354, was the most distinct species and none of the variations specific to *M. musae* was present in our *Mycosphaerella* collection. Similarly, GenBank accessions of *M. musicola* from Australia, Venezuela, Côte d'Ivoire, Indonesia, Guinea and Cameroon were included as controls to determine the presence of *M. musicola* in our collection. Species-specific sequence variations that were shared by these *M. musicola* reference checks from various origins (Fig. 3.1) were not present in any of our *Mycosphaerella* isolates, indicating a complete absence of *M. musicola* from Nigerian collection.



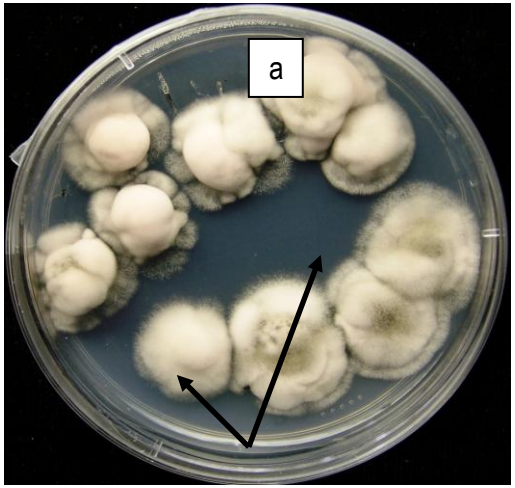
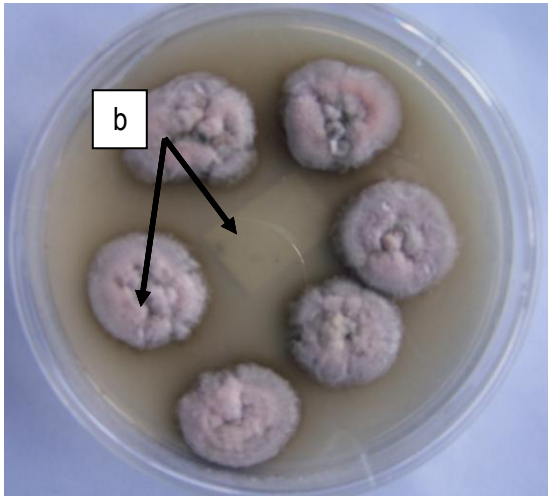


Plate 3.1 Ascospores discharged on media with two germtubes

Mycelia of *Mycosphaerella* spp. on PDA



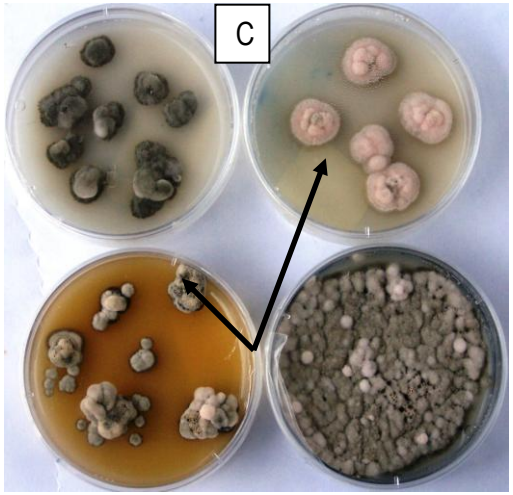


Plate 3.2 Mycelia of Sigatoka disease on PDA (a) and V8 (b) media and mycelia on the two media (C)

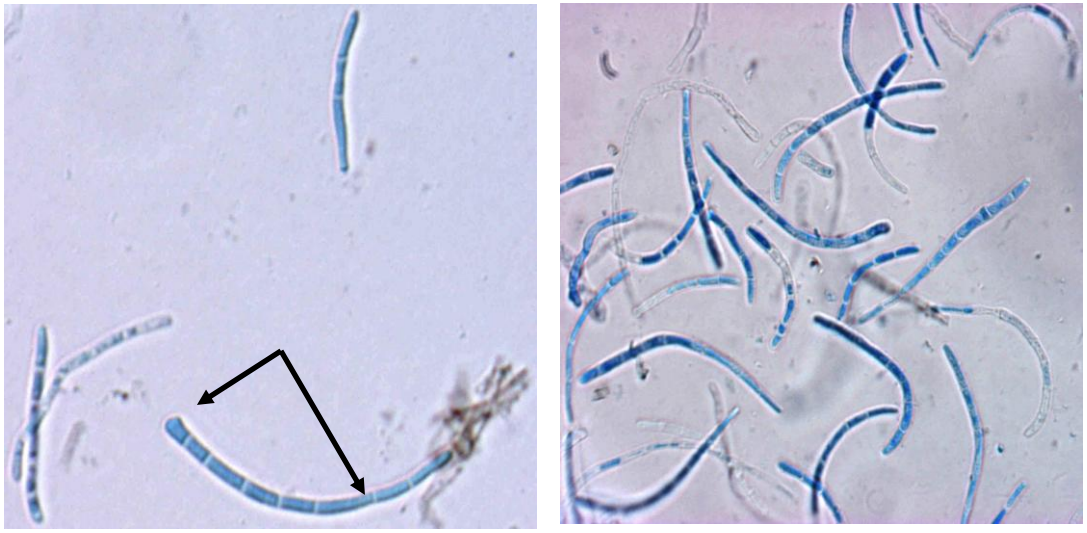


Plate 3.3.a Conidia with dumb-bell-shaped of *M. fijiensis*

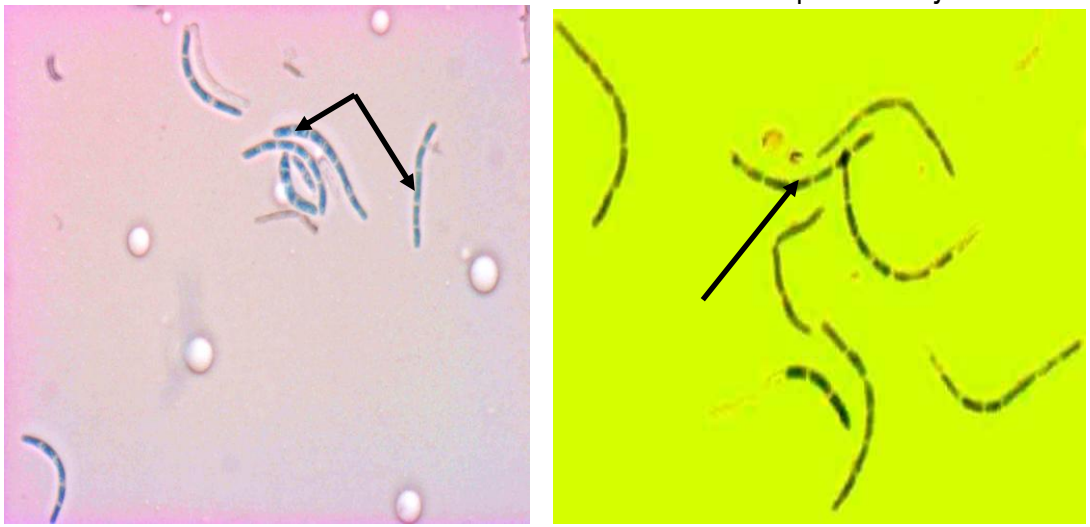


Plate 3.3.b Conidia of *M. eumusae*

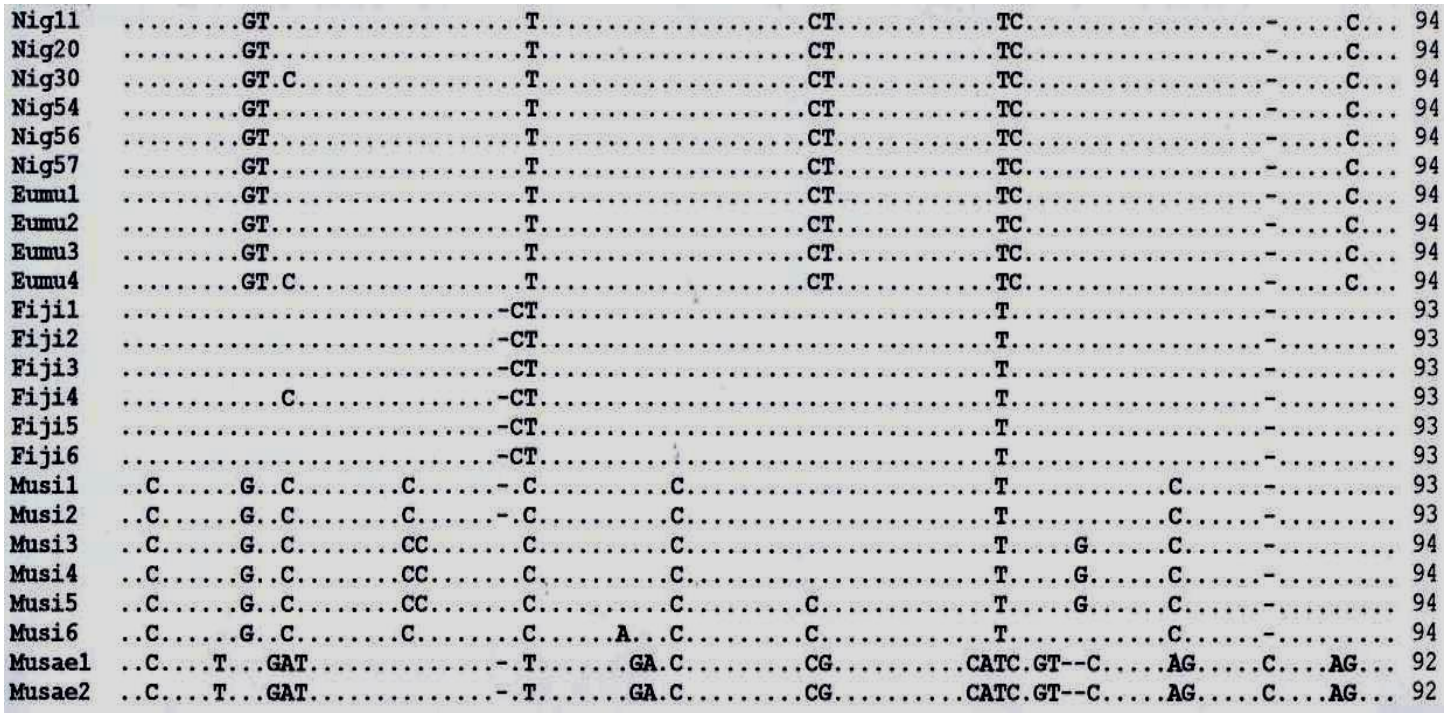


Figure 3.1 Identification of Nigerian isolates 11, 20, 30, 54, 56 and 57 (Nig11 to Nig57) as *Mycosphaerella eumusae* based on species-specific sequence variations displayed in the ribosomal DNA by *M. eumusae* reference Genbank accessions AY923757 (Eumu1), DQ019336 (Eumu2), AY923758 (Eumu3) and AY923760 (Eumu4). Reference checks for *M. fijiensis* are shown as Fiji1 (AY923763), Fiji2 (DQ865944), Fiji3 (AY923765), Fiji4 (AY266151), Fiji5 (AY923762), Fiji6 (AY923764); *M. musicola* references are Musi1 (AY646497), Musi2 (AY646494) Musi3 (AY646473), Musi4 (AY646465), Musi5 (AY646475), Musi6 (AY646480); and *M. musae* control accessions are DQ019353 (Musae1) and DQ019354 (Musae2). Shared and species-specific SNPs are highlighted as nucleotides (A, C, G and T) in the alignment; insertion-deletions are shown as dashes while shared non-polymorphic sequences are represented by dots. Numbers at the right of the alignment represent the length of sequence displayed for each isolate.

3.2.3 Phylogenetic Analysis Using SNPs and Indels

In the overall comparisons of reference checks and current Nigerian isolates combined in sequence alignments, 65 sequence variants were identified. Out of these variants, 33 variants remained when sample isolates were considered separately for sequence alignments without reference checks. This was due mostly to the absence of *M. musicola* and *M. musae* in the Nigeria collected isolates. In general, most sequence variants of *M. musae* were not shared by *M. fijiensis*, *M. eumusae* or *M. musicola* (Fig. 3.2). The assessment of the number of sequence variants (Table 3.3) showed that most SNPs occurred between species. To further confirm the genetic relationship and groupings between *Mycosphaerella* isolates in our collection and the various control accessions at a general level, a distance matrix was generated using the SNPs and Indels (including species-specific variants). At an agglomerative similarity of 87%, four major clades, each containing sub-clades, were delimited in the UPGMA dendrogram with an average of 77% bootstrap support (Fig.3.2).

Taking all the sequence polymorphisms into account, *M. fijiensis* and *M. eumusae* isolates grouped with their respective reference checks from GenBank. The major clade (I), with 59% bootstrap support, consisted of all the 84 *M. fijiensis* isolates from our collection from the four Nigerian geographical regions and GenBank checks of *M. fijiensis* from Columbia, Uganda, the Philippines, Cook Islands, Indonesia, and Papua New Guinea. This relatively low bootstrap value for the separation of *M. fijiensis* isolates indicates a close relationship within this species. The clade II, with a 67% bootstrap value, consisted of 11 *M. eumusae* species from our collection and GenBank reference species of *M. eumusae* from Sri Lanka, Taiwan, Quebec, and Vietnam. The bootstrap value indicates that *M. eumusae* isolates are relatively less differentiated from one another. Clade III with 100% bootstrap support was composed of only GenBank checks of *M. musicola* from Guinea, Cameroon, Indonesia, Australia, Venezuela, and Côte d'Ivoire. Clade IV with 100% bootstrap support contained only the two *M. musae* species (GenBank accessions DQ019353 and DQ019354).

None of the *Mycosphaerella* isolates in our collection clustered with reference species in the *M. musicola* clade (III) and the *M. musae* clade (IV). The 100% bootstrap value indicates that *M. musae* and *M. musicola* included in the analyses are relatively highly differentiated from *M. fijiensis* and *M. eumusae*. The last two species are relatively less differentiated from each other (77% bootstrap value).

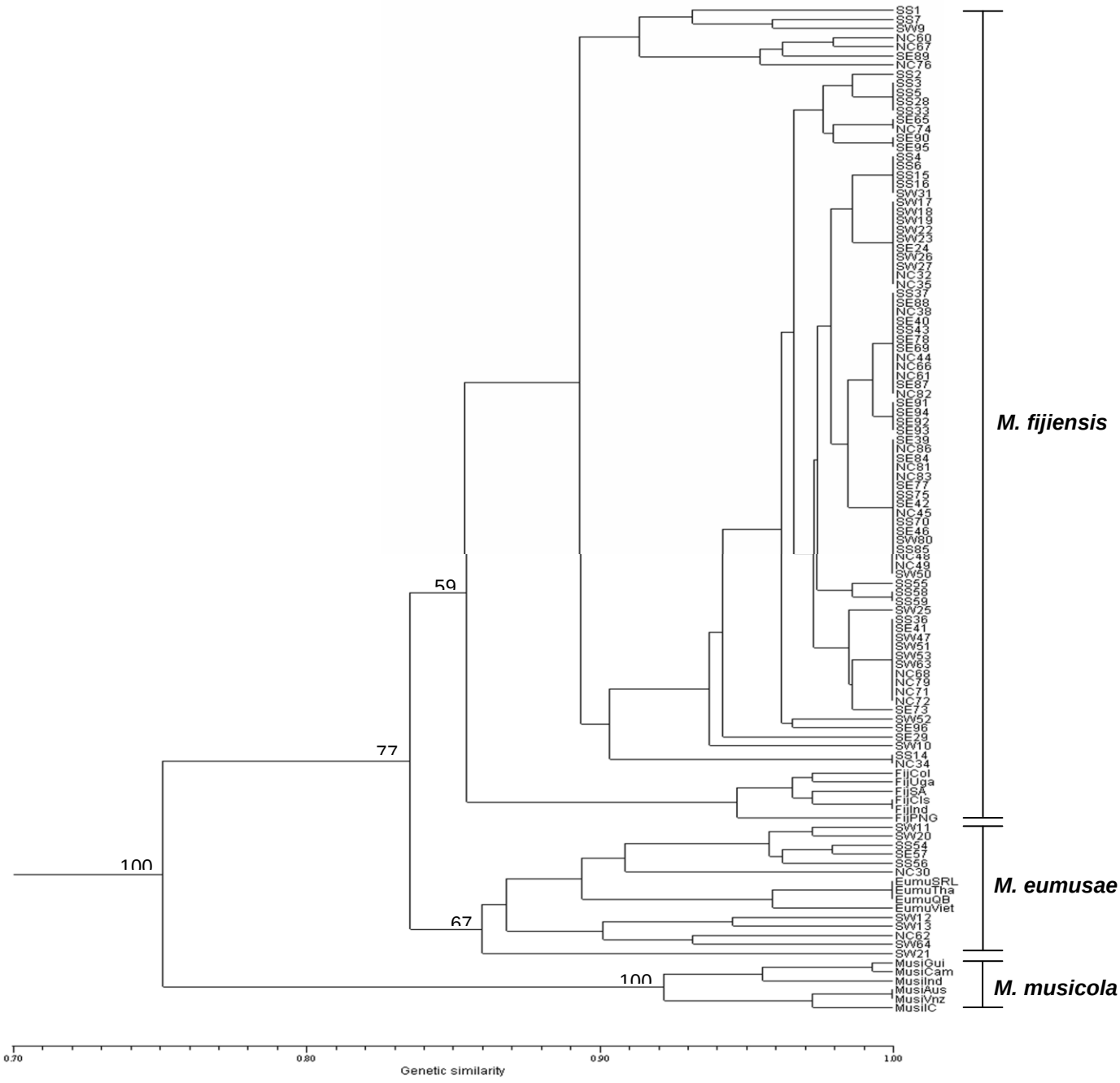


Figure 3.2 Dendrogram from unweighted paired group method with arithmetic means (UPGMA) based on SNPs and Indels analysis in the ribosomal DNA of 95 isolates of *Mycosphaerella* species obtained from *Musa* in Nigeria and GenBank references of *M. fijiensis, M. musicola, M. eumusae* and *M. musae*.(See detailed label in appendix 3.2)

Table 3.3 Intra- and inter-specific variants of SNP in rDNA of *Mycosphaerella* species collected from banana and plantain in four geographical zones in Nigeria[a]

Species	*M. fijiensis*	*M. eumusae*	*M. musicola*	*M. musae*
M. fijiensis	4			
M. eumusae	14	7		
M. musicola	7	21	1	
M. musae	21	22	17	0

[a] Values along the diagonal line (shaded) represent the intra-specific variants, while those below the diagonal lines are the inter-specific variants.

