SOUTHERN LEAF BLIGHT OF MAIZE (*Zea mays* L.) IN KENYA.

by

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D E C L A R A T I O N

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

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D E D I C A T I O N

To my daughter Christine Alum.

TABLE OF CONTENTS

TABLE OF CONTENTS (CONTD...)

TABLE OF CONTENTS (CONTD....)

LIST OF TABLES

LIST OF TABLES (CONTD....)

LIST OF FIGURES

LIST OF PLATES

LIST OF PLATES (CONT...)

LIST OF APPENDICES

A B S T R A C T

The southern leaf blight of maize caused by Helminthosporium maydis is a serious disease that has recently been introduced into Kenya. The disease is causing concern in many maize growing areas of the world. Investigations were carried out on this disease and these included studies of disease incidence, severity, symptoms, characteristics of the pathogen, factors affecting colony growth and conidial germination, screening 7 maize hybrids and/composite for resistance and host range. Where the disease was observed to occur incidence was below 5% and there was only slight to light infection possibly because of drought at the time the disease survey was carried out. Symptoms were observed to form only on leaves and consisted of greyish-tan or straw-coloured lesions showing a zonate pattern and measuring 0.5 x 0.5 to 3 x 10 mm. Colonies of the fungus formed chlamydospores in contaminated cultures along zones of anti-biotic activity between the fungus and bacteria. The conidia were curved, fusiform and pale to somewhat dark golden brown in colour. They measured 27 to 103 x 7 to 20µm and the number of septa ranged from 3 to 10. Optimum conditions for conidial germination

were when relative humidity was between 90 and 100% and when the temperature was 30°C. Conidial germination was bipolar. Germ tubes were produced within 6 hours of the inoculation of maize leaves and most appressoria formed between 6 and 18 hours after inoculation near stomata which seemed to aid penetration of the leaves by the pathogen. Fungal colony growth rate was highest when temperature was 30°C at pH 6. Attempts to obtain the perfect stage of the fungal pathogen (Cochliobolus heterostrophus) were unsuccessful. Katumani Composite maize was highly susceptible to the disease in the field. Hybrids 511, 512, 612 and 622 were only moderately susceptible while hybrids 614C, 613C and 632 were somewhat resistant to attack by southern leaf blight. In the host range study only one host plant species, Rottboellia exaltata, showed infection which was only slight, out of 13 grass and 1 sedge species. All three isolates of the fungus were similar in behaviour in all the aspects of the disease studied and were probably all race 0 of the pathogen since they infected only leaves.

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CHAPTER 1

1. INTRODUCTION

Maize is an important staple food for most of the people in Kenya. It is also the source of animal feeds. The crop grows well at altitudes up to 2200 m. Among the cereals it ranks first in hectarage under cultivation. About 1,400,000 hectares of the crop is grown annually, mostly in the highland areas. This is the largest area grown to a single crop in the country and is a large proportion of the country's limited arable land area (Kenya Seed Company, 1979). Most of the hectarage is grown by small scale farmers to meet their own subsistence maize requirements but surpluses are sold for cash. Before 1969, maize was not profitable as an export crop because of low world prices and the high costs of handling, storage and transportation. Thus Government policy was to grow maize only to meet national self-sufficiency in the crop with a small safety margin (Allan *et al.*, 1976). However, with the introduction of hybrid maize in Kenya in 1963, and with more farmers, especially the small scale, using hybrids after 1965, large surpluses of the crop began to appear on market.

As marketing, storage and transportation also improved and world prices began to rise the government changed its policy in 1969 to that of promoting large increases in maize production. The aim of this new policy was to encourage utilization of local market surpluses in livestock production, industrial processing and export (Allan et al., 1976). Between 1967 and 1976 unmilled maize accounted for an average of about 2.5% of the total value of domestic exports (Statistical Abstracts, 1977). Contributions of the various cereals including maize that are grown in Kenya to gross farm revenue between 1976 and 1981 are compared in Table 1. For most of the period reviewed contribution to gross farm revenue by maize was highest.

Against this background of increased maize production, however, there have been problems which face maize production up to now. These problems include unsuitable soils and climate, poor crop husbandry techniques, pests and diseases. Among the diseases, although the Kenya maize hybrids and composites are resistant to many diseases there are exceptions which include the widespread occurrence of the maize streak virus disease and headsmut caused by Sphacelotheca reliana. There is also new

disease, the southern leaf blight that is caused by Helminthosporium maydis Nisikado and Miyake. This disease which has probably been introduced through imported seed from abroad was first reported in Kenya in April, 1978 in the Kisii District of Nyanza Province (Singh et al., 1979). Surveys carried out during the long rainy seasons (April-June) of 1978 and 1979 confirmed the widespread occurrence of the disease in the same province and other parts of the country including Kakamega, Kitale and Coast and Central Provinces. Disease severity varied from a trace to severe blighting of leaves. The disease kills the green tissues of the leaves thus reducing the effective leaf area. Yield may be reduced, stalks weakened and the fodder value of the crop damaged (Alice Robert, 1953).

Josephson et al. (1971) reported Texas male sterile (TMS) cytoplasm plants to have greater stalk lodging, less ears per plant and lower grain quality. The disease may also attack leaf sheaths, roots and ear husks of maize (Orillo, 1949) and Robert (1956) reported it as causing blackening of silks and ear tips of maize in Florida. An epidemic of the same disease threatened the maize crops of USA in 1970

causing drastic reductions in yield of 50-75% in the southern states of the corn belt (Roberts and Boothroyd, 1972). This fungus produces microscopic vegetative spores which are blown from plant to plant to spread the disease. The spores are characteristically slender and curved. The perithecial (sexual) stage is also known and is called Cochliobolus heterostrophus (Drechsler) Drechsler.

No study has yet been carried out on the disease in Kenya and although there is yet no data on the economic importance of the disease in Kenya its reported widespread occurrence in Kenya and severe maize crop losses elsewhere especially in USA in 1970 has warranted the choice of this disease as a problem of research. This study was thus undertaken to elucidate/the facts on disease under Kenyan conditions in order to be able to check its spread and thus boost maize production. There exists a potential threat of an epidemic of this disease if the right conditions for its development, especially susceptible maize varieties, abound.

The following were the objectives of the study:-

(a) To determine the incidence and severity of the disease.

(b) To isolate the pathogen and study its characteristics.

(c) To screen various maize varieties for resistance to the disease

(d) To determine the host range of the pathogen.

TABLE 1: CONTRIBUTIONS OF THE CEREALS TO GROSS FARM REVENUE IN KENYA IN THE PERIOD 1976 - 81 IN K£'000.

	1976	1977	1978	1979	1980	1981*
Wheat	12,047	11,877	11,675	14,886	17,670	17,869
Maize	21,628	18,843	10,501	9,363	10,390	23,645
Barley	2,625	1,955	2,662	3,354	4,279	3,903
Rice	2,690	2,816	2,594	2,826	2,843	3,235
Other cereals	343	36	45	91	70	75
TOTAL	39,333	35,527	27,477	30,520	35,252	48,727

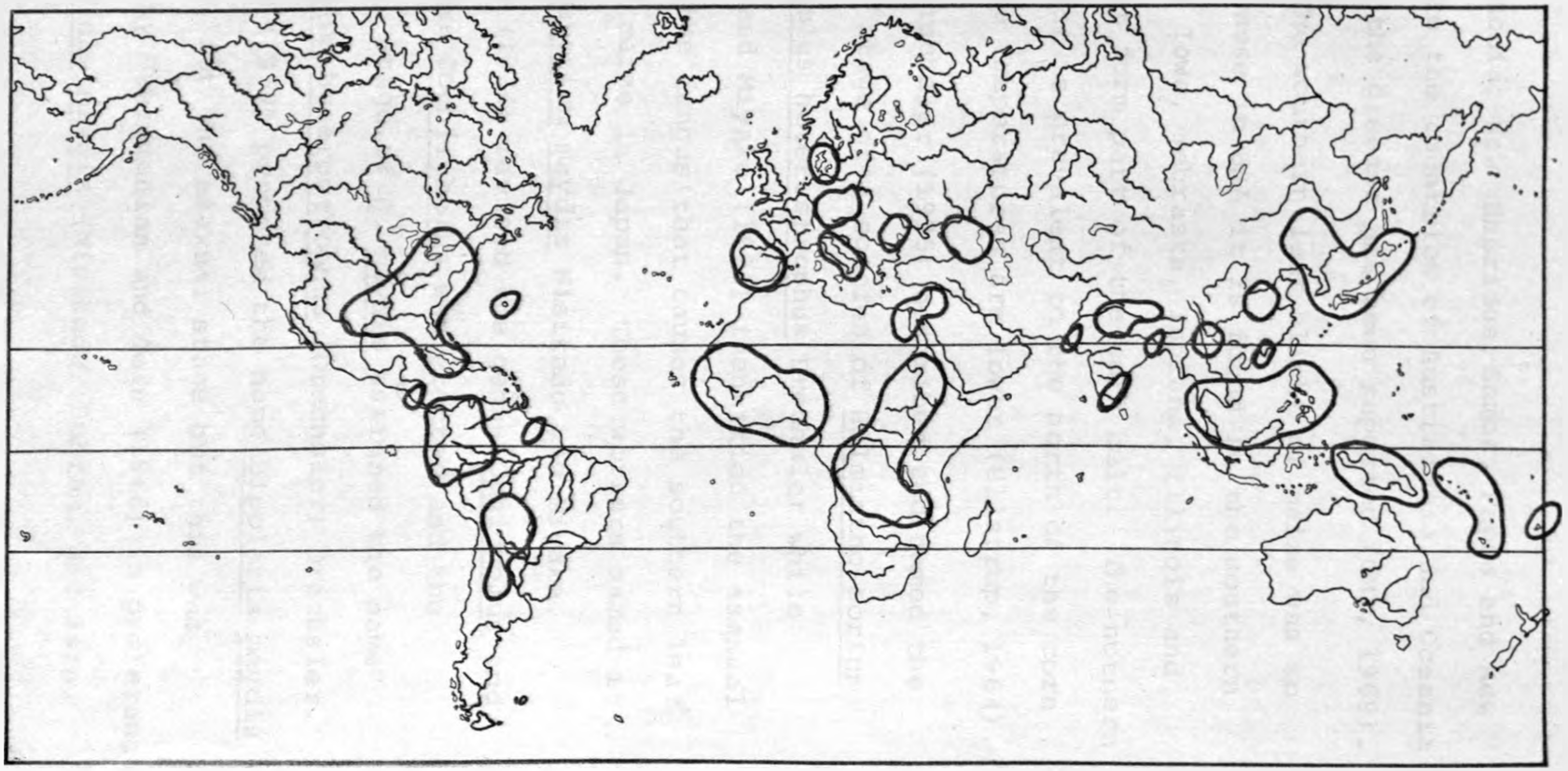
*Provisional

Source: Kenya Statistical Digest, Central Bureau of Statistics, Ministry of Economic Planning and Development.

2. LITERATURE REVIEW

2.1. HISTORY OF SOUTHERN LEAF BLIGHT OF MAIZE AND ITS CAUSAL AGENT

The southern leaf blight of maize has a worldwide distribution mainly in the tropical and sub-tropical areas. Figure 1 shows this distribution in Africa, Asia, Europe, North America, Central America and West Indies, South America, Australia and Oceania. In Africa the disease has been reported in Congo, Dahomey, Egypt, Ghana, Guinea, Ivory Coast, Malawi, Nigeria, Senegal, Sierra Leone, Sudan and Zambia while in Asia it has been reported in Cambodia, China, Hong Kong, India, Indonesia, Japan, Korea, Malaysia, Nepal, Pakistan, Philippines, South Vietnam and Thailand (Commonwealth Mycological Institute, 1969). Cyprus, Denmark, Germany, Italy, Portugal, Rumania, Spain and USSR (Caucasus) are the European countries in which the southern leaf blight of maize has been reported (CMI, 1969). In the Americas and West Indies the disease has been reported in Canada, USA, Bahamas, Cuba, Jamaica, Nicaragua, Panama, Trinidad, Argentina, Bolivia, Colombia, Ecuador and Surinam (CMI, 1969).British Columbia, Solomon Island, Fiji,



Source: Commonwealth Mycological Institute 1969

Fig. 1 Distribution of Helminthosporium maydis in the World.

New Caledonia, New Hebrides, Samoa, Papua and New Guinea are the countries of Australasia and Oceania in which the disease has been reported (CMI, 1969).

The southern leaf blight of maize was so named because in USA it is found in the southern states of Iowa, Nebraska, Indiana, Illinois and Texas that form part of the corn belt. The nothern leaf blight is prevalent to the north of the corn belt where temperatures are lower (Ullstrup, 1964).

Drechsler (1925) described and named the ascigerous stage of a species of Helminthosporium as Ophiobolus heterostrophus Drechsler while Nisikado and Miyake (1926) identified the asexual stage of the fungus that causes the southern leaf blight of maize in Japan. These workers named it Helminthosporium maydis Nisikado and Miyake. Drechsler (1934) revised the genus Ophiobolus and a new genus Cochliobolus was erected and the ascigerous stage of H. maydis assigned the name Cochliobolus heterostrophus (Drechsler) Drechsler. Shoemaker (1959) proposed the name Bipolaris maydis comb. nov. for the asexual stage but this was rejected by Subramanian and Jain (1966) in preference to Drechslera maydis (Nisikado) Subram. and Jain.

Pate and Harvey (1954) showed resistance to infection by H. maydis to be controlled by several genes that are incompletely dominant. They also concluded that the genes may be additive and/or complementary in their action. Resistance is usually expressed as small chlorotic flecks. A type of resistance expressed as chlorotic lesions was reported by Craig and Kalio (1968) and later Craig and Fajemisin (1969) showed this type of reaction to be controlled by two linked genes.

Cochliobolus heterostrophus was shown to be heterothallic with the identification of two mating types (Nelson, 1957) and single genes were identified for compatibility, for perithecial formation, ascus development and ascospore formation (Nelson, 1957; 1959).

Observation on the hypersensitivity of maize possessing Texas male-sterile (Tms) cytoplasm was reported in the Philippines by Mercado and Lantican (1961). The extreme susceptibility of Tms cytoplasm maize was observed in Iowa, Illinois, Indiana and Minnesota in the US by Scheifele et al. (1970) and it appeared that two physiological races existed within H. maydis. These races were designated as race"O" and race "T".

The fungus produces a host-specific toxin *in vitro* (Smedegard-Peterson and Nelson, 1969). The race T toxin has been used to differentiate Tms cytoplasm and normal (N) cytoplasm maize, race T being highly virulent on Tms cytoplasm maize and generally mildly virulent on maize with normal cytoplasm while race O does not show any differential pathogenicity for the two kinds of cytoplasm. There are, however, marked differences in resistance among different genotypes of maize in normal cytoplasm. Race T sporulates more abundantly and attacks ears more readily than race O which is rarely associated with rotting of maize ears in normal cytoplasm (Robert, 1956; Burton, 1968). Differences between the two races have also been observed in the leaf symptoms they incite although host genotype may modify lesion type making symptomatological distinction difficult. The 1970 southern leaf blight epiphytotics in the US were incited more by race T or *H. maydis* (Ullstrup, 1970).

Warren *et al.* (1977) made a morphological and physiological differentiation of race O and race T of *H. maydis* on Difco malt agar within 5 days using over 30 isolates of each race. Only race T produced sclerotia on the medium. Race T also hydrolysed some amino acid substrates more rapidly

and to a greater extent than did race O. Calvert and White (1972) did ultrastructural examination of the two races and found that they were quite similar in terms of sub-cellular organization, in wall structure of the conidia and in the occurrence of unidentified, osmophilic bodies in the cytoplasm.

2.2. THE ECONOMIC IMPORTANCE OF SOUTHERN LEAF BLIGHT

The southern leaf blight of maize can be very destructive. Ullstrup and Miles (1957) investigated the effect of the disease on maize. In one case a resistant variety yielded 74 hectolitres per hectare as compared with 7 hectolitres per hectare for a susceptible maize strain. Although the southern leaf blight was first observed in USA in 1923 (Robert, 1953), its development went unnoticed for several years as it moved from south to north until it suddenly reached epidemic proportions which caused near catastrophic losses of maize in 1970. In the southern states of the corn belt there were losses of 50-75% and total losses also occurred in many cases. The epidemic was due largely to the fact that many of the cultivars of maize were developed using one source of male sterility, the Texas source (Chapman and Carter, 1976). Josephson et al. (1971)

showed reductions in Tms cytoplasm maize weights to range from 30-76% in two locations in USA, all yield reductions being significant or highly so. Yield reductions of blended hybrids in relation to normal cytoplasm plants were also similarly significant, the percentage depending on the proportions of Tms plants in the hybrids.

