CHARACTERISATION OF TURCICUM LEAF BLIGHT EPIDEMICS AND PATHOGEN POPULATIONS IN THE *Exserohilum turcicum* – SORGHUM PATHOSYSTEM OF UGANDA

IDD RAMATHANI

Bsc Agric (Hons)

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF SCIENCE DEGREE IN CROP SCIENCE OF MAKERERE UNIVERSITY

SEPTEMBER, 2010

DECLARATION

This thesis is a presentation of my original research work. Wherever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature, and acknowledgement of collaborative research and discussions.

Written by

Idd Ramathani

Bsc Agriculture (MUK) ----------

Date

This thesis has been submitted with our approval as the Academic supervisors.

Approved by

Dr .Patrick Okori

Department of Crop science

Faulty of Agriculture

Makerere University ----------

Date:

Prof Dixelius Christina

Department of plant biology and Forest genetics

Uppsala, Bio-center, SlU, Sweden ----------

Date:

DEDICATION

I dedicate this thesis to my God and Lord and to my wonderful parents (To my father, who taught me that the best kind of knowledge to have is that which learned and put into practice is. To my mother, who taught me that even the largest task can be accomplished if it is done one step at a time), and who have raised me to be the person I am today. You have been with me every step of the way, through good times and bad. Thank you for all the unconditional love, guidance, and support that you have always given me, helping me to succeed and instilling in me the confidence that I am capable of doing anything I put my mind to. Thank you for everything. I love you more than I can express! Also, this thesis is dedicated to all my friends who have been a great source of motivation and inspiration. Finally, this thesis is dedicated to all those who believe in the richness of learning.

ACKNOWLEDGEMENTS

From the formative stages of this thesis, to the final draft, I owe an immense debt of gratitude to my supervisor Dr. Patrick Okori who been the ideal thesis supervisor. His sage advice, insightful criticisms, and patient encouragement aided the research and writing of this thesis in innumerable ways. I would also like to thank Prof Dixelius Christina and Dr Mulema Joseph for taking the time to read my work and BIO-EARN for providing me with the scholarship and the research funds.

I would like to formally thank. NaSARRI and MUARIK for allowing me conduct research at their research station and NaCRRI for providing me with maize seeds that I utilised in my research work

My fellow graduate students Akwero Agnes, Scovia Adikini, Emmanuel Kapande, Amuge Teddy, Namuddu Annet, Esther, Sendi, Mudonyi, Atim, Betty and Emphriah

I wish express gratitude to Sarah Serwuagi, and Elias (Laboratory technicians) for their friendships and support. Not forgetting Mrs. Eyedu Hellen a technician with NaSARRI and Mr. Opar Mario (MUARIK) whose commitments made the experiments successful.

I wish to say that the last two years have been quite an experience and you have all made it a memorable time of my life. I will miss all of you. Good luck to each of you in your future endeavours.

On a personal side, I wish to thank my parents, for their never-ending love and support in all my efforts, and for giving me the foundation to be who I am. Thank you, Mom and Dad. My brothers, and Sisters Moses, Yusufu and Faridah, Zerinah, Jamilah, Lukia respectively, for their love and support throughout the years. Thank you for the laughing and the fighting, and everything in between.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

SUMMARY

Turcicum Leaf Blight (TLB) of sorghum incited by *Exserohilum turcicum* is a major threat to sorghum production globally. This pathogen has been reported to attack both maize and sorghum resulting in yield losses as high as 70% on susceptible cultivars. Studies so far conducted on TLB have mainly focused on maize host. Little or no research has been conducted yet to try to understand the epidemiology and population structure of TLB in *Exserohilum turcicum*-sorghum pathosystem. This aspect is very important in the development of long lasting management practice. This study was therefore designed to (a) investigate the occurrence of TLB in major sorghum growing regions, (b) characterise sorghum accessions for resistance to TLB, (C) carry out epidemiological studies on temporal and spatial attributes of TLB in sorghum, (d) determine the mating types and races of *E. turcicum* that occur in Uganda as well as (e) establish variability of sorghum derived *E. turcicum* isolates in Uganda. Surveys were conducted in eight major sorghum growing agro ecologies of Uganda in order to establish the occurrence of TLB in these regions. During the survey, sorghum fields were sampled for TLB incidence and severity. Diseased leaf samples from over 200 sorghum accessions were collected from farmers' field for further studies in the field and screen house experiment. To understand the epidemiology aspect of TLB in sorghum, the collected accessions were planted in the field and challenged with *Exserohilum turcicum* isolates. The varieties that showed resistance were selected and further challenged with *Exserohilum turcicum* isolates at different locations to confirm their resistance. To establish the population structure of this pathogen, from the isolates collected from different parts in Uganda. *Exserohilum turcicum* isolate DNA was subjected to PCR using selective markers for mating types and genetic variability. The pathogen isolates were also characterised using race differentials. The survey study found out that TLB occurred in all agro-ecologies, albeit at lower levels (20-40%) than in maize (60-100%). Disease severity ranged from 24.6 to 37.8 % and were quite low on land races grown by farmers, while incidence values ranged from as high as 100% in Pallisa to as low as 20% in Tororo. The effect of agro-ecology on TLB epidemics was highly significant ($P \leq 0.05$). Areas with high levels of humid and moderate temperature had the highest severity

and incidence. Additionally cropping systems significantly influenced the patterns of spread of TLB across the region, suggesting that the upsurge in the TLB on sorghum was highly influenced by wide-spread use of susceptible genotypes such as *Epuripuri* and Sekedo. Indeed among the 202 sorghum accessions collected from farmer's fields, the majority (over 99%) were resistant to TLB. The fact that most land races grown by farmers had moderate to high level of resistance to TLB suggests that they could be used in sorghum breeding programmes to improve resistance to TLB in the improved cultivars such as *Epuripuri* and Sekedo.

The study on temporal aspect of TLB epidemics indicated that disease development in sorghum was delayed by about three weeks compared to maize. The data further indicated that the severity and incidence of TLB on sorghum were quite low as compared to maize, suggesting possible role of physiological adaptation of *E.turcicum* on maize than on sorghum. Rates of disease development were relatively low among sorghum accessions and high in maize. The data indicated that the nature of resistance being expressed by sorghum is quite different from that of maize. It also further presupposes the role of pathogen physiological specialization.

The analysis of spatial attributes of TLB epidemics in sorghum showed that disease severity and gradients were affected by wind drift. Differences in crop residues where significant in plants close to crop residue than plant found far away from the residue source. The above results where more pronounced in *Epuripuri* a susceptible plant variety as compared to selected sorghum accession MUC/007/009 and MUC/007/029. In this study flattening of the disease gradient was observed confirming the polycyclic nature of TLB of sorghum.

Mating type analysis revealed the occurrence of *MAT 1, MAT 2* and *MAT 1, 2* in Uganda on sorghum. Furthermore in Soroti, mating types *MAT 1* and *MAT* 2 were found to occur in equal proportions indicating a great potential of sexual recombination with a possibility of the emergence of new races of *E.turcicum.* In other locations *MAT* 2 was more common that *MAT 1*. The race differential study revealed

occurrence of races 0, 13, 2, and 3 in Uganda. Both the mating type and race differential study suggest a great potential of having more virulent races of *E. turcicum* in the future. Also, the genetic variability studies based on molecular tools indicated high genotypic diversity among the sorghum derived *E.turcicum* isolates. However, the similarity coefficient was high (0.64) indicating a low genetic variation among sorghum *E.turcicum* isolates collected from Uganda. A high similarity coefficient presupposes the occurrence of gene flow between populations. Therefore for disease management, the data presented here suggests that if no improvements are made on the elite released sorghum lines such as *Epuripuri* an epidemic *is* bound to happen. The variety screening study suggests that farmers grown varieties that might have alternative sources of resistances for TLB breeding. The epidemiology studies imply that the resistance mechanisms exhibited by sorghum differ from that of maize. This work further suggests that management practices should focus on reducing crop residue and use of resistant crop varieties.

CHAPTER ONE

INTRODUCTION

1.0 Sorghum: systematics and socio-economic importance

Sorghum (*Sorghum bicolour* (L.) Moench) is a cultivated tropical cereal. It is generally considered to have first been domesticated in North-Eastern Africa, possibly along the Nile or Ethiopia as recently as 1000 BC (Kimber, 2000). Sorghum belongs to the tribe Andropogonae of the grass family Poaceae. The genus is characterised by spikelets borne in pairs. Sorghum is treated as an annual plant, although it is a perennial grass. In the tropics the crop can be harvested many times. It is a source of energy, protein, vitamins and minerals for millions of the poorest people in Africa (FAO, 1990). The cultivation of sorghum played a crucial role in the spread of the Bantu people across sub-Saharan Africa (Diamond, 1998). It was introduced into Uganda by the pastoralists and the Bantu around 350 AD, who moved towards east and south along the rift valley and other extensive trade routes (land and rivers) through Uganda (Doggett, 1970).

Today, sorghum is cultivated across the world in the warmer climatic areas. It is considered the world's fifth largest and most important cereal, after wheat, maize, rice and barley (FAO, 2003). In Africa sorghum is ranked the second most important cereal were it is largely a subsistence food crop (FAO, 1990). In Uganda, it is ranked as the third most important cereal crop with national production of 456,000 tonnes from an area of 314,000 hectares (Ebiyau and Oryokot, 2001; MAAIF, 2007). Uganda is one of the major sorghum producers as well as one of the centres of diversity for sorghum (FAO, 2005).

Sorghum has a distinct advantage compared to other major cereals because it is drought-resistant and many subsistence farmers in these regions cultivate sorghum as a staple food crop for homes (ICRISAT, 2000). In the tropics, sorghum is well known for its capacity to tolerate conditions of extended drought, in

circumstances that would impede production of most other grains (Crop Plant Resources, 2000). Like maize, sorghum can be grown under a wide range of soil and climatic conditions, except that its yield under different conditions is not so varied (Crop Plant Resources, 2000).

World sorghum production has increased from 192 metric tons in 1960 to 490 metric tons in 2007 (USDA, 2007). The major sorghum producing countries are the United States (17% of the world production), Nigeria, India (each with 14%) and Mexico (11%) (FAO, 2005). Under Uganda conditions, the area under sorghum has on average increased from 282, 000 ha in 2001 to 314, 000 ha in 2007 (MAAIF, 2007). Furthermore several bottled beer brewery industries in Uganda are testing the use of sorghum as substitute to imported barley.

Uganda is divided into 33 agro-ecological zones based on land use types, population, soil and climate (Wortmann and Eledu, 1999). Sorghum is cultivated in 28 agro-ecological zones. Sorghum production occurs mostly in districts of Kabale and Kisoro in south-western highlands agro ecology; Busia, Kumi and Moroto in most of Eastern , Arua in Northern Uganda Agro-ecologies (Ebiyau and Oryokot, 2001). Cultivation is most intensive in semi arid areas and the crop usually takes 95-110 days to reach maturity (Vanderlip, 1993).

1.1 Production constraints of sorghum in Uganda

Sorghum production in most parts of the world is relatively low, estimated at 925 kg ha^{-1} compared to 5,000 kg ha^{-1} reported from experimental stations (ICRISAT, 1996). In Uganda, low yields averagely 600-800 kg ha^{-1} on farm but about 4, 500 kg ha^{-1} on station (Esele, 1988). Over the years, sorghum production increase in Uganda has been attributed to expansion of crop area rather than increased tonnage per unit area. The low yields are attributed to a number of abiotic factors like erratic and delayed rainfall, low soil fertility /poor soils, insufficient seeds; and biotic factors such as low yielding varieties, poor management systems, weeds, insect pests (termites and black ants) and diseases (Esele, 1988).

1.2.1 Abiotic and socio-economic factors

Erratic rainfall is a major problem for farmers since their agriculture is typically rain-fed. Farmers hold the view that the amount of rainfall has decreased and is insufficient. However, it appears that the problem stems from the lack of appropriate sorghum varieties that fit the current rainfall regime (Kudadjie *et al.*, 2004). Poor soils are a source of worry to all farmers. Sorghum production typically takes place in marginal areas that are prone to infertility and water stress conditions (Kudadjie *et al.*, 2004). The majority of smallholder farmers, especially in the semi-arid tropical regions of Africa, do not produce enough sorghum to meet family requirements. Furthermore sorghum is a semi-subsistence enterprise that offers smaller returns than other investments such as livestock. As a result, less attention is paid to invest in the use of seeds from improved varieties to boost production (FAO, 1996). These and other abiotic factors hamper sorghum production leading to low yields and incomes.

1.2.2 Biotic factors

Striga is a major constraint of sorghum production especially in eastern and northern Uganda (Ebiyau and Oryokot, 2001). There are at least two species of striga known to affect sorghum production in the country namely: *Striga hermonthica* and *Striga asiatica*. In districts of Tororo and Pallisa of eastern Uganda, it is estimated that Striga is present in up to 80 % of fields and causes an estimated 60-85% yield loss in infested fields (Ebiyau, 1995). Some *Striga*-resistant sorghum varieties have been developed, but these generally offer lower yields than traditional cultivars and improved (but *Striga*-susceptible) varieties (FAO, 2007). The effect of *Striga* has been found to decrease when crops are grown in conjunction with legumes (Carsky *et al.*, 1994). The most important arthropod pests of sorghum include sorghum shoot fly (*Atherigona varia soccata),* sorghum Midge *(Contarinia sorghicola) and* sorghum stem borer (*Busseola fusca)* (Sharma, 1993). Sorghum shoot fly causes substantial losses in late and off-season sorghum in Uganda (Davies and Reddy, 1981) and Stem borers are also endemic in most parts of Uganda (Gitau *et*

al., 2002). The most important species of include *Chilo partellus, Sesamia calamitis* and *Buseola sorghida*. *Chilo partellus* is mainly found in the semi arid areas of East Africa while *Sesamia calamitis* and *Buseola sorghida* are distributed throughout sorghum growing areas of Africa (Kfir, 1997). Birds are perhaps one of the most important pests of sorghum worldwide. They are capable of inflicting heavy losses and causing economic damage. In Uganda the most notorious species is *Quelea quelea*.

1.2.2.1 Sorghum diseases

The major diseases that affect sorghum include, downy mildew (*Peronoscleropora sorghi*) (Western & Uppal) Shaw); Turcicum leaf blight, TLB (*Exserohilum turcicum*); Anthracnose (*Colletrotrichium sublineolum* Henn.) (DeVries and Toeniessen, 2001) and sorghum smuts- covered kernel smut (*Sporisorium sorghi* Ehrenberg (Link); loose smut (*Sphacelotheca cruenta* (Kuhn); Langdon and Fullerton) and long smuts (*Tolyposporium entrenbargii* (Kuhn) Pattouillard).

Turcicum leaf blight (TLB) is one of the most destructive foliar diseases of maize and sorghum. It can cause yield greater than 50 % susceptible varieties and is favoured by mild temperatures and humid weather conditions with heavy dews (Bergquist, 1986; Carson, 1995). The disease occurs as long elliptic tan lesions that develop on lower leaves and progress upwards. Susceptibility to *E. turcicum* is reported to decrease with crop maturity (Frederiksen, 1980). On susceptible cultivars in Uganda, losses as high as 60 % have been recorded on maize and as high as 70% elsewhere (Yeshitila, 2003).

1.3 Justification of the study

Sorghum is distinguished among other cereals by its unusually broad range of diseases. The crop is normally attacked by more than one pathogen during its growth cycle and thus diseases are the most important constraint to sorghum production worldwide (King and Mukuru, 1994). Turcicum leaf blight is epidemic in many parts of Uganda (Adipala *et al.*, 1993). It causes severe crop losses depending on the

crop stage at infection, susceptibility of cultivar and the prevailing environmental conditions. In general, knowledge on disease epidemics and pathogen variability is particularly important for long-term disease management. This implies that the design of disease management strategies is dependent on the use of information from well-characterised path systems.

Within the TLB pathosystem, there are four major interacting factors namely the host (sorghum, maize and wild relatives), the pathogen (*Exserohilum turcicum*), the environment and the human influence (crop and farming systems). These factors contribute to the development of turcicum leaf blight epidemics in Uganda. At the moment there is contrary information on the *E. turcicum* -sorghum pathosystem of Uganda. The studies conducted as part of this thesis sought to contribute to elucidation of the *E. turcicum* -sorghum pathosystem. Accordingly, a number of related studies were conducted from 2006 –2007 under pinned by the pathology principles presented below

Sorghum is an African crop and therefore it is hypothesised that *E. turcicum* and sorghum have co-evolved over a long period leading to generation of a wide array of susceptible and resistant genotypes. Given that cereals in general have a low return to investment, deployment of resistance remains the most cost effective option for any disease management practices. It is thus necessary that stable sources of resistance be sought and be recommended for breeding programmes for improvement of elite sorghum lines. In this thesis effort has been made to characterise sorghum accessions from Uganda for resistance to TLB.

Furthermore, in this thesis efforts have been made to characterise TLB epidemics as a means of providing background data needed for designing and deploy disease management strategies. Characterisation of epidemics involves studying temporal and spatial attributes of epidemics. These two components of plant disease epidemics elucidate modes of inoculum spread over time and are in fact the basis of cultural control methods of plant diseases.

Another important component of the sorghum *E. turcicum* pathosystem is the pathogen itself. *Exserohilum turcicum* is known to have nine races with race O reported in Uganda (Bigirwa *et al.*, 1993.). Moreover, some studies indicate that the pathogen exhibits host species specialisation (Robert, 1960). Given that the maize *E. turcicum* pathosystem has been characterised (Adipala *et al.*, 1993), and the fact that host species specialisation is a possibility, studying the *E turcicum* -sorghum pathosystem becomes worthwhile. In this thesis both neutral and selectable genetic markers were employed to characterise the pathogen populations

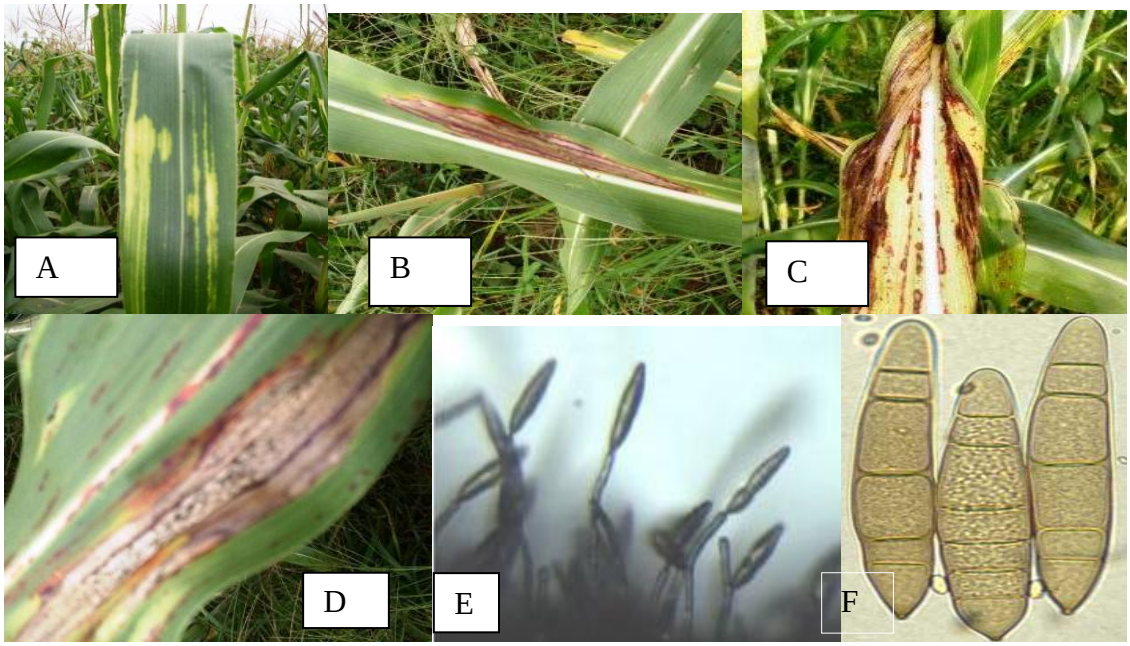


Plate 1.Turcicum leaf blight development (A-D) and *Exserohilum turcicum* spores (E sporulation ©www.ars.usda.gov/Research/docs.htm?docid=1048,and(FConidia©ss.niai.affrc.go.jp/.../contents/ehelmintho.html

1.4 Overall objective

The overall objective of the study was to characterise the turcicum leaf blight -sorghum pathosystem in Uganda by undertaking studies on the pathogen, the disease and host reaction of sorghum genotypes.

1.4.1 Specific objectives

1. To assess the severity and incidence of turcicum leaf blight in major sorghum agro-ecologies of Uganda.
2. To characterise sorghum accessions from Uganda for resistance to turcicum leaf blight.
3. To carry out epidemiological studies on temporal and spatial attributes of Turcicum leaf blight epidemics of sorghum in Uganda.
4. To determine the mating types and races of *E. turcicum* that occur in Uganda as well as establish genetic variability of sorghum derived *E. turcicum* isolates in Uganda.

1.5 Hypothesis to be tested

1. Existence of Uganda within the sorghum centre of diversity implies that local land races have co-evolved with *E. turcicum* leading to wide variation of the pathogen and disease reactions of sorghum varieties.

2. Turcicum leaf blight epidemics of sorghum are influenced by agro-ecological conditions, variety reaction and inoculum present in fields.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

Exserohilum turcicum is the pathogen that causes turcicum leaf blight. It has three major groups of hosts namely: maize (*Zea mays* L.), sorghum (*Sorghum bicolour*) and wild relatives of sorghum: Johnson grass (*Sorghum halapense*), teosinte and other grass species. The turcicum leaf blight-sorghum pathosystem in Uganda is not well characterised. Therefore this thesis focuses on elucidating *Exserohilum turcicum* – sorghum pathosystem by characterising TLB epidemics, pathogen variability and host reaction to infection.

2.1.1 Sorghum-*Exserohilum turcicum* pathosystem

2.1.1 Description of turcicum leaf blight

Turcicum Leaf Blight (TLB) is one of the major diseases affecting sorghum and maize in warm and humid parts of the world including Uganda (Ceballos *et al.*, 1991; Adipala *et al.*, 1993). It is caused by a fungal pathogen *Exserohilum turcicum.* (Syn. *Helminthosporium turcicum* (Pass) Leonard and Suggs, *Bipolaris turcica* (Pass) Shoemaker, *Drechslera turcica* (Pass.) Subram and Jain.) teliomorph Setosphaeria turcica Perfect state. In Africa, where maize and sorghum are the staple foods, TLB is reported to be widespread in the warm and humid growing regions of Ethiopia, Tanzania and Uganda (Adipala *et al.*, 1993; Nkonya *et al.*, 1998; Tilahun *et al.*, 2001). The disease also occurs whenever sorghum and maize are grown together (Ebiyau, 1995). Hosts of *E. turcicum* include sorghum, maize, Sudan grass, Johnson grass, teosinte and other grass species (Esele, 1995). It has particularly been noticed to cause significant maize yield reduction in many production regions (Latterell and Rossi, 1983) and

limiting productivity in subSaharan Africa especially in the humid mid- Attitude and highland regions (DeVries and Toeniessen, 2001).It affects foliage causing yield reduction associated with necrosis or chlorosis of leaves in the upper two-thirds of the canopy (Levy and Pataky, 1992). The disease can cause extensive defoliation during grain filling period, resulting in grain yield losses of up to 50% or more (Raymundo and Hooker, 1981). If infection is delayed by 6-8 weeks after silking or until flowering, yield losses may be minimal. Turcicum leaf blight also predisposes plants to stalk rots caused by other pathogens (Gowda *et al.*, 1992; Cardwell *et al.*, 1997)**.**

2.1.2 Symptoms and etiology

The most observed symptom of *E. turcicum* is long elliptic tan lesions that develop first on the lower leaves and progress upward. The earliest symptoms of infection are slightly oval, water-soaked, small spots on leaves. These grow into elongated, spindle-shaped necrotic lesions (Plate 2). They may appear first on lower leaves and increase in number as the plant develops and this can lead to complete blighting of the foliage (Richards and Kucharek, 2006). Typical lesions are grey-green, elliptical or cigar-shaped and are typically 12 mm wide and 3-15 cm long and have yellow to gray centres and red margins. Spore production causes the lesions to appear dark gray, olive or black (King and Mukuru, 1994).

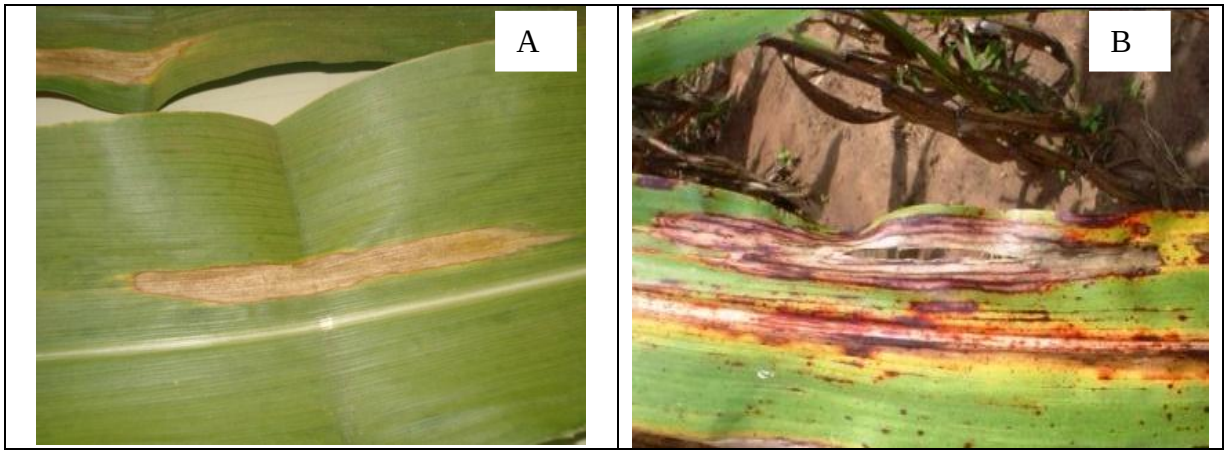


Plate 2. Turcicum leaf blight lesions on maize (A) and sorghum (B)

2.1.3 Disease cycle

Like several other common leaf blight diseases of sorghum, *E. turcicum* remains dormant during the dry period in residue of sorghum/maize infected the previous season. In the following growing season, the fungus sporulates on the residue. Conidia are wind-blown over long distances to leaves of maize and sorghum plants. Subsequently, conidial germination occurs 3-6 hours after inoculation (Hilu and Hooker, 1964). Germ tubes grow at an angle rather than parallel to the veins of the leaf producing an appressoria from which a penetration peg develops. Macroscopic symptoms appear first as minute light green to whitish flecks. Hyphae grow in the mesophyll cells in these flecks and in cells beyond the necrotic tissue of the flecks. Flecks enlarge either singly or coalesce to form long elliptical, greyish-green tan lesions ranging from 2.5 to 15 cm in length. Initial lesions develop on lower leaves. Once a dead lesion develops on a leaf, the fungus produces spores that can infect more leaf tissue (Gregory, 2004). Over time, the disease progresses up the plant, killing tissue on leaves above the ear. The dull green cast on lesions indicates sporulation of the fungus on lesions surfaces. Moderate temperature (18-27 ^{o}C), relative humidity from 90 to 100%, low luminosity , the presence of large amount of inoculum and long dew periods favour TLB epiphytotics (Hennessy *et al.*, 1990; Bentolila *et al.*, 1991; Gregory, 2004). Further spread of the disease within and between fields occurs by conidia produced abundantly on leaf lesions. In general, turcicum leaf blight is generally known to be sporadic in occurrence, depending on the environmental conditions and the level of disease resistance in the variety (Degefu, 1990).

2.1.2 Pathogen description

2.2.1 Pathogen taxonomy and biology

Exserohilum turcicum is an ascomycete pathogen of cereals. It is a heterothallic facultative parasitic fungus (Luttrell, 1958). It reproduces both sexually and asexually but the sexual / prefect stage rarely occurs in nature but in the laboratory it may occur as black, globose pseudothecia. Conidia from diseased

maize leaves are straight or slightly curved, nearly cylindrical, tapering only slightly from the middle. The conidia walls are thin and light olivaceous to light olivaceous brown in mature spores. The hilum protrudes distinctly from the conidia to bluntly rounded basal cells (Leonard and Suggs, 1974). Conidiophores are simple, cylindrical, olivaceous brown and 47-443 by 46 µm in size. Single conidium is formed terminally on the conidiophore, which then resumes growth to one side of the conidial attachment and eventually produced another condium at the new tip. Circular conidial scars are evident on the conidiophore after the abscission of the conidia (Leonard and Suggs, 1974). The ascigerous state usually occurs in culture by pairing isolates from compatible mating types. The ascocarps are globose, black uniloculate stromatic bodies 260-400 µm in diameter often with a distinct globose or cylindrical beak. Mating types appears to be controlled at a single chromosome locus (Leonard and Suggs, 1974).

The pathogen survives in unfavourable conditions over long periods within undecomposed crop residues in the fields after harvest (Levy 1995). *Exserohilum turcicum* survives longer on sorghum in host tissue in sterile soils than in non-sterile soil. *Exserohilum turcicum* is or can be seed-borne and survives in the soil saprophytically, while on debris it survives as chlamydospores. Chlamydospores may become relatively ineffective if debris is ploughed deep in the soil in autumn. Thus, *E. turcicum* can survive as mycelium, conidia and chlamydospores in and on maize / sorghum residues. In early season, new conidia are produced and then carried by wind or rain to lower leaves of young maize and sorghum plants (Agrios, 1997). Infection occurs by germinating conidia, when free water is present on leaf surface for 6-18 hours at temperatures between 18 and 27°C. Secondary infection is mediated by conidia formed on leaf tissues (Lipps and Mills, 2008).

2.2.2 Population biology of *Exserohilum turcicum*

Population biology is an integration of ecological, genetical and evolutionary principles within a population context (Okori, 2004). In the case of *E. turcicum*, most studies on population biology have

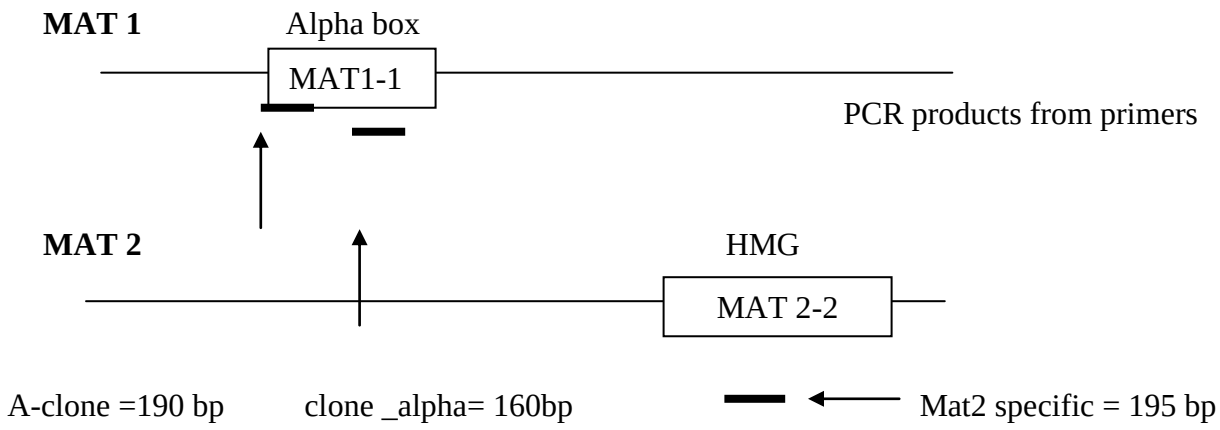
been conducted on maize derived isolates. These studies show that the fungus is highly diverse (Yongshan *et al.*, 2007).

Moreover, many physiological races of *E. turcicum* have been discovered and would probably continue to be discovered in many countries (Li *et al.*, 1999; Jingao *et al.*, 2008). In general, one of the major sources of genetic diversity is sexual recombination. However, *E. turcicum* can form sexual structures, this is seldom observed in the field except in the laboratory (Luttrell, 1958). *Exserohilum turcicum* has a single locus and two-allele mating system (Figure 1) (Nelson, 1959). The fungus exhibits basically two forms of mating type, identified by the high motility region present in the mating type locus *MAT* as *MAT 1* and *MAT 2* (Figure 1) (Arie *et al.*, 1997). The alpha box primers are normally used to amplify this locus and thus determine the mating type. The alpha box is only present in individuals of the *MAT 1* mating type (Figure 1). The MAT region contains only two genes (transcription factors) that cause major changes in the fungi leading to sexual mating. Recently, mating type alleles *MAT 1 MAT 2* and *MAT1, 2* have been reported (Yongshan *et al*., 2007). The frequency of *MAT 1* appears significantly higher than that of MAT *2* and *MAT 1, 2*. This un-equal distribution of mating types probably explains the low frequency of genetic recombination. Moreover, virulence of the three mating type groups were not significantly different (Yongshan *et al.*, 2007).

Determination of genetic diversity of fungal pathogens is of great importance in breeding for resistance as well as in studying population dynamics. Assessment of genetic diversity of *E. turcicum* is needed to determine whether races constitute genetically distinct groups and to obtain molecular markers for differentiating them (Abadi *et al.*, 1996). The genetic variability of *E. turcicum* isolates from Africa, America and Asia belonging to different races and host plants has been done using RAPDs (Abadi *et al*., 1996). The study results indicated that the levels of polymorphism among the maize derived isolates were low compared to sorghum derived isolates. Larger genetic distances were observed both among the

sorghum derived isolates and between sorghum and maize derived isolates than within maize derived isolates. It was further revealed that some sorghum infecting isolates could actually infect maize.

In another study based on RAPD, populations of *E .turcicum* from Kenya, Mexico, and southern China had an extremely high genotypic diversity but an even distribution of the two mating types suggesting, frequent sexual recombination among the populations (Dorothea *et al.*, 1997). In contrast, temperate populations from Europe and Northern China had a much lower genotypic diversity, strong gametic phase disequilibrium and an uneven distribution of mating types, indicating rare cases of sexual recombination. Populations in different continents were genetically distinct. They shared no haplotypes and carried several "private" alleles.



