CULTURAL STUDIES AND ASSESSMENT OF SEEDBORNE INOCULUM OF *Pyricularia grisea* (anamorph *Magnaporthe grisea)* ON AND IN FINGER MILLET SEED AND ITS IMPLICATION ON BLAST INCIDENCE AND SEVERITY.

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DECLARATION

This Thesis is my original work and has not been presented for a degree at any other University.

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DEDICATION

To the following persons this thesis has been dedicated:

My father Mr. Safani Julius Kigamwa and mother Mrs. Brigit Ndivi Kigamwa.

My Wife, Dr. Anisia Muriuki Ngili and my two daughters Whitney Ndivi Ngili and Fortune Yembu Ngili.

May the good God keep them for many years.

"Hard times don't last, tough people do."

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TABLE OF CONTENT

CHAPTER THREE: MATERIALS AND METHODS

Cultural characteristics and pathogenicity of *P. grisea* isolates

Seed assessments of *P. grisea* seedborne inocula.

Field Experiments

CHAPTER FOUR: RESULTS

Cultural studies

Finger millet seed health evaluation for infection/contamination by *P. grisea*

Field Experiments

CHAPTER 5 : DISCUSSION

List of appendices **Page**

List of plates

List of tables **Page**

List of figures and maps

ABSTRACT

Blast of finger millet caused by *Pyricularia grisea* anamorph *Magnaporthe grisea* has been reported to be a major constraint of finger millet (*Eleusine coracana*) production in Kenya causing 10 – 90 % losses in farmers fields. During a study on finger millet blast conducted between December 1995 and February 1997 various laboratory and field experiments were conducted to study finger millet blast disease and address the following objectives: To establish the seedborne nature of *Pyricularia grisea* anamorph *Magnaporthe grisea* in and on finger millet seeds; to establish the best cultural conditions of the pathogen, *Pyricularia grisea* anamorph *Magnaporthe grisea*; to determine the efficiency of the various seed assessment techniques for evaluation of seedborne inocula of *Pyricularia grisea* anamorph *Magnaporthe grisea*; to determine the relationship between seedborne inocula of *Pyricularia grisea* and disease incidence in the subsequent finger millet crop; to determine the relationship between time of infection of plant with *Pyricularia grisea* anamorph *Magnaporthe grisea* and level of disease severity and level of contamination of harvested seed. The methodology used included for seed assessment of farmer seeds sampled from Kisii, Bungoma and Busia/Teso were blotter, rolled paper towel and agar plate test; for cultural studies five different media, 4 different pH levels, 3 isolates and 4 different lighting regimes were used; for artificial inoculation 5 different inoculation stages were used; for planting out seeds with different levels of *P. grisea* 6 different levels were used..

P. grisea anamorph *Magnaporthe grisea* was found to sporulate best on oatmeal agar OMA) at pH 7 and a 12 hr lighting / 12 hr darkness regime and hyphal growth was best on nedia FMLEA(finger millet leaf extract agar)at pH 8 and 24 hours lighting regime. *P. grisea* namorph *Magnaporthe grisea* also exhibited concentric zonation and sectoring on finger illet seed extract agar (FMSEA) and OMA. Three *P. grisea* anamorph *Magnaporthe grisea* olates were obtained from infected seeds obtained from three districts of Busia, Bungoma

and Kisii. The Kisii isolate was found to produce most spores ($p \leq 0.05$) on media followed by isolates from Bungoma and Busia respectively. The isolate from Busia had the fastest mycelial growth rate in mm/day on media followed by the isolates from Kisii and Bungoma respectively.

Thirty four seed samples of Finger millet collected from farmers in Busia/Teso, Kisii and Bungoma and 7 samples provided by ICRISAT showed infection of *P. grisea* anamorph *Magnaporthe grisea* ranging from 0 - 14 % when assay method was blotter test. Infected seed samples showed poor germination (< 80% germination) which is below the 80 % germination requirement. Out of these farmers seeds only one would qualify for a quality certificate in Kenya. Grey/black discoloured seeds had more infection of *P. grisea* and seed infection counts were higher in the blotter than in the rolled paper or agar test methods, showing that the blotter test would be a more appropriate seed assessment test method than the other two.

Epidemics of finger millet blast caused by *P. grisea* anamorph *Magnaporthe grisea* were initiated at 5 different plant growth stages i.e. 1, 2, 3, 4 & 5 corresponding to stem elongation, inflorescence emergence, anthesis, milk development and ripening, on a finger millet genotype KNE 479 during the 1996 long rains at Kiboko and Kabete and 1996/97 short rains at Kabete. Stem elongation stage was found to be most susceptible ($p \leq 0.05$) when various disease index parameters were considered, while milk development stage had most ($p \leq 0.05$) contamination of *P. grisea* anamorph *Magnaporthe grisea* on harvested seed. In stem elongation stage, yield losses as great as 11 % were recorded from inoculated plots when seed yields were compared to those of control plots (uninoculated). The 1000 seed weight was significantly reduced in some inoculated treatments. The high yield losses, especially in 1000 seed weight were indicating that *P. grisea* anamorph *Magnaporthe grisea* could be a greater problem in seed production fields. Percent yield reductions were positively correlated ($p \leq$

0.05) to panicle, neck and leaf blast. A disease scoring system for leaf, neck and panicle blast was designed for the experiment especially for use in the field.

Infected seed samples of 0, 2.5, 4.25, 6.00, 7.75 and 14.00 percent incidences of *P. grisea* anamorph *Magnaporthe grisea* were grown in the field, exhibited less than 2 % severity on the leaves and no panicle or neck blast was recorded. Final incidences in relation to leaf blast varied from 26 to 38 %.

In recommending, *P. grisea* anamorph *Magnaporthe grisea* would sporulate best under media OMA at pH 7 and lighting regime of 12 hours lighting and 12 hours darkness. Control measures in the field for blast should start before the stem elongation stage. Farmers should be trained on production of healthy seeds, since they do use the seed from the harvested yield.

1
CHAPTER ONE

INTRODUCTION

1.1 Finger millet production and distribution

Finger millet (*Eleusine coracana* (L.) Gaertn) is a robust, free-tillering, tufted annual grass, with flattened stem and shallow fibrous root system. Although finger millet(*Eleusine coracana* L. Gaertn) represents less than 2 % of the world cereal utilization, it is an important staple food in a large number of countries of the semi-arid tropics (Marathee, 1993). Finger millet ranks fourth in the economic significance of small-seeded millets, after pearl millet, foxtail millet and proso millet. It originated in East Africa and was first domesticated by selection approximately 5000 years ago (Zeller, 2000; Odelle, 1993). It is estimated to cover approximately 8 % of the area and 11 % of the production of all millets in the world. Reliable statistics of acreage and production of finger millet are difficult or impossible to get for many countries as they are frequently included with other millets and sorghum (Rachie and Peters, 1977). Production statistics for the various millets are often lumped together (sometimes with sorghum) but most recent estimate suggests that pearl millet accounts for 50 % of the production, foxtail and proso millet account for 30 %, finger millet accounts for 10 % while a combination of eight millet species account for the rest of 10 % (Anon., 2002). The average global area which millet grain was harvested was around 38 million hectares giving a yield of 28.5 million metric tons (Anon., 2002). Hence if finger millet accounts for 10 %, that would mean 3.8 million hectares giving 2.85 million metric tons.

Finger millet is most intensively grown in regions surrounding Lake Victoria in East Africa and in the South Eastern part of Karnataka and the adjoining districts of Tamil Nadu and Andra Pradesh in Southern India. These relatively small and circumscribed regions account for nearly three-quarters of the world's production of this cereal (Rachie and Peters, 1977). Finger millet is important in Uganda, South Asia (India), China, Russia and several other African

countries, such as Kenya, Zimbabwe and others (Guiragossian and Mukuru, 1993). In East and Central Africa, around 1,000,000 hectares of land is under finger millet, with Uganda having the crop under 330,000 to 500,000 hectares (Makini, 1999; Anon., 2001b).

1.1.1 Production of finger millet in Kenya and its economic importance

In Kenya, finger millet is mainly grown in Rift valley, Western, Nyanza, Eastern and Coast provinces (Acland, 1989). The leading districts in finger millet production are Busia, Teso, Bungoma, Kisii and South Nyanza. Other districts include Kericho, Nandi, Vihiga, Kisumu, Siaya, Mount Elgon, Uasin Gishu, Meru, Embu, Machakos, Kitui and Taita-Taveta (Acland, 1989). The production statistics for finger millet include, Bungoma with 947.1 tons on 1,230 hectares, with Bumula, Kandunyi and Sirisia divisions producing the most in that order (Anon., 1996a); Busia and Teso with 4,700 tons on 6,715 hectares (Anon., 1996b); Kisii with 846 tons on 3,513 hectares (Anon., 1993) and South Nyanza with 3,704 tons on 3,450 hectares (Anon., 1988).

In the year 2000, there was an increase of 50 % on millet production from 400,000 bags to 600,000 bags (Anon., 2001a). According to FAO (Anon., 2001b) stastistics on millet production in Kenya, 55,000 metric tons i.e. 611,111 bags (90 kg bags) was produced on an area of 90,000 hectares giving 611 kg per hectare. Makini (1999) puts finger millet production on land at greater than 65,000 hectares giving approximately 39,715 metric tons.

The crop is traditionally grown without fertilizers or manure and normally by broadcasting. In Nyanza province finger millet was ranked third in importance among the cereals grown by small-scale farmers (Anon., 1988). In Kisii district the crop is quite popular and is grown mainly on small plots of about 0.1 hectares or less (Anon., 1993). In Uasin Gishu district the crop has gained popularity, especially in Moiben division because it has high

nutritive value, its low attack by diseases and pests, and it performs better than maize (Anon., 1992).

1.1.2 Marketing

The main buyers of finger millet include Unga Mills (K) Limited, The National Cereals and Produce Board (NCPB), various milling companies and cereal markets middlemen that make flour for porridge either singly or in combination with either other cereal flours or soybean flour or sell the grain as a whole.

Market prices (1$ = Ksh. 78) for the year 2001 were: Eastern province Kshs. 20.00 to 22.00 per kg of grain, Rift Valley Kshs. 20.00 to 25.00 per kg of grain, Nyanza Kshs. 24.30 to 26.10 per kg of grain and Western Kshs. 21.90 to 30.30 per kg of grain (Anon., 2001a). Current local market prices (for 90 kg bag) are: Nairobi - Kshs. 1,800; Mombasa - Kshs. 2,700; Nakuru - Kshs. 2,000; Kisumu - Kshs. 2,000 and Bungoma - Kshs. 2,000 (Anon., 2002).

1.1.3 Uses and nutritional value of finger millet

Finger millet grain is used in many forms for human food. It is most frequently ground to make flour for preparation of porridge, stiff porridge (locally called "ugali"), unleavened bread or cakes, which may be cooked, baked or fried with condiments, spices. Commonly found in the supermarkets is a blend of finger millet and soybean flours popularly used for porridge for infants. The immature grain can be roasted and eaten as a vegetable. Another favourite preparation is parching the mature grain, then grinding and mixing with some other food ingredients such as crude sugar, salt, spices or buttermilk. Finger millet is one of the few crops from which excellent malt can be prepared, and it is much appreciated for this characteristic. The malt can be used directly or in preparation of various beverages and drinks (ICAR, 1961). In Kenya and Uganda, traditional beer is made from fresh or stored grain, which is germinated,

dried, ground and mixed with water (Purseglove, 1985). Anderson (1948) reported that in China, finger millet is also used for beer making.

Straw can be used as fodder and the fields are often grazed after the panicles have been harvested. In some areas, such as in Southern Karnataka, India, the fodder is highly valued for feeding cattle and other ruminants and it makes a good supplement to the income from the grain (Rachie and Peters, 1977). In Uganda, the stover is grazed *in situ* in the field or cut and fed to the animals (Mushonga *et al.*, 1993)

Johnson and Raymond (1964) gave the average composition of the grain as Water - 13%; Protein - 8%; Fat - 1.3%; Carbohydrates - 72%; Fibre - 3%; and Ash - 2.7%. The protein content shows considerable variation ranging from 6 - 11%, and a white seeded cultivar in India had as much as 14%. The prolamine of finger millet, eleusinin, has high biological value, with a good content of cysteine, tyrosine, tryptophan and methionine, which are important in the prevention of kwashiorkor. It is important to note that this grain is low in lysine. The grain is a rich source of calcium containing 0.33%, compared with 0.01 - 0.06% in many other cereals. It is also rich in phosphorus and iron. Calcium is important for nursing mothers new-born babies and for bone and teeth formation, while iron forms haeme, the building block of haemoglobin, which carries oxygen in the blood of man (Johnson and Raymond, 1964).

Finger millet straw is used for thatching, making the walls of small granaries and making plates and various types of food containers (Rachie and Peters, 1977).

1.2 Constraints in finger millet production

1.2.1 Diseases

Early investigators and observers of finger millet had a common belief that diseases of finger millet were relatively unimportant (Coleman, 1920; Venkatakrishnaiya, 1935; Patel, 1955). Moreover, these early workers considered *Helminthosporium* spp to be more important

than other pathogens. However, more recently blast caused by *Pyricularia grisea*, has been considered extremely important owing to the high losses that can occur from panicle infection, particularly if the neck and lower earhead are infected. Literature indicates that a considerable number of different pathogenic fungi can infect and cause loss to finger millet. There are at least 14 fungal pathogens in addition to *P. grisea* and two species of *Helminthosporium* that have been reported infective on finger millet. It is also susceptible to a bacterial disease caused by *Xanthomonas coracanae* (Desai *et al.*, 1967), and two or three viral diseases causing a mosaic or mottling of the leaves as recorded in India (McRae, 1929). Some of the fungal pathogens (diseases) include *Melanopsichium eleusis*(Kulk)Mundk and Thiram cause of smut reported in India (Kulkarni, 1922) and Kenya (Mundkur, 1939); Wilt or foot rot caused by *Sclerotium rolfsii*(Sacc.)Curzi; Downy mildew caused by *Sclerophthora macrospora*(Sacc.)Thirum and coworkers (Venkatarayan, 1947); Other diseases include foot rot caused by *Gibberella saubinetti*(Mont.)Sacc. reported in Uganda (Hansford, 1933); tar spot caused by *Phyllachora eleusins* Spet reported in Uganda and Tanzania(Wallace and Wallace, 1947).

1.3 Blast of finger millet

1.3.1 Distribution and economic importance

The pathogen has been reported in Cuba, Ethiopia, Europe, Fiji, Guinea, Hong Kong, India, Japan, Kenya, Malawi, Nepal, Nigeria, N. America, Papua, Paraguay, Zimbabwe, Sierra Leone, Sudan, Tanzania, Trinidad, Uganda, Venezuela, Zambia (Ellis, 1971). Blast is the most destructive disease on finger millet in Uganda (Emechebe, 1975; Grist, 1986) where yield losses range from 10 to 80 % (Esele and Odelle, 1992). However, losses of upto 90% have been reported on highly susceptible materials at Serere Research Station (Esele, 1993). The pathogen attacks all aerial parts of the plant damaging leaves, stems, necks and panicles. Seedlings at the

tillering stage are very susceptible to seedling blast, which may lead to death of seedlings during severe attacks (Ou *et al.*, 1970; Grist, 1986).

Blast disease of finger millet causes foliar, neck and panicle (fingers) infection. Neck infection causes heavy losses to the crop and very little work has been done on this aspect. Neck infection causes considerable loss in panicle length, grain weight, grain number and thousand grains weight (Rath and Mishra, 1975; Pall, 1977). The pathogen is highly destructive and economically important, causing more than 50 % reductions in yield, particularly in wet years. Losses of upto 90 % have been recorded in field studies in Uganda and 64 % in Kenya (Makini, 1999). Farmers recognize blast as a major disease, which they correctly associate with high humidity and variety susceptibility. Nevertheless, there has been limited research on the biology and sources of finger millet blast resistance(Makini, 1999).

P. grisea is seedborne in finger millet (Mitra and Mehta, 1934; Ramakrishnan, 1963; Govindu *et al.*, 1970; Ranganathiah and Mathur, 1978; Ranganathiah and Rao, 1982) and may cause heavy losses.

Guiragossian (1988), Mukuru and coworkers (1991) have reported that one of the constraints of finger millet production in East Africa is head blast. Blast may affect finger millet at any stage of development from seedling to grain formation and that maximum losses occurred in plants inoculated 90 days after sowing (Makini, 1999). Surveys done in 1991 and 1992 in Kenya and Uganda found severe incidences of finger millet blast and blight (Pande *et al.*, 1994). Survey of finger millet diseases during January and February of 1992 in farmer's fields in South Western Uganda (125 fields), Western Kenya (62 fields) and Eastern Kenya (106 fields), revealed that neck and panicle finger millet blast caused by *P. grisea* was very common and probably caused yield losses in 272 fields. The foliar phase of this disease was severe in only 33 fields (Mukuru *et al.*, 1993).

Research on blast of finger millet should dwell on seedborne incidence and transmission of the pathogen, disease and yield-loss relationships taking into account compensatory growth, identification and evaluation of blast-resistant finger millet cultivars in context of variable pathogen species (Makini, 1999). However, progress in cytogenetics, molecular genetics and biotechnology of *Eleusine* is very scarce. Therefore efforts should be strengthened to improve tolerance to stress factors, yield and seed quality of this neglected crop (Zeller, 2000). Relatively little is known about the seed-borne phase of blast and blight diseases of finger millet in Kenya and Uganda (Pande *et al.*, 1994).

1.4 Objectives

The objectives of these research project were:

1. To conduct a field survey and establish the seedborne nature of *Pyricularia grisea* in and on finger millet seeds in Kenya.
2. To establish the best cultural conditions of the pathogen, *Pyricularia grisea*.
3. To determine the efficiency of the various seed assessment techniques for evaluation of seedborne inocula of *Pyricularia grisea*.
4. To determine the relationship between seedborne inocula of *Pyricularia grisea* and disease incidence in the subsequent finger millet crop.
5. To determine the relationship between time of infection of finger millet plant with *Pyricularia grisea* and level of disease severity and seed contamination.

CHAPTER TWO

LITERATURE REVIEW

2.1 Finger millet ecology

Plant height ranges from 0.4 - 1.5m depending on variety and prevailing environmental conditions, taking 2 ½ to 6 months to mature (Thomas, 1970; Purseglove, 1985). It is mainly grown in the tropics at altitudes between 1000 - 2000 m (Elobu and Adipala, 1993). Finger millet belongs to the family Graminae and tribe Eragrostideae (Purseglove, 1985) and it is a diploid (2n = 36) (Thomas, 1970).

Broadly there are three classes namely the African exposed grain type, the African covered grain type and the Indian covered grain type (Thomas, 1970). Mehra (1963) recognized two groups of cultivars of *Eleusine coracana*, the African highland types resembling *E. africana* with long spikelets, glumes and lamas and with grains enclosed within the florets, and the Afro-Asiatic types with a close resemblance to *E. indica*, with short spikelets, glumes and lamas and with mature grains exposed out of the florets. In India and Africa, there are highland and lowland forms, dryland and irrigation types, grain and beer types, and early and late maturing types (Purseglove, 1972).

Flowering takes place over a period of 5 -10 days with the maximum number of florets opening on the third day. Flowering begins at the top of the spike and proceeds downwards while in the spikelet it proceeds from the bottom to the top. The flowers are mainly self-pollinated and cross-pollination is rare (Purseglove, 1985).

Finger millet requires well-distributed rainfall during the growing season with an absence of prolonged droughts. Dry weather is required to dry the grains at harvest. It is not tolerant to heavy rainfall. The crop grows best where the average maximum temperature exceeds 27^0C and the average minimum does not fall below 18^0C (Thomas, 1970). Finger millet cannot tolerate competition in the early stages of growth, and early, thorough weeding is essential. The

crop grows on a variety of soils. The average yield is 600 kg/ha but yields can go as high as 1,800 kg/ha on farmers fields and upto 5 tons on experimental plots (Sastri, 1952). The seed rate is usually in the range of 2.2 kg/ha (Esele, 1986).

Against its good storage properties, finger millet has a lower yield capacity than maize and requires more labour at all stages, particularly for seedbed preparation, weeding, bird scaring, harvesting and threshing (Rachie and Peters, 1977; Acland 1989). For these reasons its production has stagnated in Kenya and Tanzania, whilst maize has become more important (Acland, 1989).

2.2 *Pyricularia grisea*

2.2.1 Etiology

The finger millet blast fungus, *Pyricularia grisea*(Cooke)Sacc., anamorph of *Magnaporthe grisea*(Hebert)Barr (Herbert, 1971; Holliday, 1980; Rossman *et al.*, 1990; Bastiaans, 1993) is spread by conidia which generally have two septa, are narrower and lighter at the apical ends and broader and darker on the middle cell. The conidia are borne at the apex of cylindrical conidiophore with 1 - 4 septa (Ou, 1985; Adipala, 1989; Bastiaans, 1993; Seetharam and Ravikumar, 1993).

The blast fungus has a wide host range and the nomenclature of the causal organism and host range shows contradictions (McRae, 1922; Ou, 1985). As a compromise, the name *Pyricularia oryzae* is retained for the fungus on rice and *Pyricularia grisea* for that on finger millet and other cereals and grasses (Ou *et al.*, 1970; Seetharam and Ravikumar, 1993).

2.2.2 History of the pathogen, *Pyricularia grisea*

There is a good deal of confusion concerning the taxonomy and the nomenclature of *P. grisea*. McRae (1922) described a species of *Pyricularia* similar to *P. oryzae*, which attacks rice,

having been found on *Eleusine coracana*. In Kenya, the records show that *Piricularia* sp was first reported on finger millet by the Agriculture Ministry of the colonial time and it is labelled as S.A.L. 1920 (Nattrass, 1961). Blast caused by *Pyricularia sp* of finger millet was prevalent at Serere in Uganda in the early 1900s (Hansford, 1933). Further investigations carried out in Uganda showed that in culture the fungus was indistinguishable from one found in *Digitaria* sp (Hansford, 1934). In Tanzania, Wallace and Wallace (1947) said the fungus was *P. oryzae*, which attacks rice. According to Ramakrishnan (1948) the *Pyricularia* isolates of *Oryzae sativa, Setaria italica* and *E. coracana* which are physiologic isolates of *Oryza sativa, Setaria italica* and *E. coracana* are infact physiologic races of *P. oryzae*. Wallace (1950) reported that the *Pyricularia* causing blast of finger millet is morphologically very similar to *P. oryzae* but is almost certainly *P. setariae*. The possibilities of existence of different strains of the pathogen that cause leaf and neck infections have been indicated by Kulkarni and Govindu (1976).

G.B. Wallace is credited in some literature as the person who first described the causal organism of blast of finger millet and the pathogen in these texts is called *Pyricularia setariae* (Wallace) Ramakrishnan (ICRISAT, 1993). In some texts the pathogen is referred to as *P. grisea* (Cooke) Sacc. (Pande *et al*., 1994), while in others *P. setariae* Nishikado (Thirumalachar and Mishra, 1953; Kulkarni and Patel, 1956).

Nisikado (1917 and 1927) recognizes four *Pyricularia* species, *P. oryzae, P.grisea, P.setariae* and *P.zingiberi* based upon infectiveness on their respective hosts. The results of physiologic studies indicate that isolates from rice, *Setaria italica*, finger millet and *Digitaria marginata* hardly differed in their behaviour in culture. The isolate from *S. italica* infects its own host and finger millet only, the finger millet isolate infects only its host and *S. italica*. The isolate from *D. marginata* was pathogenic to rice, finger millet plus its own host. The four isolates were regarded as physiologic races of *P. oryzae* by Ramakrishnan (1948).

2.2.3 Taxonomy of the pathogen

The genus *Pyricularia* was established by Saccardo in 1880 (Ellis, 1971) based upon *Trithecium griseum* Cooke on crab grass, *Digitaria sanguinalis* in North America. *P. grisea* (Cooke)Saccardo is the type species of the genus (Ou, 1985). In most of the older literature, the spelling is *Piricularia*. Saccardo himself originally used the spelling *Pyricularia* but changed it to *Piricularia*. Hughes (1958) checked the original publication of the name and found that it was spelt *Pyricularia*. According to the International Rules of Botanical Nomenclature the spelling *Pyricularia* should be used and since the publication of Hughe's paper most authors have used the spelling *Pyricularia*.

According to Ainsworth & Besby's dictionary of the fungi, 8th Ed. Mitosporic fungi the pathogen is in, the Order, Moniliales and Family, Moniliaceae. The genus *Pyricularia* was classified under the family Moniliaceae on account of its hyaline spores. However, Thirumalachar *et al*., (1956) think that the genus should be included in the family Dematiaceae owing to the occurrence of greyish to sub-hyaline and pale olivaceous conidiophores.

2.2.4 Cultural characteristics

This fungus has been found to grow well on medium with a pH of between 5.0 and 6.4(Thomas, 1940), with the optimum growth temperature being about 29.5^{0}C and with no appreciable differences in growth observed between 20 and 30^{0}C. The minimum and maximum temperatures at which the fungus was able to grow were 5 and 36^{0}C respectively, and the thermal death point of the conidia being between 48 and 49^{0}C.

2.2.4.1 Characteristics of pathogens in the genus, *Pyricularia*

Members of the genus *Pyricularia* have characteristics which according to Saccardo (1880) [cited by Ellis, 1971] include culture colonies which are effuse, thinly hairy, grey,

greyish brown or olivaceous brown; Mycelia is immersed in the medium with or without chlamydospores in culture with absent stroma, setae and hyphopodia. Conidiophores which are macronematous, mononematous, slender, thin-walled, usually emerging singly or in small groups through stomata, mostly unbranched, straight or flexuous, geniculate towards the apex, pale brown, smooth; the conidiogenous cells which are polyblastic, intergrated, terminal, sympodial, cylindrical, geniculate, denticulate; each denticle cylindrical, thin-walled, cut off as a rule by septum to form a separating cell; conidia which are solitary, dry, acropleurogenous, simple, obpyriform, obturbinate or obclavate, hyaline to pale olivaceous brown, smooth, 1 - 3 (mostly 2) septate; the hilum which is often protuberant. The type species is: *Pyricularia grisea* Sacc.