2.3. DESCRIPTION OF THE CAUSAL ORGANISM OF SOUTHERN LEAF BLIGHT

As described by Drechsler (1925) in the conidial stage the conidiophores grow out in groups of two to three or singly on the dead leaf spots. These are olivaceous, septate, 4.5 to 7 x 120 to 170µm with the points of attachment of successive conidia marked by scars on somewhat inconspicuous geniculations. When conditions are sufficiently moist conidiophores may form branching sporophoric filaments often more than 1 mm in length. They produce conidia that are fuliginuous to light, olivaceous in colour, typically curved, widest near the middle and tapering towards the round ends, 10 to 17 x 30 to 115µm, with up to 12 septa (mostly 8). The conidia have thin peripheral walls,

an inconspicuous broad basal scar contained within the rounded contour and germinate by two polar germ tubes. Luttrell (1951) gave conidial measurements as 7 to 12 x 25 to 127µm with 3 to 13 septa (average 15 x 89µm with 7 to 8 septa). Ullstrup (1954) gave an average of 90 x 115µm with 3 to 13 septa. In Israel conidiophore measured about 4 to 5 x 70 to 130µm and conidia 14 to 16 x 42 to 84µm (Kenneth, 1958).

The perithecia which develop on old disintegrating tissue are black, usually early erumpent and often bearing superficial mycelia and conidiophores. They are somewhat ellipsoid and measure about 400 x 400 to 600µm with a well defined asteolate beak about 150µm in length. The asci are numerous, short-stipitate, sub-cylindrical with rounded apices and measure 24 to 28 x 160 to 180µm. They contain 1 to 4 (typically 4) ascospores which are filamentous and thin-walled. When mature the ascospores measure 6 to 7 x 130 to 140µm with 5 to 9 cross-septa often associated with perceptible constrictions. The ascospores are usually arranged in four parallel multiple coils with approximately four turns to each ascospore. They germinate by producing up to 8 germ tubes from any of the constituent cells.

2.4. PHYSIOLOGICAL RACES OF *HELMINTHOSPORIUM MAYDIS* AND DISEASE RESISTANCE

Hooker *et al.* (1970) confirmed the existence of race O and race T of *Helminthosporium maydis* in inoculated field plots in Urbana, USA, and described race T as being specific for certain cytoplams such as the Texas type for male sterility.

Cytoplasm from South America and a few other cytoplasms are also susceptible. It is a weak parasite on resistant plants in the field and infects seedlings more easily. The pathotoxin that it produces in high amounts *in vitro* and *in vivo* is also specific for certain cytoplasms and inhibits root growth in laboratory studies (Hooker *et al.*, 1970). This toxin is probably responsible for the rapid blighting of young seedlings, blighting of leaves in the field and is perhaps associated with the drooping of ears and killing of the plant. Russell (1978) also ascribed the susceptibility to race T of *Helminthosporium maydis* to damage caused by the toxin which that pathogen produces and observed further that maize plants which do not have the Tms cytoplasm can become infected with race T of the pathogen but are not severely damaged

because their cells are not sensitive to the toxin that is produced by this race of the pathogen. Hooker et al. (1970) also reported that race T of the pathogen attacks the leaf, leaf sheath, husk, shank, ear and stalk tissue of the maize plant. It reproduces rapidly on susceptible plants and may have a lower temperature optimum than race O because it spreads rapidly throughout a major portion of the growing season.

In contrast to race T, race O shows little or no specificity to plant cytoplasms and can thus infect a wide range of plant genotypes and cytoplasms. It produces only limited amounts of pathotoxin in vitro and in vivo which is non-specific to plant cytoplams (Hooker et al., 1970; Russell, 1978). It infects the leaves primarily, producing smaller lesions with parallel sides and little chlorosis. The race seems to reproduce less rapidly than race T on susceptible plants.

Resistance to race O is mostly genetic in nature and Hooker et al. (1970) saw no evidence of cytoplasmic effects. Resistance is quantitative in expression with many inbreds expressing few, if any lesions. Genetic studies show that several genetic factors (polygenic inheritance) are

involved in resistance, the genetic action being additive, with the estimated number of genes for resistance being low. Hybrid reaction tends to be more resistant than the average of parental inbred reactions. Resistance to race T is both cytoplasmic and genetic in nature with the cytoplasmic component being of greatest importance. Most normal cytoplasms (not male sterile) give high resistance and this is also true for the G, C, CA, D, EK, F, G, H, I, IA, J, K, L, M, ME, ML, MY, PS, R, RB, S, SD, TA, TC and VG cytoplasms for male sterility. Genetic reaction is expressed in a range of reactions from tolerant to very sensitive and the best one is inferior to cytoplasmic resistance (Hooker et al., 1970). A resistant reaction of maize inbreds with J cytoplasm to race T of H. maydis was also reported by Hilty and Josephson (1971). This reaction was also similar to that of inbreds with normal cytoplasm.

2.5. DISEASE SYMPTOMS

These have been described in detail by Drechsler (1925). On maize leaves Helminthosporium maydis causes cinnamon-buff or purplish spots with darker reddish-brown margins, often delicately

variegated with brownish zonate bands. The lesions become longitudinally elongated, first elliptical, and later becoming long-rectangular. They are usually delimited by the leaf veins and measure 1 to 3 x 5 to 15 millimetres in size. The coalescence of the leaf spots may result in more extensive dead areas. The numerous small leaf spots are easily distinguishable from the fewer but much larger lesions of the northern leaf blight caused by Trichometasphaeria turcica (Passerini) Luttrell. They are less easily distinguishable from leafspots caused by Helminthosporium carbonum Ullstrup which, however, lack the purplish-brown margin that characterizes H. maydis (Ullstrup, 1944). Orillo (1949) also found H. maydis to attack the leaf sheaths, roots, and ear husks of maize, and Robert (1956) reported it as causing blackening of silks and ear tips of maize in Florida. According to Yu (1933) maize ears, leaf midribs, and stems occassionally became infected if wounded. Richardson (1942) isolated the fungus from maize rootlets in Canada, and Orillo (1949) obtained root infections when maize plants were grown in heavily inoculated soil. In culture the fungus produced a slow-acting, thermostable,

metabolic by-product toxic to maize seedlings and to sugar-cane shoots in inoculation experiments.

2.6. THE INFECTION PROCESS

Wheeler (1977) studied the ultrastructure of penetration by Helminthosporium maydis race T of susceptible leaves from Tms plants and resistant leaves from normal plants. Appressoria like structures were present but rare six hours after inoculation and there was no evidence of penetration beneath them. All penetractions seen at this stage took place between epidermal cells, most of them (two-thirds) occurring between cells adjacent to the stomata. The high frequency of penetration in this area may be accounted for by breaks in the cuticle above subsidiary cells of the stomatal apparatus. Eight to ten hours after inoculation, miniature infection cushions had formed at initial points of penetration from which hyphae radiated out beneath the cuticle of epidermal cells. Haustoria-like branches produced by the sub-cuticular hypha appeared to function in secondary infection. Hyphae of the fungus were seen occassionally within stomatal openings (apparent stomatal penetration), but these were

consistently associated with penetration which had occurred between nearby epidermal cells. No differences were observed at any stage of penetration or initial colonization between Tms and normal plants of the same inbred, or among the four inbreds studied suggesting that resistance to this pathogen develops after infection has occurred and that the selective pathotoxin produced by race T of H. maydis does not play an important role in the infection process.

2.7. DISEASE PHYSIOLOGY

Calvert and White (1972) observed an intercellular matrix to develop only in normal cytoplasm male fertile lines infected by either race O or T of Helminthosporium maydis between necrotic and healthy cells. Cytochemical and ultrastructural examinations respectively showed the matrix to be proteinaceous and fibrous.

Bhullar et al. (1975) observed that a toxin produced by H. maydis caused inhibition of dark fixation of $^{14}CO_2$ by leaf discs of susceptible Tms maize. Watrud et al. (1975) showed mitochondria from Tms and normal versions of maize to have differential sensitivity to toxins produced by race T

of H. maydis and suggested the presence of a permeability difference between the outer membranes of the Tms and normal mitochondria which affects the passage of toxins. The greatest toxin binding was associated with the inner membrane of Tms mitochondria.

Bednarski et al.(1977)found that the host selective toxin from H. maydis race T inhibited oxidative phosphorylation (AT ^{32}P formation) and stimulated ATPase activity by mitochondria from Tms but not from normal cytoplasm maize. He also got data which supported the hypothesis that the toxin affects a mitochondrial site in intact tissue.

2.8. EPIDEMIOLOGY

Warren (1976) studied the effects of temperature on lesion development and sporulation on maize after infection by race O and T of Helminthosporium maydis. Spore production by race T was sensitive to temperature whereas spore production by race O was less sensitive i.e. at 15^{o}C few race T spores were produced. The greatest number of spores of both races were produced at 30^{o}C. Race T,however, produced five times more spores at 22.5^{o}C and twice as many at

30°C than race O. More lesions formed and expanded more rapidly at 30°C than at 22.5 on 15°C.

Larsen et al. (1973) observed that fewer lesions formed on Tms or normal cytoplasm maize seedlings with less than 4 hours of high humidity in a moist chamber after infection by race O and race T of H. maydis. Lesion size increased in nearly exponential fashions on maize in a moist chamber from 4 to 8 hours and after 8 hours the rate of lesion number increase was greatly reduced. Nelson and Tung (1973) studied the colonization of a susceptible maize hybrid by an isolate of race T of H. maydis and observed that lesion size increased essentially in a straight line relationship with increasing dew and colonization temperatures and increasing dew periods. Fluctuating day/night temperatures retarded lesion expansion as compared with a constant temperature near the mean of the day/night temperatures. A lower relative humidity during colonization markedly reduced lesion size. Lesion expansion was slowed measurably when plants were removed from dew periods for infection to lighted growth chambers as compared with darkened

chambers.

Schenk and Stelter (1973) observed in Florida in the US that the southern leaf blight of maize became more severe on field maize when minimum temperatures were 20°C or above and relative humidity was 100% for 6 hours or more on several consecutive nights indicating that warm, moist weather favours southern leaf blight epidemics. Disease severity and rate of development were greater on Tms than on normal cytoplasm hybrids indicating that race T of the pathogen was predominant.

The effects of leaf age and leaf position on the development of southern maize leaf blight were studied by Nelson and Gabrielle (1973) who observed that the extent and rate of colonization were greatest on lower leaves and both decreased proportionately with increasing leaf height. There was less colonization and sporulation on the 6th leaf of a six-leaf-plant than on the 6th leaf of a twelve-leaf-plant, an indication that the physiological age of the leaf may be an important factor in disease development. The occurrence of disease and sporulation on lower leaves within the canopy may partly account for the greater spread

of disease from plant to plant rather than from one point to another.

Summer and Littrell (1974) reported the influence of tillage, planting date, inoculum survival and mixed populations on the epidemiology of the southern leaf blight of maize. Late planted maize had a greater number of lesions than early planted maize. In mixed populations of Tms and normal cytoplasm plants yield losses were directly proportional to the percentage of Tms plants. Race T of H. maydis survived more abundantly in residues from Tms plants and there were significantly more lesions on Tms plants in disced than in ploughed sub-plots but no significant yield differences occurred.

2.9. SURVIVAL OF THE PATHOGEN

In China conidia of Helminthosporium maydis remained viable for eight months on maize leaves and for over a year on seed kept in the laboratory (Yu, 1933). Nelson (1970) studying the overwintering and survival of H. maydis isolates observed that the one most fit to survive parasitically was least fit to survive in overwintered maize foliage. He also observed that relative

overwintering capacities were influenced by host substrate and that *in vitro* races O and T could survive periods of freezing. The most susceptible maize hybrid was the least compatible overwintering substrate for race T of *H. maydis*. Periods of wetting and drying resulted in a rapid decrease in the survival of this same race (T) of the pathogen and both races O and T often disappeared from tissues after 3 or more cycles. Littrell and Summer (1971) assessed the survival of *H. maydis* using the infectivity (based on the number of lesions on inoculated plants) of southern leaf blight-damaged field maize residue under natural and artificial conditions. Their results showed that infectivity was greatest from leaf and shuck residue; although more total *Helminthosporium* conidia were observed in filtrates from stalk tissue.

2.10 HOSTS OF *HELMINTHOSPORIUM MAYDIS*

Under field conditions, maize, teosinte and sorghum have been reported to be susceptible to *H. maydis* (Tar, 1962). and the tropical grass *Rottboellia exaltata* have been observed to become infected when artificially

inoculated (Orillo, 1952). Nisikado and Miyake (1926) found only Setaria glauca to be mildly attacked by H. maydis following their inoculation of 21 genera and 25 species of the Gramineae. Alice Robert (1962) also using artificial inoculation reported 31 grasses to be infected including wheat, barley and oats. Following artificial inoculation under greenhouse conditions, Nelson and Kilne (1961) reported 15 species in 14 genera of the Gramineae to be infected by H. maydis. It would seem that these inoculations were done with race O of the fungus. In inoculation experiments done at Purdue University, Indiana, USA, on 22 wild and cultivated grasses and 10 genotypes of Sorghum bicolor using two isolates of race T and one of race O no infections were observed while maize seedlings inoculated in the same test were severely infected. These inoculation experiments and other observations made on a large number of sorghum species genotypes and other genera of the Gramineae, including volunteer wheat and oats growing in close proximity of severely infected maize in Indiana during the 1970 US epiphytotic on maize seem to suggest that sorghums, the volunteer cereals or the wild grasses are not highly susceptible to H. maydis and may not function

in any important way in the epidemiology of the disease that it causes.

2.11. DISEASE CONTROL

Kumar *et al.* (1976) reported *in vitro* studies with *Helminthosporium maydis* and *H. turcicum* (*Trichometasphaeria turcica* which proved the efficiency of Dithane Z-78, Dithane M-45, Unizeb and Cuman with complete inhibition of fungal growth. The disease was effectively controlled by 3 sprays of the fungicides, which also increased yield. The copper fungicides Miltox and Fylolan proved rather phytotoxic. Vidhyasekaran *et al.* (1976) presented a paper at a plant protection symposium in which they stated that Captan and Dithane M-45 gave the best control of *H. maydis* but did not significantly increase yield. Yield was reduced only when maize was infected at 30 and 45 days. Late infection though severe, had no such effect.

Various maize inbred lines show resistance (Ullstrup, 1943). Resistance is believed to be partially dominant to susceptibility with more than one pair of factors involved, these factors being additive and complementary.

Tarr (1962) suggests that crop sanitation to reduce seasonal carry over on infected crop residues and alternate host plants and suitable crop rotations which would avoid growing susceptible crops on land which carried infected crop the previous season can also lead to effective control of the disease.

CHAPTER 3

3. MATERIALS AND METHODS

3.1. DISEASE SURVEY

Disease surveys were done through trips made to Central, Eastern and Western Provinces of Kenya between October 24 and December 11, 1980. The surveys were carried out by traversing districts within the three provinces through minor routes running through farming areas. Fields were selected by driving along the road and stopping every 20 to 30 km and examining maize fields on either side of the road. Disease incidence within each field was determined by selecting 3 maize rows at random, counting the numbers of infected and healthy plants and computing the percentage of diseased plants. Intensity was determined by examining the infected plants with respect to lesion size, abundance and distribution on the plants. A scale grouping the intensity into 6 types was defined. The six disease intensity types and their descriptions are given below.

INTENSITY	DESCRIPTION
0.5	Very slight infection; one or two restricted lesions on lower leaves.

Intensity	Description
1	Slight infection; a few scattered lesions on lower leaves.
2	Light infection; moderate number of lesions on lower leaves.
3	Moderate infection; abundant lesions on lower leaves, few on middle leaves.
4	Heavy infection; lesions abundant on lower and middle leaves extending to upper leaves.
5	Very heavy infection, lesions abundant on all leaves; plants prematurely killed.