3.2.4 Intraspecific variations and population differentiation

Analysis of *M. fijiensis* isolates in our collection revealed an average of similarity index of 98%, while the corresponding similarity index between *M. eumusae* isolates was 95.4%. Further more, the average similarity index between *M. fijiensis* and *M. eumusae* in our collection was 88% and comparison between the GenBank reference checks of *M. fijiensis* and *M. eumusae*, gave an average similarity index of 89%.

Although a certain level of interspecific diversity was observed, clones displaying 100% similarity to each other within the same species were also present. Examples of isolates collected in distinct geographical regions but sharing 100% similarity to each other within *M. fijiensis* include SE24 (site Ashala in Anambra state in the SE zone) versus SW17 (Osegere in Osun state in the SW zone), and SS36 (Mosogar in Delta state in the SS zone) versus NC72 (Agano in Abuja Federal Capital Territory in the NC zone). Based on GPS coordinates the distance between the collection sites of SE24 (Ashala) and SW17 (Osegere) is 360 km while SS36 (Mosogar) and NC72 (Gano) are 380 km apart. The two most distant sites of collection, Kwara (NC) and Rivers (SS), are 500 km apart.

The analysis of molecular variance (AMOVA) and pairwise comparisons of population genetic distances of the 4 geographical zones confirmed that there is a significant genetic differentiation between all pairs of populations, except when the NC zone was compared to the SE zone (Table 3.4) In the pairwise comparison this translated to a distribution of total gene diversity of 3% between collection sites of the NC and the SE zones, to 28.5% diversity between the sites in the SE and the SW zones. Allelic diversity within the 4 geographical locations varied from 0.02 in the SE and the SW to 0.04 in the NC zone (Table 3.5). The AMOVA of the 4 geographical collection zones indicated that 87.4% of the variation was due to differences within populations with the remaining 12.6% of total gene diversity was due to differences among populations. The genetic differentiation of each population estimated based on the F_{ST} ranged from 0.11 for the population in the SS zone to 0.14 for the SE and SW populations, with an overall F_{ST} value of 0.126.

Table 3.4 Pairwise population θ values calculated from the Weir and Cockerham differentiation estimator between *Mycosphaerella fijiensis* populations in four geographical zones in Nigeria[a]

Population	North Central	South East	South South
North Central	..	..	..
South East	0.031 (0.1261)	..	..
South South	0.094 (0.0001)	0.110 (0.009)	..
South West	0.128 (0.0001)	0.285 (0.0001)	0.144 (0.0001)

[a] Numbers in parenthesis denote *P*-values for θ, an unbiased estimator of Wright's *F* statistic.

Table 3.5 Allelic diversity in populations of *Mycosphaerella fijiensis* at 65 SNP loci[a], based on isolates collected from different geographical zones in Nigeria

Geographical zone	Sample size	Mean no. of alleles per locus	No. of polymorphic loci (%)	*H* (SE)
North Central	22	1.13	19	0.03 (0.004)
South East	23	1.11	15	0.02 (0.003)
South South	21	1.16	22	0.04 (0.004)
South West	18	1.09	13	0.02 (0.002)

[a] A locus is considered as polymorphic if more than one allele was detected. *H* is the unbiased estimation of gene diversity (Nei, 1978) and SE denotes standard error shown in parenthesis.

3.2.5 Sequence Variations Unique to Nigerian Isolates and SNP Haplotypes in the Collection

Despite the relatively low intraspecific diversity among *M. fijiensis* isolates compared with that of *M. eumusae*, *M. fijiensis* displayed three unique sequence variations compared to all the GenBank reference checks from Columbia, Uganda, the Philippines, Cook Islands, Indonesia, and Papua New Guinea. A deletion shared by all reference checks was replaced by cytosine (*C*) at position 421 and a transversion (*C* to *T*) at position 422 displayed *T* only in all the isolates used in the present study. At position 458, another transversion showed *T* in all the reference checks while all the *M. fijiensis* isolates in our collection showed 2 haplotypes at that position, with *C* in 33 isolates (39%) and *T* in those remaining. A comparison of the *M. eumusae* isolates in our collection with the reference species from Sri Lanka, Taiwan, Quebec and Vietnam showed that none of the sequence variants characterizing *M. eumusae* was specific to our isolate collection of that species. Ribosomal DNA sequences were further analyzed for the presence of shared SNP haplotypes. This grouped the 84 isolates of *M. fijiensis* into 14 haplotypes (16.7%) and the 11 isolates of *M. eumusae* into 7 haplotypes (63.6%). To crosscheck the species identification, DNA from isolates representing the above 21 haplotypes (Fig. 3.3) was amplified by the *Mycosphaerella*-specific PCR probes based on actin and β-tubulin genes (Arzanlou *et al*. 2007). The presence of *M. fijiensis* and *M. eumusae* and the absence of *M. musicola* as revealed by ribosomal DNA analyses were confirmed (Fig. 3.4 A-D).

3.2.6 Registration in the GenBank

The 14 *M. fijiensis* haplotypes were registered in GenBank (http://www.ncbi.nlm.nih.gov/) as accession numbers EF666070 to EF666083 and the *M. eumusae* haplotypes were registered under GenBank accession numbers EF670653, and EF670656 to EF670661(Appendix 3.3).

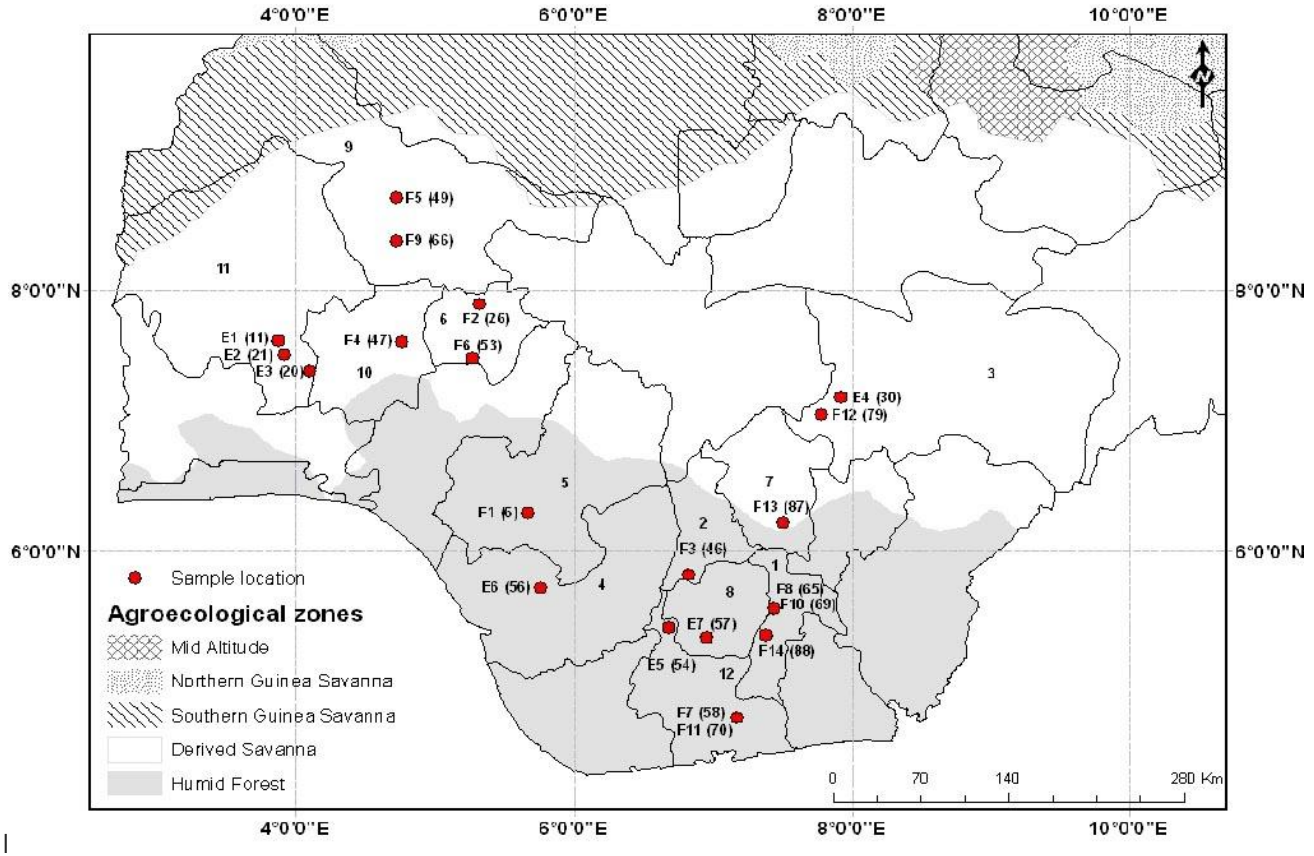


Figure 3.3 Geographical distribution of *Mycosphaerella fijiensis* and *Mycosphaerella eumusae* based on SNP haplotypes in ribosomal DNA analysis of isolates collected from *Musa* leaves in banana-producing belt of Nigeria. Stand alone numerals are states (1 = Abia, 2 = Anambra, 3 = Benue, 4 = Delta, 5 = Edo, 6 = Ekiti, 7 = Enugu, 8 = Imo, 9 = Kwara, 10 = Osun, 11 = Oyo, and 12 = Rivers) while black dots are the collection sites. Letters F and E followed by numbers are *M. fijiensis* and *M. eumusae* haplotypes, respectively. A collection number assigned to each isolate is indicated in parenthesis

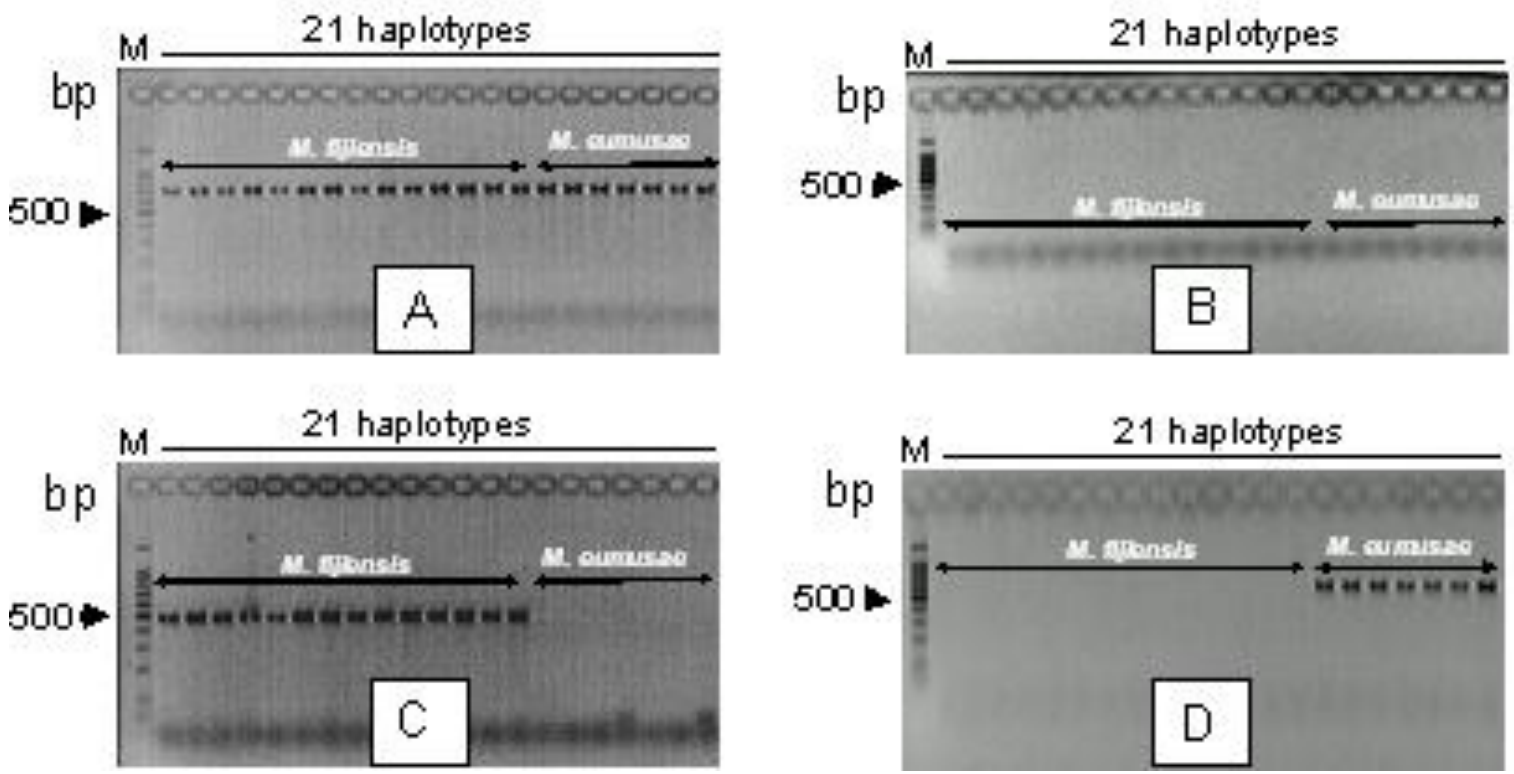


Figure 3.4 General and species-specific PCR probes designed on actin gene to confirm the presence of fungi (A), the absence of *Mycosphaerella musicola* (B), 14 haplotypes of *M. fijiensis* (C) and 7 haplotypes of *M. eumusae* (D) as revealed by the ribosomal DNA analysis. PCR amplification was performed with general primer ACTF/ACTR for the actin gene (A); M. musicola specific primer MMactF2/MMactRb (B), *M. fijiensis* specific primer MFactF/ACTR (C) and *M. eumusae* specific primer ActF/MEactR (D). The first lanes of gel indicated by M represent the 100 bp DNA Ladder (Promega Corporation, USA)

3.3 Pathogen Variability

3.3.1 Symptoms Development

All the 85 *M. fijiensis* and 11 *M. eumusae* isolates inoculated induced disease on Calcutta-4, Agbagba and Valery similar to that observation in the field. On Calcutta-4, symptom appeared generally 20 days after inoculation; streaks symptoms were visible around 30 days after inoculation (Fig. 3.5; Plate 3.4a). On Agbagba depending on the isolate, symptoms were visible already at 10 days after inoculation and necrosis was already visible at the inoculation point 20 days after inoculation (Fig. 3.5; Plate 3.4b). The infection process is almost the same with Valery. *M. eumusae* infection was high on Agbagba and Valery (Plates 3.4)

3.3.2 Aggressiveness of Mycosphaerella Species and Pathotype Determination

A significant isolate effect on disease severity, as measure by leaf percentage infected, was interpreted as evidence for differences in aggressiveness. Isolates that induced lesion express as AUDPC that are statistically longer or larger than lesions associated with other isolates on the three varieties are referred to as highly aggressive isolates, and the apparently low aggressive isolates associated with shorter and smaller lesions are referred as low or less aggressive isolates.

Significant differences ($P < 0{\cdot}0001$) were detected among cultivars and isolates. The cultivars × isolates interaction was also significant ($P < 0{\cdot}0001$) and there is a variation among responses of isolates on varieties: Calcutta-4 behaved as resistant, with the expression of low infection of the disease, while Agbagba and Valery behaved as susceptible to all isolates tested. The differential nature of isolates, indicated by significant host x pathogen interaction, was further explored using Rank-sum methods and multivariate analysis. The ranking matrix for the 96 isolates against the three Bananas varieties using the

Rank-sum method was reported (Table 3.6). This method generated three classes of aggressiveness. First class grouped isolates that were HA composed of 80% of *M. fijiensis* and 20% of *M. eumusae.* The Class II, MA and composed of 93% *M. fijiensis* and 7% *M. eumusae*. The last group III, LA composed of 100% *M. fijiensis.*

The ranking relative to agroecological zone showed that 56% of the isolates characterized as HA were from the DS and 44% were from the HF. In the group MA, 39% were from the DS and 61% from the HF respectively; 55% of isolates in the LA group were from DS and 45% were from HF.

