HOST RESISTANCE MECHANISMS AND CULTURAL PRACTICES FOR CONTROL OF GRAY LEAF SPOT (*Cercospora zeae maydis*) OF MAIZE IN TANZANIA

BY

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ABSTRACT

Studies were carried out in selected villages of the Southern and Eastern Maize Agro-ecological Zones to assess the potential variations in isolate aggressiveness, nature and genetics of host resistance and explore cultural methods suitable for the management of gray leaf spot. Southern Zone isolates (Mbeya and Iringa) grew 0.25 mm per day faster (9.3%), formed 1.1 cm longer lesions (25.9%), produced 1.1 x 10^4 more spores per cm^2 of the lesion (13.6%), formed symptoms 1 day earlier, caused 21.2% more disease and produced more toxins than the Eastern Zone isolates. Significantly ($P \leq 0.05$) more germlings of *C. zeae maydis* were established after penetration on susceptible than on resistant and moderate resistant maize genotypes. Histological studies suggest the possible involvement of inhibitory substances in host cell, a possible resistance mechanisms against *C. zeae maydis* in maize. Heritability and gene effect estimates for components of partial resistance to gray leaf revealed significant differences between generations, crosses and generation x cross and generation x location interactions. Results have shown that lesion size, lesion length, lesion numbers and disease severity could be used for selection of partial resistance to gray leaf spot based on field measurements. Composted cattle manure lowered gray leaf spot compared to CAN by 29. 4% at 60 kg N ha^{-1} and by 32.2% at 90 kg N ha^{-1} followed by composted poultry manure 24.5% and 22.9% and urea 17.6% and 18.2% at 60 kg N ha^{-1} and 90 kg N ha respectively. Grain yield in composted cattle manure, composted poultry manure and urea fertilization was 1.4 t ha^{-1}, 0.9 t ha^{-1}, 0.6 t ha^{-1} and 4.2 t ha^{-1}, 3.7 t ha^{-1}, 3.4 t ha^{-1} higher compared to CAN and none fertilized (control) treatments respectively. There was an overall yield increase of about 41 % in the intercropping compared to non-intercropping on susceptible maize cv 'Pannar (PAN 6549). It is recommended that breeders should consider the most aggressive

isolates (MBY1, MBZ1, IGAW1 and NJB1) and (DOM1, MKI1 and MGT1) when screening and breeding for resistance cultivars to gray leaf spot in the Southern highlands and Eastern agro-ecological Zone respectively.

DECLARATION

I, HERMAN JOHN FARAJI LYIMO do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work and has neither been nor submitted for a degree award in any other institution.

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DEDICATION

This thesis is dedicated to my late Dad and Mom for their encouragements and educational support they gave me.

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TERMS USED IN THE THESIS

Virulence -Ability of pathogen to cause disease on host.

Aggressiveness -Differences among multiple isolates exhibiting the same virulence on the host.

Incubation period -Period from inoculation to appearance of symptoms.

Isolate -Group of fungi producing similar symptoms on host.

Diagnosis -Procedure to establish the cause of disease

Epistasis -Non-allelic gene interaction.

Epi-phytotic -Occurrence of disease in large population of plants.

LIST OF ACRONYMS

SA -Sulphate of ammonium

CAN -Calcium ammonium nitrate

TSP -Triple super phosphate

Masl -Meters above sea level

USA -United States of America

FAO -Food and Agriculture Organization of the United Nations.

QTL -Quantitative trait loci

CHAPTER 1

1.0 INTRODUCTION

1.1 Maize production trends

Maize (*Zea mays* L.) is an important cereal of the world ranking third after wheat and rice (FAO, 2009). Over 140 million hectares world wide are estimated to be under maize cultivation with annual production output of about 630 million metric tones (FAO, 2009). The giant producers of maize are the United States of America (USA), China, Brazil, Mexico, European Union, Argentina and Africa (Meng and Ekboir, 2000). USA and China produce approximately 60% of the world maize crop (FAO, 2009).

Maize is an excellent source of carbohydrate (76.9 $g100g^{-1}$), protein (8.1 $g100g^{-1}$), fat (3.6 $g100g^{-1}$), iron (3.5 $mg100g^{-1}$), Ca (60 $mg100g^{-1}$), P (241 $mg100g^{-1}$) and Mg (127 $mg100g^{-1}$) but it is low in some essential amino acids such as lysine and tryptophan (Bressani, *et al.*, 1962; Landry and Moureaux, 1980). The protein content of maize is higher than that of polish and paddy rice and the fat content is of higher and of good quality than that of wheat, sorghum or rice (Wang and Fields, 1978). Maize can also be used to manufacture varieties of industrial products including corn starch, corn syrup, cooking oil, fructose syrup, sweeteners, dextrose and bio-fuel (Smith, 2004).

In developed countries, maize is primarily an animal feed crop, but, forms an important staple food crop in many countries of Africa, Latin America and Asia (Sprague, 1988). Maize account for 15-20% of the total daily calories in the diets of more than 20 developing countries located mainly in Latin America and Africa (Dowswell, 1996). In sub-Saharan Africa, maize is a staple food for an estimated 50% of the population consumed in a variety of dishes (Akingbala *et al.*, 1987;

Akinrelc, 1970). In Tanzania, maize is grown in all 21 regions and it is an important source of carbohydratcs for thc urban and rural people. Statistics released in 1998 (Kaliba *et al.*, 1998) indicated that per capital consumption of maize was estimated to be 112.5 kg and thc national maizc consumption was about 3 million tones per year contributing 60% of thc dictary calorics and 50 % of the utilizable protcin (FSD, 1996; Duc, 1986). In 2007/08 cropping season morc than 1.6 million ha were cstimatcd to bc undcr maizc cultivation in Tanzania with an output of about 3.6 million mctric tonc.s.

1.2 Maizc production systems in Tanzania

According to thc survcy carricd out clevcn years ago (Kaliba *et al.*, 1998), about 85% of maize produced in Tanzania was grown by thc pcasant farmcrs in small farms (1-10 ha). Ten pcrccnt was producccl in mcdium-scale farms (10-100 ha) and the rcmaining 5% in large scale farming (>100 ha). Largc and medium scale-farms were opcrated by the parastatal govcrnment organizations which collapscd in late 1990s. Currcntly, peasant farmers are the main producers of maize in Tanzania, mostly at subsistencc lcvcl. Hybrids, open pollinated synthetics and land races are the major varieties grown in pure stands or in companion with crops such as sorghum, grain legumcs, swcet potatoes, sunflower, groundnuts, cassava or millet.

Six maizc production zones can be distinguished in Tanzania; (i) The Southern Zone covering Iringa, Mbeya, Rukwa, Ruvuma, Songea, Lindi and Mtwara regions (ii) The Lake Zone covering Mwanza, Shinyanga, Kagera, and Mara regions (iii) The Northern Zone covering Arusha, Tanga and Kilimanjaro regions (iv) The Central Zone covering Dodoma and Singida regions (v) The Eastern Zone covering Dar-es salaam, Coast and Morogoro regions and (vi) The Western Zone covering Tabora and Kigoma region. The Southern Zone produces more than 60% of the total maize

produced followed by the Lake Zone and the Northern Zone. Dar-es Salaam, Singida, Coast and Kigoma are the maize deficient regions (FSD, 1992, Madadila, 1995). Deficit and surplus maize producing areas of Tanzania is shown in (Appendix 15).

1.3 Maize production constraints in Tanzania

Constraints limiting maize production in Tanzania include decline of soil fertility, low yielding varieties, drought, insect pests and diseases (Acland, 1971). Lack of constant supply of agriculture inputs and poor infrastructure have limited utilization of fertilizers, pesticides and other important agricultural inputs as such yields of maize per unit area are extremely very low, less than 1.5 tons/ha national average. In recent years, drought tolerant crops such as sorghum and millets have replaced maize in many places due to increasing rain shortages caused by global warming. The prevailing warm and humid conditions of the tropics also favors rapid multiplication of pests and diseases which are estimated to cause grain yield losses of 30- 80% (Nsemwa, 2002).

An array of diseases and insect pests plagues the maize growing areas of Tanzania. The most important diseases are downy mildew, rust, leaf blight, ear rots, leaf spots and maize streak. Important insect pests are stem and ear bores, army worms, cut worms, grain moth, beetles, weevils, grain bore, root worms, grain moths, beetles, weevils, grain bores, white grab and parasitic weed (Striga) (Acland, 1971; Kaliba *et al.*, 1998).

Gray leaf spot (GLS), incited by *Cercospora zeae-maydis*, is the most devastating yield-limiting disease of maize in Tanzania. The disease was first reported in Mbinga district, Ruvuma region, has spread in all maize producing regions of Tanzania. Most of the available local and exotic varieties are susceptible to the

disease and host-resistant breeding is hampered by wide spread isolates of the pathogen which differs in aggressiveness. Improved cultural practices and host resistance breeding offer the most cost-effective means of managing gray leaf spot in Tanzania. However, very little has been studied to reveal the prevalence isolates, their aggressiveness and distribution to explore the best effective approach for breeding of resistant genotypes to the disease. Also, the nature of host resistance and appropriate cultural methods suitable for the control of the disease are not known. The objective of this study was to investigate components of resistance and evaluate appropriate cultural practices for the management of gray leaf spot in Southern Highlands and Eastern maize agro-ecological Zones in Tanzania.

1.3.1 Overall objective of the study

To increase maize productivity through improvement of host plant resistance and cultural practices in the control of gray leaf spot of maize.

1.3.2 Specific objectives

(i) To assess the diversity and intensity of gray leaf spot in Southern Highlands and the Eastern maize agro-ecological zones.

(ii) To examine the mechanisms of resistance against *C. zeae-maydis* in maize.

(iii) To study the genetics of resistance to *C. zeae-maydis* and yield in maize.

(iv) To explore the effects of organic amendments on the development of gray leaf spot and yield of maize.

(v) To explore cultural practices that control gray leaf spot and improve yield of maize.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 History and geographical distribution of gray leaf spot

Tehol and Daniels (1925) were the first to report the occurrence of gray leaf spot in maize samples collected near the Mississippi river, Illinois, (United States of America (USA)) and later identified the causal of the disease to be (*Cercospora zeae-maydis*). This was the first documented report of gray leaf spot indicating the possible origin of the causal pathogen for gray leaf spot disease of maize. However, since its discovery, gray leaf spot has remained the disease of economic importance in USA corn industry.

Hyre (1943) was the first person to document the quantitative devastating potential of gray leaf spot in reducing the green photosynthetic leaf area of maize in Tennessee and Kentucky. Roane (1950) was the first to conduct double crossing experiments between susceptible and the resistance maize genotypes to gray leaf spot. He observed different levels of gray leaf spot severity among inbred lines, but, double crossing experiments involving genotypes with lower levels of disease resistance did not result in hybrids with higher levels of gray leaf spot resistance. Kingsland (1963) reported occurrence of gray leaf spot in Western Southern Carolina with severity ranging from 80 to100%.

In 1988 to 1995 there were dramatic increases in the prevalence and severity of gray leaf spot in the US Corn Belt (Colorado, Kansas, Nebraska, Wisconsin and Minnesota) in the fields under irrigation (Lipps, *et al.*, 1988). The maize growing area affected by gray leaf spot in US grew from 7.2 to 14.9 mill. ha during 1980 to 1990 (Sparks, 1997). The increase of gray leaf spot over 20 years in US concided with the wide spread adaptation of conservation tillage (Bhatia and Munkvold, 2000).

Gray leaf spot later expanded its geographical range and intensity to other continents of Asia, Africa, South America, North America and Australia (Ward, 1999; Lipps *et al.* 1998; Craus *et al.*, 2006). The disease has also been reported in temperate to warm humid areas including Mexico, Central America, North South America, Europe, South East Asia, India, China, Australia and phillipenes (Donald, 1999; Li-yu *et al.*, 2007).

In Africa, gray leaf spot was first reported in South Africa during 1990/91 cropping season. The disease was pandemic throughout the province of KwaZulu-Natal and quickly spread into the neighboring provinces of Eastern Cape, Free State, Gauteng, Mpumalanga and Northern province (Ward *et al.*, 1999). Contaminated maize debris in imported seeds from USA is suspected to be the source of gray leaf spot in the Republic of South African (Ward *et al.*, 1999). Gray leaf spot was later reported in all African nations neighboring South Africa and several in the North and Central Africa. In Zimbabwe, gray leaf spot was reported during 1995/96 cropping season, Kenya (1995), Uganda (1994) and Zaire (1996) (Donald, 1999; Ward *et al.*, 1999). Gray leaf spot has also been reported in Eithopia, Malawi, Mozambique, Swaziland, and Zambia (Minogue and Fry, 1983).

In Tanzania, gray leaf spot was first noted at epi-phytotic levels during 1995/96 growing season in the Southern Zone, Mbinga District, in Ruvuma Region. However, by mid 1988 the disease had spread throughout the entire Southern Zone including Songea, Mbeya and Iringa regions, and also into the Northern highland Zone, causing yield losses ranging from 50-80% (Nsemwa, 2002). Crop assessment carried in Mbinga district during 1996/97 cropping season indicated that 61 869 ha were badly affected by gray leaf spot (Nsemwa, 2002). The spread of the disease in the Eastern, Central and Western Zones has been of more recent times and gradual.

The global distribution of gray leaf spot of maize is shown in (Fig 1).

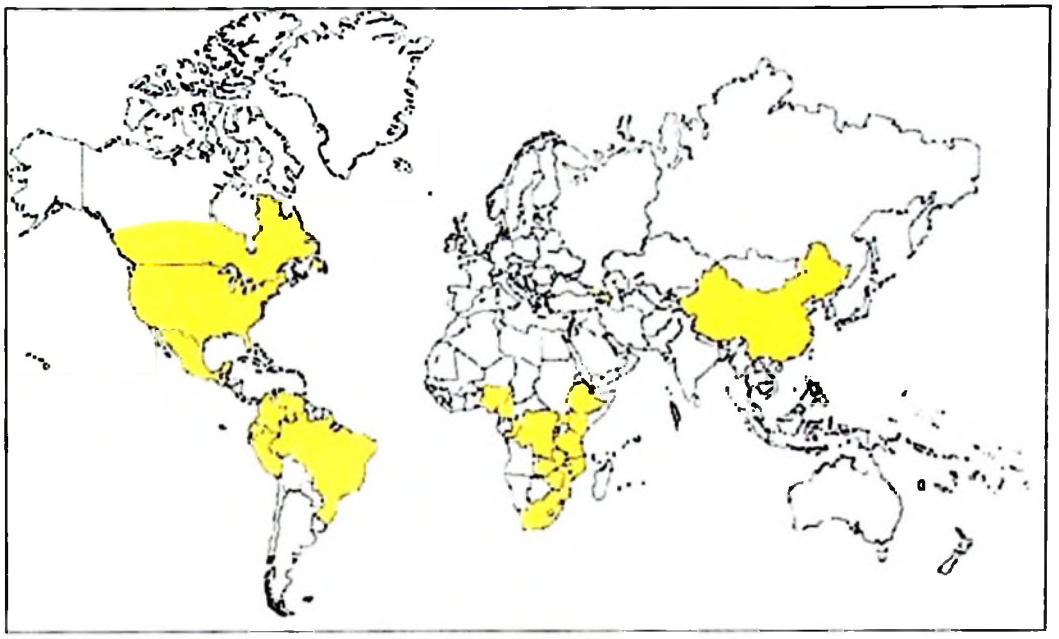


Fig 1. Global distribution of gray leaf spot of maize shown in yellow color (source: http: /maizedoctor. cimmyt.org)

2.2 Symptoms of gray leaf spot

The first symptoms of gray leaf spot appear on the leaves as small tan or brown spots of about 1 to 3 mm long, rectangular to irregular shape (Ward *et al.*, 1999; Ayodele, *et al.*, 2000) (Plate 1a). Initially, immature lesions are not easily distinguished from other foliar disease of maize (Stromberg, 1986). However, yellow hallow surrounding the gray leaf spot lesions distinguishes them with lesions of other diseases of maize (Stromberg 1986; Ward *et al.*, 1999) (Plate 1b). Mature lesions are readily distinguished due to their characteristic rectangular shapes (5-70 mm long and 2-4 mm wide) that run parallel to the leaf veins (Ward, 1999) (Plate 1c and d). As the conditions for disease development become favorable, high relative humidity and temperatures of 20 to 30 °C, the pathogen on the leaf surfaces sporulates. The sporulating lesions assume a grayish cast (Plate 1e and f), hence gray leaf spot (Laterall and Rossi, 1983; Ayers *et al.*, 1984). The disease, lesions coalesce and

blighting of the whole leaf may occur (Beckman and Payne, 1982) (Plate 1f). Severely diseased plants may die before reaches maturity (Ward *et al.*, 1999) or seriously affecting the grain filling leading to small cob sizes (Laterall and Rossi, 1983). However, the lesion size, number and type may vary greatly among genotypes (Gevers and Lake, 1994; Lipps *et al.*, 1996; Paul, 2005). Moderately susceptible genotypes may exhibit chlorotic, flex type lesions (Freppon *et al.*, 1994).

Plate 1a

Plate 1b

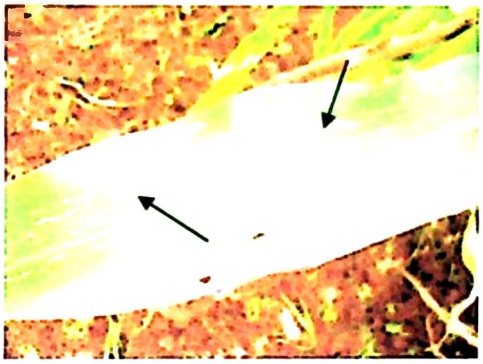


Plate 1c

Plate 1d

Plate 1e

Plate 1f

Plate 1: Field symptoms of gray leaf spot at different developmental stages; 1a, early symptoms small tan or brown spots, 1b, lesion surrounded by yellow hallo, 1c & 1d rectangular lesions run parallel to the veins, 1f & 1e gray cast symptoms on leaves.

2.2 Diagnosis of gray leaf spot.

Based on the descriptions published by Tehol and Daniels (1925). The fruiting structures of *C. zeae maydis* are amphigenous more abundant on the lower leaf surface (Plate 2f and g), stromata lacking or a few brown cells in stromatal openings; fascicles 3-12 divergent stalks (Plate 2h), conidiophores pale olivaceous brown or dark arising from in cluster and bursting out of leaf tissue stomata (Plate 2f-i), uniform in color and width or sometimes slightly wider near the tip; sparingly septate, not branched, straight to sinuous, occasionally 1-3 genulated medium spore scar at round, tip subtruncate, straight to curved, 3-10 septate, base subtruncate to long obconically truncate, tip subobtuse, 5-9 x 30-95µ. Conidia (sympodullospores), hyaline or gray, long cylindreical to filiform, obclavate, 3-7 septa (Plate 2a -e).

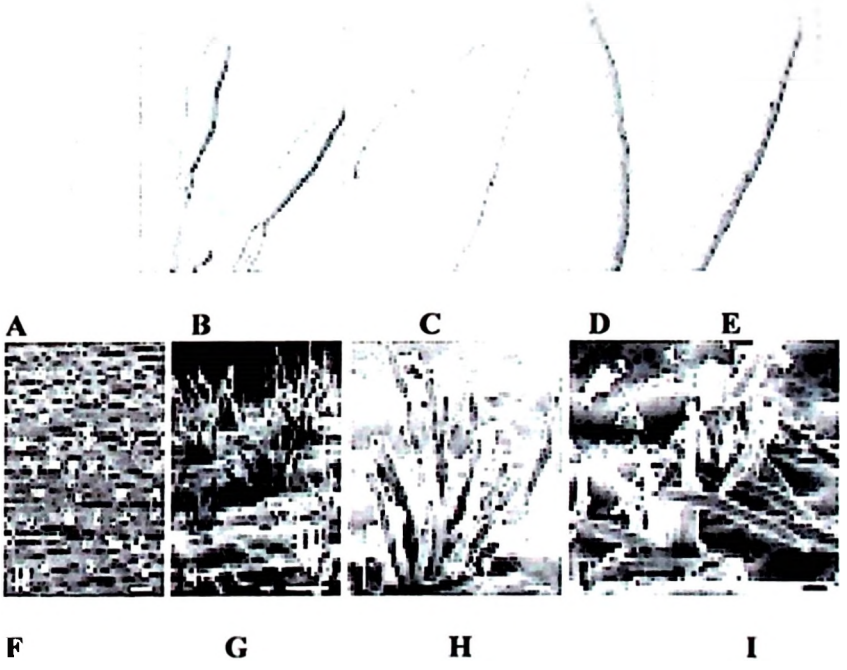


Plate 2: A, B, C, D, E conidia of *C.zeae maydis*, F, G, H I conidia and conidiophores in stroma arising from stomata (Source: Craus *et al.*, 2006).

2.4 Infection process

The source of primary inoculum for the fungus causing gray leaf spot of maize are the infected corn debris on the soil surface (DeNazareno *et al.*, 1992; Asea *et al.*, 2002) or inoculum from the neighboring fields with the disease (DeNazareno *et al.*, 1993; Stack, 1999). Conidia are deposited on the leaf either by wind or rain splash or both (1999; Bhatia and Munkovold, 2002). Spores germinate after 12-24 hrs to form germ tubes and appressoria (Beckman and Pyne, 1981; Stack, 1999). Warm temperature and high relative humidity stimulates germination of conidia on leaf surface lead to rapid multiplication of conidia and conidiophores on maize hence build up the initial inoculum (Lipps and Dennis, 2001; Paul, 2005). Germinated conidia produce germ tubes that grow by detecting humidity gradients toward stomata (Bhatia and Munkovold, 2002). Relative humidity greater than 95% is optimum for germ tube elongation and formation of appressorium (Beckman and Pyne, 1982; Thorson and Martinson, 1993). Long periods of leaf wetness favors spore germination, germ tube growth and formation of mycelia on the leaf surfaces (Beckman and Pyne, 1982; Thorson an Marinson, 1993). Temperature of 22 to 30 °C is optimum for germiation and growth of the fungi (Thorson and Martinson, 1993; Asea *et al.*, 2005; Paul and Mankvold, 2005). Direct penetration of *C. zeae maydis* has not been reported (Ward *et al.*, 1999). The pathogen enters the host plant cells through natural openings and grows intracellularly producing toxins that degrade cell membranes, cause collapse and death of the cells (Shim and Dunkle, 2002). Penetration through stomata is reported to takes 4 to7 days after inoculation (Beckman and Pyne, 1982). The incubation period can take 12 to 28 days (Stromberg, 1986; Carson *et al.*, 2002). Ringer and Grybanskas (1995) reported that the latent period of *C. zeae maydis* in the field was approximately 14 days for susceptible and 22 days for moderately resistance hybrids. DeNazareno *et al.* (1992)

observed that sporulation of *C. zeae maydis* was higher in lesions from leaves (up to 5 431 spores mm^{-2}) than from sheath (up to 10^5 spores produced per mm^2). However, when infested maize was buried 5 to 10 cm below the soil surface, sporulation was not detected until after 5 months.

2.5 Life cycle of *C. zeae maydis*

The life cycle of the *C. zeae maydis* has been illustrated by Ward *et al.* (1999) (Fig. 2). The disease begins with the spores deposited on the lower leaves that germinate, producing appressoria and infection hyphae which penetrates the host through stomates and colonize mesophyll cells producing symptoms on leaves (Fig. 2). Several cycles of the pathogen can be produced depending on the environmental conditions (Asea *et al.*, 2002). The spores on the leaf surfaces and on stroma over winter in maize residues left on the soil surface after harvest which provides inoculum for the next season and the cycle repeats. *C. zeae maydis* is a poor competitor in the soil environment, population declines quickly soon after residues are buried in the soil (Stack, 1999; Asea, *et al.*, 2002). The sexual structures of *C. zeae maydis* have not been reported although *Mycospaerella* have been reported to be associated with lesions of gray leaf spot (Ward, 1999; Goodwin *et al.*, 2001). *Cercospora zeae-maydis* is known to infect maize plant only (Stromberg and Donahue, 1986; Clements *et al.*, 2000).

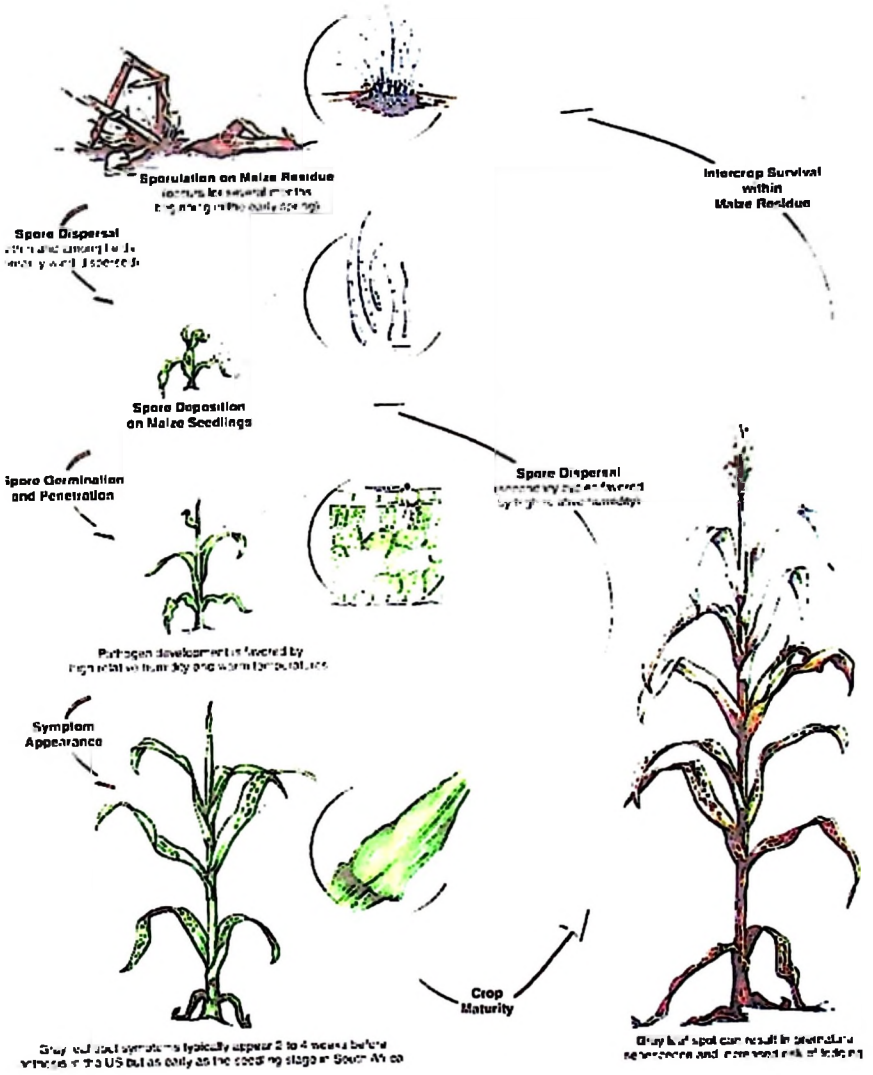


Fig 2: Life cycle of *C. zeae maydis* the causal of gray leaf spot of maize (Source: Ward *et al.*, 1999).

2.6 Epidemiology

The epidemiology of gray leaf spot of maize has been studied by several workers (Lipps, 1995; Paul, 2005). Alka (2002) reported that cultural factors such as genotype resistance, planting dates, and maize crop residues on the soil surface and weather variables such as the cumulative hrs of relative humidity and favorable temperature

were positively correlated to the severity of gray leaf spot. However, the relative humidity was more limiting factor than temperature on development of the disease.

Ringer and Grybaskas (1995) and Carson *et al.* (2002) reported that environmental conditions during the infection stages in the disease cycle were more important in determining the rate of disease progress than overall conditions during the growing season. The number of hours of daytime air temperature between 20 and 30 °C and night RH $\geq$ 90% for the period between growth stage V4 and V12, were more highly correlated with gray leaf spot severity than the overall mean temperature and RH during the growing season.

Beckman and Pyne (1983); Asea *et al.* (2002) and Paul and Munkovold (2005) observed that the severity of gray leaf spot depends on the availability of the inoculum (produced by sporulation lesions or sporulation on the maize residues on the soil surface), temperature and relative humidity. Latterell and Rossi (1983), observed that moderate to high temperature and prolonged periods of relative humidity are conditions more favorable for rapid development disease. However, the levels of gray leaf spot may differ along seasons and locations with similar weather and cropping conditions (Paul, 2005). During unfavorable condition (hot, dry weather) the fungus can remain dormant and then resumes rapid development soon as weather conditions are favorable (Thorson and Martinson, 1993). Prolonged leaf wetness increased gray leaf spot severity (Bhatia and mankovold, 2002; Paul, 2005). Beckman and Pyne (1982) observed that leaf wetness promoted spore germination, gem tube growth and formation of mycelia on leaf surfaces. However, intermittent shorter periods of wetness favored infection and resulted in more disease (Beckman and Pyne, 1982; Bhatia and mankovold, 2002). Late planted maize has greater gray leaf spot severity than early planted maize (Lipps, 1995, Paul and Munkovold, 2004).

This is because in the late maize initial infection by *C. zeae maydis* takes place at earlier plant growth stage, enabling the fungus to undergo greater number of secondary cycles (Ward *et al.*, 1999; Bhatia and mankovold, 2002). Rupe *et al.* (1982) observed that disease symptoms did not appear until plants were near anthesis and three weeks delay in planting resulted in three weeks delay in symptoms appearance. Gwin *et al.* (1987) examined stomata penetration by *C. zeae maydis* in three cultivars of corn and concludes that age-dependent resistance mechanism, which operates independently of varietal resistance, may exist.

Paul, (2005) reported that lesion expansion was significantly higher at 30 than 35 °C. The highest lesion and mean rate lesion expansion were observed at 30 °C. In this study, interaction of the effect of temperature and RH on the log of conidia per cm^2 of the disease tissue was significantly at 100% RH and the effect of temperature on sporulation was significant, with maximum spore production occurring at 25 and 30°C. Ringer and Grybauskas (1995); Bhatia and mankovold (2002) and Asea, *et al.* (2002) concluded that due to the long latent period of gray leaf spot (LP_{50}=14 to 19) and limited number of infection cycles, the amount of inoculum generated during the primary infection cycles is of more important than the number of the secondary cycles in determining final disease severity.

2.7 Taxonomy

The pathogen causing gray leaf of maize is known by its anamorphic stage *Cercospora zeae maydis*. A teliomorphic stage in the genus *Mycosphaerella* (*Ascomycota*) has been found associated with gray leaf spot in the overwintered maize specimen but its relations with *Cercospora zeae maydis* has not been confirmed or documented in definitively (Bubeck, *et al.*, 1993; Craus *et al.*, 2006). Spegazzin (1910) cited by Chupp (1953) classified groups of *Cercospora* by splitting

the genus into; *Cercospora*, with colored conidia and *Cercosporina*, with hyaline conidia. *Pseudocercospora* as Dermaticeae hyphomycetes with large phragmidium-like conidia and changed some of the *Cercospora* species to *Pseudocercospora*. However, some species of *Cercospora* species were later found to have hyaline conidia, therefore, the term *Cercosporina* became no longer valid. Deighton (1979) cited by Chupp (1953) reclassified many *Cercospora* species into several genera include; *Cercosporella, Cercosporidium, Paracercospora, Pseudocercospora* among others. This broad assemble is referred to as *Cercospora* complex, with member of *Cercospora* proper having conidia that are, acicular, hyaline and septate with conspicuous hilum produced on pigmented unbranched, septate, smooth conidiophore. Recent classification based on the phylogenic grouping has splitted *C. zeae maydis* into; Kingdom of Fungi, Genus *Cercospora*, Specie *zeae maydis* and Phylum Deuteromycota (Imperfect fungi) (Barnet and Hunter, 2003). However, combining the Hughes-Tubak-Barron system and the Saccardoan system of classification of imperfect fungi (Barnett and Hunter, 2003). *C. zeae maydis* can be classified into the following taxonomic groups:

KINGDOM: Fungi
PHYLUM: Deuteromycota
CLASS: Hyphomycetes
ORDER: Moniliales
FAMILY: Hyphomyceteceae
GENUS: *Cercospora*
SPECIE: *zeae maydis*

2.8 Pathogen variability

The variability among isolates of *C. zeae maydis* have been reported by several workers (Dunkle and Levy, 2000; Goodwin *et al.*, 2001, Okori *et al.*, 2003; 2004; Carson *et al.*, 2006). However, mechanisms generating the variability are not known,

neither the sexual stage nor para-sexuality has been reported in this pathogen (Carson *et al.*, 2002; Craus *et al.*, 2006). Variability in pathogen aggressiveness, colony morphology, relative abundance of spermagonia and the size of the spermatic have been reported among isolates of *C. zeae maydis* (Dunkle and Carson *et al.*, 2000). Latterell and Rossi (1974) reported cultural and metabolic variations among the isolates of *C. zeae maydis* which also reflected the pathogenecity and aggressiveness potential of different isolates. Blair and Ayers (1986) observed variability in the components of parasitic fitness (disease efficiency and virulence) measured as lesion size. Significant differences in the disease efficiency and lesion length also occurred among isolates.

Studies conducted in the United States revealed existence of two genetically distinct but morphologically similar sibling species of *C. zeae maydis* (Type I and Type II) (Dunkle and Levy, 2000). Type I is widely distributed in corn growing regions of the United States, whereas the second sibling specie (Type II) is confined in the Eastern United States. However, both species may be found in the field.

Dunkle and Levy (2000) compared variability of isolates from Africa with isolates from United States using Fragment Length Polymorphism (AFLP) and Restricted Digests of Internal Transcribed Space (ITS). Group Type I was more prevalent in isolates from samples collected in the United States. Group Type I was not detected from samples from Africa suggesting that Africa probably was the source of *C. zeae maydis* group Type II found in US. African and United States group Type II population were co-specific with limited variability. Crous *et al.* (2006) and Meisel *et al.* (2009) reclassified group II as distict specie now called *Cercospora zeina* endemic in Southern Africa. Okori *et al.* (2003) confirmed the widespread presence of Type II biotypes in East Africa (Kenya, Uganda, and Rwanda) and indicated gene

flow was high among African populations of *C. zeae maydis*. Mathioni *et al.* (2006) conducted experiments at different locations and concluded that isolates of Type II were more aggressive than Type I and that these types also differed in their degree of fitness in different environments in Brazil. Okori *et al.* (2004) reported variability in disease efficiency among isolates from *C. zeae-maydis* samples collected from maize in Uganda. Ayodele *et al.* (2000) distinguished isolates of C. zeae-maydis with varying levels of aggressiveness among samples collected in Nigeria. Blair and Ayers (1986) observed a wide range of aggressiveness among isolates of *C. zeae maydis*, but did not find any evidence of specificity or races, as evidenced by lack of a significant hybrid-isolate interaction.