Diseased leaves were also collected and preserved by drying between blotting papers in a wooden press for further examination in the Plant Pathology laboratory at the Faculty of Agriculture, Kabete Campus.

3.2. ISOLATION AND IDENTIFICATION OF THE PATHOGEN

Diseased leaves bearing symptoms similar to those incited by *Helminthosporium maydis* Nisikado and Miyake and collected from Gaitu and Nithi locations in Meru District and Mwala location in Machakos District were placed on moistened

filter papers in petri-dishes and placed in an incubator at 25°C to encourage growth and sporulation of the pathogen. The leaf pieces were first surface sterilized in 70% ethyl alcohol and rinsed in sterile distilled water before placing in the petri-dishes. Isolations were done in a sterile room after five days of incubation to obtain pure cultures. Under the high power of a binocular microscope surface-sterilized fine glass needles were used to pick single spores (conidia) that were used to inoculate petri-dishes containing corn meal agar as a growth medium. The spores produced on the leaf pieces were also examined under the microscope. The pure cultures that grew were kept for 10 days and spore suspensions made from them by washing the surface of the sporulating cultures with sterile distilled water. The spore suspensions were used to inoculate two-week old Katumani Composite maize seedlings by pouring about 10 mℓ of the spore suspensions into the leaf whorls or "funnels" of the plants to study symptoms. One week from the time of appearance of symptoms the fungus was re-isolated from the diseased leaves and examined under the microscope. Symptoms of the disease were also compared with those of Trichometasphaeria

turcica on the same variety of maize. Pure cultures of the fungus from all the three locations were also sent to the Commonwealth Mycological Institute for a confirmation of the identification of the fungus.

3.3. CHARACTERISTICS OF THE PATHOGEN

3.3.1 COLONIAL CHARACTERISTICS

A study of the morphology of the 3 isolates of Helminthosporium maydis was carried out on 3 different growth media that included potato dextrose agar, corn meal agar and Czapek Dox agar to determine whether the colony habits of the 3 isolates were similar. Monoconidial colonies of the isolates were grown on these media in petri-dishes from single spores transferred from the pure cultures obtained in 3:2. All the petri-dishes were kept at room temperature ($20^{o}C$). Observation of the fungal colonies were made every 7 days for 4 weeks from the time of inoculation of the plates. The morphological characteristics examined were colony shape in section, texture, zonation and the formation of sporocarps or characteristic resting structures such as micro-sclerotia or chlamydospores. Other characteristics associated with fungal colonies such as colour, odour and the diffusion of pigments into a growth

medium were also examined. Colour was examined both from above and from the reverse of the plates.

3.3.2. CONIDIAL CHARACTERISTICS

3.3.2.1. CONIDIAL COLOUR, SIZE, NUMBER OF SEPTA, LENGTH AND WIDTH

Conidia of Helminthosporium maydis were scraped from the sporulations on lesions on diseased leaf bits obtained in 3.2 and examined under the microscope for colour, shape, number of septa, length and width. With respect to the length and width of the conidia, for each isolate of the fungus 100 randomly chosen conidia were measured with an eyepiece micrometer in a 10 x ocular with a 40 x objective. The greatest diameter of the conidium was taken as the width. The numbers of septa were also counted for all the 100 randomly chosen conidia of each isolate.

3.3.2.2. MODE OF GERMINATION OF THE CONIDIA ON CORN MEAL AGAR AND ON THE HOST PLANT

Fourteen-day old cultures of the three isolates of the fungus (Gaitu, Mwala and Nithi isolates) grown on corn meal agar were flooded with sterile distilled water to obtain conidia.

The floating conidia on the water surface were then collected with a bacterial transfer loop and used to inoculate the growth medium (corn meal agar) in petri-dishes by touching the centre of the plates with the loop. Each isolate was used to inoculate three plates. The petri-dishes were left at room temperature (20°C). Observations for conidial germination were made 18 hours after inoculation under a binocular microscope. Photomicrographs of the germinating conidia were also taken.

To determine the mode of germination on the host plant, three-week old Katumani Composite maize plants were used. The leaves were inoculated by brushing with a camel's hair brush dipped in a conidial suspension of Gaita Isolate also obtained by flooding fourteen-day old cultures of the isolate grown on corn meal agar. The pots of inoculated plants were placed into plastic containers with about an inch of water and all covered with plastic bags before placing on a laboratory bench. At intervals of 6, 18, 30 and 48 hours from inoculation, inoculated leaves were cut into several squares dropped into vials containing Farmer's fluid and the times marked on the vials.

Twenty four hours after leaf materials had been added to the final vial containing Farmer's fluid, the materials were transferred to vials containing lactophenol. After another 24 hours the materials were removed from lactophenol and transferred to vials containing acid fuchsin stain. When the fungus tissue was satisfactorily stained the materials were returned to the lactophenol vials for a brief rinsing to remove excess stain from the leaf tissue. The materials were finally mounted in 50% glycerin and examined under the microscope. Photomicrographs of the germinating conidia and structures associated with the host-pathogen relationship were taken.

3.4 EFFECT OF TEMPERATURE ON GROWTH OF THE FUNGUS

To determine the effect of variations in temperature on colony growth rate monoconidial cultures of the three isolates of the fungus were grown on corn meal agar at five different temperatures: 15, 20, 25, 30 and 35^{o}C. Five plates per isolate were incubated at each temperature. The growth of the fungal colonies measured as colony diameter in millimetres were

evaluated 10 days from inoculation. The cultures were also examined for the formation of any fructifications or resting structures at the five temperatures.

3.5 EFFECT OF pH ON GROWTH OF THE FUNGUS

This experiment was carried out using the semi-synthetic medium Czapek Dox agar. One litre portions of the medium in distilled water were autoclaved for 15 minutes at a pressure of 1.01325×10^6 dynes/cm^2 (approx. 15 lb./in^2) and appropriate amounts of 10% lactic acid added to each portion to obtain pH values of 3, 4, 5, 6, and 7. Inoculation of the plates containing the medium at the various pH values were done with single spores of the three isolates of the fungus. At each pH value 5 petri-dishes of the medium were inoculated with each isolate. The monoconidial cultures were then allowed to grow for 10 days at room temperature (20^{o}C) and growth evaluated as colony diameter in millimetres.

3.6. EFFECT OF TEMPERATURE ON CONIDIAL GERMINATION

Efficiency of spore germination was evaluated for all the 3 isolates of the fungus at four different temperatures. Spore (conidial) suspensions

were prepared by washing 14-day old cultures with sterile distilled water and standardizing concentration to 2.5 x 10^6 spores/mℓ. Four drops of a suspension of each isolate were placed separately on a chemically sterile microscope slide, dried and placed at 15, 20, 25 and 30^{o}C in incubator. One slide of each isolate was placed at each temperature. Counts of the number of germinated conidia were made 14 hours later under a binocular microscope.

3.7. EFFECT OF RELATIVE HUMIDITY ON CONIDIAL GERMINATION

Spore suspensions of the three isolates of the fungus were made as described above for effect of temperature on germination. Four drops of a spore suspension of each isolate were placed separately on each of five chemically sterile glass slides, dried and placed in dessicators maintaining relative humidities of 60, 70, 80, 90 and 100%. These various relative humidities were obtained by mixing appropriate amounts of water and concentrated sulphuric acid in each dessicator. The mixtures by volume of water and sulphuric acid maintaining the relative humidities of 100, 90, 80, 70 and 60% were approximately in the ratios

0:100, 1:4, 1:3, 1:2 and 1:1.5 respectively. Three slides each bearing conidia of a particular isolate were placed in each dessicator. All the dessicators were placed in an incubator at 25°C. Counts of germinated and ungerminated conidia at the five relative humidity values were done 16 hours later under the binocular microscrope.

3.8. INVESTIGATION ON THE FORMATION OF THE ASCIGEROUS (SEXUAL OR PERFECT) STAGE OF THE FUNGUS

Adopting the method used by Nelson (1957), small mycelial pieces of the three isolates of the fungus from monoconidial cultures were placed on opposite sides of a section of sterilized dry maize leaves in pairs in all 6 combinations in the centres of petri-dishes containing corn meal agar. In three of the combinations each isolate was paired with itself and in the other three each isolate was paired with another isolate. Each pairing was replicated four times. The maize leaf sections were sterilized by autoclaving in a portable autoclave for 15 minutes at a pressure of 1.01325×10^6 dynes/cm^2 (approx. 15 lb./in^2). The sections measured approximately 6 x 6 centimetres. All the matings were incubated at 24°C for the

first 7 days and then placed at 18° and 30°C while others were left at 24°C throughout the experiment. Observations of the cultures were made under the binocular microscope at weekly intervals for 6 weeks.

3.9. REACTIONS OF 7 MAIZE HYBRIDS AND ONE COMPOSITE TO INFECTION BY *HELMINTHORPORIUM MAYDIS* IN FIELD EXPERIMENTAL PLOTS AT KABETE.

The experiment was located at Kabete Field Station of the Faculty of Agriculture, University of Nairobi. The station is about 9 kilometres north-west of Nairobi City and the altitude varies from 1777 to 1854 metres. It lies between latitudes 1° 14' 20" S and 1° 15' 15" S and between longitudes 36° 44' and 36° 45' 20" E (Wamburi, 1973). The mean annual rainfall potential at the station is 925 mm and the annual potential evapotranspiration is 1363 mm (Brown and Cochene, 1969; Wamburi, 1973). The rainfall distribution is bimodal with the long rains from late March to June and the short rains from late October to December. The soil at the station is a deep red latosol.

The experiment was begun during the long rains in 1981 but there was some drought in the middle of the season that apparently had some effect on disease development in the field experiment.

The seed source for the experiment was the Nyanza Agricultural Research Station, Kisii, in Western Kenya. The seeds used were those of hybrids 511, 512, 612, 613C, 614C, 622, 632, and Katumani Composite which were given a seed dressing of copper oxychloride before planting. Seedbed preparation was done by ploughing the land and later disc harrowing to give a fine seedbed. In the previous season (short rains) the land had been planted to Irish potatoes. The area of this land used for the experiment measured 50 by 25 metres. The design of the experiment was the completely randomized block design consisting of three blocks with each divided into 24 plots. The plots consisted of 3 three-metre rows of maize hybrids or composite. Two seeds were planted per hole and spacing was 0.6 metres between rows and 30 centimetres within rows. Planting was done on 14th April, 1981. Diammonium phosphate fertilizer (15% N) was applied in each planting

hole at a rate of about a table-spoonful to boost the nitrogen level of the soil. Three plots of each maize hybrid/composite were planted per block. Hand weeding was done at 3 weeks and later at 1½ months from planting. Inoculum was prepared by washing 14-day old cultures of the isolates of the fungus on corn meal agar in petri dishes with distilled water, homogenising the conidial suspensions in a Waring blendor,and adjusting spore concentration to 2.5×10^6 spores/mℓ. Inoculation of the plants was done 38 days from planting by pouring about 10 mℓ of a spore suspension of the fungus isolates into the leaf whorls or "funnels". All the plants in the middle row of each plot were inoculated and all 7 maize hybrids and the one composite were tested for their reaction against all the three isolates of the fungus. Observations on symptom development were begun 24 hours from inoculation and continued daily for the first week and later at weekly intervals for four weeks. Measurements of lesion size on the leaves of each hybrid or composite was done 2 weeks from inoculation as an indication of the virulence of the isolates of the fungus. For each plot an inoculated plant was selected from the middle of a row and in each case for the purpose of uniformity lesion size was measured on the seventh leaf using

a portable area meter (Ali-car, Model LI-3000, Lambda Instruments Corporation). Fifteen lesions were measured per leaf and the average lesion area computed.

3.10. REACTIONS OF 7 MAIZE HYBRIDS AND 1 COMPOSITE TO INFECTION BY HELMINTHOSPORIUM MAYDIS IN THE GREEN HOUSE.

The same hybrids and composite as in the field experiment were used in this experiment. The seed source and treatment before planting were also the same as those for the field experiment. The maize plants were grown in pots. The soil mixture consisted of coffee hulls (compost), sand, 0.6 cm ballasts and forest soil in the volume ratios 2:1:1:2 respectively. Diammonium phosphate fertilizer (15% N) was incorporated into the mixture at a rate of 1509 grams for every volume mixture of 4 buckets (tins) of coffee hulls, 2 of sand, 2 of 0.6cm ballasts, and 4 of forest soil. The soil was put in standard 24 cm plastic pots. All together 72 pots were planted, 9 to each hybrid or composite of maize and arranged to form a completely randomized block design with three blocks. The plantings were thinned to two plants per pot four weeks from planting. Inoculation of the plants was also done 38 days from

planting with the three isolates of Helminthosporium maydis. In each block a pot of each maize hybrid or composite was inoculated with a particular isolate of the fungus. Inocula were obtained as for the field experiment. Humidity control could not he exact since it was only controlled by the evaporation of water from gunny bags soaked in water and used to cover a wooden frame built over the potted plants for 48 hours. The bags were watered twice a day to keep them very moist. Observations of symptom development were begun one day from inoculation and continued daily for the first week and later weekly for four weeks. At inoculation plant heights were measured in centimetres and these measurements were continued at weekly intervals for three weeks. In each block the same randomly chosen plant of each hybrid or composite inoculated with a particular isolate of the fungus was measured throughout the experiment. Virulence of the isolates on all the hybrids and composite was also assessed by determining average lesion size as for the field experiment two weeks after inoculation. One plant was chosen per pot and the average lesion size of 15 lesions on the seventh leaf determined.

3.11. HOST RANGE

Thirteen grass species belonging to twelve genera and one sedge species were used in this study. They were grown in plastic bags in a soil mixture similar to that used in the greenhouse experiment described earlier. Triticum aestivum, Hordeum vulgare, Avena sativa, and Sorghum vulgare were grown from seed while Eragrostis pilosa, Digitaria malanjiana, Bothriochloa julcupta, Setaria sphacelata, Setaria verticillata, Pennisetum trisetum, Panicum maximum Eleusine indica, Rottboellia exaltata, and Cyperus rotundus were propagated vegetatively from stem cuttings. Three pots were grown with each species and each pot had on average four plants. The plants were inoculated one month from propagation with conidial suspensions of the isolates of Helminthosporium maydis prepared as for the inoculations in the field and greenhouse maize experiments. The spore suspensions were atomized onto the plants using a small hand atomizer. Each pot of a species was inoculated with a particular isolate of the fungus. The pots were heavily watered and covered with polythene bags for 48 hours to ensure a high relative humidity. Observations for disease symptoms were begun 24 hours

from inoculation and continued daily for a week. Re-isolation of the pathogen from infected leaves was also carried out in the end.

CHAPTER 4

4. RESULTS

4.1. DISEASE SURVEY

During the disease survey, the southern leaf blight was recorded on maize only in the Eastern Province of Kenya in three locations, one in Machakos District and two in Meru District. In the fields which had the disease, maize was planted in early October, 1980. The incidence and severity of southern leaf blight in these locations are shown in Table 2. Diseased leaves showing symptoms characteristic of southern leaf blight were collected for further studies. Symptoms of the disease are compared with those of northern leaf blight of maize in Plate 1.

4.2. ISOLATION AND IDENTIFICATION OF THE PATHOGEN

Conidia from diseased materials collected from Gaitu, Mwala and Nithi were morphologically similar and caused identical symptoms on the Katumani Composite maize seedlings. Symptoms of infected Katumani Composite seedlings were also similar to those produced on the field infected plants.

TABLE 2. OCCURRENCE OF THE SOUTHERN LEAF BLIGHT OF MAIZE IN 3 LOCATIONS IN THE EASTERN PROVINCE OF KENYA.

LOCATION AND DISTRICT	FIELDS		DISEASE INCIDENCE	DISEASE* SEVERITY
	CHECKED	INFECTED		
Gaitu Location, Meru District	3	1	<5%	2
Mwala Location, Machakos District	5	1	<5%	1
Nithi Location, Meru District	4	2	<5%	2

*Disease severity was assessed using the intensity scale defined on pages 28 & 29.

Plate 1: Leaf blights of maize. (A) lesion of northern leaf blight caused by Trichometasphaeria turcica, and (B) lesions of southern leaf blight caused by Helminthosporium maydis.