Comparison of the two species showed that the aggressiveness of the two species is similar on Agbagba and Calcutta-4, except on Valery where there is difference as revealed by the mean separation analysis (Fig.3.6).

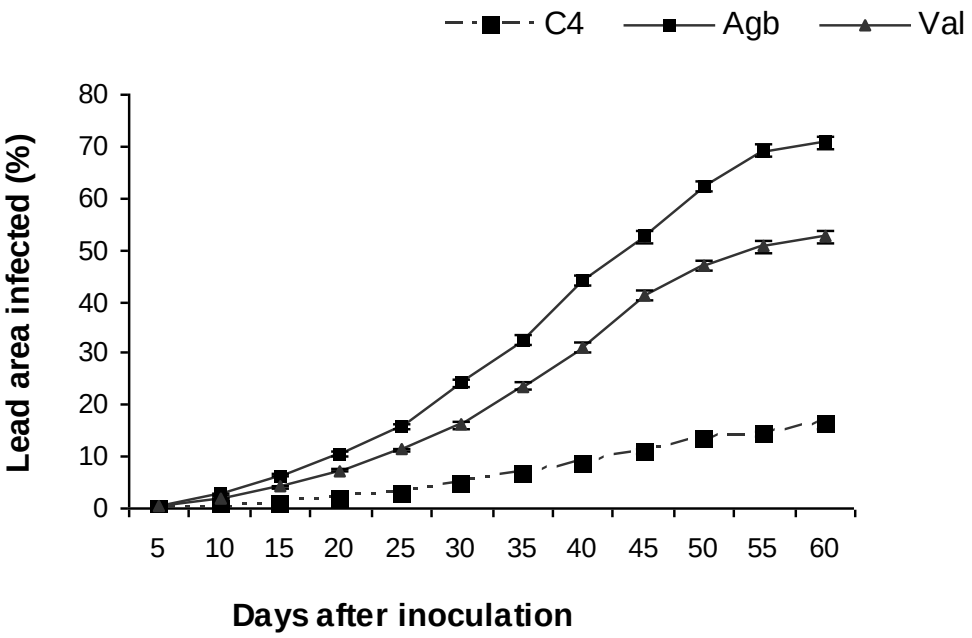
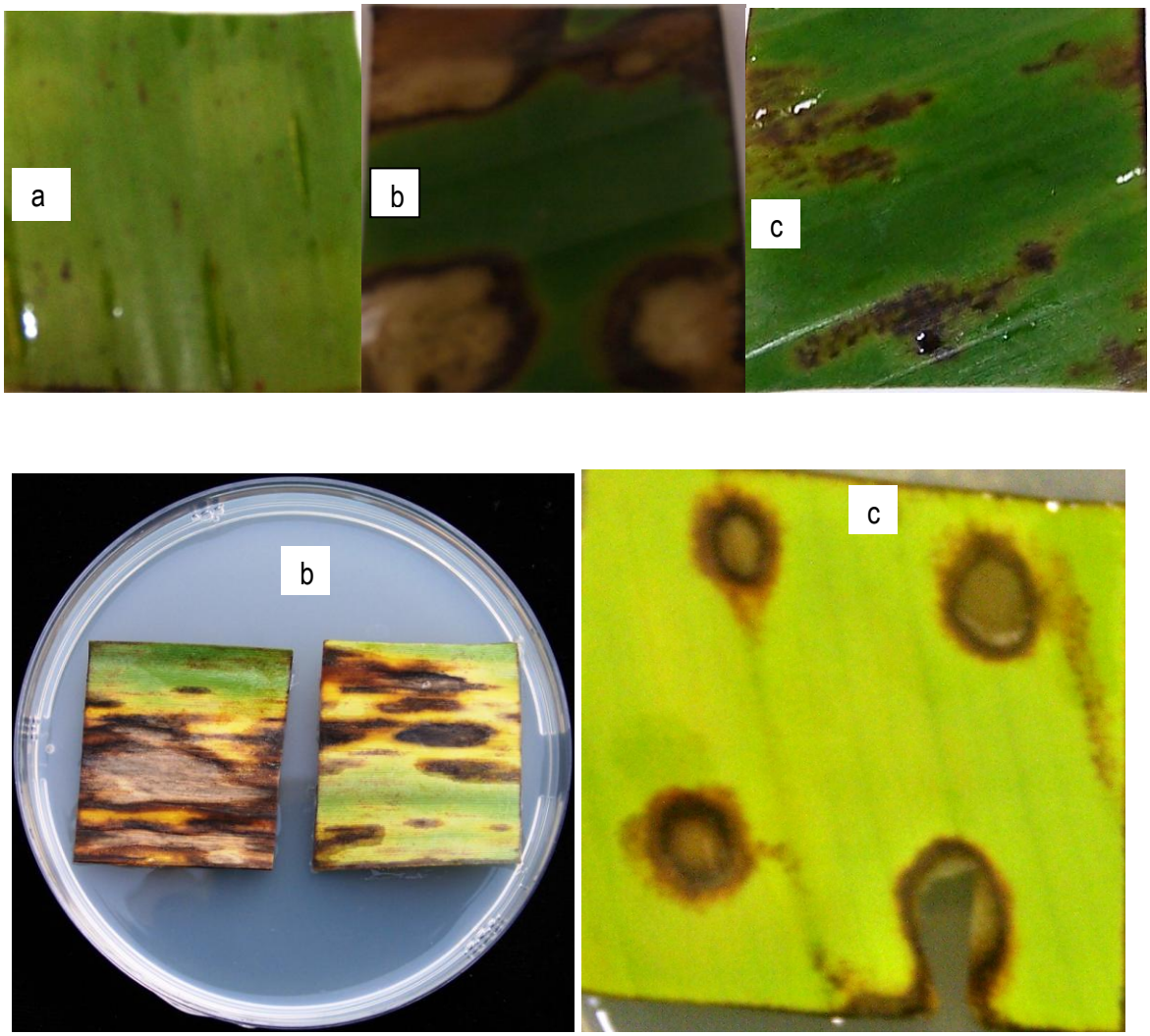


Figure 3.5 Disease progress curve (DPC) for the three varieties 60 days after inoculation on Agbagba (Agb), Valery (Val), and Calcutta-4 (C4)



Plates 3.4 Symptoms on Calcutta-4 (a), Agbagba (b) and Valery (c) when inoculated with *M. fijiensis* 20 day after inoculation (DAI) and symptoms on Agbagba and Valery when inoculated with *M. eumusae* (20 DAI)

Table 3.6 Severity of the 96 isolates as determined by ranking method based on their aggressiveness on the three varieties across the 12 scoring point in the two agroecological zones

Isolates*	Agbagba	Valery	Calcutta 4	Rank-sum	Deviation	Standardized	Classes
Iso07	1	2	2	5	-140·5	-4·08	LA
Iso19	4	10	7	21	-124·5	-3·62	LA
Iso61	8	3	13	24	-121·5	-3·53	LA
Iso09	2	6	17	25	-120·5	-3·5	LA
Iso66	7	1	19	27	-118·5	-3·44	LA
Iso02	25	9	3	37	-108·5	-3·15	LA
Iso16	11	16	11	38	-107·5	-3·12	LA
Iso22	3	7	30	40	-105·5	-3·07	LA
Iso42	12	17	12	41	-104·5	-3·04	LA
iso03	17	8	18	43	-102·5	-2·98	LA
Iso48	27	24	4	55	- 90·5	-2·63	LA
Iso49	28	25	5	58	- 87·5	-2·54	MA
Iso60	13	14	34	61	- 84·5	-2·46	MA
Iso94	32	19	10	61	- 84·5	-2·46	MA
Iso06	16	32	15	63	- 82·5	-2·4	MA
Iso82	10	29	29	68	- 77·5	-2·25	MA
Iso55	6	31	32	69	- 76·5	-2·22	MA
Iso57	43	4	25	72	- 73·5	-2·14	MA
Iso75	5	15	52	72	- 73·5	-2·14	MA
Iso38	29	36	8	73	- 72·5	-2·11	MA
Iso76	15	5	54	74	- 71·5	-2·08	MA
Iso31	34	26	16	76	- 69·5	-2·02	MA
Iso47	26	37	14	77	- 68·5	-1·99	MA
Iso04	30	13	35	78	- 67·5	-1·96	MA
Iso74	31	22	26	79	- 66·5	-1·93	MA
Iso36	21	12	49	82	- 63·5	-1·84	MA
Iso43	22	40	24	86	- 59·5	-1·73	MA
Iso37	14	79	1	94	- 51·5	-1·5	MA
Iso83	18	11	75	104	- 41·5	-1·21	MA
Iso93	38	33	33	104	- 41·5	-1·21	MA
Iso29	44	44	20	108	- 37·5	-1·09	MA
Iso86	19	27	67	113	- 32·5	-0·94	MA
Iso90	33	30	51	114	- 31·5	-0·92	MA

Table 3.6 *Continued*

Isolates*	Agbagba	Valery	Calcutta 4	Rank-sum	Deviation	Standardized	Classes
Iso01	75	21	22	118	- 27·5	-0·8	MA
Iso39	24	56	48	128	- 17·5	-0·51	MA
Iso70	76	28	28	132	- 13·5	-0·39	MA
Iso27	42	87	6	135	-10·5	-0·31	MA
Iso56	9	39	89	137	-8·5	-0·25	MA
Iso65	46	57	36	139	-6·5	-0·19	MA
Iso91	51	20	69	140	-5·5	-0·16	MA
Iso05	40	43	59	142	-3·5	-0·1	MA
Iso73	68	38	37	143	-2·5	-0·07	MA
Iso84	36	35	72	143	-2·5	-0·07	MA
Iso87	39	34	70	143	-2·5	-0·07	MA
Iso80	58	18	68	144	-1·5	-0·04	MA
Iso14	41	49	55	145	-0·5	-0·01	MA
Iso68	59	66	21	146	0·5	0·01	MA
Iso69	47	55	46	148	2·5	0·07	MA
Iso50	55	52	42	149	3·5	0·1	MA
Iso34	61	53	38	152	6·5	0·19	MA
Iso44	54	73	27	154	8·5	0·25	MA
Iso54	35	75	44	154	8·5	0·25	MA
Iso52	56	59	40	155	9·5	0·28	MA
Iso15	52	42	62	156	10·5	0·31	MA
Iso88	93	23	43	159	13·5	0·39	MA
Iso71	23	60	81	164	18·5	0·54	MA
Iso51	53	63	50	166	20·5	0·6	MA
Iso59	84	47	39	170	24·5	0·71	MA
Iso64	78	89	9	176	30·5	0·89	MA
Iso41	96	51	31	178	32·5	0·94	MA
Iso46	60	61	57	178	32·5	0·94	MA
Iso62	20	71	87	178	32·5	0·94	MA
Iso08	70	65	53	188	42·5	1·23	MA
Iso17	37	69	83	189	43·5	1·26	MA
Iso85	45	50	95	190	44·5	1·29	MA
Iso35	63	62	66	191	45·5	1·32	MA

Table 3.6 Continued

Isolates*	Agbagba	Valery	Calcutta 4	Rank-sum	Deviation	Standardized	Classes
Iso95	72	45	74	191	45·5	1·32	MA
Iso63	50	58	84	192	46·5	1·35	MA
Iso30	79	91	23	193	47·5	1·38	MA
Iso13	49	77	71	197	51·5	1·5	MA
Iso96	91	48	60	199	53·5	1·55	MA
Iso77	67	41	94	202	56·5	1·64	MA
Iso26	64	78	63	205	59·5	1·73	MA
Iso18	65	96	47	208	62·5	1·82	MA
Iso53	82	83	45	210	64·5	1·87	MA
Iso10	66	90	56	212	66·5	1·93	MA
Iso40	74	74	64	212	66·5	1·93	MA
Iso28	90	82	41	213	67·5	1·96	MA
Iso81	71	67	76	214	68·5	1·99	MA
Iso79	57	88	73	218	72·5	2·11	MA
Iso67	69	72	78	219	73·5	2·14	MA
Iso92	62	81	77	220	74·5	2·16	MA
Iso20	48	95	79	222	76·5	2·22	MA
Iso11	95	68	61	224	78·5	2·28	MA
Iso33	87	80	58	225	79·5	2·31	MA
Iso89	88	46	91	225	79·5	2·31	MA
Iso45		94	65	232	86·5	2·51	HA
Iso25	86	70	82	238	92·5	2·69	HA
Iso72	94	54	93	241	95·5	2·77	HA
Iso78	85	64	96	245	99·5	2·89	HA
Iso12	77	86	90	253	107·5	3·12	HA
Iso24	89	84	80	253	107·5	3·12	HA
Iso58	92	76	88	256	110·5	3·21	HA
Iso21	80	92	85	257	111·5	3·24	HA
Iso23	81	93	86	260	114·5	3·33	HA
Iso32	83	85	92	260	114·5	3·33	HA
Grand mean				145·5			
standard deviation			68·8				

*Isolates marked as bold are *M. eumusae*

ɲ= Sum of rank for each variety; ß= Deviation from GN; µ= Standardized mean (deviation/standard deviation)

HA= highly aggressive; MA= moderately aggressive and LA= least aggressive.

The Principal Component Analysis (PCA) with the cluster procedure showed that the 96 isolates were grouped in 9 pathotypes; the 85 *M. fijiensis* in 9 pathotypes (Fig. 3.6B) and the 11 *M*. *eumusae* isolates in 7 pathotypes. The disease severity of the isolates in the nine clusters is summarized in Table 3.7 & 3.8 using mean AUDPC calculated on the three varieties. From that table, the clusters V (7 isolates), III (16), IV (10) and II (21) representing 56% of the total isolates that caused higher levels of severity expressed as higher AUDPC across the three varieties were the most aggressive across all the varieties, followed by clusters IX (1), VI (6) and I (32) representing 41% of the total isolates and showing intermediate infection were classified MA. Clusters VII (2) and VIII (1) (3%), showing low levels of infection were LA. The separation of the 85 *M. fijiensis* and the PCA analysis showed that the grouping based on the aggressiveness of the 11 *M.eumusae* isolates could not be separated and individuals of this species fall in the *M. fijiensis* groups. Considerring the 85 *M. fijiensis*, cluster V (7) represented the HA followed with cluster III (17), cluster II (19). Cluster IV (10), IX (1) and cluster I (32), cluster VI (6) were MA and cluster VII (2) and cluster VIII (1) were LA.

Looking at the *M. eumusae*, Cluster I (2) constituted the HA. Clusters II (2), III (2) and V (1) were MA and the clusters VI and VII were grouped as LA.

The ranking method and the PCA revealed three groupings of aggressiveness. The two methods classified the isolates and there is agreement among the classifications. The overall aggressive isolates were iso 10, 23, 24, 33, 45.

The analysis based on single nucleotide polymorphism (SNP) analysis grouped the 85 *M. fijiensis* in 14 haplotypes and the 11 *M. eumusae* in seven haplotypes. An attempt to match the molecular grouping with the pathogenic grouping showed that there was no clear association between aggressive groups and haplotype groups. Three isolates in the HA group defined by PCA belonged to the same SNP cluster and the four remaining isolates were in a different cluster. Few numbers of isolates in the aggressive groups originated from the same genetic cluster. For instance, among the seven *M. fijiensis* isolates representing the HA group, three were found in the same SNP cluster, the remaining four isolates were

found in different cluster. The two *M. eumusae* members of group A determined by the Rank-sum method, belonged to the same cluster and the two individuals of the MA were also clustered together.