Cercospora sorghi Ellis & Everh. has also been reported in maize (Dunkle and Levy, 2000) and referred to as *C. sorghi var. maydis* Ellis & Everh. It is morphologically similar to *C. sorghi*, but suspected to represent a distinct species due to its lack of pathogenicity on sorghum. Dunkle and Levy (2000) confirmed that *C. sorghi* and *C. zeae maydis* are distinct and separate species.

A telemorphic stage in the genus Mycospaerrella has been found associated with gray leaf spot lesions in an over wintering field specimen of maize (Latterell and Rossi, 1977). Spermatogonia bearing spermatia considered as the fertilization structures during sexual reproduction in some ascomycetous species have been observed in culture of *C. zeae maydis* (Latterell and Rossi, 1977).

2.9 Cercosporin toxin

2.9.1 Production and mode of action

Cercospora zeae maydis produce cercosporin (Dunkle and Levy, 2000; Shim and Dankle, 2002) a phytotoxin reported to be virulent factor on the development of gray

leaf spot in maize. Cercosporin is unique as it requires light for toxicity to their host plants and use activated oxygen specie to damage the host cells (Daub and Ehrenshaft, 2000; Shim and Dunkle, 2003; Burton *et al.*, 2008). The cercosporin toxin fall under the group of photo-sensitizer compounds active by visible wave length of light and generate activated oxygen specie into living cells (Daub, 1987; Shim and Dunkle, 2005).

The toxicity of photo-sensitizes occurs after production of activated oxygen species after photo-sensitized molecule is converted to a long-lived electronically triplet state (Daub *et al.*, 2000). This singlet oxygen can catalyze membrane lipid oxidation, leading to loss of membrane integrity, leakage of cytoplasm contents and cell death (Daub, 1982). Cercosporin is highly toxic not only to plants, but also to other organisms such as mice, bacteria and many fungi (Fajola, 1978). Cercosporin and related Perylenequinones have also been shown to inhibit viruses and tumor cells in vitro and to inactivate protein kinase (Hudson *et al.*, 1991). The only cercosporin-resistant organism so far identified are *Cercospora* species themselves and other fungi that produce perylequinone (Daub, 1987) and single wild rice specie whose resistance was attributed to carotenoids and lack of cercosporin uptake (Batchvarova *et al.*, 1992).

2.9.2 Factors affecting cercosporin production

Media composition, temperature and light are reported to influence cercosporin production invitro (Daub, 1982; Burton *et al.*, 2008); however, under optimum conditions levels of toxin production are highly isolate specific (Jenns e*t al.*, 1989). Isolates that fail to produce cercosporin invitro can sometimes produce in infected plant (Upchurch *et al.*, 1991); hence, lack of cercoporin production *in-vitro* can not be taken as definite evidence that an isolate cannot produce cercosporin.

2.10 Survival of *C.zeae maydis*

Cercospora zeae maydis over-seasons on infected maize residues that remain on the soil surface after harvest and which provide the primary inoculum (DeNazareno *et al.*, 1992; Asca *et al.*, 2002). Significant positive association between the amount of infested maize residue on the soil surface and disease severity has been reported (DeNazareno *et al.*, 1992; Asea *et al.*, 2002). The percentage of maize residues in the field and the distance that residues were to the maize plants had significant positive association with severity of gray leaf spot (Asea *et al.*, 2002).

2.11 Genetics of host resistance to C. zeae maydis

Resistance to gray leaf spot is inherited quantitatively by genes that act primarily in an additive manner (Elwinger *et al.*, 1990; Donahue *et al.*, 1991; Menkir and Ayodele, 2005) and is expressed as the rate reducing resistance (Huff *et al.*, 1988; Elwinger *et al.*, 1990; Carson and Goodman, 2006). Quantitative resistance to gray leaf spot leads to prolonged latent and incubation periods, reduced infection rates, sporulation capacity and the number of lesions (Beckman and Payne, 1982: Gordon *et al.*, 2004; Menkir and Ayodele, 2005).

The resistance factors have been mapped to at least three different chromosomes, with some of quantitative trait loci (QTL) consistently expressed across environments and rating periods having large effects on gray leaf spot resistance (Clements *et al.*, 2000: Lehmensiek *et al.*, 2001, Gordon *et al.*, 2004; Asea *et al.*, 2009; Zwonitzer *et al.*, 2010). Quantitative trait loci mapping studies for gray leaf spot resistance (Bubeck *et al.*, 1993; Clements *et al.*, 2000; Gordon *et al.*, 2006; Asea *et al.*, 2009) have identified several resistance QTL that have shown consistency over diverse environments, also consistent with a lack of distinct host-pathogen interaction. Diallel analysis of gray leaf spot resistance has revealed general

combining ability (Donahue *et al.*, 1991; Lehmensiek *et al.*, 2001) and specific combining ability (Gevers *et al.*, 1994; Clements *et al.*, 2000) to be significant among inbreds. Bubeck *et al.* (1993) and Li-yu *et al.* (2007) studying the resistance of gray leaf spot found QTLs associated with dominance and additive gene action; the later being more predominant. Saghai-Maroof *et al.* (1996) found QTLs associated with additive, dominance and epistasis effect on gray leaf spot resistance. Chlorotic lesion phenotype, a form of resistance controlled by dominant allelic interaction in some inbred, have shown to decrease the rate of disease progress and may provide effective control of gray leaf spot (Freeppon, *et al.*, 1994; Frecppon *et al.*, 1996; Derera *et al.*, 2008).

2.12 The economic importance of gray leaf spot of maize

Gray leaf spot cause extensive leaf necrosis (blighting), reduce the photosynthetic leaf area essential for the grain filling hence lower the expected yield (Latterell and Rossi, 1983; Brito *et al.*, 2007). Severe blighting occurs in the 8^{th} or 9^{th} leaf which contributes 75-90% of the photosynthate for grain filling (Allison and Watson, 1996; Menkir and Ayodele, 2005). Leaves of susceptible hybrids or inbred may become severe blighten or killed as early as 30 days prior to physiological maturity (Brito *et al.*, 2007).

Yield losses attributed to gray leaf spot have been reported to be variable (Lipps *et al.*, 1996; Nsemwa, 2002), with estimated losses as high as 100% when severe epidemics contributed to increase stalk lodging and early senescence (Latterell and Ross, 1983). The critical factor that determine the extent of yield loss in gray leaf spot epidemics, include the growth stage at which the *C. zeae-maydis* infect the plant, genotype susceptibility, the presence of sufficient hours of favorable weather condition and the amount of initial inoculum in the field as affected by tillage

practice and crop rotation (Lipps, 1995; Asea *et al.*, 2002).

Lipps (1998) reported that gray leaf spot must damage at or just after silking growth stage to cause severe yield losses exceeding 50%. The relationship between the level of disease and the yield losses was more pronounced at late stages of plant growth. Stalk (1999) observed that as the effective photosynthetic leaf area is reduced due to gray leaf spot disease, the plant draws on nutrients stored in the stalk to fill in the grains, this provide favorable conditions for the predominant stalk rot pathogens such as *Fusarium*, *Diplodia* and *Colletotrichum*. Lipps and Dennis (2001) reported that gray leaf spot can cause yield losses as high as 90-100 % bushels/acre and concluded that loss of photosynthetic leaf area caused by *C. zeae maydis* decreased corn sugar production that lowered the value of corn as silage crop.

2.13 Management of Gray Leaf Spot of Maize

2.13.1 Cultural methods

2.13.1.1 Tillage and crop rotation

Increased frequency of gray leaf spot epiphytotics and their severity has been associated with reduced tillage practices that leave infected maize crop residues on soil surface (Ward and Nowell, 1998; Asea *et al.*, 2002). Infected residues are either absent or greatly reduced through deep ploughing in conventionally tilled fields; inimum tillage operations that fail to incorporate residues deep into the soil favor disease development (DeNazareno *et al.*, 1992; DeNazareno *et al.*, 1993; Asea *et al.*, 2002). Tillage practices that reduce the initial inoculum by burying infected debris have been demonstrated to be the most effective cultural method for managing gray leaf spot (Latterell and Rossi, 1983; Asea *et al.*, 2002). Payne *et al.* (1987) reported that a higher number of conidia was collected in no-till than tilled plots, lesions appeared earlier and disease was more severe in no-till than tilled plots. Mathioni *et*

al. (2006) reported that adoption of agronomic practices such as no-tillage and cultivation under central pivot irrigation systems increased the incidence and severity to the extent that gray leaf spot became one of the most important diseases of maize in Brazil.

In Tanzania, lack of proper tillage equipment pose an obstacle to combat gray leaf spot in small-scale maize farming systems where hand hoe is a main tool for cultivation. Tillage operations done by hand hoe do not adequately incorporate deep into the soil the over seasoning inoculum surviving on crop debris from previous harvests; the effect that could account for wide spread incidence of the gray leaf spot in Tanzania. Tillage practices that reduce the initial inoculum by burying infected debris have been demonstrated to be the most effective cultural method for managing gray leaf spot (Latterell and Rossi, 1983; Asea *et al.*, 2002). Moreover, land clearing practices predominant in agro-pastoral areas where animals are allowed to graze freely after maize harvest leaving bare land where maize is planted directly without tillage further intensifies the incidence of the gray leaf spot in Tanzania. Much of the infected crop debris in bare grazing lands provides primary inoculum for newly planted maize for subsequent seasons. Cultural practices such as deep ploughing that minimizes soil surface inoculum has been reported to reduce the incidence and severity of gray leaf spot (DeNazareno *et al.*, 1992).

Reduced tillage has been recommended to conserve soil moisture in maize fields; however, the advantages are frequently outweighted by risks associated with increased damage by gray leaf spot (Ward and Nowell, 1998). One year of crop rotation can significantly reduce the initial levels of *C. zeae maydis* inoculum; but, it normally takes several years of rotation to reduce the inoculum to the levels achieved by deep ploughing (Latterell and Rossi, 1983). Ploughing to incorporate debris may

not be effective to manage gray leaf spot in areas with high levels of inoculum or where gray leaf spot has already been established in other fields (Perkins *et. al.*, 1995). This is because the inoculum from neighboring fields may be wind blown to infect maize grown under convectional tillage system. Latterel and Rossi (1983) reported that the disease appeared 3 weeks earlier in no till than in conventional tilled maize, however, in season favorable for gray leaf spot there is little or no difference between no till and conventional tillage. Pyne *et al.* (1987) and Asea *et al.* (2002) compared development of gray leaf spot and spore production of *C.zeae maydis* on till and non-till plots. Disease and number of spores were higher in till than no-till plots.

2.13.1.2 Management of crop diseases by intercropping

The effect of intercropping maize with beans on diseases and yield has been reported (Pibeam, *et al.*, 1994; Chemeda, 1996; Santalla *et al.*, 2001). The mechanisms that limit disease in the intercropping system have been reviewed by Boudreau and Mindt (1997) and involve (a) reduction in the production, amount and effectiveness of the inoculum available for spread and development within the crop as the proportion of susceptible host tissue decreases within the intercrop (b) increase the space between susceptible host within the crop, resulting in the greater distance that inoculum needs to travel from one susceptible plant to another hence reduce disease development (c) interception or filtering of pathogen propagules by the non-host component of the intercrop and (d) influence of the microclimate that may lead to reduction of disease. However, little has been reported on the effect of intercropping on the development of gray leaf spot of maize.

In the tropics, it is a common practice to intercrop maize with beans (*Phaseolus vulgaris* L.) for the purpose of spreading the risks associated with crop failure; beans

also provide dietary protein that supplements the staple starches in maize. However, the effect of bean canopy cover in bean/maize intercrop on the dispersal of residue-borne primary inoculum and subsequent development of *C. zeae maydis* has not been reported.

2.13.1.3 Induced host resistance to pathogens using organic and inorganic fertilizers

Poor soil fertility of most soils in the tropics and sub-tropics pose a major constraint to sustainable small holder crop production in Sub-Sahara Africa (Meyers *et al.*, 1994; Smaling *et al.*, 1997). In small-scale subsistence farming systems, fertilizers are often not readily available or are too expensive. The improvement of soil fertility largely depends on composting of decomposable materials which are then incorporated into the soil a natural way of nutrient cycling (Inckel *et al.*, 1996). Application of composted poultry and cattle manure have shown to increase grain yield of maize; lower production costs and improve soil structure (Materechera and Salagac, 2002; Erickson *et al.*, 2001; Negassa *et al.*, 2001; Nyamangara *et al.*, 2001; 2003). Composted manures of animal origin in the tropics are generally low in N contents due to poor feeding and health of animals (Mugwira and Makurumbira, 1984; Mugwira and Murwira, 1997). Combined use of animal manures with mineral fertilizer has been recommended to overcome the negative effects caused by addition of low N-composted manure (Nyamangara *et al.*, 2003; Negassa *et al*, 2001). The interaction between mineral fertilizer and composted manure increased nutrient availability by increasing N-recovery from mineral N fertilizer (Nyamangara *et al.*, 2003).

Composted manure is widely used in organic farming as a cheap source of fertilizer (Inbar *et al.*, 1993; Znaidi *et al.*, 2002). These organic amendments provide the

required plant nutrients and at the same time have the potential to provide biological control of diseases caused by foliar, vascular and root plant pathogens (Hoitink and Fahy, 1986; Wei *et al.*, 1991; Weltzen, 1992). Beneficial bacteria and fungi present in thermophillic composts directly compete with, inhibit or kill organisms that cause plant diseases (Boehm *et al.*, 1993). Compost may also activate disease resistance genes in plants preparing them for better defense against plant pathogens (Wei *et al.*, 1991; Zhang *et al.*, 1994) or induce systemic acquired resistance to certain plant pathogens (Wei *et al.*, 1991; Zhang *et al.*, 1994). Application of urea, hog or cattle manure to the soil reduced foliar diseases in barley and wheat (deFreitas *et al.*, 2003). Addition of composted cattle manure reduced root and stem infection caused by *Fusarium oxysporium* in melon, tomato and cucumber (Yoger *et al.*, 2006). Chicken manure reduced infection by *Phytophthora cinnamomi* in *Lupinus albus* seedlings (Aryantha *et al.*, 2000).

Little has been reported on the effect of nitrogen fertilizers on the development of gray leaf spot of maize. Nitrogen applied to maize as calcium ammonium nitrate increased the severity of gray leaf spot whereas potassium and phosphorous showed little or no significant effect on the disease (Perkins *et al.*, 1995). Carrera and Grybauskas (1992) observed that gray leaf spot was not affected by increased nitrogen application in maize. Caldwell *et al.* (2002) reported that increased nitrogen (limestone ammonium nitrate) and potassium application in maize also increased the percentage leaf blighting by *C. zeae maydis*. Okori *et al.* (2004) observed that high levels of gray leaf spot of maize occurred in nitrogen treated plots; additional phosphorous had no effect on gray leaf spot but phosphorous applied with nitrogen significantly reduced the pre-disposing effects of nitrogen on gray leaf spot.

2.13.2 Host plant resistance

Host-resistance breeding is the most cost effective means of managing gray leaf spot of maize (Elwinger *et al.*, 1990; Asea *et al.*, 2009). Resistance to gray leaf spot is reported to be inherited quantitatively by genes that act primarily in an additive manner (Elwinger *et al.*, 1990; Donahue *et al.*, 1991; Cromley *et al.*, 2002) and is expressed as rate-reducing resistance (Huff *et al.*, 1988; Elwinger *et al.*, 1990; Gordon, *et al* 2006). Quantitative resistance to gray leaf spot leads to prolonged latent and incubation periods, reduced infection rates, sporulation capacity and the number and size of lesions (Beckman and Payne, 1982; Freppon *et al.*, 1994; Carson and Goodman, 2006). Methods to evaluate maize germplasm for resistance to gray leaf spot include; diallel analysis (Donahue *et al.*, 1991; Gevers *et al.*, 1994), generation mean analysis (Thompson *et al.*, 1987), statistical modeling of resistance (Elwinger *et al.*, 1990), examination of quantitative trait loci (QTL) and restriction fragment length polymorphisms (RFLPs) associated with resistance (Clements *et al.*, 2000: Lehmensiek *et al.*, 2001, Gordon *et al.*, 2004; Asea *et al.*, 2009; Zwonitzer *et al.*, 2010).

Dialell analysis of gray leaf spot resistance has revealed general combining ability (Donahue *et al.*, 1991; Lehmensiek *et al.*, 2001) and specific combining ability (Huff *et al.*, 1988; Gevers *et al.*, 1994) to be significant among inbreds. Bubeck *et al.*, (1993) studying the resistance of gray leaf spot found QTLs associated with dominance and additive gene action, the latter being more predominant. Saghai Maroof *et al.* (1996) and Li-yu *et al.* (2007) found QTLs associated with additive, dominance and epistasis effect on gray leaf spot resistance.

Breeding for resistance to *Cercospora zeae maydis* has relied on partial resistance, a form of an incomplete or quantitative resistance characterized by slow epidemic

build-up despite high infection type of a compatible host-pathogen interaction (Menkir and Ayodele, 2005; Derera *et al.*, 2008). Components associated with partial resistance to gray leaf spot of maize include prolonged latent and incubation periods, reduced infection rates, low sporulation and fewer and smaller lesions (Gordon *et al.*, 2006; Menkir and Ayodele, 2005). Understanding the genetics and heritability behaviours of components expressing partial resistance have enabled selection of this type of resistance possible in several host-pathogen systems based on component measurements (Kari and Griffiths, 1997; Kim and Dievers, 2000; Yu *et al*, 2001; Lehmensiek *et al.*, 2001; Gordon *et al.*, 2006; Deng-Feng *et al.*, 2009). However, little has been reported on the components expressing partial resistance to gray leaf spot of maize and possible applications in the selection and breeding of resistant genotypes (Gordon *et al.*, 2006).

2.13.3 Fungicides application

Fungicides are widely used in maize seed production but have proven to be uneconomical in grain maize production (Wegulo *et al.*, 1997; Munkvold *et al.*, 2001) and are too expensive to afford by low income-resource poor farmers in the tropics (Ward *et al.*, 1999; Menkir and Ayodele, 2005). Mancozeb and Propriconazole are recommended for the control of gray leaf spot of maize (Backman and Pyne, 1982). Mancozeb a protective fungicide applied at four to 14 days interval, up to 40 days before harvest gave adequate protection. Propiconazole can be applied up to tasseling.

Ward *et al.* (1997) tested the effectiveness of Benlate 50% in the control of gray leaf spot and reported that control was most effective when spraying commenced when the disease severity levels reached 2 and 3% of the leaf area blightened and when lesions were restricted to the basal five leaves of the maize plant. In these trials,

highest yields were achieved with fungicide treatments up to physiological maturity and with early infection more fungicides were necessary to provide protection up until physiological maturity.

CHAPTER 3

3.0 MATERIAL AND METHODS

3.1 Occurrence of gray leaf spot, isolate diversity and aggressiveness of *C. zeae maydis* in selected villages of the Southern Highlands and the Eastern maize agro-ecological zones.

3.1.1 Variation in aggressiveness among isolates of *C. zeae-maydis* (Controlled Studies)

3.1.1.1 Plant materials

Variations in aggressiveness of thirty isolates collected in Morogoro Iringa and Mbeya regions was studied in the greenhouse at Sokoine University of Agriculture by inoculating the susceptible maize 'Pannar' and by measuring the lesion growth (change in length over time), percentage of leaf area affected, incubation period and sporulation. Locally purchased seeds of 'Pannar' maize hybrid were sown in 15 cm diameter pots containing sterilized forest soil and then placed in a screenhouse. Triple super phosphate was applied at the rate of 1.5 g pot^{-1} during planting followed by calcium ammonium nitrate at V2 growth stage (Iowa State Univesity Extension Bulletin, 2009) at the rate of 2.5 g pot^{-1}.

3.1.1.2 Inoculation and experimental design

Plants were inoculated at V6 growth stage by spraying conidia suspension from different isolates adjusted to 2 x 10^4 conidia ml^{-1} with a hand sprayer. Prior to inoculation, pots were arranged in a Completely Randomized Design (CRD) with 3 replications. Each treatment combination comprised a region and an isolate giving a total of 30 treatment combinations (i.e. from 3 regions and 10 isolates). To prepare conidia suspensions, single well separated lesions from samples collected in the field were cut, surface sterilized in 10% sodium hypochlorite (bleach) for 30 seconds and incubated in petri dishes containing moistened filter paper at 24° C for 48-72 h to

induce sporulation. Single spores were picked by sterilized needle and placed in V8-juice agar (350 ml V8 juice, 3 g $CaCO_3$, 20g agar, and 650ml distilled water per liter) and cultures were incubated in the light chamber at 28° C ± 2 °C with 12 h of darkness and 12 h of cool-white fluorescent light (320 $\mu E/m^2/sec$) (Plate 3). The inoculum was prepared by adding 5 ml of sterile distilled water in fresh culture grown in V-8 Juice agar. The resulting conidia suspension was strained between two layers of cheese cloth and conidia concentration was adjusted to 2 x 10^4conidia/ml using a haemocytometer. The inoculated plants were placed in the humidity chamber (2.4 m. length x 1.2 m. width x 1.5 m. height) constructed using wood and polythene sheets (Morogoro Plastic Co. Ltd) (Plate 4). The humidity in the chamber was kept high (approaching 100%) by frequent wetting of newspapers spread on the floor of the chambers.

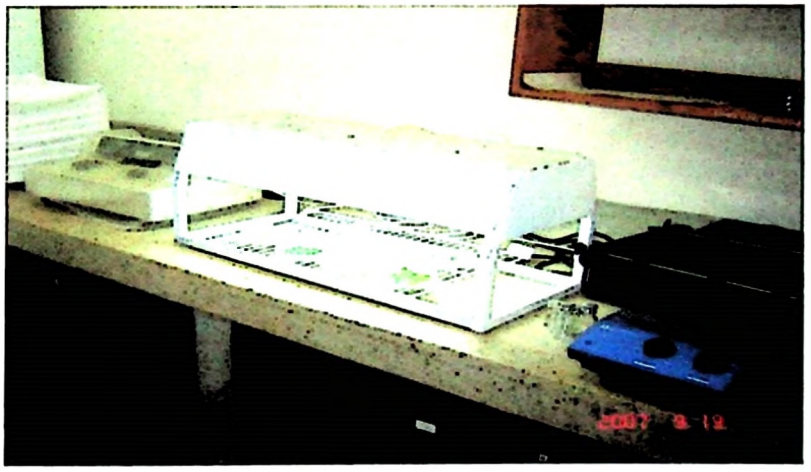


Plate 3: Light chamber used to enhance lesion sporulation

Plate 4: Humidity chamber for incubation of plants after inoculation to enhance infection.

3.1.1.3 Assessment of lesion length, spore numbers and incubation period

Thirty days after inoculation the lesion lengths were measured using a ruler (scale). The number of spores per cm^2 of leaf surface was estimated using a modified method described by Paul and Munkovold (2004). One square centimeter of leaf tissues with diseased lesions were cut, placed in vials containing 10 ml of sterile distilled water and then handshaken for 3 min to dislodge the conidia. The number of conidia/cm^2 of leaf surface was then estimated using the formula (SA=Sc x V/A) where SA=conidia/cm^2, Sc=conidia ml^{-1}, V=volume of water used (ml), and A=diseased leaf area. Conidiaml^{-1} was measured using a haemacytometer. The incubation period was recorded as days to first appearance of symptoms (small necrotic spots with chlorotic halos).

3.1.1.4 Variations in cercosporin production and growth rates among isolates of *C. zeae-maydis in-vitro* culture

Variations of isolates cercosporin production and growth rates were studied from field-collected samples from Morogoro, Iringa and Mbeya regions. Single, well

separated lesions from samples collected during field study were cut, surface sterilized in 10% sodium hypochlorite (bleach) (Reckitt Benckiser, East Africa Ltd. Nairobi) for 30 seconds and incubated in petri dishes containing moist filter paper at 24° C for 48-72 h to induce sporulation. Single spores were picked by sterilized needle and placed in V8-juice agar (350 ml V8 juice, 3 g $CaCO_3$, 20g agar, and 650ml distilled water per liter) and cultures were incubated in the light chamber at 28° C ±2 °C with 12 h of darkness and 12 h of cool-white fluorescent light (320 $\mu E/m^2/sec$) (Beckman and Payne 1983). Thereafter, the contents were maintained in the incubator (Binder, Bie & Berntsen Co. Ltd., Denmark, model 44948822) at 25°C. After spores were formed, single spores from sporulating colonies were sub-cultured on Potato Dextrose Agar (PDA) (Difco) for assessment of growth rates and cercosporin production. The rate of colony growth (mm/day) on PDA was measured using a ruler (scale) in petri dish cultures. Cercosporin production was visually estimated based on the extent of stain observed rated as +(low), ++(medium) and +++(high). A scale of 0-3 was used to grade the quantity of cercosporin production where 0= Nil, 1= +, 2= ++, 3= +++

3.1.1.5 Variations of morphological features and culture characteristics among isolates of C. zeae-maydis

Morphological features for different isolates were studied by observing the pathogen from field-collected samples using a light microscope (LeitzBiomed, Germany Type 020-507-010). Prior to observations the leaf pieces containing lesions were incubated for 48 h in petri dishes with moist filter paper to allow hyphal growth and sporulation. Six lesions were selected randomly from samples representing each location. Clear cellophane adhesive tape was placed on top of each lesion to pick the pathogen. After staining with lactophenol cotton blue, mounts were observed under

the light microscope (LeitzBiomed, Germany Type 020-507-010) using x 40 and x 100 lenses. Morphological features scored included the number of conidiophores per stroma, the nature of fascicles of conidiophores, the number of septa per conidiophore, the length and diameter of conidiophores, the number of septa per conidium, and the conidium length and diameter. Variations in culture characteristics (color and texture) and nature of fascicles of conidiophores for isolates collected in Morogoro, Iringa and Mbeya regions were also recorded.

3.1.2 Prevalence of gray leaf spot among established maize diseases (field studies)

3.1.2.1 Sites selection and field demarcation

Field studies were carried-out from 18 April, 2007 to 30 April, 2007 in the Southern Highlands Zone, (Iringa and Mbeya regions) and 13 June, 2007 to 26 June, 2007 in the Eastern Zone (Morogoro region) (Fig. 3). The study was conducted in ten villages in Morogoro region (Turiani, Mgeta, Kilosa, Mikumi, Doma, Kilombero, Mkuyuni, Mlali, Tangani and Dakawa), ten villages in Iringa region (Isimani, Tanangozi, Ihemi, Ifunda, Maguga, Malangali, Ilembula, Igawa, Ingilanyi and Njombe) and ten villages in Mbeya region (Igurusi, Chimala, Mbozi, Imuzi, Inyala, Uyole, Ileje, Semvi, Iluvi and Mbuyuni). Four farms in each village were selected randomly and plots of 10 m x 10 m were demarcated within a maize plot on each farm. One hundred and twenty observations were made (Eastern Zone 1, region 1 (Morogoro) 10 villages 4 farms = 40 observations; Southern Zone 2, region 2 (Iringa) 10 villages 4 farms = 40 observations, Southern Zone 2, region 3 (Mbeya) 10 villages 4 farms = 40 observations).

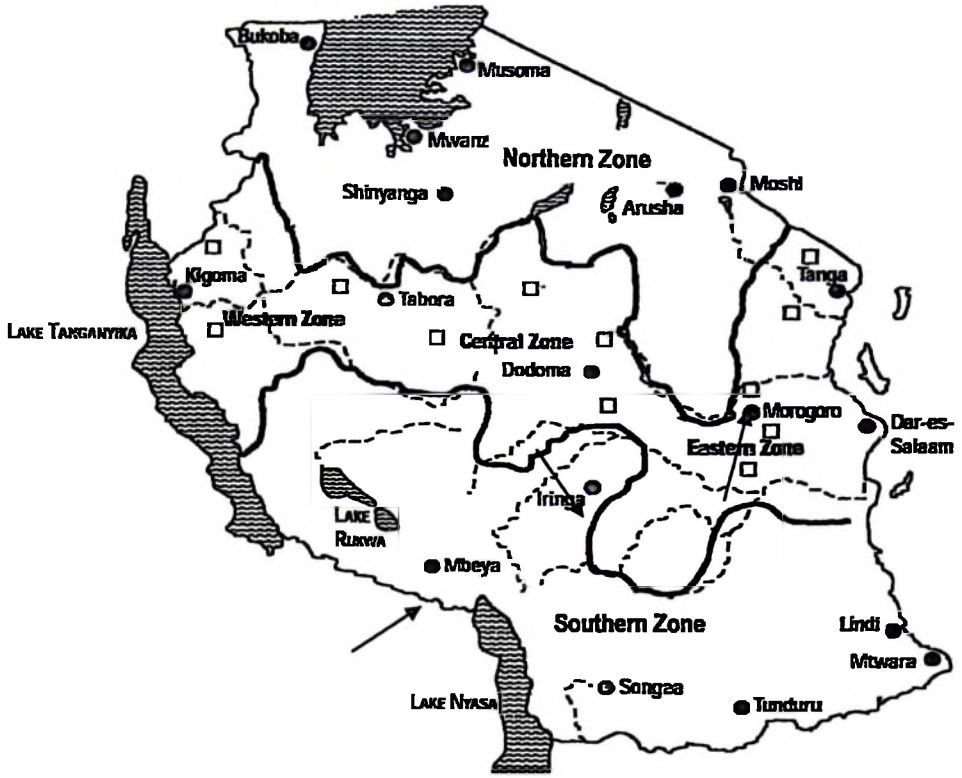


Figure 3: Map showing agro-ecological and maize production zones of Tanzania (Source: Kaliba *et al.* 1998). Mbeya and Iringa highland regions in the Southern Zone are indicated by arrows. Morogoro is located in the Eastern Zone.

3.1.2.2 Disease assessment

Severity of gray leaf spot was assessed on ten plants selected randomly in the middle of each plot. The percentage leaf area affected by the disease was assessed on four leaves (the upper most leaves including the ear leaf) per plant using a modified scale of 0-5 described by Paul and Munkovold (2004) where (0= no disease; 1= 1-20%; 2=21-40%; 3= 41-60%; 4= 61-80% and 5= 81-100%). The incidence of other diseases of maize were recorded by counting the number of plants showing symptoms of the respective diseases and expressed as a percentage of all plants in a plot i.e. disease incidence (%) = no. of diseased plants in a plot/total number of plants in a plot (healthy and diseased) x 100.

3.1.2.3 Sample collection

In every farm studied, diseased leaf specimens were collected randomly, placed between newspapers in plant pressers and transported to the laboratory at Sokoine University of Agriculture, Department of Crop Science and Production for further investigations.

3.1.2.4 Statistical model and data analysis

The data were analyzed using MSTAT-C version 2.10 (1995) statistical program for analysis of variance (ANOVA).

The following statistical model was used for ANOVA:

$Y_{ik} = \mu + t_i + \epsilon_{ik}$; Where Y_{ik} = observation in the i^{th} treatment and k^{th} plot; μ= overall mean; t_i = i^{th} treatment effect and ϵ_{ijk} = random error. All main factors were considered fixed.

3.2 Reaction of resistant and susceptible maize genotypes to *C. zeae-maydis* and associated disease resistance mechanisms

3.2.1 Plant materials

Locally purchased seeds of resistant ('UH6303'), moderate resistant ('Staha') and susceptible ('Pannar') maize genotypes were sown in 15 cm pots containing sterilized forest soil. Triple super phosphate was applied at the rate of 1.5 g pot^{-1} during planting followed by calcium ammonium nitrate at V2 growth stage at the rate of 2.5 g pot^{-1}.

3. 2. 2 Inoculation and experimental design

Plants were inoculated at V6 growth stage by spraying conidia suspension adjusted to 2 x 10^4 conidia/ml with a hand sprayer. Prior to inoculation, pots were arranged in a Complete Randomized Design (CRD) replicated four times. The inoculum was

prepared and conidia concentration adjusted to 2 x 10^4 conidia ml^{-1} as described in section 3.1.1.4. The inoculated plants were placed in the humidity chamber (8ft length x 4ft width x 5ft height) constructed using polythene sheets (Morogoro Plastics Ltd., Morogoro). The humidity in the chamber was kept high approaching 100% by constantly wetting news papers spread on the floor of the chambers.

3.2.3 Chlorophyll removal, leaf staining and microscopic studies

Leaf pieces from inoculated plants were removed at 24, 36, 48 and 72 h after inoculation and placed in sterile petri dishes and transported to the Sokoine University of Agriculture laboratory. Leaves for study of the growth of the *C.zeae maydis* in cells were removed at 72, 96, 120 and 144 h after inoculation. In the laboratory, the method of Skipp *et al.* (1974) was used to remove chlorophyll from leaf portions (Plates 5a-d). Leaf portions from resistant, moderate resistant and susceptible maize varieties were cut and placed (inoculated surface upwards) into sterile petri dishes containing two layers of filter papers. An amount of 6 ml of ethanol and 3 ml acetic acid was added into the petri dishes and incubated at room temperature (24 °C ± 1 °C) for 48hr in order to remove the chlorophyll. Cleared leaf pieces were stained with lactophenol cotton blue, mounted on microscope slides with 50% glycerin and observed under x40 and x100 lenses of the light microscope (Leitz Biomed, Type 020-507-010). Three hundred spores (100 per replicate) were scored on susceptible, moderate resistant and resistant maize genotypes for germination, germ tube growth, appressoria formation, penetration, establishment and hypha growth in cells.

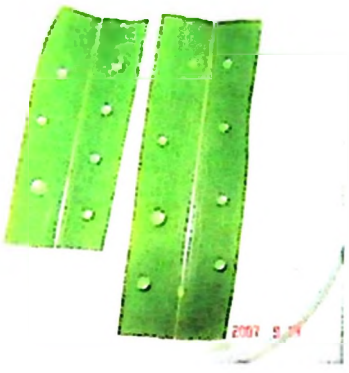


Plate 5a

Plate 5b

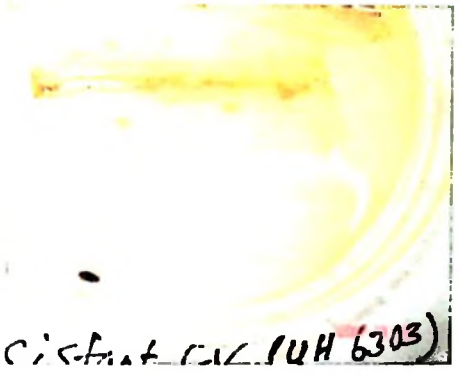


Plate 5c

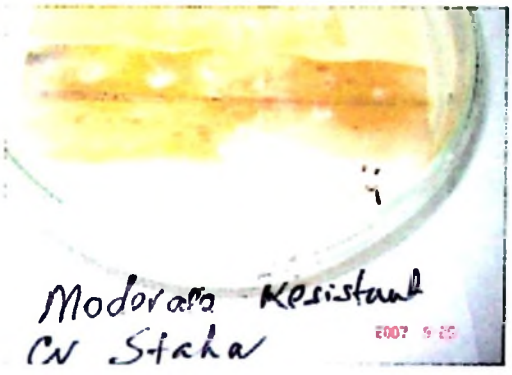


Plate 5d

Plate 5e

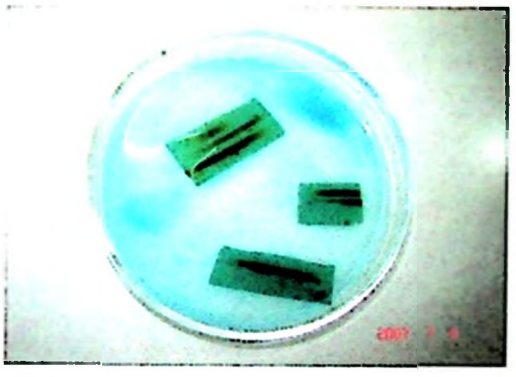


Plate 5f

Plate 5: Maize leaf inoculated with spore suspension (a & b), infected leaf segments after the removal of chlorophyll (c, d, e, & f) and leaf segments stained in lactophenol cotton blue (f).