Further microscopic examination of the fungal spores re-isolated from the inoculated maize seedlings showed that they were morphologically similar to the spores used in the inoculation of the seedlings. In all cases the spores were further shown to be similar to those of Helminthosporium maydis Nisikado and Miyake = Drechslera maydis (Nisikado) Subram. and Jain. The Commonwealth Mycological Institute (CMI) report on the fungal specimens sent there confirmed this identification.

4.3. CHARACTERISTICS OF THE PATHOGEN

4.3.1. COLONIAL CHARACTERISTICS

At room temperature (20°C) in the laboratory morphological characteristics for the three isolates from Gaitu, Mwala and Nithi locations were similar. On potato dextrose agar, colony shape in section was roughly convex. On both corn meal agar and Czapek Dox agar the shape in section was at first flat later becoming irregular with the growth of mycelial tufts from the surface especially on Czapek Dox agar. On corn meal agar, coarse, wiry aerial mycelium that closely adhered to the medium surface grew while on Czapek Dox agar and potato dextrose agar there was a flush, smooth, wiry

aerial mycelium that was matted. No zonation was observed in cultures. The colour of the cultures of the 3 isolates varied with the medium and age of culture from almost white to charcoal grey. On Czapek Dox agar the colour of the culture was at first almost white, later turning light grey and finally charcoal grey. On potato dextrose agar culture were generally a light grey in colour, while on corn meal agar cultures produced were charcoal grey. On both corn meal agar and potato dextrose agar culture colour variation with age was minimal. On all the media and for all the isolates the culture colour from the reverse of the plate was bluish-black. No diffusion of pigments into the medium was observed in all cases although hyphal masses growing into the medium surface appeared as pigments diffusing into the medium. A mushroom odour was produced by the cultures. With the media used, no sporocarps or characteristic resting structures such as chlamydospores or micro-sclerotia were observed even after a period of four weeks of culture growth at room temperature in the laboratory. Chlamydospore formation was, however, observed in bacterium-contaminated cultures along zones of anti-biotic activity between the

fungus and bacterium. Chlamydospores of the fungus are shown in Plate 2.

4.3.2. CONIDIAL CHARACTERISTICS

The conidia were distinctly curved, fusiform, and pale to somewhat dark golden brown in colour. A comparison of the conidia with respect to curvature is shown in Plate 3. Length, width and septation among 100 spores of each isolate selected at random are presented in Table 3. These data are compared by means of frequency polygons in Figures 2, 3 and 4. There were no significant differences among the mean spore lengths of the three isolates. The mean spore widths of the isolates also showed no significant differences. Mean number of septa of the isolates were also not significantly different.

4.3.3. MODE OF GERMINATION OF THE CONIDIA ON HOST PLANT AND ON CORN MEAL AGAR

Observations made under the binocular microscope 18 hours from inoculation of the petri-dishes containing corn meal agar showed that conidia of the three isolates of Helminthosporium maydis germinated by producing two polar germ tubes.

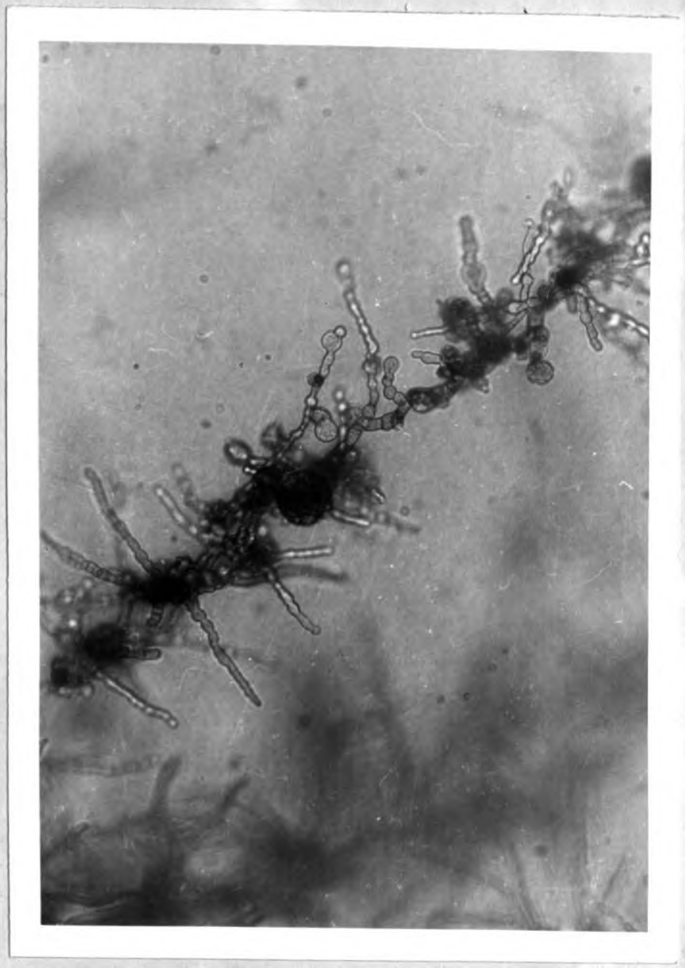


Plate 2: Photomicrograph of chlamydospores of Helminthosporium maydis formed on corn meal agar medium after 10 days' incubation at 20°C (magnification x 200).

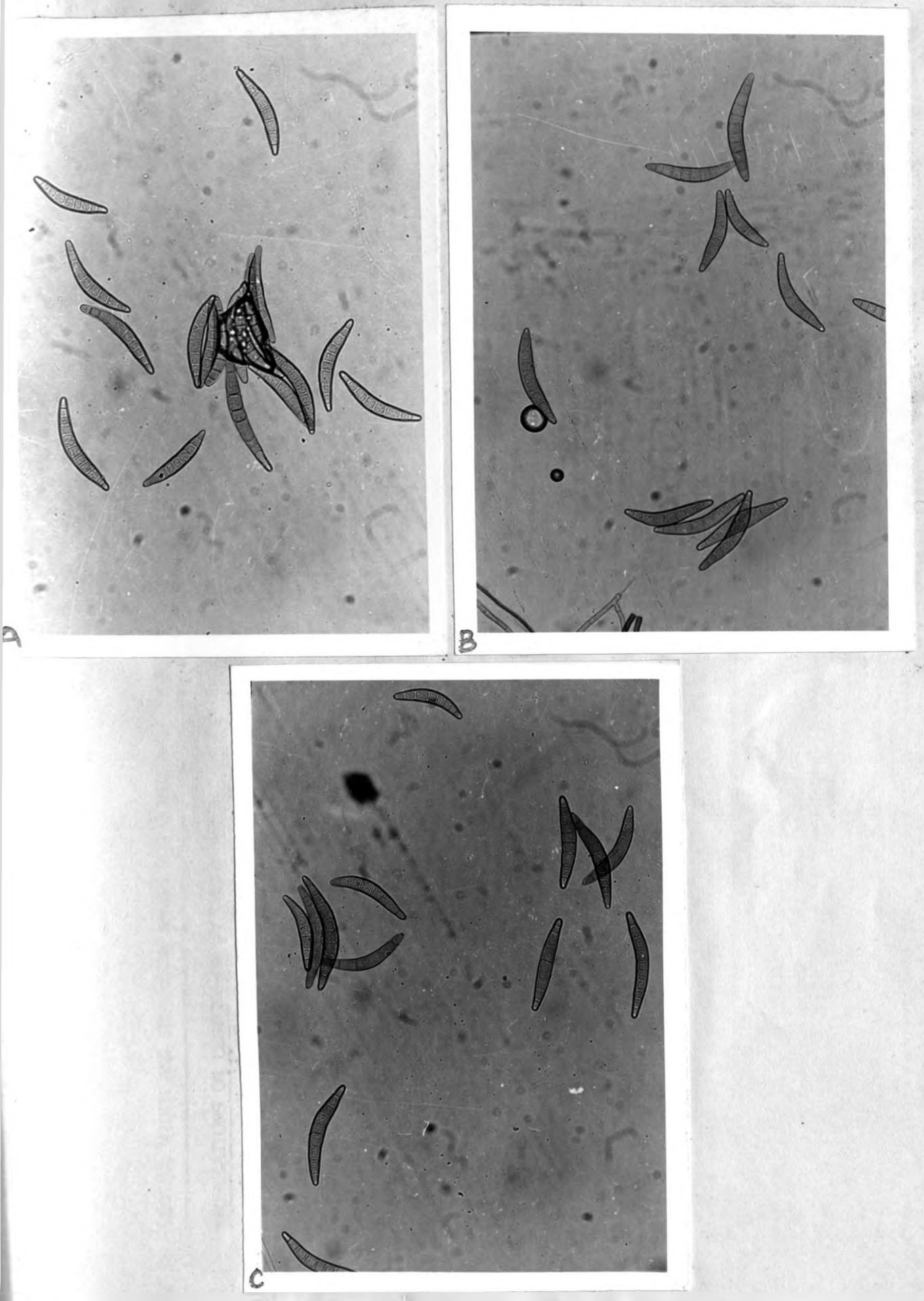


PLATE 3: Photomicrographs of conidia of three isolates of Helminthosporium maydis (magnification x 200). (A) Gaitu isolate (B) Mwala isolate and (C) Nithi isolate.

TABLE 3. LENGTH, WIDTH AND SEPTATION AMONG 100 CONIDIA OF THREE ISOLATES OF *HELMINTHOSPORIUM MAYDIS* OBTAINED FROM SPORULATIONS ON LESIONS ON DISEASED MAIZE.

ISOLATE	SPORE LENGTH IN µm			SPORE WIDTH IN µm			SEPTA		
	Range	Mean & Standard Deviation (SD)	CV	Range	Mean & Standard Deviation (SD)	CV	Range	Mean & Standard Deviation (SD)	CV
Gaitu	27-100	74.59 ± 13.14	17.61%	7-20	13.25 ± 2.17	16.38%	3-10	7.21 ± 1.28	17.75%
Mwala	15-103	71.43 ± 19.40	27.16%	7-18	12.87 ± 1.64	12.74%	3-10	6.78 ± 1.76	25.98%
Nithi	30-93	73.44 ± 15.56	21.18%	7-18	13.22 ± 2.09	15.81%	4-10	7-11 ± 1.29	18.14%

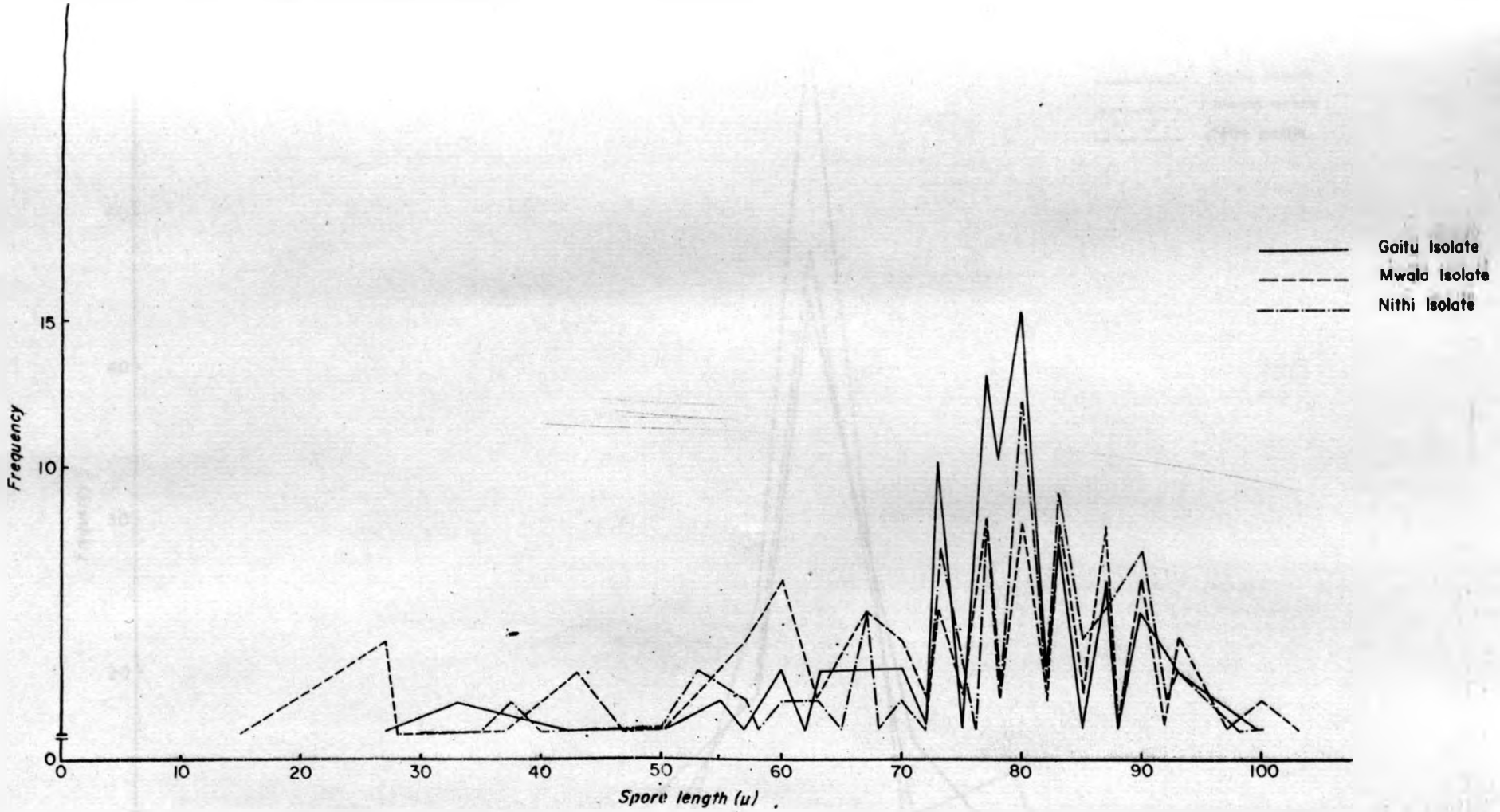


Fig. 2 Frequency distribution of spore(conidial) length of three isolates of Helminthosporium maydis

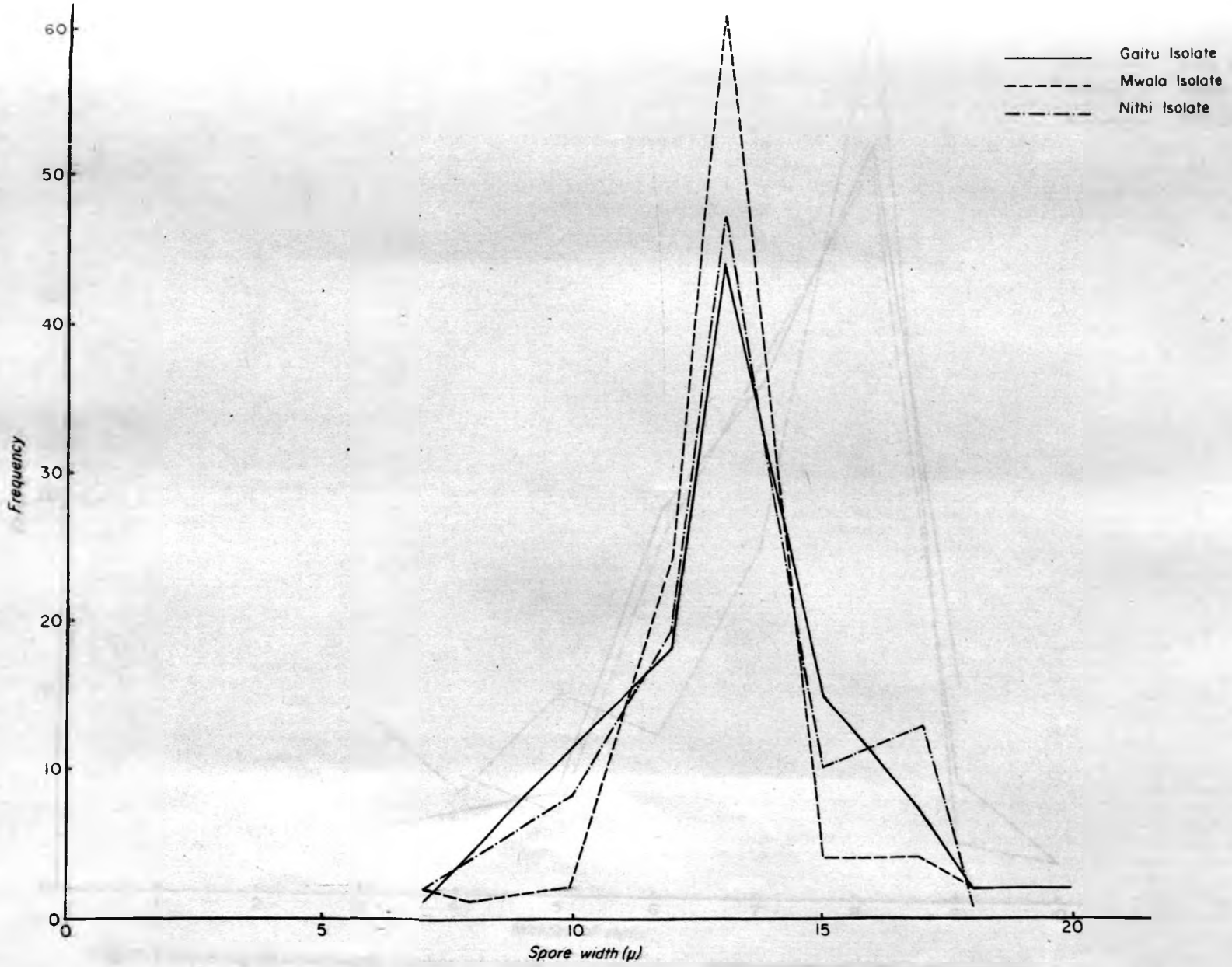


Fig. 3 Frequency distribution of spore widths of three isolates of Helminthosporium maydis