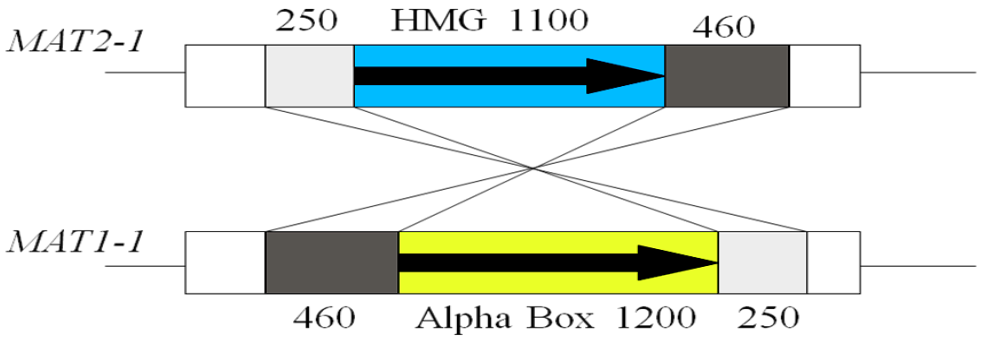


Figure 1. The proposed region of the MAT Locus. The region can be distinguished by the presence of the alpha box in the *MAT1* and HMG in *MAT2.* The mating type locus consists of a single gene flanked by inverted conserved regions at either end. The gene has either an HMG or alpha box domain containing gene. .the numbers indicates the band size of each section. Flanking regions are inverted.

The number of migrants between continents and between regions (between Northern and Southern China, Western and Central Kenya, and Europe West and East of the Alps) was estimated to be less than one per generation (Dorothea *et al*., 1997). Multivariate statistical analysis suggested a greater relatedness of populations from the same continents than from different continents (Dorothea *et al*., 1997). Within agro-ecological zones, migration appeared extensive. The potential within populations of *E. turcicum* for adaptation is thought to be high especially in tropical climates (Dorothea *et al*., 1997).

2.2.3 *Exserohilum turcicum* physiological races

Exserohilum turcicum races are defined based on their phenotypic reactions when inoculated onto a set of differential maize lines in this system (Berguist and Masias 1974, Leonard *et al.* 1989). Race designations are based on resistance genes that their virulence matches. For example *E. turcicum* race 0 is ineffective (avirulent) against all *Ht* genes described above whereas race 1 is only effective (virulent) against *Ht* 1. Both races are effective against maize genotypes lacking all resistance genes. So under this nomenclature there are nine known races of *E. turcicum* (Table 1) (Leonard *et al.*, 1989). The *Ht1*, *Ht2* and *Ht3* resistant gene occurs as chlorotic lesions with minimum sporulation, while the *HtN* induced resistance is expressed as a delay in disease development until after pollination (Leonard *et al.*, 1989).

Table 1. Race designation of *Exserohilum turcicum* pathotypes.

Number	Race	Resistance Formula	Author cited
1	0	*Ht1, Ht2, Ht3, HtN/,*	(Welz *et al.*, 1993)
2	1	*Ht2, Ht3, HtN / Ht1*	(Jordan *et al.*, 1983)
3	2	*Ht1, Ht3, HtN/Ht2*	(Smith and Kinsey, 1980)
4	2N	*Ht 1,Ht 3/Ht2, HtN*	(Welz *et al.*, 1993)
5	3	*Ht1 / Ht2 Ht3 HtN*	(Welz *et al.*, 1993)
6	4	*Ht 1/Ht 2,Ht 3,HtN*	(Welz *et al.*, 1993)
7	12	*Ht3, HtN / Ht1, Ht2*	(Welz *et al.*, 1993)
8	23	*Ht2, Ht3 / Ht1, HtN*	(Welz *et al.*, 1993)
9	23N	*Ht2, Ht3, HtN / Ht1*	(Welz *et al.*, 1993)

Five races of *E. turcicum* have been reported to overcome specific *Ht* resistance genes in the United States (Windes and Pedersen, 1991) and others have been reported from crosses of races in the laboratory (Fallah and Pataky, 1994). Races 0 and 1 are most prevalent, whereas races 23, 2N, and 23N are rare (Fallah and Pataky, 1994). Welz *et al*. (1993) reported that races 0, 2, 2N, 23, and 23N existed in East Africa, but race 0 is most prevalent (Bigirwa *et al.*, 1993). So far, among cultivated crops, the vast majority of *E. turcicum* races have been isolated from maize; but the same pathogen has been isolated from several grass crop species. The early studies on *E. turcicum* suggest that isolates from Johnson grass do not infect maize and conversely isolates from maize do not infect Johnson grass, suggesting host species specialisation, (Robert, 1960). There is need to further test this hypothesis on sorghum given that sorghum and Johnson grass (*Sorghum halapense*) belong to the same genus.

2.2.4 Genetics of resistance to *Exserohilum turcicum*

The earliest sources of resistance to TLB were first found in ladyfinger popcorn in the 1940's (Hilu and Hooker, 1963). The *Ht1* gene identified from popcorn cv. ladyfinger and field corn inbred GE440 was characterised by chlorotic lesions, reduced sporulation and smaller necrotic lesions (Hooker, 1963). It was further characterised by development of a green halo around the point of infection. Later, studies showed that this type of resistance reaction was conditioned by a single gene called *Ht* (*Helminthosporium turcicum*), which was the name of the pathogen at the time (Hooker, 1963). A gene–for-gene relation was found and with the discovery of several new races, more *Ht* resistance loci have been reported (Carson, 1995).

The degree of resistance expressed by lines with the *Ht* gene are influenced by the level of partial resistance in the line (Pataky, 1994). Incorporation of the *Ht* gene into a background with partial resistance confers the most effective resistance to *E. turcicum*, as displayed by reduced sporulation, number and size of lesions (Jiansheng and Jilin, 1984). Polygenic / partial resistance is considered to be more durable since

single-gene resistance is vulnerable to the development of new races (Lipps, 1982). Partial resistance is not easily overcome by new races (Parlevliet, 1993). A combination of monogenic *Ht* resistance with partial resistance permits additive or complementary interallelic interactions that may enhance the overall level of resistance (Hughes and Hooker, 1971).

Quantitatively, partial resistance ranges from a high level with few, small lesions to a low level with many, large sporulating lesions (Raymundo and Hooker, 1982). Whereas several quantitative genes have been found, resistance break down is quite common. The development of new races shortens durability of the *Ht* based resistance (Ceballos *et al.*, 1991). Durable resistance is characterised by reduced number of lesions and decrease in lesion size and amount of sporulation normally which is typical of polygenic resistance (Ullstrup, 1970).

2.2.5 Tools and past studies of pathogen population

Genetic variation of a population can be measured on the basis of ecologically important traits or selectively neutral genetic markers (Okori, 2004). Ecologically important traits are those traits that affect fitness and are therefore under selection. For example, the use of race differentials is regarded as dependant on ecologically selective traits because it is based on differences in pathogenicity among pathotypes (Okori, 2004). Pathogenicity itself is a result of pathogen-host interaction, clearly a product of co-evolution. However, estimation of genetic variation based on ecologically selective traits is constrained by biased evolutionary signature especially selection (Okori, 2004). Pathotype analysis based on race differentials may also be influenced by the environment, or may have unknown resistance factors compounding analysis such as the judgemental biases of the investigator (Okori, 2004)

Selectively neutral variation refers to variation in traits that are not under selection (Milgroom and Fry, 1997). Selectively neutral traits are however influenced by evolutionary forces that includes; mutation, genetic drift, gene flow, mating system and selection, if linked to a selectable gene (Okori, 2004). Neutral

genetic markers are found largely in non-coding regions of the genome that are less conserved and prone to mutations and are also less conservative, hence highly variable (McDonald, 2004). Inference of phylogeny and other population genetic characteristics of a pathogen rely on presence of polymorphism that relates to the evolutionary history of a species. Neutral genetic markers may be regarded as dominant or co-dominant depending on the ability to detect heterozygosity. Co-dominant markers are DNA sequences that allow unequivocal distinction of homozygous and heterozygous genotypes during analysis. In contrast, dominant markers cannot distinguish between homozygous and heterozygous individuals and will require previous knowledge of the sequence.

Based on selective neutral DNA-based genetic markers, a wide range of genome characterisation techniques exist for analysis of genetic variation of an organism. Commonly used techniques include, restriction fragment length polymorphism (RFLP) (Bonierbale *et al.*, 1988), random amplified polymorphic DNA (RAPD) (Williams *et al.*, 1990), amplified fragment length polymorphism (AFLP) (Vos *et al.*, 1995), inter simple sequence repeats (ISSR) (Reddy *et al.*, 2002), cleaved amplified polymorphic markers (CAPs) (Konieczny and Ausubel, 1993), microsatellites, single nucleotide polymorphisms (SNPs) (Brinkman and Leipe, 2001) and species specific primers designed based on Internal transcribed ribosomal DNA (ITS) and 5.8s rDNA sequences (Stromberg *et al.*, 2003). These markers or techniques may also be used for DNA finger printing (Stromberg *et al.*, 2003). In this study, both selective (race differentials) and neutral genetic marker (SSR-Simple Sequence Repeats and RAPDs (Randomly Amplified polymorphic DNA) were used to study and monitor *E. turcicum* species variation in Uganda.

2.3 Epidemiology of Turcicum Leaf Blight on maize and sorghum

2.3.1 Spatial and temporal studies

The dispersal potential of a pathogen is often quantified in field studies where gradients of inoculum or diseases are measured at various distances from a point source of inoculum. Inoculum gradients have the advantage of measuring directly the number of propagules transported to different distances from the source. Disease gradients have the advantage of accounting for all sub- processes leading to the spread of disease including the release, transport and deposition of inoculums, as well as the infectiveness of that inoculum (Mundt *et al.*, 1998). Primary gradients measure dispersal potential in a monocycle, whereas secondary and subsequent gradients are to be interpreted in the context of the more complex relations between temporal and spatial increase of disease (Minogue and Fry, 1983; Campbell and Madden, 1990).

Both inoculum and disease gradients are often fitted to simple empirical models so as to quantify the steepness of the gradient with a slope (Gregory, 1968) and this has been measured for many plant pathogens/diseases, most of which are fungal (Fitt *et al.*, 1987). Decrease in spore concentration over distance away from the inoculum foci may result in decrease in spore deposition on the ground or crop. Local sources of inoculum, secondary dispersal, population of susceptible hosts, background contamination flattens primary gradient and when disease incidence is expressed as fraction of individuals infected, the multiple infection transformation has to be applied. These are some of the basic principles in dispersal gradient in plant pathosystem (Gregory, 1968).

The role of residue-borne pathogens in the epidemiology of maize diseases has been extensively documented especially in the USA. *Exserohilum turcicum* infested maize leaves has been used to induce turcicum leaf blight epidemic and *Colletotrichum graminicola* infested maize residues have been shown to play critical role in anthracnose epidemics (Lipp, 1983). Other pathogens reported to survive on maize residues and influence epidemics include *Phyosticta maydis* which causes yellow maize leaf blight,

Physoderma maydis, which causes brown leaf spot (Shurtleff 1980), and *Cercospora zea-maydis*, which induces gray leaf spot (Payne and Waldron, 1983). These literature reports indicate that infested maize residues present on soil surface is an important factor in the epidemics of maize diseases. The role of infested residues spread of TLB on sorghum is not yet well documented.

Nevertheless for both maize and sorghum residue –borne pathogens, the final disease severity can be influenced by the amount of infested residue on soil surface, which in turn can be influenced by the cropping system and tillage practices. Diseases such as anthracnose, turcicum leaf blight, grey leaf spot and brown leaf spot are more severe under minimal tillage than in conventional tillage where maize debris is buried (de Nazareno *et al.,* 1993). It is therefore possible that deep ploughing and crop rotation may control some of the residue borne pathogens. Under continuous cereal cultivation, deep ploughing may reduce inoculum levels and the incidence of early season disease development, but this may not necessarily result into complete disease control (Lipp, 1983). Farmers in Uganda rarely practice deep cultivation and it is likely that infested sorghum residues is an important factor in the initiation of epidemics of turcicum leaf blight of sorghum.

Past studies on TLB of maize in Uganda, have reported significant effect of crop residue on epidemics of the disease (Takan *et al.*, 1994). The study reported that the final percentage leaf area blighted and area under disease progress curve (AUDPC) were significantly higher in the residue infested plots as compared to the residue free plots (Takan *et al.,* 1994). Disease severity decreased with increase in distance from the maize residue area where as the apparent infection rate remained relatively constant. Levels of maize residues significantly influence gradients of disease-spread curves. The gradients flattened as the seasons progressed (Takan *et al.*, 1994; Adipala *et al.*, 1995).

Plant disease epidemics may also be described by analysing disease spread over time (Campbell and Madden, 1990). Such analysis often referred to as temporal studies. Several disease progress models have

been proposed for characterising increase in disease over time for polycyclic diseases with the logistic and Gompertz models being most frequently used (Campbell and Madden, 1990). These models define disease progress in terms of rate of disease increase and estimated disease level at the observed start of the epidemic. A pathogen with ability to complete several generations in the course of the epidemics can best be described by the logistic model (Vander Plank, 1963). A related approach is to calculate the area under disease progress curve (AUDPC), which describes disease progress in terms of disease levels, integrated over the assessment time (Campbell and Madden, 1990).

Previous studies in western Kenya on the temporal dynamics of the development of sorghum anthracnose and leaf blight have been used to determine the parameters that best described the disease progress in *C. sublineolum*–sorghum and *E. turcicum*–sorghum pathosystems, under the effects of different treatments. The use of logistic model allowed direct comparisons to be drawn between disease epidemics caused by the two pathogens. There were clear differences in time of disease onset for the two epidemics and, in most cases, leaf blight severity was low. It was therefore unlikely that the two pathogens were competing for host resources such as green leaf tissues. This conclusion was supported by the pattern of leaf blight progress in the absence of anthracnose in 1996. It was relatively easy to discriminate between anthracnose and leaf blight disease symptoms on the same plant, reducing the likelihood that errors in estimated severity for the two diseases would be correlated (Madden *et al*., 1987).

Furthermore host resistance was consistently associated with delayed onset of the two diseases with lower rates of progress and with reduced disease severity at crop maturity. Delayed disease onset was taken to indicate longer latent periods of the pathogens in resistant cultivars, while reduced rate of progress and lower disease levels were taken to indicate inhibition of pathogen development or host colonization.

The earlier disease onset associated with delayed planting for both anthracnose and leaf blight was thought to be due to increased inoculum from infected plants in adjacent plots. Plants from the third planting date, i.e. a 20-day delay in planting, were therefore expected to develop the highest disease severity, particularly for leaf blight as this is more severe on younger plants (Julian *et al*., 1994). The presence of significant interactions between planting date and cultivar on parameter estimates for both anthracnose and leaf blight indicated the effects of environment on disease progress. Although large amounts of inoculum may be essential for early disease onset, climatic conditions, for example dry weather, could also profoundly affect disease progress. The results presented in their study illustrated that planting date should be a critical consideration in developing screening programmes for resistance to foliar diseases in sorghum. It is therefore proposed that, when screening for resistance to anthracnose and leaf blight, test entries should be planted at least 15 days later than the normal planting time, usually defined by the onset of seasonal rains in eastern Africa (Ngugi *et al*., 2000).

2.4 Sectional Conclusion

Turcicum leaf blight is one of the most important diseases of maize and sorghum in Uganda. Several studies have been conducted on the pathogen in maize. These studies have facilitated the screening and breeding of varieties resistant to TLB in maize. Though this has been done extensively in maize, there is limited information on TLB-sorghum pathosystem to guide disease management. This calls for extensive characterisation of the sorghum-TLB pathosystem. Moreover, whereas maize is an America crop, sorghum is an African crop. It is therefore conceivable that both pathogen and host have co-evolved to produce the current pathosystem. The purpose of this study was to characterise the TLB-sorghum pathosystem specifically studying the pathogen variability, host reaction and disease development over distance and time on sorghum in different Ugandan agro-ecologies.

CHAPTER THREE

MATERIAL AND METHODS

3.1 Description of the study area

Diseased sorghum leaf samples were collected from sorghum fields in 23 districts in Eastern, Western and North-Western parts of Uganda where sorghum is commonly cultivated. These included the districts of Kaberamaido, Lira, Amuria, Dokolo, Apac, Katakwi, Masindi, Homia, Arua, Nebbi, Koboko, Marahca, Pakwach, Iganga, Kumi, Mbale, Gulu, Pallisa, Tororo, Soroti, Serere, Kiboga and Wakiso (Figure 2). The districts were selected to represent different agro-ecological zones (Wortmann and Eledu, 1999), differentiated from each other by farming systems, edaphic factors; climatic factors, altitude and major vegetation cover (Figure 3) (Wortmann and Eledu, 1999). It is important to note that farming systems tend to overlap between districts and agro-ecological zones.

The districts of Lira, Serere, Kaberamaido and Soroti are found in the North-Eastern-Central Grass-Bush Farmlands. This agro-ecological zone is characterised by mean annual temperatures of about 20^0C and an annual precipitation of about 1200 mm received during one long rain season (Ruecker *et al.*, 2003). The agro-ecological zone is on average 1073 meters above sea level. Two very similar farming systems exist in this agro-ecological zone; the annual cropping and cattle northern system, practiced in Lira, and the annual cropping and cattle Teso system, practiced in Soroti (Ruecker *et al.*, 2003). Both farming systems are characterised by unimodal seasonality/agricultural potential (Ruecker *et al.*, 2003) with annual precipitation potentials of 900 – 1200 mm of rainfall and temperatures ranging between 28 – 31^0C. The major cereals cultivated in this agro-ecological zone include finger millet (*Eleusine coracana*), maize, and sorghum. Cultivation of crops and rearing of livestock is also practiced and the use of crop residues is very common in these systems as feed.

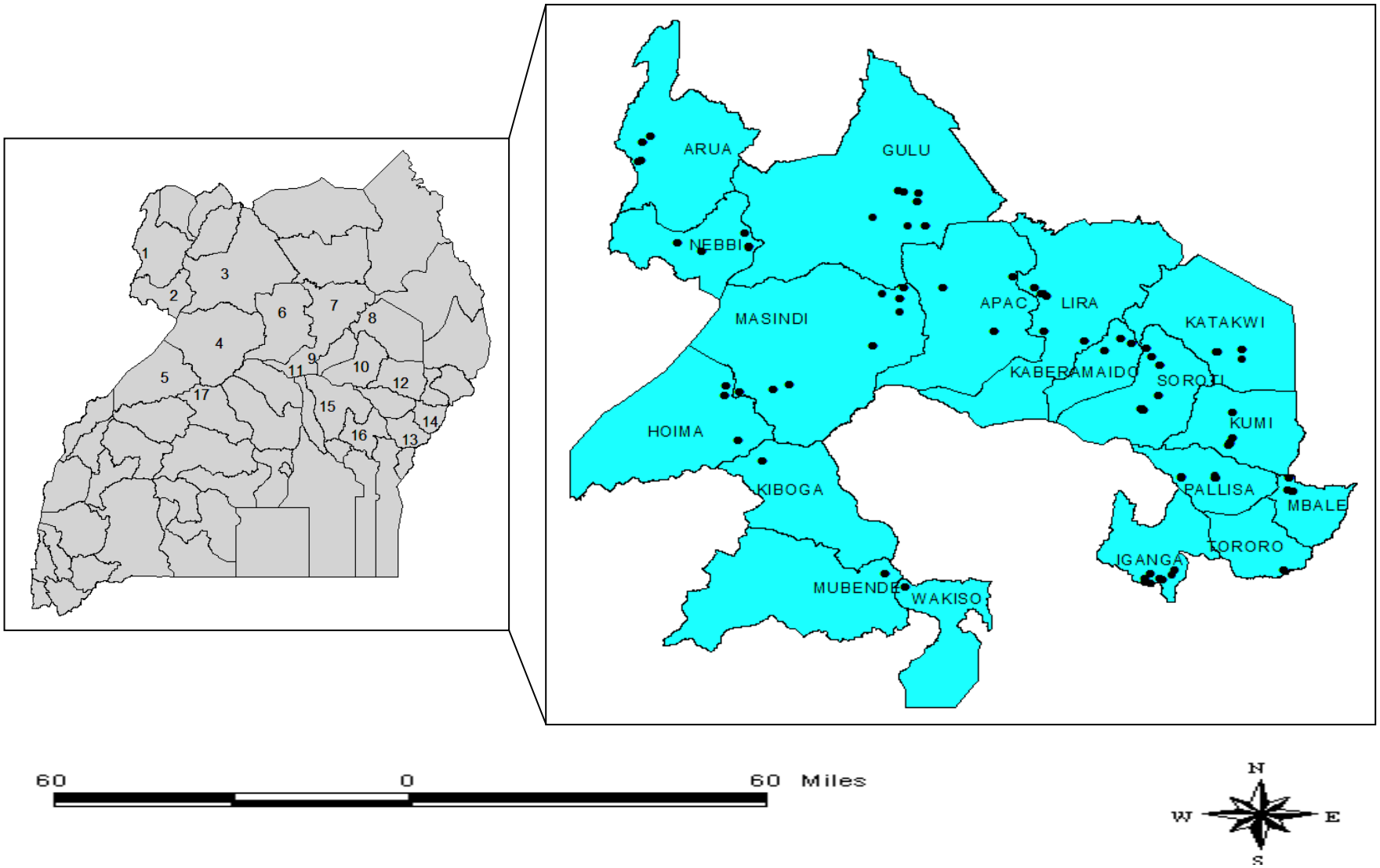
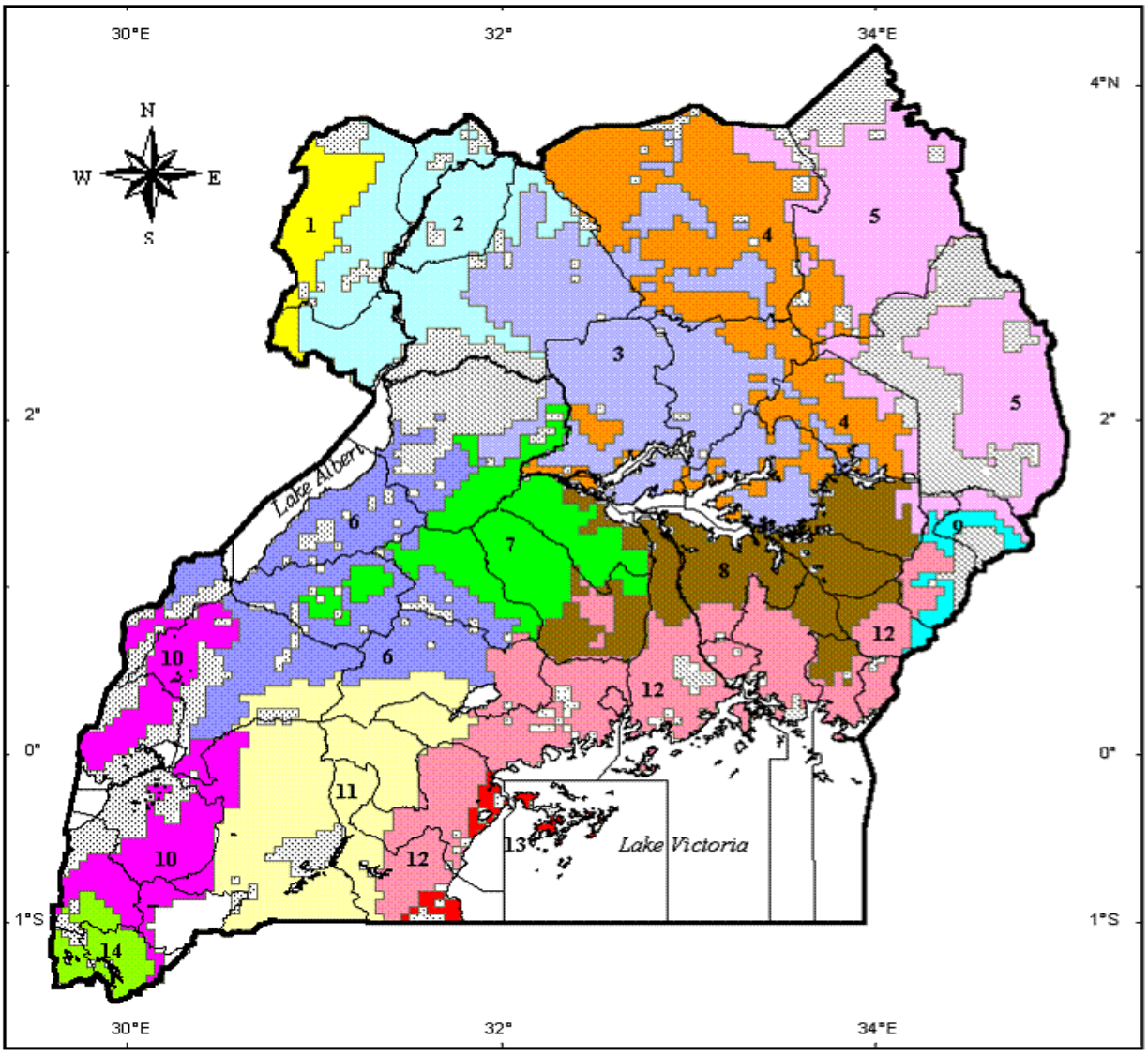


Figure 2. A Map of Uganda showing areas were Turcicum leaf blight infested leaves were collected. Sampling points are indicated by dots 1-Arua, 2 –Nebbi, 3-Gulu, 4-Masindi, 5-Hoima, 6-Apac, 7-Lira, 8-Katakwi, 9-Kaberamaido, 10-Soroti,-Dokolo, 12-Kumi, 13-Tororo, 14-Mbale, 16-Iganga and 17-Kiboga

The districts of Kumi and Pallisa are found in the Southern and Eastern Lake Kyoga Basin (Figure 3). This agro-ecological zone receives an annual precipitation of 1200 mm of rainfall, with mean temperatures of 28 – 31^{0}C and about 1143 meters above sea level. The farming system prevalent in Kumi district is the annual cropping and cattle Teso system whereas, the farming system practiced in Pallisa is the Banana-millet-cotton system. Vegetation in the banana-millet-cotton system is moist *Combetrum* savanna with moderate biomass production. The major cereals cultivated include millet, sorghum and maize. In the drier areas, livestock keeping is the main activity.

Iganga, Wakiso and Tororo districts represent the Lake Victoria Crescent and Mbale Farmlands (Figure 3). This agro-ecological zone is about 1106 meters above sea level with mean annual precipitation between 1200 – 1400 mm of rainfall received during two rain seasons (Ruecker *et al.*, 2003). The banana-millet-cotton system is the farming system prevalent in Tororo. Conversely, the farming system in Iganga district is the intensive banana coffee lakeshore system. The Lake Victoria Crescent and Mbale Farmlands agro-ecology is characterised by soils of medium to high productivity. The vegetation is mainly forest-savanna mosaic with pastures suitable for intensive livestock production. Maize and bananas are the major crops in this agro-ecological zone; sorghum, millet, and sweet potatoes are secondary food security crops. Livestock are generally not integrated into the system but dairy cattle are gaining prominence.



LEGEND

1. West Nile Farmlands
2. Northwestern Farmlands-Wooded Savanna
3. Northern Moist Farmlands
4. Northeastern-central Grass-Bush-Farmlands
5. Northeastern Semi-arid Short Grass Plains
6. Western Mid-Altitude Farmlands and the Semliki Flats
7. Central Wooded Savanna
8. Southern and Eastern Lake Kyoga Basin
9. Mt. Elgon Farmlands
10. Western Medium-High Farmlands
11. Southwestern Grass-Farmlands
12. Lake Victoria Crescent and Mbale Farmlands
13. Ssese Islands and Sango Plains
14. Southwestern Highlands

Gazetted (protected) areas

Figure 3. A map of Uganda showing aggregation of agro-ecologies in the country (Wortmann and Eledu, 1999**).**

3.2 Study 1. Assessment of incidence and severity of turcicum leaf blight on sorghum in different agro ecologies of Uganda

The objective of this study was to assess the severity and incidence of Turcicum leaf blight in the major sorghum agro-ecologies of Uganda

3.2.1 Sampling strategy

A hierarchical sampling structure was used to collect TLB infested sorghum leaf samples from fields in 23 districts found within eight agro-ecological zones (Figure 2). The sampling structure consisted of two hierarchical levels; agro-ecological zones and districts within agro-ecological zones. From each district, at least five farmer fields, each averaging 2 acres were visited. In each field, at least 6 leaf samples bearing characteristic TLB symptoms were picked. Global Positioning Systems (GPS) readings were taken at each sampling point using a GPS receiver model 315 (Magellan Navigation, Inc. Tulsa, Oklahoma, U.S.A) and the coordinates used to generate maps using the Geographic Information Systems (GIS) software Arc View 3.2a and spatial analyst 1.1 (Environmental Systems Research Institute, Inc. Seattle, WA, U.S.A). No information was taken of the sorghum varieties grown in the different agro-ecological zones, since farmers had limited knowledge of the varieties cultivated.

3.2.2 Disease assessment

Turcicum leaf blight disease incidence in each field was assessed as the proportion of plants showing symptoms in a field. In each field 20 plants in middle of each plot were randomly selected and the number of plants having TLB symptoms on a whole plant basis counted and expressed as a percentage of the plant population. Severity of TLB on whole plant basis was rated using a scale of 0, 0.5, 1.5, 10, 25, 50 and > 75% leaf area affected (Adipala *et al.,* 1993). At each sampling point, GPS coordinates were taken. The data were subsequently used to generate disease maps.

3.2.3 Data analysis: Survey data

Disease maps were generated using the GIS software Arc View 3.2a with spatial analyst by interpolating the surface from GPS points and the associated field severity data using the inverse distance weighted (IDW) interpolation method. Prior to interpolation, a power parameter of 10 was set to control the influence of surrounding points at each location (Environmental Systems Research Institute, Inc. Seattle, U.S.A). Field incidence and severity data were subjected to analysis of variance (ANOVA) (Steel *et al.*, 1997). Turcicum leaf blight severity ratings were also subjected to nested analysis of variance at two levels of hierarchy that is, district and agro-ecological zone to determine the impact of *E. turcicum* species variability on TLB severity. Where significant differences were detected, means were compared using Turkey's (Turkey-Kramer) simultaneous tests for unbalanced data at the 95% confidence interval (Steel *et al.*, 1997). All statistical analyses were performed using Genstat 7 version 3.2, 2007 (Lawes Agricultural Trust: Rothamsted Experimental Station, UK) and MINITAB release 14 versions 14.20, 2005 (Minitab Inc, Pennsylvania, USA).

3.3 Study 2. Reaction of sorghum accessions from Uganda to turcicum leaf blight

The objective of this study was to characterise sorghum accessions from Uganda for resistance to Turcicum leaf blight

3.3.1 Experimental set-up

For the identification of sources of resistance to *E. turcicum*, 202 accessions of sorghum from farmers' fields were screened at field level. These included both the local land races and the newly released varieties from National Agricultural Semi Arid Resources Research Institute (NaSARRI) in Serere, Soroti. The experiment was carried out at NaSARRI, located at latitude 1° 29' 39N, longitude 33° 27' 19E and an altitude of 1085 metres above sea level. The experiment was established in the second crop-growing

season of 2007, following a randomised complete block design (RCBD) with two replications. Test lines were planted in 4 row plots bordered by susceptible disease spreader rows on either side. A total of 82 plants /plot were established at a plant spacing of 60 X 30 cm.

3.3.2 Inoculum preparation and application

Seedlings were inoculated at the 5-leaf stage. To prepare the inoculum, lesions were cut from infested leaves, placed on moist paper towels in petri dishes for 48 hours to allow sporulation. Single spores were picked from lesions with the aid of a sterile microscope and placed on potato dextrose agar (PDA) plate and incubated at room temperature. Individual colonies of *E. turcicum* were subsequently sub-cultured to fresh PDA plates and used to inoculate autoclaved sorghum kernels and allowed to colonize the grains for about 10 days. The colonized sorghum kernels were air-dried prior to field inoculation. Inoculation was done at the four- to six-leaf stage by placing 20 to 30 seeds of colonized sorghum kernels into the leaf whorls. Inoculation was done in the evening to allow successful infection when dew and ambient temperature is optimal (Carson, 1995).

3.3.3 Disease assessment

Disease assessment commenced 51 days after planting (DAP) and assessment of disease severity continued on weekly basis for 6 weeks. A scale of 0 – 5 (Adipala *et al*., 1993) was used where 0 = no disease (no lesions identifiable on any of the leaves), 1 = 0.5 to 1.0 % of leaf surface diseased (a few restricted lesions on a few leaves); 2 = 5 to 10 % of leaf area diseased (several small or big lesions on many leaves); 3 = 10 to 15 % of leaf surface diseased (numerous small and large lesions on many leaves); 4 = 20 - 35 % of leaf surface diseased (many large and coalesced lesions on many leaves) and 5 = 45-75 % of leaf surface diseased ; representing multitudes of coalesced lesions resulting in leaf wilting and tearing and blotching. Disease severity data later were used to compute areas under disease progress curves

(AUPDC), as described by Campbell and Madden (1990) and Adipala *et al.* (1993). Data were also taken on sorghum race, sorghum head colour, plant height (cm) and days to flowering.

3.3.4 Data analysis

The AUPDC data were subjected to analysis of variance (ANOVA) and means separated using Turkey's simultaneous tests for unbalanced data at the 95% confidence interval (Steel *et al.*, 1997). Furthermore data were subjected to cluster analysis on the basis of accession performances. These were classified basing on disease reaction type i.e. Resistant (a score of 0-2.08), moderately resistant (2.08-3.13), susceptible (3.13-4.17), and highly susceptible (> 4.17). Qualitative data (sorghum races) was based on sorghum phenotypic descriptors (IBPGR/ICRISAT, 1993) and used to classify sorghum accession. All data analyses were performed using the software described in section 3.2.3.

3.4 Study 3. Characterisation of temporal attributes of turcicum leaf blight epidemics of sorghum

The objective of this study was to carryout epidemiological studies on the temporal attributes of Turcicum leaf blight on both Maize and Sorghum raised at the same time in Uganda under two agro-ecological zones.