Table 3.7 Mean and standard deviation (in parenthesis) of disease severity (AUDPC) on three bananas varieties for all 96 isolates of *Mycosphaerella* spp. in nine groupings derived by cluster analysis

Pathotype clusters	Number of isolates	Agbagba	Calcutta-4	Valery
I	32	1400 (226)	247 (88)	1026 (252)
II	21	2025 (279)	294 (80)	1256 (261)
III	16	2153 (304)	591 (145)	1423 (135)
IV	10	1963 (331)	287 (102)	1844 (297)
V	7	2125 (182)	623 (98)	1988 (239)
VI	6	1388 (205)	520 (195)	1345 (171)
VII	2	1176 (12)	219 (26)	143 (202)
VIII	1	153 (0)	85 (0)	80 (0)
IX	1	2109 (0)	276 (0)	1011 (0)
Mean		1610 (0)	349 (0)	1124 (0)
Std		663 (0)	185 (0)	661 (0)

Table 3.8 Mean and standard deviation (in parenthesis) of disease severity (AUDPC) on Three banana varieties for all 85 isolates of *Mycosphaerella fijiensis* in nine groupings derived by cluster analysis

Pathotype Clusters	Number of isolates	Agbagba	Calcutta-4	Valery
I	30	1349 (204)	229 (78)	982 (229)
II	19	2010 (286)	299 (77)	1286 (189)
III	17	2068 (278)	571 (155)	1389 (135)
IV	7	1909 (390)	292 (102)	1779 (339)
V	6	2189 (12)	544 (141)	1910 (236)
VI	2	1176 (12)	219 (26)	143 (202)
VII	2	1324 (379)	605 (10)	1483 (47)
VIII	1	153 (0.0)	85 (0)	80 (0)
IX	1	2109 (0.0)	1011 (0)	276 (0)
Mean		1588 (0.0)	428 (0)	1036 (0)
Std		662 (0.0)	283 (0)	707 (0)

Table 3.9 Mean and standard deviation (in parenthesis) of disease severity (AUDPC) on three banana varieties for all 11 isolates of *Mycosphaerella eumusae* in seven groupings derived by cluster analysis

Pathotypes	Nb of isolates	Agbagba	Calcutta - 4	Valery
I	2	2163.5 (54.8)	444.1 (259.4)	2089.1 (69.4)
II	2	2454.7 (476.9)	308.1 (208.6)	1729.4 (308.5)
III	2	1673.4 (133.9)	440.6 (104.3)	1565 (15.9)
IV	2	1266.6 (108.3)	660.9 (44.6)	1350.6 (253.7)
V	1	1746.9 (0.0)	571.9 (0.0)	2228.1 (582.5)
VI	1	1680.3 (0.0)	271.3 (0.0)	582.5 (0.0)
VII	1	2113.8 (0.0)	716.9 (0.0)	1745.6 (0.0)

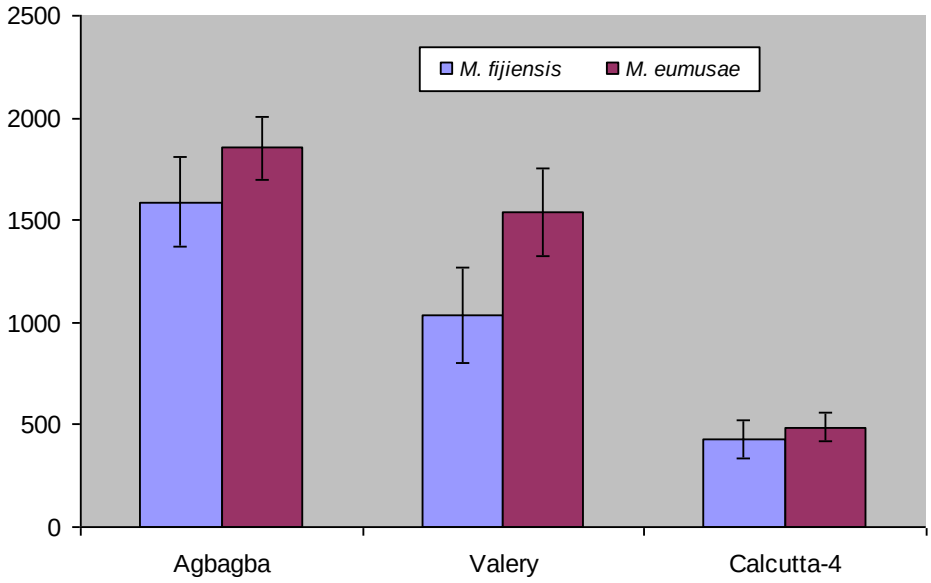


Figure 3.6 Distribution of aggressiveness of the two *Mycosphaerella* species (*M. fijiensis* & *M. eumusae*) on the three varieties (Agbagba, Valery, Calcutta- 4)

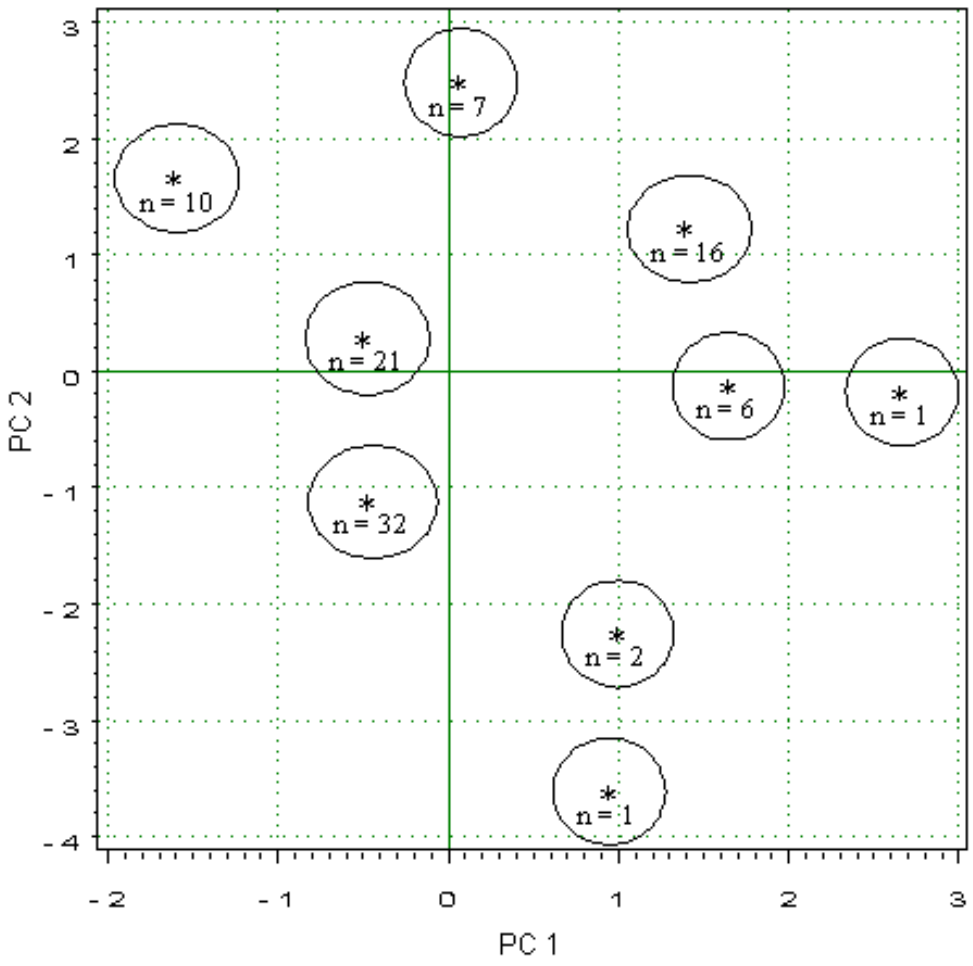


Figure 3.7.A Plots of clusters obtained from PCA and cluster analysis of the 96 Isolates of *Mycosphaerella* spp

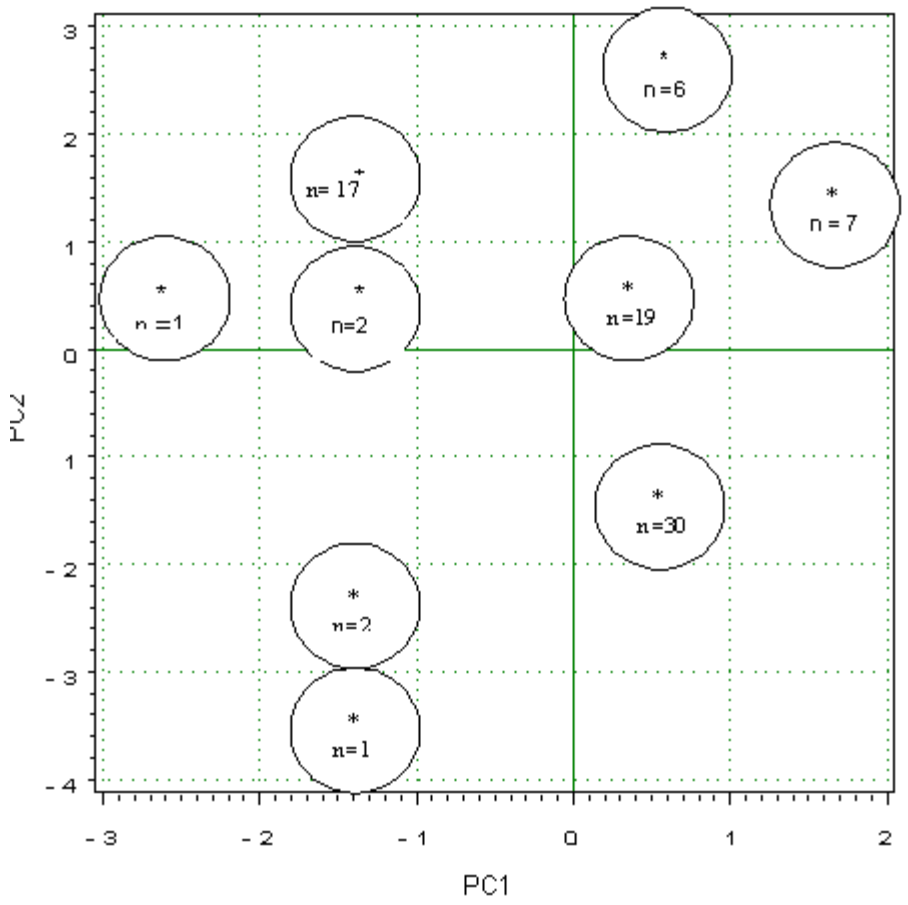


Figure 3.7B Plots of clusters obtained from PCA and cluster analysis of the 85 isolates of *M. fijiensis*

3.3.3. Geographical Distribution of Pathotype Clusters

The geographical distribution of the clusters showed that the clusters were distributed within the two agroecological zones. The frequency of individuals in the first three pathotypes is higher in the HF while the frequency from IV to VI is higher in the DS. Higher frequency of isolates belonging to the most aggressive (cluster V) is found in the DS and the cluster IV, while clusters VIII and IX, the least virulent, were found in the HF (Fig. 3.7). Shannon's index for pathogenic diversity within the agroecological zones, indicated high variability with *H'* ranging from 1·80 to 1·50 for the DS and the HF respectively. At species level, *H'* varies from 1·2 to 0·73 for *M. eumusae* in the DS and the HF respectively while with *M. fijiensis,* it varies from 1·73 to 1·20 in DS and HF zones, respectively.

In general Shannon's index is high in the DS compared to the HF (Table 3.9). The relative abundance of the different types in the sample (Evenness) of the two species is higher (0·46) for the DS and lower (0·39) for the HF zone. The richness, representing the number of different types divided by the number of individuals in a sample, is high for *M. eumusae*. Compared to *M. fijiensis, M. eumusae* displayed a higher evenness and higher richness in the two zones.

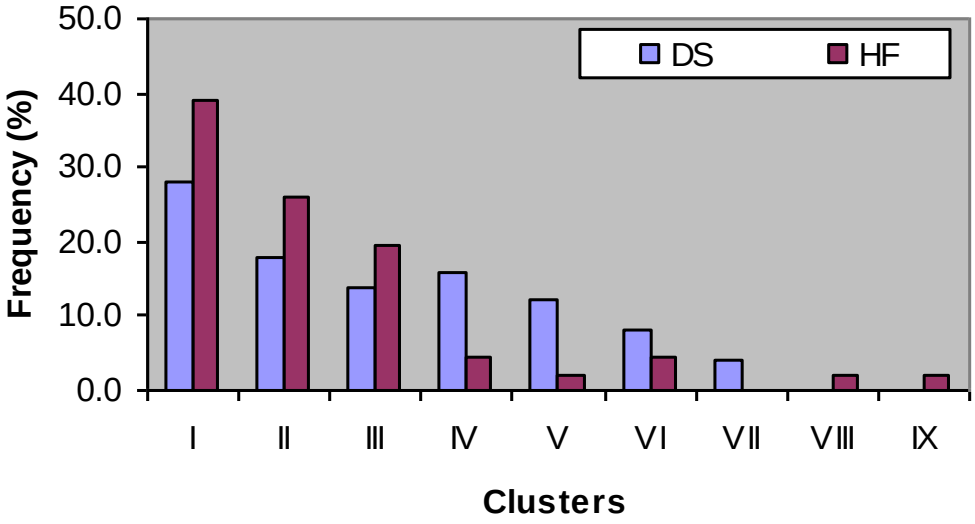


Figure 3.8 Clusters distribution of the 96 isolates in the two agroecological zones

Table 3.10 Normalized Shannon's index and other parameters for pathogenic diversity within the two agroecological zones

	Derived savanna			Humid forest		
	M. spp.	*M. fijiensis*	*M. eumusae*	*M*. spp.	*M. fijiensis*	*M. eumusae*
Shannon's index (*H'*)	1·81	1·73	1·2	1·50	1·20	0·73
Es	0·46	0·46	0·58	0·39	0·32	0·66
D	0·18	0·22	0·75	0·20	0·22	2·00

DS= Derived Savanna; HF= Humid Forest

Es = Evenness; *D* = Richness

DISCUSSION

This work examines and increases knowledge on the distribution, identification, population structure, as well as pathogen variability of *Mycosphaerella* spp. in a pathogen collection isolated from bananas in four geographic zones of Nigeria located in three agroecological zones. Banana fields infected with leaf spot disease were represented more in the HF than in the DS. There was a variation in disease severity in the different regions within the three years of survey. The overall disease severity was similar in the HF (27·1%) and the DS (25·4%), meaning the climatic conditions prevailing in the two zones in that period of collection were the same. The two zones have a bimodal rainfall regime (1500 mm and 2000mm) with mean temperature between 25 to 35 °C and from 27·4°C to 31·89°C for the DS and the HF respectively, conditions known to be suitable for the development of the disease. Report has shown that the temperature most favourable for the incubation period and disease severity was 26°C (Romero & Sutton, 1997). This observation is in opposition with the study of genetic diversity of *Fusarium graminearum* and *F. asiaticum* on wheat spikes throughout China where differences in the disease severity were observed among locations and have been attri buted mostly to differences in the amount of inoculation and variation among rainfall, temperature and humidity (Li *et al*., 2008).

Using molecular tools, *Mycosphaerella fijiensis* (black sigatoka) was the most predominant pathogen followed by *M. eumusae* (*Septoria* leaf spot), but *M. musicola* (yellow sigatoka) and *M. musae* (leaf speckle) were not found in the collection. Intraspecific variations were less in *M. fijiensis* compared to *M. eumusae*. To the best of our knowledge, the isolate collection used in the present study represents the largest population ever used to determine the genetic diversity of *Mycosphaerella* infecting banana and plantain within any given country. Furthermore, the inclusion of ribosomal DNA sequences from GenBank accessions from a wide range of geographical locations further broadened comparisons, thus adding an intercontinental dimension to this study. Previous studies involving intercontinental

comparisons of *Mycosphaerella* isolates (Neu *et al.,* 1999; Carlier *et al.,* 2000a; Goodwin *et al.,* 2001; Rivas *et al.,* 2004) chose a few Nigerian isolates as representative of *Mycosphaerella* from Africa.

Species-specific SNPs and Indels identified isolates of *M. fijiensis* and *M. eumusae* available in our collection without ambiguity, with the latter species displaying a greater inter-specific differentiation. The identification of species based on sequence polymorphisms in ribosomal DNA was confirmed by species-specific probes designed on the actin and the β-tubulin genes (Arzanlou *et al.,* 2007). However, because the PCR probes produced DNA bands of the same size within species (around 500 bp for *M. fijiensis* isolates and 650 bp for *M. eumusae* isolates) the probes could not distinguish among haplotypes of the same species, as revealed through the ribosomal DNA sequence analysis. These probes can probably reveal differences among haplotypes if SNPs and Indels specific to strains within species are located at the 3'-end of the primers. Haplotypes may relate to strain behavior if they are correlated with epidemiological or pathogenicity traits. *Mycosphaerella* species were introduced in Africa during the 1930s (*M. musicola*) and the 1960s (*M. fijiensis*) (Rhodes, 1964) and since then, they have been continuously spreading to new *Musa* growing areas. The absence of *M. musicola* in our random sample may be due to chance alone or to the fact that *M. musicola* is being effectively replaced by *M. fijiensis* in the collection zones, as previously reported in several other banana and plantain producing areas (Leach, 1964; Mouliom-Pefoura *et al.,* 1996; Carlier *et al.,* 2000a, b). These findings are also consistent with the predominance of *M. fijiensis* species at lower altitudes, such as in Nigeria. The weak bootstrap value (59%) for the separation of *M. fijiensis* isolates indicates close relationships within that species.

The greater differentiation observed in *M. eumusae* compared to *M. fijiensis* suggests that *M. eumusae* has been present in Nigeria for a longer period of time than *M. fijiensis* and/or the species is evolving, mostly through sexual reproduction and frequent recombination. The extent of the representation of *M. eumusae* in our collection and its geographical distribution show that this newly reported species may be more widespread

than previously thought, since the collection base on which *M. eumusae* was discovered included isolates from India (2), Sri Lanka (2), Thailand (4) Malaysia (1), Vietnam (1), Mauritius (1) and Nigeria (2) (Carlier *et al*., 2000b).