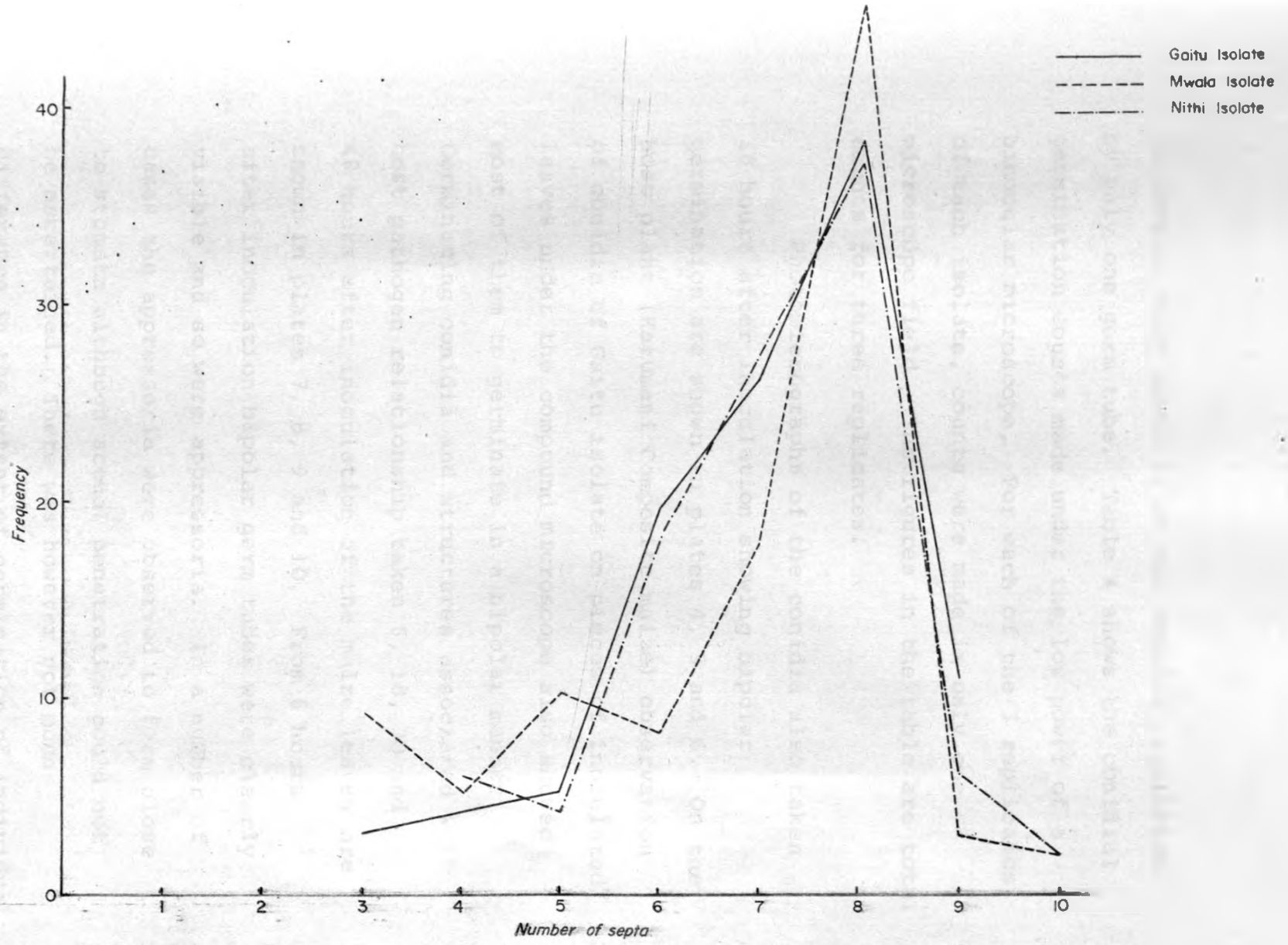


Fig. 4 Frequency distribution of number of septa of spores of three isolates of Helminthosporium maydis

In general only about 6% of the conidia germinated by only one germ tube. Table 4 shows the conidial germination counts made under the low power of a binocular microscope. For each of the 3 replicates of each isolate, counts were made in only one microscope field. The figures in the table are total counts for three replicates.

Photomicrographs of the conidia also taken 18 hours after inoculation showing bipolar germination are shown in plates 4, 5 and 6. On the host plant (Katumani Composite maize) observation of conidia of Gaitu isolate on pieces of inoculated leaves under the compound microscope also showed most of them to germinate in a bipolar manner. Germinating conidia and structures associated with host pathogen relationship taken 6, 18, 30 and 48 hours after inoculation of the maize leaves are shown in plates 7, 8, 9 and 10. From 6 hours after inoculation bipolar germ tubes were clearly visible and so were appressoria. In a number of cases the appressoria were observed to form close to stomata although actual penetration could not be ascertained. There was however not much difference in the extent of germination of individual conidia at 6, 18, 30 and 48 hours although most

TABLE 4. CONIDIAL GERMINATION COUNTS

Isolate	Number showing bipolar germination	Number showing (uni) polar germination	Number not germinated
Gaitu	67	4	8
Mwala	74	2	3
Nithi	65	7	5

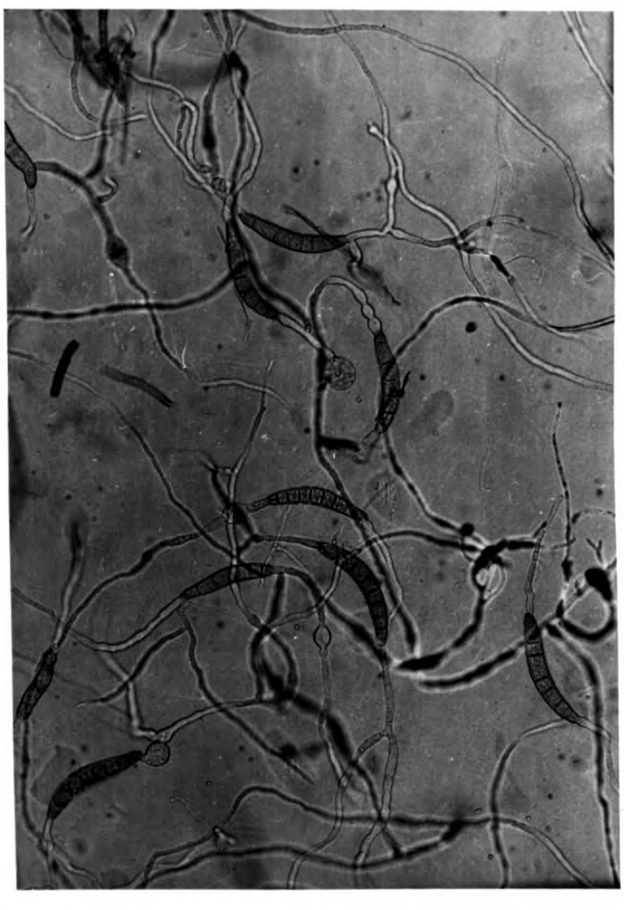


PLATE 4: Photomicrograph of germinating conidia of Gaitu isolate of Helminthosporium maydis on corn meal agar medium 18 hours after inoculation (magnification x 200).

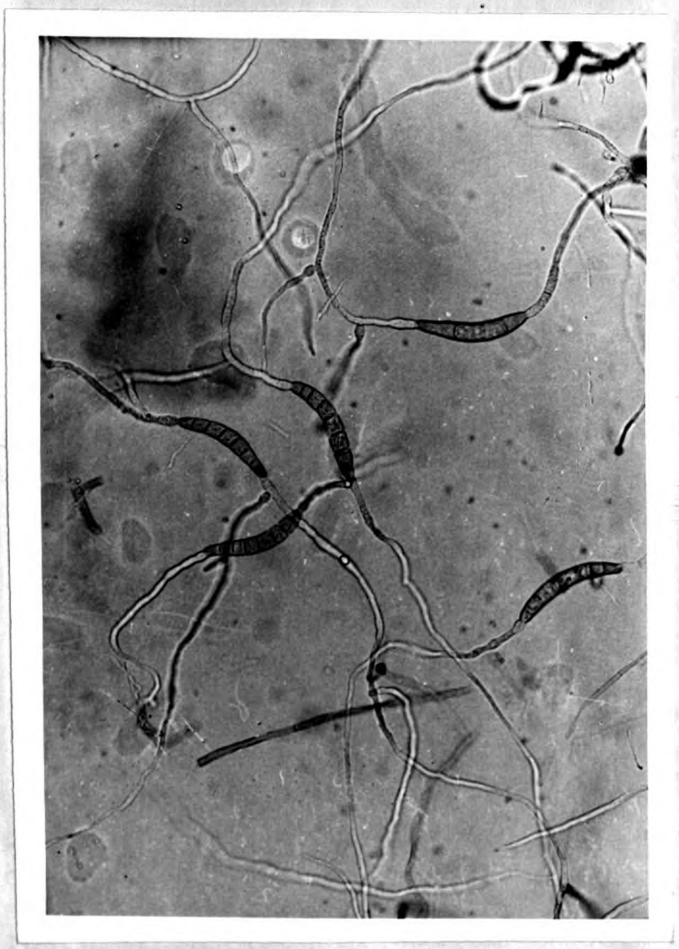


PLATE 5: Photomicrograph of germinating conidia of Mwala isolate of Helminthosporium maydis on corn meal agar medium 18 hours after inoculation (magnification x 200).

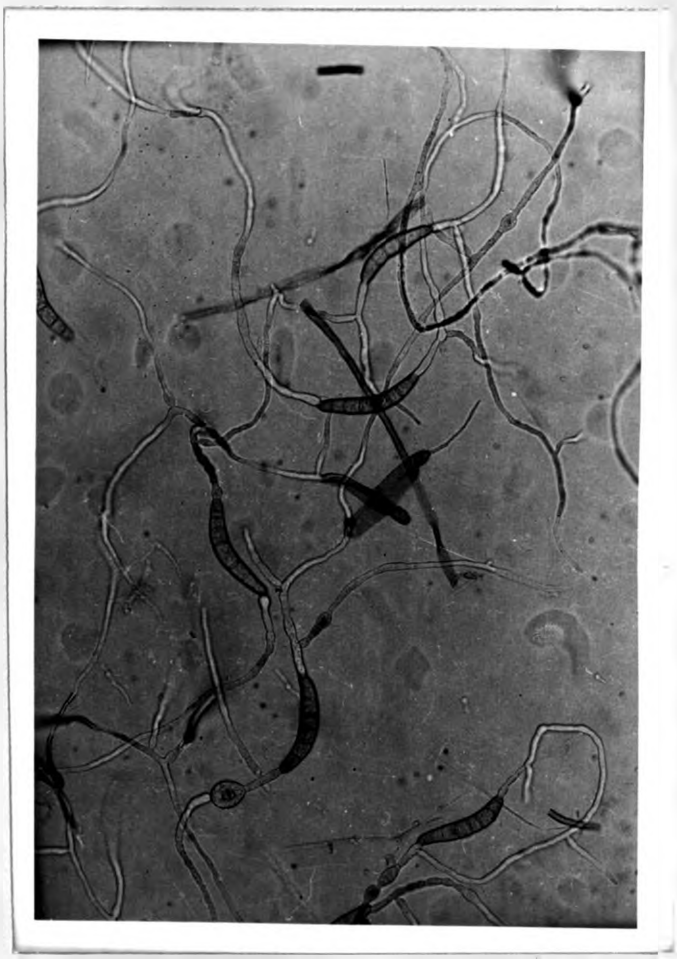


PLATE 6: Photomicrograph of germinating conidia of Nithi isolate of Helminthosporium maydis on corn meal agar 18 hours after inoculation (magnification x 200).

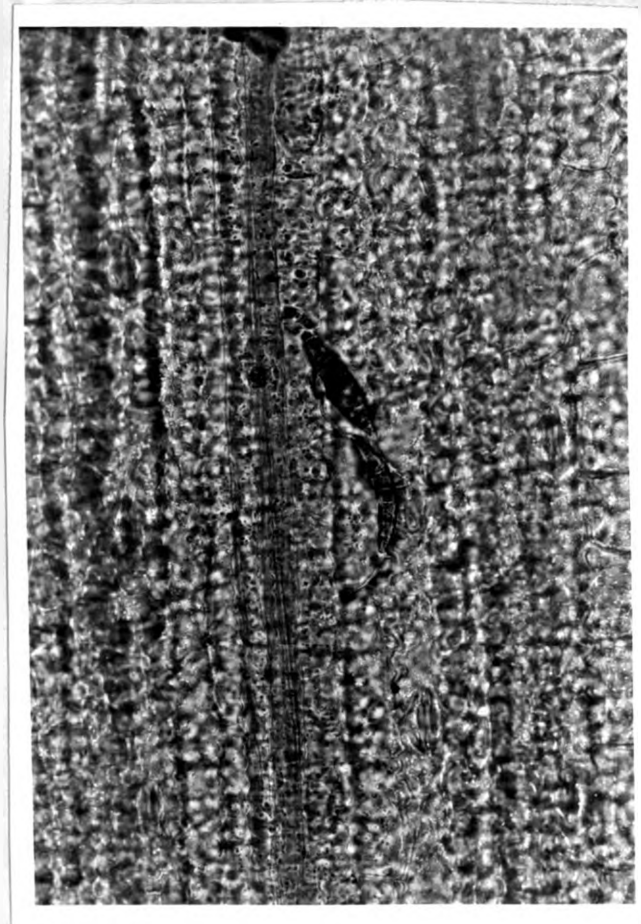


PLATE 7: Photomicrograph showing germinating conidia of Gaitu isolate of Helminthosporium maydis on Katumani Composite maize leaf 6 hours after inoculation of the leaf. (magnification x 200).