3.4.1 Host genotypes

Three accessions were selected from the sorghum land races collected from farmer's fields. These constituted *Epuripuri*, which is highly susceptible to TLB, MUC007/009 and MUC007/029 moderately resistant and resistant lines respectively. *Epuripuri* is a commercial cultivar developed at NaSARRI, while MUC007/009 and MUC007/029 are local land races that were picked from farmers fields during the survey in Homia and Masindi respectively. Two open pollinated maize cultivars, Longe 4 and Longe 5 moderately resistant and highly susceptible to TLB, respectively, were also incorporated in this study

because the spatio-temporal dynamics TLB of maize has been previously characterised in Uganda and Maize served as a control crop. Longe 4 and Longe 5 are commercial cultivars developed at National Crop Resource Research Institute (NaCRRI), Namulonge, Wakiso in Uganda.

3.4.2 Field plots

Experiments were established at two locations: Makerere University Agricultural Research Institute Kabanyolo (MUARIK), Wakiso district, and National Agriculture Semi Arid Resource Research Institute (NaSARRI), Serere, Soroti district during the second rains (August-November) of 2007 and first rains of (February-April) of 2008. Land not previously cultivated with sorghum or its close relative was used. In NaSARRI, land used for cotton production the previous year was used while in Kabanyolo, land under fallow was opened up for the experimental work. At both locations, the plots were tractor ploughed and disc harrowed twice before planting. The trials were established on 26th October and 1st November 2007, and 6th April and 17th April 2008 at NaSARRI and MUARIK, respectively in the two respective cropping seasons. The plots were arranged following a randomised complete block design (RCBD) with two and three replications for maize and sorghum, respectively. Sorghum and maize rows were planted in a north-south direction at MUARIK and in an East-west direction in NaSARRI. Each experimental unit measured 15 X 15 m with 15 sorghum rows planted at a spacing of 60 by 30cm and 15 x 15 m with 15 maize rows planted at a spacing of 75 X 30 cm. All plots for maize were planted by hand with two or three seeds per hole while for sorghum 5-10 seeds were planted to ensure germination in all the plots. Maize plots were weeded at V3 and V8 growth stages (Ritchie *et al.*, 1989) while weeding was carried out at stage 1 and stage 3 for sorghum (Vanderlip, 1993).

3.4.3 Inoculation and disease assessment

Inoculation was carried out on 28th December 2007 at NaSARRI, Soroti and on 4th January 2008 at MUARIK, Wakiso in season one and 25th April at NaSARRI, Soroti and 2nd May 2008 at MUARIK,

Wakiso in the second season. Plots were inoculated by placing approximately 5-10 *Exserohilum turcicum* colonised sorghum kernels into the whorl of each plant at V6 growth stage (Ritchie *et al.*, 1989) and stage 2 (Vanderlip, 1993) for maize and sorghum, respectively. Three weeks after inoculation, plots where assessed for disease severity using a 0 to 75 % scale (Adipala *et al.*, 1993). In additional, other parameters such as disease incidence (%), percentage ear leaf area affected (PELAA) as described by (Freppon *et al.*, 1996), and the number of TLB lesions on the ear leaf of the tagged plants were also considered. Data on severity was taken as described by Adipala *et al.* (1993). Incidence, lesion sizes and sporulation during latent period were assessed. Disease assessment commenced 40 days after inoculation which corresponded to approximately the R1 growth stage in maize (Ritchie *et al.*, 1989) and stage 6 in sorghum (Vanderlip, 1993). Six assessments were made at 7-10 day intervals.

3.4.4 Modelling temporal disease spread and data analysis

Turcicum leaf blight symptoms were recorded on each test plant by visual assessment of disease symptoms on the leaves. Plants were assessed for five weeks. Data on severity, lesion number, lesion sizes, latent period and plot means were subjected to ANOVA. Severity data were used to compute areas under disease progress curves (AUDPC), as well as fitted to the logistic and Gompertz models. Areas under disease progress curves (AUDPC) were computed according to Campbell and Madden (1990). Where there was variation in dates of disease assessment, the data were standardised by dividing AUDPC values by the total duration of the epidemics (Campbell and Madden, 1990). The formulae for computing AUDPC was given as

$$AUDPC = \sum_{i=1}^{n-1}\left[\left(\frac{y_i + y_{i+1}}{2}\right)t_{t+1} + t_t\right] \text{---------- (1)}$$

Where t is the time in days of each reading, Y is the % of affected foliage at each reading and n is the number of readings

Fitting of the data to growth curve models of Logistic and Gompertz were performed to characterise the polycyclic nature of epidemics. The slope of the curve, (r) depicts rate of disease increase over time, and y, the theoretical estimates of initial amount of epidemic y_0 (y-axis intercept. The Logistic model used was given by

$$In\left(y/(1-y)\right) = In\left(y_o/1-y_0\right) + rt \text{ ---------- (2)}$$

Computation of y_0 and r (apparent rate of infection) was performed by plots of logits over time

Data were also fitted to the Gompertz model (Berger, 1981) as described for the logistic models expect that the linearised formula for the Gompertz model was different, i.e. it was given by

$$-In[-In(y)] = -In[(-Iny_0)] + \text{rt.} \text{ ---------- (3)}$$

These models were selected because of their common usage and preliminary pots indicated their suitability. In these models y is proportion of disease severity at time (t) due to inoculum application and background infection and r is the slope. The appropriateness of each model was evaluated on the basis of coefficient of determination (R^2) and plot of residuals. As appropriate, data were subjected to ANOVA and where significant differences were found, means were compared using Fisher's Protected Least Significant Differences (LSD) at $P \leq 0.05$ (Steel *et al.,* 1997).

3.5 Study 4. Characterisation of spatial attributes of turcicum leaf blight epidemics of sorghum in Uganda

The objective of this study was to carryout epidemiological studies on the spatial attributes of Turcicum leaf blight infected crop residues on the epidemics of sorghum in two agro-ecological zones of Uganda

3.5.1 Host genotype used and experimental set-up

The three accessions used in section 3.4.1 were used in the study i.e. namely *Epuripuri*, which is highly susceptible to TLB, MUC007/009 and MUC007/029 which are moderately resistant and resistant lines, respectively. Field plots were established as described in section 3.4.2. The experiment was set-up as a factorial experiment following a RCBD design. The first factor was cultivar with varying levels of disease reaction and the second amount of plant residues available in the plots. Other factors were direction (North, east, west and south) and distances 1.2, 1.8, 2.4, 3.0, 3.6 and 4.2 m away from the inoculum sources. These distances are based on earlier studies of TLB and grey leaf spot of maize (Adipala *et al.*, 1995; Ayiga, 2000).

3.5.2 Residue application and disease assessment

Dry sorghum residues parasitized by *Exserohilum turcicum* the previous season was used as an inoculum source. The amount of residues applied to the soil was varied to provide 0 (control), 40 (covering an area of 1 m x 1 m) and 80 % soil cover (covering an area of 2 m x 2 m) (for different plots), prior to disease assessment, which occurred three week later. Residues were applied in the centre of each plot in 2007 (second season), and 2008 (first season).

3.5.3 Modelling dispersion /disease gradient

For each distance from the inoculum foci, the mean severity, TLB lesion spot counts, severity, lesion sizes and latent period were calculated for each cultivar; data point and direction of disease assessment were taken. Data from the plots were used to compute areas under disease progress curve (AUPDC), slope or gradient (b), apparent infection rates (r), and intercept (a). The linearised power model, (Gregory, 1968)

$$y=ax^{b} \quad \text{----------(4)}$$

and Exponential model, (Kiyosawa and Shiyomi, 1972) were fitted to the pooled data.

$$y = a \exp(-b) \text{ ----------} (5)$$

These models were selected because of their common usage and similar studies on the spread of TLB from infested residues caused by *E. turcicum* indicating their suitability (Ayiga 2000; Asea, 2001).The spatial data were further subjected to ANOVA as described in section 3.4.4 for this study. However additional parameters such as effect of distances from residue area, time of disease assessment: initial severity (Y_i) and final severity (Y_f) and direction were included.

Two additional models were also tested. This included the inverse-power model as given by

$$y = ax - b. \text{ ----------} (6)$$

Where x is the distance from the source of the inoculum, *y* is the spore concentration, *b* is the coefficient of the slope or gradient, and *a* is the value of *y* at *x* = 1 unit from the source.

Secondly, the exponential model as given by

$$y = a \exp(-bx). \text{ ----------} (7)$$

Where *x* and *y* are as above, *a* is the spore concentration source and *b* is the coefficient for the slope or gradient.

3.6 Study 5. Characterisation of *Exserohilum Turcicum* sorghum populations in Uganda

The objective of this study was to characterise the *Exserohilum turcicum* sorghum populations in Uganda in terms of confirming the existences of two mating types namely *MAT 1* and *MAT 2* of the pathogen, nature of races of that exist in Uganda based on sorghum *E. turcicum* isolates as well as the genetic variability of *E. turcicum* populations in Uganda

3.6.1 Fungal culture

Leaf samples obtained from study 1 were air dried for 7 days and thereafter stored at room temperature. Two lesions were cut out from each leaf sample, surface sterilised using 0.5% sodium hypo chlorite solution, rinsed twice in sterile distilled water, placed on moist filter paper in a Petri dish and incubated for 48 to 72 hrs to stimulate sporulation. Conidia were dislodged from the lesions by placing the leaf tissues in 1.5 ml micro centrifuge tubes containing 100 µl of sterile water and vortexed. The spore suspension was inspected using a compound microscope. Positive samples were plated on Potato Dextrose Agar plates (Difco Laboratories, Becton Dickinson and company Sparks, MD 21152 U.S.A) and cultured under natural light and darkness regimes for 48 to 72 hours at room temperature. Mono-conidial cultures of the isolates were then established by sub-culturing germinated spore bearing distinct colony characteristics of *E. turcicum* to fresh potato dextrose agar plates amended with 100 mg/ml of ampicillin.

3.6.2 DNA isolation

Genomic DNA was extracted directly from fungal cultures growing on potato dextrose agar plates using the hot Cetyl-trimethylammonium bromide (CTAB) method (Okori *et al.,* 2003). The extraction buffer used contained 1.4 M NaCl, 2% CTAB (w/v), and 100 mM Tris-HCL pH 8.0. The extraction was performed as follows; a two week old fungal culture were crushed in liquid nitrogen using a mortar and a pestle and then homogenized in 1000 µl of CTAB extraction buffer (2% (w/v) CTAB (Cetyl-trimethylammonium bromide), 1.4m sodium chloride, 0.2% (w/v) β-mercaptoethanol, 20mM EDTA, 100mM Tris –HCL and 1% polyvinylpyrodilone (PVP-25). The content was then incubated at 65^{o}C for 60 minutes. Afterwards, the mixture was cooled to room temperature followed by addition of an equal volume of chloroform: isoamyl alcohol (24:1). The mixture was inverted several times and centrifuged at 604 g for 10 min. The supernatant was transferred into a new tube. DNA was precipitated with 900 ml of ice-cold isopropanol and then placed in the -20^{0}c for 20 min. for proper precipitation of the DNA and then

centrifuged at 604 g for 10 min. The supernatant was removed and the pellet washed with 70% (v/v) ethanol by vortexing and centrifuged at 419 x g for 10 minutes. The pellet was re-suspended in 100 µl of TE buffer (10 mM Tris-HCl pH 7.4 and 1 mM EDTA pH 8.0) and finally stored at -20^{o}C. Genomic DNA extracted was quantified for each sample by NanoDrop spectrophotometer (NanoDrop Technologies, Inc Wilmington, U.S.A) and dilutions made to a final concentration of 10 ng.

3.6.3 Genetic variability based on mating type genes

The objective of this study was to confirm the existence of mating types of *Exserohilum turcicum* based on sorghum derived isolates. It involved polymerase chain reaction (PCR) reactions performed in a final volume of 25 µl. The reaction mixture contained 20 ng genomic quantified using a NanoDrop Spectrophotometer DNA, 5 µl buffer 1X (200 mM Tris (pH 8.4), 500 mM KCl), gelatin 0.01%, 2.0 mM MgCl2, 0.2 mM of each dNTP, 10 pmol/µl of both forward and reverse primers (Table 2) and 0.06 Unit *Taq* polymerase (Promega Corporation, 2800 Woods Hollow Road Madison. U.S.A) The amplifications were performed in a thermo cycler (Gene Amp PCR system 9700 Applied bio systems, 850 Lincoln Centre Drive Foster City, CA 94404 USA) using the following program: 1 cycle of 4 min at 94ºC followed by 35 cycles of 30 sec at 94ºC, 30 sec at 55ºC and 30 sec at 72ºC, with a final extension of 9 min at 72ºC. Amplification products were stopped with 2.0 µl loading-dyes (500 µl ml^{-1} glycerol, 20 mM EDTA, 0.6 mg ml^{-1} bromophenol blue) (Promega) .

Amplification products were separated by electrophoresis in a horizontal gel system (Bio-Rad, model 96, Bio-Rad Laboratories, Inc. Life Science Research Group 2000 Alfred Nobel Drive Hercules, CA 94547 USA) at 110 V for 2.0 h on 1.5 % agarose gels stained with ethidium bromide (0.5 µg ml^{-1}) using 1.0 × TBE buffer (89 mM Tris, 89 mM borate, 2 mM EDTA pH 8.3). Finally, the gels were photographed under UV Transilluminator 2000 (Bio rad laboratories, Sagrate (Milan) Italy).

Table 2. Sequences of Internal Transcribed Spacer Ribosomal DNA (ITS) and *Exserohilum turcicum* mating type diagnostic primers used in this study

Name	Nature	Gene Type	Band Size/bp	Sequence
ITS1	Forward	Specie specific	344	TGTGTGTGTGTGTGTGTGTGT
	Reverse			ATAAGACGGCCAACACCAAG
Mat2-specific	Forward	Mat 1-2 & Mat2-2	195	ACCGATTGCTTCG
	Reverse			CAAACATCTCAAGGCGGAA
a-clone	Forward	Mat 1-1 gene	190	GTGAACCGACCCTCAAC
	Reverse			GTCCATGGGATACGCTACG
Clone alpha	Forward	Mat 1-1 gene	160	GCTGTGGGGTACTTGGTCTG
	Reverse			AGCGACAGGCAGCTAGAGCC

3.6.4 Genetic variability based on RAPDS and microsatellites

Randomly amplified polymorphic DNAs (RAPDS)

Ten RAPDs markers were tested for polymorphism and only two gave good polymorphism namely (A9-GGGGTCTTG and A5 –GGGTAACGCC). These were included in this study. Polymerase chain reaction reactions were performed accordingly in a final volume of 15 µl containing 20 ng genomic DNA, 2.5 µl buffer 1X (200 mM Tris (pH 8.4), 500 mM KCl), gelatin 0.01%, 1.3 µl of 2.5 mM MgCl2, 0.20 mM of each dNTP, 10pmol/ul of primers and 0.2 U *Taq* polymerase. The amplifications were performed in a thermo-cycler (Gene Amp PCR system 9700 Applied bio-systems 850 Lincoln Centre Drive Foster City, CA 94404 USA) using the following program: 1 cycle of 2 min at 94ºC followed by 33 cycles of 1 min at 94ºC, 2 min at 47ºC and 2min at 72ºC, with a final extension of 10 min at 72ºC. Amplification products were stopped with 2 µL loading-dye. Amplification products were separated by electrophoresis in a horizontal gel system as previously described.

Simple Sequence Repeats (Microsatellites SSRs)

The micro-satellites used were obtained from a number of related loci ascomycetes (Table 3). These were first screened for fidelity and only primer combinations that amplified the right genotype were included in the study. Polymerase chain reaction reactions were performed accordingly in a final volume of 25 µl

containing 20 ng genomic DNA, 5 µl buffer 1X (200 mM Tris (pH 8.4), 500 mM KCl), gelatin 0.01%, 1.5 mM MgCl2, 0.20 mM of each dNTP, 30pmol/ul of both forward and reverse primers and 0.4 U *Taq* polymerase. The amplifications were performed in a thermo-cycler (Gene Amp PCR system 9700 Applied bio-systems 850 Lincoln Centre Drive Foster City, CA 94404 USA) using the following program: 1 cycle of 1 min at 95ºC followed by 30 cycles of 30 sec at 95ºC, 30 sec at 45-50ºC and 45 sec at 72ºC, with a final extension of 7 min at 72ºC. Amplification products were stopped with 2 µL loading-dye. Amplification products were separated by electrophoresis in a horizontal gel system as previously described.

3.6.4.1 RAPDS and SSR Data Analysis

Gel photographs were scored manually. The bands were binary coded by 1 or 0 for their presence or absence in each genotype. Estimates of similarity among isolates were calculated from the data matrices in the form of dissimilarity units and expressed as euclidean genetic distance (Hintze, 1998). Cluster analysis was performed to generate a dendrogram using the Unweighted Pair Group Method with Arithmetic Average (UPGMA) as implemented in the NTSYs software.

Table 3. SSR primers used in the study to characterise populations

Name	**Sequence**	**Origin** *Mycosphaerella fijiensis* **EMBL Accession No**	**Author**
SSR01	TAGTTGCAACCGAACAGG CTCCGTAGGTATGATGGTGT	AJ303015	(Neu *et al.*, 1999)
SSR06	CGAACAGGACGAAAGAATAG GTTTGTTCCAGTTCGTCAAG	AJ303023	(Neu *et al.*, 1999)
SSR24	TCAAGAGGAGAAGTTGA GGTTCTGATCAAGAGGAGGA	AJ303034	(Neu *et al.*, 1999)
SSR36	ATTCCAGGTACGGCTACAC ATTCAGATCTGGTCTGGTTG	AJ303040	(Neu *et al.*, 1999)
SSR10	GAGAGCATGAAAAGTGGAAA CGTGACACTCGTCAGTTACA	AJ303026	(Neu *et al.*, 1999)
SSR14	ATTTGGTGAATGGGGTAAG ACAGAGGGAAGCAAGTTTTT	AJ303027	(Neu *et al.*, 1999)

3.6.5 Determination of sorghum - *E. turcicum* races

The objective if this study was to determine the nature of races that occur in Uganda based on Sorghum – *E.turcicum* isolates by inoculating each isolate on a set of race differentials namely A619, A619 *Ht 1*, A619 *Ht 2*, and A619 *Ht 3*. These were obtained from Maize Genetics Cooperation Stock Centre maize USDA/ARS & Crop Science /UIUC, S -123 Turner Hall, 1102. Urban IL, USA 61801-4730). These race differentials in total exhibit four single resistance genes (Ht $_{1}$, *Ht* $_{2}$, *Ht* $_{3}$ and *Ht* $_{N}$) which mean that a potential of 16 races of the pathogen (2^4=16) can be found in nature. These race differentials can identity races such as 0, 1, 2, 23, and 23N.

The isolated cultures were grown in petri plates for 10 days prior to testing. The plates were moistened with 200µl of sterile water using a sterile pipette. The conidia were dislodged from the surface of the colonies with a microscope slide and then drained into a beaker with 10 ml of sterile distilled water. The suspension was filtered through double cheese folded cloth and the concentration adjusted to 2.5 x 10^4 spores/ml using a haemocytometer. The test spores cultures were used to inoculate seedlings as explained below. A mixture of sterilised forest loam soil was used to grow the test plants in the screen house at Makerere University Agricultural Research Institute Kabanyolo (MUARIK). Five seeds per differential were planted and then thinned to three plants per pot. The treatments were replicated three times. The seedlings were inoculated 12 days after planting using a small hand-sprayer. Inoculum was sprayed to runoff in the screen house. The seedlings were placed on a bench within a room covered with polythene to increase the relative humidity for 48 hours.

Plants were evaluated for disease severity 15 days after inoculation using a 0 to 5 scale (Adipala *et al.*, 1993). The reactions were examined and classified as resistant or susceptible based on lesion type (Hooker, 1963). The lesions were examined and sporulating lesion without evidence of chlorosis was taken as susceptible types and non-sporulating lesions bearing chlorosis were the resistant types. Length and width of selected lesions was determined at the time of symptom evaluation.

CHAPTER FOUR

OCCURRENCE OF TURCICUM LEAF BLIGHT OF SORGHUM IN DIFFERENT AGRO-ECOLOGIES OF UGANDA

4.1 Agronomic practices in the different agro-ecologies

Fields were typically less than 1 ha. They varied from averagely maintained to near –abandonment with similar patterns of management found across districts especially in northern Uganda. Agronomic practices such as plant spacing across all agro ecologies were poor. Basing on the cultivar characteristics such as head shape, the sorghum cultivars grown varied from land races to new released cultivars such as *Epuripuri* and *Sekedo*. Most of the farmers interviewed could not distinguish various foliar diseases in their fields and with very little knowledge on Turcicum leaf blight. They associated symptoms of TLB with crop maturity or onset of dry period. Farmers considered weeds such as *Striga* and hailstorms as the major threats to their livelihoods. Some farmers in Lira identified sooty mould and considered grain anthracnose as a new emerging problem for sorghum.

4.2 Incidence and severity of turcicum leaf blight

Nested analysis of variance was performed at two hierarchical levels that is, agro-ecological zone and districts. Significant differences ($P \leq 0.05$) were observed in TLB severity in agro-ecological zones and districts within agro-ecological zones (Table 4). Disease was more severe in the most humid farm lands as compared to moderately dry agro-ecologies. Turcicum leaf blight was more severe in West Nile farmland, North-western farmland-wooded savanna and Central Wooded Savanna.

Table 4. Mean squares for severity of turcicum leaf blight assessed during the 2007 second rains derived from nested analysis of variance

Source	**df**	**SS**	**MS**	**F**[2]
Agro ecology	5	28.69	5.16	9.45*
District	6	10.69	1.79	3.26*
Error	200	109.13	0.55	
Total	**211**	**148.50**		

[2] Statistical significant differences* =P ≤ 0.05, ** P≤0.01, and *** = P≤ 0.001.

In all the fields visited there was concomitant infestation of TLB and anthracnose on sorghum except on the improved cultivar *Epuripuri* (which was resistant to anthracnose but very susceptible to TLB). Turcicum leaf blight was found in all districts visited. The analysis of variance revealed highly significant influence of agro-ecology (P ≤ 0.001) on TLB severity. Within each agro-ecology, there was a significant difference at district level for both severity (P = 0.006) and incidence (P ≤ 0.001) of TLB. Incidence of TLB was high, being in the southern and eastern Lake Kyoga basin (89.1%) and lowest in the northern – central-grass bush farmlands (65.8%). Severity of TLB was generally low across agro-ecological zones with a mean of 28.5 % (Table 6). At district level, TLB incidence ranged from 100.0 % in Pallisa, to 20.0 % in Tororo; the associated severities ranged from 41.6 % to 25.5%, respectively (Table 5). The lowest severity was recorded in districts of Mbale (13.8%), Tororo (20%) and Wakiso (13%) which incidentally are found in Lake Victoria crescent Mbale farmland agro-ecology (Table 6). In Pallisa district a very high incidence of TLB was found. In this district there was cultivation of commercial but highly susceptible sorghum variety *Epuripuri.* In general, districts in the northern moist farmland and north-western farmland agro-ecologies had very high incidence of TLB, for example Kaberamaido, Gulu and Kumi all found in theses agro-ecologies had incidences of over 75 % and above.

Table 5. Mean severity and incidence of Turcicum leaf blight on sorghum in major sorghum growing districts in Uganda.

	Means	
Location (Districts)	**Severity**	**% Incidence**
Amuria	20.6	62.5
Apac	32.1	88.4
Arua	35.0	64.1
Dokolo	24.6	66.1
Gulu	26.0	78.7
Homia	35.1	81.1
Iganga	16.8	75.8
Kaberamaido	27.2	82.3
Katakwi	24.3	70.5
Kiboga	16.5	86.1
Koboko	50.7	90.0
Kumi	19.4	85.9
Lira	38.6	60.8
Maraca	50.0	50.0
Masindi	38.9	71.4
Mbale	13.8	63.6
Nebbi	31.3	87.4
Pakwach	16.7	50.0
Pallisa	21.5	100.0
Serere	27.6	89.4
Soroti	23.4	82.5
Tororo	11.8	20.0
Wakiso	13.1	85.7
$LSD_{(0.05)}$	**23.8**	**36.8**
C.V (%)	**93.3**	**34.4**

L.S.D = Fishers Protected Least Significant Difference test at ($P \leq 0.05$) (Steel *et al.*, 1997)
CV = Percentage Coefficient of variation

Table 6. Mean severity and incidence of Turcicum leaf blight in major sorghum growing agro-ecologies, Uganda

Agro-ecological zones	**%Severity**	**% Incidence**
Central Wooded Savanna (Msd, Kbg)	16.5	86.1
North-eastern –central Grass-Bush-Farmland (Ka, Am & M)	22.1	65.8
Northern Moist Farmland (Ap, Li, K, Gu & Do)	30.5	73.7
North-western Farmland –Wooded Savanna (Ne & Pak)	30.0	84.1
Western Mid-Altitude Farmland and The Semiliki Flat (Ho &Ku)	37.0	76.4
West Nile Farmland (Ar & Ko)	37.6	67.0
Lake Victoria Crescent and Mbale farmland (Ig, Mb &Wks)	14.8	66.2
Southern and Eastern Lake Kyoga Basin (Pa, So & Se)	23.3	89.1
$L.S.D_{0.05}$	**7.9**	**12.6**
CV $_{(\%)}$	**57.3**	**34.6**

L.S.D = Fisher's Protected Least Significant Difference test at ($P \leq 0.05$) (Steel *et al*., 1997) Acronyms used: Msd-Masindi, K-Kaberamaido, Ka-Katakwi, A-Amuria, M-Maraca, Ap-Apac, Li-Lira, Do-Dokolo, Ne-Nebbi, Ar-Arua, Ig-Iganga, Ku-Kumi, Mb-Mbale, Gu-Gulu, Pa-Pallisa, So-Soroti, Se-Serere, Pak-Pakwach, Kbg-Kiboga, Wks-Wakiso and Ko-Koboko.

4.3 Disease maps of turcicum leaf blight of sorghum

The disease severity/incidence data and GPS recordings were used to construct a disease map. The disease map illustrates severity and incidence levels over the agro-ecologies and was used to study epidemic patterns at the time of the study. The data shows that highest disease severities were recorded in the wet humid regions such as Lira and Apac districts (Figure 6). The cooler agro ecologies had relatively lower severity. In terms of incidence, virtually all districts recorded very high levels of disease. The vast majority of districts studied recorded over 60% incidence in fields. There was no field where TLB was not recorded / encountered.

The disease map also illustrates spatial pattern of epidemics with some districts showing high variation in disease severity, for example in Apac, severity ranged from as low as 8% to as high as 60% in areas close to Lake Kyoga and Lake Kwania humid areas (Figure 6). In the districts of Masindi, the disease map shows more or less similar patterns of disease severity of between 22.3-30.0%. Incidence patterns were more similar to severity patterns. In general, most humid areas around Lake Kyoga such as Soroti, Kaberamaido, Pallisa, and Apac had very high incidences of TLB. In Masindi, incidence was generally lower in the warmer areas of the district bordering Homia, perhaps due to cultivar effects. In Masindi and Homia the highly susceptible *Epuripuri* variety was not cultivated.

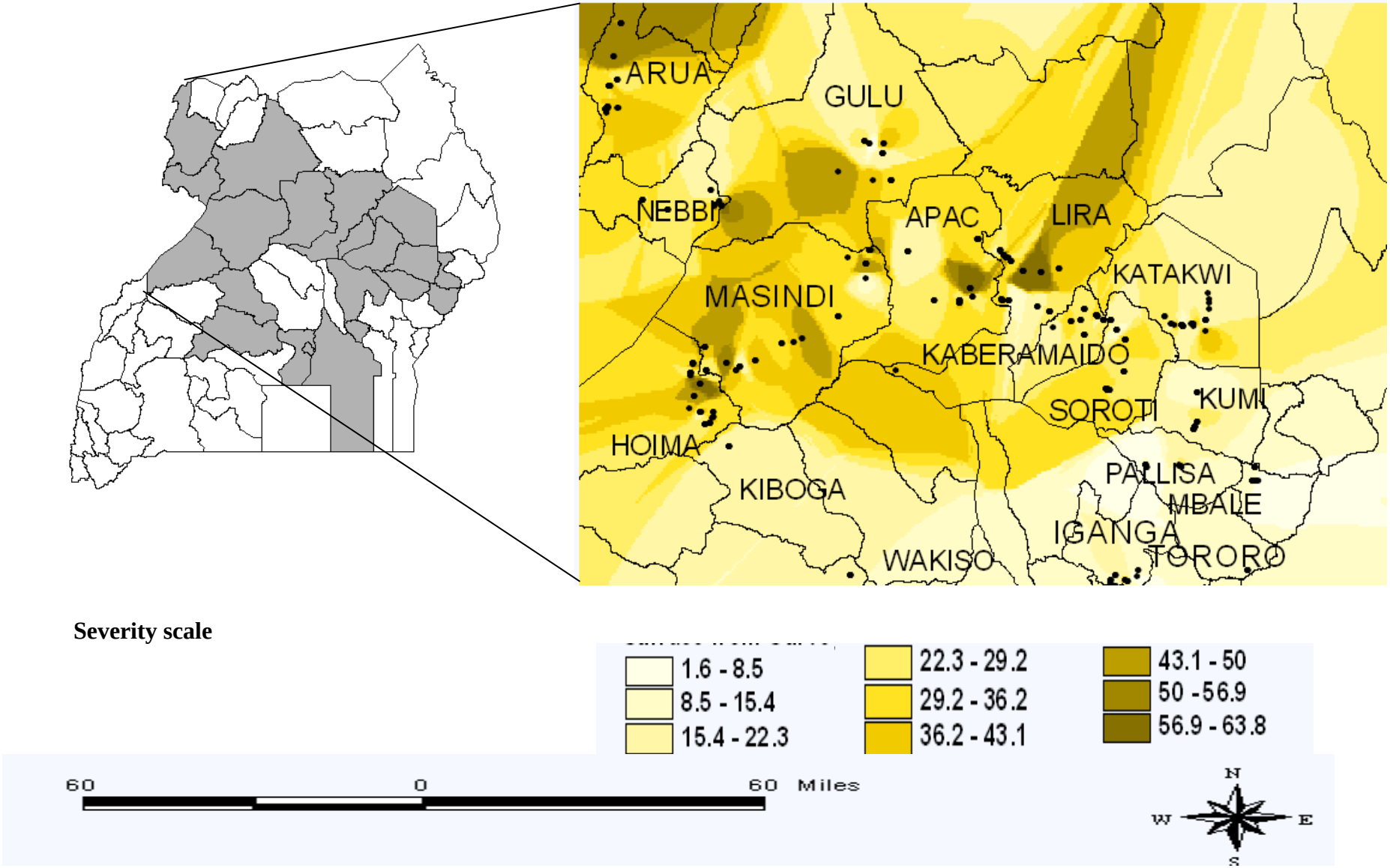


Figure 4 Disease map based on severity of Turcicum leaf blight in 23 districts (8 different agro ecologies) of Uganda. The study sites are shaded in grey.

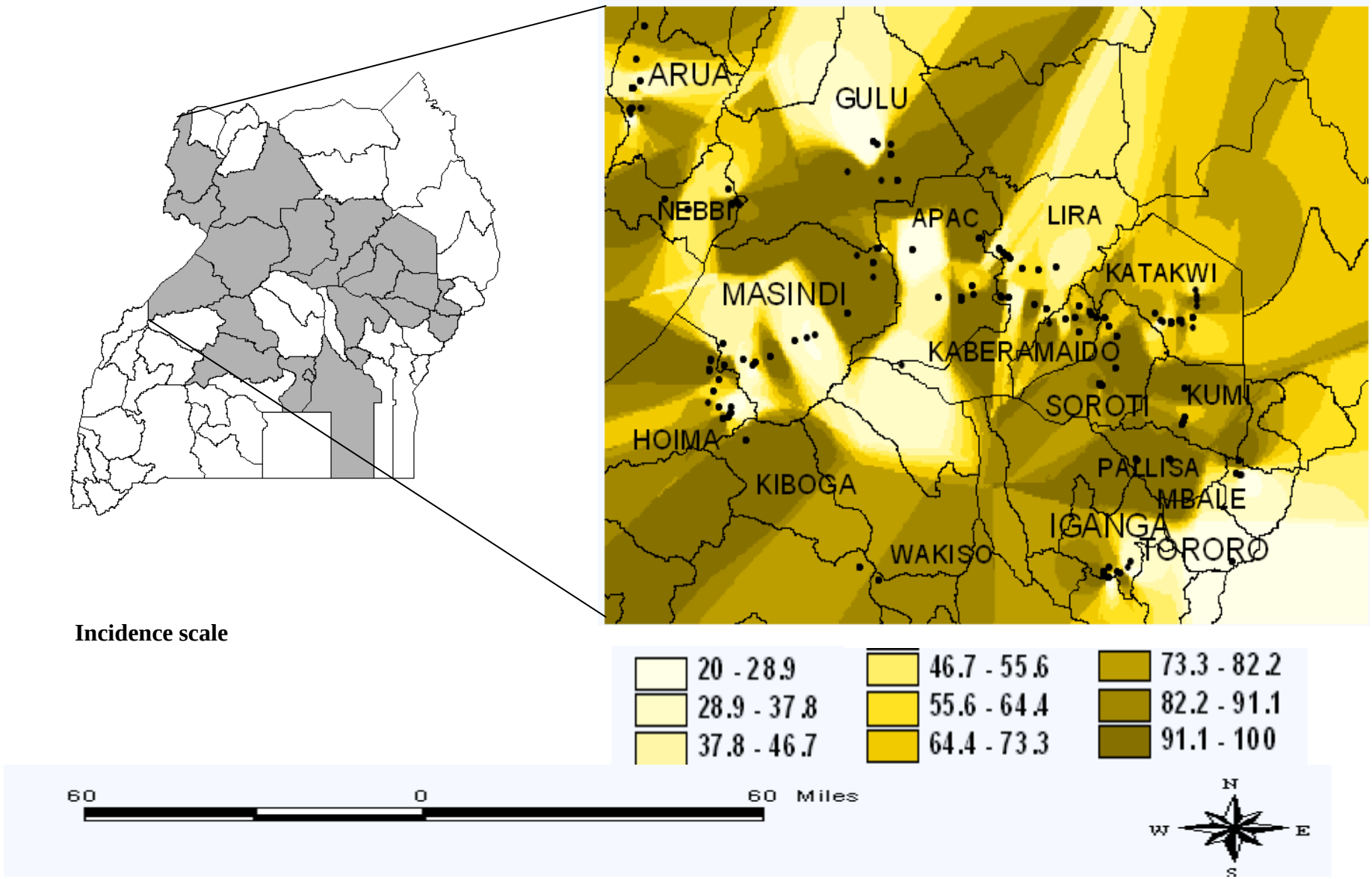


Figure 5 Disease map based on incidence of Turcicum leaf blight in 23 districts (8 different agro ecologies) of Uganda. The study sites are shaded in grey.