Visual symptoms of leaf spot disease caused by *M. eumusae* are similar to those of black Sigatoka caused by *M. fijiensis,* except at the early stages of lesion development (Crous and Mourichon, 2002). Therefore, it is possible that *M. eumusae* has been mistaken in the past for *M. fijiensis*. It is important to note that *M. eumusae* isolates constituted only 11% of the entire collection, as opposed to *M. fijiensis* isolates which were the majority (88%) in our collection. However, significant damage due to *Septoria* leaf spot disease caused by *M. eumusae* was observed in Southeast Asia (Carlier *et al.,* 2000b). Due to the difficulty in separating *M. fijiensis* from *M. eumusae* based on leaf symptoms, SNPs that identify and differentiate these two *Mycosphaerella* species and strains (haplotypes) within species ensure that banana breeding populations are evaluated for resistance against a desired and known pathogen population. Where a specific *Mycosphaerella* species is of quarantine importance, such as in Australia (Henderson *et al*., 2006), the success of an eradication campaign relies on early and accurate detection and identification of the pathogen of interest while the disease spread is still limited. Correct identification of the species involved is highly important as this determines whether or not to destroy infected plantations.

The 96 isolates analyzed in this study merged into 21 haplotypes only. It has been reported that most colonization involves founder effects that can lead to reduced haplotype diversity from the original population (Nei *et al.,* 1975). Founder effects, windborne ascospores and gene flow through the movements of infected suckers and leaves may have contributed to the genetic structuring observed in this report. In view of changing agricultural trade and cultivation practices, the presence of genetically distinct but less diverse populations should be of concern for plant pathologists and considered in disease management strategies, particularly in resistance breeding and the movement of germplasm. If the low intraspecific genetic diversity has any relationship with pathogenicity and aggressiveness genes, one of the consequences would be that a few genetically diverse

isolates of *M. fijiensis* and *M. eumusae,* representing several agroecologies, can be used to challenge a range of banana breeding populations, including those with black sigatoka resistance, for developing banana clones with stable resistance to both *Mycosphaerella* species in several banana growing regions. The isolates used in our study were collected from several banana cultivars, primarily landraces, several of which are susceptible to leaf spot diseases. It is likely that more growers will adopt black Sigatoka resistant cultivars with support from national governments and donor agencies (Faturoti *et al*., 2007), and this change in cultivar scenario will affect *Mycosphaerella* population structure through selection pressure, as demonstrated in other pathosystems (McDonald and Linde, 2002). A combination of approaches using sequence diversity at several loci and pathogenicity assays might be ideal in determining the intra-specific and inter-specific evolutionary potential of *Mycosphaerella* population as a result of the adoption of various disease management practices.

Phylogenetic analysis revealed the composition of sub-clades by isolates from different geographical regions in Nigeria. This may be a consequence of intermatings between such isolates introduced from distant regions and the resident isolates of the respective regions. In addition, the presence of identical isolates may represent clones introduced by transfer of *Musa* suckers from one region to another by farmers or breeders, by diseased leaves that were used as wraps for food and goods, as suggested previously by Rivas *et al.,* (2004), or through long distance aerial dispersal of *Mycosphaerella* ascospores (Stover, 1962). The geographical map of haplotypes (Fig. 3.3) generated in this study provides a visual and practical aid to breeders, scientists and quarantine services in targeting specific disease management options. Such a map when complemented with the pathogenicity ratings can aid in monitoring the dynamics of virulent strains and haplotypes at regional levels. The finding of sequence variations that are unique to Nigerian isolates of *M. fijiensis* indicates that large collections are necessary to capture the diversity and population complexities within countries or regions. SNP and Indel of ribosomal DNA sequences were used because of their efficiency to discriminate at the intra- and inter-specific levels

compared to a number of molecular markers and classical morphology descriptors. The presence of registered ribosomal DNA sequences of worldwide origins from *Mycosphaerella* species allows crosschecking and use of sequence variations that are shared among worldwide isolates of the same species.
However SNPs while permitting good assessment of pathogen population structure do not necessarily provide information on pathogenic variation.

Three banana varieties were used to classify pathogenic variation in the 85 identified isolates of *M. fijiensis* and the 11 identified *M. eumusae*. Summary of the Rank-sum method and the PCA of each isolate on each of the varieties partitioned the isolates into different aggressiveness levels (HA, MA and LA groups). It is important to mention that classification based on the Rank-sum methods cannot replace the PCA classification but the two methods complemented each other. The result showed that most of the isolates are aggressive on Agbagba and Valery, two commonly used varieties, while aggressiveness is very low with Calcutta-4. The same reaction has been reported by Twizeyimana *et al.,* (2007) when Agbagba and Calcutta-4 were inoculated with mycelia and conidial suspension. Based on the similarity in reaction to *M. fijiensis* and *M. eumusae* infection, these two cultivars might not be used as references for further analysis of host differential selection. According to Fullerton & Olsen (1995), to detect pathogenic diversity, the host genotypes used must be able to discriminate among strains of the organism. Cultivars which are consistently susceptible are of limited value, other than as susceptible checks. In comparison with the aggressiveness of the two species, significant differences were observed among isolates within each species. The significant genetic differentiation was observed in the population of *M. fijiensis* and *M. eumusae* with higher intra-specific dissimilarity in the *M. eumusae* group (Zandjanakou-Tachin *et al*., 2009). This may be a factor that determines the different level of aggressiveness due to limited gene flow between populations. The significant differences may also be explained by the (i) variation in host responses with interaction of the two species, (ii) the historic cultivation of highly susceptible cultivars and the susceptibility in wild bananas, exerting little selection pressure for adaptation on the *Mycosphaerella* population.

The multivariate analysis using PCA revealed 9 pathotypes to represent the existing range of pathogen aggressiveness, confirming the pathogenic variation within the two agroecological zones in Nigeria. Except for pathotypes VIII and IV that were exclusively found in the HF, there is a distribution of the other group in the two zones, although most of the isolates belonging to the aggressive group were found in the DS. The variation in humidity that usually characterized the DS may induce aggressiveness in the isolates of that region, as an adaptation faculty making them more aggressive than the homologue from the HF. This distribution can also be explained by the host-pathogen co-evolution in the two zones. *Mycosphaerella* spp. was considered to be a highly variable organism and having a high rate of mutation. It has been reported that with isolates such as *Septoria tritici*, that possess a high level of genetic variability, the presence of new cultivars that carry different resistance gene arrays determines the selection of new aggressive factors in the pathogen. Also, the development of new strains of the pathogen may appear, bearing the ability to break the resistance of the new cultivar. These new strains will then disseminate at the same place in which the new cultivars are extensively planted, allowing them in a short period of time to become susceptible (Gieco *et al*., 2004).

Genetic diversity of *Mycosphaerella* spp are known to be highly variable, and also there is a pathogenic variation, meaning breeding for resistance should be continuous process either with single strain or multiple strains for durable resistant. However, there is no strong association between aggressiveness and genetic grouping, meaning that the aggressiveness gene might have arisen independently within each genetic group. It has been reported that association between molecular markers and virulence patterns in plant pathogens can be perfect, partial, or absent (Leung *et al*., 1993) and close association between virulence and selection-neutral molecular markers, such as RAPD, has been recorded in some pathogens.

CONCLUSION

From the different surveys, it was confirmed that leaf spot diseases caused by *Mycosphaerella spp* are present in two different agroecological zones in Nigeria. The disease severity is high in the HF compared to the DS.

The Nigerian isolates were identified without ambiguity using Species-specific SNP or Indels. All the isolates grouped with the reference checks of *M. fijiensis* or the reference checks of *M. eumusae,* showing that only two species were present in our collection.

Species-specific sequence variations that were shared by the reference checks of *M. musicola* from different origins were not present in any of our *Mycosphaerella* spp. collection. That allows the conclusion that no *M. musicola* is present in our collection.

The assessment of sequence variants showed that most SNP occurred among species based on SNP and Indels.

Four major clades, each with sub-clade were delimited in the UPGMA dendrogram. Although a certain level of inter-specific diversity was observed, some clones displayed a total similarity to one another within the same species with *M. eumusae* displaying a higher intraspecific diversity than *M. fijiensis*.

The analysis of molecular variance and pairwise comparison of genetic distances of the four geographical zones confirmed that significant genetic differentiation exists.

In conclusion, the finding of sequence variations that are unique to Nigerian isolates of *M. fijiensis* indicates that large collections are necessary to capture the diversity and population complexities within countries or regions.

SNP and Indel of ribosomal DNA sequences were used because of their efficiency to discriminate at the intra- and inter-specific levels compared to a number of molecular markers and classical morphology descriptors.

The presence of registered ribosomal DNA sequences of worldwide origins from *Mycosphaerella* spp. and the elaboration of the map allows crosschecking and the use of sequence variations that are shared among worldwide isolates of the same species.

The sequence variants revealed in this report can be used in diagnostics for the detection of the pathogens at inter- and intraspecific levels, as well as at the geographical levels.

All the 96 isolates were pathogenic to the three varieties of banana selected. A significant difference was detected among cultivars and isolates and the interaction cultivars X isolates.

The level of aggressiveness is high with Agbagba and Valery and low with Calcutta-4, making Calcutta-4 to behave as if resistant and the two first as if susceptible.

PCA and cluster analysis revealed different aggressiveness groups: The 85 *M. fijiensis* isolates grouped in nine pathotypes and the 11 *M. eumusae* isolates grouped in 7 pathotypes.

Investigations to assess the extent of the genetic variability, the geographical spread, and the pathogenicity of the fungus are necessary in banana-growing regions worldwide.

In addition, the current emphasis of breeding for black Sigatoka resistance should be expanded to include resistance to *M. eumusae*.

The information based on pathotypes provided in this study should enable West African pathologists and breeders to screen breeding material more accurately for resistance to *M. fijiensis* and *M. eumusae*.

It is anticipated that this study will form the basis of selecting and releasing sigatoka resistant banana cultivars in West Africa, and that the severe yield and quality losses currently experienced will be minimized. It should be emphasized, however, that continual monitoring of these pathogens is necessary to provide meaningful contributions to banana sigatoka control.

In another word, the diagnostic tools will allow (a) early detection and intervention, (b) less fungicide used in banana production, (c) better protection of the environment and farmers' health, (d), better quarantine decisions and (e) better selection pressure in breeding programs.

REFERENCES

Alexander B, Mkandawire C, Robert B Mabagala, Pablo Guzmán, Paul Gepts, Gilbertson RL, 2004. Genetic diversity and pathogenetic diversity and pathogenic variation of common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) suggest pathogens coevolution with the common bean. *American Phytopathology* **94,** 593–603.

Alexoupoulos CJ, Mims CW, Blackwell M, 1996. Introductory Mycology. Four Edition John Wiley & Sons, Inc., New York pp 440–442.

Anonymous, 1995. Musanews. *Infomusa* **4**, 26–30.

Anonymous, 2005. All is good with the banana tree. *Spore* (FRA) 118:3

Aptroot, A., 2006. *Mycosphaerella* and its anamorphs:2. Conspectus *Mycosphaerella* and its anamorphs: 2. Conspectus of M*ycosphaerella*. *CBS Biodiv. Series* **5**, 1–231.

Arias P, Dankers C, Liu P, Pilkauskas P, 2003. Technology developments In: Arias P, Dankers C, Liu P, Pilkauskas P, eds. *The world banana economy 1985–2002*.

Arzanlou M, Abeln ECA, Kema GHJ, Waalwijk C, Carlier J, de Vries I, Guzman M, Crous PW, 2007. Molecular diagnostics for the Sigatoka disease complex of banana. *Phytopathology* **97**, 1112–1118.

Balint-Kurti PJ, May JD, Churchill ACL, 2001. Development of a transformation system for *Mycosphaerella* pathogens of banana: a tool for the study of host/pathogen interactions, *FEMS Microbiology Letters* **195**, 9–15.

Banke S, Peschon A, McDonald BA, 2004. Phylogenetic analysis of globally distributed *Mycosphaerella graminicola* populations based on three DNA sequence loci. Fungal Genetic Biology **41,** 221–238.

Barr, ME, 1972. Preliminary studies on the Dothideales in temperate North America. Contributions from the University of Michigan Herbarium **9,** 523–638.

Brown JKM, Hovmoller MS, 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* **297**, 537–541.

Brun J, 1962. Etudes preliminaries sur l'utilisation des varietes de bananiers resistants dans la lutte contre la cercosporiose. *Fruit* **10**, 113–119.

Bucheli EB, Gautschi B, Shykoff JA, 2001. Differences in population structure of the anther smut fungus *Microbotryum violaceum* on two closely related host species, Silene *latifolia* and *S. dioica*. *Molecular ecology* **10**, 285–294.

Buddenhagen IW, 1962. Bacterial wilt of certain seed bearing *Musa* spp. Caused by the tomato strain of *Pseudomonas solanacearum*. *Phytopathology* **52**, 286.

Campbell CL, Madden LV, 1990. Introduction to Plant Disease Epidemiology New York: John Wiley and Sons NY 532 pp.

Carlier J, Foure E, Gauhl F, Jones DR, Lepoivre P, Mourichon X, Pasberg-Gauhl C, Romero RA, 2000a. Sigatoka leaf spots. In: Jones DR, eds. *Diseases of Banana*, *Abaca and Ensete*. New York: *CABI Publishing*, 37–92.

Carlier J, Hayden H, Rivas G, Zapater M-F, Abadie C, Aitken E, 2002. Genetic differentiation in *Mycosphaerella* leaf spot pathogens In: Jacôme L, Lepoivre P, Marin DH, Ortiz R, Romero RA, Escalant JV, eds. *Mycosphaerella Leaf Spot Diseases of Bananas: Present Status and Outlook,* 2002. Montpellier, France: *INIBAP*, 123–129.

Carlier J, Lebrun MH, Zapater MF, Dubois C, Mourichon X, 1996. Genetic structure of the global population of banana black leaf streak fungus *Mycosphaerella fijiensis*. *Molecular Ecology* **5**, 499–510.

Carlier J, Mourichon X, Gonzales de Leon D, Zapater MF and Lebrun MH, 1994. DNA restriction fragment length polymorphisms in *Mycosphaerella* species causing banana leaf spot diseases. *Phytopathology* **84,** 751–756.

Carlier J, Zapater MF, Lapeyre F, Jones DR, Mourichon X, 2000b. *Septoria* leaf spot of banana: A newly discovered disease caused by *Mycosphaerella eumusae* (anamorph *Septoria eumusae*). *Phytopathology* **90,** 884–890.

Cheesman EE 1947. Classification of the bananas II. The genus *Musa* L. *Kew Bulletin* **2**, 106–117.

Cheesman EE, Wardlaw CW, 1937. Specific and varietal susceptibility of bananas to *Cercospora* leaf spot. *Tropical Agricuture* (Trinidad) **12**, 335–336.

Chukwu UE, 1996. Effects of postharvest injury on shelf-life and extrusion processing of *Musa* spp. fruits. PhD thesis, University of Ibadan, Nigeria.

Cooke DEL, Drenth A, Duncan JM, Wagels G, Brasier CM, 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* **30,** 17–32.

Cooke DEL, Schena LI, Cacciola SO, 2007. Tools to detect identify and monitor *Phytophthora* species in natural ecosystems. *Journal of Plant Pathology* **89,** 13–28.

Cordeiro ZJM, 1990. Economic impact of Sigatoka disease in Brazil Pp. 56–60 in Sigatoka leaf spot diseases Fullerton RA, Stover RH (eds.). Montpellier, Inibap, France.

Cordeiro ZJM, Pires de Matos, 2002. Impact of *Mycosphaerella* spp. in Brazil, In: Jacôme L, Lepoivre P, Marin DH, Ortiz R, Romero RA, Escalant JV, eds. *Mycosphaerella Leaf Spot Diseases of Bananas: Present Status and Outlook,* 2002. Montpellier, France: *INIBAP*, 91–97.

Côte FX, Tomekpe K, Staver C, Depigny S, Lescot T, Markham R, 2008. Agroecological Intensification in Banana & Plantain: approach to develop more sustainable cropping system. Banana & Plantain in Africa, International Conference Monbassa, Kenya. October 5-9. (Oral presentation)

Craenen K, 1998. Black Sigatoka disease of banana and plantain: A reference manual IITA-Ibadan. 60pp.

Crous PW, 1998. *Mycosphaerella* spp. and their anamorphs associated with leaf spot diseases of *Eucalyptus*. *Mycologia Memoir* **21**, 1–170.

Crous PW, Braun U, Groenewald JZ, 2007. *Mycosphaerella* is polyphyletic. *Studies in Mycology* **58**, 1–32.

Crous PW, Groenewald JZ, Aptroot A, Braun U, Mourichon X, Carlier J, 2002. Integrating morphological and molecular data sets on *Mycosphaerella*, with specific reference to species occurring on *Musa*. In: Jacôme L, Lepoivre P, Marin DH, Ortiz R, Romero RA,

Escalant JV, eds. *Mycosphaerella Leaf Spot Diseases of Bananas: Present Status and Outlook,* 2002. Montpellier, France: INIBAP, p 43–57.