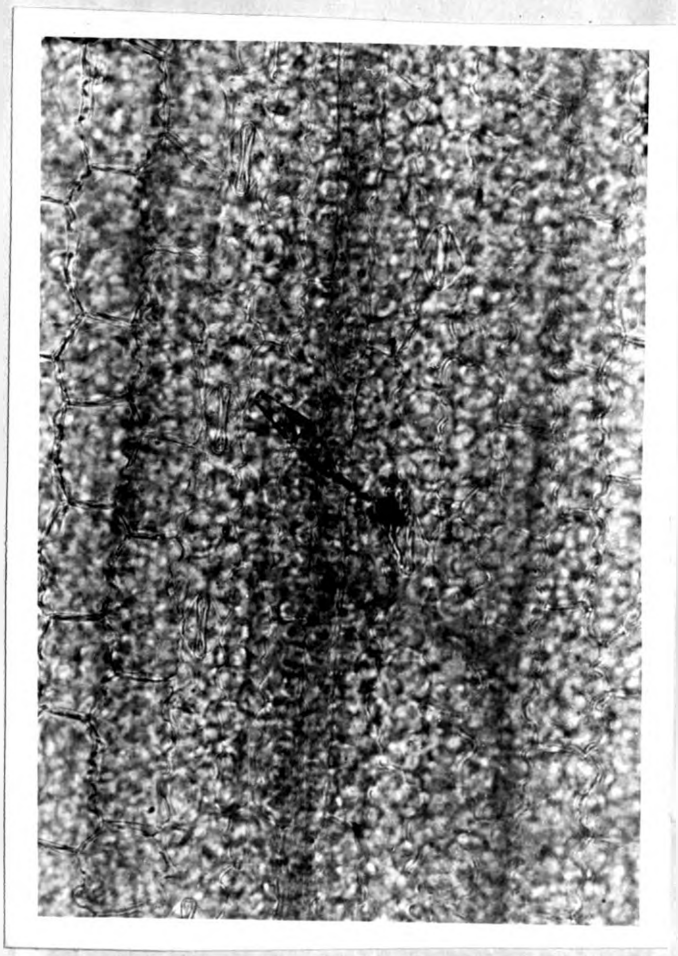


PLATE 8: Photomicrograph showing a germinating conidium of Gaitu isolate of Helminthosporium maydis on Katumani Composite maize leaf 18 hours after inoculation of the leaf. Note the formation of the appressorium very close to a stoma (magnification x 200).

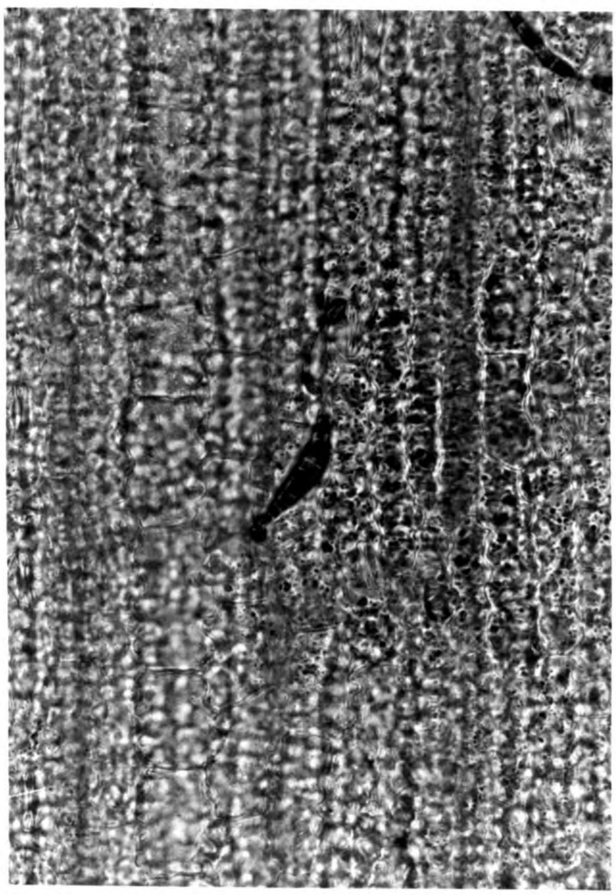


PLATE 9: Photomicrograph showing a germinating conidium of Gaitu isolate of Helminthosporium maydis on Katumani Composite maize leaf 30 hours after inoculation of the leaf. Note the formation of one of the appressoria very close to a stoma (magnification x 200).

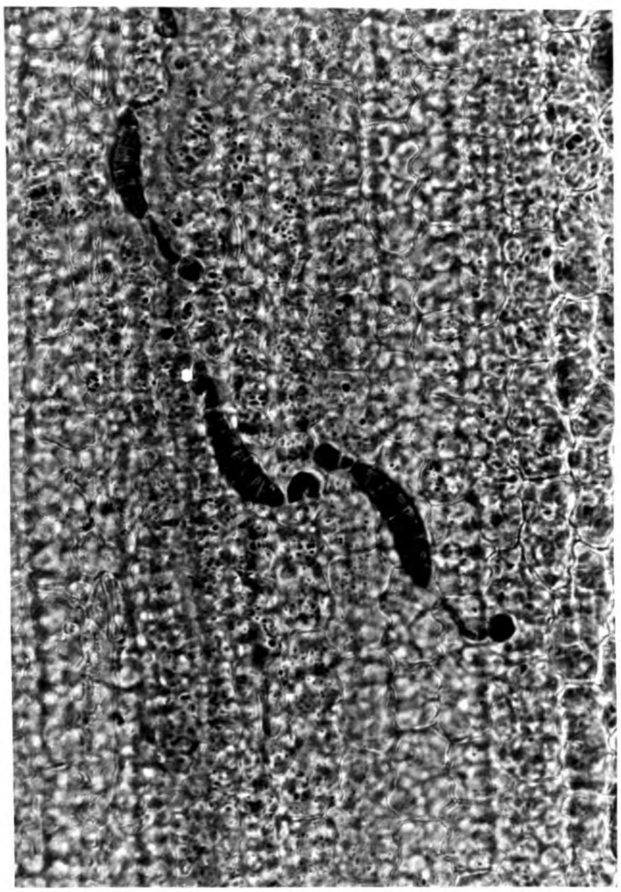


PLATE 10: Photomicrograph showing germinating conidia of Gaitu isolate of Helminthosporium maydis on Katumani Composite maize leaf 48 hours after inoculation of the leaf (magnification x 200).

conidia had germinated after 18 hours.

4.4. EFFECT OF TEMPERATURE ON GROWTH OF THE FUNGUS

The colony diameters of the three isolates on corn meal agar in petri dishes measured 10 days after inoculation are given in Table 5, and presented graphically in Figure 5. Growth occurred at all temperatures used except at 35°C. The highest growth rate was recorded at 30°C (83 mm) followed by growth at 25°C (60 mm), then at 20°C (53 mm) with the lowest growth rate occurring at 15°C (31 mm). Analysis of variance for the three isolates from Gaitu, Mwala, and Nithi showed no significant differences in radial growth among them at the four different temperatures (Appendix 1). Temperature however, had a highly significant effect on the radial growth rate. A comparison of the means of the temperature effects showed a significant difference between the highest and lowest temperature effect means i.e. between radial growth rate at 15°C and 30°C. Growth rates at 15, 20 and 25°C were not significantly different and neither were growth rates at 20, 25 and 30°C. The optimum growth occurred at 30°C

TABLE 5: EFFECT OF TEMPERATURE ON COLONY GROWTH OF THREE ISOLATES OF *HELMINTHOSPORIUM MAYDIS* ON CORN MEAL ACAR(COLONY DIAMETERS MEASURED IN MM 10 DAYS AFTER INOCULATION)

Treatments	Replicates I	II	III	IV	V	Treatment totals	Treatment means
T_1 I_1	39	31	32	37	31	170	34.00
I_2	36	27	33	38	38	172	34.40
I_3	34	32	35	37	38	176	35.20
T_2 I_1	52	46	53	47	50	248	49.60
I_2	49	50	48	51	45	243	48.60
I_3	51	49	49	52	47	248	49.60
T_3 I_1	54	55	59	58	59	285	57.00
I_2	56	53	57	58	60	284	56.80
I_3	56	57	45	57	55	270	54.00
T_4 I_1	64	67	77	82	83	373	74.60
I_2	51	73	67	52	66	309	61.80
I_3	70	69	74	38	70	321	64.20
Replicate totals	612	609	629	607	642	3099	

T_1 = 15°C; T_2 = 20°C; T_3 = 25°C; T_4 = 30°C; I_1 = Gaitu isolate; I_2 = Mwala isolate
I_3 = Nithi isolate.

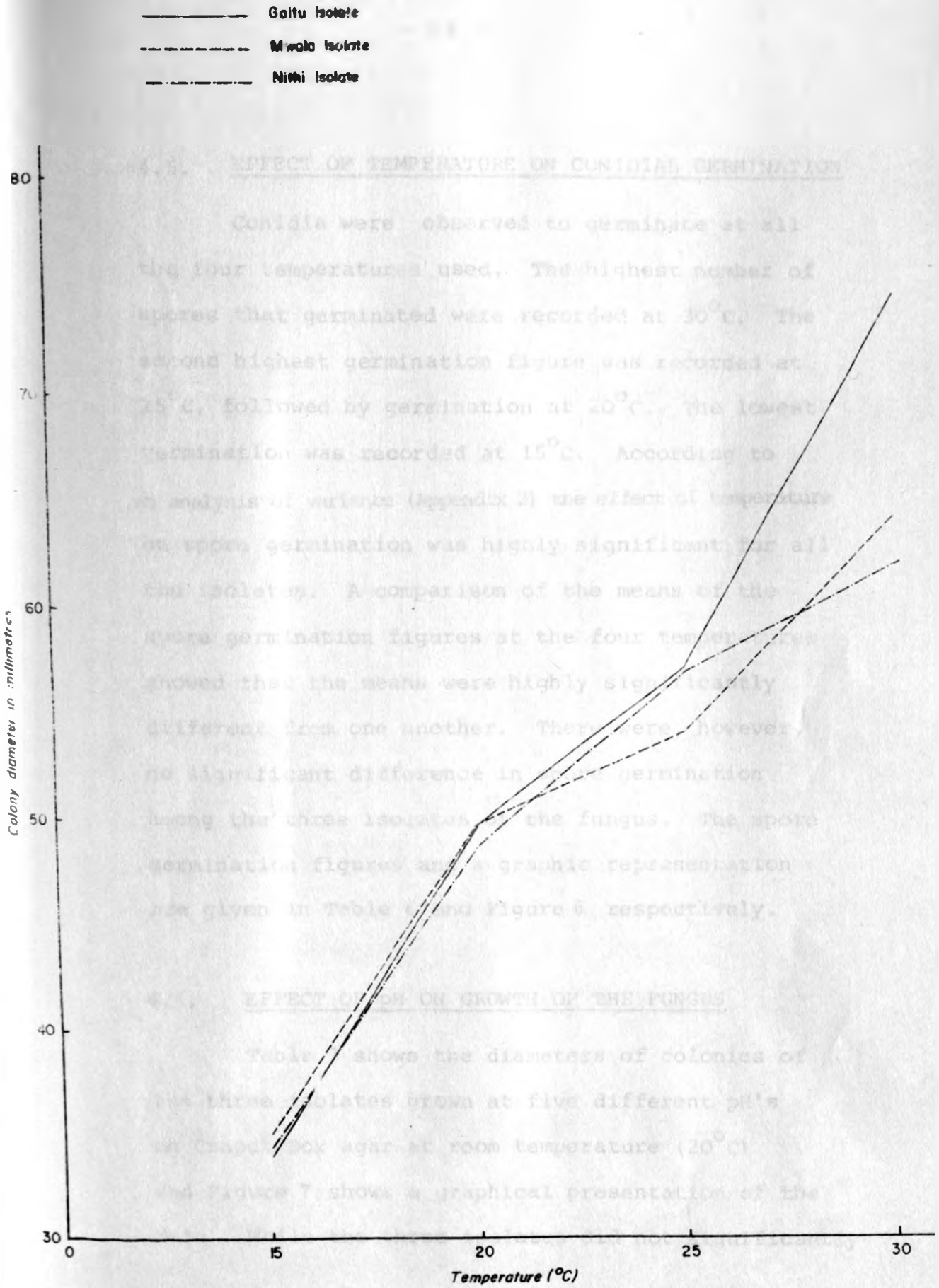


Fig.5 Effect of temperature on colony growth of three isolates of Helminthosporium maydis on corn meal agar (Colony diameters measured 10 days after inoculation)

4.5. EFFECT OF TEMPERATURE ON CONIDIAL GERMINATION

Conidia were observed to germinate at all the four temperatures used. The highest number of spores that germinated were recorded at 30°C. The second highest germination figure was recorded at 25°C, followed by germination at 20°C. The lowest germination was recorded at 15°C. According to an analysis of variance (Appendix 2) the effect of temperature on spore germination was highly significant for all the isolates. A comparison of the means of the spore germination figures at the four temperatures showed that the means were highly significantly different from one another. There were, however, no significant difference in spore germination among the three isolates of the fungus. The spore germination figures and a graphic representation are given in Table 6 and Figure 6 respectively.

4.6. EFFECT OF pH ON GROWTH OF THE FUNGUS

Table 7 shows the diameters of colonies of the three isolates grown at five different pH's on Czapek Dox agar at room temperature (20°C) and Figure 7 shows a graphical presentation of the data. While the three isolates did not significantly

TABLE 6: EFFECT OF TEMPERATURE ON CONIDIAL GERMINATION OF THREE ISOLATES OF *HELMINTHOSPORIUM MAYDIS* IN DISTILLED WATER (COUNTS OF CONIDIA THAT GERMINATED AFTER 14 HOURS IN STERILE DISTRILLED WATER).

Treatments	Replicates I	II	III	Treatment totals	Treatment means
T_1 I_1	51	53	50	154	51.33
I_2	48	51	48	147	49.00
I_3	50	49	46	145	48.33
T_2 I_1	55	53	54	162	54.00
I_2	52	56	55	163	54.33
I_3	53	50	52	155	51.67
T_3 I_1	78	80	79	337	79.00
I_2	75	79	77	231	77.00
I_3	77	81	75	233	77.67
T_4I_1	96	97	93	286	95.33
I_2	97	94	98	289	96.33
I_3	94	98	96	288	96.00
Replicate totals	826	841	823	2490	

T_1 = 15°C; T_2= 20°C; T_3 = 25°C; T_4= 30°C; I_1 = Gaitu isolate; I_2 = Mwala isolate; I_3 = Nithi isolate.

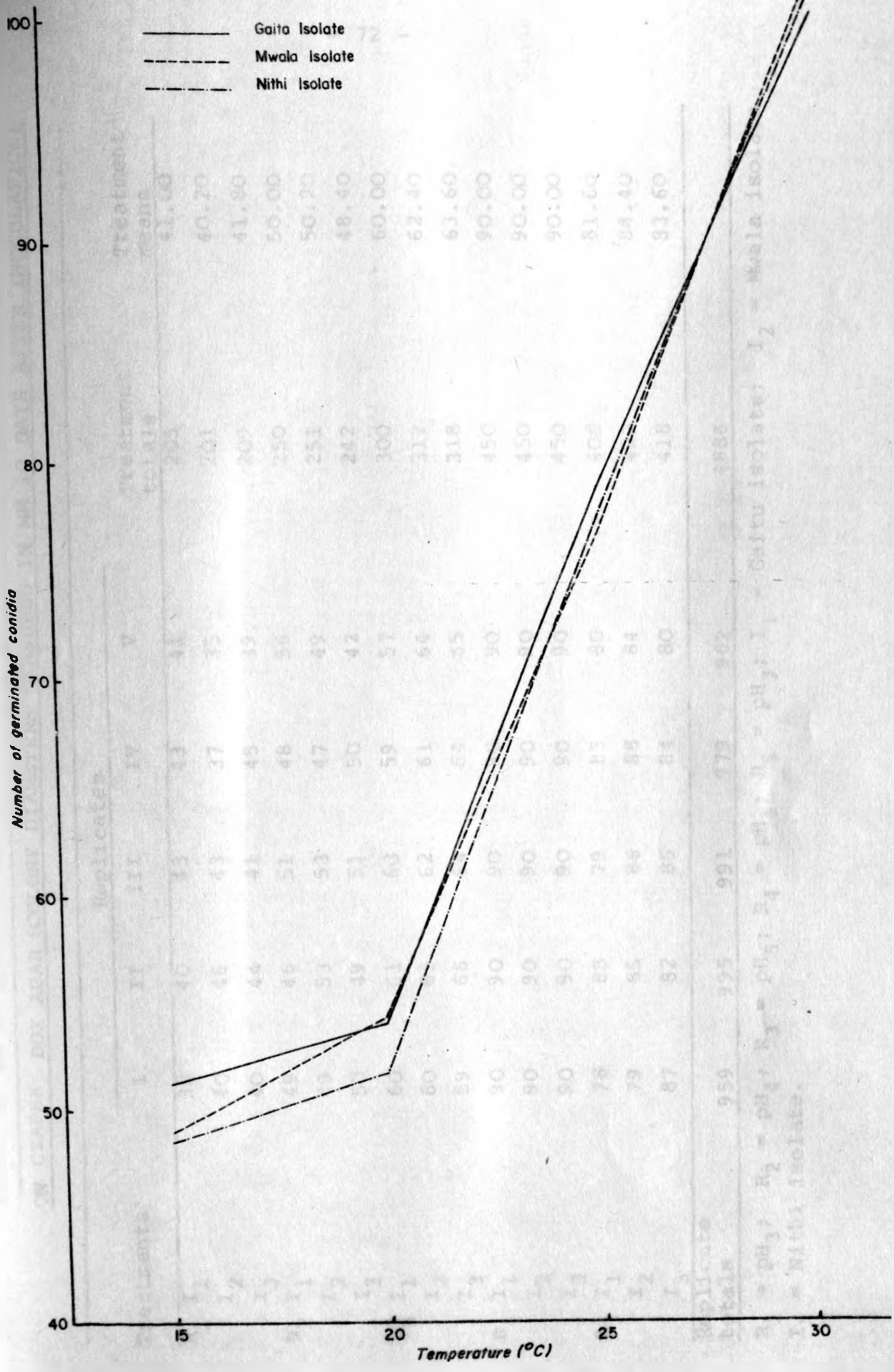


Fig.6 Effect of temperature on conidial germination of three isolates of Helminthosporium maydis (Counts of conidia that germinated after 14 hours in sterile distilled water)