4.4 Discussion

The objective of this study was to establish the incidence and severity of TLB in different sorghum growing agro-ecologies of Uganda. Turcicum Leaf Blight was prevalent in all the agro ecologies albeit at varying levels. In general severity was low averaging about 28% while incidence about 78%. Concomitant infection of sorghum with TLB and anthracnose was also common. Turcicum leaf blight incidence and severity was high such as in Pallisa district (Figure 5). The low severity value suggests that a number of varieties grown by farmers appear to be resistant. A second possible explanation for the observed data could be host species specificity of *E. turcicum* pathogenic on sorghum may not be pathogenic on maize. Thus while TLB of maize has been well characterised with high severity and incidence patterns, the role of pathogen species adaptation in the case of sorghum cannot be precluded (Adipala *et al.*, 1993)

The data also demonstrates a strong effect of agro-ecology on TLB epidemics. Agro-ecologies in this case represent the collective effects of weather and farming systems (Robinson, 1987). Disease severity was highest in warm-humid areas around Lake Kyoga. In contrast, the area around Lake Victoria had very low severity and incidence, perhaps due to farming systems, which have less sorghum and thus reduce host tissue availability. Indeed, in the Lake Victoria crescent, the highly susceptible commercial variety *Epuripuri* was very rare. Thus, while TLB is favoured by moderate temperature (20-25^{o}C) and high relative humidity as is common around lake Victoria crescent, cultivar effects, availability and susceptibility to TLB appears to play a more leading role (Lipp, 1991).

Nested ANOVA revealed a significant role of districts on TLB epiphytotics. In each district, the different ethnic groups that live there use sorghum for various purposes. In large measure, cultivating varieties with specific end-user preferences. Therefore the significant role of districts on TLB epidemics could in part allude to farmer preferences for varieties that react differently to TLB. The district effects may also allude

to the predominant farming systems effects that influence selection pressure and thus consequently epidemics in agro- ecology.

In general, in east and northern parts of the country, sole cropping of sorghum is common. Furthermore at harvest, most of the stovers are left on fields and these serve as sources of inoculum for the next season. Earlier studies on sorghum epidemics management and control, indicate that crop varieties affects disease epidemics (Duncan and De Milliano, 1995). These suggestions are in line with general principles of plant disease epidemics, which show that in any pathosystem, the host-pathogen relation is a reflection of the effects of how human activities have influenced agriculture (Zadoks and Schein, 1979). In the case of TLB of sorghum, the pathosystem has not been characterised and therefore a number of information gaps still exist. To support disease management, six methods of disease control for sorghum have been suggested i.e. avoidance, exclusion and eradication of pathogen, protection of the crop through breeding for resistance against the pathogen and therapy (Frederiksen, 1986). For disease control, farmers especially in sub-Saharan Africa employ methods that do not require high investment. Thus for sorghum farmers in Africa, there is need to elucidate the TLB pathosystem so as to avail information on the pathogen, the epidemics, nature of resistance and begin to develop resistant sorghum lines (Duncan and De Milliano, 1995). These areas are the focus of the next three chapters of this thesis.

CHAPTER FIVE

REACTION OF SORGHUM ACCESSIONS FROM DIFFERENT AGRO-ECOLOGIES OF UGANDA TO *Exserohilum turcicum* INFECTION

Introduction

The objective of this study was to screen 202 sorghum germplasm from Uganda for their reaction to turcicum leaf blight infection. The study was carried out at two locations with diverse environmental conditions namely Serere, Soroti and Kabanyolo, Wakiso.

5.1 Reaction and disease progress

Analysis of variance revealed highly significant variation in disease severity ($P \leq 0.001$) and AUDPC ($P \leq .001$) for the 202 sorghum accessions (divided into 5 races of sorghum under field conditions) included in the study (Table 7 and Annex 1). Out of the 202 accessions of *Sorghum bicolour* inoculated with *E. turcicum*, 194 accessions exhibited a moderate resistant reaction (Table 8). Most resistant accessions were: MUC007/1-9, MUC007/011-69, and MUC007/70 A, MUC007/700 B, MUC007/071-161, MUC007/163-192 MUC007/193A-D, MUC007/194A-D and MUC007/194. Accessions MUC007/016, MUC00/017, MUC007/025, MUC007/045, MUC007/048, MUC007/067, MUC007/160, MUC007/163, and MUC007/188 which displayed mild visual symptoms to TLB during the fourth to the sixth week of assessment (booting stage). A susceptible response was observed on two sorghum accessions MUC007/010 and MUC007/162 from the second week after inoculation up to the last assessment date and these were considered highly susceptible to leaf blight. Susceptibility reaction varied among accessions. Two out of 202 accessions developed typical TLB symptoms 12 days after inoculation and 9 accessions which were later classified as susceptible, developed symptoms 28 days after inoculation. Variation in

turcicum leaf blight severity and incidence became more apparent ($P<0.01$) from the 3rd week up to the last assessment date (Table 7).

The classification of the different accessions using cluster analysis based on final severity and AUDPC values revealed four major groups, i.e. resistant (a score of 0-2.08), moderately resistant (a score of 2.08-3.13), susceptible (a score of 3.13-4.17) and highly susceptible , which had a score greater than 4.17 (Table 8).

Table 7. Mean squares for final severity and AUDPC values due to Turcicum Leaf Blight on 202 sorghum accessions. Screened for disease reaction at NASARRI in 2007 second rain season

Parameter	Accessions		Races	
	Final Severity	AUDPC	Final severity	AUDPC
d.f	195	195	4	4
m.s	107.0***	21.63***	166.38*	26496.7***
Grand Mean	8.17	0.94	8.17	32.4
L.S.D	1.85	0.70	4.00	63.1
C.V (%)	-	-	80.1	378.3

Table 8. Classification of sorghum accessions into four major clusters based on AUDPC values as computed using Cluster Analysis

Resistance class	[a]Sorghum Accessions: MUC007 series
Resistant	17,25,26,3,4,6,7,8,9,14,16,21,22,27,28,29,30,31,32,33,34,35,36,38,40, 41,43,45,46,47,48,49,50,51,67,68,69,71,72,73,74,75,76,77,78,79,80, 81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100,101,10 2,103,104,105,106,107,108,110,111,112,113,114,115,116,117,118,119 ,120,121,122,123,124,125,126,127,128,129,130,131,132,133, 134,135, 139, 191
Moderately resistant	10 and 24
Susceptible	1,2,12,13,18,19,20,23,37,39,42,44,53,54,55,56,57,58,59,60,61,62,63,6 4,65,66,136,137,138,140,141,142143,145,146,147,148,149,150,151,15 2,153,154,155,156,157,158,159,160,161,162,163,164,166,167,168,169 ,170,171,172,173,174,175,176,179,180,181,182,183,184,185,186,187, 188,189,190,192,193,194,195,196,197,198,200,201, and, 202
Highly susceptible	52,15,5,165 ,11

AUDPC was computed according to Campbell and Madden (1990) .For more details see Annex 2
a MUC007 refers to the Makerere University Collection 2007. It is the prefix for all the accessions studied.

5.1.1 Reaction of sorghum accession to *Exserohilum turcicum* infection

The germplasm studied comprised of 202 sorghum accessions collected in 2007 from 23 districts of Uganda. The numbers of accessions collected per district were up to 10. The accessions were categorised as Kafir (34.1%), Guinea (30.6%), Caudatum (27.0%), Durra (<0.1%) and Bicolour (0.1%). This was done basing on their head shape (plant morphology).

The analysis of variance revealed that final severity and AUDPC values were not significant ($P<0.05$) influenced by races under study (Table 9) though the data revealed that bicolour race had the lowest level of severity and AUDPC while Caudatum race had the highest (Figure 9). Attempts were made to identify important gene pools as sources of TLB resistance. It was observed that the races with greatest resistances sources followed this trend: Kafir > Guinea > Caudatum > Bicolour > Dura as presented in Table 9.

5.2 Assessment for multiple resistance to biotic and a biotic

After the assessment of 202 accessions, ten sorghum accessions were selected based on multiple disease resistance (TLB and anthracnose), drought resistance and good yield attribute (Data not shown) for further testing on their reaction to turcicum leaf blight infection as previous done with the 202 accessions. These included: MU007/129, MU007/193A, MU007/123, MU007/52, MU007/009, MU007/124, MU007/059, MU007/028, MU007/096, and, MU007/058. The variation in TLB infection response was highly significant ($P<.001$) for the ten sorghum accessions (Table 10).

Table 9. Reaction of sorghum races to turcicum leaf blight infection

	Sorghum Race classification	Number of test materials			
		Resistant	Moderately resistant	Susceptible	Highly susceptible
1	Kafir	35	0	29	3
2	Guinea	31	0	29	0
3	Caudatum	26	2	23	2
4	Bicolour	8	0	1	0
5	Dura	3	0	4	0

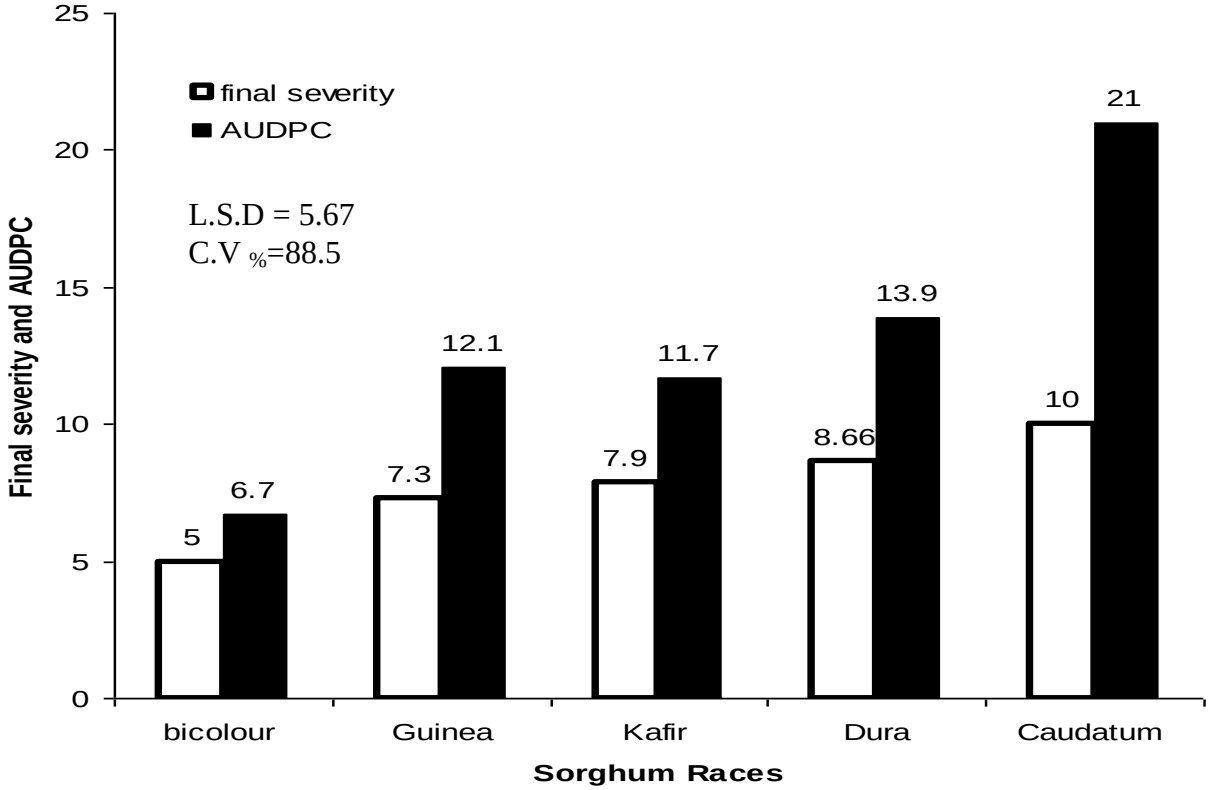


Figure 6. Disease reaction of five different sorghum races based on final severity and AUDPC to turcicum leaf blight infection.

During the initial evaluation at MUARIK the mean final severity was 41.8 % for all the accessions. Two accessions MU007/052 and MU007/059 had AUDPC values below 20.0% i.e. 16.0% and 16.8% respectively. In the second season, the accessions were tested at two locations, i.e., NaSARRI and MUARIK. In MUARIK, the accessions exhibited considerable variations in mean final severity and AUDPC. The mean AUDPC value was 38.90. The lowest AUDPC values at MUARIK were exhibited in MU007/052 (8.04) and MU007/059 (17.86) with 8.04. At NaSARRI the accessions still exhibited the lowest AUDPC values of 22.42 and 22.46, respectively. These two sorghum accessions belonged to the Caudatum race. The interactive effect of accession and location for final severity and AUDPC were found to be highly significant ($P \leq 0.001$), while for accession and season non-significant ($P \leq 0.05$) (Table 10).

Table 10. Mean squares for turcicum leaf blight initial severity (Yi) final severity (Yf) and Area Under Disease Progress Curve (AUDPC) on ten sorghum accessions

Source of variation	df	Initial severity (y_i)	Final Severity (y_f)	AUDPC
Location	1	10.05***	0.93ns	147.23ns
Accessions	9	20.35***	995.49***	1247.89***
Season	1	19.26**	23.97ns	5.59ns
Location .x Accession	9	4.06ns	219.96***	124.10**
Accession .x Season	9	1.92ns	5.73ns	48.90ns
Residual	58	2.10ns	13.75	39.50
Total	89			

AUDPC: Area under disease progress curve was standardized by dividing by the total number of days for period of disease assessment. (Campbell and Madden, 1990).

5.3 Disease progress in the ten sorghum accessions

A logistic model was used to study disease progress in the ten sorghum accessions. Selection of the logistic model was based on the fact that it is one of the most widely used plant disease epidemiology techniques (Campbell and Madden, 1990). The logistic model was also selected because it provided the best fit for the data based on the high coefficient of determination. Apart from accession MU007/52 and MU007/059, the rest of the accessions had projected disease severity values ranging from 3.23 to 3.54, while the two accessions had ranging from, 4.10 to 4.24 (Table 11). The rates of disease development were relatively low for accessions MU007/052 and MU007/059 having 0.54 and 0.61 logits per week (Table 11).

Table 11. Predicted parameters depicting initial disease level $\hat{y}_0$, (intercept), the slope representing the apparent rate of infection (r) and coefficient of determination (R^2) for 10 sorghum accessions for reaction to turcicum leaf blight

Accessions	$\hat{y}_0$	r	R^2	Race
MU007/129	-3.51	0.68	0.999	Guinea
MU007/193A	-3.46	0.65	0.996	Guinea
MU007/123	-3.54	0.69	0.990	Kafir
MU007/052	-4.10	0.54	0.993	Caudatum
MU007/009	-3.27	0.67	0.994	Caudatum
MU007/124	-3.38	0.67	0.998	Caudatum
MU007/059	-4.24	0.61	0.994	Caudatum
MU007/028	-3.51	0.65	0.999	Caudatum
MU007/096	-3.23	0.66	0.996	Kafir
Mu007/085	-3.24	0.65	0.989	Caudatum

$\hat{y}_0$: initial severity at time zero, and r; apparent rate of disease development (where computed based on logistic model (Campbell and Madden, 1990) and R^2 coefficient of determination.

5.4 Discussion

Host plant resistance provides an economical approach to stabilizing crop production and enhancing profitability (Erpelding and Prom, 2004). High variable pathogens such as *E.turcicum* require additional sources of host resistance for pathogen management and ensuring stable /high yields. The objective of this study was to assess sorghum germplasm from Uganda for resistances to TLB. The majority of the accessions (196 out of 202) screened were resistant to TLB when challenged at seedling stage. Over 99% (194) accessions showed a resistant reaction during the first four weeks of disease assessment and only a few showed mild symptoms during the fourth to the sixth week of disease assessment. Two accessions including a commercially popular open pollinated variety *Epuripuri* which showed severe disease infection and this were observed from the second week up to the last week of assessment. These results suggest that the varieties grown by farmers have a high level of resistance to TLB as compared to the recently released varieties such as *S*ekedo and *Epuripuri* from research stations.

This study also revealed a variation in disease reaction among sorghum races. Of the races, *Kafir and Guinea* races were most resistant to TLB but interestingly had also the largest number of susceptible genotypes. This suggests that among these two races, there is a wide array of host resistance to *E. turcicum*. These two races are widely grown in the country (Leslie, 2003) and most of the sorghum improvement work in the country has not targeted them. Contrastingly the vast majority of improved varieties are Caudatum race with moderate- high susceptibility to TLB. Taken together, this study shows that for TLB there is a vast amount of germplasm present that can be used as sources of resistance to TLB infection. This wide variation of resistance to TLB alludes to long term co-evolution of *E. turcicum* and sorghum in the sub-region. Eastern African is the one of the centres of diversity to sorghum especially Kafir race, with Guinea and Caudatum being introduced races (Leslie, 2003). It is thus conceivable that among both host and pathogen, there is a wide array of pathogenicity and resistance genes respectively. Long term disease management especially against TLB will need to draw from these germplasm.

Assessment for multiple stress tolerance revealed availability of such germplasm even among land races. In this study at least 10 such accessions were found modelling for disease progress using logistic model generated relatively steep curves with apparent rate of infection averagely 0.63. These data suggest that in these germplasm the form of resistance displayed inhibits start of epidermis as is typical of major gene resistance (Vander plank, 1963; Campbell and Madden, 1990). Indeed, in a vast majority of resistant accessions, symptoms developed wildly after 28 days post inoculation. Resistance to TLB has been characterised largely based on *Ht* sources (Pataky, 1992). Partial or rate reducing or minor gene resistance has also been reported in maize to TLB (Jiansheng and Jilin, 1984). In sorghum, resistance to TLB has not been characterised before as compared to maize however the fact that both maize and sorghum are members of the Poaceae, with high synteny (Paterson *et al.*, 1995) suggest that some resistance typology may be operational in sorghum. Confirmation of this requires characterisation indirectly of disease progress in maize and sorghum thus the focus of the next chapters as well as detailed comparative genomics.

CHAPTER SIX

ANALYSIS OF TEMPORAL ATTRIBUTES OF TURCICUM LEAF BLIGHT OF SORGHUM FOR TWO DIVERSE AGRO-ECOLOGIES IN UGANDA

Introduction

The objective of this study was to characterise the temporal attributes of turcicum leaf blight of sorghum under field conditions. It involved artificial inoculation of three sorghum and two maize cultivars by placing 5-10 *E. turcicum* colonised sorghum grains kernel within the whorls of the plant when they had reached V6 and Stage 2 of their growth for maize and sorghum respectively. Severity data was collected depending per crop depending on the onset on the disease symptoms.

6.1 Disease development

This study was carried out at two locations i.e. NaSARRI and MUARIK as well as two cropping seasons 2nd cropping season 2007 (August –December) and 1st cropping season 2008 (February –April). Disease development was detected earlier on maize plots as compared to sorghum. Accordingly data collection on maize commenced much earlier, that is, three weeks before sorghum. The ANOVA showed that the effect of genotype was highly significant ($P< 0.001$) on severity of TLB during the two growing seasons at both locations (Table 12). Generally there was more disease development on maize as compared to sorghum with variation among the varieties. During the first season, there was a significant difference in the reaction of sorghum and maize varieties.

In both locations, accessions MU007/029 expressed the lowest severity and *Epuripuri* had the highest in sorghum. In maize more disease was observed on Longe 5 as compared to Longe 4 (Table 13). During the second rains, at MUARIK and NaSARRI, less disease development was detected with accession

MU007/009 having the least severity as compared to MU007/029 while *Epuripuri* still had the highest severity scores Disease development followed the same trends in the second trail (1st cropping season 2008) of experimentation. Analysis of variance on selected plant attributes revealed a significant difference (P<. 001) between sorghum and maize varieties (Table 14). More diseased leaves where observed on maize as compared to sorghum. The ANOVA results revealed a non-significance difference (P=0.05) on lesion length, width and area.

Table 12. Mean squares of final severity and AUDPC for the effect of turcicum leaf blight infection on maize and sorghum response at MUARIK and NaSARRI

		September –December 2007		March –June 2008)	
		Mean squares		Mean squares	
	Df	Final Severity[b]	AUDPC[a]	Final Severity[b]	AUDPC
Kabanyolo					
Maize (A)	1	7840.8***	20.01[ns]	5535.2***	4657.5***
Sorghum (B)	2	10838.7***	298.79***	1196.3***	3539.9***
A* B	1	12877.3***	11441.59***	14758.4***	46203.7***
Serere					
Maize (A)	1	132.1[ns]	158.9[ns]	872.4***	236.7***
Sorghum (B)	2	4558.57***	527.29 ****	20819.0***	6884.2***
A*B	1	3036.1***	1478.1***	11206.7***	6480.3***

*** Significant at P<0.05, ns = Non-significant at P<0.05 [a]AUDPC values were standardized by dividing the number of days from first to last disease assessment times. [b]Final severity score taken at 72 days after inoculum application. 1st trial 2007 and 2nd trial 2008 corresponds to the first (September, October and December 2007) and second (April, May June, July 2008) seasons respectively.

Table 13. Severity means of turcicum leaf blight on sorghum accessions and maize varieties during two cropping seasons of experimentation

	2nd cropping season 2007-Sept-December				1st cropping season 2008-March-June			
	Kabanyolo		Serere		Kabanyolo		Serere	
Variety	[a]Final severity	[b]AUDPC	[a]Final Severity	[b]AUDPC	[a]Final severity	[b]AUDPC	[a]Final severity	[b]AUDPC
MU007/029	18.0	23.4	4.9	5.4	19.3	15.2	52.4	27.1
MU007/009	25.0	18.8	6.2	6.7	17.6	14.9	53.4	68.1
Epuripuri	49.3	39.9	45.1	35.1	27.8	32.3	65.0	49.8
Longe 4	32.7	26.9	16.1	13.9	28.4	23.7	57.9	47.3
Longe 5	38.5	27.9	21.5	17.5	14.8	17.9	55.9	49.4
s.e.d	3.4	1.7	1.7	1.1	3.0	1.8	1.6	19.9
l.s.d	6.6	3.3	3.4	2.2	5.9	3.5	3.2	39.2
$C.V_{(\%)}$	53.6	29.5	44.4	34.70	68.2	41.5	14.00	NS

[a]Final severity score taken at 72 days after inoculum application. 1st Trial 2007 and 2nd Trial 2008 corresponds to the first (September to December 2007) and second (April to July 2008) seasons respectively. [b]AUDPC values were standardized by dividing the number of days from first to last disease assessment times.

Among sorghum accessions, *Epuripuri* exhibited the largest number of lesions but the smallest lesion area. MU007/009 and MU007/029 sorghum accessions exhibited the lowest number of lesions and smallest lesion length as compared to susceptible lines. Among maize varieties, the analysis of variance revealed a non-significant differences ($P \leq 0.05$) between varieties in their disease reactions as assessed using the numbers of diseased leaves, lesion number, lesion length and lesion width were detected, (Table 14). MU007/029 and Longe 4 exhibited the lowest number of conidial count as compared to *Epuripuri* and Longe 5 (susceptible varieties of sorghum and maize respectively) and it was significant ($P \leq 0.05$), Duration from inoculation to symptomatic expression (latent period) was highly significant among the sorghum accessions as compared to the maize varieties (Table 15).

Table 14. Mean squares of selected Turcicum leaf blight disease assessment indices on maize and sorghum.

		[a]Variety		[b]Crop
Source of variation	d.f	Mean squares	d.f	Mean squares
Number of diseased leaves	4	35.5***	1	33.8***
Number of lesions	4	1647.3***	1	261.6^{ns}
Lesion length (cm)	4	41.23^{ns}	1	317.9**
Lesion width (cm)	4	2.65^{ns}	1	11.0*
Lesion area(cm^2)	4	264.0^{ns}	1	3141.1***
Conidal count	4	14199.0^{ns}	1	59050.0**
Latent period	4	458.8^{***}	1	7.4^{ns}

[a]Variety: MU007/029, MU007/0009 and *Epuripuri* and [b]Crop: maize and sorghum. Conidial counts data were transformed using their square root values to normalize the data

Table 15. Means for Turcicum leaf blight disease assessment indices on maize and sorghum

	Treatment						
	Crop		**Maize**		**Sorghum Accessions**		
Disease indices	**Maize**	**Sorghum**	**Longe 4**	**Longe 5**	**9**	**29**	***Epuripuri***
Number of diseased leaves	5.8	4.5	5.5	5.3	4.0	3.8	8.5
Number of lesions	14.1	17.7	15.7	14.9	6.0	3.7	36.2
Lesion length (cm)	6.9	10.9	8.2	8.2	6.2	7.0	11.5
Lesion width (cm)	0.9	1.7	1.3	1.1	1.5	1.6	0.4
Lesion Area (cm^2)	8.2	20.7	13.3	11.5	6.3	20.2	10.5
Conidal count [a]	216	170	167	213	176	169	220
Latent period	35.5	36.2	37.6	33.9	40.3	42.1	25.2

[a] conidial count data were transformed to their square root values in order to normalize the data

6.2 Modelling disease progress over time

Disease progress on the sorghum genotypes were less pronounced from 21 days after inoculum application. Gompertz and logistic models were fitted to temporal study data. Both gave adequate description of disease development over time as depicted by the higher R^2 values in the two seasons of the trial (Figure 7-10) as good residual scatters. There was some significant difference in the rate of disease increase among plots with different sorghum accessions at NaSARRI and MUARIK. Differences in the rate of disease increase were not consistent between plots and experimental seasons. Rates of disease progress among sorghum accessions ranged from 0.068 to 0.019 logits/day in study season one and 0.078 to 0.020 logits/day in study season two in both locations. In season one of the study, MU007/029 and MU007/009 had the lowest rates of disease development at NaSARRI and MUARIK, respectively. In season two, the same observation was obtained. *Epuripuri* had the highest apparent rate of infection in all seasons and at both locations (Table 16). Maize varieties had a moderate rate of disease development ranging from 0.1 to 0.023 logits/day in season one and 0.097 to 0.017 logits/day in season two at both locations. Generally, the rate of disease development was quite high in maize and low in sorghum with the exception of *Epuripuri* (Table 16).

Table 16. Rates (logits/day) of Turcicum leaf blight disease development on three sorghum accessions and two maize varieties as influenced

	2nd cropping season 2007 September-December		1st cropping season 2008 March -June	
Variety/accession	NaSARRI	MUARIK	NaSARRI	MUARIK
MU007/029	0.023	0.019	0.039	0.022
MU007/009	0.052	0.015	0.078	0.020
Epuripuri	0.068	0.029	0.071	0.023
Longe 4	0.035	0.023	0.064	0.017
Longe 5	0.101	0.050	0.097	0.043

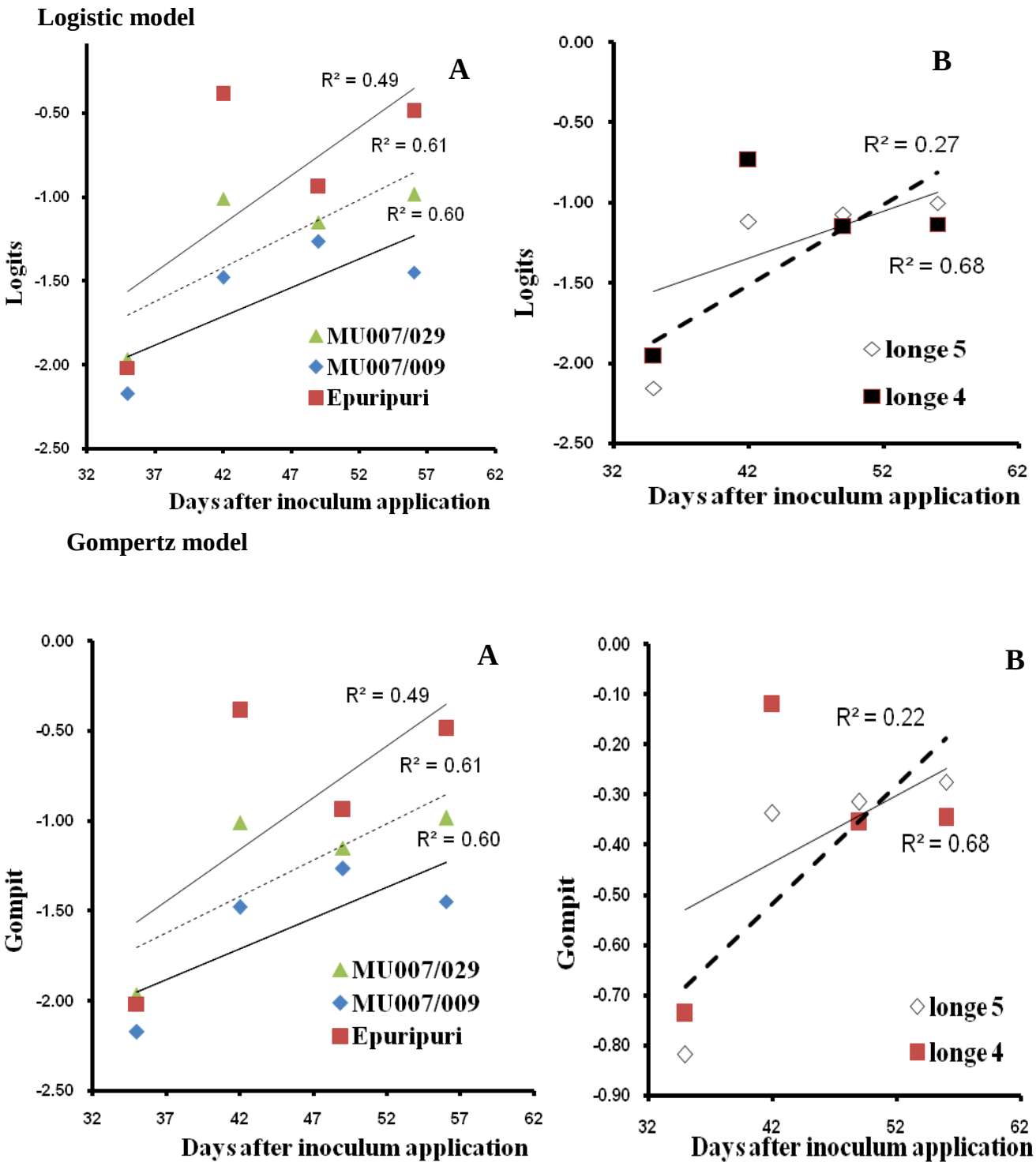


Figure 7. Logistic and Gompertz models of turcicum leaf blight epidemics on sorghum and maize in at MUARIK. Figure A is for sorghum and B for maize during the first experimental season (2007 B rains- September –December).

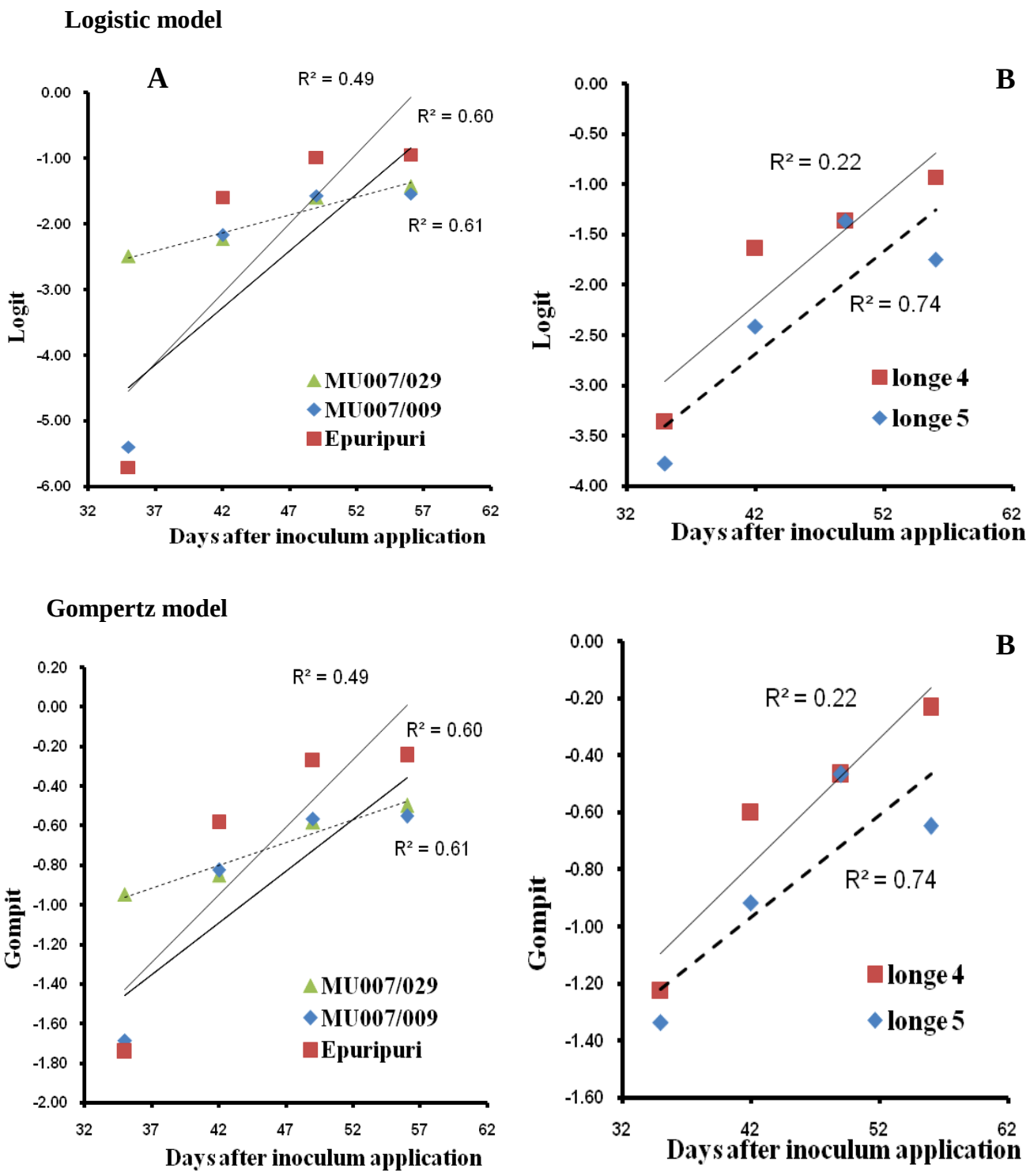


Figure 8. Logistic and Gompertz models of turcicum leaf blight epidemics on sorghum and maize in at NaSARRI. Figure A is for sorghum and B for maize during the first experimental season (2007 B rains-September –December).