3.2.4 Data analysis and statistical model

Data were analysed using statistical program described in section 3.1.2.4.

The following statistical model was used for ANOVA:

$Y_{ij}=\mu+ Gi + \epsilon_{ij}$; where Y_{ij}=observation in the i^{th} genotype and j^{th} plot; μ=overall mean; G_i =is the i^{th} genotypic effect and ϵ_{ij} the random error.

3.3 Heritability and gene effect estimates for components of partial resistance to gray leaf spot and yield of maize.

3.3.1 Germplasm development and generation of crosses

Two gray leaf spot resistant maize inbred lines K36 and P103 were crossed to susceptible maize inbred lines CML 395-5 and L37 in the field. The F_1 generations were selfed to produce F_2 generations and F_1 generations were crossed to both parents (susceptible parents (P_1) and resistant parents (P_2)) to produce backcrosses of F_1 for each parent (BCP_1) and (BCP_2) generations respectively. Experimental units comprised populations of six generations for each set of crosses as shown on (Table 1). Source and other information of inbred lines are shown in (Table 2).

Table 1: Inbred lines used to develop three sets of crosses

Set 1	Set 2	Set 3
P_1= P103	P_1= K36	P_1=K36
P_2= CML 395-5,	P_2= L37	P_2= CML395-5,
F_1=P103 x CML395-5	F_1= K36 x L37	F_1= K36 x CML395-5
F_2= P103/ CML395-5 (selfed)	F_2= K36/L37 (selfed)	F_2= K36 /CML395-5 (selfed)
BCP_1= P103/CML395-5 x P103	BC_1= K36/L37 x K36	BC_1= K36 /CML395-5 x K36
BCP_2= P103/CML395-5 x CML395-5	BC_2= K36/L37 x L37	BC_2= K36/CML395-5 x CML395-5

P_1 =Resistant Parent; P_2 =Susceptible parent; F1 =First filial generation; F_2 =Second filial generation; BC_1 =First backcross generation; BC_2 =Second backcross generation

Table 2: Source, maturity, grain type and heterotic group of inbred lines used to develop crosses

Inbred line	Origin	Maturity	Grain Type	Heterotic group
K37	ARI-Uyole	Intermediate	Semi-flint	A
P103	ARI-Uyole	Late	Flint	B
CML395-5	CIMMYT	Intermediate	Hard Dent	A/B
L37	ARI-Uyole	Late	Flint	B

ARI=Agriculture Research Institute; CIMMY=International Center for Maize Research, Mexico.

3.3.2 Field evaluation of generations and experimental design

The six basic generations for each set of crosses (Table 1) were evaluated in the field at two locations viz. Sokoine University of Agriculture (SUA) experimental field plots, Bigwa Kisiwani and Bigwa Mlimani on the foot hills of Uluguru mountains during 2008/09 cropping seasons. In all sites, experimental plots were ploughed and harrowed twice. Seeds were hand-sown one per hill. Spacing between rows was 70 cm and 40cm between plants. Planting in both locations was done on the same day. The method described by Mather and Jinks (1982) was adopted for experimental set up in the evaluation of experimental material. The experimental design used in both locations was a Complete Randomized Block Design (CRBD) in two replicates. Treatments were assigned using randomization table. Triple-super-phosphate (TSP) was applied to the entire field at the rate of 50 kg/ha during planting followed by top dressing of calcium ammonium nitrate (CAN) in maize at the rate of 50kg/ha at growth stage V4.

3.3.3 Inoculation

Plants were inoculated at V5 growth stage (Iowa State University Extension Bulletin, 2009) by spraying the conidia suspension of *C. zeae maydis* adjusted to 2×10^4 conidia/ml with a hand sprayer to supplement the natural infection. The conidia

suspension was prepared as described in section 3.1.1.4. The resulting conidia suspension was strained between two layers of cheese cloth and conidia concentration was adjusted to 2 x 10^4 conidia ml^{-1} using a haemocytometer prior field inoculation. The environment favorable for infection after inoculation in the first 15 days was maintained using overhead sprinkler irrigation that produces very fine mist 10h daily over the foliage. One of the major constraint after inoculation of *C. zeae maydis* is that the environmental conditions has to be near-perfect for infection to take place (Thompson and Martinson, 1993; Ward and Nowell, 1998)

3.3.4 Assessment of disease and other components of resistance

In each experiment, 40 plants from homogenous generations (P_1, P_2, and F_1), 80 plants from backcross generations (BC_1, BC_2) and 120 plants from F_2 generations were assessed for disease severity, lesion size, lesion length, lesion number and incubation as follows:

3.3.5 Disease severity assessment

The three top most leaves of each plant including the flag leaf were tagged and disease severity was assessed as described in section 3.1.2.2. Four assessments were carried out at 7 days interval. Same leaves were assessed in all subsequent different assessments recorded.

3.3.6 Lesion length assessment

Three leaves selected randomly from each plant were tagged and lesion length was measured as described in section 3.1.1.3 from 10 lesions per leaf. Four assessments were carried out in similar lesions at 7 days interval. Non-toxic marker pen was used to put identity marks on selected lesions so that the same lesions were assessed throughout.

3.3.7 Lesion size assessment

Three leaves selected randomly from each plant were tagged and lesion size was determined by measuring the length and the width of 10 lesions per leaf using a ruler (scale). Lesion size (mm^2) was obtained by multiplying the length and the width of each lesion measured. Four assessments were carried out in similar lesions at 7 days interval. Non-toxic marker pen was used to put identity marks on selected lesions so that the same lesions were assessed throughout.

3.3.8 Lesion number assessment

Three leaves selected randomly from each plant were tagged and the numbers of lesions were counted in 45 x 9 cm^2 of each leaf. Four assessments were carried out in leaves at 7 days interval. Same leaves were assessed in all subsequent different assessments.

3.3.9 Incubation period assessment

The incubation period was assessed by counting the number of days from inoculation to first appearance of symptoms.

3.3.10 Yield determinations

Yield per plot was determined following manually harvesting and shelling operations. Yield data were expressed at 15.5% (wet basis) moisture content.

3.3.11 Analysis of variance

In each location, data for disease severity, lesion length, lesion size, incubation period and yield (average for 10 plants per plot) were subjected to analysis of variance (ANOVA). Data were analysed using statistic program described in section 3.1.2.4 Generations, crosses and locations were the main effects. All factors were considered random.

The following statistical model was used for ANOVA. $Y_{ijklmn}= \mu + R_i + G_j + C_k + GC_{jk} + L_l + GL_{jl} + CL_{kl} + GCL_{jkl} + \epsilon_{ijklm}$. Where; Y_{ijklm}=observation in the i^{th} replication, j^{th} generation, k^{th} cross, l^{th} location, m^{th} plot; μ= general mean; $R_i = i^{th}$ replication effect; $G_j = j^{th}$ generation effect; $C_k = k^{th}$ cross effect; GC_{jk}= interaction effect of j^{th} generation and k^{th} cross; $L_l = l^{th}$ location effect; GL_{jl} =interaction effect of j^{th} generation and l^{th} location; CL_{kl} =interaction effect of k^{th} cross and l^{th} location; GCL_{jkl} =interaction effect of j^{th} generation, k^{th} cross and l_{th} location; ϵ_{ijklm} = random error.

3.3.12 Gene effect

A three parameter model (Individual-scaling test) ($A = 2BC_1 - P_1 - F_1$; $B = 2BC_2 - P_2 - F_1$; $C = 4F_2 - 2F_1 - P_1 - P_2$) described by Mather and Jinks (1982) was used to test for conformity of additive-dominance model using the proccdure of weighted least square as inverse of the variance of generation means. When these quantities A, B and C will each be equal to zero the additive-dominance model is adequate for analysis of variations. A joint-scale test described by Cavalli (1952) using chi-square goodness of fit with three degrees of freedom was performed to compare the results of the Individual-scaling test. When three parameter-Individual scaling model did not show conformity of additive-dominance (i.e. with values different from zero), a six-parameter scaling model ($m=½P_1 + ½P_2 + 4F_2 - 2B_1 - 2B_2$; $a=½P_1 - ½P_2$; $d=6B_1 + 6B_2 - 8F_2 - F_1 - 1½P_1 - 1½P_2$; $aa=2B_1 + 2B_2 - 4F_2$; $ad=2B_1 - P_1 - 2B_2 + P_2$; $dd=P_1 + P_2 + 2F_1 + 4F_2 - 4B_1 - 4B_2$) was performed to include the contribution of a digenic epistasis (non-allelic interaction). The test provides estimates for three parameters (mid-parent (m)), additive effect (a), dominance effect (d) but also provide estimates for three epistatic parameters; additive x additive (aa); additive x dominance (ad) and dominance x dominance (dd). The significant t-rest for each component was

calculated as t=[x]/sx where x=magnitude of the component and s= the standard error. The standard errors were obtained by finding the square root of respective component variances. The significance of individual components within the complete model was first evaluated from which simple models were chosen after omitting the non-significant components so as to increase the precision. A significant level ($P \leq 0.05$) was used to compare all components. Data were not transformed in any of the analysis since the ranges of variation in the data were relatively low. Moreover, scale of millimeter used in this study becomes unequal when the data are log transformed, unless the range of variation in the data is relatively large (Mather and Jinks, (1982). The three and six-parameter models were developed as described by Mather and Jinks (1982). The genetic models applied to estimate the genetic effects are shown on Table 14 below.

Table 3. Genetic models applied to estimate the genetic effects of mean generations

Generation	m	a	d	aa	ad	dd
P1	1	1	0	1	0	0
P2	1	-1	0	1	0	0
F1	1	0	1	0	0	1
F2	1	0	1/2	0	0	¼
BC1	1	1/2	1/2	1/4	1/4	1/4
BC2	1	-1/2	1/2	1/4	-1/4	1/4

P_1 =Resistant Parent; P_2 =Susceptible parent; F1 =First filial generation; F_2 =Second filial generation; BC_1 =First backcross generation; BC_2 =Second backcross generation

3.3.13 Heritability estimate

Broad-sense heritability was estimated using the method described by Wright (1968) as $h^2_b=\sigma_g^2 /(\sigma_g^2 + \sigma_e^2)$. The estimate of genetic variance (σ_g^2) is equal to the variance of F_2 generation (σ^2_{F2}) minus the environmental variance (σ_e^2). In this formula, $\sigma_e^2= [n_{P1}\ \sigma_{P1}^2 + n_{P2}\ \sigma_{P2}^2 + n_{F1}\ \sigma_{F1}^2]/$ Ne ; where n_{P1}, n_{P2} and n_{F1} refer to the number of

plants of susceptible parents (P_1), resistant parents (P_2) and F_1 generations respectively; The term Ne refers to effective population size, where Ne= $nP_1 + nP_2 + nF_1$ i.e. number of P_1, P_2 and F_1 respectively (Wright, 1968).

The method used to estimate narrow-sense heritability was adapted from Fehr (1991).

$h^2_n = [2(\sigma^2_{F2}) - (\sigma^2_{BC1} + \sigma^2_{BC2})] / \sigma^2_{F2}$

Where σ^2_{F2} is the variance among F_2 individuals, σ^2_{BC1} and σ^2_{BC2} are the variances of BCP_1 and BCP_2 generations respectively.

3.3.14 Heterosis

Heteroic effects as determined by deviation of F_1, BCP_1, BCP_2 and F_2 from the mid parents and its relative % heterosis for all generations and variables was performed (Fehr, 1991).

3.4 The effects of organic fertilizers (composted cattle and poultry manure) on development of gray leaf spot and yield of maize

3.4.1 Description of the study area

The study was conducted during the 2006/07 and 2007/08 cropping seasons at Sokoine University of Agriculture, Morogoro, Tanzania located 6° South and 37° 37'East with an altitude of about 550 masl. Total monthly mean rainfall, wind speed, relative humidity, maximum and minimum temperatures in the area was 120 mm, 63.4 km day^{-1}, 74.6%, 30.8°C, 20.6°C for 2006/07 cropping season and 136mm, 64.2 $kmday^{-1}$, 83.7%, 29.2°C, 18.2°C for 2007/08 cropping season. The experiments were planted in 2nd and 3rd March and harvested in 27th and 29th June in first and second season respectively. The soils in the experimental area were classified as sandy clay oxisols with pH 6.3, total nitrogen 0.1%, organic carbon 1.4%, exchangeable P= 3.19

mg kg^{-1} and exchangeable K= 3.33 mg kg^{-1}. Soil pH, nitrogen, organic carbon, exchangeable P and K were determined as described in section 3.4.2.

3.4.2 Soil analysis of the study area

Six composite soil samples were collected from the study area. Sampling was carried out at the depth of 0-30 cm. The composite samples were air dried, grounded to pass through 2mm sieve prior laboratory analysis. Soil pH was determined using glass electrode pH meter in a 1:2.5 mixture (v/v) of soil and water. Soil organic matter was determined by Walkley-Black wet oxidation method (Sparks, 1996). Total nitrogen was determined by micro-Kjeldahl method (Bremmer and Mulvaney, 1982). Soil available Phosphorous was determined by Bray-1 method (Bray and Kurtz, 1945). Cation exchange capacity (CEC) of the soil was determined by neutral ammonium acetate (pH 7) saturated method (Sparks, 1996). Exchangeable bases (K^+, Ca^+, Mg^+ and Na^+) were extracted by ammonium acetate solution and Ca^+ and Mg^+ were quantified using Atomic Absorption Spectrophotometer and K^+ and Na^+ by flame photometer. The Percent base saturation (% BS) of the soil was calculated from the sum of the exchangeable bases as the % of the CEC of the soil.

3.4.3 Composting of cattle and poultry manure

Cattle and poultry manure were collected from Sokoine University of Agriculture farm transported and pilled in heaps outside near the experimental site and left for six weeks where they underwent continuous aerobic decomposition. The Carbon nitrogen (C:N) ratio of the compost was determined to test the maturity of the compost and value of $\leq 25\%$ was taken as well mature compost ready for use. The piles were also monitored (by constant recording the temperature with a thermometer) to ensure that they were no longer overheating prior to use indicating a low microbial activity hence well matured compost. The expected mineralization rate

of composts was not determined but they were expected to be in the range of 13-18% for such types of composted manure (Eghball, 2000).

3.4.4 Nutrient analysis of composted cattle and poultry manure

Twelve dried samples (two kilograms each) of composted poultry and cattle manure were collected randomly from pile lots and packed in polythene bags and transported to the laboratory for nutrient analysis. Oven dried samples at 70°C were milled to pass through 2 mm sieve. Samples were digested following the method described by (Yoger *et al.*, 2006) and total Nitrogen (N) and Potassium (K) in the digest was determined by Spectrophotometer (Hach, 1988). Phosphorous (P) was determined by Olsen method (Olsen *et al.*, 1954). The organic mater was determined after the sample was ashed at 550°C. The concentration of soluble ions (NO_3^- and NH_4^+) was determined in water extracts of composts (1:10 v/v compost and water). The Potassium concentration was measured with Corning Flame Photometer, Ammonium was determined follow method described by (Kempers and Zweers, 1986). Nitrate was determined follow method described by (Carlson *et al.*, 1990). Ca^+, Mg^+ and K^+ were determined by Atomic Absorption Spectrophotometer. The pH of the composted manure was measured by glass electrode pH meter in a 1:2.5 mixture (v/v) of compost and water.

3.4.5 Nutrient analysis of maize leaf tissues

Maize leaf samples from different fertilizer treatments collected at the end of the experiment were analyzed for tissue nutrient contents based on method described by Konieczynski and Wesolowski (2007). Samples were dried at 60°C for 48h and milled to pass through 1.0 mm sieve and were analyzed for total dry matter (TDM), protein, and total nitrogen and phosphorous. Total dry matter was obtained by drying the leaves at 80 °C for 12h to remove water. Total nitrogen was determined by micro-

Kjeldahl method (Bremner and Mulvaney, 1982). Phosphorus was determined calometricaly by method described by (Thomas *et al.*, 1967). Ammonium nitrogen and nitrate nitrogen were determined by method described by Carlson, *et al.* (1990).

3.4.6 Plant material and experimental set-up

In both seasons, locally purchased seeds of maize cvs 'Pannar' (susceptible to gray leaf spot), 'Staha' (moderate resistant) and 'UH6303' (resistant) were sown two seeds per hole at a spacing of 70 cm between rows and 40 cm between plants and thinned to one plant per hole fifteen days after planting. Experimental design adopted was a split plot replicated three times. Sub-plots dimensions were 4.4 m x 4.2 m with 6 rows, 11 maize plants per row and plant density of 66 maize plants plot^{-1}. Main plot dimensions were 4.5 m x 27 m. Main plot treatments consisted of maize varieties (resistant, moderate resistant and susceptible to gray leaf spot) and sub-plot treatments were fertilizers (composted poultry manure, composted cattle manure, urea, calcium ammonium nitrate and sulphate of ammonium applied at recommended rates 60 kg N h^{-1} and heavy application 90 kg N h^{-1}). Three rows of resistant maize variety 'UH6303' were sown between sub-plots to prevent inter-plot interference. Treatment were allocated in main and sub-plots.

3.4.7 Mineral fertilizer and composted manure application

In both seasons, fertilizers were applied once at V4 growth stage (Iowa State University Extension Bulletin, 2009) when the disease is at the lowest level. Urea, CAN and SA were top dressed at the rates of 0.22, 0.57 and 0.45 kg sub-plot^{-1} respectively each delivering (60 kg N ha^{-1}); fertilizer recommended for Eastern Maize Growing Zone (Kaliba *et al.*, 1988). Heavily fertilized plots were applied 0.4, 0.87 and 0.68 kg of Urea, CAN and SA sub-plot^{-1} respectively each delivering 90 kg N ha^{-1}. 0.5 kg of Triple Super Phosphate (TSP) (23% P) was applied sub-plot^{-1}

during planting. Composted cattle and composed poultry manure were incorporated in soil the same day mineral fertilizers were applied to provide equivalent amount of N as applied in mineral fertilizer treatments (60 kg N ha^{-1} at recommended rate and 90 kg N ha^{-1} as heavy application). Mineral fertilizers, composted cattle and poultry manure were applied to provide the same amounts of nitrogen at two levels of application. 6.2 kilogram of composted cattle manure and 5.3 kg of composted poultry manure were applied sub-$plot^{-1}$ respectively as recommended rates each delivering 60 kg N ha^{-1}. 9.3 kg of composted cattle manure and 8.0 kg of composted poultry manure were applied sub-$plot^{-1}$ respectively as heavy application rates each delivering 90 kg N ha^{-1}. Composted cattle and manure manure provided an additional of 0.24, 0.36 0.23, 0.35 kg of P at lower and higher rate of N treatments respectively.

3.4.8 Inoculation

In both seasons, plants were inoculated at V6 growth stages by spraying conidia suspension adjusted to 2 x 10^4conidia ml^{-1} using hand sprayer to supplement natural infection. Inoculum suspension was prepared as described in section 3.1.1.4. The resulting conidia suspension was strained between two layers of cheese cloth and conidia concentration was adjusted to 2 x 10^4 conidia/ml using a haemocytometer prior field inoculation. The environment favorable for infection after inoculation in the first 15 days was maintained using overhead sprinkler irrigation that produces very fine mist 10h daily over the foliage. One of the major constraint after inoculation of *C. zeae maydis* is that the environmental conditions has to be near-perfect for infection to take place (Thompson and Martinson, 1993; Ward and Nowell, 1998)

3.4.9 Disease severity assessment

In both seasons, ten plants in the middle row of each plot were marked for disease

scoring at V8, V10, V12, V15 and V18 growth stages. Disease severity (leaf area affected) was recorded in four middle leaves as described in section 3.1.2.2. Similar leaves were used for subsequent different assessments, repeated five times on the same plants at seven day intervals.

3.4.10 Yield determinations

Yield per plot was determined following manually harvesting and shelling operations. Yield data were expressed at 15.5% (wet basis) moisture content. Yield components (days to 50%, silk, ear height and 100 seed weight) were also assessed.

3.4.11 Statistical analysis procedures

Data were analysed using statistic program described in section 3.1.2.4 for mean separation and correlation matrix for yield and disease data. A significance level ($P \leq 0.05$) was used throughout the study.

The following statistical model was used for ANOVA.

$Y_{ijklmn} = \mu + R_i + G_j + Y_k + GY_{jk} + D_l + GD_{jl} + YD_{kl} + GYD_{jkl} + \epsilon_{ijkl}$. Where; Y_{ijkl}=observation in the i^{th} replication, j^{th} genotype, k^{th} year, l^{th} disease severity, and n^{th} plot; μ= general mean; R_i =i^{th} replication effects; G_j= j^{th} genotypic effect; GY_{jk}=interaction effect of j^{th} genotype and k^{th} year; D_l =l^{th} disease severity effect; GD_{jl} =interaction effect of j^{th} genotype and l^{th} disease severity; YD_{kl} =interaction effect of kth year and l^{th} disease severity; GYD_{jkl} =interaction effect of j^{th} genotype and k^{th} year and l^{th} disease severity; ϵ_{ijkl} = random error.

3.5 The effect of intercropping maize with beans on inoculum dispersal, development of GLS and yield of maize in minimum tillage operations

3.5.1 Field trials

3.5.1.1 Description of the study area

The site descriptions, planting and harvesting dates and weather conditions of the experimental area is as described in section 3.4.1.

3.5.1.2 Land preparation and fertilizer application

The experimental area was under maize cultivation for the past two years after two years of beans in the rotation. Infested maize residues were spread in the experimental field prior to cultivation to supplement the available inoculum. The amount of maize crop residue was applied to provide 80% of the soil ground cover prior cultivation. The minimum tillage plots were cultivated by hand-hoeing partially incorporating the infected maize residue into the soil. The conventional till-plots were ploughed (15 cm) and harrowed twice by a tractor deeply incorporating the infected maize residues into the soil.

3.5.1.3 Plant material and field design

In 2006/07 and 2007/08 cropping season, locally purchased seeds of susceptible cultivar (cv. 'Pannar') and resistant ('UH6303') hybrids and open pollinated moderately resistant cultivar (cv. 'Staha') in sole and intercrop plantings were hand-sown two seeds per hill at a spacing of 70 cm between rows and 40 cm between plants and thinned to one plant per hill 15 days after planting. Triple super phosphate (TSP) was applied to the entire field at the rate of 50 kg/ha during planting followed by top dressing of Calcium ammonium nitrate (CAN) in maize at the rate of 50 kg ha^{-1} at growth stage V4 (Iowa State University Extension Bulletin, 2009). A split-split plot experimental design with three replications was adopted. Main plot treatments were maize varieties, sub-plot treatments were tillage operations (no-till, minimum and conventional tillage) and the sub-sub plot treatment was intercropping. Main plots dimensions were 27 m x 3.5 m, sub-plots 9 m x 3.5 m and sub-sub plots

4.5 m x 3.5 m. At the same planting date, red seeded bush type (ex-market) beans were hand-planted by dibbling one seed per hole in the intercropped plots at a spacing of 30 cm between rows and 20 cm between plants. Narrower bean rows were planted across the maize rows in a perpendicular fashion. Maize and bean densities in the intercrop were 49 603 maize plants ha^{-1} and 231 481 bean plants ha^{-1} respectively for all treatments (no-till, minimum tillage and conventional tillage). Maize density was the same in sole and intercrop plots (49 603 plants ha^{-1}). Bean canopy level was similar for all treatments providing 60-90% cover of the soil surface from early stages of maize plant growth until senescing. Maize was planted without cultivation in no-till plots leaving the infected maize crop residue on the soil surface. Three rows of resistant hybrid 'UH6303' were sown between plots to prevent inter-plot interference. Plots were kept weed-free by hand-hoeing whenever necessary. Weeding in no-till plots was done carefully not to disturb the surface residue.

3.5.1.4 Disease severity assessment and logistic models

Fifty-four days after planting (V14), ten plants in the middle row of each plot were marked for disease scoring. Disease severity (percent leaf area affected by disease) was recorded on four middle leaves using method described in section 3.1.2.2. Severity assessments were repeated five times at an interval of seven days using the same plants. Disease progress curves for different treatments were compared by the logistic model (Van de Plank, 1963). The rate of disease increase over time (r) was estimated as the regression coefficient (b) of the logit x time in days where x is the proportion of host tissue infected by the pathogen and logit x being $\log_e [(x/1-x)]$. The coefficient of determination (R^2) (square of correlation coefficients) was calculated for each treatment to determine the appropriateness of the logistic model. The relative importance of single and multiple practices were compared using Wald

statistical test. The areas under disease progress curves (AUDPC) for different treatments were estimated using the equation suggested by Shanner and Finney (1977). AUDPC=$\sum$ $[(x_{i+1} + x_i) / 2] [t_{i+1} - t_i]$; where xi = the proportion of the host tissue damaged at i^{th} day, t_i =the time in days after the appearance of the disease at i^{th} day and n = the total number of observations.

3.5.1.5 Determination of airborne conidia

Three days after planting, four spore traps (microscope slides coated with petroleum jelly) were placed in each sub-plot in the field (in a double diagonal fashions) on raised stools. Stools were placed on concrete blocks that raised the traps (microscope slides) 0.75 m high above the ground surface in each plot. After 24 h the slides were removed and transported in covered boxes to the laboratory and examined under the light microscope. Conidia were counted with the aid of a hand-counter. Field conidia assessments began 7 days after planting and continue up to 84 days after planting at 7 days intervals. Weather data (total rainfall, wind speed, relative humidity, minimum and maximum temperatures) were recorded and related to spore trapped.

3.5.1.6 Yield determinations

Yield per plot and yield components were determined following hand-harvesting and shelling operations. Yield data were expressed at 15.5% (wet basis) moisture content.

3.5.1.7 Statistical analysis procedures

Data were analysed using statistic program described in section 3.1.2.4 for mean separation and correlation matrix for yield and disease variable data. Wald statistical test using SPSS statistical program (2008) version 12 for window was used to compare the relative importance of single and multiple cultural practices. A

significance level ($P \leq 0.05$) was used throughout the study.

The following statistical model was used for ANOVA.

$Y_{ijklmn} = \mu + R_i + G_j + Y_k + GY_{jk} + T_l + GT_{jl} + YT_{kl} + GYT_{jkl} + I_m + GI_{jm} + YI_{km} + GYI_{jkm} + TI_{lm} + GTI_{jlm} + YTI_{klm} + GYTI_{jklm} + \epsilon_{ijklmn}$ Where; Y_{ijklmn}=observation in the i^{th} replication, j^{th} genotype, k^{th} year, l^{th} tillage, m^{th} intercropping and n^{th} plot; μ= general mean; R_i =i^{th} replication effects; G_j= j^{th} genotypic effect; GY_{jk}=interaction effect of j^{th} genotype and k^{th} year; T_l =l^{th} tillage effect; GT_{jl} =interaction effect of j^{th} genotype and l^{th} tillage; YT_{kl} =interaction effect of k^{th} year and l^{th} tillage; GYT_{jkl} =interaction effect of j^{th} genotype and k^{th} year and l^{th} tillage; I_m =m^{th} intercropping effect; GI_{jm} = interaction effect of j^{th} genotype and m^{th} intercropping; YI_{km} =interaction of k^{th} year and m^{th} intercropping; GYI_{jkm} = interaction of j^{th} genotype and k^{th} year and m^{th} intercropping; TI_{lm} = interaction of l^{th} tillage and m^{th} intercropping; GTI_{jlm} = interaction of j^{th} genotype and l^{th} tillage and m^{th} intercropping; YTI_{klm} = interaction of k^{th} year and l^{th} tillage and m^{th} intercropping; $GYTI_{jklm}$ = interaction of j^{th} genotype and k^{th} year, l^{th} tillage and m^{th} intercropping; ϵ_{ijklmn} = random error.

CHAPTER 4

4.0 RESULTS

4.1 Occurrence of gray leaf spot, isolate diversity and aggressiveness of *C. zeae maydis* in selected villages of the Southern Highlands and the Eastern maize agro-ecological zones.

4.1.1 Variations in aggressiveness among isolates of *C. zeae maydis*

The analysis of variance (mean squares) for variables used to assess aggressiveness of isolates collected in Morogoro, Iringa and Mbeya is shown on (Appendix 1). There were highly significant differences ($P \leq 0.001$) between isolates for lesion length, percent leaf area affected by gray leaf spot and the number of spores formed on inoculated maize plants. Lesion length, percent leaf area affected and spore number variations in the three regions are shown in Table 4. Lesion length ranged between 1.3 to 5.8 cm. Isolates with the longest lesions arose from the Southern Zone. The shortest lesion lengths were associated with isolates from the Eastern Zone. Mean lesion length for isolates from Southern Zone was 1.1 cm longer (25.9%) than the Eastern Zone. The isolate with the highest percent area affected (PLAA) with GLS was NJB1 (36.8%) from the Iringa region, Southern Zone. The lowest percent leaf area affected by gray leaf spot resulted from isolates representing both zones. Mean scores indicated Southern Zone isolates caused 21.2% more disease than Eastern Zone isolates. Isolates with highest spore numbers were obtained from Southern Zone isolates whereas the isolate displaying the lowest spore number was obtained from the Eastern Zone. Mean scores indicated that Southern Zone isolates produced 1.05×10^4 more spores per cm^2 of the lesion per day (13.6%) than Eastern Zone isolates. Incibation period between isolates did not differ significantly. Isolates from the Southern Zone produced symptoms 1 day earlier than isolates from the Eastern Zone (Table 4).

Table 4: Aggressiveness variables for isolates collected in Morogoro, Iringa and Mbeya regions (Studies conducted at SUA in 2008).

Region	Isolate	Village	Variable			
			LL[1]cm	PLAA[2]	SPN[3]	IP[4]
Morogoro	MKY1	Mkuyuni	1.3^{p}	11.5^{l}	6.2^{jk}	12.9^{a}
"	KLM1	Kilombero	2.9^{lmn}	16.1^{h}	7.3^{hi}	10.1^{a}
"	KIL1	Kilosa	1.8^{o}	12.6^{jkl}	6.6^{hijk}	11.3^{a}
"	MKI1	Mikumi	3.8^{fgh}	26.7^{d}	8.4^{bcd}	13.4^{a}
"	DOM1	Doma	4.3^{de}	28.3^{cd}	9.2^{ab}	13.5^{a}
"	TRN1	Turiani	3.3^{ijkl}	13.4^{jk}	5.8^{kl}	13.6^{a}
"	MGT1	Mgeta	3.7^{ghi}	12.5^{jkl}	6.6^{hijk}	13.7^{a}
"	MLI1	Mlali	3.1^{jklm}	16.5^{h}	6.2^{jk}	12.4^{a}
"	TGN1	Tangeni	2.6^{n}	13.7^{jk}	6.1^{jk}	13.1^{a}
"	DKW1	Dakawa	2.8^{mn}	12.4^{jkl}	3.2^{n}	12.1^{a}
Mean			**3.0**	**16.4**	**6.6**	**12.6**
std±			**0.9**	**6.1**	**1.6**	**1.2**
Iringa	ISMA1	Ismani	4.5^{cde}	16.9^{gh}	8.7^{bc}	10.1^{a}
"	TANA1	Tanangozi	4.1^{efg}	17.1^{gh}	7.6^{efg}	13.8^{a}
"	IHE1	Ihemi	3.0^{klmn}	15.6^{hi}	6.9^{ghij}	11.2^{a}
"	IFUN1	Ifunda	3.6^{hi}	14.1^{ij}	7.1^{ghi}	12.9^{a}
"	MAG1	Maguga	3.7^{ghi}	12.1^{kl}	5.0^{lm}	10.8^{a}
"	MALAG1	Malangali	2.9^{lmn}	19.0^{f}	8.2^{cdef}	10.8^{a}
"	ILE1	Ilembula	3.8^{fgh}	18.5^{fg}	7.7^{defg}	12.0^{a}
"	IGAW1	Igawa	5.1^{b}	22.7^{e}	9.7^{a}	12.5^{a}
"	MTWAG1	Mtwango	3.7^{ghi}	28.5^{c}	9.0^{abc}	11.9^{a}
"	NJB1	Njombe	4.6^{cd}	36.8^{a}	8.5^{bcd}	13.1^{a}
Mean			**3.9**	**20.1**	**7.8**	**11.9**
std±			**0.7**	**7.5**	**1.3**	**1.2**
Mbeya	IGR1	Igurusi	3.7^{ghi}	19.4^{f}	7.4^{fgh}	11.3^{a}
"	CHL1	Chimala	4.1^{efg}	22.6^{e}	8.7^{bc}	9.7^{a}
"	MBZ1	Mbozi	4.8^{bc}	23.5^{e}	6.2^{jk}	9.5^{a}
"	IMUZ1	Imuzi	3.8^{fgh}	16.8^{gh}	7.7^{defg}	10.5^{a}
"	INYL1	Inyala	4.2^{def}	31.4^{b}	6.9^{ghij}	12.0^{a}
"	ILEJ1	Ileje	3.5^{hij}	18.5^{fg}	4.7^{m}	10.4^{a}
"	SEV1	Semvi	4.2^{def}	10.9^{l}	7.3^{ghi}	12.8^{a}
"	UYL1	Uyole	3.4^{hijk}	11.3^{l}	6.5^{ijk}	12.4^{a}
"	ILV1	Iluvi	4.4^{cde}	28.7^{c}	9.7^{a}	13.3^{a}
"	MBY1	Mbuyuni	5.8^{a}	32^{b}	9.6^{a}	11.5^{a}
Mean			**4.2**	**21.5**	**7.5**	**11.3**
std±			**0.7**	**7.6**	**1.6**	**1.3**
Overall Mean			3.7	19.3	7.3	12
Over all std ±			1	7.2	1.5	1.3
CV (%)			12.67	10.72	12.92	14.81
SE±			0.27	1.14	0.53	1.02

Means followed by the same letter within columns do not differ significantly according to DMRT; [1]LL=Lesion length (cm); [2]PLAA=Percent leaf area affected; [3]SPN=Spore number x 10^4 /cm^2; IP=Incubation period; std=Standard deviation.

Table 5: Correlations of parameters used to assess aggressiveness of *C. zeae maydis* isolates from samples collected in Morogoro, Iringa and Mbeya regions.