TABLE 7: EFFECT OF pH ON THE GROWTH OF THREE ISOLATES OF HELMINTHOSPORIUM MAYDIS GROWN ON CZAPEK DOX AGAR (COLONY DIAMETERS MEASURED IN MM 10 DAYS AFTER INOCULATION)

Treatments	Replicates I	II	III	IV	V	Treatment totals	Treatment means
R_1 I_1	38	40	43	43	41	205	41.00
I_2	40	46	43	37	35	201	40.20
I_3	40	44	41	45	39	209	41.80
R_2 I_1	49	46	51	48	56	250	50.00
I_2	49	53	53	47	49	251	50.20
I_3	50	49	51	50	42	242	48.40
R_3 I_1	60	61	63	59	57	300	60.00
I_2	60	65	62	61	64	312	62.40
I_3	59	66	64	64	65	318	63.60
R_4 I_1	90	90	90	90	90	450	90.00
I_2	90	90	90	90	90	450	90.00
I_3	90	90	90	90	90	450	90.00
R_5 I_1	78	88	79	83	80	408	81.60
I_2	79	85	86	88	84	422	84.40
I_3	87	82	85	84	80	418	83.60
Replicate totals	959	995	991	979	962	4886	

R_1 = pH_3; R_2 = pH_4; R_3 = pH_5; R_4 = pH_6; R_5 = pH_7; I_1 = Gaitu isolate; I_2 = Mwala isolate I_3 = Nithi isolate.

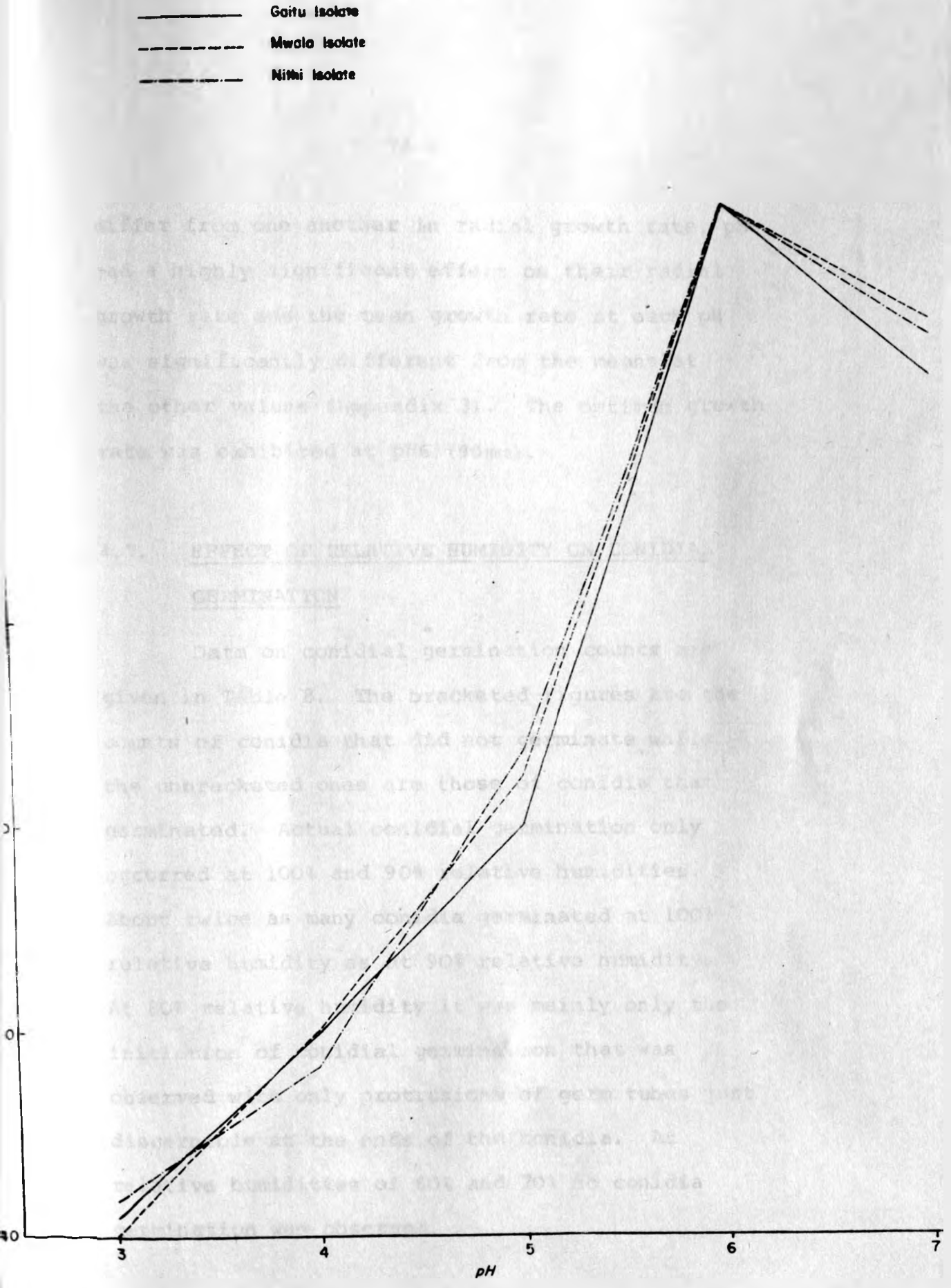


Fig.7 Effect of pH on growth of three isolates of Helminthosporium maydis grown on Czapek Dox agar (Colony diameters measured 10 days after inoculation)

differ from one another in radial growth rate, pH had a highly significant effect on their radial growth rate and the mean growth rate at each pH was significantly different from the means at the other values (Appendix 3). The optimum growth rate was exhibited at pH6 (90mm).

4.7. EFFECT OF RELATIVE HUMIDITY ON CONIDIAL GERMINATION

Data on conidial germination counts are given in Table 8. The bracketed figures are the counts of conidia that did not germinate while the unbracketed ones are those of conidia that germinated. Actual conidial germination only occurred at 100% and 90% relative humidities. About twice as many conidia germinated at 100% relative humidity as at 90% relative humidity. At 80% relative humidity it was mainly only the initiation of conidial germination that was observed with only protrusions of germ tubes just discernible at the ends of the conidia. At relative humidities of 60% and 70% no conidia germination was observed.

TABLE 8: COUNTS OF GERMINATED AND UNGERMINATED CONIDIA OF THREE HELMINTHOSPORIUM MAYDIS AT 5 DIFFERENT RELATIVE HUMIDITY VALUES.

Relative Humidity	Isolate		
	Gaitu	Mwala	Nithi
100	78(50)	84(56)	81(59)
90	32(156)	41(123)	35(100)
80	1(168)	6(200)	3(180)
70	0(170)	0(168)	0(160)
10	0(193)	0(155)	0(230)

4.8. FORMATION OF THE SEXUAL (ASCIGEROUS OR PERFECT) STAGE OF THE FUNGUS (*Cochliobolus heterostrophus* (Drechsler) Drechsler).

At the end of five weeks there was still no perithecial formation and hence no asci production on the maize leaf sections along the zone where the colonies of the paired isolates met. On the corn meal agar medium on which the maize leaf sections were placed there was also no perithecial formation. This observation applied to all the four replicates of each of the six pairings of the isolates.

4.9. REACTIONS OF 7 MAIZE HYBRIDS AND 1 COMPOSITE TO INFECTION BY *HELMINTHOSPORIUM MAYDIS* UNDER FIELD CONDITIONS.

Symptoms began to show on the leaves within 24 hours as tiny water-soaked spots. The spots later enlarged to form elliptical lesions delimited by leaf veins. Lesion colour varied with the maize hybrids and composite from greyish-tan to straw coloured. Some lesions showed a zonate pattern with purplish-brown margins. The lesions measured 0.5 x 0.5 to 3 x 10 mm.

Measurements of lesion size in square millimetres for all the maize hybrids and composite tested against all the isolates of the fungus are presented in Table 9. Analysis of variance showed no significant differences in lesion size due to isolates. Hybrids, however, had highly significant effects on lesion size (Appendix 4a). On Katumani Composite maize lesion size was larger compared to those of the hybrids. A separate analysis of variance was carried out which also showed no significant differences in lesion size due to the isolates (Appendix 4b). Among the hybrids average lesion size was highest for hybrid 512 and this was significantly different from the average of all the other six hybrids. Hybrids 622, 612 and 511 were not significantly different in average lesion size and neither were hybrids 632, 613C, 622 and 612. Average lesion size for hybrid 614C was not significantly different from those of hybrids 632 and 613C but differed significantly from the averages of all the other four hybrids. Taking lesion size as a measure of susceptibility to the disease, therefore, Katumani Composite was highly susceptible to the southern leaf blight while hybrids 511, 512, 612 and 622 were only moderately susceptible. Hybrids 613C,

TABLE 9: AVERAGE LESION SIZE ON LEAVES IN MM^2 AMONG 7 HYBRIDS AND 1 COMPOSITE OF MAIZE ARTIFICIALLY INOCULATED WITH THREE ISOLATES OF HELMINTHOSPORIUM MAYDIS IN THE FIELD

Treatments		Blocks I	II	III	Treatment totals	Treatment means
H632	I_1	3.49	1.95	2.96	8.40	2.80
	I_2	3.53	3.00	2.73	9.26	3.09
	I_3	3.00	3.13	2.81	8.94	2.98
H614	I_1	2.90	1.82	2.33	7.05	2.35
	I_2	3.11	2.00	1.75	6.86	2.29
	I_3	2.14	1.80	2.54	6.48	2.16
H512	I_1	5.01	5.37	6.11	16.49	5.50
	I_2	4.18	7.07	5.62	16.87	5.62
	I_3	5.51	5.23	6.25	16.99	5.66
H612	I_1	3.79	3.12	3.56	10.47	3.49
	I_2	3.76	4.67	3.27	11.70	3.90
	I_3	3.82	4.25	4.05	12.12	4.04
H613	I_1	4.00	2.42	3.15	9.57	3.19
	I_2	3.44	3.50	2.83	9.77	3.26
	I_3	3.31	2.44	3.00	8.75	2.92
H511	I_1	4.60	3.70	4.55	12.85	4.28
	I_2	4.18	5.10	4.77	14.05	4.68
	I_3	4.20	3.00	4.45	11.65	3.88
KAT	I_1	13.28	9.07	12.98	35.33	11.78
	I_2	13.40	15.40	10.05	38.85	12.95
	I_3	11.47	12.02	14.50	37.99	12.66
H622	I_1	3.49	3.37	2.94	9.80	3.27
	I_2	3.96	4.37	3.00	11.33	3.78
	I_3	3.45	3.20	4.23	10.88	3.63
Block totals		117.02	111.00	114.43	342.45	

I_1 = Gaitu isolate; I_2 = Mwala isolate; I_3 = Nithi isolate.

614 and 632 were somewhat resistant to the disease.

4.10. REACTIONS OF 7 MAIZE HYBRIDS AND 1 COMPOSITE TO INFECTION BY HELMINTHOSPORIUM MAYDIS IN THE GREEN HOUSE.

Symptom development in this experiment was the same as that in the field experiment described above with the symptoms becoming visible within 24 hours as tiny water-soaked spots. Analyses of variance were carried out for data collected on lesion size and plant height increases which are presented in Tables 10 and 11 respectively. The hybrids and composite did not have any significant difference in average lesion size (Appendix 5). Isolates of the fungus did not also effect any significant differences in average lesion size. Plant height increases determined 3 weeks after inoculation of the plant, however, showed highly significant differences due to hybrids and the composite (Appendix 6).

4.11. HOST RANGE

The results of the inoculation tests are presented in Table 12. Of all the twelve grass species and one sedge species used in the test only

TABLE 10: AVERAGE LESION SIZE ON LEAVES IN MM^2 AMONG 7 HYBRIDS AND 1 COMPOSITE OF MAIZE ARTIFICIALLY INOCULATED WITH THREE ISOLATES OF HELMINTHOSPORIUM MAYDIS IN THE GREENHOUSE

Treatment		Blocks I	Blocks II	Blocks III	Treatment totals	Treatment totals
H632	I_1	3.80	3.50	2.75	10.05	3.35
	I_2	3.16	3.45	3.00	9.61	3.20
	I_3	2.65	2.99	3.21	8.85	2.95
KAT.	I_1	4.41	4.24	2.11	10.76	3.59
	I_2	3.02	3.71	3.52	10.25	3.42
	I_3	3.24	3.46	3.40	10.10	3.37
H612	I_1	5.09	3.47	3.04	10.60	3.53
	I_2	2.82	3.60	3.98	10.40	3.47
	I_3	3.54	3.16	3.73	10.43	3.48
H614	CI_1	4.65	3.55	3.36	11.56	3.85
	I_2	2.72	3.44	3.99	10.15	3.38
	I_3	3.77	3.63	3.25	10.65	3.55
H511	I_1	4.23	3.06	3.16	10.45	3.48
	I_2	3.32	3.67	3.72	10.71	3.57
	I_3	3.03	3.25	3.45	9.73	3.24
H512	I_1	4.58	3.68	2.57	10.83	3.61
	I_2	2.96	3.58	3.87	10.41	3.47
	I_3	3.68	3.78	4.15	11.61	3.87
H613	CI_1	3.69	3.45	3.43	10.57	3.52
	I_2	3.83	3.74	3.55	11.12	3.71
	I_3	3.70	3.33	3.46	10.49	3.50
H622	I_1	3.98	3.68	3.56	11.22	3.74
	I_2	3.66	3.45	3.70	10.81	3.60
	I_3	3.50	3.36	3.83	10.69	3.56
Block totals		87.03	83.23	81.79		

I_1 = Gaitu isolate; I_2 = Mwala isolate; I_3 = Nithi isolate.

TABLE 11: PLANT HEIGHT INCREASES IN CM AMONG 7 MAIZE HYBRIDS AND 1 COMPOSITE THREE WEEKS AFTER INOCULATION WITH THREE ISOLATES OF HELMINTHOSPORIUM MAYDIS IN THE GREENHOUSE

Treatments	Blocks I	II	III	Treatment totals	Treatment means
H632 I_1	12.2	11.5	11.9	35.6	11.87
I_2	12.2	12.7	16.0	40.9	13.63
I_3	15.5	14.1	15.8	45.4	15.13
KAT. I_1	32.0	25.2	22.1	79.3	26.43
I_2	18.1	26.7	29.0	73.8	24.60
I_3	19.5	20.4	17.5	57.4	19.13
H612 I_1	22.1	21.8	17.2	61.1	20.37
I_2	20.05	20.3	16.2	57.0	19.00
I_3	27.3	24.6	22.8	74.7	24.90
H614CI I_1	20.06	19.7	17.5	57.8	19.27
I_2	18.8	15.9	12.6	47.3	15.77
I_3	18.0	22.8	26.9	67.7	22.57
H511 I_1	18.0	24.9	8.0	50.9	16.97
I_2	27.0	26.5	24.2	77.7	25.90
I_3	35.3	28.5	27.7	91.5	30.50
H512 I_1	23.0	22.7	28.8	74.5	24.83
I_2	25.1	20.3	21.9	67.3	24.43
I_3	24.8	27.1	30.3	82.2	27.4
H613CI I_1	15.5	21.8	20.2	57.5	19.17
I_2	26.7	23.6	20.5	70.8	23.60
I_3	17.5	19.2	23.4	60.1	20.03
H622 I_1	16.3	19.5	18.3	54.1	18.03
I_2	20.1	20.6	18.5	59.2	19.73
I_3	10.2	16.7	11.0	37.9	12.63
Block totals	496.30	507.10	478.30	1481.70	

I_1 = Gaitu isolate; I_2 = Mwala isolate; I_3 = Nithi isolate.