2.2.4.2 Morphology of *Pyricularia grisea*.

According to Wallace(1950) and Ramakrishnan (1963 morphological characteristics of *P. grisea* include colonies that are colourless to light olive green, and grows in tufts or a continuous mass on PDA (Potato Dextrose Agar) and OMA (Oatmeal Agar); the mycelium are profuse and darkens at maturation; individual hyphae are hyaline, brown with hyphal cells somewhat swollen and 1.5 - 6.0 μm long; conidiophores are simple, septate, darker at the base and paler towards the apex; conidia are typically obpyriform, sub-hyaline, acrogenous, or produced one after another by the sympodial growth of the conidiophore; spores have 3 - 4 cells, the middle cell being broader and darker than the others, and measure 19 - 31 x 10 - 15 μm. According to Ellis (1971), *P. grisea* Sacc. has conidiophores measuring 150 μm long and 2.5 - 4.5(usually 3-4)μm thick, while the conidia mostly are 17 - 28(20.9) x 6 -9(7.6)μm.The darker colour of the middle cell is only observed in a few conidia. Chlamydospores are thick walled, olive brown or dark brown, terminal or intercalary, and 4 - 10 μm in diameter (Wallace, 1950; Ramakrishnan, 1963).

2.2.5 Physiology of *Pyricularia grisea*

Great pathogenic variability has been reported both among monoconidial isolates from single lesions and among monoconidial sub-cultures from single monospore cultures of *P. grisea* (Ou and Ayad, 1968; Giatgong and Frederiksen, 1969; Ou *et al*, 1970). *Pyricularia grisea* is also highly variable in culture. Cultural morphology varies greatly with isolates and with media used. The amount of aerial mycelium varies from scanty to a thick cottony mass; colour varies from whitish or cream, through pink, grey to dark olivaceous (Ou, 1985). Various workers have shown that the blast fungus undergoes changes easily on successive mycelial transfers to produce variants, which may differ from the parental type in cultural behaviour and pathogenicity (Ren-jong *et al*., 1965). The phenomenon makes the maintenance of the blast fungus difficult and complicates race studies (Ren-jong *et al*., 1965).

There is a great deal of controversy over the pathogenic diversity in *P. grisea*. Recent studies using DNA fingerprints of some isolates from U.S. indicate clonal lineages, which tend to conserve pathotypes (Levy *et al*., 1991). Another school of thought based on genetic analysis of host cultivar specifity in *Magnaporthe grisea* (teleomorph), suggests a simple Mendelian inheritance of virulence genes to control such specificity (Valent *et al*., 1991). Goto and Sakai (1963) observed significant intra-clonal variation in pathogenicity and stated that segregation seems to occur in vegetative isolates from a clone. Vegetative and conidial cells of *P. grisea* are multinucleate, containing 2 - 6 nuclei (Ou, 1985), and migration and fusion of nuclei has been observed in both mycelia and conidia (Ou *et al*., 1970). Anastomosis has been observed in monoconidial cultures suggesting that changes in gene characteristics in respective sites may bring about genetic changes, hence variation in pathogenic efficiency (Giatgong and Frederiksen, 1969; Ou, 1985). Leung and co-workers (1988) observed that chromosomal movement at meiotic and mitotic anaphase was synchronous, which might account for the variable chromosome number previously reported by Giatgong and Frederiksen (1969). Local

appearance of new races of the blast fungus after the release of a new cultivar is attributed to virulent mutations that frequently occur at a rate of 10^{-2} to 10^{-5} (Leung *et al.*, 1988; Castano *et al.*, 1989; Notteghem, 1993). Chromosomal aberration such as deletions also occur, sometimes at the rate of 0.5% with some isolates (Notteghem, 1993).

Kulkarni and Govindu (1976) experimented with 12 media and oat meal agar medium was found to be the best in respect to growth rate and production of spores and mycelium, although potato dextrose and bean meal agar media gave as good growth as oat meal agar, with poor sporulation. Least growth was achieved on Czapek's agar. On potato dextrose agar (PDA), mycelial growth of the pathogen reached their peak on the 14th day of seeding (Kulkarni and Govindu, 1976).

The amount of growth of leaf and neck isolates was measured on 14 liquid media and the average mycelial weight recorded, 14 days after inoculation. Maximum growth was recorded on liquid media made with finger millet meal followed by oatmeal, and they supported significantly more growth than the other media (Kulkarni and Govindu, 1976). Leaf and neck isolates of the pathogen were grown in Richard's medium at different pH levels and dry mycelial weight and shift in pH levels were recorded after 14 days. A pH of 6.9 was found to be the optimum, supporting maximum mycelial growth (Kulkarni and Govindu, 1976). The leaf and neck isolates were grown on Richard's media and incubated at different temperatures for 14 days. The maximum growth of both the isolates occurred at 30^0C while least growth was observed at 5^0C. The growth of the neck isolate was significantly more than that of the leaf isolate. Neither isolate grew at 0 or 45^0C. The culture incubated at 45^0C for 7 days were assumed dead since they did not resume growth when incubated at 30^0C (Kulkarni and Govindu, 1976). These results are in agreement with the findings of Ramakrishnan (1948) who obtained the maximum growth of the *Pyricularia* isolates on finger millet medium at 30^0C. Similar results were obtained by Thomas (1941) at 29.5^0C.

Maximum growth of leaf and neck isolates was in soluble starch and the least growth in mannitol. Asparagine was found to be the best nitrogen source for growth of both isolates (Kulkarni and Govindu, 1976). The response to vitamins was significantly more in the neck isolate than the in leaf isolate. The growth of both the isolates was more in biotin, thiamine, pyridoxine and in ositol combination than in other vitamins and their combinations studied (Kulkarni and Govindu, 1976).

The effect of artificial nutrition media and temperatures on the spore size of *P. setariae* showed that temperature had practically no effect on the width of the spore but length was shortened at 10^0C and 30^0C and increased at 15, 20 and 25^0C (Kulkarni and Patel, 1956). Nisikado (1927) found that the optimum temperature for mycelial growth was 26-28^0C, with the maximum and minimum at 36-37^0C and 8-9^0C, while thermal death occurred at 51-52^0C.

2.3 Host - pathogen interactions

2.3.0 Infection process

Infection by *P. grisea* follows germination of conidia, formation of appressoria, production of infection tubes from the appressorium, and penetration through cuticle and epidermis. The infection hyphae may also enter through stomata (Ou, 1985). Given adequate moisture the time required for conidial germination and invasion of the host cells varies with temperature. Hashioka (1965) found that a minimum of 10 hours at 32^0C, 8 hours at 28^0C, and 6 hours at 24^0C was required for invasion of host cells. Free water and high relative humidity (near to saturation) is necessary for germination and infection (Hashioka *et al.*, 1968).

The incubation period varies with temperature with 13 - 18 days at 10^0C, 7 - 9 days at 17 - 18^0C, 5 - 6 days at 24 - 25^0C and 4 - 5 days at 26 - 28^0C (Hashioka, 1950; Hashioka, 1965; Ou, 1985). The conditions favourable for infection are similar to those for mycelial growth, sporulation and conidial germination (Hashioka, 1965; Ou, 1985). Light affects the infection

process. Infection occurs most readily in darkness and is suppressed under diffused light. However, disease development decreases under extended darkness (Hashioka, 1965; Ou, 1985).

2.3.1 Symptoms associated with finger millet blast

Rachie and Peters (1977) studied the symptomatology of blast of finger millet and documented it as follows. The disease affects finger millet in all stages of growth from seedling to the time of grain formation. Large oval or elongated spindle-shaped brown spots occur on the leaves and often the neck below the earhead is attacked, resulting in failure of grain set or shrivelled seeds. Spindle shaped lesions of varying sizes are formed on seedling leaves. These are usually greyish- olive green in the centre with a yellowish margin, but later the central portion turns white and gradually disintegrates. In the early stages of the infection, the lesions are isolated but often coalesce and cover extensive areas. The distal portions of leaves beyond the lesions may dry up, break up or drop off. Infection in the mature plant is very similar to that of the seedling stage. Sometimes one may not see lesions. Lesions may measure 1 - 2 cm in length and 0.3 - 0.5 cm in width. The greatest damage is caused when the infection occurs in the panicle. Often the upper peduncle immediately below the head becomes infected over an area of 3 - 5 cm and individual portions of the head or fingers can be infected and break away from the stalk. These infected portions become discoloured and shrunken.

The fungus invades and causes the breakdown of parenchymatous, sclerenchymatous and vascular tissues of the neck region thereby impeding the flow of nutrients into the grain. In effect, grain formation is partially or totally inhibited (Keshi, 1966; Ou, 1985; Ekwamu, 1991; Seetharam and Ravikumar, 1993).

Attacks on sheaths and leaves cause lesions with the upper young leaves being more susceptible than the lower leaves while foliar infection is less common as the plant matures (Adipala, 1989; Bastiaans, 1993). Panicle and neck blasts cause yield losses (Ekwamu, 1991).

There is a linear relationship between panicle infection and yield loss and the magnitude of loss depends on time of infection, with the earlier the infection the greater the loss (Ou, 1985). Somasekhara *et al.*, (1991) found a very low correlation between leaf and neck blast (r = 0.04) and between leaf and finger blast (r = 0.27).

2.3.1.1 Signs associated with the pathogen

The leaf lesions, under humid conditions, turn to an olive grey due to the growth of the fungus, which covers the central portions of the lesions, especially on the upper surfaces of the leaves. This overgrowth consists of the conidiophores and the conidia of the fungus. After the head becomes discoloured and shrunken, a hollowed grey fungus covers the area (Rachie and Peters, 1977). Upon incubation, infected seeds show profuse growth of mycelium and spores. Colonies are colourless to light olive green, aand grow in tufts or a continuos mass (Wallace, 1950; Ramakrishnan, 1963).

2.3.2 Host range

P. grisea attacks are common on leaves of many different grasses and sometimes other plants, causing round elliptical, pale tan or brown spots often with a purple or dark brown border. Hosts include *Bambusa, Brachiara, Commelina, Cynodon, Digitaria, Echinochloa, Eleusine, Eragrostis, Nicotiana, Panicum, Pennisetum, Secale, Setaria, Sorghum,* and *Zea.* Thomas (1940,1941) found that *P. setariae* fails to infect rice and ginger but does infect wheat, barley and oats quite readily. He also found that *Pyricularia* strains from rice and *Panicum repens* would infect only its own host, and the strains from finger millet and *Setaria italica* were capable of infecting wounded leaves of each other but would not infect rice or *Panicum repens.*

Pande and co-workers (1993), conducted two cross inoculation tests among grass species and finger millet. One involved the various grasses with two isolates of *P. grisea* from finger

millet. In this case, typical blast symptoms formed on both the leaves and panicles of grasses (*Eleusine coracana, E. jaegeri, E. indica, E. africana, E. multiflora, Digitaria aegyptium, Digitaria macroblephara, Digitaria sclarum, Setaria verticillata)* and what appeared to be a hypersensitive reaction was found in two other species of the same genus *Cynadon dactylon and Cyadon plectostachyum).* A questionable response was observed in *Pennisetum massicum* and no blast symptoms developed in three species (*Eragrostis superba, Panicum maximum, and Rottboellia exaltata*). In the second cross inoculation test, five grasses namely, *E. africana, E. coracana, D. aegyptium, D. macroblephara*, and *S. verticillata,* were each inoculated with *P. grisea* isolates obtained from each of the five grasses. Each of the five isolates were able to produce typical blast symptoms in each of the five grasses, except for unclear symptom expression on *E. africana* inoculated with *P. grisea* from *D. aegyptium*, and on *D. eagyptium* inoculated with *P. grisea* from *E. coracana*. These results suggest that a number of grasses that occur in and near finger millet fields in Kenya and Uganda could serve as sources of *P. grisea* inoculum for finger millet, and as a bridge for inoculum maintenance between seasons and inoculum increase within seasons (Pande *et al.,* 1993).

2.3.3 Survival and spread

Pyricularia grisea survives as conidia and mycelia for up to 1 and 3 years, respectively (Ou and Ayad, 1968; Ou *et al*., 1970; Kato, 1977). In tropical areas such as Uganda and western Kenya, airborne conidia are present throughout the year thus providing a ready source of inoculum to initiate epidemics (Adipala, 1989; Esele, 1993; Pande *et al.,* 1993; Makini, 1999). Where the microclimate is highly conducive, epidemics of blast occur frequently and often with disastrouos (dilapidating) effects on rice and finger millet (McRae, 1923; Adipala, 1989; Esele, 1993). The fungus is seedborne and may be found within the embryo, endosperm and glumes (Ranganathaiah and Mathur, 1978; Adipala, 1992; Pande *et al.*, 1994). Thus, infected seed is

considered a major source of finger millet inoculum (Adipala, 1992; Pande *et al.*, 1994). The conidia of *P. grisea* are mostly disseminated by air and rain splash, although the fungus may also be disseminated on infected seed (Pande *et al.*, 1994).

Development of the blast disease is favoured by alternating night (16 - 20^{0}C) and day temperatures (26 - 30^{0}C), relative humidity of greater than 93%, and high rainfall (Hashioka, 1965; Pall, 1987). Relative humidity of greater than 93% induces conidial production in leaf lesions (Hashioka, 1965). Optimum temperature for mycelial growth is about 28^{0}C. Sporulation is optimal at 24^{0}C but lasts longer under slightly lower temperatures. Conidia germinate best at 25 - 30^{0}C and no germination occurs at less than 15^{0}C (Hashioka, 1965; Ou, 1985).

2.4 Seed as a source of primary inoculum for fungal pathogens

The extent to which fungi occur in seeds depends on their capability to survive under the extreme dry conditions of seed as a carrier (Neergaard, 1988). Climatic conditions leading to severe field attacks of seed-borne pathogens usually result in increased seed-borne inoculum. Parenchymatous diseases like leaf blights, leaf spots and downy mildews require high levels of humidity (Neergaard, 1988). Frederiksen (1974) found that the seed-borne diseases of sorghum he dealt with occurred in about equal frequency and severity in sub-tropical regions and tropical lowlands. This indicated that humidity is more important than temperature in determining distribution and severity of these diseases.

Incase of *P. grisea,* seeds are the principal overwintering organs. The fungus may be found within the embryo, endosperm and glumes of the seed and sometimes between the glumes and the kernel(Suzuki, 1930). *P. oryzae* is frequently seed-borne in all rice producing countries(Noble and Richardson, 1968) and surveys conducted in Nigeria detected infected seed samples (Awoderu and Onuarah, 1974).

The relationship between degree of seed infection and incidence of blast disease in finger millet cv Purna in the field was studied at Jabalpur, India using seeds infected with *P. setariae* at the rate of 0-5%. The study reported that one infected seed could cause an epidemic (Pall, 1988). Similarly, Shetty (1985) reported that epidemics of blast (*P. grisea)* resulted from using seed of *E. coracana* with very low levels of seed-borne infection. It appeared that a single infected plant could bring about an epidemic and he further stated that laboratory seed tests capable of detecting 1 diseased seed/10,000 are able to predict successfully the field severity of blast and that it is necessary to adopt zero tolerance in the seed certification programme. This obviously is very difficult to achieve and is impractical anyway since *P. grisea* inoculum is available from other sources in the field situation. In Japan in 1973, during field trials on finger millet an outbreak of blast occurred which yielded 5 - 46% diseased seed (Kato *et al*., 1977).

Heald (1921) found that a minimum of 100 spores of *Tilletia caries* per grain of wheat cultivar Jenkins club were necessary to produce a smutted spike, whereas 500-5,000 spores per grain were needed to produce a corresponding attack in the smut resistant cultivar Marquis. Up to a certain point (0.5g inoculum per 100-g grain) increasing amounts of inoculum increased degree of infection. Beck (1920) and Glaeser (1961) also established the validity of this correlation, although some authors found other threshold values.

Munerati (1922) demonstrated that the location of inoculum of *Tilletia caries* on the grain is of importance. The nearer the spores are to the embryo, the greater the prospects of infection. Leblond (1948) found clear positive correlation between the number of spores of *Ustilago avenae* and *U. hordei* and the percentage of infected oat plants in the field. Studies were done to determine the fungi infecting 400 finger millet samples collected from different areas in Bihar, India. *Pyricularia* spp were found in 18 samples with incidences ranging from 2 to 25 % (Rajiv *et al*, 2000).

2.4.1 Inoculum Thresholds of Seedborne pathogen

Inoculum thresholds of seedborne pathogens are the levels of infection on or in seed that will significantly affect disease development and result in economic loss. The threshold level must be zero for a disease that is not in an area protected by an established quarantine (Baker and Smith, 1966). To establish a threshold level, the amount of seed infected must be determined. Ideally, the seed testing method should be sensitive, quick, inexpensive, accurate and reproducible. Seed health tests for specific host-pathogens have been developed and evaluated by the Plant Disease Committee (PDC) of the International Seed Testing Association [ISTA] (Baker and Smith, 1966). In any test, it is important to guard against both false positives and false negatives. An example of a false positive is the identification of a dead fungal structure. A false positive can be avoided by including a pathogenicity test. A false negative occurs when the conditions of a test fail to reveal a virulent pathogen in the seed. Including a known infected control in each test can guard against this. In addition it is important that sample size and replication are adequate to measure significant amounts of infection or infestation (Baker and Smith, 1966). Threshold levels must be developed for the average environmental conditions in which seed is sown. Threshold levels will be influenced by all factors affecting the epidemiology of each host-pathogen combination, such as inoculum level, host susceptibility, pathogen virulence, environment, biology of the pathogen, potential for secondary spread, cropping practices, other sources of inoculum and relationship of inoculum to seed (Baker and Smith, 1966). Some seedborne pathogens may rot infected seed before the seedling reaches the soil surface and primary inoculum is effectively buried. For most pathogens in this category, much less inoculum reaches the soil surface to become infection loci than would be indicated by seed health tests (Baker and Smith, 1966). When zero tolerance for a pathogen is established, it is hoped that the seed will be pathogen-free (Gabrielson, 1988). However, any seed health test evaluating individual seed based on sampling a portion of a seedlot can never predict that the

seed lot will be pathogen-free (Gabrielson, 1988). As the sample tested grows larger, the predicted infection percentage becomes lower, but never zero. For this reason, a seed health test is not a suitable method of enforcing quarantine. It can, however, define an inoculum tolerance level for disease management (Gabrielson, 1988). Little work has been done to establish tolerance levels where effective chemical eradicant treatments are used. Establishment of valid tolerances for local areas coupled with accurate seed health tests provides a powerful disease management tool that can minimize the use of fungicides in crop production (Gabrielson, 1988).

2.5 Control measures of the blast disease on finger millet

The primary aim of disease control in finger millet, as in other crops, is to disrupt the combination of factors necessary for disease development. This must be based on an understanding of the plant parts attacked, the pathogen's disease cycle, and the dissemination of the causal organisms (Agrios, 1988). Plant resistance and chemical control have been used with varying levels of success.

2.5.1 Chemical control

Rao and Chennamma (1983) reported that in field trials, spraying with carbendazim at flowering and at the milk stage effectively controlled *Pyricularia grisea* on finger millet (*Eleusine coracana).*

In Uganda, Adipala and Mukiibi (1985) reported that foliar diseases of finger millet can be controlled by applying weekly sprays of Benomyl (Benlate) at 1 kg a.i/ha or Maconzeb (Dithane M-45) at 2 kg a.i/ha. Under Ugandan conditions, three sprays are vital which include spraying four weeks before flowering, at flowering, and three weeks after flowering i.e. 6, 10 and 13 weeks after planting (Adipala and Mukiibi, 1985; Adipala, 1990). On 10 seed treatment fungicides tested, carbendazim + iprodione, and carbendazim + thiram at the rate of 0.3 %

proved to be the most effective in controlling seedborne fungi and improving seed germination (Godke *et al*, 2000).

Finger millets plants were sprayed with 8 fungicidal treatments, comprising of four fungicides (0.05 % carbendazim, 0.1 % chlorothalonil, 0.10 % edifenphos and 0.25 % mancozeb) and their combinations, against *P. grisea* in a field experiment conducted during 1996 – 1997 in Madhya Pradesh, India. The fungicides were sprayed at tillering and ear emergence stages. All of the tested fungicides were found equally efficient in controlling blast disease. The chlorothalonil + mancozeb treatment recorded the highest yield and was the most economical treatment (Patel *et al*, 2001).

2.5.2 Biological control

Reddy and Subbayya (1981) reported that in tests with 38 fungal and 52 bacterial isolates from phyllosphere, sterilised culture filtrates of some of the bacteria reduced growth of the pathogen in culture and suppressed spore germination, and fungal isolates were less inhibitory. Inioculation sprays of culture filtrates of three bacterial isolates were highly effective in reducing the number of lesions caused by *Pyricularia* sp on *Eleusine coracana.*

2.5.3 Host resistance

In Uganda and Kenya, efforts to control finger millet blast have been directed toward breeding for resistance to blast because it is cost-effective, and are with no environmental hazards (Esele, 1993; Odelle, 1993). Although there is a lot of literature on the rice blast disease, literature on finger millet blast disease is relatively limited. There is considerable literature on the genetics of resistance to *P. oryzae* (Leung *et al.*, 1988; Levy *et al.*, 1991; Valent *et al.*, 1991; Urashima *et al.*, 1993), the work of Seetharam and Ravikumar (1993) provides some insight on the genetics of resistance to *P. grisea* in finger millet. Sixteen finger millet genotypes grown

during the kharif seasons of 1996 and 1997 in Karnataka, India were evaluated for resistance to neck and finger millet blast, *P. grisea* by recording the percentage disease incidence at the grain filling stage. A higher disease incidence was observed in 1997 than in 1996 due to higher relative humidity, temperature and rainfall (Rajanna *et al*, 2000).

Sources of resistance to finger millet blast are not yet fully known. In Uganda, breeding for resistance to finger millet blast started in 1965 (Esele and Odelle, 1992). The evaluation of finger millet for various traits whether disease resistance or early maturity, on a regional basis began at Serere Agricultural and Animal Research Institute (SAARI) in 1966 (Peters *et al.*, 1968). Subsequently, research at SAARI has concentrated on breeding for resistance to blast and screening for resistance sources of resistance among existing germplasm (1058 accessions collected from various parts of the world act as a genebank). The preliminary blast resistance-screening programme was initiated under conditions of natural infection in the field, but in recent years, an infector row system has been employed in screening for resistance to blast. This consists of planting rows of very susceptible entries mainly obtained from the Ethiopian and Indian collections among rows of entries to be tested (Esele and Odelle, 1992; Esele, 1993). The results obtained showed that open-headed, early maturing, white-seeded varieties are more susceptible to blast than darker seeded varieties with compact heads. The resistant varieties so far identified in Uganda under this program include Engeny, Gulu-E, Serere 1, P224, P227 and S X 10. Makini (1999) identified P224 as blast tolerant and Gulu E as moderately resistant during a study on epidemiology and control of finger millet blast using farmer participatory methods. In the breeding programme crosses are generated using both the hot water emasculation (HWE) and male sterile (MS) techniques (Esele and Odele, 1992; Esele, 1993; Odelle, 1993).

Released in 1985, KAT/FM-1 (*Eleusine coracana*, P1578006) was derived from plants selected from drought tolerance within a local cultivar collected from Ekalakala in Machakos,

Kenya. In 1991, 191 single plants that survived water stressed field conditions were selected and subjected to 5 cycles of mass selection for improved grain yield, blast tolerance and drought tolerance. KAT/FM-1 is self-pollinated. In Kenya it reaches approximately equal to 60 cm in height, flowers in 75 days and matures in 90 – 115 days, depending on season and altitude. Grain yield of KAT/FM-1 averaged 1400 kg/ha over 12 environments in Kenya, 45 % more than the average of the best local cultivars. KAT/FM-1 can be grown from 50 – 2000 m altitude and is capable of avoiding water stress, which is prevalent in semiarid areas in Kenya (M'Ragwa and Watson, 1994).

The following varieties from various parts of the world have shown resistance to blast: Mozambique variety MO 359, a poor yielder but of possible parental value, remained immune (Anon, 1959); varieties PR 722 and PR 202 are moderately resistant (Deshkar *et al.*, 1973); varieties TAH 91-1 and TAH 14-8 are moderately resistant (Pall and Nema, 1979); cultivar AP-27 is field resistant to leaf blast and AKP-2 to neck blast of finger millet (Rath and Swain, 1978). According to Pall (1991) finger millet cultivars GE 746, GE 1009 and GE 378 had high levels of resistance to blast disease. The entries GE 2362, GE 232 and GE 769 showed high levels of resistance to neck infection; GE 232, which also had moderate resistance, was most suited to the conditions at Jabalpur, India.

2.5.4 Certified seeds

2.5.4.1 Seed health testing procedures

Ultimately, the aims of seed health testing are related to the actual policies toward seed improvement, seed trade and plant protection. Testing techniquess depend invariably on the pathogen carried in the seed and the potential of this pathogen in a given situation (Neergaard, 1988). Practically any health test of a seedlot is based on examination of a sample, which usually is an extremely small part of the seedlot. The procedures applied in drawing a representative

sample and in dividing it into working samples for analysis are therefore fundamental in order to get uniform, accurate and reproducible results [(ISTA: 1966, 1976); cited by Neergard, 1988].