	LL[1]	PLLA[2]	IP[3]	CP[4]	SPN[5]
LL	-				
PLAA	0.574**	-			
IP	-0.028	-0.048	-		
CP	0.563**	0.578**	-0.1.94	-	
SPN	0.024	0.203	-0.154	0.030	-

**P≤0.01; df=18; [1]LL=Lesion length (cm); [2]PLAA=Percent leaf area affected; [3]IP=Incubation period; [4]CP=Cercosporin production; [5]SPN=Spore number x 10^4 /cm^2

4.1.2 Variations in growth rates and cercosporin production among isolates of *C. zeae-maydis* in *invitro* culture

4.1.2.1 Variations of isolate growth rates

The analysis of variance (mean squares) for isolate growth rates revealed significant differences between regions and isolates (Appendix 2). Significant differences (P≤0.05) in growth rates of isolates, ranging from 1.2 to 3.9 mm/day, were observed (Table 6). The fastest growing isolates were obtained from the Southern Zone as was the slowest growing isolate. Mean values for the regions indicated that Southern Zone isolates grew 0.25 mm per day faster (9.3%) than the Eastern Zone isolates.

4.1.2.2 Variations of isolates in cercosporin production

Cercosporin toxin was visible on eight to 12 days old cultures as reddish purple compound (Plate 6a-d) which gave an absorption spectrum characteristic after extraction from the media into KOH (Balis and Payne, 1971; Jeans *et al.* 1989). Some isolates released the toxin slowly and it was visible after 23 days, while others didn't produce the toxin at all (Table 7). Production of cercosporin was high for most isolates but the amount of cercosporin production varied between isolates. Isolates from Mbeya located in higher altitudes produced more toxin invitro followed by

Morogoro and Iringa regions (Table 6). Mean cercosporin production was higher in the Southern Zone than Eastern Zone. Cercosporin production was significantly positively correlated to percent leaf area affected by disease (r=0.578) and lesion lenght (r=0.563) ($P \leq 0.01$) (Table 5).

Correlations between aggressiveness variables are shown on (Table 2). Lesion length was significantly ($P \leq 0.01$) positively correlated (r=0.57) with PLAA. Cercosporin production was positively correlated (r=0.58) to PLAA and lesion length (r=0.56) ($P \leq 0.01$).

Table 6: Growth rates for isolates collected in villages of Morogoro, Iringa and Mbeya regions (Studies conducted at SUA 2008).

Region	Isolate	Village	GR[1](mm/day)	Cercosporin QTE[2]
Morogoro	MKY1	Mkuyuni	3.3^{de}	2
"	KLM1	Kilombero	$3.1c^{fg}$	3
"	KIL1	Kilosa	3.3^{de}	1
"	MKI1	Mikumi	2.5^{kl}	1
"	DOM1	Doma	1.8^{mn}	0
"	TRN1	Turiani	2.8^{hij}	1
"	MGT1	Mgeta	3.7^{ab}	3
"	MLI1	Mlali	1.6^{n}	2
"	TGN1	Tangeni	1.9^{m}	1
"	DKW1	Dakawa	2.7^{ijk}	3
Mean			**2.7**	**1.7**
std±			**0.7**	**1.1**
Iringa	ISMA1	Ismani	3.4^{cd}	2
"	TANA1	Tanangozi	1.2^{o}	0
"	IHE1	Ihemi	1.9^{m}	2
"	IFUN1	Ifunda	2.7^{ijk}	2
"	MAG1	Maguga	2.9^{ghi}	1
"	MALAG1	Malangali	2.6^{jk}	0
"	ILE1	Ilembula	2.6^{jk}	3
"	IGAW1	Igawa	3.6^{bc}	2
"	MTWAG1	Mtwango	3.8^{ab}	3
"	NJB1	Njombe	3.9^{a}	0
Mean			**2.9**	**1.5**
std±			**0.9**	**1.2**
Mbeya	IGR1	Igurusi	3.0^{fgh}	3
"	CHL1	Chimala	3.2^{def}	2
"	MBZ1	Mbozi	3.8^{ab}	2
"	IMUZ1	Imuzi	2.5^{kl}	3
"	INYL1	Inyala	3.0^{fgh}	1
"	ILEJ1	Ileje	3.2^{def}	3
"	SEV1	Semvi	2.3^{l}	3
"	UYL1	Uyole	2.7^{ijk}	3
"	ILV1	Iluvi	2.6^{jk}	2
"	MBY1	Mbuyuni	3.9^{a}	3
Mean			**3.0**	**2.5**
std±			**0.5**	**0.7**
Overall mean			2.9	1.9
CV (%)			36.83	
SE±			0.61	

Means followed by the same letter within columns do not differ significantly according to DMRT. [1]GR=Growth rate (mm/day); std=Standard deviation; [2]QT2 (0-3) scale= Cercosporin Quantity Estimates, 0= Nill, 1= +, 2=++, 3=+++

Plate 6a

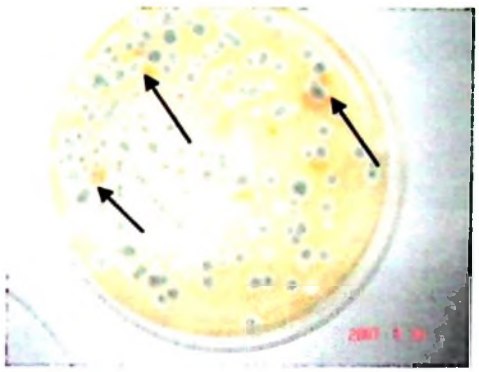


Plate 6b

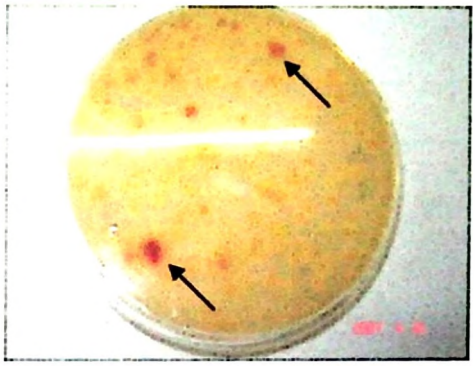


Plate 6c

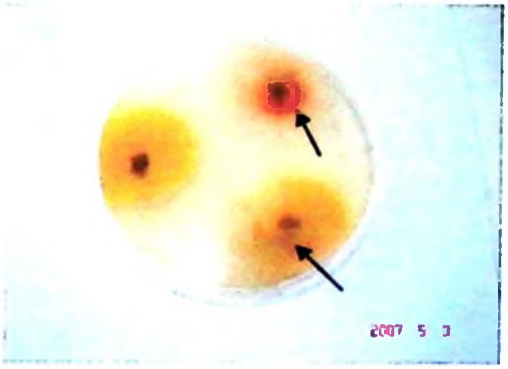


Plate 6d

Plate 6a-d: Arrows shows reddish purple cercosporin toxins from in cultures of *C. zeae maydis* on PDA.

4.1.3 Variations on isolate morphological variables

The analysis of variance (mean squares) for isolates morphological variables from samples collected in Morogoro, Iringa and Mbeya regions are shown on (Appendix 3). There were highly significant differences on morphological variables between isolates (Table 7). The number of conidiophores per stroma ranged between 3.3 for MBY1 (Mbeya region) to 9.2 for ILV1 also in Mbeya region (Table 4). The number of septa per conidiophore ranged between 2.2 for IFUN1 (Iringa region) to 7.8 for TRN1 (Morogoro region). Conidiophore diameter ranged from 2.6µ to 5.8µ.

Table 7: Pathogen morphological variables for isolates collected in Morogoro, Iringa and Mbeya regions (Studies conducted at SUA in 2008).

Region	Isolate	Village	CPN[1]	SN[2]	CPD[3] (u)	CPL[4]	SNC[5]	CND[6] (u)	CNL[7] (u)
Morogoro	MKY1	Mkuyuni	7.3^{cdefgh}	3.6^{lm}	3.2^{hij}	56.1^{hi}	8.4^{cdef}	3.7^{gh}	34.5^{m}
"	KLM1	Kilombero	8.1^{bcd}	4.6^{ijk}	5.1^{bc}	62.4^{fghi}	8.0^{def}	5.5^{cde}	47.5^{jk}
"	KIL1	Kilosa	4.3^{n}	4.6^{ijk}	4.8^{cd}	58.2^{ghi}	9.5^{ab}	3.5^{h}	17.7^{o}
"	MKI1	Mikumi	8.7^{ab}	6.3^{cde}	5.2^{abc}	69.4^{ef}	8.6^{bcde}	6.5^{abc}	55.8^{cde}
"	DOM1	Doma	8.2^{bc}	4.6^{ijk}	4.6^{cde}	80.7^{cd}	7.5^{fgh}	5.9^{bc}	54.9^{def}
"	TRN1	Turiani	7.6^{cdefg}	7.8^{a}	2.6^{j}	81.4^{cd}	6.8^{ghi}	5.9^{bc}	51.6^{gh}
"	MGT1	Mgeta	8.1^{bcd}	6.4^{cde}	4.3^{def}	69.7^{ef}	6.1^{ijk}	6.5^{abc}	45.6^{k}
"	MLI1	Mlali	4.6^{n}	3.7^{kl}	4.1^{efg}	52.9^{ij}	5.9^{ijk}	4.8^{def}	50.0^{hi}
"	TGN1	Tangeni	4.7^{mn}	4.8^{ghi}	5.2^{abc}	54.7^{hij}	6.7^{ghi}	4.6^{efg}	56.2^{cd}
"	DKW1	Dakawa	6.7^{ghij}	5.6^{efgh}	3.3^{hi}	83.3^{bcd}	7.6^{cfg}	5.6^{cde}	53.6^{efg}
Mean			**6.8**	**5.2**	**4.2**	**66.9**	**7.5**	**5.3**	**46.7**
std±			**1.7**	**1.3**	**0.9**	**11.7**	**1.2**	**1.1**	**12.1**
Iringa	ISMA1	Ismani	5.9^{jkl}	3.6^{lm}	3.3^{hi}	83.3^{bcd}	5.6^{ijk}	5.5^{cde}	60.9^{a}
"	TANA1	Tanangozi	7.6^{cdefg}	6.4^{cde}	5.2^{abc}	57.1^{ghi}	4.5^{lm}	6.9^{ab}	53.2^{fg}
"	IHE1	Ihemi	5.6^{klm}	5.1^{fghi}	4.8^{cd}	38.7^{k}	3.3^{m}	6.0^{bc}	48.9^{ij}
"	IFUN1	Ifunda	4.9^{mn}	2.2^{n}	3.4^{hi}	22.8^{l}	3.8^{mn}	4.4^{fgh}	37.0^{l}
"	MAG1	Maguga	5.0^{lmn}	4.7^{hij}	5.2^{abc}	41.7^{k}	3.4^{n}	3.7^{gh}	22.7^{n}
"	MALAG1	Malangali	6.0^{ijk}	3.8^{jkl}	3.3^{hi}	53.7^{ij}	8.8^{bcd}	3.8^{fgh}	35.0^{lm}
"	ILE1	Ilembula	6.3^{ijk}	5.2^{fghi}	5.8^{a}	66.1^{fg}	8.5^{bcdef}	5.7^{cd}	48.7^{ij}
"	IGAW1	Igawa	6.8^{fghij}	4.8^{ghi}	5.2^{abc}	57.6^{ghi}	10.4^{a}	3.7^{gh}	18.7^{o}
"	MTWAG1	Mtwango	7.7^{cdef}	6.8^{bc}	5.8^{a}	63.9^{fgh}	9.3^{bc}	6.8^{ab}	56.6^{bcd}
"	NJB1	Njombe	7.9^{bcde}	5.8^{def}	4.7^{cde}	83.8^{abcd}	7.6^{efg}	6.3^{abc}	49.2^{ij}
Mean			**6.4**	**4.8**	**4.7**	**56.9**	**6.5**	**5.3**	**43.1**
std±			**1.1**	**1.4**	**1.0**	**19.1**	**2.7**	**1.3**	**14.2**
Mbeya	IGR1	Igurusi	7.2^{defghi}	3.7^{kl}	3.7^{fgh}	80.9^{cd}	6.5^{bij}	5.7^{cd}	61.0^{a}
"	CHL1	Chimala	7.1^{efghi}	7.7^{ab}	2.8^{ij}	93.2^{a}	6.8^{ghi}	6.2^{abc}	53.7^{efg}
"	MBZ1	Mbozi	6.4^{hijk}	6.8^{bc}	4.7^{cde}	63.7^{fgh}	6.4^{ij}	6.9^{ab}	47.4^{jk}
"	IMUZ1	Imuzi	5.6^{klm}	5.7^{defg}	3.7^{fgh}	89.3^{abc}	4.3^{lmn}	6.8^{ab}	58.8^{ab}
"	INYL1	Inyala	7.0^{efghi}	6.6^{cd}	5.6^{ab}	64.5^{fgh}	5.3^{kl}	7.1^{a}	53.8^{efg}
"	ILEJ1	ilejc	7.8^{bcde}	5.5^{efhgi}	5.2^{abc}	45.3^{jk}	3.6^{mn}	6.8^{ab}	49.8^{hi}
"	SEV1	Semvi	7.0^{efgbj}	2.7^{mn}	3.6^{gb}	22.4^{l}	3.9^{mn}	4.7^{defg}	34.6^{m}
"	UYL1	Uyole	7.5^{cdefg}	3.8^{jkl}	3.4^{hi}	53.5^{ij}	8.5^{bcdef}	3.9^{fgh}	35.8^{lm}
"	ILV1	Iluvi	9.2^{a}	4.8^{ghi}	2.6^{j}	92.1^{ab}	6.7^{ghi}	5.9^{bc}	52.4^{g}
"	MBY1	Mbuyuni	3.3^{o}	5.5^{efghi}	3.5^{gh}	77.6^{de}	3.6^{mn}	6.8^{ab}	57.8^{bc}
Mean			**6.8**	**5.3**	**3.9**	**68.3**	**5.6**	**6.1**	**50.5**
Std±			**1.5**	**1.6**	**1.0**	**23.0**	**1.7**	**1.1**	**9.0**
Overall std±			1.4	1.3	1.0	18.6	2.0	1.3	12
CV (%)			15.49	18.97	13.17	16.96	16.25	18.17	13.39
SE±			0.58	0.56	0.44	6.53	0.66	0.64	4.69

Means followed by the same letter within columns do not differ significantly according to DMRT. [1]CPN/SRT=Conidiophores number per stroma; [2]SN=Septa number per conidiophore; [3]CPD=Conidiophore diameter; [4]CPL=Conidiophore length; [5]SNC=Septa number per conidia; [6]CND=Conidium diameter; [7]CNL=Conidium length.

4.1.4 Variations of isolates in colony culture characteristics

Most isolates were gray to light gray with compact, semi compact or loose texture with or without aerial growth (Plates 7a-f). Fascicles of conidiophore between isolates ranged from loose to compact (Table 8).

Table 8: Culture characteristics (color and texture), facsicles of conidiophores and cercosporin production for isolates collected Morogoro, Iringa and Mbeya regions (Studies conducted at SUA in 2008).

Region	Isolate Designation	Sampled area/village	Variable		
			Color[1]	Texture[1]	Fascicles[2]
Morogoro	MKY1	Mkuyuni	gray	semi compact	Loose
"	KLM1	Kilombero	dark gray	aerial	Loose
"	KIL1	Kilosa	light gray	compact	Compact
"	MKI1	Mikumi	gray	very aerial	Compact
"	DOM1	Doma	light gray	compact	Loose
"	TRN1	Turiani	gray	compact	Loose
"	MGT1	Mgeta	gray	compact	Compact
"	MLI1	Mlali	light gray	aerial	Loose
"	TGN1	Tangeni	light gray	compact	Compact
"	DKW1	Dakawa	gray	compact	Loose
Iringa	ISMA1	Ismani	gray	very aerial	Loose
"	TANA1	Tanangozi	gray	loose	Compact
"	IHE1	Ihemi	gray	aerial	Loose
"	IFUN1	Ifunda	gray	loose	Compact
"	MAG1	Maguga	gray	compact	Loose
"	MALAG1	Malangali	gray	semi compact	Loose
"	ILE1	Ilembula	gray	compact	Compact
"	IGAW1	Igawa	gray	very aerial	Loose
"	MTWAG1	Mtwango	gray	very aerial	Compact
"	NJB1	Njombe	gray	compact	Compact
Mbeya	IGR1	Igurusi	gray	compact	Compact
"	CHL1	Chimala	gray	loose	Compact
"	MBZ1	Mbozi	light gray	very aerial	Compact
"	IMUZ1	Imuzi	dark gray	compact	Loose
"	INYL1	Inyala	gray	very aerial	Compact
"	ILEJ1	ileje	light gray	loose	Loose
"	SEV1	Semvi	gray	compact	Loose
"	UYL1	Uyole	gray	very aerial	Loose
"	ILV1	Iluvi	light gray	loose	Loose
"	MBY1	Mbuyuni	light gray	loose	Loose

[1]On Potato Dextrose Agar; [2]Fascicles of conidiophores;

Plate 7a

Plate 7b

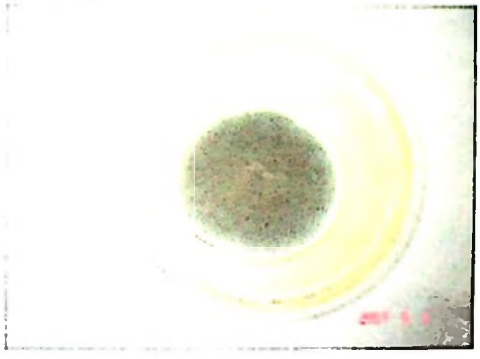


Plate 7c

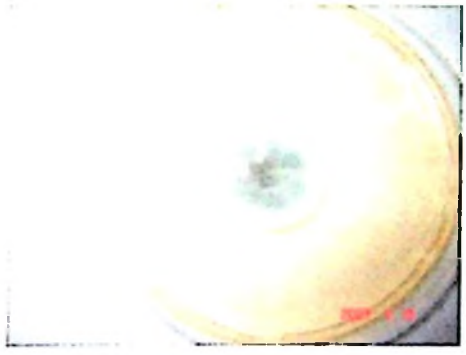


Plate 7d

Plate 7e

Plate 7f

Plate 7: A-F: Gray to light gray colonies of *C. zeae maydis* on PDA media.

4.1.5 Prevalence of gray leaf spot among established maize diseases

There were significant differences between locations for all diseases (Appendix 4). The severity of gray leaf spot and incidence of other diseases of maize assessed in Morogoro, Iringa and Mbeya regions is shown in Table 6. Higher levels of gray leaf spot severity were recorded in Iluvi (4.6); Imuzi (4.5) in Mbeya region and Njombe

(4.4) in Iringa region (Table 6). Generally, gray leaf spot was significantly ($P \leq 0.05$) more severe in sites surveyed in Mbeya and Iringa regions than in Morogoro region. Several other diseases were associated with gray leaf spot in the field, some were localized in certain areas and others present throughout production regions (Table 9). Leaf rust (*Puccinia polysora*) was the most common disease followed by southern leaf blight (*Bipolaris maydis*), northern leaf blight (*Exserohilum turcicum*), downy mildew (*Perenoslerospora maydis*); head smut (*Spathelotheca reilliana*) and stalk rot (*Gibberella zeae*) in that order. The incidence levels of common leaf rust (*Puccinia polysora*), northern leaf blight (*Exserohilum turcicum*) and southern leaf blight (*Bipolaris maydis*) were significantly higher ($P \leq 0.05$) at sites located in Morogoro than in the Iringa or Mbeya regions. The incidence levels of stalk rot (*Gibberella zeae*) were lower in sites located in Morogoro region than at sites located in Iringa and Mbeya regions ($P \leq 0.05$). The levels of incidence of downy mildew (*Perenoslerospora maydis*), head smut (*Spathelotheca reilliana*) and common leaf rust (*Puccinia polysora*) also varied between regions and locations (Table 9).

Table 9: Severity of gray leaf spot and incidence of other diseases of maize assessed in villages of Morogoro, Iringa and Mbeya regions in 2007/2008 cropping season.

Region	Villages	GLS[1] Severity (0-5)[2]	Incidence (%) C/Rust[3]	SLS[4]	NLB[5]	DMLD[6]	Smut[7]	S/rot[8]	Variety grown
Morogoro	Mkuyuni	2.3[l]	73.3c	75.4[ab]	68.1[ab]	15.1[lmj]	3.9[opqr]	7.9[opq]	Staha, Local
	Kilombero	2.0[m]	80 8[bc]	65.9[cde]	71.9[a]	12.0[lq]	2.9[pqr]	4.9[q]	Stuka, Local, TMV1
	Kilosa	3.2[ij]	83.7[b]	57.2[fgh]	59.2[ede]	19.6[op]	8.7[mn]	6.8[pq]	Kilima, Local
	Mikumi	2.3[l]	91.5[a]	79.7[a]	70.2[ab]	21.2[no]	12.6[klm]	6.8[pq]	Katumani, Kito,
	Doma	3.3[kl]	62.9[g]	57.8[fgh]	68.3[ab]	26.0[mn]	29 2[bcd]	12.2[klm]	TMV1, Paner, Local
	Turiani	4.2[bc]	77.3[d]	34 9[opq]	37.8[kl]	40.5[fghi]	20.2[ghi]	17.1[ghi]	Stuka, Local, KH628
	Mgeta	3.4[ghi]	36.8[mn]	48.8[ikl]	48.8[fgh]	45.0[def]	7.9[mno]	21.6[def]	Local, KH628
	Mlali	2 1[kl]	48.1[ij]	53.0[ghij]	50.9[fg]	25.7[mn]	2.2[qr]	7.6[opq]	Local, Stuka, Staha
	Tangeni	3.0[j]	53.5[g]	48 0[ikl]	60.3[cde]	17.7[opq]	1.6[r]	15.1[ijk]	Kilima, Kito, Paner
	Dakawa	2.6[k]	62.8[ij]	61.7[def]	67.9[b]	16 3[opq]	1.6[r]	18.3[fghi]	Local, Kito, Staha
	Mean	2.8	67.1	58.2	60.3	23.9	9.1	11.8	
	std±	0.7	17.3	13.3	11.3	10.9	9.2	5.9	
Iringa	Ismani	3.9de	70.6[e]	45.5[klm]	55.8[kl]	38.8[ghi]	20.0[ghi]	29.2[ab]	Local, UH6303,
	Tanangozi	3.4[ghi]	79.1[cd]	52.8[ghij]	72.2[jk]	41.2[efgh]	23.7[efg]	7.7[opq]	Local,hybrids, H628
	Ihemi	3.3[hi]	38.5[lm]	52.3[hijk]	66.4[hi]	46.8[cde]	34.1[a]	11.8[klmn]	Local, KH625, H628
	Ifunda	4.2[bc]	50 2[fg]	39.3[mnop]	49.4[lm]	35.5[hijk]	24 6[defg]	21.9[de]	Local, KH628, H625
	Maguga	3 5[fgh]	37.5[lmn]	59.3[efg]	63.9[jk]	41.4[efg]	14.0[ijk]	18.8[efg]	KH625, local, H625
	Malangali	3.6[fg]	72.5[e]	25.5[r]	57.3[hi]	45.6[cdef]	15.5[ijk]	16.1[hi]	Stuka, Local, KH625
	Ilembula	4.2[bc]	66.4[fg]	31.2[qr]	34.2[kl]	37.8[ghij]	33.3[ab]	30.2[a]	Local, UH615, H625
	Igawa	3.5[fgh]	44.7j[k]	41.0[mno]	43 1[ij]	43.0[efg]	27.4[cde]	23 4[cd]	Paner,Local, KH628
	Mtwango	4.2[bc]	35.6[mn]	49 2[ijkl]	52.3[f]	28.4[klm]	12.4[klm]	9.3[mnop]	Kilima, Local
	Njombe	4.4[ab]	27.4[o]	53.6[ghij]	49.0[fgh]	18 4[op]	9.2[lmn]	7.4[opq]	local, Paner, UH614
	Mean	3.8	52.3	45.0	54.4	37.7	21.4	17.6	
	std±	0.4	18.4	10.7	11.3	8.6	8.7	8.5	
Mbeya	Igurusi	3.6[fg]	43.9[k]	60.7[def]	57.8[de]	56.5[b]	25.7[def]	25.3[c]	Local, TMV, KH625
	Chimala	4.2[bc]	69.9[f]	55.8[fghi]	48.0[gh]	28.8[lm]	17.2[hij]	24.0[cd]	Local, Kilima, H615
	Mbozi	3.9[de]	63.0[g]	35.0[opq]	32.5[m]	30.1[klm]	24.2[efg]	15.7[hij]	UH6303, Local,
	Imuzi	4.5[a]	81.9[bc]	38.6[nop]	62.4[e]	62.5[a]	21.8[fgh]	32.5[a]	TMV1, Local Staha
	Inyala	3.2[ij]	48.3[ij]	32.9[pq]	56.9[e]	38.3[ghij]	30.9[abc]	10.01[mnop]	Paner,Local, KH625
	Ileje	3.6[fg]	53.3[h]	69.2[bc]	61.1[ef]	35.1[ijk]	7.2[nop]	19.6[efg]	UH 6303, Local, 625
	Semvi	4.1[cd]	34.5[n]	52 4[hij]	38.2[kl]	32.5[jkl]	5.5[nopqr]	12.7[jkl]	Local, Kilima
	Uyole	3.7[ef]	28.3[o]	67.2[cd]	59.3[cde]	26.4[mn]	6.8[nopq]	26.2[bc]	Local, Kito, TMV1
	Iluvi	4.6[a]	41.2[kl]	42 9[lmn]	62.0[e]	51.2[bc]	12.8[ikl]	10.41[mno]	Paner, Staha, Local,
	Mbuyuni	3.2[ij]	52.9[h]	56.2[fgh]	52.6[f]	49.1[cd]	22.1[fg]	8.7[nop]	Local, UH 615, H625
	Mean	3.9	51.7	51.1	53.1	41.1	17.4	18.5	
	std±	0.5	16.3	13.1	10.4	12.8	8.9	8.2	
Overall mean		3.5	57	51.4	56	34.2	16	16	
Overall std±		0.7	18.2	13.2	11.1	13	10.1	8	
CV (%)		6.62	7.47	14.39	7.82	18.47	29.61	22.6	
SE±		0.13	2.5	4.42	2.58	3.76	2.99	2.16	

Means followed by the same letter within columns do not differ significantly according to DMRT. [1]Gray leaf spot ; [2]0=no disease 1=1-20% ;2=21-40%; 3=41-60% 4=61-80% and 5=81-100%; [3]C/Rust=Common leaf rust (*Puccinia sorghi*); [4]SLS=Southern leaf spot (Bipolaris maydis); [5]NLS=Northern leaf blight (*Exerohilum turcicum*); [6]DMLD=Downy mildew (*Perenosclerospora maydis*); [7]Smut=Head smut (*Sphaerotheca reilliana*); [8]S/rot=Stalk rot (*Gibberella zeae*); std=Standard deviation

Correlations among diseases of maize in the field are shown on (Table 10). Downy mildew was significantly (P≤0.01) positively correlated to smut (r=0.648) and stalk rot (r=0.562). Similarly, southern leaf blight was positively correlated to turcicum leaf blight (r=0.538; P≤0.05). Gray leaf spot had the most positive correlation with downy mildew, smut and stalk rot although non significant relationship.

Table 10: Correlations among diseases of maize from field assessed data in villages of Morogoro, Iringa and Mbeya regions.

	C/RUST[1]	SLS[2]	NLS[3]	DMLD[4]	SMUT[5]	S/ROT[6]	GLS[7]
C/RUST	-						
SLS	0.162	-					
TLS	0.293	0.538*	-				
DMLD	-0.062	-0.284	0.033	-			
SMUT	0.059	-0.346	-0.039	0.648**	-		
S/ROT	-0.016	-0.229	-0.170	0.562**	0.372	-	
GLS	-0.197	-0.237	-0.030	0.434	0.435	0.370	-

*P≤0.05; **P≤0.01; df=18; [1]C/Rust=Common leaf rust (*Puccinia sorghi*); [2]SLS=Southern leaf spot (*Bipolaris maydis*); [3]NLS=Northern leaf spot (*Exerohilum turcicum*); [4]DMLD=Downy mildew (*Perenosclerospora maydis*); [5]Smut=Head smut (*Sphaerotheca reilliana*); [6]S/ rot=Stalk rot (*Gibberella zeae*); [7]Gray leaf spot.

4.2 Reaction of resistant and susceptible maize genotypes to *C. zeae-maydis* and associated disease resistance mechanisms

4.2.1 Symptom development

Symptoms of gray leaf spot appear in the leaves and were observed 10 to 13 days after inoculation as small tan or brown spots (Plate 8). Susceptible maize hybrid 'Paner' displayed necrotic rectangular lesions after infection that runs parallel to the leaf veins (Plate 9) while moderately resistant 'Staha' displayed chlorotic type of lesions (Plate 10). Resistant cv 'UH6303' showed mild symptoms (Plate 11).

Plate 8: Arrow showing early symptoms of gray leaf spot (tan to brown tiny spots) on leaves of cv 'Pannar'.

Plate 9: Arrow showing rectangular lesion of gray leaf spot running parallel to the leaf vain on leaf of susceptible maize 'Pannar'.

Plate 10: Arrow showing chlorotic lesions on leaf of moderately resistant cv 'Staha'.

Plate 11: Mild symptoms of gray leaf spot on leaves of resistant cv 'UH6303'.

4.2.2 Microscopic examination

Microscopic studies revealed no significant ($P \leq 0.05$) differences among genotypes in the number of spores germinated after 24, 36, 48, and 72 h after inoculation (Table 11).

Table 11: Percent spore germination 24, 36, 48 and 72h after inoculation (Studies conducted in 2009).

Genotype	% spore germination			
	24h	36h	48h	72h
UH6303	18.3	38.4	64.0	84.4
STAHA	18.2	38.9	64.1	84.1
PANNAR	18.5	38.4	64.5	84.4
Mean	18.3	38.6	64.2	84.3
CV(%)	2.01	1.87	2.14	1.01
$LSD_{0.05}$	0.51	1.00	1.94	1.20
SE±	0.210	0.412	0.794	0.493

The length of the germ tubes and the percent of germ tubes that formed mature appressorium at 24, 36, 48 and 72 h after inoculation also did not differ significantly ($P \leq 0.05$) between genotypes (Fig. 4 and Table 12).

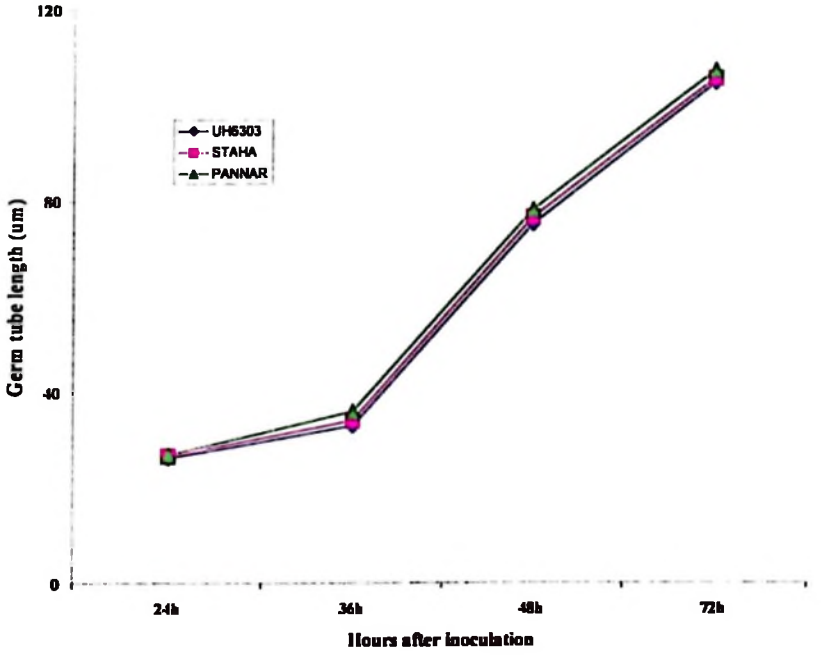


Fig. 4: The length of germ tubes 24, 36, 48 and 72h after inoculation on resistant ('UH6303'), moderately resistant ('Staha') and susceptible ('Pannar') maize genotypes.

Table 12: Percent germ tubes with mature appressorium 24, 36, 48 and 72h after Inoculation (Studies conducted in 2009).

Genotype	% germ tubes with mature appressoria			
	24h	36h	48h	72h
UH6303	47.6	57.4	73.1	84.1
STAHA	47.3	57.1	72.6	83.3
PANNAR	47.4	57.2	73.4	84.1
Mean	47.4	57.2	73.0	83.8
CV (%)	1.60	0.72	1.62	0.66
$LSD_{0.05}$	1.06	0.58	1.67	0.78
SE±	0.437	0.238	0.684	0.320

Pathogen units that established successfully after penetration differed significantly between genotypes (Table 13). Out of 60 germlings that penetrated the host 144 h after inoculation (Table 13), 24.6% produced hyphae greater than 30µ on resistant genotype 'UH6303' compared to 78.1% on susceptible genotype 'Paner', more than two-fold reduction of pathogen establishment on resistant vs susceptible maize genotypes (Table 13). In this study, an appresorium producing secondary hyphae greater than 30µ after penetrating the host 72, 96, 120 and 144 h after inoculation was considered to have established successfully.

Table 13: Percent germlings established in cells 72, 96, 120 and 144h after inoculation (Studies conducted in 2009).

Genotype	% germlings established[1]			
	72h	96h	120h	144h
UH6303	12.3	18.1	22.3	24.6
STAHA	26.7	40.2	47.3	50.5
PANNAR	55.4	67.4	72.5	78.1
Mean	31.5	41.9	47.4	51.1
CV (%)	2.46	1.45	1.13	0.96
$LSD_{0.05}$	1.09	0.97	0.85	0.78
SE±	0.448	0.351	0.310	0.284

[1]Percent germilings with extending hyphae >30µ in cells

Longer wefts were recorded in susceptible than resistant and moderate resistant genotypes. At 144h after inoculation hyphae wefts in cells of resistant genotype

"UH6303" were significantly (P≤0.05) shorter (14.1μ) than in moderate resistant genotype 'Staha' (39.1 μ) and susceptible genotype 'Pannar' (171.7 μ) (Fig 5).