TABLE 12: HOST REACTION TO INOCULATION WITH THREE ISOLATES OF HELMINTHOSPORIUM MAYDIS.

Host plant species inoculated	Isolate		
	Gaitu	Mwala	Nithi
Eragrostis pilosa	O	O	O
Triticum aestivum	O	O	O
Hardeum vulgare	O	O	O
Avena sativa	O	O	O
Sorghum vulgare	O	O	O
Digitaria malanjiana	O	O	O
Bothriochloa julcupta	O	O	O
Panicum maximum	O	O	O
Pennisetum trisetum	O	O	O
Setaria sphacelata	O	O	O
Setaria verticillata	O	O	O
Rottboellia exaltata	+	+	+
Eleusine indica	O	O	O
Cyperus rotundus	O	O	O

Descriptions of abbreviations: O = Healthy plant (no reaction);

+ = Slight infection (one or two lesions on infected leaves).

one grass species, Rottboellia exaltata was infected. All three isolates of the fungus infected this grass. The reaction of R. exaltata was very slight showing only one or two lesions developing on some of the lower leaves. Helminthosporium maydis that was used for inoculation was re-isolated from the lesions on the infected leaves.

CHAPTER 5

5. DISCUSSION

The results of the disease survey carried out during a drought did not show the same widespread occurrence reported in Kenya by Singh et al. (1979) who carried out their surveys during rainy periods. Disease incidence was also very low and there was only slight infection. These results seem to support the observations of Schenk and Stelter (1973) which indicated that southern leaf blight epidemics are favoured by warm, moist weather. Disease symptoms on maize leaves were similar to those described by Drechsler (1925) and Ullstrup (1944). Symptoms were identical for all isolates.

The colonial characteristics of Helminthosporium maydis that were studied which included colony shape, texture, zonation and formation of sporocarps or characteristic resting structures were similar for all isolates. This together with the disease symptom observations would suggest that the three isolates were similar and probably of the same race. The existence of two different races (T and O) of the fungus was first suggested by the observations

of Scheifele et al. (1970) on male sterile cytoplasm (Tms) maize in the US and later confirmed by Hooker et al. (1970). Observations on conidial colour, length, width and number of septa supported those reported by Drechsler (1925), Luttrell (1951) and Kenneth (1958). These observations were similar for all the isolates studied thus suggesting again that all the isolates were similar. The bipolar germination of the conidia of Helminthosporium maydis as observed during this research had earlier been reported by several researchers including Drechsler (1925), Ullstrup (1944), and Shoemaker (1959). The observations on the formation of appressoria is in line with those of Wheeler (1977). The appressoria form within six hours from inoculation and this probably explains why the pathogen is able to infect a susceptible host and produce visible symptoms within just twenty four hours. Although it was not possible through the microscopic examinations of inoculated plant tissue carried out in this research to ascertain whether or not there was actual penetration of host tissue by the pathogen below the appressoria, the common occurrence of these appressoria close to the stomata clearly suggests that these appertures probably influence

penetration by the pathogen. Proximity of conidia to stomata alone does not seem to explain the formation of the appressoria close to the stomata. In fact there were several instances when elongating germ tubes of conidia located far from stomata did not form appressoria until they reached some point on or near a stoma.

Temperature, humidity and pH had the same effects on all three isolates of Helminthosporium maydis studied. The optimum temperatures for colony growth and conidial germination were the same ($30^{\circ}C$) while the optimum pH for colony growth was 6. The greatest number of spores germinated between 90 and 100% relative humidities. These observations show the need for warm and moist weather for disease development. There was no literature available on the effects of these factors on the fungus. Nevertheless, the similarity of observations of their effects on the three isolates of the fungus further suggests that the isolates were probably of the same race.

The failure of perithecia to form on the leaf sections even after five weeks of incubation in a moist chamber probably shows how difficult it is to

obtain the perfect (ascigerous) stage of H. maydis under artificial conditions. It would also seem to suggest that all the pairings of the isolates on the maize leaf sections were of incompatible mating types as observed by Nelson (1957). Schenck (1970) was also unsuccessful in his attempts to obtain perithecia on the leaf blade after leaving blighted maize leaves ranging from the earliest infection up to senescence for up to four weeks in moist chambers and although he had reported the common occurrence of perithecia on maize leaves in the field these were found only at the junction of the leaf sheath and the blade but not on the leaf blade.

Disease symptom development as observed in this study both in the field and in the greenhouse on artificially inoculated maize plants was the same as that reported by Ullstrup (1944) and Hilty and Josephson (1971). Throughout the disease symptom observations in the field up to plant maturity no symptoms developed on any other parts other than the leaves. Even during the disease surveys in all infected fields lesions were observed only on leaves. The isolates of the fungus used in this study could thus belong to race 0 of the fungus which

according to Ullstrup (1972) and Russel (1978) is mainly a leaf pathogen.

Lesion colour and size varied with the maize hybrid/composite or variety as reported by Hilty and Josephson (1971) but not with the fungus isolate. The high susceptibility of Katumani Composite maize to H. maydis has not been reported before in Kenya but another composite, Coast Composite, has earlier been reported to have been seriously damaged Singh et al., 1979). The relative resistance of hybrid 632, however, contrasts the reports of Singh et al. (1979) of severe damage on the hybrid. The high susceptibility of Katumani Composite maize to H. maydis may not be a very serious threat in Kenya since the composite is one of those bred for the drier areas of the country where most of the year conditions of moisture are not favourable for the disease development. The significant differences in plant height increases in the greenhouse inoculation experiment seem to be due more to variety differences in growth rates than to susceptibility differences among the hybrids and composite since there were no significant differences in lesion size and hence virulence among them.

The infection of Rottboellia exaltata by

H. maydis as observed in the host range study had earlier been reported by Orillo (1952) in artificial inoculation studies. The failure of this fungus to infect Triticum aestivum, Hordeum vulgare, Avena sativa, and Sorghum vulgare, however, contrasts the report of Robert (1962) which indicated the susceptibility of these species to the fungus. Robert (1962) also reported Helminthosporium maydis infections on Pennisetum typhoides and Setaria italica while Nisikado and Miyake (1926) reported the fungus infection on Setaria glauca but related grass species including P. trisetum, S. sphacelata and S. verticillata used in this study were not infected. There have been no reports of studies on the susceptibility of Cyperus rotundus, Digitaria malanjiana, Bothriochloa julcupta, Panicum maximum, and Eleusine indica which were not infected by H. maydis in this study. The non-susceptibility of the sedge and grass species in this study together with the contrasting reports of host range studies indicate that the cereals named above and the wild grasses and sedge species are probably not highly susceptible to H. maydis and so may not be important as hosts of the pathogen.

CHAPTER 6

6. CONCLUSIONS

The disease survey carried out during a hot dry period shows that epidemics of southern leaf blight of maize caused by Helminthosporium maydis are not favoured by dry weather. The greatest number of conidia germinate at 30°C and between 90 and 100% relative humidities. The three isolates of the fungus studied are probably of the same race since they incited identical disease symptoms on the maize hybrids and composite inoculated and showed similar colonial characteristics. The optimum temperature for colony growth was also 30°C while the optimum pH was 6. The race of the pathogen could be O which attacks mainly leaves of susceptible maize varieties. The conidia germinate by two polar germ tubes and penetration of maize leaf tissue seems to be influenced by stomata near which appressoria form.

The perithecial (ascigerous or perfect) stage of the fungus is very difficult to obtain under artificial conditions in the laboratory. The virulence of H. maydis varies with the maize variety

and this is due to the maize genotype. If it can be confirmed that race O of the pathogen is the race that is prevalent in Kenya then the inclusion of maize inbreds with good resistance in maize breeding programs will effectively check the disease. Under light infections such as was the case during this study plant height increase is probably influenced more by varietal differences in plant growth rates than by disease infection. Other cereals like wheat, barley and oats, and wild grasses do not seem to pose a serious danger of being alternate hosts to the fungus as almost all of those inoculated were not susceptible to the pathogen in this study. Due to very limited time that was available for this research it was not possible to determine the effect of the disease on maize yield and there is also need for a proper national survey of the disease at the right time and right places.

APPENDICES

APPENDIX 1: EFFECT OF TEMPERATURE ON GROWTH OF THREE ISOLATES OF HELMINTHOSPORIUM MAYDIS ON CORN MEAL AGAR.

ANALYSIS OF VARIANCE

Source of variation	df	SS	MSS	Fcal.
Total	60	170737.00		
Level	1	160063.35		
Replicates	4	76.57		
Treatments	11	8726.45	793.31	18.66**
Temperature	3	8228.32	2742.77	64.52**
Isolate	2	139.90	69.95	1.65 NS
Temperature x Isolate	6	358.23	59.71	1.40 NS
Error	44	1870.63	42.51	

**Significant at 1% level (highly significant); NS = not significant.

DUNCAN'S MULTIPLE RANGE TEST (1%)

$S\bar{x}$ = $\sqrt{\text{Error mean square}/r} = \sqrt{42.51/5} = 8.5$

Values of p:	2	3	4
SSR	3.82	3.99	4.10
LSR	32.47	33.27	34.85

Temperature (^{o}C):	15	20	25	30
Means of colony diameter:	34.53	49.27	55.93	66.87

DUNCAN'S MULTIPLE RANGE TEST (5%)

Values of p:	2	3	4
SSR	2.86	3.01	3.10
LSR	24.31	25.59	26.35

Temperature (^{o}C):	15	20	25	30
Means of colony diameter:	34.53	49.27	55.93	66.87

APPENDIX 2: EFFECT OF TEMPERATURE ON CONIDIAL GERMINATION OF THREE ISOLATES OF HELMINTHOSPORIUM MAYDIS IN DISTILLED WATER.

ANALYSIS OF VARIANCE

Source of Variation	df	SS	MSS	Fcal.
Total	36	185178.00		
Level	1	172225.00		
Replicates	2	15.49		
Treatments	11	12864.33	1169.48	351.20**
Temperature	3	2829.00	4276.33	1284.18**
Isolates	2	13.50	6.75	2.03 NS
Isolate x temperature	6	21.83	3.64	1.09 NS
Error	22	73.18	3.33	

**Significant at 1% level (highly significant); NS = not significant.

DUNCAN'S MULTIPLE RANGE TEST (1%)

$S\bar{x}$ = √Error mean square/r = $\sqrt{\frac{3.33}{3}}$ = 1.05

Value of p :	2	3	4
SSR :	3.99	4.17	4.28
LSR :	4.19	4.38	4.49

Temperature (^{O}C):	15	20	25	30
Conidial germination means:	49.55	53.33	77.89	95.89

APPENDIX 3: EFFECT OF pH ON THE GROWTH OF THREE ISOLATES OF HELMINTHOSPORIUM MAYDIS ON CZAPEK DOX AGAR.

ANALYSIS OF VARIANCE

Source of Variation	df	SS	MSS	Fcal.
Total	75	345552.00		
Level	1	318306.61		
Replicates	4	71.52		
Treatments	14	26775.79	1912.56	269.00**
pH	4	26705.26	6676.32	939.00**
Isolates	2	14.75	7.38	1.04 NS
Isolates x pH	8	55.78	6.97	0.98 NS
Error	56	398.08	7.11	

**Significant at 1% level (highly significant); NS = not significant.

DUNCAN'S MULTIPLE RANGE TEST (1%)

$S\bar{x}$ = √error mean square/r $= \sqrt{\frac{7.11}{2}} = 1.19$

Value of p :	2	3	4	5
SSR :	3.76	3.92	4.03	4.12
LSR :	4.47	4.66	4.80	4.90

pH :	3	4	5	6	7
Colony diameter means	41	49	62	90	83

APPENDIX 4a: AVERAGE LESION SIZE ON LEAVES AMONG 7 HYBRIDS AND 1 COMPOSITE OF MAIZE ARTIFICIALLY INOCULATED WITH THREE ISOLATES OF HELMINTHOSPORIUM MAYDIS IN THE FIELD.

ANALYSIS OF VARIANCE

Source of Variation	df	SS	MSS	Fcal.
Total	63	922.07		
Level	1	841.73		
Blocks	2	0.45		
Treatments	20	64.34	3.22	8.26**
Hybrids	6	62.05	10.34	26.51**
Isolates	2	0.71	0.36	0.92^{NS}
Hybrid x Isolates	12	1.58	0.13	0.33^{NS}
Error	40	15.55	0.39	

**Significant at 1% level (highly significant); NS = not significant.

DUNCAN'S MULTIPLE RANGE TEST (1%)

$S\bar{x}$ = error mean square/r = $\frac{0.39}{3}$ = 0.36

Value of p :	2	3	4	5	6	7
SSR :	3.82	3.99	4.10	4.17	4.24	4.30
LSR :	1.38	1.44	1.46	1.50	1.53	1.55

Maize hybrid:	614C	632	613c	622	612	511	512
Mean lesion size:	2.27	2.96	3.12	3.56	3.81	4.28	5.59

DUNCAN'S MULTIPLE RANGE TEST (5%)

Value of p :	2	3	4	5	6	7
SSR :	2.86	3.01	3.10	3.17	3.22	3.27
LSR :	1.03	1.08	1.12	1.14	1.16	1.18

Maize hybrid:	614C	632	613C	622	612	511	512
Mean lesion size:	2.27	2.96	3.12	3.56	3.81	4.28	5.59

APPENDIX 4b. AVERAGE LESION SIZE ON LEAVES OF KATUMANI COMPOSITE MAIZE PLANTS INOCULATED WITH THREE ISOLATES OF HELMINTHOSPORIUM MAYDIS IN THE FIELD.

ANALYSIS OF VARIANCE

Source of Variation	df	SS	MSS	Fcal.
Total	9	1431.12		
Level	1	1398.01		
Blocks	2	0.47	0.25	0.03 NS
Treatments	2	2.25	1.13	0.15 NS
Error	4	30.39	7.60	

NS = not significant.

APPENDIX 5. AVERAGE LESION SIZE ON LEAVES AMONG 7 MAIZE HYBRIDS AND 1 COMPOSITE ARTIFICIALLY INOCULATED WITH THREE ISOLATES OF HELMINTHOSPORIUM MAYDIS IN THE GREENHOUSE.

ANALYSIS OF VARIANCE

Source of Variation	df	SS	MSS	Fcal.
Total	12	900.14		
Level	1	882.35		
Blocks	2	0.61		
Treatments	23	2.78	0.12	0.39 NS
Hybrid/Composite	7	1.55	0.22	0.71 NS
Isolate	2	0.27	0.14	0.45 NS
Hybrid/Composite x Isolate	14	0.96	0.07	0.23 NS
Error	46	14.4	0.31	

NS = Not significant

APPENDIX 6: PLANT HEIGHT INCREASES AMONG 7 MAIZE HYBRIDS AND 1 COMPOSITE 3 WEEKS AFTER INOCULATION WITH THREE ISOLATES OF HELMINTHOSPORIUM MAYDIS IN THE GREENHOUSE.

ANALYSIS OF VARIANCE

Source of Variation	df	SS	MSS	Fcal.
Total	72	32689.83		
Level	1	30492.15		
Blocks	2	17.64		
Treatments	23	1637.93	71.21	6.04**
Hybrid/Composite	7	972.02	138.86	11.78**
Isolate	2	44.28	22.14	1.88 NS
Hybrid/Composite x Isolate	14	621.63	44.40	3.77**
Error	46		11.79	

**Significant at 1% level (highly significant); NS = not significant

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