Seed health tests are usually carried out under seed certification schemes and quarantine stations. Routine methods to be adopted for seed health testing must fulfill the following demands of efficiency and economy as outlined by Neergard (1988):

- A test must give reliable information pertaining to field performance and quarantine requirements.
- The results must be reproducible within statistical limits.
- The time, labour and equipment for carrying through a test must be kept within economic limits.
- In tests requiring incubation, the results must be made available quickly (Neergaard, 1988).

2.5.4.2 Seed certification schemes.

The purpose of seed health certification schemes is the elimination of pathogens. Consequently, testing procedures applied in this connection must aim at maximum recording, when detecting pathogens (Neergaard, 1988). Indicator tests capable of revealing traces should also be taken into consideration. For many fungi the agar plate test may reveal traces more readily than the blotter test (Neergaard, 1988). But, this is not always so, as exemplified by *Pyricularia oryzae* (Neergaard, 1988).

2.5.4.3 Quarantine procedures.

Closed quarantine in glasshouses especially equipped for post entry growing-on tests is used in order to exclude dangerous pathogens (Neergaard, 1988). This seed health testing procedure is very expensive and is limited to valuable seeds. For seed to be certified by an exporting country as 'substantially free' from a particular pathogen, a sufficiently large

representative sample must be taken and the testing procedure must be sufficiently sensitive to reveal even traces of infection (Neergaard, 1988). The agar plate test is more sensitive than the blotter test for many fungi for instance *Fusarium oxysporum* f.sp. *lini* in flax (Neergaard, 1988). Another category of pathogens observed in inspection of seeds for quarantine are those seed-borne pathogens that have not been specified in the quarantine regulations of importing countries requiring a general phytosanitary certificate (Neergaard, 1988).

Interpretation of results, especially when the testing techniques are not specified, may vary substantially from country to country. Therefore, tolerance levels may be set and followed at the discretion of the certifying agency of the exporting country. For this category of pathogens, versatile, standard procedures are appropriate. Infact, most quarantine seed inspection is devoted to these kinds of pathogens (Neergaard, 1965). The tolerance traditionally accepted by inspection services vary [(Neergaard, 1962; EPPO, 1966) cited by Neergaard, 1988].

2.5.4.4 Evaluation of planting value.

Assessing the effect of pathogens on planting value of seeds is extremely complex because of innumerable and often unpredictable factors in the field. High correlation between laboratory tests and field performance may be difficult to get from tests of a few seedlots, but clearer when many test results are subjected to statistical analysis (Neergaard, 1988). The correlation between laboratory and field results depends on two major factors according to Neergaard (1988):

1) The testing procedure and;

2) The transmission rate and rate of pathogen increase.

Some diseases, such as barley loose smut and barley leaf stripe, show a ratio of 1:1 between percentage of infected seeds and percentage of yield reduction.

2.5.4.5 Methods used in seed health testing.

The methods used in seed health testing are summarised into the following principal categories (Neergaard, 1988) namely:

(1) Direct inspection of the seed with unaided eye, hand lens or under low power stereoscopic microscope. The examination may be assisted by such simple procedures as cutting through individual seeds followed by staining submerging seeds in water drops or placing them for a few hours or one day on moist filter paper to get swelling of pycnidia, etc.

(2) Microscopic examination of suspensions obtained by washings from the seed.

(3) Microscopic examination of infected seed after application of a clearing and staining procedure.

(4) Examination of seed and seedlings after incubation of the seed sown on blotter paper, within blotting paper, on agar media, or on any other essentially sterile media.

(5) Examination of symptoms developed on seedlings grown in soil, sand or similar material.

(6) Examination of growing-on tests carried out in greenhouse, environmentally controlled chambers or in the field.

(7) Tests based on biochemical reactions (serological tests).

(8) Tests performed as bioassays (indicator test, phage plague, etc)

(9) Tests conducted under field conditions.

CHAPTER THREE

MATERIALS AND METHODS

Cultural characteristics and pathogenicity of *P. grisea* isolates

3.1 Isolation of *Pyricularia grisea* from infected seeds

P. grisea was obtained from suspected diseased farmer seed samples from Busia, Bungoma and Kisii. The seeds were surface sterilised in 2% available NaOCL for 5 minutes and put through 5 changes of sterile distilled water. Then 20 seeds were plated on oatmeal agar (Ranganathiah and Mathur, 1978; Pande *et al.*, 1994). The seeds were incubated at room temperature ($20^0C \pm 4^0C$) for 7 days until growth was observed on media. The pathogen grew as a dark cottony growth around the seed. This was reisolated onto oatmeal again and maintained by subculturing to a maximum of two times. If more of the pathogen was required the procedure was repeated.

3.2 Pathogenicity of finger millet *Pyricularia grisea* isolates

Pathogenicity tests were conducted for all three isolates obtained from Busia/Teso, Bungoma and Kisii districts (Refer 3.4.1). Inoculations were done on healthy finger millet plants of variety KNE 479 grown in 20cm pots. The pots were filled with steam sterilised potting mixture composed of soil, manure and coffee husk in the ratio of 2:2:1. The plantlets were transplanted into these pots. Calcium Ammonium Nitrate (CAN) was added after 4 weeks according to Muthuswamy and co-workers (1985) who reported that increasing nitrogen in the soil increased the severity of blast on finger millet. The experiments were conducted at the field station glasshouses, Kabete Campus.

3.2.1 Inoculum preparations and inoculations

The inoculum was prepared by harvesting the spores from culture media after 14 to 16 days from seeding. This was done by pouring 10ml of sterile distilled water (SDW) on growth culture, scrapping off the culture with a microscopic slide and sieving it with a double cheesecloth and making a homogenous spore suspension in sterile distilled water (SDW). The suspension was calibrated using a haemocytometer and adjusted to 2.8 X 10^6 conidia/ml concentration (Adipala, 1989).

The inoculum was applied to 7-week-old plants (Adipala, 1989) using a 1 litre hand sprayer (E.A. Seed Company), until the whole plant was uniformly covered. Then a moistened polythene bag was used to cover the plant to ensure there was high relative humidity as the pathogen requires high relative humidity of greater than 85 % (Pall, 1987). After 24 hours these polythene bags were removed and the observation for the typical blast symptoms started 4 days (for the chlorotic speck) later and 7 days for fully developed lesions and continued upto 21 days when symptoms expression was at the maximum lesion. The glasshouse was kept humid by application of water onto the benches, walls and floor.

An equal number of control plants were sprayed with SDW for comparison. Graphs were drawn for the different isolates in relation to lesion expansion (LE) and severity on leaves. The average change for the 3 weeks on a daily basis was measured.

3.3 Slide Cultures

The slide technique described by Riddell (1950) was used for observation of fungal morphological characteristics without disruption, all of which were important features for fungal identification. Two sheets of filter paper, a bent glass rod, microscopic slide (on the rod) and a cover slip were placed into a petri a dish in that order and sterilised. Sterilised oatmeal agar was poured into a sterile 9cm glass petri dish to form a layer of about 2mm deep. After the medium

had solidified, a block was cut out and aseptically placed on a sterile microscopic slide. Using an inoculating needle, the centre of the block was seeded with mycelia/conidia of the fungus and then the cover slip was centrally placed on the agar block. The petri dishes were incubated at room temperature (20- 24^{0}C). To maintain humidity and keep the paper moist sterile distilled water was added periodically to the filter papers in the petri dishes.

After 7-14 days of incubation the cover slips were lifted carefully and the agar block was discarded. The cover slips were mounted on different slides, some on a drop of clear lactophenol and others on lactophenol in cotton blue. Photographs were taken; drawings were made with the aid of a drawing tube to help in identification of the fungus from the seeds.

To determine the size of the conidia and conidiophores, over 100 readings of the sizes were recorded using a microscope having a calibrated ocular lens in accordance with recommendations by Kulkarni and Patel (1956).

3.4 Cultural studies of finger millet blast pathogen, *Pyricularia grisea.*

The main objective of the experiment was to test environmental conditions of lighting and pH for culturing *P. grisea* with the aim of identifying a culture medium and environments that encourages sporulation and fast hyphal growth for three isolates of *P. grisea*. Since *P. grisea* has been found to sporulate poorly in most artificial culture media (Kulkami and Govindu, 1976), experiments were conducted using 3 isolates and 5 media under different conditions of pH and lighting.

3.4.1 Isolates and cultural media.

The three isolates were obtained from the farmers' seeds of the three districts of Bungoma, Kisii and Busia, hence they were named: Bungoma isolate; Kisii isolate and Busia isolate. The five culture media used were: Potato Dextrose Agar (PDA); Oat Meal Agar (OMA); Finger Millet

leaf Extract Agar (FMLEA); Finger Millet Seed Extract Agar (FMSEA); Bean Meal Agar (BMA). Details of media ingredients are given in the appendix. Media were adjusted to pHs of 5, 6, 7 and 8 and tested. The pH was adjusted (by use of 0.1M NaOH or 0.1M HCL depending if it is alkaline or acidic) using a pH meter before autoclaving (sterilisation). Four lighting regimes were tested in an incubator for their effect on *P. grisea*: A cycle of 12 hours of flourescent light and 12 hours darkness (in the incubation chamber); A cycle of 12 hours near ultra violet light (NUV) and 12 hours darkness (in the incubation chamber); Continuous fluorescent lighting (in the incubation chamber); Continuous darkness (was achieved by covering the petri dish with aluminium foil and kept in the incubation chamber).

In all these cases the temperature was room temperature, which was 20 ± 4°C. The experimental design was completely randomised design (CRD). The experiment replicated three times.

3.4.2 Data collection

i) Rate of mycelial growth (radial growth rate):

Four days after seeding, diameters of the *P. grisea* colony on media surface were measured on a 3-day interval. The diameter was measured using a ruler, with two lines at right angles drawn at the bottom of the plate. The growth rate was measured upto 16 days after seeding. Growth rate was measured only for the exponential phase of growth as suggested by Pande and coworkers (1991) and Bigirwa (1992).

ii) Sporulation:

After 16 days from seeding the sporulation count was done. This was done by pouring 10ml of SDW on growth culture, scrapping off the culture with a microscopic slide and sieving

it with double cheesecloth. The counts were done by getting a few drops from the suspension obtained and placing on a haemocytometer using a micropipette, then recording readings (on average thirty) from each replicate which later were averaged.

3.4.3 Statistical analysis and data presentation.

The data obtained were analysed by analysis of variance to determine the effects of different conditions on *P. grisea* sporulation and growth diameter. The Fisher's protected least significant difference (LSD) was computed at $P \leq 5\%$ to determine the best media, pH and lighting regime for *P. grisea* growth among those tested (Steel and Torrie, 1980). Also correlation analysis was conducted to determine the degree of association between sporulation and hyphal growth.

3.5 Assessment of farmers' finger millet seeds for infection and contamination by *Pyricularia grisea*

3.5.1 Farmers' seeds

Finger millet seeds used in these experiments were obtained from farmers during surveys conducted in Kisii, Bungoma and Busia/Teso districts in January 1996. In each district, sampling areas were chosen at random purposely selecting areas with finger millet production and incidence of blast disease (*Pyricularia grisea*). Ten sites were sampled in Bungoma district, 12 in Kisii district and 12 in Busia/Teso district. Within each sampling site, grain was obtained from 8 - 16 randomly chosen farmers. Later, 100g grain from each of these 8 - 16 samples were mixed together to form a representative sample of approximately 800 - 1600 g per sampling area (Neergaard, 1979). In addition to these 34 samples, seven samples, each weighing 150 grams were kindly provided by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) – Nairobi office, making a total of 41 samples for the various experiments and tests.

Samples were kept in paper seed envelopes in a dry place in the Plant Pathology Laboratory, Kabete Campus, University of Nairobi. The seed health assessment tests were done within a month of the seed sampling procedure.

Samples collected from farmers, in each of the three districts and ICRISAT's samples were subjected to various procedures to determine the incidence of *P. grisea* in each seedlot. The objective was to identify a rapid assessment method for analysis of seedborne inoculum of *Pyricularia grisea* in and on finger millet

3.5.1.1 Seed health assessment methods

Five methods were used in this study to determine the seed health of the seed samples. The methods included seed washings, agar test, blotter test, rolled paper towel test and growing-on test (Neergard, 1988).

3.5.1.2 Examination of seed washings

A sample of 1000 seeds (approximately 3 grams) from each seed sample was divided into 4 subsamples (replications) of 250 seeds each. Each subsample was placed into a test tube containing 5 ml of SDW where a drop of liquid detergent was added, and shaken vigorously for 5 minutes. The test tube contents were filtered through one layer of cheesecloth and the filtrate was examined under the microscope for fungal spores i.e. surface infestation (Adipala, 1992). Ten readings were taken for each subsample. To ascertain if the conidia were viable, a germination test was done for the filtrate by the putting a drop of the filtrate on a germination slide and leaving it then for 6 days. This procedure was repeated for each of the 41 samples. The mean concentration, i.e. conidia per ml was subsequently calculated for each sample using the figures obtained from above.

3.5.1.3 Seed assay tests

The seed assay tests included: The rolled paper towel test; the agar plate test; the blotter test. In each test, 400 seeds per sample were used in four replicates of 100 seeds each. The completely randomised design (CRD) was used. Incidence of *P. grisea* was noted in each replicate. Slide preparations were made for confirmation of presence or absence of *P. grisea*. The percent germination was noted in each replicate.

3.5.1.3.1 The rolled paper towel test

The seeds were placed on moist paper towels (made moist by SDW) and then wrapped-up into a scroll and labelled. The scrolls were then hung in a fabricated moist chamber, which was kept moist by applying a mist of SDW from a hand sprayer. The moist chamber was a cubical structure measuring 50 x 50 x 50 cm made of polythene sheet with a small opening (door) to put or remove the samples and spray the mist. The moist chamber was placed on the bench. Incubation was done at room temperature (20-24^{0}C) with a normal day/night regime for 7 days. After 7 days the paper towels were removed from the moist chamber and opened. The 100 seeds (four replications) were carefully transferred onto moist blotter paper into two clean plastic petri dishes each with 50 seeds. The seeds were individually observed under compound binocular microscope at 10 x 40 magnification for the presence and growth of *P. grisea* on the as seed described by Ranganathaiah and Mathur (1976).

3.5.1.3.2 The Agar Plate test

One hundred seeds per replicate (four replications) were surface sterilised using sodium hypochlorite solution (containing 2% available chlorine) for 5 minutes and then rinsed with 5 changes of SDW. This procedure is useful for internal infection. The seeds were then plated 20 seeds per petri plate on oatmeal agar (OMA). The OMA had streptomycin incorporated for the

control of bacterial growth. The plates were incubated at room temperature for 7 days under 12 hours alternating cycles of near ultra violet light (NUV) and darkness, after which colonies of *P. grisea* were examined for mycelial growth, colour (pigmentation) and sporulation.

3.5.1.3.3 The Blotter test.

One hundred seeds per replicate (four replications) were divided into batches of 50 seeds and placed onto two layers of moistened filter paper in a plastic petri dish using forceps. They were incubated at room temperature (20-24°C) for 7 days under 12 hours alternating cycles of near ultra violet light (NUV) and darkness. After 7 days, each seed was examined for fungal growth under the compound binocular microscope and the incidence of *P. grisea* was recorded.

3.5.1.3.4 Statistical analysis

Data were analysed by analysis of variance to determine the effects of different seedborne inocula assessment methods. Standard error of the means was calculated and the least significant difference (LSD) was computed at $P \leq 0.05$. This was done to determine which method was superior. Correlation analysis was run to correlate percentage germination with the presence of *P. grisea* in the seeds to see if there is a relationship between percentage *P. grisea* incidence and % seed germination.

3.6 Growing-on tests in the glasshouse

The objective of this experiment was to determine the percent incidence of *P. grisea* in a particular sample of finger millet seed after germination. Steam sterilised sandy soil was put in four containers and 100 seeds per replicate (4 replicates) were planted into each container (15 cm deep pots). Emergence was recorded one week after planting but the plants were observed for an additional two weeks for seedlings with leaf blast symptoms of blast disease were

observed on the first leaf as oval to elliptical lesions. The design used was completely randomised design (CRD) with four replicates. The lesions previously observed were cut out, surface sterilised and incubated. Isolations were carried out for the confirmation of the presence of *P. grisea*.

3.6.1 Statistical analysis

The incidence of *Pyricularia grisea* obtained was correlated to percent emergence of the seed samples. Also the percent incidence obtained from the various methods in the laboratory incubation tests was correlated to percent incidence obtained in this glasshouse experiment.

3.7 Relationship of seedborne *Pyricularia grisea* and blast incidence under field conditions

The major objective in this experiment was to determine the relation between seedborne *P. grisea* and finger millet blast incidence under field conditions.

The experiment was conducted at two locations namely the Kabete campus field station farm, Faculty of Agriculture, University of Nairobi during May to October 1996 and August 1996 to February 1997 and the Kenya Agricultural Research Institute (KARI)/ICRISAT Dryland Crops Research Station at Kiboko, Makueni District during period of April 1996 to August 1996. Kabete field station is about 1800 metres above sea level (asl) and lies within latitude 1° 14' 20"S and 1° 15' 15"S and longitude 36° 44'E and 36° 45' 20"E (Wamburi, 1973). Kabete receives about 1046 mm of rainfall per annum with mean temperatures of 23.4°C (Maximum) and 12.6°C (Minimum). The soils are deep, friable, clay resistant to soil erosion (Keya and Mukunya, 1979) with acid humic to humic nitrosols developed from Limuru trachyte soils (Michieka, 1979). Kiboko station is about 960 metres ASL (above sea level) at latitude 2°S

and the longitude 37°E. It is located 170 km SE of Nairobi on an erosional plain. The soils are deep, dark, reddish brown or dark red, friable, clayey loam. The soil moisture regime is aridic. On average the area receives 621 mm of rainfall per year (ICRISAT, 1992).

The seed samples were part of the larger samples from six locations. The seed health assessment had already been conducted and using the blotter method (refer section 3.5.1.3.3) the *P. grisea* incidence on seed was as follows: Mwagichana, L_c - 0.00%; Suo, L_1- 2.50%; KNE479-Alupe, L2 - 4.25%; East Bukusu, L_3- 6.00%; South Teso, L_4- 7.75% ; Majoge Chacha, L_5- 14.00%.

The trial consisted of four replications in each location arranged in a randomised complete block design (RCBD). One thousand four hundred seeds (approximately 4 grams per plot i.e. 4 kg per hectare sowing rate) were planted per plot. Seedlings were thinned randomly to 20 plants per 4-metre row. The thinning was done randomly to ensure that the disease incidence was not influenced by selection. Plot size was 4 by 2 metres and contained 5 rows of finger millet plants with 40 cm interrow and 20 cm intrarow spacing giving a plant population of 125,000 plants per hectare (100 plants per plot). A distance of 1 metre was left between the plots.

Basal Diammonium phosphate (DAP) fertilizer (0:18:46) was applied at a rate of 50 kg/ha during planting. Urea (46 % N) was applied 6 weeks after plant emergence at a rate of 40 kg/ha.

To prevent insect damage insecticides Decis® 2.5EC (25g/l Deltamelthrin) and Rogor®L 40 (Dimethoate 40%EC) were used. One week after germination Rogor was applied, while Decis was applied when the pests were seen (It was done three times in a season). The insects encountered especially during the dry spell were aphids (*Aphis spp, Rhopalosiphum maidis*) and caterpillars (*Heliothis armigera*) during the wet times. The pest infection was minimal. The plots were kept weed free by hand weeding.

3.7.1 Data collection and analysis

3.7.1.1 Blast incidence

Individual plants were assessed for blast infection in the field on a once in two weeks basis, starting 14 days after emergence (DAE) upto 154 DAE. The whole plot was examined and the number of plants with typical lesions were noted and expressed as percent blast incidence per plot.

3.7.1.2 Blast severity

3.7.1.2.1 Blast severity on leaves

On each of the ten tagged tillers five top leaves (as they are the ones that make a major contribution to grain dry matter content in cereals, Thorne (1966), were assessed for the percent area with blast symptoms. The scale of 1 to 6 (Cited by Thomas and co-workers, 1996) was used, where: 1 = no symptoms; 2 = upto 5%; 3 = 6 to 25%; 4 = 26 to 50%; 5 = 51 to 75% and 6 = more than 75% of the leaf area covered by disease.

The mean disease ratings for the 10 tagged plants per plot were used in the analysis of variance.

3.7.1.3 Data analysis

Blast disease index i.e. final incidence was transformed using square root of $\sqrt{}+ X$ and compared statistically by analysis of variance for each location and period. The least significant difference (LSD) was computed at $P \leq 0.05$ and the treatment means separated (Steel and Torrie 1981).

Blast disease index i.e. severity on leaves were plotted over time to illustrate disease progress curves. To correlate the effect of blast disease index, the area under disease progress curves (AUDPC) for severity on leaves was calculated using the formula of Shanner and Finney (1977), as;

$$AUDPC = \sum_{i=1}^{n} [(Y_{i+n1} + Y_i) / 2] [(X_{i+1} - X_i)]$$

Where: -

Y_i = Blast severity at the ith observation,

X_i = Time, in days at the ith observation and

n = total number of observations

AUDPC (area under disease progress curves) for blast severity on leaves for the six treatments was compared statistically by analysis of variance for each location and period and the treatment means were separated using least significant difference (LSD) computed at $P \leq 0.05$ (Steel and Torrie 1981).

Also the relationship between seed *P. grisea* inoculum load and disease incidence and severity was analysed. This was supposed to establish the functional relationship i.e. linear, quadratic or cubic.

Correlation coefficients were calculated for the % incidence noted for blotter assay test (refer 3.5.1.3.3) to the % leaves severity in the field experiment.

3.7.2 Artificial inoculation of finger millet plants at various growth stages

The main objective of this experiment was to establish the critical time of blast infection on the finger millet plants that would result in any detectable blast infection on harvested seed. Once established, the concept could be used in disease control program for finger millet seed production in Kenya.

The experiment was conducted at two locations namely Kabete campus field station farm, Faculty of Agriculture, University of Nairobi during May to October 1996 and August 1996 to February 1997 and the Kenya Agricultural Research 'Institute (KARI)/ICRISAT Dryland Crops Research Station at Kiboko, Makueni District during April 1996 to August 1996.

The trial consisted of four replications in a randomised complete block design (RCBD), with one control per plot. The plot size was 4 x 2.8 m with 5 rows. The spacing was 40cm interrow and 20 cm intrarow spacing.

Pathogen free seed of the variety KNE 479 harvested at KARI/ICRISAT, Kiboko station and tested at the Kabete plant Pathology Laboratory using the blotter, rolled paper towel and agar test, and found to have no contamination of *P. grisea* was used. One thousand four hundred seeds (approximately 4 grams per plot or 4kg per hectare) were planted per plot. Seeds were drilled in rows and seedlings were later thinned to a spacing of 20 cm between plants.

Fertiliser application was done as per section 3.7 and insect pest and weed control was done as per section 3.7.

Inoculum was prepared from cultures grown on oatmeal agar for 14 days (refer section 3.4.1). The cultures were flooded with sterile distilled water (SDW) and using a sterile microscopic slide, spores were gently scrapped off the colony cultures and sieved through cheesecloth. The conidial suspension was adjusted to 2.8×10^6 spores/ml after counting under the haemocyotometer (Adipala, 1989).

Plants were subsequently inoculated at different developmental growth stages (as per a decimal code for the growth stage of cereals, Zadoks *et al.,* 1974).

Growth stage	One digit code(Zadoks *et al.*, 1974)	Feekes' scale (1941)
Stage one: Stem elongation	3	9
Stage two: Inflorence emergence	5	10.4
Stage three: Anthesis	6	10.51
Stage four: Milk Development	7	11.1
Stage five: Ripening	9	11.3

Each plot of the five growth stages was inoculated using the inoculum suspension and covered for 14hours, while the control plot was inoculated using SDW and covered using a

polythene bag for 14 hours. Both ends of the plots had one guard row. In both cases, the plants were inoculated in the evening hours i.e. 4.00 - 7.00 p.m. using a conidial suspension of *P. grisea* (2.8 X 10^6 spores/ml) or SDW applied to the plants until runoff.

3.7.2.1 Data collection

3.7.2.1.1 Blast assessment

Ten plants (since one plant has many tillers, only the main tiller was tagged) were tagged for blast assessment on a weekly basis starting from 39 days after emergence (DAE) upto 102 DAE, for Kiboko long rain (1996), 64 – 141 DAE for Kabete long rains (1996) and 58 – 149 DAE for Kabete short rains (1996/1997) from which the following data was collected: Blast incidence on the tillers per plant, severity on panicles, severity on neck and severity on leaves. Data collection started one week after inoculation.

3.7.2.1.1.1 Blast incidence on the tillers per plant

On the tagged plant the panicles showing blast symptoms were noted against the total number of tillers for that plant and the percent infected tillers computed.

3.7.2.1.1.2 Severity on panicles

Blast severity on the panicles was taken on a weekly basis. Ten plants were tagged for this purpose. Blast severity data was taken depending on the number of panicle fingers infected against the total number of fingers on the panicle and then expressed as a percentage.

Blast severity was assessed on a scale of 1 to 6 (Cited by Thomas and co-workers, 1996 – refer section 3.7.1.2.1). The mean disease severity ratings for the 10 tagged plants per plot were used in the analysis of variance.

3.7.2.1.1.3. Severity on neck

Blast severity on the neck was recorded as the length of blast lesion. A ruler was used and lesion length was recorded in centimeters. The measurements were taken from the base of the end of all the fingers along the neck upto the end of the black band (the end of the spread of blast on the neck is charactised by a black band). This was done for all the 10 tagged plants that had developed neck blast.