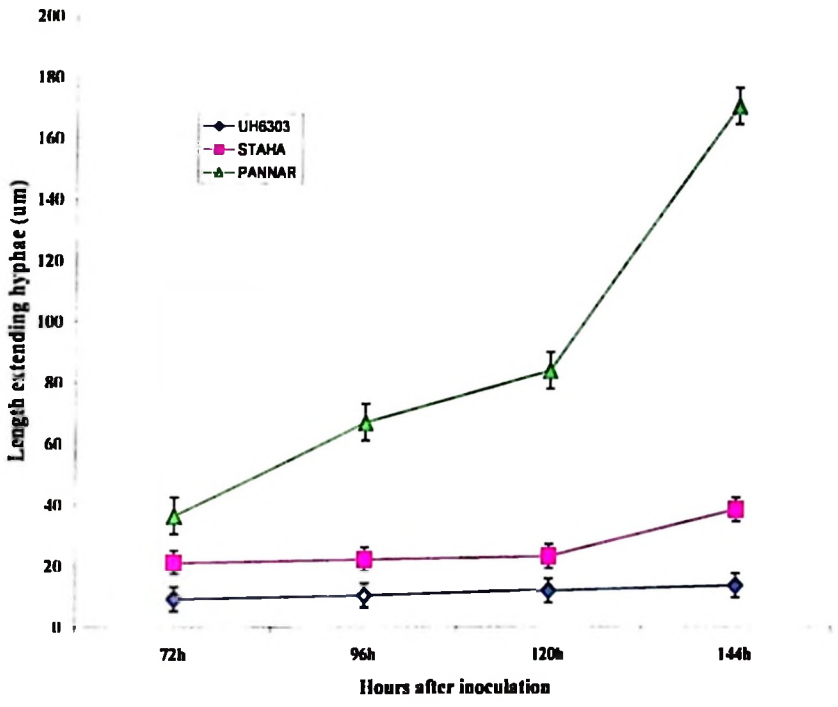


Fig. 5: The length of extending secondary hyphae in cells 72, 96, 120 and 144h after inoculation on resistant ('UH6303'), moderately resistant (Staha) and susceptible ('Pannar') maize genotypes.

The Lesion length, number of conidiophores per stroma and spore numbers/ cm^2 between genotypes differed (P≤0.05) ranging from 0.7 to 4.6 cm, 3.6 to 9.6, 2.7 to 6.1 respectively (Table 14). Susceptible maize hybrid 'Pannar' displayed necrotic rectangular lesions after infection while moderately resistant 'Staha' displayed chlorotic lesions.

Table 14: Lesion size, number of conidiophores per stroma and sporulation 16 days after inoculation (Studies conducted in 2009).

Genotype	LL (cm)[1]	No. conidiophore/stroma	SPN[2] x 10^4/Cm^2
UH6303	0.7	3.6	2.7
STAHA	1.6	4.8	4.0
PANNAR	4.6	9.6	6.1
Mean	2.3	6.0	4.3
CV (%)	12.34	8.43	10.25
$LSD_{0.05}$	0.40	0.71	0.62
SE±	0.166	0.293	0.254

[1] LL=Lesion length; [2]SPN=Spore number

4.3 Heritability and gene effect estimates for components of partial resistance to gray leaf spot and yield of maize.

4.3.1 Analysis of variance

The analysis of variance (mean squares) for gray leaf spot, components of resistance (lesion length, lesion size, lesion number, incubation period, disease severity) and yield for parents (P_1, P_2) and F_1, F_2, BC_1 and BC_2 is shown on Appendix 5. The main effects (generations, crosses, location) and generation x crosses were significant for all traits. Similarly generations x location interactions were significant except for incubation period. Components for partial resistance and yield differed significantly between generations (Appendix 5 and Table 15). Lesion size ranged from 8.6 cm^2 to 26.4 cm^2, lesion length from 3.6 to 12.8 cm, lesion number from 7.2 to 30.7 per 45 x 9 cm^2 of the leaf and incubation period from 7.7 to 11.3 days. Means of lesion size, disease severity, lesion length, lesion number, incubation period and yield for BC_1 generations were skewed toward the susceptible parent (P_1); and BC_2 towards the resistant parent (P_2). Mean yield for F_1 were superior to the better parent. Resistant maize inbred lines registered the lowest disease severity levels (Table 15).

Table 15: Means for gray leaf spot severity, components of resistance, yield of P_1, P_2 and F_1, F_2, BC_1 BC_2 generations conducted in 2008/09 cropping season (Means for two locations SUA and Bigwa)

Generation	No. of plants	Variable					
		[1]LS(mm^2)	[2]DS	[3]LL (mm)	[4]LN	[5]IP (days)	Yield (t ha^{-1})
P^1	40	26.4^a	3.2^a	12.8^a	30.7^a	7.6^d	3.9^e
P^2	40	8.6^f	1.7d^e	3.6^e	8.0^e	11.3^a	7.2^b
F^1	40	14.0^c	2.3^c	6.7^c	13.8^c	11.2^b	8.3^a
F^2	120	11.5^d	1.6^e	5.0^d	7.2^d	8.3^{cd}	7.1^b
BC^1	80	20.1^b	2.8^b	9.4^b	20.8^b	10.9^b	5.8^d
BC^2	80	10.5^e	2.0^{cd}	4.5^{de}	11.4^{de}	8.7^c	6.8^c
Mean		15.16	2.26	7.02	15.31	9.66	6.52
CV (%)		0.20	0.09	0.29	0.70	0.87	0.16
SE±		0.098	0.045	0.147	0.345	0.431	0.081
Mid-parent (m)		17.5	2.5	8.2	19.4	9.5	5.6

Means followed by the same letter within columns do not differ significantly according to DMRT; [1]LS=Lesion size; [2]DS=Disease severity; 0=no disease 1=1-20% ;2=21-40%; 3=41-60% 4=61-80% and 5=81-100%; DS=Disease severity; [3]LL=Lesion length; 4LN=Lesion number; [5]IP=Incubation period; P_1 =Resistant Parent; P_2 =Susceptible parent; F1 =First filial generation; F_2=Second filial generation; BC_1 =First backcross generation; BC_2=Second backcross generation; SE± = Standard error of the mean

The effect of crosses on gray leaf spot, components of partial resistance and yield are shown in Table 16. Cross 2 had lowest values for all components except incubation period and it displayed the highest grain. Highest values for all components except incubation period were recorded in cross 3 which also registered the lowest yields. F_2 and BC_1 generations had lowest disease severity records with higher yields (Table 15).

Table 16: Effect of crosses on gray leaf spot severity, components of resistance and yield studies conducted in 2008/09 cropping season (Means regardless of generations in two locations SUA and Bigwa).

Crosses	Variable					
	[1]LS (mm^2)	[2]DS	[3]LL (mm)	[4]LN	[5]IP (days)	Yield (t ha^{-1})
1. (P103 x CML395-5)	15.1	2.0	6.9	14.3	10.2	6.0
2. (K36 x L37)	14.6	1.2	5.2	10.2	11.4	7.8
3. (K36 x CML395-5)	15.8	2.4	7.3	15.90	8.2	5.1
Mean	15.17	2.26	7.07	15.31	10.15	6.51
CV (%)	3.18	6.98	7.23	7.81	14.71	4.34
Lsd $_{(0.05)}$	0.28	0.07	0.21	0.49	0.61	1.18
SE±	0.139	0.032	0.105	0.24	0.305	0.58

Generation x cross interactions were significant ($P \leq 0.05$) for all variables. The longest incubation period was associated with the highest yields. F^1 registered the highest yields in all crosses while the lowest yields were registered in P_1 for cross 3 (Table 17).

Table 17: Combinations of cross x generation on gray leaf spot severity, components of resistance and yield conducted in 2008/09 cropping season (Means for two locations SUA and Bigwa).

		Variable					
crosses	Generation	[1]LS (mm^2)	[2]DS	[3]LL (mm)	[4]LN	[5]IP (days)	Yield ($t\ ha^{-1}$)
1. (P103 x CML395-5)	P^1	27.2^{a}	3.0^{bc}	12.4^{b}	27.8^{b}	8.3^{k}	3.7^{i}
	P^2	7.3^{m}	1.6^{ij}	3.7^{ij}	8.4^{g}	15.0^{a}	7.0^{d}
	F^1	12.1^{g}	1.6^{ij}	5.4^{f}	7.6^{gh}	11.6^{f}	8.4^{a}
	F^2	14.1^{e}	2.4^{e}	6.6^{e}	13.2^{e}	8.7^{j}	6.8^{d}
	BC^1	19.9^{d}	2.9^{cd}	9.3^{d}	19.7^{d}	9.7^{i}	5.7^{g}
	BC^2	11.0^{i}	2.0^{g}	4.2^{gh}	11.5^{f}	8.4^{ijk}	6.7^{de}
2. (K36 x L37)	P^1	25.2^{b}	3.1^{b}	12.8^{a}	6.3^{ij}	12.8^{e}	4.9^{h}
	P^2	8.5^{l}	1.5^{j}	3.4^{j}	7.3^{ghi}	14.4^{bc}	7.7^{bc}
	F^1	10.8^{i}	1.7^{hi}	2.2^{l}	6.5^{hij}	14.2^{c}	8.4^{a}
	F^2	13.6^{f}	0.2^{k}	2.9^{k}	6.6^{hij}	13.4^{d}	8.0^{b}
	BC^1	20.4^{c}	1.8^{h}	3.9^{hi}	10.6^{f}	12.6^{e}	6.3^{f}
	BC^2	9.1^{k}	1.8^{h}	4.4^{g}	5.6^{j}	14.6^{b}	7.4^{c}
3. (K36 x CML395-5)	P^1	26.9^{a}	3.5^{a}	13.1^{a}	33.1^{a}	7.9^{j}	3.3^{j}
	P^2	10.0^{j}	1.8^{h}	3.8^{j}	8.3^{g}	13.5^{d}	6.9^{d}
	F^1	11.5^{h}	2.3^{ef}	5.4^{f}	7.5^{gh}	10.7^{g}	8.0^{b}
	F^2	14.3^{e}	1.6^{ij}	6.8^{e}	13.7^{e}	7.8^{l}	6.3^{f}
	BC^1	20.1^{cd}	2.8^{d}	10.0^{c}	22.0^{c}	10.3^{h}	5.8^{g}
	BC^2	11.9^{g}	2.2^{f}	5.2^{f}	11.1^{f}	8.1^{kl}	6.4^{ef}
Mean		15.2	2.26	7.08	15.31	10.16	6.52
CV (%)		3.18	6.98	7.23	7.81	14.71	4.34
SE±		0.241	0.079	0.256	0.598	0.746	0.141

Means followed by the same letter within columns do not differ significantly according to DMRT; [1]LS=Lesion size; [2]DS=Disease severity 0=no disease 1=1-20% ;2=21-40%; 3=41-60% 4=61-80% and 5=81-100%; [3]LL=Lesion length; [4]LN=Lesion number; [5]IP=Incubation period; P_1 =Resistant Parent; P_2 =Susceptible parent; F_1 =First filial generation; F2 =Second filial generation; BC_1 =First backcross generation; BC_2 =Second backcross generation; SE± = Standard error of the mean.

Only lesion length (LL) showed consistent ranking of generations in all crosses while other variables indicated rank changes across generations. However, lesion size indicated consistent ranking in generations in crosses 1 and 2 while lesion number showed consistency in generations 1 and 3 (Table 17). Highest grain yields (7.9t/ha)

were obtained at Bigwa (Table 18) which was also associated with higher incubation period and lower lesion size, disease severity, lesion length, and lesion number. Lesion length in crosses 1 and 2 showed consistency of ranking at SUA while lesion size in crosses 2 and 3 showed consistency of ranking at Bigwa. Lesion size gave highest mean variances for cross 1 at SUA and Bigwa while the lowest mean variance was recorded in cross 3 for disease severity at SUA and Bigwa (Tables 19 and 20).

Table 18: Effect of location on gray leaf spot severity, components of resistance and yield conducted in 2008/09 cropping season.

	Variable					
Location	[2]LS (mm^2)	[3]DS	[4]LL (mm)	[5]LN	[6]IP (days)	Yield ($t\ ha^{-1}$)
SUA farm	15.7	2.6	7.3	16.1	9.8	6.4
Bigwa farm[1]	14.2	2.2	6.7	14.4	10.5	7.9
Mean	15.17	2.27	7.08	15.32	10.16	6.51
CV (%)	3.18	6.98	7.23	7.81	14.71	4.34
Lsd $_{(0.05)}$	0.16	0.05	0.17	0.40	0.50	0.16
SE±	0.08	0.026	0.085	0.199	0.249	0.08

[1]SUA=Sokoine University of Agriculture Farm; [2]LS=Lesion size; [3]DS=Disease severity 0=no disease 1=1-20%; 2=21-40%; 3=41-60% 4=61-80% and 5=81-100%; [4]LL=Lesion length; [5]LN=Lesion number; [6]IP=Incubation period; LSD=Least significant difference; SE± = Standard error of the mean

Table 19: Generation means, standard errors and variances for P_1, P_2, and F_1, F_2, BC_1 BC_2 generations of three crosses evaluated at SUA farm (Studies conducted in 2008/09 cropping season).

Generation	Cr1 (P103 x CML395-5)		Cr2 (K36 x L37)		Cr3 (K36 x CML395-5)	
	Mean+SE±	Variance	Mean+SE±	Variance	Mean+SE±	
Lesion size			Lesion size		Lesion size	
P_1	29.9± 0.27	2 82	P_1	26.3± 0.09	0.32	P_1 28.3±0.31
P_2	7.3± 0 24	2.34	P_2	9.8± 0 25	2.54	P_2 5.4± 0.09
F_1	13 3± 0.36	5.3	F_1	12.5± 0.23	2.13	F_1 14.4± 0.03
F_2	12 3± 0.85	6.1	F_2	10 5± 0.12	3.7	F_2 11.7± 0.12
BC_1	10.6± 0 17	2.7	BC_1	20.1± 0.19	4.1	BC_1 20.8± 0.16
BC_2	19 8± 0.35	5.7	BC_2	10.6± 0.10	0.83	BC_2 8.1± 0.05
Mean		4.16			2.27	
Disease[2]			Disease[2]		Disease[2]	
P_1	2.2± 0.06	0.14	P_1	2.9± 0.07	0.17	P_1 3.9± 0.04
P_2	1 6± 0 15	0 89	P_2	1 8± 0 026	0.03	P_2 1.6± 0.03
F_1	2 0± 0.09	0 29	F_1	2.1± 0.05	0 09	F_1 2.3± 0.02
F_2	1.5± 0.02	2.4	F_2	2.2± 0 03	1.6	F_2 1.9± 0.03
BC_1	2.4± 0 04	1.5	BC_1	2.9± 0 04	0.4	BC_1 2.2± 0.03
BC_2	1 8± 0 03	1.3	BC_2	2.0± 0.04	0.12	BC_2 1.8± 0.02
Mean		1.09			1.4	
Lesion length			Lesion length		Lesion length	
P_1	11 3± 0 11	0.45	P_1	12 8± 0 08	0.23	P_1 13.6± 0.60
P_2	3 3± 0.06	0.15	P_2	3.0± 0 07	0.19	P_2 3.4± 0.13
F_1	6.9± 0 18	1 35	F_1	6.1± 0.11	0.5	F_1 6.8± 0.09
F_2	5 1± 0 06	3.2	F_2	5 0± 0.06	2.7	F_2 5.2± 0.29
BC_1	8 3± 0.10	0.78	BC_1	9 3± 0.11	1.02	BC_1 9.4± 0 12
BC_2	4 5± 0 08	0.48	BC_2	4.9± 0 07	1.4	BC_2 4.3± 0.13
Mean		1.07			1.01	
Lesion number			Lesion number		Lesion number	
P_1	29.5± 0.28	3 13	P_1	22.1± 0.31	3.82	P_1 35.4± 0.52
P_2	7 9± 0.23	2.81	P_2	6.1± 0 14	0.79	P_2 7.6± 0.15
F_1	14 1± 0.12	0.57	F_1	13.5± 0.16	1 07	F_1 14.1± 0.11
F_2	7 1± 0.12	4.3	F_2	8 1± 0 11	4.3	F_2 6.8± 0.13
BC_1	20 8± 0 26	5.36	BC_1	18.8± 0.01	3.6	BC_1 21.1± 0.63
BC_2	10 6± 0 15	1.4	BC_2	10 0± 0.12	0.8	BC_2 7.2± 0.07
Mean		2.93			2.4	
Incubation[3]			Incubation[3]		Incubation[3]	
P_1	7 99 0 04	0.08	P_1	9.2± 0.18	1.3	P_1 7.0± 0.10
P_2	13.0± 0.13	0.67	P_2	11.8± 0.21	1.69	P_2 14.5± 0.11
F_1	10 9± 0.13	0.56	F_1	8.8± 0 13	0.67	F_1 10.8± 0.10
F_2	8.0± 0 05	1.7	F_2	7.4± 0.08	2.9	F_2 7.7± 0.06
BC_1	11.7± 0.010	1.71	BC_1	10.2± 0.11	1.9	BC_1 9.1± 0.09
BC_2	9.2± 0.11	1.02	BC_2	8.2± 0.07	1.42	BC_2 9.6± 0.08
Mean		0.96			1.65	

[1]SUA=Sokoine University of Agriculture Farm; [2]Disease severity; [3]Incubation period (days); P_1 =Resistant Parent; P_2 =Susceptible parent; F_1 =First filial generation; F_2 =Second filial generation; BC_1 =First backcross generation; BC_2 =Second backcross generation

Table 20: Generation means, standard errors and variances for P_1, P_2, and F_1, F_2, BC_1 BC_2 generations of three crosses evaluated at Bigwa farm conducted in 2008/09 cropping season. (Avarage of two locations SUA and Bigwa).

Generation	Cr1 (P103 x CML395-5)		Cr2 (K36 x L37)			Cr3 (K36 x CML395-5)		
	Mean+SE±	Variance		Mean+SE±	Variance		Mean+SE±	Variance
Lesion size			Lesion size			Lesion size		
P_1	25.5± 0 39	6.02	P_1	27.5± 0.34	4.63	P_1	27.5± 0.13	0.7
P_2	8 2± 0.41	0.79	P_2	9.3± 0 34	2.53	P_2	9.0± 0.29	3.45
F_1	15.3± 0 17	1.15	F_1	15.5± 0.16	0.96	F_1	15.1± 0.15	0.85
F_2	10.2± 0.12	6 1	F_2	11 8± 0.11	3.7	F_2	11.4± 0.16	3.8
BC_1	20.8± 0.19	2 6	BC_1	19.9± 0.46	4	BC_1	21.5± 0.38	3.7
BC_2	10.5± 0.41	5.6	BC_2	8.7± 0.13	1	BC_2	7.8± 0.15	1
Mean		**3.71**			**2.8**			**2.25**
Disease[1]			Disease[1]			Disease[1]		
P_1	3 2± 0.05	0.09	P_1	3.1± 0.07	0.18	P_1	3.3± 0..09	0.31
P_2	1.7± 0.03	0.03	P_2	1.8± 0.03	0.04	P_2	1.7± 0.03	0.05
F_1	2 4± 0.05	0.11	F_1	2.5± 0.05	0.11	F_1	2.6± 0.05	0.1
F_2	1 6± 0.02	2 4	F_2	1.6± 0.02	1.6	F_2	1.5+0.02	0.25
BC_1	2 5± 0.02	1 3	BC_1	2.7± 0.51	0.4	BC_1	2.7± 0.03	0.26
BC_2	1 7± 0 02	1 2	BC_2	1 4± 0.02	0.04	BC_2	1 8± 0.02	0.04
Mean		**0.86**			**0.4**			**0.17**
Lesion length			Lesion length			Lesion length		
P_1	15 7± 0.16	1.05	P_1	15.3± 0.10	0.43	P_1	15.5± 0.14	0.78
P_2	3 6± 0 15	0.88	P_2	4 7± 0.10	0.42	P_2	4.3± 0.12	0.53
F_1	8 5± 0.19	1.46	F_1	8.0± 0.17	1.14	F_1	6.4± 0.19	1.38
F_2	5 1± 0.08	3 3	F_2	5 1± 0.09	2.7	F_2	5.1± 0.08	2
BC_1	10.7± 0.15	3 5	BC_1	9.5± 0.19	2.1	BC_1	8 7± 0.13	1.3
BC_2	6 0± 0 14	1 7	BC_2	6.0± 0.07	1 4	BC_2	4.4± 0.09	1.03
Mean		**1.98**			**1.37**			**1.17**
Lesion number			Lesion number			Lesion number		
P_1	32 4± 0.28	3.09	P_1	34.7± 0 35	4.84	P_1	36.2± 0.20	1.52
P_2	10 4± 0 17	1 19	P_2	11.0± 0.19	1.48	P_2	8.9± 0.14	0.82
F_1	13.4± 0.29	3.43	F_1	15 3± 0.14	0.78	F_1	13.1± 0.14	0.74
F_2	5 5± 0.09	4.2	F_2	8 0± 0.14	4.3	F_2	6.3± 0.10	2.98
BC_1	20 7± 0.021	4 4	BC_1	23 5± 0.37	3 6	BC_1	22..9± .15	2.8
BC_2	10.7± 0 19	1 4	BC_2	14.6± 0 17	0.8	BC_2	11.9± 0.11	0.74
Mean		**2.95**			**2.63**			**1.6**
Incubation[2]			Incubation[2]			Incubation[2]		
P_1	9.1± 0.08	0.28	P_1	10.0± 0 15	0.93	P_1	9 1± 0.09	0.34
P_2	14 8± 0.12	0.58	P_2	14.9± 0.09	0.35	P_2	15.4± 0.09	0.29
F_1	11.0± 0 15	0.92	F_1	12.4± 0.53	2.56	F_1	12.1± 0.07	0.22
F_2	8 6± 0 10	1 7	F_2	7.9± 0.09	2.9	F_2	9.3± 0.06	0.8
BC_1	13.4± 0.08	1.7	BC_1	10.4± 0.09	1.9	BC_1	0.9± 0.09	0.74
BC_2	7.8± 0.11	0.8	BC_2	7.1± 0.07	1.4	BC_2	8.1± 0.06	0.39
Mean		**1.0**			**1.7**			**0.5**

[1]Disease severity; [2]Incubation period; P_1 =Resistant Parent; P_2 =Susceptible parent; F_1 =First filial generation; F_2 =Second filial generation; BC_1 =First backcross generation; BC_2 =Second backcross generation.

4.3.2 Test of the additive-dominance model

The results of the three parameter model (Individual-scaling test) and Joint-scaling-test (chi-square goodness of fit test) are shown on Table 21. Estimates of gene effects based on six parameter model are shown on Table 22. Additive-dominance model by the Individual-scaling test was inadequate for all components except for disease severity and lesion number in cross 2 (Table 21). The Joint-scaling test (chi-square) confirmed the results of individual-scaling test. The model gave significant χ^2 values in all components except for disease severity and lesion number in cross 2 as in the individual scaling test hence judged inadequate to explain for the variation in the former groups.

Table 21: Joint-scaling test (chi-square) and Individual-scaling tests (A, B, C) of the fit of the additive-dominance model for heritability of components of partial resistance to gray leaf spot conducted in 2008/09 cropping season. (Average of two locations SUA and Bigwa)

Variable[3]	Procedure	Cr1 (P103 x CML395-5)	Cr2 (K36 x L37)	Cr3 K36 x CML 395-5
Lesion size	Individual scaling-test			
	A	-3.67+0.56**	1.36+0.84	-1.65
	B	19.02+0.82**	-16.47+0.40**	-3.60+0.14**
	C	-3.83	19.28+0.73**	2.20+0.89
	Joint scaling-test $\chi^2(3)^1$	7.9*	12.4**	8.6*
Disease severity	Individual scaling-test			
	A	0.71+0.51	0.70+0.40	0.83+0.12**
	B	0.02	0.10+0.61	-0.07
	C	1.55+0.25**	0.60+0.95	0.18+0.03**
	Joint scaling-test $\chi^2(3)^1$	7.9*	2.8 (NS)	8.2*
Lesion length	Individual scaling-test			
	A	-1.52+0.29*	-0.09	-1.70+0.08**
	B	-1.18+0.25**	0.74+0.53	-0.76
	C	-7.83+0.46**	-7.39+0.34**	-9.50+0.14**
	Joint scaling-test $\chi^2(3)^1$	17.7***	21.3***	18.7***
Lesion number	Individual scaling-test			
	A	-1.13	-1.13	-6.00+0.75**
	B	-0.54	0.41+0.30	-12.00+0.24**
	C	3.06+0.66*	-0.95	7.00+0.79**
	Joint scaling-test $\chi^2(3)^1$	7.9*	3.2 (NS)	12.8**
Incubation period	Individual scaling-test			
	A	2.17+0.95	2.24+1.31	-6.33+0.36**
	B	-3.48+0.08**	-0.29	-1.64
	C	8.79+0.38**	-9.86+0.38**	-7.81+0.64**
	Joint scaling-test $\chi^2(3)^1$	9.1*	8.6*	11.6**

*P≤0.05; **P≤ 0.01; ***P≤0.001; NS= Non-significant; [1](3) = 3 degree of freedom;
[3]Variables= Components of partial resistance

The six-parameter model test (Table 22) performed where the additive-dominance model did not show conformity revealed significant higher level of gene interactions for individual components as follows.

4.3.3 The six parameter model test

4.3.3.1 Lesion size

The additive effect was significant for all crosses while dominance effect was significant for only two crosses (2 and 3). The significance of digenic interactions was not consistent in all crosses. Crosses 2 and 3 displayed significant negative dominance effects (Table 22).

4.3.3.2 Disease severity

The additive and dominance effects were significant in all crosses while additive x additive digenic interaction was significant for crosses 1 and 3. In cross 1 all types of gene effects tested were significant for this variable. Crosses 1 and 2 showed significant negative dominance effects (Table 22).

4.3.3.3 Lesion length

The additive and dominance effects were significant in all crosses while the additive x additive digenic interaction was significant in crosses 1 and 2 and additive x dominance interaction in crosses 2 and 3. Crosses 1 and 2 displayed significant negative dominance effects (Table 22).

4.3.3.4 Lesion number

Additive and dominance effects were significant in all crosses. Additive x additive digenic interaction was significant for crosses 1 and 3 while dominance x dominance interaction was significant in cross 3. Crosses 2 and 3 displayed significant negative dominance effects (Table 22).

4.3.3.5 Incubation period

Dominance effect was significant and negative in all crosses while the additive effect was negative and significant only in cross 1. Additive x additive digenic interaction was significant for crosses 1 and 2 while dominance x dominance and additive x dominance were significant in crosses 1 and 3 (Table 22).

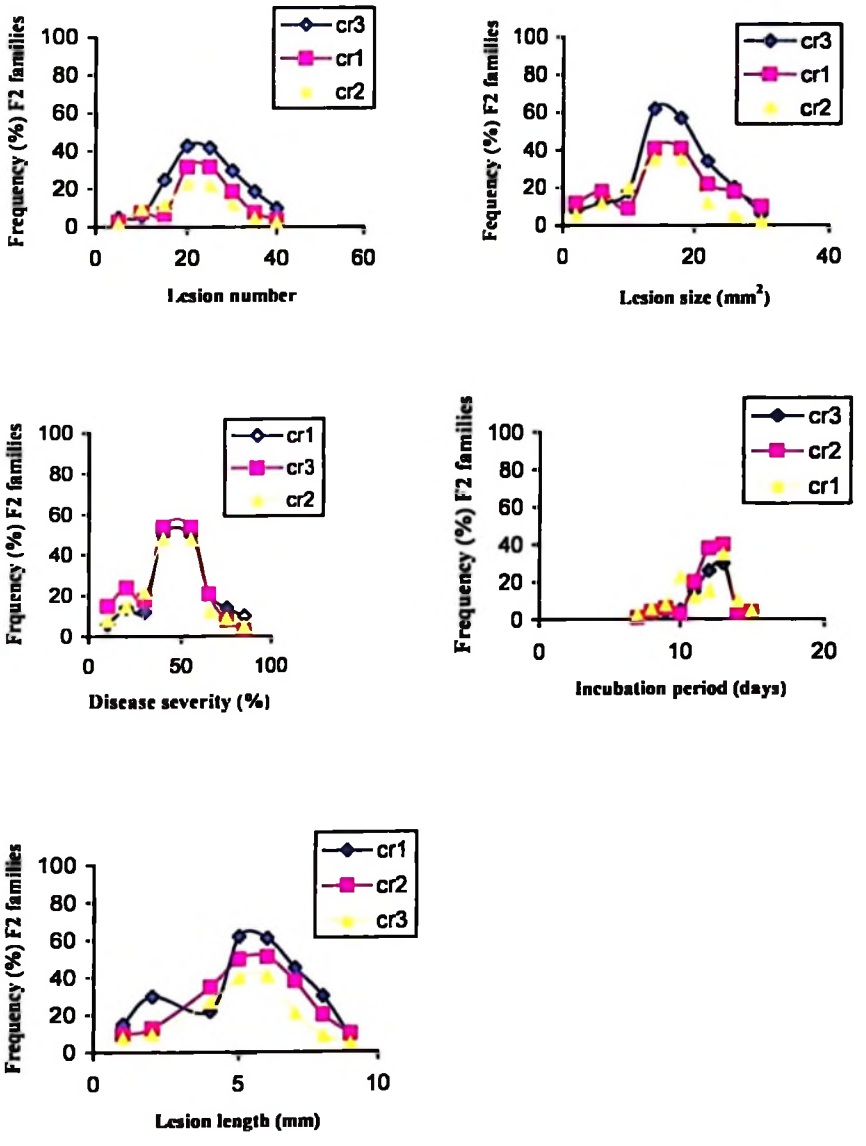
Table 22: Estimate of additive, dominance and epistatic effects (and standard errors) for components of partial resistance to gray leaf spot in three crosses conducted in 2008/09 cropping season (Avarage of two locations SUA and Bigwa).

		Cr1	Cr2	Cr3
Trait	Gene effect	(P103 x CML395-5)	(K36 x L37)	K36 x CML 395-5
Lesion size	m	7.0± 3.53	-1.3± 0.68	6.6± 0.37**
	a	11.3± 0.18**	8.2± 0.19*	12.7± 0.16**
	d	-14.5± 7.17	-8.9± 1.67**	-12.6± 1.48**
	aa	8.6± 3.40	19.4± 0.65**	11.5± 0.58**
	dd	8.1± 0.52**	2.6± 0.50*	-2.7± 0.96
	ad	8.6± 3.75	19.6±1.32**	4.5± 0.89
Disease severity	m	-0.3± 0.02**	2.3± 0.17**	-0.8± 0.15**
	a	0.3± 0.01***	2.6± 0.03**	0.5± 0.02**
	d	-5.3± 0.41**	-6.8± 0.43**	6.5± 0.35**
	aa	2.3± 0.13**	-	2.6± 0.16**
	dd	0.7± 0.18*	-	0.8± 0.69
	ad	3.0± 0.32**	-	3.6± 1.22
Lesion length	m	2.2± 0.36**	-0.5± 0.03**	2.0± 0.84
	a	3.9± 0.06**	4.9± 0.31**	5.2± 0.04**
	d	-7.9± 0.94**	-15.9± 0.94**	8.5± 0.77**
	aa	5.1± 0.35**	8.6± 0.03**	2.6 ± 0.88
	dd	0.4± 0.18	-1.0± 0.35	-0.1± 0.07
	ad	5.8± 2.46	9.0± 0.62**	3.4± 0.39**
Lesion number	m	-15.5± 0.80**	-10.9± 0.33**	16.6± 0.63-**
	a	10.8± 0.18**	14.9± 0.02**	13.8± 0.03**
	d	14.8± 1.49**	-22.0± 1.40**	-21.2± 1.17**
	aa	-8.3± 0.77**	-	17.0± 0.45**
	dd	1.3± 0.66	-	8.2± 0.22**
	ad	1.1± 0.35	-	1.2± 0.62
incubation	m	1.1± 0.39	2.9± 0.45**	9.2± 0.44**
	a	-2.9± 0.07**	-1.3± 0.84	-2.5± 0.89
	d	-17.8± 1.02**	-11.3± 1.12**	-7.7± 1.07**
	aa	-26.9± 0.36**	-24.6± 0.46**	3.4± 1.72
	dd	11.1± 0.33**	2.1± 0.78	11.5± 0.29**
	ad	6.60± 0.70**	3.6± 1.72	12.1± 0.86**

**P≤P 0.01; m= Mid-parent; a=Additive effect; d=Dominance effect; aa= Additive x additive; dd= Dominance x dominance; ad= Additive x dominance

4.3.4 Frequency distribution

The relative distributions of F_2 individuals fairly fitted a continuous distribution curve for all crosses in all variables (Fig. 6). Cross 1 displayed a form of two peak curves for lesion length and lesion number, lesion size and disease severity.



Figures 6: Frequency distribution curves of F_2 families for lesion number, lesion size, disease severity, incubation period and lesion length

4.3.5 Heritability

Broad sense heritability (h^2_b) and narrow sense heritability (h_2n) for components of partial resistance are shown on Table 23. Heritability estimates ranged from 0.41 to 0.88 for broad sense heritability and 0.21 to 0.76 for narrow sense in all crosses. High values of broad and narrow sense heritability were recorded on disease severity and lesion length. Moderate to high heritability values were recorded in all components for all three crosses. High heritability estimates (>50%) were consistently recorded for disease severity with narrow sense heritability being higher ranging 80-88% for all crosses. On the other hand incubation period registered low to medium heritability, except for the broad sense heritability (54%) in cross 3. Other variables indicated high heritability except for narrow sense (45%) of lesion size in cross 2 and cross 1 for lesion length (42%) and lesion number (43%).

Table 23. Broad (h^2b) and narrow (h^2n) sense heritability estimates for components of partial resistance to gray leaf spot in three crosses (Studies conducted in 2008/09 cropping season).

	Cr1 (P103 x CML395-5)		Cr2 (K36 x L37)		Cr3 K36 x CML 395-5	
Variable	h^2b	h^2n	h^2b	h^2n	h^2b	h^2n
Lesion size	0.65	0.50	0.64	0.45	0.76	0.58
Disease severity	0.88	0.71	0.88	0.54	0.80	0.76
Lesion length	0.58	0.42	0.83	0.70	0.84	0.75
Lesion number	0.62	0.43	0.62	0.52	0.81	0.52
Incubation period	0.41	0.21	0.41	0.38	0.54	0.38

4.3.6 Heterosis

Mid-parent heterosis between generations ranged from -3.5 to -2.6 for lesion size, 0.3 to 0.9 for disease severity, -3.7 to 1.2 for lesion length, -8.0 to 12.2 for lesion number, -0.8 to 1.7 for incubation period and 0.2 to 2.7 for yield and the % heterosis between generations ranged from -40 to 14.9 for lesion size, -36.0 to 12.0 for disease severity, -39.0 to 14.6 for lesion length, -62.9 to 7.2 for lesion number, -12.6 to 17.9 for incubation period and 3.6 to 48.2 for yield (Table 24). Significant heterosis was

observed in all generations and for all variables (Table 24).

Table 24. Heterotic effects and relative % heterosis for various variables in different generations conducted in 2008/09 cropping season (Means for two locations SUA and Bigwa).