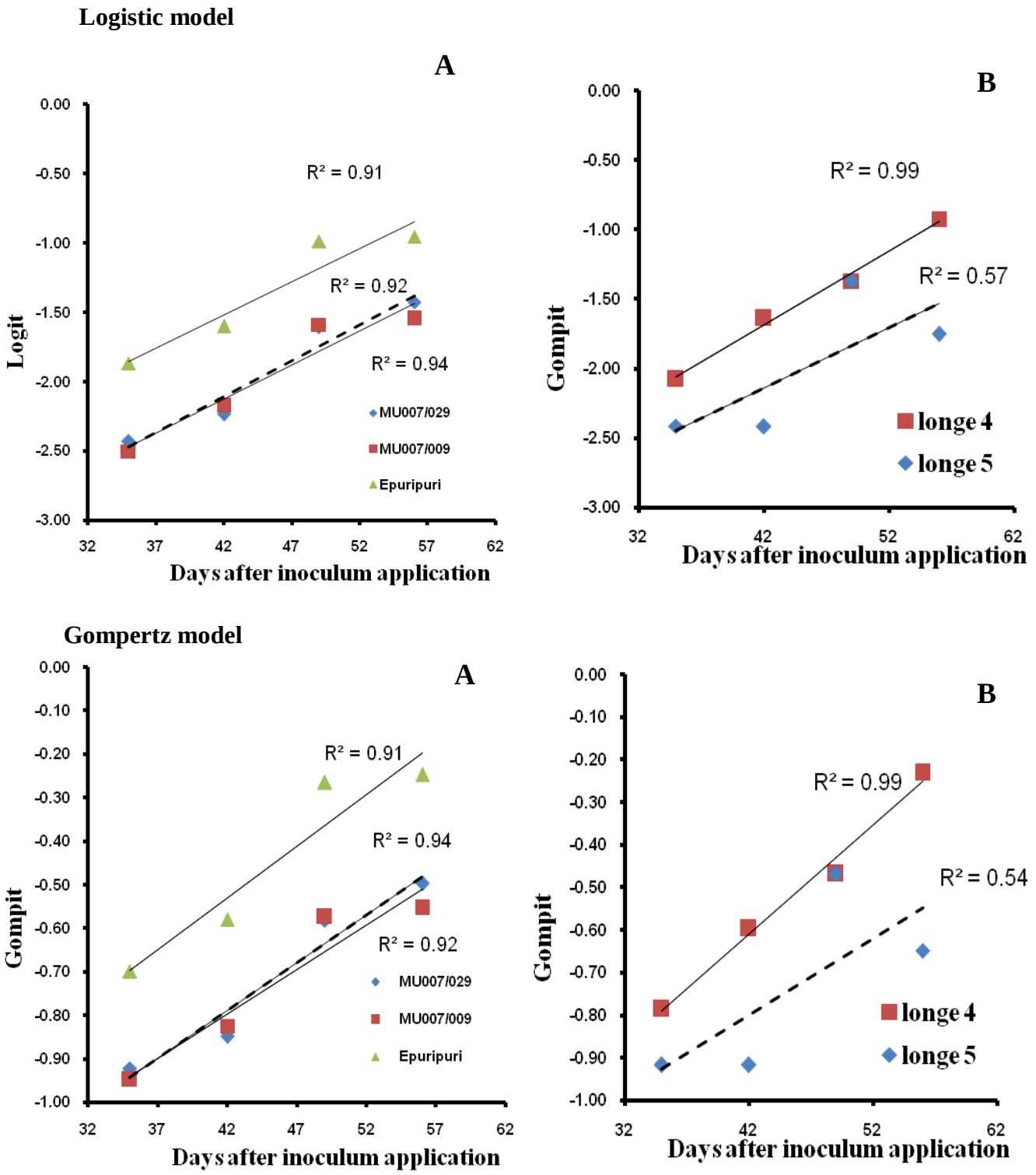


Figure 9. Logistic and Gompertz models of turcicum leaf blight epidemics on sorghum and maize in at MUARIK. Figure A is for sorghum and B for maize during the second experimental (2008 A rains-March –June).

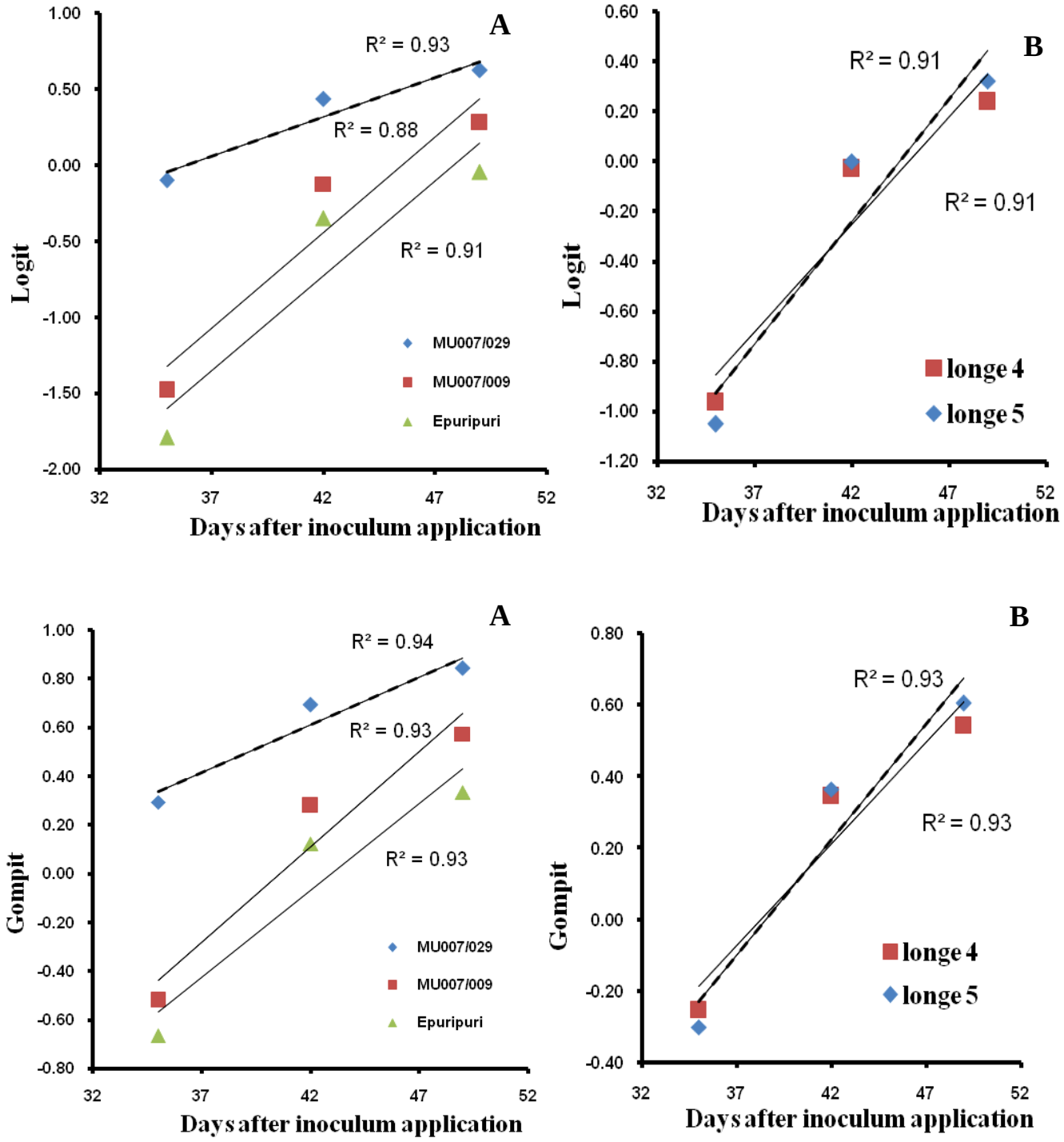


Figure 10. Logistic and Gompertz models of turcicum leaf blight epidemics on sorghum and maize in at NaSARRI. Figure A is for sorghum and B for maize during the second experimental (2008 A rains-March –June).

Rates of turcicum leaf blight progress, ranged from 0.02-0.07 logits /day for the susceptible sorghum and maize genotypes while for the resistant genotypes they ranged from 0.003-0.02 logits/day. Generally, the resistant test lines in both crops had lower rates of disease progress. Whereas disease developed faster on maize, the apparent rates of infection on sorghum were in some instances comparable.

6.3 Discussions

The objective of this study was to elucidate the temporal attributes of turcicum leaf blight on sorghum and maize. The study found out that disease development occurred much earlier in maize as compared to sorghum. It was further revealed that during epidemics, the rates of disease development in sorghum accession MU007/029 and MU007/009 was relatively low as compared to *Epuripuri*, Longe 4 and Longe 5. These results allude to more physiological adaptation of *E. turcicum* to maize than sorghum. In the TLB-sorghum pathosystem, there is evidence of physiological adaptation of *E. turcicum* with at least 7 races of the pathogen known (Leonard, 1989). In this study, 4 races of the pathogen were identified (chapter 8). Therefore the role of pathogen variability and adaptation in the observed epidemics cannot be precluded and may in fact account for the observations.

These results also suggest that sorghum accessions do exhibit qualitative and quantitative (rate-reducing) resistance which limit the spread of TLB. The results further suggest that the level of resistance within genotypes also affects the disease development over time as there was limited résistance on *Epuripuri* as compared to MU007/009 and MU007/029. The data further demonstrated that there was significant difference in latent period among the sorghum accessions. It is known that long latent period is closely related to partial resistance, which suggests that selection of breeding materials would require selecting materials with increased latent period than selecting for reduced severity. Sorghum accession MU007/029 had the longest latent period of 42 days as compared with *Epuripuri* with just 25 days almost half the days in the susceptible variety. It is likely that these sorghum accessions MU007/009 and MU007/029 possess

partial resistance. This form of resistance is characterised by an increase in latent period, a reduction in lesion number and size. In the maize partial resistance ranges from a high level with few small lesions to a low level with many large sporulating lesions as observed with Longe 5 (Raymundo and Hooker, 1982). It is thus conceivable that the form of resistance observed in these materials is rate reducing (Carson, 1995).

In this study both logistic and Gompertz models described adequately disease progress over time both maize and sorghum at two locations. These models are most suitable in cases where plant tissues becomes limiting and therefore what is observed in the apparent infection rather than the absolute disease levels. The apparent infection rates of this study were indeed indicative of the nature of disease development. For example in the susceptible *Epuripuri* sorghum variety and susceptible Longe 5 the apparent rates of infections though different were similar in trend. In *Epuripuri* which characteristically had smaller lesions the apparent rate of infection was also lower than in maize but markedly higher than the resistant sorghum accessions. A comparison of the well characterized maize –TLB pathosystem revealed a similar trend but with higher values for rates of disease development and apparent rates of infection. These data thus allude to some form of *E. turcicum* physiological specialisation presented in Chapter 5.

In conclusion, these results suggest that the TLB development in sorghum is much slower progressing and perhaps with a rate reducing resistance being operational in the test accessions. In the Maize TLB pathosystem rate reducing resistance has been reported and, given that both maize and sorghum are all poaceae with synteny for some chromosomes, the role of this type of resistance cannot be precluded. Being one of the reports of its kind however raises a number of questions including the need characterize and map the resistance loci.

CHAPTER SEVEN

ANALYSIS OF SPATIAL ATTRIBUTES OF TURCICUM LEAF BLIGHT EPIDEMICS IN SORGHUM IN TWO AGRO-ECOLOGIES OF UGANDA

Introduction

The objective of this study was to characterise the spatial attributes of turcicum leaf blight epidemics in sorghum. It involved infecting three sorghum accessions with turcicum leaf blight infected crop residues kept from the previous season under field conditions. The essences of the study was to assess the effect of varying levels of crop residues level (80%, 40 % and 0 of the plot size covered with crop residues), distance away from crop residue source and direction of movement of inoculum due to wind drift on disease development within the plot .

7.1 Prevailing weather conditions during the study period

The weather conditions were not favourable during the first season of experimentation (second rains of 2007) at NaSARRI however at MUARIK the prevailing weather was milder and more favourable. Maximum and minimum temperatures averaged 31.1^{o}C and 18.8 ^{o}C at NaSARRI during the first experimental season at NaSARRI while 29.3 ^{o}C and 15.9 ^{o}C at MUARIK respectively. In the second cropping seasons, at both sites the conditions were all favourable. During this period of experimentation (first rains of 2008) at NaSARRI the maximum and minimum temperatures were 29.9 ^{o}C and 18.9 ^{o}C. These conditions were favourable for the development of turcicum leaf blight in both locations.

7.2 Disease development as influenced by distance from inoculum sources

At both locations the disease was restricted to the inoculated plots with crop residues and it was observed that plots with varying amounts of residues exhibited varying amounts of the disease. Turcicum leaf blight was detected initially only on plants near the inoculum source, but with time disease symptoms were also observed on plants at extreme end of the plot far from the inoculum source (crop residues), (Table 17). Analysis of variance showed that the direction of spread did not significantly ($P \leq 0.05$) affect the development of the disease in the different plots apart from the final week of disease assessment at NaSARRI during season two (Table 17). As a result, data for the severity of the disease in the four directions (North, East, South and West) were pooled and used to compute AUDPC and apparent infection rates.

At NaSARRI during the first cropping season (March –June, 2008), the amount of crop residues significantly ($P \leq .001$) affected the initial severity (Y_i) and AUDPC values but did not significantly affect the final severity (Y_f). In the second cropping season, the amount of crop residue did not significantly ($P \geq 0.05$) affect the initial severity (Y_i), final severity (Y_f) and AUDPC values (Table 17). At MUARIK the amount of crop residue significantly ($P \leq .001$) affected the final severity (Y_f) but did not affect the initial severity (Y_i) and AUDPC values during the first cropping season (March –June) of 2008 (Table 18).

During the initial disease assessment, disease severity was positively correlated with varying amounts of crop residues. However by the last week termites had invaded some plots causing a reduction in the inoculum load especially at NaSARRI. At both NaSARRI and MUARIK the highest disease severity was obtained with 40% crop residues cover (Table 18). A comparison of treatment means revealed a varied response. During the warmer and short rains of 2007 at NaSARRI, distance of the host from crop residue only significantly affected AUDPC ($P \leq 0.05$) (Table 18). During the cooler and longer rains of 2008

(March to July) at both NaSARRI and MUARIK, no significant effect of host distance from residue was observed ($P \geq 0.05$). At NaSARRI however significant effect of distance was observed for all disease assessment indices (Table 19). Overall disease epidemics were more severe at NaSARRI than at MUARIK. Furthermore, comparison of main effect of cultivar and distance from inoculum sources on disease development revealed that indeed disease incidence and severity was being influenced by inoculum sources (Table 19). In both agro ecologies, during both study periods plants at a spacing of 1.2 m from inoculum sources had the highest severity and overall AUDPC. At longer distances from inoculation i.e. 4.2 m disease severity was low.

7.3 Disease progress as influenced by distance from inoculum sources

Logistic and Gompertz models were used to study the disease progress as influenced by crop residues. The main effects of crop residues on the intercept (a) and apparent infection rate were significant for both locations. The interactive effect of cultivars by crop residues was significant on the apparent rate of infection (r) ($P < 0.01$) and predicted initial amount of disease ($P < 0.05$) at MUARIK. At MUARIK and NaSARRI the apparent rates of infection were higher for *Epuripuri* than for accession MU007/009 and MU007/029 (Table 20). The residual level of 80 % had the highest apparent infection rate. The rates of disease increase per day were not significantly influenced by time of disease assessment (Table 21) but by the amount of crop residues, (Table 22). The apparent rates of disease development in season one in NaSARRI ranged from 0.056-0.066 logits/day and in season two from 0.1153-0.1178 logits/day (Table 18). In MUARIK the rates ranged from 0.055-0.083 logits/day. Rates of disease development were quite high in NaSARRI as compared to MUARIK (Table 18). The disease level was linearly correlated with crop residue level. Rates of disease development were relatively higher in the second trail than one in NaSARRI compared to MUARIK. Also r values were higher where crop residues (0.1178 at 80 % coverage as compared to 0.1153 at 0 levels at NaSARRI during the second trail) were applied compared to the control where no residue was applied (Table 18).

Table 17. Mean squares of the effect of crop residue level, cultivar, direction and distance from the inoculum sources on severity and Area Under Disease progress Curves (AUDPC) of turcicum leaf blight of sorghum at two agro-ecologies (MUARIK and NaSARRI)

Treatment	2007(September –December)				2008 (March –June)					
	df	Y_i	Y_f	AUDPC	Y_i	Y_f	AUDPC	Y_i	Y_f	AUDPC
		NaSARRI			MUARIK			NaSARRI		
Variety (A)	2	23.15***	0.59***	22.87***	1039.0***	1147.0***	16516.3***	246.1***	266.3***	606.7***
ResidueLevel B	2	12.14***	0.16^{ns}	11.16***	7.9^{ns}	961.9***	484.6**	0.02ns	50.6^{ns}	8.4^{ns}
Distances (C)	5	0.77^{ns}	0.25**	11.10***	160.2**	475.8***	1928.6***	1.2ns	134.5^{ns}	4.0^{ns}
Direction (D)	3	1.53^{ns}	0.05^{ns}	3.96^{ns}	18.4^{ns}	114.5^{ns}	102.7^{ns}	1.1ns	307.4*	10.8**
A*B	4	2.85***	0.61***	3.97*	26.8^{ns}	701.8***	441.2**	2.0ns	1104.9***	33.4***
A*C	10	0.44^{ns}	0.13^{ns}	2.20^{ns}	42.0**	50.0^{ns}	319.4**	1.6^{ns}	77.6^{ns}	1.9^{ns}
B*C	10	0.56^{ns}	0.16**	3.21**	52.4***	18.1^{ns}	223.7^{ns}	0.9^{ns}	98.8^{ns}	3.9^{ns}
A*D	6	0.51^{ns}	0.15*	3.03^{ns}	9.8^{ns}	37.3^{ns}	50.6^{ns}	1.2^{ns}	173.0^{ns}	3.5^{ns}
B*D	6	0.44^{ns}	0.30***	1.87^{ns}	9.3^{ns}	32.4^{ns}	35.0^{ns}	1.3^{ns}	192.1^{ns}	5.7^{ns}
C*D	15	0.62^{ns}	0.16***	0.67^{ns}	2.5^{ns}	37.0^{ns}	30.5^{ns}	1.4^{ns}	100.1^{ns}	3.4^{ns}
A*B*C	20	11.36^{ns}	0.26***	1.68^{ns}	24.8*	43.3^{ns}	121.9^{ns}	0.9^{ns}	78.0^{ns}	19^{ns}
A*B*D	12	7.41^{ns}	0.24***	2.12^{ns}	2.9^{ns}	40.0^{ns}	21.5^{ns}	1.5^{ns}	245.4*	9.6***
A*C*D	30	21.13^{ns}	0.15***	1.80^{ns}	3.7^{ns}	26.9^{ns}	27.6^{ns}	1.4^{ns}	59.5^{ns}	1.0^{ns}
B*C*D	30	18.77^{ns}	0.22***	1.28^{ns}	3.3^{ns}	27.5^{ns}	16.3^{ns}	1.1^{ns}	68.4^{ns}	1.2^{ns}
A*B*C*D	60	49.53^{ns}	0.17***	1.18^{ns}	4.6^{ns}	23.4^{ns}	28.8^{ns}	1.0^{ns}	79.6^{ns}	1.9^{ns}

Asterisks indicate *, **, and ***, = significant at 0.05, 0.01 and 0.001 probability levels, respectively. ns indicate not significant at $P \leq 0.05$ probability level. AUDPC. Area under Disease progress curve calculated as described by Campbell and Madden (1990) and standardised by dividing by the number of days from the first to the last assessment date. Y_i and Y_f mean first and last score.

Table 18. The effect of varying levels of crop residue of infested sorghum residues on initial Turcicum leaf blight severity (yi), final severity (yf), Area under Disease Progress Curve and apparent infection rates (r)

	2007 (September –December)				2008 (March – June)							
	NASARRI				MUARIK				NASARRI			
Residual Level	y_i	y_f	AUDPC	r	y_i	y_f	AUDPC	r	y_i	y_f	AUDPC	r
0% (none)	3.75	4.88	4.39	0.066	2.17	6.08	7.97	0.055	1.55	40.66	40.12	0.1153
40% (medium)	3.81	4.90	4.30	0.063	2.44	11.07	11.55	0.072	1.54	41.67	39.03	0.1155
80% (High)	4.28	4.95	4.83	0.056	1.99	10.07	10.44	0.083	1.56	41.39	41.45	0.1178
Grand Mean	3.95	4.91	4.51	0.061	2.20	9.07	9.99	0.072	1.55	41.24	40.20	0.1163
LSD $_{(P\leq 0.05)}$	0.198	0.06	0.29	0.041	1.00	2.60	3.23	0.047	0.31	2.84	2.68	0.0774
C.V$_{(\%)}$	21.6	5.3	27.3		191.1	123.7	140.0		85	29.7	28.7	

LSD= Fishers Protected Least Significant Difference Test at P<0.05 Steel et al., 1997). CV = Percentage coefficient of variation. Initial disease (y_i) assessed 21days after residues application at Serere, and Kabanyolo respectively. Final (y_f) assessed 49 days after residue application.[a]AUDPC was calculated as described by Campbell and Madden (1990) and standardised by dividing by the number of days from the first to the last assessment date.

7.4 Modelling disease gradient

Two epidemiology models were used to study the plant disease gradient. The exponential and power models were examined on the basis of R^2 values. Both models gave good fit for all the two, cropping seasons and were thus used to characterise the disease spread (Figure 11). The amount of infested significant ($P \leq 0.05$) affected b and Y_o during both seasons on the test sorghum varieties. Effect of time on disease gradient was influenced by the rate of disease progress (Table 21). For example at MUARIK, during the first study season (September –December 2007), the mean disease gradient was –0.86 on 23rd June, -0.2 on 8th July and -0.26 on 16 July which indicates a steep to flatter to steeper kind of disease gradient. The crop residual level influenced the disease gradient, being initially steeper but flattening thereafter. The disease gradient based on the means from the residue source between 23rd June and 16th July at MUARIK (March –June 2008) ranged from -1.35 to 0.004 and at NASARRI ranged from -0.08 to 0.005 and -0.24 to 0.09 in second rains of 2007 and first rains of 2008 respectively (Table 21). Gradient values based on means derived from variety performance during the same period at MUARIK (March – June 2008) ranged from -1.19 to -0.20 and at NASARRI the values ranged from -0.33 to 0.17 and -0.09 to 0.03 during second rains of 2007 and first rains of 2008, respectively (Table 22).

Table 19. The effect of increasing distance from residue source on initial Turcicum leaf blight severity (yi), final severity (yf), standardized Area under Disease Progress Curve (AUDPC) and apparent infection rates (r) at MUARIK and NASARRI in 2007 and 2008

	2007-September -December			2008-March –June					
	NaSARRI			MUARIK			NaSARRI		
Distance (m)	y_i	y_f	AUDPC	y_i	y_f	AUDPC	y_i	y_f	AUDPC
1.2	30.0	61.8	86.9	1.7	57.0	26.9	4.8	13.3	18.9
1.8	30.4	62.9	87.4	1.4	56.5	25.8	2.7	10.7	12.5
2.4	30.4	62.8	88.3	1.6	57.4	26.1	2.2	9.4	9.9
3.0	30.2	64.4	84.2	1.5	58.4	26.1	2.0	7.5	7.7
3.6	26.8	60.4	74.6	1.7	57.9	26.4	1.0	7.0	6.1
4.2	28.6	60.6	68.9	1.5	57.7	25.5	0.4	6.4	4.8
$LSD_{(P\leq0.05)}$	5.5	9.0	11.5	0.7	4.8	8.5	1.3	3.7	4.3
$CV_{\%}$	53.0	19.7	43.1	104.4	18.9	32.1	186.4	123.7	133.0

Initial disease (Y_i) assessment was carried out 21 days after residue application at Kabanyolo and Serere respectively. Final disease (Y_f) assessment carried out after 49 days after residue application. AUDPC was calculated as described by Campbell and Madden (1990) and standardized by dividing by the number of days from the first to the last assessment date.

Table 20. Effect of cultivar and distance from inoculum source on initial severity (Yi), final severity (Yf), Area under Disease Progress Curve (AUDPC) on Turcicum Leaf Blight Severity on three sorghum varieties at NaSARRI and MUARIK in 2007 and 2008

	Cultivars											
	MU007/009				MU007/029				*Epuripuri*			
Distance (m)	Y_i	Y_f	AUDPC	r	Y_i	Y_f	AUDPC	r	Y_i	Y_f	AUDPC	r
Kabanyolo 2008 B												
1.2	1.86	7.49	8.71	0.51	2.96	5.72	10.47	0.19	9.46	26.46	37.63	0.44
1.8	0.67	6.63	5.67	0.72	0.58	4.79	4.47	0.66	6.83	20.29	27.63	0.46
2.4	0.46	5.77	3.41	0.85	0.50	2.92	3.25	0.56	5.63	19.79	22.96	0.50
3.0	0.21	4.69	2.56	0.94	0.21	0.64	1.55	0.38	5.71	17.08	18.94	0.44
3.6	0.04	3.31	2.43	1.39	0.08	1.96	0.81	1.60	3.00	15.83	14.97	0.60
4.2	0.08	2.61	1.88	1.09	0.12	0.71	0.47	0.46	1.13	15.83	12.12	0.89
S.E.D	0.27	1.50	1.09	0.54	0.44	1.50	1.20	0.35	1.50	3.59	4.39	0.30
Serere 2007 A												
1.2	38.54	58.66	50.83	0.26	17.71	58.66	34.79	0.60	33.75	67.67	52.15	0.44
1.8	33.75	62.35	47.85	0.37	12.41	58.06	36.21	0.51	34.67	70.08	54.41	0.48
2.4	32.92	60.56	49.30	0.35	21.25	57.27	37.95	0.50	36.75	68.90	52.64	0.43
3.0	33.37	59.71	45.10	0.34	18.69	59.23	32.45	0.56	35.83	66.85	55.87	0.41
3.6	27.65	54.10	37.88	0.36	19.34	54.53	29.34	0.52	31.01	68.58	50.41	0.48
4.2	32.27	56.67	31.63	0.34	21.24	52.69	29.03	0.46	32.26	70.46	48.58	0.52
S.E.D	2.04	4.33	3.75	0.25	2.58	3.24	3.68	0.09	1.74	2.39	2.86	0.07
Serere 2008 B												
1.2	14.08	33.54	37.17	0.32	13.33	33.54	31.15	0.40	18.54	58.75	63.97	0.57
1.8	10.54	35.42	26.46	0.43	12.50	35.42	31.19	0.44	17.50	56.04	61.09	0.57
2.4	11.38	35.42	27.40	0.44	13.13	35.42	31.36	0.43	17.29	56.04	59.66	0.57
3.0	10.63	37.08	28.06	0.43	12.92	37.08	1.52	0.45	17.29	58.33	59.43	0.60
3.6	14.38	36.87	32.83	0.34	13.75	36.87	32.15	0.42	16.29	56.25	57.67	0.58
4.2	12.08	34.37	30.03	0.40	12.29	34.37	29.15	0.43	16.04	55.83	56.83	0.59
S.E.D	4.29	3.35	5.36	-0.1	3.61	3.40	4.09	0.00	4.09	2.61	4.96	-0.1

SED = Standard Error of the difference among means (Steel *et al*., 1997). Initial severity scored 21 days after inoculum application. Final severity scored after 49 days after inoculum application, AUDPC was calculated as described by Campbell and Madden (1990) and standardized by dividing by the number of days from the first to the last assessment date.

Table 21. Regression (n) of the logits (In(y/ (1-y)) transformation of Turcicum leaf blight severity on transformed distances from inoculum foci with infested sorghum residues in NaSARRI and MUARIK in 2007 and 2008

	0% soil Cover			40% soil Cover			80% soil cover		
Assessment Time	**b**	**Y_o***	**R^2**	**b**	**Y_o***	**R^2**	**B**	**Y_o***	**R^2**
Kabanyolo									
2nd Season									
23 June 2008	-1.19	-2.69	0.89	-0.86	-1.70	0.98	-1.35	-0.99	0.96
30 June 2008	0.004	-3.67	0.00	-0.80	-1.29	0.93	-0.72	-1.74	0.97
8 July 2008	-0.39	-2.00	0.90	-0.60	-1.35	0.98	-0.63	-1.13	0.94
16 July 2008	-0.26	-2.10	0.74	-0.26	-1.41	0.97	-0.28	-1.46	0.95
Serere									
1st Season									
28 January 2008	-0.07	-0.41	0.36	-0.06	-0.86	0.29	-0.01	-1.05	0.07
6 February 2008	-0.02	-0.05	0.08	-0.04	-0.36	0.21	-0.03	-0.39	0.38
13 February 2008	-0.03	0.35	0.67	0.05	-0.60	0.60	-0.04	-0.01	0.22
20 February 2008	-0.08	0.83	0.49	0.01	0.42	0.02	-0.05	0.50	0.17
2nd Season									
23 June 2008	0.09	-2.10	0.63	-0.01	-1.93	0.00	-0.13	-1.32	0.76
30 June 2008	0.06	-1.11	0.37	-0.01	-0.89	0.18	-0.10	-0.67	0.71
8 July 2008	0.01	-0.42	0.08	-0.01	-0.64	0.21	-0.24	0.15	0.66
16 July 2008	0.01	-1.42	0.05	-0.02	-0.26	0.19	0.03	-0.42	0.17

b=rate of disease decrease over time, Y_o* =intercept or logit (Y) and R^2= coefficient of determination. Data are averages for four directions (North, East, West and South of Disease assessment from Inoculated foci

Table 22. Regression of logit transformation for turcicum leaf blight severity and transformed distance for three sorghum varieties as affected by *Exserohilum turcicum* Infested crop residues

	Cultivar								
	MU007/009			**MU007/029**			***Epuripuri***		
Assessment time	**b**	**Y_o**	**R^2**	**b**	**Y_o**	**R^2**	**B**	**Y_o**	**R^2**
Kabanyolo 2008 B									
23rd June 2008	-1.19	-2.69	0.89	-1.09	-2.70	0.87	-0.65	-1.32	0.86
30th June 2008	-0.52	-2.98	0.83	-1.23	-1.50	0.96	-0.45	-1.50	0.96
8th July 2008	-0.60	-2.34	0.83	-0.47	-2.48	0.05	-0.64	-0.35	0.95
16th July 2008	-0.38	-1.97	0.97	-0.71	-1.94	0.70	-0.20	-0.90	0.87
Serere 2007 A									
28th January 2008	-0.33	0.02	0.74	0.02	-1.44	0.03	-0.04	-0.55	0.23
6th February 2008	-0.11	-0.02	0.50	0.06	-0.94	0.65	-0.04	0.12	0.36
13th February 2008	-0.05	0.02	0.32	0.05	-0.60	0.20	-0.02	0.32	0.06
20th February 2008	-0.07	0.54	0.42	-0.07	0.47	0.64	0.17	0.74	0.08
Serere 2008 B									
23rd June 2008	0.01	-1.99	0.00	-0.01	-1.88	0.03	-0.05	-1.43	0.92
30th June 2008	0.02	-1.47	0.02	-0.01	-1.39	0.05	-0.07	-0.05	0.75
8th July 2008	0.03	-1.15	0.04	-0.04	-0.93	0.61	-0.09	0.28	0.90
16th July 2008	0.02	-0.83	0.16	0.02	-0.66	0.16	-0.02	0.34	0.23

b=rate of disease decrease over time, Y_o* =intercept or logit (Y) and R^2= coefficient of determination. Data are averages for four directions (North, East, West and South of Disease assessment from Inoculated foci.

.