3.7.2.1.1.4 Severity on leaves

On the tagged tiller, for each of the five top leaves (they are the ones that make a major contribution to grain dry matter content in cereals, Thorne (1966), as percent area affected was noted and averaged over the five leaves. The scale of 1 to 6 (Cited by Thomas and co-workers, 1996 – refer section 3.7.1.2.1). The mean disease ratings for the 10 tagged plants per plot were used in the analysis of variance.

3.7.2.1.1.5 Yield data

The following yield data were taken:

a. 100 panicles threshed weight,

b. 1000 seed weight,

c. Total yield per plot (in kg/ha),

d. % Yield loss in inoculated plots =

$$\frac{\text{Sterile Distilled Water treated (Control) plot - Inoculum treated plot}}{\text{Sterile Distilled Water treated (Control) plot}} \times 100\ \%$$

3.7.2.2 Presence of *P. grisea* on harvested seed

The seeds harvested from the various sub plots in the inoculation experiment were assessed for the presence of *P. grisea* using the blotter method. For each sub plot 400 seeds were assayed. The procedure described in section 3.5.1.3.3. was followed .

3.7.2.3 Analysis of data

Blast disease index i.e. final incidence on tillers was transformed using square root of 1 + X subjected to analysis of variance for each location and period, and mean separation using the least significant difference (LSD) at $P \leq 0.05$, the treatment means were separated (Steel and Torrie 1981).

Blast severities on panicles, neck and leaves were plotted over time to illustrate the progress of the disease. To correlate the effect of blast severity on panicles, neck and leaf components. Likewise, AUDPC for blast severity on panicles, neck and leaves were computed using the formula of Shanner and Finney (1977) and analysed using analysis of variance for each location and period, and the least significant difference (LSD) computed at $P \leq 0.05$, was used to separate the treatment means (Steel and Torrie 1981).

ANOVA was also conducted to establish effect of blast infection on yield in kg/ha, 1000 seed weight, and 100 panicle threshed weights were analysed using analysis of variance for each location and time and using the least significant difference (LSD) computed at $P \leq 0.05$, means were separated (Steel and Torrie, 1981).

The relationship between time of inoculation and disease incidence and severity was analysed using orthogonal polynomials. This was supposed to establish the functional relationship i.e. linear, quadratic or cubic.

CHAPTER FOUR

RESULTS

CULTURAL STUDIES

4.1 Cultural characteristics

4.1.1 The effect of different media, pH and lighting on growth and sporulation of *P. grisea* isolates

Isolates of *P. grisea* grew slowly on PDA and had cottony white, whitish grey to ashy grey, flat and very thick mycelial growth. The reverse side of the colonies was dark grey. The margins were smooth with a whitish tinge. The darkening (greyish) or sporulation process took longest to start. Isolates of *P. grisea* on FMLEA had light grey colour at the centre and showed medium thickness of mycelial growth. Isolates of *P. grisea* on BMA grew very fast (5.19 mm/day) giving a dark mycelial growth of medium thickness. Isolates of *P. grisea* on OMA grew at medium pace (4.72 mm/day) giving a thick mycelial mat with grey/black centre, while the outside was white. The culture is very noticeable on this media. Isolates of *P. grisea* on FMSEA (5.06 mm/day) grew relatively fast showing a dark centered, scant mycelial growth. FMLEA was found the best with respect to rate of growth (5.22 mm/day), but OMA gave the best sporulation (671,600 spores/ml). OMA had one major advantage over FMLEA in that; the white medium colour ensured quick identification of the pathogen, since the pathogen is darker in colour. Plate 1 shows Busia isolate growing on the different media. There was a considerable variation in the colony morphology under the different media. The colony colours on media ranged from whitish or cream, through grey and dark olivaceous, with grey being the most common with the mycelial growth at the margins remaining in most cases white. The colonies on media also exhibited zonation (plate 2) and sectoring (plate 2) patterns.

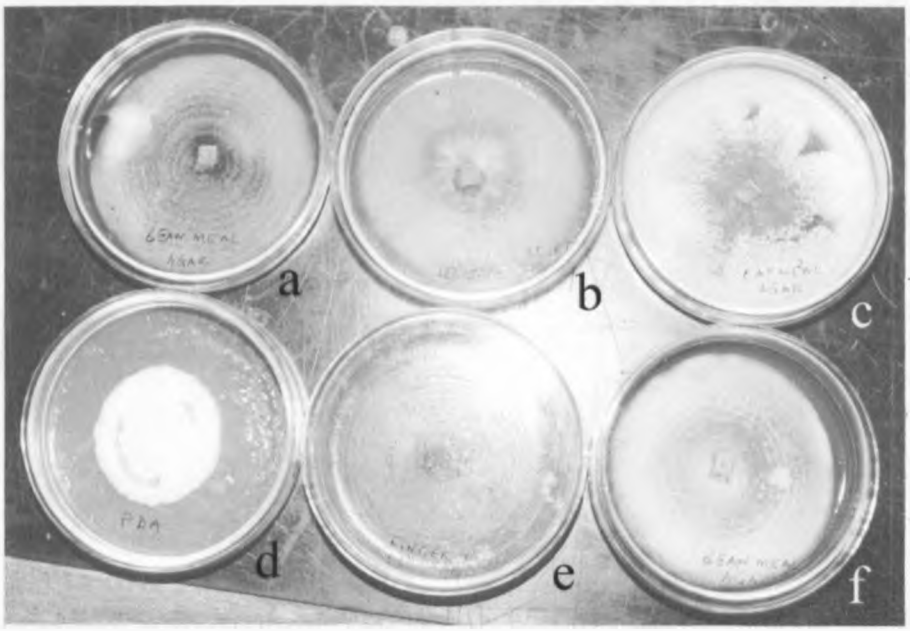


Plate 1 Growth of Busia Isolate, under pH 7, 12 hours lighting / 12 hours darkness in different media;
a. Bean Meal Agar (BMA), b. Finger Millet Leaves Extract Agar (FMLEA), c. Oat Meal Agar (OMA), d. Potato Dextrose Agar (PDA), e. Finger Millet Seeds Extract Agar (FMSEA) and f. BMA.

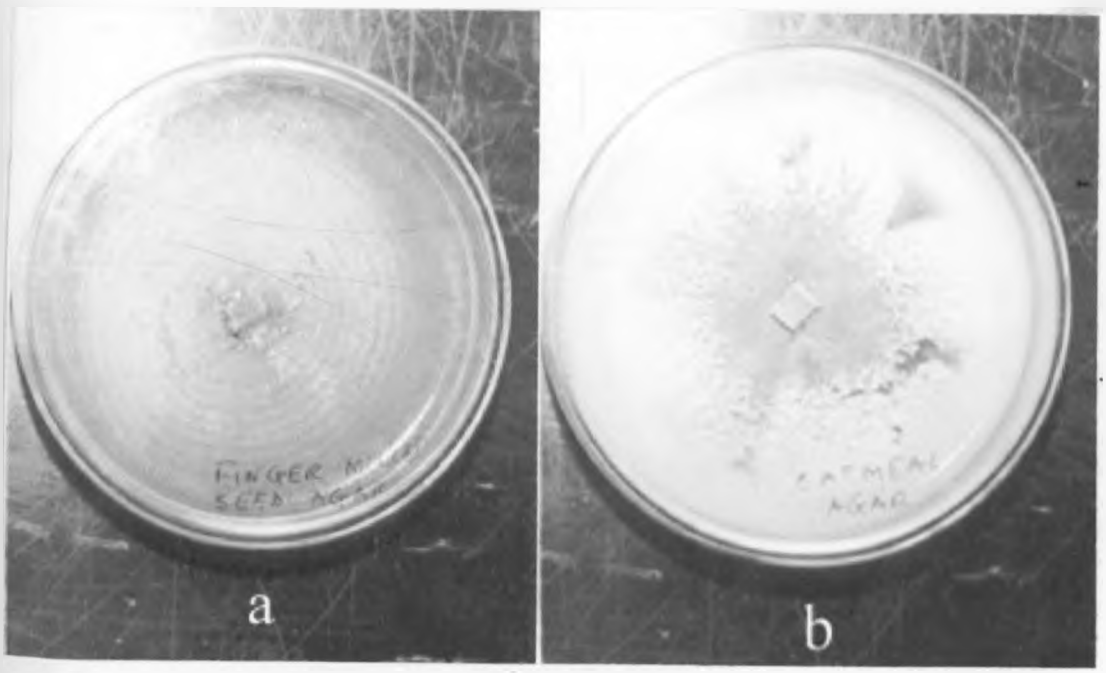


Plate 2 : Zonation (a) and sectoring (b) of *Pyricularia grisea* on media (FMLEA [a]and OMA [b]).

4.2 Mycelial growth rates

Mycelial growth observations were made on the five media (FMLEA, BMA, FMSEA, PDA and OMA), at all the four lighting regimes (12 hrs lighting / 12 hrs darkness, 24 hrs darkness, 24 hrs lighting and 12 hrs NUV light / 12 hrs darkness) and the four pH levels (pH 5, 6, 7 and 8) and three isolates, starting four days after seeding *P. grisea* on media.

For all lighting regimes, the isolate from Busia was statistically ($P \leq 0.05$) different from isolates from Kisii and Bungoma, except under 12hours NUV / 12 hours darkness, where there was no statistical differences between the isolates (Table 4.1).

For all pHs, the isolate from Busia was significantly different ($P \leq 0.05$) from the Kisii and Bungoma isolates (Table 4.2).

For all media BMA, PDA na OMA, the isolate from Busia was statistically different ($P \leq 0.05$) from the Kisii and Bungoma isolates; under media FMLEA and FMSEA isolates from Busia and Kisii were statistically different ($P \leq 0.05$) from the isolate of Bungoma (Table 4.3).

The fastest growth rate was FMLEA with 5.22 mm (disregarding other factors) per day and this was significantly different ($P \leq 0.05$) from the other four media. Decreasing growth rate in the other media followed this order: BMA, FMSEA, OMA and PDA (Table 4.4).

The lighting regime that recorded the fastest growth rate was 24 hours lighting which gave a growth rate of 4.56 mm (disregarding other factors) per day and this was statistically different ($P \leq 0.05$) from the other lighting regimes. Decreasing growth rate in the other lighting regimes followed this order: 12 hrs lighting / 12 hrs darkness, 24 hrs darkness and 12 hrs NUV light / 12 hrs darkness (Table 4.4).

The pH that recorded the fastest growth rate was pH 8 with 4.89 (disregarding other factors) mm per day and this was significantly different ($p \leq 0.05$) from the other pH. Decreasing growth rate in the other followed this order, pH : 7, 5 and 6 (Table 4.4).

4.3 Sporulation

Sporulation in general was associated with darkening of the cultures, starting from the inner layers of the culture. The OMA media supported earliest sporulation and that was two days after plating. PDA generally remained white and took eight days to start sporulating albeit poorly still.

On all lighting regimes, the isolate from Kisii was statistically different from ($P \leq 0.05$) Bungoma and Busia isolates except under 24 hours of lighting when the isolate from Bungoma was statistically different ($P \leq 0.05$) from the Kisii and Busia isolates (Table 4.5).

On all pH, the isolate from Kisii was statistically different ($P \leq 0.05$) from the isolates of Bungoma and Kisii, except under pH 8 when the isolate from Bungoma was different statistically ($P \leq 0.05$) from the isolates from Kisii and Busia (Table 4.6).

On the various media, the isolate from Kisii was statistically different ($P \leq 0.05$) for the media FMLEA and PDA from the isolates from Bungoma and Busia. On the media BMA, the isolate from Busia was statistically different ($P \leq 0.05$) from the isolates from Kisii and Bungoma. While on the media FMSEA and OMA, the isolate from Bungoma was statistically different the isolates from Kisii and Busia (Table 4.7).

Table 4.1 Influence of lighting regime on mean radial growth rates (mm/day) of the three isolates of *Pyricularia grisea.*

Isolate	12hrs daylight / 12hrs darkness	24 hrs lighting	24 hrs darkness	12 hrs NUV / 12 hrs darkness	Mean
Kisii	4.53 b	4.58 b	4.46 b	4.47 a	4.51 b
Busia	4.63 a	4.68 a	4.60 a	4.50 a	4.60 a
Bungoma	4.43 c	4.59 b	4.40 c	4.44 a	4.46 c
LSD (5%)	0.036	0.036	0.036	0.036	0.018

Table 4.2 Influence of pH on mean radial growth rates (mm/day) of the three isolates of *Pyricularia grisea.*

Isolate	pH 5	pH 6	pH 7	pH 8	Mean
Kisii	4.36 b	4.29 c	4.45 b	4.95 a	4.51 b
Busia	4.41 a	4.44 a	4.60 a	4.96 a	4.60 a
Bungoma	4.29 c	4.34 b	4.47 b	4.75 b	4.46 c
LSD (5%)	0.036	0.036	0.036	0.036	0.018

Table 4.3 Influence of media on mean radial growth rates (mm/day) of the three isolates of *Pyricularia grisea.*

Isolate	FMLEA	BMA	FMSEA	PDA	OMA	Mean
Kisii	5.29 a	5.28 a	5.12 a	2.28 c	4.56 c	4.51 b
Busia	5.30 a	5.20 b	5.12 a	2.49 a	4.90 a	4.60 a
Bungoma	5.12 b	5.13 c	4.95 b	2.42 b	4.70 b	4.46 c
LSD (5%)	0.036	0.036	0.036	0.036	0.036	0.018

Table 4.4 Overall influence of media, lighting regimes and pH on mean radial growth rates (mm/day) of *Pyricularia grisea* .

Media	Overall radial means (mm/day)	Lighting regimes*	Overall radial means (mm/day)	pH	Overall radial means (mm/day)
FMLEA	5.22 a	1	4.53 b	5	4.36 c
BMA	5.19 b	2	4.47 d	6	4.29 d
FMSEA	5.06 c	3	4.49 c	7	4.51 b
PDA	2.36 e	4	4.56 a	8	4.89 a
OMA	4.72 d				
LSD (5%)	0.000045		0.00036		0.00036

Lighting regime * : 1. 12 hours lighting / 12 hours darkness ; 2. 12 hours NUV light / 12 hours darkness; 3. 24 hours darkness; 4. 24 lighting.

Table 4.5 Influence of lighting regimes on sporulation count (spores x 10^3/ml/mm^2) of three isolates of *Pyricularia grisea.*

Isolate	12hrs daylight / 12 hrs darkness	24 hours lighting	24 hours darkness	12 hrs NUV / 12 hrs Darkness	Mean
Kisii	994.6 a	199.6 b	51.2 a	425.9 a	417.8 a
Busia	620.6 c	150.0 c	28.7 c	235.8 c	258.7 c
Bungoma	790.6 b	254.7 a	36.4 b	383.8 b	366.4 b
LSD (0.05)	3.9	3.9	3.9	3.9	2.0

Table 4.6 Influence of pH on sporulation count (spores x 10^3/ml/mm^2) of three isolates of *Pyricularia grisea.*

Isolate	pH 5	pH 6	pH 7	pH 8	Mean
Kisii	250.5 a	347.2 a	707.8 a	365.9 b	417.9 a
Busia	142.0 c	218.1 c	514.1 c	160.8 c	258.8 c
Bungoma	176.0 b	255.1 b	632.6 b	401.9 a	366.4 b
LSD (0.05)	3.9	3.9	3.9	3.9	2.0

Table 4.7 Influence of media on sporulation count (spores x 10^3/ml/mm^2) of three isolates of *Pyricularia grisea.*

Isolate	FMLEA	BMA	FMSEA	PDA	OMA	Mean
Kisii	644.9 a	105.1 b	442.0 b	236.1 a	661.1 b	417.9 a
Busia	235.0 c	120.6 a	353.0 c	108.5 b	476.7 c	258.8 c
Bungoma	284.5 b	94.3 c	472.2 a	103.7 c	877.0 a	366.4 b
LSD (0.05)	3.9	3.9	3.9	3.9	3.9	2.0

Table 4.8 Overall influence of media, lighting regimes and pH on spore counts (spores x 10^3/ml/mm^2) of *Pyricularia grisea.*

Media	Overall spore count (spores x 10^3/ml/mm^2)	Lighting regimes*	Overall spore count (spores x 10^3/ml/mm^2)	pH	Overall spore count (spores x 10^3/ml/mm^2)
FMLEA	388.2 c	1*	801.8 a	5	189.5 d
BMA	106.6 e	2*	345.5 b	6	270.5 c
FMSEA	418.7 b	3*	38.8 d	7	618.0 a
PDA	149.4 d	4*	201.5 c	8	309.6 b
OMA	671.6 a				
LSD (5%)	1.20		0.73		0.73
CV(%)	0.58		0.58		0.58

Lighting regime * : 1*. 12 hours lighting / 12 hours darkness; 2*. 12 hours NUV light / 12 hours darkness; 3*. 24 hours darkness; 4*. 24 lighting.

The OMA media supported the highest overall spore count (671,600 spores per ml) and this was significantly different ($P \leq 0.05$) from the other media that followed as: FMSEA (418,700 spores/ml), FMLEA (388,200 spores/ml), PDA (149,400 spores/ml) and BMA (106,600 sspores/ml)(Table 4.8). The lighting regime that recorded the highest overall spore count was 12 hrs lighting / 12 hrs darkness with 801,800 spores per ml and was statistically different ($P \leq 0.05$) from the other lighting regimes that followed as 12 hrs NUV light/ 12 hrs darkness (345,500 spores/ml), 24 hours lighting (201, 500 spores/ml) and 24 hrs darkness (38,800 spores/ml) (Table 4.8). The pH that recorded the highest overall spore count was pH 7 with 618,000 spores per ml/mm^2 and was significantly ($P \leq 0.05$) different from the other pH. that followed as pH: 8 (309, 600 spores/ml), 6 (270, 500 spores/ml) and 5 (189,500 spores/ml)(Table 4.8).

The correlation coefficient, r, between radial growth and spore count for media was 0.345, for lighting regime was 0.203 and pH was 0.174, showing almost no strong relationship between these two traits.

4.4 Morphology of *P. grisea*

Plates 3 and 4 show morphological characteristics of *P. grisea* . The conidia were hyaline to light brown, 1 - 5 septate but commonly 2 septate. The conidia were obypriform, with the middle cell being broader and darker than the others and measured in length 17.6μm - 33.0μm with the average being 25.2μm and the widest part being 7.5μm - 12.0μm (9.2μm). The darker colour of the middle cell of the conidia was observed in only a few conidia. The conidia were produced on olivaceous brown, mostly unbranched, straight or flexuos conidiophores, measuring 140μm - 160μm (154μm) in length with the average being 154μm. The width of the conidiophore was between 3μm - 4μm (3.5μm).

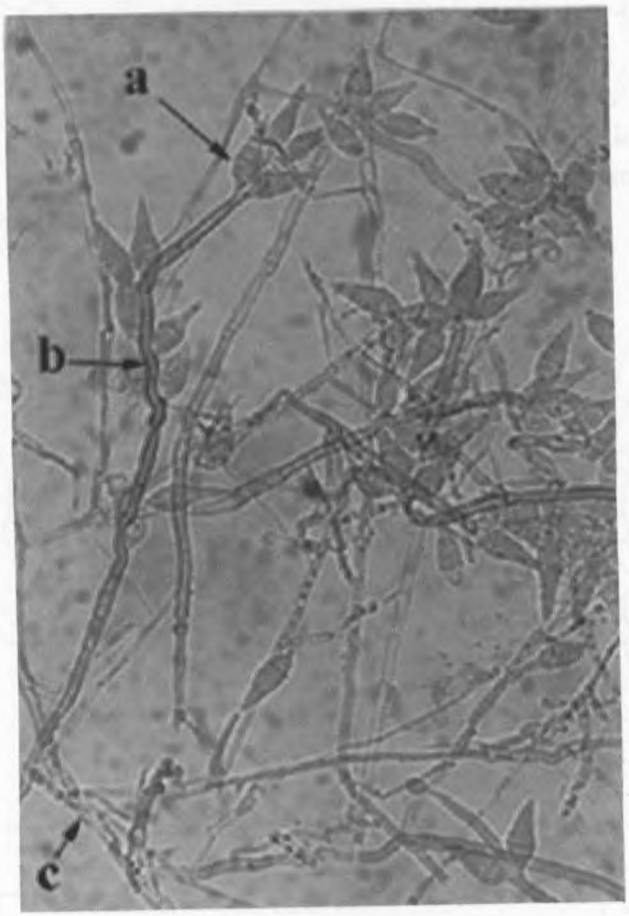


Plate 3 Conidia (a), conidiophore (b) and mycelium (c) of *Pyricularia grisea* in a slide culture.

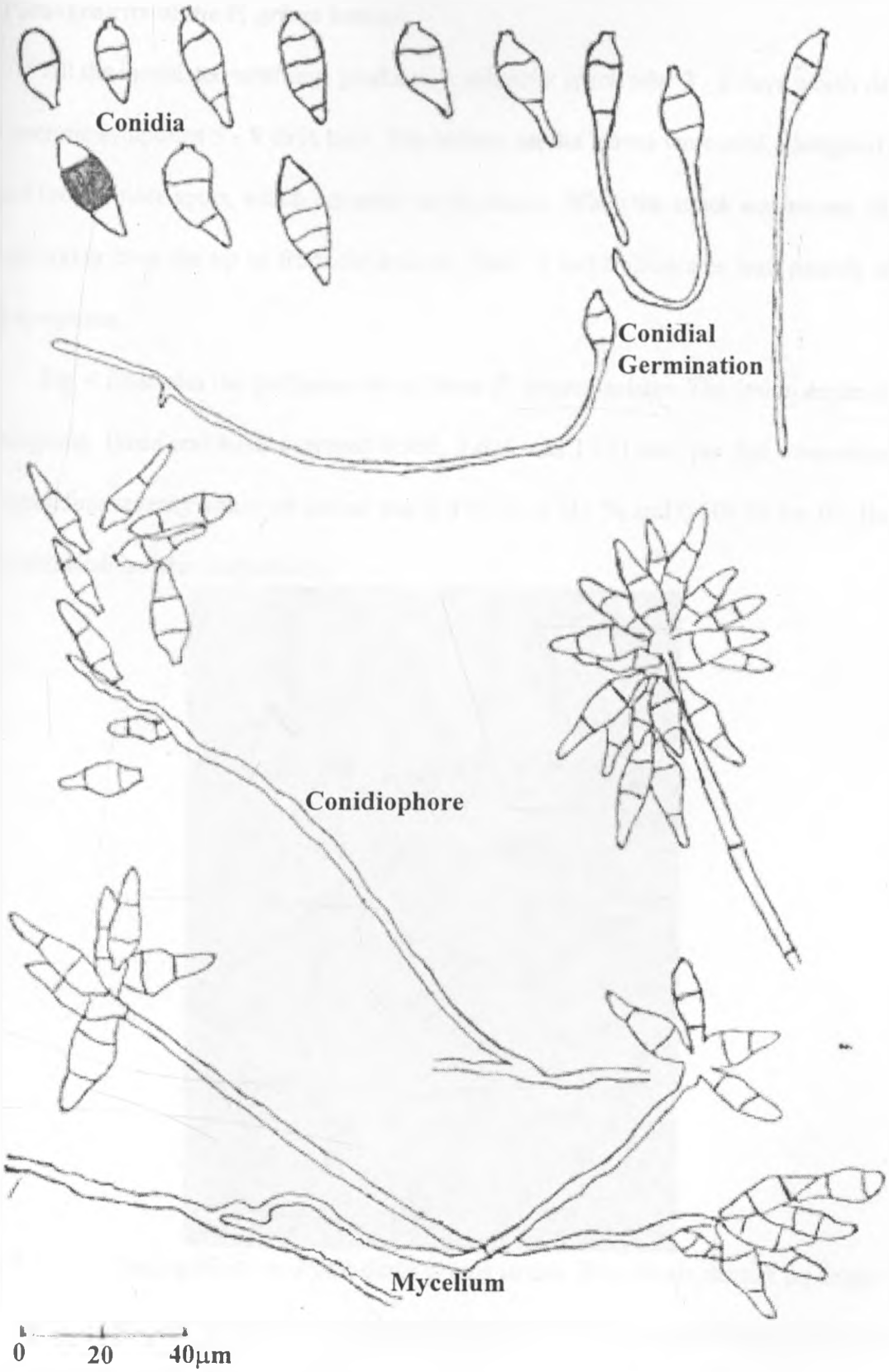


Plate 4 Illustrations of *Pyricularia grisea* showing various taxonomical aspects drawn using a drawing tube fitted to a microscope

4.5 Pathogenicity of the *P. grisea* isolates

All the inoculated seedlings produced a chlorotic speck after 3 - 6 days which developed into necrotic symptoms 5 - 8 days later. The lesions on the leaves were oval, elongated, spindle shaped brown-black spots, which occurred on the leaves. When the attack was severe, the leaves started drying from the tip or from the lesions. Plate 5 and 6 illustrates leaf, panicle and neck blast symptoms.

Fig. 4 illustrates the pathogenicity of three *P. grisea* isolates. The lesion expansion rates of Bungoma, Busia and Kisii averaged 0.905, 1.625 and 1.131 mm per day, respectively. The corresponding severity value on leaves was 0.475 %, 0.741 % and 0.804 % for the Bungoma, Busia and Kisii isolates respectively.