Crous PW, Johannes Z, Groenewald J, Mansilla P, Hunter GC, Wingfield MJ, 2004. Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*. *Studies in Mycology* **50,** 195–214.

Crous PW, Mourichon X, 2002. *Mycosphaerella eumusae* and its anamorph *Pseudocercospora eumusae* spp. novel: causal agent of eumusae leaf spot disease of banana. *Syndowia* **54,** 35–43**.**

Crous PW, Wingfield MJ, Mansilla JP, Alfenas AC, Groenewald JZ, 2006. Phylogenetic reassessment of *Mycosphaerella* spp., and their anamorphs occurring on *Eucalyptus* II. *Studies in Mycology* **55,** 99–131.

Crous, P. W., & Braun, U. 2003. *Mycosphaerella* and its anamorphs. 1. Names published in *Cercospora* and *Passalora*. *CBS Biodiversity, Series* **1**, 1–571.

Crous, P. W., Aptroot, A., Kang, J. C., Braun, U., & Wingfield, M. J. 2000. The genus *Mycosphaerella* and its anamorphs. *Studies in Mycology* **45**, 107–121.

Crous, P. W., Kang, J. C., Braun, U. 2001. A phylogenetic redefinition of anamorph genera in *Mycosphaerella* based on ITS rDNA sequence and morphology. *Mycologia* **93**, 1081–1101.

Daniells JW, Peterson RA, Reid DJ, Bryde NJ, 1996. Screening of 165 Papua New Guinea banana accessions for resistance to yellow Sigatoka (*Mycosphaerella musicola* Leach) in North Queensland. *Infomusa* **5**, 31–34.

Dassanayake EM, Rathnabharathi BM, 2002. Development of virus free banana foundation stock. *Annals of the Sri Lanka department of Agriculture* **4**, 255–267.

Essiaba RO, 1987. Economic importance of plantains and bananas. Pp 18–21. In: Black Sigatoka disease of plantain and banana in Nigeria. Proceedings of an Interdisciplinary Workshop held at the Federal Secretariat, Port-Harcourt 7th -10th Dec. 1987.

Etebu E, Pasberg-Gaugh F, Gauhl F, Daniel Kalio A, 2005. Effect of light and sealing pattern on sporulation and growth of *Mycosphaerella fijiensis*. *Infomusa* **14**, 24–25.

Excoffier L, Laval G, Schneider S, 2005. Arlequin version 3.01 an integrated software package for population genetics data analysis. *Evolution and Bioinformatics* Online [http://www.cmpg.unibe.ch/software/arlequin3/#HowToCite].

Excoffier L, Smouse PE, Quattro JM, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes; Application to human mitochondrial DNA restriction sites. *Genetics* **131,** 479–491.

FAO AGROSTAT Database, 2004. Food and Agriculture Organization of the United Nations. Production yearbook. FAO Rome.

FAOSTAT, 2005. FAO Statistics Division 2006. Food and Agriculture Organization of the United Nations. Production yearbook. FAO Rome.

FAO 2004, Faostat homepage. www.faostat.fao.org/faostat/collection Food and Agriculture Organisation.

FAOSTAT 2006, FAO Statistics Division 2006. Food and Agriculture Organization of the United Nations. Production yearbook. FAO Rome.

FAO 2007. Faostat homepage www.faostat.fao.org/faostat/collection

Faturoti BO, Madukwe MC, Tenkouano A, Agwu AE, 2007. A review of policy acts and initiatives in plantain and banana innovation system in Nigeria. *African Journal of Biotechnology* **6**, 2297–2302.

Feau N, Hamelin RC, Bernier L, 2006. Attributes and congruence of three molecular data sets: inferring phylogenies among *Septoria*-related species from woody perennial plants. *Molecular Phylogenetic Evolution* **40**, 808–829.

Felsenstein J, 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39,** 783–791.

Fortune MP, Gosine S, Chow S, Dilbar A, Hill AS, Gibbs H. Rambaran N, 2005. First report of black sigatoka disease (causal agent *Mycosphaerella fijiensis)* from Trinidad *Plant Pathology* **54**, 246.

Fraaije B.A., Lovell D.J, Rohel E.A., Hollomon D.W., 1999. Rapid detection and diagnosis of *Septoria tritici* epidemics in wheat using a polymerase chain reaction/Pico green assay. *Journal of Applied Microbiology* **86,** 701–708.

Franchet AR. 1889. Un nouveau type de *Musa*, *Musa lasiocarpa*. *Journal de Botanique* **3**, 329.

Frison E, Sharrock S, 1998. The economic, social and nutritional importance of banana in the world. *In* Picq C, Fouré E, Frison, and EA eds. Bananas and Food Security:Les productions bananières : un enjeu économique majeur pour la sécurité alimentaire. International symposium, Douala, Cameroon, 10-14 November 1998. 21–35.

Frossard P, 1980. Apparition d'une nouvelle et grave maladie foliaire des bananiers et plantains au Gabon: la maladie des raies noires: *Mycosphaerella fijiensis* Morelet, *Fruit* **45**, 339–345.

Fullerton RA, Olsen TL, 1991. Pathogenic variability in *Mycosphearella fijiensis* Morelet. *In* Valmayor RV, Umali BE, Bejosano CP, eds. *Banana diseases in Asia and the Pacific*: Proceedings of a technical meeting on diseases affecting banana and plantain in Asia and the Pacific. Montpellier, France: INIBAP, 105–115.

Fullerton RA, Olsen TL, 1995. Pathogenic variability in *Mycosphaerella fijiensis* Morelet, cause of black sigatoka in banana and plantain. *New Zealand Journal of Crop and Horticultural Science* **23**, 39–48.

Fusari CM, Verónica VL, Esteban Hopp H, Heinz RA, Norma B P, 2008. Identification of Single Nucleotide Polymorphisms and analysis of Linkage Disequilibrium in sunflower elite inbred lines using the candidate gene approach BMC *Plant Biology*; **8**, 7. Published online 2008 January 23. doi: 10.1186/1471-2229-8-7.

Gauhl F, Pasberg-Gauhl C, Vuylsteke D, Ortiz R, 1993. Multilocational evaluation of black sigatoka resistance in banana and plantain. Research Guide 47, IITA, Ibadan Nigeria, 60pp.

Gieco JO, Dubcovsky J, Eduardo L, Camargo A, 2004. Aggressiveness and physiological specialization of *Septoria tritici* Rob. Isolates. *Scienta Agriola* **61,** 414–421.

Goodwin SB, Dunkle LD, Zismann VL, 2001. Phylogenetic analysis of *Cercospora* and *Mycosphaerella* based on the internal transcribed spacer region of ribosomal DNA. *Phytopathology* **91,** 648–658.

Graham KM, 1969. A simple way to distinguish black leaf streak from sigatoka disease on bananas. *Bulletin of the Department of Agriculture*, *Fiji*, 49, 4.

Groppe K, Boller T, 1997. PCR assay based on a microsatellite markers-containing locus for detection and quantification of epichloe endophytes in grass tissue. *Applied and Environment Microbiology* **63**, 1543–1550.

Häkkinen M, 2009. *Musa chunii* Häkkinen, a new species (Musaceae) from Yunnan, China and taxonomic identity of *Musa rubra*, *Journal of Systematics and Evolution* **47**, 1–5.

Hayden HL, Carlier J, Aitken EAB, 2003. Population differentiation in the banana leaf spot *Mycosphaerella musicola*, examined at a global scale. *Plant Pathology* **52**, 713–719.

Hayden HL, Carlier J, Aitken EAB, 2005. The genetic structure of Australian populations of *Mycosphaerella musicola* suggests restricted gene flow at the continental scale. *Phytopathology* **95,** 489–498.

Hayward AC, 1964. Characteristics of *Pseudomonas solanacearum*. *Journal of Applied Bacteriology* **27**, 265–277.

He LY, Sequeira L, Kelman A, 1983. Characteristics of strains of *Pseudomonas solanacearum* from China. *Plant Disease* **67**, 1357–1361.

Henderson J, Grice K, Pattemore J, Peterson R, Aitken E, 2003. Improved PCR-based detection of sigatoka disease and black leaf streak disease in Australian banana crops. In: Jacôme L, Lepoivre P, Marin DH, Ortiz R, Romero RA, Escalant JV, eds. *Mycosphaerella Leaf Spot Diseases of Bananas: Present Status and Outlook,* 2002. Montpellier, France: INIBAP, 59–64.

Henderson J, Pattemore JA, Porchun SC, Hayden HL, Van Brunschot S, Grice KRE, Peterson RA, Thomas-Hall SR, Aitken EAB, 2006. Black sigatoka disease: new technologies to strengthen eradication strategies in Australia. *Australasian Plant Pathology* **3**, 181–193.

Horanonow PF, 1862. Prodomus Monographiae Scitaminarum. Petropoli: Academiae Caesareae Scientiarum (in Latin).

Hoog GS de, Hijwegen T, Batenburg-Van der Vegte WH, 1991. A new species of *Dissoconium*. *Mycological Ressource* **95,** 679–682.

Hunter GC, Wingfield BD, Crous PW, Wingfield MJ, 2006. A multi-gene phylogeny for species of *Mycosphaerella* occurring on *Eucalyptus* leaves. *Studies in Mycology* **55**, 147–161.

IITA, 1990. (International Institute of Tropical Agriculture) Annual reports 1989/1990 Ibadan, Nigeria.

Ikotun T, 1987. The biology and life cycle of *Mycosphaerella fijiensis* and *M. musicola* and the symptoms of the Sigatoka diseases. In black sigatoka disease of plantains and bananas in Nigeria. Proceedings of an interdisciplinary workshop held at the Federal Secretariat, Port Harcourt, 7-10 December. Pp. 42–49.

Inyang E, 1987. Black sigatoka and the plantain industry in Nigeria. In: Black sigatoka disease of plantains and bananas in Nigeria. Proceedings of an interdisciplinary workshop held at the Federal Secretariat, Port Harcourt, 7 -10December. Pp. 11–15.

Jackson, SL., Maxwell A., Neumeister-Kemp HG, Dell B, Hardy GE StJ. 2004. Infection, hyperparasitism and conidiogenesis, of *Mycosphaerella lateralis* on *Eucalyptus globulus* in Western Australia. *Australian. Plant Pathology***. 33**, 49–53.

Jacôme LH, 2002. Population biology and epidemiology In: Jacôme L, Lepoivre P, Marin DH, Ortiz R, Romero RA, Escalant JV, eds. *Mycosphaerella Leaf Spot Diseases of Bananas: Present Status and Outlook,* 2002. Montpellier, France: INIBAP, 107–110.

Jacôme LH, Schuh W, 1993. Effect of temperature on growth and conidia production in vitro, and comparison of infection and aggressiveness in vitro among isolates of *Mycosphaerella fijiensis* var difformis. *Tropical Agriculture* (Trinidad) **70**, 51–59.

Jeger MJ, Eden-Green S, Thresh JM, Johanson A, Waller JM, Brown AE, 1995. Banana diseases, in: Gowen, S. (Ed.), Bananas and Plantains. Chapman & Hall, London, UK, pp. 317–381

Johanson A, Crowhurst RN, Rikkerink EHA, Fullerton RA, Templeton MD 1994. The use of species-specific DNA probes for the identification of *Mycosphaerella fijiensis* and *M. musicola*, the causal agents of Sigatoka disease of banana. *Plant Pathology* **44**, 701–707.

Johanson A, Jeger MJ, 1993. Use of PCR for detection of *Mycosphaerella fijiensis* and *M. musicola*, the causal agents of Sigatoka leaf spots in banana and plantain. *Mycological Research* **97,** 670–674.

Jones DR, 2002. The distribution and importance of the *Mycosphaerella* leaf spot diseases of banana. In: Jacôme L, Lepoivre P, Marin DH, Ortiz R, Romero RA, Escalant JV, eds. *Mycosphaerella Leaf Spot Diseases of Bananas: Present Status and Outlook,* 2002. Montpellier, France: INIBAP, 25–42.

Jones DR, 2000. Introduction to banana, abaca and enset: In Jones DR, editor. Diseases of Banana, Abaca and Enset. Wallingford (UK): *CABI Publishing*, 1–36.

Jones DR, 2003. The distribution and importance of the *Mycosphaerella* leaf spot diseases of banana. Pages 25–42 in: Proceedings of 2nd international workshop on *Mycosphaerella* leaf spot disease of bananas, Costa Rica. Jacome L, Lepoivre P, Marin D, Ortiz R, Romero R, Escalant JV eds. 20–23 May 2002, San José, Costa Rica, INIBAP.

Kobenan K, Gnonhouri GP, Akyeampong E, Tenkouano A, Tomekpe K, 2004. Evaluation of *Musa* hybrids in Côte d'Ivoire In Proceedings of the 1st International Congress on Banana: *Harnessing Research to Improve Livelihoods*, Penang, Malaysia, 6–9 July.

Leach R, 1946. Banana leaf spot ( ) on the Gros Michel Variety in Jamaica, Investigations into the Aetiology of the Disease and the Principles of Control by spraying. Bulletin, Government Printer, Kingston, Jamaica.

Leach R, 1964. A new form of banana leaf spot in Fiji, black leaf streak. *World Crops* **16**, 60–64.

Lescot T, Ganry J, 2008. Plantain cultivation in Africa: Production and research in the past two decades. Banana and plantain in Africa: Harnessing international partnerships to

increase research impact Leisure Lodge Resort, Mombasa, Kenya - October 5–9, 2008.

Leung H, Nelson R, Leach JE, 1993. Population structure of plant pathogenic fungi and bacteria. *Advances in Plant Pathology* **10**, 157–205.

Li HP, Zhang JB, Xu YB, Huang T, Wu AB, Zhao CS, Carter J, Nicholson P, Liao YC, 2008. Geographic distribution and genetic diversity of *Fusarium graminearum* and *F. asiaticum* on wheat spikes throughout China. *Plant pathology* **54**, 15–24.

Lievens B, Claes L, Vanachter ACRC, Cammue BPA, Thomma BPHJ, 2006. Detecting single nucleotide polymorphisms using DNA arrays for plant pathogen diagnosis. *FEMS Microbiology Letters* **255,** 129–139.

Lievens B, Thomma BPHJ, 2005. Recent developments in pathogen detection arrays: *Journal of Botany* **41**, 1531–1532.

Lockhart BEI, 1986. Purification and serology of a bacilliform virus associated with a streak disease of banana, *Phytopathology* **76**, 995**–**999.

Ludwig JA, Reynolds JF, 1988. Statistical ecology: a primer on methods and computing. John Wiley and Sons. New York, USA.

Massee G, 1914. *Fungi exotici*: XVIII. Kew bulletin, 159.

Manzo-Sanchez G, Guzman-Gonzalez S, 2004. Biologia de *Mycosphaerella fijiensis* Morelet y su Interaccion con *Musa* spp. *Revista Mexicana de Fitopathologia* **23**, 87–96

Marín DH, Romero RA, Guzman M, Sutton TB, 2003. Black sigatoka: An increasing threat to banana cultivation. *Plant Disease* **87,** 208–222.

Marriot J, Lancaster PA, 1983. Handbook of Tropical food. Marcel Dekker Inc. NY, USA. 86–234.

Maxwell A, Jackson SL, Dell B, & Hardy GE, StJ. 2005. PCR-identification of *Mycosphaerella* species associated with leaf diseases of *Eucalyptus*. *Mycol. Res*. **109**, 992–1004.

McDonald BA, Linde C, 2002. Pathogen population genetics, evolutionary potential and durable resistance. *Annual Review of Phytopathology* **40**, 349–379.

Meredith DS, Lawrence JS, 1969. Black leaf streak disease of bananas (*Mycosphaerella fijiensis*): Symptoms of disease in Hawaii, and notes on the conidial state of the causal fungus. *Transactions of the British Mycology Society* **52**, 459 –476.

Meredith DS, Lawrence JS, 1970. Black leaf streak disease of bananas (*Mycosphaerella fijiensis*); susceptibility of cultivars. *Tropical Agriculture* **47**, 275–287.

Milgroom MG, Peever TL, 2003. Population biology of plant pathogens: The synthesis of plant disease epidemiology and population genetics. *Plant Disease* **87**, 608–617.

Mobambo KN, Gauhl F, Adeniji MO, Pasberg-Gauhl C and Swennen R. 1993. Yield loss in plantain from black Sigatoka leaf spot and field performance of resistant hybrids. *Field Crop Research* **35**, 35–42.

Molina C, Fabregar E, 2002. Management of black streak disease in Tropical Asia. In: Jacôme L, Lepoivre P, Marin DH, Ortiz R, Romero RA, Escalant JV, eds. *Mycosphaerella Leaf Spot Diseases of Bananas: Present Status and Outlook,* 2002. Montpellier, France: INIBAP, 85–90

Molina C, Kaemmer D, Aponte S, Weising K, Kahl G, 2001. Microsatellite markers for the fungal banana pathogen *Mycosphaerella musicola*. *Molecular Ecology Notes* **1,** 137–139.