Generations	LS[1]	DS[2]	LL[3]	LN[4]	IP[5]	Yield t/ha
	[6]dfm	dfm	dfm	dfm	dfm	dfm
F_1	-3.5***	-0.5***	-1.5***	-5.6***	1.7**	2.7***
	(-20.0)	(-8.0)	(-18.3)	(-28.9)	(-17.9)	(-48.8)
BC_1	2.6**	0.3***	1.2**	1.4***	1.4**	0.2**
	(-14.9)	(-12.0)	(-14.6)	(-7.2)	(-14.7)	(-3.6)
BC_2	-7.0***	-0.5***	-3.7***	-8.0***	-0.8*	1.2**
	(-40.0)	(-20.0)	(-45.1)	(-41.2)	(-8.4)	(-21.4)
F_2	-6.0***	-0.9***	-3.2***	12.2***	-1.2**	1.5***
	(-34.3)	(-36.0)	(-39.0)	(-62.9)	(-12.6)	(-26.8)

[1]LS=Lesion size; [2]DS=Disease severity; [3]LL=Lesion length; [4]LN=Lesion number; [5]IP=Incubation period; [6]dfm=Deviation from mid-parent; Means in parentheses are Percent deviation from mid-parent; *P≤0.05; **P≤ 0.01; ***P≤0.001

4.4 The effects of organic fertilizers (composted cattle and poultry manure) on development of gray leaf spot and yield of maize

4.4.1 Soil and compost manure nutrient composition

The result of soil analysis of the study area at the beginning of the experiment is shown in Table 25. The organic carbon (1.4 %), organic matter (0.6%) and total nitrogen (0.1%) were rated as very low according to London, (1991) criterion of classification; CEC (12.8), available P (3.2%), and % BS (34.5%) were rated as low. The soil pH (5.8) was rated as acidic. Exchangeable cations ranged from 0.15 mg kg^{-1} for Mg^+ to 3.2 mg kg^{-1} for K^+.

Soil properties and soil properties x fertilizer interaction were highly significant (P≤0.001) for soil analyzed at the end of the experiment (Appendix 6). The soil organic matter, organic carbon, total nitrogen and available P for soil analyzed at the end of the experiments were higher in composted poultry and composted cattle

manure treatments than in mineral fertilizers ($P \leq 0.001$) (Table 26). The Exchangeable cations, CEC and % BS had similar trend. Most of the nutrients except K in composted poultry manure were higher than in composted cattle manure (Table 27).

Table 25: Soil properties of the study area (test conducted at the beginning of the experiment). During 2006/2007 cropping season at SUA

OM (%)	OC (%)	N (%)	P (mg kg^{-1})	pH (H_2O)	EC (dSm^{-1})	Cat ions (mg kg^{-1})				CEC	BS %	C:N ratio
						Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}			
0.6	1.4	0.1	3.2	5.8	0.04	0.31	0.15	3.22	0.73	12.8	34.5	14:1

OM=Organic matter; OC=Organic carbon; N=Total nitrogen; P=Available Phosphorous extracted by Bray I method; Ca=Calcium; Mg=Magnesium; K=Potassium Na=Sodium; EC=Electrical conductivity; BS%=Percent base saturation; C: N ratio=Carbon nitrogen ratio.

Table 26: Soil properties (test conducted at the end of the experiment for mineral fertilizers and composted manure treatments. During 2006/2007 cropping season at SUA

Type of fertilizer	OM (%)	OC (%)	N (%)	P mg kg_{-1}	pH (H_2O)	EC (dS m^{-1})	Cations (mg kg^{-1})				CEC	BS (%)	CN: Ratio
							Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}			
Mineral[1]	0.52	1.48	0.13	3.11	5.9	0.08	0.32	0.02	3.1	0.71	11.9	34.9	11:1
cattle manure	0.78	2.12	0.24	3.91	6.7	0.12	0.45	0.04	3.9	0.73	16.3	31.4	9:1
poultry manure	0.95	2.91	0.36	4.36	7.1	0.18	0.64	0.07	4.5	0.96	17.5	35.3	8:1
Control	0.7	0.3	0.1	3.0	5.6	0.04	0.2	0.1	3.1	0.6	12.0	33.3	13.1
Mean	0.74	1.70	0.21	3.60	6.33	0.11	0.40	0.06	3.65	0.75	14.43	33.73	
Lsd $_{0.05}$	0.05	0.12	0.02	0.18	0.37	0.02	0.02	0.01	0.28	0.03	0.32	2.65	

[1]Mineral fertilizers (Mean value for urea, CAN and SA); OM=Organic matter; OC=Organic carbon; N=Total nitrogen; P=Available Phosphorous; Ca=Calcium; Mg=Magnesium; K=Potassium Na=Sodium; EC=Electrical conductivity; BS%=Percent base saturation; C:N ratio=Carbon nitrogen ratio.

Table 27: Nutrient analysis of composted cattle and composted poultry manure for trials conducted during 2006/2007 cropping season at SUA

Composted manure	pH (H2O)	P(mg kg^{-1})	TN (%)	OC (%)	CN: Ratio	EC ($dScm^{-1}$)	OM (%)	NH-N mgg-1	NO3-N mgg-1	% Total		
										Ca	Mg	K
Poultry	7.18	0.33	2.28	29.84	6:01	5.69	13.75	2.8	73.6	7.37	0.96	1.05
Cattle	9.35	0.3	1.96	59.31	14:01	6.07	27.33	282.52	12.5	5.38	0.47	2.08

EC=Electrical conductivity; OM=Organic matter; OC=Organic carbon; N=Total nitrogen; P=Available Phosphorous extracted by Olsen method; K=Potassium; $N\text{-}NH_4$=Ammonium nitrogen; $N\text{-}NO_3$=Nitrate nitrogen; C:N ratio=Carbon nitrogen ratio

4.4.2 Tissue nutrient concentration

Concentration of nutrients in maize leaf tissues analyzed at the end of the experiment differed between fertilizer types (P≤0.001) (Appendix 7). Total dry matter, P, N, NH_4-N and NO_3-N differed between fertilizer types. Significantly higher concentration of NH4 –N was recorded in leaf tissues from composted cattle manure, composted poultry and urea than other fertilizer treatments. Higher NO_3 -N and lower NH_4 –N were recorded in SA and CAN fertilizer treatments (Table 28). Total dry matter between treatments ranged from 71.3 to 80.3%.

Table 28: Residue leaf tissue nutrient concentration for different fertilizer treatments (leaf tissues samples collected at the end of the experiment) studies conducted at SUA.

Type of fertilizer	TDM (%)	N (%)	NH_4-N mg g-1	NO_3-N mg g-1	P mg kg-1
No fertilizer	76.6^{d}	0.83^{e}	0.01^{d}	2.47^{cd}	0.15^{a}
Poultry manure[1]	79.1^{b}	2.33^{a}	0.26^{b}	2.42^{d}	0.17^{a}
Cattle manure[2]	75.2^{e}	0.63^{e}	0.38^{a}	2.68^{c}	0.18^{a}
Urea	80.3^{a}	1.8^{b}	0.18^{c}	0.12^{e}	0.15^{a}
CAN	77.8^{c}	2.4^{a}	0.03^{d}	4.85^{a}	0.16^{a}
SA	71.3^{f}	0.56^{e}	0.02^{d}	3.83^{b}	0.15^{a}
Mean	76.7	1.43	0.13	2.90	0.16
CV %	0.05	13.04	12.31	3.63	7.31
SE±	0.02	0.11	0.01	0.06	0.01

Means followed by the same letter within columns do not differ significantly according to DMRT; [1]Composted poultry manure; [2]Composted cattle manure; TDM=Dry matter; N=Total nitrogen; P=Phosphorous; NH_4 -N=Ammonium nitrogen; NO_3-N=Nitrate nitrogen; CAN=Calcium ammonium nitrate; SA=Sulphate of ammonium.

4.4.3 Effect of treatments on disease, yield and yield components

Significant differences (P≤0.001) between genotypes on the severity of gray leaf spot were observed in all scored dates (Appendix 8). Main effect of genotype on disease severity at various maize growth (Table 29) indicated consistently high levels of gray leaf spot was recorded on susceptible maize 'Pannar' followed by moderately resistant cv 'Staha'. Resistant maize 'UH6303' had the lowest levels of gray leaf spot in all scored dates. Differences of gray leaf spot severity between years were not significant (Appendix 8).

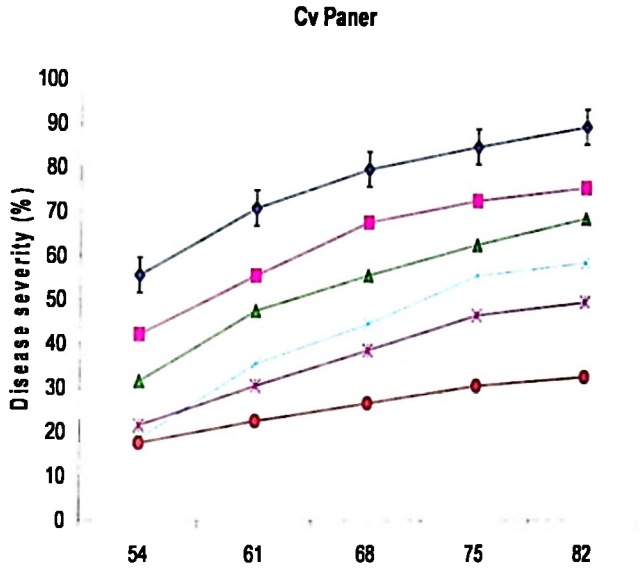
Table 29: Effects of genotype on gray leaf spot severity averaged over two growing seasons (2006/07 and 2007/08) at SUA farm

Genotype	GLS[1]				
	V8[2]	V10	V12	V15	V18
UH6303 (R)	0.86	1.01	1.19	1.29	1.47
Staha (MR)	1.13	1.15	1.82	2.00	2.31
Pannar (S)	1.37	1.62	2.19	3.06	3.33
Mean	1.12	1.26	1.73	2.12	2.37
CV%	32.16	22.45	20.46	19.71	20.48
$Lsd_{0.005}$	0.086	0.084	0.092	0.098	0.110
SE±	0.044	0.043	0.047	0.05	0.059

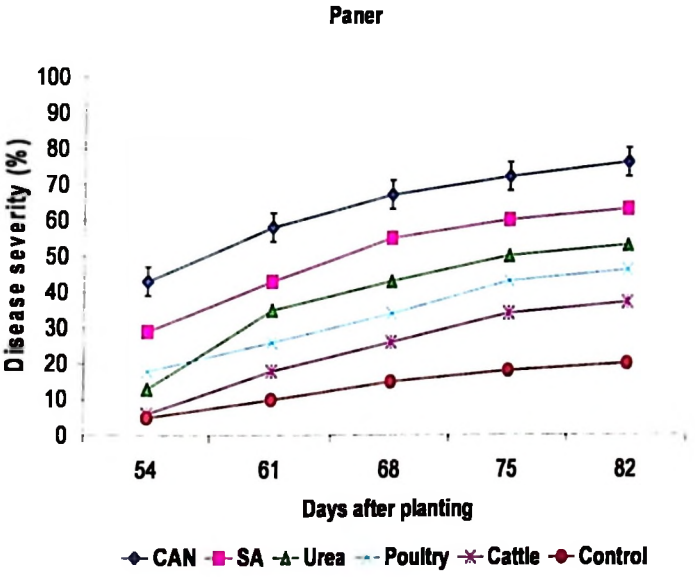
[1]Gray leaf spot severity 0=No disease; 1=10-20%; 2=21-41%; 3=41-61%; 4=61-80%; 5=81-100%; [2]V8 to V18 are maize growth stages; R=Resistant MR=Moderate resistant S=Susceptible.

The progress of gray leaf spot on susceptible maize 'Pannar' in 2006/07 and 2007/08 cropping seasons under different fertilizer treatments is shown on Fig 7. In both seasons, maize plants fertilized with composted cattle and composted poultry manure displayed lower levels of gray leaf spot than mineral fertilizers; within this group urea was the best, followed by SA and CAN in that order. The highest severity levels of gray leaf spot were recorded in CAN treated plants. Non- fertilized plants (control) experienced the lowest levels of disease severity across all scored dates (Fig 7). Similar trend was observed in other cultivars (Appendixes 11 and 12).

A



B



CAN=Calcium ammonium nitrate: SA=Sulphate of ammonia; Poultry=Poultry manure; Cattle=Cattle manure; Control=Not fertilized; DAP=Days after planting.

Figures 7: Progress of gray leaf spot on cv 'Pannar' on different fertilizer treatments (during 2006/07 (A) and 2007/08 (B) cropping seasons) at SUA farm.

Effect of fertilizer on disease was highly significant (P≤0.001) in all scored dates (Appendix 8). Main effect of fertilizer on disease is shown on Table 30. Plants heavily fertilized were more affected by gray leaf spot than plants receiving the recommended rate while non-fertilized plants (control) had significantly lowest levels of gray leaf spot than other fertilizer treatments. Assessment carried-out at V15 growth stage showed that composted cattle manure lowered gray leaf spot compared to CAN by 29.4% at recommended rate and by 32.2% at heavy application (Table 30). On other hand, composted poultry manure lowered gray leaf spot compared to CAN by 24.5% at recommended rate and by 22.9% at heavy application. Urea produced intermediate reaction lowering gray leaf spot compared to CAN by 17.6% at recommended rate and by 18.2% at heavy application. Similar trends prevailed on scores recorded at V8, V10, V12 and V18 growth stages.

Table 30: Effect of fertilizers on gray leaf spot averaged over two growing seasons (2006/07 and 2007/08) at SUA farm

	GLS[1] on growth stage				
Fertilizer	V8[5]	V10	V12	V15	V18
CAN[2]	1.31^{b}	2.09^{a}	2.32^{b}	2.45^{b}	2.84^{ab}
	(1.67)^{a}	(2.15)^{a}	(2.50)^{a}	(2.58)^{a}	(2.96)^{a}
SA[3]	1.21^{b}	1.75^{bc}	2.01^{c}	2.24^{c}	2.54^{cd}
	(1.24)^{b}	(1.85)^{b}	(2.25)^{b}	(2.30)^{c}	(2.61)^{bc}
Urea	1.02^{c}	1.62^{cd}	1.82^{d}	2.02^{de}	2.33^{de}
	(1.20)^{b}	(1.66)^{c}	(1.96)^{c}	(2.11)^{d}	(2.46)^{cd}
Poultry manure	0.99^{c}	1.40^{ef}	1.64^{ef}	1.85^{f}	2.14^{ef}
	(1.01)^{c}	(1.52)^{de}	(1.74)^{de}	(1.99)^{g}	(2.15)^{ef}
Cattle manure	0.78^{d}	1.04^{g}	1.57^{f}	1.73^{g}	1.86^{g}
	(0.93)^{e}	(1.27)^{f}	(1.63)^{ef}	(1.75)^{fg}	(1.90)^{fg}
Control[4]	0.53^{e}	0.89^{h}	1.28^{g}	1.71^{g}	1.78^{g}
Mean	1.14	1.60	1.91	2.08	2.37
CV%	32.16	22.45	20.46	19.71	20.48
SE±	0.0858	0.0852	0.0904	0.095	0.1147

Means followed by the same letter within columns do not differ significantly according to DMRT; [1]Gray leaf spot severity 0=No disease; 1=10-20%; 2=21-41%; 3=41-61%; 4=61-80%; 5=81-100%; [2]CAN=calcium ammonium nitrate; [3]SA=Sulphate of ammonia; [4]Control=Not fertilized; Mean in parentheses are values under heavy fertilizer application. [5]V8 to V18 are maize growth stages; Mean in parentheses are values under heavy fertilizer.

Genotype x Fertilizer interaction on gray leaf spot severity was significant ($P \leq 0.001$) on all scored dates (Appendix 8). The combination of Genotype x Fertilizer interaction on disease severity (Table 31) indicated that in all scored dates, the effect of composted cattle manure, composted poultry manure, urea, SA and CAN on gray leaf spot was not significant on resistant maize cv 'UH6303' and moderately resistant cv 'Staha' but, significant differences ($P \leq 0.05$) were observed on susceptible maize cv 'Pannar' where heavily fertilized plants with CAN, SA, Urea, composted poultry and composted cattle manure were more affected by gray leaf spot than plants receiving recommended rates (Table 31)

Table 31: Combination of Genotype x Fertilizers on gray leaf spot severity averaged over two growing seasons (2006/07 and 2007/08) at SUA farm

Genotype	Fertilizer	GLS[1]				
		V8[5]	V10	V12	V15	V18
UH6303 (R)	CAN[2]	0.96^{fg}	1.24^{hi}	1.31^{hmn}	1.42^{jl}	1.50^{kl}
		(1.01)^{efg}	(1.25)^{hi}	(1.34)^{klm}	(1.43)^{jl}	(1.53)^{kl}
	SA[3]	0.90^{g}	1.21^{hi}	1.30^{lmno}	1.30^{jk}	1.45^{jl}
		(0.91)^{g}	(1.24)^{hi}	(1.30)^{klmno}	(1.41)^{jl}	(1.46)^{kl}
	Urea	0.83^{g}	1.19^{hi}	1.26^{klmno}	1.28^{jk}	1.34^{kl}
		(0.84)^{g}	(1.20)^{hi}	(1.28)^{klmno}	(1.28)^{jk}	(1.36)^{kl}
	Poultry manure	0.79^{gh}	1.06^{ij}	1.11^{mno}	1.22^{jk}	1.32^{kl}
		(0.83)^{g}	(1.13)^{hi}	(1.25)^{klmno}	(1.26)^{jk}	(1.34)^{kl}
	Cattle manure	0.73^{gh}	0.99^{ij}	1.03^{n}	1.16j^{k}	1.24^{kl}
		(0.77)^{gh}	(0.99)^{ij}	(1.07)^{mno}	(1.19)^{jk}	(1.28)j
	Control[4]	0.51^{h}	0.02^{k}	1.05^{mno}	1.13^{k}	1.17^{l}
Staha (MR)	CAN[2]	1.55^{bc}	1.90^{de}	2.10^{cdefg}	2.21^{cdef}	2.60^{cdef}
		(1.56)^{bc}	(2.02)^{d}	(2.12)^{cdef}	(2.32)^{cde}	(2.86)^{cde}
	SA[3]	1.48^{bcd}	1.70^{def}	1.93^{cdefgh}	2.12^{cdefg}	2.33^{cdefg}
		(1.55)^{bc}	(1.82)^{def}	(1.95)^{cdefgh}	(2.13)^{cdefg}	(2.55)^{cdefg}
	Urea	1.34^{cd}	1.62^{f}	1.87^{defgh}	1.94^{defgh}	2.22^{defgh}
		(1.40)^{bcd}	(1.63)^{def}	(1.90)^{cdefgh}	(2.02)^{defgh}	(2.24)^{cdefgh}
	Poultry manure	1.30^{cd}	1.60^{ef}	1.74^{def}	1.92^{defgh}	2.03^{defgh}
		(1.30)^{cd}	(1.62)^{f}	(1.86)^{defgh}	(1.93)^{defgh}	(2.22)^{defgh}
	Cattle manure	1.29^{cde}	1.55^{fg}	1.61^{efg}	1.81^{def}	1.91^{defgh}
		(1.30)^{cd}	(1.56)^{efg}	(1.70)^{defg}	(1.88)^{cdefg}	(2.00)^{defgh}
	Control[4]	0.76^{gh}	0.74j	1.33^{klm}	1.71^{ijk}	1.88^{defgh}
Pannar (S)	CAN[2]	1.64^{a}	3.00^{a}	3.33^{a}	3.71^{ab}	4.00^{a}
		(2.36)^{b}	(3.04)^{a}	(3.36)^{a}	(3.81)^{a}	(4.02)^{a}
	SA[3]	1.20^{def}	2.66^{b}	3.01^{b}	3.18^{c}	3.52^{bc}
		(1.21)^{def}	(2.80)^{ab}	(3.01)^{b}	(3.48)^{b}	(3.62)^{ab}
	Urea	0.99^{fg}	2.30^{c}	2.72^{cd}	2.97^{cd}	3.3^{cd}
		(1.20)^{def}	(2.66)^{b}	(2.92)^{bc}	(3.14)^{c}	(3.40)^{bcd}
	Poultry manure	0.97^{fg}	1.67^{ef}	2.17^{e}	2.31^{e}	2.96^{e}
		(0.99)^{fg}	(1.69)^{ef}	(2.51)^{d}	(2.78)^{d}	(3.29)^{d}
	Cattle manure	0.75^{gh}	1.06^{ij}	1.70^{defg}	1.96^{defgh}	2.11^{cdefgh}
		(0.90)^{g}	(1.34)^{gh}	(1.83)^{defgh}	(2.05)^{cdefgh}	(2.12)^{cdefgh}
	Control[4]	0.81^{g}	1.98^{d}	1.44^{jkl}	1.82^{def}	1.92^{defgh}
Mean		1.12	1.63	1.90	2.11	2.34
CV%		32.16	22.45	20.46	19.71	20.48
SE±		0.148	0.147	0.156	0.168	0.198

Means followed by the same letter within columns do not differ significantly according to DMRT; [1]Gray leaf spot severity 0=No disease; 1=10-20%; 2=21-41%; 3=41-61%; 4=61-80%; 5=81-100%; [2]CAN=calcium ammonium nitrate; [3]SA=Sulphate of ammonia; [4]Control=Not fertilize d; application. [5]V8 to V18 are maize growth stages; R=Resistant; MR=Moderate resistant; S=Susceptible; Mean in parentheses are values under heavy fertilizer

Analysis of variance (mean squares) on yield and yield components (days to 50% silk, car height and 100 seed wt) for different fertilizer treatments is shown on (Appendix 9). Main effects of genotype, fertilizer and Genotype x Fertilizer interaction on days to 50% silk, ear height, 100 seed wt and grain yield were significant ($P \leq 0.05$). The main effect of genotypes on yield and yield components (days to 50% silk, ear height and 100 seed wt) (Table 32) indicated that longer car heights, higher 100 seed wt and higher yield were recorded on resistant maize cv 'UH6303' than moderately resistant cv 'Staha' and susceptible maize cv 'Pannar' ($P \leq 0.05$).

Table 32: Effects of genotypes on yield and yield components averaged over two growing seasons (2006/07 and 2007/08) at SUA farm

Genotype	Variable			
	Days to 50% Silk	Ear height (cm)	100 seed wt (g)	Yield(t/ha)
UH6303 (R)	72.92	125.37	45.20	7.78
Staha (MR)	82.01	115.09	40.27	6.23
Pannar (S)	89.84	112.01	36.33	5.10
Mean	84.92	84.16	40.60	6.70
CV%	4.11	6.46	12.52	14.84
$Lsd_{0.005}$	0.84	1.89	1.22	0.23
SE±	0.429	0.969	0.625	0.122

R=Resistant; MR=Moderate resistant; S=Susceptible;

Main effect of fertilizer on yield and yield component is shown in (Table 33). Plots receiving recommended rates of fertilizer displayed higher yields ($P \leq 0.05$) than those receiving higher rates. Days to 50 % silk, ear height and 100 seed wt had a similar trend. Plots fertilized with composted cattle manure had the highest yield ($P \leq 0.05$) followed by composted poultry manure and urea in that order. Lowest grain yields were recorded in CAN and SA treated plots.

Table 33: Main effect of fertilizer on yield and yield components averaged over two growing seasons (2006/07 and 2007/08) at SUA farm

	Variables			
Fertlizers	Days to 50% silk	Ear height (cm)	100 seed wt (g)	Yield (t ha^{-1})
CAN[1]	85.1^{bc}	127.9^{ab}	41.6^{bc}	6.4^{cd}
	(83.2)^{de}	(123.8)^{cd}	(38.7)^{d}	(5.8)^{e}
SA[2]	83.2^{de}	121.4^{ef}	34.6^{e}	6.6^{e}
	(80.3)^{f}	(117.3)^{fg}	(31.2)^{f}	(5.8)^{e}
Urea	83.6^{cd}	119.4^{ef}	39.4^{cd}	7.0^{b}
	(81.6)^{ef}	(111.6)^{h}	(34.7)^{e}	(6.1)^{de}
Poultry manure	88.9^{a}	118.6^{ef}	46.0^{a}	7.3^{b}
	(85.7)^{b}	(114.6)^{gh}	(41.5)^{bc}	(6.3)^{cd}
Cattle manure	89.5^{a}	129.4^{a}	46.6^{a}	7.8^{a}
	(86.7)^{b}	(125.0)^{bc}	(43.1)^{b}	(7.0)^{b}
Control[3]	61.2^{c}	86.3^{i}	28.6^{g}	0.6^{f}
Mean	82.6	117.8	38.7	6.1
CV%	4.11	6.46	12.52	13.99
SE±	0.822	1.855	1.198	0.221

Means followed by the same letter within columns do not differ significantly according to DMRT; [1]CAN=calcium ammonium nitrate; [2]SA=Sulphate of ammonia; [3]Control=Not fertilized; Mean in parentheses are values under heavy fertilizer application.

Combination of Genotype x Fertilizer interaction on yield and yield component variables (Table 34) indicated that for all genotypes, composted cattle manure treated plots had higher grain yield, 100 seed wt and ear height than any other treatments followed by composted poultry, urea and SA in that order ($P \leq 0.05$). On the other hand, the lowest yield levels were recorded in non-fertilized followed by CAN treated plots. Superior yields were recorded on resistant maize cv. 'UH6303' under treatment of composted cattle manure (9.2t/ha) and composted poultry manure (8.4 t/ha). Compared to CAN treatment, there was yield increases of 30.8% and 28.5% following normal and heavy applications of composted cattle manure respectively on resistant maize cv 'UH6303'; 39.7% and 42.1% respectively on moderately resistant cv 'Staha' and 40.8% and 52.9% respectively on susceptible maize cv 'Pannar'. Consistently, in all genotypes, significantly lower yield were recorded in non-fertilized (control) than in fertilizer treated plots.

Table 34: Combination of Genotype x Fertilizers for yield and yield components averaged over two growing seasons (2006/2007 and 2007/2008) at SUA farm.

Genotype	Fertilizer	Variable			
		Days to 50% Silk	Ear height (cm)	100 seed wt (g)	Yield(t ha^{-1})
	CAN[1]	83.25^{d}	104.38^{kl}	32.21^{lmn}	6.35^{fgh}
UH6303 (R)		(79.90)^{e}	(96.31)^{m}	(28.26)^{op}	(6.28)^{fghi}
	SA[2]	92.60^{a}	114.21^{ghi}	35.03j^{kl}	7.65^{cd}
		(85.00)^{cd}	(112.66)^{hij}	(33.86)^{klm}	(6.80)^{ef}
	Urea	79.46^{ef}	127.10^{cd}	38.33^{hij}	8.00^{cd}
		(77.33)^{efgh}	(115.40)^{fgh}	(36.95)ijk	(7.68)^{cd}
	Poultry manure	76.96^{fgh}	131.13^{bc}	40.73^{fgh}	8.40^{bc}
		(74.70)^{hij}	(130.23)^{bc}	(39.40)^{mno}	(8.13)^{bcd}
	Cattle manure	73.60^{ijk}	139.66^{a}	48.20^{bc}	9.18^{a}
		(71.16)^{klm}	(133.50)^{b}	(43.91)^{def}	(8.78)^{ab}
	Control[3]	53.60^{no}	68.70^{o}	21.30^{r}	3.20^{n}
Staha (MR)	CAN[1]	93.80^{a}	102.53^{kl}	28.35o^{p}	5.05^{klm}
		(78.60)^{efg}	(98.90)^{lm}	(24.91)^{pq}	(4.38)m
	SA[2]	78.26^{efg}	108.23^{ijk}	30.00^{no}	5.60^{hijk}
		(78.23)^{efg}	(107.53)^{jk}	(29.03)^{no}	(5.20)kl
	Urea	77.60^{efg}	113.40^{ghij}	34.00^{kl}	6.16^{fghi}
		(76.06)^{ghi}	(112.20)^{hij}	(31.76)l^{mno}	(5.73)^{ghijk}
	Poultry manure	73.30j^{k}	117.90^{efgh}	40.83^{efgb}	6.46^{fg}
		(72.98)^{jkl}	(117.46)^{efgh}	(34.95)^{jkl}	(6.30)^{fghi}
	Cattle manure	72.80^{kl}	121.60^{de}	44.33^{de}	8.38^{bc}
		(71.63)^{klm}	(119.13)^{efg}	(40.91)^{efgh}	(7.56)^{de}
	Control[3]	51.60^{o}	67.80^{o}	23.80^{qr}	3.00^{no}
Pannar (S)	CAN[1]	93.46^{a}	119.05^{efg}	34.93^{jkl}	4.30^{m}
		(88.13)^{b}	(98.86)^{lm}	(32.26)^{lmn}	(3.05)^{n}
	SA[2]	86.46^{bc}	121.45^{def}	40.26^{ghi}	5.35^{jkl}
		(85.88)^{bcd}	(119.18)^{efg}	(40.00)^{ghi}	(4.66)^{lm}
	Urea	72.76^{jklm}	122.36^{de}	41.93^{efg}	6.01^{ghij}
		(72.50)^{jklm}	(122.21)^{de}	(41.83)^{efgh}	(5.56)^{hijk}
	Poultry manure	71.90^{klm}	126.16^{cd}	45.96^{cd}	6.48^{fg}
		(71.66)^{klm}	(122.48)^{de}	(44.20)^{def}	(6.15)^{fghi}
	Cattle manure	70.30^{lm}	135.36^{ab}	54.96^{a}	7.26^{o}
		(70.06)^{m}	(126.96)^{cd}	(49.93)^{b}	(6.48)^{fg}
	Control[3]	56.20^{n}	82.30^{n}	21.60^{qr}	2.70^{no}
Mean		84.93	121.83	40.60	6.72
CV%		4.11	6.46	12.52	14.84
SE±		1.424	3.214	2.075	0.406

Means followed by the same letter within columns do not differ significantly according to DMRT; [1]CAN=calcium ammonium nitrate; [2]SA=Sulphate of ammonia; [3]Control=Not fertilized; R=Resistant; MR=Moderate resistant; S=Susceptible; Mean in parentheses are values under heavy fertilizer application.

Yield was negatively correlated to disease severity (-0.435) (P≤0.01) (Table 36). Days to 50% silk was positively correlated to ear height (0.463) and 100 seed wt (0.439) (P≤0.01) and disease severity (0.541) (P≤0.001).
(Table 35)

Table 35: Correlation matrix for ear height, 100 seed weight, 50% silk, disease severity and yield of maize.

	Ear height	100 Seed wt	50% Silk	Disease	Yield
Ear height	-				
Seed wt.	0.504***	-			
50% Silk	0.463**	0.439**	-		
Disease	-0.078	-0.106	0.541***	-	
Yield	0.270	0.420**	0.244	-0.435**	-

***P≤0.001; **P≤0.01; df=32

4.5 The effect of intercropping maize with beans on inoculum dispersal, development of GLS and yield of maize in minimum tillage operations

4.5.1 Effect of treatments on components of resistance and disease severity

The main effects (genotype, tillage, and intercropping) on disease, spores trapped during the season and grain yield was significant (Appendix 10). In all genotypes, AUDPC, rate of disease increase and spore trapped during the season were higher in no-till than minimum and conventional tillage operations with lower grain yields (Table 36).

Table 36: Effects of tillage on gray leaf spot severity, components of resistance and yield of maize produced under no-till, minimum tillage and conventional tillage average of two growing seasons (2006/07and 2007/08) at SUA farm

Tillage	Variables				
	AUDPC[a]	r[b]	R^2(%)[c]	NST[d]	Yield(t ha^{-1})
No-tillage	266.6	10.6	90.5	13.2	5.1
Min-tillage	222.3	7.6	86.2	8.3	6.3
Conv-tillage	184.4	2.6	78.1	3.2	7.4
Mean	224.40	6.90	84.90	8.20	6.30
CV (%)	9.53	28.03	13.49	13.75	12.16
$LDS_{0.05}$[e]	6.89	0.64	3.81	0.37	0.25
SE±	3.46	0.33	1.91	0.19	0.13

Means followed by the same letter within columns do not differ significantly according to DMRT; [a]Area under disease progress curve; [b]Apparent infection rate x 100; [c]R^2=Coefficient of determination; [d]NST=Number of spores trapped x 100; Min-tillage=Minimum tillage; Conv-tillage=Conventional tillage.

The two-way interaction of genotype x tillage on variables that had significant interactions (Table 37) showed that, except for yield, all studied variables had higher values in no-till than minimum and conventional tillage operations. Year x tillage interaction was significantly on the number of spores trapped and coefficient of determination (R^2) (Appendix 10). The coefficient of determination (R^2) for treatments was high ranging from 69.2-95.5% (Table 37).

Table 37: Genotype x Tillage interaction on gray leaf spot, components of resistance and yield of maize produced under sole cropped during two growing seasons (2006/07and 2007/08) (variables with significant interactions) at SUA farm.

		Variables				
Genotype	Tillage	AUDPC[a]	r[b]	R^2 (%)[c]	NST[d]	Yield (t ha^{-1})
UH6303	No-tillage	144.2[g]	3.3[f]	95.5[a]	6.3[f]	6.5[b]
	Min-tillage	107.4[h]	3.2[f]	81.9[b]	7.1[e]	6.8[b]
	Conv-tillage	110.9[h]	1.6[g]	69.2[d]	5.3[g]	7.2[a]
Staha	No-tillage	283.5[d]	13.4[b]	80.1[bc]	22.3[b]	4.4[d]
	Min-tillage	243.5[e]	7.8[c]	74.6[cd]	16.5[d]	5.7[c]
	Conv-tillage	194.4[f]	5.3[e]	95.0[a]	4.1[h]	6.6[b]
Pannar	No-tillage	409.5[a]	16.9[a]	91.8[a]	24.9[a]	3.3[f]
	Min-tillage	344.3[b]	13.7[b]	92.3[a]	17.1[c]	3.9[e]
	Conv-tillage	301.5[c]	6.6[d]	73.5[d]	3.9[h]	6.7[b]
Mean		237.70	8.00	83.80	11.90	5.70
CV (%)		9.50	28.00	13.40	13.70	12.10
SE±		5.99	0.56	3.31	0.33	0.20

Means followed by the same letter within columns do not differ significantly according to DMRT; [a]Area under disease progress curve; [b]Apparent infection rate x 100; [c] R^2=Coefficient of determination; [d]NST=Number of spores trapped x 100; Min-tillage=Minimum tillage; Conv-tillage=Conventional tillage.

Genotype x intercropping interactions on AUDPC, rate (r) of disease increase, R^2 and yield were significant (Appendix 10). In all genotypes, except 'UH6303' higher AUDPC was recorded in no-till plots where maize was intercropped with beans than other treatments (Table 38) while the rate (r) of disease progress was highest in no-till intercropped with beans in all genotypes.