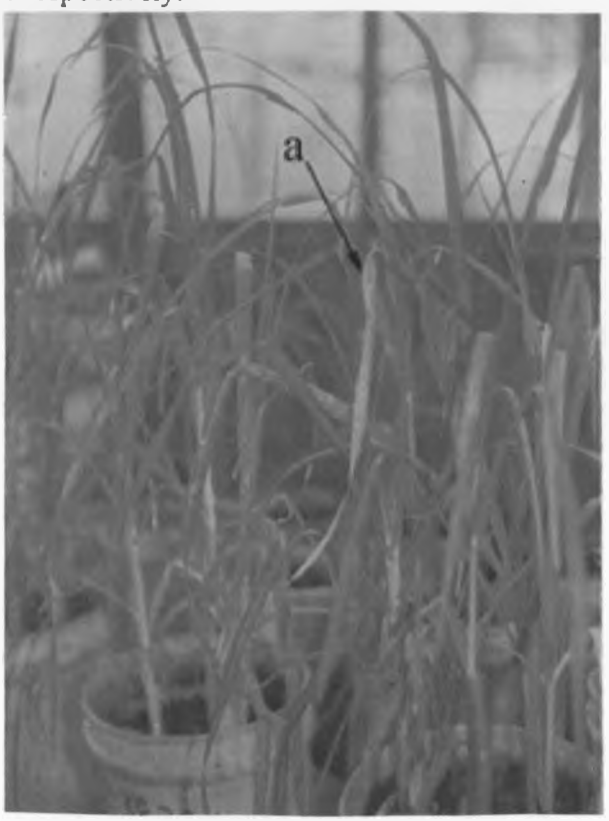


Plate 5 Pathogenicity of *Pyricularia grisea* isolate from Busia district on finger millet, (Variety KNE 479). Typical elliptical blast lesions were formed on the leaves for instance the one marked a.

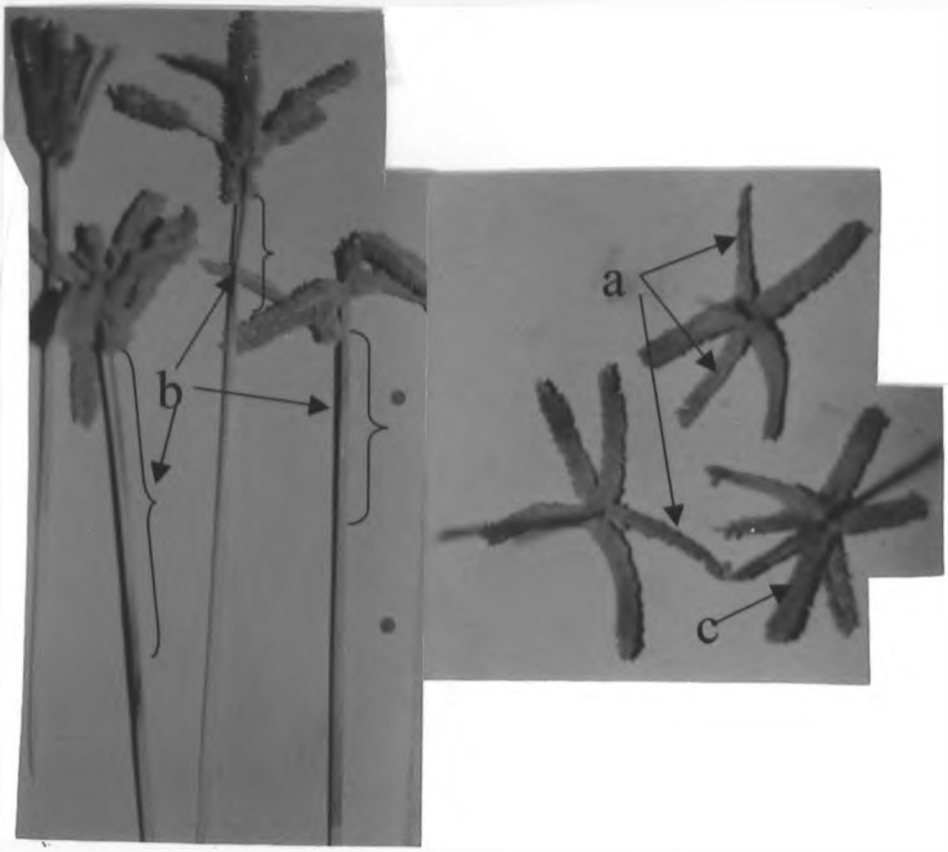
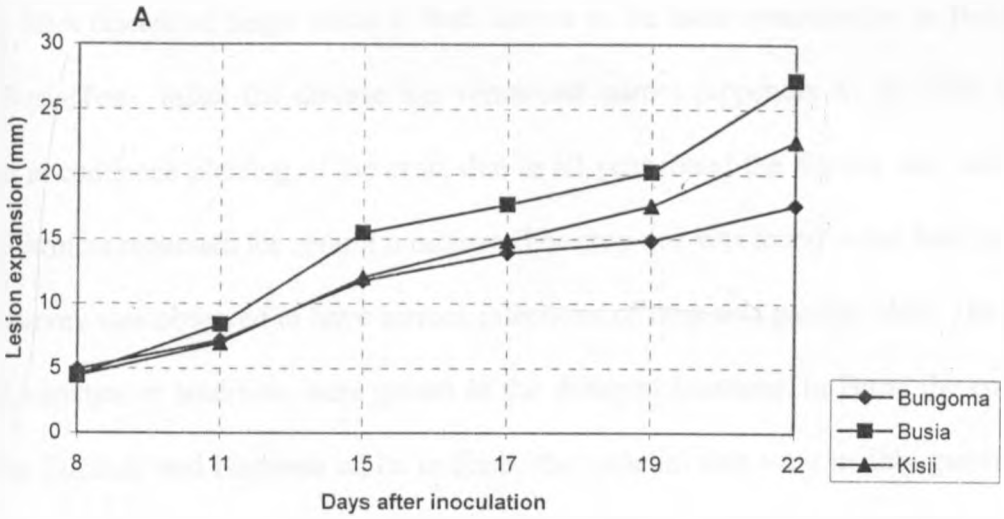
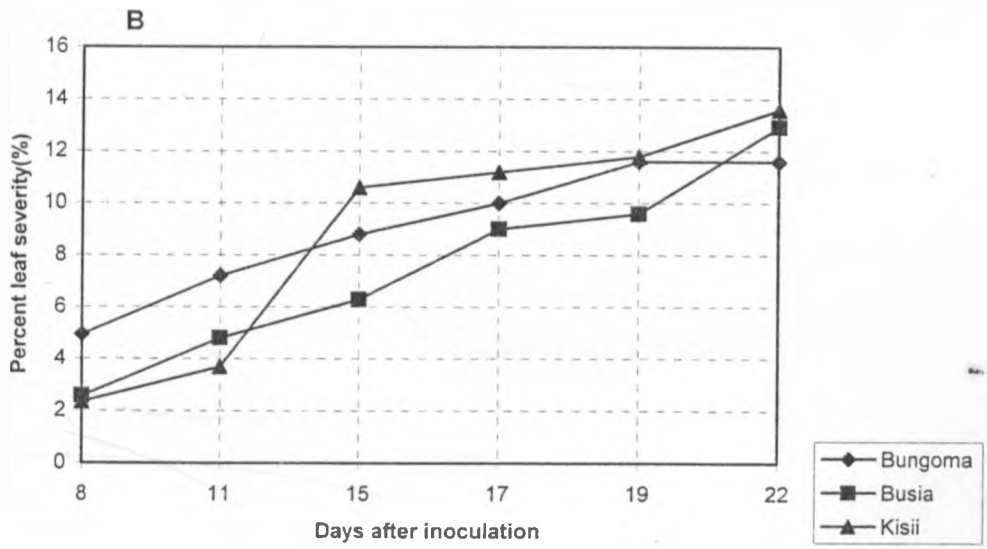


Plate 6 Blast symptoms on Finger millet, variety KNE 479 showing panicle – shrivelled panicles (a) and neck (b) blast. A normal sized panicle (c)

Fig 4 Influence of Bungoma, Busia and Kisii *P. grisea* isolates on leaf blast expansion (A) and severity (B)

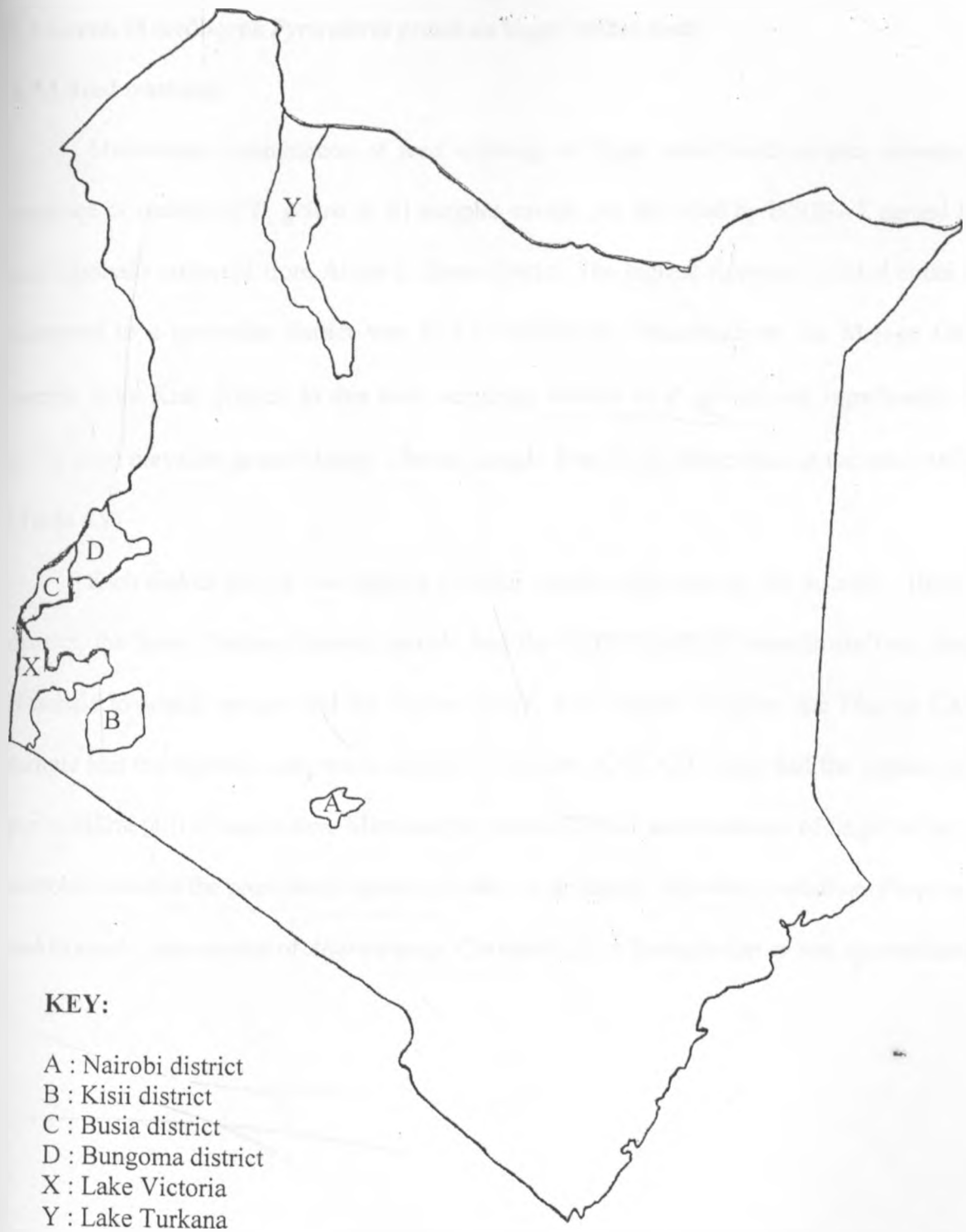




4.6 Finger millet seed health evaluation for infection/contamination by *P. grisea*

4.6.1 Survey findings

The blast disease of finger millet is well known to the local communities in Bungoma, Kisii and Busia/Teso. Infact the disease has vernacular names (appendix 5). In Kisii district where there is continous planting of the crop, due to all year round the disease was said to be serious and farmers requested for control measures. The crop that was found in the field growing during the survey was observed to have serious infections of neck and panicle blast. The finger millet local varieties or landraces were grown in the different locations. In Busia the common landrace was Ikhulule and Namaala while in Kisii, the varieties that were mainly grown were Enyaikuro (A brown seeded variety) and Enyandabu (A white seeded variety). Others included Egetengenyi, Omokomoni, Enyamarambe, Endere and Egetui. These varieties were said to be low yielding and late maturing, especially in the case of Kisii.



Map 1: Map of Kenya showing the districts where sampling of finger millet seeds was done (B=Kisii, C= Busia/Teso and D=Bungoma)

4.7 Levels of seedborne *Pyricularia grisea* on finger millet seeds

4.7.1 Seed washings

Microscopic examination of seed washings of finger millet seed samples revealed the presence of conidia of *P. grisea* in all samples except one provided by ICRISAT named P224 and originally collected from Alupe in Busia district. The highest recorded conidial count with disregard to a particular district was 81,579 conidia/ml, obtained from the Majoge Chacha sample from Kisii district. In this seed washings, conidia of *P. grisea* was significantly ($P \leq 0.05$) more prevalent in the Majoge Chacha sample from Kisii district than in the other samples (Table 4.9)

Each district had its own highest conidial concentration per ml, for instance, Bungoma district, the South Bukusu-Bumula sample had the highest conidial count,Busia/Teso district, Nambale township sample had the highest count, Kisii district samples, the Majoge Chacha sample had the highest count while ICRISAT samples KNE 479-Alupe had the highest count, per millilitre (ml) of suspension. Microscopic examination of seed washings of finger millet seed samples revealed the presence of spores of other fungi namely *Bipolorus nodulosa, Fusarium sp* and in some cases species of *Alternaria sp, Curvularia sp, Cladosporium sp* and *Aspergillus sp.*

Table 4.9 *P. grisea* conidial concentrations (conidia / ml) from different parts of Kenya in finger millet seed washings (LSD [$p \leq 0.05$] = 81.92).

	Sampling area	District	Number of conidia per ml
1.	Majoge Chacha	Kisii	81,579 A
2.	Bassi Regina	Kisii	75,938 B
3.	S.Bukusu-Bumula	Bungoma	72,368 C
4.	East Bukusu	Bungoma	58,824 D
5.	Bassi Masige	Kisii	47,619 E
6.	Bassi Borabu Bogetaorio	Kisii	46,875 F
7.	Mwamonari	Kisii	28,935 G
8.	Nambale Township	Busia/Teso	24,509 H
9.	Kaujakito	Busia/Teso	22,556 I
10.	KNE 479-Alupe	ICRISAT	21,898 J
11.	Bungoma township	Bungoma	20,619 K
12.	Kocholia	Busia/Teso	18,328 L
13.	Bobasi Bogetaorio	Kisii	17,606 M
14.	South Teso	Busia/Teso	16,878 N
15.	Kabuchai	Bungoma	15,957 O
16.	Syekumulo-Bumula	Bungoma	15,000 P
17.	Buluani	Busia/Teso	13,100 Q
18.	Kibabii	Bungoma	12,640 R
19.	Sengera	Kisii	12,295 S
20.	Kamuriai	Busia/Teso	9,901 T
21.	Kotur	Busia/Teso	9,102 U
22.	Syekumulo-S.Bukusu	Bungoma	8,772 V
23.	Suo	Busia/Teso	7,772 W
24.	Musikoma	Bungoma	7,653 X
25.	Bukhayo North	Busia/Teso	7,538 Y
26.	Bukhayo Central	Busia/Teso	6,329 Z
27.	Bukhayo West	Busia/Teso	5,464 a
28.	Bukembe	Bungoma	5,000 b
29.	North Bukusu	Bungoma	4,934 b
30.	Bassi Central	Kisii	4,310 c
31.	Nyakeiri	Kisii	2,964 d
32.	U15	ICRISAT	2,769 e
33.	GULU-E	ICRISAT	2,475 f
34.	KAT FM 1	ICRISAT	2,174 g
35.	Kakapel	Busia/Teso	1,932 h
36.	Sensi	Kisii	1,569 i
37.	Gesangero Ngenyi	Kisii	1,431 j
38.	KNE 808	ICRISAT	1,168 k
39.	KNE 479-Kiboko	ICRISAT	845 l
40.	Mwagichana	Kisii	527 m
41.	P224	ICRISAT	0 n
	Mean		17,517
	LSD ($P < 0.05$)		81.92
	CV (%)		0.33 %

4.7.2 Incubation tests

Most of the infected seeds did not germinate. Several seedlings were heavily colonised and those that emerged had discoloured, dull brown coleoptiles with some etiolation at the tip. No seed rot or quick death of the seedlings was observed, although with time, browning and etiolation of the coleoptile increased and seedlings collapsed after 9 - 12 days.

4.7.2.1 Rolled paper towel test

The identification of the blast fungus on seed was based on the observation under the compound binocular microscope using sporulation with respect to conidiophores and conidia on the seed and shoot (plate 3). *P. grisea* produced effuse growth of whitish grey mycelium. Conidiophores arose singly or in groups and covered part of the seed and in a few cases the whole seed. Conidiophores were slender, straight and bore clusters of conidia. The conidia were typically obpyriform or obclavate, hyaline and two-septate usually, with the central cell larger than the two terminal cells.

The highest recorded incidence under this method was 10.25% for the Majoge Chacha sample from Kisii and it was significantly higher than for the rest of the samples except Bassi Masige sample. The lowest *P. grisea* of 0 % was detected in 9 samples, namely Syekumulo-S.Bukusu, KNE 479-Kiboko, P224, GULU-E, Bukhayo North, Bukhayo West, Nyakeiri, Mwagichana and Sensi (Table 4.10).

The highest *P. grisea* incidence for the Bungoma district samples was observed in the Kibabii sample which had 2.0 % this was significantly ($P \leq 0.05$) higher than from samples of Syekumulo-Bumula, Musikoma, Syekumulo-S. Bukusu, Kabuchai and N. Bukusu (Table 4.11). Syekumulo - S. Bukusu sample recorded 0 % incidence of *P. grisea*. The highest incidence for the Busia/Teso district samples was observed in the South Teso sample which had 5.00 % and was significantly ($P \leq 0.05$) different from the other samples except Kamuriai and

Kotur.There was no *P. grisea* in Bukhayo North and Bukhayo West samples (Table 4.13). The highest incidence for the Kisii district samples was observed in the Majoge Chacha sample which had 10.25 % and was significantly different ($P \leq 0.05$) from the other samples except the Bassi Masige sample. There was no *P. grisea* in Nyakeiri, Mwigichana and Sensi (Table 4.14).

The highest incidence for the ICRISAT samples was observed in the KNE 479 - Alupe sample which had 1.00 % and was significantly ($P \leq 0.05$) different from all other samples except KAT FM-1. There was no *P. grisea* in three samples namely KNE 479 - Kiboko, P224 and GULU-E (Table 4.12).

4.7.2.2 Agar plate test

Noting the characteristic dark grey woolly growth around the seed (Plate 7) carried out the identification for the blast fungus on media.

The highest recorded *P. grisea* incidence under the agar plate test method was 5 % in the Majoge Chacha sample from Kisii and was significantly different ($P \leq 0.05$) from the other samples. The lowest was 0 % for 25 samples (Table 4.10).

In Bungoma district samples, Kibabii, Syekumulo - S. Bukusu, S. Bukusu - Bumula and N. Bukusu had 0.25 % *P. grisea* incidence with the rest recording 0 % incidence. None of the samples had significantly different ($P \leq 0.05$) *P. grisea* incidence from the others (Table 4.11). In Busia/Teso district, the Bukhayo West sample had the highest *P. grisea* incidence of 3 % and was significantly ($P \leq 0.05$) different from the other samples. No *P. grisea* was recorded in 6 samples namely Kocholia, Kaujakito, Kamuriai, Buluani, Nambale Township and Suo (Table 4.13). In the Kisii district samples the Majoge Chache sample had the highest recorded *P. grisea* incidence with 5.00 % and was significantly different ($P \leq 0.05$) from the other samples. However, no *P. grisea* was observed in 8 samples out of a total of 12 samples (Table 4.14).

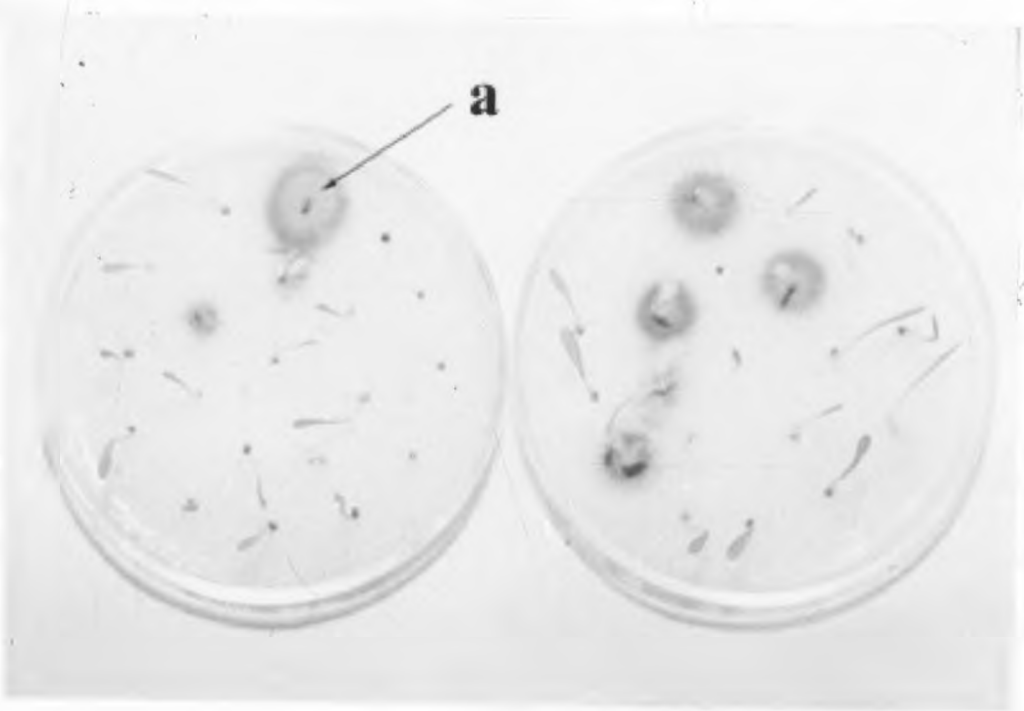


Plate 7 Growth of *P. grisea* from infected seeds, marked a (Bukhayo West sample, 5 days after plating).

In the ICRISAT samples, KNE 479 – Alupe sample had the highest recorded *P. grisea* incidence of 0.50%, and was significantly different ($P \leq 0.05$) from the other samples except KAT-FM 1 (Table 4.12).

4.7.2.3 Blotter test

Many of the infected seeds did not germinate. The roots and shoots of most seeds that did germinate were colonised by *P. grisea*. Such seedlings often died during the test period, and the fungus sporulated on the dead tissue. Many germinated seeds produced seedlings with variations, which included decayed roots and shoots, no primary roots, poorly developed shoots, spirally twisted, and stunted plumules.

The highest recorded incidence in all samples in all districts was 14.00 % in the Majoge Chacha sample from Kisii district which was significantly ($P \leq 0.05$) different from other samples except Bassi Masige. No *P. grisea* incidence was recorded in 5 samples (Table 4.10).

In the Bungoma district samples, the East Bukusu sample had the highest *P. grisea* incidence of 6.00 % and was significantly different ($P \leq 0.05$) from the other samples except N. Bukusu. The lowest *P. grisea* incidence was 0.75 % in the Kabuchai sample (Table 4.11). Among the Busia/Teso district samples, the Kotur and South Teso samples had the highest *P. grisea* incidences of 7.75 % and were significantly different ($P \leq 0.05$) from the other samples, while the lowest *P. grisea* incidence recorded was 1.00 % for the Bukhayo West sample (Table 4.13). Among the Kisii district sample, Majoge Chacha had the highest *P. grisea* incidence of 14.00 % and was significantly different ($P \leq 0.05$) from the other samples, Mwagichana sample recorded no *P. grisea* incidence (Table 4.14).

In the ICRISAT samples, KNE 479 (Alupe) sample had the highest *P. grisea* incidence of 4.25 % and was significantly different ($P \leq 0.05$) from the other samples, while for four samples had no *P. grisea* incidence (Table 4.12).

4.7.2.4 Rolled paper towel , agar plate and blotter tests

The summarised results for the various incubation tests are given in table 4.15. The blast fungus was recorded from 78 % of the finger millet samples when the rolled paper towel test was used, 88% when the blotter test was used and 39% when the agar plate test was used. The blotter test recorded the highest incidence for the samples in general and was the easiest to set up.

Percent germination varied from 88.5% for the Mwagichana sample from Kisii to 42.5% in the Bukembe sample from Bungoma district. Seeds that were positively identified to be infected by *P. grisea* were put aside and divided into two categories ungerminated and germinated (this was only for the blotter method). Among germinated seeds it was found that *P. grisea* was associated at a frequency of 0 - 50 %.

The highest recorded incidence was for the Majoge Chacha sample under the blotter test. Three samples did not record any incidence under the three methods of analysis and these were KNE 479-Kiboko, P224 and Mwagichana. The blotter method was found to be most sensitive as it revealed the most seed contamination/infection by *P. grisea* and was statistically ($P \leq 0.05$) different from the others (refer table 4.15).

4.7.2.5 Seed assay methods

The method of analysis that recorded the highest incidences in general was the blotter method, which had accummulated *P. grisea* incidence of 473 seeds in 41samples. The rolled paper towel had 268 seeds and the agar plate method had 78 seeds in the four replications under all the 41 samples. In terms of percent *P. grisea* incidences in seeds translates to 2.88 %, 1.63 % and 0.48 % for the blotter, rolled paper towel and agar plate test respectively. The blotter test was significantly different ($P \leq 0.05$) from rolled paper towel and agar plate test (Table 4.15).