Mouliom-Perfoura A, Lassoudière A, Foko J, Fontem DA, 1996. Comparison of development of *Mycosphaerella fijiensis* and *Mycosphaerella musicola* on banana and plantain in the various ecological zones in Cameroon. *Plant Disease* **80,** 950–954.

Mouliom Perfoura A, 1999. First observation of the breakdown of high resistance in Yangambi Km 5 (*Musa* sp.) to the black leaf streak disease in Cameroon. *Plant Disease* **83**, 78.

Mourichon X, Fullerton RA, 1990. Geographical distribution of the two species *Mycosphaerella musicola* Leach (*Cercospora musae*) and *M. fijiensis* Morelet (*C. fijiensis*), respectively agents of Sigatoka disease and black leaf streak disease in bananas and plantains. *Fruits* **45,** 213–218.

Mourichon X, Zapater MF, 1990. Obtention in vitro du stade forme parfaite de *Cercospora fijiensis. Fruits* **45**, 553–557.

Mülder JL, Holliday M, 1974. *Mycosphaerella fijiensis*. Descriptions of Pathogenic fungi and bacteria. Commonwealth Mycological Institute. Kew, Surrey, UK, No. 413.

Müller R, Pasberg-Gauhl C, Gauhl F, Ramser J, Kahl G, 1997. Oligonucleotide fingerprinting detects genetic variability at different levels in Nigerian *Mycosphaerella fijiensis*. *Journal of Phytopathology* **145,** 25–30.

Mullis KB, Faloona FA, 1987. Specific synthesis of DNA in vitro via a polymerase-catalysed chain reaction. *Methods in Enzymol*ogy **155**: 335–350.

Mutasa ES, Chwarszczynska DM, Asher MJC, 1996. Single-tube nested PCR for the diagnosis of *Polymyxa betae* infection in sugar beet roots and colorimetric analysis of amplified products. *Phytopathology* **86**: 493–497

Nei M, 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590.

Nei M, Maruyama T, Chakraborty R, 1975. The bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.

Neu C, Kaemmer D, Kahl G, Fischer D, Weising K, 1999. Polymorphic microsatellite markers for the banana pathogen *Mycosphaerella fijiensis*. *Molecular Ecology* **8,** 523–525.

Njoku JE, 1990. Socio economic aspects of plantain in the humid tropics. Plantain research and technology transfer, IITA, Ibadan, 13 pp.

Nkendah R, Akyeampong E, 2003. Socio economic data on the plantain commodity chain in West and Central Africa, *InfoMusa*, **12** (1).

Nweke FI, Njoku JE, Wilson GF, 1988. Productivity and limitations of plantain (*Musa* spp. cv. AAB) Production in compound gardens in Southern Nigeria. *Fruit* **43**, 161–166.

Nürnberger T, Scheel D, 2001. Signal transmission in the plant immune response. *Trends Plant Science*. **8**, 372–379.

Okechukwu RU, Dixon AGO, Akoroda M, Mwangi M, Bandyopadyay R, 2008. Root rot resistance in new cassava varieties introduced to farmers in Nigeria. *Experimental agriculture* **44**, 1–10.

Ogazi PO, 1986. Cottage industry for the production of plantain chips in Nigeria. Papers presented at the 8th Annual Conference of the Horticultural Society of Nigeria. (Horton) Nov. 10–14, 1986, Ibadan.

Ortiz R, Vuylsteke D, 1994. Inheritance of black Sigatoka disease in plantain-banana (*Musa* spp.) hybrids. *Theoretical and Applied Genetics* **89**,146–156.

Ortiz R, Vuylsteke D, 1995. Genotype-by –environment interaction in Musa germplasm revealed by multi-site evaluation in sub-Saharan Africa. *HortScience* **30**, 795.

Palleroni NJ, 1994. Pseudomonaceae. In: Krieg NR and Holt JG (eds) Bergey's Manual of systematic bacteriology 1. Williams and Wilkins, Baltimore, pp 143–219.

Palo MA, Calinisan MR, 1939. The bacterial wilt of the Abacá (manila hemp) plant in Davo: I Nature of the disease and pathogenicity tests. *Philippine Journal of Agriculture* **10,** 373–395.

Parham BEV, 1935. Annual report on banana disease investigations, 1934. In Report of the department of Agriculture of Fiji for 1934. Department of Agriculture, Fiji, Suva.

Park RF, Keane PJ, Wingfield MJ, Crous PW, 2000. Fungal diseases of eucalypt foliage. In: Diseases and pathogens of eucalypts. Keane, P. J., Kile, G. A.,Podger, F. D., & Brown, B. N. eds. CSIRO Publishing, Collingwood, Australia. Pp 153–259.

Pasberg-Gauhl C, 1989. Untersuchungen zur symptomentwicklung and bekämphung der schwarzen sigatoka-krankheit (*Mycosphaerella fijiensis* Morelet) an bananen (*Musa* spp.) in vitro and in freiland. Goïhinger Beitrage zur Land-und Froswirstschaft in den tropen und subtropen, Heft 40.

Perea E, Rodriguez A, Marquez E, Arango R, 2005. Genetic diversity of Colombian isolates of *Mycosphaerella fijiensis* Morelet based on microsatellite. *InfoMusa* **14,** 18–21.

Pereira LV, Cordeiro ZJM, Figueira A, Hinz RH, Matos AP. 1999. Doencas da bananeira. informe agropecuario. *Belo Horizonte* **20**, 37–47.

Pérez Vicente L, Alvarez JM, Pérez M, 2002. Economic impact and management of black leaf streak disease in Cuba. In: Jacôme L, Lepoivre P, Marin DH, Ortiz R, Romero RA, Escalant JV, eds. *Mycosphaerella Leaf Spot Diseases of Bananas: Present Status and Outlook,* 2002. Montpellier, France: *INIBAP*, 71–84

Persley GJ, George P, 1999. Commodity Advances through Banana Improvement Research, 1994-1998. Environmentally and socially sustainable development agricultural research and extension group series, The World Bank, Washington, DC, USA. 62pp.

Philpott J, Knowles GH, 1913. Report on a visit to Sigatoka. Pamphlet of the Department of Agriculture, Fiji 3, Suva.

Ploetz R, 2000. Black Sigatoka on bananas. *Pesticide Outline* **11**, 19–23.

Ploetz RC, 2001. The most important disease of a most important fruit. Reviewed feature article. APSnet education Center DOI:10.1094/PHI-I-2001-0126-01

Ploetz RC, Mourichon X, 1999. First report of black sigatoka in Florida. *Plant Disease* **83**, 300.

Pontier D, Balague C, and Roby D, 1998. The hypersensitive response. A programmed cell death associated with plant resistance. *C R Acad Sci III* **9**, 721-34.

Pons N, 1990. Taxonomy of *Cercospora* and related genera. In: Sigatoka leaf spot disease of bananas. Proceedings of an International Workshop held at San Jose, Costa Rica, 28 March-1 April 1989 (ed RA Fullerton & RH Stover) Pp 360-367. INIBAP, Momtpellier, France.

Punithalingam E, 1983. *Phaeoseptora musae*. Description of pathogenic fungi and bacteria No. 772, Commonwealth Mycological Institute, Kew, Surrey, UK.

Research, 1994-1998. Environmentally and socially sustainable development, agricultural research and extension group series, The World Bank, Washington, D.C. 62 pp.

Rhodes PL, 1964. A new banana disease in Fiji. *Commonwealth Phytopathology News* **10,** 38– 41.

Rivas GG, Zapater MF, Abadie C, Carlier J, 2004. Founder effects and stochastic dispersal at the continental scale of the fungal pathogen of bananas *Mycosphaerella fijiensis*. *Molecular Ecology* **13,** 471– 482.

Robinson JC, 1996. Bananas and Plantains. Crop Protection Science in Horticulture 5, CAB International, Wallingford, UK, 238 pp.

Rohlf FJ, 1998. NTSYS-pc version 2.02j. Numerical taxonomy and multivariate analysis system. Exeter software, Setauket, New York, USA.

Romero RA, Sutton TB, 1997a. Reaction of four *Musa* genotypes at three temperatures to isolates of *Mycosphaerella fijiensis* from different geographical regions. *Plant Disease* **81**, 1139–1142.

Romero RA, Sutton TB 1997b. Sensitivity of *Mycosphaerella fijiensis*, causal agent of black Sigatoka, to propiconazole. *Phytopathology* **87**, 96–100.

Roux N, Baurens FC, Dolezel J, Hribova E, Heslop-Harrison JS, Town C, Sasaki T, Matsumoto T, Aert R, Remy S, Souza M, Lagoda PJL, 2008. Genomics of banana and plantain (*Musa* spp.), Major Staple Crops in the Tropics. Genomics of Tropical Crop Plants (Moore, P. and Ming, R. Eds.). *Plant Genetics and Genomics: Crops and Modles* **1**, 83–111.

Rukazambuga NM, 1996. The effects of Banana Weevil *Cosmopolites sordidus* Germar on the growth and productivity of bananas (*Musa* AAA EA) and the influence of host vigour on Weevil attack. PhD thesis, University of Reading, UK.

SAS, 2003. SAS/STAT software, version 9.1. for Windows. Cary, NC, USA.

Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW, 2006. A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia* **98**, 1043–1054.

Shaner G, Finney RE, 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. *Phytopathology* **67,** 1051–1056.

Shanner G, Stronberg EL, Lacy GH, Barker KR, Pirone TP, 1992. Nomenclature and concepts of pathogenicity and virulence. *Annual Review Phytopathology* **30,** 47–66.

Shannon CE, Weaver W, 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana, IL, USA.

Simmonds NW, 1962. The evolution of bananas, Longman, London, UK. 282pp.

Simmonds NW, 1966. Bananas, 2nd ed.. Longmam, Green, London, UK, 512pp.

Simmonds NW, Shepherd, 1955. The taxonomy and origins of cultivated bananas. Journal of the Linnean Society of London, *Botany* **55**, 302–3112.

Stewart EL, Liu Z, Crous PW, Szabo LJ, 1999. Phylogenetic relationships among some cercosporoid anamorphs of *Mycosphaerella* based on rDNA sequence analysis. *Mycological Research* **103**, 1491–1499.

Stover RH, 1962. Intercontinental spread of banana leaf spot (*Mycosphaerella musicola* Leach). *Tropical Agriculture (Trinidad)* **29**, 327–338.

Stover RH, 1963. Sexuality and heterothallism in *Mycosphaerella musicola. Canadian journal of Botany* **41**, 1481–1485

Stover RH, 1971. A proposed international scale for estimating intensity of banana leaf spot. *Tropical Agriculture* (*Trinidad*) **48**, 185–186.

Stover RH, 1972. *Banana, Plantain, and Abaca Diseases*. Commonwealth Mycological Institute Kew, Surrey, UK.

Stover RH, 1976. Distribution and cultural characteristics of the pathogens causing banana leaf spot. *Tropical Agriculture (Trinidad)* **53**, 111–114

Stover RH, 1978. Distribution and probable origin of *Mycosphaerella fijiensis* in Southern Asia. *Tropical Agriculture* (Trinidad) **55**, 65–68

Stover RH, 1983. Effet du *Cercospora* noir sur les plantains en Amerique centrale. *Fruit* **38**, 326–329.

Stover RH, Dickson JD, 1970. Leaf spots of banana caused by *Mycosphaerella musicola*: methods of measuring spotting prevalence and severity. *Tropical Agriculture* (Trinidad) **47**, 289–302.

Stover RH, Simmonds NW, 1987. *Bananas*, 3rd edition Longman Scientific and Technical, Harlow, Essex, UK. 468 pp.

Stover RH, Slabaugh WR, Grove MD, 1978 Effect of chlorothalonil on a severe outbreak of banana leafspot caused by benomyl tolerant *Mycosphaerella fijiensis* var. *difformis Phytopathology News* **12**, 268.

Stukenbrock EH, Banke S, Javan-Nikkham M, McDonal BA, 2007. Origin and domestication of the fungal wheat pathogen *Mycosphaerella graminola* via sympatric speciation. Molecular. Biology. Evolution. **24**, 398–411.

Swennen R, 1990. Plantain cultivation under West African conditions. A reference manual, IITA, Ibadan, Nigeria. 24 pp.

Swennen R, Ortiz R (1997). Morphology and growth of plantain and banana, IITA Research Guide 66. Training Program, International Institute of Tropical Agriculture, Ibadan, Nigeria.

Su HJ, Hwang SC, Ko WH, 1986. *Fusarium* wilt of Cavendish bananas in Taiwan. *Plant Disease* **70**, 814–818.

Su HJ, Tsao LY, Wu ML, Hung TH, 2 2003. Biological and molecular categorization of strains of banana bunchy top virus. *Journal of Phytopathology* **151**, 290–296.

Thomas JE, Iskra-Caruana LV, Magnaye, Jones DR, 2000. Bract mosaic In: Jones DR (ed) Diseases of banana, Abaca, and Ensete, CAB International, Wallingford, UK, 253–255.

Thwaites R, Eden-Green SJ Black R, 2000. In: Diseases of Banana, Abaca and Enset (D.R. Jones, ed.). CAB International, Wallingford, UK. Pp. 213–239.

Tripathi L, 2009. Biotechnology and nematodes. http://r4dreview.org/2009/03/biotechnology-and-nematodes/.

Twizeyimana M, Ojiambo PS, Tenkouano A, Ikotun T, Bandyopadhyay R, 2007. Rapid screening of *Musa* species for resistance to black leaf streak using in vitro plantlets in tubes and detached leaves. *Plant Disease* **91,** 308–314.

Udugama S, 2002. Septoria-spot disease of banana *Mycosphaerella eumusae*: A new record for Sri Lanka. *Annals of the Sri Lanka Department of Agriculture* **4**, 337–343.

Vakili NG, 1968. Responses of *Musa acuminata* species and edible cultivars to infection by *Mycosphaerella musicola*. Tropical Agriculture (Trinidad) **45**, 13–32.

Valmayor RV, Silayoi B, Jamaluddin SH et al., 1991. Banana classification and commercial cultivars in Southeast Asia. *PCARRD information bulletin* No.24, Los Banos, the Philippines.

Verkley GJM, Starink-Willense M, 2004. A phylogenetic study of some *Septoria* species pathogenic to Asteracae based on ITS ribosomal DNA sequences. *Mycological Progress* **3**, 315–322.

Vuylesteke D, Ortiz R, Foure E, 1993. Genotype by environment interaction and black Sigatoka resistance in the humid forest zone of West and Central Africa. *Musafrica* **2**, 6–7.

Waalwijk C, Heide R van der, Vries I de, Lee T van der, Schoen C, Costrel-de Corainville G, Hauser-Hahn I, Kastelein P, Kohl J, Lonnet P, Demarquet T, Kema GHJ, 2004. Quantitative detection of *Fusarium* species in wheat using TaqMan. *European Journal of Plant Pathology* **110,** 481–494.

Ward E., Foster S.J., Fraaije B.A., McCartney H.A., 2004. Plant pathogen diagnostics: immunological and nucleic acid-based approaches. *Annals of Applied Biology* **145**: 1–16.

Wardlaw CW, 1972. Banana diseases including plantains and abaca, 2nd ed., Longman, London, UK, 878 p.

Washington JR, Crutz J, Lopez F, Fajardo M, 1998. Infection studies of *Mycosphaerella fijiensis* on banana and the control of black sigatoka with chlorothalonil. *Plant disease* **82**, 1185–1190.

Weir BS, Cockerham CC, 1984. Estimating F-statistic for the analysis of population structure. *Evolution* **38,** 1358–1370.

White, T. J., Bruns, T., Lee, S., & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 in: PCR Protocols: a guide to methods and applications. Innis, M. A., Gelfand, D. H., Sninsky, J. J., & White, T. J. eds. Academic Press, San Diego, California.

Wilson GF, Buddenhagen I, 1986. The black Sigatoka threat to plantain and banana in West Africa. *IITA Research Briefs.* **7**, 3.

Wright S, 1969. *Evolution and the genetics of populations: A treatise in Three Volumes. Volume 2, The Theory of Gene Frequencies*. University of Chicago Press Chicago, USA.

Wu CY, 1978. *Musella lasiocarpa. Acta Phytotaxonomica Sinica* **16**, 56–57.

Yabuuchi, E, Kosako Y, Yano I, Hota H, Nishiuchi Y, 1995. Transfer of two *Burkhhoderia* and an *Alcaligene*s species to *Ralstonia* Gen. Nov.: proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) Comb. Nov.: *Ralstonia solanacearum* (Smith 1896) Comb Nov.

Yirgou D, Bradbury JF, 1968. Bacterial wilt of enset (*Ensete ventricosum*) incited by *Xanthomonas musacearum* sp. n. *Phytopathology* **58**, 111—112.

Zandjanakou-Tachin M, Vroh-Bi I, Ojiambo PS, Tenkouano A Gumedzoe YM, Bandyopadhyay R, 2009. Identification and genetic diversity of *Mycosphaerella* species of banana and plantain in Nigeria, 2009. *Plant Pathology*, Doi: 10.1111/j.1365-3059.2008.01988.x.