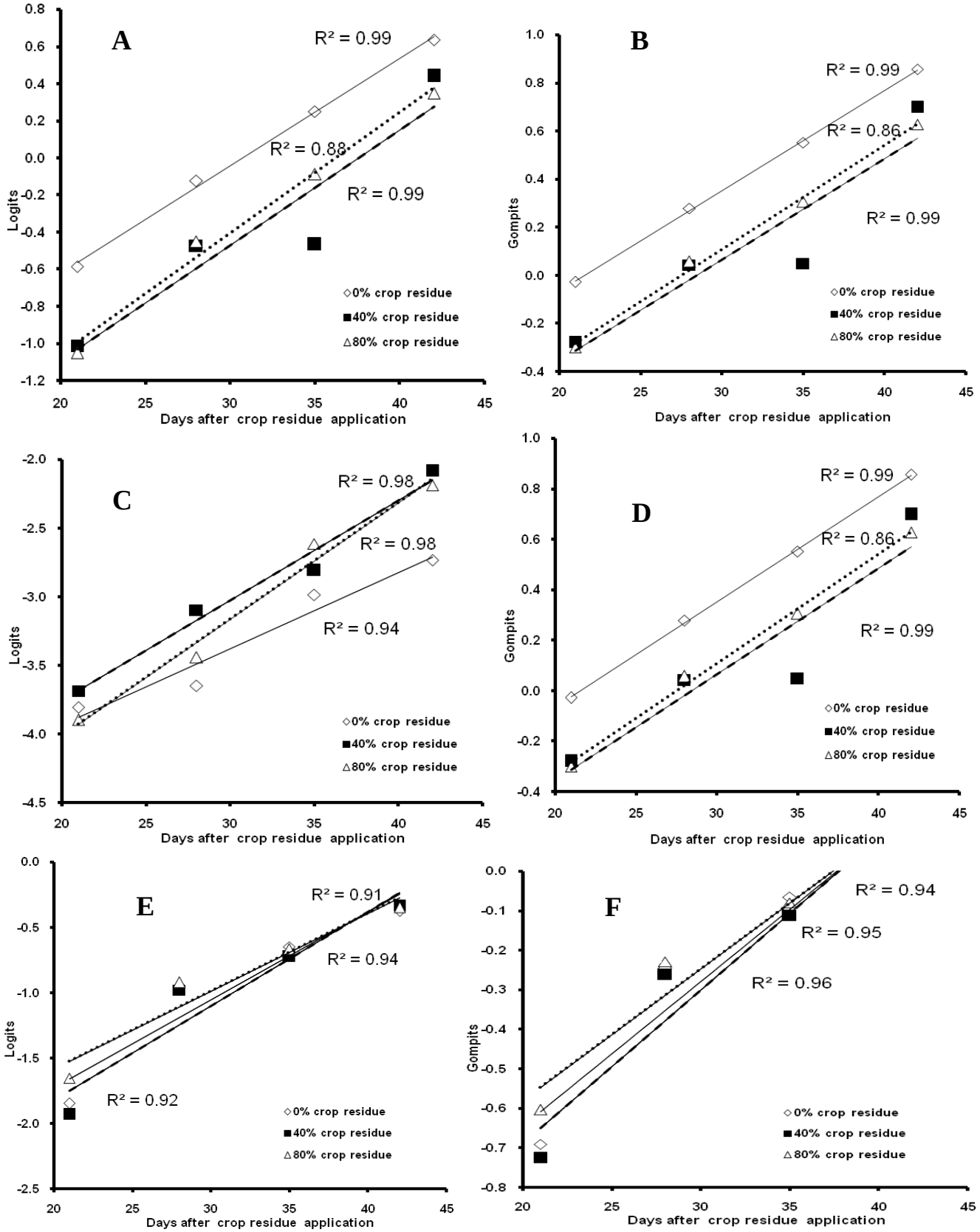


Figure 11. Logistic and Gompertz models of turcicum leaf blight epidemics on sorghum at NaSARRI (A, B, E and F) and MUARIK (C and D)

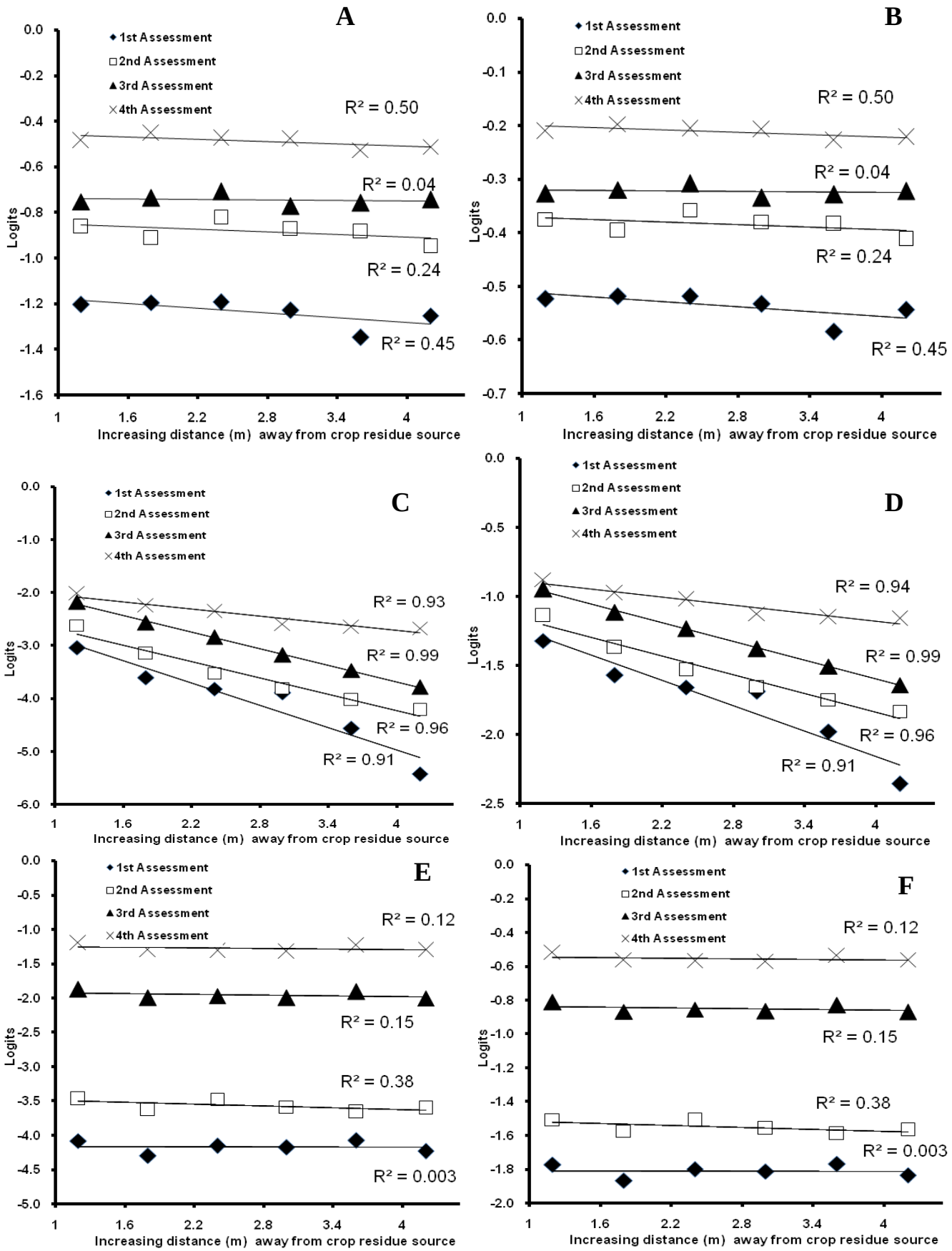


Figure 12. Spread of turcicum leaf blight over distance investigated using the power (A, C & E) and exponential (B, D & F) model over four assessment dates at NaSARRI (A, B, E and F) and MUARIK (C and D)

7.5 Discussion

The objective of this study was to examine the biological significance of crop residue as initial source of inoculum on the development of TLB of sorghum. The study indicated that plots with higher levels of crop residues exhibited higher disease levels as compared to the control suggesting that these crop residues influenced epidemics. In the TLB of maize epidemics similar reports have been made (Takan *et al*., 1994). The significant role of surface inoculum on epidemics suggests that successful disease management will have to rely on methods that reduce inoculum levels. These include among others deep ploughing as is the case in conventional tillage. Thus for TLB of sorghum minimum tillage, would only exacerbate disease development (de Nazareno *et al.*, 1993). Studies on TLB on maize indicate that maize residues is an important factor for the survival of the pathogen and initiation of the epidemic (Fullerton and Fletcher, 1974).

In this study it was also observed that in majority of the plots, disease severity and AUDPC decreased with distance from the inoculum source, apparent infection rates in some cases increased with distance from crop residue source. This is in agreement with Minogue and Fry (1983), but contrary to Alderman *et al*. (1989) and Takan *et al* (1994) who observed constant apparent infection rates with increase in distance from inoculum loci. De Nazareno (1992 observed a decrease in apparent infection rates from inoculum source in leaf blight of maize in Ohio, U.S.A. The variation in disease progress as estimated using AUDPC and other parameters suggests possible crop species effects. Indeed in Chapter 6, temporal aspects of epidemics were markedly different being influenced by crop species. Moreover, there appears to be pathogen species specialization. As such under a dual host single pathogen pathosystem, disease will tend to develop faster on the host species on which the pathogen is best adapted. In this study the combined effects of host species and *E. turcicum* pathogen variability cannot be precluded.

Disease gradients measured on plants in the different plots suggest that dispersal of this pathogen is influenced greatly by the mechanics of splash dispersal, although wind conditions also influence the shape of disease gradients (Ferrandino and Elmer, 1996). Direction differences in disease development suggest that wind-driven rain, as well as rain splash influence the dispersal of secondary inoculum (Parker *et al.*, 1995). During the second period of experimentation especially at NaSARRI the gradients were flatter suggesting the effect of background inoculum. The trend was less pronounced at MUARIK. These data suggest that during the longer and more humid experimental period, disease development could have been influenced by accumulated inoculum from the previous seasons and the high inoculum load available during a major cropping season. In any case the experiments rains have resulted in similar results being reported on septoria leaf spot of wheat (Ferrandino and Elmer, 1996).

Disease occurrence in the control plants could have been attributed to contaminants from neighbouring sorghum line close by or due to wind drift which could have blown inoculum spore from infested fields onto the control plots. Furthermore it could also be attributed to rain events that could have dispersed the spores from infected plants placed on the soil surface (Sujkowski *et al.*, 2000). Presence of TLB lesions on plants at the plot boundary during initial assessment suggests the presence of incoming inoculum from other source such as alternative hosts probably due to long distance spread (Takan *et al.*, 1994).

It was noted that disease increased with time. This could have been attributed to the polycyclic nature of TLB on sorghum as multiple cycles of inoculum production and dispersal do occur during epidemic development resulting in propagules spread from crop residues onto immediate plants (Takan *et al.*, 1994). The increase in disease with time and the closeness of the gradient values towards zero could be attributed to secondary inoculum followed by released conidia in the air during saturation periods, (Gregory, 1968). It can further be attributed to background contamination or due to proximity to inoculum source (Takan *et al.*, 1994).

In this study TLB disease gradients were relatively high (1.19 to 0.02 per day) compared to those reported in other path systems (Minogue and Fry, 1983; Aldermann *et al.*, 1989; de Nazareno *et al.*, 1993). Secondary spread, background contamination, or proximity to a large area inoculum source could have caused the flattening of the primary gradient (Gregory, 1968), Disease gradients also vary depending on the method of assessment and time at which the assessments are made (Cammack, 1985). The rapid flattening of the gradient that occurs in the initial stage of the epidemic implies that, until a steady state is reached, the apparent infection rate increases with distance from the focal centre. At the steady state apparent infection rate is constant.

Generally, the gradients flattened with time, which supports earlier observations. This flattening has been attributed to secondary spread in polycyclic diseases such as this pathosystems (Adipala *et al.*, 1993). The flattening effect may have been further accentuated by background contamination which probably arose from other fields near the vicinity of the study area as sorghum is grown whole year around and farmers under the experimental site do practice this form of farming. Gradient flattening also occurs much fast with intensification of epidemics as a result of secondary spread, especially in polycyclic diseases with short monocycles (Vander Plank, 1963; Mackenzie, 1976). This study was conducted in two areas where turcicum leaf blight is endemic (Adipala *et al.*, 1993) and therefore it was not uncommon to obtain lesions on the plants far away from the crop residues before any lesions developed on the plants within the residue area.

CHAPTER EIGHT

CHARACTERISATION OF UGANDAN *Exserohilum turcicum* SORGHUM POPULATIONS

8.1 Mating types of sorghum derived *Exserohilum turcicum*

A preliminary screening for *E. turcicum* was performed using species specific primers. In total 300 isolates were screened using the internal transcribed spacer ribosomal DNA (ITS rDNA). Positive isolates gave a bright band at 344 base pairs (Figure 13). *Exserohilum. turcicum* isolates identified using the ITS species specific primers were then used in the population genetic analyses. Firstly they were screened for the mating type using mating type specific primers (Table 23.).Both *MAT 1* and *MAT 2* mating types were identified. Twenty five isolates carried the *MAT 2* (1-2 gene and 2-2 gene), whereas 23 isolate carried *MAT 1* (1-1 gene) (Annex 3; Figure 14, A, B, C) and sixteen isolates carried the *MAT1, 2* gene (Figure 14D). In some cases both mating types were found in the same location for example in the districts of Soroti, Kumi, Kaberamaido, Amuria, Apac, Homia, Dokolo, Iganga, Koboko Arua, and Kiboga.

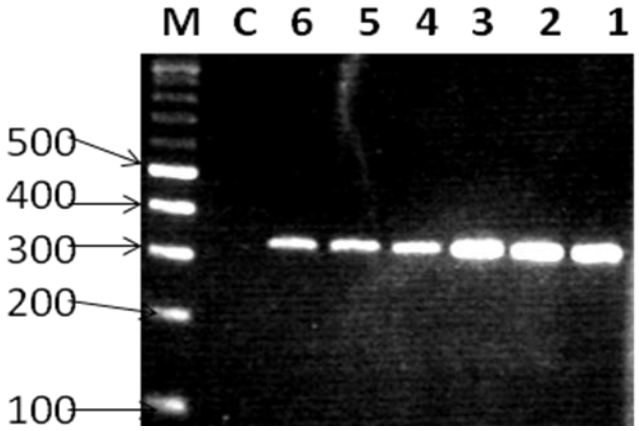


Figure 13. PCR amplicons of *E. turcicum* derived by amplification using rDNA ITS species specific primers: Lane descriptions 1-Iganga14, 2-Soroti 9, 3-Masindi 37, 4-Kaberamadio 32, 5-Kabale 30 6-Hoima 12, c-Control M- 100bp DNA ladder

Distributions of *MAT1* and *MAT-2* isolates among samples from individual districts were compared to determine the potential for sexual recombination. Mating type frequencies were found in near-equal proportions in Soroti, Kumi, Kaberamaido, Amuria, Apac, Homia, Dokolo, Iganga, Arua and Kiboga. In other districts, one mating type was more prevalent than the other (Figure 15). The districts of Katakwi, Gulu, Lira, Kabale, Pakwach and Maracha had only one mating type that is, *MAT 2.*

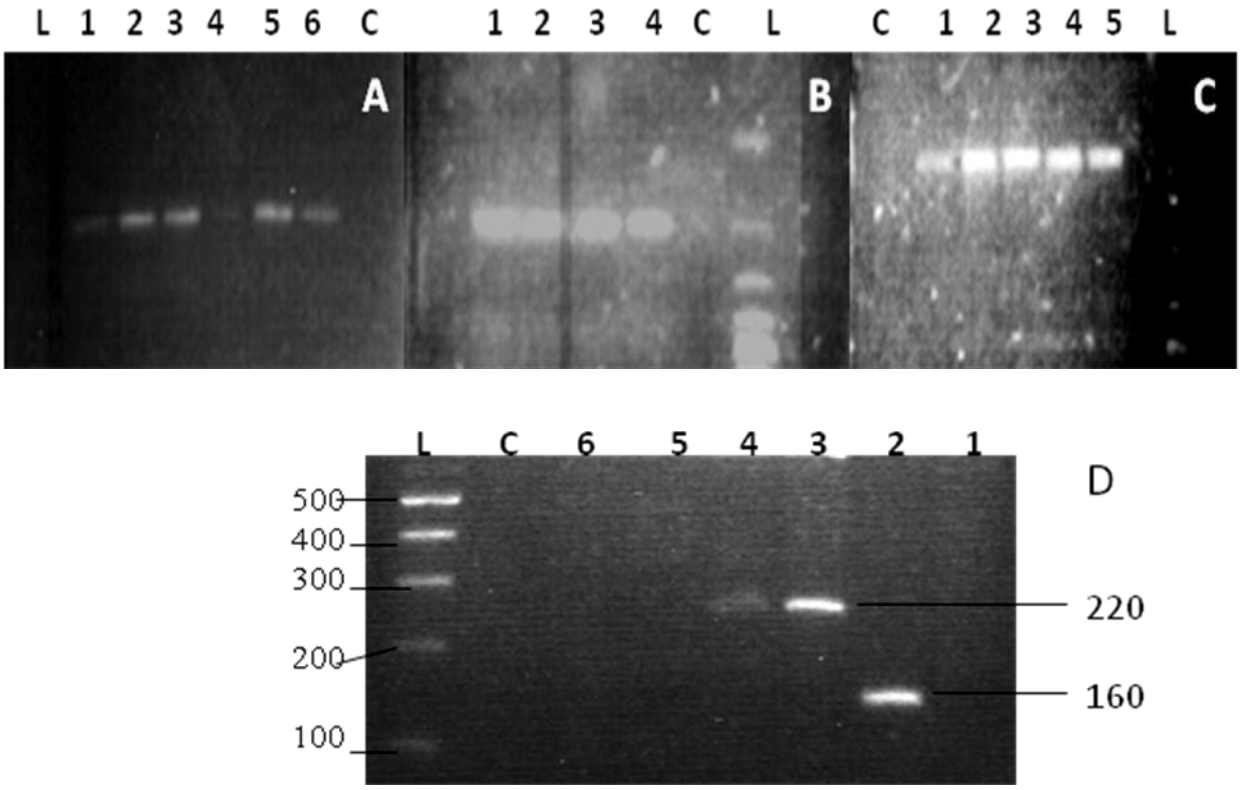


Figure 14. A-Alpha clone gene (160 bp-Mat 1), B-Mat 2 –specific gene |195bp) and C-A-clone gene (190bp-Mat 1). PCR amplicons of sorghum derived *E. turcicum* isolates using mating type primers. Figures A and C indicate MAT *1;* Figure B indicates MAT *2* isolates. Assignment of isolates to lanes in each figure are such that: lane 1 is the isolate Iganga-14,2 Soroti-9, 3 Masindi-37, 4 Kaberamadio-32, 5 Kabale-30, 6Hoima-12, c-Control and Ladder- 100bp DNA ladder. Figure D are amplicons of the *MAT 1*, *2* gene and *MAT* 2-2 genes; lanes in each figure are such 1 = Iganga-14, 2 Amuria- 8, 3 Amuria-8, 4 Kaberamaido32, 5 Kabale-30, 6 Hoima-12, c-Control, L-100 bp DNA ladder.

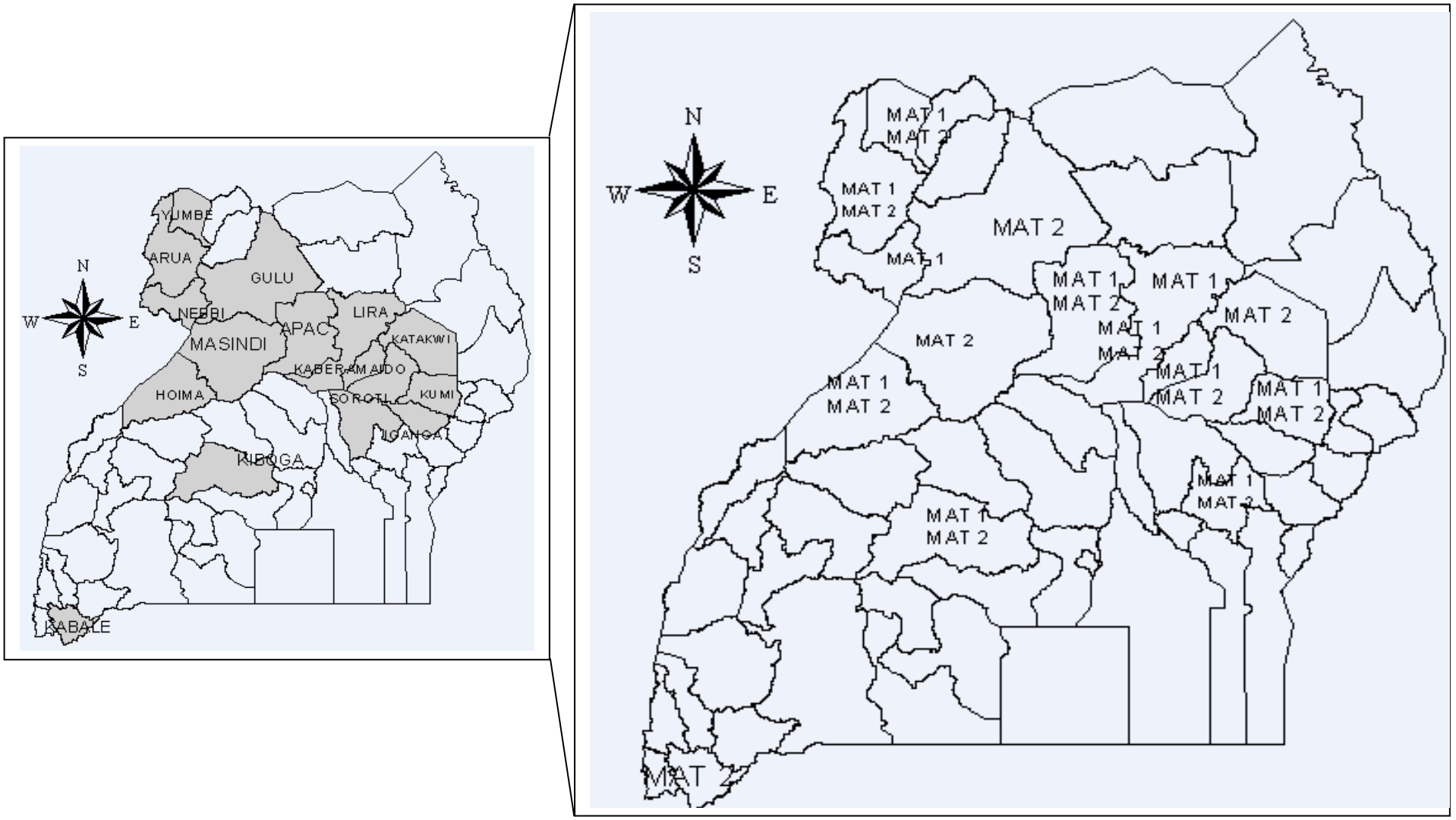


Figure 15. Sketch map of Uganda showing distribution of mating types MAT 1 and MAT2 of sorghum derived *Exserohilum turcicum* isolates of Uganda

8.2 Population characterisation using neutral genetic markers

8.2.1 Characterisation based on RAPDs analysis

Ten primer sets were tested for polymorphism but only two primers (A9 and A5 –GGGTAACGCC and GGGGTCTTG) gave good polymorphism while the remaining 8 markers had very few RAPD amplicons. The two primer sets were consistent and generated reproducible RAPDS patterns (Figure 16). Each primer produced an average of 7 bands ranging in size between 0.1-1 kb. A total 271 bands were scored. It was revealed by the analysis that 10% of the amplicons were common to all individual isolates while 90% were polymorphic. Phenetic analysis of the RAPD data revealed four broad clusters. Cluster 4 comprised of a single isolates from Dokolo, Cluster 3 had an isolate from Masaka while the remaining isolates formed two major groups' cluster 1 and cluster 2 (Figure 17). From the phenogram there was no apparent clustering together of isolates from a particular region. Most isolates clustered together from various regions.

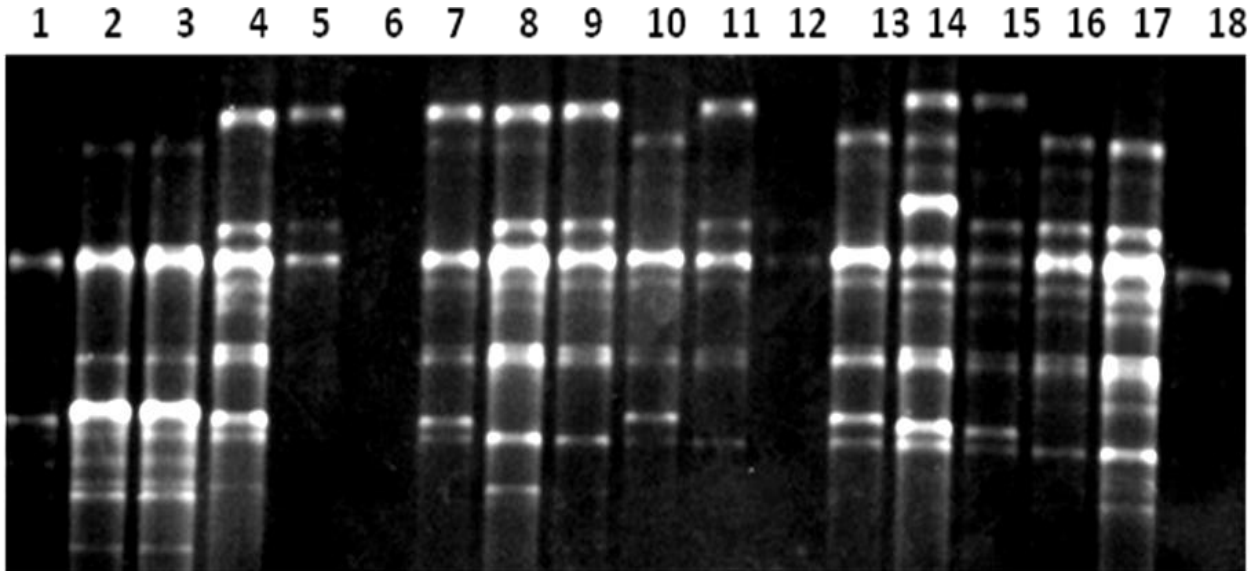


Figure 16. Banding pattern of 18 *Exserohilum turcicum* isolates derived from sorghum generated by a RAPD primer A9. Samples are in lanes 1 – 18 and L DNA 100bp Ladder

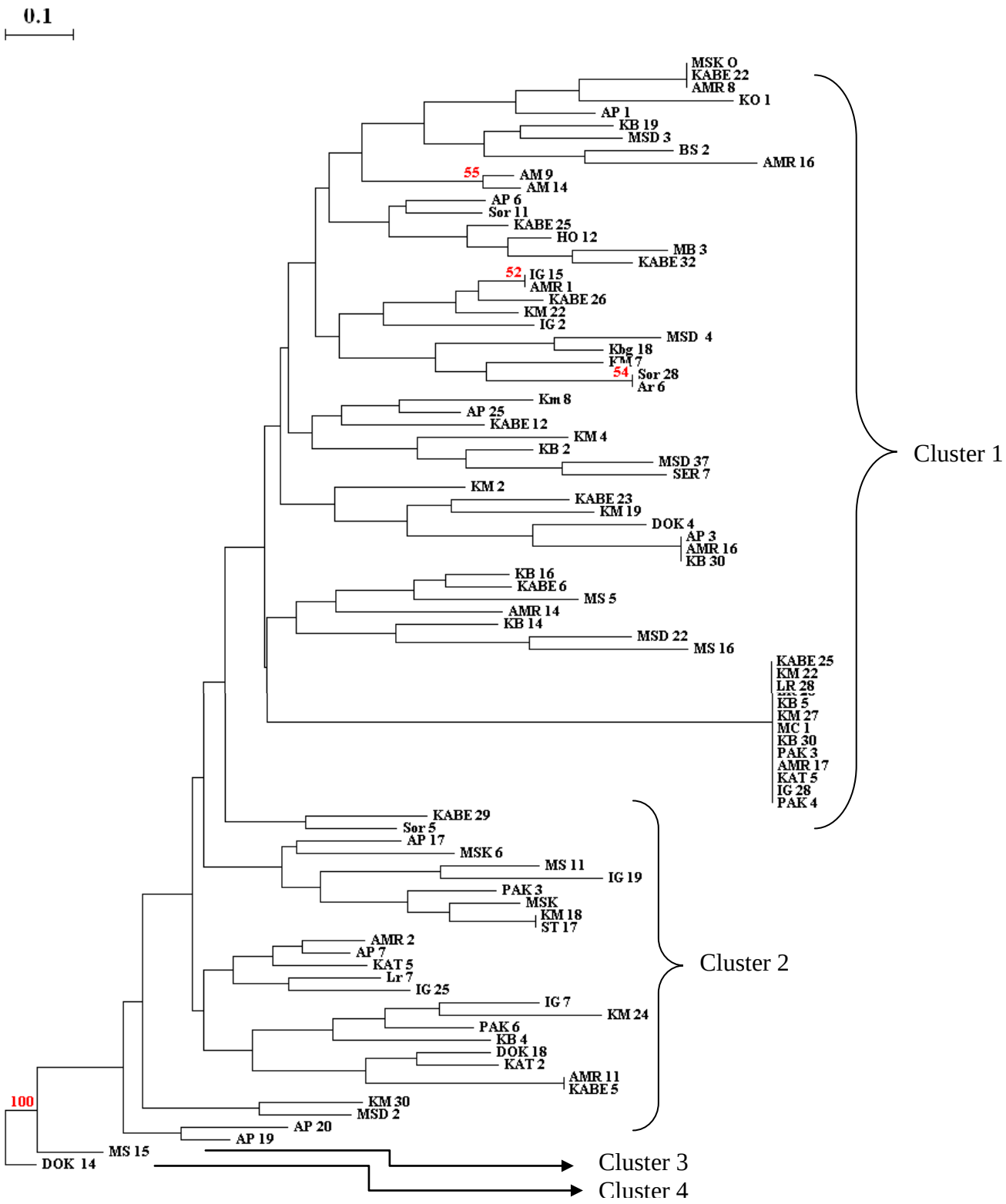


Figure 17. Phenogram of *E. turcicum* sorghum derived isolates based on RAPDs. The phenogram was constructed using neighbour joining method (Nei and Li, 1979).

8.2.2 Characterisation based on SSR analysis

Ten primer sets were tested on 80 *E. turcicum* isolates (Table 23) and five of the primers sets gave polymorphic PCR patterns (Figure 19). Each primer produced on average of 6 bands ranging in size from 100 to 1000 bp and a total of 486 bands were scored. The genetic distances between isolates ranged from 0.68 to 1 similarity coefficient. Two major clusters (I and II) where formed at 0.676 similarity coefficient. Cluster I comprised of five isolates namely from Kumi, Kaberamaido and Apac. The rest of the isolates formed cluster II. Some isolates were 100% genetic similar though obtained from different locations and agro-ecologies. The isolates formed four minor clusters with group I comprised of isolates from Masindi, Kaberamaido, Amuria and Apac; Group II Pakwach and Kumi; group III Lira and Kumi and group IV isolates from Masindi and Amuria.

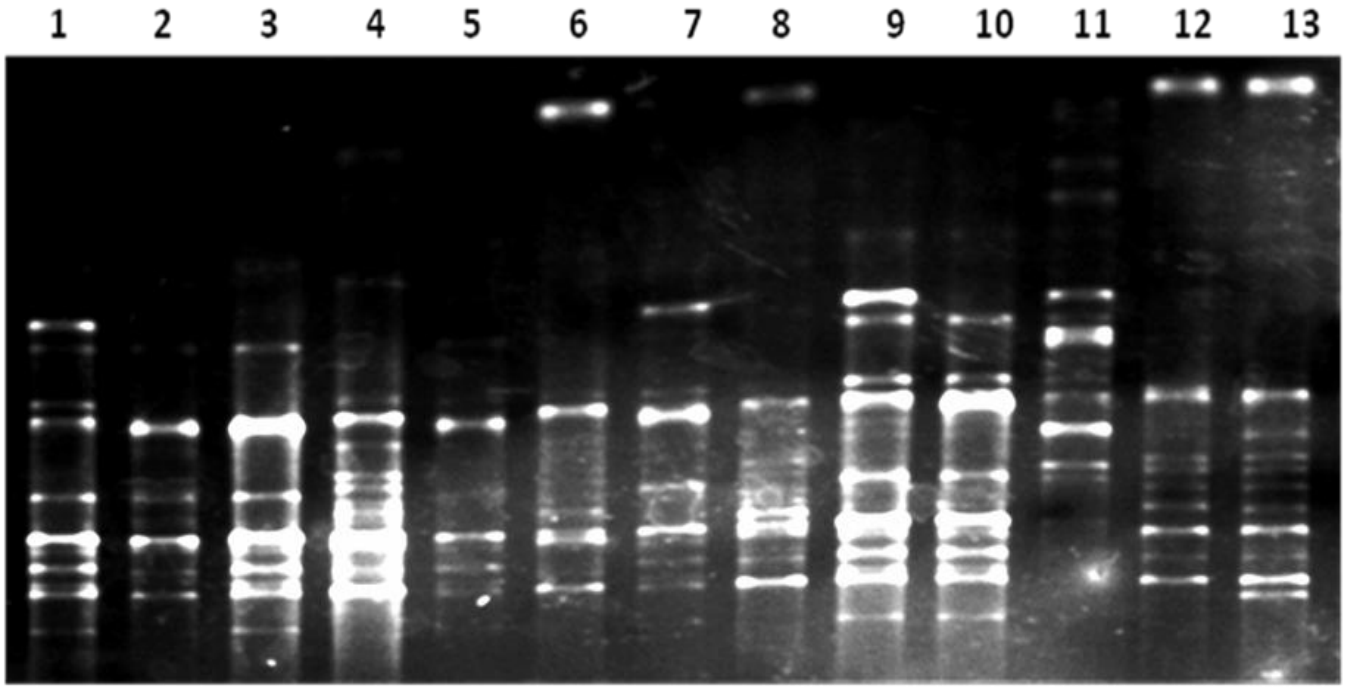


Figure 18. Banding pattern of 13 *E. turcicum* isolates derived from sorghum generated by a SSRs primer. Samples 1 – 13 and L DNA 100bp Ladder

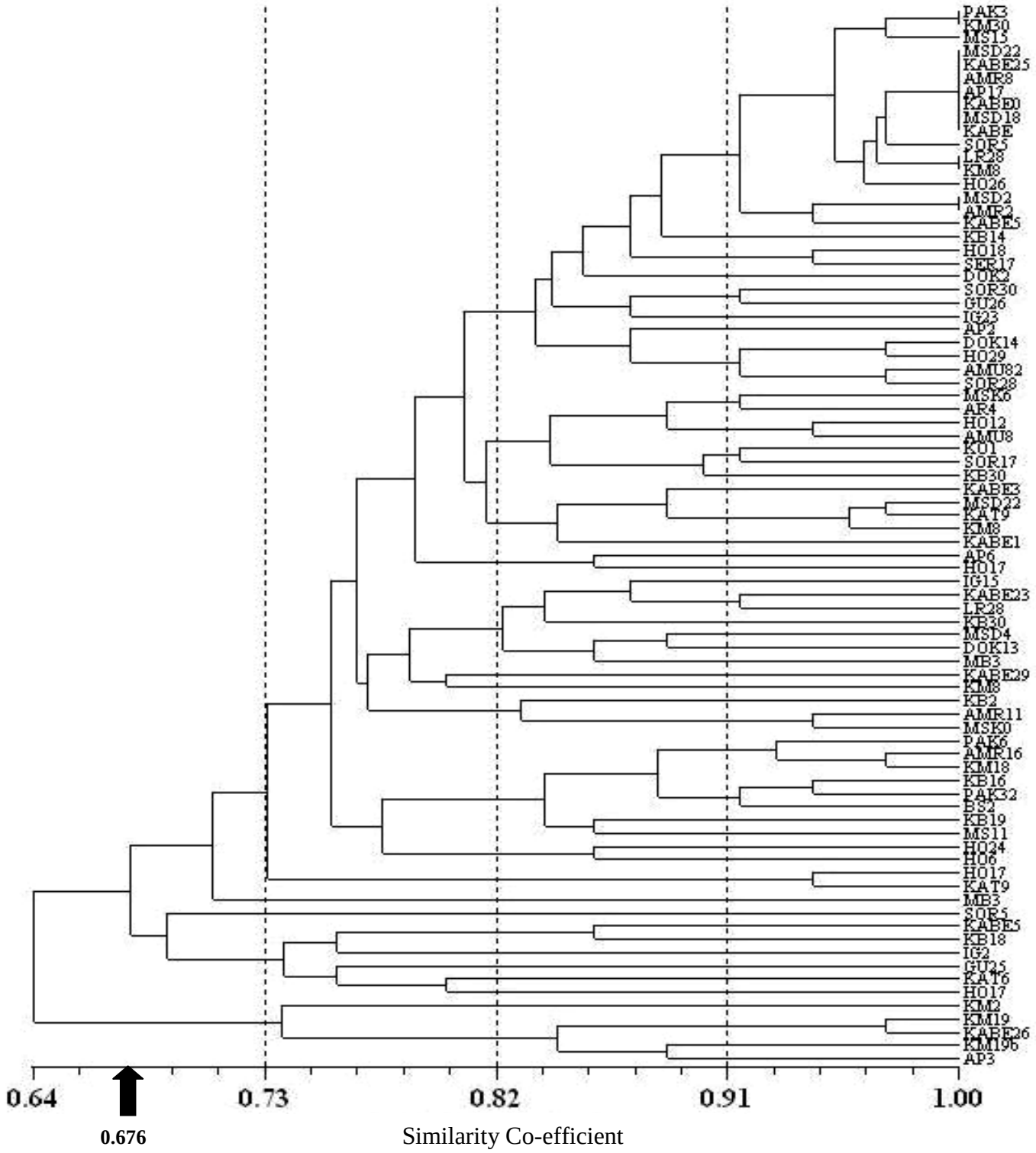


Figure 19. Phenogram of *E. turcicum* sorghum derived isolates based on SSRs data. The phenogram constructed using neighbour joining method option of SAHN programme of the NTSYs-Population genetics soft ware. Scale value of 1 indicates 100% genetic similarity

8.3 Characterisation based on race differentials

Eighteen sorghum derived *E.turcicum* isolates were used to inoculate the differential inbred lines carrying the *Ht* genes and their reactions recorded. All plants expressed a hypersensitive reaction 24 hours after inoculations. They exhibited yellowish chlorotic coloration along the margin of the maize leaves. Resistant plants exhibited chlorotic spots while susceptible plants exhibited small chlorotic lesions with elliptical gray necrotic lesions and sporulation. Assessment was carried out to a certain the reaction of the differentials (Table 24). Two isolates from Apac and Kaberamaido Pakwach and Arua were race 0 with the virulence formulae of *Ht 1, Ht 2 Ht 3*. Ten were race 123 from Kaberamaido, Soroti, Kumi, Pallisa, Lira and Amuria Iganga with the virulence formulae *Ht 2, Ht 3/Ht1*. Only one isolate from Kaberamaido was race 3. Only a single isolate failed to cause disease on all lines tested. Isolates where not tested on HtN reaction, as seed was not available.