The blotter test method recorded the highest incidences for the blast pathogen (*P. grisea)* on seeds and was statistically ($P \leq 0.05$) superior from the other two methods (Table 4.15). Incidence values for *P. grisea* obtained using this seed health assessment technique were used to select the samples used in the field experiment to test the relationship of seed-borne *P. grisea* with *P. grisea* infections under field conditions.

Table 4.10 Incidences (percent) of *P. grisea* in and or on finger millet seed samples as assessed using three assay tests.

Sampling area	Assay tests					
	Rolled paper towel test		Blotter test		Agar plate test	
Bungoma township	1.75	defg	2.75	defgh	0	f
Kibabii	2	def	1.50	fghijk	0.25	ef
Bukembe	1	efgh	2	fghij	0	f
East Bukusu	1.75	defg	6	bc	0	f
Syekumulo-Bumula	0.25	gh	1.25	ghijk	0	f
Musikoma	0.75	efgh	2.75	defgh	0	f
Syekumulo-S.Bukusu	0	h	2.50	defg	0.25	ef
S. Bukusu-Bumula	1.25	defgh	3	defg	0.25	ef
Kabuchai	0.25	gh	0.75	ijk	0	f
North Bukusu	0.75	efgh	4.50	cd	0.25	ef
KNE 479-Kiboko	0	h	0	k	0	f
KNE 479-Alupe	1	efgh	4.25	cde	0.5	ef
KAT FM 1	0.75	efgh	1.75	ijk	0.25	ef
U15	0.25	gh	1	hijk	0	f
P224	0	h	0	k	0	f
KNE 808	0.25	gh	0	k	0	f
GULU-E	0	h	0	k	0	f
Kocholia	2	def	2.50	efghi	0	f
Kaujakito	2	def	2.75	defgh	0	f
Kamuriai	4	bc	5.75	c	0	f
South Teso	5	b	7.75	b	0.50	ef
Kakapel	2.25	de	2.75	defgh	1	de
Kotur	4	bc	7.75	b	1	de
Buluani	1.50	defgh	1.75	fghijk	0	f
Nambale Township	0.50	fgh	1.25	ghijk	0	f
Bukhayo North	0	h	1.75	fghijk	0.50	ef
Bukhayo Central	0.50	fgh	1.50	fghijk	1	de
Bukhayo West	0	h	1	hijk	3	b
Suo	0.25	gh	2.50	efghi	0	f
Majoge Chacha	10.25	a	14	a	5	a
Sengera	2.75	cd	2.75	efghi	0	f
Bassi Masige	8.75	a	12.50	a	2.25	bc
Bassi Central	0.75	efgh	0.50	jk	0	f
Bobasi Bogetaorio	1.50	defgh	3.25	def	1.50	cd
Bassi Borabu Bogetaorio	5.25	b	5.75	c	2	c
Bassi-Regina	1.50	defgh	3	defg	0	f
Nyakeiri	0	h	1	hijk	0	f
Mwamonari	1.25	defgh	1.75	fghijk	0	f
Mwagichana	0	h	0	k	0	f
Sensi	0	h	0.25	jk	0	f
Gesangero Ngenyi	0.75	efgh	0.50	jk	0	f
Mean	1.63		2.88		0.48	
LSD (5%)	1.71		1.89		0.88	
CV %	75.0		46.92		132.0	

Table 4.11 Incidences (percent) of *P. grisea* in and or on finger millet seed samples from Bungoma district as assessed using three assay tests.

Sampling area		Assay tests		
		Rolled paper towel test	Blotter test	Agar plate test
1.	Bungoma Township	1.75 ab	2.75 bc	0.00 a
2.	Kibabii	2.00 a	1.50 cd	0.25 a
3.	Bukembe	1.00 abcd	2.00 cd	0.00 a
4.	East Bukusu	1.75 ab	6.00 a	0.00 a
5.	Syekumulo - Bumula	0.25 cd	1.25 cd	0.00 a
6.	Musikoma	0.75 bcd	2.75 bc	0.00 a
7.	Syekumulo - S. Bukusu	0.00 d	2.50 cd	0.25 a
8.	S. Bukusu - Bumula	1.25 abc	3.00 bc	0.25 a
9.	Kabuchai	1.00 cd	0.75 d	0.00 a
10.	North Bukusu	0.75 bcd	4.50 ab	0.25 a
	Mean	0.975	2.700	0.075
	LSD (5%)	1.029	1.827	0.3955
	CV (5%)	73.13	46.85	365.15

Table 4.12 Incidences (percent) of *P. grisea* in and or on finger millet seed samples from ICRISAT as assessed using three assay tests.

Sampling area		Assay tests		
		Rolled paper towel test	Blotter test	Agar plate test
1.	KNE 479- Kiboko	0.00 c	0.00 c	0.00 b
2.	KNE 479 - Alupe	1.00 a	4.25 a	0.50 a
3.	KAT FM - 1	0.75 ab	1.75 b	0.25 ab
4.	U15	0.25 bc	1.00 b	0.00 b
5.	P224	0.00 c	0.00 c	0.00 b
6.	KNE 808	0.25 bc	0.00 c	0.00 b
7.	GULU - E	0.00 c	0.00 c	0.00 b
	Mean	0.321	1.00	0.107
	LSD (5%)	0.6609	0.8786	0.4236
	CV %	139.96	59.76	269.43

Table 4.13 Incidences (percent) of *P. grisea* in and or on finger millet seed samples from Busia/Teso district as assessed using three assay tests.

Sampling area		Assay tests		
		Rolled paper towel test	Blotter test	Agar plate test
1.	Kocholia	2.00 bcd	2.50 c	0.00 c
2.	Kaujakito	2.00 bcd	2.75 c	0.00 c
3.	Kamuriai	4.00 ab	5.75 b	0.00 c
4.	South Teso	5.00 a	7.75 a	0.50 bc
5.	Kakapel	2.25 bc	2.75 c	1.00 b
6.	Kotur	4.00 ab	7.75 a	1.00 b
7.	Buluani	1.50 cd	1.75 c	0.00 c
8.	Nambale Township	0.50 cd	1.25 c	0.00 c
9.	Bukhayo North	0.00 d	1.75 c	0.50 bc
10.	Bukhayo central	0.50 cd	1.50 c	1.00 b
11.	Bukhayo West	0.00 d	1.00 c	3.00 a
12.	Suo	0.25 cd	2.50 c	0.00 c
	Mean	1.833	3.25	0.583
	LSD (5%)	2.171	1.804	0.9556
	CV %	82.57	38.72	114.29

Table 4.14 Seed samples showing the incidences (percent) of *P. grisea* associated with 12 finger millet grain samples from Kisii district using three assay tests.

Sampling area		Assay tests		
		Rolled paper towel test	Blotter test	Agar plate test
1.	Majoge Chacha	10.25 a	14.00 a	5.00 a
2.	Sengera	2.75 c	2.75 cde	0.00 c
3.	Bassi Masige	8.75 a	12.50 a	2.25 b
4.	Bassi Central	0.75 cd	0.50 def	0.00 c
5.	Bobasi Bogetaorio)	1.50 cd	3.25 bc	1.50 b
6.	Bassi Borabu Bogetaorio	5.25 b	5.75 b	2.00 b
7.	Bassi Regina	1.50 cd	3.00 cd	0.00 c
8.	Nyakeiri	0.00 d	1.00 cdef	0.00 c
9.	Mwamonari	1.25 cd	1.75 cdef	0.00 c
10.	Mwagichana	0.00 d	0.00 f	0.00 c
11.	Sensi	0.00 d	0.25 ef	0.00 c
12.	Gesangero Ngenyi	1.00 cd	0.50 def	0.00 c
	Mean	2.75	3.771	0.896
	LSD (5%)	2.144	2.538	1.259
	CV %	54.38	46.93	98.01

Table 4.15 Incidences of *P. grisea* in and or on seeds under the various assay tests.

No.	Assay test	Infected seed Incidence[x] in 16,400 seeds	% Incidence of infected seeds in 16,400 seeds	Mean no. of infected seeds/ replicate
1.	Rolled paper towel test	268	1.63	67.00 b
2.	Blotter test	473	2.88	118.25 a
3.	Agar plate test	78	0.48	19.50 c
	Mean			68.25
	LSD (5 %)			11.51
	CV %			10.54

x - *P. grisea* incidence for the 41 samples X 400 seeds = 16,400 seeds

4.8 Grow-on test in the glasshouse

The symptoms included an elliptical lesion on the first leaf i.e. cotyledon leaf (Plate 8). The highest incidence in this test was 3.5 % for the Majoge Chacha sample, while 18 samples recorded no *P. grisea* (Table 4.16). The percent germination varied from 82.75 % for the KNE 479 - Kiboko sample to 36.25 % for the Kiobegi Masige sample.

Among Bungoma district samples, the S. Bukusu-Bumula sample had the highest *P. grisea* incidence of 1.50% and this was significantly ($P \leq 0.05$) different from the other samples. No *P. grisea* incidence was recorded in five samples (Table 4.17). Among the Busia/Teso district samples, the S. Teso and Kotur samples had the highest *P. grisea* incidence of 2.25 % and were significantly different ($P \leq 0.05$) from the other samples. No *P. grisea* was recorded in four samples (Table 4.19). Among the Kisii district samples, the Majoge Chacha sample had the

highest *P. grisea* incidence of 3.5 % which was significantly higher ($P \leq 0.05$) from the other samples. No *P. grisea* was recorded in five samples (Table 4.20).

Among the ICRISAT samples, the KNE-Alupe and U15 samples had the highest *P. grisea* incidence of 1% and were significantly different ($P \leq 0.05$) from the other samples. No *P. grisea* incidence was recorded in four samples (Table 4.18).

The correlation (r =-0.102), between percent *P. grisea* incidence observed in the glasshouse experiment and percent germination was not significant at $P \leq 0.05$). Contrasting, the correlation between percent *P. grisea* incidence observed in the glasshouse experiment and percent incidence observed in the rolled paper towel (0.788), blotter (0.807) and agar tests (0.621) were all significant at $P \leq 0.05$.

Plate 8 Symptoms of blast (marked a) on finger millet seedlings in the grow-on test in the glasshouse at Kabete field station.

Table 4.16 Incidence of *P. grisea* of finger millet samples as detected by grow-on test and subsequent seed germination.

Sampling area	Grow-on test P. grisea Incidence[a]	% germination in glass house exp.	% germination as per blotter test
Majoge Chacha	3.5 a	42	44.75
South Teso	2.25 b	73	67
Kotur	2.25 b	75.5	71
Bassi Masige	2.25 b	36.25	56.5
S.Bukusu-Bumula	1.50 bc	68.75	50.5
Kaujakito	1.50 bc	58.5	70.25
Kocholia	1.25 cd	48	60.5
KNE-Alupe	1 cde	79.5	79.5
U15	1 cde	57.5	91.75
Buluani	0.75 cdef	45.75	75.5
Bungoma Township	0.50 def	69.5	77
Bukembe	0.50 def	52.5	42.5
East Bukusu	0.50 def	57	69.25
North Bukusu	0.50 def	63.25	72
Bukhayo North	0.50 def	71.5	68
Bukhayo West	0.50 def	51.5	65
Sengera	0.50 def	47.5	60.5
Bassi Central	0.50 def	57.75	78
Bobasi Bogetaorio	0.50 def	59.75	82
Bassi Borabu Bogetaorio	0.50 def	48.25	54
Gesangero Ngenyi	0.50 def	60.75	70.5
GULU-E	0.25 ef	48.5	88.25
Bukhayo Central	0.25 ef	49	71.5
Kibabii	0 f	47.75	60.5
Syekumulo-Bumula	0 f	59.25	82
Musikoma	0 f	62.25	58.25
Syekumulo-S.Bukusu	0 f	73.5	73
Kabuchai	0 f	45.25	58
KNE 479-Kiboko	0 f	82.75	82.25
KAT FM 1	0 f	49	92.25
P224	0 f	82	95
KNE 808	0 f	82	96
Kamuriai	0 f	51.5	71.5
Kakapel	0 f	59.25	62
Nambale Township	0 f	46.75	60
Suo	0 f	55.5	63
Bassi Regina	0 f	48.5	76.5
Nyakeiri	0 f	54.25	85.5
Mwamonari	0 f	55.25	86.5
Mwagichana	0 f	73.75	88.5
Sensi	0 f	56.5	83.5
Mean	0.567		
LSD (5%)	0.9717		
CV %	122.39		

a - Incidence for 400 seeds

Table 4.17 Finger millet seed samples obtained from Bungoma district incidences (percent) of *P. grisea* in grow-on test.

	Sampling area	Grow-on test P.grisea Incidence (%)	% germination in glass house exp.	% germination as per blotter test
1.	Bungoma Township	0.5 b	69.5	77
2.	Kibabii	0 c	47.75	60.5
3.	Bukembe	0.5 b	52.5	42.5
4.	East Bukusu	0.5 b	57	69.25
5.	Syekumulo - Bumula	0 c	59.25	82
6.	Musikoma	0 c	62.25	58.25
7.	Syekumulo - S. Bukusu	0 c	73.5	73
8.	S. Bukusu - Bumula	1.50 a	68.75	50.5
9.	Kabuchai	0 c	45.25	58
10.	North Bukusu	0.5 b	63.25	72
	Mean	0.10		
	LSD (5%)	0.22		
	CV (5%)	149.85 %		

Table 4.18 Finger millet seed samples obtained from ICRISAT showing incidences (percent) of *P. grisea* in grow-on test.

	Sampling area	Grow-on test P. grisea Incidence (%)	% germination in glass house exp.	% germination as per blotter test
1.	KNE 479- Kiboko	0 b	82.75	82.25
2.	KNE 479 - Alupe	1 a	79.5	79.5
3.	KAT FM - 1	0 b	49	92.25
4.	U15	1 a	57.5	91.75
5.	P224	0 b	82	95
6.	KNE 808	0 b	82	96
7.	GULU - E	0.25 b	48.5	88.25
	Mean	0.08		
	LSD (5%)	0.32		
	CV %	266.56		

Table 4.19 Finger millet seed samples obtained from Busia/Teso district showing incidences (percent) of *P. grisea* in grow-on test.

	Sampling area	Grow-on test P. grisea Incidence (%)	% germination in glasshouse exp.	% germination as per blotter test
1.	Kocholia	1.25 b	48	60.5
2.	Kaujakito	1.50 b	58.5	70.25
3.	Kamuriai	0 e	51.5	71.5
4.	South Teso	2.25 a	73	67
5.	Kakapel	0 e	59.25	62
6.	Kotur	2.25 a	75.5	71
7.	Buluani	0.75 c	45.75	75.5
8.	Nambale Township	0 e	46.75	60
9.	Bukhayo North	0.50 cd	71.5	68
10.	Bukhayo central	0.25 de	49	71.5
11.	Bukhayo West	0.50 cd	51.5	65
12.	Suo	0 e	55.5	63
	Mean	0.19		
	LSD (5%)	0.28		
	CV %	101.47 %		

Table 4.20 Finger millet seed samples obtained from Kisii district showing incidences (percent) of *P. grisea* in grow-on test.

	Sampling area	Grow-on test P. grisea Incidence (%)	% germination in glass house exp.	% germination as per blotter test
1.	Majoge Chache	3.5 a	42	44.75
2.	Sengera	0.50 c	47.5	60.5
3.	Bassi Masige	2.25 b	36.25	56.5
4.	Bassi Central	0.50 c	57.75	78
5.	Bobasi Bogetaorio)	0.50 c	59.75	82
6.	Bassi Borabu Bogetaorio	0.50 c	48.25	54
7.	Bassi Regina	0 d	48.5	76.5
8.	Nyakeiri	0 d	54.25	85.5
9.	Mwamonari	0 d	55.25	86.5
10.	Mwagichana	0 d	73.75	88.5
11.	Sensi	0 d	56.5	83.5
12.	Gesangero Ngenyi	0.50 c	60.75	70.5
	Mean	0.17		
	LSD (5%)	0.21		
	CV %	85.94 %		

4.9 Field Experiments

In this section the results shown are for three seasons viz Kabete season I, long rains (for 141 days after emergence for inoculation experiment); Kabete season II, short rains (149 days after emergence for inoculation experiment, while 154 days after emergence for transmission experiment) and Kiboko season I, long rains (102 days after emergence for inoculation experiment). The different dates for harvesting of the two experiments is due to the fact that the transmission experiment had later maturing varieties (Land races). Finger millet took 8 to 14 days to germinate.

4.9.1 The effect of different levels of seedborne *Pyricularia grisea* incidence / infection on incidence of subsequent crop under field conditions

The seeds were collected from a varied background and across many districts resulting in the mixture of varieties and hence no yield data was considered except percent incidence (as related to plants with leaf blast) and percent severity leaf blast was recorded. Due to the observation under blotter test that some infected seeds do not germinate, transmission from seed to seedling was not observed for Kiboko season I and Kabete season I. There was no observed panicle blast or neck blast.

The data collected was in Kabete season II and the following data was collected: Blast incidence in relation to leaf blast on tillers and leaf blast severity.

Data collection for the transmisson experiment in Kabete season II, was done on the following days after emergence (DAE) – 14, 28, 42, 56, 70, 84, 98, 112, 126, 140 and 154 DAE.

4.9.1.1 Blast incidence

In general leaf blast incidence on tillers varied from 0.261 to 0.377 i.e. 26.1 % to 37.7

%. (Table 4.21). L_4 (7.75 % *P. grisea* incidence) was found to be statistically ($P \leq 0.05$) different from other levels of inoculum (Table 4.21).

Table 4.21: Means of incidence on tillers of leaf blast on affected tillers for different treatments (different levels of inoculum), Kabete season II [Experiment of planting farmer's finger millet seeds with different levels of *P. grisea* inoculum].

Treatment (% *P. grisea* incidence)	Days after emergence (DAE)											Transformed Final incidence (day 154)
	14	28	42	56	70	84	98	112	126	140	154	
L_c (0%)	0	0	0	0	0.01	0.052	0.103	0.15	0.181	0.223	0.261	1.123 e
L_1(2.5%)	0	0	0	0	0.02	0.093	0.181	0.222	0.291	0.346	0.367	1.169 ab
L_2(4.25%)	0	0	0	0	0.028	0.123	0.201	0.247	0.286	0.331	0.363	1.167 b
L_3(6%)	0	0	0	0	0	0.102	0.170	0.222	0.262	0.318	0.347	1.160 c
L_4(7.75%)	0	0	0	0	0.027	0.066	0.178	0.224	0.284	0.336	0.377	1.173 a
L_5(14 %)	0	0	0	0	0	0.072	0.141	0.184	0.226	0.277	0.322	1.149 d
											LSD (5 %)	0.00477

Note : The incidence values can reach a maximum of 1.00

4.9.1.2 Blast severity on leaves

The amount of leaf blast recorded under transmission experiment was generally less than 2 % (Table 4.22). There was no development of leaf blast into panicle or neck blast. The tiny elliptical lesions appeared on leaves and never grew into full-blown blast.

L_5, L_4 and L_2 were statistically different ($P \leq 0.05$) from the other treatments (Table 4.22).

Table 4.22 : Means of percent leaf blast for different treatments (different levels of inoculum), Kabete season II [Experiment of planting farmer's finger millet seeds with different levels of *P. grisea* inoculum].

Treatment	Days after emergence (DAE)											AUDPC
	14	28	42	56	70	84	98	112	126	140	154	
L_c	0	0	0	0.002	0.005	0.014	0.03	0.25	0.445	0.7	0.95	13.45 c
L_1	0	0.0008	0.002	0.006	0.02	0.06	0.13	0.30	0.55	0.8	1.05	16.68 bc
L_2	0	0.001	0.004	0.018	0.042	0.21	0.42	0.59	0.88	1.12	1.37	27.63 a
L_3	0	0	0.003	0.009	0.043	0.11	0.27	0.46	0.68	0.91	1.19	21.45 b
L_4	0	0	0.002	0.013	0.048	0.15	0.38	0.65	0.89	1.14	1.53	28.15 a
L_5	0	0	0.002	0.007	0.055	0.19	0.43	0.67	0.91	1.20	1.59	29.75 a
											LSD (5 %)	5.791

4.9.2 The effect of time of inoculation on the finger millet seed infection/contamination by *P. grisea* of harvested seed thereafter

Kiboko season I & Kabete season II

There was on average a yield reduction of between 1.98 % - 11.14 % due to blast in these sites described (Table 4.37).

Kabete season I

Due to the cold spell that prevailed from June to August 1996, there was very poor seed set for the crop that was in the field and hence no data yield could be obtained, however since the crop was inoculated with *P. grisea*, disease progress was monitored hence the data below.

Inoculation dates

The days are recorded for days after emergence (DAE)

Site/period	I_1	I_2	I_3	I_4	I_5
Kiboko I	32	39	53	67	81
Kabete I	57	78	92	113	120
Kabete II	51	65	79	93	107

Data collection

Kiboko season I, data was collected on the following days after emergence (DAE) 39, 46, 53, 60, 67, 74, 81, 88, 95 and 102. For Kabete season I, data was collected on the following DAE 78, 85, 92, 99, 106, 113, 120, 127, 134 and 141. While for Kabete season II, data was collected on the following DAE 58, 65, 72, 79, 86, 93, 100, 107, 114, 121, 128, 135, 142 and 149.

4.9.2.1 Blast incidence

In Kiboko long rains, stage one of inoculation was found to be with the highest transformed incidence of 1.23 and was statistically ($p \leq 0.05$) different from other stages, in the order of stages 3, 2, 4 and 5 (Table 4.23). In Kabete season I, long rains, stage one and two were statistically ($p \leq 0.05$) different from other stages of growth (Table 4.24). In Kabete season II, short rains, stage three of inoculation had with the highest transformed incidence of 1.214, which was statistically ($p \leq 0.05$) higher than those other stages in the order of stages 1, 2, 5 and 4 (Table 4.25).

Generally, inoculation at stage 1, had the highest transformed incidence of 1.128 in all sites and seasons, and was statistically different ($P \leq 0.05$) from other stages in the order 2, 3, 4 and 5 (Table 4.35). In general the incidences observed in Kiboko I were highest while those

under Kabete II were the lowest. The relationship between stage of inoculation and AUDPC for incidence was found to be linear. The linear function was statistically significant ($P \leq 0.05$) over the quadratic and cubic function (Appendix 3).

Table 4.23 Means of blast incidence for Kiboko season I after inoculation with *P. grisea* at different stages of growth of finger millet.

Treat-ment	(Days after emergence) DAE										Transformed Final incidence
	39	46	53	60	67	74	81	88	95	102	
I_1	0.095	0.143	0.188	0.243	0.280	0.320	0.408	0.445	0.485	0.515	1.23 a
I_2	0	0	0.008	0.020	0.029	0.040	0.075	0.118	0.165	0.198	1.094 b
I_3	0	0	0	0.021	0.044	0.063	0.121	0.170	0.208	0.253	1.119 b
I_4	0	0	0	0	0	0.045	0.075	0.105	0.120	0.135	1.063 bc
I_5	0	0	0	0	0	0	0	0.008	0.023	0.043	1.021 c
										LSD (5 %)	0.0674

Table 4.24 Means of blast incidence for Kabete season I after inoculation with *P. grisea* at different stages of growth of finger millet.

Trea t-ment	(Days after emergence) DAE												Transformed Final incidence
	64	71	78	85	92	99	106	113	120	127	134	141	
I_1	0	0	0.150	0.173	0.195	0.203	0.213	0.213	0.233	0.245	0.250	0.253	1.118 ab
I_2	0	0	0	0.138	0.168	0.213	0.235	0.223	0.230	0.235	0.245	0.253	1.119 ab
I_3	0	0	0	0	0	0	0.038	0.048	0.065	0.070	0.083	0.119	1.057 bc
I_4	0	0	0	0	0	0	0	0	0.121	0.155	0.205	0.236	1.111 ab
I_5	0	0	0	0	0	0	0	0	0	0.115	0.273	0.313	1.144 a
												LSD (5 %)	0.0674

Table 4.25 Means of blast incidence on tillers for Kabete season II after inoculation with *P. grisea* at different stages of growth of finger millet.

Treat-ment	(Days after emergence) DAE														Transformed Final incidence
	58	65	72	79	86	93	100	107	114	121	128	135	142	149	
I_1	0	0	0	0	0	0	0	0	0.013	0.026	0.04	0.043	0.05	0.075	1.034 b
I_2	0	0	0	0	0	0	0	0.011	0.018	0.021	0.028	0.036	0.048	0.073	1.033 b
I_3	0	0	0	0	0	0	0	0	0.088	0.133	0.153	0.165	0.185	0.245	1.214 b
I_4	0	0	0	0	0	0	0	0	0	0	0.002	0.002	0.003	0.003	1.001 b
I_5	0	0	0	0	0	0	0	0	0	0	0	0.002	0.004	0.008	1.004 b
														LSD (5 %)	0.09532

4.9.2.2 Blast severity

4.9.2.2.1 Blast severity on panicles

Panicle blast was observed to reduce the panicle capacity to yield grains. In some instances there was no grain in certain heads. Normally blast would start at the tips of the fingers of the panicle and would move upto the centre of the panicle. If the conditions were ideal (warm and humid), the panicle blast would quickly cover the entire head within even one week.

There were very few instance that neck blast was present if there was no panicle blast. The panicle blast covered the whole panicle then proceeded down the head. The panicle dried up as the disease covered the entire panicle, leaving a black colouration on the mid rib of the panicle. In the presence of moisture, the panicle was covered with a greyish woolly blanket of mycelial growth and sporulation of *P. grisea*. Panicle and neck blast would form 5 – 13 days after inoculation depending on weather conditions.