Table 38: Genotype x Intercropping interaction on gray leaf spot, components of resistance for intercropped maize during two growing seasons (2006/07and 2007/08) (variables with significant interactions) at SUA farm

		Variables			
Genotype	Intercropping	AUDPC[a]	r[b]	R^2(%)[c]	Yield(t ha^{-1})
UH6303	No-till/beans	113.6[g]	3.1[ef]	77.9[c]	7.3[b]
	Min-till/beans	121.0[g]	2.2[fg]	90.0[b]	7.0[bc]
	Conv-till/beans	114.1[g]	0.9[g]	91.3[b]	7.8[a]
Staha	No-till/beans	220.4[d]	10.5[b]	90.4[b]	5.8[dc]
	Min-till/beans	207.3[e]	4.3[d]	96.7[a]	6.8[c]
	Conv-till/beans	152.0[f]	1.8[gh]	74.0[c]	7.3[b]
Pannar	No-till/beans	360.5[a]	15.1[a]	93.9[ab]	4.9[f]
	Min-till/beans	294.0[b]	8.1[c]	95.2[ab]	5.5[e]
	Conv-till/beans	231.9[c]	3.5d[e]	66.2[d]	6.1[d]
Mean		201.60	5.50	86.20	6.70
CV (%)		9.50	28.00	13.40	12.10
SE±		4.89	0.46	2.70	0.18

Means followed by the same letter within columns do not differ significantly according to DMRT; [a]Area under disease progress curve; [b]Apparent infection rate x 100; [c] R^2=Coefficient of determination; [No]-till/beans=No tillage maize intercropped with beans; Min-till/beans= Minimum tillage maize intercropped with beans; Conv-till/beans=Conventional tillage maize intercropped with beans

Significantly higher AUDPC was recorded on susceptible cv 'Pannar' than moderately resistant 'Staha' and resistant 'UH6303' (Table 37). Differences of AUDPC between seasons were significant (Appendix 8). Large AUDPC were recorded in 2006/07 than 2007/08 cropping season (Table 39). The AUDPC was larger in no-till (266.6) than the other tillage operations viz. minimum tillage (222.3) while conventional tillage registered the lowest levels (184.4) ($P \leq 0.05$) (Table 36). Non-intercropped maize exhibited significantly higher AUDPC than maize intercropped with beans (Table 40). The mean rate (r) of disease increase was higher on susceptible maize hybrid 'Pannar' than on 'Staha' and 'UH6303' (Table 37). The (r) value for 2006/07 was higher than 2007/08 ($P \leq 0.05$) (Table 39). The mean (r) value in the no-till treatment was signifiantly ($P \leq 0.05$) higher than in minimum and conventional tillage (Table 37). The mean (r) value in bean/maize intercrop was significantly lower than in non-intercropped system; all other studied variables had

similar trend (Table 40). The rate (r) of disease increase was positively correlated with AUDPC, the number of spores trapped during the season, but negatively correlated with yield (Table 42).

Table 39: Effects of years on different variables at SUA farm

Years	Variables				
	AUDPC[a]	r[b]	R^2(%)[c]	SPN[d]	Yield(t ha^{-1})
2006/07	220.8	7.8	86.9	12.8	6.1
2007/08	214.8	6.1	83.0	3.8	6.7
Mean	217.8	7.0	85.0	8.3	6.4
CV (%)	9.53	28.03	13.49	13.75	12.16
$LDS_{0.05\pm}$	5.63	0.52	3.11	0.30	0.20
SE±	2.824	0.265	1.559	0.154	0.104

[a]Area under disease progress curve; [b]Apparent infection rate x 100; [c]Coefficient of determination (%); [d]Spore number trapped x 100

Table 40: Effects of intercropping on gray leaf spot severity, components of resistance and yield of maize produced under sole crop and intercrop conditions during two growing seasons (2006/07and 2007/08) at SUA farm

Intercropping	Variable				
	AUDPC[a]	r[b]	R^2(%)[c]	NST[d]	Yield(t ha^{-1})
Non-intercropping	237.0	8.8	86.1	10.2	5.4
Intercropping	198.4	5.0	83.7	6.2	6.8
Mean	217.70	6.9	84.90	8.20	6.10
CV (%)	9.53	28.03	13.49	13.75	12.16
[f]$LDS_{0.05\pm}$	5.63	0.52	3.11	0.30	0.20
SE±	2.82	0.27	1.56	0.15	0.10

[a]Area under disease progress curve; [b]Apparent infection rate x 100; [c]R^2=Coefficient of determination; [d]NST=Number of spores trapped x 1; [f]LSD= Least significant difference

Tillage x Intercropping interaction was significant on rate of disease increase and number of spores trapped during the season (Appendix 10). Lower rate of disease increase was recorded in convectional tillage and intercropping and the highest values were recorded no-tillage non-intercropped (Table 41). The number of spores

produced during the season had similar trend.

Table 41: Tillage x Intercropping interaction on gray leaf spot, components of resistance for sole cropped maize and intercropped during two growing seasons (2006/07and 2007/08) (variables with significant interactions) at SUA farm

Tillage and	Intercropping	Variables	
		r[a]	NST[b]
No-tillage	Non-intercropped	12.8[a]	15.5[a]
	Intercropped	8.2[c]	10.8[c]
Minimum tillage	Non-intercropped	10.3[b]	11.8[b]
	Intercropped	4.9[e]	4.8[e]
Conventional tillage	Non-intercropped	6.3[d]	8.4[d]
	Intercropped	2.0[f]	2.9[f]
Mean		7.40	9.00
CV (%)		28.03	13.75
SE±		0.46	0.27

Means followed by the same letter within columns do not differ significantly according to DMRT; [a]Apparent infection rate x 100; [b]NST=Number of spores trapped x 100;

AUDPC was significantly positively correlated with the rate (r) of disease progress (0.806), the numbers of spores trapped during the season (0.157) and negatively correlated with yield (-0.822) (Table 42).

Table 42: Correlation matrix for disease severity, rate of disease progress, number of spore trapped and yield

	Spores	Rate (r)	AUDPC	Yield
Spores	-			
Rate (r)	0.642**	-		
AUDPC	0.157	0.806***	-	
Yield	-0.353	-0.807***	-0.822***	-

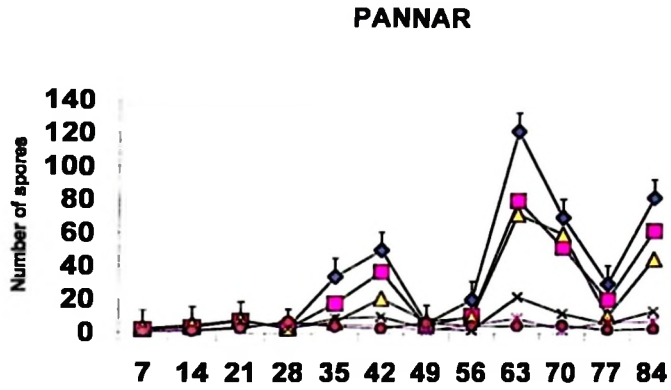
Rate (r) = Apparent infection rate; AUDPC=Area under disease progress curve;**P≤0.01; ***P≤0.001, df=18

4.5.2 Effect of treatments on air-borne conidia

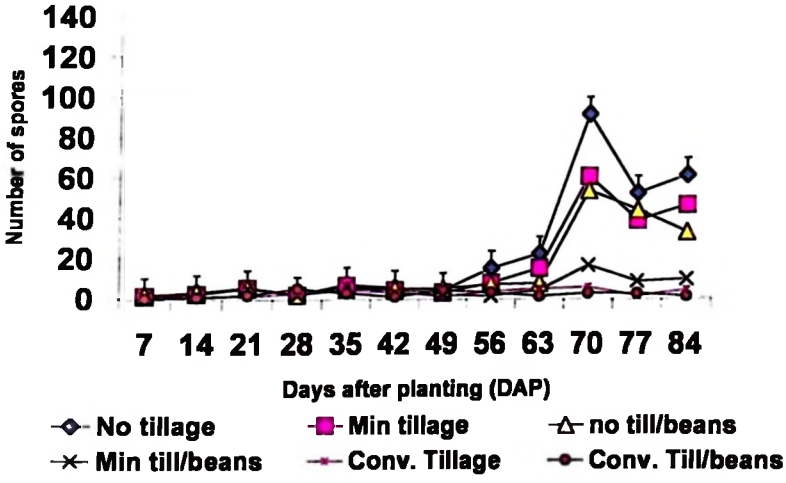
Spore counts on susceptible genotype "Pannar" above maize crop residues fluctuated between sampling dates with peaks varying between treatments during 2006/07 and 2007/08 cropping seasons (Fig. 8). Other genotypes followed a similar pattern

(Appendixes 13 and 14). The mean number of spores trapped between genotypes and years differed significantly (Appendix 10). More spores were trapped on the susceptible maize genotype ('Pannar') than on the moderately resistant ('Staha') and resistant ('UH6303') maize genotypes ($P \leq 0.05$); similarly, more spores were trapped in 2006/07 than in the 2007/08 cropping season ($P \leq 0.05$) (Table 39). A higher number of spores were observed in no-till plots than in minimum and conventional tilled plots (Table 38). Mean values of spores trapped in the intercropped plots were lower than those trapped in non-intercropped plots ($P \leq 0.05$) (Table 40).

A



B



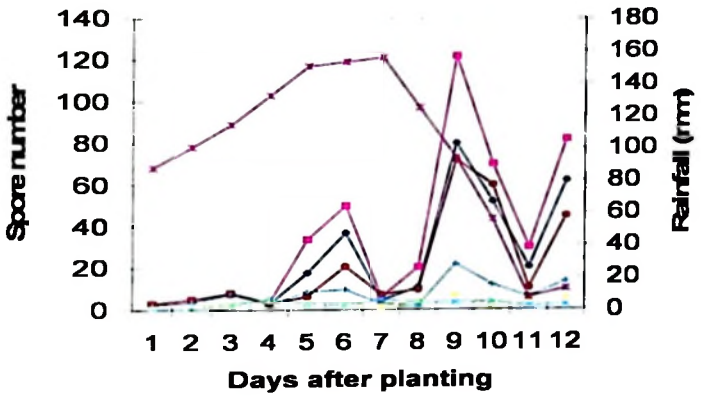
No till= no-tillage; min till= minimum tillage; no-till/ beans=no-tillage maize intercropped with beans; min till/beans= minimum tillage maize intercropped with beans; conv. tillage=conventional tillage; conv. till/beans= conventional tillage maize intercropped with bean.

Figures 8: Number of spores x 10^3 of C. zeae maydis trapped on maize hybrid 'Pannar' during 2006/07 (A) and 2007/08 (B) cropping seasons at SUA farm.

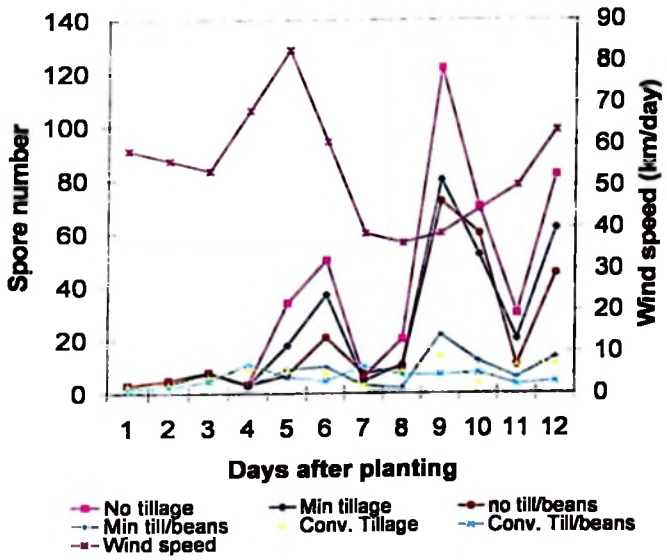
4.5.3 Effect of weather on spore production

The relationship between spore production and weather conditions during growing season is shown on (Fig 9-11). Peak spore production occurred at lower rainfall (30mm), higher R.H (>80%) and at lower wind speed (40 km/day). Max temperature of 30 °C and minimum temperature of 17 °C favored higher spore production.

A

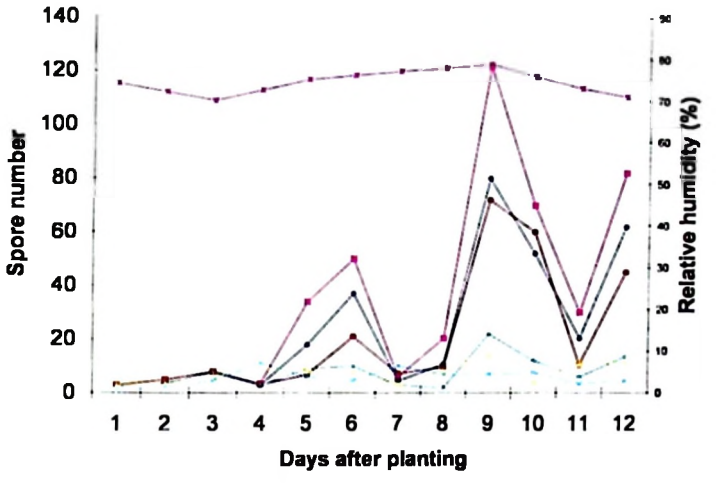


B

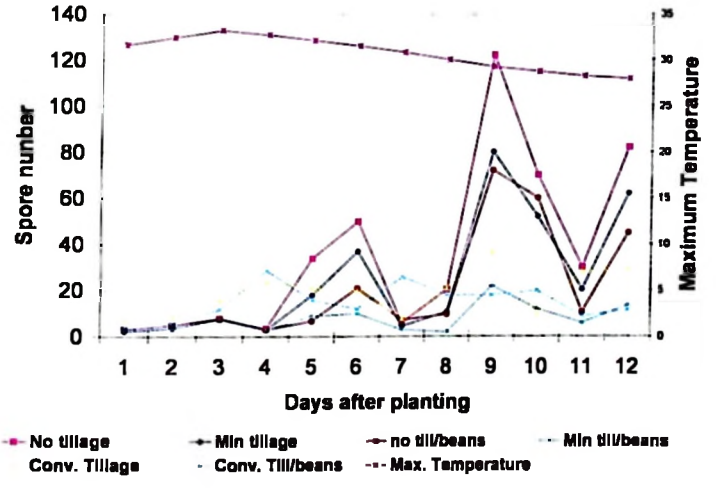


Figgures 9: Effect of rainfall (A) and Wind speed (B) on *C. zeae maydis* spore production (mean for two cropping seasons on cv 'Pannar').

B



B



Figures 10: Effect of relative humidity (A) and Maximum Temperature (B) on *C. zeae maydis* spore production (mean for two cropping seasons
on cv 'Pannar').

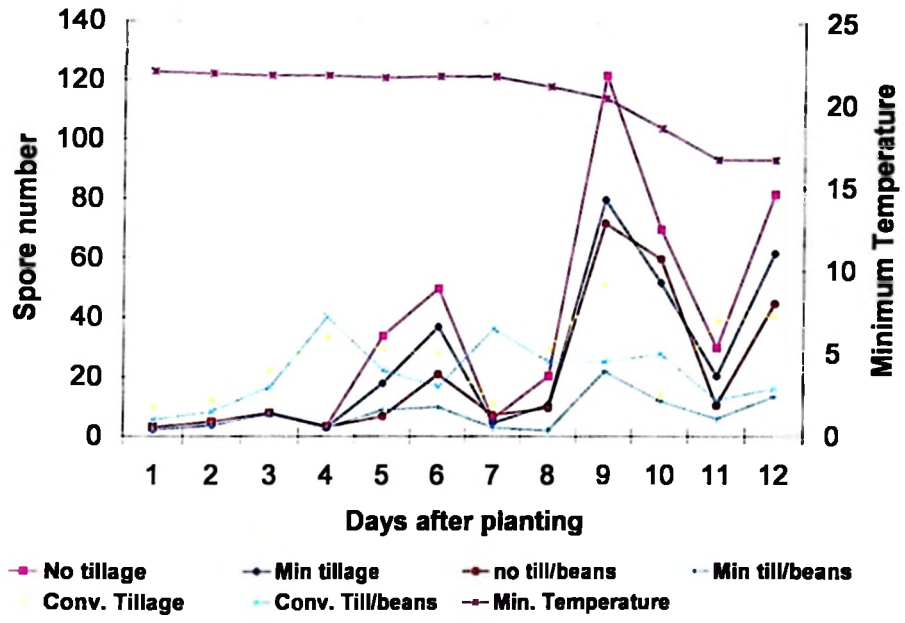


Figure 11: Effect of minimum temperature on *C. zeae maydis* spore production (mean for two cropping seasons on cv 'Pannar').

4.5.4 Relative importance of single and multiple cultural practices

The Wald statistical test for comparing single and multiple cultural practices is shown on (Table 43). Except no-till, all treatments showed significance Wald chi-square statistic. Larger Wald chi-square statistic values were recorded in convectional tillage and minimum tillage where maize was intercropped with beans

Table 43: Wald statistical test for relative comparison of single and multiple practices

Effect	B	SE_B	Wald	df	Sig	Exp(B)
No-till	0.909	0.129	49.653	1	0.060	1.10E+03
Min-till	1.171	0.132	78.698	1	0.030	9.80E+02
Conv. till	1.699*	0.170	99.882	1	0.002	6.70E+02
No-till /intercrop	0.760*	0.154	24.354	1	0.016	1.90E+03
Min-till /intercrop	0.876**	0.167	27.515	1	0.014	1.20E+03
Conv. / intercrop	1.921***	0.123	243.918	1	0.001	1.60E+03

B=Regression coefficient; SE=standard error; Wald=Wald chi-square statistic; df=Degree of freedom; Sig=Significance; Exp (B) =Expected regression coefficient; No-till/beans=No tillage maize intercropped with beans; Min-till/beans= Minimum tillage maize intercropped with beans; Conv-till/beans=Conventional tillage maize intercropped with beans; *Significant at P≤0.05; ** Significant at P≤ 0.01; *** Significant at P≤0.001

4.5.5 Effect of treatments on yield

Mean grain yield in minimum tillage was higher on 'UH6303' (6.8 t/ha) than 'Staha' (5.7 t/ha) and 'Pannar' (3.9 t/ha) genotypes ($P \leq 0.05$) (Table 37) while average yield for 2006/08 (6.7t/ha) was higher than 2007/08 (6.1 t/ha) ($P \leq 0.05$) (Table 39). Grain yield was higher in the conventional tillage than minimum and no-tillage treatments ($P \leq 0.05$) (Table 36) and in the intercropped than non-intercropped treatments (Table 40). Yield differences between intercropping treatments on resistant genotype ('UH6303') were not significant except for conventional tillage (Table 37).

CHAPTER 5

5.0 DISCUSSION

5.1 Occurrence of gray leaf spot, isolate diversity and aggressiveness of *C. zeae maydis* in selected villages of the Southern Highlands and the Eastern maize agro-ecological zones.

The results have revealed significant variations in pathogen population existing in the Southern and Eastern maize agro-ecological Zones of Tanzania. The aggressiveness variables were higher as reflected in lesion length, spore numbers and percent leaf area affected by disease for Southern Zone isolates than Eastern Zone suggesting that the former group was more aggressive than the latter. The data also suggest that the Southern highland Zone is more conducive to greater pathogen variability than the low-mid Eastern Zone. Differences in cropping systems, climate and farming practices between the two agro-ecological zones may account for the observed pathogen variations. However, adaptation of pathogen aggressiveness mediated by climatic parameters, global pathogen population structure changes, selection for quantitative traits and differential adaptation of host cultivars in Agricultural pathosystems have also been reported as the causes of pathogen variations (Pariau *et al.*, 2009; Eugene *et al.* (2009). The prevalence of more aggressive isolates in the Southern Highland Zone compared to the Eastern Zone suggest a more natural adaptation of the pathogen to cooler areas hence call for urgent needs to supply resistant varieties of maize in these areas. However, the challenge of global climate change will likely lead to increased adaptation of the virulent gray leaf pathogen to warmer areas as it was evident in some areas in these studies. This also suggests that ready adaptation of the pathogen may ensue with increased temperatures due to global warming. Eugene *et al.* (2009) observed that wheat rust fungi (*Puccinia striiformis* f. sp. *tritici*) adapted to warmer temperatures and caused severe disease in

previously unfavorable environments.

Variation among isolates of *C. zeae maydis* observed in this study is consistent with the reports of other workers (Blair and Ayers 1986; Wang *et al.*, 1998; Dunkle and Carson 1999). Carson *et al.* (2002) observed that when isolates from four test locations were compared in common environments, significant differences in aggressiveness of those isolates were apparent. Okori *et al.* (2004) observed variability in pathogenicity and aggressiveness of isolates from samples collected in Uganda. In this study, isolates from the Southern Zone were more aggressive than isolates from the Eastern Zone suggesting that many of the Southern Zone groups might belong to the Type II and the Eastern Zone to Type I. Mathioni *et al.* (2006) observed that isolates of Type II were more aggressive than those of Type I which also differed in their fitness under different environments. However, confirmation using molecular methods will be required which will separate the two types more accurately for isolates in the study area. Significant positive correlations between isolate cercosporin production with disease and the lesions length suggest that cercosporin toxin plays a key role on disease development and lesion expansion in agreement with the report of (Wang *et al.*, 1998; Dunkle and Levy, 2000).

Differences in morphological variables were not significantly related to pathogen aggressiveness contrary to the reports of other workers on other pathogens. Ageel *et al.* (2008) observed significant variations on conidia and microsclerotia width and length of *Colletotrichum coccoides* which were significantly associated with pathogen aggressiveness. Asad *et al.* (2009) reported significant variations in conidial width, length and septa numbers among isolates of *Bipolaris sorokiniana* which were related to pathogen aggressiveness. However, significant positive associations of aggressiveness variables (Table 5) suggest that they are of more

important than morphological variables in the expression of pathogen aggressiveness for gray leaf spot.

Differential adaptation of individual causal organisms resulting to strains adaptation to the local environments could be the cause of variations in the incidences of other diseases of maize across regions and locations. Certain environments would appear to favor certain diseases and tolerance of local varieties and landraces endemic to the areas may also differ. For example, mildews, stalk rots and smuts were more severe in the cooler humid areas of the southern highlands (Iringa and Mbeya regions) while rusts, turcicum and southern leaf spots were severe in the lesser humid areas of the Eastern Zone (Morogoro region). Rupe *et al.* (1982) reported that gray leaf spot predisposes plants to fungi that are capable of causing stalk rot. However, incidences of gray leaf spot was not related to stalk rot during 2006 suggesting that factors other than predisposition effects of stalk rot could be a determining factor. Utilization of maize stables could have effected the development of gay leaf spot in the studied regions. In the southern highlands Zone, maize is usually left to dry in the farm for a long time and infected maize stables are left in the field after harvest provide sources of primary inoculum lead to high incidence of gray leaf spot compared to the Eastern Zone, where maize is harvested soon after maturity and stables are removed and fed to cattle hence remove the infected leaf trashes, lower primary inoculum and the disease incidence.

5.2 Reaction of resistant and susceptible maize genotypes to *C. zeae maydis* and associated disease resistance mechanisms

Spore germination, germ tubes growth and formation of mature appressorium did not differ between genotypes. These results are consistent with the reports of (Beckman and Payne, 1983; Thorson and Martinson, 1993) that spore germination and

penetration of *C. zeae maydis* is not influenced by genotypes inoculated and subjected to similar environmental conditions. However, there are some plants that release inhibitory compounds on leaf surfaces that prevent spore germination and subsequent penetration of plant pathogens as one of the disease defense mechanisns. For example, the red skin onions release Phenolic compounds (Protocatechuic acid and Catechol) that inhibits the germination and cause spore burst of *Colletotrichum circinus*) on onion leaves preventing infection (Walker, 1921; Agrios, 1988). Lack of significant differences between genotypes on early stages of *C. zeae maydis* development on leaf surface suggest that the leaf chemical environment between genotypes didn't have marked effects on development of *C. zeae maydis* on leaf surfaces. However, the reduced hyphae wefts growth in cells of resistant genotype ('UH6503) compared to moderately resistant ('Staha') and susceptible genotype ('Pannar') suggest that once the pathogen penetrate the host cells its growth is significantly been impaired as one of the host disease resistance mechanism against *C. zeae maydis* in maize. Gwin *et al.* (1987) found no difference between hybrids of varying resistance on penetration in inoculated leaf discs and speculated that the relative resistance among hybrids may involve difference in pathogen growth within tissues. The results of cytological studies reported here confirm significant pathogen reductions as possible resistant mechanisms against *C. zeae maydis* pathogen hence answer the speculations of Gwin (1987). However, as hypersensitivity cell reaction and pathogen impeding structures have shown not to be defence mechanisms likely for necrophyllic fungi such as *C.zeae mayidis*. Other disease resistant mechanisms possibly of chemical nature may be involved and could explain the slow hyphae growth of *C.zeae mayidis* observed after the pathogen had penetrated the resistant compared to susceptible maize genotypes (Fig. 3). Plant cells may also contain hydrolytic enzymes such as glucanase and chitinases which can cause breakdown of

pathogen cell wall components limiting growth in host cells (Agrios, 1988) or releases of toxic phenolic compounds and phytoalexins in cells upon infection capable of inhibiting the growth of pathogenic fungi (Farkas and Kiraly, 1963; Bell, 1981). A change in cell chemical environment in relation to host resistance following infection by *C. zea maydis* is poorly known and has not been reported. *Cercospora zeae maydis* is reported to produces cercosporin; a phototoxin virulent factor on the development of gray leaf spots in maize (Shim and Dunkle, 2002). However, host resistance to Cercospora diseases including *Cercospora zeae maydis* in maize has not been correlated with resistance to cercosporin or interference with cercosporin production or action, except for one report in rice (Batchvarova *et al.*, 1992), thus, suggesting that several other disease resistance mechanisms may play a significant role in the resistance of maize against *C. zeae maydis*. Call for more research on this area.

Reduced levels of components of resistance (numbers of conidiophores per stroma, spores per unit area and lesion sizes and number) were associated with reduced pathogen growth suggesting that the factor that was responsible for reducing mycelia growth in cells also reduced the growth of other morphological components of *C. zeae maydis*. Ringer and Grybauskas (1995) studying the primary and secondary infection cycles in gray leaf spot epidemics reported that moderately resistant hybrids had fewer lesions and lower sporulation capacity per unit leaf area than susceptible hybrids due to lower infection efficiency of *C. zeae maydis* on moderate resistant hybrids than susceptible hybrids. However, slow rate of pathogen growth and multiplication could have a positive effect in reducing disease epidemic.

5.3 Heritability and gene effect estimates for components of partial resistance to gray leaf spot and yield of maize.

Generation mean analysis provide useful information for designing breeding strategies to take the advantage of gene interaction that exist in the succession breeding generations. The test of additive-dominance model either by individual scaling test or by joint scaling test allow the detection and estimation of useful non-allelic interactions to be included in the breeding programmes. Lack of conformity with the additive-dominance model in all components except for disease severity and lesion number in cross 2 indicates the presence of epistatic gene interactions in many of the components expressing partial resistance to gray leaf spot of maize. The dominance effects were more wide spread followed by additive effects while stronger effects were observed more with the additive gene action especially in disease severity in cross 1 suggesting that both additive and dominance effects are important in the expression of partial resistance traits in maize. The importance of additive effects from breeder's point of view is essential for predictability in gene expression as genes contribute to traits in additive manner especially for quantitative ones while with dominance effect one can easily identify hybrids with promising performance due to dominance nature of the traits. However, un desirable data transformation in some cases has resulted to failure of the additive-dominance model, where inadequate cases were considered as adequate and vice versa (Mather and Jinks, 1982). This however, might affect the selection scheme relative to the assumption of models that evoke epistasis considering the fact that breeders main opportunity is to take advantage of additive effects. The heterotic effects and the relative % heterosis for all variables indicate involvement of dominance in the expression of the variables. Thus the use of hybrids, composites and synthetics can maximize the expression of these traits in the maize genotypes. Estimation of heterosis based on

additive-dominance model has well been described by Mather and Jinks (1982). The occurrence of significant additive effects for disease severity in all crosses compares with the reports of other workers (Elwinger *et al.*, 1990; Donahue *et al.*, 1991) that gray leaf spot is largely inherited additively. The significant additive x additive effect observed in many crosses compared to the dominant x dominant effect coupled with complementary epistasis suggests more favorable situation from breeder's point of view for greater chances of breeding success especially for the quantitatively inherited traits (Fig. 6).

The significant genetic variations among crosses in different components which were consistent at both locations suggests possible involvement of several genes in the expression of the components that confer partial resistance to gray leaf spot of maize and that maximization of expression of a trait depends on the specific combination of parental genotypes. Breeders can as well maximize selection for suitable parents. For example, maximization of yield, minimization of disease (less lesion size, disease severity, lesion length, lesion number and longer incubation period) will be obtained in crosses or progenies emanating from cross 2 (K36 x L37).

A difference in sign between the a and aa parameters, and the a and ad parameters, the d and ad parameters, and the d and dd parameters frequently occurs. This is interpreted as evidence for dispersal of genes. In other words, genes from both parents contribute to resistance and the choice of susceptible parent with good background will be an important consideration for maximizing resistance.

The relative frequency distribution for F_2 generations in these crosses fitted a continuous distribution curve indicating that these characters behave in quantitative manner and they are governed by polygenes. From breeders point of view this

situation favors success in the breeding process in the sense that resistance breakdown is not easy and thus, offers a sustainable resistance mechanism due to many components involved in the expression of partial resistance, each with its own system of genetic control. Thus, evolution of aggressive races or pathotypes would be expected to slow-down due to multiple gene interactions that offer a continuous form of durable resistance. Ahmed and Singh (2003) working on the components expressing partial resistance to rice blast (*Pyricularia oryzae*) observed similar results.

The additive effect was significant and more important for lesion size while dominant effect was significant and more important in lesion number in cross 1. El-Hissewy *et al.* (1992) working on rice blast observed that additive gene effect was significant and more important in the number of lesions and disease severity and the dominant effect was significant and more important for lesion length. However, the importance of dominant or additive effect is variable and depends on the character itself and crosses on the other hand (Higash and Kushibuchi, 1978; Ezuka, 1979; Lin, 1985) as it was observed in this study.

Additive effects were consistent and positively significant in all crosses for lesion size while other gene effects varied with a cross suggesting additivity is more important for this trait. The direction of gene effects was variable where for instance in cross 1, F_1 leaned towards resistant parent for all variables except for lesion number suggesting that resistance is dominant for these characters while in crosses 2 and 3 susceptibility was dominant for disease severity and lesion length.

The moderate to higher heritability values for components of partial resistance to gray leaf spot observed provide an evidence of higher degree by which these

characters can be passed from parents to off springs during breeding. Gordon *et al.* (2006) observed higher heritability for disease severity and lower for incubation period as it was the case of this study and concluded that the former could be suitable than the latter in the selection during breeding. The results of this study have also shown that lesion length, lesion sizes as disease severity too have reasonably high heritability values suggesting they may be handled easily during selection. Early selection for these variables is possible during screening for partial resistance to gray leaf spot. However, inorder to maximize resistance, later generation selection will also be necessary. The later generation selection will probably be more effective and important in some crosses. Heritability and gene effect estimates from this study have shown that lesion length, lesion size, and disease severity measurements are controlled in a quantitative manner, with high heritability. They can serve as estimators of partial resistance during selection and breeding for host-resistance to gray leaf spot of maize. Incubation period could be less useful due to low heritability and also more time required assessing the trait.

5.4 The effects of organic fertilizers (composted cattle and poultry manure) on development of gray leaf spot and yield of maize

Mean monthly rainfall, relative humidity and minimum and maximum temperatures were favorable for disease development in both cropping seasons. Gray leaf spot is favored by moderate to high temperatures (20-30°C) and high relative humidity (Ringer and Grybaskas, 1995; Paul and Munkovold, 2005).

The nutrient status of the soil in the study area was considered to be low for maize production with respect to N and P for recommendations in the Eastern Maize Growing Zone; at least 60 kg N ha^{-1} is required to obtain more than 3 t ha^{-1} grain yield of maize under local growing conditions (Kaliba *et al.*, 1988). Bray P-1 of less

than 8.0 mg kg^{-1} is considered to be sub-optimum for maize and requires P application of at least 20 kg ha^{-1} for more than 3 t ha^{-1} grain yield of maize. Bray 1-P value of 25 mg kg^{-1} of soil and above is considered adequate for maize production in Morogoro region where the experiments were conducted (Urio *et al.*, 1977). Potassium was not applied since K leaching in the area is minimal and observations confirmed that plants grew well without any K deficient symptoms.

The organic carbon and nitrogen contents of soils of the study area were very low reflecting infertile soils. The % BS (34.5%) of the soils was also low. According to London (1991) criterion of classification, % BS >50 % indicates fertile soils and % BS <50% indicates infertile soils. The soil pH of the study area was acid but was within the acceptable range for maize production. According to Sprague and Dudley (1988) maize performs well in soils with pH range from 5.0-8.0. Low soil pH favors low soil organic mater decomposition, promote P fixation, increases solubility of the bases, hence greater leaching capacity and low CEC (Hausenbuiller, 1985).

Concentration of total nitrogen in composted poultry manure was higher than in composted cattle manure consistent with reported results of Materechera *et al.* (2002) and Rosen and Bierman (2005). Materechena *et al.* (2002) also found that poultry manure resulted in higher maize grain yield than composted cattle manure. However, in the present study, composted cattle manure in the presence of gray leaf spot gave higher maize grain yield than composted poultry manure possibly due to the higher disease reducing effects observed. Nutrient uptake by plants from mineral fertilizer treatments appeared to be higher than in manure treated plants as reflected by significantly higher nutrient contents in composted manures than in mineral fertilizer treatments for soil sample analyzed at harvest. These data suggest that there would be little or no deficiency in nutrients after the compost application compared to mineral

fertilization and, therefore, compost would be a good substitute for the conventional mineral fertilization for maize. Gill *et al.* (2008) observed that total soil K, Ca, pH, OM, CEC and Na at harvest were higher in composted manure treatments than in mineral fertilizer treatments in the first year of application but NO_3-N, NH_4-N, P and Mg did not differ significantly between treatments. Residue tissue concentration of NO_3-N and NH4-N in this study differed significantly between fertilizer treatments suggesting differences in the uptake and assimilation of the nutrients by maize plants. Clark (1979) observed that higher concentration of NH_4-N was found in leaves, stems and roots of ammonium treated tomato plants. A higher concentration of ammonium in the solution resulted in the higher concentration in the plant. However, the absolute amount of the NH_4-N in the ammonium treated plants did not approach that of the NO_3-N in nitrate treated plants.

Yield responses of maize in the presence of gray leaf spot disease varied significantly between fertilizer treatments indicating fertilizer type dependent responses. Higher nitrogen rates increases susceptibility of maize to gray leaf spot infection but different forms of fertilizers showed different levels of disease progress. Nitrogen availability may differ with form of fertilizer such that fertilizers with higher nitrogen availability to plants resulted to more disease and vice versa (Kupper *et al.*, 2006; Tavernier *et al.*, 2007). However, it has been reported that the form in which nitrogen is available to plants (NO_3^-, NH_4^+) will influence the increase or decrease of disease severity (Huber and Watson, 1974; Utkhede and Smith, 1995) and that response of nitrogen on plant diseases depends on pathogen metabolic requirements and different ways in which pathogens acquire nutrients (Snoeijjers *et al.*, 2000; Tavernier *et al.*, 2007). In soils urea, CAN, SA, composted cattle and poultry manures mineralizes to NH_4-N and NO_3-N forms available to plants.