Zapater M-F, Abadie C, Pignolet L, Carlier J, Mourichon X, 2008. Diagnosis of *Mycosphaerella* spp., responsible for *Mycosphaerella* leaf spot diseases of bananas and plantains, through morphotaxonomic observations. *Fruits,* **63**, 389—393.

Zimmermann A, 1902. Uber einige tropischer Kulturpflansen beobachtete Pilze. Zentralblatt fur Bacteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene 8, 219.

LIST OF PUBLICATIONS & CONFERENCE ATTENDED

- M. Zandjanakou-Tachin, I. Vroh-Bi, P. S. Ojiambo, A. Tenkouano, Y. M. Gumedzoe and R. Bandyopadhyay (2009) Identification and genetic diversity of *Mycosphaerella* species on banana and plantain in Nigeria. *Plant Pathology* **58,** 536–546.

- I. Vroh-Bi, M. Zandjanakou-Tachin, W. M'bah, A. Tenkouano, P. Ojiambo and R. Bandyopadhyay (2009). Resistance of F1 Segregating Populations Derived from Crosses between Wild Banana Accessions *Musa acuminata* spp. *burmannicoides* 'Calcutta 4' and *M. balbisiana* 'Montpellier' to Black Leaf Streak Disease. Proceeding International Symposium on Recent Advances in Banana crop Protection for Sustainable Production & Improve Livehoods Eds: D. Jones and I. Van den berg. *ISHS Acta Horticulture* 828.

- M. Zandjanakou-Tachin, Irie Vroh-Bi, Peter Ojiambo, Abdou Tenkouano, Mawuena Gumedzoe, and Ranajit Bandyopadhyay 2008. Genetic diversity of *Mycosphaerella* spp. on bananas in Nigeria. Banana and plantain in Africa: Harnessing international partnerships to increase research impact Leisure Lodge Resort, Mombasa, Kenya - October 5-9, 2008. Poster presentation (Best poster)

- Zandjanakou-Tachin M, Fanou A, LeGall P, Wydra K (2007). Detection, survival and transmission of *Xanthomonas axonopodis* pv. *manihotis* and *X. axonopodis* pv. *vignicola*, causal agents of cassava and cowpea bacterial blight, respectively, in / by insect vectors. *Journal of Phytopathology* **155**,159-169.

APPENDICES

.

Appendix 1.1 Sigatoka disease stages

The disease stages are characterized as follows:

Stage 1: The first symptoms that are visible are yellowish specks of less than 1 mm diameter on the abaxial surface of the leaf. Specks are often most abundant near the margin of the left side of the leaf, particularly towards the tip. This is due to the unrolling of the leaf, whereby the left side unfurls first in the heart-leaf stage.

Stage 2: The initial specks elongate, becoming slightly wider to form a reddish brown streak up to 2 mm long, parallel with the veins of the leaf. They are visible in translucent light and recognizable from some distance. At this stage, the streaks are more visible on the abaxial side than on the adaxial surface. The distribution of the streaks is not consistent. Sometimes they appear more numerous near the edge of the left side of the leaf. Otherwise, they are equally numerous on both right and left sides of the abaxial surface. Sometimes, they appear as a band. The length of the streaks can overlap to become large.

Stage 3: The brown streak grows longer and reaches 20 to 30 mm in length. The change in color of the streak from reddish brown to dark brown and almost black is characteristic of this stage, sometimes with a purple tint. The streak is now clearly visible on the adaxial surface of the leaf.

Stage 4: The streak broadens and develops into an elliptical spot, brown underneath and often black on the top. The spot is surrounded by a light brown, water-soaked border.

Stage 5: The central area of the dark spot becomes totally black and necrotic often slightly depressed. The brown, water-soaked border becomes more pronounced with a yellow halo surrounding this border. This is due to the yellowing of the tissue surrounding the border.

Stage 6: The center of the spot dries out and fades to a clear grey. This grey center is encircled by a clear black ring, which is surrounded by a bright yellow halo. The spots are separated from each other. Due to the contrast between the grey centre and the surrounding black ring the spots remain visible after the leaf has dried up completely.

Appendix 2.1 DNA extraction ptotocol

200mg of dry mycelia were weighed in a mortar

Quickly ground to fine powder with liquid nitrogen using a micro pestle

Transfer to 1.5ml microcentifuge tubes

Add 600µl of nucleic lysis

Add to each tube 3µl Mercaptoethanol

Incubated at 60°C for 1 hour in a water bath

Add 3 µl RNAase solutions

Vortex three times

Incubated at 37°C for 15 minutes

Cool for 2 minutes at room temperature

Add 250µl protein precipitation

Vortex the tubes and incubate for 30 minutes on ice

Centrifuge at 14,000 g for 20 minutes

Decant supernatant to clean tube containing 600µl isopropanol and invert

Precipitate DNA for 15 minutes at -20°C

Centrifuge for 10 min at 14,000

Pour supernant and washed with 70% ethanol

Centrifuge at 14,000 for five minutes

Wash the pellet with 70% isopropanol

Dry pellet at room temperature

Add 100µl rehydratation solution to the DNA and at 4°C overnight

Gel agarose

Visualise the gel by staining with ethidium bromide

Distaine for 10 minutes, print using a camera connected to a computer

Quantification of the DNA using spectrophotometer

Appendix 3.1. Isolates and locations visited during 2004, 2005 and 2006 surveys and their GPS

Isolate Nb	Geog. Zone	Isolate code	Location	Alt	Lat	Long	Host	Village
1	SS	3L20P13-1	Delta	5	5.9083	5.7257	AAB	Mosogar
2	SS	3L20P20-2	Delta	5	5.9083	5.7257	AAB	Mosogar
3	SS	2L1P9-1	Edo	74	6.2870	5.6694	AAB	SANKPOBA
4	SS	2L8P4-2	River	-7	5.4051	6.6824	AAB	OBRIKOM
5	SS	2L8P3-2	River	-7	5.4051	6.6824	AAB	OBRIKOM
6	SS	2L1P19-3	Edo	74	6.2870	5.6694	AAB	SANKPOBA
7	SS	2L1P12-3	Edo	74	6.2870	5.6694	AAB	SANKPOBA
8	SS	2L1P3-4	Edo	74	6.2870	5.6694	AAB	SANKPOBA
9	SW	3L15P3	Ekiti	555	7.7833	5.2421	AAB	IFAKI
10	SW	2L28P15-2	Osun	231	7.4676	4.3529	AAB	ABUBI
11	SW	2L30P5-2	Oyo	220	7.5056	3.90953	AAB	IITA
12	SW	2L30P6-1	Oyo	210	7.4922	3.9044	AAB	IITA
13	SW	2L30P6-2	Oyo	210	7.4922	3.9044	AAB	IITA
14	SS	3L17P9-3	Delta	1	5.7110	5.7578	AAB	OKWEJEBA
15	SS	3L17P1-3	Delta	1	5.7110	5.7578	AAB	OKWEJEBA
16	SS	3L17P1e-1	Delta	1	5.7110	5.7578	AAB	OKWEJEBA
17	SW	3L25P10-3	Osun	199	7.375	4.0949	AAB	Osegere
18	SW	3L25P10-1	Osun	199	7.375	4.0949	AAB	Osegere
19	SW	3L25P10-2	Osun	199	7.375	4.0949	AAB	Osegere
20	SW	3L30P4-1	Oyo	210	7.4922	3.9044	AAB	IITA
21	SW	3L30P4-2	Oyo	251	7.4985	3.9079	AAA	IITA
22	SW	3L25P10-4	Osun	199	7.375	4.0949	AAB	Osegere
23	SW	2L28P7-1	Osun	231	7.4676	4.3529	AAB	ABUBI
24	SE	2L15P10-5	Anambra	34	6.2541	7.1450	AAA	ASHALA
25	SW	4L24P2-3	Oyo	306	7.6812	3.9604	AAA	Iware
26	SW	3L15P10-a	Ekiti	555	7.7833	5.2421	AAB	IFAKI

Appendix 3.1 Continued

Isolate Nb	Geog. Zone	Isolate code	Location	Alt	Lat	Long	Host	Village
27	SW	3L15P10-5	Ekiti	555	7.7833	5.2421	AAB	IFAKI
28	SS	3L17P8-2	Delta	1	5.7110	5.7578	AAB	OKWEJEBA
29	SE	2L13P16-4	Anambra	46	5.8526	6.8639	AAB	UMUAHA
30	NC	4L11P-4	Benue	272	7.1564	7.7511	AAA	Ugbokolo
31	SW	3L25P1	Osun	199	7.375	4.0949	AAB	Osegere
32	NC	4L18P9-1	Kwara	149	8.4160	4.6126	AAA	Ilorin
33	SS	3L17P9-4	Delta	1	5.7110	5.7578	AAB	OKWEJEBA
34	NC	4L18P7-1	Kwara	149	8.4160	4.6126	AAA	Ilorin
35	NC	4L18P7-3	Kwara	149	8.4160	4.6126	AAA	Ilorin
36	SS	3L20P9	Delta	5	5.9083	5.7257	AAB	Mosogar
37	SS	3L16P13a	Edo	-2	6.0572	5.6637	AAB	Olugbo
38	NC	4L10P1-1	Kwara	Nd	Nd	Nd	Nd	Gambani
39	SE	4L2P7-3	Abia	119	5.4983	7.4475	AAB	NSUKWE
40	SE	4L1P3-1	Abia	173	5.5512	7.4361	Nd	Umuopara
41	SE	2L14P4-1	Anambra	46	0.5812	6.8213	AAA	Nd
42	SE	2L14P11-1	Anambra	46	0.5812	6.8213	AAA	Nd
43	SS	3L20P1-3	Delta	5	5.9083	5.7257	AAB	Mosogar
44	NC	4L10P2-1	Kwara	Nd	Nd	Nd	Nd	Gambani
45	NC	4L19P-2	Kwara	386	8.706	4.7138	AAA	Idofian
46	SE	2L14P17-3	Anambra	46	0.5812	6.8213	AAA	Nd
47	SW	3L24P4-1	Osun	388	7.6056	4.7596	AAB	IROJO
48	NC	4L17P8-x	Kwara	386	8.3741	4.7138	AAA	Idofian
49	NC	4L19P3-1	Kwara	386	8.706	4.7138	AAA	Idofian
50	SW	3L14P13-4	Ekiti	388	7.6790	5.0938	AAB	AJAYE
51	SW	3L14P18-3	Ekiti	388	7.6790	5.0938	AAB	AJAYE
52	SW	3L14P13-x	Ekiti	389	7.6791	5.0939	AAB	AJAYE

Appendix 3.1 Continued

Isolate Nb	Geog. Zone	Isolate code	Location	Alt	Lat	Long	Host	Village
53	SW	3L14P3-x	Ekiti	390	7.6792	5.0940	AAB	AJAYE
54	SS	3L17P2-3	River	-7	5.4051	6.6824	AAB	OBRIKOM
55	SS	3L24P16	Osun	388	7.6056	4.7596	AAB	IROJO
56	SS	L30P3-1	Delta	5	7.4985	3.9079	AAA	Mosogar
57	SE	2L12P15-1	Imo	24	5.3307	6.9580	AAA	Umuagwo
58	SS	2L7P17-2	River	27	4.719	7.1745	AAB	Onne
59	SS	2L7P16-2	River	27	4.719	7.1745	AAB	Onne
60	NC	4L18P10-1	kwara	149	8.4160	4.6126	AAA	Ilorin
61	NC	4L11P5-4f	Benue	272	7.1564	7.7511	AAA	Ugbokolo
62	NC	4L11P7	Benue	272	7.1564	7.7511	AAA	Ugbokolo
63	SW	4L24P13-3	Oyo	27	4.719	7.1745	AAB	Onne
64	SW	2L8P4-1	River	-7	5.4051	6.6824	AAB	OBRIKOM
65	SE	4L1P2-1	Abia	173	5.5512	7.4361	Nd	Umuopara
66	NC	4L10P7-1	Kwara	Nd	Nd	Nd	Nd	Gambani
67	NC	4L17P2-2	Kwara	386	8.3741	4.7138	AAA	Idofian
68	NC	3L2P9-1	Abuja	423	9.0035	7.4249	AAB	Ganevillage
69	SE	4L1P10-1	Abia	173	5.5512	7.4361	Nd	Umuopara
70	SS	2L7P2-1	River	27	4.719	7.1745	AAB	Onne
71	NC	4L10P9-2	Kwara	Nd	Nd	Nd	Nd	Gambani
72	NC	3L2P1	Abuja	423	9.0035	7.4249	AAB	AGANO
73	SE	4L3P3	Abia	138	5.4223	7.4034	AAB	IMEREME
74	NC	4L17P8-2	Kwara	386	8.3741	4.7138	AAA	Idofian
75	SS	2L8P4-2	River	-7	5.4051	6.6824	AAB	OBRIKOM
76	NC	3L2P3-2	Abuja	423	9.0035	7.4249	AAB	Ganevillage
77	SE	4L4P2-1	Abia	122	5.3868	7.4352	AAB	MBAOSA
78	SE	4L2P13-1	Abia	119	5.4983	7.4475	AAB	NSUKWE
79	NC	4L19P2-3	Benue	440	7.1441	7.6713	AAA	Otukpa

Appendix 3.1 Continued

Isolate Nb	Geog. Zone	Isolate code	States	Alt	Lat	Long	Host	Village
80	SW	4L24P7-3	Oyo	306	7.6812	3.9604	AAA	Iware
81	NC	4L11P3-1	Benue	272	7.1564	7.7511	AAA	Ugbokolo
82	Nc	4L10P7-1	Kwara	Nd	Nd	Nd	Nd	Gambani
83	NC	3L2P3-1	Abuja	423	9.0035	7.4249	AAB	AGANO
84	SE	4L4P4	Abia	122	5.3868	7.4352	AAB	MBAOSA
85	SS	Onne4	River	27	4.719	7.1745	AAB	Onne
86	NC	4L19P2-2	Benue	440	7.1441	7.6713	AAA	Otukpa
87	SE	2L16P1-1	Enugu	20	6.2140	7.5005	AAA	IGUGU
88	SE	4L3P15-4	Abia	119	5.4223	7.4034	AAB	IMEREME
89	SE	2L13P16-3	Anambra	46	5.8526	6.8639	AAB	UMUAHA
90	SE	2L13P12-3	Anambra	46	5.8526	6.8639	AAB	UMUAHA
91	SE	4L3P7-1	Abia	119	5.4223	7.4034	AAB	IMEREME
92	SE	4L3P8-1	Abia	119	5.4223	7.4034	AAB	IMEREME
93	SE	2L13P12-3	Anambra	46	5.8526	6.8639	AAB	UMUAHA
94	SE	2L13P19-2	Anambra	46	5.8526	6.8639	AAB	UMUAHA
95	SE	2L13P4-2	Anambra	46	5.8526	6.8639	AAB	UMUAHA
96	SE	4L3P15-4	Abia	119	5.4223	7.4034	AAB	IMEREME

Appendix 3.2 Label for Fig. 3.2

Fig. 3.2 Dendrogram from unweighted paired group method with arithmetic means (UPGMA) based on SNPs and Indels analysis in the ribosomal DNA of 95 isolates of Mycosphaerella species obtained from Musa *in Nigeria and GenBank references of* M. fijiensis, M. musicola, M. eumusae *and* M. musae.

NC = North Centre, SS = South South, SE = South-East, SW = South-West geographical zones and the numerals are isolate numbers. GenBank references are indicated by Fij for M. fijiensis (Col = Columbia, CIs = Cook Islands, Ind = Indonesia, PNG = Papua New Guinea, Ph = Philippines, and Uga = Uganda); Eumu for M. eumusae (QB = Quebec, SRL = Sri Lanka, Tha = Thailand, and Viet = Vietnam); Musi for M. musicola (Aust = Australia, Cam = Cameroon Gui = Guinea, IC = Ivory Coast, Ind = Indonesia, and Vnz = Venezuela); and Musae for M. musae (QB = Quebec).

The scale bar indicates the horizontal distance corresponding to genetic similarity as measured by the dice similarity coefficient.

Appendix 3.3 Registered isolates

Organism Species	GeneBank Accession #	Reference #	Origin (AEZ)
	EF666070	SS6-IITA	HF
	EF666071	SW26-IITA	DS
	EF666072	SE46-IITA	DS
	EF666073	SW47-IITA	DS
	EF666074	NC49-IITA	DS
	EF666075	SW53-IITA	DS
	EF666076	SS58-IITA	HF
M. fijiensis	EF666077	SE65-IITA	DS
	EF666078	NC66-IITA	DS
	EF666079	SE69-IITA	DS
	EF666080	SS70-IITA	HF
	EF666081	NC79-IITA	DS
	EF666082	SE87-IITA	DS
	EF666083	SE88-IITA	DS
	EF670653	IITA-SW11	DS
	EF670656	IITA-SW20	DS
	EF670657	IITA-SW21	DS
M. eumusae	EF670658	IITA-NC30	DS
	EF670659	IITA-SS54	HF
	EF670660	IITA-SS56	HF
	EF670661	IITA-SE57	DS

Mycosphaerella fijiensis isolate and internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence
SS= South-south, SS= south west, NC= North-centre, SE= South-east
HA: Humid forest, DS: Derived savanna