A percent panicle blast severity scoring scale was developed (plate 9) to help in evaluating blast severity on finger millet panicles. The severity on panicles observed was plotted over dates indicated and the AUDPC calculated. At Kiboko during long rains, stage one of inoculation was found to have the highest AUDPC of 9.56 and statistically ($P \leq 0.05$) different from the other stages in the order of stage 3, 2, 4 and 5 (Table 4.26). For Kabete season I during long rains, stage two of inoculation was found to be with the highest AUDPC of 13.81 and was statistically ($p \leq 0.05$) different from other stages, except stage one (Table 4.27). For Kabete season II during short rains, stage three was found to be statistically ($p \leq 0.05$) different from the other stages in the order of stages 2, 1, 4 and 5 (Table 4.28).

In general, considering all sites and seasons the highest AUDPC for blast severity on panicle was in stage one with a figure of 8.795 followed by stages 3 and 2 which were all statisticaly different from stages 4 and 5 (Table 4.35). In general, Kabete I had the highest severity values, while Kabete II had the lowest. The relationship between stage of inoculation

and AUDPC for blast severity on panicles was found to be linear. The linear function was statistically significant ($P \leq 0.05$) over the quadratic and cubic function (Appendix 2).

Table 4.26 Means of blast severity on panicles for Kiboko season I after inoculation with *P. grisea* at different stages of growth of finger millet.

Treat-ment	(Days after emergence) DAE										
	39	46	53	60	67	74	81	88	95	102	AUDPC
I_1	0.008	0.022	0.06	0.108	0.133	0.160	0.210	0.238	0.275	0.315	9.563 a
I_2	0	0	0.007	0.011	0.018	0.032	0.051	0.080	0.115	0.153	2.735 bc
I_3	0	0	0	0.046	0.058	0.100	0.142	0.163	0.185	0.230	5.659 b
I_4	0	0	0	0	0	0.018	0.040	0.068	0.100	0.143	2.074 bc
I_5	0	0	0	0	0	0	0	0.007	0.010	0.013	0.161 c
										LSD (5 %)	3.762

Table 4.27 Means of blast severity on panicles for Kabete season I after inoculation with *P. grisea* at different stages of growth of finger millet.

Treat-ment	(Days after emergence) DAE												
	64	71	78	85	92	99	106	113	120	127	134	141	AUDPC
I_1	0	0	0.15	0.173	0.195	0.203	0.213	0.213	0.233	0.245	0.250	0.253	13.466 a
I_2	0	0	0	0.138	0.168	0.213	0.235	0.223	0.230	0.235	0.245	0.253	12.679 a
I_3	0	0	0	0	0	0	0.038	0.048	0.065	0.070	0.083	0.119	2.537 b
I_4	0	0	0	0	0	0	0	0	0.121	0.155	0.205	0.236	4.196 b
I_5	0	0	0	0	0	0	0	0	0	0.115	0.273	0.313	3.806 b
												LSD (5 %)	4.449

Table 4.28 Means of blast severity on panicles for Kabete season II after inoculation with *P. grisea* at different stages of growth of finger millet.

Treat-ment	(Days after emergence) DAE														
	58	65	72	79	86	93	100	107	114	121	128	135	142	149	AUDPC
I_1	0	0	0	0	0	0	0.005	0.015	0.025	0.054	0.077	0.103	0.135	0.155	3.435 b
I_2	0	0	0	0	0.005	0.015	0.02	0.03	0.045	0.078	0.09	0.12	0.198	0.225	4.988 b
I_3	0	0	0	0	0	0.025	0.058	0.093	0.145	0.215	0.315	0.405	0.525	0.59	14.525 a
I_4	0	0	0	0	0	0	0	0	0	0	0	0	0.013	0.02	0.157 b
I_5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 b
														LSD (5 %)	5.875

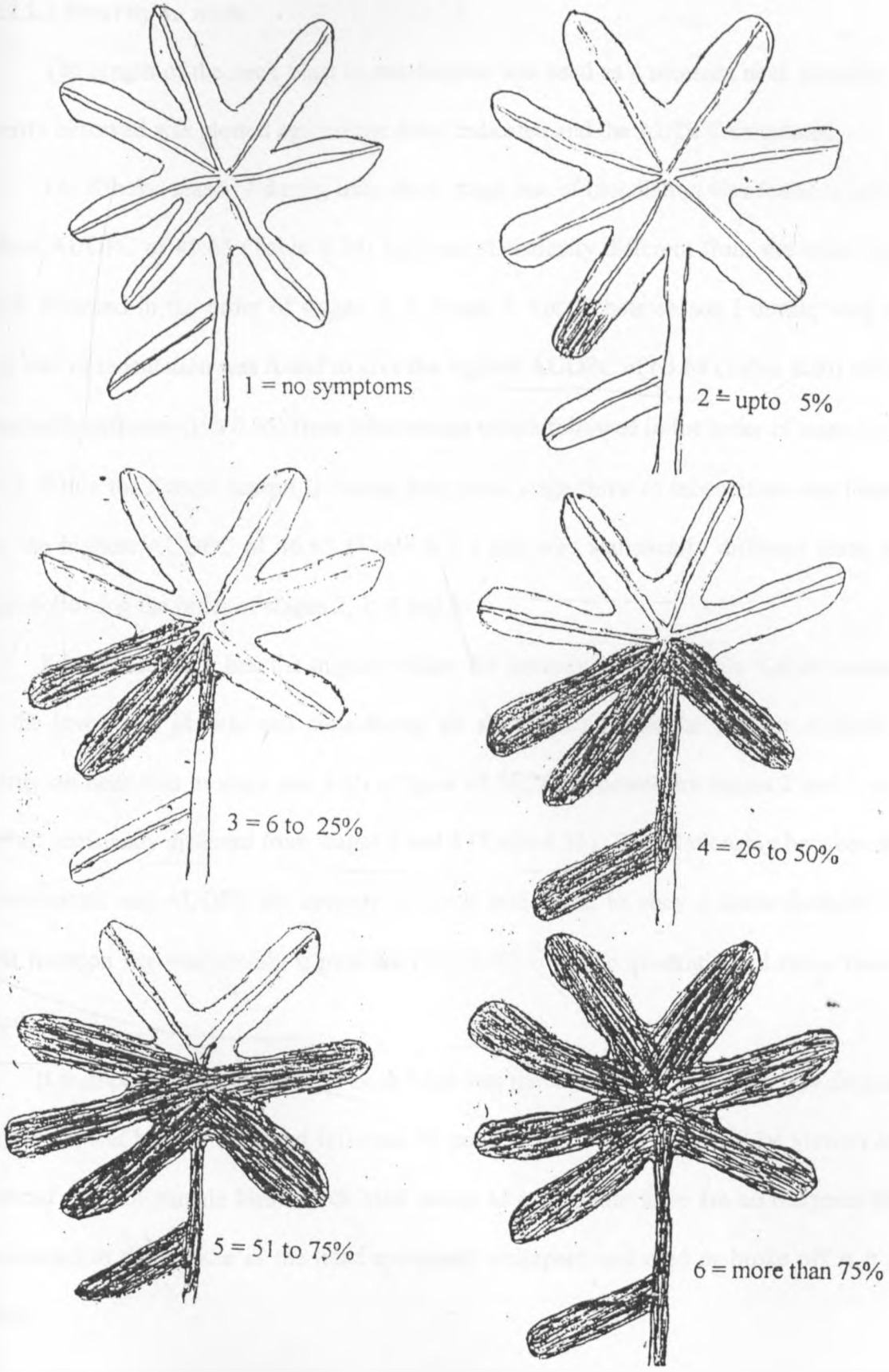


Plate 9: A sketch diagram showing percent blast severity scale used in evaluating finger millet for blast severity on panicles.

4.9.2.2.2 Severity on neck

The length of the neck blast in centimetres was used as a measure neck severity. The severity observed was plotted against the dates indicated and the AUDPC calculated.

For Kiboko season I during long rains, stage one of inoculation was found to give the highest AUDPC of 49.65 (Table 4.29) and was statistically different from the other stages, which followed in the order of stages 3, 2, 4 and 5. For Kabete season I during long rains stage two of inoculation was found to give the highest AUDPC of 63.68 (Table 4.30) and was statistically different ($P \leq 0.05$) from other stages which followed in the order of stages 1, 3, 4 and 5. While for Kabete season II during long rains stage three of inoculation was found to give the highest AUDPC of 46.63 (Table 4.31) and was statistically different from other stages following the order of stages 2, 1, 4 and 5.

Kiboko season I, had the highest values for severity on neck while Kabete season II had the lowest. In general and considering all sites and seasons the highest AUDPC for severity on neck was in stage one with a figure of 36.266 followed by stages 2 and 3 which were all statisticaly different from stages 4 and 5 (Table 4.35). The relationship between stage of inoculation and AUDPC for severity on neck was found to obey a linear function. The linear function was statistically significant ($P \leq 0.05$) over the quadratic and cubic function (Appendix 3).

It is important to note that the neck blast was the last to appear, since a few days after inoculation, leaf blast is observed followed by panicle blast. Neck blast can be viewed as an advanced stage of panicle blast. Neck blast seems to ensure that there are no nutrients being translocated to the panicle as the head eventually collapsed and died or broke off if it was mature.

Table 4.29 Means of blast severity on neck for Kiboko season I after inoculation with *P. grisea* at different stages of growth of finger millet.

Treat-ment	(Days after emergence) DAE										
	39	46	53	60	67	74	81	88	95	102	AUDPC
I_1	0	0.018	0.048	0.093	0.218	0.608	1.165	1.578	1.983	2.77	49.648 a
I_2	0	0	0	0	0	0.23	0.36	0.563	0.813	1.193	17.929 b
I_3	0	0	0	0.04	0.088	0.168	0.453	0.675	0.920	1.165	20.475 b
I_4	0	0	0	0	0	0.095	0.213	0.365	0.530	0.605	10.535 b
I_5	0	0	0	0	0	0	0	0.123	0.388	0.673	5.924 b
										LSD (5 %)	3.762

Table 4.30 Means for blast severity on neck for Kabete season I after inoculation with *P. grisea* at different stages of growth of finger millet.

Treat-ment	(Days after emergence) DAE												
	64	71	78	85	92	99	106	113	120	127	134	141	AUDPC
I_1	0	0	0	0	0.085	0.4	0.653	0.883	1.035	1.215	1.343	1.343	43.986 b
I_2	0	0	0	0	0.073	0.485	0.98	1.478	1.633	1.728	1.815	1.815	63.683 a
I_3	0	0	0	0	0	0	0.195	0.383	0.575	0.655	0.7	0.7	20.003 c
I_4	0	0	0	0	0	0	0	0.285	0.56	0.645	0.743	0.878	18.699 c
I_5	0	0	0	0	0	0	0	0	0	0.038	0.13	0.153	1.706 d
												LSD (5 %)	15.70

Table 4.31 Means of blast severity on neck for Kabete season II after inoculation with *P. grisea* at different stages of growth of finger millet.

Trea t-ment	(Days after emergence) DAE														
	58	65	72	79	86	93	100	107	114	121	128	135	142	149	AUDP C
I_1	0	0	0	0	0	0	0	0	0	0.135	0.335	0.468	0.738	0.983	15.164 b
I_2	0	0	0	0	0	0	0	0.03	0.038	0.095	0.218	0.52	0.883	1.213	16.721 a
I_3	0	0	0	0	0	0	0	0	0	0.243	0.815	1.433	2.558	3.232	46.646 b
I_4	0	0	0	0	0	0	0	0	0	0	0	0	0.063	0.09	0.752 b
I_5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 b
														LSD (5 %)	19.65

4.9.2.2.3 Severity on leaves

A percent leaf blast severity scoring scale was developed (plate 10) to help in evaluating blast severity on finger millet leaves. The severity on leaves observed was plotted against dates indicated and the AUDPC calculated. For Kiboko season I during long rains, stage one of inoculation was found to give the highest AUDPC of 136.911 and was statistically ($P \leq 0.05$) different from other stages in the order of stages 3, 2, 4 and 5 (Table 4.32). For Kabete season I during long rains, stage one of inoculation was found to give the highest AUDPC of 61.579 and was statistically ($P \leq 0.05$) different from other stages, that followed the order of stages 2,3,4 and 5 (Table 4.33). For Kabete season II during short rains, stage one of inoculation was found to give the highest AUDPC of 21.88 and was statistically ($P \leq 0.05$) different from other stages except stage two in the order of stages 3, 4 and 5 (Table 4.34).

Kabete season II, had the highest blast severity on leaves while Kabete season I had the lowest. From this it is clear that foliar infection is more evident before flowering and heading, while older plants had more head and neck blast.

When the season and site were considered together, stage one of inoculation with an AUDPC of 92.481 was found to be statistically ($P \leq 0.05$) different from the other stages of inoculation in the order of stages 2, 3, 4 and 5 (Table 4.35). The relationship between stage of inoculation and AUDPC for severity on leaves was found to obey a linear function. The linear function was statistically different ($P \leq 0.05$) from the quadratic and cubic function (Appendix 3). After inoculation the chlorotic speck would appear 3 –5 days later, while the typical elliptical lesions after 5 – 8 days. When the leaf sheath was infected the leaf dried off very fast.

Table 4.32 Means of blast severity on leaves for Kiboko season I after inoculation with *P. grisea* at different stages of growth of finger millet.

Treat - ment	(Days after emergence) DAE										
	39	46	53	60	67	74	81	88	95	102	AUDPC
I_1	0.078	0.165	0.303	0.585	0.953	1.345	2.325	4.26	5.983	7.205	136.911 a
I_2	0	0.015	0.027	0.052	0.039	0.263	0.458	0.665	0.89	1.285	21.348 b
I_3	0	0	0	0.044	0.145	0.27	0.395	0.63	1.005	1.27	21.868 b
I_4	0	0	0	0	0	0.002	0.038	0.055	0.115	0.195	2.147 b
I_5	0	0	0	0	0	0	0	0.002	0.004	0.008	0.068 b
										LSD (5 %)	39.30

Table 4.33 Means of blast severity on leaves for Kabete season I after inoculation with *P. grisea* at different stages of growth of finger millet.

Treat - ment	(Days after emergence) DAE												
	64	71	78	85	92	99	106	113	120	127	134	141	AUDPC
I_1	0.075	0.15	0.21	0.34	0.415	0.653	0.825	0.985	1.278	1.445	1.64	1.64	61.579 a
I_2	0	0	0	0.145	0.188	0.215	0.293	0.428	0.533	0.705	0.863	1.175	27.685 b
I_3	0	0	0	0	0	0.045	0.045	0.048	0.05	0.06	0.08	0.093	2.616 c
I_4	0	0	0	0	0	0	0	0	0	0	0	0	0 c
I_5	0	0	0	0	0	0	0	0	0	0	0	0	0 c
												LSD (5 %)	18.18

Table 4.34 Means of blast severity on leaves for Kabete season II after inoculation with *P. grisea* at different stages of growth of finger millet.

Treat -ment	(Days after emergence) DAE														
	58	65	72	79	86	93	100	107	114	121	128	135	142	149	AUDPC
I_1	0.04	0.055	0.08	0.145	0.25	0.45	0.63	0.855	1.035	1.28	1.625	1.903	1.968	1.968	78.951 a
I_2	0	0	0.073	0.083	0.163	0.369	0.493	0.660	0.861	1.04	1.263	1.485	1.49	1.50	61.063 ab
I_3	0	0	0	0	0.088	0.113	0.143	0.215	0.435	0.67	0.925	1.095	1.38	1.61	41.073 bc
I_4	0	0	0	0	0	0	0.053	0.083	0.128	0.285	0.62	0.98	1.22	1.52	28.892 c
I_5	0	0	0	0	0	0	0	0	0.12	0.165	0.355	0.67	0.925	1.22	19.915 c
														LSD (5 %)	25.11

Table 4.35 Summary of all disease measuring indexes for all seasons and sites

Stage of inoculation	AUDPC			
	Incidence on tillers	Leaf blast	Neck blast	Panicle blast
1	1.128 a	92.481 a	36.266 a	8.795 a
2	1.082 bc	36.699 b	32.778 a	7.177 a
3	1.097 ab	21.852 bc	29.041 a	8.004 a
4	1.058 c	10.347 c	9.995 b	2.593 b
5	1.056 c	6.661 c	2.543 b	0.565 b
LSD (5 %)	0.0367	16.00	13.69	3.113

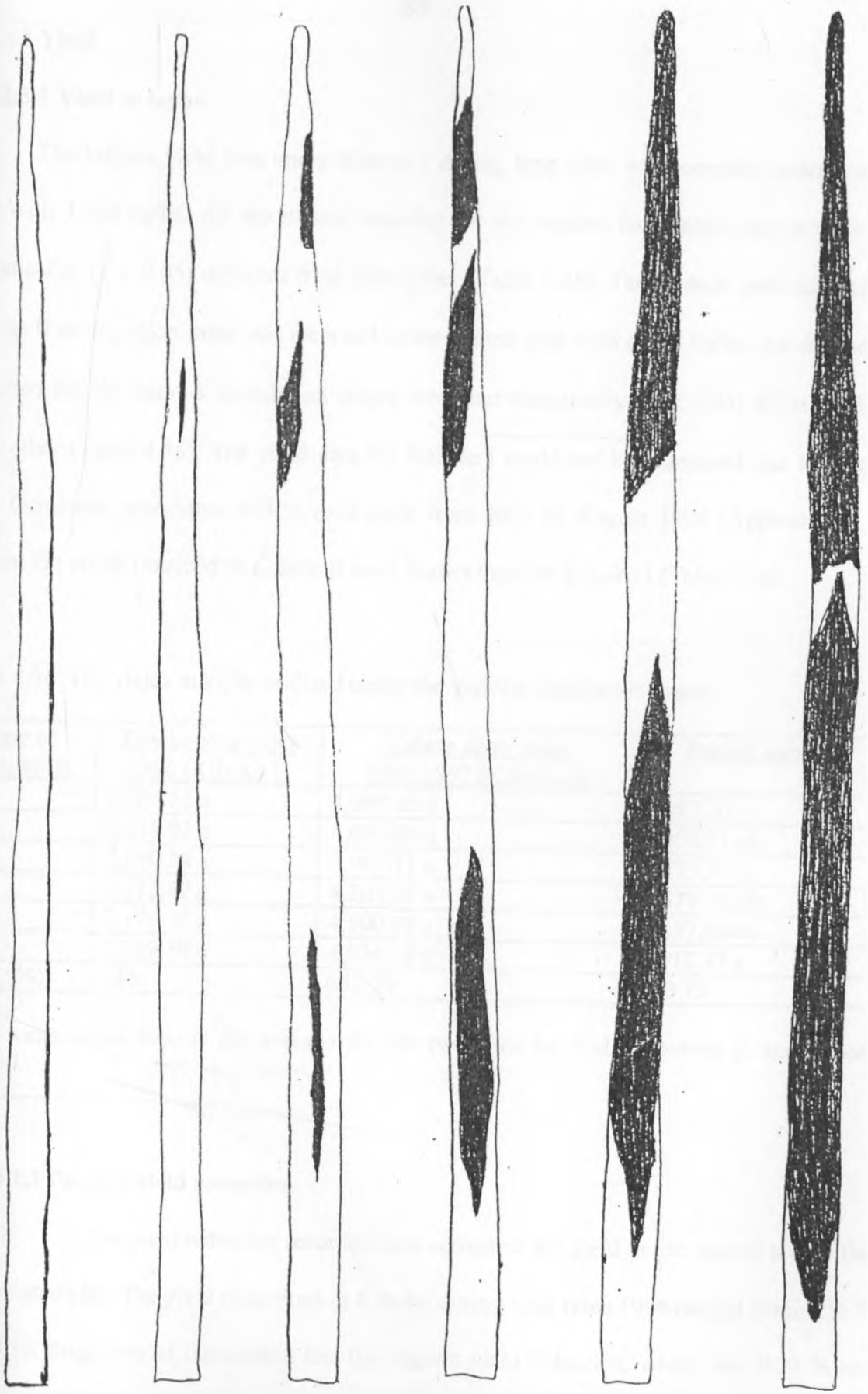


Plate 10: Developed percent severity scale used in evaluating finger millet for blast severity on leaves

4.9.2.3 Yield

4.9.2.3.1 Yield in kg/ha

The highest yield data under Kiboko I during long rains was recorded under control plot with 3,269 kg/ha. All the means recorded for the various inoculation stages were not statistically ($P \geq 0.05$) different from each other (Table 4.36). The highest yield data under Kabete II during short rains was recorded in the control plot with 4,556 kg/ha. All the means recorded for the various inoculation stages were not statistically ($P \geq 0.05$) different from each other (Table 4.36). The yield data for Kabete I could not be computed due to lack of seed formation, associated with a cold spell from June to August 1996 (Appendix 2). In general the yields obtained in Kabete II were higher than for Kiboko I (Table 4.36).

Table 4.36 The yields in kg/ha realised under the various inoculation stages

Time of inoculation	Kiboko long rains 1996 (Kiboko I)	Kabete short rains 1996/1997 (Kabete II)	Pooled means[a]
I_1	2,964.55 a	4,009.85 a	3,487.20 b
I_2	3,218.93 a	4,090.49 a	3,654.71 ab
I_3	2,990.38 a	3,961.11 a	3,475.75 b
I_4	3,155.17 a	4,203.03 a	3,679.10 ab
I_5	3,175.12 a	4,500.08 a	3,837.60 ab
I_c	3,269.38 a	4,556.13 a	3,912.87 a
LSD (5%)	385	657.19	363.75

[a] The mean stated here is the average for the two sites i.e. Kabete season II and Kiboko season I.

4.9.2.3.1.1 Percent yield reduction

The yield reduction recorded here compares the yield in the control plot to that in inoculated plot. The yield reductions at Kiboko during long rains 1996 ranged from 1.55 % to 9.33 %. Stage one of inoculation had the highest yield reduction, which was 9.33 % and was statistically ($P \leq 0.05$) different from the other yield reductions except that of stage three

of growth (Table 4.37). The yield reductions for Kabete during short rains 1996/97 ranged from 1.23 % to 13.06 %. Stage three of inoculation had the highest yield reduction which was 13.06 % and statistically ($P \leq 0.05$) different from the others stages of growth except stages one, two and four (Table 4.37). The highest yield reductions were observed for the stage three inoculations closely followed by stage one while the lowest yield reductions were observed for the stage five inoculations (Table 4.37). There was no data for yield reduction for Kabete long rains 1996 as there was no seed formation due to cold spell in June to August 1996 (Appendix 2).

Table 4.37 The % yield losses under various inoculations compared to the control plot.

	Kiboko	Kabete	Pooled means
I_1	9.33 a	11.99 a	10.88^{2}
I_2	1.55 b	10.22 a	6.93^{3}
I_3	8.54 a	13.06 a	11.14^{1}
I_4	3.50 b	7.75 ab	6.11^{4}
I_5	2.89 b	1.23 b	1.98^{5}
Lsd (5 %)	6.67	9.77	

The superscript 1,2,3,4 & 5 are the various rankings as per the disease parameter, with the number 1 being the most affected by disease.

4.9.2.3.2 Yield: 100 threshed panicles weight

At Kiboko during long rains, the 100 threshed panicles weight for the control plot was statistically ($P \leq 0.05$) different from the others (Table 4.38). The highest reduction in weight was under stage one of 15.63 %, which was statistically ($P \leq 0.05$) different from the other stages of growth (Table 4.38). While at Kabete duringshort rains there were no statistical ($P \geq 0.05$) differences between the various stages of inoculation. Stage one of growth had the highest yield reduction of 5.93 % which was statistically ($P \leq 0.05$) different from other stages of growth (Table 4.38).

Table 4.38 The 100 panicles weights (in grams) for seeds obtained from various inoculations and percent weight reduction against the individual controls of finger millet

Time of inoculation	Kiboko long rains 1996	% Yield reduction relative to the control	Kabete short rains 1996/1997	% Yield reduction relative to the control	Pooled means	% Loss of pooled means (relative to the control)
I_1	317.102 c	15.63 a	167.925 a	5.93 a	242.514 b	12.51[1]
I_2	371.863 b	1.06 b	173.369 a	2.88 b	272.616 a	1.75[4]
I_3	363.030 bc	3.41 b	177.672 a	0.47 b	270.351 a	2.32[2]
I_4	370.059 b	1.54 b	176.654 a	1.04 b	273.356 a	1.35[5]
I_5	367.041 b	2.13 b	175.750 a	1.54 b	271.796 a	1.89[3]
I_6	375.847 a	0	178.511 a	0	277.179 a	0
LSD (5%)	46.65	7.94	16.28	3.30	27.07	

The superscript 1,2,3,4 & 5 are the various rankings as per the disease parameter, with the number 1 being the most affected by disease.

4.9.2.3.3 Yield : 1000 seed weight

Grains from infected plants were smaller and shrievelled than for those that were not infected by *P. grisea*, hence the weight reduction.

At Kiboko during long rains, the control plot had the highest 1000 seed weight but was statistically ($P \geq 0.05$) not different from other stages of growth. Stage one of growth had the highest weight reduction of 16.27 %, which was statistically ($P \leq 0.05$) different from the other growth stages (Table 4.39). At Kabete during short rains, the control plot had the highest 1000 seed weight, which was not statistically ($P \leq 0.05$) different from other growth stages. The highest weight reduction of 17.31 % was under stage two that was statistically different from stages one, three and five but not stage four (Table 4.39). General weight reduction in all sites and seasons for 1000 seed weight varied from 2.16 to 12.04 % (Table 4.39).

Table 4.39 The 1000 seeds weights (in grams) for seeds obtained from various inoculations and percent weight reduction against the individual controls of finger millet.