Table 23. Races of sorghum derived *Exserohilum turcicum* isolates based on reaction on differential maize lines carrying Ht resistance genes

	Maize race differential with *Ht* resistance genes					
Tester Isolates	**A619/0**	**A619/Ht1**	**A619/Ht 2**	**A619/Ht 3**	***Race designation**	**Race name**
Kaberamaidio	S	S	S	S	*Ht 1,Ht2 Ht3*	123
Soroti	S	S	S	S	Ht 1, Ht2 Ht3	123
Usuku	S	S	S	S	*Ht 1,Ht2 Ht3*	123
Kumi	S	S	S	S	*Ht 1,Ht2 Ht3*	123
Soroti-Atiira	S	S	S	S	*Ht 1,Ht2 Ht3*	123
Kumi 2	S	R	S	S	*Ht 1/Ht2 Ht3*	23
Kaberamaido 2	S	R	R	S	*Ht 1,Ht2/ Ht3*	3
Kaberamaido 3	S	S	S	S	*Ht 1,Ht2 Ht3*	123
Kaberamaido	R	R	R	R		X
Kaberamaido 4	S	S	S	S	*Ht 1,Ht2 Ht3*	123
Serere	S	S	S	S	*Ht 1,Ht2 Ht3*	123
Pallisa	S	S	S	S	*Ht 1,Ht2 Ht3*	123
Serere	S	S	R	S	*Ht2/ Ht 1Ht3*	13
Lira	S	S	S	S	*Ht 1,Ht2 Ht3*	123
Amuria	S	S	S	S	*Ht 1,Ht2 Ht3*	123
Kaberamaido	S	R	R	R	*Ht 1,Ht2 Ht3*	0
C-Usuku	S	S	S	S	*Ht 1,Ht2 Ht3*	123
Apac	S	R	R	R	*Ht 1,Ht2 Ht3*	0

*From (Leonard *et al.*, 1989) S=Susceptible (necrotic /sporulating lesions), R=Resistant

8.4 Discussion

Globally, little is known about the genetic variability of *E. turcicum* populations in Uganda especially from sorghum derived isolates. In general only limited studies have been done using selective genetic tools such as race differentials (Adipala 1993; Bigirwa et al., 1993). No neutral genetic markers which provide better signature of evolutionary history of populations have been identified. Accordingly the objective of this study was to characterise sorghum derived *E. turcicum* using both selective and neutral genetic markers. The selectable markers used were mating types while the neutral markers were RAPDs and SSR markers.

Mating type analysis revealed occurrence of both *MAT 1* and *MAT 2* types in sorghum derived isolates as has been reported earlier on TLB of maize (Adipala, 1993). Between the various agro-ecologies, *Mat 1* and *Mat 2* occurred in unequal proportions. Some isolates tested positive for both *Mat 1* and *Mat 2* and were assigned *MAT 1, 2* genotype. In some locations such as Soroti, the mating types *Mat 1 and Mat 2* occurred in equal proportions. The occurrence of both mating types in equal proportion indicates a good potential of sexual recombination. Previous studies have shown that tropical climates may be most suitable for sexual reproduction of *E. turcicum* as evidence supports the fact that recombination does occur particularly in tropical populations (Abadi and Levy, 1993). These results further suggest that sexual reproduction may be more likely to occur in the most humid areas of Soroti, Lira, and Katakwi than in drier and less humid areas of Apac and Masindi (Pedersen and Brandenburg, 1986.; Abadi and Levy 1993; Borchardt *et al.,* 1998.). Moreover, under severe epidemics, which are more likely under tropical conditions especially where susceptible varieties such as *Epuripuri* has been planned, higher pathogen population densities and more frequent contact of sexually compatible isolates may occur (Borchardt *et al.,* 1998). Under such circumstances local inoculum production and dispersal become important factors influencing epidemics of TLB. Consequently under conditions that are highly favourable for epidemics

multiple cycles of asexual reproduction are possible, producing clones and possibly mutants and this can easily occur in the Uganda setting.

The occurrence of both mating types suggests that during years with favourable environmental conditions, susceptible hosts, or appropriate substrates for mating, genotypic diversity may increase, even in tropical climates. It is plausible that the asexual conidia may reach various parts of Uganda where sorghum and maize is grown via short -distance movement of plant materials among farmers and through windblown conidia from various locations where the sexual stage may occur, this will likely result in a diverse pool of individuals that reflects sexual recombination occurring in those areas. Additionally, the genotypic diversity may also increase due to sexual recombination (Stoddart and Taylor, 1988). However, when the severity of epidemics increases, the distribution of mating types within fields may become more random and enhance the chance of sexual reproduction as was observed from the survey (Welz *et al.,* 1996).

In general, genetic diversity is expected to be high near the centre of origin, or in areas in where an organism has had a long history of colonization, or under conditions that are highly favourable for its growth and reproduction (Harlan, 1971; Fehr, 1991). At the outer limits of spread of the organism, or in environments "new" to it, the levels of diversity are expected to be considerably lower. Sorghum is an African crop that may have co-evolved over long time with *E. turcicum*. Based on studies of intercontinental genetic structure, it has been proposed that East Africa could be another possible centre of origin (or perhaps a centre of diversity) *(*Borchardt *et al.,* 1998)

Whereas no direct estimation for gene flow was taken during this study, indirect deductions can be made from the phenetic and mating type analysis. Both data (phenetics and mating type analysis) provide evidence of gene flow between populations in different agro-ecologies. For example isolates from different origins frequently clustered together and overall genetic variability was detected (0.64-100) for most isolates. In studies elsewhere such data have been interpreted to mean presence of extensive gene

flow (Jingao *et al.*, 2008). However, the high similarity coefficient (0.64-1.0) among isolates leads to low genetic variation among isolates from various locations.

Characterisation of isolates based on race differentials revealed the existence of four types of races namely race 0, race 3, race 13 and race 123 within the *E. turcicum* sorghum derived isolates. Race 3 and 0 were also reported to exist in Uganda on maize derived *E.turcicum* isolates (Welz *et al.*, 1993). Majority of the isolates collected from Uganda tested positive for Race 123 not as was observed in turcicum leaf blight of maize, (Adipala *et al*., 1993). Race 0 and 3 were found to occur in the Northern moist farmlands, race 123 was widely distributed in the southern and eastern Lake Kyoga basin agro-ecology. Regions bordering each other shared the same race while regions far apart had distinctive races. These results suggest that race 0 is still the most predominant race in Uganda in relation to studies carried out on maize though races 13, 123, and 3 have also been confirmed to exist. Indeed, it has been proposed that more aggressive populations of *E.turcicum* may occur in Uganda (Adipala *et al.*, 1993). All what remains is to test their level of aggressiveness on selected sorghum lines to support breeding work.

CHAPTER NINE

GENERAL DISCUSSION AND CONCLUSION

9.0 General Discussion

Turcicum Leaf Blight of sorghum incited by *Exserohilum turcicum* is a major threat to sorghum production globally. Yield losses as high as 60% have been recorded on susceptible cultivars (Adipala *et al*, 1993). Previous studies on TLB have been mainly focused on maize host and a few on sorghum. Therefore the aforementioned studies were focused on understanding the sorghum–*Exserohilum turcicum* pathosystem. This was achieved by undertaking the following studies namely (a) investigating the occurrence of TLB in major sorghum growing regions, (b) screening sorghum accessions for reaction to TLB, (C) carrying out epidemiological studies, (d) determining the mating types and races of *E. turcicum* that occur, and (e) establishing variability sorghum derived *E. turcicum* isolates in Uganda.

The first study involved a survey conducted in 23 districts and 8 agro ecologies of major sorghum growing regions of Uganda. The study found that TLB does occur in all agro-ecologies on sorghum, albeit at lower levels than in maize. Severity values ranged from 24.6 to 37.8 % and quite low on land races grown by farmers. The study revealed that TLB was more common and severe on recently release elite sorghum varieties such as *Epuripuri* and *Sekedo,* as compared to land races. Furthermore the results indicated a strong effect of agro ecology on TLB epiphytotics. Areas with high levels of humid and moderate temperature had the highest incidence and severity. Cropping systems also significantly influenced the patterns of spread of TLB across the region. Taken together these results suggest that the flare-up in the TLB on sorghum is very much being influenced by wide-spread use of susceptible genotypes such as *Epuripuri* and Sekedo that are being cultivated in disease promoting humid tropical weather.

The analysis of disease reactions of sorghum germplasm collected from farmer's fields to TLB infection indicated that majority of genotypes grown by farmers were resistant to TLB. Of the 202 accession screened, 8 accession shows susceptibility while 194 accession had some level of resistance. It seemed that the accessions exhibited some form of polygenic kind of resistance which is highly needed inbreeding for TLB resistance in sorghum (Marley *et al.*, 2001). Studies in West Africa have earlier indicated the existence of horizontal resistance in most sorghum varieties characterised by slow disease development (Thomas and Smart 1993). The fact that most accessions screened had moderate to high level of resistance to TLB means that further sorghum breeding work should make use of this rich sources of resistance to improve elite lines.

The present study on temporal aspect of turcicum leaf blight indicated that disease development in sorghum was delayed by about three weeks compared to maize. The data further indicated that the incidence and severity of TLB on sorghum were quite low as compared to maize. Rates of disease development were relatively low among sorghum accessions and high in maize. The data indicate that the nature of resistance being expressed by sorghum may be different from that of maize. This has been reported over 40 years ago (Robert, 1960) but has not be confirmed more recently. This study thus leaves a gap in knowledge on TLB pathogenesis on maize and sorghum including the nature of resistance.

Analysis of spatial attributes of TLB epidemics in sorghum showed that disease severity and gradients were affected by wind drift. These results are in agreement with those observed by de Nazareno *et al*., (1993) and Asea *et al*., (2001). Differences in crop residues were significant in that plants close to the residue source developed more disease compared to those far away from the inoculum sources. The above results where more pronounced in *Epuripuri*. The data emphasize the role of *E.turcicum* infested residues in initiating epidemics of TLB (Takan *et al*., 1994). They also corroborate the findings of Asea *et al*. (2001) on grey leaf spot of Maize (*Cercospora zeae-maydis*). The study further indicates that in some cases, apparent infection rate increased with distance from the residue source as observed by Minogue and

Frey (1983) but contrary to Takan *et al.,* (1994) who observed constant apparent infection rate and De Nazareno *et al.*, (1992) who observed decreasing apparent infection rates for turcicum leaf blight on maize. The flattening of the disease gradient during the initial assessment further confirms the polycyclic nature of TLB (Gregory, 1968).

Mating type analysis revealed the occurrence of *MAT 1, MAT 2* and *MAT 1, 2* in Uganda on sorghum. These results confirm earlier finding of Adipala *et al*., (1993). Furthermore, in Soroti both mating types were found to occur in equal proportions indicating a great potential of sexual recombination with a possibility of emergence of new races of *E.turcicum.* In other locations *MAT* 2 was more prominent than *MAT 1*. The race differential work revealed that occurrence of races 0, 13, 123, and 3 in Uganda. Both the mating types and race differential study indicate a great potential of having more virulent races of *E.turcicum* in the future.

Genetic variability studies based on molecular analysis indicated high genotypic diversity among the sorghum derived *E. turcicum* isolates. This is typical of *E.turcica* populations in tropical climates (Jingao *et al.*, 2008). However, the coefficient of similarity of 0.64 was high indicating low genetic variation among isolates from various locations. A high similarity coefficient presupposes the occurrence of gene flow between populations. In this case of Uganda this is possible due the increased sorghum seed trade. The pathogen may be transported over long distance in contaminated seed especially on susceptible varieties such as *Epuripuri.* For disease management, the data suggests that similar varieties may be used across agro-ecologies.

9.1 Conclusions and recommendations

The following conclusions can be drawn from this thesis.

a) Turcicum leaf blight does occur in all the major sorghum growing regions. Incidence and severity of the disease is comparatively lower on sorghum than in maize although on susceptible sorghum elite varieties *Epuripuri* and Sekedo exhibited high disease levels.

b) Sources of resistance to TLB possibly do exist (about 194 of the 202 accession showed some level of resistance) within the farmer preferred varieties and thus the hypothesis that there's possible resistant genotype among the farmer grown varieties is accepted. However the identified accessions need further evaluations under more disease pressure as well as under diverse environments.

c) Analysis of the temporal spread of TLB epidemics showed the TLB pathosystem of sorghum was significantly different from that in maize. Epiphytotics were slower and at less severity level as indicated by fewer lesion numbers, longer latent period on sorghum as compare to maize. It is recommended that the sorghum pathosystem be studied more especially in order to fully characterises it especially aspects of the resistance mechanism being expressed.

d) Analysis of the spatial spread of TLB epidemics emphasized the importance of infested crop residues on the spread of TLB in Uganda. However it was noticed that in accessions such as MU007/009 and MU007/029 which expressed some level of resistance, that in presence of host resistances the effect of crop residues as inoculum source is minimised. This is good news for management of the TLB because deployment of resistant lines is the least laborious disease management method. Resistant lines should thus be developed and promoted.

e) The mating type and race differential studies confirmed the existence of both mating types (*MAT1, MAT 2*) and occurrence of race 0, 13, 123, and 3 in Uganda. It is expected that there may have more races emerging in locations such as Soroti with equal proportion of both mating types.

f) The molecular variability study indicated the non-existence of population structure among the sorghum –*Exserohilum turcicum* derived isolates from various locations. The genetic similarity coefficient was 0.64 that is to say that the isolates/races from Uganda are uniform and this implies that varieties bred at one location can perform more less the same at other locations.

9.2 Research gaps

The following research gaps areas do require address in order to fully understand the sorghum – *Exserohilum turcicum* pathosystem in Uganda.

1. Elucidating the effect of plant density of sorghum on the spread and progress of turcicum leaf blight from *Exserohilum turcicum* Infested crop residues
2. Establishing the number of genes controlling resistance in sorghum to turcicum leaf blight
3. Studying variability of Exserohilum *turcicum* isolates collected from different hosts (Sorghum, maize, wild sorghum, Sudan grass, Johnson grass and teosinte) in different locations.
4. Testing the possibility of cross infection between isolates from sorghum, maize and wild relatives: Sudan grass, wild sorghum and teosinte.
5. Deducing the pathogenicity of sorghum *E.turcicum* isolates obtained from farmer's fields in Uganda

REFERENCES

Abadi, R, and Levy, Y 1993. Mating types of *Exserohilum turcicum* in Israel. *Phytoparasitica* 21:315-320.

Abadi, R., Perl-Treves, R and Levy, Y. 1996. Molecular variability among *Exserohilum turcicum* isolates using RAPD (Random amplified polymorphic DNA). *Canadian Journal of Plant Pathology* 18:29-34.

Adipala, E, Lipps, E, P and Madden, L.V. 1993. Occurrence of *Exserohilum turcicum* on maize in Uganda. *Plant Disease* 77:202-205.

Adipala, E, Lipps E, P and. Madden. L.V. 1993. Reaction of maize cultivars from Uganda to *Exserohilum turcicum. Phytopathology* 83:217-223.

Adipala, E, Takan, J.P and Ogenga-latigo. M.W. 1995. Effect of plant density of maize on the progress and spread of northern leaf blight from *Exserohilum turcicum* infested residue source. *European Journal of Plant Pathology* 101:23-33.

Agrios, G.N. 1997. *Plant Pathology* Fourth Ed. New York: Academic Press.

Aldermann, S.C, Nutter, E.W. and. Labrinos. J.L. 1989. Spatial and temporal analysis of spread of late spot of peanut. *Phytopathology* 79:837-844.

Arie, T, Christiansen, S. K. Yoder, O. C. and Turgeon. B. G.1997. Efficient cloning of ascomycete mating type genes by PCR amplification of the conserved MAT HMG box. *Fungal Genetics and Biology* 21:118-130.

Asea, G. 2001. Reaction of maize genotypes in young stages to *Cercospora zeae-maydis* and influence of infested maize residue on progress and spread of gray leaf spot in central Uganda, Master's degree thesis ,Crop science Department ,Faculty of Agriculture, Makerere University Kampala.

Ayiga, A.J.A.O. 2000. Sources of resistance to and spread of *Cercospora zeae-maydis* from inoculum source in maize, Master's degree thesis Crop science, Department, Faculty of Agriculture Makerere University Kampala.

Bentolila, S, C Guitton, N Bouvet, A. Sailand, Nykaza, S and Freyssinet. G.1991. Identification of RFLP marker tightly linked to the *Ht*1 gene in maize. *Theoretical Applied Genetics* 82:393-398.

Berger, R.D. 1981. Comparison of Gompertz and logistic equations to describe plant disease progress. . *Phytopathology* 69:1199-1201.

Bergquist, R. 1986. Leaf blight. In: *Compendium of Sorghum Diseases*. Edited by R. A. Frederiksen. St. Paul, MN, USA: American Phytopathological Society

Berguist, R and. Masias. O.R. 1974. Physiologic specialisation in *Trichometasphaeria turcicum f.sp. Zeae* and *T. turcicum sp sorghi* in Hawaii. *Phytopathology* 83:612-614.

Bigirwa, A. G, Julian, A. M. and Adipala. E. 1993. Characterization of Ugandan isolates of *Exserohilum turcicum* from maize. *African Crop Science Journal* 1:69-72.

Bonierbale, M.W, Plaisted, R.L .and Tanksley. S.D. 1988. RFLP maps based on a common set of clones reveals modes of chromosomal evolution in a potato and tomato. *Genetics* 120:1095-1103.

Borchardt, D. S. Welz, H. G and Geiger. H.H. 1998. Genetic structure of *Setosphaeria turcica* populations in temperate and tropical climates. *Phytopathology* 88:322-329.

Borchardt, D. S. Welz, H. G. and Geiger. H. H. 1998. Molecular marker analysis of European *Setosphaeria turcica* populations. *European Journal Plant Pathology* 104:611-617.

Brinkman, F. S. and Leipe. D. D.2001. *Phylogenetic analysis.* In*: Bioinformatics*. Edited by A. D. Baxevanis and B. F. F. Oellette, *A Practical Guide to the Analysis of Genes and Proteins.* England, UK: Wiley Inter-Science.

Cammack, R.H. 1985. Factors affecting infection gradient from a point source of *Puccinia polysora* in a plot of *Zea mays*. *Annals of Applied biology* 46:186-197.

Campbell, C.L. and Madden. L.M.1990. *Introduction to Plant Disease Epidemiology* Newyork: John Wiley and Sons

Cardwell, K.F, Schulthess. F, Ndemah, R. and Ngoko. Z. 1997. A system approach to assess crop health and maize yield losses due to pests and diseases in Cameroon. *Agricultural Ecosystem Environment* 65:33-47.

Carsky, R.J, Sigh, I and Ndikawa. R 1994. Suppression of *Striga hermonthica* on sorghum using a cowpea intercrop. *Experimental. Agriculture* 30:349-358.

Carson, M. L. 1995. A new gene in maize conferring the chlorotic halo reaction to infection by *Exserohilum turcicum*. *Plant Disease* 79:717-720.

Carson, M. L. 1995. Inheritance of latent period length in maize infected with *Exserohilum turcicum*. *Plant Disease* 79:581-585.

Ceballos, H., Deutsch, J. A and Gutierrez. H. 1991. Recurrent selection for resistance to *Exserohilum turcicum* in eight subtropical populations. *Crop Science* 31:964-971.

Crop Plant Resources. 2000. "Sorghum: *Sorghum bicolour*.

Davies, J, C. and Reddy. K. 1981. Shootfly species and their graminaceous hosts in Andhra Pradesh. *Insect Science and its Application*: 33-37.

De Nazareno, N.R.X. Madden, L.V and Lipp. P.E.1992. Survival of *Cercospora zeae-maydis* in corn residue in Ohio. *Plant Disease* 76:67-70.

De Nazareno, N.R.X, Lipps, P .E and Madden. L.V. 1993. Effects of levels of corn residue on the epidemiology of gray leaf spot of corn in Ohio. *Plant Disease* 77:67-70.

Degefu, Y. 1990. Evaluation of Ethiopian maize germplasms for resistance against leaf blight caused by *Helminthosporium turcicum*.

DeVries, J. and Toeniessen. G.2001. Securing the harvest Biotechnology, Breeding and Seed System for Africa Crops. *CABI publishing*.

Diamond, J. 1998. Guns, germs and steel, vintage. London.

Doggett, H. 1970. *Sorghum*. London: Longman's.

Dorothea, S, Borchardt, H.Welz, G.and Geiger. H.H.1997. Genetic structure of *Setosphaeria turcica* populations in tropical and temperate climates. *Phytopathology* 88:322-329.

Duncan, R. R. and De Milliano. W. A.J.1995. *Plant disease control*. Edited by Leslie J. F and R. A. Frederiksen, *Diseases Analysis Through Genetics and Biotechnology: Interdisciplinary Bridges to Improved Sorghum and Millet Crops*. Iowa: Lowa State University Press.

Ebiyau, J, and Oryokot. J.E. 2001. *Sorghum (Sorghum Bicolour (L) Moench .In Agriculture in Uganda*. Vol. Volume II Crops: National Agricultural Research Organisation and Fountain Publisher.

Ebiyau, J. 1995. Screening of resistance of *Striga* in some of the advanced sorghum hybrid lines.20 August 4th November, 1995.

Erpelding, J, E, and Prom. L K.2004. Evaluation of Malian sorghum germplasm for resistance against Anthracnose. *Plant Pathology* 3 (2):65-71.

Esele, J.P. 1988. Current Status of Sorghum and Millets Research in Uganda. In: Proceedings of the 6th Regional Workshop on East African Sorghum and Millets. , held at Mogadishu, Somalia.

Esele, J.P.E. 1995. Foliar and head diseases of sorghum. *African Crop Science Journal* 3:185-189.

Fallah, M, P. and. Pataky. J. K 1994. Reactions of isolates from mating of races 1 and 23N of *Exserohilum turcicum*. *Plant Disease* 78:767-771.

FAO (Food and Agriculture organisation). 1990. FAOSTAT.

FAO (Food and Agriculture organisation). 1990. Sorghum and millets in human nutrition. *FAO Food and Nutrition*.

FAO (Food and Agriculture organisation). 1996. The World Sorghum and Millet Economies: Facts, Trends and Outlook: Paper read at a joint study by the basic foodstuffs service FAO commodities and trade division and the socioeconomics and policy division international crops research institute for the semi-arid tropics international crops research institute for the semi-arid tropics, Patancheru, India at Rome, Italy

FAO (Food and Agriculture organisation). 2003. FAOSTAT.

FAO (Food and Agriculture organisation). 2005. FAOSTAT data,

FAO (Food and Agriculture organisation). 2007. FAOSTAT.

Fehr, W. R. 1991. *Principles of Cultivar Development:* Vol. 1, *Theory and Technique*. Amsterdam, IA: Iowa State University.

Ferrandino, F. J, and Elmer. W. H.1996. Septoria leaf spot lesion density on trap plants exposed at varying distances from infected tomatoes. *Plant Disease* 80:1059-1062.

Fitt, B. D. L, Gregory, P. H. Todd, A. D. McCartney H. A and MacDonald. O. C.1987. Spore dispersal and plant disease gradients: A comparison between two empirical models. *Phytopathology* 118:227-242.

Frederiksen, R.A. 1980. *Sorghum leaf blight*. Edited by R. J. Williams , R. A. Frederiksen , L. K. Mughogho and G. D. Bengtson, *Sorghum Disease; A world Review*. Patancheru, India: ICRISAT.

Freppon, J, T, Pratt, R.C. and Lipps P.E. 1996. Chlorotic lesion response of maize to *Cercospora zeae-maydis* and its effects on gray leaf spot disease. *Phytopathology* 86:790-794.

Fullerton, R. A. and Fletcher. J. D.1974. Observations on the survival of *Drechslera turcica* in maize debris in New Zealand. *N. Z. J. Agric.Res.* 17:153-155.

Gitau, C.W. Ngi-Song A.J, Overholt, W.A. and Otieno. S.A. 2002. Pupal parasitoids of sub-Saharan African cereal stem borers and the potential for introduction of an exotic species-*Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichnuemonidae). Paper read at Integrated Pest Management Conference, at Kampala, Uganda.

Gowda, K, T.P, Shetty, H.S. Gowada, B.J. Prakash, H.S. and Snagam. L. 1992. Comparsion of two methods of assessment of yield loss due to Turcicum leaf blight of maize. *Indian Phytopathology:* 45:316-320.

Gregory, P. H. 1968. Interpreting plant disease dispersal gradients. *Annual Reviews: Phytopathology* 6:189-212.

Gregory, S. 2004. Northern Corn Leaf Blight on Corn. *Plant Disease: Pests and Crop* 23:4.

Harlan, J. R. 1971. Agricultural origins: Centers and noncenters. *Science* 174:468-474.

Hennessy, G.G, De Milliano .W.A.J, and McLaren. C .G.1990. Influence of primary weather variables on sorghum leaf blight in southern Africa. *Phytopathology* 80:943-5.

Hilu, H. M. and Hooker. A. L 1963. Monogenic chlorotic lesion resistance to *Helminthosporium turcicum* in corn seedlings. *Phytopathology* 53:909-912.

Hilu, H.M. and Hooker. A.L. 1964. Host-pathogen relationship of *Helminthosporium turcicum* in resistant and susceptible corn seedlings. *Phytopathology* 54:570-575

Hintze, J. L. 1998. *Statistical System for Windows. Number Chuncher Statistical Systems*. Kayville, Utah.

Hooker, A. L. 1963. Mongenic resistance in *Zea mays* .L to *Helminthosporium turcicum*. *Crop Science* 3:381-383.

Hughes, G. R, and Hooker. A. L.1971. Gene action conditioning resistance to northern leaf blight in Maize *Crop science* 11:180-184.

ICRISAT (International Centre for Research In Semi- Arid Tropics) .1996. Technical Advisory Committee: CGIAR Priorities and Strategies for Resource Allocation during 1998-2000 at Patancheru, India.

ICRISAT (International Centre for Research In Semi- Arid Tropics). 2000. Annual Report.

IBPGR/ICRISAT .1993. Descriptors for sorghum (Sorghum bicolor (L.) Moench). Rome/Patancheru: International Board of Plant Genetic Resources/International Crop Research Institute for Semi-Arid Tropics.

Jenkins, M. T, and Robert. A.L.1952. Inheritance of resistance to leaf blight of corn caused by *Helminthosporium turcicum*. *Agronomy* 44:136-140.

Jiansheng, L. and Jilin. L. 1984. Study on the interaction of monogenic and polygenic resistance to *Helminthosporium turcicum* in maize (*Zea mays* L.). *Acta Agron. Sin* 10:154-162.

Jingao, D. F. Yongshan, G. Xiumei, A. Xinlong, M. Jifang, and Zhiping. D. 2008. Geographic Distribution and Genetic Analysis of Physiological races of *Setosphaeria turcica* in Northern China. *American journal of Agricultural and Biological Sciences* 3 (1):389-398.

Jordan, E. G. Perkins, J. M. Schal, l.R. A and Pedersen. W. L.1983. Occurrence of race 2 of *Exserohilum turcicum* on corn in the central United States. *Plant Disease* 67:1163-1165.

Julian AM, Crowson HL, and de Milliano WJ, 1994. Factors influencing epidemiology of *Exserohilum turcicum* on sorghum. *African Crop Science, Conference proceedings* **1**, 263–8.

Kfir, R. 1997. Competitive displacement of *Busseola fusca* (Lepidoptera: Noctuidae) by *Chilo partellus* (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America* 90:619-624.

Kimber, C.T. O. 2000. *Origins of domesticated sorghum and its early diffusion into India and China.* In *'Sorghum: Origin, History, Technology, and Production',* Edited by C. Wayne Smith and R. A. Frederiksen. New York: John Wiley & Sons.

King, S.B. and Mukuru. S.Z. 1994. An overview of sorghum finger millet and pearl millet in Eastern Africa with special attention to diseases. *Breeding for disease resistance with emphasis on durability* Wageningen, Netherlands: Wagening Agricultural University.

Kiyosawa, S. and Shiyomi. M. 1972. A theoretical evaluation of mixing resistant and susceptible varieties for the control of plant disease. *Annals of Phytopathological Society* 38:41-51.

Konieczny, A, and Ausubel. F. M. 1993. A procedure for mapping Arabidopsis mutations using co-dominant markers. *Plant Journal* 4:403-410.

Kudadjie, C.Y, P.C Struik, P Richards, and S.K. Offei. 2004. Assessing production constraints, management and use of sorghum diversity in north-east Ghana: a diagnostic study.

Latterell, F. M. and Rossi. A. E. 1983. Gray leaf spot of corn: A disease on the move. *Plant Disease* 67:842-847.

Leonard, K. J. and Suggs. E. G. 1974. *Setosphaeria prolata,* the Ascigerous state of *Exserohilum prolatum*. *Mycological Society of America* 66:281-297.

Leonard, K. J, Levy, Y. and Smith. D. R.1989. Proposed nomenclature for pathogenic races of *Exserohilum turcicum* on corn. *Plant disease* 73:776-777.

Leonard, K. J. 1993. Durable resistance in the path systems: Maize - northern and southern leaf blights. Edited by Jacobs. T. and J. E. Parlevliet, *Durability of Disease Resistance*. Dordrecht, the Netherlands. Kluwer Academic.

Leslie, J. F. 2003. *Sorghum and Millet*. Edited by B. Maunder. , *Sorghum worldwide*: Iowa State Press

Levy, Y. 1995. Inoculum survival of *Exserohilum turcicum* on corn between and during growing periods. *Canadian Journal of Plant Pathology* 17:144-146.

Levy, Y. and Pataky. J. K. 1992. Epidemiology of Northern leaf blight on sweet corn. *Phytoparasitica* 20 (1):53-66.

Li, X., X. Yang, W. He and Yang. J. 1999. Studies on constitutional variation of physiologic races of leaf blight. *Southeast Agricultural University* 21:37-39.

Lipp, P.E. 1983. Survival of *Colletrotrichum graminicola* in infested corn residue in Ohio. *Plant disease* 67:102-104.

Lipp, Y. 1991. Variation in fitness among field isolates of *Exserohilum turcicum* in Israel. *Plant disease* 75:163-166.

Lipps, P, and Mills, D.2008. Northern leaf blight, Fact sheet. *Ohio State University*

Lipps, P. E. 1982. New fungus race threatens Ohio's corn crop. *Ohio Rep* 67:80-82.

Luttrell, E, S. 1958. The prefect stage of *Helminthosporium turcicum*. *Phytopathology* 48:281-287.

Ministry of Agriculture, Animal Industry and Fisheries (MAAIF). 2007. Basic facts on agricultural activities in Uganda: Sorghum production levels.

Mackenzie, DR. 1976. Application of two epidemiological models for the identification of slow stem rusting in wheat. *Phytopathology* 66 55-59.

Marley, P.S, Thakur, R.P. and Ajayi. O. 2001. Variation among foliar isolates of *Colletrotrichum sublineolum* of sorghum in Nigeria. *Field Crop Research* 69:133-142.

McDonald, B.A. 2004. The population genetics of fungi tools and techniques *Phytopathology* 87:448-453.