However, the rate of mineralization and uptake will depend on the type of fertilizer and soil conditions (Eghball, 2000). Maize leaf tissue nutrients at harvest indicated significant variations of nutrients including NH_4-N and NO_3-N that possibly could have directly or indirectly affected the development of the pathogen in maize leaf tissues. Treatments with lower levels of gray leaf spot (composted cattle manure, composted poultry manure and urea) had higher residue leaf tissue concentration of NH_4-N with lower diseases severity suggesting that higher NH_4-N leaf tissues concentration might have inhibited or interfered with the development of gray leaf pathogen in leaf tissues hence lowered the disease; a similar trend was observed in all other treatments with higher concentrations of NH_4-N giving an indication of possible involvement of this nutrient in the development of the gray leaf spot pathogen that affected the disease.

Differential micro and macronutrients preferences including NH_4-N on growth of different types of fungi have been reported. Smiley and Cook (1973) observed that wheat plants fertilized with NH_4-N were more resistant to take-all disease caused by *Ophiobolus graminis* var *titrici* whereas plants fertilized with NO_3-N were more diseased. Huber and Watson, (1974) listed *Pythium*, *Diplodia*, *Verticillium* and *Puccinia* among other pathogens which decrease in virulence on various hosts in the presence of NH_4-N and increase in the presence of NO_3-N and the reverse applied for *Rhizoctonia*, *Fusarium*, *Sclerotinia*, *Botrytis* and *Drenchslera*. On the other hand, animal manures such as composted cattle manure are rich in urea which has been reported to inhibit some crops diseases; an effect that could also account for disease reduction observed in composted cattle manure treatments in this study.

Lyimo and Kasuga (1994) tested the effect of urea, SA, CAN and composted poultry manure on bean diseases and observed urea fertilization significantly lowered bean

rust (*Uromyces phaseoli* Reben) Wint.) and increased yield significantly. Sulphate of ammonium suppressed bean angular leaf spot (*Phaseoisariopsis griseola* Sacc.) while CAN increased bean angular leaf spot and suppressed rust significantly. Composted poultry manure decreased the severity of bean rust and angular leaf spot. Mixture of urea silicofloride and Bayleton (Triadimefon) significantly reduced rust and mildew in wheat (Wei *et al.*, 1984). Composted cattle manure is also reported to reduce root and stem infection in cucumber caused by *Fusarium oxysporium* (Yoger *et al.*, 2006) and leaf gray mold in strawberries caused by *Botrytis cinera* (Sylvia, 2004). Compost added in soils have also been reported to suppress *Phytophthora* wilt in pepper, *Rhizoctonia* root rot in black eye, *Fusarium* wilt and gammy stem blight in squash (Rynk, 1992).

Composted cattle and poultry manure appeared to induce some degree of resistance on susceptible cv 'Pannar' suggesting the potential of these organic fertilizers in suppressing gray leaf spot of susceptible maize genotypes compared to inorganic fertilizers (urea, SA and CAN). The best genotype fertilizer combination was found in UH6303 + composted cattle manure with lower levels of disease possibly due to the inherent resistant trait of the cultivar coupled with the disease reducing effects of cattle manure. Non-fertilized plots (control) had lower levels of disease. Plots receiving supraoptimal rates of fertilizers were more diseased and lower yielding than plots receiving the recommended amount of fertilizers; these findings indicated that excessive use of the nitrogen incurs not only higher costs, but also results in reduced yield.

5.5 The effect of intercropping maize with beans on inoculum dispersal, development of GLS and yield of maize in minimum tillage operations

More disease was recorded in hand hoe till plots compared to convectional till by a

tractor possibly due to the failure of hand hoe to incorporate the infected residues deep into the soil to the level achieved by tractor that lead to higher disease incidence. Tractor ploughing followed by harrowing buried all infected crop residues into the soil compared to hand cultivation. *Cercospora zeae maydis* is reported to produces spores (conidia) within infected crop residues lying on soil surface which are wind and or rain-splash acting as the main source of primary inoculum (Ward *et al.*, 1999). *Cercospora zeae maydis* also produce dehydrated conidia under low relative humidity through microcycle conidiation (Lapaire and Dunkle, 2003) ensures constant supply of spores on infected residues lying on the soil surface.

Tillage practices that reduce the initial inoculum by burying infected debris deep into the soil has been demonstrated to be the most effective cultural methods for managing gray leaf spot of maize (Latterell and Rossi, 1983). However, it is likely that farmers will not stop doing reduced tillage operations for some reasons including the needs of soil conservation or/and lack of suitable tillage equipment as in the case of maize subsistence farmers in Tanzania. Therefore, it will be essential to prioritize host-resistance breeding or identify other cultural practices to reduce yield losses caused by gray leaf spot in reduced tillage operations like hand cultivation.

In this study, a significant disease reduction and higher yields was obtained by intercropping maize with beans compared to non-intercropping suggesting that intercropping could provide an alternative cultural practice for the management of gray leaf spot in reduced tillage operations; the practice has shown to be quite effective when beans and maize are planted on the same day at the spacing that will allow beans to provide dense canopy creating barrier that prevents upward movement of soil surface inoculum by wind and rain splash to maize leaves. Moreover, it is a common practice in the tropics to intercrop maize with beans. Therefore, it would be

easily accepted by farmers as it is already a familiar practice to them. The obvious additional advantage of this practice will be reduction of gray leaf spot as demonstrated in this study. Superior yield under intercropping was obtained on resistant cultivar under deep ploughing (7.8t ha^{-1}) possibly attributed to inherent resistance of the cultivar coupled with deep ploughing and intercropping practices; susceptible cultivar under similar cultural practices also gave good yields (5.5 t ha^{-1}) suggesting that multiple cultural practice combination (tolerant cultivars, deep ploughing and intercropping) when practiced together gave superior results than single cultural practice done alone. On other hand, intercropping also gave significant disease reduction in no-till suggesting that gray leaf spot could as well be reduced to a significant level in no-till operations by adopting intercropping practice hence solve the problem of high incidences of gray leaf spot in no-till maize cultivation.

The contribution of no-till without infected trashes was not resolved in this study; hence, lower yield in no tillage treatments could partly be contributed by no-till practice on the other side. However, Rattan (1974) observed that the grain yield of maize in no-tillage were equivalent to those of conventionally plowed treatments, moreover, no-tillage had higher organic matter, controlled soil erosion, with higher silt and clay contents than plowed plots. DeFelice *et al.* (2009) observed that no-till had greater corn yield than conventional tillage on moderate to well drained soils, but slightly lower yield than conventional tillage on poorly drained soils.

In the early stages of plant growth, the disease resistant parameters (AUDPC, r and spore numbers) were lower in the intercropped than non-intercropped indicating the potential of intercropping in preventing spore movement and rate of disease development. Spore traps that were placed outside the experimental area indicated no outside spore interference in these experiments hence the observed effects were

largely caused by the inoculum within the field. In the first 12 days after planting, spore trapped increased with increasing rainfall especially in non intercropped indicating the importance of rain splash in the disseminating of the spores of *C. zeae maydis* from infected debris that was spread on soil surface to the upper zones of maize crop canopy. Spore traps fluctuated with wind speed where more spores were trapped at lower than at higher wind speed suggesting that at reduced wind speed more spores fell on the traps due to gravitational forces than at higher speed which accelerated the horizontal spore movement. No attempts were made to study spore movements below bean canopy; however, we hypothesized that as the canopy of bean grew denser during the season, it produced an effective barrier to spore movement, reducing the number of propagules reaching the maize canopy from infected debris that was spread on the soil surface. Therefore, disease reduction under maize/beans intercropping treatments could possibly be due to the mechanical interactive effects of bean canopy on spore movements from the soil crop residue inoculum that were spread on soil surface. Canopy interactions that affect inoculum movement and changes in microclimate within crop canopy that favor pathogen development are among the mechanisms reported to influence disease development in the intercropping system (Allen, 1983). The interaction of spore number, spore viability and conditions favorable for infection will determine the severity of disease development in a crop land (Van de Plank, 1963). However, spore movement of *C. zeae maydis* could be minimal in mixed cropping systems common in the tropics compared to monoculture Corn Belt systems. Mixture of crop species may provide barrier on local spore movement leading to low disease when long distance wind spore dispersal is minimum. Asea *et al.* (2002) observed that both the amount of the crop residues and the distance of residues to the plant in a reduced tillage operation have significant influence on the level of infection by *C. zeae maydis* and on the

development of gray leaf spot. Payne *et al.* (1987) observed that more conidia were trapped in the air on no-till plots than on plowed plots which were reflected on no-till plots having higher disease severity than tilled plots.

Spore peak production coincided with higher R.H (>80%), and temperature of 23 °C to 32 °C corroborates early reports that gray leaf spot is favored by moderate to high temperatures (20-30°C) and high relative humidity (Beckman and Pyne, 1982; Ringer and Grybauskas 1995; Paul and Munkovold, 2005). The coefficient of determination was higher for all treatments indicating the suitability of the liner regression model used being capable of explaining 69-95% of the variance in the data.

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Current study has revealed useful information in the control of gray leaf spot of maize in Tanzania. Diverse of pathogen population in the Southern and Eastern maize growing Zones which differ in aggressiveness informs breeders the need to consider the existence of such diverse population of isolates in their breeding work. Breeders need to consider breeding cultivars with durable and broad resistance to gray leaf spot due to the existence of many isolates of the *C. zeae maydis*. It is recommended that the most virulent isolates such as (MBY1, MBZ1, IGAW1 and NJB1) and (DOM1, MKI1 and MGT1) be used when screening and development of improved varieties with resistance to gray leaf spot in the Southern highlands and Eastern Agro-ecological Zones respectively. Breeders should also be aware of increasing adaptation of gray leaf spot pathogen to warmer areas that likely to increase spread of disease in these areas. The selection for quantitative traits and differential adaptation to host cultivars in agricultural pathosystems has to be a priority in breeding programmes due to global climate change.

A histological study for gray leaf spot pathogen has revealed very useful information and the needs for further research in the mechanisms of control of gray leaf spot in the future. The result has shown possible involvement of chemical compound(s) produced follow infection that inhibited the growth of *C. zeae maydis*, a likely host disease resistance mechanism against this pathogen. Structural defense mechanisms and hypersensitivity cell reaction have shown not to be involved. More research is needed to identify and characterize possible cell compounds associated with the slow growth of gray leaf spot pathogen in the host cells. Also gene(s) involved in the

production of these compound(s) needs to be identified so that they can be incorporated into maize genotypes to improve resistance to gray leaf spot.

The heritability and gene effect study has revealed useful information that breeders could use in the selection and breeding of gray leaf spot of maize in Tanzania. The results has shown that it is possible to use information of lesion size, lesion length, lesion number and disease severity in the selection process during breeding process for resistance to gray leaf spot in maize for isolates exists in Tanzania. These characters are controlled in quantitative manner with high heritability values thus recommended as potential estimators of partial resistance during selection and breeding genotypes for resistance to gray leaf spot in maize. It may also be advantageous to select for complimentary components of resistance in the respective parents of a hybrid (e.g. emphasizing selection for one component in one parent and a different component in the other).

The additive and additive x additive interaction suggests the identification of complimentary QTL in respective may also be desirable for the resistance improvement. Presence of dominance effects also holds promise for development of hybrids, composites and synthetics in maize for resistance to the disease. The presence of gene interactions also indicates that background effects in particular crosses will be unpredictable. Suitable cultural practices for control of gray leaf spot of maize have been identified in this study that could be quite useful for maize growers in Tanzania. Intercropping maize with beans in minimum tillage by hand hoe has shown to reduced gray leaf spot to the range of 15 to 40.9% with overall increase yield of about 41 % compared to non-intercropped maize. This is a big save of what would otherwise been lost due disease attack and a super gain when converted and considered in monetary terms. Proper fertilizer management regimes

that will ensure low levels of gray leaf spot and higher yield of maize have been identified in this study. The use of composted cattle and poultry manure have shown to lower gray leaf spot and increased maize yield by 30% compared to untreated. This is a positive gain considering there will be no fungicides application and the low cost of manure compared to chemical industrial fertilizers. Moreover, nutrients from compost manure are released slowly into the soil which builds a stable soil nutrient reserves bound and cycled within the soil humus (Hepperly *et al.*, 2009). Hence, compost builds a more self-sustaining soil nutrient cycle that provides nutrients with a long term advantages of soil improvement. Composts manure also increase soil C and N contents compared to synthetic chemical fertilizer treatment which has shown to have little or no effect on soil nutrient contents (Hepperly *et al.*, 2009). Over short and medium-term use, synthetic chemical fertilizers may be attractive due to their convenience, ease of application, and reliable high yield. However, chemical fertilizers have been shown to accelerate nitrate leaching, breakdown of soil organic matter and soil acidification compared to composted manure treatment (Hepperly *et al.*, 2009). Composts treatments also required no liming over time, while with chemical fertilization lime applications is necessary as it acidifies the soil. However, with chemical fertilizers, urea treatment has shown to be the best in managing gray leaf spot followed by SA and least for CAN, these results needs to be considered in the management of disease with fertilizer use although the choice of fertilizer will also depend on other soil characteristics. These low cost technologies need to be disseminated as appropriate Integrated Pest Management Strategies for maize growers in Tanzania to meet the challenges of millennium development goals.

6.2 Recommendations

Since the inception of the bioinformatics by Hogeweg (1978) for the study of

informatics processes in biotic systems, useful information in genetics and genetics engineering have been discovered. Its primary use since late 1980s has been in genomics and genetics, particularly in those areas of genomics involving large-scale DNA sequencing. It entails the creation and advancement of databases, algorithms, computational and statistical techniques and theory to solve formal and practical problems and application of mathematical and computing approaches to increase understanding of biological processes. For example, analysis of gene expression through measuring mRNA levels with multiple techniques including microarrays, expressed cDNA sequence tag (EST) sequencing, serial analysis of gene expression (SAGE) tag sequencing or massively parallel signature sequencing (MPSS); analysis of regulation of complex extracellular signal such as a hormone and protein activity and modeling of biological systems involving the use of computer simulations of cellular subsystems (such as the networks of metabolites and enzymes which comprise metabolism, signal transduction pathways and gene regulatory networks) to both analyze and visualize the complex connections of these cellular processes. Bioinformatics on gene regulation for resistance to gray leaf spot did not form part of this research but it is recommended for future work which may include studies such as:

(i) Microarray analysis aimed at identifying the genetic and molecular components involved in the infection of gray leaf spot isolates in Tanzania.

(ii) Genotyping, development of biological database for disease, QTL mapping for resistance in various maize varieties, development of QTL-mapping software and statistical methods suitable for Tanzania.

(iii) Population and evolutionary genetics and genomics of maize varieties and gray leaf spot isolates in Tanzania.

(iv) Analysis of genome-scale DNA polymorphism datasets in order to infer the relative importance of processes such as mutation, recombination, genetic drift, and natural selection for gray leaf spot pathogen in Tanzania.

(iv) Develop methods of natural selection at the DNA level in order to identify adaptive genetic variation of gray leaf spot pathogen in various Agro-ecological Zones in Tanzania.

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8.0 APPENDIXES

Appendix 1. Analysis of variance (mean squares) on aggressiveness variables for isolates collected in villages of Morogoro, Iringa and Mbeya regions

Source variation	df	Mean squares			
		LL	PLAA	IP	SPN
Regions	2	0.257*	3.894*	6.058*	1.006
Isolates	29	4.266***	145.640***	4.951	7.116***
Error	58	0.233	3.919	3.152	0.869
Total	89				

[1]LL=Lesion length (cm); [2]PLAA=Percent leaf area affected; [3]IP=Incubation period
[4]SPN=Spore number x 10^4 /cm^2

Appendix 2. Analysis of variance (mean squares) for isolates growth rates

Source variation	Mean square	
	df	[1]GR
Replication	2	15.109
Isolates	29	2.115**
Error (a)	58	1.116
Total	89	

[1]GR=Growth rate (mm/day)

Appendix 3. Analysis of variance (mean squares) on pathogen morphological variables for isolates of C. zeae maydis collected in villages of Morogoro, Iringa and Mbeya regions

Source variation	df	Mean squares						
		CPN[1]	SN[2]	CPD[3]	CPL[4]	SN[5]	CNL[6]	CND[7]
Regions	2	1.107	0.234	4.087*	999.201*	15.324*	11.757*	1512.534**
Isolates	29	3.573***	3.9991***	2.030***	478.331***	3.618*	3.751**	280.289***
Error	58	1.017	0.953	0.594	128.026	1.328	1.238	66.056
Total	89							

[1]CPN=Conidiophores number per stroma; [2]SN=Septa number per conidiophore; [3]CPD=Conidiophore diameter; [4]CPL=Conidiophore length; [5]SN=Septa number per conidia; [6]CNL=Conidium length; [7]CND=Conidium diameter.

Appendix 4. Analysis of variance (mean squares) for severity of gray leaf spot and incidence of other diseases of maize assessed in villages of Morogoro, Iringa and Mbeya regions

Source variation	df	GLS[1]	Mean squares[2]					
		Severity (0-5)[3]	C/Rust[4]	SLS[5]	NLS[6]	DMLD[7]	Smut[8]	S/rot[9]
Regions	2	0.012**	22.279**	113.457**	18.803**	78.405**	51.094*	15.329*
Villages	29	1.528***	1006.983***	560.942***	420.836***	615.156***	334.671***	189.291***
Error	58	0.055	18.804	58.634	19.999	42.546	26.82	14.001
Total	89							

[1]Gray leaf spot ; [2]% incidence [3]0=no disease 1=1-20% ;2=21-40%; 3=41-60% 4=61-80% and 5=81-100%; [4]C/Rust=Common leaf rust (*Puccinia sorghi*); [5]SLS=Southern leaf spot (*Bipolaris maydis*); [6]NLS=Northern leaf spot (*Exerohilum turcicum*); [7]DMLD=Downy mildew (*Perenosclerospora maydis*); [8]Smut=Head smut (*Sphaerotheca reilliana*); [9]S/rot=Stalk rot (*Gibberella zeae*).

Appendix 5. Analysis of variance (mean squares) for gray leaf spot severity, components of resistance, yield of P_1, P_2 and F_1, F_2, BC_1 BC_2 generations evaluated at SUA and Bigwa Kisiwani and Kilimani.

		Mean square					
Source of variation	df	LL[1]	LS[2]	LN[3]	IP[4]	DS[5]	Yield(kg/ha)
Replication	1	0.32	0.02	11.63	12.92	0.03	0.44
Generations	5	221.72***	860.69***	1581.91***	97.77**	0.49***	39.92***
Crosses	2	3.16***	10.35***	4.38*	6.54*	0.19**	8.66***
Gen x Crosses	10	0.42	3.31***	6.37*	5.12*	0.13***	0.32**
Locations	2	4.41***	10.42***	29.52**	4.93*	0.27*	0.24*
Gen x Loc	10	0.79	2.69***	29.00***	2.17	0.51***	0.75**
Cross x Loc	4	0.36	0.27	7.25	0.10	0.09	1.23***
Gen x Cross x Loc	20	0.46	0.88**	2.18	1.80	0.04	0.12
Error	53	0.23	0.27	3.16	1.67	0.02	0.15
Total	107						

SUA=Sokoine University of Agriculture Farm; [1]LL=Lesion length; [2]LS=Lesion size; [3]LN=Lesion number; [4]IP=Incubation period; [5]DS=Disease severity; *Significant at P≤0.05; ** Significant at P≤ 0.01; *** Significant at P≤0.001

Appendix 6. Analysis of variance (mean square) soil properties and fertilizer treatments (test conducted at the end of the experiment).

Source of variation	df	Mean square
Replication	1	0.012
Soil properties (A)[1]	12	602.082***
Fertilizer types (B)	2	6.673***
A x B	24	1.648***
Error	38	0.031
Total	76	

Appendix 7. Analysis of variance (mean square) for residue leaf tissue nutrient concentration for different fertilizer treatments (test conducted at the end of the experiment) studies conducted at SUA.

Source of variation	df	Mean squares				
		TDM (%)	P (%)	N (%)	$N\text{-}NH_4$	$N\text{-}NO_3$
Replication	2	0.001	0.004	0.021	0.000	0.007
Fertilizer types	5	30.737***	89.528***	2.190***	0.043***	7.738***
Error	10	0.001	0.002	0.035	0.000	0.011
Total	17					

TDM=Total dry matter; N=Total nitrogen; P=Phosphorous; $N\text{-}NH_4$=Ammonium nitrogen; $N\text{-}NO_3$=Nitrate nitrogen; ***$P \leq 0.001$

Appendix 8. Analysis of variance (mean squares) for gray leaf severity in different fertilizer treatments averaged over two growing seasons (2006/07 and 2007/08) studies conducted at SUA.

Source of variation	df	Mean squares				
		V8[1]	V10	V12	V15	V18
Replication	2	10.891	9.846	12.330	7.216	4.257
Genotype	2	4.375***	23.214***	38.509***	57.726***	56.881***
Year	1	0.002	0.009	0.387	0.000	0.002
Genotype x Year	2	0.000	0.007	0.223	0.000	0.000
Fertilizers	10	1.025***	2.994***	1.663***	1.539***	2.287***
Genotype x Fertilizers	20	0.426***	0.740***	0.507***	0.430**	0.341***
Year x Fertilizers	10	0.001	0.005	0.026	0.001	0.004
Genotype x Year x Fertilizers	20	0.001	0.005	0.026	0.001	0.004
Error	130	0.132	0.131	0.147	0.169	0.237
	197					

[1]V8 to V18 are maize growth stages

Appendix 9. Analysis of variance (mean squares) for yield and yield components in different fertilizer treatments averaged over two growing seasons (2006/07 and 2007/08) studies conducted at SUA.

Source of variation	df	Mean squares			
		Days to 50% Silk	Ear height (cm)	100 seed wt	Yield(t/ha)
Replication	2	7.470	337.883	125.783	1.444
Genotype	2	1211.078***	2247.358***	1303.974***	57.901***
Year	1	0.090	0.158	1.047	0.002
Genotype x Year	2	0.012	0.176	0.181	0.048
Fertilizers	10	140.975***	685.467***	551.111***	7.375***
Genotype x Fertilizers	20	54.901*	295.814*	86.635*	4.461*
Year x Fertilizers	10	0.024	0.162	0.240	0.007
Genotype x Fert x Year	20	0.025	0.142	0.387	0.029
Error	130	12.178	61.986	25.853	0.992
Total	197				

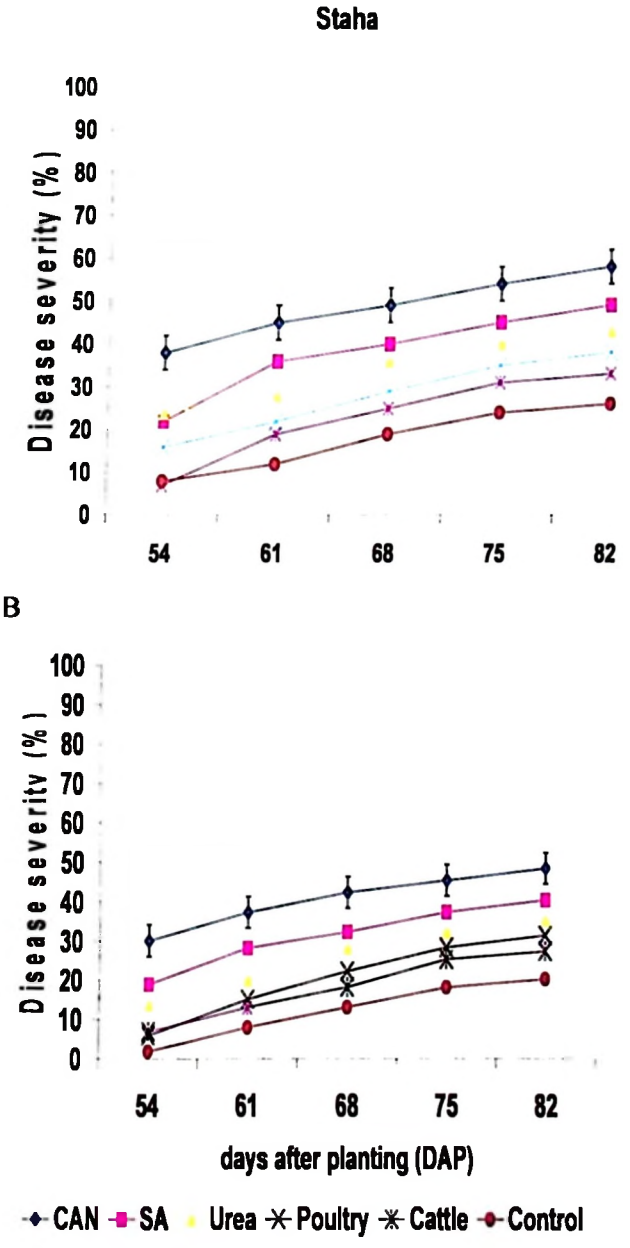
Appendix 10. Analysis of variance (mean squares) for gray leaf spot severity, components of resistance and yield of maize produced under sole crop and intercrop conditions during two growing seasons (2006/07and 2007/08) conducted at SUA.

		Mean squares				
Source	df	$(r)^2$	NST[3]	R^2	AUDPC[4]	YIELD(t/ha)
Replications	2	0.99	1.63	409.18	1664.25	6.36
Genotype	2	390.40***	29.67***	17.79	400173.20***	43.71***
Year	1	82.93***	2198.13***	408.33	960.63*	23.61***
Genotype x Year	2	0.12	18.88***	117.794***	1184.66	1.19
Tillage	2	571.19***	898.65***	1430.70***	35325.16***	12.38***
Genotype x Tillage	4	42.15***	6.02*	886.52*	10231.27***	4.15***
Year X Tillage	2	7.03	513.33***	151.23**	1264.84	0.04
Genotype x Year x Tillage	4	8.17	6.56***	225.11	1078.54*	0.78
Intercropping	1	389.27***	442.30***	161.33*	40209.62***	26.90***
Genotype x Intercropping	2	8.46**	1.42	1313.26*	10199.53***	1.90*
Year x Intercropping	1	5.62	297.21***	333.56	3118.38**	1.36
Genotype x Year x Inter	2	3.78	1.74	234.31	3986.38*	0.24
Tillage x Intercropping	2	41.86***	97.45***	175.66	304.86	0.11
Genotype x Tillage x Inter	4	5.83	0.94	261.04	184.50	0.25
Year x Tillage x Inter	2	0.462	78.86***	920.50***	312.54	0.42
Genotype x Year x Till x Inter	4	1.03	1.45	278.41	748.32	0.29
Error	70	3.82	1.29	131.33	430.70	0.59
TOTAL	107					

[1]GLS=Gray leafspot; [2]Rate=apparent infection rate; [3]NST=Number of spores trapped; R^2=Coefficient of determination; [4]AUDPC=Area under disease progress curve; *P≤0.05; **P≤0.01; ***P≤0.001

Appendix 11. Effect of composted manure and mineral fertilizers on the development of gray leaf spot cv 'Staha' during 2006/07 (A) and 2007/08 (B) cropping seasons (Trials conducted at SUA).

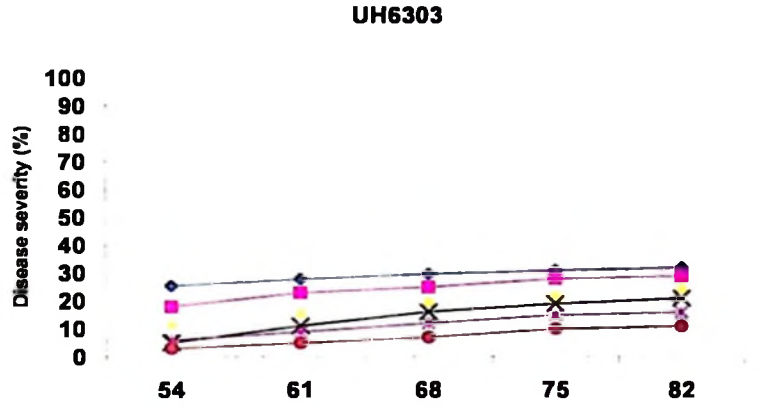
A.



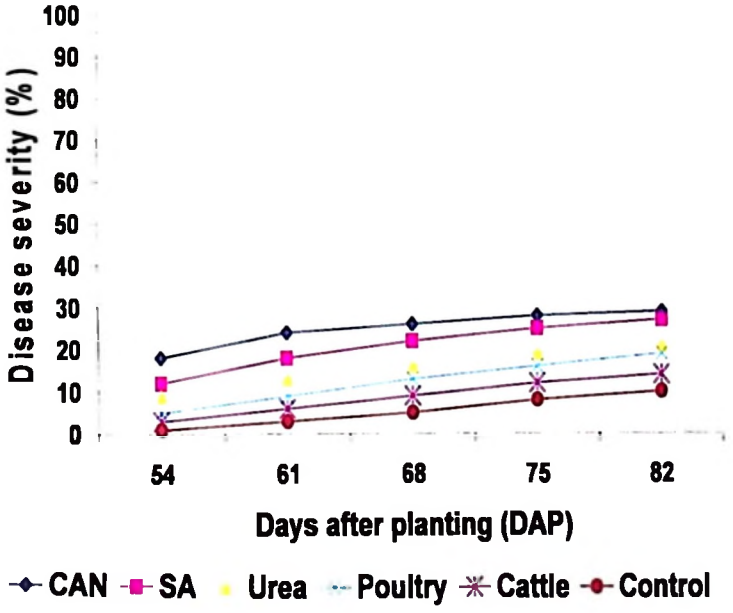
CAN=Calcium ammonium nitrate: SA=Sulphate of ammonia; Poultry=Poultry manure; Cattle=Cattle manure; Control=Not fertilized; DAP=Days after planting

Appendix 12. Effect of composted manure and mineral fertilizers on the development of gray leaf spot cv 'UH6303' during 2006/07 (A) and 2007/08 (B) cropping seasons (Trials conducted at SUA).

A



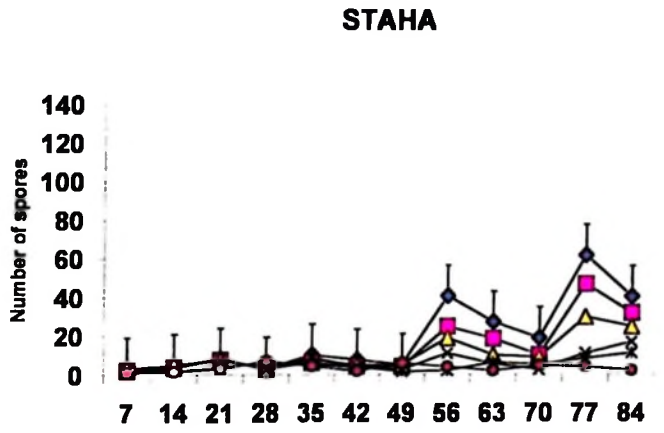
B



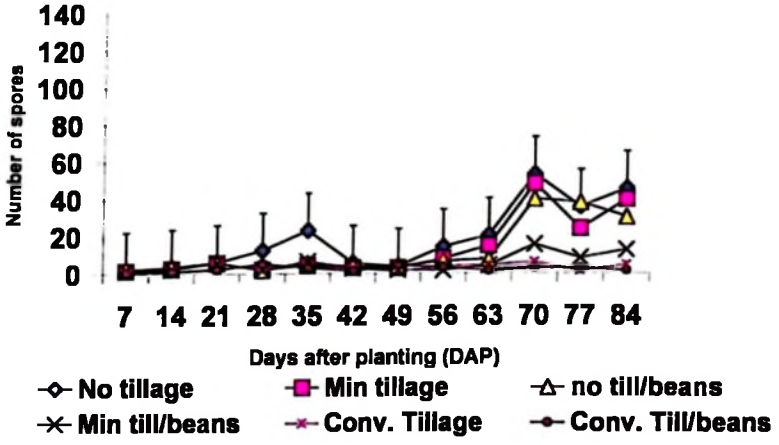
CAN=Calcium ammonium nitrate: SA=Sulphate of ammonia; Poultry=Poultry manure; Cattle=Cattle manure; Control=Not fertilized; DAP=Days after planting.

Appendix 13. Number of spores x 10^3 of *C. zeae maydis* trapped on cv 'Staha' during 2006/07 (A) and 2007/08 (B) cropping seasons (Trials conducted at SUA).

A.



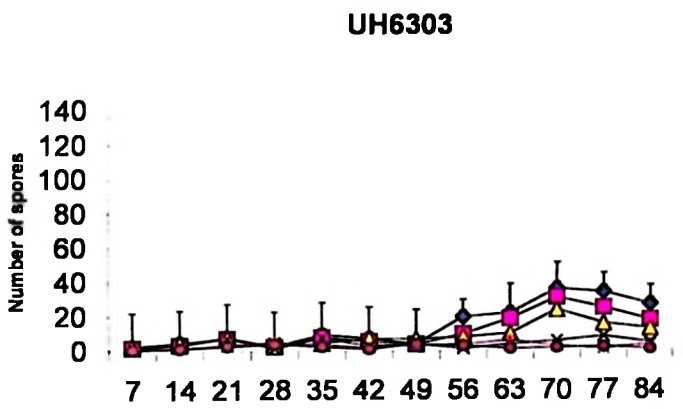
B.



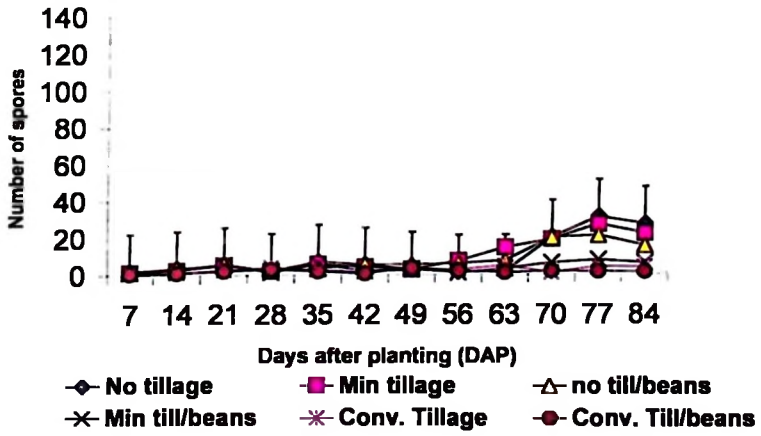
No till= no-tillage; min till= minimum tillage; no-till/ beans=no-tillage maize intercropped with beans; min till/beans= minimum tillage maize intercropped with beans; conv. tillage=conventional tillage; conv. till/beans= conventional tillage maize intercropped with beans.

Appendix 14. Number of spores x 10^3 of *C. zeae maydis* trapped on cv 'UH6303' during 2006/07 (A) and 2007/08 (B) cropping seasons (Trials conducted at SUA).

A.



B.



No till= no-tillage; min till= minimum tillage; no-till/ beans=no-tillage maize intercropped with beans; min till/beans= minimum tillage maize intercropped with beans; conv. tillage=conventional tillage; conv. till/beans= conventional tillage maize intercropped with beans.

Appendix 15. Map showing deficit and surplus maize producing areas of Tanzania