Time of inoculation	Kiboko long rains 1996	% Yield reduction relative to control	Kabete short rains 1996/1997	% Yield reduction relative to control	Pooled means	% loss of pooled means (relative to controls)
I_1	3.475 a	16.27 a	2.718 a	6.02 b	3.097 b	12.04[1]
I_2	4.102 a	0.44 b	2.388 a	17.31 a	3.245 ab	8.18[3]
I_3	4.118 a	0.06 b	2.727 a	6.30 b	3.412 ab	2.81[4]
I_4	3.956 a	3.98 b	2.466 a	14.61 a	3.211 ab	8.39[2]
I_5	4.012 a	2.63 b	2.758 a	4.5 b	3.385 ab	2.16[5]
I_c	4.120 a	0	2.888 a	0	3.504 a	
LSD (5%)	0.691	10.91	0.751	4.40	0.395	

The superscript 1,2,3,4 & 5 are the various rankings as per the disease parameter, with the number 1 being the most affected by disease.

4.9.2.3.4 Laboratory assessment for seedborne *P. grisea* of harvested seed thereafter using blotter test

For Kiboko I, stage four of inoculation had the highest blast incidence of 4.25 %, which was statistically ($P \leq 0.05$) different from the other stages (Table 4.40). For Kabete II, stage one of inoculation had the highest blast incidence of 3.75 %, which was statistically ($P \leq 0.05$) different from the other stages except stage three (Table 4.40). The lowest blast incidence was 0.417 % for Kiboko I and at stage five of inoculation. In general the highest pooled blast incidence mean was under stage four which was statistically ($P \leq 0.05$) different from the other stages except stage one, while the lowest was stage two (Table 4.40).

Table 4.40 Percent seed infection for seed harvested under inoculation experiment.

Time of inoculation	Kiboko I	Kabete II	Pooled means for the two sites	Ranking
I_1	2.580 b	3.750 a	3.165 a	2
I_2	0.500 c	0.750 c	0.625 c	5
I_3	1.580 bc	1.750 b	1.665 b	3
I_4	4.250 a	3.250 a	3.750 a	1
I_5	0.417 c	1.000 bc	0.709 c	4
I_c	0.250 c	0.25 c	0.25 c	6
LSD (5 %)	1.352	0.793	0.700	

4.9.2.3.6 Correlations and rankings of scored parameters

After ranking all inoculation stages under the various diseases indexes and yield data, the most susceptible stage was found to be stage one of artificial inoculation, while the least susceptible was stage five of artificial inoculation. The ranking was arrived at by putting the different diseases indexes measured in the experiments of artificial inoculation by *P. grisea* in a table form (table 4.41) and averaging the rank achieved under each diseases index.

A correlation matrix table (Table 4.42) was computed and is considered a useful predictor of expected disease and yield reductions using various disease indexes. For instance the correlation coefficient between percent yield reduction and incidence on tillers, panicle blast, neck blast, leaf blast and 1000 seed weight were all statistically highly significant ($P \leq 0.01$).

The correlation matrix that summarises all the correlations, shows that yield reduction (the most important aspect to the farmer) is directly affected by panicle, neck and leaf blast (Table 4.42). Percent yield reduction was negatively correlated to 1000 seed weight; while percent yield reduction was correlated positively ($p \leq 0.05$) to panicle, neck and leaf severity. This for instance, indicates that the severity on panicles disease index explained 80.8% of the total yield reduction.

There was a negative correlation between yield per hectare and the following disease indexes: Incidence observed on the tillers, panicle severity, neck severity, leaf severity and incidence observed on harvested seeds. Head blast caused yield reduction as a result of reduction in spikelet length, grain weight and number of grains per head.

Table 4.41 The overall ranking showing the most susceptible growth stage

Treatment	I_1	I_2	I_3	I_4	I_5
Incidence observed	1	3	2	4	5
Panicle severity	1	3	2	4	5
Neck severity	1	2	3	4	5
Leaf severity	1	2	3	4	5
% Weight reduction for 100 panicle	1	3	2	5	4
% Weight reduction for 1000 seed weight	1	3	4	2	5
% Yield reduction	2	3	1	4	5
Incidence for harvested seed	2	5	3	1	4
Overall ranking	1	3	2	4	5

Table 4.42 The correlation matrix for the various parameters under the artificial inoculation of finger millet with *P. grisea* at different stages of growth.
The correlation coefficient, r is the value in the table

Y ↓ X →	Incidence observed	Panicle severity	Neck severity	Leaf severity	100 panicles weight	1000 seed weight	Yield per hectare	% yield reduction	Incidence for harvested seeds
Incidence observed	_	0.972**	0.986**	0.991**	-0.249^{ns}	-0.714*	-0.448^{ns}	0.880**	0.622^{ns}
Panicle severity	0.972**	_	0.970**	0.963**	-0.116^{ns}	-0.735*	-0.309^{ns}	0.899**	0.537^{ns}
Neck severity	0.986**	0.970**		0.986**	-0.290^{ns}	-0.734*	-0.378^{ns}	0.866**	0.594^{ns}
Leaf seveity	0.991**	0.963**	0.986**		-0.261^{ns}	-0.683*	-0.414^{ns}	0.820**	0.563^{ns}
25 panicles weight	-0.412^{ns}	-0.455^{ns}	-0.489^{ns}	-0.468^{ns}	0.511^{ns}	0.267^{ns}	0.167^{ns}	-0.261^{ns}	-0.221^{ns}
100 panicles weight	-0.249^{ns}	-0.116^{ns}	-0.290^{ns}	-0.261^{ns}	_	-0.027^{ns}	0.691*	-0.091^{ns}	-0.431^{ns}
1000 seed weight	-0.714*	-0.735*	-0.734*	-0.683*	-0.027^{ns}	_	-0.061*	-0.831**	-0.705*
Yield per hectare	-0.448^{ns}	0.309^{ns}	-0.378^{ns}	0.414^{ns}	0.691*	0.061^{ns}	_	-0.336^{ns}	-0.554^{ns}
% yield reduction	0.880**	0.899**	0.866**	0.820**	-0.091^{ns}	-0.831**	-0.336*	_	0.748*
Incidence for harvested seeds	0.622^{ns}	0.537^{ns}	0.594^{ns}	0.563^{ns}	-0.431^{ns}	-0.705*	-0.554^{ns}	0.748*	_

Key :

ns Implies that the parameters in consideration are statistically not significant at 5 % and 1 % level of significance.
* Implies that the parameters in consideration are statistically significant at only 5 % level of significance.
** Implies that the parameters in consideration are statistically significant at 5 % and 1 % level of significance.

CHAPTER FIVE

DISCUSSION

5.1 Identification of the pathogen

5.1.1 Morphological characteristics

The morhological characteristics of *Pyricularia grisea* observed and recorded are in close agreement with other reports (Wallace, 1950; Ramakrishnan 1963 and Ellis, 1971), although they give different names to the same pathogen. *P. grisea* was positively identified using the slide culture technique described by Ridell (1950).

5.1.2 Pathogenicity tests

Confirmation was done using the pathogenicity test on finger millet plants. Typical elliptical blast lesions were observed. The pathogenicity test showed that the Busia isolate seemed to induce more damage than others as per the lesion expansion. However the Kisii isolate induced more damage when the % severity on leaves was considered. The aggressiveness of the Busia isolate may be attributed to the fact that since there is more acreage of the finger millet, maybe more virulent strains have evolved in that region. In addition Busia district is nearer to Uganda, where the bulk of finger millet is grown in E. Africa and the world at large and thus more virulent strains might drift across the border.

From the results presented here, there seemed to be a relationship between the various isolates lesion expansion rate and mycelial growth rate. This may be an area of study that can be pursued in later researches to ascertain if there is a concrete relationship.

5.2 Cultural studies

The best media in terms of mycelial growth was FMLEA, while in terms of sporulation was OMA. Both media were made in the laboratory from easily obtainable and cheap materials. FMLEA is actually a combination of finger millet leaf extract and agar while

OMA is a combination of oatmeal flour and agar. Both FMLEA and OMA can be used to grow *P. grisea*, but OMA is better especially in relation to easy identification of *P. grisea* on media, since OMA is white while FMLEA is light green. *P. grisea* is grey-black on OMA. FMLEA as a media shows good prospects and can be used as an alternative especially where oatmeal is not available on the market. The best pH in terms of hyphal growth was pH 8, while for sporulation was pH 7. Kulkarni and Govindu (1976) also reported best hyphal growth occurring between pH 7 to 8. The best lighting regime in terms of hyphal growth was 24 hours lighting and 12 hours lighting / 12 hours darkness. This would actually imply that good growth would be obtained on a normal day i.e. normal day and night cycle and if need be use expensive equipment to ensure 24 hours lighting. This result fits very well for the pathogen of a tropical crop because the day and night lenghts are almost equal. In general hyphal growth is important but sporulation is more important especially when you require high sporulation for inoculation purposes.

This study demonstrated that there are differences in the cultural characteristics of *P. grisea* isolates. Similar findings have been reported elsewhere for the rice blast fungus, *P. oryzae* (Ren - jong *et al.*, 1965). Further observations revealed that even within a given isolate the cultural morphology varied considerably. Sporulation of *P. grisea* is a process influenced by several factors. Given an isolate's inherently capable of sporulating other factors are pH, lighting regime and media. The requirements for sporulating seem to be more exacting than the requirements for hyphal growth. The intensity of sporulation was in certain instances negatively correlated to radial growth, while in others positively correlated. The results obtained generally showed that the Kisii isolate which sporulated most has a relatively slower growth than the Busia isolate which grew faster. The characterisation of the various isolates of *P. grisea* in the country has not been done this could be good research area especially in the light that there were clear differences in reaction of the isolates to the

cultural factors. It is apparent that certain isolates sporulate more than others, while others have a faster mycelial growth rate. It would be important to ascertain which of the two, high sporulation or faster mycelial growth rate is a more measure of aggressiveness of the pathogen.

5.3 Assessments of farmers seed samples

From the survey carried out in the three districts, it became clear that blast on finger millet is fully recognised by various tribes who grow finger millet and have given the disease vernacular names, showing that the disease is ancient and also that the farmers are clear about the symptoms. The fact that the crop in Kisii had high levels of disease means that continous cropping seems to contribute to the build up of inoculum and hence crop rotation is necessary in such areas.

As an indicator the seed washing experiment was not an appropriate one, as some seeds with low levels of *P. grisea* under the various assay tests, were found to be high with this method. Also broken or maybe non-viable strains conidia of *P. grisea* were observed. Spores of *P. grisea* were found in finger millet seed washings, suggesting that the fungus is loosely carried on the seed surface. Although conidial and mycelial germination was done, the results did not tally very well since other organisms were also present, hence it tended to confuse the eye when doing the assessment.

The growth of *P. grisea* from surface sterilized seeds (on the agar plate method) suggested further that this fungal species was internally seed-borne. It would appear that *P. grisea* may contribute to the observed decline in viability with time. In the present study, *P. grisea* seemed to be mostly externally seedborne, since more inoculum was found with the blotter method than agar plate method. Under the various incubation techniques the blotter test was found best, although the rolled paper towel technique followed closely. The blotter method was most sensitive as the method recorded in 88 % of the samples tested had *P.*

grisea and the differences were statistically ($P \leq 0.05$) different from the others. The other methods have advantages like for instance the agar plate test you can detect *P. grisea* (using oatmeal agar as the pathogen is grey to black growth on media) that is internally seedborne after surface seed sterilization. The high positive correlation, $r = 0.914$ between % reduction in germination due to *P. grisea* and % seed infected by *P. grisea* shows that the pathogen has effect on seed viability. Adipala (1992) observed that there was a reduction in seed viability due to various fungal infections and more so *P. grisea* and this study confirms this. The correlation, $r = 0.807$ proves the sensitivity of the blotter test and its suitability as a seed health testing technique with respect to *P. grisea* on finger millet. Blotter method gave higher figures than either method, although rolled paper towel test had comparable results. The seedborne nature of *P. grisea* was substantially lower in the agar plate method and this is consistent with Ranganathiah and Mathur (1978) but not consistent with findings of Pande *et al.*, (1994), who reported that the agar plate method and blotter tests were comparable although the agar plate method was uneconomical, cumbersome and does not restrict the growth of saprophytes. Also the shoots and roots growth complicate observation, since they open the top of the petri dish.

Low seed germination was associated with high seed infection by the fungus. Seed infection by species of *Aspergillus, Curvularia, Fusarium, Alternaria* and other fungi suggests that detailed investigations are warranted to establish the role of these fungi in the health of finger millet seeds. Seedborne fungi of *E. coracana* have previously been studied by Grewal and Pal (1965), Ranganathaiah and Mathur (1978) and Pande *et al.*, (1994). In all cases the blotter method was found most suitable method for seed health testing on finger millet.

Only 12 samples of all the 41 seed samples examined would qualify for quality certificates for germination (above 80 %), yet out of these 12 only 5 would be allowed to pass

the seed health test at 0 % *P. grisea* tolerance in seed samples i.e KNE 479 - Kiboko, P224, KNE 808, GULU - E and Mwagichana. It is important to note that only one is a farmer's sample the rest are ICRISAT samples.

5.4 Green house assessments

The grow-on test tests indicatd that *P. grisea* is seed-transmitted. The pathogen sporulated on ungerminated, infected seeds and on rotten or necrotic tissues of seedlings and was able to kill the seedling. Mitra and Mehta (1934) and Ranganathaiah and Mathur (1978) also reported seedling mortality. The data supports previous reports on the seedborne nature of the fungus (Pande *et al.*, 1994).

5.5 Field trials using seeds obtained during the survey

Although there was no serious epidemic, the little disease observed goes to show that there was very little transmission from seed to plant (SP), as earlier reported (Gabrielson, 1988) that some seedborne pathogens may rot infected seed before the seedling reaches the soil surface and thus primary inoculum is effectively buried. For most pathogens in this category, much less inoculum reaches the soil surface to become primary infection foci than would be indicated by seed health tests.

An experiment on the seeds tested under blotter test using 99 seeds showed infection by *P. grisea* which sporulated heavily on the seed with only 13 germinated seeds, representing 13.1 % seeds. This shows that 86.9 % of the seeds did not germinate and hence seeds could not transmit inoculum to the plant, which meant that the inoculum had been buried. This condition was related to the field. The fact that only 13.1 % of seeds germinated from 99 seeds observed to be infected with *P. grisea,* goes to show that for *P. grisea* to really become epidemic this has to be accompanied by higher germination levels in the seed.

Establishment of valid tolerances for the areas sampled and the country in general could not be reached. There is a need for more insight into the disease, especially that phase after germination and when the actual transmission starts.

Baker and Smith (1966) reported that even though the pathogen is carried in or on the seed, it does not ensure transmission to the subsequent crop stand. Other factors such as environment may restrict infection and disease expression. During the experiment at both sites it was evident that just after planting and even germination, it was hot and dry, which is not conducive to sporulation and even movement of the pathogen to creat epidemics. In some cases it became just too cold (Appendix 2) like at Kabete during the long rains (April – August 1996) where temperatures stayed below 18^0C most of the time. This resulted in little disease progress as *P. grisea* grows best when temperatures are between 20 - 30°C with humidity above 90 %. Other factors that could be against pathogen establishment at the initial stages include the pathogen in or on the seed may die before the seed loses satisfactory commercial germinability, the soil microflora may be inhibitory or antagonistic to the pathogen when seed is planted in it. Shetty and co-workers(1985) emphasised that there is a need for a laboratory method that can detect one diseased seed in ten thousand to be able to predict successfully the field severity of blast.This is because epidemics of blast resulted from using seed of *Eleusine coracana* with very low levels of seedborne infection and that a single infected plant could bring about an epidemic. These results are in line with those of Pall (1988). Hence they propose an adoption of zero tolerance in the seed certification programme. Although from the experiment, this was not evident, it would be good to repeat this part of the experiment with a more wide variety of seeds sampled from across the country.

For Kabete season II (short rains) an inoculum threshold may not have been reached even with the high *P. grisea* infections to show any transmission and maybe a higher level of

viability of seeds infected by *P. grisea* is required to elicit some transmission. The amount of leaf blast recorded under transmission experiment was generally less than 2 %. The disease possibly did not become visible due to:

(a) Weather conditions as the development of blast requires temperatures of 25 – 30^0C and relative humidity of over 90 % (Pall, 1987).

(b) The inoculum could have been buried with the diseased seeds, which did not germinate.

(c) Indications during field experimentation were that the disease would start to develop and then be arrested in development leaving only elliptical lesions.

5.6 The effect of time of inoculation on blast development in the field and seed infection by *P. grisea*

In the inoculation experiment, the evidence obtained shows a significant linear positive relationship between percent yield reduction and the following parameters namely panicle blast severity, neck blast severity, leaf blast severity, panicle blast incidence observed on tillers and seedborne incidence of *P. grisea* on harvested seed with a negative relationship with 1000 seed weight. The significant linear relationships established in this study between the various blast severities above and percent yield reduction may be important in determining the economic threshold level of the disease that can justify control measures for adoption. Panicle and neck blast caused loss in panicle length, grain weight and 1000 grain weight and these results are in line with those of Pall(1977), Rath and Mishra (1975).

The significant positive correlation (r = 0.748*) between harvested seeds incidence of seedborne *P. grisea* and percent yield reduction would point to a situation whereby a certain amount of inoculum of *P. grisea* on planting seed would mean a certain amount of yield losses would be expected.

The correlation coeffiecients derived from the experiment are useful, but due to the fact that not very many seasons and sites were considered, they should be used with caution. Although the yield reductions did not reach the set economic threshold of 30 %, the inferences gathered are important since finger millet is an important crop in the East African region and that blast is a well known constraint of finger millet production (Guiragossian, 1988). Scoring scales were developed for scoring leaf and panicle. Such scales would be useful especially in the field to help in scoring of blast on finger millet germplasm.

The failure of the finger millet to develop seeds or develop seeds poorly during Kabete season I, long rains, could be realted to the fact that finger millet grows best in temperatures between 18 and 27^0C (Thomas, 1970). During the months of May and July average temperatures did not go above 18^0C.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

The best media that supported good growth and sporulation was OMA. Such media is important not just for cultural studies but also when *P. grisea* is required in large quantities for germplasm evaluation for disease resistance.

In case of seed health testing such a medium is crucial for the detection of the pathogen in culture. OMA is white and the pathogen (*P. grisea*) grows as a greyish colour, which is easily noticed on a white background. Thus OMA was the best medium for this pathogen while pH 7 is best pH, lighting regime would be 12 hours lighting / 12 hours darkness is best.

To favour maximum sporulation of *P. grisea*, in addition to medium, pH, lighting regime and possibly temperature are very important.

According to results obtained under various seed assay tests for seedborne *P. grisea* the blotter test gave better results for testing this fungus in and on finfer millet.

The high seedborne *P. grisea* levels did not translate to high blast epidemics under field conditions, as most seeds seemed to have lost viability. This effectively buried the inoculum and reduced primary infection foci. There is a need to consider more sites, more seasons and higher inoculum levels.

Stage one of growth was found to be the most susceptible growth stage to blast leading to high blast severity, incidence and yield reduction. When blast was introduced at stage four i.e. at milk stage of seed development, the seed ended with a lot of seedborne inocula of *P. grisea*. High neck, leaf, panicle blast severity translated to high seedborne *P. grisea* of harvested seed.

The following are the recommendations:

1. Future studies should include more locations and uniform land races. This would permit more inferences on the relation of weather and disease development and on the relation of yield loss to disease development.
2. It would be important to repeat the experiment of transmission in more sites to ascertain some already noted facts from earlier experiments like the fact that if there is only one seed that is infected then an epidemic is possible (Pall, 1988).
3. There is a need for further study to ascertain the role of the nematodes, *Alternaria sp. Curvularia sp., Aspergillus sp., Cladosporium sp., Helminthosporium sp.* and *Fusarium sp.* all found in and on the seed.
4. Survey to document prevalence and severity of blast in the field in districts growing finger millet.
5. Sample seeds from farmer field under a number to establilsh the seedborne *P. grisea* incidences and correlate the data to field blast data.
6. Establish the *P. grisea* tolerance levels, as the pathogen is cleary seedborne.
7. Train farmers in the production of healthy seeds as the farmers used their own saved seeds for planting.
8. Setup field experiments, which can finally come up with cost-effective blast control measures, which can be adopted by the smallscale farmer growing finger millet.
9. For routing seed health studies, the blotter test would be the ideal method.
10. When the pathogen is needed in large quantities oatmeal agar should be the media of choice and in combination with a pH of 7 and lighting regime of 12 hours lighting and 12 hours darkeness.

CHAPTER SEVEN

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CHAPTER EIGHT

APPENDICES

Appendix 1

Preparation of media used in culturing *P. grisea* during cultural studies.

Oatmeal Agar (OMA)

Into 1 litre of sterile distilled water (SDW) was added 38 gms of oatmeal, plus 20 gms of agar and autoclaved at 121^0C, 1 Atm., pH obtained was 6.15.

Potato Dextrose Agar (PDA)

Into 1 litre of SDW was put 39 gm of PDA and autoclaved at 121^0C, 1 Atm. The pH obtained was 5.80.

Finger Millet Leaves Extract Agar (FMLEA)

300 gms of chopped-up finger millet leaves were put into a conical flask and the volume made upto 1 litre. The contents were boiled for thirty minutes. The contents were streamed through a cheese cloth and then 20 gm of agar added. The volume was made upto one liter and then autoclaved at 121^0C, 1 Atm. The pH obtained was 6.20.

Bean Meal Agar (BMA)

100 gms of dry beans were put into a conical flask and made upto 1 litre and boiled for 30 minutes. The contents were streamed through a cheesecloth. 20 grams of agar was added and the volume made upto 1 litre and then autoclaved at 121^0C, 1 Atm. The pH was 6.25.

Finger Millet Seed Extract Agar (FMSEA)

100 grams of dry finger millet seed were put into a conical flask and made upto 1 litre and boiled for 30 minutes. The contents were streamed through a cheesecloth. 20 grams of agar was added to the sieved content and the volume made upto 1 litre and the autoclaved at 121^0C, 1 Atm. The pH was 7.00.

Appendix 2

Meteriological data

Mean monthly temperatures and rainfall for Kabete and Kiboko field experiment sites

Kabete

	1996									1997
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
Rainfall (mm)	32.2	29.7	17.2	11.9	11.8	12.3	44.4	34.5	0	0
Temp. (^{0}C)	16.0	18.3	16.6	15.6	15.4	17.4	19.2	23.2	22.6	23.1

Kiboko

	1996									1997
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
Rainfall (mm)	10.2	12.7	2.2	1.9	1.8	2.1	17.4	14.0	0	.0
Temp. (^{0}C)	26.0	28.3	28.6	26.6	31.4	32.4	30.2	33.2	32.6	33.1

Appendix 3

Sum of squares (SS) and F values for various functions for the disease measuring indexes for AUDPC (Polynomial ANOVA separations).

Function	Incidence on tillers	Severity on leaves	Severity on neck	Severity on panicles
Linear	683.503	47040.998	9769.526	531.420
F value	59.10**	124.756**	35.406**	37.212**
Quadratic	60.624	9911.624	462.821	42.699
F value	5.242*	26.286**	1.677^{ns}	2.990^{ns}
Cubic	10.358	1316.003	168.308	1.056
F value	0.896^{ns}	3.490^{ns}	0.610^{ns}	0.074^{ns}
SS for inoculation from the ANOVA table	762.706	58571.413	10702.244	632.589
Df	4	4	4	4

Sum of squares (SS) and F values for various yield data (Orthogonal Polynomial ANOVA separations).

Yield component	Function	Kiboko	Kabete	Pooled means
Total wt	ss Linear	14197.135**	103320*	97090.443**
	ss Quadratic	182.086^{ns}	12215.308^{ns}	7701.38^{ns}
	ss Cubic	3124.779^{ns}	2770.621^{ns}	5.476^{ns}
100 panicles wt	ss Linear	444387.459**	199.285^{ns}	3454.940*
	ss Quadratic	250304.956**	55.837^{ns}	1389.201^{ns}
	ss Cubic	173446**	36.154^{ns}	
1000 seed wt	ss Linear	0.487ns	0.169^{ns}	0.620*
	ss Quadratic	0.312ns	0.208*	0.004^{ns}
	ss Cubic	0.476ns	0.010^{ns}	0.166^{ns}

Key

** Highly significant (P = 0.01).

* Significant (P = 0.05)

ns Not Significant.

Appendix 4

Field layout for transmission and artificial inoculation experiments on the field.

Experiment 1: Growing seeds with different levels of *P. grisea* inoculum.

Block 1	L_5	L_3	L_1	L_c	L_2	L_4
Block 2	L_2	L_c	L_4	L_5	L_3	L_1
Block 3	L_3	L_1	L_5	L_4	L_c	L_2
Block 4	L_3	L_4	L_1	L_c	L_5	L_2

Design: RCBD

Experiment 2: Inoculation at different stages of growth

Block 1	I_5	I_2	I_4	I_c	I_1	I_3
Block 2	I_3	I_1	I_c	I_4	I_5	I_2
Block 3	I_c	I_4	I_1	I_2	I_5	I_3
Block 4	I_2	I_3	I_5	I_1	I_4	I_c

Design: RCBD

Key:

L implies the different levels of inoculum.

I implies the different times of inoculation.

C implies control e.g. L_c or I_c

Appendix 5

This are the vernacular names of the finger millet blast disease as reported by the farmers in areas seed samples were collected:

Language	Name of blast
i. Kiluhya	*Bwaburukha* and *Bwalala* (Kibukusu)
	Milaka (Kisamia and Kikhayo)
ii. Kikisii	*Egetabo, Egebabo, Rikuba* (Especially in Nyamira), *Chiyoni and Oboke.*
iii. Kiteso	*Emaeleke.*

The finger millet crop has the following names in vernacular language in Kiteso it is called *Akima;* in Kiluhya especially in Kikhayo and Kisamia dialects - *(O)bule* while in Kibukusu dialects – *Vuro* ; in Kikisii it is *Obori.*