Milgroom, M.G. and Fry. W.E 1997. Contributions of population genetics to plant epidemiology and management. *Advances in Botanical Research* 24:1- 25.

Minogue, K. P. and Fry. W. E.1983. Models for the spread of disease: Model description. *Phytopathology* 73:1168-1173.

Mundt, C.C. Hafiz, U.A Finckh, M.R and Alfonso .R.F. 1998. Primary disease gradient of bacterial blight of rice. *Phytopathology* 89:64-67.

Nei, M. and Li. W. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Academy Science, USA.* 76: 5269-5273.

Nelson, R.R. 1959. A major gene locus for compatibility in *Trichometasphaeria turcica*. *Phytopathology* 49:159-161.

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

Ngugi, H, K, Julian .A.M, King.S.B and Peacocke .B.J .2000. Epidemiology of sorghum anthracnose (*Colletotrichum sublineolum*) and leaf blight (*Exserohilum turcicum*) in Kenya. Plant Pathology (2000) 49,129-140

Nkonya, E, Xavery, E.P. Akonaay, H. Mwangi, W. Anandajasekeram .P, Verkuijl, H. Martella, D. and Moshi. A. 1998. Adoption of maize production technologies in Northern Tanzania.56.

Okori, P. 2004. Population studies of *Cercospora zeae-maydis* and related cercospora fungi Plant biology and Forest genetics department, Swedish University of Agricultural Sciences Uppsala.

Okori, P, Fahleson, J. Rubaihayo, P. R. Adipala E. and Dixelius. C.2003. Assessment of genetic variation among East African Cercospora *zeae-maydis* populations using AFLP and RFLP. *African Crop Science Journal* 11:75-86.

Parker, S. K, Gleason, M. L and Nutter, F.W. Jr. 1995. Influence of rain events on spatial distribution of Septoria leaf spot of tomato. *Plant Disease* 79:148-152.

Parlevliet, J.E. 1993. Selecting components of partial resistance. Paper read at plant breeding in the 1990s, at Wallingford, UK.

Pataky, J. K. 1992. Relationship between yield of sweet corn and Northern leaf blight caused by *Exserohilum turcicum*. *Phytopathology* 82:379-375.

Pataky, J, K. 1994. Effects of races 0 and 1 of *Exserohilum turcicum* on sweet corn hybrids differing for *Ht*- and partial resistance to northern leaf blight. *Plant disease* 78:1189-1193.

Pataky, J. K, Perkins, J. M. and Leath, S. 1986. Effects of qualitative and quantitative resistance on the development and spread of northern leaf blight of maize caused by *Exserohilum turcicum* races 1 and 2. *Phytopathology* 76:1349-1352.

Paterson, A.H., Lin, Y.R Schertz, Z. Li, K.F. Doebley, J.F. Pinson, S.R.M Liu, S.C. Stansel, J.W. and Irvine. J.E. 1995. Convergent domestication of cereal crops by independent mutations at corresponding loci. *Science* 269:1714-1718.

Payne, G.A., and Waldron. J.K.1983. Over wintering and spore release of *Cercospora zeae-maydis* in corn debris in North Carolina *Plant Disease* 67:329-332.

Pedersen, W. L. and Brandenburg. L. J.1986. Mating type, virulence, and cultural characteristics of *Exserohilum turcicum* race 2. *Plant Disease* 70:290-292.

Raymundo, A. D. and Hooker. A. L.1982. Single and combined effects of monogenic and polygenic resistance on certain components of northern leaf blight development. *Phytopathology* 72:99-103.

Raymundo, A. D. and Hooker. A. L.1981. Measuring the relationship between northern corn leaf blight and yield losses. *Plant Disease* 65:325-327.

Reddy, M.P, Sarla, N. and Siddiq E.A. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* 128:9-17.

Richards, R. and Kucharek. T. 2006. Florida plant disease management guide. *University of Florida*.

Ritchie, S, W, Hanway J.J and Benson. G.O. 1989. How a corn plant develops. *Iowa state University* 48.

Robert, A.L. 1960. Physiologic specialisation in *Helminthosporium turcicum Phytopathology* 50:599-601.

Robinson, R.A. 1987. Host management in crop pathosystems. *Macmillan Publishers.*

Ruecker, G. R, Park, S. J. Ssali, H. and Pender. J. 2003. Strategic targeting of development policies to a complex region. A GIS-based stratification applied to Uganda. ZEF - Discussion Papers on Development Policy No. 69.41.

Sharma, H.C. 1993. Host plant resistance to insects in sorghum and its role in integrated pest management. *Crop Protection* 12: I 1-34.

Shurtleff, M.C. 1980. Compendium of corn diseases. *American Phytopathological Society*: 105.

Simcox, K. D, Pedersen, K. D and Nickrent. D. L. 1993. Isozyme diversity in *Setosphaeria turcica*. *Canadian Journal of Plant Pathology* 15:91-96.

Smith, D. R. and J. G. Kinsey. 1980. Further physiologic specialization in *Helminthosporium turcicum*. *Plant Disease* 64:779-781.

Steel, R.G.D. Torrie, J.H and. Dickey. D.A. 1997. *Principles and procedures of statistics: A biometrical approach*. New York: McGraw-Hill.

Stoddart, J. A. and Taylor. J. F. 1988. Genotypic diversity: Estimation and prediction in samples. . *Genetics* 118:705-711.

Stromberg, E. L, Stromberg V. K and Hammond. G. 2003. Gray leaf spot: Identifying sibling species of *Cercospora zeae-maydis* using PCR-DNA amplification. *International Society of Plant Pathology* 2.

Sujkowski, L.S. Parra, G.R Gumpertz, M.L and Ristaino. J.B.2000. Temporal dynamics of phytophthora blight on bell pepper in relation to the mechanisms of dispersal of primary inoculum of *Phytophthora capsici* in Soil. *Phytopathology* 90:148-156.

Takan, J.P, Adipala, E. and Ogenga-latigo. M. W. 1994. Northern leaf blight progress and spread from *Exserohilum turcicum* infested maize residue. *African Crop Science Journal* 2 (2):197-205.

Thomas, H, and Smart C.M.1993. Crop that stay green. *Annual of applied biology* 123:193-219.

Tilahun, T, Ayana, G. Abebe, F. and Wegary. D. 2001. *Maize pathology research in Ethiopia*. Edited by N. Mandefro, D. Tanner and S. Twumasi-Afriyie. *Enhancing the contribution of maize to food security in Ethiopia. Proceedings of the Second National workshop of Ethiopia*. Addis Ababa, Ethiopia.

Ullstrup, A.J. 1970. A comparison of monogenic and polygenic resistance to *Helminthosporium turcicum*. *Phytopathology* 60:1597-1599.

United State Department of Agriculture (USDA). 2007. Uganda Yearly Sorghum Production.

Vander Plank, J.E. 1963. *Plant disease: Epidemics and control* New York: Academic press.

Vanderlip, R.L. 1993. *How a sorghum plant develops*. Volume. Contribution No. 1203, *Cooperative extension service*. Manhattan, Kansas: Kansas Agricultural Experiment Station.

Vos, P, Hogers, R Bleeker, M. Rejians, M. van de Lee, T. Hornes, M Frijters, A. Peleman, J Pot, J. Kiuper, M and Zabeau. M. 1995. AFLP: A new technique for DNA finger printing. *Nucleic Acids Research* 23:4407-4414.

Welz, H. G, Bassetti, P. and Geiger. H. H.1996. Turcicum-Blattdürre und Aleppohirse: Zwei Schaderreger auf dem Vormarsch. *Mais* 24:66-68.

Welz, H. G. Wagner, R. and Geiger. H. H.1993. Virulence in *Setosphearia turcica* populations collected from maize in China, Mexico, Uganda, and Zambia. *Phytopathology* 83:1856.

Williams, J.G.K, Kunelik A, Livak, K.J Rajalski, J.A and Tingey. S.V.1990. DNA polymorphisms amplified by arbitrary priers are useful as genetic markers. *Nucleic Acida Res* 18:6531-6535.

Windes, J.M. and Pedersen. W.L.1991. An isolate of *Exserohilum turcicum* virulent on maize inbreeds with resistance gene *HtN*. *Plant Disease* 75:430.

Wortmann, C. S. and Eledu. C. A.1999. Uganda's Agro ecological zones: A guide for policy makers and planners.

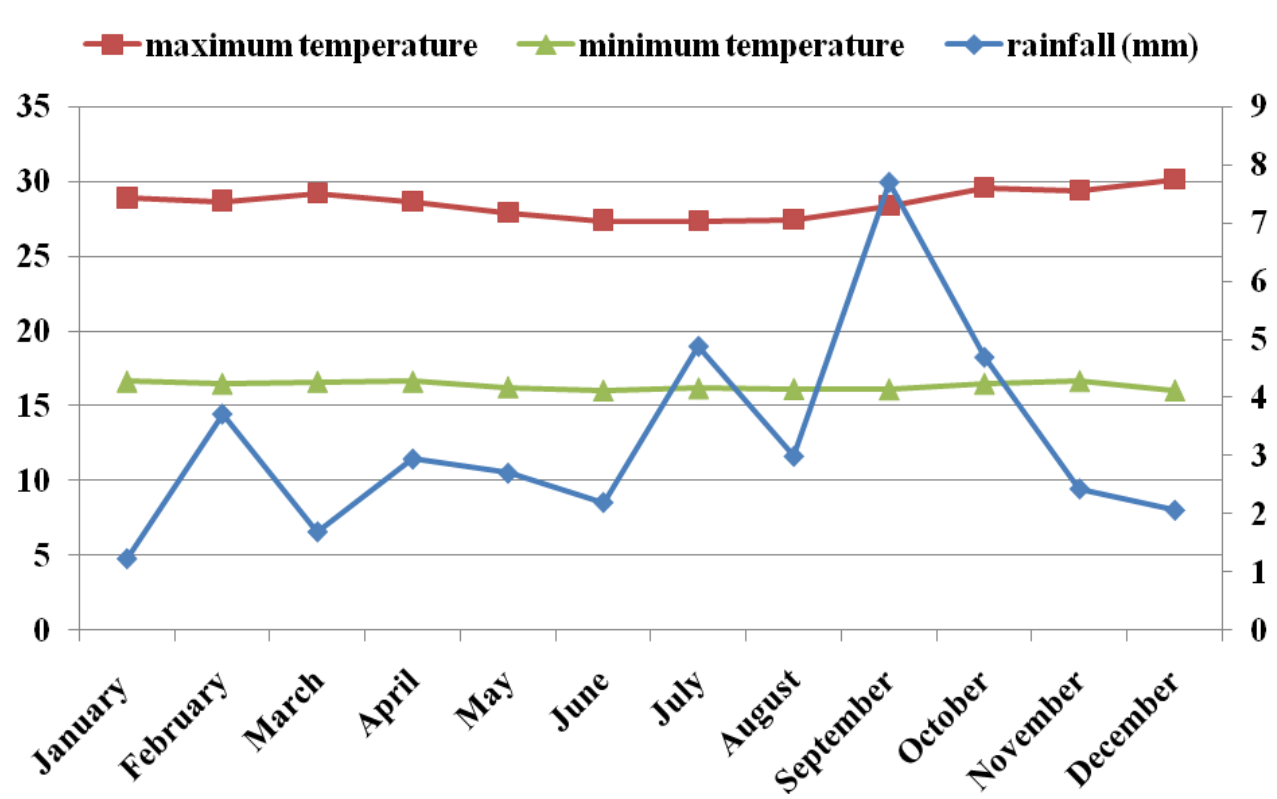
Yeshitila, D. 2003. Cloning and characterization of Xylanase genes from phytopathogenic fungi with a special reference to *Helminthosporium turcicum* the cause of Northern Leaf Blight of maize.

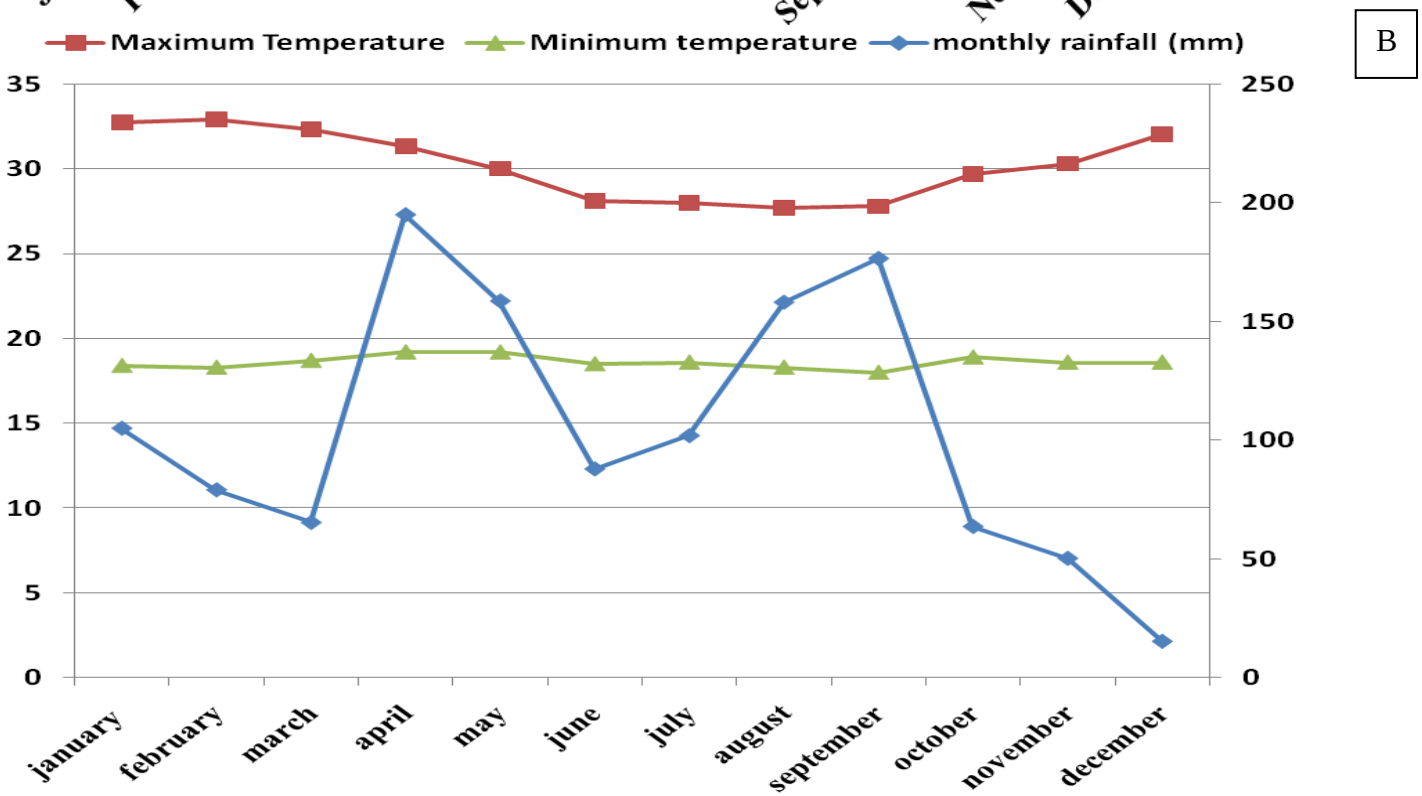
Yongshan, F, Jifang, M. Xiumei, G. Xinlong, A. Shuqin, S and Jingao .D. 2007. Distribution of mating types and genetic diversity induced by sexual recombination in *Setosphaeria turcica* in northern China. *Higher Education Press and Sprinfer -Verlag* 1 (4):368-376.

Zadoks, J. C. and Schein. R. D.1979. *Epidemiology and Plant Disease Management*. New York: Oxford University Press.

ANNEX

Annex1





Graphical representation of daily maximum and minimum temperature and rainfall data recorded for MUARIK for the year 2007 at Kabanyolo, Wakiso, MUARIK (A) and Serere, Soroti, NASARRI (B)

Annex 2. Analysis of variance of 202 accessories collected from the farmer's fields for Severity. Scored as (R) Resistance and (S) susceptibility ((MS) moderate susceptible and (MR) moderately resistant after 6 weeks

Accession	District	Region	Agro ecological zone	Race	AUPDC	Evaluation
MU007/001	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Guinea	4.17	MS
MU007/002	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Caudatum	4.17	MS
MU007/003	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Kafir	2.08	R
MU007/004	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Kafir	2.08	R
MU007/005	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Kafir	16.67	S
MU007/006	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Caudatum	2.08	R
MU007/007	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Guinea	2.08	R
MU007/008	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Guinea	2.08	R
MU007/009	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Caudatum	2.08	R
MU007/010	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Caudatum	3.13	MR
MU007/011	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Kafir	31.25	S
MU007/012	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Kafir	4.17	MS
MU007/013	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Guinea	4.17	MS
MU007/014	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Caudatum	2.08	R
MU007/015	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Caudatum	8.33	S
MU007/016	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Guinea	2.08	R

MU007/017	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Guinea	0	R
MU007/018	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Guinea	4.17	MS
MU007/019	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Caudatum	4.17	MS
MU007/020	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Guinea	4.17	MS
MU007/021	Homia	Western	Western Mid-Altitude Farmland and the Semliki Flats	Kafir	2.08	R
MU007/022	Masindi	Western	Central Wooded Savanna	Caudatum	2.08	R
MU007/023	Masindi	Western	Central Wooded Savanna	Kafir	4.17	MS
MU007/024	Masindi	Western	Central Wooded Savanna	Caudatum	3.13	MR
MU007/025	Masindi	Western	Central Wooded Savanna	Kafir	1.04	R
MU007/026	Masindi	Western	Central Wooded Savanna	Guinea	1.04	R
MU007/027	Masindi	Western	Central Wooded Savanna	Kafir	2.08	R
MU007/028	Masindi	Western	Central Wooded Savanna	Caudatum	2.08	R
MU007/029	Masindi	Western	Central Wooded Savanna	Caudatum	2.08	R
MU007/030	Masindi	Western	Central Wooded Savanna	Caudatum	2.08	R
MU007/031	Masindi	Western	Central Wooded Savanna	Caudatum	2.08	R
MU007/032	Masindi	Western	Central Wooded Savanna	Caudatum	2.08	R
MU007/033	Masindi	Western	Central Wooded Savanna	Bicolor	2.08	R
MU007/034	Masindi	Western	Central Wooded Savanna	Caudatum	2.08	R

MU007/035	Masindi	Western	Central Wooded Savanna	Caudatum	2.08	R
MU007/036	Masindi	Western	Central Wooded Savanna	Dura	2.08	R
MU007/037	Masindi	Western	Central Wooded Savanna	Kafir	4.17	MS
MU007/038	Masindi	Western	Central Wooded Savanna	Kafir	2.08	R
MU007/039	Masindi	Western	Central Wooded Savanna	Bicolor	4.17	MS
MU007/040	Masindi	Western	Central Wooded Savanna	Guinea	2.08	R
MU007/041	Masindi	Western	Central Wooded Savanna	Dura	2.08	R
MU007/042	Masindi	Western	Central Wooded Savanna	Guinea	4.17	MS
MU007/043	Masindi	Western	Central Wooded Savanna	Bicolor	2.08	R
MU007/044	Masindi	Western	Central Wooded Savanna	Caudatum	4.17	MS
MU007/045	Masindi	Western	Central Wooded Savanna	Guinea	2.08	R
MU007/046	Masindi	Western	Central Wooded Savanna	Caudatum	2.08	R
MU007/047	Masindi	Western	Central Wooded Savanna	Caudatum	2.08	R
MU007/048	Masindi	Western	Central Wooded Savanna	Kafir	2.08	R
MU007/049	Masindi	Western	Central Wooded Savanna	Guinea	2.08	R
MU007/050	Masindi	Western	Central Wooded Savanna	Kafir	2.08	R
MU007/051	Masindi	Western	Central Wooded Savanna	Caudatum	2.08	R
MU007/052	Masindi	Western	Central Wooded Savanna	Caudatum	5.21	S

MU007/053	Masindi	Western	Central Wooded Savanna	Guinea	4.17	MS
MU007/054	Masindi	Western	Central Wooded Savanna	Guinea	4.17	MS
MU007/055	Nebbi	North west	Northwestern farmland—Wooded savanna	Guinea	4.17	MS
MU007/056	Nebbi	North west	Northwestern farmland—Wooded savanna	Kafir	4.17	MS
MU007/057	Nebbi	North west	Northwestern farmland—Wooded savanna	Caudatum	4.17	MS
MU007/058	Nebbi	North west	Northwestern farmland—Wooded savanna	Kafir	4.17	MS
MU007/059	Nebbi	North west	Northwestern farmland—Wooded savanna	Caudatum	4.17	MS
MU007/060	Nebbi	North west	Northwestern farmland—Wooded savanna	Caudatum	4.17	MS
MU007/061	Nebbi	North west	Northwestern farmland—Wooded savanna	Guinea	4.17	MS
MU007/062	Nebbi	North west	Northwestern farmland—Wooded savanna	Caudatum	4.17	MS
MU007/063	Nebbi	North west	Northwestern farmland—Wooded savanna	Caudatum	4.17	MS
MU007/064	Nebbi	North west	Northwestern farmland—Wooded savanna	Guinea	4.17	MS
MU007/065	Nebbi	North west	Northwestern farmland—Wooded savanna	Kafir	4.17	MS
MU007/066	Nebbi	North west	Northwestern farmland—Wooded savanna	Caudatum	4.17	MS
MU007/067	Arua	North west	Northwestern farmland—Wooded savanna	Guinea	2.08	R
MU007/068	Arua	North west	Northwestern farmland—Wooded savanna	Bicolor	2.08	R
MU007/069	Arua	North west	Northwestern farmland—Wooded savanna	Bicolor	2.08	R
MU007/071	Arua	North west	Northwestern farmland—Wooded savanna	Caudatum	2.08	R

MU007/072	Arua	North west	Northwestern farmland—Wooded savanna	Guinea	2.08	R
MU007/073	Arua	North west	Northwestern farmland—Wooded savanna	Guinea	2.08	R
MU007/074	Arua	North west	Northwestern farmland—Wooded savanna	Caudatum	2.08	R
MU007/075	Arua	North west	Northwestern farmland—Wooded savanna	Caudatum	2.08	R
MU007/076	Arua	North west	Northwestern farmland—Wooded savanna	Guinea	2.08	R
MU007/077	Arua	North west	Northwestern farmland—Wooded savanna	Kafir	2.08	R
MU007/078	Arua	North west	Northwestern farmland—Wooded savanna	Bicolor	2.08	R
MU007/079	Koboko	North west	West Nile Farmland	Kafir	2.08	R
MU007/080	Koboko	North west	West Nile Farmland	Kafir	2.08	R
MU007/081	Koboko	North west	West Nile Farmland	Kafir	2.08	R
MU007/082	Koboko	North west	West Nile Farmland	Guinea	2.08	R
MU007/083	Koboko	North west	West Nile Farmland	Guinea	2.08	R
MU007/084	Koboko	North west	West Nile Farmland	Caudatum	2.08	R
MU007/085	Koboko	North west	West Nile Farmland	Caudatum	2.08	R
MU007/086	Koboko	North west	West Nile Farmland	Kafir	2.08	R
MU007/087	Koboko	North west	West Nile Farmland	Kafir	2.08	R
MU007/088	Koboko	North west	West Nile Farmland	Kafir	2.08	R
MU007/089	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R

MU007/090	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Caudatum	2.08	R
MU007/091	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R
MU007/092	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R
MU007/093	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Caudatum	2.08	R
MU007/094	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Bicolor	2.08	R
MU007/095	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Dura	2.08	R
MU007/096	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/097	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/098	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/099	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R
MU007/100	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/101	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R
MU007/102	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Caudatum	2.08	R
MU007/103	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/104	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R
MU007/105	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/106	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R
MU007/107	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R

MU007/108	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/110	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R
MU007/111	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/112	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Caudatum	2.08	R
MU007/113	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/114	Katakwi	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/115	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/116	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/117	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R
MU007/118	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R
MU007/119	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Bicolor	2.08	R
MU007/120	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/121	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/122	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Bicolor	2.08	R
MU007/123	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/124	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Caudatum	2.08	R
MU007/125	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/126	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Caudatum	2.08	R

MU007/127	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R
MU007/128	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/129	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R
MU007/130	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R
MU007/131	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R
MU007/132	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	2.08	R
MU007/133	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Guinea	2.08	R
MU007/134	Kaberamaido	Eastern	Northern moist farmlands	Caudatum	2.08	R
MU007/135	Kaberamaido	Eastern	Northern moist farmlands	Guinea	2.08	R
MU007/136	Kaberamaido	Eastern	Northern moist farmlands	Caudatum	4.17	MS
MU007/137	Kaberamaido	Eastern	Northern moist farmlands	Guinea	4.17	MS
MU007/138	Kaberamaido	Eastern	Northern moist farmlands	Dura	4.17	MS
MU007/139	Kaberamaido	Eastern	Northern moist farmlands	Kafir	2.08	R
MU007/140	Kaberamaido	Eastern	Northern moist farmlands	Kafir	4.17	MS
MU007/141	Kaberamaido	Eastern	Northern moist farmlands	Kafir	4.17	MS
MU007/142	Kaberamaido	Eastern	Northern moist farmlands	Caudatum	4.17	MS
MU007/143	Kaberamaido	Eastern	Northern moist farmlands	Kafir	4.17	MS
MU007/145	Kaberamaido	Eastern	Northern moist farmlands	Guinea	4.17	MS

MU007/146	Kaberamaido	Eastern	Northern moist farmlands	Caudatum	4.17	MS
MU007/147	Kaberamaido	Eastern	Northern moist farmlands	Kafir	4.17	MS
MU007/148	Kaberamaido	Eastern	Northern moist farmlands	Caudatum	4.17	MS
MU007/149	Kaberamaido	Eastern	Northern moist farmlands	Caudatum	4.17	MS
MU007/150	Kaberamaido	Eastern	Northern moist farmlands	Caudatum	4.17	MS
MU007/151	Kaberamaido	Eastern	Northern moist farmlands	Caudatum	4.17	MS
MU007/152	Kaberamaido	Eastern	Northern moist farmlands	Kafir	4.17	MS
MU007/153	Kaberamaido	Eastern	Northern moist farmlands	Dura	4.17	MS
MU007/154	Kaberamaido	Eastern	Northern moist farmlands	Caudatum	4.17	MS
MU007/155	Kaberamaido	Eastern	Northern moist farmlands	Dura	4.17	MS
MU007/156	Kaberamaido	Eastern	Northern moist farmlands	Guinea	4.17	MS
MU007/157	Kaberamaido	Eastern	Northern moist farmlands	Guinea	4.17	MS
MU007/158	Kaberamaido	Eastern	Northern moist farmlands	Guinea	4.17	MS
MU007/159	Kaberamaido	Eastern	Northern moist farmlands	Guinea	4.17	MS
MU007/160	Kaberamaido	Eastern	Northern moist farmlands	Guinea	4.17	MS
MU007/161	Kaberamaido	Eastern	Northern moist farmlands	Guinea	4.17	MS
MU007/162	Kaberamaido	Eastern	Northern moist farmlands	Caudatum	4.17	MS
MU007/163	Arua	North west	Northwestern farmland—Wooded savanna	Guinea	4.17	MS

MU007/164	Arua	North west	Northwestern farmland—Wooded savanna	Caudatum	4.17	MS
MU007/165	Lira	North	Northern moist farmlands	Kafir	28.13	S
MU007/166	Lira	North	Northern moist farmlands	Guinea	4.17	MS
MU007/167	Kaberamaido	Eastern	Northern moist farmlands	Kafir	4.17	MS
MU007/168	Dokolo	Eastern	Northern moist farmlands	Kafir	4.17	MS
MU007/169	Dokolo	Eastern	Northern moist farmlands	Caudatum	4.17	MS
MU007/170	Dokolo	Eastern	Northern moist farmlands	Kafir	4.17	MS
MU007/171	Dokolo	Eastern	Northern moist farmlands	Guinea	4.17	MS
MU007/172	Dokolo	Eastern	Northern moist farmlands	Kafir	4.17	MS
MU007/173	Dokolo	Eastern	Northern moist farmlands	Kafir	4.17	MS
MU007/174	Dokolo	Eastern	Northern moist farmlands	Guinea	4.17	MS
MU007/175	Dokolo	Eastern	Northern moist farmlands	Guinea	4.17	MS
MU007/176	Dokolo	Eastern	Northern moist farmlands	Caudatum	4.17	MS
MU007/179	Apac	North	Northern moist farmlands	Kafir	4.17	MS
MU007/180	Apac	North	Northern moist farmlands	Kafir	4.17	MS
MU007/181	Apac	North	Northern moist farmlands	Caudatum	4.17	MS
MU007/182	Lira	North	Northern moist farmlands	Kafir	4.17	MS
MU007/183	Lira	North	Northern moist farmlands	Kafir	4.17	MS

MU007/184	Lira	North	Northern moist farmlands	Guinea	4.17	MS
MU007/185	Lira	North	Northern moist farmlands	Guinea	4.17	MS
MU007/186	Lira	North	Northern moist farmlands	Guinea	4.17	MS
MU007/187	Lira	North	Northern moist farmlands	Guinea	4.17	MS
MU007/188	Lira	North	Northern moist farmlands	Guinea	4.17	MS
MU007/189	Lira	North	Northern moist farmlands	Kafir	4.17	MS
MU007/190	Lira	North	Northern moist farmlands	Kafir	4.17	MS
MU007/191	Lira	North	Northern moist farmlands	Kafir	2.08	R
MU007/192	Lira	North	Northern moist farmlands	Kafir	4.17	MS
MU007/193	Lira	North	Northern moist farmlands	Guinea	4.17	MS
MU007/194	Lira	North	Northern moist farmlands	Kafir	4.17	MS
MU007/195	Lira	North	Northern moist farmlands	Kafir	4.17	MS
MU007/196	Lira	North	Northern moist farmlands	Kafir	4.17	MS
MU007/197	Lira	North	Northern moist farmlands	Kafir	4.17	MS
MU007/198	Lira	North	Northern moist farmlands	Kafir	4.17	MS
MU007/200	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Kafir	4.17	MS
MU007/201	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Caudatum	4.17	MS
MU007/202	Amuria	Eastern	Northeastern-central Grass-Bush Farmland	Dura	4.17	MS

Annex 3. Result of ITS specie specificity (ITS) and mating types as per each isolate

Isolate Identification	ITS 1 – 344bps	Alpha clone 160 bps	195 bps	Clone 190 bps	*Mat 1*	*Mat 2*	*Mat 1,2*
Amuria 12	✓	✓	X	✓	1	0	0
Amuria 14	✓	X	✓	X	0	1	0
Amuria 16	✓	X	✓	X	0	1	0
Amuria 17	✓	✓	X	X	1	0	0
Amuria 8	✓	✓	✓	✓	0	0	1
Amuria 9	✓	✓	X	X	1	0	0
Apac 1	✓	X	✓	✓	0	0	1
Apac 2	✓	✓	✓	✓	0	0	1
Apac 19	✓	X	✓	X	0	1	0
Apac 7	✓	X	X	✓	1	0	0
Arua 4	✓	✓	✓	✓	0	0	1
Arua 6	✓	X	✓	X	0	1	0
Dokolo 14	✓	X	✓	X	0	1	0
Dokolo 8	✓	X	✓	X	0	1	0
Ho 12	✓	X	✓	✓	0	0	1
Iganga 23	✓	X	✓	X	0	1	0
Iganga 17	✓	✓	✓	X	0	0	1
Iganga 18	✓	✓	✓	✓	0	0	1
Iganga 19	✓	✓	X	✓	1	0	0
Kaberamaido 1	✓	✓	X	✓	1	0	0
Kaberamaido 19	✓	X	✓	X	0	1	0
Kaberamaido 22	✓	✓	✓	X	0	0	1
Kaberamaido 30	✓	X	✓	X	0	1	0
Kaberamaido 32	✓	X	X	✓	1	0	0
Kaberamaido 5	✓	X	X	X	0	0	0
Kaberamaido 7	✓	✓	X	✓	1	0	0
Katakwi 2	✓	X	✓	X	0	1	0
Katakwi 9	✓	X	✓	X	0	1	0
Kabale 14	✓	X	✓	X	0	1	0
Kabale 30	✓	✓	✓	✓	0	0	1
Kiboga 18	✓	X	✓	X	0	1	0
Kiboga 19	✓	X	✓	X	0	1	0
Kumi 22	✓	X	✓	X	0	1	0

Kumi 25	✓	X	✓	X	0	1	0
Kumi 27	✓	✓	X	X	1	0	0
Kumi 12	✓	X	X	X	0	0	0
Kumi 3	✓	✓	✓	X	0	0	1
Kumi 5	✓	✓	✓	X	0	0	1
Kumi 8	✓	✓	X	✓	1	0	0
Lira 28	✓	✓	X	✓	1	0	0
Lira 7	✓	X	✓	X	0	1	0
Marahca 1	✓	X	✓	X	0	1	0
Masaka 5	✓	X	X	X	0	0	0
Masindi 17	✓	X	✓	✓	0	0	1
Pakwach 3	✓	X	X	✓	1	0	0
Serere 17	✓	✓	X	✓	1	0	0
Serere 9	✓	X	✓	X	0	1	0
Soroti 11	✓	✓	X	✓	1	0	0
Soroti 17	✓	✓	X	X	1	0	0
Soroti 27	✓	X	✓	X	0	1	0
Soroti 28	✓	X	✓	X	0	1	0
Soroti 30	✓	✓	✓	✓	0	0	1
Soroti 5	✓	✓	X	✓	1	0	0
Soroti 9	✓	✓	X	✓	1	0	0
Homia 18	✓	X	✓	X	0	1	0
Dokolo 2	✓	✓	X	✓	1	0	0
Homia 29	✓	✓	X	✓	1	0	0
Homia 17	✓	X	✓	X	0	1	0
Kaberamaido 2	✓	✓	X	✓	1	0	0
Homia 26	✓	X	✓	X	0	1	0
Dokolo 4	✓	X	✓	X	0	1	0
Homia 12	✓	X	X	✓	1	0	0
Dokolo 13	✓	✓	X	X	1	0	0
Kaberamaido 9	✓	✓	X	X	1	0	0
Homia 12	✓	X	X	✓	1	0	0
Koboko 1	✓	✓	✓	✓	0	0	1
Gulu 26	✓	✓	X	X	1	0	0